ISSN 1007-9327 (print)
ISSN 2219-2840 (online)

World Journal of *Gastroenterology*

World J Gastroenterol 2019 August 7; 25(29): 3838-4042

Published by Baishideng Publishing Group Inc

World Journal of Gastroenterology

Contents

Weekly Volume 25 Number 29 August 7, 2019

Contents

World Journal of Gastroenterology

Volume 25 Number 29 August 7, 2019

ABOUT COVER

Editorial board member of *World Journal of Gastroenterology*, Herwig Cerwenka, MD, Professor, Department of Surgery, Medical University of Graz, Graz A-8036, Austria

AIMS AND SCOPE

World Journal of Gastroenterology (*World J Gastroenterol*, *WJG*, print ISSN 1007-9327, online ISSN 2219-2840, DOI: 10.3748) is a peer-reviewed open access journal. The *WJG* Editorial Board consists of 701 experts in gastroenterology and hepatology from 58 countries.

The primary task of *WJG* is to rapidly publish high-quality original articles, reviews, and commentaries in the fields of gastroenterology, hepatology, gastrointestinal endoscopy, gastrointestinal surgery, hepatobiliary surgery, gastrointestinal oncology, gastrointestinal radiation oncology, *etc*. The *WJG* is dedicated to become an influential and prestigious journal in gastroenterology and hepatology, to promote the development of above disciplines, and to improve the diagnostic and therapeutic skill and expertise of clinicians.

INDEXING/ABSTRACTING

The *WJG* is now indexed in Current Contents®/Clinical Medicine, Science Citation Index Expanded (also known as SciSearch®), Journal Citation Reports®, Index Medicus, MEDLINE, PubMed, PubMed Central, and Scopus. The 2019 edition of Journal Citation Report® cites the 2018 impact factor for *WJG* as 3.411 (5-year impact factor: 3.579), ranking *WJG* as 35th among 84 journals in gastroenterology and hepatology (quartile in category Q2). CiteScore (2018): 3.43.

RESPONSIBLE EDITORS FOR THIS ISSUE

Responsible Electronic Editor: *Yu-Jie Ma*

Proofing Production Department Director: *Yun-Xiaojian Wu*

NAME OF JOURNAL
World Journal of Gastroenterology

ISSN
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

LAUNCH DATE
October 1, 1995

FREQUENCY
Weekly

EDITORS-IN-CHIEF
Subrata Ghosh, Andrzej S. Tarnawski

EDITORIAL BOARD MEMBERS
http://www.wjgnet.com/1007-9327/editorialboard.htm

EDITORIAL OFFICE
Ze-Mao Gong, Director

PUBLICATION DATE
August 7, 2019

INSTRUCTIONS TO AUTHORS
https://www.wjgnet.com/bpg/gerinfo/204

GUIDELINES FOR ETHICS DOCUMENTS
https://www.wjgnet.com/bpg/GerInfo/287

GUIDELINES FOR NON-NATIVE SPEAKERS OF ENGLISH
https://www.wjgnet.com/bpg/gerinfo/240

PUBLICATION MISCONDUCT
https://www.wjgnet.com/bpg/gerinfo/208

ARTICLE PROCESSING CHARGE
https://www.wjgnet.com/bpg/gerinfo/242

STEPS FOR SUBMITTING MANUSCRIPTS
https://www.wjgnet.com/bpg/GerInfo/239

ONLINE SUBMISSION
https://www.f6publishing.com

World Journal of Gastroenterology

Submit a Manuscript: https://www.f6publishing.com

World J Gastroenterol 2019 August 7; 25(29): 3838-3841

DOI: 10.3748/wjg.v25.i29.3838

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

EDITORIAL

Healthy axis: Towards an integrated view of the gut-brain health

Federico Boem, Amedeo Amedei

ORCID number: Federico Boem (0000-0003-4339-6431); Amedeo Amedei (0000-0002-6797-9343).

Author contributions: Boem F conceived the study and wrote the manuscript; Amedei A conceived the study and drafted the manuscript. Both authors approved the final version of the article.

Conflict-of-interest statement: The authors have no conflict of interest to declare.

Manuscript source: Invited manuscript

Received: May 7, 2019
Peer-review started: May 7, 2019
First decision: June 9, 2019
Revised: June 14, 2019
Accepted: June 22, 2019
Article in press: June 23, 2019
Published online: August 7, 2019

P-Reviewer: Abalo R, Orel R, Rostami-Nejad M
S-Editor: Ma RY
L-Editor: A

Federico Boem, Department of Oncology and Hemato-Oncology, University of Milan, Milano 20122, Italy

Amedeo Amedei, Department of Experimental and Clinical Medicine, University of Florence, Firenze 50134, Italy

Amedeo Amedei, Department of Biomedicine, Azienda Ospedaliera Universitaria Careggi, Firenze 50134, Italy

Corresponding author: Amedeo Amedei, BSc, Associate Professor, Department of Experimental and Clinical Medicine, University of Florence, Largo Brambilla 03, Firenze 50134, Italy. amedeo.amedei@unifi.it
Telephone: +39-55-2758330
Fax: +39-55-2758330

Abstract

Despite the lack of precise mechanisms of action, a growing number of studies suggests that gut microbiota is involved in a great number of physiological functions of the human organism. In fact, the composition and the relations of intestinal microbial populations play a role, either directly or indirectly, to both the onset and development of various pathologies. In particular, the gastrointestinal tract and nervous system are closely connected by the so-called gut–brain axis, a complex bidirectional system in which the central and enteric nervous system interact with each other, also engaging endocrine, immune and neuronal circuits. This allows us to put forward new working hypotheses on the origin of some multifactorial diseases: from eating to neuropsychiatric disorders (such as autism spectrum disorders and depression) up to diabetes and tumors (such as colorectal cancer). This scenario reinforces the idea that the microbiota and its composition represent a factor, which is no longer negligible, not only in preserving what we call "health" but also in defining and thus determining it. Therefore, we propose to consider the gut-brain axis as the focus of new scientific and clinical investigation as long as the locus of possible systemic therapeutic interventions.

Key words: Microbiota; Gut-brain axis; Dysbiosis; Symbiosis; Person-centered medicine; Personalized medicine

Core tip: The interest for gut-microbiota is rapidly increasing due to its impact on many physiological and pathological functions. In particular, gut-brain axis, in which commensal microorganisms' impact, in interplay with immune and endocrine systems,

E-Editor: Ma YJ

might be a tool and a focus of both scientific investigation and therapeutic interventions. Accordingly, here, by focusing on some examples of multifactorial conditions, such as obesity, we advocate for a redefined health account, in eco-systemic terms, in order to promote a new way of considering the detection of and the approach to diseases. A healthy axis could become part of a more effective perspective towards both person-centered medicine and personalized medicine.

Citation: Boem F, Amedei A. Healthy axis: Towards an integrated view of the gut-brain health. *World J Gastroenterol* 2019; 25(29): 3838-3841
URL: https://www.wjgnet.com/1007-9327/full/v25/i29/3838.htm
DOI: https://dx.doi.org/10.3748/wjg.v25.i29.3838

INTRODUCTION

The term microbiota usually refers to the community of different microorganisms populating specific ecological niches within the human body (*e.g.*, in the gastro-enterological system, the gut microbiota), thus forming a mutually advantageous relationship, often called symbiosis, with the host[1]. Especially in the gut (the most investigated area), microbiota contributes to the maintenance of its integrity, taking part into energy harvesting from food, constituting the first barrier against pathogens and modulating the immune responses[2].

The composition of gut microbiota (GM) is quite complex and can undergo changes (more or less radical) in response to both exogenous (*e.g.*, life style and habits, interactions with pathogens and/or chemicals, environmental agents) and endogenous factors (such as genetic profile)[2-4].

Notably, the number of microorganisms living in the intestines outnumber the cells of our organism. Additionally, more than 1000 bacterial species reside in the human gut, primarily situated within distal ileum and colon, predominantly belonging to Bacteroidetes and Firmicutes phyla[1]. Nevertheless, as already mentioned, the composition could be highly variable, dynamic, and susceptible to rapid changes in response to external factors or perturbations in health.

In fact, an increasing number of studies suggests that the microbiota (and especially the GM) plays a role in shaping a vast number of physiological functions of the human organism.

For example, the dysbiosis, a structural and compositional imbalance in intestinal microbial populations, can contribute, either directly or indirectly, to both the onset and development of various pathologies. This allows us to put forward new working hypotheses on the origin of some multifactorial diseases: From eating to neuropsychiatric disorders (such as autism spectrum disorders and depression)[5], up to diabetes and tumors (such as colorectal cancer)[6-8].

This scenario reinforces the idea that the microbiota and its composition represent a factor, which is no longer negligible, not only in preserving what we call "health" but also in defining and thus determining it[9].

Indeed, from the perspective of the gastroenterologist, the increasing relevance of the microbiota impact in the understanding some pathological disorders, offer also a chance to reconsider the boundaries of current clinical analysis, towards the embrace of a more systemic mindset on both health and disease, with an eye to therapeutic interventions[9].

STUDY ANALYSIS

It is known that biological functions are modulated by the interaction with the environment. However, the very notion of symbiosis and its implications challenge the mainstream view concerning the sharp distinction between external and internal factors[10]. Since decades, ecologists warned us that the environment is not just a container or simply a background in which biological species live and exist. The relationship between the host and its microbiota does not simply take place within the environment. It rather constructs it. Furthermore, at the microbiota level, both commensal and non-commensal microbe species take part (either cooperating or struggling among each other's) to specific niches construction, thus constituting a crucial node of that intricate ecosystem that is the human body. If we accept an

ecological view of health, it seems reasonable to reconsider clinical approaches in a more organismic (*i.e.*, the body is more than the sum of its parts/organs) and systemic way[10,11].

Indeed, novel studies confirm that the gastrointestinal tract and nervous system (particularly the encephalon) are closely connected by the so called gut–brain axis, a complex bidirectional system in which the central and enteric nervous system interact with each other, also engaging endocrine, immune and neuronal circuits[1,4,12]. Indeed, the articulation of these relationships and the functions of this axis are also modulated by the gut microbiota, thus pushing to reconsider the idea that microbial activity is circumscribed only to the intestines. Because of that the concept of microbiota -gut-brain axis has been introduced to highlight the relevance of this interplay in the development of both metabolic and neurological conditions, thus challenging a sharp taxonomy of diseases, primarily based on organ situation[9,10].

In line with these conjectures, the obesity is somehow paradigmatic since it is definitely a metabolic disorder, but it can be seen also from a psychopathological angle[13]. This fact, combined with the view of some scholars[14] who claim, challenging the reductionist approach of a vast part of contemporary biomedical research, points out that causal trajectories are neither linear nor one-way in the life sciences. It is crucial to recognize that the way by which diseases and disorders are classified, understood and therapeutically addressed, is more often the result of disciplinary interests and history rather than "carving nature at its joints".

Indeed, recent data indicate a relevant relationship between microbiota composition and the obesity development. This connection should be surely evaluated as a dynamic interplay between microbial activities and human physiology but also be seen considering usual obesity-associated (*e.g.*, anxiety and depression) "comorbidities" [13].

This healthy axis perspective aims at reconsidering this frame by arguing that an ecological, systemic view on health should stop seeing the problem in a mere "additive sense", by privileging one side (*i.e.*, obesity) over other factors (*i.e.*, comorbidities). Rather, the entire question, obviously without simplifying or neglecting specific issues associated with localized phenomena, should be seen as a "network disease".

Therefore this suggests that clinical approaches, if not coordinated, should always be performed in the awareness that therapeutic interventions can rarely neglect the presence of different (either cooperating or in contrast) forces acting on the system, *i.e.*, our healthy state.

On the practical side, such an approach implies a twofold change. On one hand, both researchers and clinicians should be more aware that the their way approaching a particular condition is partial and runs the risk of neglecting important factors pertaining to other specialists. Thus, an open attitude towards integration should be encouraged. On the other hand, disciplinary boundaries are not always a direct responsibility of single researchers but rather reflect the way scientific programs are designed and thought at the institutional level, which often mirrors political and economic factors. Therefore our aspiration is that science, at a regulatory level, would become more open, inclusive, fostering the need of promoting a more systemic and integrated perspectives[9]. I hope that this might also further, among researchers, the importance of conceptual issues and terms once central within the life sciences (such as "organism") and now lost within disciplinary boundaries[15]. Of course, science is also the difficult combination of innovation and caution. New ideas are important but in order to be "scientific" something more is needed.

CONCLUSION

The increasing studies about the microbiota impact in human healthy generated a great enthusiasm, but runs also the risk of a big hype[16]. The idea that microbiota could be the new "Holy Grail" of biology is not only wrong and reductive but it also contradicts the systemic and ecological perspective we support. This is why, in the light of the well know adversities in settling precise causality in biology, it is fundamental to recall extreme caution, avoiding the seducing idea of a privileged point of view that will explain anything else[9].

Bearing this in mind, the importance of microbiota-gut-brain axis should be considered, primarily as a methodological stance, in order to develop new systemic procedures. We hope that this perspective would promote a more satisfactory and definite framework for person-centered medicine[9,17], whereas healthy axis will become not only a research tool but also an active locus for therapeutic interventions.

REFERENCES

1 **Schroeder BO**, Bäckhed F. Signals from the gut microbiota to distant organs in physiology and disease. *Nat Med* 2016; **22**: 1079-1089 [PMID: 27711063 DOI: 10.1038/nm.4185]
2 **De Almeida CV**, de Camargo MR, Russo E, Amedei A. Role of diet and gut microbiota on colorectal cancer immunomodulation. *World J Gastroenterol* 2019; **25**: 151-162 [PMID: 30670906 DOI: 10.3748/wjg.v25.i2.151]
3 **Ursell LK**, Metcalf JL, Parfrey LW, Knight R. Defining the human microbiome. *Nutr Rev* 2012; **70** Suppl 1: S38-S44 [PMID: 22861806 DOI: 10.1111/j.1753-4887.2012.00493.x]
4 **Belkaid Y**, Hand TW. Role of the microbiota in immunity and inflammation. *Cell* 2014; **157**: 121-141 [PMID: 24679531 DOI: 10.1016/j.cell.2014.03.011]
5 **Huang TT**, Lai JB, Du YL, Xu Y, Ruan LM, Hu SH. Current Understanding of Gut Microbiota in Mood Disorders: An Update of Human Studies. *Front Genet* 2019; **10**: 98 [PMID: 30838027 DOI: 10.3389/fgene.2019.00098]
6 **Niccolai E**, Ricci F, Russo E, Nannini G, Emmi G, Taddei A, Ringressi MN, Melli F, Miloeva M, Cianchi F, Bechi P, Prisco D, Amedei A. The Different Functional Distribution of "Not Effector" T Cells (Treg/Tnull) in Colorectal Cancer. *Front Immunol* 2017; **8**: 1900 [PMID: 29375559 DOI: 10.3389/fimmu.2017.01900]
7 **De Almeida CV**, Lulli M, di Pilato V, Schiavone N, Russo E, Nannini G, Baldi S, Borrelli R, Bartolucci G, Menicatti M, Taddei A, Ringressi MN, Niccolai E, Prisco D, Rossolini GM, Amedei A. Differential Responses of Colorectal Cancer Cell Lines to Enterococcus faecalis' Strains Isolated from Healthy Donors and Colorectal Cancer Patients. *J Clin Med* 2019; 8 [PMID: 30897751 DOI: 10.3390/jcm8030388]
8 **Zitvogel L**, Ayyoub M, Routy B, Kroemer G. Microbiome and Anticancer Immunosurveillance. *Cell* 2016; **165**: 276-287 [PMID: 27058662 DOI: 10.1016/j.cell.2016.03.001]
9 **Amedei A**, Boem F. I've Gut A Feeling: Microbiota Impacting the Conceptual and Experimental Perspectives of Personalized Medicine. *Int J Mol Sci* 2018; **19** [PMID: 30486338 DOI: 10.3390/ijms19123756]
10 **Bordenstein SR**, Theis KR. Host Biology in Light of the Microbiome: Ten Principles of Holobionts and Hologenomes. *PLoS Biol* 2015; **13**: e1002226 [PMID: 26284777 DOI: 10.1371/journal.pbio.1002226]
11 **Mallee H**. The evolution of health as an ecological concept. *Curr Opin Environ Sus-tain* 2017; **25**: 28–32 [DOI: 10.1016/j.cosust.2017.04.009]
12 **Johnson KV**, Foster KR. Why does the microbiome affect behaviour? *Nat Rev Microbiol* 2018; **16**: 647-655 [PMID: 29691482 DOI: 10.1038/s41579-018-0014-3]
13 **Niccolai E**, Boem F, Russo E, Amedei A. The GutBrain Axis in the Neuropsychological Disease Model of Obesity: A Classical Movie Revised by the Emerging Director "Microbiome". *Nutrients* 2019; **11** [PMID: 30642052 DOI: 10.3390/nu11010156]
14 **Noble D**. Claude Bernard, the first systems biologist, and the future of physiology. *Exp Physiol* 2008; **93**: 16-26 [PMID: 17951329 DOI: 10.1113/expphysiol.2007.038695]
15 **Nicholson DJ**. The return of the organism as a fundamental explanatory concept in biology. *Philosophy Compass* 2014; **9**: 347–359 [DOI: 10.1111/phc3.12128]
16 **Hanage WP**. Microbiology: Microbiome science needs a healthy dose of scepticism. *Nature* 2014; **512**: 247-248 [PMID: 25143098 DOI: 10.1038/512247a]
17 **Zmora N**, Zeevi D, Korem T, Segal E, Elinav E. Taking it Personally: Personalized Utilization of the Human Microbiome in Health and Disease. *Cell Host Microbe* 2016; **19**: 12-20 [PMID: 26764593 DOI: 10.1016/j.chom.2015.12.016]

World Journal of Gastroenterology

Submit a Manuscript: https://www.f6publishing.com

World J Gastroenterol 2019 August 7; 25(29): 3842-3848

DOI: 10.3748/wjg.v25.i29.3842

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

EDITORIAL

Hepatocellular carcinoma and metabolic syndrome: The times are changing and so should we

Georgios Tsoulfas

ORCID number: Georgios Tsoulfas (0000-0001-5043-7962).

Author contributions: Tsoulfas G interpreted the literature and wrote the manuscript.

Conflict-of-interest statement: Georgios Tsoulfas reports no conflict of interest.

Manuscript source: Invited manuscript

Received: April 28, 2019
Peer-review started: April 28, 2019
First decision: May 24, 2019
Revised: June 3, 2019
Accepted: June 25, 2019
Article in press: June 26, 2019
Published online: August 7, 2019

P-Reviewer: Chen GX, Kamimura K, Lleo A, Vorobjova T
S-Editor: Yan JP
L-Editor: A
E-Editor: Ma YJ

Georgios Tsoulfas, Department of Surgery, Aristotle University of Thessaloniki, Thessaloniki 54124, Greece

Corresponding author: Georgios Tsoulfas, FACS, FICS, MD, PhD, Associate Professor, Department of Surgery, Aristotle University of Thessaloniki, University Campus, Thessaloniki 54124, Greece. tsoulfasg@auth.gr
Telephone: +30-697189190
Fax: +30-2310-3320222

Abstract

Although hepatocellular carcinoma (HCC) is as prevalent as ever as a cancer-related mortality, and some would even argue that it is increasing, the pattern of its etiologies has been changing. Specifically, the domination of viral hepatitis C virus is being overcome, partly because of the emergence of the antiviral treatments, and partly because of the significant increase, especially in developed countries, of the combination of obesity, diabetes, metabolic syndrome, non-alcoholic fatty liver disease and non-alcoholic steatohepatitis. This editorial will explore the interconnection of this group of diseases and how they are linked to HCC. More importantly, it will argue that this shift in HCC etiology essentially means that we have to change how we approach the treatment of HCC, by changing our focus (and resources) to earlier stages of the disease development in order to prevent the appearance and progression of HCC.

Key words: Hepatocellular carcinoma; Diabetes; Obesity; Steatosis; Non-alcoholic fatty liver disease; Body-mass index; Non-alcoholic steatohepatitis

Core tip: There is a changing landscape whereby metabolic syndrome and non-alcoholic fatty liver disease and non-alcoholic steatohepatitis have replaced hepatitis viral infections and alcohol as the predominant causes of cirrhosis and hepatocellular carcinoma (HCC) on the global scale. As such, we need to change the treatment focus and address metabolic syndrome and its elements in an effort to intervene more timely in the development of cirrhosis and HCC.

Citation: Tsoulfas G. Hepatocellular carcinoma and metabolic syndrome: The times are changing and so should we. *World J Gastroenterol* 2019; 25(29): 3842-3848
URL: https://www.wjgnet.com/1007-9327/full/v25/i29/3842.htm

DOI: https://dx.doi.org/10.3748/wjg.v25.i29.3842

INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common primary cancer of the liver, whereas among all types of cancers HCC is the fifth most common with an aggressive nature that had it ranking second in 2012 in terms of causes of cancer-related death in the world[1,2]. The prevalence and aggressiveness of HCC have led to a world-wide interest and an increasingly multidisciplinary approach with the use of new technologies and molecular analysis with the hope of achieving a more patient-targeted approach. From a surgical standpoint, the armamentarium available has been increasing with examples such as hepatic resection, microwave or radiofrequency ablation, transarterial chemoembolization, irreversible electroporation, and of course liver transplantation (LT). The latter is especially important as HCC frequently coexists with cirrhosis and LT represents a treatment for both. Unfortunately, the lack of donors has led to efforts to expand the donor pool with the use of Donors after Cardiac Death, split grafts, living related and expanded criteria grafts, all of which necessitate careful donor and recipient selection and matching. Despite all these efforts, HCC remains a formidable opponent and the only significant victory that we have been able to enjoy in this last decade is the advent of the latest all oral, ribavirin- and interferon-free regimens of direct acting antivirals against the hepatitis C virus (HCV) which have achieved 90% sustained virological response, which is essentially a cure[3]. The fact that this is for all genotypes, has led to LT actually providing a cure for HCV, rather than a short interlude from an aggressive recurrence; at the same time, it is part of a big change in the landscape of HCC etiology and management.

CHANGING LANDSCAPE OF HCC

Specifically, the progress having to do with the HCV epidemic and the persistent increase in obesity, diabetes, non-alcoholic steatohepatitis (NASH), and non-alcoholic fatty liver disease (NAFLD) have allowed metabolic syndrome (MetS) to take the reins regarding factors and diseases affecting the liver and eventually leading to HCC[4].

Defining NAFLD and NASH

Before proceeding any further it is important to present some of the definitions of the terms used. The reason is that very frequently the term NAFLD is associated and may be used interchangeably with the other terms such as NASH or "hepatic steatosis", which is not correct as there are significant differences with clinical implications. The term "hepatic steatosis" refers to the presence of micro- or macro- or mixed vesicular fat in the cytoplasm of the hepatocytes[5]. Using the American Association for the Study of Liver Diseases guidelines for the definition of NAFLD we need to establish primary hepatic steatosis (confirmed either by imaging or biopsy), while at the same time exclude any secondary causes of hepatic steatosis (medications, alcohol, hereditary)[6]. NASH represents an extension of NAFLD, whereby the presence of primary hepatic steatosis (need more than 5%) leads to inflammation and hepatocellular injury (ballooning), and is the form that can actually progress to fibrosis, cirrhosis and HCC[7]. Today, NAFLD represents the most common chronic liver disease worldwide. It constitutes an epidemic with prevalence in adults in developed countries somewhere between 30%-50%, with the main obstacle in finding a more concise measurement being the difficulties in the diagnostic methods between the different studies[8-10]. NAFLD is frequently associated with obesity, type II diabetes mellitus (T2DM), and dyslipidemia, all of which are components of the MetS[11,12]. The definition of MetS developed over time and through different medical associations, such as the International Diabetes Federation (IDF), World Health Organization and the United States National Cholesterol Education program Adult Treatment Panel. Eventually this led to the Harmonized (consensus) definition in 2009 incorporating those of the IDF and the American Heart Association, which includes any three of the following: (1) Waist circumference: According to population and country-specific definitions; (2) Triglycerides: ≥ 150 mg/dL (1.7 mmol/L); (3) High density lipoprotien cholesterol: < 40 mg/dL (1.03 mmol/L) in men and < 50 mg/dL (1.29 mmol/L) in women; (d) Blood pressure: ≥ 130 mmHg systolic; ≥ 85 mmHg diastolic; and (5)

Fasting glucose: ≥ 100 mg/dL (5.6 mmol/L) or use of medication[13].

Epidemiology of NAFLD and NASH

The relation between NAFLD, and as an extension NASH, and MetS is a complex one. In the past, NAFLD was viewed as the hepatic component of MetS given its relationship with obesity and insulin resistance. Over time we have essentially seen that this is a two-way street, as on the one hand NAFLD can lead to T2DM and its relation to obesity and the lipid abnormalities combined with the hepatic inflammation can lead to MetS. On the other hand, the various manifestations of MetS can lead to a deterioration of NAFLD and move towards NASH, fibrosis, cirrhosis and eventually HCC[13,14]. As complex as the relationship between NAFLD/NASH and MetS may seem, that of NAFLD/NASH to HCC is a much more straightforward one. Currently, NAFLD-related cirrhosis or NAFLD-related HCC are the second cause of LT in the United States, whereas NAFLD is responsible for somewhere between 5%-20% of HCC cases in the Western world[15,16]. This is depicted in an excellent study by Younossi *et al*[17] who aim to identify the global prevalence of NAFLD and NASH, while at the same time describing their natural history and progression. By looking at reports between the years 1989 and 2015, they arrive at three main conclusions: (1) There is a significant global burden of NASH and a global prevalence of NAFLD of 25% with a geographical variation. This last point could have to do with genetic and cultural differences which can certainly play a role in shaping body mass; (2) The progression of fibrosis that can be seen in NAFLD and NASH is very slow with these patients having a > 50% chance of non-liver related mortality[17]. The incidence of HCC among NAFLD patients is very low at a frequency of 0.44/1000 person-years; however, the prevalence of NAFLD in the population makes up for that, and as a result NAFLD by affecting over 1 billion adults world-wide remains a basic cause of LT[18]; and (3) Despite the fact that liver-related events may be responsible for only a small fraction of deaths in NAFLD and NASH patients, NASH is rapidly becoming the most common etiology of liver-related death globally.

The above findings present an association between a metabolic disease predominantly and a type of cancer. This is quite intriguing, especially if we consider that the mechanism is not completely clear. Alterations in gene expressions may play a significant role, as a high number of them were observed during the progression from steatosis to NASH, with special emphasis on the fibrosis and inflammation aspects[19]. As part of this progression towards cirrhosis and, eventually, HCC, extracellular matrix and angiogenesis genes are up regulated, whereas others that affect iron homeostasis are down regulated[20]. A central part of the evolution of NASH, at the molecular level, is the down regulation of the Wnt signaling pathway, as Wnt inhibitors are up regulated[21]. This is directly related to HCC, as dysregulated activation of Wnt signaling has been linked to HCC subclasses[21].

OBESITY

Obesity represents a common denominator between NAFLD/NASH and MetS, and as such deserves special mention. At first its role seems quite straightforward as the association of obesity with T2DM and cardiovascular disease are expected to present a risk to a person's health. This may lead us to believe that the mere presence of obesity should lead to higher morbidity and mortality; yet, there have been several studies using data from the National Surgical Quality Improvement Program of the American College of Surgeons which have failed to find a correlation between obesity and mortality in surgical patients[22,23]. This has also been the case with studies in general or colorectal surgery, leading to the term "obesity paradox", in order to describe the unexpected protective effect of obesity[24,25]. Part of the explanation for this may be the existence of different definitions for obesity and corpulence, as well as the different distributions of fat in either adipose subcutaneous tissue or visceral obesity[26,27]. Either way, the above should not distract from the fact that abdominal obesity is directly linked to MetS, with its variables including visceral obesity, insulin resistance, dyslipidemia and systemic hypertension[28]. Furthermore, obesity is linked to NASH, which is also closely associated with MetS, thus bringing everything to a full circle. The relation between MetS and NASH with obesity as the "go-between" has led to NASH becoming the fastest growing indication for LT in the US, with a prediction that by 2025 approximately 25 million Americans will have developed NASH, a fifth of whom may need to undergo transplantation[29,30]. If that were not enough, in those patients undergoing LT, the prevalence of NASH after 6 months is around 50%-60%, as opposed to 23% in the general population[31,32]. The main explanation for this is the immunosuppressive medications and their side-effects. However, what is significant

is the fact that the presence of MetS post-transplantation is predictive of NASH recurrence, which can jeopardize the graft and the patient's life[33,34].

Overall, we are seeing a paradigm shift where NAFLD/NASH and MetS are steadily replacing hepatitis viral infections (usually HCV) as the main cause of HCC and the second most frequent one for LT. Although the underlying mechanism of the progression from MetS and NAFLD/NASH to HCC is not fully understood, possibilities include the generation of reactive oxygen species, the presence of leptin (a proinflammatory cytokine with angiogenic abilities), the mild yet persistent inflammation state seen in obesity, which may all affect cellular transcription and signaling, thus leading to the appearance of HCC[35,36].

TREATMENT

This paradigm shift that we have seen, which essentially signifies that MetS, through NAFLD/NASH, now represents the main pathway to HCC and cirrhosis, has several connotations for treatment. Specifically, it means that a significant part of our efforts should be towards preventing HCC and cirrhosis, rather than waiting for them to happen and then have to deal with complicated and costly treatments. Efforts should start focusing at dealing with MetS, which mean addressing its main components such as DM, hypertension, dyslipidemia, obesity and through those the effects of NASH and NAFLD. The following are some important parts of this treatment plan and include:

Lifestyle changes

Weight loss is key in managing all the different elements of MetS, such as obesity, hypertension, dyslipidemia and T2DM, as well as in helping to control NAFLD and its progression to NASH[37-39]. This implies a combination of decreased caloric intake, as well as increased physical activity, especially walking. Although there is no consensus as to the specifics of the weight loss, there is agreement that it should be steady.

Pharmacologic therapy

The intimate causal relationship (possibly in all directions) between MetS, NAFLD/NASH and T2DM has caused a lot of interest in medications, such as metformin and pioglitazone. Metformin, together with the lifestyle changes, is believed to be especially appropriate for patients with T2DM and NAFLD or early NASH, although it has not been shown to have a beneficial effect on liver histology[40,41]. Pioglitazone, belonging in the thiazolidinediones category of medications that cause an upregulation of the genes involved in glucose metabolism, resulting in decreased hepatic lipogenesis, thus leading to improved glucose tolerance and decreased hepatic inflammation and avoidance of NASH[42,43]. The main limitations have been the need for long-term treatment and the side-effects which include congestive heart failure and stroke among others[44]. Well-established medical treatments currently exist also for hypertension and dyslipidemia, which in certain instances, such as the use of statins, have been shown to affect in a positive manner the prevention and progression of cirrhosis and HCC[45].

Nutrition therapy

Although the question of whether NAFLD and the progression to NASH is a matter of overnutrition or simply the result of a "different" nutritional pattern with different responses from the metabolic system, there is no good data on what the proper diet specifically for NAFLD/NASH patients should be. The closest to a recommendation are those originating from the American Diabetes Association and the American Heart Association, given the prevalence and importance in the whole process of T2DM and cardiovascular disease[46].

Bariatric surgery

There have been significant advances in bariatric surgery, especially pertaining to identifying the best type of surgery for the specific patient. The recognition of MetS, as well as the effect that we have witnessed bariatric surgery having on T2DM and hypertension, have led to bariatric surgery taking a central role in the management of MetS. There are several procedures such as the adjustable gastric banding, the sleeve gastrectomy, the Roux-en-Y gastric bypass, the duodenal switch or biliopancreatic diversion with all of them having different amounts of restrictive and malabsorptive elements[47]. The advances in minimally invasive surgery have also made these procedures more physiologically "attractive" for these patients. As potentially useful as bariatric surgery can be, it needs to be stressed that it is not enough by itself to

avoid the combined ill effects of MetS and especially those pertaining to NAFLD/NASH and the HCC progression; the reason is that the main therapy for MetS remains more a matter of lifestyle adjustments/change, rather than surgical treatment.

CONCLUSION

The goal of this editorial is to hopefully change the mindset of how we approach cirrhosis and HCC. Specifically, by recognizing the importance of MetS, NAFLD and NASH and the combined role that they play in the progression to fibrosis, cirrhosis and eventually HCC, can help us shift the focus from the management of HCC once it has appeared with challenging and costly procedures and interventions, to the avoidance or management of MetS and its elements with the methods previously described. Additionally, we need to change the way that we have been approaching obesity as the result of bad lifestyle choices and realize that it is a multidimensional disease affecting several organ systems and where successful management requires a spectrum of interventions ranging from public education and preventive care to medications and bariatric surgery. In summary, MetS and NAFLD and their association with NASH, T2DM, hypertension, obesity and cardiovascular disease are all part of an equation which explains today (more than any other cause) the progression of chronic liver disease to cirrhosis and, eventually, to HCC. Once we understand this, we can start changing or adjusting the focus of our interventions for cirrhosis and HCC by placing emphasis on an earlier part of the disease spectrum where all these factors are at play; ultimately, the goal is to prevent than to have to treat.

REFERENCES

1 **Ferlay J**, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer* 2010; **127**: 2893-2917 [PMID: 21351269 DOI: 10.1002/ijc.25516]

2 **GLOBOCAN**. Estimated cancer incidence: Mortality and prevalence worldwide in 2012. Available from: http:// globocan.iarc.fr/Pages/fact_sheets_cancer.aspx

3 **Fobi M**. Metabolic surgery: Procedures vary internationally. Metabolic Surgery and International Practice by ACS. *Bull Am Coll Surg* 2019

4 **Caldwell SH**, Crespo DM, Kang HS, Al-Osaimi AM. Obesity and hepatocellular carcinoma. *Gastroenterology* 2004; **127**: S97-103 [PMID: 15508109 DOI: 10.1053/j.gastro.2004.09.021]

5 **Burt AD**, Mutton A, Day CP. Diagnosis and interpretation of steatosis and steatohepatitis. *Semin Diagn Pathol* 1998; **15**: 246-258 [PMID: 9845426]

6 **Chalasani N**, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, Charlton M, Sanyal AJ; American Gastroenterological Association; American Association for the Study of Liver Diseases; American College of Gastroenterologyh. The diagnosis and management of non-alcoholic fatty liver disease: Practice guideline by the American Gastroenterological Association, American Association for the Study of Liver Diseases, and American College of Gastroenterology. *Gastroenterology* 2012; **142**: 1592-1609 [PMID: 22656328 DOI: 10.1053/j.gastro.2012.04.001]

7 **Hellerbrand C**. Pathophysiological similarities and synergisms in alcoholic and non-alcoholic steatohepatitis. *Dig Dis* 2010; **28**: 783-791 [PMID: 21525763 DOI: 10.1159/000324286]

8 **Browning JD**, Szczepaniak LS, Dobbins R, Nuremberg P, Horton JD, Cohen JC, Grundy SM, Hobbs HH. Prevalence of hepatic steatosis in an urban population in the United States: Impact of ethnicity. *Hepatology* 2004; **40**: 1387-1395 [PMID: 15565570 DOI: 10.1002/hep.20466]

9 **Williams CD**, Stengel J, Asike MI, Torres DM, Shaw J, Contreras M, Landt CL, Harrison SA. Prevalence of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis among a largely middle-aged population utilizing ultrasound and liver biopsy: A prospective study. *Gastroenterology* 2011; **140**: 124-131 [PMID: 20858492 DOI: 10.1053/j.gastro.2010.09.038]

10 **Bellentani S**, Scaglioni F, Marino M, Bedogni G. Epidemiology of non-alcoholic fatty liver disease. *Dig Dis* 2010; **28**: 155-161 [PMID: 20460905 DOI: 10.1159/000282080]

11 **Fujioka K**. Current and emerging medications for overweight or obesity in people with comorbidities. *Diabetes Obes Metab* 2015; **17**: 1021-1032 [PMID: 26040215 DOI: 10.1111/dom.12502]

12 **VanWagner LB**, Rinella ME. Extrahepatic Manifestations of Nonalcoholic Fatty Liver Disease. *Curr Hepatol Rep* 2016; **15**: 75-85 [PMID: 27218012 DOI: 10.1007/s11901-016-0295-9]

13 **Anstee QM**, Targher G, Day CP. Progression of NAFLD to diabetes mellitus, cardiovascular disease or cirrhosis. *Nat Rev Gastroenterol Hepatol* 2013; **10**: 330-344 [PMID: 23507799 DOI: 10.1038/nrgastro.2013.41]

14 **Naik A**, Belič A, Zanger UM, Rozman D. Molecular Interactions between NAFLD and Xenobiotic Metabolism. *Front Genet* 2013; **4**: 2 [PMID: 23346097 DOI: 10.3389/fgene.2013.00002]

15 **Bellentani S**. The epidemiology of non-alcoholic fatty liver disease. *Liver Int* 2017; **37** Suppl 1: 81-84 [PMID: 28052624 DOI: 10.1111/liv.13299]

16 **White DL**, Kanwal F, El-Serag HB. Association between nonalcoholic fatty liver disease and risk for hepatocellular cancer, based on systematic review. *Clin Gastroenterol Hepatol* 2012; **10**: 1342-1359.e2 [PMID: 23041539 DOI: 10.1016/j.cgh.2012.10.001]

17 **Younossi ZM**, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes.

Hepatology 2016; **64**: 73-84 [PMID: 26707365 DOI: 10.1002/hep.28431]
18 **Angulo P**, Kleiner DE, Dam-Larsen S, Adams LA, Bjornsson ES, Charatcharoenwitthaya P, Mills PR, Keach JC, Lafferty HD, Stahler A, Haflidadottir S, Bendtsen F. Liver Fibrosis, but No Other Histologic Features, Is Associated With Long-term Outcomes of Patients With Nonalcoholic Fatty Liver Disease. *Gastroenterology* 2015; **149**: 389-97.e10 [PMID: 25935633 DOI: 10.1053/j.gastro.2015.04.043]
19 **Lake AD**, Novak P, Fisher CD, Jackson JP, Hardwick RN, Billheimer DD, Klimecki WT, Cherrington NJ. Analysis of global and absorption, distribution, metabolism, and elimination gene expression in the progressive stages of human nonalcoholic fatty liver disease. *Drug Metab Dispos* 2011; **39**: 1954-1960 [PMID: 21737566 DOI: 10.1124/dmd.111.040592]
20 **Clarke JD**, Novak P, Lake AD, Shipkova P, Aranibar N, Robertson D, Severson PL, Reily MD, Futscher BW, Lehman-McKeeman LD, Cherrington NJ. Characterization of hepatocellular carcinoma related genes and metabolites in human nonalcoholic fatty liver disease. *Dig Dis Sci* 2014; **59**: 365-374 [PMID: 24048683 DOI: 10.1007/s10620-013-2873-9]
21 **Hoshida Y**, Nijman SM, Kobayashi M, Chan JA, Brunet JP, Chiang DY, Villanueva A, Newell P, Ikeda K, Hashimoto M, Watanabe G, Gabriel S, Friedman SL, Kumada H, Llovet JM, Golub TR. Integrative transcriptome analysis reveals common molecular subclasses of human hepatocellular carcinoma. *Cancer Res* 2009; **69**: 7385-7392 [PMID: 19723656 DOI: 10.1158/0008-5472.CAN-09-1089]
22 **Al-Refaie WB**, Parsons HM, Henderson WG, Jensen EH, Tuttle TM, Rothenberger DA, Kellogg TA, Virnig BA. Body mass index and major cancer surgery outcomes: Lack of association or need for alternative measurements of obesity? *Ann Surg Oncol* 2010; **17**: 2264-2273 [PMID: 20309642 DOI: 10.1245/s10434-010-1023-2]
23 **Davenport DL**, Xenos ES, Hosokawa P, Radford J, Henderson WG, Endean ED. The influence of body mass index obesity status on vascular surgery 30-day morbidity and mortality. *J Vasc Surg* 2009; **49**: 140-147, 147.e1; discussion 147 [PMID: 19028047 DOI: 10.1016/j.jvs.2008.08.052]
24 **Mullen JT**, Moorman DW, Davenport DL. The obesity paradox: Body mass index and outcomes in patients undergoing nonbariatric general surgery. *Ann Surg* 2009; **250**: 166-172 [PMID: 19561456 DOI: 10.1097/SLA.0b013e3181ad8935]
25 **Dindo D**, Muller MK, Weber M, Clavien PA. Obesity in general elective surgery. *Lancet* 2003; **361**: 2032-2035 [PMID: 12814714 DOI: 10.1016/S0140-6736(03)13640-9]
26 **Yusuf S**, Hawken S, Ounpuu S, Bautista L, Franzosi MG, Commerford P, Lang CC, Rumboldt Z, Onen CL, Lisheng L, Tanomsup S, Wangai P, Razak F, Sharma AM, Anand SS; INTERHEART Study Investigators. Obesity and the risk of myocardial infarction in 27,000 participants from 52 countries: A case-control study. *Lancet* 2005; **366**: 1640-1649 [PMID: 16271645 DOI: 10.1016/S0140-6736(05)67663-5]
27 **Blackett PR**, Sanghera DK. Genetic determinants of cardiometabolic risk: A proposed model for phenotype association and interaction. *J Clin Lipidol* 2013; **7**: 65-81 [PMID: 23351585 DOI: 10.1016/j.jacl.2012.04.079]
28 **Cornier MA**, Després JP, Davis N, Grossniklaus DA, Klein S, Lamarche B, Lopez-Jimenez F, Rao G, St-Onge MP, Towfighi A, Poirier P; American Heart Association Obesity Committee of the Council on Nutrition; Physical Activity and Metabolism; Council on Arteriosclerosis; Thrombosis and Vascular Biology; Council on Cardiovascular Disease in the Young; Council on Cardiovascular Radiology and Intervention; Council on Cardiovascular Nursing, Council on Epidemiology and Prevention; Council on the Kidney in Cardiovascular Disease, and Stroke Council. Assessing adiposity: A scientific statement from the American Heart Association. *Circulation* 2011; **124**: 1996-2019 [PMID: 21947291 DOI: 10.1161/CIR.0b013e318233bc6a]
29 **Singal AK**, Guturu P, Hmoud B, Kuo YF, Salameh H, Wiesner RH. Evolving frequency and outcomes of liver transplantation based on etiology of liver disease. *Transplantation* 2013; **95**: 755-760 [PMID: 23370710 DOI: 10.1097/TP.0b013e31827afb3a]
30 **Agopian VG**, Kaldas FM, Hong JC, Whittaker M, Holt C, Rana A, Zarrinpar A, Petrowsky H, Farmer D, Yersiz H, Xia V, Hiatt JR, Busuttil RW. Liver transplantation for nonalcoholic steatohepatitis: The new epidemic. *Ann Surg* 2012; **256**: 624-633 [PMID: 22964732 DOI: 10.1097/SLA.0b013e31826b4b7e]
31 **Bianchi G**, Marchesini G, Marzocchi R, Pinna AD, Zoli M. Metabolic syndrome in liver transplantation: Relation to etiology and immunosuppression. *Liver Transpl* 2008; **14**: 1648-1654 [PMID: 18975273 DOI: 10.1002/lt.21588]
32 **Ford ES**, Giles WH, Dietz WH. Prevalence of the metabolic syndrome among US adults: Findings from the third National Health and Nutrition Examination Survey. *JAMA* 2002; **287**: 356-359 [PMID: 11790215 DOI: 10.1001/jama.287.3.356]
33 **Malik SM**, Devera ME, Fontes P, Shaikh O, Sasatomi E, Ahmad J. Recurrent disease following liver transplantation for nonalcoholic steatohepatitis cirrhosis. *Liver Transpl* 2009; **15**: 1843-1851 [PMID: 19938117 DOI: 10.1002/lt.21943]
34 **El Atrache MM**, Abouljoud MS, Divine G, Yoshida A, Kim DY, Kazimi MM, Moonka D, Huang MA, Brown K. Recurrence of non-alcoholic steatohepatitis and cryptogenic cirrhosis following orthotopic liver transplantation in the context of the metabolic syndrome. *Clin Transplant* 2012; **26**: E505-E512 [PMID: 23061759 DOI: 10.1111/ctr.12014]
35 **Stickel F**, Hellerbrand C. Non-alcoholic fatty liver disease as a risk factor for hepatocellular carcinoma: Mechanisms and implications. *Gut* 2010; **59**: 1303-1307 [PMID: 20650925 DOI: 10.1136/gut.2009.199661]
36 **Vinciguerra M**, Carrozzino F, Peyrou M, Carlone S, Montesano R, Benelli R, Foti M. Unsaturated fatty acids promote hepatoma proliferation and progression through downregulation of the tumor suppressor PTEN. *J Hepatol* 2009; **50**: 1132-1141 [PMID: 19398230 DOI: 10.1016/j.jhep.2009.01.027]
37 **Suzuki A**, Lindor K, St Saver J, Lymp J, Mendes F, Muto A, Okada T, Angulo P. Effect of changes on body weight and lifestyle in nonalcoholic fatty liver disease. *J Hepatol* 2005; **43**: 1060-1066 [PMID: 16140415 DOI: 10.1016/j.jhep.2005.06.008]
38 **Lazo M**, Solga SF, Horska A, Bonekamp S, Diehl AM, Brancati FL, Wagenknecht LE, Pi-Sunyer FX, Kahn SE, Clark JM; Fatty Liver Subgroup of the Look AHEAD Research Group. Effect of a 12-month intensive lifestyle intervention on hepatic steatosis in adults with type 2 diabetes. *Diabetes Care* 2010; **33**: 2156-2163 [PMID: 20664019 DOI: 10.2337/dc10-0856]
39 **Sullivan S**, Kirk EP, Mittendorfer B, Patterson BW, Klein S. Randomized trial of exercise effect on intrahepatic triglyceride content and lipid kinetics in nonalcoholic fatty liver disease. *Hepatology* 2012; **55**: 1738-1745 [PMID: 22213436 DOI: 10.1002/hep.25548]
40 **Nair S**, Diehl AM, Wiseman M, Farr GH, Perrillo RP. Metformin in the treatment of non-alcoholic

steatohepatitis: A pilot open label trial. *Aliment Pharmacol Ther* 2004; **20**: 23-28 [PMID: 15225167 DOI: 10.1111/j.1365-2036.2004.02025.x]

41 **Haukeland JW**, Konopski Z, Eggesbø HB, von Volkmann HL, Raschpichler G, Bjøro K, Haaland T, Løberg EM, Birkeland K. Metformin in patients with non-alcoholic fatty liver disease: A randomized, controlled trial. *Scand J Gastroenterol* 2009; **44**: 853-860 [PMID: 19811343 DOI: 10.1080/00365520902845268]

42 **Smith U**. Pioglitazone: Mechanism of action. *Int J Clin Pract Suppl* 2001; 13-18 [PMID: 11594239 DOI: 10.1016/S1567-5769(01)00109-6]

43 **Belfort R**, Harrison SA, Brown K, Darland C, Finch J, Hardies J, Balas B, Gastaldelli A, Tio F, Pulcini J, Berria R, Ma JZ, Dwivedi S, Havranek R, Fincke C, DeFronzo R, Bannayan GA, Schenker S, Cusi K. A placebo-controlled trial of pioglitazone in subjects with nonalcoholic steatohepatitis. *N Engl J Med* 2006; **355**: 2297-2307 [PMID: 17135584 DOI: 10.1056/NEJMoa060326]

44 **Lu CJ**, Sun Y, Muo CH, Chen RC, Chen PC, Hsu CY. Risk of stroke with thiazolidinediones: A ten-year nationwide population-based cohort study. *Cerebrovasc Dis* 2013; **36**: 145-151 [PMID: 24029780 DOI: 10.1159/000353679]

45 **Vargas JI**, Arrese M, Shah VH, Arab JP. Use of Statins in Patients with Chronic Liver Disease and Cirrhosis: Current Views and Prospects. *Curr Gastroenterol Rep* 2017; **19**: 43 [PMID: 28752475 DOI: 10.1007/s11894-017-0584-7]

46 **Conlon BA**, Beasley JM, Aebersold K, Jhangiani SS, Wylie-Rosett J. Nutritional management of insulin resistance in nonalcoholic fatty liver disease (NAFLD). *Nutrients* 2013; **5**: 4093-4114 [PMID: 24152749 DOI: 10.3390/nu5104093]

47 **Ayloo S**, Armstrong J, Hurton S, Molinari M. Obesity and liver transplantation. *World J Transplant* 2015; **5**: 95-101 [PMID: 26421262 DOI: 10.5500/wjt.v5.i3.95]

World Journal of Gastroenterology

Submit a Manuscript: https://www.f6publishing.com

World J Gastroenterol 2019 August 7; 25(29): 3849-3856

DOI: 10.3748/wjg.v25.i29.3849

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

OPINION REVIEW

Improving cirrhosis care: The potential for telemedicine and mobile health technologies

Matthew Jonathon Stotts, Justin Alexander Grischkan, Vandana Khungar

ORCID number: Matthew Jonathon Stotts (0000-0003-1108-8653); Justin Alexander Grischkan (0000-0003-2610-7802); Vandana Khungar (0000-0001-8720-533X).

Author contributions: Stotts MJ, Grischkan JA, and Khungar V all contributed to the literature review and the writing of this manuscript.

Conflict-of-interest statement: All the Authors have no conflict of interest related to the manuscript.

Manuscript source: Invited manuscript

Received: March 2, 2019
Peer-review started: March 2, 2019
First decision: May 16, 2019
Revised: June 14, 2019
Accepted: July 1, 2019
Article in press: July 3, 2019
Published online: August 7, 2019

P-Reviewer: Guo XZ, Zhang ZH
S-Editor: Yan JP
L-Editor: A

Matthew Jonathon Stotts, Vandana Khungar, Department of Medicine, Division of Gastroenterology and Hepatology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, United States

Justin Alexander Grischkan, Department of Medicine, Massachusetts General Hospital, 55 Fruit Street, Boston, MA 02114, United States

Corresponding author: Vandana Khungar, MD, MSc, Assistant Professor, Department of Medicine, Division of Gastroenterology and Hepatology, Perelman School of Medicine, University of Pennsylvania, 3400 Spruce Street 2 Dulles, Philadelphia, PA 19104, United States. vandana.khungar@pennmedicine.upenn.edu
Telephone: +1-312-3168415

Abstract

Decompensated cirrhosis is a condition associated with significant morbidity and mortality. While there have been significant efforts to develop quality metrics that ensure high-value care of these patients, wide variations in clinical practice exist. In this opinion review, we discuss the quality gap in the care of patients with cirrhosis, including low levels of compliance with recommended cancer screening and other clinical outcome and patient-reported outcome measures. We posit that innovations in telemedicine and mobile health (mHealth) should play a key role in closing the quality gaps in liver disease management. We highlight interventions that have been performed to date in liver disease and heart failure-from successful teleconsultation interventions in the care of veterans with cirrhosis to the use of telemonitoring to reduce hospital readmissions and decrease mortality rates in heart failure. Telemedicine and mHealth can effectively address unmet needs in the care of patients with cirrhosis by increasing preventative care, expanding outreach to rural communities, and increasing high-value care. We aim to highlight the benefits of investing in innovative solutions in telemedicine and mHealth to improve care for patients with cirrhosis and create downstream cost savings.

Key words: Cirrhosis; Liver disease; Quality improvement; Telemedicine; Telemonitoring; Mobile health

Core tip: Telehealth and mobile health technologies have been used in other disease states with great success to reduce morbidity, mortality and cost while employing

E-Editor: Ma YJ

innovative design. Providers caring for patients with cirrhosis have not widely adopted these technologies but could benefit greatly from doing so. More resources need to be devoted to using innovative telemedicine strategies to improve the care of patients with liver disease. In turn, policy change will be necessary to allow all centers to implement these solutions in a cost-effective manner.

Citation: Stotts MJ, Grischkan JA, Khungar V. Improving cirrhosis care: The potential for telemedicine and mobile health technologies. *World J Gastroenterol* 2019; 25(29): 3849-3856
URL: https://www.wjgnet.com/1007-9327/full/v25/i29/3849.htm
DOI: https://dx.doi.org/10.3748/wjg.v25.i29.3849

INTRODUCTION

Patients with cirrhosis are at risk for a variety of complications including ascites, hepatic encephalopathy, esophageal or gastric varices, and hepatocellular carcinoma (HCC). The development of ascites, encephalopathy, or bleeding varices defines the transition from compensated to decompensated cirrhosis, a change that is associated with a marked decrease in survival, from 12 years to approximately 2 years after initial diagnosis[1]. A pressing need exists to develop strategies to prevent or slow the transition to decompensated cirrhosis, improve management of complications including HCC, and ensure patients are referred for liver transplantation when appropriate.

Recently, the Practice Metrics Committee of the American Association for the Study of Liver Disease (AASLD) developed quality measures in the care of patients with cirrhosis, identifying process and outcome measures for the management of ascites, gastric and esophageal varices, hepatic encephalopathy, HCC screening, and evaluation for liver transplantation[2]. The final 46 metrics were intended to drive quality improvement and allow providers to deliver high-value care to patients with cirrhosis. Based on this assessment, there is considerable room for physicians to improve on the metrics laid out by the AASLD.

Prior studies have also supported the need for improvement. While several published cost-effectiveness models have reported that performing screening for HCC is cost effective, the screening rate for HCC in the United States is under 20%, and substantial disparities exist in screening for those followed by primary care physicians compared to hepatology/gastroenterology subspecialists (16.9% *vs* 51.7%)[3-5]. This screening rate is likely lower in developing regions of the world, where many countries do not have national screening programs for the early detection of HCC and cost effectiveness has not been evaluated in these populations[6]. Retrospective studies in Veterans Health Administration cohorts show that less than one third of patients receive all recommended care in the management of cirrhosis-related ascites and even fewer receive all recommended care related to the screening and management of varices[7,8]. Readmission rates among patients with cirrhosis are approximately 30% at thirty days and 50% at ninety days from hospital discharge[9,10].

There are many potential reasons for these shortcomings. The limited supply of hepatologists, particularly in rural and underserved locations in the United States and worldwide, can make it difficult for patients with cirrhosis to access specialized care. Patients with cirrhosis require multidisciplinary, coordinated care for titration of their medications, frequent laboratory monitoring and vaccinations, and scheduling screening endoscopies and imaging. Yet, the shortage of hepatologists and limited appointment availably of primary care providers and gastroenterologists leaves many patients and their families with much of the burden of managing their disease.

There are significant challenges to the implementation of successful and wide-reaching quality improvement initiatives for patients with cirrhosis. While much of the care of individuals with cirrhosis in the United States is done through the care of hepatologists at academic medical centers, there are fewer than 600 board certified hepatologists in the United States, roughly one for every 550000 persons[11]. Although certified hepatologists may be best suited to implement change, their scarcity means that the majority of the burden of medical care for patients without access to hepatologists likely falls on primary care providers and gastroenterologists. As noted above, outcomes may be improved for patients with cirrhosis who have access to care under the guidance of hepatologists or gastroenterologists. These challenges are similar in other countries with many more patients with liver disease than specialized

physicians available to provide such care. Innovative health care solutions will be critical to improve the care of patients with cirrhosis. Specifically, telemedicine (a broad term for medicine practiced at a distance) and mobile health (mHealth, the use of interactive and mobile devices such as mobile phones and tablets to improve health) could play a key role in closing the quality gap in the care of patients with liver disease by expanding the ability of hepatologists to provide care.

TELEMEDICINE IN THE CARE OF CHRONIC DISEASES AND LIVER DISEASE

Telemedicine is defined by the World Health Organization as the delivery of health care services from a distance by the use of telecommunications and virtual technology to provide health care outside of traditional health-care facilities. Three promising types of telemedicine for patients with cirrhosis are teleconsultation, televisits and telemonitoring, each of which have been used in the care of patients with liver disease[12].

Teleconsultation, in which a practitioner in one location presents a case to an expert in another location, has been used in some settings with encouraging results. One of the most well-known telemedicine interventions is through the Veterans Health Administration (VA) system, which implemented the Specialty Access Network-Extension for Community Healthcare Outcomes (SCAN-ECHO) model to provide specialty consultation to practitioners in underserved areas regarding new treatment options for hepatitis C in case-based formats[13]. Developed at the University of New Mexico, the ECHO model has been successfully implemented in other locations, including Argentina[14]. It has also been used in the VA system for managing patients with chronic liver disease, with promising results suggesting increased screening rates for liver cancer and varices and a lower mortality in those that received the intervention[15]. Teleconsultation has also proved useful in determining which patients may be candidates for liver transplantation and who should proceed to formal evaluation[16].

Televisits, in which the patient has direct contact with a provider in another location, have been shown to be a feasible model in the treatment of hepatitis C (Table 1), although they have not been well described in caring for patients with liver disease in other settings. Face-to-face telemedicine encounters for the treatment of hepatitis C have been successfully implemented in rural populations in California and Canada, and for helping patients with opioid use disorder during their attendance at an opioid substitution program[17-19].

A hybrid between a teleconsultation and televisit model is that of a provider to provider consultation, with the patient physically present with the less specialized provider. The consultant can advise the general gastroenterologist or primary care provider to elicit a particular history, to perform certain physical exam maneuvers, or advise on a treatment plan. The physician physically present with the patient is responsible for the visit. While these hybrid consultations may be beneficial, this model has potential for difficulties with payment models and provider reimbursement.

Through telemonitoring, patients are monitored remotely for signs and symptoms of disease progression as well as objective data that may inform management. This approach has been described using smart tablets in patients in the perioperative period after liver transplantation and as a modality to monitor weight, vital signs, and laboratory values for pediatric liver transplant patients[20,21] (Table 1). A smartphone-based Stroop test has been validated for the diagnosis of covert hepatic encephalopathy[22]. Similarly, a "Patient Buddy App" that monitors symptoms such as weight gain along with medication adherence and daily sodium intake has shown potential to prevent hospital readmissions secondary to hepatic encephalopathy[23]. Additionally, an innovative program utilizing a telehealth platform with 4-G tablets, wireless blood pressure monitors, pulse oximeters and scales demonstrated efficacy in remotely monitoring patients for signs and symptoms of decompensation including hepatic encephalopathy, fluid overload, bleeding, and infections. Preventable readmissions were reduced from 33.8% in the standard of care arm to 0% at 30 and 90 days in the intervention arm. This intervention showed the ability for telemonitoring to reduce 30-d and 90-d readmissions while promoting patient-centered care[24] (Table 1).

Studies performed in cirrhosis and liver transplant populations highlight the potentials of telemedicine and mHealth in liver disease, and yet, relative to other specialties and disease states, there is a paucity of literature implementing these innovative technologies with patients. Comparatively, the role of telemedicine in

Table 1 Interventions targeting hepatitis C treatment, cirrhosis care and readmissions, and liver transplant recipients

Study	Population	Modality	Findings
Interventions targeting hepatitis C treatment			
Arora *et al*[40], 2011	Patients with hepatitis C in rural areas and prisons in New Mexico (n = 261), compared to in-person visits at a University clinic (n = 146)	Videoconferences at ECHO site between community physicians and specialists, compared to in person visits at a University clinic	Comparable rates of SVR were seen between ECHO model and those seen in person at the University HCV clinic (58.2% *vs* 57.5%, P = 0.89)
Marciano *et al*[14], 2017	Providers treating hepatitis C in the Patagonia Region in South America (n = 14)	Videoconferences at ECHO sites between community physicians and those at a University Hospital in Argentina	Survey data focused on skills and competence in hepatitis C before and after 6 months of participating in the project, ultimately showing significant improvement in provider confidence regarding their ability to stage fibrosis, determine appropriate candidates for treatment, and select appropriate HCV treatment
Rossaro *et al*[17], 2008	Patients with hepatitis C in rural California (n = 103)	Videoconference between patients and specialists	23% of patients were candidates for therapy, 15 patients were evaluated for liver transplant
Talal *et al*[19], 2018	Patients with hepatitis C undergoing an opioid substitution therapy program (n = 62)	Biweekly telemedicine sessions between the patient and a specialty provider during the treatment course	Of 45 treated patients, 42 (93.3%) achieved SVR
Cooper *et al*[18], 2017	Patients with hepatitis C in Canada receiving care from the Ottawa Hospital Viral Hepatitis Outpatient Clinic, comparing telemedicine (n = 157) and non-telemedicine (n = 1130)	Videoconference between patients and specialists	Significantly fewer telemedicine patients initiated antiviral therapy compared to non-telemedicine patients (27.4% *vs* 53.8%, P < 0.001). Among those treated with DAA they noted similar SVR rates (94.7% *vs* 94.8%, P = 0.99)
Interventions targeting cirrhosis care and readmissions			
Su *et al*[15], 2018	Patients with liver disease in the Veterans Health Administration (VA) system receiving ECHO visits (n = 513) compared to all patients in the VHA with liver disease (n = 62237)	Virtual Consultations (through the VA SCAN-ECHO Project) compared to usual care	Propensity-adjusted mortality rates showed improved survival in the SCAN-ECHO cohort (HR of 0.54, 95%CI 0.36-0.81)
Khungar *et al*[24], 2017	Patients with cirrhosis received 4G tablets with wireless devices to monitor blood pressure, heart rate, weight, symptoms, and medication administration. Telehealth nurses in conjunction with primary hepatology team intervened to prevent readmissions. (n = 19 intervention, 143 control)	Remote monitoring with telehealth based early intervention	The remote monitoring/ telehealth arm had 0% of readmissions due to potentially preventable causes (fluid overload or hepatic encephalopathy) due to early outpatient interventions whereas 31% of readmissions were due to these causes in the control arm
Konjeti *et al*[16], 2019	Potential Liver Transplant Candidates in the VA system (n = 19091 through SCAN-ECHO and 99 seen in-person)	Virtual Consultations (through the VA SCAN-ECHO Project) compared to in-person visits	The telehealth-based triage reduced futile transplant evaluations by approximately 60%
Ganapathy *et al*[23], 2017	Cirrhotic patients with caregivers after hospital discharge (n = 40)	Home monitoring using an iPad with the Patient Buddy App (monitoring medication adherence, sodium intake and weights, and cognition)	17 of 40 patients were readmitted within 30 d. 8 potential readmissions related to hepatic encephalopathy were prevented via early outpatient interventions
Interventions targeting liver transplant recipients			
Ertel *et al*[20], 2016	Post Liver Transplantation Patients (n = 20)	Telehealth home monitoring (vital sign tracking) and an educational video program	19 of the 20 patients responded to a survey, with 95% watching all videos and 100% finding them effective. 90-d readmission rate of 30% (42% lower than historical controls)
Song *et al*[21], 2013	Pediatric Post Liver Transplant Patients, International (n = 4)	Home monitoring and decision support using a tablet PC and a specially developed software	Four international patients/families transferred 38 records of blood tests, demonstrating that this software is technically feasible
Le *et al*[37], 2018	Post Liver Transplant Patients	Televisits (n = 21) versus in clinic visits (n = 21)	Similar patient satisfaction. Less commute and waiting times in the televisit group

SCAN-ECHO: Specialty Access Network-Extension for Community Healthcare Outcomes; CI: Confidence interval; HR: Hazard ratio; HCV: Hepatitis C virus; VA: Veterans Health Administration.

monitoring and facilitating treatment of patients with heart failure has been widely studied. Similar to patients with cirrhosis, patients with heart failure frequently require emergency hospitalizations and can have prolonged hospital admissions and frequent readmissions. Many of these hospitalizations could be avoided if patients received more education and had access to remote interactions with their medical teams, thereby empowering them to participate in the management of their own disease including modifications to their sodium intake or titration of medications.

The heart failure literature is robust with randomized control trials, systematic reviews, and meta-analyses showing associations with reductions in mortality and hospitalizations for heart failure[25]. In addition, telemedicine in congestive heart failure can be economically beneficial; studies show savings that ranged from $5000 to over $50000 per year per patient[26]. Examples of innovative technologies that facilitate remote monitoring and treatment of patients with heart failure include telemonitoring devices that track hemodynamics, video-based nursing visits after hospital discharge, and a mobile application to set physical activity goals and provide feedback to individuals undergoing cardiac rehabilitation[27-29].

By contrast, the use of telemedicine in liver disease is limited to a handful of individual interventions and limited publications. Overall, the medical field has been slow in adapting telemedicine to interact with patients. According to data from the American Medical Association's 2016 Patient Practice Benchmark Survey, only 15.4% of physicians use telemedicine to interact with patients. Of all specialties, gastroenterologists were lowest-only 7.9% use telemedicine to interact with patients[30].

THE POTENTIAL UNMET NEEDS

Effectively managing cirrhosis requires titrating medications, closely monitoring symptoms including changes in weight and cognitive abilities (as a surrogate for hepatic encephalopathy), and establishing regular reminders to schedule imaging, labs, and procedures. As such, cirrhosis is a medical condition ripe for telemedicine and mHealth interventions, with a myriad of potential targets for improvement.

IMPROVING SCREENING AND PREVENTATIVE CARE

Some of the most innovative uses of telemedicine and mHealth have been in dermatology and skin cancer screening, including use of smartphone applications for skin monitoring and melanoma detection[31]. Text message interventions have shown increases in screening rates for other cancers, including breast, cervical, and colorectal cancers[32]. Likewise, newly-developed smartphone applications aim to improve patient and provider education regarding screening for cancers, including colorectal and prostate cancer[33,34]. For the care of patients with liver disease, the implementation of the SCAN-ECHO program for chronic liver disease by the Ann Arbor Veterans Affair Healthcare System found marked improvement in the frequency of HCC screening (42% *vs* 25%) and variceal surveillance (25% *vs* 15%) in patients whose providers consulted virtually with a liver specialist, compared to those who had no consultation at all[15]. Relatively simple, low cost interventions like text messaging, smartphone applications and teleconsultations could improve the rates of HCC screening and variceal screening-two interventions that have been shown to be cost-effective in the care of patients with advanced liver disease-and ultimately improve outcomes for patients[4,5,35].

IMPROVING ACCESS TO SPECIALTY CARE IN DISADVANTAGED POPULATIONS

Certain populations have difficulties engaging with specialized care for liver disease, including those who suffer from substance abuse and those living in rural locations. In a retrospective cohort study of over 16000 persons with chronic liver disease, those who live more than 150 miles from a liver transplant center were shown to have a higher mortality and transplant-free mortality, highlighting significant geographic disparities that could be addressed by telemedicine[36]. Prior studies examining the use of teleconsultation in the treatment of hepatitis C and patients with opioid use disorder on methadone show the efficacy of these interventions in reaching groups that lack access or do not seek out medical care[19]. Strategies such as video conferencing with patients, primary care providers, and general gastroenterologists

could play a significant role in increasing the reach of liver specialists to improve outcomes in patients with cirrhosis.

PROVIDING VALUE BASED HEALTHCARE

Approximately 30% of patients with cirrhosis are readmitted within 30 d of discharge, posing a significant cost burden to the United States healthcare system[9]. Given the promising cost-savings shown from using telemedicine among persons with heart failure, similar models should target patients with cirrhosis to reduce costs in the healthcare system, allow monitoring of patients in between visits, and facilitate communication for patients and providers between hospital discharge and clinic follow up. In one study in the care of patients after liver transplantation, general patient satisfaction of those who had telemedicine visits via video connection was similar to that of patients who had in-person visits. Moreover, telemedicine patients reported significantly less commute and waiting times compared to patients seen in-person[37]. Above all, the improved survival rates, as seen in the VA system with virtual consultations, indicate strong potential benefits to investing in telemedicine.

MONITORING INDIVIDUALS IN THEIR NATURAL ENVIRONMENTS

Patients suffering from chronic disease spend only a few hours with providers each year. This means that much of the burden of their disease management falls on the individual patients and their families during the remaining 5000 waking hours each year-including decisions on taking medications, following dietary restrictions, and making other choices that can significantly affect their health[38]. When patients with cirrhosis are seen in clinic, they often feel the need to hold their lactulose or diuretics to facilitate travel without frequent bathroom breaks. This disruption in medication dosing can lead to mild encephalopathy during their clinical assessment, and they may not be aware of everything conveyed to them during a visit. The use of televisits and telemonitoring strategies can give providers the opportunity to obtain assessments of patients in their home environment and gather more useful information than what they would otherwise obtain in a clinic visit.

BARRIERS TO OVERCOME

While telemedicine is a promising field, there are several barriers that will need to be overcome before its use can become widespread. Reimbursement remains an ongoing challenge, as payment varies for private payers and according to state laws, and Medicare currently reimburses for video consultation only for individuals in designated Health Professional Shortage Areas[12]. In addition, concerns regarding the quality of healthcare have been raised in telehealth, particularly with the limitations of the remote physical exam, the difficulty in establishing patient-physician trust remotely, and the fragmentation of care among multiple providers[39]. Consideration will also need to be given to ensuring adherence to state and national regulations and to establishing the appropriate infrastructure for patients with limited access or ability to use telecommunication technologies.

Additionally, the majority of interventions described to date are single arm interventions without control groups, making it difficult to estimate the true benefit of any intervention or to have a clear understanding of cost effectiveness. To understand downstream cost savings of such interventions, considerations will need to be given to defining clinical outcomes, clearly stating costs, and carefully defining control groups to better assess the potential benefits of an intervention.

CONCLUSION

The available literature suggests we are falling short of meeting a variety of quality metrics in the care of patients with cirrhosis-including preventative strategies such as cancer screening and treatment strategies such as the management of variceal bleeding and ascites. Interventions using telemedicine and mHealth provide logical solutions to improve screening rates, to reach disadvantaged rural populations, and to provide value-based care. Telemedicine may prove to be the guiding force in the

coordination of care between episodes for patients with cirrhosis. There is a need for more resources to evaluate telemedicine interventions and to develop infrastructure to care for patients with cirrhosis. If executed effectively, telemedicine and mHealth technologies can provide cost savings and improve outcomes for patients with cirrhosis.

REFERENCES

1 **D'Amico G**, Garcia-Tsao G, Pagliaro L. Natural history and prognostic indicators of survival in cirrhosis: A systematic review of 118 studies. *J Hepatol* 2006; **44**: 217-231 [PMID: 16298014 DOI: 10.1016/j.jhep.2005.10.013]

2 **Kanwal F**, Tapper EB, Ho C, Asrani SK, Ovchinsky N, Poterucha J, Flores A, Ankoma-Sey V, Luxon B, Volk M. Development of Quality Measures in Cirrhosis by the Practice Metrics Committee of the American Association for the Study of Liver Diseases. *Hepatology* 2019; **69**: 1787-1797 [PMID: 30586188 DOI: 10.1002/hep.30489]

3 **Singal AG**, Yopp A, S Skinner C, Packer M, Lee WM, Tiro JA. Utilization of hepatocellular carcinoma surveillance among American patients: A systematic review. *J Gen Intern Med* 2012; **27**: 861-867 [PMID: 22215266 DOI: 10.1007/s11606-011-1952-x]

4 **Sarasin FP**, Giostra E, Hadengue A. Cost-effectiveness of screening for detection of small hepatocellular carcinoma in western patients with Child-Pugh class A cirrhosis. *Am J Med* 1996; **101**: 422-434 [PMID: 8873514 DOI: 10.1016/S0002-9343(96)00197-0]

5 **Lin OS**, Keeffe EB, Sanders GD, Owens DK. Cost-effectiveness of screening for hepatocellular carcinoma in patients with cirrhosis due to chronic hepatitis C. *Aliment Pharmacol Ther* 2004; **19**: 1159-1172 [PMID: 15153169 DOI: 10.1111/j.1365-2036.2004.01963.x]

6 **Lemoine M**, Thursz MR. Battlefield against hepatitis B infection and HCC in Africa. *J Hepatol* 2017; **66**: 645-654 [PMID: 27771453 DOI: 10.1016/j.jhep.2016.10.013]

7 **Kanwal F**, Kramer JR, Buchanan P, Asch SM, Assioun Y, Bacon BR, Li J, El-Serag HB. The quality of care provided to patients with cirrhosis and ascites in the Department of Veterans Affairs. *Gastroenterology* 2012; **143**: 70-77 [PMID: 22465432 DOI: 10.1053/j.gastro.2012.03.038]

8 **Buchanan PM**, Kramer JR, El-Serag HB, Asch SM, Assioun Y, Bacon BR, Kanwal F. The quality of care provided to patients with varices in the department of Veterans Affairs. *Am J Gastroenterol* 2014; **109**: 934-940 [PMID: 24989087 DOI: 10.1038/ajg.2013.487]

9 **Okafor PN**, Nnadi AK, Okoli O, Huang AE, Nwaiwu O. Same- vs Different-Hospital Readmissions in Patients With Cirrhosis After Hospital Discharge. *Am J Gastroenterol* 2019; **114**: 464-471 [PMID: 30676364 DOI: 10.14309/ajg.0000000000000050]

10 **Bajaj JS**, Reddy KR, Tandon P, Wong F, Kamath PS, Garcia-Tsao G, Maliakkal B, Biggins SW, Thuluvath PJ, Fallon MB, Subramanian RM, Vargas H, Thacker LR, O'Leary JG; North American Consortium for the Study of End-Stage Liver Disease. The 3-month readmission rate remains unacceptably high in a large North American cohort of patients with cirrhosis. *Hepatology* 2016; **64**: 200-208 [PMID: 26690389 DOI: 10.1002/hep.28414]

11 **American Board of Internal Medicine**. 2018, April. Candidates Certified by US State/Territory. Available from: https://www.abim.org/~/media/ABIM%20Public/Files/pdf/statistics-data/candidates-certified-by-state.pdf

12 **Serper M**, Volk ML. Current and Future Applications of Telemedicine to Optimize the Delivery of Care in Chronic Liver Disease. *Clin Gastroenterol Hepatol* 2018; **16**: 157-161.e8 [PMID: 29389489 DOI: 10.1016/j.cgh.2017.10.004]

13 **Arora S**, Kalishman S, Thornton K, Dion D, Murata G, Deming P, Parish B, Brown J, Komaromy M, Colleran K, Bankhurst A, Katzman J, Harkins M, Curet L, Cosgrove E, Pak W. Expanding access to hepatitis C virus treatment--Extension for Community Healthcare Outcomes (ECHO) project: Disruptive innovation in specialty care. *Hepatology* 2010; **52**: 1124-1133 [PMID: 20607688 DOI: 10.1002/hep.23802]

14 **Marciano S**, Haddad L, Plazzotta F, Mauro E, Terraza S, Arora S, Thornton K, Ríos B, García Dans C, Ratusnu N, Calanni L, Allevato J, Sirotinsky ME, Pedrosa M, Gadano A. Implementation of the ECHO® telementoring model for the treatment of patients with hepatitis C. *J Med Virol* 2017; **89**: 660-664 [PMID: 27551942 DOI: 10.1002/jmv.24668]

15 **Su GL**, Glass L, Tapper EB, Van T, Waljee AK, Sales AE. Virtual Consultations Through the Veterans Administration SCAN-ECHO Project Improves Survival for Veterans With Liver Disease. *Hepatology* 2018; **68**: 2317-2324 [PMID: 29729194 DOI: 10.1002/hep.30074]

16 **Konjeti VR**, Heuman D, Bajaj JS, Gilles H, Fuchs M, Tarkington P, John BV. Telehealth-Based Evaluation Identifies Patients Who Are Not Candidates for Liver Transplantation. *Clin Gastroenterol Hepatol* 2019; **17**: 207-209.e1 [PMID: 29723691 DOI: 10.1016/j.cgh.2018.04.048]

17 **Rossaro L**, Aoki C, Yuk J, Prosser C, Goforth J, Martinez F. The evaluation of patients with hepatitis C living in rural California via telemedicine. *Telemed J E Health* 2008; **14**: 1127-1129 [PMID: 19119836 DOI: 10.1089/tmj.2008.0029]

18 **Cooper CL**, Hatashita H, Corsi DJ, Parmar P, Corrin R, Garber G. Direct-Acting Antiviral Therapy Outcomes in Canadian Chronic Hepatitis C Telemedicine Patients. *Ann Hepatol* 2017; **16**: 874-880 [PMID: 29055923 DOI: 10.5604/01.3001.0010.5277]

19 **Talal AH**, Andrews P, Mcleod A, Chen Y, Sylvester C, Markatou M, Brown LS. Integrated, Co-located, Telemedicine-based Treatment Approaches for Hepatitis C Virus (HCV) Management in Opioid Use Disorder Patients on Methadone. *Clin Infect Dis* 2018 [PMID: 30329042 DOI: 10.1093/cid/ciy899]

20 **Ertel AE**, Kaiser TE, Abbott DE, Shah SA. Use of video-based education and tele-health home monitoring after liver transplantation: Results of a novel pilot study. *Surgery* 2016; **160**: 869-876 [PMID: 27499142 DOI: 10.1016/j.surg.2016.06.016]

21 **Song B**, Schulze M, Goldschmidt I, Haux R, Baumann U, Marschollek M. Home monitoring and decision support for international liver transplant children. *Stud Health Technol Inform* 2013; **192**: 268-272 [PMID: 23920558 DOI: 10.3233/978-1-61499-289-9-268]

22 **Bajaj JS**, Heuman DM, Sterling RK, Sanyal AJ, Siddiqui M, Matherly S, Luketic V, Stravitz RT, Fuchs M, Thacker LR, Gilles H, White MB, Unser A, Hovermale J, Gavis E, Noble NA, Wade JB. Validation of

EncephalApp, Smartphone-Based Stroop Test, for the Diagnosis of Covert Hepatic Encephalopathy. *Clin Gastroenterol Hepatol* 2015; **13**: 1828-1835.e1 [PMID: 24846278 DOI: 10.1016/j.cgh.2014.05.011]
23 **Ganapathy D**, Acharya C, Lachar J, Patidar K, Sterling RK, White MB, Ignudo C, Bommidi S, DeSoto J, Thacker LR, Matherly S, Shaw J, Siddiqui MS, Puri P, Sanyal AJ, Luketic V, Lee H, Stravitz RT, Bajaj JS. The patient buddy app can potentially prevent hepatic encephalopathy-related readmissions. *Liver Int* 2017; **37**: 1843-1851 [PMID: 28618192 DOI: 10.1111/liv.13494]
24 **Khungar V**, Serper M, Peyton D, Mehta S, Norris A, Huffenberger A, Siddique SM, Forde KA. Use of an Innovative Telehealth Platform to Reduce Readmissions and Enable Patient-Centered Care in Cirrhotic Patients. *Hepatology* 2017; **66**: 94A-95A
25 **Anker SD**, Koehler F, Abraham WT. Telemedicine and remote management of patients with heart failure. *Lancet* 2011; **378**: 731-739 [PMID: 21856487 DOI: 10.1016/S0140-6736(11)61229-4]
26 **Andrès E**, Talha S, Zulfiqar AA, Hajjam M, Ervé S, Hajjam J, Gény B, Hajjam El Hassani A. Current Research and New Perspectives of Telemedicine in Chronic Heart Failure: Narrative Review and Points of Interest for the Clinician. *J Clin Med* 2018; **7**: pii: E544 [PMID: 30551588 DOI: 10.3390/jcm7120544]
27 **Tse G**, Chan C, Gong M, Meng L, Zhang J, Su XL, Ali-Hasan-Al-Saegh S, Sawant AC, Bazoukis G, Xia YL, Zhao JC, Lee APW, Roever L, Wong MC, Baranchuk A, Liu T; International Health Informatics Study (IHIS) Network. Telemonitoring and hemodynamic monitoring to reduce hospitalization rates in heart failure: A systematic review and meta-analysis of randomized controlled trials and real-world studies. *J Geriatr Cardiol* 2018; **15**: 298-309 [PMID: 29915620 DOI: 10.11909/j.issn.1671-5411.2018.04.008]
28 **Jerant AF**, Azari R, Martinez C, Nesbitt TS. A randomized trial of telenursing to reduce hospitalization for heart failure: Patient-centered outcomes and nursing indicators. *Home Health Care Serv Q* 2003; **22**: 1-20 [PMID: 12749524 DOI: 10.1300/J027v22n01_01]
29 **Beatty AL**, Magnusson SL, Fortney JC, Sayre GG, Whooley MA. VA FitHeart, a Mobile App for Cardiac Rehabilitation: Usability Study. *JMIR Hum Factors* 2018; **5**: e3 [PMID: 29335235 DOI: 10.2196/human-factors.8017]
30 **Kane CK**, Gillis K. The Use Of Telemedicine By Physicians: Still The Exception Rather Than The Rule. *Health Aff (Millwood)* 2018; **37**: 1923-1930 [PMID: 30633670 DOI: 10.1377/hlthaff.2018.05077]
31 **Chao E**, Meenan CK, Ferris LK. Smartphone-Based Applications for Skin Monitoring and Melanoma Detection. *Dermatol Clin* 2017; **35**: 551-557 [PMID: 28886812 DOI: 10.1016/j.det.2017.06.014]
32 **Uy C**, Lopez J, Trinh-Shevrin C, Kwon SC, Sherman SE, Liang PS. Text Messaging Interventions on Cancer Screening Rates: A Systematic Review. *J Med Internet Res* 2017; **19**: e296 [PMID: 28838885 DOI: 10.2196/jmir.7893]
33 **Khan Z**, Darr U, Khan MA, Nawras M, Khalil B, Abdel-Aziz Y, Alastal Y, Barnett W, Sodeman T, Nawras A. Improving Internal Medicine Residents' Colorectal Cancer Screening Knowledge Using a Smartphone App: Pilot Study. *JMIR Med Educ* 2018; **4**: e10 [PMID: 29535080 DOI: 10.2196/mededu.9635]
34 **Owens OL**, Beer JM, Reyes LI, Thomas TL. Systematic Review of Commercially Available Mobile Phone Applications for Prostate Cancer Education. *Am J Mens Health* 2019; **13**: 1557988318816912 [PMID: 30526243 DOI: 10.1177/1557988318816912]
35 **Arguedas MR**, Heudebert GR, Eloubeidi MA, Abrams GA, Fallon MB. Cost-effectiveness of screening, surveillance, and primary prophylaxis strategies for esophageal varices. *Am J Gastroenterol* 2002; **97**: 2441-2452 [PMID: 12358270 DOI: 10.1016/s0002-9270(02)04357-5]
36 **Goldberg DS**, Newcomb C, Gilroy R, Sahota G, Wallace AE, Lewis JD, Halpern SD. Increased Distance to a Liver Transplant Center Is Associated With Higher Mortality for Patients With Chronic Liver Failure. *Clin Gastroenterol Hepatol* 2017; **15**: 958-960 [PMID: 28246053 DOI: 10.1016/j.cgh.2017.02.023]
37 **Le LB**, Rahal HK, Viramontes MR, Meneses KG, Dong TS, Saab S. Patient Satisfaction and Healthcare Utilization Using Telemedicine in Liver Transplant Recipients. *Dig Dis Sci* 2019; **64**: 1150-1157 [PMID: 30519848 DOI: 10.1007/s10620-018-5397-5]
38 **Asch DA**, Muller RW, Volpp KG. Automated hovering in health care--watching over the 5000 hours. *N Engl J Med* 2012; **367**: 1-3 [PMID: 22716935 DOI: 10.1056/NEJMp1203869]
39 **Dorsey ER**, Topol EJ. State of Telehealth. *N Engl J Med* 2016; **375**: 154-161 [PMID: 27410924 DOI: 10.1056/NEJMra1601705]
40 **Arora S**, Thornton K, Murata G, Deming P, Kalishman S, Dion D, Parish B, Burke T, Pak W, Dunkelberg J, Kistin M, Brown J, Jenkusky S, Komaromy M, Qualls C. Outcomes of treatment for hepatitis C virus infection by primary care providers. *N Engl J Med* 2011; **364**: 2199-2207 [PMID: 21631316 DOI: 10.1056/NEJMoa1009370]

World Journal of Gastroenterology

Submit a Manuscript: https://www.f6publishing.com

World J Gastroenterol 2019 August 7; 25(29): 3857-3869

DOI: 10.3748/wjg.v25.i29.3857

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

OPINION REVIEW

Lumen-apposing metal stents for malignant biliary obstruction: Is this the ultimate horizon of our experience?

Andrea Anderloni, Edoardo Troncone, Alessandro Fugazza, Annalisa Cappello, Giovanna Del Vecchio Blanco, Giovanni Monteleone, Alessandro Repici

ORCID number: Andrea Anderloni (0000-0002-1021-0031); Edoardo Troncone (0000-0003-4520-6865); Alessandro Fugazza (0000-0003-0485-4903); Annalisa Cappello (0000-0002-3333-6833); Giovanna Del Vecchio Blanco (0000-0002-6327-563X); Giovanni Monteleone (0000-0003-1339-9076); Alessandro Repici (0000-0002-1621-6450).

Author contributions: Anderloni A, Troncone E and Fugazza A designed the study; Troncone E and Fugazza A wrote the paper; Del Vecchio Blanco G, Monteleone G and Cappello A critical revision of the work; Anderloni A and Repici A supervised the work.

Conflict-of-interest statement: Dr. Anderloni reports personal fees from Boston Scientific, during the conduct of the study; personal fees from Boston Scientific, outside the submitted work; Dr. Repici reports personal fees from Boston Scientific, personal fees from Fujifilm, during the conduct of the study; personal fees from Boston Scientific, personal fees from Fujifilm, outside the submitted work; Dr. Troncone, Dr. Fugazza, Dr. Del Vecchio Blanco, Dr. Cappello, Dr. Monteleone have nothing to disclose.

Andrea Anderloni, Alessandro Fugazza, Annalisa Cappello, Alessandro Repici, Digestive Endoscopy Unit, Division of Gastroenterology, Humanitas Research Hospital, Milan 20089, Italy

Edoardo Troncone, Giovanna Del Vecchio Blanco, Giovanni Monteleone, Department of Systems Medicine, University of Rome "Tor Vergata", Rome 00133, Italy

Alessandro Repici, Digestive Endoscopy Unit, Division of Gastroenterology, Humanitas University, Milan 20089, Italy

Corresponding author: Andrea Anderloni, MD, PhD, Chief Doctor, Digestive Endoscopy Unit, Division of Gastroenterology, Humanitas Research Hospital, Via Manzoni 56, Milan 20089, Italy. andrea.anderloni@humanitas.it
Telephone: +39-02-82247308

Abstract

In the last years, endoscopic ultrasonography (EUS) has evolved from a purely diagnostic technique to a more and more complex interventional procedure, with the possibility to perform several type of therapeutic interventions. Among these, EUS-guided biliary drainage (BD) is gaining popularity as a therapeutic approach after failed endoscopic retrograde cholangiopancreatography in distal malignant biliary obstruction (MBO), due to the avoidance of external drainage, a lower rate of adverse events and re-interventions, and lower costs compared to percutaneous trans-hepatic BD. Initially, devices created for luminal procedures (*e.g.*, luminal biliary stents) have been adapted to the new trans-luminal EUS-guided interventions, with predictable shortcomings in technical success, outcome and adverse events. More recently, new metal stents specifically designed for transluminal drainage, namely lumen-apposing metal stents (LAMS), have been made available for EUS-guided procedures. An electrocautery enhanced delivery system (EC-LAMS), which allows direct access of the delivery system to the target lumen, has subsequently simplified the classic multi-step procedure of EUS-guided drainages. EUS-BD using LAMS and EC-LAMS has been demonstrated effective and safe, and currently seems one of the most performing techniques for EUS-BD. In this Review, we summarize the evolution of the EUS-BD in distal MBO, focusing on the novelty of LAMS and analyzing the unresolved questions about the possible role of EUS as the first therapeutic option to achieve BD in this setting of patients.

Key words: Interventional endoscopic ultrasonography; Endoscopic ultrasonography-

Manuscript source: Invited manuscript

Received: March 2, 2019
Peer-review started: March 3, 2019
First decision: April 30, 2019
Revised: June 20, 2019
Accepted: July 1, 2019
Article in press: July 3, 2019
Published online: August 7, 2019

P-Reviewer: Sugimoto M
S-Editor: Yan JP
L-Editor: A
E-Editor: Ma YJ

guided biliary drainage; Endoscopic ultrasonography-guided choledocho-duodenostomy; Biliary metal stent; Lumen-apposing metal stent

Core tip: Endoscopic ultrasonography (EUS)-guided choledocho-duodenostomy represents one of the possible therapeutic options to achieve biliary drainage after failed endoscopic retrograde cholangiopancreatography. Lumen-apposing metal stent (LAMS) are fully covered metal stents specifically designed for EUS-guided transluminal interventions, such as peripancreatic fluid collection or gallbladder drainage, that have been proposed for biliary drainage in the setting of distal malignant biliary obstruction, in order to overcome the limits of non-dedicated devices. This Review focuses on the new role of LAMS in the complex scenario of EUS-guided biliary drainage.

Citation: Anderloni A, Troncone E, Fugazza A, Cappello A, Blanco GDV, Monteleone G, Repici A. Lumen-apposing metal stents for malignant biliary obstruction: Is this the ultimate horizon of our experience? *World J Gastroenterol* 2019; 25(29): 3857-3869
URL: https://www.wjgnet.com/1007-9327/full/v25/i29/3857.htm
DOI: https://dx.doi.org/10.3748/wjg.v25.i29.3857

INTRODUCTION

Management of obstructive jaundice is of paramount importance in patients with malignant biliary obstruction (MBO), as impaired biliary drainage dramatically affects the possibility of systemic therapy in unresectable disease, reduces quality of life and increases morbidity and mortality[1]. The most frequent causes of distal MBO are adenocarcinoma of the head of the pancreas, distal cholangiocarcinoma, ampullary carcinomas and adenopathy or metastasis from other cancers. It is estimated that more than half of patients with unresectable ductal adenocarcinoma of the head of the pancreas presents with obstructive jaundice, and nearly 80% of these patients will develop jaundice in absence of therapy or interventions[2,3]. Regardless of the cause, unresolved biliary obstruction increases the risk of cholangitis and liver failure; determines fat and fat-soluble vitamins malabsorption, contributing to malnutrition and cachexia; associates in up to 25% with pruritus, which poorly responds to medical therapy and dramatically compromises quality of life. It is responsible, directly or indirectly, for death of a great proportion of non-palliated patients[1,4]. For many years, palliation of obstructive jaundice has been achieved with open surgery, by performing surgical choledocho-enterostomy, cholecysto-enterostomy or hepatico-jejunostomy, with or without gastrojejunostomy in case of concomitant gastric outlet obstruction (GOO). Operative biliary bypass has shown high rate of technical success and low rate of jaundice recurrence, but at the expense of significant post-operative morbidity and mortality, which range from 27%-60% and 5.4%-23% respectively in some series[4-6]. More recent studies that compared endoscopic biliary stenting and operative biliary bypass found a higher post-operative morbidity in the operative group, while endoscopic drainage was associated with lower costs, shorter duration of hospital stay and a better quality of life[7,8]. Due to these evidences, less invasive approaches to achieve biliary drainage, namely percutaneous transhepatic biliary drainage (PTBD) and endoscopic retrograde cholangiopancreatography (ERCP) with biliary stenting, have progressively spread, with a concomitant reduction of the patients undergoing operative palliation over the years[2].

ENDOSCOPIC AND PERCUTANEOUS BILIARY DRAINAGE IN DISTAL MALIGNANT BILIARY OBSTRUCTION

Currently, ERCP with placement of plastic or self-expanding metal stent (SEMS) is widely recognized as the first strategy to achieve biliary drainage in distal MBO and, when feasible, should be preferred over PTBD and surgery[9]. Reaching the papillary region in the second portion of the duodenum and cannulating the bile duct represent the first fundamental steps to perform endoscopic operative procedures on the biliary system. The success of such steps depends on several factors related, among the

others, to the patient's anatomy and to the experience of the endoscopist. The success rate of ERCP for all indications reported in literature is high, ranging from 86%-99%. However, an underlying neoplastic process could predict a lower success rate, a higher need of advanced cannulation techniques (*i.e.*, needle knife pre-cut, double guidewire (DGW) technique, pancreatic septotomy) with consequent higher risk of adverse events (AE)[10-12]. During ERCP, malignant biliary stricture could be very hard to pass, even for experienced endoscopists; neoplastic diseases involving the distal common bile duct (CBD) can determinate infiltration and distortion of the ampulla, thus making very difficult the identification and the subsequent attempt to cannulate the papilla (Figure 1). Moreover, advanced neoplastic disease could associate with concomitant biliary and duodenal obstruction, determining the inaccessibility of the ampullary region. In addition to the aforementioned possibilities, also common benign conditions such as intradiverticular papilla or gastroduodenal surgically altered anatomy could make ERCP difficult. For several years, a common accepted therapeutic algorithm after a failed ERCP has provided these options: in cases of an accessible papilla, a possible new attempt at the same institution or after referral to a tertiary-care hospital in 3-5 d, after the resolution of the edema of the ampulla; in case of inaccessible papilla, or after definitely failed ERCP, a PTBD performed by interventional radiologists. First described in the seventies, PTBD is performed under fluoroscopic or ultrasonographic guidance, and allows to place an external biliary catheter with subsequent drainage internalization with placement of plastic or metal stent, in a one-step or two-step procedure[13-17]. PTBD is a highly effective procedure, but is burdened of significant morbidity, with a high rate of procedure-related or drainage- related AE[18]. Most frequent AE reported are occlusion or dislocation of the catheter, cholangitis, bile leakage alongside the drain[18-21]. A retrospective study of more than 2000 PTBD procedures in 385 patients reported that 40% of patients presented at least one drainage-related AE, with malignant disease being a risk factor for drainage occlusion and cholangitis[18]. A recent retrospective study from Sarwar and co-workers on 266 PTBD procedures in 266 patients reported a 45.9% of readmission at 30 d, 63.9% of which were unplanned[22]. The high rate of AE and readmissions, in addition to the presence of the external drainage, could heavily impair the patient's quality of life and, at the same time, significantly increases the costs. Thus far, the widespread and easy availability have confirmed PTBD as the first option to drain MBO after ERCP failure. However, alternative techniques based on interventional endoscopic ultrasonography (EUS), such as EUS-guided biliary drainage (EUS-BD) have progressively demonstrated feasibility and high effectiveness, providing useful alternatives for jaundice palliation.

EUS-GUIDED BILIARY DRAINAGE

Since the early 2000's, the development of echoendoscope with larger operative channel allowing devices up to 10 French, opened the way for an increasingly interventional role for EUS procedures. Over the year, several types of EUS-guided procedures such as biliary and pancreatic drainage, peri-pancreatic fluid collections (PFC) drainage, gastro-enteral anastomosis, vascular interventions and ablative treatment of neoplasms have been successfully reported[23]. EUS-guided biliary access and drainage procedures are primarily performed as alternative of percutaneous or surgical drainage after failed ERCP, but the rapid widespread of interventional EUS is currently challenging the role of ERCP as primary approach for MBO[24-27]. EUS-BD can be achieved through different approaches, depending on the experience and preference of the endoscopist, the availability of specific devices, the localization of the biliary obstruction and the accessibility of the papilla: Rendez-vous (RV) technique, EUS-guided antegrade stenting; EUS-guided choledocho-duodenostomy (EUS-CD) or choledocho-gastrostomy; EUS-guided hepato-gastrostomy (EUS-HGS); EUS-guided cholecysto-gastrostomy (as a last resort). To date, which is the best technique is still a matter of debate among interventional endoscopists[28]. A recent systematic review on EUS-CD versus EUS-HGS including 10 studies with 434 patients showed a very high technical [94.1% *vs* 93.7%, pooled odds ratio (OR) = 0.96, 95% confidence interval (CI) = 0.39-2.33, I = 0%] and clinical success (88.5% *vs* 84.5%, pooled OR = 0.76, 95%CI = 0.42-1.35, I = 17%) without difference for AE (OR = 0.97, 95%CI = 0.60-1.56, I = 37%) for these procedures[29].

Regardless of the preferred approach, the first step is the access to the biliary system. Using a curved linear array echoendoscope, the bile duct is punctured with a 19 Gauge needle and the correct positioning of the needle is confirmed by aspiring bile and injecting contrast to fluoroscopically visualize the biliary tree. Then, a 0.035 inch or 0.025 inch guidewire is passed through the needle and manipulated in the

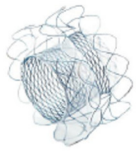
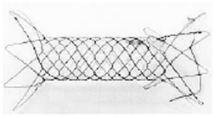
Stent type	Diameter and length (mm)	Figure
AXIOS (bostonScientific) Hot-Axios (electrocautery-enhanced-lumen apposing metal stent (EC-LAMS) Cold axios (non-electro-cauteryenhanced)	6 × 8 8 × 8 10 × 10 15 × 10 20 × 10	
SPAXUS (Taewoong medical)	8 × 20 10 × 20 16 × 20	
NAGI (Taewoong medical)	10 × 10/20/30 12 × 10/20/30 14 × 10/20/30 16 × 10/20/30	
AIXSTENT (Leufen medical)	10 × 10/20/30 15 × 30	
HANAROSTENT (M.I.Tech)	10 × 30/40 12 × 30/40	

Figure 1 Currently lumen-apposing metal stent and fully covered self-expanding metal stent with peculiar anti-migratory shape available on the market.

desired direction. The biliary system can be accessed through a trans-hepatic route, by which the intrahepatic biliary ducts are usually punctured at the third segment with the scope positioned at the gastro-esophageal junction, or through the extra-hepatic bile duct, generally from the bulb or the stomach[23,30]. In case of RV or antegrade stenting, the guidewire is manipulated toward the papilla across the obstruction, and then coiled in the duodenum. On the contrary, the wire is directed toward the hepatic hilum in case of CD or choledocho-gastrostomy. For the RV procedure, the echoendoscope is subsequently exchanged over the guidewire and a duodenoscope is inserted in the duodenum. Then, biliary cannulation is attempted alongside the guidewire previously placed, or, after grasping the guidewire with a snare or forceps and pulling back through the operative channel of the duodendoscope, performed over the guidewire. As already mentioned, directing the guidewire toward the papilla, negotiating the obstruction and reaching the duodenum are key steps for a successful RV procedure or antegrade stenting, and are usually facilitated by keeping the scope in "short" position when puncturing the bile duct[23]. Although the high clinical success rate once these steps are achieved, they could fail in up to 25% of cases[31]. For the other EUS-BD technique, tract dilation with cystotome, needle-knife or balloon is needed before plastic stent or SEMS placement, with a sequence of over-the-wire procedural steps which are crucial for the success of the procedure as well as critical for possible AE[23]. Since the first EUS-CD has been described by Giovannini *et al*[32], several studies have investigated technical and clinical success of different EUS-BD. A systematic review and meta-analysis from Wang *et al*[33] reported the technical success rate, final success rate and AE rate in 1192 patients treated with EUS-BD, including transluminal drainages, RV procedures and antegrade stenting from 42 studies (14 prospective, 25 retrospective single-centre studies and 3 retrospective multi-centre studies). The overall technical and final success rate were 94.71% and

91.66% respectively, while technical and final success rate of EUS-guided transluminal biliary drainage procedures from 29 studies were 95.68% and 90.32%. This study was not able to compare the outcome between all types of EUS drainages, however a comparison between the trans-gastric and trans-duodenal approach did not show significant differences in success or AE. The same systematic review reported a cumulative risk of AE of 23.32% (278 patients), being bleeding (4.03%), bile leakage (4.03%), pneumoperitoneum (3.02%), stent migration (2.68%), cholangitis (2.43%), abdominal pain (1.51%), and peritonitis (1.26%) the most frequent. Strikingly, the use of metal stent *vs* plastic stents was associated with a lower risk of AE (17.52 *vs* 31.03, $P = 0.013$), with no significant differences in technical and clinical success rate[33]. The difference is probably due to the radial force exerted by the SEMS during the expansion, which seals the fistula between the gastrointestinal wall and the bile duct wall, reducing the risk of bile leakage and bile peritonitis. In addition, the larger calibre of metal stent compared to plastic stent probably reduces the risk of occlusion and subsequent cholangitis. Currently, SEMS instead of plastic stent should be preferred for EUS-BD[24]. The efficacy of EUS-BD questioned the primary role of PTBD after failed ERCP in MBO (Figure 2). In 2017, a systematic review and meta-analysis from Sharaiha *et al*[34] including 9 studies (483 patients) aimed to compare EUS-BD and PTBD outcome and safety. Despite a similar technical success, a slightly higher clinical success rate [although data from 3 randomized clinical trials (RCTs) reported no significant differences] and a lower risk of AE were found in EUS-BD compared to PTBD. Bile leak, bleeding, cholangitis, sepsis and peritonitis were the most frequent AE reported, and were all more frequent in the PTBD group. Moreover, EUS-BD was associated with less re-intervention and lower costs. A retrospective study comparing EUS-BD and PTBD in 60 patients also reported lower post-procedures pain score in EUS-BD group[35]. Analysing the issue from a different point of view, Nam *et al*[36] aimed to evaluate the patient's preference in case of failed ERCP conducting a multicentre survey in 7 tertiary referral centers. Among 313 patients who responded about a simulated scenario of failed ERCP, 251 (80.2%) preferred EUS-BD, mainly for the possibility of internal drainage. Taken together, these data promoted a novel therapeutic algorithm, which favours EUS-BD, where the expertise is available, as primary approach after failed ERCP in MBO.

LUMINAL-APPOSING METAL STENTS FOR EUS-GUIDED BILIARY DRAINAGE

Despite the exciting reports of clinical efficacy and the favourable safety data over PTBD, the AE rate for EUS-BD is not negligible and is up to 24%[33]. In the last years, interventional EUS has spread rapidly, but the devices available have remained that adapted from other interventional procedures for a long time. In fact, all the devices commonly used come from luminal indications (*i.e.*, biliary dilation balloon, biliary stent, needle-knife), have adapted for transluminal indication and, even if the results have been motivating, it was reasonable that they could be improved. As already discussed, the use of fully covered SEMS (FCSEMS) partially resolved the issue of bile leaks and bile peritonitis due to expanding radial force of the stent that seals the transluminal fistula. However, in absence of specific anti-migratory properties, all the biliary stent designed for luminal indication present a significant risk of dislocation when used for transluminal drainage due to their tubular shape, with possible subsequent peritonitis, perforation and cholangitis. With this regard, lumen-apposing metal stent (LAMS) are fully covered "dumbbell"-shaped short stent made up of braided nitinol, specifically designed for interventional trans-luminal EUS-guided procedures, with distal anti-migratory flanges which provide the lumen-to-lumen apposition effect[37]. The device is pre-loaded in a 9 French or 10.8 French catheter with a through-the-scope delivery system compatible with therapeutic echoendoscope with a working channel of 3.7 mm or larger. Currently, two different LAMS are available on the market: Axios stent (Boston Scientific); Spaxus (Taewoong Medical). The 16 mm Spaxus stent has the largest flange (31 mm), followed by the 20 mm Axios stent (29 mm). Moreover, short FCSEMS with peculiar anti-migratory shape have been commercialized for similar indications: NAGI (Taewoong Medical); Aixstent (Leufen Medical); Hanarostent (M.I. Tech)[38] (Figure 1). LAMS have been originally designed for EUS-guided PFC drainage, as they provided large calibre to drain solid components of walled-off necrosis, low risk of leak alongside the stent and of migration, allowing trans-stent interventional procedures, such as endoscopic necrosectomy[34,39-42]. In 2011, Binmoeller and Shah first described transluminal stenting between two non-adherent lumens of the gastrointestinal tract using LAMS in an *ex-vivo* model[43]. Soon after, in 2012, Itoi *et al*[44] reported the first experience of LAMS in

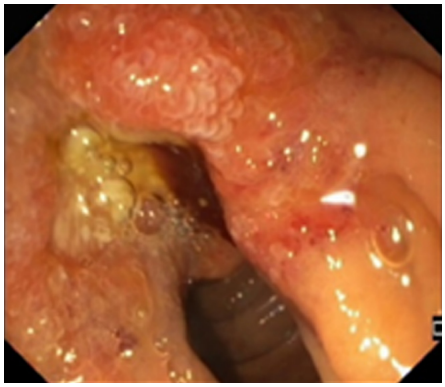


Figure 2 Endoscopic view of infiltration of the papilla by invasive pancreatic cancer.

humans, describing the successful drainage of 15 symptomatic pancreatic pseudocyst and 5 acute cholecystitis in patients unfit for surgery. Since then, several reports have confirmed the feasibility and efficacy of LAMS in these settings, and the indication has expanded to biliary drainage, where the smaller target [*i.e.*, the bile duct instead of PFC or gallbladder (GB)] lead to the development of smaller LAMS. In 2014, the first EUS-CD with LAMS was successfully performed by Itoi and Binmoeller[45] in a patient with unresectable pancreatic cancer and obstructive jaundice. Despite the innovative and dedicated design, the LAMS delivery system was the same of the "old" non-specific plastic stent or SEMS, and still included the same several steps: (1) Puncture of bile duct with FNA needle; (2) Guidewire introduction; (3) Tract dilation; (4) Introduction and delivery of the LAMS. As discussed above, a multi-steps procedure carries *per se* the risk of AE due to multiple exchanges (*e.g.*, losing the wire and/or the scope position, bile leakage during tract dilation). To overcome these shortcomings, a LAMS delivery system has further evolved with the addition of an electrocautery tip [electrocautery-enhanced (EC)-LAMS-HOT-AXIOS, Boston Scientific Corp., Marlborough, Massachusetts, United States] which allows a single-stage technique with the access to the target lumen in one-step procedure, without the need of multiple exchanges and with reduced fluoroscopy and procedure time[46-49] (Figures 3-7). Data from the main studies on biliary LAMS are summarized in Table 1. In 2016, Kunda *et al*[50] reported a retrospective analysis of 57 patients who underwent EUS-CD with LAMS (27 patients) and EC-LAMS (30 patients). The overall technical and clinical success were 98.2% and 94.6% respectively. The major AE rate was 7%, with 2 duodenal perforation (one caused by the tip of the scope and not related to the delivery of the stent; the other during tract dilation for subsequent LAMS placement without cautery), 1 bleeding and 1 transient cholangitis. During the mean follow-up of 151 ± 145 d, 5 out 54 patients (9.3%) need a re-intervention (1 LAMS migration; 4 sump syndrome). A prospective study from Tsuchiya *et al*[51] evaluated 19 patients who underwent EUS-CD with EC-LAMS for MBO after failed ERCP. The stent was deployed using the electro-enhanced catheter over a guidewire previously placed with a 19 Gauge FNA needle puncture. The Authors reported a 100% and 95% technical and clinical success rate, with an AE rate of 36.3% (5/19), mostly with mild severity. Five patients experienced stent obstruction due to occlusion by food residue (n = 2), kinking (n = 1), tumour progression (n = 1) and spontaneous dislodgement (n = 1), and 4 patients underwent a successful re-intervention. Recently, our group reported a retrospective analysis of 46 patients with MBO treated with EC-LAMS after failed ERCP with a single-stage procedure, that is with a direct access to the bile duct with electro-enhanced catheter without a previously placed guidewire[48]. In our series, the technical and clinical success rate were 93.5% (43/46) and 97.1% (42/43), with a major AE rate of 11.6% (3 stent obstruction; 1 stent migration; 1 fatal bleeding). The only case of stent migration was a mild AE that occurred after 148 d from the procedure, and was successfully treated with a RV technique through the remaining fistula and placement of a trans-papillary biliary SEMS. Stent obstruction were also successfully managed with endoscopic interventions. Currently, no specific effective measures have been identified to avoid AE in the setting of biliary LAMS. As far as the risk of LAMS obstruction is concerned, it could be reasonable to manage concomitant duodenal obstruction in the same session, as we reported higher rate of LAMS obstruction in this sub-group of patients[48]. Technical failures with misdeployment of the first flange of the stent occurred in case of endoscope instability in the duodenal bulb, or due to a smaller CBD diameter. For such reason, we now recommend to proceed with single-stage EUS-CD in more dilated CBD (*i.e.*, 15 mm)

and to pre-load the delivery system with a guidewire in difficult cases, in order to perform an over-the-wire stent placement in case of misdeployment of the LAMS. Of note, these cases were all successfully treated during the same endoscopic session, by performing a RV technique through the fistula with subsequent transpapillary drainage or with a successful second attempt with EC-LAMS. Finally, nine patients (19.6%) with concomitant duodenal obstruction were treated in the same session with EUS-BD and subsequent duodenal stent placement, confirming the feasibility of a complete endoscopic palliation in this subgroup of patients[48,52-54]. A recent retrospective study of 52 patients treated with EC-LAMS for MBO confirmed the high rate of technical and clinical success (88.5% and 100% respectively)[55]. The Authors reported a 3.8% short-term (1 stent occlusion and 1 bleeding from pre-cut site of previous failed ERCP) and 13.5% long-term AE rate (including stent obstruction due to tumor progression or food impaction and stent migration). Various technique for EC-LAMS placement have been used in this work, and the single-stage technique, in addition to bile duct diameter > 15 mm and EC-LAMS 6 mm, was found to be significantly associated to technical success. AE related to the use of LAMS for biliary drainage in the setting of MBO are summarized in Table 2. Taken together, the cited works highlight a high efficacy and a good safety of EUS-BD with LAMS. Of note, AE reported were mostly successfully managed with endoscopic re-intervention (stent cleansing, stent-in-stent placement), and the risk of bile leakage, bile peritonitis, perforation or pneumoperitoneum compared to classic multi-steps EUS-BD was definitely lower. With an easier deployment technique, a good safety and a very short procedural time, EUS-CD with LAMS seems currently one of the most performing EUS-BD approaches[48,55].

FUTURE PERSPECTIVES AND UNRESOLVED QUESTIONS

The impressive results of EUS-BD have recently questioned the role of interventional EUS as a mere second option after failed ERCP, advancing the hypothesis of a possible primary role in MBO alternative to ERCP. It is clear that a trans-papillary approach is difficult in case of duodenal obstruction, and EUS-BD could be the best solution, but which is actually the best drainage strategy in a patient with MBO and an accessible papilla remains debatable. ERCP for MBO can be associated with a significant morbidity, including acute pancreatitis and cholangitis, and pre-operative drainage is not indicated in patients with obstructive jaundice and surgical indication[9,10]. Moreover, some studies reported that advanced techniques of cannulation (*i.e.*, DGW techniques, pre-cut sphincterotomy, trans-pancreatic septotomy) are associated with increased risk of AE[11,56]. These observations stressed the need of high quality evidences comparing ERCP and EUS-BD as primary approach for MBO. Recently, 3 RCT trying to address the issue have been published, and all concluded that EUS-BD has comparable outcome to ERCP in this setting[25-27]. However, it should be noted that these RCT have been powered on different outcomes: AE rate for the study from Bang *et al*[25]; stent patency for the study from Park *et al*[27]; technical success (designed as non-inferiority RCT) for the study from Paik *et al*[26]. Despite the good design and the importance of the data provided, these studies did not offered conclusive information about EUS-BD in primary biliary drainage. Moreover, for the study from Paik *et al*[26], it should also be noted that the AE rate in the ERCP group was higher than EUS-BD group, but extremely high in absolute (39.1%), probably also because of the lack of prophylactic measure to prevent post-ERCP pancreatitis[57]. However, the risk of pancreatitis in EUS-BD groups from all studies was 0%, as expected for a procedure in which the papilla is not manipulated and the pancreatic parenchyma is always spared. None of the aforementioned RCT used LAMS for EUS-BD, and a future challenge will be to address in a RCT the outcome of EUS-CD with LAMS compared to ERCP for primary biliary drainage.

Patency of biliary stents is a crucial issue in jaundice palliation, as stent occlusion determines morbidity (*e.g.*, cholangitis) and increases the need of re-interventions, thus impacting on quality of life and costs. Biliary SEMS have demonstrated a longer patency compared to plastic stents[9,58]. However, even SEMS carries a risk of occlusion due to tumor ingrowth (for uncovered SEMS), and overgrowth (for FCSEMS)[59]. EUS-CD is performed in a CBD segment above the obstruction and the stent does not cross the neoplastic tissue. On the other hand, EUS-CD carries the risk of occlusion due to food impaction or biliary sludge deposits, and this has been reported as particularly relevant for patients with duodenal obstruction[48,50,51]. The long-term patency of LAMS for EUS-CD has yet to be evaluated, and possible technical precautions aimed to extend the patency duration needs further studies.

Table 1 Comparison of the main studies reporting patients treated with lumen-apposing metal stent for distal malignant biliary obstruction

Author	*n*, patients	EC-LAMS, *n* (%)	Technical success (%)	Clinical success (%)	Adverse events (%)
Kunda *et al*[50]	57	27 (47.4)	98.2	96.4	7
Tsuchiya *et al*[51]	19	19 (100)	100	94.7	36.8
Anderloni *et al*[48]	46	46 (100)	93.5	97.7	11.6
Jacques *et al*[55]	52	52 (100)	88.5	100	17.3

Clinical success is reported as percentage among patients with technical success. EC-LAMS: Electrocautery lumen-apposing metal stent.

Another point that should be addressed is whether EUS-CD with LAMS is feasible in patients candidate for pancreatic surgery. Almost all mentioned studies included patients with unresectable malignancies, and very few information are available on performing Whipple procedures in patients with an indwelling duodenal LAMS. In the retrospective study from Jacques and colleagues[55], 2 patients underwent EUS-CD with LAMS for pre-operative drainage, and a Whipple procedure was subsequently performed without complications. Recently, a case series of patients who underwent EUS-CD with LAMS before pancreatic surgery confirmed that duodenal LAMS did not interfere with surgery[60]. Due to the small numbers, the question remain to be clarified, but data are encouraging about the possibility of LAMS placement for patient possibly candidate for surgery.

In patients with MBO and failed ERCP, an alternative to EUS-BD through the traditional approaches (*i.e.*, EUS-CD or hepatogastrostomy) is GB drainage. EUS-guided GB drainage has emerged as an alternative treatment for acute cholecystitis in patients unfit for surgery due to relevant comorbidity[61,62]. In this setting, GB drainage with LAMS has been demonstrated as safe and effective[47,63]. A retrospective study evaluated EUS-guided GB drainage with SEMS for jaundice palliation in 12 patients with MBO[64]. The study reported a high technical and clinical success rate (100% and 91.7% respectively), with AE in 16.7% and stent dysfunction in 8.3%. Currently, further studies are needed to evaluate if LAMS could offer a better safety and efficacy in this setting. Finally, technical innovation in design and delivery system could be improve LAMS performance in the future, especially to increase the duration of patency and to reduce AE during deployment.

CONCLUSION

In a few years, advances in knowledge and technology radically changed the role of interventional EUS in clinical practice and, at the same time, questioned therapeutic algorithms which have been unchanged for several years. The last technological advance has been represented by LAMS, whose innovative design contributed to improve the already exciting results of EUS-BD. A consistent body of evidence highlights the advantages of EUS-BD over PTBD: lower AE; fewer re-interventions; lower costs; internal drainage with a better quality of life; lower post-procedural pain; different routes of drainage (trans-hepatic or extra-hepatic); the possibility to proceed to drainage during the same session and with the same operator after failed ERCP; concomitant jaundice and GOO palliation. With this background, LAMS contributed to make easier and faster the drainage, to reduce the shortcomings of complex multi-step procedures and probably will be responsible for further widespread of EUS-BD. RCT including LAMS to compare EUS-BD and ERCP for primary drainage are lacking, and conducting high quality studies in this field will be one of the hardest challenges in the next future.

Table 2 Comparison of the adverse events reported in the main studies with lumen-apposing metal stent for distal malignant biliary obstruction

Author	Migration	Bleeding	Obstruction	Cholangitis	Others
Kunda *et al*[50]	0	1.7% (1/57)	0	1.7% (1/57)	3.5% (2/57)
Tsuchiya *et al*[51]	0	0	26.3% (5/19)	10.5% (2/19)	10.5% (2/19)
Anderloni *et al*[48]	2.2% (1/46)	2.2% (1/46)	6.5% (3/46)	0	0
Jacques *et al*[55]	1.9% (1/52)	1.9% (1/52)	13.5% (7/52)	11.5% (6/52)	0

Others: Include perforation, pneumoperitoneum, fever. In the study from Jacques *et al*[55], 6 patients presented both stent obstruction and cholangitis (total adverse events: 9).

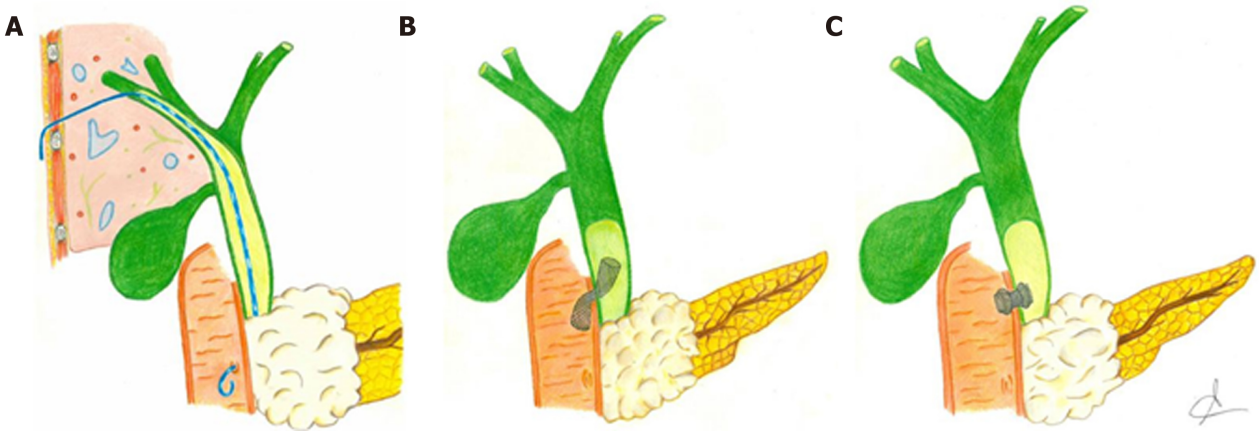


Figure 3 Graphic representation of the main interventional techniques applied to perform biliary drainage after failed endoscopic retrograde cholangiopancreatography in malignant biliary obstruction. A: Percutaneous transhepatic biliary drainage; B: Endoscopic ultrasonography-guided choledocho-duodenostomy with placement of bilary fully covered self-expanding metal stent; C: Endoscopic ultrasonography-guided choledocho-duodenostomy with placement of lumen-apposing metal stent.

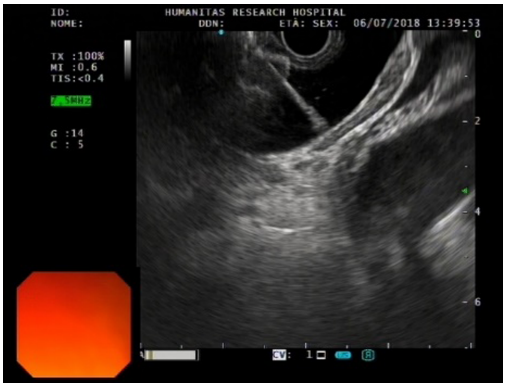


Figure 4 Echoendoscopic view of the first flange deployment of electrocautery-enhanced lumen-apposing metal stent in a dilated common bile duct.

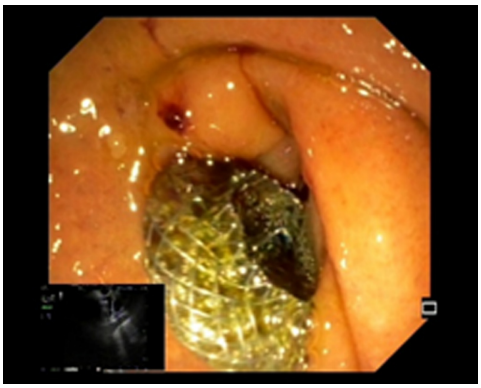


Figure 5 Final endoscopic appearance of electrocautery-enhanced lumen-apposing metal stent deployed in the duodenal bulb.

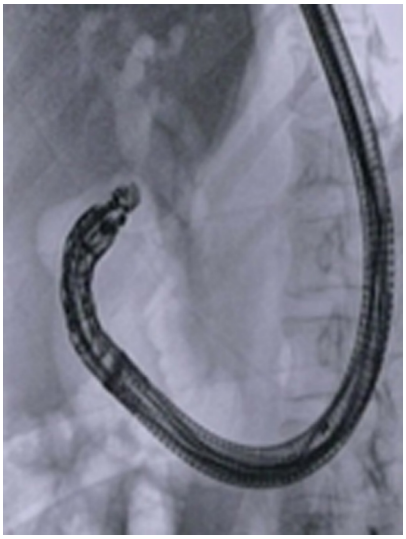


Figure 6 Final RX appearance of electrocautery-enhanced lumen-apposing metal stent deployed across the duodenal bulb into the common bile duct.

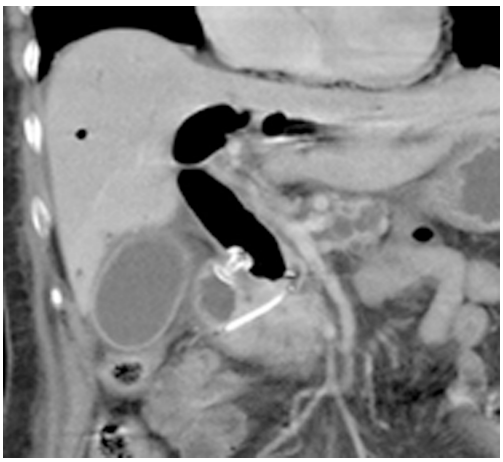


Figure 7 Computed tomography scan appearance of electrocautery-enhanced lumen-apposing metal stent deployed across the duodenal bulb and plastic pancreatic stent previously placed during failed endoscopic retrograde cholangiopancreatography.

REFERENCES

1 **Stark A**, Hines OJ. Endoscopic and operative palliation strategies for pancreatic ductal adenocarcinoma. *Semin Oncol* 2015; **42**: 163-176 [PMID: 25726060 DOI: 10.1053/j.seminoncol.2014.12.014]
2 **Kneuertz PJ**, Cunningham SC, Cameron JL, Torrez S, Tapazoglou N, Herman JM, Makary MA,

Eckhauser F, Wang J, Hirose K, Edil BH, Choti MA, Schulick RD, Wolfgang CL, Pawlik TM. Palliative surgical management of patients with unresectable pancreatic adenocarcinoma: Trends and lessons learned from a large, single institution experience. *J Gastrointest Surg* 2011; **15**: 1917-1927 [PMID: 21913044 DOI: 10.1007/s11605-011-1665-9]

3 **Maire F**, Hammel P, Ponsot P, Aubert A, O'Toole D, Hentic O, Levy P, Ruszniewski P. Long-term outcome of biliary and duodenal stents in palliative treatment of patients with unresectable adenocarcinoma of the head of pancreas. *Am J Gastroenterol* 2006; **101**: 735-742 [PMID: 16635221 DOI: 10.1111/j.1572-0241.2006.00559.x]

4 **Singh SM**, Reber HA. Surgical palliation for pancreatic cancer. *Surg Clin North Am* 1989; **69**: 599-611 [PMID: 2471281 DOI: 10.1097/00000658-199008000-00003]

5 **Huguier M**, Baumel H, Manderscheid JC, Houry S, Fabre JM. Surgical palliation for unresected cancer of the exocrine pancreas. *Eur J Surg Oncol* 1993; **19**: 342-347 [PMID: 7689488]

6 **Rosemurgy AS**, Burnett CM, Wasselle JA. A comparison of choledochoenteric bypass and cholecystoenteric bypass in patients with biliary obstruction due to pancreatic cancer. *Am Surg* 1989; **55**: 55-60 [PMID: 2464297 DOI: 10.1097/00000478-198901000-00016]

7 **Artifon EL**, Sakai P, Cunha JE, Dupont A, Filho FM, Hondo FY, Ishioka S, Raju GS. Surgery or endoscopy for palliation of biliary obstruction due to metastatic pancreatic cancer. *Am J Gastroenterol* 2006; **101**: 2031-2037 [PMID: 16968509 DOI: 10.1111/j.1572-0241.2006.00764.x]

8 **Bliss LA**, Eskander MF, Kent TS, Watkins AA, de Geus SW, Storino A, Ng SC, Callery MP, Moser AJ, Tseng JF. Early surgical bypass versus endoscopic stent placement in pancreatic cancer. *HPB (Oxford)* 2016; **18**: 671-677 [PMID: 27485061 DOI: 10.1016/j.hpb.2016.05.008]

9 **Dumonceau JM**, Tringali A, Papanikolaou IS, Blero D, Mangiavillano B, Schmidt A, Vanbiervliet G, Costamagna G, Devière J, García-Cano J, Gyökeres T, Hassan C, Prat F, Siersema PD, van Hooft JE. Endoscopic biliary stenting: Indications, choice of stents, and results: European Society of Gastrointestinal Endoscopy (ESGE) Clinical Guideline - Updated October 2017. *Endoscopy* 2018; **50**: 910-930 [PMID: 30086596 DOI: 10.1055/a-0659-9864]

10 **van der Gaag NA**, Rauws EA, van Eijck CH, Bruno MJ, van der Harst E, Kubben FJ, Gerritsen JJ, Greve JW, Gerhards MF, de Hingh IH, Klinkenbijl JH, Nio CY, de Castro SM, Busch OR, van Gulik TM, Bossuyt PM, Gouma DJ. Preoperative biliary drainage for cancer of the head of the pancreas. *N Engl J Med* 2010; **362**: 129-137 [PMID: 20071702 DOI: 10.1056/NEJMoa0903230]

11 **Wang P**, Li ZS, Liu F, Ren X, Lu NH, Fan ZN, Huang Q, Zhang X, He LP, Sun WS, Zhao Q, Shi RH, Tian ZB, Li YQ, Li W, Zhi FC. Risk factors for ERCP-related complications: A prospective multicenter study. *Am J Gastroenterol* 2009; **104**: 31-40 [PMID: 19098846 DOI: 10.1038/ajg.2008.5]

12 **Enochsson L**, Swahn F, Arnelo U, Nilsson M, Löhr M, Persson G. Nationwide, population-based data from 11,074 ERCP procedures from the Swedish Registry for Gallstone Surgery and ERCP. *Gastrointest Endosc* 2010; **72**: 1175-1184, 1184.e1-1184.e3 [PMID: 20970787 DOI: 10.1016/j.gie.2010.07.047]

13 **Pereiras RV**, Rheingold OJ, Huston D, Mejia J, Viamonte M, Chiprut RO, Schiff ER. Relief of malignant obstructive jaundice by percutaneous insertion of a permanent prosthesis in the biliary tree. *Ann Intern Med* 1978; **89**: 589-583 [PMID: 82416]

14 **Krokidis M**, Fanelli F, Orgera G, Bezzi M, Passariello R, Hatzidakis A. Percutaneous treatment of malignant jaundice due to extrahepatic cholangiocarcinoma: covered Viabil stent versus uncovered Wallstents. *Cardiovasc Intervent Radiol* 2010; **33**: 97-106 [PMID: 19495871 DOI: 10.1007/s00270-009-9604-9]

15 **Molnar W**, Stockum AE. Relief of obstructive jaundice through percutaneous transhepatic catheter--a new therapeutic method. *Am J Roentgenol Radium Ther Nucl Med* 1974; **122**: 356-367 [PMID: 4419510 DOI: 10.2214/ajr.122.2.356]

16 **Thornton RH**, Frank BS, Covey AM, Maybody M, Solomon SB, Getrajdman GI, Brown KT. Catheter-free survival after primary percutaneous stenting of malignant bile duct obstruction. *AJR Am J Roentgenol* 2011; **197**: W514-W518 [PMID: 21862781 DOI: 10.2214/AJR.10.6069]

17 **Nennstiel S**, Treiber M, Faber A, Haller B, von Delius S, Schmid RM, Neu B. Comparison of Ultrasound and Fluoroscopically Guided Percutaneous Transhepatic Biliary Drainage. *Dig Dis* 2019; **37**: 77-86 [PMID: 30253406 DOI: 10.1159/000493120]

18 **Nennstiel S**, Weber A, Frick G, Haller B, Meining A, Schmid RM, Neu B. Drainage-related Complications in Percutaneous Transhepatic Biliary Drainage: An Analysis Over 10 Years. *J Clin Gastroenterol* 2015; **49**: 764-770 [PMID: 25518004 DOI: 10.1097/MCG.0000000000000275]

19 **Günther RW**, Schild H, Thelen M. Percutaneous transhepatic biliary drainage: Experience with 311 procedures. *Cardiovasc Intervent Radiol* 1988; **11**: 65-71 [PMID: 2455599 DOI: 10.1007/BF02577061]

20 **Born P**, Rösch T, Triptrap A, Frimberger E, Allescher HD, Ott R, Weigert N, Lorenz R, Classen M. Long-term results of percutaneous transhepatic biliary drainage for benign and malignant bile duct strictures. *Scand J Gastroenterol* 1998; **33**: 544-549 [PMID: 9648997 DOI: 10.1080/00365529850172142]

21 **Mueller PR**, van Sonnenberg E, Ferrucci JT. Percutaneous biliary drainage: Technical and catheter-related problems in 200 procedures. *AJR Am J Roentgenol* 1982; **138**: 17-23 [PMID: 6976698 DOI: 10.2214/ajr.138.1.17]

22 **Sarwar A**, Hostage CA, Weinstein JL, Kim G, Novack V, Chakrala N, Park Y, Brook OR, Ahmed M. Causes and Rates of 30-day Readmissions after Percutaneous Transhepatic Biliary Drainage Procedure. *Radiology* 2019; **290**: 722-729 [PMID: 30599096 DOI: 10.1148/radiol.2018180279]

23 **ASGE Technology Committee**. Maple JT, Pannala R, Abu Dayyeh BK, Aslanian HR, Enestvedt BK, Goodman A, Komanduri S, Manfredi M, Navaneethan U, Parsi MA, Smith ZL, Thosani N, Sullivan SA, Banerjee S. Interventional EUS (with videos). *Gastrointest Endosc* 2017; **85**: 465-481 [PMID: 28117034 DOI: 10.1016/j.gie.2016.11.021]

24 **Teoh AYB**, Dhir V, Kida M, Yasuda I, Jin ZD, Seo DW, Almadi M, Ang TL, Hara K, Hilmi I, Itoi T, Lakhtakia S, Matsuda K, Pausawasdi N, Puri R, Tang RS, Wang HP, Yang AM, Hawes R, Varadarajulu S, Yasuda K, Ho LKY. Consensus guidelines on the optimal management in interventional EUS procedures: Results from the Asian EUS group RAND/UCLA expert panel. *Gut* 2018; **67**: 1209-1228 [PMID: 29463614 DOI: 10.1136/gutjnl-2017-314341]

25 **Bang JY**, Navaneethan U, Hasan M, Hawes R, Varadarajulu S. Stent placement by EUS or ERCP for primary biliary decompression in pancreatic cancer: A randomized trial (with videos). *Gastrointest Endosc* 2018; **88**: 9-17 [PMID: 29574126 DOI: 10.1016/j.gie.2018.03.012]

26 **Paik WH**, Lee TH, Park DH, Choi JH, Kim SO, Jang S, Kim DU, Shim JH, Song TJ, Lee SS, Seo DW, Lee SK, Kim MH. EUS-Guided Biliary Drainage Versus ERCP for the Primary Palliation of Malignant Biliary Obstruction: A Multicenter Randomized Clinical Trial. *Am J Gastroenterol* 2018; **113**: 987-997

[PMID: 29961772 DOI: 10.1038/s41395-018-0122-8]
27 **Park JK**, Woo YS, Noh DH, Yang JI, Bae SY, Yun HS, Lee JK, Lee KT, Lee KH. Efficacy of EUS-guided and ERCP-guided biliary drainage for malignant biliary obstruction: Prospective randomized controlled study. *Gastrointest Endosc* 2018; **88**: 277-282 [PMID: 29605722 DOI: 10.1016/j.gie.2018.03.015]
28 **Guo J**, Giovannini M, Sahai AV, Saftoiu A, Dietrich CF, Santo E, Fusaroli P, Siddiqui AA, Bhutani MS, Bun Teoh AY, Irisawa A, Arturo Arias BL, Achanta CR, Jenssen C, Seo DW, Adler DG, Kalaitzakis E, Artifon E, Itokawa F, Poley JW, Mishra G, Ho KY, Wang HP, Okasha HH, Lachter J, Vila JJ, Iglesias-Garcia J, Yamao K, Yasuda K, Kubota K, Palazzo L, Sabbagh LC, Sharma M, Kida M, El-Nady M, Nguyen NQ, Vilmann P, Garg PK, Rai P, Mukai S, Carrara S, Parupudi S, Sridhar S, Lakhtakia S, Rana SS, Ogura T, Baron TH, Dhir V, Sun S. A multi-institution consensus on how to perform EUS-guided biliary drainage for malignant biliary obstruction. *Endosc Ultrasound* 2018; **7**: 356-365 [PMID: 30531022 DOI: 10.4103/eus.eus_53_18]
29 **Uemura RS**, Khan MA, Otoch JP, Kahaleh M, Montero EF, Artifon ELA. EUS-guided Choledochoduodenostomy Versus Hepaticogastrostomy: A Systematic Review and Meta-analysis. *J Clin Gastroenterol* 2018; **52**: 123-130 [PMID: 29095426 DOI: 10.1097/MCG.0000000000000948]
30 **Boulay BR**, Lo SK. Endoscopic Ultrasound-Guided Biliary Drainage. *Gastrointest Endosc Clin N Am* 2018; **28**: 171-185 [PMID: 29519330 DOI: 10.1016/j.giec.2017.11.005]
31 **Iwashita T**, Lee JG, Shinoura S, Nakai Y, Park DH, Muthusamy VR, Chang KJ. Endoscopic ultrasound-guided rendezvous for biliary access after failed cannulation. *Endoscopy* 2012; **44**: 60-65 [PMID: 22127960 DOI: 10.1055/s-0030-1256871]
32 **Giovannini M**, Moutardier V, Pesenti C, Bories E, Lelong B, Delpero JR. Endoscopic ultrasound-guided bilioduodenal anastomosis: A new technique for biliary drainage. *Endoscopy* 2001; **33**: 898-900 [PMID: 11571690 DOI: 10.1055/s-2001-17324]
33 **Wang K**, Zhu J, Xing L, Wang Y, Jin Z, Li Z. Assessment of efficacy and safety of EUS-guided biliary drainage: A systematic review. *Gastrointest Endosc* 2016; **83**: 1218-1227 [PMID: 26542374 DOI: 10.1016/j.gie.2015.10.033]
34 **Sharaiha RZ**, Khan MA, Kamal F, Tyberg A, Tombazzi CR, Ali B, Tombazzi C, Kahaleh M. Efficacy and safety of EUS-guided biliary drainage in comparison with percutaneous biliary drainage when ERCP fails: A systematic review and meta-analysis. *Gastrointest Endosc* 2017; **85**: 904-914 [PMID: 28063840 DOI: 10.1016/j.gie.2016.12.023]
35 **Sharaiha RZ**, Kumta NA, Desai AP, DeFilippis EM, Gabr M, Sarkisian AM, Salgado S, Millman J, Benvenuto A, Cohen M, Tyberg A, Gaidhane M, Kahaleh M. Endoscopic ultrasound-guided biliary drainage versus percutaneous transhepatic biliary drainage: Predictors of successful outcome in patients who fail endoscopic retrograde cholangiopancreatography. *Surg Endosc* 2016; **30**: 5500-5505 [PMID: 27129552 DOI: 10.1007/s00464-016-4913-y]
36 **Nam K**, Kim DU, Lee TH, Iwashita T, Nakai Y, Bolkhir A, Castro LA, Vazquez-Sequeiros E, de la Serna C, Perez-Miranda M, Lee JG, Lee SS, Seo DW, Lee SK, Kim MH, Park DH. Patient perception and preference of EUS-guided drainage over percutaneous drainage when endoscopic transpapillary biliary drainage fails: An international multicenter survey. *Endosc Ultrasound* 2018; **7**: 48-55 [PMID: 29451169 DOI: 10.4103/eus.eus_100_17]
37 **Mussetto A**, Fugazza A, Fuccio L, Triossi O, Repici A, Anderloni A. Current uses and outcomes of lumen-apposing metal stents. *Ann Gastroenterol* 2018; **31**: 535-540 [PMID: 30174389 DOI: 10.20524/aog.2018.0287]
38 **Stier MW**, Waxman I. Lumen-Apposing Metal Stents: Which One and Why? *Gastrointest Endosc Clin N Am* 2018; **28**: 207-217 [PMID: 29519333 DOI: 10.1016/j.giec.2017.11.008]
39 **Rinninella E**, Kunda R, Dollhopf M, Sanchez-Yague A, Will U, Tarantino I, Gornals Soler J, Ullrich S, Meining A, Esteban JM, Enz T, Vanbiervliet G, Vleggaar F, Attili F, Larghi A. EUS-guided drainage of pancreatic fluid collections using a novel lumen-apposing metal stent on an electrocautery-enhanced delivery system: A large retrospective study (with video). *Gastrointest Endosc* 2015; **82**: 1039-1046 [PMID: 26014960 DOI: 10.1016/j.gie.2015.04.006]
40 **Shah RJ**, Shah JN, Waxman I, Kowalski TE, Sanchez-Yague A, Nieto J, Brauer BC, Gaidhane M, Kahaleh M. Safety and efficacy of endoscopic ultrasound-guided drainage of pancreatic fluid collections with lumen-apposing covered self-expanding metal stents. *Clin Gastroenterol Hepatol* 2015; **13**: 747-752 [PMID: 25290534 DOI: 10.1016/j.cgh.2014.09.047]
41 **Siddiqui AA**, Kowalski TE, Loren DE, Khalid A, Soomro A, Mazhar SM, Isby L, Kahaleh M, Karia K, Yoo J, Ofosu A, Ng B, Sharaiha RZ. Fully covered self-expanding metal stents versus lumen-apposing fully covered self-expanding metal stent versus plastic stents for endoscopic drainage of pancreatic walled-off necrosis: Clinical outcomes and success. *Gastrointest Endosc* 2017; **85**: 758-765 [PMID: 27566053 DOI: 10.1016/j.gie.2016.08.014]
42 **Siddiqui AA**, Adler DG, Nieto J, Shah JN, Binmoeller KF, Kane S, Yan L, Laique SN, Kowalski T, Loren DE, Taylor LJ, Munigala S, Bhat YM. EUS-guided drainage of peripancreatic fluid collections and necrosis by using a novel lumen-apposing stent: A large retrospective, multicenter U.S. experience (with videos). *Gastrointest Endosc* 2016; **83**: 699-707 [PMID: 26515956 DOI: 10.1016/j.gie.2015.10.020]
43 **Binmoeller KF**, Shah J. A novel lumen-apposing stent for transluminal drainage of nonadherent extraintestinal fluid collections. *Endoscopy* 2011; **43**: 337-342 [PMID: 21264800 DOI: 10.1055/s-0030-1256127]
44 **Itoi T**, Binmoeller KF, Shah J, Sofuni A, Itokawa F, Kurihara T, Tsuchiya T, Ishii K, Tsuji S, Ikeuchi N, Moriyasu F. Clinical evaluation of a novel lumen-apposing metal stent for endosonography-guided pancreatic pseudocyst and gallbladder drainage (with videos). *Gastrointest Endosc* 2012; **75**: 870-876 [PMID: 22301347 DOI: 10.1016/j.gie.2011.10.020]
45 **Itoi T**, Binmoeller KF. EUS-guided choledochoduodenostomy by using a biflanged lumen-apposing metal stent. *Gastrointest Endosc* 2014; **79**: 715 [PMID: 24424399 DOI: 10.1016/j.gie.2013.11.021]
46 **Walter D**, Will U, Sanchez-Yague A, Brenke D, Hampe J, Wollny H, López-Jamar JM, Jechart G, Vilmann P, Gornals JB, Ullrich S, Fähndrich M, de Tejada AH, Junquera F, Gonzalez-Huix F, Siersema PD, Vleggaar FP. A novel lumen-apposing metal stent for endoscopic ultrasound-guided drainage of pancreatic fluid collections: A prospective cohort study. *Endoscopy* 2015; **47**: 63-67 [PMID: 25268308 DOI: 10.1055/s-0034-1378113]
47 **Walter D**, Teoh AY, Itoi T, Pérez-Miranda M, Larghi A, Sanchez-Yague A, Siersema PD, Vleggaar FP. EUS-guided gall bladder drainage with a lumen-apposing metal stent: A prospective long-term evaluation. *Gut* 2016; **65**: 6-8 [PMID: 26041748 DOI: 10.1136/gutjnl-2015-309925]

48 **Anderloni A**, Fugazza A, Troncone E, Auriemma F, Carrara S, Semeraro R, Maselli R, Di Leo M, D'Amico F, Sethi A, Repici A. Single-stage EUS-guided choledochoduodenostomy using a lumen-apposing metal stent for malignant distal biliary obstruction. *Gastrointest Endosc* 2019; **89**: 69-76 [PMID: 30189198 DOI: 10.1016/j.gie.2018.08.047]

49 **Anderloni A**, Leo MD, Carrara S, Fugazza A, Maselli R, Buda A, Amato A, Auriemma F, Repici A. Endoscopic ultrasound-guided transmural drainage by cautery-tipped lumen-apposing metal stent: Exploring the possible indications. *Ann Gastroenterol* 2018; **31**: 735-741 [PMID: 30386125 DOI: 10.20524/aog.2018.0299]

50 **Kunda R**, Pérez-Miranda M, Will U, Ullrich S, Brenke D, Dollhopf M, Meier M, Larghi A. EUS-guided choledochoduodenostomy for malignant distal biliary obstruction using a lumen-apposing fully covered metal stent after failed ERCP. *Surg Endosc* 2016; **30**: 5002-5008 [PMID: 26969661 DOI: 10.1007/s00464-016-4845-6]

51 **Tsuchiya T**, Teoh AYB, Itoi T, Yamao K, Hara K, Nakai Y, Isayama H, Kitano M. Long-term outcomes of EUS-guided choledochoduodenostomy using a lumen-apposing metal stent for malignant distal biliary obstruction: A prospective multicenter study. *Gastrointest Endosc* 2018; **87**: 1138-1146 [PMID: 28843583 DOI: 10.1016/j.gie.2017.08.017]

52 **Anderloni A**, Buda A, Carrara S, Di Leo M, Fugazza A, Maselli R, Repici A. Single-session double-stent placement in concomitant malignant biliary and duodenal obstruction with a cautery-tipped lumen apposing metal stent. *Endoscopy* 2016; **48**: E321-E322 [PMID: 27706541 DOI: 10.1055/s-0042-117425]

53 **Anderloni A**, Fugazza A, Auriemma F, Maia L, Maselli R, Troncone E, D'Amico F, Repici A. Cautery-Tipped Lumen Apposing Metal Stent Placement Through the Mesh of an Indwelling Duodenal Self-Expanding Metal Stent. *Am J Gastroenterol* 2018; **113**: 644 [PMID: 29681628 DOI: 10.1038/s41395-018-0040-9]

54 **Glessing BR**, Mallery S, Freeman ML, Newcomb MD, Arain MA. EUS-guided choledochoduodenostomy with a lumen-apposing metal stent before duodenal stent placement for malignant biliary and duodenal obstruction. *Gastrointest Endosc* 2015; **81**: 1019-1020 [PMID: 25450606 DOI: 10.1016/j.gie.2014.09.061]

55 **Jacques J**, Privat J, Pinard F, Fumex F, Valats JC, Chaoui A, Cholet F, Godard B, Grandval P, Legros R, Kerever S, Napoleon B. Endoscopic ultrasound-guided choledochoduodenostomy with electrocautery-enhanced lumen-apposing stents: A retrospective analysis. *Endoscopy* 2019; **51**: 540-547 [PMID: 30347424 DOI: 10.1055/a-0735-9137]

56 **Masci E**, Mariani A, Curioni S, Testoni PA. Risk factors for pancreatitis following endoscopic retrograde cholangiopancreatography: A meta-analysis. *Endoscopy* 2003; **35**: 830-834 [PMID: 14551860 DOI: 10.1055/s-2003-42614]

57 **Spadaccini M**, Fugazza A, Troncone E, Craviotto V, D'Amico F, Lamonaca L, Anderloni A, Repici A. EUS-Guided Biliary Drainage Versus ERCP for the Primary Palliation of Malignant Biliary Obstruction: An Unfair Comparison. *Am J Gastroenterol* 2019; **114**: 174 [PMID: 30333537 DOI: 10.1038/s41395-018-0371-6]

58 **Adams MA**, Anderson MA, Myles JD, Khalatbari S, Scheiman JM. Self-expanding metal stents (SEMS) provide superior outcomes compared to plastic stents for pancreatic cancer patients undergoing neoadjuvant therapy. *J Gastrointest Oncol* 2012; **3**: 309-313 [PMID: 23205306 DOI: 10.3978/j.issn.2078-6891.2011.050]

59 **Tringali A**, Hassan C, Rota M, Rossi M, Mutignani M, Aabakken L. Covered vs. uncovered self-expandable metal stents for malignant distal biliary strictures: A systematic review and meta-analysis. *Endoscopy* 2018; **50**: 631-641 [PMID: 29342491 DOI: 10.1055/s-0043-125062]

60 **Fabbri C**, Fugazza A, Binda C, Zerbi A, Jovine E, Cennamo V, Repici A, Anderloni A. Beyond palliation: using eus-guided choledochoduodenostomy with a lumen-apposing metal stent as a bridge to surgery. *J Gastrointest Liver Dis* 2019; **51**: e126 [DOI: 10.1016/S1590-8658(19)30236-1]

61 **Law R**, Baron TH. Endoscopic Ultrasound-Guided Gallbladder Drainage. *Gastrointest Endosc Clin N Am* 2018; **28**: 187-195 [PMID: 29519331 DOI: 10.1016/j.giec.2017.11.006]

62 **Anderloni A**, Buda A, Vieceli F, Khashab MA, Hassan C, Repici A. Endoscopic ultrasound-guided transmural stenting for gallbladder drainage in high-risk patients with acute cholecystitis: A systematic review and pooled analysis. *Surg Endosc* 2016; **30**: 5200-5208 [PMID: 27059975 DOI: 10.1007/s00464-016-4894-x]

63 **Dollhopf M**, Larghi A, Will U, Rimbaş M, Anderloni A, Sanchez-Yague A, Teoh AYB, Kunda R. EUS-guided gallbladder drainage in patients with acute cholecystitis and high surgical risk using an electrocautery-enhanced lumen-apposing metal stent device. *Gastrointest Endosc* 2017; **86**: 636-643 [PMID: 28259594 DOI: 10.1016/j.gie.2017.02.027]

64 **Imai H**, Kitano M, Omoto S, Kadosaka K, Kamata K, Miyata T, Yamao K, Sakamoto H, Harwani Y, Kudo M. EUS-guided gallbladder drainage for rescue treatment of malignant distal biliary obstruction after unsuccessful ERCP. *Gastrointest Endosc* 2016; **84**: 147-151 [PMID: 26764194 DOI: 10.1016/j.gie.2015.12.024]

World Journal of Gastroenterology

Submit a Manuscript: https://www.f6publishing.com

World J Gastroenterol 2019 August 7; 25(29): 3870-3896

DOI: 10.3748/wjg.v25.i29.3870

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

REVIEW

Pharmacogenetics of the systemic treatment in advanced hepatocellular carcinoma

Elena De Mattia, Erika Cecchin, Michela Guardascione, Luisa Foltran, Tania Di Raimo, Francesco Angelini, Mario D'Andrea, Giuseppe Toffoli

ORCID number: Elena De Mattia (0000-0003-4948-8767); Erika Cecchin (0000-0001-7517-7490); Michela Guardascione (0000-0001-5052-3502); Luisa Foltran (0000-0002-2313-2247); Tania Di Raimo (0000-0002-2896-8211); Francesco Angelini (0000-0002-0052-6956); Mario Rosario D'Andrea (0000-0003-4585-7473); Giuseppe Toffoli (0000-0002-5323-4762).

Author contributions: De Mattia E performed the literature review and analysis, contributed to writing the manuscript, and elaborated the tables; Cecchin E conceptualized and edited the manuscript; Guardascione M, Foltran L and D'Andrea M contributed to writing the manuscript; Di Raimo T and Angelini F contributed to writing the manuscript and elaborated the figures; and Toffoli G was the guarantor. All authors reviewed the manuscript.

Supported by the European Union's Horizon 2020 Research and Innovation Programme, No. 668353.

Conflict-of-interest statement: The authors declare no conflict of interest.

Elena De Mattia, Erika Cecchin, Michela Guardascione, Tania Di Raimo, Francesco Angelini, Giuseppe Toffoli, Clinical and Experimental Pharmacology, Centro di Riferimento Oncologico di Aviano (CRO) IRCCS, Aviano (PN) 33081, Italy

Luisa Foltran, Department of Medical Oncology, Centro di Riferimento Oncologico di Aviano (CRO) IRCCS, Aviano (PN) 33081, Italy

Tania Di Raimo, Francesco Angelini, Medical Oncology and Anatomic Pathology Unit, "San Filippo Neri Hospital", Rome 00135, Italy

Mario D'Andrea, Department of Oncology, "San Filippo Neri Hospital", Rome 00135, Italy

Corresponding author: Elena De Mattia, PhD, Clinical and Experimental Pharmacology, Centro di Riferimento Oncologico di Aviano (CRO) IRCCS, Aviano (PN) 33081, Italy. edemattia@cro.it.
Telephone: +39-434-659765
Fax: +39-434-659799

Abstract

Hepatocellular carcinoma (HCC) accounts for the majority of primary liver cancers. To date, most patients with HCC are diagnosed at an advanced tumor stage, excluding them from potentially curative therapies (*i.e.*, resection, liver transplantation, percutaneous ablation). Treatments with palliative intent include chemoembolization and systemic therapy. Among systemic treatments, the small-molecule multikinase inhibitor sorafenib has been the only systemic treatment available for advanced HCC over 10 years. More recently, other small-molecule multikinase inhibitors (*e.g.*, regorafenib, lenvatinib, cabozantinib) have been approved for HCC treatment. The promising immune checkpoint inhibitors (*e.g.*, nivolumab, pembrolizumab) are still under investigation in Europe while in the US nivolumab has already been approved by FDA in sorafenib refractory or resistant patients. Other molecules, such as the selective CDK4/6inhibitors (*e.g.*, palbociclib, ribociclib), are in earlier stages of clinical development, and the c-MET inhibitor tivantinib did not show positive results in a phase III study. However, even if the introduction of targeted agents has led to great advances in patient response and survival with an acceptable toxicity profile, a remarkable inter-individual heterogeneity in therapy outcome persists and constitutes a significant problem in disease management. Thus, the identification of biomarkers that predict which patients will benefit from a specific intervention could significantly affect decision-making and therapy planning. Germ-line

Manuscript source: Invited manuscript

Received: March 17, 2019
Peer-review started: March 18, 2019
First decision: May 14, 2019
Revised: May 23, 2019
Accepted: July 1, 2019
Article in press: July 3, 2019
Published online: August 7, 2019

P-Reviewer: Jin B, Luo GH, Patel JN, Sun JH, Sun XT, Wang GY
S-Editor: Gong ZM
L-Editor: A
E-Editor: Ma YJ

variants have been suggested to play an important role in determining outcomes of HCC systemic therapy in terms of both toxicity and treatment efficacy. Particularly, a number of studies have focused on the role of genetic polymorphisms impacting the drug metabolic pathway and membrane translocation as well as the drug mechanism of action as predictive/prognostic markers of HCC treatment. The aim of this review is to summarize and critically discuss the pharmacogenetic literature evidences, with particular attention to sorafenib and regorafenib, which have been used longer than the others in HCC treatment.

Key words: Hepatocellular carcinoma; Pharmacogenetics; Genetic markers; Sorafenib; Regorafenib; Immune checkpoint inhibitors; Cytochromes; UDP glucuronosyltransferase 1A

Core tip: Patients with advanced hepatocellular carcinoma (HCC) have few effective therapeutic options. Although multikinase inhibitors-such as sorafenib as first-line treatment and regorafenib in sorafenib progressors-show some overall survival benefit, unmet needs persist in the treatment of advanced HCC. Particularly, the identification of potential prognostic and predictive biomarkers for better stratifying and personalizing the treatment remains a challenge. Germ-line polymorphisms have been suggested to contribute significantly to inter-individual variability in HCC therapy outcome in terms of both toxicity and effectiveness, opening new avenues for pharmacogenetic investigation.

Citation: De Mattia E, Cecchin E, Guardascione M, Foltran L, Di Raimo T, Angelini F, D'Andrea M, Toffoli G. Pharmacogenetics of the systemic treatment in advanced hepatocellular carcinoma. *World J Gastroenterol* 2019; 25(29): 3870-3896
URL: https://www.wjgnet.com/1007-9327/full/v25/i29/3870.htm
DOI: https://dx.doi.org/10.3748/wjg.v25.i29.3870

INTRODUCTION

Liver cancer incidence is approximately 850000 new cases per year, and about 90% of liver tumors are hepatocellular carcinoma (HCC)[1]. The dominant risk factors for HCC vary worldwide. For most countries in Asia and Africa, hepatitis B virus infection and aflatoxin B1 exposure are the major risk factors. In contrast, hepatitis C virus infection, alcoholism, and metabolic syndrome play more important roles in other areas in the world[2]. The Barcelona Clinic Liver Cancer (BCLC) staging is the main clinical classification that stratifies patients from A to C stages, according to prognosis, to inform treatment decisions. Early-stage cancers are potentially suitable for therapies with curative intent such as surgical resection, liver transplantation, or local ablation. Chemoembolization and systemic therapy represent the only therapeutic options for intermediate or advanced HCC[1].

Surgical resection is the standard option for patients with solitary HCC at BCLC A stage. Other criteria for selecting the best surgical candidates are absence of portal hypertension along with well-preserved liver function. A surgical strategy is associated with 5-year survival rates of 70%, and adjuvant therapies have yet to show a survival advantage[1]. Liver transplantation is the best option for BCLC A tumors with respect to Milan criteria (single tumor ≤ 5 cm or up to three nodules ≤ 3 cm in size and no vascular invasion)[3]. Furthermore, local ablation with radiofrequency represents a good alternative to surgery in patients with single tumors < 2 cm[4]; however, no randomized clinical trials (RCTs) have been conducted to specifically address whether ablation is non inferior to surgery[5].

For patients with HCC at BCLC B stage, transarterial chemoembolization (TACE) is recommended based on results from RCTs and a systematic review showing survival benefits with TACE as compared with the best supportive care[6]; more recently TACE was found to give an objective response of 52.5%[7]. Better selection of candidates and improvement in the procedure, such as supra-selective embolization and the use of drug-eluting beads, have led to median survival times beyond 40 mo in referral

centers[1]. Radioembolization is an alternative embolization approach with a favorable safety and efficacy profile, but well-designed, properly powered RCTs are still needed to demonstrate a real benefit[1].

In advanced HCC or in intermediate HCC when chemoembolization is no longer indicated, systemic treatment is the standard therapy. Conventional chemotherapeutic agents (*e.g.*, doxorubicin, fluoropyrimidines, platinum derivates, irinotecan) are minimally effective in HCC, with significant toxicity, and do not improve patient survival[8-10]. HCC is also rarely amenable to radiation therapy[10].

Targeted agents based on an improved molecular characterization of HCC have opened a new era for the treatment of patients with HCC (Figure 1). A number of small-molecule tyrosine kinase inhibitors and immune checkpoint inhibitors have demonstrated some survival benefit in intermediate/advanced disease (BCLC B-C); more recently, preliminary promising data are emerging on the use of CDK4/6inhibitors[11]. At present, the approved drugs in Europe for advanced HCC indication are the small-molecule multikinase kinase inhibitors sorafenib, lenvatinib, regorafenib, and cabozantinib. In particular, sorafenib and lenvatinib are approved as first-line therapy and regorafenib and cabozantinib in patients who have progressed or are intolerant to sorafenib. Other molecules, such as the immune checkpoint inhibitors (*e.g.*, nivolumab, pembrolizumab) and selective CDK4/6inhibitors (*e.g.*, palbociclib, ribociclib), are still under investigation in the HCC setting in Europe while in the US nivolumab received accelerated approval for HCC patients previously treated with sorafenib. Among other molecules tested, the c-MET inhibitor tivantinib has not shown positive results[12].

Based on the results of major studies conducted to date, several unmet needs persist in the management of intermediate/advanced HCC that might be addressed through new therapies and biomarkers for therapy stratification and a patient-tailored approach. In this context, genetic polymorphisms, with their well-established role in liver carcinogenesis[13,14], could be important and contribute, in combination with clinical and molecular parameters, to predicting HCC therapy outcomes for efficacy and for toxicity risk. The aim of this review is to critically report and discuss current literature on the effect of germ-line variants as predictive markers of HCC systemic therapy outcome and how they can aid in stratifying patients according to toxicity risk, as well as the likelihood of benefit from administration of specific anti-tumor agents.

SYSTEMIC TREATMENT OF ADVANCED HCC

The phase III SHARP trial evaluating sorafenib in previously untreated patients with advanced HCC reported a median overall survival (OS) of 10.7 mo for the sorafenib-treated group compared to 7.9 mo in patients who received placebo[15]. The most common adverse effects observed in the trial included fatigue, hand–foot skin reaction (HFSR), alopecia, gastrointestinal, and liver dysfunction. A number of studies have investigated the role of clinical and/or biological markers in HCC patients treated with sorafenib[15,16]. Results from the SHARP trial showed that baseline alpha fetoprotein plasma levels > 200 ng/mL had a negative impact on OS, a finding that has been recently confirmed in a pooled analysis[17]. A recent meta-analysis demonstrated that the occurrence of sorafenib-related side effects (*e.g.*, hypertension, skin toxicities, and diarrhea) is associated with a better OS in sorafenib-treated HCC patients[18]. In addition to the abovementioned markers, other clinical parameters have been evaluated, such as macroscopic vascular invasion, BCLC stage and etiology of cirrhosis[17], and Child–Pugh subgroups[19]. Some biological markers have been also suggested as potentially related to sorafenib outcome. For instance, in the SHARP trial[15], baseline angiopoietin-2 (Ang-2) and vascular endothelial growth factor (VEGF)-A plasma levels independently predicted survival in the entire patient population and in the placebo cohort; conversely, none of the tested biomarkers significantly predicted response to sorafenib[15]. Additionally, high insulin-like growth factor 1 pre-treatment levels are associated with better progression-free survival (PFS) and OS in patients with advanced HCC receiving first-line antiangiogenic therapy[20]. More recently, a study recruiting 80 HCC patients prospectively treated with sorafenib showed that independent risk factors for poor OS were high serum concentration of Ang-2 and hepatocyte growth factor (HGF) as well as poor performance status before treatment[21].

In patients who tolerated but progressed on sorafenib, the other multikinase inhibitor regorafenib has been reported to provide an OS benefit compared with placebo of 10.6 mo *vs* 7.8 mo. The most common grade 3 or 4 treatment-related events were hypertension, HFSR, fatigue, and diarrhea[22]. Preliminary data on potential

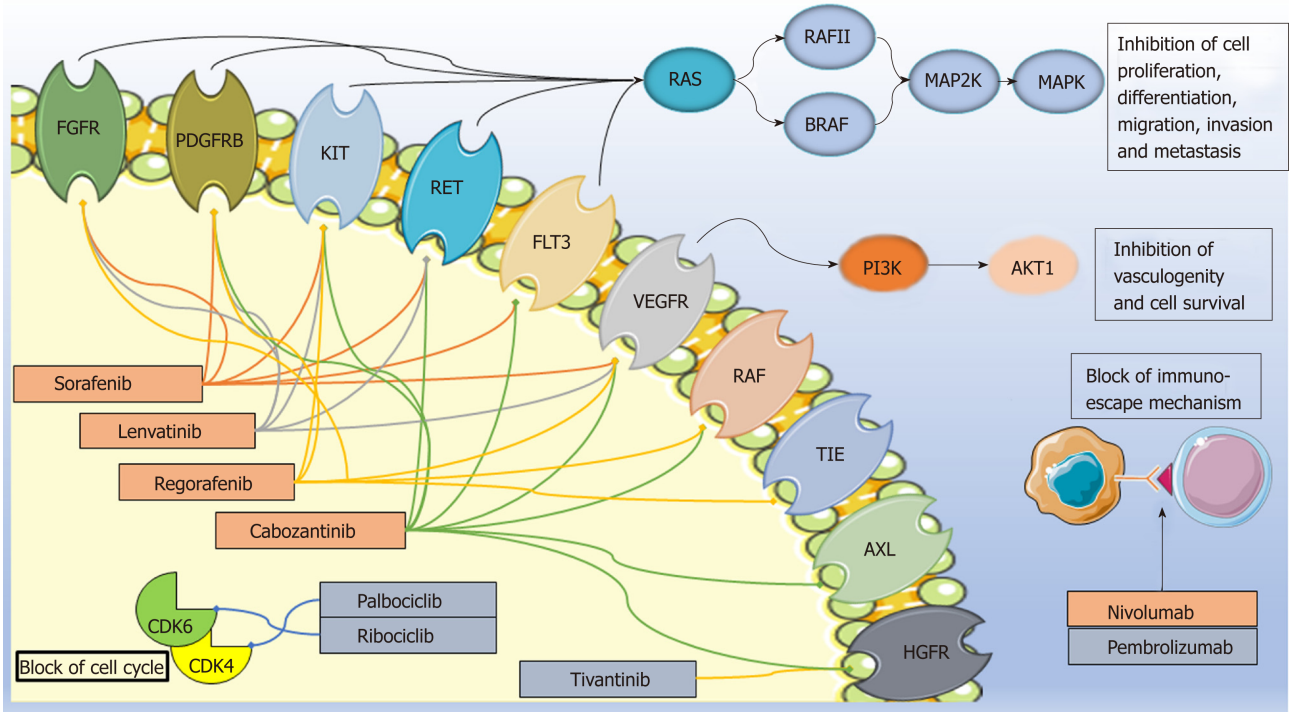


Figure 1 Mechanisms of action of the drugs covered in the text. Approved drugs are in the orange box while those under approval are in the grey box. Image created with Servier Medical Art (https://smart.servier.com/). FGFR1: Fibroblast growth factor receptor; PDGFR: Platelet-derived growth factor receptor; FLT3: Fms-related tyrosine kinase 3; VEGFR: Vascular endothelial growth factor receptor; TIE: Tyrosine kinase with immunoglobulin-like and EGF-like domains; HGFR: Hepatocyte growth factor receptor; PD1: Programmed cell death protein-1; PDL1/2: programmed cell death protein ligand 1/2; CDK: Cyclin-dependent kinases.

biomarkers of response to regorafenib in patients with HCC have been recently published. Particularly, a study involving a large cohort of patients enrolled in the phase III RESORCE trial showed a significant association of OS with plasma concentrations of some proteins involved in inflammation and/or HCC pathogenesis as well as a number of plasma miRNAs. In addition, a somatic profile of tumor tissues was described that suggested a potential mutational pattern associated with response to regorafenib[23].

More recently, a phase III trial comparing lenvatinib to sorafenib in the first-line setting showed non-inferiority of lenvatinib to sorafenib for the primary endpoint OS and statistically significant improvement for secondary end-point PFS. The most common any-grade adverse events described for lenvatinib were hypertension, diarrhea, and appetite and weight reduction. In addition, there were fewer dermatological adverse events but more hypertension for lenvatinib compared to sorafenib[24]. Finally, the small-molecule multikinase inhibitor cabozantinib was associated with longer OS than placebo in a phase III trial involving patients already treated for advanced disease. In that study, incidence of grade 3 or 4 adverse events was higher (predominantly grade 3) in the cabozantinib arm, including palmar–plantar erythrodysesthesia and HFSR, hypertension, increased aspartate aminotransferase (AST), fatigue, and diarrhea[25].

Other molecules not yet approved in Europe for the treatment of liver cancer are under investigation in the HCC setting, with promising preliminary results. Particularly, the novel class of immune checkpoint inhibitors has demonstrated significantly improved survival outcomes for patients with HCC. A phase I/II study trial investigated the role of the immunotherapeutic agent nivolumab in patients whose disease progressed while receiving at least one previous line of systemic therapy, including sorafenib, or who were intolerant to sorafenib. In this trial 262 eligible patients were treated, 48 in the dose-escalation phase and 214 in the dose-expansion phase. During dose escalation, 12 (25%) patients had grade 3 or 4 adverse events while 3 (6%) patients had serious adverse events (*i.e.*, pemphigoid, adrenal insufficiency, liver disorder); the objective response rate was 15% (95%CI: 6%-28%). For dose expansion, the objective response rate was 20% (95%CI: 15%-26%) with nivolumab 3 mg/kg[26]. Based on the results of this study, the U.S. Food and Drug Administration (FDA) granted accelerated approval of nivolumab on September 2017. A phase III randomized trial of first-line nivolumab compared with sorafenib is ongoing (ClinicalTrials.gov Identifier: NCT02576509). Another phase II trial focused

on another immune checkpoint inhibitor, pembrolizumab, in patients with HCC pre-treated with sorafenib. These results showed that pembrolizumab was effective and tolerable, with fatigue and increased AST as the most frequent adverse events[27]. Several phase II/III clinical trials with immunotherapeutic agents are currently recruiting HCC patients worldwide. One is an ongoing phase III randomized, active-controlled trial to evaluate the safety and efficacy of lenvatinib in combination with pembrolizumab compared with lenvatinib plus placebo in first-line therapy for advanced HCC (ClinicalTrials.gov Identifier: NCT03713593). A phase II trial with sorafenib and nivolumab as first-line therapy is also in progress (ClinicalTrials.gov Identifier: NCT03439891).

Among the molecules at an earlier stage of clinical development in HCC, the selective CDK4/6 inhibitors stand out. In an early trial, palbociclib demonstrated activity in patients with advanced HCC after failure of first-line sorafenib. This trial enrolled 21 patients, 4 being non-evaluable. In evaluable patients median OS was 19 wk and median time to progression was 24 wk; prolonged stability was seen in 3 patients. The most common grade 3 or 4 adverse events were neutropenia and thrombocytopenia, and non-serious adverse events were anemia, pain, ascites, and fatigue[11]. A phase Ib/II study of another CDK4/6 inhibitor, ribociclib, in association with chemoembolization in advanced HCC is currently recruiting patients (ClinicalTrials.gov Identifier: NCT02524119).

Another molecule under investigation that should be cited for completeness is tivantinib, a selective inhibitor of the proto-oncogene MET, belonging to the class of the small-molecule kinase inhibitors. A phase II randomized trial evaluated the administration of tivantinib as the second-line therapy for patients with HCC. The study showed improved PFS for tivantinib compared with placebo in a subset of patients with high MET expression tumors, and the most common grade 3 or worse adverse events in the tivantinib group were neutropenia and anemia[28]. On 13 November 2013, orphan designation (EU/3/13/1202) was granted for tivantinib for the treatment of HCC in patients whose disease has stopped responding or is resistant to sorafenib. However, a subsequent phase III trial evaluating the use of tivantinib for second-line treatment of MET-high expressing advanced HCC showed no OS improvement for tivantinib compared with placebo in patients previously treated with sorafenib[12].

PHARMACOGENETICS OF APPROVED DRUGS

Sorafenib

Sorafenib (NEXAVAR®) is an orally administered multi-targeted tyrosine kinase inhibitor. This small molecule inhibits a number of serine/threonine and tyrosine kinases [*e.g.*, VEGF receptors (VEGFR1–3), platelet-derived growth factor receptor (PDGFR), fibroblast growth factor receptor 1 (FGFR1), KIT proto-oncogene receptor tyrosine kinase (KIT), ret proto-oncogene (RET], and fms-related tyrosine kinase 3 (FLT3)] and downstream oncogenic Raf signaling players (*e.g.*, Raf-1 and B-Raf). Thus, it affects multiple tumor-related signaling pathways, such as those involved in angiogenesis, tumor proliferation, and cell apoptosis[29,30]. Although survival improvement has been achieved with this targeted agent, only a limited number of patients have experienced a real and long-term benefit. Moreover, a high resistance rate and some significant and expensive toxicities further restrict the advantages of sorafenib therapy and constitute a crucial problem in HCC management.

In recent years, some pharmacogenetic studies have focused on identifying genetic markers that could predict risk for severe adverse events (Table 1) and discriminate sorafenib-responsive patients from non-responders (Table 2). Details regarding the pharmacogenetic panel analyzed, the study population (*e.g.*, sample size, ethnicity) and therapy (*e.g.*, dose and schedule) characteristics, the clinical end-points evaluated along with the main findings (*e.g.*, statistical results) of the studies are shown in Tables 1 and 2.

Markers of pharmacokinetics/toxicity: (1) Sofarenib metabolism: The metabolism[29,30] of sorafenib is well-established and occurs mainly in the liver through two pathways: Phase I oxidation mediated by cytochrome P450 3A4 (CYP3A4), and phase II conjugation mediated by UDP glucuronosyltransferase 1A9 (UGT1A9) (Figure 2). In people, specifically, the glucuronidation contributes to about 15% of the clearance of sorafenib while the oxidation accounts for only 5%. Eight metabolites of sorafenib have been identified (M1–M8). The most abundant in the plasma is sorafenib N-oxide (M2), which is produced by CYP3A4 and exhibits an *in vitro* potency similar to the parental drug. M2 together with the sorafenib derivatives M4, obtained by

Table 1 Published works on germ-line variants and pharmacokinetic and toxicity profiles of sorafenib in hepatocellular carcinoma patients

Pharmacogenetic panel	Study population	Therapy	Clinical endpoint	Main findings	Ref.
CYP3A4*1, CYP3A5*3, CYP2C19*2, CYP2D6*10	Advanced HCC (n = 51) (Chinese) Aflatoxin-induced HCC rat models (n = 105)	Sorafenib	Toxicity	Rat models: CYP3A4*1 (rs2740574) and CYP3A5*3 (rs776746) were associated with the lowest (8 ± 2.5 ng/mL) and highest (67 ± 4.8 ng/mL) levels of sorafenib plasma concentration, respectively (P < 0.001). CYP3A5*3 correlated with the most severe liver [change in ALT and AST blood concentration [(IU/L) over time] and renal injury (change in BUN [nmol/L) and Cr [umol/L] blood concentration [IU/L] over time) and CYP3A4*1 with the least severe injury (P < 0.001) Clinical setting: CYP3A5*3 was associated with increased severe hepatic toxicity (change in ALT and AST blood concentration [IU/L] over time, P < 0.001)	[32]
9 SNPs in CYP3A5, UGT1A9, ABCB1, ABCG2	Advanced solid cancer (n = 54; 37% HCC) (whites)	Sorafenib 400 or 200 mg, twice daily	PK Toxicity	UGT1A9 rs17868320-T allele was associated with increased grade ≥ 2 diarrhea (OR: 14.33, P = 0.015, multivariate analysis) ABCG2 rs2622604-TT genotype exhibited a greater exposure compared to the CC (sorafenib AUC, 131.8 *vs* 82.4 mg/L.h, P = 0.093 univariate analysis, not confirmed in multivariate)	[34]
8 SNPs in SLCO1B1, SLCO1B3, ABCC2, ABCG2, UGT1A1, UGT1A9	Advanced solid cancer (n = 114; 87% HCC) (mainly whites)	Sorafenib	Toxicity PFS OS	UGT1A1*28 (rs8175347) was associated with increased risk of acute hyperbilirubinemia (*28/*28 *vs* other, OR:5.413, P = 0.016) and of interrupting treatment (*28 *vs* other, OR: 3.397, P = 0.002) by multivariate analysis. The *28 allele also showed a trend towards a higher risk for any toxicity at grade 3 or higher (P = 0.088) SLCO1B1*1b (rs2306283-G) allele was associated with inferior risk of diarrhea (OR: 0.125, P = 0.007) and increased risk of hyperbilirubinemia (OR: 1.230, P = 0.002), and the SLCO1B1*5 (rs4149056-C) allele with higher risk of thrombocytopenia (OR: 4.219, P = 0.045) in univariate but not multivariate analysis No SNP was associated with OS or PFS	[35]
49 SNPs in UGT1A9, UGT1A1, CYP3A4, CYP2B6, TNFA, VEGFA, IGF2, HIF1A	Intermediate stage HCC (n = 59) (Korean)	Sorafenib 400 mg twice daily in combination with TACE	Toxicity	VEGFA 1991-CC (OR: 45.68, P = 0.011), TNFA rs1800629-GG (OR: 44.06, P = 0.023), and UGT1A9 rs7574296-AA (OR: 18.717, P = 0.015) were independent risk factors for the development of high-grade HFSR (multivariate analysis).	[36]
5 SNPs in ABCB1, ABCG2	Advanced HCC (n = 47) (Caucasians)	Sorafenib 400 mg twice daily	PK	ABCB1 rs2032582 (CT: 0.7 ± 0.6 kg/L *vs* TT:2.3 ± 2.2 kg/L, P = 0.035), ABCG2 rs2231137 (AG: 0.8 ± 0.4 kg/L *vs* GG: 1.4 ± 1.5 kg/L, P = 0.02), ABCG2 rs2231142 (CA: 0.5 ± 0.5 kg/L *vs* CC: 1.4 ± 1.4 kg/L, P = 0.007) heterozygous genotypes were associated with the lowest sorafenib plasma levels	[42]

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; AUC: Area under the curve; BUN: Blood urea nitrogen; Cr: Creatinin; HCC: Hepatocellular carcinoma; HFSR: Hand–foot skin reaction; OR: Odds ratio; OS: overall survival; PFS: Progression-free survival; PK: Pharmacokinetic; SNP: Single nucleotide polymorphism; *vs*: Versus.

Table 2 Published works on germ-line variants and response to sorafenib in hepatocellular carcinoma patients

Pharmacogenetic panel	Study population	Therapy	Clinical endpoint	Main findings	Ref.
17 SNPs in VEGFA, VEGFC, FLT1 (VEGFR1), KDR (VEGFR2), FLT4 (VEGF3)	Advanced or intermediate-stage HCC (*n* = 148) (whites) (ALICE-1)	Sorafenib 400 mg, twice daily	PFS OS Objective Response	Univariate analysis VEGFA (rs25648-C, rs833061-T, rs699947-C, rs2010963-C), VEGFC (rs4604006-T), VEGFR1 (rs664393-G), and VEGFR2 (rs2071559-C, rs2305948-C) alleles were associated with longer PFS and OS Multivariate analysis VEGFA rs2010963-C allele (PFS, 6.9 mo *vs* 4.0 mo, HR: 0.25, p = 0.0376; OS, 17.0 *vs* 9.3 mo, HR: 0.28, *P* = 0.0201), VEGFC rs4604006-T allele (PFS, 10.1 mo *vs* 4.3 mo, HR: 0.22, p = 0.004; OS, 22.0 mo *vs* 13.0 mo, HR: 0.25, *P* = 0.04) and BCLC C stage (PFS, 7.6 mo *vs* 4.5 mo, HR: 0.17, *P* = 0.0163; OS, 21.0 mo *vs* 10.7 mo, HR: 0.36, *P* = 0.0015) were independent prognostic factors predicting PFS and OS Patients with both the favorable alleles of VEGFA rs2010963 and VEGFC rs4604006 showed improved PFS and OS compared to those with only one or none (PFS: *P* = 0.0004; two favorable alleles: 11.4 mo, one favorable and one unfavorable: 5.6 mo, two unfavorable: 3.4 mo; OS: *P* = 0.0001, two favorable alleles: 22.7 mo, one favorable and one unfavorable, 15.1 mo, two unfavorable, 8.8 mo) VEGFA rs2010963-C (*P* = 0.0343) and VEGFC rs4604006-T (*P* = 0.0028) alleles were also associated with a better objective response	[48]
18 SNPs in KDR (VEGFR2)	Advanced HCC (*n* = 78) (Chinese)	First-line sorafenib 400, mg twice daily	TTP OS Response	Univariate analysis VEGFR2 rs1870377-AA (5.8 mo *vs* 4.0 mo, *P* = 0.001) and rs2305948-AA (5.8 mo *vs* 4.5 mo, *P* = 0.016) genotypes were associated with longer TTP VEGFR2 rs1870377-AA genotype (15.0 mo *vs* 9.6 mo, *P* = 0.001) and rs2071559-T allele (13.0 mo *vs* 9.0 mo, *P* = 0.007) were associated with longer OS VEGFR2 rs1870377-AA (*P* = 0.011) and rs2305948-AA (*P* = 0.047) genotypes were associated with a better response Multivariate analysis Major vascular invasion (HR: 2.51, *P* = 0.021) and VEGFR2 rs1870377-AA (HR: 0.68, *P* = 0.005) were independent factors in TTP; performance status (HR: 2.36, *P* = 0.017), VEGFR2 rs1870377-AA (HR: 0.35, *P* = 0.003) and rs2071559-CC (HR: 2.25, *P* = 0.036) were independent factors in OS	[50]

3 SNPs in eNOS	Advanced HCC (n = 41 training set; n = 87 validation set (whites)	First-line sorafenib 400 mg, twice daily	PFS OS	Univariate analysis Training set Patients homozygous for the eNOSHT1 haplotype (HT1: T-4b by combining eNOS rs2070744 T > C and eNOS VNTR 27bp 4a/b** variants) had a lower median PFS (2.6 mo *vs* 5.8 mo, HR: 5.43, P < 0.0001) and OS (3.2 mo vs 14.6 mo, HR: 2.35 P = 0.024) than those with other haplotypes Validation set Patients homozygous for HT1 had a lower median PFS (2.0 mo vs 6.7 mo, HR: 5.16, P < 0.0001) and OS (6.4 mo *vs* 18.0 mo, HR: 3.01, P < 0.0001) than those with other haplotypes Multivariate analysis eNOS haplotype HT1 is confirmed as the only independent prognostic factor ** "4a" allele with 4 repeats; "4b" allele with 5 repeats	[51]
9 SNP in ANG2	HCC (n = 158) (whites)	Sorafenib	PFS OS	ANG2 rs55633437-GG genotype was associated with a better PFS (median PFS: 4.67 mo *vs* 2.94 mo, P = 0.03) and OS (median OS: 16.9 mo *vs* 6.5 mo, p = 0.016) with respect to the T-allele. Data were confirmed in multivariate analysis	[52]
8 SNPs in HIF1A	HCC (n = 210) (whites) (ALICE-2)	Sorafenib	PFS OS	Univariate analysis HIF1A rs1951795, rs10873142, and rs12434438 emerged as significant predictors of PFS and OS. The extended analysis of VEGF/VEGFR SNPs confirms the results of ALICE-1 study (see above) Multivariate analysis HIF1A rs12434438, VEGFA rs2010963, and VEGFC rs4604006 were confirmed as independent prognostic factors The combination of the favorable alleles of rs2010963 and rs4604006 compared to only one or to none, identifies three populations with different PFS (respectively: 10.8 mo *vs* 5.6 mo *vs* 3.7 mo, P < 0.0001) and OS (respectively: 19.0 mo *vs* 13.5 mo *vs* 7.5 mo, P < 0.0001) HIF1A rs12434438-GG genotype was associated with a poor outcome independently of VEGF markers (PFS: 2.6 mo, P < 0.0001; OS: 6.6 mo, P < 0.0001)	[49]

HCC: Hepatocellular carcinoma; HFSR: Hand–foot skin reaction; mo., months; OR: Odds ratio; OS: Overall survival; PFS: Progression-free survival; SNP: Single nucleotide polymorphism; TTP: Time to progression.

Table 3 Published works on germ-line variants and pharmacokinetics, toxicity, and efficacy of regorafenibin solid cancer patients

Pharmacogenetic panel	Study population	Therapy	Clinical endpoint	Main findings	Ref.
3 SNPs in SLCO1B1, ABCG2	Advanced solid cancer (*n* = 37; no HCC) (Japanese)	Regorafenib	Toxicity	*SLCO1B1**1b (rs2306283-G) allele was associated with lower incidences of increased grade ≥ 2 AST (8% *vs* 42%, *P* = 0.03) and anemia (16% *vs* 50%, *P* = 0.048). A similar tendency was observed for the incidence of increased grade ≥ 2 ALT (4% *vs* 25%, *P* = 0.09) and total bilirubin (12% *vs* 25%, *P* = 0.37).	[60]
3 SNPs in SLCO1B1, ABCG2	Advanced solid cancer (*n* = 28; no HCC) (Japanese)	Regorafenib	PK	ABCG2 rs2231142 was associated with different blood platelet counts (Plt, CC: 29.4 ± 10.7 *104/μL, CA + AA: 21.4 ± 11.3*104/μL, *P* = 0.03) SLCO1B1*5 (rs4149056-C) allele [17.3 (ng/mL)/mg *vs* 11.5 (ng/mL)/mg, p = 0.167] and SLCO1B1*1b (rs2306283-G/rs4149056-T) non-carriers [14.0 (ng/mL)/mg *vs* 12.1 (ng/mL)/mg, *P* = 0.226] demonstrated a tendency toward higher concentration-to-dose ratio Drug concentrations were higher in the group with grade ≥ 2 total bilirubin elevation (3.45 μg/mL *vs* 1.76 μg/mL, *P* = 0.01) and thrombocytopenia (3.45 μg/mL *vs* 1.76 μg/mL, p = 0.02) compared with the group with grades 0-1	[61]
Sequencing ofCYP34, UGT1A9	refractory mCRC (*n* = 93)1 (whites)	Regorafenib	Toxicity	UGT1A9*22 (rs383204-T10) allele was associated with acute hepatitis (descriptive study)	[57]
17 SNPs in VEGFA, VEGFC, FLT1 (VEGFR1), KDR (VEGFR2), *FLT4* (VEGF3)	mCRC (*n* = 59) (whites)	Regorafenib	PFS OS DCR	**Univariate analysis** *VEGFA* rs2010963-CC *vs* to CG + GG genotype was associated with both longer OS (9.0 mo *vs* 6.5 mo, HR: 0.52, *P* = 0.049) and PFS (2.2 mo *vs* 1.8 mo, HR: 0.49, *P* = 0.0038). The same genotype was also associated with better DCR (progression rate, 47% *vs* 74%, *P* = 0.02) VEGFR2 rs1870377 (TT: 1.97 mo, AT: 1.84 mo, AA: 1.12 mo; *P* = 0.0061) and VEGFR3 rs307805 (AA: 1.91 mo, AG: 2.07 mo, GG: 2.3 mo; *P* = 0.0492) were associated with OS only VEGFR1 rs664393 was associated with PFS only (CC: 2.01 mo, CT: 1.84 mo, TT: 1.48 mo; *P* < 0.0001) **Multivariate analysis** *VEGFA* rs2010963 [Exp(B): 2.77, *P* = 0.009] and ECOG performance status [Exp (B): 2.80, *P* = 0.004] were independent predictors of OS. The combination of these two parameters further stratified patients into three groups with progressively different OS (*P* < 0.0001) VEGFA rs2010963 was the only independent predictor of PFS (*P* = 0.005).	[63]
9 SNPs in CCL3, CCL4, CCL5, CCR5, PRKCD, KLF13, and HIF1A	mCRC (*n* = 79 Japanese patients–evaluation set; *n* = 150 Italian patients–validation set)	Regorafenib	PFS OS Toxicity	CCL4 rs1634517-CC and CCL3 rs1130371-GG were associated with longer PFS in the evaluation set (2.5 mo *vs* 2.0 mo, HR: 1.54, *P* = 0.043; 2.5 mo *vs* 2.0 mo, HR: 1.48, *P* = 0.064) and longer PFS (2.3 mo *vs* 1.8 mo, HR:1.74, *P* < 0.001; 2.3 mo *vs* 1.8 mo, HR: 1.66, *P* = 0.002) and OS (7.9 mo *vs* 4.4 mo, HR: 1.65, *P* = 0.004; 7.9 mo *vs* 4.4 mo, HR: 1.65, *P* = 0.004) in the validation set. These associations were confirmed by multivariate analysis In the evaluation set, the CCL5 rs2280789-GG genotype *vs* the A allele (12.9 mo *vs* 7.9 mo, HR: 0.45, p = 0.032) and the rs3817655-TT genotype respect to A allele (12.9 mo *vs* 7.9 mo, HR: 0.50, *P* = 0.055) was associated with longer OS by multivariate analysis. The analysis in the validation set was not possible because of the low SNP frequencies In the evaluation set, the CCL5 rs2280789-GG genotype was associated with higher incidence of grade ≥ 3 HFRS compared to the A allele (53% *vs* 27%, *P* = 0.078), and similarly, the CCL5 rs3817655-TT genotype *vs* the A allele (56% *vs* 26%, *P* = 0.026). The same variants in addition to the CCL5 rs1799988 were also associated with different distribution by genotype of the incidence of grade ≥ 3 hypertension In the validation set, the CCL5 rs2280789-G allele was associated with inferior incidence of grade ≥ 3 diarrhea *vs* AA genotype (0% *vs* 10%, p = 0.034) and KLF13 rs2241779-A allele with inferior incidence of grade ≥ 3 rash respect to CC genotype (10% *vs* 30%, *P* = 0.010). CCL3 rs1130371 and CCL4 rs1634517 were associated with different distributions by genotype of the incidence of grade ≥ 3 AST/ALT	[62]

[1]Focused on 3 patients presented severe toxic hepatitis. ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; DCR: Disease control rate; HCC: Hepatocellular carcinoma; HFSR: Hand–foot skin reaction; mo., months; OR: Odds ratio; OS: Overall survival; PFS: Progression-free survival; PK: Pharmacokinetic; SNP: Single nucleotide polymorphism.

demethylation, and M5, an oxidative metabolite, inhibit VEGFR and PDGFR signaling and members of the MAPK pathway. Given the key role of CYP3A4 and UGT1A9 in sorafenib metabolism, inducers or inhibitors of these enzymes, such as some foods and co-administered drugs (*e.g.*, carbamazepine, dexamethasone, phenobarbital, phenytoin, rifampin, rifabutin, St. John's wort), could modify bioavailability of the agent. Moreover, even if sorafenib is not a substrate for the cytochrome isoforms CYP2B6, CYP2C8, and CYP2C9 and the UDP glucuronosyltransferase UGT1A1, the biological agent *in vivo* inhibits activity of these enzymes with potential pharmacological consequences and drug-interaction events. Membrane translocation of sorafenib and its metabolites, including the inactive sorafenib-glucuronide (SG) derivative, has been reported to be carried out by the coordinated activity of ATP-binding cassette (ABC) and solute carrier (SLC) transporters, not y*et al*l identified[31] (Figure 2). An enterohepatic recirculation of sorafenib has specifically been suggested[31]; according to this hypothesis, the drug glucuronide-conjugated SG is extensively extruded from the hepatocytes into the bile through a process mediated mainly by the multidrug resistance protein (MRP) 2 (encoded by *ABCC2*). However, under physiological conditions, a considerable fraction of intracellular SG can also be secreted back into the blood by some sinusoidal transport mechanisms, including MRP3 (encoded by *ABCC3*). From the circulation, downstream hepatocytes can efficiently take up SG again *via* the organic anion transporter family member 1B (OATP1B1 and OATP1B3, encoded by *SLCO1B1* and *SLCO1B3*)-type carriers, resulting in only low SG concentrations reaching the general circulation. This secretion-and-reuptake loop may help prevent saturation of MRP2-mediated biliary SG secretion in hepatocytes located upstream within liver lobules, resulting in more efficient drug detoxification. Once secreted into the bile, SG enters the intestinal lumen, where it can be a substrate for bacterial β-glucuronidases that regenerate the parental drug sorafenib. This sorafenib can then undergo intestinal absorption, thus reentering the circulation. This ongoing enterohepatic recirculation of sorafenib has been inferred to contribute to the long-lasting sorafenib plasma levels observed in patients. In addition to these transporters, preclinical *in vitro* studies have identified other membrane carriers that might translocate sorafenib and its metabolites, such as the hepatic uptake pump organic cation transporter-1 (OCT1, encoded by *SLC22A1*) and the efflux transporters P-glycoprotein (p-gp or MDR1, encoded by *ABCB1*) and breast cancer resistance protein (BCRP, encoded by *ABCG2*)[30].

Functional polymorphic variants in genes encoding the phase I and II enzymes and ABC/SLC transporters involved in the sorafenib pathway have been described and could contribute to the inter-individual variability in the pharmacokinetics and toxicity profile observed in patients treated with sorafenib. Some studies have evaluated the role of genetic polymorphisms in predicting the bioavailability and toxicity of sorafenib administered to patients with HCC (Table 1). The most consistent data concern the predictive contribution of germ-line genetic variants in the oxidative and glucuronidative pathways on outcome with sorafenib.

(2) Oxidation pathway: Guo *et al*[32] recently focused on some CYP450 polymorphisms. In preclinical aflatoxin-induced HCC rat models, *CYP3A4**1B (rs2740574; located in the 5′ untranslated region [5′UTR]) and *CYP3A5**3 (rs776746; located in the intron 3) variants were associated with the lowest and highest sorafenib plasma concentrations, respectively. This difference in drug disposition was consistent with a different toxicity risk; *CYP3A5**3-carrier rats had the most severe liver (measured as a change in alanine aminotransferase [ALT] and AST blood concentration [IU/L] over time) and renal (measured as a change in blood urea nitrogen [nmol/L] and creatinin [umol/L] blood concentration [IU/L] over time) injury, whereas *CYP3A4**1-carrier rats had the mildest toxicity outcome. This author group analyzed other *CYP* family genetic variants in the same study, using additional engineered rat models. Carriers of *CYP2C19**2 (rs4244285; Pro227Pro) or *CYP2D6**10 (rs1065852, Pro34Ser) had sorafenib plasma levels and associated liver/renal toxicity that were intermediate between those of rats carrying *CYP3A5**3 or *CYP3A4**1 genetic variants[32]. This preclinical observation on rat models was confirmed in a small group of Chinese patients with advanced hepatitis B and C viral-associated HCC treated with sorafenib. In these patients, the *CYP3A5**3 polymorphism was associated with rapid worsening of hepatic damage, but *CYP3A4**1 carriers showed only a small effect. The findings therefore suggested that the *CYP3A5**3 variant that determines decreased CYP3A5 enzymatic activity[33] could influence hepatic and renal exposure to sorafenib, with severe associated damage.

(3) Glucuronidation pathway: Other investigations generated positive preliminary data on the predictive contribution of genetic variants in the glucuronidation pathway on sorafenib treatment outcome[34-36]. A study in a cohort of white patients with advanced solid cancer, including HCC, identified the rs17868320 variant in the promoter region of the *UGT1A9* gene as a predictive factor for grade ≥ 2 diarrhea

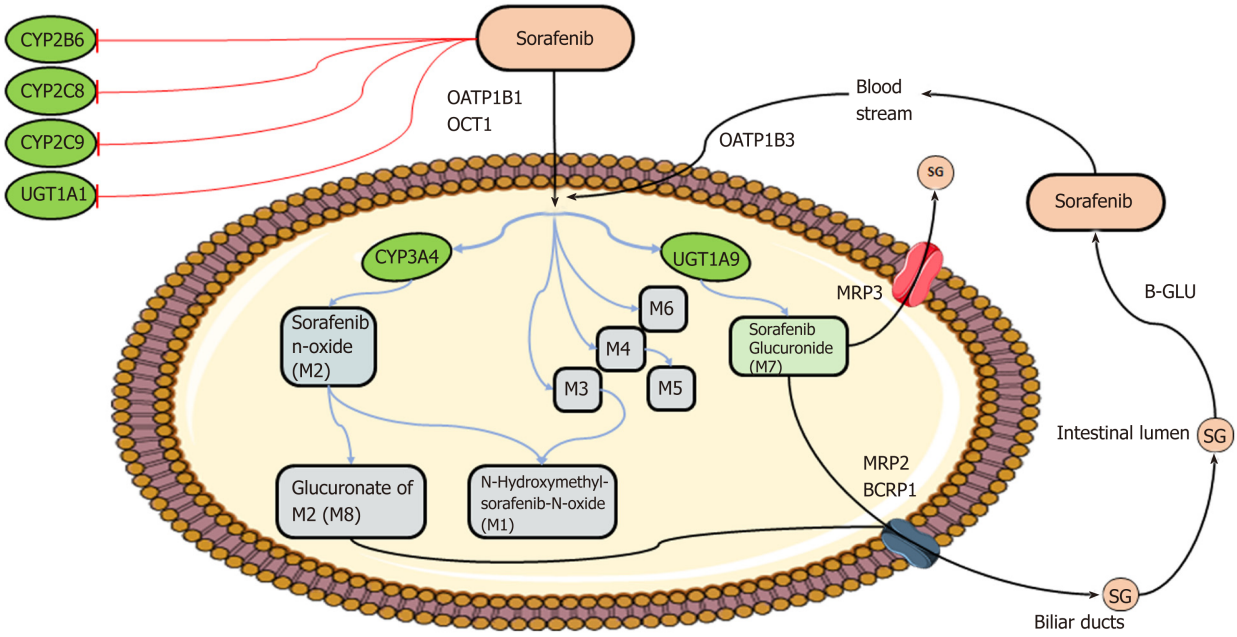


Figure 2 Schematic overview of sorafenib metabolism. Briefly, after oral administration, sorafenib enters hepatocytes by anion transporter family member (OATP1B, encoded by *SLCO1B*)-type carriers and cation transporter-1 (OCT1, encoded by *SLC22A1*). Within the hepatocytes, sorafenib undergoes phase I cytochrome P450 3A4 (CYP3A4)- and phase II UDP glucuronosyltransferase 1A9 (UGT1A9)-mediated metabolism to form M1-8 metabolites and sorafenib glucuronide (SG). After conjugation, SG is extensively secreted into the bile by a process that is mainly mediated by multidrug resistance protein (MRP) 2 (encoded by *ABCC2*) and breast cancer resistance protein BCRP (encoded by *ABCG2*) and into the bloodstream by MRP3 (encoded by ABCC3). A fraction of SG enters the intestinal lumen, where it could be a substrate for bacterial β-glucuronidases (B-GLU) that regenerate the parental drug sorafenib, which reenters the systemic circulation through the OATP1B3 carrier. CYP2B6, CYP2C8, CYP2C9, and UGT1A1 may interfere with sorafenib metabolism, being inhibited by sorafenib (see text for details). Image created with Servier Medical Art (https://smart.servier.com/).

occurrence. Carriers of the polymorphic rs17868320-T allele were exposed to a higher toxicity risk, without any impact on systemic drug exposure[34]. To explain this result, the authors suggested that the increased intestinal expression of UGT1A9, linked to the rs17868320 polymorphism[37,38], could cause a higher glucuronidation rate of the sorafenib metabolite M6. M6 is the major sorafenib derivative found in the feces, and when it is converted by UGT1A9 to the glucuronidated form, it exerts a damaging action on enterocytes, provoking diarrhea. The discovery of novel predictive factors of sorafenib-induced diarrhea is of particular interest, not only for the effect on patient quality of life but also for a potential interference with oral absorption of the drug, leading to decreased anti-tumor efficacy.

Results of another study involving Korean patients with intermediate-stage HCC receiving sorafenib in combination with TACE suggested that the genetic polymorphisms in *UGT1A9* could also influence the development of HFSR[36]. This common side-effect shows an ethnicity-specific incidence (*i.e.*, higher incidence in Asian trials compared with Western trials) and can affect treatment efficacy, causing dose reduction or treatment discontinuation[36]. Particularly, the A allele of the intronic variant *UGT1A9* rs7574296, whose functional impact is not yet known[39], is associated with increased HFSR risk. This preliminary result is of great clinical interest because early detection of patients at risk for HFSR would allow for continuation of life-prolonging therapy with minimal morbidity. Positive data also have been reported for an additional UGT1A isoform, UGT1A1, and its promoter polymorphism *UGT1A1**28 (rs8175347). A study involving predominantly white patients with advanced solid tumor, mostly HCC, identified the *UGT1A1**28 variant as a clinically significant predictive factor in hyperbilirubinemia risk during the first 2 months of sorafenib treatment and consequently of treatment interruption risk[35]. The *UGT1A1**28 allele also showed a trend to increased risk of developing any kind of toxicity of grade 3 or higher. These results are consistent with a previous case report reporting severe unconjugated hyperbilirubinemia in a sorafenib-treated patient carrying one *UGT1A1**28 polymorphic allele[40]. This genetic variant is associated with a remarkable reduction in bilirubin glucuronidation activity of the UGT1A1 enzyme, leading to significantly increased bilirubin concentrations[14], and also sorafenib inhibits the same enzyme UGT1A1[41]. Thus, use of sorafenib in patients who are homozygous for *UGT1A1**28 could lead to acute hyperbilirubinemia and a related risk of treatment interruption. Clinicians might need to be aware of their patient's *UGT1A1**28 status to

adequately consider sorafenib therapy in cases of hereditary genetic predisposition to hyperbilirubinemia development (*e.g.*, patients with Gilbert's syndrome)[40].

(4) Transporter mechanism: Genetic variants in the sorafenib transporter mechanism also appear to influence drug availability and toxicity risk, although data are quite preliminary. Particularly, some exploratory studies involving white patients with advanced solid cancer, including HCC, and receiving sorafenib reported an association of some functionally relevant genetic variants in *ABCG2*, *ABCB1*, and *SLCO1B1* genes with sorafenib pharmacokinetics and pharmacodynamics[34,35,42]. The TT genotype for the intronic *ABCG2* rs2622604 polymorphism was associated with decreased protein expression[43], and patients treated with sorafenib and carrying the TT genotype showed a tendency toward higher drug exposure at the plasma level. This tendency was not, however, confirmed in the multivariate analysis probably because of the small population[34]. Tandia and colleagues also reported an impact of *ABCG2* variants on sorafenib bioavailability[42]. In their analysis, the heterozygous genotypes of *ABCG2* rs2231137 (Val12Met), *ABCG2* rs2231142 (Lys141Gln), and *ABCB1* rs2032582 (lle1145Ile) polymorphisms were associated with lower drug plasma levels in comparison to the wild-type genotype carriers. Another group focused instead on sorafenib-related toxicity and reported significant differences in toxicity incidence according to two *SLCO1B1* polymorphisms that alter the transport activity of OATP1B1 in a substrate-specific manner[44]: *SLCO1B1* rs2306283 (*1b, Asn130Asp) and *SLCO1B1*-rs4149056 (*5, Val174Ala). Patients carrying at least one *SLCO1B1**1b (rs2306283-G) allele showed a reduced incidence of diarrhea and increased risk for hyperbilirubinemia; patients with the *SLCO1B1*5* (rs4149056-C) allele were more likely to develop thrombocytopenia, but only in a univariate and not in a multivariate model[35].

For background, we note that variants in MRP2- and OCT1-encoding genes also have been suggested to modulate sorafenib bioavailability and related adverse reactions, although mostly in other cancers. Studies performed in cancer settings other than HCC reported a significant involvement in the modulation of sorafenib plasma level and toxicity risk (*e.g.*, erythema) for the promoter variant rs717620 in the *ABCC2* gene (encoding MRP2)[45,46]. Particularly, a preliminary investigation, which involved mainly white patients (n=120) with solid cancer receiving sorafenib, suggested that the *ABCC2* rs717620-TT polymorphic genotype was associated with the lowest sorafenib plasma concentration (*i.e.*, AUC, area under the curve) compared with CT or CC genotype; interestingly this polymorphism seemed to modify AUC phenotype only in patients with *UGT1A1**28/*28 status. Another study, including 55 Japanese patients with advanced renal cell carcinoma treated with sorafenib, indicated that the *ABCC2* rs717620-CC genotype was associated with significantly increased risk of developing grade 3 or higher HFSR respect to CT genotype (35 vs. 0%, P=0.032). For what concerns OCT1 genetic variants, an ex vivo investigation of HCC tumor samples demonstrated that two novel exonic polymorphisms in the *SLC22A1* (gene encoding OCT1) (*i.e.*, Arg61Ser fs*10 and Cys88Ala fs*16) were associated with decreased expression of the OCT1 transporter and dramatically affected the ability of sorafenib to reach active intracellular concentrations[47].

Markers of response: (1) Mechanism of action: Sorafenib exerts its pharmacological effect through inhibition of cell surface and downstream intracellular kinases involved in several tumor cell signaling pathways, including proliferation, angiogenesis, and apoptosis. Therefore, data from *in vitro* analysis and animal models have demonstrated that sorafenib exerts its anticancer activity by repressing proliferation of HCC cells and tumor growth, inducing HCC cell apoptosis, and reducing tumor angiogenesis and related pathways (*e.g.*, inflammation)[29,30]. In addition to kinase inhibition, other mechanisms implicated in the activity of sorafenib include MAPK-independent apoptosis induction and immunomodulatory effects. Thus, primary and acquired resistance to sorafenib represent complex and multifaceted phenomena for which underlying mechanisms are not completely defined. At present, few pharmacogenetic studies have investigated the role of inherited genetic variability in determining the response to sorafenib (Table 2).

(2) VEGF-dependent pathways: The retrospective multicenter study ALICE1 (Angiogenesis Liver CancEr) evaluated a panel of functionally relevant polymorphisms in genes encoding VEGF and its receptor VEGFR for their role in clinical outcomes among white patients with advanced or intermediate-stage HCC receiving sorafenib[48]. On univariate analysis, the rs25648-C, rs833061-T, rs699947-C, and rs2010963-C alleles in *VEGFA*, rs4604006-T allele in *VEGFC*, rs664393-G allele in *FLT1* (encoding the receptor VEGFR1), and rs2071559-C and rs2305948-C alleles in *KDR* (encoding the receptor VEGFR2) emerged as potential predictive markers of longer PFS and OS. At the multivariate level, *VEGFA* rs2010963-C and *VEGFC* rs4604006-T alleles, together with BCLC stage, were confirmed as the only independent prognostic

factors predicting outcome in terms of PFS and OS. Moreover, the combination of *VEGFA* rs2010963 and *VEGFC* rs4604006 markers further improved patient stratification according to recurrence risk and survival probability. Patients expressing both favorable alleles showed longer PFS and OS compared to those expressing only one or none. The same favorable alleles were also significantly associated with a better objective response. The significant impact of *VEGFA* rs2010963 and *VEGFC* rs4604006 genetic variants, alone and in combination, on PFS and OS was also confirmed in the subsequent multicenter study ALICE-2[49]. Collectively, these findings suggest an impact of polymorphisms that might influence the level of circulating VEGF, such as rs2010963, located in the 5'UTR region of the *VEGFA* gene, and rs4604006, located in one of the intronic sequences of the *VEGFC* gene. The result would be a crucial effect on a drug such as sorafenib that targets this pathway. Another study also confirmed the key involvement of the angiogenesis process in modulating sorafenib treatment. Results from this Chinese cohort with advanced HCC suggested positive results with polymorphisms in *KDR* encoding the receptor VEGR2, whose dysfunction is correlated with decreased antiapoptotic effects of VEGF among other vascular alterations[50]. Particularly, the AA genotype of the rs1870377 variant was associated with longer time to progression and with OS as well as with better objective response. The T allele of the rs2071559 variant was associated with longer OS. Both polymorphisms were reported to affect VEGFR2 functionality and/or expression level, thus potentially interfering with sorafenib's mechanism of action[50]. The rs1870377 allele is a missense variant (Gln472His) located in the fifth NH2-terminal Ig-like domains within the extracellular region, which are important for ligand binding. Rs1870377, which is linked to a significant decrease in VEGF binding efficiency to VEGFR2, causes an altered protein phosphorylation pattern. Rs2071559 is a promoter variant that alters the binding affinity of this regulatory region for the transcriptional factor E2F, leading to decreased expression of the VEGF receptor. The same group reported preliminary data for another functionally relevant missense polymorphism, rs2305948 (Val297Ile), located in the third NH2-terminal Ig-like domains of the receptor. This variant was associated with differences in progression risk, with longer time to progression for the AA genotype, but only in the univariate and not in the multivariate model.

(3) Other pathways: Pharmacogenetic interest also has focused on different genetic targets in VEGF-dependent pathways. In particular, the Italian multicenter ePHAS study[51] focused on polymorphisms in the endothelial nitric oxide synthase (*eNOS*) gene, given the direct correlation between activation of the VEGF signaling pathway and stimulation of the vasodilator nitric oxide. This study, including training and validation populations of white patients with HCC undergoing sorafenib treatment, found in both cohorts a significant association of lower PSF and OS with a specific *eNOS* haplotype (*i.e.*, HT1:T-4b), derived by the combination of a rs2070744 T-to-C substitution in the 5'UTR region and the intronic VNTR 27bp 4a/4b polymorphism (*i.e.*, "4a" the allele with 4 repeats and "4b" the allele with 5 repeats). The rs2070744 variant was suggested to coordinate with the VNTR 27bp 4a/4b variant and directly affect gene transcription efficiency, resulting in altered eNOS expression levels that could in turn affect activation of VEGF signaling, and eventually sorafenib cytotoxicity. Particularly, the rs2070744-T and VNTR 27bp 4b alleles seemed to be associated with higher eNOS protein levels and activity, and consequently with increased basal NO production that could contribute to the sorafenib resistance. On the other hand, more recent preliminary results of another multicenter study, the ALICE-2[49], have highlighted a predictive role of polymorphisms in the gene encoding hypoxia–inducible factor α subunit (HIF1α) on sorafenib efficacy. HIF1α stabilization in hypoxic conditions upregulates VEGF expression by binding the VEGFA promoter, increasing angiogenesis. For this reason, HIF1α represents another player in the VEGF-dependent pathway that could be involved in sorafenib efficacy. Moreover, overexpression of HIF-1α in HCC is associated with tumor angiogenesis, invasion, metastasis, treatment resistance, and poor prognosis. The ALICE-2 study, which involved white patients with HCC treated with sorafenib, showed that *HIF1A* rs1951795, rs10873142, and rs12434438 variants contribute to discriminating patients according to different progression and survival probabilities. Multivariate analysis confirmed the predictive role only for the *HIF1A* rs124344308 polymorphism with the GG genotype, associating it with poorer PFS and OS independently from VEGF markers (*i.e.*, *VEGFA* rs2010963; *VEGFC* rs4604006). An additional clinical study[52] with a similar patient cohort generated positive data for genetic markers in another key angiogenic factor, Ang-2. By binding to its receptor Tie2, Ang-2 cooperates with the VEGF pathway in regulating angiogenesis and maintaining normal physiological vascular functions. In cancer, this protein is suggested to contribute to determining tumor aggressiveness and metastatic phenotype. In addition, a high baseline level of Ang-2 correlates with shorter OS in patients with advanced HCC without affecting

clinical response to sorafenib[53]. A preliminary study by Marisi *et al*[52] explored for the first time the role of an *Ang-2* genetic variant in sorafenib therapy outcome. These authors found that in particular, the GG genotype of the synonymous polymorphism rs55633437 (Thr238Thr) was associated with significantly longer PFS and OS compared to other genotypes.

Regorafenib

Regorafenib (STIVARGA®) is an oral small molecule inhibitor with an almost identical structure to sorafenib with which it shares most of the pharmacokinetic and pharmacodynamic properties[54]. Regorafenib, similarly to sorafenib, blocks multiple membrane-bound and intracellular kinases involved in normal cellular functions and pathologic processes such as tumor angiogenesis (VEGFR1, -2, -3, TIE2), oncogenesis (KIT, RET, RAF-1, BRAF), and modulation of the tumor microenvironment (PDGFR, FGFR). However, the small but significant difference in the chemical structure confers on regorafenib a stronger inhibition power of the targeted angiogenic and oncogenic kinases than sorafenib, resulting in higher pharmacological potency[54]. The liver metabolism of regorafenib, even if less well-characterized, is comparable with that of sorafenib and occurs through an oxidative process mediated by CYP3A4 and glucuronidation mediated by UGT1A9[54]. Two major and six minor metabolites of regorafenib have been identified in human plasma. The main circulating metabolites are M2 (N-oxide) and M5 (N-oxide and N-desmethyl), which show similar steady-state plasma concentrations and efficacy compared to the parental drug, as studied in *in vitro* and *in vivo* models[54-56]. Moreover, regorafenib and its metabolites M2 and M5 are suggested substrates of some ABC/SLC membrane transporters, such as MDR1, BCRP, MRP2, and OATP1B1, and thought to undergo enterohepatic recycling similar to that of sorafenib[54-56]. Regorafenib and its major metabolites are also reported to inhibit a number of cytochromes (CYP2C8, CYP2C9, CYP2B6, CYP3A4, CYP2D6), UGT1A enzymes (UGT1A9, UGT1A1), and transporters (BCRP) and induce others (CYP1A2, CYP2B6, CYP2C19, CYP3A4) with potential alteration in the exposure of co-administered drugs[55-58].

Since the recent introduction of regorafenib as a second-line treatment for HCC, no pharmacogenetic data have been published regarding potential genetic markers that could predict the risk of severe toxicity and response to the targeted drug in patients with liver cancer. However, given the similar metabolism and mechanism of action between regorafenib and sorafenib, the same genes and related variants suggested to modulate sorafenib therapy may also influence regorafenib. In support of this hypothesis are preliminary results from recent studies performed in other cancer settings, where regorafenib has been used for a long time. Details regarding the pharmacogenetic panel analyzed, the study population (*e.g.*, disease, sample size, ethnicity) and therapy (*e.g.*, dose and schedule) characteristics, the clinical end-points evaluated along with the main findings (*e.g.*, statistical results) of the studies are shown in Table 3.

Markers of pharmacokinetics/toxicity: Regarding potential markers of regorafenib toxicity, preliminary positive data have been generated for variants in genes encoding the metabolic enzymes UGT1A9, BCRP, and OATP1B1. A descriptive study[57] assessed *CYP3A4* and *UGT1A9* genetic variability by sequencing the germline DNA of three patients with metastatic colorectal cancer (mCRC) experiencing severe toxic hepatitis after sorafenib treatment and reported that two patients were heterozygous for the *UGT1A9**22 (rs3832043) polymorphism. This variant consisted of a single base insertion of thymidine in the promoter region and it is likely to increase gene expression and enzymatic function[59]. The high-activity *UGT1A9**22 allele probably affects hepatic metabolism of regorafenib, setting the stage for hepatotoxicity. This finding warrants strict liver monitoring during regorafenib treatment for patients with unfavorable *UGT1A9* genotypes. However, further investigations are needed to explore the exact mechanism by which an altered activity of UGT1A9 could contribute to the occurrence of hepatotoxicity. Another preliminary investigation of a small cohort of Japanese patients with solid cancer and receiving regorafenib[60] showed that the presence of the *SLCO1B1**1b (rs2306283-G) allele protected against the development of grade ≥ 2 hepatic injury and anemia, two of the most important regorafenib-related adverse drug reactions. The authors speculated that these associations could arise from a change in the pharmacokinetic profile of the biological agent, resulting from an inherited alteration in transporter activity of OATP1B1, as determined by the functional *1b variant haplotype[44]. The same work also showed that the loss-of-function rs2231142-A allele of the *ABCG2* gene correlated with inferior blood platelet counts (Plt) without an effect on risk for treatment-related grade ≥ 2 adverse reactions. A subsequent study from the same group[61], monitoring a small cohort of Japanese patients with solid cancer for 28 days after regorafenib

administration, showed a tendency, although not significant, to a higher drug concentration-to-dose ratio for the *SLCO1B1*5* (rs4149056-C) allele and for *SLCO1B1**1b (rs2306283-G/rs4149056-T) non-carriers. However, further investigations are required to confirm this association and to understand the biological mechanism underlying the observed genotype/phenotype correlation. Of interest, the results of the same study also included a strong association between serum regorafenib concentrations and total bilirubin levels, which could be used as a potential marker for estimating regorafenib pharmacokinetics. In fact, liver cells take up unconjugated bilirubin through OATP1B1, and in the hepatic cell, it is conjugated to glucuronic acid by UGT1A1. Considering that serum bilirubin is suggested to increase because of competitive inhibition *via* OATP1B1, bilirubin plasma level could be considered a surrogate marker of drug exposure. However, further analyses are needed to clarify the exact mechanism of competition between regorafenib and bilirubin with respect to OATP1B1.

Beside polymorphisms in metabolic enzymes encoding genes, genetic variants affecting the VEGFA-related pathway are also hypothesized to contribute to inter-individual differences in toxicity risk. Particularly, a recent study[62], including an evaluation (Japanese mCRC patients) and validation (Italian mCRC patients) cohort, reported significant differences in toxicity incidence according to genetic variants in the C-C motif chemokine ligand 5/C-C motif chemokine receptor 5 (CCL5/CCR5) pathway. This pathway modulate VEGFA production *via* endothelial progenitor cell migration. The investigation showed that in the evaluation set, the *CCL5* rs2280789-GG and rs3817655-TT genotypes were associated with higher incidence of grade ≥ 3 HFRS. The replication of these associations according to the recessive model in the validation set was not possible because of the low frequency of the homozygote genotype. With respect to the risk for HFRS, the observed differences in the frequency distribution of the rs2280789 and rs3817655 variants between the Japanese and Italian cohorts could also explain the different incidence of severe HFRS by ethnicity noted in clinical practice[36]. An exploratory analysis of the other toxicity types was also performed and highlighted that, in the evaluation set, the *CCL5* rs2280789, rs3817655, and rs1799988 variants could have a predictive effect on risk for grade ≥ 3 hypertension. In the validation set, the *CCL5* rs2280789 variant emerged as a predictive marker of grade ≥ 3 diarrhea while the *CCL4* rs1634517 and *CCL5* rs1130371 markers were differently distributed in genotype frequencies relative to incidence of grade ≥ 3 AST/ALT variation. Another marker of the CCL5/CCR5 pathway, the *KLF13* rs2241779, seemed to influence risk for grade ≥ 3 rash. Although these findings should be considered exploratory, they suggest a promising candidate targets for future pharmacogenetic studies aimed at discovering novel predictive markers to improve the management of regorafenib-associated toxicity (*e.g.*, personalized dosing and other strategies to support patient care).

Markers of response: Other studies have focused on the potential role of polymorphisms in the VEGF/VEGFR cascade and related mechanisms in modulating the response to regorafenib treatment. The work of Giampieri and colleagues[63], involving a small cohort of white patients with mCRC, reported that the *VEGFA* rs2010963 variant is an independent predictive marker of regorafenib efficacy in terms of disease control rate, PFS, and OS, with the CC genotype associated with a better outcome. The integration of patient Eastern Cooperative Oncology Group (ECOG) performance status with the *VEGFA* rs2010963 genotype improved stratification by survival rate. On univariate analysis, other markers, such as *VEGFR2* rs1870377, *VEGFR3* rs307805, and *VEGFR1* rs664393, were suggested to contribute to determining regorafenib outcome. In particular, the *VEGFA* rs2010963 variant, located in the 5'UTR of the gene, has a potential effect on VEGFA expression and tumor angiogenesis. The observed association is consistent with the results of other studies reporting significant involvement of VEGFA rs2010963 in influencing other biological agents targeting the VEGF/VEGFR cascade, such as sorafenib and bevacizumab[48,64].

Another recent investigation[62] evaluated the role on regorafenib therapy outcome of a panel of variants from the CCL5/CCR5 pathway that is involved in the modulation of VEGFA production. The study comprised 79 Japanese patients with mCRC as the evaluation cohort and 150 Italian patients with mCRC as the validation cohort. The results showed that in the evaluation set, the *CCL5* rs2280789-GG and rs3817655-TT genotypes were associated with longer OS. The replication of these associations according to the recessive model in the validation set was not possible because of the low frequency of the homozygote genotype. Functional analyses have demonstrated that the G allele of the rs2280789 polymorphism, located in the promoter region of *CCL5*, negatively affects transcriptional activity of RANTES, resulting in a lower serum level of CCL5 and VEGFA[62]. A similar phenotypic effect on CCL5 and VEGFA expression level was also suggested for the T allele of the

intronic *CCL5* rs3817655 variant[62]. These functional data could help explain the clinical impact on regorafenib outcome observed for the *CCL5* rs2280789 and rs3817655 markers. Of interest, the same study generated positive data for polymorphisms in genes encoding other CCR5 ligands, such as CCL4 (rs1634517, intronic variation) and CCL3 (rs1130371, synonymous variation, Pro60Pro) that were associated with PFS and OS in both evaluation and validation cohorts. These variants also displayed similar allelic distribution between the two ethnic groups, unlike CCL5. From a functional point of view, the *CCL4* rs1634517-C and *CCL3* rs1130371-G alleles, associated with longer PSF and OS, seemed to correlate with higher CCL5 level without any impact on VEGFA level[62].

Taken together, these data highlighted the importance of the VEGF/VEGFR cascade and related pathway (*i.e.*, CCL5/CCR5) in modulating the effectiveness of regorafenib therapy. Polymorphisms in gene encoding the several members of these pathways should be the target of future pharmacogenetic studies aimed at optimizing regorafenib treatment outcomes.

Other approved drugs

Cabozantinib (XL-184, COMETRIQ®) is an oral tyrosine kinase inhibitor that can block multiple oncogenic and angiogenic pathways implicated in tumor progression, worse prognosis, and metastasis, such as PDGFR, HGFR, VEGFR2, AXL, RET, KIT, and FLT3[65-69]. Following oral administration, the median time to peak plasma concentrations (Tmax) of cabozantinib ranged from 2 to 5 hours post-dose. This drug undergoes hepatic metabolism by CYP3A4 and, to a minor extent, by CYP2C9[66]. In addition, the major metabolites of cabozantinib identified in human plasma, after a single dose oral intake (140 mg), are EXEL-1646 (M9), obtained from M16 sulfation; EXEL-5162 (M19), obtained from the oxidation at the nitrogen of the quinolone portion; EXEL-5366 (M7), derived from the hydrolysis at the amide bond; and EXEL-1644 (M2a), the M7 sulfate conjugate[66,70]. Considering excretion, cabozantinib is eliminated mostly by the feces (54%) and urine (27%)[66]. Between 2012 and 2013, the FDA and the European Medicines Agency initially approved cabozantinib as a treatment for patients with medullary thyroid cancer. In 2016, the drug received a new indication as a treatment for patients with advanced renal cell carcinoma following one prior anti-angiogenic therapy[71-73]. Recently, several clinical trials have demonstrated that cabozantinib exhibits encouraging clinical activity in multiple human cancers, including HCC, with manageable side-effects[25,74-77]. Based on this evidence, cabozantinib represents an efficient alternative in the management of sorafenib-resistant HCC. In 2018, it received FDA approval for HCC treatment[76,78].

Lenvatinib (E7080 or LENVIMA®) is an orally active multikinase inhibitor that selectively inhibits receptors related to pro-angiogenic and oncogenic pathways such as VEGFR1-3, FGFR 1–4, PDGFRα, and RET, and KIT proto-oncogenes[79-83]. After oral administration, lenvatinib is rapidly absorbed, and time to peak plasma concentration occurs from 1 to 4 hours postdose[84]. However, even if administration with food does not affect the extent of absorption, it can decrease the rate of absorption and delay median Tmax from 2 to 4 hours. Both *in vitro* plasma and *in vivo* serum protein-binding assays demonstrated that lenvatinib protein binding ranges from 96.6 to 98.2%[84]. Lenvatinib is metabolized in liver microsomes mostly through CYP3A4 (> 80%) and, to a minor extent, by aldehyde oxidase and acts as a substrate for ABC transporters, encoded by the ABCB1 and ABCG2 genes, such as BCRP and P-glycoprotein[84-86]. Regarding excretion, the percentage of unchanged lenvatinib found in urine and feces is 2.5% of the administered dose, suggesting that lenvatinib is highly metabolized.

The principal metabolites of lenvatinib are derived from decyclopropylation (M1), demethylation (M2), N-oxidation (M3), and O-dearylation (M5)[84]. The formed metabolites are mainly excreted, approximately 64% *via* the biliary route in the feces, and 25% of the metabolites formed in the liver are released into the circulation and excreted *via* urine[84,87]. At first, lenvatinib was approved for the treatment of radioiodine-refractory differentiated thyroid cancer, as a single agent, and for the treatment of advanced renal cell carcinoma in combination with everolimus[79,88-90]. On August 2018, based on positive results of the REFLECT trial (NCT01761266), the FDA approved lenvatinib as a first-line treatment in patients with advanced and unresectable HCC[91,92].

To the best of our knowledge, no studies still have investigated the correlation between genetic polymorphisms and cabozantinib or lenvatinib treatment outcome for either toxicity or efficacy in HCC patients. However, a very recent study by Ozeki *et al*[93] on Japanese patients with thyroid cancer, demonstrated for the first time an impact of *CYP3A4/5* and *ABC* transporter genetic variants on lenvatinib pharmacokinetics[93]. Particularly, the *CYP3A4**1G (rs2242480, intronic variation) and *ABCC2* rs717620 polymorphisms were suggested to have an effect on the steady-state

mean plasma [*i.e.*, mean dose-adjusted C_0, (ng/mL/mg)] trough concentrations of lenvatinib. The mean dose-adjusted C_0 values of lenvatinib in patients with the *CYP3A4**1/*1 genotype and *ABCC2* rs717620-T allele were significantly higher than those in patients with the *CYP3A4**1G allele and *ABCC2* rs717620-CC genotype, respectively (effect size: 0.863, P = 0.018 and effect size: 0.605, P=0.036, respectively). Moreover, the dose-adjusted C_0 of lenvatinib in patients with both the *CYP3A4**1/*1 genotype and *ABCC2* rs717620-T allele (median 6.70 ng/mL/mg) was about 1.5-fold higher than that in patients with both the *CYP3A4**1G/*1G and ABCC2 rs717620-CC genotypes (median 4.42 ng/mL/mg; P = 0.007)[93]. These results demonstrated that functionally relevant genetic variants in proteins involved in the metabolism, translocation, and mechanism of action of cabozantinib or lenvatinib could be important determinants of therapy outcome and represent good candidates for future pharmacogenetic studies. With increasing therapeutic opportunities, the identification of markers that help clinicians choose the drug most suited to that patient becomes an urgent need. On this ground, Takeda *et al*. recently said that "approval of lenvatinib opened the new era of molecular targeting therapy for HCC. It requires the use of several molecular targeted agents appropriate for each HCC patient. To realize this personalized medicine, the establishment of genetic or transcriptional biomarkers needed to select the appropriate regimen is eagerly awaited''[94].

PHARMACOGENETICS OF DRUGS UNDER INVESTIGATION

The genomic understanding of HCC and the development of molecularly targeted therapies represent a promising stepping-stone for increasing the number of effective drugs for HCC patients. In recent years, many new drugs have been tested or are still under investigation as an alternative to sorafenib or, most important, after sorafenib failure. However, even if the survival benefit improvement and adverse drug event reduction are still the main focus, the identification of predictors of good responders could allow application of these new drugs in personalized treatments for HCC[95-98]. Furthermore, a deep understanding of the proteins involved in the metabolic pathway and mechanism of action of these novel molecularly targeted agents could suggest potential candidate targets (*i.e.*, genes and polymorphisms) for future pharmacogenetic studies. Therefore, this paragraph is focused on drugs currently under investigation for HCC therapy by providing general information on their metabolism, pharmacokinetics, mechanisms of action and, where available, pharmacogenetics data.

Nivolumab

The presence of tumor-infiltrating lymphocytes expressing programmed cell death protein-1 (PD-1, encoded by *PDCD1*) in HCC lesions and their correlation with outcome paved the way for immunotherapeutic approaches for HCC treatment[98-101]. The immune checkpoint inhibitor nivolumab (MDX-1106,OPDIVO®) is a fully human immunoglobulin (Ig) G4 (IgG4) monoclonal antibody. It binds the PD-1 receptor, expressed on activated T-cells, blocking interaction with its ligands PD-L1 and PD-L2 on tumor cells. This inhibition leads to downregulation of the T-cell–promoted tumor immune-escape mechanism, restoring the antitumor activity of T-cells[102,103]. Nivolumab is intravenously administered and thus is completely bioavailable. After initiation of the infusion, its median time to peak concentration is 1–4 hours[104,105]. As stated on the drug label, no formal studies were conducted to characterize the specific nivolumab metabolic pathway. However, it is thought to be degraded into small peptides and aminoacids through canonical pathways, such as endogenous IgG, and not by CYPP450. Similarly, no studies have addressed the specific elimination route of nivolumab. The phase I/II CHECKMATE-040 trial (NCT01658878) demonstrated the efficacy, safety, and tolerability of nivolumab in HCC treatment leading, on September 2017, to its accelerated FDA approval for the treatment of HCC in patients who previously have been treated with sorafenib[26,98]. At present, the multicenter phase III randomized controlled CHECKMATE-459 trial (NCT02576509) is ongoing to determine if nivolumab or sorafenib is more effective as first-line treatment for advanced HCC. In term of pharmacogenetics, it has been demonstrated, in lung adenocarcinoma, non–small cell lung cancer (NSCLC) and squamous cell carcinoma, that PD-1/PD-L1 gene polymorphisms may alter the immune checkpoint functions and affect the clinical response to nivolumab[106,107]. Patients with the CC or CG *PD-L1* genotypes (rs4143815) and the GG or GT *PD-L1* genotypes (rs2282055) experience a significantly longer median PFS (2.6 months) with nivolumab treatment than patients with the GG and TT genotypes (2.1 and 1.8 months respectively)[106]. Furthermore, none of the patients obtained a treatment effect with the GG genotype of *PD-L1*

rs4143815 and the TT genotype of rs2282055. In addition, it has been demonstrated that rs2297136, rs4143815, and rs17718883 polymorphisms of the *PD-L1* gene are associated with HCC risk and prognosis[107,108]. Even if the functional and biological effect of *PD-L1* genetic variants are still under investigation and debate, taking together, these results reinforce the role of these polymorphisms as possible prognostic markers for HCC development as well as markers of outcomes in nivolumab-treated patients[108-110].

Another study analyzed 322 nivolumab-treated patients with NSCLC and assessed the association between toxicities and polymorphisms in genes considered as contributors to PD-1–directed T-cell responses, such as the PD-1 gene (*PDCD1*), tyrosine-protein phosphatase non-receptor type 11 (*PTPN11*) and interferon gamma (*IFNG*). The TT genotype in the *PDCD1* rs2227981 polymorphism was associated with less nivolumab toxicity. On the contrary, patients presenting one G allele in the *PTPN11* rs2301756 polymorphism or who are homozygous CC for the *IFNG* rs2069705 polymorphism were at increased risk for developing any grade toxicity[111]. Further investigations are required to confirm these preliminary data and to test their validity also in the HCC setting.

Pembrolizumab

Pembrolizumab (lambrolizumab or MK-3475 or KEYTRUDA®) is a high-affinity humanized IgG4 monoclonal antibody that can bind with to the cell surface receptor PD-1, antagonizes receptor interaction with its known ligands PD-L1 and PD-L2, and allows the immune system to destroy cancer cells[98]. The antibody, intravenously administered, is immediately and completely bioavailable, does not bind to plasma proteins, and undergoes catabolism to small peptides and single aminoacids *via* general protein degradation routes[112]. In terms of clearance, a correlation has been demonstrated between clearance rate and increasing body weight, explaining the rationale for dosing on an mg/kg basis, whereas age, sex, race, and tumor burden have no clinically important effect on clearance. Furthermore, mild or moderate renal and hepatic impairments do not differ in clinically important way in clearance compared to patients with normal functions[112]. In 2016, Truong *et al*. published the first case report of a 75-year-old man with advanced HCC responsive to pembrolizumab, on a compassionate use basis, after failure of sorafenib therapy[113]. Since 2016, several observational and interventional phase I/II/III studies, such as the KEYNOTE-224 and the KEYNOTE-240 trials, continue investigating the safety and efficacy of pembrolizumab, alone or in combination with other drugs/procedures, in patients with advanced HCC who progressed on or were intolerant to first-line systemic therapies (*e.g*., NCT02940496, NCT02658019, NCT03062358, NCT03753659, NCT02702401)[83,114]. In 2016, considering a cohort of patients with metastatic melanoma treated with pembrolizumab or nivolumab, it has been demonstrated that 28% of responsive tumors were significantly enriched in non-synonymous single-nucleotide variations in disparate breast cancer type 2 susceptibility protein (BRCA2) domains. Specifically, one in the N-terminal nucleophosmin–interacting region (rs775903570, Val950Leu), one in the DNA polymerase eta–interacting domain (Ser1792Phe), four in the helical domain critical for Fanconi anemia group D2 (FANCD2) interaction (His2361Tyr [rs786203493], Pro2505Ser, Ser2522Phe, His2537Tyr), and one between these two interacting domains (Glu2115Lys)[115]. The authors, according to the disposition of the highlighted loss-of-function mutations and known role of BRCA2 in DNA repair, suggested that enhanced responsiveness could arise from cellular stress resulting from defective DNA repair that leads to increased cell death and anti-tumor immunity[115,116].

Furthermore, Al-Samkari *et al*[117] recently published a case report of a 58-year-old woman with aggressive metastatic breast cancer who developed hemophagocytic lymphohistiocytosis (HLH) while undergoing experimental treatment with pembrolizumab, resulting in critical illness and multi-organ system failure. Next-generation sequencing revealed that she was heterozygous for germ-line perforin-1 (*PRF1*) c.272C>T (rs35947132, p.Ala91Val). Several studies have demonstrated that *PRF1* rs35947132 is aberrantly post-translationally processed and results in reduced perforin expression together with partial loss of lytic activity. The rs35947132 polymorphism is a genetic risk factor for the development of HLH in patients exposed to certain environmental triggers. Taking all these findings together, the authors postulated that in the presence of the PRF1 polymorphism, pembrolizumab treatment could ignite a dramatic adverse drug event such as HLH[117]. Once again, these interesting pharmacogenetic results stress the hypothesis that the presence of genetic variations could affect, in this case, pembrolizumab therapy outcome, giving the possibility to investigate and, so, to extend their spectrum of action to other oncological fields, such as HCC therapy.

Palbociclib and ribociclib

Palbociclib (PD-0332991, IBRANCE®) and ribociclib (LEE-011,KISQALI®) are oral, specific inhibitors of the cyclin-dependent kinases CDK4 and CDK6[118,119]. Through CDK inhibition, both drugs prevent the formation of the cyclin D-CDK4/6 complex and retinoblastoma protein phosphorylation. Accordingly, cells cannot switch from R to G1 phase and proceed through the cell cycle[120,121]. In addition to canonical CDK4/6 retinoblastoma signaling, palbociclib shows *in vitro* and *in vivo* antiHCC activity by inducing cell autophagy and apoptosis *via* a mechanism involving 5′ AMPactivated protein kinase activation and protein phosphatase 5 inhibition[122]. Palbociclib is slowly absorbed, with a median Tmax generally observed between 6 to 12 hours, while ribociclib is rapidly absorbed, with median Tmax ranging from 1 to 5 hours. Binding of palbociclib to human plasma proteins *in vitro* is approximately 85%, while binding of ribociclib is approximately 70%, with no concentration dependence in either case. Following oral administration, palbociclib and ribociclib undergo extensive hepatic metabolism mainly by CYP3A; palbociclib also is metabolized through the sulfotransferase enzyme SULT2A1[123,124]. The major primary metabolic pathways for palbociclib involve oxidation and sulfonation, with acylation and glucuronidation contributing as minor pathways. For ribociclib, the primary metabolic pathways involve oxidation (dealkylation, C and/or N-oxygenation, oxidation (-2H)) and combinations thereof. Phase II conjugates of ribociclib phase I metabolites involved N-acetylation, sulfation, cysteine conjugation, glycosylation, and glucuronidation. Palbociclib and ribociclib are the major circulating drug-derived entities in plasma (23% and 46%, respectively), and their clinical activity traces primarily to the parent drug, with negligible contribution from circulating metabolites. Both drugs are eliminated mostly (69%–74%) *via* the feces, but also (17%–23%) *via* the urine. Following encouraging results from clinical trials, palbociclib and ribociclib have been approved, between 2015 and 2017, by the FDA and European Medicines Agency for hormone receptor-positive, human epidermal growth factor receptor 2-negative (HR+/HER2-) advanced or metastatic breast cancer therapy in combination with an aromatase inhibitor, such letrozole, or with fulvestrant (FASLODEX®), a selective estrogen receptor degrader, in women with disease progression after endocrine therapy[125-134]. The effects of palbociclib and ribociclib as a treatment for other malignancies, including HCC, are of great clinical interest and under current investigation (NCT01356628, NCT02524119).

Tivantinib

Tivantinib (ARQ197) is a selective, orally available, non-ATP competitive c-MET inhibitor currently under clinical investigation in patients with cancer[135,136]. Indeed, upregulation of the c-MET pathway, including its only known ligand HGF, is found in multiple cancers, such as HCC, and is associated with poor prognosis and metastases[136-139]. Conversely, tivantinib also revealed an anti-proliferative activity that was not restricted to only c-MET–dependent cell lines[140]. In fact, several *in vitro* and *in vivo* studies have demonstrated that tivantinib can affect microtubule dynamics by disrupting mitotic spindles. It also can promote G2/M cell cycle arrest and apoptosis by inhibiting the anti-apoptotic molecules myeloid cell leukemia-1 and B-cell lymphoma-extra large and increasing Cyclin B1 expression[140-144]. Indeed, despite controversies regarding its mechanism of action, two phase II clinical trials (NCT01575522, NCT00988741) have demonstrated that tumors with high levels of MET present a high degree of response to tivantinib treatment[145,146]. In term of pharmacokinetics, tivantinib is metabolized by CYP2C19, CYP3A4/5, UGT1A9, and alcohol dehydrogenase isoform 4[147]. CYP2C19 shows catalytic activity for the formation of the hydroxylated metabolite (M5), whereas CYP3A4/5 catalyzes formation of M5 and its stereoisomer (M4). Moreover, CYP3A4/5 represents the major cytochrome isoform involved in the elimination of M4, M5, and the keto-metabolite (M8), and together with UGT1A9, involved in the glucuronidation of M4 and M5[147]. Finally, the alcohol dehydrogenase isoform 4, through a sequential keto-metabolite of M4 and M5 and through M8, leads to the formation of M6[147]. Between 2010 and 2014, two phase I trials (NCT01069757, NCT01656265) in Japanese patients with advanced solid tumors examined the safety, pharmacokinetics, and preliminary efficacy of tivantinib as a single agent to determine recommended phase II dose according to CYP2C19 polymorphisms[148,149]. Recently, two Phase III trials, the METIV-HCC (NCT02029157) and the JET-HCC (NCT01755767), were conducted to determine if tivantinib is effective as a second-line treatment MET in patients with diagnostic-high HCC who have already been treated once with another systemic therapy. This trial also further evaluated the safety profile of the experimental drug in this population[12,150]. Unfortunately, no statistically significant differences between tivantinib and placebo in terms of OS or PFS were identified in either trial.

CONCLUSION AND FUTURE DIRECTIONS

Despite the advantage in patient survival resulting from the introduction of targeted agents in the therapeutic scenario of HCC, a significant inter-individual heterogeneity in therapy outcome persists and constitutes a crucial problem in HCC management. Moreover, with the increasing number of therapeutic options, selection of the most appropriate treatment for each patient is a great challenge. The identification of genetic markers that predict which patients will benefit from a specific intervention could significantly affect decision-making and therapy planning

Most of the pharmacogenetic studies published to date have focused on sorafenib, which has the longest track record in HCC treatment. However, these investigations, conducted for the most part in small cohorts, have generated only quite sparse, unreplicated data that do not permit drawing conclusions about their clinical validity. Moreover, comparison among studies is difficult because of the high heterogeneity in regard to the ethnic and clinical-demographic characteristics of the cohorts, study design (*e.g.*, retrospective/prospective analyses, low statistical power, adoption of an internal data validation), panel of investigated genes and related variants, method of controlling for confounding and environmental factors, and parameters for measuring clinical outcomes. For these reasons, the pharmacogenetic data published to date should be considered only hypothesis-generating. These data could be useful, however, for indicating specific genes and related pathways as potential candidate predictors of sorafenib therapy, guiding future research efforts.

Regarding the discovery of potential markers of toxicity risk after sorafenib administration, *CYP* and *UGT1A* polymorphisms are associated with different risks for severe adverse events. Although these findings should be considered preliminary because of small sample sizes, lack of replication, and some negative results[151], they surely support the need for additional pharmacogenetic research efforts to deeply understand the real clinical utility of *CYP* and *UGT1A* markers. Other published data, although mainly hypothesis-generating, have pointed out the clinical contribution in determining the sorafenib-related toxicity risk of polymorphisms in *ABC/SLC* membrane transporter genes. Some caveats are necessary, however. Given the complex enterohepatic recirculation in which sorafenib appears to be involved (see above)[31], it could be supposed that the evaluation of the combinatorial effect of multiple ABC/SLC carriers with respect to single gene/variant analysis is a more effective strategy for identifying inter-individual differences in the pharmacological profile of sorafenib. Moreover, based on the results of a pilot study, evaluating *UGT1A1**28 carriers with two distinct phenotypes in relation to sorafenib exposure based on *ABCC2* rs717620 genotypes[46], the integration of inherited variability in multiple metabolic processes, such as phase I and II metabolism and membrane translocation, could further improve prediction of therapy outcome. Moreover, the investigation of other pathways with closer links to the drug mechanism of action, such as angiogenesis (*e.g.*, VEGFA) and inflammation (*e.g.*, tumor necrosis factor-α), could be useful for discovering additional novel genetic markers that could contribute to stratifying patients based on individual toxicity risk[36].

Concerning potential genetic determinants of sorafenib efficacy, recent studies have highlighted the potential utility of inherited variability in the VEGF/VEGFR cascade and related pathways to identify patients who could benefit from sorafenib administration. This information can help clinicians in the selection of the most appropriate treatment and improve clinical outcome. For example, patients with a favorable genetic background could be administered sorafenib as soon as clinically indicated, instead of delaying it with other therapy; patients with an unfavorable background could be preferably included in clinical trials exploring new or upcoming treatment options. At present, the multicenter prospective INNOVATE study is ongoing to validate the contribution of polymorphisms in genes encoding VEGF, eNOS, Ang-2, and HIF-1α in determining clinical outcomes in patients with advanced HCC receiving sorafenib (NCT02786342)[152].

Only a few pharmacogenetic data, obtained for the most part in a non-HCC tumor setting, have been published regarding the recently approved regorafenib. Globally, these data, are only of hypothesis-generating value, but they have indicated potential candidate genes related to the regorafenib metabolism (*e.g.*, ABC/SLC transporters, UGT1As) and mechanism of action (*e.g.*, VEGFA and its receptors; the CCL5/CCR5 pathway). Their predictive power for therapy outcome could be useful to investigate in the HCC setting. Moreover, the similar pharmacological proprieties of regorafenib and sorafenib suggest that the genetic determinants of therapy outcome described for sorafenib could apply for regorafenib treatment and should be further investigated.

For the more recently developed multikinase kinase inhibitors (*e.g.*, lenvatinib, cabozantinib), immune checkpoint inhibitors (*e.g.*, nivolumab, pembrolizumab), and selective CDK4/6inhibitors (*e.g.*, palbociclib, ribociclib), no pharmacogenetic markers

have been identified in the HCC setting. Research efforts should respond to this lack of information.

The discovery of biomarkers, subsequently validated in large prospective studies, is a compelling need because they are expected to allow for more accurate selection of patients with HCC who are potential candidates for a specific targeted agent. This stratification could mean the ability to limit treatment to potentially responding patients and sparing unnecessary toxicity to those who are unlikely to benefit.

REFERENCES

1 **Llovet JM**, Zucman-Rossi J, Pikarsky E, Sangro B, Schwartz M, Sherman M, Gores G. Hepatocellular carcinoma. *Nat Rev Dis Primers* 2016; **2**: 16018 [PMID: 27158749 DOI: 10.1038/nrdp.2016.18]

2 **McGlynn KA**, Petrick JL, London WT. Global epidemiology of hepatocellular carcinoma: an emphasis on demographic and regional variability. *Clin Liver Dis* 2015; **19**: 223-238 [PMID: 25921660 DOI: 10.1016/j.cld.2015.01.001]

3 **Mazzaferro V**, Regalia E, Doci R, Andreola S, Pulvirenti A, Bozzetti F, Montalto F, Ammatuna M, Morabito A, Gennari L. Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. *N Engl J Med* 1996; **334**: 693-699 [PMID: 8594428 DOI: 10.1056/NEJM199603143341104]

4 **Livraghi T**, Meloni F, Di Stasi M, Rolle E, Solbiati L, Tinelli C, Rossi S. Sustained complete response and complications rates after radiofrequency ablation of very early hepatocellular carcinoma in cirrhosis: Is resection still the treatment of choice? *Hepatology* 2008; **47**: 82-89 [PMID: 18008357 DOI: 10.1002/hep.21933]

5 **Bruix J**, Sherman M; American Association for the Study of Liver Diseases. Management of hepatocellular carcinoma: an update. *Hepatology* 2011; **53**: 1020-1022 [PMID: 21374666 DOI: 10.1002/hep.24199]

6 **Llovet JM**, Bruix J. Systematic review of randomized trials for unresectable hepatocellular carcinoma: Chemoembolization improves survival. *Hepatology* 2003; **37**: 429-442 [PMID: 12540794 DOI: 10.1053/jhep.2003.50047]

7 **Lencioni R**, de Baere T, Soulen MC, Rilling WS, Geschwind JF. Lipiodol transarterial chemoembolization for hepatocellular carcinoma: A systematic review of efficacy and safety data. *Hepatology* 2016; **64**: 106-116 [PMID: 26765068 DOI: 10.1002/hep.28453]

8 **Abou-Alfa GK**, Venook AP. The impact of new data in the treatment of advanced hepatocellular carcinoma. *Curr Oncol Rep* 2008; **10**: 199-205 [PMID: 18765149 DOI: 10.1007/s11912-008-0031-x]

9 **Rougier P**, Mitry E, Barbare JC, Taieb J. Hepatocellular carcinoma (HCC): an update. *Semin Oncol* 2007; **34**: S12-S20 [PMID: 17449346 DOI: 10.1053/j.seminoncol.2007.01.007]

10 **Verslype C**, Van Cutsem E, Dicato M, Arber N, Berlin JD, Cunningham D, De Gramont A, Diaz-Rubio E, Ducreux M, Gruenberger T, Haller D, Haustermans K, Hoff P, Kerr D, Labianca R, Moore M, Nordlinger B, Ohtsu A, Rougier P, Scheithauer W, Schmoll HJ, Sobrero A, Tabernero J, van de Velde C. The management of hepatocellular carcinoma. Current expert opinion and recommendations derived from the 10th World Congress on Gastrointestinal Cancer, Barcelona, 2008. *Ann Oncol* 2009; **20** Suppl 7: vii1-vii6 [PMID: 19497945 DOI: 10.1093/annonc/mdp281]

11 **Littman SJ**, Brus C, Burkart A. A phase II study of palbociclib (PD-0332991) in adult patients with advanced hepatocellular carcinoma. *J Clin Oncol* 2015; **33**: 277-277 [DOI: 10.1200/jco.2015.33.3_suppl.277]

12 **Rimassa L**, Assenat E, Peck-Radosavljevic M, Pracht M, Zagonel V, Mathurin P, Rota Caremoli E, Porta C, Daniele B, Bolondi L, Mazzaferro V, Harris W, Damjanov N, Pastorelli D, Reig M, Knox J, Negri F, Trojan J, López López C, Personeni N, Decaens T, Dupuy M, Sieghart W, Abbadessa G, Schwartz B, Lamar M, Goldberg T, Shuster D, Santoro A, Bruix J. Tivantinib for second-line treatment of MET-high, advanced hepatocellular carcinoma (METIV-HCC): a final analysis of a phase 3, randomised, placebo-controlled study. *Lancet Oncol* 2018; **19**: 682-693 [PMID: 29625879 DOI: 10.1016/S1470-2045(18)30146-3]

13 **De Mattia E**, Cecchin E, Polesel J, Bignucolo A, Roncato R, Lupo F, Crovatto M, Buonadonna A, Tiribelli C, Toffoli G. Genetic biomarkers for hepatocellular cancer risk in a caucasian population. *World J Gastroenterol* 2017; **23**: 6674-6684 [PMID: 29085212 DOI: 10.3748/wjg.v23.i36.6674]

14 **De Mattia E**, Cecchin E, Polesel J, Lupo F, Tiribelli C, Crovatto M, Buonadonna A, Toffoli G. UGT1A polymorphisms as genetic biomarkers for hepatocellular carcinoma risk in Caucasian population. *Liver Int* 2017; **37**: 1345-1353 [PMID: 28294511 DOI: 10.1111/liv.13411]

15 **Llovet JM**, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, de Oliveira AC, Santoro A, Raoul JL, Forner A, Schwartz M, Porta C, Zeuzem S, Bolondi L, Greten TF, Galle PR, Seitz JF, Borbath I, Häussinger D, Giannaris T, Shan M, Moscovici M, Voliotis D, Bruix J; SHARP Investigators Study Group. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* 2008; **359**: 378-390 [PMID: 18650514 DOI: 10.1056/NEJMoa0708857]

16 **Marisi G**, Cucchetti A, Ulivi P, Canale M, Cabibbo G, Solaini L, Foschi FG, De Matteis S, Ercolani G, Valgiusti M, Frassineti GL, Scartozzi M, Casadei Gardini A. Ten years of sorafenib in hepatocellular carcinoma: Are there any predictive and/or prognostic markers? *World J Gastroenterol* 2018; **24**: 4152-4163 [PMID: 30271080 DOI: 10.3748/wjg.v24.i36.4152]

17 **Bruix J**, Cheng AL, Meinhardt G, Nakajima K, De Sanctis Y, Llovet J. Prognostic factors and predictors of sorafenib benefit in patients with hepatocellular carcinoma: Analysis of two phase III studies. *J Hepatol* 2017; **67**: 999-1008 [PMID: 28687477 DOI: 10.1016/j.jhep.2017.06.026]

18 **Abdel-Rahman O**, Lamarca A. Development of sorafenib-related side effects in patients diagnosed with advanced hepatocellular carcinoma treated with sorafenib: a systematic-review and meta-analysis of the impact on survival. *Expert Rev Gastroenterol Hepatol* 2017; **11**: 75-83 [PMID: 27882800 DOI: 10.1080/17474124.2017.1264874]

19 **Marrero JA**, Kudo M, Venook AP, Ye SL, Bronowicki JP, Chen XP, Dagher L, Furuse J, Geschwind JH, de Guevara LL, Papandreou C, Takayama T, Sanyal AJ, Yoon SK, Nakajima K, Lehr R, Heldner S, Lencioni R. Observational registry of sorafenib use in clinical practice across Child-Pugh subgroups: The

GIDEON study. *J Hepatol* 2016; **65**: 1140-1147 [PMID: 27469901 DOI: 10.1016/j.jhep.2016.07.020]
20 **Su WW**, Lee KT, Yeh YT, Soon MS, Wang CL, Yu ML, Wang SN. Association of circulating insulin-like growth factor 1 with hepatocellular carcinoma: one cross-sectional correlation study. *J Clin Lab Anal* 2010; **24**: 195-200 [PMID: 20486202 DOI: 10.1002/jcla.20320]
21 **Adachi T**, Nouso K, Miyahara K, Oyama A, Wada N, Dohi C, Takeuchi Y, Yasunaka T, Onishi H, Ikeda F, Nakamura S, Shiraha H, Takaki A, Takabatake H, Fujioka SI, Kobashi H, Takuma Y, Iwadou S, Uematsu S, Takaguchi K, Hagihara H, Okada H; Okayama Liver Cancer Group. Monitoring serum proangiogenic cytokines from hepatocellular carcinoma patients treated with sorafenib. *J Gastroenterol Hepatol* 2019; **34**: 1081-1087 [PMID: 30402928 DOI: 10.1111/jgh.14535]
22 **Bruix J**, Qin S, Merle P, Granito A, Huang YH, Bodoky G, Pracht M, Yokosuka O, Rosmorduc O, Breder V, Gerolami R, Masi G, Ross PJ, Song T, Bronowicki JP, Ollivier-Hourmand I, Kudo M, Cheng AL, Llovet JM, Finn RS, LeBerre MA, Baumhauer A, Meinhardt G, Han G; RESORCE Investigators. Regorafenib for patients with hepatocellular carcinoma who progressed on sorafenib treatment (RESORCE): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet* 2017; **389**: 56-66 [PMID: 27932229 DOI: 10.1016/S0140-6736(16)32453-9]
23 **Teufel M**, Seidel H, Köchert K, Meinhardt G, Finn RS, Llovet JM, Bruix J. Biomarkers Associated With Response to Regorafenib in Patients With Hepatocellular Carcinoma. *Gastroenterology* 2019; **156**: 1731-1741 [PMID: 30738047 DOI: 10.1053/j.gastro.2019.01.261]
24 **Kudo M**, Finn RS, Qin S, Han KH, Ikeda K, Piscaglia F, Baron A, Park JW, Han G, Jassem J, Blanc JF, Vogel A, Komov D, Evans TRJ, Lopez C, Dutcus C, Guo M, Saito K, Kraljevic S, Tamai T, Ren M, Cheng AL. Lenvatinib versus sorafenib in first-line treatment of patients with unresectable hepatocellular carcinoma: a randomised phase 3 non-inferiority trial. *Lancet* 2018; **391**: 1163-1173 [PMID: 29433850 DOI: 10.1016/S0140-6736(18)30207-1]
25 **Abou-Alfa GK**, Meyer T, Cheng AL, El-Khoueiry AB, Rimassa L, Ryoo BY, Cicin I, Merle P, Chen Y, Park JW, Blanc JF, Bolondi L, Klümpen HJ, Chan SL, Zagonel V, Pressiani T, Ryu MH, Venook AP, Hessel C, Borgman-Hagey AE, Schwab G, Kelley RK. Cabozantinib in Patients with Advanced and Progressing Hepatocellular Carcinoma. *N Engl J Med* 2018; **379**: 54-63 [PMID: 29972759 DOI: 10.1056/NEJMoa1717002]
26 **El-Khoueiry AB**. Sangro B, Yau T, Crocenzi TS, Kudo M, Hsu C, Kim TY, Choo SP, Trojan J, Welling TH Rd, Meyer T, Kang YK, Yeo W, Chopra A, Anderson J, Dela Cruz C, Lang L, Neely J, Tang H, Dastani HB, Melero I. Nivolumab in patients with advanced hepatocellular carcinoma (CheckMate 040): an open-label, non-comparative, phase 1/2 dose escalation and expansion trial. *Lancet* 2017; **389**: 2492-2502 [PMID: 28434648 DOI: 10.1016/S0140-6736(17)31046-2]
27 **Zhu AX**, Finn RS, Edeline J, Cattan S, Ogasawara S, Palmer D, Verslype C, Zagonel V, Fartoux L, Vogel A, Sarker D, Verset G, Chan SL, Knox J, Daniele B, Webber AL, Ebbinghaus SW, Ma J, Siegel AB, Cheng AL, Kudo M; KEYNOTE-224 investigators. Pembrolizumab in patients with advanced hepatocellular carcinoma previously treated with sorafenib (KEYNOTE-224): a non-randomised, open-label phase 2 trial. *Lancet Oncol* 2018; **19**: 940-952 [PMID: 29875066 DOI: 10.1016/S1470-2045(18)30351-6]
28 **Borbath I**, Santoro A, Laethem JV, Daniele B, Cicalese L, Zeuzem S, Buggish P, Bolondi L, Strosberg JR, Abbadessa G. ARQ 197-215: A randomized, placebo-controlled phase II clinical trial evaluating the c-Met inhibitor, ARQ 197, in patients (pts) with hepatocellular carcinoma (HCC). *J Clin Oncol* 2010; **28**: TPS215-TPS215 [DOI: 10.1200/jco.2010.28.15_suppl.tps215]
29 **Keating GM**. Sorafenib: A Review in Hepatocellular Carcinoma. *Target Oncol* 2017; **12**: 243-253 [PMID: 28299600 DOI: 10.1007/s11523-017-0484-7]
30 **Gong L**, Giacomini MM, Giacomini C, Maitland ML, Altman RB, Klein TE. PharmGKB summary: sorafenib pathways. *Pharmacogenet Genomics* 2017; **27**: 240-246 [PMID: 28362716 DOI: 10.1097/FPC.0000000000000279]
31 **Vasilyeva A**, Durmus S, Li L, Wagenaar E, Hu S, Gibson AA, Panetta JC, Mani S, Sparreboom A, Baker SD, Schinkel AH. Hepatocellular Shuttling and Recirculation of Sorafenib-Glucuronide Is Dependent on Abcc2, Abcc3, and Oatp1a/1b. *Cancer Res* 2015; **75**: 2729-2736 [PMID: 25952649 DOI: 10.1158/0008-5472.CAN-15-0280]
32 **Guo XG**, Wang ZH, Dong W, He XD, Liu FC, Liu H. Specific CYP450 Genotypes in the Chinese Population Affect Sorafenib Toxicity in HBV/HCV-associated Hepatocellular Carcinoma Patients. *Biomed Environ Sci* 2018; **31**: 586-595 [PMID: 30231963 DOI: 10.3967/bes2018.080]
33 **Aouam K**, Kolsi A, Kerkeni E, Ben Fredj N, Chaabane A, Monastiri K, Boughattas N. Influence of combined CYP3A4 and CYP3A5 single-nucleotide polymorphisms on tacrolimus exposure in kidney transplant recipients: a study according to the post-transplant phase. *Pharmacogenomics* 2015; **16**: 2045-2054 [PMID: 26615671 DOI: 10.2217/pgs.15.138]
34 **Boudou-Rouquette P**, Narjoz C, Golmard JL, Thomas-Schoemann A, Mir O, Taieb F, Durand JP, Coriat R, Dauphin A, Vidal M, Tod M, Loriot MA, Goldwasser F, Blanchet B. Early sorafenib-induced toxicity is associated with drug exposure and UGTIA9 genetic polymorphism in patients with solid tumors: a preliminary study. *PLoS One* 2012; **7**: e42875 [PMID: 22912756 DOI: 10.1371/journal.pone.0042875]
35 **Bins S**, Lenting A, El Bouazzaoui S, van Doorn L, Oomen-de Hoop E, Eskens FA, van Schaik RH, Mathijssen RH. Polymorphisms in SLCO1B1 and UGT1A1 are associated with sorafenib-induced toxicity. *Pharmacogenomics* 2016; **17**: 1483-1490 [PMID: 27533851 DOI: 10.2217/pgs-2016-0063]
36 **Lee JH**, Chung YH, Kim JA, Shim JH, Lee D, Lee HC, Shin ES, Yoon JH, Kim BI, Bae SH, Koh KC, Park NH. Genetic predisposition of hand-foot skin reaction after sorafenib therapy in patients with hepatocellular carcinoma. *Cancer* 2013; **119**: 136-142 [PMID: 22736425 DOI: 10.1002/cncr.27705]
37 **Girard H**, Court MH, Bernard O, Fortier LC, Villeneuve L, Hao Q, Greenblatt DJ, von Moltke LL, Perussed L, Guillemette C. Identification of common polymorphisms in the promoter of the UGT1A9 gene: evidence that UGT1A9 protein and activity levels are strongly genetically controlled in the liver. *Pharmacogenetics* 2004; **14**: 501-515 [PMID: 15284532 DOI: 10.1097/01.fpc.0000114754.08559.27]
38 **Stingl JC**, Bartels H, Viviani R, Lehmann ML, Brockmöller J. Relevance of UDP-glucuronosyltransferase polymorphisms for drug dosing: A quantitative systematic review. *Pharmacol Ther* 2014; **141**: 92-116 [PMID: 24076267 DOI: 10.1016/j.pharmthera.2013.09.002]
39 **Touma JA**, McLachlan AJ, Gross AS. The role of ethnicity in personalized dosing of small molecule tyrosine kinase inhibitors used in oncology. *Transl Cancer Res* 2017; **6**: S1558-S1591 [DOI: 10.21037/tcr.2017.09.09]
40 **Meza-Junco J**, Chu QS, Christensen O, Rajagopalan P, Das S, Stefanyschyn R, Sawyer MB. UGT1A1 polymorphism and hyperbilirubinemia in a patient who received sorafenib. *Cancer Chemother Pharmacol*

2009; **65**: 1-4 [PMID: 19672597 DOI: 10.1007/s00280-009-1096-4]
41 **Qosa H**, Avaritt BR, Hartman NR, Volpe DA. In vitro UGT1A1 inhibition by tyrosine kinase inhibitors and association with drug-induced hyperbilirubinemia. *Cancer Chemother Pharmacol* 2018; **82**: 795-802 [PMID: 30105461 DOI: 10.1007/s00280-018-3665-x]
42 **Tandia M**, Mhiri A, Paule B, Saffroy R, Cailliez V, Noé G, Farinotti R, Bonhomme-Faivre L. Correlation between clinical response to sorafenib in hepatocellular carcinoma treatment and polymorphisms of P-glycoprotein (ABCB1) and of breast cancer resistance protein (ABCG2): monocentric study. *Cancer Chemother Pharmacol* 2017; **79**: 759-766 [PMID: 28289864 DOI: 10.1007/s00280-017-3268-y]
43 **Poonkuzhali B**, Lamba J, Strom S, Sparreboom A, Thummel K, Watkins P, Schuetz E. Association of breast cancer resistance protein/ABCG2 phenotypes and novel promoter and intron 1 single nucleotide polymorphisms. *Drug Metab Dispos* 2008; **36**: 780-795 [PMID: 18180275 DOI: 10.1124/dmd.107.018366]
44 **Lee HH**, Ho RH. Interindividual and interethnic variability in drug disposition: polymorphisms in organic anion transporting polypeptide 1B1 (OATP1B1; SLCO1B1). *Br J Clin Pharmacol* 2017; **83**: 1176-1184 [PMID: 27936281 DOI: 10.1111/bcp.13207]
45 **Tsuchiya N**, Narita S, Inoue T, Hasunuma N, Numakura K, Horikawa Y, Satoh S, Notoya T, Fujishima N, Hatakeyama S, Ohyama C, Habuchi T. Risk factors for sorafenib-induced high-grade skin rash in Japanese patients with advanced renal cell carcinoma. *Anticancer Drugs* 2013; **24**: 310-314 [PMID: 23237922 DOI: 10.1097/CAD.0b013e32835c401c]
46 **Peer CJ**, Sissung TM, Kim A, Jain L, Woo S, Gardner ER, Kirkland CT, Troutman SM, English BC, Richardson ED, Federspiel J, Venzon D, Dahut W, Kohn E, Kummar S, Yarchoan R, Giaccone G, Widemann B, Figg WD. Sorafenib is an inhibitor of UGT1A1 but is metabolized by UGT1A9: implications of genetic variants on pharmacokinetics and hyperbilirubinemia. *Clin Cancer Res* 2012; **18**: 2099-2107 [PMID: 22307138 DOI: 10.1158/1078-0432.CCR-11-2484]
47 **Herraez E**, Lozano E, Macias RI, Vaquero J, Bujanda L, Banales JM, Marin JJ, Briz O. Expression of SLC22A1 variants may affect the response of hepatocellular carcinoma and cholangiocarcinoma to sorafenib. *Hepatology* 2013; **58**: 1065-1073 [PMID: 23532667 DOI: 10.1002/hep.26425]
48 **Scartozzi M**, Faloppi L, Svegliati Baroni G, Loretelli C, Piscaglia F, Iavarone M, Toniutto P, Fava G, De Minicis S, Mandolesi A, Bianconi M, Giampieri R, Granito A, Facchetti F, Bitetto D, Marinelli S, Venerandi L, Vavassori S, Gemini S, D'Errico A, Colombo M, Bolondi L, Bearzi I, Benedetti A, Cascinu S. VEGF and VEGFR genotyping in the prediction of clinical outcome for HCC patients receiving sorafenib: the ALICE-1 study. *Int J Cancer* 2014; **135**: 1247-1256 [PMID: 24510746 DOI: 10.1002/ijc.28772]
49 **Faloppi L**, Gardini AC, Masi G, Silvestris N, Loretelli C, Ulivi P, Vivaldi C, Bianconi M, Giampieri R, Bittoni A, Andrikou K, Prete MD, Scartozzi M, Cascinu S. Angiogenesis polymorphisms profile in the prediction of clinical outcome of advanced HCC patients receiving sorafenib: Combined analysis of VEGF and HIF-1α—Final results of the ALICE-2 study. *Journal of Clinical Oncology* 2016; **34**: 280-280 [DOI: 10.1200/jco.2016.34.4_suppl.280]
50 **Zheng YB**, Zhan MX, Zhao W, Liu B, Huang JW, He X, Fu SR, Zhao Y, Li Y, Hu BS, Lu LG. The relationship of kinase insert domain receptor gene polymorphisms and clinical outcome in advanced hepatocellular carcinoma patients treated with sorafenib. *Med Oncol* 2014; **31**: 209 [PMID: 25182707 DOI: 10.1007/s12032-014-0209-z]
51 **Casadei Gardini A**, Marisi G, Faloppi L, Scarpi E, Foschi FG, Iavarone M, Lauletta G, Corbelli J, Valgiusti M, Facchetti F, Della Corte C, Neri LM, Tamberi S, Cascinu S, Scartozzi M, Amadori D, Nanni O, Tenti E, Ulivi P, Frassineti GL. eNOS polymorphisms and clinical outcome in advanced HCC patients receiving sorafenib: final results of the ePHAS study. *Oncotarget* 2016; **7**: 27988-27999 [PMID: 27058899 DOI: 10.18632/oncotarget.8569]
52 **Marisi G**, Scartozzi M, Faloppi L, Foschi GF, Lauletta G, Valgiusti M, Aprile G, Fornaro L, Iavarone M, Ercolani G, Frassineti GL, Amadori D, Gardini AC. Ang-2 polymorphisms in relation to outcome in advanced HCC patients receiving sorafenib. *J Clin Oncol* 2017; **35**: e15666-e15666 [DOI: 10.1200/JCO.2017.35.15_suppl.e15666]
53 **Llovet JM**, Peña CE, Lathia CD, Shan M, Meinhardt G, Bruix J; SHARP Investigators Study Group. Plasma biomarkers as predictors of outcome in patients with advanced hepatocellular carcinoma. *Clin Cancer Res* 2012; **18**: 2290-2300 [PMID: 22374331 DOI: 10.1158/1078-0432.CCR-11-2175]
54 **Thillai K**, Srikandarajah K, Ross P. Regorafenib as treatment for patients with advanced hepatocellular cancer. *Future Oncol* 2017; **13**: 2223-2232 [PMID: 28766967 DOI: 10.2217/fon-2017-0204]
55 STIVARGA® EU label.
56 STIVARGA® FDA label.
57 **Sacré A**, Lanthier N, Dano H, Aydin S, Leggenhager D, Weber A, Dekairelle AF, De Cuyper A, Gala JL, Humblet Y, Sempoux C, Van den Eynde M. Regorafenib induced severe toxic hepatitis: characterization and discussion. *Liver Int* 2016; **36**: 1590-1594 [PMID: 27500989 DOI: 10.1111/liv.13217]
58 **Ohya H**, Shibayama Y, Ogura J, Narumi K, Kobayashi M, Iseki K. Regorafenib is transported by the organic anion transporter 1B1 and the multidrug resistance protein 2. *Biol Pharm Bull* 2015; **38**: 582-586 [PMID: 25739790 DOI: 10.1248/bpb.b14-00740]
59 **Yamanaka H**, Nakajima M, Katoh M, Hara Y, Tachibana O, Yamashita J, McLeod HL, Yokoi T. A novel polymorphism in the promoter region of human UGT1A9 gene (UGT1A9*22) and its effects on the transcriptional activity. *Pharmacogenetics* 2004; **14**: 329-332 [PMID: 15115919 DOI: 10.14896/jssxmeeting.19.0.190.0]
60 **Maeda A**, Ando H, Ura T, Komori A, Hasegawa A, Taniguchi H, Kadowaki S, Muro K, Tajika M, Kobara M, Matsuzaki M, Hashimoto N, Maeda M, Kojima Y, Aoki M, Kondo E, Mizutani A, Fujimura A. Association between ABCG2 and SLCO1B1 polymorphisms and adverse drug reactions to regorafenib: a preliminary study. *Int J Clin Pharmacol Ther* 2017; **55**: 409-415 [PMID: 28157071 DOI: 10.5414/CP202788]
61 **Maeda A**, Irie K, Ando H, Hasegawa A, Taniguchi H, Kadowaki S, Muro K, Tajika M, Aoki M, Inaguma K, Kajita M, Fujimura A, Fukushima S. Associations among regorafenib concentrations, severe adverse reactions, and ABCG2 and OATP1B1 polymorphisms. *Cancer Chemother Pharmacol* 2019; **83**: 107-113 [PMID: 30368586 DOI: 10.1007/s00280-018-3710-9]
62 **Suenaga M**, Schirripa M, Cao S, Zhang W, Yang D, Ning Y, Cremolini C, Antoniotti C, Borelli B, Mashima T, Okazaki S, Berger MD, Miyamoto Y, Gopez R, Barzi A, Lonardi S, Yamaguchi T, Falcone A, Loupakis F, Lenz HJ. Gene Polymorphisms in the CCL5/CCR5 Pathway as a Genetic Biomarker for Outcome and Hand-Foot Skin Reaction in Metastatic Colorectal Cancer Patients Treated With

Regorafenib. *Clin Colorectal Cancer* 2018; **17**: e395-e414 [PMID: 29606345 DOI: 10.1016/j.clcc.2018.02.010]
63 **Giampieri R**, Salvatore L, Del Prete M, Prochilo T, D'Anzeo M, Loretelli C, Loupakis F, Aprile G, Maccaroni E, Andrikou K, Bianconi M, Bittoni A, Faloppi L, Demurtas L, Montironi R, Scarpelli M, Falcone A, Zaniboni A, Scartozzi M, Cascinu S. Angiogenesis genotyping and clinical outcome during regorafenib treatment in metastatic colorectal cancer patients. *Sci Rep* 2016; **6**: 25195 [PMID: 27117754 DOI: 10.1038/srep25195]
64 **Cecchin E**, De Mattia E, Ecca F, Toffoli G. Host genetic profiling to increase drug safety in colorectal cancer from discovery to implementation. *Drug Resist Updat* 2018; **39**: 18-40 [PMID: 30075835 DOI: 10.1016/j.drup.2018.07.001]
65 **Grüllich C**. Cabozantinib: Multi-kinase Inhibitor of MET, AXL, RET, and VEGFR2. *Recent Results Cancer Res* 2018; **211**: 67-75 [PMID: 30069760 DOI: 10.1007/978-3-319-91442-8_5]
66 **Lacy SA**, Miles DR, Nguyen LT. Clinical Pharmacokinetics and Pharmacodynamics of Cabozantinib. *Clin Pharmacokinet* 2017; **56**: 477-491 [PMID: 27734291 DOI: 10.1007/s40262-016-0461-9]
67 **Xiang Q**, Chen W, Ren M, Wang J, Zhang H, Deng DY, Zhang L, Shang C, Chen Y. Cabozantinib suppresses tumor growth and metastasis in hepatocellular carcinoma by a dual blockade of VEGFR2 and MET. *Clin Cancer Res* 2014; **20**: 2959-2970 [PMID: 24700742 DOI: 10.1158/1078-0432.CCR-13-2620]
68 **Grüllich C**. Cabozantinib: a MET, RET, and VEGFR2 tyrosine kinase inhibitor. *Recent Results Cancer Res* 2014; **201**: 207-214 [PMID: 24756794 DOI: 10.1007/978-3-642-54490-3_12]
69 **Yakes FM**, Chen J, Tan J, Yamaguchi K, Shi Y, Yu P, Qian F, Chu F, Bentzien F, Cancilla B, Orf J, You A, Laird AD, Engst S, Lee L, Lesch J, Chou YC, Joly AH. Cabozantinib (XL184), a novel MET and VEGFR2 inhibitor, simultaneously suppresses metastasis, angiogenesis, and tumor growth. *Mol Cancer Ther* 2011; **10**: 2298-2308 [PMID: 21926191 DOI: 10.1158/1535-7163.MCT-11-0264]
70 **Lacy S**, Hsu B, Miles D, Aftab D, Wang R, Nguyen L. Metabolism and Disposition of Cabozantinib in Healthy Male Volunteers and Pharmacologic Characterization of Its Major Metabolites. *Drug Metab Dispos* 2015; **43**: 1190-1207 [PMID: 26015560 DOI: 10.1124/dmd.115.063610]
71 **Viola D**, Cappagli V, Elisei R. Cabozantinib (XL184) for the treatment of locally advanced or metastatic progressive medullary thyroid cancer. *Future Oncol* 2013; **9**: 1083-1092 [PMID: 23902240 DOI: 10.2217/fon.13.128]
72 **Fallahi P**, Ferrari SM, Di Bari F, Materazzi G, Benvenga S, Miccoli P, Antonelli A. Cabozantinib in Thyroid Cancer. *Recent Pat Anticancer Drug Discov* 2015; **10**: 259-269 [PMID: 26152149 DOI: 10.2174/1574892810666150708110816]
73 **Singh H**, Brave M, Beaver JA, Cheng J, Tang S, Zahalka E, Palmby TR, Venugopal R, Song P, Liu Q, Liu C, Yu J, Chen XH, Wang X, Wang Y, Kluetz PG, Daniels SR, Papadopoulos EJ, Sridhara R, McKee AE, Ibrahim A, Kim G, Pazdur R. U.S. Food and Drug Administration Approval: Cabozantinib for the Treatment of Advanced Renal Cell Carcinoma. *Clin Cancer Res* 2017; **23**: 330-335 [PMID: 27793960 DOI: 10.1158/1078-0432.CCR-16-1073]
74 **Schöffski P**, Gordon M, Smith DC, Kurzrock R, Daud A, Vogelzang NJ, Lee Y, Scheffold C, Shapiro GI. Phase II randomised discontinuation trial of cabozantinib in patients with advanced solid tumours. *Eur J Cancer* 2017; **86**: 296-304 [PMID: 29059635 DOI: 10.1016/j.ejca.2017.09.011]
75 **Kim DY**. [New Systemic Therapies for Advanced Hepatocellular Carcinoma]. *Korean J Gastroenterol* 2019; **73**: 10-15 [PMID: 30690953 DOI: 10.4166/kjg.2019.73.1.10]
76 **de Jesus VHF**, Dettino ALA. Update on hepatocellular carcinoma from the 2018 Gastrointestinal Cancer Symposium (ASCO GI). *J Hepatocell Carcinoma* 2018; **5**: 87-90 [PMID: 30324097 DOI: 10.2147/JHC.S171396]
77 **Kelley RK**, Verslype C, Cohn AL, Yang TS, Su WC, Burris H, Braiteh F, Vogelzang N, Spira A, Foster P, Lee Y, Van Cutsem E. Cabozantinib in hepatocellular carcinoma: results of a phase 2 placebo-controlled randomized discontinuation study. *Ann Oncol* 2017; **28**: 528-534 [PMID: 28426123 DOI: 10.1093/annonc/mdw651]
78 **Bakouny Z**, Assi T, El Rassy E, Nasr F. Second-line Treatments of Advanced Hepatocellular Carcinoma: Systematic Review and Network Meta-Analysis of Randomized Controlled Trials. *J Clin Gastroenterol* 2019; **53**: 251-261 [PMID: 30575632 DOI: 10.1097/MCG.0000000000001160]
79 **Tohyama O**, Matsui J, Kodama K, Hata-Sugi N, Kimura T, Okamoto K, Minoshima Y, Iwata M, Funahashi Y. Antitumor activity of lenvatinib (e7080): an angiogenesis inhibitor that targets multiple receptor tyrosine kinases in preclinical human thyroid cancer models. *J Thyroid Res* 2014; **2014**: 638747 [PMID: 25295214 DOI: 10.1155/2014/638747]
80 **Yamamoto Y**, Matsui J, Matsushima T, Obaishi H, Miyazaki K, Nakamura K, Tohyama O, Semba T, Yamaguchi A, Hoshi SS, Mimura F, Haneda T, Fukuda Y, Kamata JI, Takahashi K, Matsukura M, Wakabayashi T, Asada M, Nomoto KI, Watanabe T, Dezso Z, Yoshimatsu K, Funahashi Y, Tsuruoka A. Lenvatinib, an angiogenesis inhibitor targeting VEGFR/FGFR, shows broad antitumor activity in human tumor xenograft models associated with microvessel density and pericyte coverage. *Vasc Cell* 2014; **6**: 18 [PMID: 25197551 DOI: 10.1186/2045-824X-6-18]
81 **Ikeda M**, Okusaka T, Mitsunaga S, Ueno H, Tamai T, Suzuki T, Hayato S, Kadowaki T, Okita K, Kumada H. Safety and Pharmacokinetics of Lenvatinib in Patients with Advanced Hepatocellular Carcinoma. *Clin Cancer Res* 2016; **22**: 1385-1394 [PMID: 26500236 DOI: 10.1158/1078-0432.CCR-15-1354]
82 **Tamai T**, Hayato S, Hojo S, Suzuki T, Okusaka T, Ikeda K, Kumada H. Dose Finding of Lenvatinib in Subjects With Advanced Hepatocellular Carcinoma Based on Population Pharmacokinetic and Exposure-Response Analyses. *J Clin Pharmacol* 2017; **57**: 1138-1147 [PMID: 28561918 DOI: 10.1002/jcph.917]
83 **Kudo M**. Systemic Therapy for Hepatocellular Carcinoma: Latest Advances. *Cancers (Basel)* 2018; **10** [PMID: 30380773 DOI: 10.3390/cancers10110412]
84 **Hussein Z**, Mizuo H, Hayato S, Namiki M, Shumaker R. Clinical Pharmacokinetic and Pharmacodynamic Profile of Lenvatinib, an Orally Active, Small-Molecule, Multitargeted Tyrosine Kinase Inhibitor. *Eur J Drug Metab Pharmacokinet* 2017; **42**: 903-914 [PMID: 28236116 DOI: 10.1007/s13318-017-0403-4]
85 **Inoue K**, Asai N, Mizuo H, Fukuda K, Kusano K, Yoshimura T. Unique metabolic pathway of [(14)C]lenvatinib after oral administration to male cynomolgus monkey. *Drug Metab Dispos* 2012; **40**: 662-670 [PMID: 22207053 DOI: 10.1124/dmd.111.043281]
86 **Gupta A**, Jarzab B, Capdevila J, Shumaker R, Hussein Z. Population pharmacokinetic analysis of lenvatinib in healthy subjects and patients with cancer. *Br J Clin Pharmacol* 2016; **81**: 1124-1133 [PMID: 26879594 DOI: 10.1111/bcp.12907]
87 **Leonetti A**, Leonardi F, Bersanelli M, Buti S. Clinical use of lenvatinib in combination with everolimus

for the treatment of advanced renal cell carcinoma. *Ther Clin Risk Manag* 2017; **13**: 799-806 [PMID: 28721060 DOI: 10.2147/TCRM.S126910]
88 **Hewett Y**, Ghimire S, Farooqi B, Shah BK. Lenvatinib - A multikinase inhibitor for radioiodine-refractory differentiated thyroid cancer. *J Oncol Pharm Pract* 2018; **24**: 28-32 [PMID: 27856921 DOI: 10.1177/1078155216680119]
89 **Roviello G**, Corona SP, Bozza G, Aieta M, Generali D, Rodriquenz MG, Mileo AM, Imperatori M, Ianza A, Conca R, Sobhani N. Lenvatinib for the treatment of renal cell carcinoma. *Expert Opin Investig Drugs* 2018; **27**: 507-512 [PMID: 29718721 DOI: 10.1080/13543784.2018.1472235]
90 **De Lisi D**, De Giorgi U, Lolli C, Schepisi G, Conteduca V, Menna C, Tonini G, Santini D, Farolfi A. Lenvatinib in the management of metastatic renal cell carcinoma: a promising combination therapy? *Expert Opin Drug Metab Toxicol* 2018; **14**: 461-467 [PMID: 29557694 DOI: 10.1080/17425255.2018.1455826]
91 **Baxter MA**, Glen H, Evans TR. Lenvatinib and its use in the treatment of unresectable hepatocellular carcinoma. *Future Oncol* 2018; **14**: 2021-2029 [PMID: 29783903 DOI: 10.2217/fon-2017-0689]
92 **Spallanzani A**, Orsi G, Andrikou K, Gelsomino F, Rimini M, Riggi L, Cascinu S. Lenvatinib as a therapy for unresectable hepatocellular carcinoma. *Expert Rev Anticancer Ther* 2018; **18**: 1069-1076 [PMID: 30220234 DOI: 10.1080/14737140.2018.1524297]
93 **Ozeki T**, Nagahama M, Fujita K, Suzuki A, Sugino K, Ito K, Miura M. Influence of CYP3A4/5 and ABC transporter polymorphisms on lenvatinib plasma trough concentrations in Japanese patients with thyroid cancer. *Sci Rep* 2019; **9**: 5404 [PMID: 30931962 DOI: 10.1038/s41598-019-41820-y]
94 **Takeda H**, Nishikawa H, Osaki Y. The new era of precision medicine in hepatocellular carcinoma: the urgent need for promising biomarkers. *Hepatobiliary Surg Nutr* 2018; **7**: 490-491 [PMID: 30652097 DOI: 10.21037/hbsn.2018.08.06]
95 **Woo HY**, Yoo SY, Heo J. New chemical treatment options in second-line hepatocellular carcinoma: what to do when sorafenib fails? *Expert Opin Pharmacother* 2017; **18**: 35-44 [PMID: 27849399 DOI: 10.1080/14656566.2016.1261825]
96 **Waidmann O**, Trojan J. Novel drugs in clinical development for hepatocellular carcinoma. *Expert Opin Investig Drugs* 2015; **24**: 1075-1082 [PMID: 26108356 DOI: 10.1517/13543784.2015.1058776]
97 **Ikeda M**, Morizane C, Ueno M, Okusaka T, Ishii H, Furuse J. Chemotherapy for hepatocellular carcinoma: current status and future perspectives. *Jpn J Clin Oncol* 2018; **48**: 103-114 [PMID: 29253194 DOI: 10.1093/jjco/hyx180]
98 **Siu EH**, Chan AW, Chong CC, Chan SL, Lo KW, Cheung ST. Treatment of advanced hepatocellular carcinoma: immunotherapy from checkpoint blockade to potential of cellular treatment. *Transl Gastroenterol Hepatol* 2018; **3**: 89 [PMID: 30603725 DOI: 10.21037/tgh.2018.10.16]
99 **Shi F**, Shi M, Zeng Z, Qi RZ, Liu ZW, Zhang JY, Yang YP, Tien P, Wang FS. PD-1 and PD-L1 upregulation promotes CD8(+) T-cell apoptosis and postoperative recurrence in hepatocellular carcinoma patients. *Int J Cancer* 2011; **128**: 887-896 [PMID: 20473887 DOI: 10.1002/ijc.25397]
100 **Prieto J**, Melero I, Sangro B. Immunological landscape and immunotherapy of hepatocellular carcinoma. *Nat Rev Gastroenterol Hepatol* 2015; **12**: 681-700 [PMID: 26484443 DOI: 10.1038/nrgastro.2015.173]
101 **Brizzi MP**, Pignataro D, Tampellini M, Scagliotti GV, Di Maio M. Systemic treatment of hepatocellular carcinoma: why so many failures in the development of new drugs? *Expert Rev Anticancer Ther* 2016; **16**: 1053-1062 [PMID: 27548441 DOI: 10.1080/14737140.2016.1227706]
102 **Brahmer JR**, Hammers H, Lipson EJ. Nivolumab: targeting PD-1 to bolster antitumor immunity. *Future Oncol* 2015; **11**: 1307-1326 [PMID: 25798726 DOI: 10.2217/fon.15.52]
103 **Medina PJ**, Adams VR. PD-1 Pathway Inhibitors: Immuno-Oncology Agents for Restoring Antitumor Immune Responses. *Pharmacotherapy* 2016; **36**: 317-334 [PMID: 26822752 DOI: 10.1002/phar.1714]
104 **Topalian SL**, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, Powderly JD, Carvajal RD, Sosman JA, Atkins MB, Leming PD, Spigel DR, Antonia SJ, Horn L, Drake CG, Pardoll DM, Chen L, Sharfman WH, Anders RA, Taube JM, McMiller TL, Xu H, Korman AJ, Jure-Kunkel M, Agrawal S, McDonald D, Kollia GD, Gupta A, Wigginton JM, Sznol M. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N Engl J Med* 2012; **366**: 2443-2454 [PMID: 22658127 DOI: 10.1056/NEJMoa1200690]
105 **Wong AC**, Ma B. An update on the pharmacodynamics, pharmacokinetics, safety and clinical efficacy of nivolumab in the treatment of solid cancers. *Expert Opin Drug Metab Toxicol* 2016; **12**: 1255-1261 [PMID: 27548326 DOI: 10.1080/17425255.2016.1223047]
106 **Nomizo T**, Ozasa H, Tsuji T, Funazo T, Yasuda Y, Yoshida H, Yagi Y, Sakamori Y, Nagai H, Hirai T, Kim YH. Clinical Impact of Single Nucleotide Polymorphism in PD-L1 on Response to Nivolumab for Advanced Non-Small-Cell Lung Cancer Patients. *Sci Rep* 2017; **7**: 45124 [PMID: 28332580 DOI: 10.1038/srep45124]
107 **Yeo MK**, Choi SY, Seong IO, Suh KS, Kim JM, Kim KH. Association of PD-L1 expression and PD-L1 gene polymorphism with poor prognosis in lung adenocarcinoma and squamous cell carcinoma. *Hum Pathol* 2017; **68**: 103-111 [PMID: 28851662 DOI: 10.1016/j.humpath.2017.08.016]
108 **Zou J**, Wu D, Li T, Wang X, Liu Y, Tan S. Association of PD-L1 gene rs4143815 C>G polymorphism and human cancer susceptibility: A systematic review and meta-analysis. *Pathol Res Pract* 2019; **215**: 229-234 [PMID: 30552042 DOI: 10.1016/j.prp.2018.12.002]
109 **Nomizo T**, Ozasa H, Tsuji T, Funazo T, Yasuda Y, Yoshida H, Sakamori Y, Hirai T, Kim YH. PD-L1 rs2282055 is associated with opposite treatment effect between platinum-based chemotherapy and nivolumab treatment [abstract]. In: Proceedings of the American Association for Cancer Research Annual Meeting 2018; 2018 Apr 14-18; Chicago, IL. Philadelphia (PA): AACR;. *Cancer Res* 2018; **78**: 2604-2604 [DOI: 10.1158/1538-7445.AM2018-2604]
110 **Salmaninejad A**, Khoramshahi V, Azani A, Soltaninejad E, Aslani S, Zamani MR, Zal M, Nesaei A, Hosseini SM. PD-1 and cancer: molecular mechanisms and polymorphisms. *Immunogenetics* 2018; **70**: 73-86 [PMID: 28642997 DOI: 10.1007/s00251-017-1015-5]
111 **Bins S**, Basak EA, El Bouazzaoui S, Koolen SLW, Oomen-de Hoop E, van der Leest CH, van der Veldt AAM, Sleijfer S, Debets R, van Schaik RHN, Aerts JGJV, Mathijssen RHJ. Association between single-nucleotide polymorphisms and adverse events in nivolumab-treated non-small cell lung cancer patients. *Br J Cancer* 2018; **118**: 1296-1301 [PMID: 29695768 DOI: 10.1038/s41416-018-0074-1]
112 **Longoria TC**, Tewari KS. Evaluation of the pharmacokinetics and metabolism of pembrolizumab in the treatment of melanoma. *Expert Opin Drug Metab Toxicol* 2016; **12**: 1247-1253 [PMID: 27485741 DOI: 10.1080/17425255.2016.1216976]
113 **Truong P**, Rahal A, Kallail KJ. Metastatic Hepatocellular Carcinoma Responsive to Pembrolizumab.

Cureus 2016; **8**: e631 [PMID: 27433410 DOI: 10.7759/cureus.631]
114 **Mody K**, Abou-Alfa GK. Systemic Therapy for Advanced Hepatocellular Carcinoma in an Evolving Landscape. *Curr Treat Options Oncol* 2019; **20**: 3 [PMID: 30635732 DOI: 10.1007/s11864-019-0601-1]
115 **Hugo W**, Zaretsky JM, Sun L, Song C, Moreno BH, Hu-Lieskovan S, Berent-Maoz B, Pang J, Chmielowski B, Cherry G, Seja E, Lomeli S, Kong X, Kelley MC, Sosman JA, Johnson DB, Ribas A, Lo RS. Genomic and Transcriptomic Features of Response to Anti-PD-1 Therapy in Metastatic Melanoma. *Cell* 2016; **165**: 35-44 [PMID: 26997480 DOI: 10.1016/j.cell.2016.02.065]
116 **Roy R**, Chun J, Powell SN. BRCA1 and BRCA2: different roles in a common pathway of genome protection. *Nat Rev Cancer* 2011; **12**: 68-78 [PMID: 22193408 DOI: 10.1038/nrc3181]
117 **Al-Samkari H**, Snyder GD, Nikiforow S, Tolaney SM, Freedman RA, Losman JA. Haemophagocytic lymphohistiocytosis complicating pembrolizumab treatment for metastatic breast cancer in a patient with the PRF1A91V gene polymorphism. *J Med Genet* 2019; **56**: 39-42 [PMID: 30287596 DOI: 10.1136/jmedgenet-2018-105485]
118 **Rocca A**, Farolfi A, Bravaccini S, Schirone A, Amadori D. Palbociclib (PD 0332991): targeting the cell cycle machinery in breast cancer. *Expert Opin Pharmacother* 2014; **15**: 407-420 [PMID: 24369047 DOI: 10.1517/14656566.2014.870555]
119 **Clark AS**, Karasic TB, DeMichele A, Vaughn DJ, O'Hara M, Perini R, Zhang P, Lal P, Feldman M, Gallagher M, O'Dwyer PJ. Palbociclib (PD0332991)-a Selective and Potent Cyclin-Dependent Kinase Inhibitor: A Review of Pharmacodynamics and Clinical Development. *JAMA Oncol* 2016; **2**: 253-260 [PMID: 26633733 DOI: 10.1001/jamaoncol.2015.4701]
120 **Johnson J**, Thijssen B, McDermott U, Garnett M, Wessels LF, Bernards R. Targeting the RB-E2F pathway in breast cancer. *Oncogene* 2016; **35**: 4829-4835 [PMID: 26923330 DOI: 10.1038/onc.2016.32]
121 **Spring L**, Bardia A, Modi S. Targeting the cyclin D-cyclin-dependent kinase (CDK) 4/6-retinoblastoma pathway with selective CDK 4/6 inhibitors in hormone receptor-positive breast cancer: rationale, current status, and future directions. *Discov Med* 2016; **21**: 65-74 [PMID: 26896604]
122 **Hsieh FS**, Chen YL, Hung MH, Chu PY, Tsai MH, Chen LJ, Hsiao YJ, Shih CT, Chang MJ, Chao TI, Shiau CW, Chen KF. Palbociclib induces activation of AMPK and inhibits hepatocellular carcinoma in a CDK4/6-independent manner. *Mol Oncol* 2017; **11**: 1035-1049 [PMID: 28453226 DOI: 10.1002/1878-0261.12072]
123 **Dhillon S**. Palbociclib: first global approval. *Drugs* 2015; **75**: 543-551 [PMID: 25792301 DOI: 10.1007/s40265-015-0379-9]
124 **Thill M**, Schmidt M. Management of adverse events during cyclin-dependent kinase 4/6 (CDK4/6) inhibitor-based treatment in breast cancer. *Ther Adv Med Oncol* 2018; **10**: 1758835918793326 [PMID: 30202447 DOI: 10.1177/1758835918793326]
125 **Beaver JA**, Amiri-Kordestani L, Charlab R, Chen W, Palmby T, Tilley A, Zirkelbach JF, Yu J, Liu Q, Zhao L, Crich J, Chen XH, Hughes M, Bloomquist E, Tang S, Sridhara R, Kluetz PG, Kim G, Ibrahim A, Pazdur R, Cortazar P. FDA Approval: Palbociclib for the Treatment of Postmenopausal Patients with Estrogen Receptor-Positive, HER2-Negative Metastatic Breast Cancer. *Clin Cancer Res* 2015; **21**: 4760-4766 [PMID: 26324739 DOI: 10.1158/1078-0432.CCR-15-1185]
126 **Schmidt M**, Sebastian M. Palbociclib-The First of a New Class of Cell Cycle Inhibitors. *Recent Results Cancer Res* 2018; **211**: 153-175 [PMID: 30069766 DOI: 10.1007/978-3-319-91442-8_11]
127 **Kim ES**, Scott LJ. Palbociclib: A Review in HR-Positive, HER2-Negative, Advanced or Metastatic Breast Cancer. *Target Oncol* 2017; **12**: 373-383 [PMID: 28488183 DOI: 10.1007/s11523-017-0492-7]
128 **Iwata H**, Im SA, Masuda N, Im YH, Inoue K, Rai Y, Nakamura R, Kim JH, Hoffman JT, Zhang K, Giorgetti C, Iyer S, Schnell PT, Bartlett CH, Ro J. PALOMA-3: Phase III Trial of Fulvestrant With or Without Palbociclib in Premenopausal and Postmenopausal Women With Hormone Receptor-Positive, Human Epidermal Growth Factor Receptor 2-Negative Metastatic Breast Cancer That Progressed on Prior Endocrine Therapy-Safety and Efficacy in Asian Patients. *J Glob Oncol* 2017; **3**: 289-303 [PMID: 28831437 DOI: 10.1200/JGO.2016.008318]
129 **McShane TM**, Wolfe TA, Ryan JC. Updates on managing advanced breast cancer with palbociclib combination therapy. *Ther Adv Med Oncol* 2018; **10**: 1758835918793849 [PMID: 30202448 DOI: 10.1177/1758835918793849]
130 **Shah A**, Bloomquist E, Tang S, Fu W, Bi Y, Liu Q, Yu J, Zhao P, Palmby TR, Goldberg KB, Chang CJG, Patel P, Alebachew E, Tilley A, Pierce WF, Ibrahim A, Blumenthal GM, Sridhara R, Beaver JA, Pazdur R. FDA Approval: Ribociclib for the Treatment of Postmenopausal Women with Hormone Receptor-Positive, HER2-Negative Advanced or Metastatic Breast Cancer. *Clin Cancer Res* 2018; **24**: 2999-3004 [PMID: 29437768 DOI: 10.1158/1078-0432.CCR-17-2369]
131 **Rascon K**, Flajc G, De Angelis C, Liu X, Trivedi MV, Ekinci E. Ribociclib in HR+/HER2- Advanced or Metastatic Breast Cancer Patients. *Ann Pharmacother* 2019; **53**: 501-509 [PMID: 30522347 DOI: 10.1177/1060028018817904]
132 **Meattini I**, Desideri I, Scotti V, Simontacchi G, Livi L. Ribociclib plus letrozole and concomitant palliative radiotherapy for metastatic breast cancer. *Breast* 2018; **42**: 1-2 [PMID: 30118901 DOI: 10.1016/j.breast.2018.08.096]
133 **Burris HA**. Ribociclib for the treatment of hormone receptor-positive, human epidermal growth factor receptor 2-negative advanced breast cancer. *Expert Rev Anticancer Ther* 2018; **18**: 201-213 [PMID: 29457921 DOI: 10.1080/14737140.2018.1435275]
134 **Sonke GS**, Hart LL, Campone M, Erdkamp F, Janni W, Verma S, Villanueva C, Jakobsen E, Alba E, Wist E, Favret AM, Bachelot T, Hegg R, Wheatley-Price P, Souami F, Sutradhar S, Miller M, Germa C, Burris HA. Ribociclib with letrozole vs letrozole alone in elderly patients with hormone receptor-positive, HER2-negative breast cancer in the randomized MONALEESA-2 trial. *Breast Cancer Res Treat* 2018; **167**: 659-669 [PMID: 29058175 DOI: 10.1007/s10549-017-4523-y]
135 **Munshi N**, Jeay S, Li Y, Chen CR, France DS, Ashwell MA, Hill J, Moussa MM, Leggett DS, Li CJ. ARQ 197, a novel and selective inhibitor of the human c-Met receptor tyrosine kinase with antitumor activity. *Mol Cancer Ther* 2010; **9**: 1544-1553 [PMID: 20484018 DOI: 10.1158/1535-7163.MCT-09-1173]
136 **Rimassa L**, Personeni N, Simonelli M, Santoro A. Tivantinib: a new promising mesenchymal-epithelial transition factor inhibitor in the treatment of hepatocellular carcinoma. *Future Oncol* 2013; **9**: 153-165 [PMID: 23414466 DOI: 10.2217/fon.12.188]
137 **Gherardi E**, Birchmeier W, Birchmeier C, Vande Woude G. Targeting MET in cancer: rationale and progress. *Nat Rev Cancer* 2012; **12**: 89-103 [PMID: 22270953 DOI: 10.1038/nrc3205]
138 **Gao JJ**, Inagaki Y, Xue X, Qu XJ, Tang W. c-Met: A potential therapeutic target for hepatocellular

carcinoma. *Drug Discov Ther* 2011; **5**: 2-11 [PMID: 22466090 DOI: 10.5582/ddt.2011.v5.1.2]
139 **Bottaro DP**, Rubin JS, Faletto DL, Chan AM, Kmiecik TE, Vande Woude GF, Aaronson SA. Identification of the hepatocyte growth factor receptor as the c-met proto-oncogene product. *Science* 1991; **251**: 802-804 [PMID: 1846706 DOI: 10.1126/science.1846706]
140 **Calles A**, Kwiatkowski N, Cammarata BK, Ercan D, Gray NS, Jänne PA. Tivantinib (ARQ 197) efficacy is independent of MET inhibition in non-small-cell lung cancer cell lines. *Mol Oncol* 2015; **9**: 260-269 [PMID: 25226813 DOI: 10.1016/j.molonc.2014.08.011]
141 **Xiang Q**, Zhen Z, Deng DY, Wang J, Chen Y, Li J, Zhang Y, Wang F, Chen N, Chen H, Chen Y. Tivantinib induces G2/M arrest and apoptosis by disrupting tubulin polymerization in hepatocellular carcinoma. *J Exp Clin Cancer Res* 2015; **34**: 118 [PMID: 26458953 DOI: 10.1186/s13046-015-0238-2]
142 **Basilico C**, Pennacchietti S, Vigna E, Chiriaco C, Arena S, Bardelli A, Valdembri D, Serini G, Michieli P. Tivantinib (ARQ197) displays cytotoxic activity that is independent of its ability to bind MET. *Clin Cancer Res* 2013; **19**: 2381-2392 [PMID: 23532890 DOI: 10.1158/1078-0432.CCR-12-3459]
143 **Katayama R**, Aoyama A, Yamori T, Qi J, Oh-hara T, Song Y, Engelman JA, Fujita N. Cytotoxic activity of tivantinib (ARQ 197) is not due solely to c-MET inhibition. *Cancer Res* 2013; **73**: 3087-3096 [PMID: 23598276 DOI: 10.1158/0008-5472.CAN-12-3256]
144 **Lu S**, Török HP, Gallmeier E, Kolligs FT, Rizzani A, Arena S, Göke B, Gerbes AL, De Toni EN. Tivantinib (ARQ 197) affects the apoptotic and proliferative machinery downstream of c-MET: role of Mcl-1, Bcl-xl and Cyclin B1. *Oncotarget* 2015; **6**: 22167-22178 [PMID: 26259250 DOI: 10.18632/oncotarget.4240]
145 **Tolaney SM**, Tan S, Guo H, Barry W, Van Allen E, Wagle N, Brock J, Larrabee K, Paweletz C, Ivanova E, Janne P, Overmoyer B, Wright JJ, Shapiro GI, Winer EP, Krop IE. Phase II study of tivantinib (ARQ 197) in patients with metastatic triple-negative breast cancer. *Invest New Drugs* 2015; **33**: 1108-1114 [PMID: 26123926 DOI: 10.1007/s10637-015-0269-8]
146 **Santoro A**, Rimassa L, Borbath I, Daniele B, Salvagni S, Van Laethem JL, Van Vlierberghe H, Trojan J, Kolligs FT, Weiss A, Miles S, Gasbarrini A, Lencioni M, Cicalese L, Sherman M, Gridelli C, Buggisch P, Gerken G, Schmid RM, Boni C, Personeni N, Hassoun Z, Abbadessa G, Schwartz B, Von Roemeling R, Lamar ME, Chen Y, Porta C. Tivantinib for second-line treatment of advanced hepatocellular carcinoma: a randomised, placebo-controlled phase 2 study. *Lancet Oncol* 2013; **14**: 55-63 [PMID: 23182627 DOI: 10.1016/S1470-2045(12)70490-4]
147 **Nishiya Y**, Nakai D, Urasaki Y, Takakusa H, Ohsuki S, Iwano Y, Yasukochi T, Takayama T, Bazyo S, Oza C, Kurihara A, Savage RE, Izumi T. Stereoselective hydroxylation by CYP2C19 and oxidation by ADH4 in the in vitro metabolism of tivantinib. *Xenobiotica* 2016; **46**: 967-976 [PMID: 26899628 DOI: 10.3109/00498254.2016.1144896]
148 **Yamamoto N**, Murakami H, Nishina T, Hirashima T, Sugio K, Muro K, Takahashi T, Naito T, Yasui H, Akinaga S, Koh Y, Boku N. The effect of CYP2C19 polymorphism on the safety, tolerability, and pharmacokinetics of tivantinib (ARQ 197): results from a phase I trial in advanced solid tumors. *Ann Oncol* 2013; **24**: 1653-1659 [PMID: 23413279 DOI: 10.1093/annonc/mdt014]
149 **Okusaka T**, Aramaki T, Inaba Y, Nakamura S, Morimoto M, Moriguchi M, Sato T, Ikawa Y, Ikeda M, Furuse J. Phase I study of tivantinib in Japanese patients with advanced hepatocellular carcinoma: Distinctive pharmacokinetic profiles from other solid tumors. *Cancer Sci* 2015; **106**: 611-617 [PMID: 25711511 DOI: 10.1111/cas.12644]
150 **Pievsky D**, Pyrsopoulos N. Profile of tivantinib and its potential in the treatment of hepatocellular carcinoma: the evidence to date. *J Hepatocell Carcinoma* 2016; **3**: 69-76 [PMID: 27896243 DOI: 10.2147/JHC.S106072]
151 **Jain L**, Woo S, Gardner ER, Dahut WL, Kohn EC, Kummar S, Mould DR, Giaccone G, Yarchoan R, Venitz J, Figg WD. Population pharmacokinetic analysis of sorafenib in patients with solid tumours. *Br J Clin Pharmacol* 2011; **72**: 294-305 [PMID: 21392074 DOI: 10.1111/j.1365-2125.2011.03963.x]
152 **Casadei Gardini A**, Faloppi L, Aprile G, Brunetti O, Caparello C, Corbelli J, Chessa L, Bruno D, Ercolani G, Leonetti A, de Stefano G, Farella N, Foschi FG, Lanzi A, Dadduzio V, Marisi G, Masi G, Negri FV, Pagan F, Santini D, Scarpi E, Silletta M, Silvestris N, Tamburini E, Tassinari D, Vivaldi C, Gentilucci UV, Zagonel V, Calvetti L, Cascinu S, Frassineti GL, Scartozzi M. Multicenter Prospective Study of Angiogenesis Polymorphism Validation in HCC Patients Treated with Sorafenib. An INNOVATE Study Protocol. *Tumori* 2017; Epub ahead of print [PMID: 29739298 DOI: 10.5301/tj.5000704]

World Journal of Gastroenterology

Submit a Manuscript: https://www.f6publishing.com

World J Gastroenterol 2019 August 7; 25(29): 3897-3919

DOI: 10.3748/wjg.v25.i29.3897

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

REVIEW

Consensus on management of hepatitis C virus infection in resource-limited Ukraine and Commonwealth of Independent States regions

Massimo Giuseppe Colombo, Erkin Isakovich Musabaev, Umed Yusupovich Ismailov, Igor A Zaytsev, Alexander V Nersesov, Igor Anatoliyevich Anastasiy, Igor Alexandrovich Karpov, Olga A Golubovska, Kulpash S Kaliaskarova, Ravishankar AC, Sanjay Hadigal

ORCID number: Massimo Giuseppe Colombo (0000-0001-8295-7508); Erkin Isakovich Musabaev (0000-0001-5124-4353); Umed Yusupovich Ismailov (0000-0002-8256-8251); Igor A Zaytsev (0000-0003-1943-3511); Alexander V Nersesov (0000-0002-8601-3966); Igor Anatoliyevich Anastasiy (0000-0003-2882-9905); Igor Alexandrovich Karpov (0000-0001-5816-2166); Olga A Golubovska (0000-0003-3455-8718); kulpash S Kaliaskarova (0000-0002-2708-0042); Ravishankar AC (0000-0002-2612-5616); Sanjay Hadigal (0000-0001-9072-2303).

Author contributions: All authors contributed equally to this paper with the conception and design of the study, literature review and analysis, and drafting, critical revision and editing of the manuscript, and provided approval of the final version.

Conflict-of-interest statement: Colombo MG received grants/research/clinical trial support from Bristol-Meyers Squibb [Hepatology, viral hepatitis C (Daclatasvir)], Gilead Sciences [Hepatology, viral hepatitis C (Sofosbuvir)], and Merck [Hepatology, viral hepatitis C (Boceprevir)]. Colombo MG is a part of the speaker's bureau at Bayer, Gilead Sciences, and Roche and is a consultant and advisory panel at Bayer, AbbVie, Gilead

Massimo Giuseppe Colombo, Research and Clinical Center, Department of Medicine, Humanitas Hospital, Rozzano 20089, MI, Italy

Erkin Isakovich Musabaev, Research Institute of Virology, Scientific Research Institute of Virology, Tashkent 100194, Uzbekistan

Umed Yusupovich Ismailov, Hepatocenter, Research Institute of Virology, Scientific Research Institute of Virology, Tashkent 100194, Uzbekistan

Igor A Zaytsev, Department of Therapy, Infectious Diseases and Dermatology, Bogomolets National Medical University, Kyiv 01601, Ukraine

Alexander V Nersesov, Department of Gastroenterology and Hepatology, National Research Institute of Cardiology and Internal Diseases, Almaty 050000, Kazakhstan

Igor Anatoliyevich Anastasiy, Olga A Golubovska, Department Infectious Diseases, Bogomolets National Medical University, Kyiv 01601, Ukraine

Igor Alexandrovich Karpov, Department Infectious Diseases, Belarus State Medical University, Minsk 220116, Belarus

Kulpash S Kaliaskarova, Hepatology, Ministry of Health, Astana 010000, Kazakhstan

Ravishankar AC, Sanjay Hadigal, Medical Affairs, Mylan Pharmaceuticals Private Limited, Kadubeesanahalli, Bengaluru 560103, India

Corresponding author: Massimo Giuseppe Colombo, MD, Director, Research and Clinical Center, Department of Medicine, Humanitas Hospital, Via Alessandro Manzoni 56, Rozzano 20089, MI, Italy. massimo.colombo@humanitas.it
Telephone: +39-335-5719512

Abstract

Globally, 69.6 million individuals were infected with hepatitis C virus (HCV) infection in 2016. Of the six major HCV genotypes (GT), the most predominant one is GT1, worldwide. The prevalence of HCV in Central Asia, which includes most of the Commonwealth of Independent States (CIS), has been estimated to be 5.8% of the total global burden. The predominant genotype in the CIS and Ukraine regions has been reported to be GT1, followed by GT3. Inadequate HCV

Sciences, Janssen, Merck, Roche, Mylan, and Boehringer Ingelheim. Nersesov AV supports research at Janssen, Merck, AbbVie, Abbott, and Sanofi. Nersesov AV is a part of the speaker's bureau at Abbott, AbbVie, Bayer, Gilead Sciences, Janssen, Merck, Roche, and Sanofi and is a board member/advisory panel at Abbott, AbbVie, Gilead Sciences, Janssen, Merck, Roche, and Mylan. Ravishankar AC and Hadigal S are employees at Mylan Pharmaceuticals Private Limited. The remaining authors have no conflict of interests.

Manuscript source: Unsolicited manuscript

Received: March 23, 2019
Peer-review started: March 25, 2019
First decision: April 11, 2019
Revised: June 4, 2019
Accepted: June 8, 2019
Article in press: June 6, 2019
Published online: August 7, 2019

P-Reviewer: Cheungpasitporn W, Farshadpour F, Goral V, Rezaee-Zavareh MS, Tabll AA, Yang SS
S-Editor: Ma RY
L-Editor: Filipodia
E-Editor: Ma YJ

epidemiological data, multiple socio-economic barriers, and the lack of region-specific guidelines have impeded the optimal management of HCV infection in this region. In this regard, a panel of regional experts in the field of hepatology convened to discuss and provide recommendations on the diagnosis, treatment, and pre-, on-, and posttreatment assessment of chronic HCV infection and to ensure the optimal use of cost-effective antiviral regimens in the region. A comprehensive evaluation of the literature along with expert recommendations for the management of GT1-GT6 HCV infection with the antiviral agents available in the region has been provided in this review. This consensus document will help guide clinical decision-making during the management of HCV infection, further optimizing treatment outcomes in these regions.

Key words: Antiviral agents; Commonwealth of Independent States; Genotype; Hepatitis C virus; Sustained virologic response; Ukraine

Core tip: A high prevalence of hepatitis C virus (HCV) infection has been reported in Ukraine and most of the Commonwealth of Independent States regions. The scarcity of adequate epidemiological data, the lack of national guidelines, and multiple socio-economic barriers hinder the effective management of HCV infection in these regions. The current consensus document intends to guide clinicians and healthcare providers on the diagnosis, treatment, and pre-, on-, and posttreatment assessment of HCV infection and to help optimize the treatment outcomes in the region.

Citation: Colombo MG, Musabaev EI, Ismailov UY, Zaytsev IA, Nersesov AV, Anastasiy IA, Karpov IA, Golubovska OA, Kaliaskarova KS, AC R, Hadigal S. Consensus on management of hepatitis C virus infection in resource-limited Ukraine and Commonwealth of Independent States regions. *World J Gastroenterol* 2019; 25(29): 3897-3919
URL: https://www.wjgnet.com/1007-9327/full/v25/i29/3897.htm
DOI: https://dx.doi.org/10.3748/wjg.v25.i29.3897

INTRODUCTION

Epidemiology of hepatitis C virus infection in Ukraine and Commonwealth of Independent States countries

Hepatitis C is a liver disease caused by hepatitis C virus (HCV), which manifests clinically as acute and chronic hepatitis[1,2]. There are six different genotypes of HCV (GT1-GT6)[1].

In the latest nationwide HCV disease burden estimation by the Polaris Observatory HCV collaborators in about 113 countries, the global prevalence of HCV infection was estimated to be about 1.0% in 2015 (71.1 million viremic HCV-infected individuals)[3]. In a separate analysis of the prevalence data from 109 countries estimated by the World Health Organization, the global epidemic size of HCV infection was found to be 69.6 million HCV-infected individuals in 2016[4]. In another recent, systematic review, the global genotype distribution pattern revealed the predominance of GT1 (49.1%), followed by GT3 (17.9%), GT4 (16.8%), GT2 (11.0%), GT5 (2%), mixed (1.8%), and GT6 (1.4%)[5]. In the same review, the prevalence of HCV infection in Central Asia, which included the Commonwealth of Independent States (CIS) regions of Armenia, Azerbaijan, Kazakhstan, Kyrgyzstan, Tajikistan, Turkmenistan, and Uzbekistan, besides Mongolia and Georgia, was found to be 5.8%[5]. The predominant genotype in this region was reported to be GT1 (70.4%), followed by GT3 (19.6%) and GT2 (8.6%). The prevalence of mixed GTs was noted to be rare in this region, with a complete absence of cases of GT4, GT5, and GT6. In the Eastern European zone, which includes, among other countries, Ukraine and the three CIS regions of Belarus, Moldova, and Russia, the prevalence of HCV infection was found to be 3.1%. GT1 was the most predominant genotype (68.1%), followed by GT3 (26.6%), GT2 (4.3%), mixed GTs (0.5%), and GT4 (0.5%). No GT5 and GT6 cases were reported in this region[5]. The lack of robust epidemiological data at a national level and in some extended regions of Central Asia was cited as one of the major setbacks in this review[5].

Another survey was conducted by the Alliance for Public Health (Alliance, Ukraine) in collaboration with the Saint Petersburg-based International Treatment Preparedness Coalition in 11 Eastern Europe and Central Asian countries (including Armenia, Azerbaijan, Belarus, Georgia, Kazakhstan, Kyrgyzstan, Moldova, Russia, Tajikistan, Ukraine, and Uzbekistan)[6]. Among the CIS regions and Ukraine, the highest prevalence of HCV infection was reported in Uzbekistan (6.5%), followed by Ukraine (5%); Russia, Armenia, and Kyrgyzstan (4% each); Azerbaijan (3.2%), Tajikistan (2.3%), Belarus (2%-3%), Moldova (1.7%-4.0%), and Kazakhstan (1.5%-3.0%)[6]. Furthermore, the survey reported the lack of adequate HCV epidemiological data required to plan services and resources in the CIS region[6].

Regional unmet needs in the management of HCV infection

The dearth of data pertaining to HCV epidemiology, coupled with the disparity in the genotype distribution across Ukraine and the various CIS regions, highlights a clear unmet need in the optimal management of HCV infection in this region[5,6]. Several other unmet needs in the management of HCV infection in the CIS region have also been described in the literature. These include: (1) Lack of awareness on the disease and modes of transmission and weak epidemiological surveillance[7,8]; (2) Barriers in providing access to diagnostics and surveillance systems[7,9]; (3) Lack of adequate and updated national guidelines/strategies or other regulatory directives on the diagnosis and management of viral hepatitis and HCV infection[6-10]; (4) Fear of treatment side effects[8]; and (5) High treatment cost and lack of reimbursement coverage for treatment[8,9].

This primary aim of this consensus document is to guide physicians on the diagnosis and treatment of chronic HCV infection and to ensure the optimal use of cost-effective regimens in resource-limited settings in Ukraine and CIS countries.

METHODOLOGY OF CONSENSUS DEVELOPMENT

On 9 April 2018, on the sidelines of the European Association for the Study of the Liver 2018 conference, a panel of experts in the field of hepatology from four countries in the Ukraine/CIS region (Uzbekistan, Ukraine, Belarus, and Kazakhstan) convened at Holiday Inn Paris-St. Germain des Près to review the updated literature on the management of HCV infection and to provide recommendations to optimize the: (1) Diagnosis of HCV infection; (2) Use of cost-effective treatment regimens for the management of HCV infection in resource-limited settings in Ukraine and CIS regions; and (3) Pre-, on-, and posttreatment assessments during HCV management.

The recommendations for the use of optimal treatment regimens in the management of HCV infection in Ukraine and the CIS region were graded by the expert panel as "Preferred," "Alternative," or "Not Recommended" (Table 1).

OPTIMIZING THE DIAGNOSIS OF HCV INFECTION

Diagnosis of HCV infection

Consensus recommendations on the diagnosis of HCV infection: (1) Anti-HCV testing is recommended for the screening/initial testing of HCV infection. If the result is positive, the current infection should be confirmed with a sensitive HCV ribonucleic acid (RNA)/core antigen test; (2) Qualitative HCV RNA testing is a reasonable, good, and cost-effective method; it can replace quantitative testing in most patients; (3) It is important to consider quantitative viremia in immunocompromised patients; and (4) Genotyping is recommended to guide appropriate selection of the antiviral regimen.

Key international guidelines recommend initial HCV serological testing for the detection of anti-HCV antibodies and the diagnosis of HCV infection[11-13]. In case of a positive HCV test result, the diagnosis of chronic HCV infection may be established with a nucleic acid test or a sensitive nucleic acid diagnostic assay that detects HCV RNA[11-13]. In low- and middle-income countries, the use of a qualitative HCV RNA assay has been found to be feasible for providing broader access to HCV diagnosis and care[12]. A less sensitive alternative to the HCV RNA test for the diagnosis of HCV infection is the detection of the HCV core antigen[12]. The results of initial HCV serological testing may be negative in some HCV-infected cases (*e.g.*, in case of early acute infection, in immunocompromised patients, or in patients on hemodialysis). In these patients, HCV RNA testing should be a part of the initial assessment[12].

Whenever the staging of hepatitis C is deemed necessary, the degree of liver fibrosis/cirrhosis should be assessed using liver biopsy or other noninvasive tests[1,13].

Table 1 Definitions of the grading of the recommendations

Grading	Definition
Preferred	Treatment can be used in most patients and recommendation is based on optimal efficacy, favorable tolerability, toxicity profiles, treatment duration, and pill burden
Alternative	Treatment can be the one that is effective but with potential disadvantages/limitations in certain patient populations or with less supporting data as compared with the recommended regimens. In certain situations, an alternative regimen may be an optimal regimen for a specific patient population
Not recommended	Treatment is clearly inferior compared with the recommended or alternative regimens because of factors such as lower efficacy, unfavorable tolerability, toxicity, longer duration, and/or higher pill burden. Unless otherwise indicated, such regimens should not be administered in HCV-infected patients

HCV: Hepatitis C virus.

In resource-limited settings, however, the use of liver biopsy may be limited due to cost, invasiveness, and plausible complications, whereas the use of noninvasive tests, such as transient elastography, may be limited by cost and availability constraints. In these settings, serum noninvasive tests, such as the aminotransferase/platelet ratio index (APRI) or fibrosis-4 score, may be useful[11,13]. The APRI has been found to have sufficient sensitivity and specificity for predicting cirrhosis[14]. Besides the detection of liver fibrosis/cirrhosis, testing and detection of the HCV genotype should also be conducted to guide decisions on the choice of treatment[1,13].

Screening of HCV infection

Owing to the high prevalence of HCV infection in Ukraine and the CIS region, periodic screening programs should be conducted to detect infected individuals and to ensure a timely management of the disease. According to the Centers for Disease Control and Prevention, routine HCV screening is not recommended for the general population, pregnant women, healthcare workers, or nonsexual contacts of HCV-positive individuals[2]. Serological testing for HCV may be offered to adults born between 1945 and 1965, high-risk individuals, and those with a history of HCV risk exposure or behavior[2,11,13]. In individuals with a positive anti-HCV test result, further confirmation of the diagnosis of HCV infection should be made with an HCV RNA or HCV core antigen assay. Rapid diagnostic tests using serum, plasma, fingerstick whole blood, or saliva may be considered as alternatives to standard enzyme immunoassays[12].

OPTIMIZING THE MANAGEMENT OF HCV INFECTION

The treatment of HCV infection should focus on: (1) Achievement of sustained virologic response (SVR); (2) Education in liver-associated adverse effects, such as hepatic cirrhosis, end-stage liver disease, and hepatocellular carcinoma (HCC); (3) Management of extrahepatic manifestations; and (4) Reduction in mortality rate[11]. SVR is defined as the continued absence of detectable HCV RNA and/or HCV core antigen for at least 12 wk after the completion of therapy[11].

Pretreatment assessments

Consensus recommendations on pre-treatment assessments: (1) Liver fibrosis assessment: The use of liver biopsy and/or noninvasive markers is recommended for deciding on the regimen and the need for initiating additional measures for the management of cirrhosis (*e.g.*, HCC screening); (2) Assessment for potential drug-drug interactions with concomitant medications is recommended; and (3) Recommended laboratory tests: Complete blood count; hepatic function tests [albumin, total and direct bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase, and alkaline phosphatase levels], international normalized ratio, calculated glomerular filtration rate (GFR), creatinine levels, hepatitis B surface antigen (HBsAg) test, tests for hepatitis B surface antibody (anti-HBs) and antibody to hepatitis B core antigen, additional test for PCR hepatitis B virus (HBV) DNA (quantitative, if the qualitative test yields positive results) in patients with HBsAg and/or antibody to hepatitis B core antigen positivity, and alpha-fetoprotein in

patients with cirrhosis.

Pretreatment assessments for optimizing the choice of therapy should include the assessment of virologic parameters and the severity of liver disease. Other important parameters that must be assessed to guide treatment selection include alcohol intake, HBV/human immunodeficiency virus (HIV) co-infection, renal impairment, diabetes mellitus, autoimmunity, and cardiac diseases[12]. Alcohol consumption should be assessed, and, if needed, counseling should be provided to correct the same[12].

Treatment of HCV infection with direct-acting antivirals (DAAs) may result in reactivation of HBV infection in patients with HCV-HBV co-infection[13,15-18]. Patients with HCV-HBV co-infection have been noted to have accelerated progression of liver disease and an increased risk of HCC[11,13,19,20]. However, reactivation of HBV and subsequent hepatitis has been found to be rare in HCV-HBV co-infected patients who are HBsAg-negative or those who have baseline HBV DNA < 2000 IU/mL prior to DAA therapy[21-24]. Therefore, the expert panel recommended that all HCV patients with positive HBsAg/anti-HBs should be tested for HBV DNA (quantitative, if the qualitative test yields positive results). Patients who fulfil the standard treatment criteria for HBV should be initiated on HBV antiviral treatment. Other patients should be monitored periodically by the assessment of HBV DNA and ALT during HCV DAA therapy. Antiviral therapy for HBV infection should be initiated if patients develop HBV reactivation (presence of HBsAg and HBV DNA plus elevation in ALT more than the upper limit of normal[12]. A recent systematic review and meta-analysis has suggested that anti-HBV prophylaxis with tenofovir or entecavir may significantly reduce the risk of HBV reactivation in patients receiving DAA-based treatment[24].

Rapid progression of fibrosis has been noted in individuals with HCV-HIV co-infection. Persistent elevation of liver enzymes, especially aspartate aminotransferase, has been found to be a useful marker to predict the progression of fibrosis in these individuals[25]. Therefore, all individuals with HCV infection should be evaluated for HIV infection prior to deciding on the choice of therapy[11-13]. The plausibility of drug-drug interactions between DAAs and anti-retroviral therapy should be carefully considered in HCV-HIV-co-infected patients, and the choice and dose of DAAs should be optimized accordingly[11,12].

Several extrahepatic manifestations may occur in patients with HCV infection. Hence, these individuals should be assessed for plausible comorbidities, such as renal impairment, diabetes mellitus, autoimmunity, and cardiac diseases[12]. Additionally, assessment of HCV RNA or HCV core antigen and staging of fibrosis/cirrhosis are also important prior to the initiation of treatment for HCV infection. Furthermore, HCV genotype testing may be useful in guiding treatment selection and optimizing treatment outcomes[11,12].

Who should be treated?

Treatment should be initiated in all individuals with chronic HCV infection, except in patients with a limited life expectancy that cannot be improved by treatment or transplantation. Patients with decompensated cirrhosis should be managed by an expert with relevant clinical experience[11].

DAAs available in Ukraine and the CIS region

Pegylated interferon (peg-IFN) and ribavirin are still used and listed as first-line medications in Ukraine and some CIS countries. First-generation DAAs, such as boceprevir and telaprevir, that are no longer recommended are also registered in most CIS countries. One or more second-generation DAAs are available in Ukraine and in the majority of CIS regions[6]. A summary of the DAA regimens available in Ukraine and in some CIS regions, as compiled by the expert panel, is presented in Table 2. The pharmacological features of the DAAs available in this region have been described in Figure 1[26-29].

Treatment of patients with HCV GT1 infection

The regimens proposed for the treatment of patients with chronic HCV GT1 infection are listed in Table 3.

Sofosbuvir + ledipasvir ± ribavirin: Sofosbuvir in combination with ledipasvir, with or without ribavirin, has been evaluated for the treatment of HCV GT1 infection in several clinical studies worldwide. The phase III ION-1 trial studied the efficacy of this regimen taken for 12 wk or 24 wk in previously untreated, chronic HCV GT1-infected patients (n = 865). About 67% of the patients had GT1a infection, and 16% had cirrhosis. Eligible patients were randomized in a 1:1:1:1 ratio to receive ledipasvir and sofosbuvir fixed-dose combination once daily for 12 wk or 24 wk, or ledipasvir-sofosbuvir + ribavirin for 12 wk or 24 wk. The primary endpoint of SVR at 12 wk after

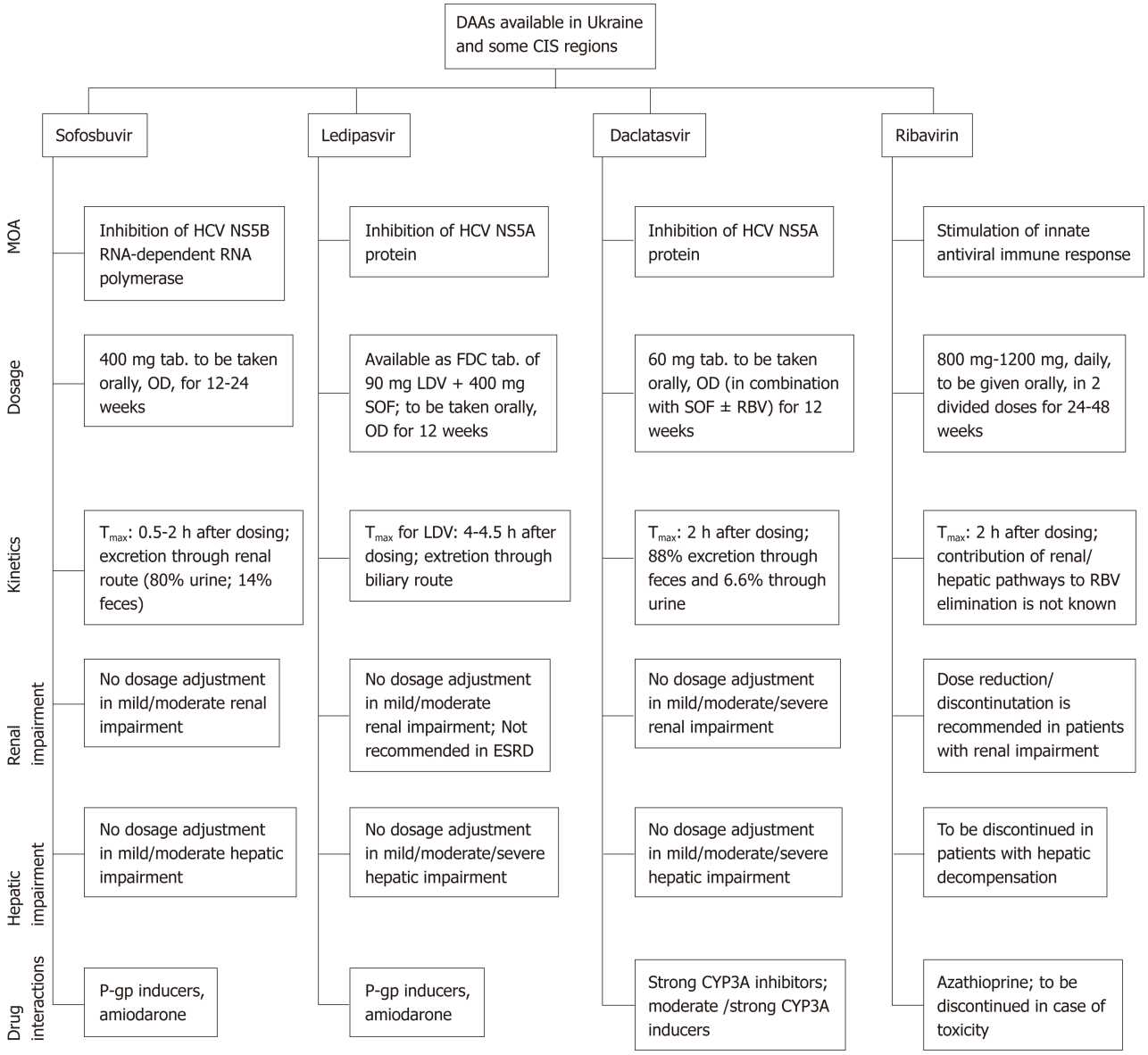


Figure 1 Pharmacological features of direct-acting antiviral agents available in Ukraine and some Commonwealth of Independent States regions. DAA: Direct-acting antiviral agents; CIS: Commonwealth of Independent States; HCV: Hepatitis C virus; RNA: Ribonucleic acid; Tmax: Time required to reach the peak plasma concentration of the drug; LDV: Ledipasvir; RBV: Ribavirin; SOF: Sofosbuvir; ESRD: End-stage renal disease; OD: Once-daily; CYP: Cytochrome P; MOA: Mechanism of action.

the end of treatment was 99%, 98%, 97%, and 99%, respectively, in the four treatment groups. In patients with cirrhosis, the rates of SVR ranged from 94% to 100% in the four treatment groups[30]. Several other clinical and real-world studies and meta-analyses have also reported the efficacy of this regimen in treating HCV GT1-infected patients, including: (1) Both treatment naïve and treatment-experienced patients[31-49]; (2) Patients with compensated cirrhosis or advanced liver disease[31,36,38,40,44-46,48,50-52]; and (3) Liver transplantation cases (the transplantation cases studied included treatment-naïve as well as treatment-experienced and those with cirrhosis and HCC prior to transplantation)[50,53-59]. The presence of fibrosis, cirrhosis, or HCC has been found to lower the SVR rates with sofosbuvir and ledipasvir combination in HCV GT1-infected patients in a few studies[56,58-62].

The phase III, open-label, randomized, ION-3 trial demonstrated that sofosbuvir in combination with ledipasvir given for a shorter duration of 8 wk to treatment- naïve HCV GT1-infected patients without cirrhosis achieved a 94% SVR rate, comparable to the same regimen given for 12 wk, or given in combination with ribavirin for 8 wk[63]. The effectiveness of the 8-wk regimen in the specified population has also been proven in other clinical and real-world studies[31,64-68].

The ION-4 trial was a multicenter, single-group, open-label study conducted to assess the effectiveness of sofosbuvir and ledipasvir fixed-dose combination in patients co-infected with HIV-1 and HCV GT1 or GT4 infection (n = 335; 55% were

Table 2 Direct-acting antivirals available in Ukraine and specific Commonwealth of Independent States countries

Country	SOF	LDV/SOF	DCV
Uzbekistan	√	√	√
Ukraine	√	√	
Belarus	√	√	√
Kazakhstan	√	√	√

SOF: Sofosbuvir; DCV: Daclatasvir; LDV: Ledipasvir.

previously treated for HCV infection, and 20% had cirrhosis). The study demonstrated a 96% SVR rate at 12 wk after the treatment in patients with HCV GT1a and a 96% SVR rate in patients with HCV GT1b infection. The SVR rates were not affected by previous treatment or cirrhosis status[69]. High SVR rates have also been reported with this regimen in several other clinical and real-world studies in HCV GT1 individuals co-infected with HIV, including: (1) Both treatment-naïve and treatment-experienced patients[42,70-72] and (2) Those with cirrhosis[73,74].

The use of a ribavirin-free sofosbuvir and ledipasvir combination regimen has been found to be associated with a significant improvement in the quality of life in HCV GT1-infected patients, regardless of the treatment history or the presence of cirrhosis or HIV co-infection[75-78]. An increase in toxicity has been noted with the inclusion of ribavirin in the treatment regimen[75,79].

The efficacy and safety of sofosbuvir and ledipasvir combination has also been tested in HCV GT1-infected patients with severe renal insufficiency, including those undergoing dialysis and kidney transplantation with favorable tolerability and SVR rates[56,80-85]. Of note, the safety and SVR rates with this regimen have been noted to be better among noncirrhotic *versus* cirrhotic HCV GT1-infected patients with renal conditions, in a few studies[56,84].

Sofosbuvir + daclatasvir ± ribavirin: Sofosbuvir + daclatasvir with or without ribavirin has been evaluated in several clinical studies in varied HCV GT1-infected patient populations. This regimen, provided for 12 wk or 24 wk to treatment-naïve (n = 126) and for 24 wk to treatment-experienced (n = 41) HCV GT1-infected patients, has been found to result in high SVR rates (98%) in the open-label AI444040 trial[86]. Another open-label, phase III trial, viz. ALLY-1, included 76% HCV GT1-infected patients with: (1) Cirrhosis (compensated/decompensated) or (2) Postliver transplantation recurrence. The study evaluated the sofosbuvir, daclatasvir, and ribavirin combination regimen for 12 wk. In patients with cirrhosis interrupted by liver transplantation, treatment was extended for an additional period of 12 wk after transplantation. The SVR rates were 82% and 95% in patients with cirrhosis and liver transplant recipients, respectively. The regimen was well-tolerated, with no treatment-related serious adverse events[87]. In real-world settings and large-scale, multicentric studies, an optimal duration of 12 wk and 24 wk has been suggested with this regimen in noncirrhotic and cirrhotic HCV GT1-infected patients, respectively, for achieving favorable SVR rates[47,88]. The efficacy and safety of this regimen have also been proven in other clinical and real-world studies and meta-analyses that enrolled HCV GT1-infected patients, including treatment-experienced patients, patients with cirrhosis or advanced liver disease, and liver transplant recipients[46,49,57,89-95]. The SVR rates in a few studies were found to be lower in cirrhotic *versus* noncirrhotic HCV GT1-infected patients treated with this regimen[46,96].

The daclatasvir + sofosbuvir regimen has also been found to be effective, with high SVR rates in HCV GT1 patients co-infected with HIV, including treatment-experienced patients, patients with advanced liver disease, and patients undergoing liver transplantation[74,97-103].

Several studies have evaluated the use of this regimen in HCV GT1-infected patients with renal conditions. The combination of sofosbuvir and daclatasvir has been found to be well-tolerated and effective for the treatment of HCV GT1-infected patients with severe renal insufficiency, including those on dialysis or undergoing renal transplantation[80,85,104-107]. Furthermore, a pangenotypic regimen of daclatasvir and half-daily dose of sofosbuvir has been found to be effective for the treatment of HCV GT1-infected patients with an estimated GFR (eGFR) < 30 mL/min with favorable SVR rates (SVR12: 90%-100%)[105,108]. In pharmacokinetic studies, it has been noted that an impaired eGFR (30-60 mL/min) may not lead to the dose accumulation of sofosbuvir in HCV-positive kidney transplant recipients or hemodialysis

Table 3 Recommended treatment regimens for hepatitis C virus GT1 infection

Recommendation category	Treatment option/s	Treatment regimens
Preferred	LDV + SOF ± RBV	**LDV + SOF for 12 wk**
		In treatment-naïve patients having HCV RNA < 6 million IU/mL in whom cirrhosis has been conclusively ruled out by transient elastography (FibroScan) or biopsy: LDV + SOF for 8 wk
		In treatment-experienced cirrhotic patients/patients with decompensated liver disease/postliver transplant patients: LDV + SOF + RBV for 12 wk (or) LDV + SOF for 24 wk if RBV is ineligible
Alternative	SOF + DCV ± RBV	SOF + DCV for 12 wk (addition of RBV may be considered if cirrhosis has not been conclusively ruled out)
		In patients with compensated cirrhosis: SOF + DCV ± weight-based RBV for 24 wk
		In patients with decompensated cirrhosis: SOF + DCV + RBV for 12 wk (or) SOF + DCV for 24 wk if RBV is ineligible
Not recommended	Due to the advent of newer DAAs, pegylated interferon, boceprevir, and telaprevir-based regimens are not recommended.	

SOF: Sofosbuvir; LDV: Ledipasvir; DAAs: Direct-acting antivirals; DCV: Daclatasvir; RBV: Ribavirin; RNA: Ribonucleic acid.

patients[109,110]. Studies may be needed in future to understand further the kinetic profile of sofosbuvir-based treatment in HCV-positive end-stage kidney disease patients or renal transplant recipients.

Treatment of patients with HCV GT2 infection

The preferred regimens recommended by the expert panel for the treatment of patients with chronic HCV GT2 infection are given in Table 4.

Sofosbuvir + daclatasvir ± ribavirin: The AI444040 trial (cited earlier) also included 26 treatment-naïve HCV GT2-infected patients who were treated with the sofosbuvir + daclatasvir regimen with or without ribavirin for 24 wk. SVR was attained in about 92% of these patients[86]. In the ALLY-1 trial, the SVR rate in HCV GT2-infected patients with cirrhosis treated with the sofosbuvir, daclatasvir, and ribavirin combination for 12 wk was 80%[87]. One hundred percent SVR rate was noted in another retrospective study conducted in HCV GT2-infected patients treated with the sofosbuvir and daclatasvir regimen (n = 13), regardless of the degree of baseline fibrosis. The treatment was also found to induce improvement in fibrosis in these patients[111]. The effectiveness of this regimen in treating HCV GT2-infected patients has been proven in routine clinical settings, with an SVR of 88.1%-100% and 94.5%-100% with daclatasvir and sofosbuvir combination with and without ribavirin, respectively[112,113]. Studies have also evaluated the efficacy and safety of this regimen in patients with recurrent HCV GT2 infection post liver transplantation and have reported favorable SVR rates, but the number of patients tested is very low to draw any clinically relevant conclusions in this setting[103].

In HCV GT2-infected patients who cannot tolerate ribavirin, the use of sofosbuvir and daclatasvir for 12 wk in noncirrhotic patients, and for 24 wk in cirrhotic patients, including those with decompensated disease, has been found to achieve high SVR rates 12 wk after the treatment[114].The efficacy of the 12 wk sofosbuvir + daclatasvir regimen has also been proven in patients with HCV GT2 infection, co-infected with HIV-1[97,98].

One hundred percent SVR rate was achieved and no deterioration of renal function was noted in HCV GT2-infected patients with chronic kidney disease treated with sofosbuvir + daclatasvir ± ribavirin regimen[113], and 100% SVR rate was noted in HCV GT2-infected patients with end-stage renal disease (eGFR < 30 mL/min) with daclatasvir full dose plus low-dose sofosbuvir regimen[105]. However, the number of patients evaluated in these studies is too small, and the results need to be substantiated with larger, well-designed studies in future.

Sofosbuvir + ribavirin: The VALENCE trial enrolled HCV GT2- or GT3-infected patients (n = 419; 58% were previously treated with an IFN-based regimen and 21% had cirrhosis). Of the 419 patients, about 91 HCV GT2-infected patients were

Table 4 Recommended preferred treatment regimens for hepatitis C virus GT2 infection

Recommendation category	Treatment option(s)	Treatment regimen
Preferred	SOF + DCV ± RBV	SOF + DCV for 12 wk in noncirrhotics
		In decompensated cirrhosis and previous failures:
		SOF + DCV + RBV for 12 wk
	SOF + RBV	SOF + RBV for 12 wk in noncirrhotics
		To be extended to 24 wk in cirrhotics and treatment failures (Data are not available for patients with decompensated cirrhosis.)
		Should be considered as an alternative regimen when DCV is not available
Not recommended	Due to the advent of newer DAAs, pegylated interferon, boceprevir, and telaprevir-based regimens are not recommended.	

DAAs: Direct-acting antivirals; DCV: Daclatasvir; RBV: Ribavirin; SOF: Sofosbuvir.

randomized in a 4:1 ratio to receive sofosbuvir + ribavirin or placebo for 12 wk. The primary endpoint was SVR at 12 wk after the therapy. The study findings revealed that the SVR rate was 93% in HCV GT2-infected patients treated with the sofosbuvir + ribavirin regimen[115]. Several other randomized and real-world studies have also reported high SVR rates with the sofosbuvir and ribavirin regimen (12 wk or 16 wk duration) in HCV GT2-infected patients, regardless of the treatment history or the presence of cirrhosis[96,116-120]. However, the presence of cirrhosis or a history of HCC was found to influence negatively the SVR rates in some real-world studies[121-124].

The efficacy of 48 wk of sofosbuvir and ribavirin combination regimen, given prior to liver transplantation (due to HCC), on the prevention of HCV recurrence post transplantation was assessed in an open-label study in 61 HCV-infected patients (GT2; n = 8). A total of 46 liver transplantations were done, of which 43 had HCV RNA level < 25 IU/mL at the time of transplantation (GT2; n = 6). The primary endpoint of HCV RNA level < 25 IU/mL at 12 wk after transplantation was achieved by all GT2-infected patients, with no evidence of HCV recurrence[125]. In a separate case study, a patient with liver transplant graft re-infected with HCV GT2 infection was safely and successfully treated with sofosbuvir and ribavirin combination regimen[126].

In randomized phase III studies, a treatment extension of about 24 wk with sofosbuvir and ribavirin regimen has been found to result in 100% SVR rates in treatment-experienced, cirrhotic HCV GT2-infected patients[118]. An extended-duration regimen has also been tested in real-world settings; treatment of treatment-naïve or treatment-experienced HCV GT2-infected patients with cirrhosis for up to 20 wk with this regimen resulted in 94.9% SVR rates[127].

The use of this regimen for the treatment of previously untreated and treatment-experienced HCV GT2-infected patients co-infected with HIV-1 for 12 wk and 12 wk or 24 wk, respectively, has been found to yield high SVR rates[128,129].

A recent study evaluated the efficacy and safety of sofosbuvir and ribavirin regimen in 231 HCV GT2-infected patients with renal dysfunction (82.8% and 17.2% with chronic kidney disease stage G1/2, and G3, respectively). While the overall SVR rate was 97%, the SVR rate in chronic kidney disease stages G1, G2, G3a, and G3b were 98.1%, 98.6%, 87.9%, and 100%, respectively. Multivariate analysis revealed that baseline renal dysfunction significantly and negatively influenced the SVR rates, thus suggesting the need for monitoring of baseline renal function in HCV GT2-infected patients treated with this regimen[130].

Treatment of patients with HCV GT3 infection

The preferred and alternative regimens for the treatment of HCV GT3 infection are listed in Table 5.

Sofosbuvir + daclatasvir ± ribavirin: While the AI444040 trial reported an 89% SVR rate in 18 treatment-naïve HCV GT3-infected patients treated with this regimen for 24 wk, the ALLY-1 trial that enrolled both treatment-naïve and treatment-experienced patients reported an 83% SVR rate in HCV GT3-infected patients with cirrhosis (n = 6) and a 91% SVR rate in liver transplant recipients with posttransplant recurrence of HCV GT3 infection (n = 11)[86,87]. The phase III ALLY-3 trial evaluated the once-daily, 12 wk sofosbuvir + daclatasvir regimen in HCV GT3-infected patients [previously untreated (n = 101) and treatment-experienced (n = 51)]. The SVR rates were 90% and 86% in treatment-naïve and treatment-experienced patients, respectively. About 19%

Table 5 Recommended treatment regimens for hepatitis C virus GT3 infection

Recommendation category	Treatment option(s)	Treatment regimen
Preferred	SOF + DCV ± RBV	SOF + DCV for 12 wk (addition of RBV may be considered if cirrhosis has not been conclusively ruled out)
		In patients with compensated cirrhosis
		Treatment-naïve patients: SOF + DCV + RBV for 24 wk if patients can tolerate ribavirin well, if not SOF+DCV for 24 wk
		Treatment-experienced patients: SOF + DCV + RBV for 24 wk if patients tolerated ribavirin well, if not SOF + DCV for 24 wk
		In patients with decompensated cirrhosis:
		SOF + DCV + RBV for 12 wk
		If RBV is ineligible: SOF + DCV for 24 wk
Alternative	SOF + RBV	SOF + RBV for 24 wk (should be considered only when preferred regimens are not available)
	LDV + SOF + RBV	LDV + SOF + RBV for 12 wk (should be considered only when preferred regimens are not available)
Not recommended	Due to the advent of newer DAAs, pegylated interferon, boceprevir, and telaprevir-based regimens are not recommended.	

DAAs: Direct-acting antivirals; DCV: Daclatasvir; LDV: Ledipasvir; RBV: Ribavirin; SOF: Sofosbuvir.

of patients in the treatment-naïve and 25% of patients in the treatment-experienced groups had cirrhosis in this study. The SVR rates were 96% and 63% in patients without and with cirrhosis, respectively[131]. The ALLY-3+ trial was a randomized, phase III trial that included both treatment-naïve and treatment-experienced GT3-infected patients with advanced fibrosis or compensated cirrhosis. The efficacy and safety of daclatasvir + sofosbuvir with ribavirin given for either 12 wk or 16 wk were assessed in this trial. The SVR rates were 88% and 92% in the groups treated with the 12-wk regimen and the 16-wk regimen, respectively. In patients with cirrhosis, the corresponding SVR rates were 83% and 89%, respectively. Previous treatment had no influence on the SVR rates[132]. The ALLY-3C trial was a single-arm, phase III study that evaluated the efficacy and safety of sofosbuvir + daclatasvir + ribavirin regimen given for 24 wk in HCV GT3-infected patients with compensated cirrhosis. While the SVR12 rate was 87% in the primary analysis, the rates in treatment-naïve and treatment-experienced patients were 93% and 79%, receptively. The regimen was well-tolerated[133]. In real-world settings, treatment of HCV GT3-infected patients, including cirrhotic and treatment-experienced patients, and liver transplant recipients (with a history of HCC prior to transplantation) with recurrent cirrhosis, with this regimen has been found to result in high SVR rates[46,56,57,91,112,134-144].

The efficacy and safety of the daclatasvir + sofosbuvir regimen have also been proven in treatment-naïve and treatment-experienced HCV GT3-infected patients co-infected with HIV-1, including those with advanced liver disease and recurrent HCV after liver transplantation[73,97-99,101,103,140].

High SVR rates have been reported with the use of this regimen in HCV GT3-infected patients with advanced chronic kidney disease with an eGFR < 30 mL/min or those on dialysis[80-82,104]. Further, a regimen comprising low-dose sofosbuvir and full dose daclatasvir has been found to be safe and effective in achieving high SVR rates in HCV GT3-infected patients with eGFR < 30 mL/min or those on maintenance hemodialysis[105,108,145]. Full or half-dose sofosbuvir + daclatasvir regimen has also been evaluated in HCV GT3-infected renal transplant recipients with 100% SVR rates[85]. However, the number of patients included in all these studies are small, and large-scale studies may be needed to corroborate the significance of these findings.

Sofosbuvir + ribavirin: The VALENCE trial enrolled HCV GT2 (n = 91) or GT3 (n = 328)-infected patients and randomized them in a 4:1 ratio to receive sofosbuvir-ribavirin or placebo. The duration of treatment for GT3-infected patients was 24 wk. The study findings revealed high SVR rates (85%) in HCV GT3-infected patients[115]. The response rates were 91% and 68% in GT3-infected patients without and with cirrhosis, respectively[115]. Another prospective study reported an overall SVR rate of 99.2% in GT3-infected HCV patients who received sofosbuvir and ribavirin for 24

wk[146]. In a Russian phase IIIb study, treatment of HCV GT3-infected patients with sofosbuvir + ribavirin for 16 wk or 24 wk was found to be safe and associated with 87% and 90% SVR12 rates, respectively[147]. In a recent real-world study, treatment of HCV GT3-infected patients (n = 110) (51 with compensated and 59 with decompensated cirrhosis) with sofosbuvir + ribavirin for 24 wk resulted in achievement of SVR in 83.3% and 71.4% of treatment-naïve and treatment-experienced patients with compensated cirrhosis, respectively; and 77.8% and 75% of treatment-naïve and treatment-experienced patients with decompensated cirrhosis, respectively. The combination was well-tolerated; however, the outcomes in decompensated and treatment-experienced patients were noted to be suboptimal[148]. This combination regimen given for 24 wk was safe and effective in achieving 95% SVR12 and 94% SVR24 in HCV GT3-infected liver transplant recipients with recurrent HCV infection[149]. Administration of this regimen 48 wk before liver transplantation resulted in 80% of HCV GT3-infected patients achieving HCV RNA < 25 IU/mL 12 wk post transplantation[125].

In HCV GT3-infected patients co-infected with HIV, sofosbuvir and ribavirin regimen has been reported to be associated with significantly lower SVR12 when compared to daclatasvir and sofosbuvir regimen[150]. Literature is sparse on the safety and efficacy of this combination in HCV GT3-infected patients with renal conditions.

Sofosbuvir + ledipasvir + ribavirin: In an open-label trial, 12 wk of the sofosbuvir, ledipasvir, and ribavirin regimen administered to treatment-naïve (n = 26) and treatment-experienced (n = 50) HCV GT3-infected patients resulted in 100% and 82% SVR rates, respectively[151]. In another open-label trial, treatment-naïve HCV patients with and without compensated cirrhosis were treated with sofosbuvir, ledipasvir, and weight-based ribavirin for 12 wk. About 95% of the patients had GT3a infection. The overall SVR rate was 89%, with 79% and 94% SVR rates in patients with and without cirrhosis, respectively[152]. Real-world studies have reported ≥ 90% SVR12 rate with this regimen in HCV GT3-infected patients, including those with cirrhosis and advanced or compensated liver disease[46,134,140-142,153].

Evidence on the efficacy and safety of this regimen in HCV GT3-infected patients co-infected with HIV is limited to a few real-world studies[73,140,153], in which the SVR rates have been reported to be 100%, > 90%, and 80% in patients without and with compensated or decompensated cirrhosis, respectively[73].

Studies on the efficacy and safety of this regimen in HCV GT3-infected liver transplant recipients or patients with renal conditions are limited.

Treatment of patients with HCV GT4 infection

The treatment options for HCV GT4-infected patients listed in Table 6 have been recommended by the expert panel.

Sofosbuvir + ledipasvir + ribavirin: Several phase II studies have established the efficacy of the 12-wk sofosbuvir and ledipasvir regimen in the treatment of HCV GT4-infected patients, regardless of the treatment history or the presence of cirrhosis[154,155]. Administering the combination of this regimen with ribavirin for 12 wk or 24 wk also resulted in high SVR rates in phase II studies on HCV GT4-infected patients with advanced liver diseases[77]. Similarly, high SVR rates have been noted in a phase III study that used a 12-wk sofosbuvir and ledipasvir regimen with or without ribavirin for the treatment of HCV GT4-infected and cirrhotic patients (including both treatment-naïve and treatment-experienced patients)[156]. In another cohort study, the sofosbuvir and ledipasvir combination administered for 12 wk was associated with a 99% SVR rate in HCV GT4-infected patients[157]. In real-world studies and meta-analyses, favorable SVR rates have been noted with the sofosbuvir and ledipasvir regimen with or without ribavirin given for 12 wk or 24 wk in HCV GT4-infected patients, including treatment-naïve and treatment-experienced patients[49,158,159] and patients with advanced liver fibrosis and compensated and decompensated cirrhosis[158,159]. The addition of ribavirin has not been found to improve the efficacy of the combination regimen[158,159]. In a recent real-world study, an 8-wk regimen of ledipasvir + sofosbuvir was found to be well-tolerated and effective in treatment-naïve and noncirrhotic HCV GT4-infected patients (n = 45) (SVR12: 97.8%)[160]. Studies evaluating the efficacy of sofosbuvir + ledipasvir + ribavirin regimen in HCV GT4-infected liver transplant recipients are limited.

The ION-4 trial was a large phase III trial that enrolled HCV GT1- or GT4-infected patients co-infected with HIV-1 who were treated with the sofosbuvir and ledipasvir regimen for 12 wk. About 55% of the patients were treatment-experienced, and 20% had cirrhosis. The SVR rate was noted to be 100% in the GT4-infected patients[69]. In real-world studies, treatment of HCV GT4-infected patients co-infected with HIV with ledipasvir and sofosbuvir regimen was associated with 96%, 94%, and 80% SVR rate

Table 6 Recommended treatment regimens for hepatitis C virus GT4 infection

Recommendation category	Treatment option(s)	Treatment regimen
Preferred	LDV + SOF ± RBV	LDV + SOF for 12 wk [Addition of RBV may be considered based on the physician's discretion in treating difficult-to-treat patients (treatment-experienced patients, patients with cirrhosis)].
		In case of previous SOF treatment failure: LDV + SOF + RBV for 12 wk
Alternative	SOF + DCV ± RBV	SOF + DCV for 12 wk (Addition of RBV may be considered if cirrhosis has not been conclusively ruled out.)
		Cirrhosis of any class: SOF + DCV + RBV for 12 wk
		If RBV is ineligible, SOF + DCV for 24 wk
Not recommended	Due to the advent of newer DAAs, pegylated interferon, boceprevir, and telaprevir-based regimens are not recommended.	

DAAs: Direct-acting antivirals; DCV: Daclatasvir; LDV: Ledipasvir; RBV: Ribavirin; SOF: Sofosbuvir.

in patients without cirrhosis and with compensated and decompensated cirrhosis, respectively[73].

Very few studies have evaluated the efficacy of sofosbuvir + ledipasvir ± ribavirin in HCV GT4-infected renal transplant recipients. One hundred percent SVR12 rates have been noted in these studies with good safety profile of the regimen[83,85]. However, the number of patients evaluated in these studies is too small, and results from studies in larger patient populations may be needed to translate these findings to clinical practice.

Sofosbuvir + daclatasvir ± ribavirin: In the ALLY-1 trial, the combination of sofosbuvir + daclatasvir with ribavirin for 12 wk or 24 wk was associated with a 100% SVR rate in GT4-infected patients with cirrhosis[87]. In another cohort study, the sofosbuvir and daclatasvir combination for 12 wk was associated with a 96% SVR in HCV GT4-infected patients[161]. A separate prospective study categorized HCV GT4-infected patients into two groups: Group 1 included treatment-naïve patients treated with sofosbuvir + daclatasvir for 12 wk; and group 2 included treatment-experienced patients treated with sofosbuvir + daclatasvir + ribavirin for 12 wk (sofosbuvir-experienced patients were treated for 24 wk). The SVR12 rate was 93.3% and 87.5% in groups 1 and 2, respectively. A significant improvement in liver fibrosis was also noted with the treatment in this study[162]. Real-world studies have also supported the efficacy of this combination regimen (with or without ribavirin) with high SVR rates in HCV GT4-infected patients[103,163,164], including those with decompensated cirrhosis and HCV recurrence after liver transplantation[103,163].

Daclatasvir + sofosbuvir has also been found to result in favorable SVR rates in HCV GT4-infected patients co-infected with HIV-1, including those with cirrhosis and advanced liver disease[73,97-101].

Studies evaluating the efficacy and safety of this regimen in HCV GT4-infected patients with renal conditions are limited.

Treatment of patients with HCV GT5 or GT6 infections

The preferred and alternative regimens for the treatment of HCV GT5 or GT6 infections are listed in Table 7.

Sofosbuvir + ledipasvir ± ribavirin: The regimen of sofosbuvir and ledipasvir without ribavirin was evaluated in a single-arm, open-label, phase II trial, in GT5-infected treatment-naïve and treatment-experienced patients, including those with cirrhosis (n = 41). The treatment was provided for 12 wk. While the overall SVR rate with the combination was found to be 95%, the SVR rates in patients with and without cirrhosis were 89% and 97%, respectively[165]. Another prospective, open-label, multicentric study evaluated the efficacy and safety of ledipasvir and sofosbuvir combination given for 8 wk or 12 wk in 60 HCV GT6-infected patients. There were two patients with decompensation, three with liver cancer, and 14 with prior treatment exposure in the 12-wk group. The SVR12 rate was 95% in both the 8- wk and 12-wk treatment groups, and the regimen was found to be safe[166]. In another study conducted by Gane *et al*[151], the combination was evaluated with or without ribavirin for 12 wk in 126 treatment-naïve or treatment-experienced patients with

Table 7 Recommended treatment regimens for hepatitis C virus GT5 or GT6 infections

Recommendation category	Treatment option(s)	Treatment regimen
Preferred	LDV + SOF ± RBV	LDV + SOF for 12 wk [Addition of RBV may be considered based on the physician's discretion in treating difficult-to-treat patients (treatment-experienced patients, patients with cirrhosis)].
		In case of previous SOF treatment failure: LDV + SOF + RBV for 12 wk
Alternative	SOF + DCV ± RBV	SOF + DCV for 12 wk (Addition of RBV may be considered if cirrhosis has not been conclusively ruled out.)
		Cirrhosis of any class: SOF + DCV + RBV for 12 wk
		If RBV is ineligible, SOF + DCV for 24 wk
Not recommended	Due to the advent of newer DAAs, pegylated interferon, boceprevir, and telaprevir-based regimens are not recommended.	

DAAs: Direct-acting antivirals; DCV: Daclatasvir; LDV: Ledipasvir; RBV: Ribavirin; SOF: Sofosbuvir.

HCV GT3 or GT6 infection (n = 25 for GT6). The SVR rate in patients with HCV GT6 infection was 96%. Recent real-world studies, systematic reviews, and meta-analyses have also reported high SVR rates (up to 100%) in HCV GT6-infected patients treated with this combination[47,49,167].

There is limited evidence on the efficacy and safety of this combination in HCV GT6-infected patients undergoing liver transplantation or with concomitant renal conditions.

Sofosbuvir + daclatasvir ± ribavirin: Data on the use of this regimen in the treatment of HCV GT5- or GT6-infected patients are limited. In the open-label ALLY-1 study, SVR was achieved in a single GT6-infected liver transplant recipient treated with the combination[87]. Real-world studies and systematic reviews have reported 94%-100% SVR rate with daclatasvir and sofosbuvir combination in HCV GT6-infected patients[47,167,168]. Data on the efficacy of this combination in HCV GT6-infected kidney transplant recipients are limited to studies with very limited patient population[169].

On- and posttreatment assessments

The expert panel recommended several on- and posttreatment assessments that should be conducted during the management of HCV infection (Table 8).

On-treatment assessments help monitor treatment efficacy and safety, evaluate drug-drug interactions, and ensure medication adherence. In all HCV-infected patients receiving DAA-containing regimens (with or without ribavirin or peg-IFN), complete blood count, renal function tests, and hepatic function panel test should be conducted 4 wk after therapy initiation. Assessment for any side effects or drug-drug interactions, and treatment adherence, is also recommended in the fourth week of treatment[13]. In patients treated with ribavirin-containing regimens, complete blood count should be conducted at 4 wk and 8 wk of therapy to assess for any significant drop in hemoglobin levels[12,13]. Considering the resource-limited settings in the CIS and Ukraine regions, and the lack of any standard recommendations, the expert panel did not recommend HCV RNA testing during treatment[12,13]. However, the panel recommended HCV RNA testing at the end of therapy[11].

Posttreatment follow-up and assessment help confirm the elimination of the virus and prevent relapses. The expert panel recommended the assessment of HCV RNA at 12 wk or 24 wk after completion of therapy, for evaluation of SVR12 or SVR24, in line with the other international recommendations[11-13]. Furthermore, the panel provided posttreatment assessment recommendations for two categories of patients: (1) Those who have failed the therapy and (2) Those who achieved SVR.

Patients who fail the therapy not only remain carriers of the virus but also experience continued liver injury and fibrosis progression[170]. The incidence of death or liver transplantation can be as high as 12.2% in patients with advanced fibrosis and 31.5% among patients with cirrhosis[170]. Therefore, it is important to systematically assess reasons for failure of therapy in these patients. Such patients should also be followed up regularly to assess disease progression. Furthermore, patients with advanced fibrosis (Metavir stage F3 or F4) should be evaluated for HCC every 6 mo using ultrasound surveillance[12].

Among patients who have achieved SVR, those with advanced fibrosis (Metavir

Table 8 On- and posttreatment assessments during the management of hepatitis C virus infection

Assessments	Expert recommendations
On-treatment	In patients with cirrhosis, CBC, creatinine level, estimated GFR, and hepatic function panel may be repeated after 4 wks
	All patients on RBV should have CBC done at four and 8 wk to monitor for hemolysis
	HCV RNA testing (qualitative/quantitative) may not be required, as there are no current recommendations for response-guided therapy. Testing at the end of treatment is mandatory
	Assessment of potential drug-drug interactions with concomitant medications is recommended
	A periodic review of therapy compliance and the general condition of the patient is recommended
Posttreatment	SVR should be assessed at 12 wk or 24 wk after the end of treatment
	In patients who have failed therapy:
	Disease progression (hepatic function panel, CBC, and INR) should be assessed once in 6-12 mo
	In patients with advanced fibrosis (Metavir stages F3 or F4), screening for hepatocellular carcinoma with ultrasound is recommended every 6 mo
	Endoscopic screening for esophageal varices is recommended in cirrhotic patients
	In patients who achieve SVR:
	In patients with advanced fibrosis (Metavir stage F3 or F4), screening for hepatocellular carcinoma with ultrasound is recommended in every 6 mo
	Endoscopic screening for esophageal varices is recommended in cirrhotic patients with pretreatment varices or portal hypertensive gastropathy
	AFP as a screening test for HCC is recommended in cirrhotic patients

CBC: Complete blood count; GFR: Glomerular filtration rate; RBV: Ribavirin; RNA: Ribonucleic acid; SVR: Sustained virologic response; AFP: Alpha-fetoprotein; HCC: Hepatocellular carcinoma.

stage F3 or F4) should be evaluated for HCC every 6 mo using ultrasound surveillance. Furthermore, patients with cirrhosis should be screened endoscopically for esophageal varices and evaluated with alpha-fetoprotein test to screen for HCC[12,13].

A SHORT NOTE ON HCV DRUG RESISTANCE

The emergence of HCV variants with substitutions associated with resistance to DAAs is critical and is particularly noted with NS5A inhibitor-containing regimens. These substitutions are termed resistance-associated substitutions (RAS). Resistant HCV viruses that are enriched in patients with DAA therapy failure contain substitutions termed treatment-emergent RAS. Both baseline and treatment-selected RAS may negatively impact the response rate and treatment outcomes[11,13]. The RAS in the NS5A position for HCV genotypes 1a and 3 are currently considered clinically significant. Methods to detect RAS include population (Sanger) sequencing and deep sequencing [next generation sequencing], with 15% prevalence of RAS as the recommended cutoff[11].

Access to reliable HCV resistance testing techniques is limited in the resource constraint settings of Ukraine and CIS regions. Hence, no consensus could be formed on the methods of HCV resistance testing and reporting, and no recommendations could be made on the systematic testing for HCV resistance prior to DAA treatment or monitoring for HCV drug resistance during or after therapy. However, the following approaches were proposed to help overcome resistance: (1) Optimal risk stratification of patients, based on prior treatment, or degree of cirrhosis; (2) Determination of HCV genotype and subtype so as to help optimize the treatment approach; (3) Optimization of treatment duration and careful selection of patients for short-duration therapy; (4) Addition of ribavirin in selected populations, such as those with prior DAA failure or at risk of treatment failure, and those with baseline NS5A RAS; and (5) Optimal selection of DAA therapy combinations[11,13].

CONCLUSION

In Ukraine and the CIS regions, several challenges hinder the optimal management of HCV infection, including the lack of sufficient epidemiological data, the disparity in genotype distribution, barriers in access to diagnostics, lack of updated national guidelines, and financial constraints. The use of peg-IFN, ribavirin, and first-generation DAAs is still prevalent in these regions, with very few second-generation DAAs being available in most of the regions. There is a clear unmet need for the development of a guidance document for the optimal screening, diagnosis, monitoring, and management of HCV infection with the use of cost-effective, available DAA regimens. The current consensus document compiles the evidence-based recommendations on the diagnosis and management of HCV infection provided by key opinion leaders (from Ukraine and the CIS regions) in the field of hepatology. This document will help guide clinical decision-making on the diagnosis, treatment, and pre-, on-, and posttreatment assessments of HCV infection, further optimizing the treatment outcomes in these regions.

REFERENCES

1 **World Health Organization**. Hepatitis C. 2018. Available from: http://www.who.int/en/news-room/fact-sheets/detail/hepatitis-c
2 **Centers for Disease control and prevention**. *Hepatitis C. 2018*. Accessed on: 01 August 2018; Available from: https://www.cdc.gov/hepatitis/hcv/index.htm
3 **Polaris Observatory HCV Collaborators**. Global prevalence and genotype distribution of hepatitis C virus infection in 2015: a modelling study. *Lancet Gastroenterol Hepatol* 2017; **2**: 161-176 [PMID: 28404132 DOI: 10.1016/S2468-1253(16)30181-9]
4 **Hill AM**, Nath S, Simmons B. The road to elimination of hepatitis C: analysis of cures versus new infections in 91 countries. *J Virus Erad* 2017; **3**: 117-123 [PMID: 28758018]
5 **Petruzziello A**, Marigliano S, Loquercio G, Cozzolino A, Cacciapuoti C. Global epidemiology of hepatitis C virus infection: An up-date of the distribution and circulation of hepatitis C virus genotypes. *World J Gastroenterol* 2016; **22**: 7824-7840 [PMID: 27678366 DOI: 10.3748/wjg.v22.i34.7824]
6 **Maistat L**, Kravchenko N, Reddy A. Hepatitis C in Eastern Europe and Central Asia: a survey of epidemiology, treatment access and civil society activity in eleven countries. *Hepatol Med Policy* 2017; **2**: 9 [PMID: 30288322 DOI: 10.1186/s41124-017-0026-z]
7 **World Health Organization**. Assessment of the viral hepatitis response in Ukraine. 2017; Available from: http://www.euro.who.int/data/assets/pdf_file/0007/372697/ukr-hepatitis-report-eng.PDF
8 **Bivol S**, Sarang A. Hepatitis C in Russia: an epidemic of negligence. The Andrey Rylkov Foundation for Health and Social Justice. 2013.. Available from: http://en.rylkov-fond.org/wp-content/uploads/2014/07/ARF-HCV-report-2013-final_eng.pdf
9 **Leblebicioglu H**, Arends JE, Ozaras R, Corti G, Santos L, Boesecke C, Ustianowski A, Duberg AS, Ruta S, Salkic NN, Husa P, Lazarevic I, Pineda JA, Pshenichnaya NY, Tsertswadze T, Matičič M, Puca E, Abuova G, Gervain J, Bayramli R, Ahmeti S, Koulentaki M, Kilani B, Vince A, Negro F, Sunbul M, Salmon D; ESGHV (part of ESCMID). Availability of hepatitis C diagnostics and therapeutics in European and Eurasia countries. *Antiviral Res* 2018; **150**: 9-14 [PMID: 29217468 DOI: 10.1016/j.antiviral.2017.12.001]
10 **Buscarons AC**. Strategies to Increase Access to Hepatitis C Treatment: A Question of Price or Public Health? Study based on the situation in the Eastern European selected countries Russia, Ukraine and Georgia. 2015. 2017; Available from: https://www.isglobal.org/documents/10179/3408669/Strategies+to+Increase+Access+to+Hepatitis+C+Treatment/2024ff58-b41a-41d1-9a96-7c19343ffe6c
11 **American Association for the Study of Liver Disease**. Recommendations for Testing, Managing, and Treating Hepatitis C. 2018; Available from: https://www.hcvguidelines.org/sites/default/files/full-guidance-pdf/HCVGuidance _May_24_2018a.pdf
12 **European Association for the Study of the Liver**. Recommendations on Treatment of Hepatitis C 2018. Available from: http://www.easl.eu/medias/cpg/2018/EASL%20Recommendations%20on%20Treatment%20of%20Hepatitis%20C%202018/English-report.pdf
13 **World Health Organization**. Guidelines for the screening, care and treatment of persons with chronic hepatitis C infection Updated version, April 2016. Available from: http://apps.who.int/iris/bitstream/handle/10665/ 205035/9789241549615_eng.pdf;jsessionid=3CEAF78983CE7CDCC8CD39E2990145C7?sequence=1
14 APRI calculator.. Available from: https://www.hepatitisc.uw.edu/page/clinical-calculators/apri
15 **Calvaruso V**, Craxì A. HBV recurrence after HCV clearance on DAAs: Sometimes they come back. *J Hepatol* 2017; **67**: 898-901 [PMID: 28864279 DOI: 10.1016/j.jhep.2017.08.017]
16 **Aggeletopoulou I**, Konstantakis C, Manolakopoulos S, Triantos C. Risk of hepatitis B reactivation in patients treated with direct-acting antivirals for hepatitis C. *World J Gastroenterol* 2017; **23**: 4317-4323 [PMID: 28706414 DOI: 10.3748/wjg.v23.i24.4317]
17 **Liu CJ**, Chuang WL, Sheen IS, Wang HY, Chen CY, Tseng KC, Chang TT, Massetto B, Yang JC, Yun C, Knox SJ, Osinusi A, Camus G, Jiang D, Brainard DM, McHutchison JG, Hu TH, Hsu YC, Lo GH, Chu CJ, Chen JJ, Peng CY, Chien RN, Chen PJ. Efficacy of Ledipasvir and Sofosbuvir Treatment of HCV Infection in Patients Coinfected With HBV. *Gastroenterology* 2018; **154**: 989-997 [PMID: 29174546 DOI: 10.1053/j.gastro.2017.11.011]
18 **Fabbri G**, Mastrorosa I, Vergori A, Mazzotta V, Pinnetti C, Grisetti S, Zaccarelli M, Ammassari A, Antinori A. Reactivation of occult HBV infection in an HIV/HCV Co-infected patient successfully treated with sofosbuvir/ledipasvir: a case report and review of the literature. *BMC Infect Dis* 2017; **17**: 182 [PMID: 28249574 DOI: 10.1186/s12879-017-2287-y]

19 **Jamma S**, Hussain G, Lau DT. Current Concepts of HBV/HCV Coinfection: Coexistence, but Not Necessarily in Harmony. *Curr Hepat Rep* 2010; **9**: 260-269 [PMID: 21258658 DOI: 10.1007/s11901-010-0060-4]

20 **Konstantinou D**, Deutsch M. The spectrum of HBV/HCV coinfection: epidemiology, clinical characteristics, viralinteractions and management. *Ann Gastroenterol* 2015; **28**: 221-228 [PMID: 25830779]

21 **Tamori A**, Abiru S, Enomoto H, Kioka K, Korenaga M, Tani J, Enomoto M, Sugiyama M, Masaki T, Kawada N, Yatsuhashi H, Nishiguchi S, Mizokami M. Low incidence of hepatitis B virus reactivation and subsequent hepatitis in patients with chronic hepatitis C receiving direct-acting antiviral therapy. *J Viral Hepat* 2018; **25**: 608-611 [PMID: 29194858 DOI: 10.1111/jvh.12840]

22 **Ogawa E**, Furusyo N, Murata M, Toyoda K, Hayashi T, Ura K. Potential risk of HBV reactivation in patients with resolved HBV infection undergoing direct-acting antiviral treatment for HCV. *Liver Int* 2018; **38**: 76-83 [PMID: 28618152 DOI: 10.1111/liv.13496]

23 **Gane EJ**, Hyland RH, An D, Svarovskaia ES, Brainard D, McHutchison JG. Ledipasvir and sofosbuvir for HCV infection in patients coinfected with HBV. *Antivir Ther* 2016; **21**: 605-609 [PMID: 27367295 DOI: 10.3851/IMP3066]

24 **Jiang XW**, Ye JZ, Li YT, Li LJ. Hepatitis B reactivation in patients receiving direct-acting antiviral therapy or interferon-based therapy for hepatitis C: A systematic review and meta-analysis. *World J Gastroenterol* 2018; **24**: 3181-3191 [PMID: 30065564 DOI: 10.3748/wjg.v24.i28.3181]

25 **Konerman MA**, Mehta SH, Sutcliffe CG, Vu T, Higgins Y, Torbenson MS, Moore RD, Thomas DL, Sulkowski MS. Fibrosis progression in human immunodeficiency virus/hepatitis C virus coinfected adults: prospective analysis of 435 liver biopsy pairs. *Hepatology* 2014; **59**: 767-775 [PMID: 24436062 DOI: 10.1002/hep.26741]

26 Sofosbuvir tablets for oral use. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2015/204671s002lbl.pdf

27 Ledipasvir and sofosbuvir tablets for oral use. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2014/205834s000lbl.pdf

28 Daclatasvir tablets for oral use. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2015/206843Orig1s000lbl.pdf

29 Ribavirin tablets. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2011/021511s023lbl.pdf

30 **Afdhal N**, Zeuzem S, Kwo P, Chojkier M, Gitlin N, Puoti M, Romero-Gomez M, Zarski JP, Agarwal K, Buggisch P, Foster GR, Bräu N, Buti M, Jacobson IM, Subramanian GM, Ding X, Mo H, Yang JC, Pang PS, Symonds WT, McHutchison JG, Muir AJ, Mangia A, Marcellin P; ION-1 Investigators. Ledipasvir and sofosbuvir for untreated HCV genotype 1 infection. *N Engl J Med* 2014; **370**: 1889-1898 [PMID: 24725239 DOI: 10.1056/NEJMoa1402454]

31 **Lawitz E**, Poordad FF, Pang PS, Hyland RH, Ding X, Mo H, Symonds WT, McHutchison JG, Membreno FE. Sofosbuvir and ledipasvir fixed-dose combination with and without ribavirin in treatment-naive and previously treated patients with genotype 1 hepatitis C virus infection (LONESTAR): an open-label, randomised, phase 2 trial. *Lancet* 2014; **383**: 515-523 [PMID: 24209977 DOI: 10.1016/S0140-6736(13)62121-2]

32 **Gane EJ**, Stedman CA, Hyland RH, Ding X, Svarovskaia E, Subramanian GM, Symonds WT, McHutchison JG, Pang PS. Efficacy of nucleotide polymerase inhibitor sofosbuvir plus the NS5A inhibitor ledipasvir or the NS5B non-nucleoside inhibitor GS-9669 against HCV genotype 1 infection. *Gastroenterology* 2014; **146**: 736-743.e1 [PMID: 24262278 DOI: 10.1053/j.gastro.2013.11.007]

33 **Mizokami M**, Yokosuka O, Takehara T, Sakamoto N, Korenaga M, Mochizuki H, Nakane K, Enomoto H, Ikeda F, Yanase M, Toyoda H, Genda T, Umemura T, Yatsuhashi H, Ide T, Toda N, Nirei K, Ueno Y, Nishigaki Y, Betular J, Gao B, Ishizaki A, Omote M, Mo H, Garrison K, Pang PS, Knox SJ, Symonds WT, McHutchison JG, Izumi N, Omata M. Ledipasvir and sofosbuvir fixed-dose combination with and without ribavirin for 12 weeks in treatment-naive and previously treated Japanese patients with genotype 1 hepatitis C: an open-label, randomised, phase 3 trial. *Lancet Infect Dis* 2015; **15**: 645-653 [PMID: 25863559 DOI: 10.1016/S1473-3099(15)70099-X]

34 **Chuang WL**, Chien RN, Peng CY, Chang TT, Lo GH, Sheen IS, Wang HY, Chen JJ, Yang JC, Knox SJ, Gao B, Garrison KL, Mo H, Pang PS, Hsu YC, Hu TH, Chu CJ, Kao JH. Ledipasvir/sofosbuvir fixed-dose combination tablet in Taiwanese patients with chronic genotype 1 hepatitis C virus. *J Gastroenterol Hepatol* 2016; **31**: 1323-1329 [PMID: 26841930 DOI: 10.1111/jgh.13305]

35 **Lim YS**, Ahn SH, Lee KS, Paik SW, Lee YJ, Jeong SH, Kim JH, Yoon SK, Yim HJ, Tak WY, Han SY, Yang JC, Mo H, Garrison KL, Gao B, Knox SJ, Pang PS, Kim YJ, Byun KS, Kim YS, Heo J, Han KH. A phase IIIb study of ledipasvir/sofosbuvir fixed-dose combination tablet in treatment-naïve and treatment-experienced Korean patients chronically infected with genotype 1 hepatitis C virus. *Hepatol Int* 2016; **10**: 947-955 [PMID: 27198664 DOI: 10.1007/s12072-016-9726-5]

36 **Wei L**, Xie Q, Hou JL, Tang H, Ning Q, Cheng J, Nan Y, Zhang L, Li J, Jiang J, McNabb B, Zhang F, Camus G, Mo H, Osinusi A, Brainard DM, Gong G, Mou Z, Wu S, Wang G, Hu P, Gao Y, Jia J, Duan Z. Ledipasvir/sofosbuvir for treatment-naive and treatment-experienced Chinese patients with genotype 1 HCV: an open-label, phase 3b study. *Hepatol Int* 2018; **12**: 126-132 [PMID: 29637511 DOI: 10.1007/s12072-018-9856-z]

37 **Afdhal N**, Reddy KR, Nelson DR, Lawitz E, Gordon SC, Schiff E, Nahass R, Ghalib R, Gitlin N, Herring R, Lalezari J, Younes ZH, Pockros PJ, Di Bisceglie AM, Arora S, Subramanian GM, Zhu Y, Dvory-Sobol H, Yang JC, Pang PS, Symonds WT, McHutchison JG, Muir AJ, Sulkowski M, Kwo P; ION-2 Investigators. Ledipasvir and sofosbuvir for previously treated HCV genotype 1 infection. *N Engl J Med* 2014; **370**: 1483-1493 [PMID: 24725238 DOI: 10.1056/NEJMoa1316366]

38 **Lim JK**, Liapakis AM, Shiffman ML, Lok AS, Zeuzem S, Terrault NA, Park JS, Landis CS, Hassan M, Gallant J, Kuo A, Pockros PJ, Vainorius M, Akushevich L, Michael L, Fried MW, Nelson DR, Ben-Ari Z; HCV-TARGET Study Group. Safety and Effectiveness of Ledipasvir and Sofosbuvir, With or Without Ribavirin, in Treatment-Experienced Patients With Genotype 1 Hepatitis C Virus Infection and Cirrhosis. *Clin Gastroenterol Hepatol* 2018; **16**: 1811-1819.e4 [PMID: 29306043 DOI: 10.1016/j.cgh.2017.12.037]

39 **Akuta N**, Sezaki H, Suzuki F, Fujiyama S, Kawamura Y, Hosaka T, Kobayashi M, Kobayashi M, Saitoh S, Suzuki Y, Arase Y, Ikeda K, Kumada H. Ledipasvir plus sofosbuvir as salvage therapy for HCV genotype 1 failures to prior NS5A inhibitors regimens. *J Med Virol* 2017; **89**: 1248-1254 [PMID: 28079269 DOI: 10.1002/jmv.24767]

40 **Bourlière M**, Bronowicki JP, de Ledinghen V, Hézode C, Zoulim F, Mathurin P, Tran A, Larrey DG,

Ratziu V, Alric L, Hyland RH, Jiang D, Doehle B, Pang PS, Symonds WT, Subramanian GM, McHutchison JG, Marcellin P, Habersetzer F, Guyader D, Grangé JD, Loustaud-Ratti V, Serfaty L, Metivier S, Leroy V, Abergel A, Pol S. Ledipasvir-sofosbuvir with or without ribavirin to treat patients with HCV genotype 1 infection and cirrhosis non-responsive to previous protease-inhibitor therapy: a randomised, double-blind, phase 2 trial (SIRIUS). *Lancet Infect Dis* 2015; **15**: 397-404 [PMID: 25773757 DOI: 10.1016/S1473-3099(15)70050-2]

41 **Aqel B**, Leise M, Vargas HE, Watt KD, Keaveny AP, Zhang N, Zhang N, Pungpapong S. Multicenter Experience using Ledipasvir/Sofosbuvir ± RBV to Treat HCV GT 1 Relapsers after Simeprevir and Sofosbuvir Treatment. *Ann Hepatol* 2018; **17**: 815-821 [PMID: 30145562 DOI: 10.5604/01.3001.0012.3142]

42 **Tam E**, Luetkemeyer AF, Mantry PS, Satapathy SK, Ghali P, Kang M, Haubrich R, Shen X, Ni L, Camus G, Copans A, Rossaro L, Guyer B, Brown RS; RESCUE and ACTG A5348 study investigators. Ledipasvir/sofosbuvir for treatment of hepatitis C virus in sofosbuvir-experienced, NS5A treatment-naïve patients: Findings from two randomized trials. *Liver Int* 2018; **38**: 1010-1021 [PMID: 29091342 DOI: 10.1111/liv.13616]

43 **Backus LI**, Belperio PS, Shahoumian TA, Loomis TP, Mole LA. Real-world effectiveness of ledipasvir/sofosbuvir in 4,365 treatment-naive, genotype 1 hepatitis C-infected patients. *Hepatology* 2016; **64**: 405-414 [PMID: 27115523 DOI: 10.1002/hep.28625]

44 **Mehta V**, Mahajan R, Midha V, Narang V, Kaur K, Singh A, Malhotra A, Parvez A, Sood A. Impact of Direct Acting Antiviral Therapy for Treatment of Hepatitis C Genotypes 1, 3 and 4: A Real Life Experience from India. *J Clin Exp Hepatol* 2018; **8**: 7-14 [PMID: 29743791 DOI: 10.1016/j.jceh.2017.06.003]

45 **Flisiak R**, Łucejko M, Mazur W, Janczewska E, Berak H, Tomasiewicz K, Mozer-Lisewska I, Kozielewicz D, Gietka A, Sikorska K, Wawrzynowicz-Syczewska M, Nowak K, Zarębska-Michaluk D, Musialik J, Simon K, Garlicki A, Pleśniak R, Baka-Ćwierz B, Olszok I, Augustyniak K, Stolarz W, Białkowska J, Badurek A, Piekarska A. Effectiveness and safety of ledipasvir/sofosbuvir±ribavirin in the treatment of HCV infection: The real-world HARVEST study. *Adv Med Sci* 2017; **62**: 387-392 [PMID: 28554119 DOI: 10.1016/j.advms.2017.04.004]

46 **Del Rio-Valencia JC**, Asensi-Diez R, Villalobos-Torres L, Muñoz Castillo I. Direct-acting antiviral agents in patients with hepatitis C genotype 1-4 infections in a tertiary hospital. *Rev Esp Quimioter* 2018; **31**: 226-236 [PMID: 29771105]

47 **Hu C**, Yuan G, Liu J, Huang H, Ren Y, Li Y, Chen X, Li W, Wu T, Deng H, Peng Y, Zhang YY, Zhou Y. Sofosbuvir-Based Therapies for Patients with Hepatitis C Virus Infection: Real-World Experience in China. *Can J Gastroenterol Hepatol* 2018; **2018**: 3908767 [PMID: 30538973 DOI: 10.1155/2018/3908767]

48 **He QF**, Zhang QF, Zhang DZ. Efficacy and Safety of Ribavirin with Sofosbuvir Plus Ledipasvir in Patients with Genotype 1 Hepatitis C: A Meta-Analysis. *Dig Dis Sci* 2016; **61**: 3108-3117 [PMID: 27619394 DOI: 10.1007/s10620-016-4291-2]

49 **Ferreira VL**, Leonart LP, Tonin FS, Borba HHL, Pontarolo R. Sustained Virological Response in Special Populations with Chronic Hepatitis C Using Interferon-Free Treatments: A Systematic Review and Meta-analysis of Observational Cohort Studies. *Clin Drug Investig* 2018; **38**: 389-400 [PMID: 29435907 DOI: 10.1007/s40261-018-0624-6]

50 **Charlton M**, Everson GT, Flamm SL, Kumar P, Landis C, Brown RS, Fried MW, Terrault NA, O'Leary JG, Vargas HE, Kuo A, Schiff E, Sulkowski MS, Gilroy R, Watt KD, Brown K, Kwo P, Pungpapong S, Korenblat KM, Muir AJ, Teperman L, Fontana RJ, Denning J, Arterburn S, Dvory-Sobol H, Brandt-Sarif T, Pang PS, McHutchison JG, Reddy KR, Afdhal N; SOLAR-1 Investigators. Ledipasvir and Sofosbuvir Plus Ribavirin for Treatment of HCV Infection in Patients With Advanced Liver Disease. *Gastroenterology* 2015; **149**: 649-659 [PMID: 25985734 DOI: 10.1053/j.gastro.2015.05.010]

51 **Tsuji K**, Kurosaki M, Itakura J, Mori N, Takaki S, Hasebe C, Akahane T, Joko K, Yagisawa H, Takezawa J, Nakata R, Kusakabe A, Kojima Y, Kimura H, Tamada T, Kobashi H, Mitsuda A, Kondou M, Ogawa C, Uchida Y, Sohda T, Narita R, Izumi N. Real-world efficacy and safety of ledipasvir and sofosbuvir in patients with hepatitis C virus genotype 1 infection: a nationwide multicenter study by the Japanese Red Cross Liver Study Group. *J Gastroenterol* 2018; **53**: 1142-1150 [PMID: 29626296 DOI: 10.1007/s00535-018-1455-1]

52 **Ahmed H**, Elgebaly A, Abushouk AI, Hammad AM, Attia A, Negida A. Safety and efficacy of sofosbuvir plus ledipasvir with and without ribavirin for chronic HCV genotype-1 infection: a systematic review and meta-analysis. *Antivir Ther* 2017; **22**: 369-379 [PMID: 27588749 DOI: 10.3851/IMP3083]

53 **Saab S**, Rheem J, Jimenez MA, Fong TM, Mai MH, Kachadoorian CA, Esmailzadeh NL, Bau SN, Kang S, Ramirez SD, Grotts J, Choi G, Durazo FA, El-Kabany MM, Han SB, Busuttil RW. Effectiveness of Ledipasvir/Sofosbuvir with/without Ribavarin in Liver Transplant Recipients with Hepatitis C. *J Clin Transl Hepatol* 2017; **5**: 101-108 [PMID: 28660147 DOI: 10.14218/JCTH.2016.00070]

54 **Ueda Y**, Ikegami T, Akamatsu N, Soyama A, Shinoda M, Goto R, Okajima H, Yoshizumi T, Taketomi A, Kitagawa Y, Eguchi S, Kokudo N, Uemoto S, Maehara Y. Treatment with sofosbuvir and ledipasvir without ribavirin for 12 weeks is highly effective for recurrent hepatitis C virus genotype 1b infection after living donor liver transplantation: a Japanese multicenter experience. *J Gastroenterol* 2017; **52**: 986-991 [PMID: 28138756 DOI: 10.1007/s00535-017-1310-9]

55 **Shoreibah M**, Orr J, Jones D, Zhang J, Venkata K, Massoud O. Ledipasvir/sofosbuvir without ribavirin is effective in the treatment of recurrent hepatitis C virus infection post-liver transplant. *Hepatol Int* 2017; **11**: 434-439 [PMID: 28083718 DOI: 10.1007/s12072-016-9778-6]

56 **Saxena V**, Khungar V, Verna EC, Levitsky J, Brown RS, Hassan MA, Sulkowski MS, O'Leary JG, Koraishy F, Galati JS, Kuo AA, Vainorius M, Akushevich L, Nelson DR, Fried MW, Terrault N, Reddy KR. Safety and efficacy of current direct-acting antiviral regimens in kidney and liver transplant recipients with hepatitis C: Results from the HCV-TARGET study. *Hepatology* 2017; **66**: 1090-1101 [PMID: 28504842 DOI: 10.1002/hep.29258]

57 **Rupp C**, Hippchen T, Neuberger M, Sauer P, Pfeiffenberger J, Stremmel W, Gotthardt DN, Mehrabi A, Weiss KH. Successful combination of direct antiviral agents in liver-transplanted patients with recurrent hepatitis C virus. *World J Gastroenterol* 2018; **24**: 1353-1360 [PMID: 29599610 DOI: 10.3748/wjg.v24.i12.1353]

58 **Liao HT**, Tan P, Huang JW, Yuan KF. Ledipasvir + Sofosbuvir for Liver Transplant Recipients With Recurrent Hepatitis C: A Systematic Review and Meta-analysis. *Transplant Proc* 2017; **49**: 1855-1863 [PMID: 28923637 DOI: 10.1016/j.transproceed.2017.04.014]

59 **Liu J**, Ma B, Cao W, Li M, Bramer WM, Peppelenbosch MP, Pan Q. Direct-acting antiviral agents for liver transplant recipients with recurrent genotype 1 hepatitis C virus infection: Systematic review and meta-analysis. *Transpl Infect Dis* 2019; **21**: e13047 [PMID: 30615227 DOI: 10.1111/tid.13047]

60 **Ji F**, Wei B, Yeo YH, Ogawa E, Zou B, Stave CD, Li Z, Dang S, Furusyo N, Cheung RC, Nguyen MH. Systematic review with meta-analysis: effectiveness and tolerability of interferon-free direct-acting antiviral regimens for chronic hepatitis C genotype 1 in routine clinical practice in Asia. *Aliment Pharmacol Ther* 2018; **47**: 550-562 [PMID: 29327780 DOI: 10.1111/apt.14507]

61 **Backus LI**, Belperio PS, Shahoumian TA, Loomis TP, Mole LA. Real-world effectiveness and predictors of sustained virological response with all-oral therapy in 21,242 hepatitis C genotype-1 patients. *Antivir Ther* 2017; **22**: 481-493 [PMID: 27934775 DOI: 10.3851/IMP3117]

62 **Itokawa N**, Atsukawa M, Tsubota A, Ikegami T, Shimada N, Kato K, Abe H, Okubo T, Arai T, Iwashita AN, Kondo C, Mikami S, Asano T, Matsuzaki Y, Toyoda H, Kumada T, Iio E, Tanaka Y, Iwakiri K. Efficacy of direct-acting antiviral treatment in patients with compensated liver cirrhosis: A multicenter study. *Hepatol Res* 2019; **49**: 125-135 [PMID: 30307682 DOI: 10.1111/hepr.13256]

63 **Kowdley KV**, Gordon SC, Reddy KR, Rossaro L. Ledipasvir and Sofosbuvir for 8 or 12 Weeks for Chronic HCV without Cirrhosis. *N Engl J Med* 2014; **370**: 1879-1888 [PMID: 24720702 DOI: 10.1056/NEJMoa1402355]

64 **Kowdley KV**, Sundaram V, Jeon CY, Qureshi K, Latt NL, Sahota A, Lott S, Curry MP, Tsai N, Chaiyakunapruk N, Lee Y, Petersen J, Buggisch P. Eight weeks of ledipasvir/sofosbuvir is effective for selected patients with genotype 1 hepatitis C virus infection. *Hepatology* 2017; **65**: 1094-1103 [PMID: 28027579 DOI: 10.1002/hep.29005]

65 **Lai JB**, Witt MA, Pauly MP, Ready J, Allerton M, Seo S, Witt DJ. Eight- or 12-Week Treatment of Hepatitis C with Ledipasvir/Sofosbuvir: Real-World Experience in a Large Integrated Health System. *Drugs* 2017; **77**: 313-318 [PMID: 28078644 DOI: 10.1007/s40265-016-0684-y]

66 **Yanny B**, Saab S, Durazo F, Latt N, Mitry A, Mikhail MM, Hanna RM, Aziz A, Sahota A. Eight-Week Hepatitis C Treatment with New Direct Acting Antivirals Has a Better Safety Profile While Being Effective in the Treatment-Naïve Geriatric Population Without Liver Cirrhosis and Hepatitis C Virus-RNA < 6 Million IU/mL. *Dig Dis Sci* 2018; **63**: 3480-3486 [PMID: 30259281 DOI: 10.1007/s10620-018-5283-1]

67 **Buggisch P**, Vermehren J, Mauss S, Günther R, Schott E, Pathil A, Boeker K, Zimmermann T, Teuber G, Vornkahl HP, Simon KG, Niederau C, Wedemeyer H, Zeuzem S. Real-world effectiveness of 8-week treatment with ledipasvir/sofosbuvir in chronic hepatitis C. *J Hepatol* 2018; **68**: 663-671 [PMID: 29133244 DOI: 10.1016/j.jhep.2017.11.009]

68 **Latt NL**, Yanny BT, Gharibian D, Gevorkyan R, Sahota AK. Eight-week ledipasvir/sofosbuvir in non-cirrhotic, treatment-naïve hepatitis C genotype-1 patients with hepatitis C virus-RNA < 6 million: Single center, real world effectiveness and safety. *World J Gastroenterol* 2017; **23**: 4759-4766 [PMID: 28765697 DOI: 10.3748/wjg.v23.i26.4759]

69 **Naggie S**, Cooper C, Saag M, Workowski K. Ledipasvir and Sofosbuvir for HCV in Patients Coinfected with HIV-1. *N Engl J Med* 2015; **373**: 705-713 [PMID: 26196665 DOI: 10.1056/NEJMoa1501315]

70 **Townsend K**. High Efficacy of Sofosbuvir/Ledipasvir for the Treatment of HCV Genotype 1 in Patients Coinfected With HIV on or off Antiretroviral therapy: Results from The NIAID ERADICATE Trial. AASLD LiverLearningÂ®. 2014; 60133..

71 **Osinusi A**, Townsend K, Kohli A, Nelson A, Seamon C, Meissner EG, Bon D, Silk R, Gross C, Price A, Sajadi M, Sidharthan S, Sims Z, Herrmann E, Hogan J, Teferi G, Talwani R, Proschan M, Jenkins V, Kleiner DE, Wood BJ, Subramanian GM, Pang PS, McHutchison JG, Polis MA, Fauci AS, Masur H, Kottilil S. Virologic response following combined ledipasvir and sofosbuvir administration in patients with HCV genotype 1 and HIV co-infection. *JAMA* 2015; **313**: 1232-1239 [PMID: 25706232 DOI: 10.1001/jama.2015.1373]

72 **Bhattacharya D**, Belperio PS, Shahoumian TA, Loomis TP, Goetz MB, Mole LA, Backus LI. Effectiveness of All-Oral Antiviral Regimens in 996 Human Immunodeficiency Virus/Hepatitis C Virus Genotype 1-Coinfected Patients Treated in Routine Practice. *Clin Infect Dis* 2017; **64**: 1711-1720 [PMID: 28199525 DOI: 10.1093/cid/cix111]

73 **Berenguer J**. Gil-Martin Á, Jarrin I, Moreno A, Dominguez L, Montes M, Aldámiz-Echevarría T, Téllez MJ, Santos I, Benitez L, Sanz J, Ryan P, Gaspar G, Alvarez B, Losa JE, Torres-Perea R, Barros C, Martin JVS, Arponen S, de Guzmán MT, Monsalvo R, Vegas A, Garcia-Benayas MT, Serrano R, Gotuzzo L, Menendez MA, Belda LM, Malmierca E, Calvo MJ, Cruz-Martos E, González-García JJ. All-oral direct-acting antiviral therapy against hepatitis C virus (HCV) in human immunodeficiency virus/HCV-coinfected subjects in real-world practice: Madrid coinfection registry findings. *Hepatology* 2018; **68**: 32-47 [PMID: 29377274 DOI: 10.1002/hep.29814]

74 **Navarro J**, Laguno M, Vilchez HH, Guardiola JM, Carrion JA, Force L, Cairó M, Cifuentes C, Vilaró J, Cucurull J, Marco A, Roget M, Erice E, Crespo M; Catalano-Balear Study Group. Efficacy and safety of direct antiviral agents in a cohort of cirrhotic HCV/HIV-coinfected patients. *J Antimicrob Chemother* 2017; **72**: 2850-2856 [PMID: 29091196 DOI: 10.1093/jac/dkx223]

75 **Younossi ZM**, Stepanova M, Omata M, Mizokami M, Walters M, Hunt S. Quality of life of Japanese patients with chronic hepatitis C treated with ledipasvir and sofosbuvir. *Medicine (Baltimore)* 2016; **95**: e4243 [PMID: 27537553 DOI: 10.1097/MD.0000000000004243]

76 **Younossi ZM**, Stepanova M, Chan HL, Lee MH, Yu ML, Dan YY, Choi MS, Henry L. Patient-reported Outcomes in Asian Patients With Chronic Hepatitis C Treated With Ledipasvir and Sofosbuvir. *Medicine (Baltimore)* 2016; **95**: e2702 [PMID: 26945356 DOI: 10.1097/MD.0000000000002702]

77 **Manns M**, Samuel D, Gane EJ, Mutimer D, McCaughan G, Buti M, Prieto M, Calleja JL, Peck-Radosavljevic M, Müllhaupt B, Agarwal K, Angus P, Yoshida EM, Colombo M, Rizzetto M, Dvory-Sobol H, Denning J, Arterburn S, Pang PS, Brainard D, McHutchison JG, Dufour JF, Van Vlierberghe H, van Hoek B, Forns X; SOLAR-2 investigators. Ledipasvir and sofosbuvir plus ribavirin in patients with genotype 1 or 4 hepatitis C virus infection and advanced liver disease: a multicentre, open-label, randomised, phase 2 trial. *Lancet Infect Dis* 2016; **16**: 685-697 [PMID: 26907736 DOI: 10.1016/S1473-3099(16)00052-9]

78 **Stokes W**, Fenton C, Clement F, James M, Ronksley P, Tang KL. The Efficacy and Safety of 12 Weeks of Sofosbuvir and Ledipasvir versus Sofosbuvir, Ledipasvir, and Ribavirin in Patients with Chronic Hepatitis C, Genotype 1, Who Have Cirrhosis and Have Failed Prior Therapy: A Systematic Review and Meta-Analysis. *Can J Gastroenterol Hepatol* 2017; **2017**: 6468309 [PMID: 28367429 DOI: 10.1155/2017/6468309]

79 **Tao T**, Jiang X, Chen Y, Song Y. Efficacy and Safety of Ledipasvir/Sofosbuvir with and without Ribavirin in Patients with Chronic Hepatitis C Virus Genotype 1 Infection: a meta-analysis. *Int J Infect Dis* 2017; **55**: 56-71 [PMID: 28040553 DOI: 10.1016/j.ijid.2016.12.023]

80 **Singh A**, Kumari S, Kumar P, De A, Singh V. Sofosbuvir with NS5A inhibitors in hepatitis C virus infection with severe renal insufficiency. *J Viral Hepat* 2018; **25**: 1501-1506 [PMID: 30113115 DOI: 10.1111/jvh.12983]

81 **Akhil MS**, Kirushnan B, Martin M, Arumugam K, Ganesh Prasad NK, Ravichandran R. Sofosbuvir-based treatment is safe and effective in Indian hepatitis C patients on maintenance haemodialysis: A retrospective study. *Nephrology (Carlton)* 2018; **23**: 446-452 [PMID: 28339162 DOI: 10.1111/nep.13050]

82 **Manoj Kumar**. Nayak SL, Gupta E, Kataria A, Sarin SK. Generic sofosbuvir-based direct-acting antivirals in hepatitis C virus-infected patients with chronic kidney disease. *Liver Int* 2018; **38**: 2137-2148 [PMID: 29676846 DOI: 10.1111/liv.13863]

83 **Colombo M**, Aghemo A, Liu H, Zhang J, Dvory-Sobol H, Hyland R, Yun C, Massetto B, Brainard DM, McHutchison JG, Bourlière M, Peck-Radosavljevic M, Manns M, Pol S. Treatment With Ledipasvir-Sofosbuvir for 12 or 24 Weeks in Kidney Transplant Recipients With Chronic Hepatitis C Virus Genotype 1 or 4 Infection: A Randomized Trial. *Ann Intern Med* 2017; **166**: 109-117 [PMID: 27842383 DOI: 10.7326/M16-1205]

84 **Fernández I**, Muñoz-Gómez R, Pascasio JM, Baliellas C, Polanco N, Esforzado N, Arias A, Prieto M, Castells L, Cuervas-Mons V, Hernández O, Crespo J, Calleja JL, Forns X, Londoño MC. Efficacy and tolerability of interferon-free antiviral therapy in kidney transplant recipients with chronic hepatitis C. *J Hepatol* 2017; **66**: 718-723 [PMID: 28039098 DOI: 10.1016/j.jhep.2016.12.020]

85 **Taneja S**, Duseja A, De A, Kumar V, Ramachandran R, Sharma A, Dhiman RK, Gupta KL, Chawla Y. Successful treatment of chronic hepatitis C infection with directly acting antivirals in renal transplant recipients. *Nephrology (Carlton)* 2018; **23**: 876-882 [PMID: 28703905 DOI: 10.1111/nep.13109]

86 **Sulkowski MS**, Gardiner DF, Rodriguez-Torres M, Reddy R. Daclatasvir plus Sofosbuvir for Previously Treated or Untreated Chronic HCV Infection. *N Engl J Med* 2014; **370**: 211-221 [PMID: 24428467 DOI: 10.1056/NEJMoa1306218]

87 **Poordad F**, Schiff ER, Vierling JM, Landis C, Fontana RJ, Yang R, McPhee F, Hughes EA, Noviello S, Swenson ES. Daclatasvir with sofosbuvir and ribavirin for hepatitis C virus infection with advanced cirrhosis or post-liver transplantation recurrence. *Hepatology* 2016; **63**: 1493-1505 [PMID: 26754432 DOI: 10.1002/hep.28446]

88 **Pol S**, Bourliere M, Lucier S, Hezode C, Dorival C, Larrey D, Bronowicki JP, Ledinghen VD, Zoulim F, Tran A, Metivier S, Zarski JP, Samuel D, Guyader D, Marcellin P, Minello A, Alric L, Thabut D, Chazouilleres O, Riachi G, Bourcier V, Mathurin P, Loustaud-Ratti V, D'Alteroche L, Fouchard-Hubert I, Habersetzer F, Causse X, Geist C, Rosa I, Gournay J, Saillard E, Billaud E, Petrov-Sanchez V, Diallo A, Fontaine H, Carrat F; ANRS/AFEF HEPATHER study group. Safety and efficacy of daclatasvir-sofosbuvir in HCV genotype 1-mono-infected patients. *J Hepatol* 2017; **66**: 39-47 [PMID: 27622858 DOI: 10.1016/j.jhep.2016.08.021]

89 **Welzel TM**, Petersen J, Herzer K, Ferenci P, Gschwantler M, Wedemeyer H, Berg T, Spengler U, Weiland O, van der Valk M, Rockstroh J, Peck-Radosavljevic M, Zhao Y, Jimenez-Exposito MJ, Zeuzem S. Daclatasvir plus sofosbuvir, with or without ribavirin, achieved high sustained virological response rates in patients with HCV infection and advanced liver disease in a real-world cohort. *Gut* 2016; **65**: 1861-1870 [PMID: 27605539 DOI: 10.1136/gutjnl-2016-312444]

90 **Boglione L**, Pinna SM, Cardellino CS, De Nicolò A, Cusato J, Carcieri C, Cariti G, Di Perri G, D'Avolio A. Treatment with daclatasvir and sofosbuvir for 24 weeks without ribavirin in cirrhotic patients who failed first-generation protease inhibitors. *Infection* 2017; **45**: 103-106 [PMID: 27854063 DOI: 10.1007/s15010-016-0962-3]

91 **Calvaruso V**, Mazzarelli C, Milazzo L, Badia L, Pasulo L, Guaraldi G, Lionetti R, Villa E, Borghi V, Carrai P, Alberti A, Biolato M, Piai G, Persico M, Santantonio T, Felder M, Angelico M, Montalbano M, Mancusi RL, Grieco A, Angeli E, D'Offizi G, Fagiuoli S, Belli L, Verucchi G, Puoti M, Craxì A. Daclatasvir-based regimens in HCV cirrhosis: experience from the Italian early access program. *Sci Rep* 2019; **9**: 585 [PMID: 30679515 DOI: 10.1038/s41598-018-36734-0]

92 **Fontana RJ**, Brown RS, Moreno-Zamora A, Prieto M, Joshi S, Londoño MC, Herzer K, Chacko KR, Stauber RE, Knop V, Jafri SM, Castells L, Ferenci P, Torti C, Durand CM, Loiacono L, Lionetti R, Bahirwani R, Weiland O, Mubarak A, ElSharkawy AM, Stadler B, Montalbano M, Berg C, Pellicelli AM, Stenmark S, Vekeman F, Ionescu-Ittu R, Emond B, Reddy KR. Daclatasvir combined with sofosbuvir or simeprevir in liver transplant recipients with severe recurrent hepatitis C infection. *Liver Transpl* 2016; **22**: 446-458 [PMID: 26890629 DOI: 10.1002/lt.24416]

93 **Liao H**, Tan P, Zhu Z, Yan X, Huang J. Sofosbuvir in combination with daclatasvir in liver transplant recipients with HCV infection: A systematic review and meta-analysis. *Clin Res Hepatol Gastroenterol* 2017; **41**: 262-271 [PMID: 28082137 DOI: 10.1016/j.clinre.2016.12.001]

94 **Lionetti R**, Calvaruso V, Piccolo P, Mancusi RL, Mazzarelli C, Fagiuoli S, Montalbano M, Lenci I, Carrai P, Guaraldi G, Visco-Comandini U, Milana M, Biolato M, Loiacono L, Valente G, Craxì A, Angelico M, D'offizi G. Sofosbuvir plus daclatasvir with or without ribavirin is safe and effective for post-transplant hepatitis C recurrence and severe fibrosis and cirrhosis: A prospective study. *Clin Transplant* 2018; 32 [PMID: 29193356 DOI: 10.1111/ctr.13165]

95 **Mucenic M**, Bandeira de Mello Brandao A, Marroni CA, Medeiros Fleck A, Zanotelli ML, Kiss G, Meine MH, Leipnitz I, Soares Schlindwein E, Martini J, Costabeber AM, Sacco FKF, Cracco Cantisani GP. Daclatasvir and Sofosbuvir With or Without Ribavirin in Liver Transplant Recipients: A Single-Center Real-World Study. *Transplant Proc* 2018; **50**: 769-771 [PMID: 29661434 DOI: 10.1016/j.transproceed.2018.02.001]

96 **Miotto N**, Mendes LC, Zanaga LP, Lazarini MSK, Goncales ESL, Pedro MN, Goncales FL, Stucchi RSB, Vigani AG. All-oral direct antiviral treatment for hepatitis C chronic infection in a real-life cohort: The role of cirrhosis and comorbidities in treatment response. *PLoS One* 2018; **13**: e0199941 [PMID: 29990371 DOI: 10.1371/journal.pone.0199941]

97 **Wyles DL**, Ruane PJ, Sulkowski MS, Dieterich D, Luetkemeyer A, Morgan TR, Sherman KE, Dretler R, Fishbein D, Gathe JC, Henn S, Hinestrosa F, Huynh C, McDonald C, Mills A, Overton ET, Ramgopal M, Rashbaum B, Ray G, Scarsella A, Yozviak J, McPhee F, Liu Z, Hughes E, Yin PD, Noviello S, Ackerman P; ALLY-2 Investigators. Daclatasvir plus Sofosbuvir for HCV in Patients Coinfected with HIV-1. *N Engl J Med* 2015; **373**: 714-725 [PMID: 26196502 DOI: 10.1056/NEJMoa1503153]

98 **Luetkemeyer AF**, McDonald C, Ramgopal M, Noviello S, Bhore R, Ackerman P. 12 Weeks of

Daclatasvir in Combination With Sofosbuvir for HIV-HCV Coinfection (ALLY-2 Study): Efficacy and Safety by HIV Combination Antiretroviral Regimens. *Clin Infect Dis* 2016; **62**: 1489-1496 [PMID: 27025835 DOI: 10.1093/cid/ciw163]

99 **Lacombe K**, Fontaine H, Dhiver C, Metivier S, Rosenthal E, Antonini T, Valantin MA, Miailhes P, Harent S, Batisse D, Pageaux GP, Chas J, Aumaitre H, Dominguez S, Allegre T, Lafeuillade A, Billaud E, De Truchis P, Perre P, Leroy V, De Ledinghen V, Sogni P, Dabis F, Zhao Y, Filipovics A, Fedchuk L, Akremi R, Bennai Y, Salmon Ceron D. Real-World Efficacy of Daclatasvir and Sofosbuvir, With and Without Ribavirin, in HIV/HCV Coinfected Patients With Advanced Liver Disease in a French Early Access Cohort. *J Acquir Immune Defic Syndr* 2017; **75**: 97-107 [PMID: 28272163 DOI: 10.1097/QAI.0000000000001342]

100 **Mandorfer M**, Schwabl P, Steiner S, Scheiner B, Chromy D, Bucsics T, Stättermayer AF, Aichelburg MC, Grabmeier-Pfistershammer K, Trauner M, Reiberger T, Peck-Radosavljevic M. Interferon-free treatment with sofosbuvir/daclatasvir achieves sustained virologic response in 100% of HIV/hepatitis C virus-coinfected patients with advanced liver disease. *AIDS* 2016; **30**: 1039-1047 [PMID: 26760453 DOI: 10.1097/QAD.0000000000001020]

101 **Rockstroh JK**, Ingiliz P, Petersen J, Peck-Radosavljevic M, Welzel TM, Van der Valk M, Zhao Y, Jimenez-Exposito MJ, Zeuzem S. Daclatasvir plus sofosbuvir, with or without ribavirin, in real-world patients with HIV-HCV coinfection and advanced liver disease. *Antivir Ther* 2017; **22**: 225-236 [PMID: 27845298 DOI: 10.3851/IMP3108]

102 **Campos-Varela I**, Moreno A, Morbey A, Guaraldi G, Hasson H, Bhamidimarri KR, Castells L, Grewal P, Baños I, Bellot P, Brainard DM, McHutchison JG, Terrault NA. Treatment of severe recurrent hepatitis C after liver transplantation in HIV infected patients using sofosbuvir-based therapy. *Aliment Pharmacol Ther* 2016; **43**: 1319-1329 [PMID: 27098374 DOI: 10.1111/apt.13629]

103 **Castells L**, Llaneras J, Campos-Varela I, Bilbao I, Crespo M, Len O, Rodríguez-Frías F, Charco R, Salcedo T, Esteban JI, Esteban-Mur R. Sofosbuvir and daclatasvir in mono- and HIV-coinfected patients with recurrent hepatitis C after liver transplant. *Ann Hepatol* 2017; **16**: 86-93 [PMID: 28051797 DOI: 10.5604/16652681.1226819]

104 **Gupta A**, Arora P, Jain P. Sofosbuvir Based Regimen in Management of Hepatitis C for Patients With End Stage Renal Disease on Hemodialysis: A Single Center Experience from India. *J Clin Exp Hepatol* 2018; **8**: 116-120 [PMID: 29892172 DOI: 10.1016/j.jceh.2017.10.001]

105 **Taneja S**, Duseja A, De A, Mehta M, Ramachandran R, Kumar V, Kohli HS, Gupta KL, Dhiman RK, Chawla Y. Low-Dose Sofosbuvir Is Safe and Effective in Treating Chronic Hepatitis C in Patients with Severe Renal Impairment or End-Stage Renal Disease. *Dig Dis Sci* 2018; **63**: 1334-1340 [PMID: 29484572 DOI: 10.1007/s10620-018-4979-6]

106 **Duerr M**, Schrezenmeier EV, Lehner LJ, Bergfeld L, Glander P, Marticorena Garcia SR, Althoff CE, Sack I, Brakemeier S, Eckardt KU, Budde K, Halleck F. A prospective study of daclatasvir and sofosbuvir in chronic HCV-infected kidney transplant recipients. *BMC Nephrol* 2019; **20**: 36 [PMID: 30717681 DOI: 10.1186/s12882-019-1218-0]

107 **Huang H**, Tang H, Deng H, Shen J, Zhou Q, Xie W, Wu J, Chen J. Treatment of chronic hepatitis C viral infection with sofosbuvir and daclatasvir in kidney transplant recipients. *Transpl Infect Dis* 2019; **21**: e13018 [PMID: 30369001 DOI: 10.1111/tid.13018]

108 **Goel A**, Bhadauria DS, Kaul A, Verma P, Mehrotra M, Gupta A, Sharma RK, Rai P, Aggarwal R. Daclatasvir and reduced-dose sofosbuvir: An effective and pangenotypic treatment for hepatitis C in patients with estimated glomerular filtration rate <30 mL/min. *Nephrology (Carlton)* 2019; **24**: 316-321 [PMID: 29327401 DOI: 10.1111/nep.13222]

109 **Schrezenmeier E**, Hoffmann F, Jaeger C, Schrezenmeier J, Lisec J, Glander P, Algharably E, Kreutz R, Budde K, Duerr M, Halleck F. Pharmacokinetics of Daclatasvir, Sofosbuvir, and GS-331007 in a Prospective Cohort of Hepatitis C Virus-Positive Kidney Transplant Recipients. *Ther Drug Monit* 2019; **41**: 53-58 [PMID: 30422962 DOI: 10.1097/FTD.0000000000000567]

110 **Desnoyer A**, Pospai D, Lê MP, Gervais A, Heurgué-Berlot A, Laradi A, Harent S, Pinto A, Salmon D, Hillaire S, Fontaine H, Zucman D, Simonpoli AM, Muret P, Larrouy L, Bernard Chabert B, Descamps D, Yazdanpanah Y, Peytavin G. Pharmacokinetics, safety and efficacy of a full dose sofosbuvir-based regimen given daily in hemodialysis patients with chronic hepatitis C. *J Hepatol* 2016; **65**: 40-47 [PMID: 26952005 DOI: 10.1016/j.jhep.2016.02.044]

111 **Lv DD**, Wang ML, Chen EQ, Wu DB, Tao YC, Zhang DM, Tang H. A retrospective study of the efficacy of sofosbuvir plus NS5A inhibitors for patients with hepatitis C virus genotype-2 chronic infection. *Eur J Gastroenterol Hepatol* 2019; **31**: 382-388 [PMID: 30383554 DOI: 10.1097/MEG.0000000000001299]

112 **Belperio PS**, Shahoumian TA, Loomis TP, Mole LA, Backus LI. Real-world effectiveness of daclatasvir plus sofosbuvir and velpatasvir/sofosbuvir in hepatitis C genotype 2 and 3. *J Hepatol* 2019; **70**: 15-23 [PMID: 30266283 DOI: 10.1016/j.jhep.2018.09.018]

113 **Cheng PN**, Chiu YC, Chien SC, Chiu HC. Real-world effectiveness and safety of sofosbuvir plus daclatasvir with or without ribavirin for genotype 2 chronic hepatitis C in Taiwan. *J Formos Med Assoc* 2019; **118**: 907-913 [PMID: 30316677 DOI: 10.1016/j.jfma.2018.09.016]

114 **Mangia A**, Arleo A, Copetti M, Miscio M, Piazzolla V, Santoro R, Squillante MM. The combination of daclatasvir and sofosbuvir for curing genotype 2 patients who cannot tolerate ribavirin. *Liver Int* 2016; **36**: 971-976 [PMID: 26786792 DOI: 10.1111/liv.13069]

115 **Zeuzem S**, Dusheiko GM, Salupere R, Mangia A, Flisiak R, Hyland RH, Illeperuma A, Svarovskaia E, Brainard DM, Symonds WT, Subramanian GM, McHutchison JG, Weiland O, Reesink HW, Ferenci P, Hézode C, Esteban R; VALENCE Investigators. Sofosbuvir and ribavirin in HCV genotypes 2 and 3. *N Engl J Med* 2014; **370**: 1993-2001 [PMID: 24795201 DOI: 10.1056/NEJMoa1316145]

116 **Lawitz E**, Mangia A, Wyles D, Rodriguez-Torres M, Hassanein T, Gordon SC, Schultz M, Davis MN, Kayali Z, Reddy KR, Jacobson IM, Kowdley KV, Nyberg L, Subramanian GM, Hyland RH, Arterburn S, Jiang D, McNally J, Brainard D, Symonds WT, McHutchison JG, Sheikh AM, Younossi Z, Gane EJ. Sofosbuvir for previously untreated chronic hepatitis C infection. *N Engl J Med* 2013; **368**: 1878-1887 [PMID: 23607594 DOI: 10.1056/NEJMoa1214853]

117 **Jacobson IM**, Gordon SC, Kowdley KV, Yoshida EM, Rodriguez-Torres M, Sulkowski MS, Shiffman ML, Lawitz E, Everson G, Bennett M, Schiff E, Al-Assi MT, Subramanian GM, An D, Lin M, McNally J, Brainard D, Symonds WT, McHutchison JG, Patel K, Feld J, Pianko S, Nelson DR; POSITRON Study; FUSION Study. Sofosbuvir for hepatitis C genotype 2 or 3 in patients without treatment options. *N Engl J Med* 2013; **368**: 1867-1877 [PMID: 23607593 DOI: 10.1056/NEJMoa1214854]

118 **Foster GR**, Pianko S, Brown A, Forton D, Nahass RG, George J, Barnes E, Brainard DM, Massetto B, Lin

M, Han B, McHutchison JG, Subramanian GM, Cooper C, Agarwal K; BOSON Study Group. Efficacy of sofosbuvir plus ribavirin with or without peginterferon-alfa in patients with hepatitis C virus genotype 3 infection and treatment-experienced patients with cirrhosis and hepatitis C virus genotype 2 infection. *Gastroenterology* 2015; **149**: 1462-1470 [PMID: 26248087 DOI: 10.1053/j.gastro.2015.07.043]

119 **Welzel TM**, Nelson DR, Morelli G, Di Bisceglie A, Reddy RK, Kuo A, Lim JK, Darling J, Pockros P, Galati JS, Frazier LM, Alqahtani S, Sulkowski MS, Vainorius M, Akushevich L, Fried MW, Zeuzem S; HCV-TARGET Study Group. Effectiveness and safety of sofosbuvir plus ribavirin for the treatment of HCV genotype 2 infection: results of the real-world, clinical practice HCV-TARGET study. *Gut* 2017; **66**: 1844-1852 [PMID: 27418632 DOI: 10.1136/gutjnl-2016-311609]

120 **Tacke F**, Günther R, Buggisch P, Klinker H, Schober A, John C, Lutz T, Pfeiffer-Vornkahl H, Niederau C, Cornberg M, Sarrazin C, Mauss S. Treatment of HCV genotype 2 with sofosbuvir and ribavirin results in lower sustained virological response rates in real life than expected from clinical trials. *Liver Int* 2017; **37**: 205-211 [PMID: 27428297 DOI: 10.1111/liv.13206]

121 **Ogawa E**, Furusyo N, Nomura H, Takahashi K, Higashi N, Kawano A, Dohmen K, Satoh T, Azuma K, Nakamuta M, Koyanagi T, Kato M, Shimoda S, Kajiwara E, Hayashi J; Kyushu University Liver Disease Study (KULDS) Group. Effectiveness and safety of sofosbuvir plus ribavirin for HCV genotype 2 patients 65 and over with or without cirrhosis. *Antiviral Res* 2016; **136**: 37-44 [PMID: 27789224 DOI: 10.1016/j.antiviral.2016.10.012]

122 **Akahane T**, Kurosaki M, Itakura J, Tsuji K, Joko K, Kimura H, Nasu A, Ogawa C, Kojima Y, Hasebe C, Wada S, Uchida Y, Sohda T, Suzuki H, Yoshida H, Kusakabe A, Tamada T, Kobashi H, Mitsuda A, Kondo M, Shigeno M, Ide Y, Morita A, Kitamura T, Abe T, Izumi N. Real-world efficacy and safety of sofosbuvir + ribavirin for hepatitis C genotype 2: A nationwide multicenter study by the Japanese Red Cross Liver Study Group. *Hepatol Res* 2019; **49**: 264-270 [PMID: 30171740 DOI: 10.1111/hepr.13246]

123 **Han SY**, Woo HY, Heo J, Park SG, Pyeon SI, Park YJ, Kim DU, Kim GH, Kim HH, Song GA, Cho M. The predictors of sustained virological response with sofosbuvir and ribavirin in patients with chronic hepatitis C genotype 2. *Korean J Intern Med* 2019 [PMID: 30879288 DOI: 10.3904/kjim.2018.329]

124 **Yada M**, Miyazaki M, Tanaka K, Masumoto A, Motomura K. Hepatocellular carcinoma or interferon-based therapy history attenuates sofosbuvir/ribavirin for Japanese genotype 2 hepatitis C virus. *World J Gastroenterol* 2018; **24**: 1478-1485 [PMID: 29632428 DOI: 10.3748/wjg.v24.i13.1478]

125 **Curry MP**, Forns X, Chung RT, Terrault NA, Brown R, Fenkel JM, Gordon F, O'Leary J, Kuo A, Schiano T, Everson G, Schiff E, Befeler A, Gane E, Saab S, McHutchison JG, Subramanian GM, Symonds WT, Denning J, McNair L, Arterburn S, Svarovskaia E, Moonka D, Afdhal N. Sofosbuvir and ribavirin prevent recurrence of HCV infection after liver transplantation: an open-label study. *Gastroenterology* 2015; **148**: 100-107.e1 [PMID: 25261839 DOI: 10.1053/j.gastro.2014.09.023]

126 **Sasaki R**, Kanda T, Ohtsuka M, Yasui S, Haga Y, Nakamura M, Yokoyama M, Wu S, Nakamoto S, Arai M, Maruyama H, Miyazaki M, Yokosuka O. Successful Management of Graft Reinfection of HCV Genotype 2 in Living Donor Liver Transplantation from a Hepatitis B Core Antibody-Positive Donor with Sofosbuvir and Ribavirin. *Case Rep Gastroenterol* 2016; **10**: 366-372 [PMID: 27721720 DOI: 10.1159/000447423]

127 **Mangia A**, Susser S, Piazzolla V, Agostinacchio E, De Stefano G, Palmieri V, Spinzi G, Carraturo I, Potenza D, Losappio R, Arleo A, Miscio M, Santoro R, Sarrazin C, Copetti M. Sofosbuvir and ribavirin for genotype 2 HCV infected patients with cirrhosis: A real life experience. *J Hepatol* 2017; **66**: 711-717 [PMID: 27965158 DOI: 10.1016/j.jhep.2016.12.002]

128 **Sulkowski MS**, Naggie S, Lalezari J, Fessel WJ, Mounzer K, Shuhart M, Luetkemeyer AF, Asmuth D, Gaggar A, Ni L, Svarovskaia E, Brainard DM, Symonds WT, Subramanian GM, McHutchison JG, Rodriguez-Torres M, Dieterich D; PHOTON-1 Investigators. Sofosbuvir and ribavirin for hepatitis C in patients with HIV coinfection. *JAMA* 2014; **312**: 353-361 [PMID: 25038354 DOI: 10.1001/jama.2014.7734]

129 **Molina JM**, Orkin C, Iser DM, Zamora FX, Nelson M, Stephan C, Massetto B, Gaggar A, Ni L, Svarovskaia E, Brainard D, Subramanian GM, McHutchison JG, Puoti M, Rockstroh JK; PHOTON-2 study team. Sofosbuvir plus ribavirin for treatment of hepatitis C virus in patients co-infected with HIV (PHOTON-2): a multicentre, open-label, non-randomised, phase 3 study. *Lancet* 2015; **385**: 1098-1106 [PMID: 25659285 DOI: 10.1016/S0140-6736(14)62483-1]

130 **Sho T**, Suda G, Nagasaka A, Yamamoto Y, Furuya K, Kumagai K, Uebayashi M, Terashita K, Kobayashi T, Tsunematsu I, Onodera M, Meguro T, Kimura M, Ito J, Umemura M, Izumi T, Kawagishi N, Ohara M, Ono Y, Nakai M, Natsuizaka M, Morikawa K, Ogawa K, Sakamoto N; NORTE Study Group. Safety and efficacy of sofosbuvir and ribavirin for genotype 2 hepatitis C Japanese patients with renal dysfunction. *Hepatol Res* 2018; **48**: 529-538 [PMID: 29316051 DOI: 10.1111/hepr.13056]

131 **Nelson DR**, Cooper JN, Lalezari JP, Lawitz E, Pockros PJ, Gitlin N, Freilich BF, Younes ZH, Harlan W, Ghalib R, Oguchi G, Thuluvath PJ, Ortiz-Lasanta G, Rabinovitz M, Bernstein D, Bennett M, Hawkins T, Ravendhran N, Sheikh AM, Varunok P, Kowdley KV, Hennicken D, McPhee F, Rana K, Hughes EA; ALLY-3 Study Team. All-oral 12-week treatment with daclatasvir plus sofosbuvir in patients with hepatitis C virus genotype 3 infection: ALLY-3 phase III study. *Hepatology* 2015; **61**: 1127-1135 [PMID: 25614962 DOI: 10.1002/hep.27726]

132 **Leroy V**, Angus P, Bronowicki JP, Dore GJ, Hezode C, Pianko S, Pol S, Stuart K, Tse E, McPhee F, Bhore R, Jimenez-Exposito MJ, Thompson AJ. Daclatasvir, sofosbuvir, and ribavirin for hepatitis C virus genotype 3 and advanced liver disease: A randomized phase III study (ALLY-3+). *Hepatology* 2016; **63**: 1430-1441 [PMID: 26822022 DOI: 10.1002/hep.28473]

133 **Poordad F**, Shiffman ML, Ghesquiere W, Wong A, Huhn GD, Wong F, Ramji A, Shafran SD, McPhee F, Yang R, Noviello S, Linaberry M; ALLY-3C study team. Daclatasvir and sofosbuvir with ribavirin for 24 weeks in chronic hepatitis C genotype-3-infected patients with cirrhosis: a Phase III study (ALLY-3C). *Antivir Ther* 2019; **24**: 35-44 [PMID: 30382942 DOI: 10.3851/IMP3278]

134 **Del Rio-Valencia JC**, Asensi-Diez R, Madera-Pajin R, Yunquera-Romero L, Muñoz-Castillo I. Interferon-free treatments in patients with hepatitis C genotype 3 infection in a tertiary hospital. *Rev Esp Quimioter* 2018; **31**: 35-42 [PMID: 29376623]

135 **Hézode C**, De Ledinghen V, Fontaine H, Zoulim F, Lebray P, Boyer N, Larrey D, Silvain C, Botta-Fridlund D, Leroy V, Bourliere M, D'Alteroche L, Hubert-Fouchard I, Guyader D, Rosa I, Nguyen-Khac E, Di Martino V, Carrat F, Fedchuk L, Akremi R, Bennai Y, Bronowicki JP. Daclatasvir plus sofosbuvir with or without ribavirin in patients with HCV genotype 3 infection: Interim analysis of a French multicenter compassionate use program. *J Hepatol* 2015; **62** Suppl 2: S265-266 [DOI: 10.1016/S0168-8278(15)30159-8]

136 **Leroy V**, Hezode C, Metivier S, Tateo M, Conti F, Nguyen-Khac E, Lacoste D, Vergniol J, Truchi R, Guyader D, Riachi G, Michau C, Blaison D, Oberti F, Fontaine H, Di Martino V, Bronowicki JP, Akremi R, Bennai Y, Filipovics A, Pageaux GP. Daclatasvir plus sofosbuvir with or without ribavirin in patients with HCV infection and decompensated cirrhosis: Interim analysis of a French multicentres, compassionate use programme. *J Hepatol* 2016; **64**: S829

137 **Hézode C**, Lebray P, De Ledinghen V, Zoulim F, Di Martino V, Boyer N, Larrey D, Botta-Fridlund D, Silvain C, Fontaine H, D'Alteroche L, Leroy V, Bourliere M, Hubert-Fouchard I, Guyader D, Rosa I, Nguyen-Khac E, Fedchuk L, Akremi R, Bennai Y, Filipovics A, Zhao Y, Bronowicki JP. Daclatasvir plus sofosbuvir, with or without ribavirin, for hepatitis C virus genotype 3 in a French early access programme. *Liver Int* 2017; **37**: 1314-1324 [PMID: 28177199 DOI: 10.1111/liv.13383]

138 **Petersen J**, Welzel TM, Herzer K, Ferenci P, Gschwantler M, Cornberg M, Ingiliz P, Berg T, Spengler U, Weiland O, van der Valk M, Klinker H, Rockstroh J, Peck-Radosavljevic M, Zhao Y, Jimenez-Exposito MJ, Zeuzem S. Daclatasvir plus sofosbuvir with or without ribavirin for the treatment of chronic HCV infection in patients with decompensated cirrhosis: Results of a European multicentre compassionate use programme. *J Hepatol* 2016; **64**: S781-S782 [DOI: 10.1016/S0168-8278(16)01524-5]

139 **Tao YC**, Deng R, Wang ML, Lv DD, Yuan M, Wang YH, Chen EQ, Tang H. Satisfactory virological response and fibrosis improvement of sofosbuvir-based regimens for Chinese patients with hepatitis C virus genotype 3 infection: results of a real-world cohort study. *Virol J* 2018; **15**: 150 [PMID: 30285800 DOI: 10.1186/s12985-018-1066-8]

140 **Wehmeyer MH**, Ingiliz P, Christensen S, Hueppe D, Lutz T, Simon KG, Schewe K, Boesecke C, Baumgarten A, Busch H, Rockstroh J, Schmutz G, Kimhofer T, Berger F, Mauss S, Schulze Zur Wiesch J. Real-world effectiveness of sofosbuvir-based treatment regimens for chronic hepatitis C genotype 3 infection: Results from the multicenter German hepatitis C cohort (GECCO-03). *J Med Virol* 2018; **90**: 304-312 [PMID: 28710853 DOI: 10.1002/jmv.24903]

141 **Alonso S**. Riveiro-Barciela M, Fernandez I, Rincón D, Real Y, Llerena S, Gea F, Olveira A, Fernandez-Carrillo C, Polo B, Carrión JA, Gómez A, Devesa MJ, Baliellas C, Castro Á, Ampuero J, Granados R, Pascasio JM, Rubín A, Salmeron J, Badia E, Planas JM, Lens S, Turnes J, Montero JL, Buti M, Esteban R, Fernández-Rodríguez CM. Effectiveness and safety of sofosbuvir-based regimens plus an NS5A inhibitor for patients with HCV genotype 3 infection and cirrhosis. Results of a multicenter real-life cohort. *J Viral Hepat* 2017; **24**: 304-311 [PMID: 27935168 DOI: 10.1111/jvh.12648]

142 **Dalgard O**, Weiland O, Noraberg G, Karlsen L, Heggelund L, Färkkilâ M, Balslev U, Belard E, Øvrehus A, Skalshøi Kjær M, Krarup H, Thorup Røge B, Hallager S, Madsen LG, Lund Laursen A, Lagging M, Weis N. Sofosbuvir based treatment of chronic hepatitis C genotype 3 infections-A Scandinavian real-life study. *PLoS One* 2017; **12**: e0179764 [PMID: 28704381 DOI: 10.1371/journal.pone.0179764]

143 **Pellicelli A**, Messina V, Giannelli V, Distefano M, Palitti VP, Vignally P, Tarquini P, Izzi A, Moretti A, Babudieri S, Dell'Isola S, Marignani M, Scifo G, Iovinella V, Cariti G, Pompili M, Candilo FD, Fontanella L, Ettorre GM, Vennarecci G, Ippolito AM, Barbarini G. High efficacy and safety of flat-dose ribavirin plus sofosbuvir/daclatasvir in genotype 3 cirrhotic patients. *Gut Liver* 2019 [PMID: 30970444 DOI: 10.5009/gnl18269]

144 **Teegen EM**, Globke B, Schott E, Pratschke J, Eurich D. A Closing Chapter: Hepatitis C Genotype 3 Elimination in Liver Transplant; Sofosbuvir/Daclatasvir in a Hard-to-Treat Population. *Exp Clin Transplant* 2018; **16**: 61-67 [PMID: 29137590 DOI: 10.6002/ect.2016.0296]

145 **Sperl J**, Frankova S, Kreidlova M, Merta D, Tothova M, Spicak J. Combination of sofosbuvir and daclatasvir in the treatment of genotype 3 chronic hepatitis C virus infection in patients on maintenance hemodialysis. *Ther Clin Risk Manag* 2017; **13**: 733-738 [PMID: 28790832 DOI: 10.2147/TCRM.S133983]

146 **Iqbal S**, Yousuf MH, Yousaf MI. Dramatic response of hepatitis C patients chronically infected with hepatitis C virus genotype 3 to sofosbuvir-based therapies in Punjab, Pakistan: A prospective study. *World J Gastroenterol* 2017; **23**: 7899-7905 [PMID: 29209131 DOI: 10.3748/wjg.v23.i44.7899]

147 **Isakov V**, Zhdanov K, Kersey K, Svarovskaia E, Massetto B, Zhu Y, Knox SJ, Bakulin I, Chulanov V. Efficacy of sofosbuvir plus ribavirin in treatment-naive patients with genotype-1 and -3 HCVinfection: results from a Russian Phase IIIb study. *Antivir Ther* 2016; **21**: 671-678

148 **Butt N**, Akbar A, Abbasi A, Reema S, Baqar JB, Shaikh QH. Safety and Efficacy of Sofosbuvir with Ribavirin® in Hepatitis C, Genotype 3 Patients with Cirrhosis: A Real-world Experience. *Cureus* 2019; **11**: e4012 [PMID: 31001466 DOI: 10.7759/cureus.4012]

149 **Anand AC**, Agarwal SK, Garg HK, Khanna S, Gupta S. Sofosbuvir and Ribavirin for 24 Weeks Is An Effective Treatment Option for Recurrent Hepatitis C Infection After Living Donor Liver Transplantation. *J Clin Exp Hepatol* 2017; **7**: 165-171 [PMID: 28970701 DOI: 10.1016/j.jceh.2017.06.007]

150 **Swallow E**, Song J, Yuan Y, Kalsekar A, Kelley C, Peeples M, Mu F, Ackerman P, Signorovitch J. Daclatasvir and Sofosbuvir Versus Sofosbuvir and Ribavirin in Patients with Chronic Hepatitis C Coinfected with HIV: A Matching-adjusted Indirect Comparison. *Clin Ther* 2016; **38**: 404-412 [PMID: 26839044 DOI: 10.1016/j.clinthera.2015.12.017]

151 **Gane EJ**, Hyland RH, An D, Svarovskaia E, Pang PS, Brainard D, Stedman CA. Efficacy of ledipasvir and sofosbuvir, with or without ribavirin, for 12 weeks in patients with HCV genotype 3 or 6 infection. *Gastroenterology* 2015; **149**: 1454-1461.e1 [PMID: 26261007 DOI: 10.1053/j.gastro.2015.07.063]

152 **Feld JJ**, Ramji A, Shafran SD, Willems B, Marotta P, Huchet E, Vachon ML, Svarovskaia ES, Huang KC, Hyland RH, Yun C, Massetto B, Brainard DM, McHutchison JG, Tam E, Bailey R, Cooper C, Yoshida EM, Greenbloom S, Elkhashab M, Borgia S, Swain MG. Ledipasvir-Sofosbuvir Plus Ribavirin in Treatment-Naive Patients With Hepatitis C Virus Genotype 3 Infection: An Open-Label Study. *Clin Infect Dis* 2017; **65**: 13-19 [PMID: 28535298 DOI: 10.1093/cid/cix289]

153 **Moser S**, Kozbial K, Laferl H, Schütz A, Reiberger T, Schwabl P, Gutic E, Schwanke C, Schubert R, Luhn J, Lang T, Schleicher M, Steindl-Munda P, Haltmayer H, Ferenci P, Gschwantler M. Efficacy of ledipasvir/sofosbuvir plus ribavirin for 12 weeks in patients with chronic hepatitis C genotype 3 and compensated liver disease. *Eur J Gastroenterol Hepatol* 2018; **30**: 291-295 [PMID: 29120906 DOI: 10.1097/MEG.0000000000001027]

154 **Kohli A**, Kapoor R, Sims Z, Nelson A, Sidharthan S, Lam B, Silk R, Kotb C, Gross C, Teferi G, Sugarman K, Pang PS, Osinusi A, Polis MA, Rustgi V, Masur H, Kottilil S. Ledipasvir and sofosbuvir for hepatitis C genotype 4: a proof-of-concept, single-centre, open-label phase 2a cohort study. *Lancet Infect Dis* 2015; **15**: 1049-1054 [PMID: 26187031 DOI: 10.1016/S1473-3099(15)00157-7]

155 **Abergel A**, Metivier S, Samuel D, Jiang D, Kersey K, Pang PS, Svarovskaia E, Knox SJ, Loustaud-Ratti V, Asselah T. Ledipasvir plus sofosbuvir for 12 weeks in patients with hepatitis C genotype 4 infection.

Hepatology 2016; **64**: 1049-1056 [PMID: 27351341 DOI: 10.1002/hep.28706]
156 **Shiha G**, Esmat G, Hassany M, Soliman R, Elbasiony M, Fouad R, Elsharkawy A, Hammad R, Abdel-Razek W, Zakareya T, Kersey K, Massetto B, Osinusi A, Lu S, Brainard DM, McHutchison JG, Waked I, Doss W. Ledipasvir/sofosbuvir with or without ribavirin for 8 or 12 weeks for the treatment of HCV genotype 4 infection: results from a randomised phase III study in Egypt. *Gut* 2019; **68**: 721-728 [PMID: 29666174 DOI: 10.1136/gutjnl-2017-315906]
157 **Ahmed OA**, Kaisar HH, Badawi R, Hawash N, Samir H, Shabana SS, Fouad MHA, Rizk FH, Khodeir SA, Abd-Elsalam S. Efficacy and safety of sofosbuvir-ledipasvir for treatment of a cohort of Egyptian patients with chronic hepatitis C genotype 4 infection. *Infect Drug Resist* 2018; **11**: 295-298 [PMID: 29535545 DOI: 10.2147/IDR.S153060]
158 **Crespo J**, Calleja JL, Fernández I, Sacristan B, Ruiz-Antorán B, Ampuero J, Hernández-Conde M, García-Samaniego J, Gea F, Buti M, Cabezas J, Lens S, Morillas RM, Salcines JR, Pascasio JM, Turnes J, Sáez-Royuela F, Arenas J, Rincón D, Prieto M, Jorquera F, Sanchez Ruano JJ, Navascués CA, Molina E, Moya AG, Moreno-Planas JM; Spanish Group for the Study of the Use of Direct-acting Drugs Hepatitis C Collaborating Group. Real-World Effectiveness and Safety of Oral Combination Antiviral Therapy for Hepatitis C Virus Genotype 4 Infection. *Clin Gastroenterol Hepatol* 2017; **15**: 945-949.e1 [PMID: 28238958 DOI: 10.1016/j.cgh.2017.02.020]
159 **Sanai FM**, Altraif IH, Alswat K, AlZanbagi A, Babatin MA, AlMousa A, Almutairi NH, Aljawad MS, Alghamdi AS, Aljumah AA, Alalwan AM, Al-Hamoudi WK, Assiri AM, Dahlan Y, Alsahafi A, Alothmani HS, AlSaleemi MS, Mousa WA, Albenmousa A, Awny A, Albiladi H, Abdo AA, AlGhamdi H. Real life efficacy of ledipasvir/sofosbuvir in hepatitis C genotype 4-infected patients with advanced liver fibrosis and decompensated cirrhosis. *J Infect* 2018; **76**: 536-542 [PMID: 29742470 DOI: 10.1016/j.jinf.2018.04.001]
160 **Babatin MA**, AlGhamdi AS, Assiri AM, AlBiladi H, AlOthmani HS, Mogharbel MH, Mahallawi W, Asselah T, Sanai FM. Treatment efficacy of ledipasvir/sofosbuvir for 8 weeks in non-cirrhotic chronic hepatitis C genotype 4 patients. *Saudi J Gastroenterol* 2019; **25**: 55-60 [PMID: 30117490 DOI: 10.4103/sjg.SJG_189_18]
161 **Ahmed OA**, Safwat E, Khalifa MO, Elshafie AI, Fouad MHA, Salama MM, Naguib GG, Eltabbakh MM, Sherief AF, Abd-Elsalam S. Sofosbuvir Plus Daclatasvir in Treatment of Chronic Hepatitis C Genotype 4 Infection in a Cohort of Egyptian Patients: An Experiment the Size of Egyptian Village. *Int J Hepatol* 2018; **2018**: 9616234 [PMID: 29755792 DOI: 10.1155/2018/9616234]
162 **Abdel-Aziz AM**, Ibrahim MA, El-Sheikh AA, Kamel MY, Zenhom NM, Abdel-Raheim S, Abdelhaleem H. Effect of Sofosbuvir Plus Daclatasvir in Hepatitis C Virus Genotype-4 Patients: Promising Effect on Liver Fibrosis. *J Clin Exp Hepatol* 2018; **8**: 15-22 [PMID: 29743792 DOI: 10.1016/j.jceh.2017.06.006]
163 **Abdel-Moneim A**, Aboud A, Abdel-Gabaar M, Zanaty MI, Ramadan M. Efficacy and safety of sofosbuvir plus daclatasvir with or without ribavirin: large real-life results of patients with chronic hepatitis C genotype 4. *Hepatol Int* 2018; **12**: 348-355 [PMID: 29754329 DOI: 10.1007/s12072-018-9868-8]
164 **Shiha G**, Soliman R, ElBasiony M, Hassan AA, Mikhail NNH. Sofosbuvir plus Daclatasvir with or without ribavirin for treatment of chronic HCV genotype 4 patients: real-life experience. *Hepatol Int* 2018; **12**: 339-347 [PMID: 29663115 DOI: 10.1007/s12072-018-9861-2]
165 **Abergel A**, Asselah T, Metivier S, Kersey K, Jiang D, Mo H, Pang PS, Samuel D, Loustaud-Ratti V. Ledipasvir-sofosbuvir in patients with hepatitis C virus genotype 5 infection: an open-label, multicentre, single-arm, phase 2 study. *Lancet Infect Dis* 2016; **16**: 459-464 [PMID: 26803446 DOI: 10.1016/S1473-3099(15)00529-0]
166 **Nguyen MH**, Trinh H, Do S, Nguyen T, Nguyen P, Henry L. Open Label Study of 8 vs. 12 Weeks of Ledipasvir/Sofosbuvir in Genotype 6 Treatment Naïve or Experienced Patients. *Am J Gastroenterol* 2017; **112**: 1824-1831 [PMID: 29087397 DOI: 10.1038/ajg.2017.399]
167 **Mettikanont P**, Bunchorntavakul C, Reddy KR. Systematic review: epidemiology and response to direct-acting antiviral therapy in genotype 6 chronic hepatitis C virus infection. *Aliment Pharmacol Ther* 2019; **49**: 492-505 [PMID: 30687952 DOI: 10.1111/apt.15100]
168 **Wu DB**, Jiang W, Wang YH, Chen B, Wang ML, Tao YC, Chen EQ, Tang H. Safety and efficacy of sofosbuvir-based direct-acting antiviral regimens for hepatitis C virus genotype 6 in Southwest China: Real-world experience of a retrospective study. *J Viral Hepat* 2019; **26**: 316-322 [PMID: 30380166 DOI: 10.1111/jvh.13033]
169 **Xue Y**, Zhang LX, Wang L, Li T, Qu YD, Liu F. Efficacy and safety of sofosbuvir and daclatasvir in treatment of kidney transplantation recipients with hepatitis C virus infection. *World J Gastroenterol* 2017; **23**: 5969-5976 [PMID: 28932089 DOI: 10.3748/wjg.v23.i32.5969]
170 **Dienstag JL**, Ghany MG, Morgan TR, Di Bisceglie AM, Bonkovsky HL, Kim HY, Seeff LB, Szabo G, Wright EC, Sterling RK, Everson GT, Lindsay KL, Lee WM, Lok AS, Morishima C, Stoddard AM, Everhart JE; HALT-C Trial Group. A prospective study of the rate of progression in compensated, histologically advanced chronic hepatitis C. *Hepatology* 2011; **54**: 396-405 [PMID: 21520194 DOI: 10.1002/hep.24370]

World Journal of Gastroenterology

Submit a Manuscript: https://www.f6publishing.com

World J Gastroenterol 2019 August 7; 25(29): 3920-3928

DOI: 10.3748/wjg.v25.i29.3920

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

MINIREVIEWS

Immunotherapy in colorectal cancer: Available clinical evidence, challenges and novel approaches

Joseph Tintelnot, Alexander Stein

ORCID number: Joseph Tintelnot (0000-0003-4619-9433); Alexander Stein (0000-0002-9768-0086).

Author contributions: Tintelnot J and Stein A performed literature research; Tintelnot J drafted the manuscript and performed the revision; Stein A designed the outline, coordinated and corrected the writing of the paper.

Conflict-of-interest statement: Dr. Stein reports institutional research grants from Merck, BMS, Roche, Sanofi, and Servier, personal fees from BMS, MSD, Merck, Roche, and Amgen during the conduct of the study.

Manuscript source: Invited manuscript

Received: March 18, 2019
Peer-review started: March 19, 2019
First decision: May 9, 2019
Revised: May 21, 2019
Accepted: July 2, 2019
Article in press: July 3, 2019

Joseph Tintelnot, Alexander Stein, Department of Internal Medicine II (Oncology Center), University Medical Center Hamburg-Eppendorf, Hamburg 20246, Germany

Corresponding author: Alexander Stein, MD, Assistant Professor, Department of Internal Medicine II (Oncology Center), University Medical Center Hamburg-Eppendorf, Martinistr. 52, Hamburg 20246, Germany. a.stein@uke.de
Telephone: +49-40-741056882
Fax: +49-40-741053563

Abstract

In contrast to other tumor types, immunotherapy has not yet become a relevant part of the treatment landscape of unselected colorectal cancer. Beside the small subgroup of deficient mismatch repair or microsatellite instable tumors (about 5%) as a surrogate for high mutational burden and subsequently high neoantigen load and immunogenicity, inhibitors of programmed death 1 (PD-1), programmed death ligand 1 (PD-L1) and/or cytotoxic T lymphocyte-associated antigen-4 were not or only modestly effective in metastatic colorectal cancer. Thus, a variety of combination approaches with chemotherapy, targeted therapy, toll-like receptor agonists, local ablation or oncolytic viruses is currently being evaluated in different disease settings. Despite several encouraging single arm data already presented or published, available randomized data are unimpressive. Adding PD-1/PD-L1 inhibitors to fluoropyrimidines and bevacizumab maintenance showed no beneficial impact on delaying progression. In refractory disease, the combination of PD-1/PD-L1 and MEK inhibitor was not different from regorafenib, whereas a PD-1/PD-L1 and cytotoxic T lymphocyte-associated antigen-4 inhibitor combination demonstrated better overall survival compared to supportive care alone. Clinical trials in all disease settings applying different combination approaches are ongoing and may define the role of immunotherapy in colorectal cancer.

Key words: Immunotherapy; Combination; Colorectal cancer; Metastatic; Adjuvant

Core tip: Colorectal cancer is not responsive to single agent programmed death 1/ligand 1 or cytotoxic T lymphocyte-associated antigen-4 inhibitors. Thus, a variety of combination approaches with chemotherapy, targeted therapy, toll-like receptor agonists, local ablation or oncolytic viruses are currently being evaluated to enhance immunogenicity of mismatch repair proficient colorectal cancers. Here we review

Published online: August 7, 2019

P-Reviewer: Fiorentini G, Guo XZ, Guo ZS, Kamimura K, Kim J, Maher J, Rangel-Corona R
S-Editor: Yan JP
L-Editor: Filipodia
E-Editor: Ma YJ

current clinical evidence, challenges and novel approaches in ongoing clinical programs.

Citation: Tintelnot J, Stein A. Immunotherapy in colorectal cancer: Available clinical evidence, challenges and novel approaches. *World J Gastroenterol* 2019; 25(29): 3920-3928
URL: https://www.wjgnet.com/1007-9327/full/v25/i29/3920.htm
DOI: https://dx.doi.org/10.3748/wjg.v25.i29.3920

INTRODUCTION

The first clinical evidence for the effectiveness of immunotherapy in solid tumors dates back to 1891 when Coley *et al*[1] first injected streptococcal bacteria into patients with inoperable sarcomas and observed shrinkage of some tumors. Despite this early success it took over a century and the breakthrough discovery of immune checkpoints, namely programmed death 1 (PD-1), programmed death ligand 1 (PD-L1) and cytotoxic T lymphocyte-associated antigen-4 (CTLA–4), to relevantly change the treatment of different malignancies like melanoma, renal, bladder and lung cancer or Hodgkin's disease. This breakthrough discovery was recently rewarded by the Nobel Prize for James P Allison and Tasuku Honjo in 2018.

In metastatic colorectal cancer (MCRC), only a minority of patients respond to immune checkpoint inhibition[2]. However patients who had a high tumor mutational burden and a high infiltration of T cells expressing checkpoint receptors (*e.g.*, PD-1, PD-L1 or CTLA–4) mainly found in the subset of mismatch repair-deficient (dMMR) tumors with high levels of microsatellite-instability (MSI-H) responded to the immunotherapy[3-5]. Intriguingly, activated T cells directly recognized neoantigens that evolved from somatic mutations in gastrointestinal malignancies[6] or melanoma[7,8]. The highest rates of neoantigen load were observed in frameshift mutated tumors through insertions or deletions[9].

In an attempt to define molecular subgroups of CRC, dMMR and MSI-H tumors cluster into the consensus molecular subtype (CMS) 1 of CRC[10], named as the immune subtype due to the high infiltration of lymphocytes. However, some CMS1 CRCs show a proficient mismatch repair (pMMR) system but harbor polymerase proofreading domain mutations that lead to even greater tumor mutational burden. Therefore they may be susceptible to immunotherapy as well[11,12]. These findings led to the approval of both PD-1 inhibitors pembrolizumab and nivolumab for the treatment of dMMR or MSI-H treatment-refractory MCRC in 2017.

Immunotherapy has no relevance in the canonical (CMS2), metabolic (CMS3) or mesenchymal subgroup (CMS4) of CRC[13]. Therefore the majority of CRC patients will not respond to this therapy. Combinatorial strategies to enhance the immunogenicity and infiltration by lymphocytes (*e.g.*, combination with EGFR antibodies, radiotherapy, oncolytic viruses, adoptive cell therapy, tyrosine kinase inhibitors or toll-like receptor (TLR) agonists) have been recently addressed although their clinical relevance has yet to be determined. The present article summarizes current clinical evidence of immunotherapy in CRC and reviews ongoing clinical challenges and novel approaches. Published clinical data from PubMed, ESMO (Annual Symposium, World Congress on Gastrointestinal Cancer) and ASCO (Annual Meeting, Gastrointestinal Cancers Meeting) have been systematically collected and reviewed for the years 2012–2019.

IMMUNOTHERAPY IN DMMR/MSI-H MCRC

Monotherapy with PD-1 inhibitors (pembrolizumab or nivolumab)

The first interim data reported from the KEYNOTE (KN)-016 trial of pembrolizumab in treatment-refractory dMMR/MSI-H MCRC showed an overall response rate (ORR) of 40% (n = 10) in comparison to a missing response in pMMR or microsatellite stable (MSS) tumors (n = 18)[5]. In total, 40 MCRC patients (dMMR/MSI-H) were treated in KN-016 as part of 86 patients (dMMR/MSI-H) with 11 tumor types that led to the first ever agnostic (*i.e.* histology and tumor-site independent) approval of a cancer drug in 2017 in the United States[14]. The second cohort came from a part of the Check-Mate (CM)-142 phase II clinical trial with 74 MCRC patients (dMMR/MSI-H) after at least one prior systemic treatment who were treated with nivolumab. This trial led to the approval of nivolumab in 2017 in the United States[15]. These studies together with

data from 61 treatment-refractory MSI-H patients (KN-164)[16] provided the current clinical evidence for treating dMMR/MSI-H MCRC patients with PD-1 inhibitors. An ORR of 31% in CM-142 or 28% in KN-164 were observed. Strikingly, progression free survival (PFS) at 12 mo was high with 50% and 34%, respectively as well as 1-year overall survival (OS) of 73% and 72%, respectively. Although generally well tolerated, the clinical response came at a cost of all grade drug-related adverse events (AEs) like fatigue (23%), diarrhea (21%), pruritus (14%) or rash (11%). Increase in lipase (8%) or amylase (3%) were the most frequent observed grade 3 or 4 AEs[15].

Combination of PD-1 (nivolumab) and CTLA-4 inhibitors (ipilimumab)

Recent evidence for the combination of nivolumab and ipilimumab in MCRC patients (dMMR/MSI-H) came from two further cohorts of the CM-142 study. In the treatment refractory cohort comprising of 119 patients with at least two prior therapies, an ORR of 55% and a 12-wk disease control rate of 80% were reported. Moreover, PFS rates of 71% and OS of 85% after one year were reached independent of KRAS or BRAF mutational status, PD-L1 tumor expression or family history of Lynch syndrome. Although no randomized data is currently available, ORR of 55% and 1-year OS of 85% compares favorably to an ORR of 31% and 1-year OS of 72% in single-agent PD-1 inhibitor treatment. Despite the expected increase in treatment-related AEs that occurred as grade 3 or 4 AEs in 32% of all patients, all AEs were manageable in the chosen regimen of nivolumab (3 mg/kg week 1 and every 2 wk from week 13) and ipilimumab (1 mg/kg on week 1, 4, 8 and 11). Recently, a third cohort with 45 previously untreated dMMR/MSI-H MCRC patients was reported showing an ORR of 60% (27/45), 1-year PFS of 77% and 1-year OS of 83%[17]. Notably, a low dose ipilimumab regimen was applied with 1 mg/kg every 6 wk continuously in combination with nivolumab 3mg/kg every 2 wk resulting in a very low rate of treatment-related grade 3/4 AEs of 16%.

In the nonmetastatic setting, 4 wk of neoadjuvant treatment with nivolumab 3 mg/kg (day 1 and 15) and ipilimumab 1 mg/kg (day 1) was tested in seven dMMR/MSI-H (stage II and III) CRC patients[18]. Four out of those seven patients showed non-vital tumor cells after resection whereas all remaining patients showed relevant downstaging and histological regression of their tumors (1%-2% vital tumor cells). Although limited by the small number, this dramatic pathological regression after 4 wk of treatment questions the objective response rates determined by imaging with checkpoint inhibition in dMMR/MSI-H MCRC. Data for selected PD-1 inhibitor +/- CTLA–4 inhibitor trials are displayed in Table 1.

Combination of PD-L1 inhibitor (atezolizumab) with anti-angiogenic VEGF-antibody (bevacizumab)

The combination of PD-L1 inhibitors and anti-angiogenic antibody bevacizumab showed favorable results in renal cancer. Recently, this combination was tested in ten dMMR/MSI-H MCRC patients in a phase Ib study. The combination was well tolerated and disease control was reached in 90% of patients with an ORR of 30%[19].

Ongoing clinical trials

A phase III study is currently observing the combination of atezolizumab, 5-fluorouracil, folinic acid and oxaliplatin (FOLFOX) in the adjuvant setting of stage III lymph node metastasized CRC (ATOMIC trial-NCT02912559). In MCRC, the KN-177 trial is investigating the addition of pembrolizumab to the first line standard of care therapy with FOLFOX or 5-fluorouracil, folinic acid and irinotecan alone or in combination with bevacizumab or cetuximab (NCT02563002). After completing the recruitment, results are expected this year. Furthermore, another phase II study is evaluating FOLFOX and bevacizumab and/or atezolizumab compared to FOLFOX and bevacizumab in the first line treatment of CRC (NCT02997228). In the second line treatment setting, the FFCD SAMCO trial is investigating the PD-L1 inhibitor avelumab compared to standard chemotherapy (NCT03186326).

IMMUNOTHERAPY IN PMMR/MSS MCRC

Monotherapy or combination of PD-1 (nivolumab and pembrolizumab) or PD-L1 (durvalumab) inhibitors and CTLA–4 (ipilimumab and tremelimumab) inhibitors

The above mentioned studies (KN-016 and CM-142) that evaluated pembrolizumab (KN-016) or the combination of nivolumab and ipilimumab (CM-142) also included some pMMR/MSS MCRC patients[5,15]. In contrast to the appealing results in dMMR/MSI-H MCRC, pMMR/MSS MCRC patients did not respond to checkpoint inhibition, highlighting the predictive value of the dMMR/MSI-H status.

Table 1 Selected trials on immunotherapy in mismatch repair-deficient/microsatellite-instability metastatic colorectal cancer

Setting	Clinical trial	Drugs and regimen	*n*	ORR	PFS rate at 12 mo	Median PFS, mo	OS rate at 12 mo
Neoadjuvant	[18]	Nivolumab (3) + Ipilimumab (1)	7	pCR 57% (4/7)	NR	NR	NR
First line	CM-142[17]	Nivolumab (3) + Ipilimumab (1/6 wk)	45	60%	77%	NR	83%
≥ Second line	CM-142[14]	Nivolumab	74	31%	50%	14.3	73%
	KN-164[16]	Pembrolizumab	61	28%	34%	2.3	72%
	CM-142[15]	Nivolumab (3) + Ipilimumab (1/3 wk)	119	55%	71%	NR	85%

ORR: Overall response rate; pCR: Pathological complete response rate; PFS: Progression free survival; OS: Overall survival; NR: Not reported.

A phase I study further evaluated the combination of durvalumab (PD-L1) and tremelimumab (CTLA–4) in 18 unselected MCRC patients. Although an ORR of 11% (2/18) was observed results can hardly be interpreted due to missing MMR/MSI-status[20]. Recently, a randomized trial comparing best supportive care +/- durvalumab and tremelimumab in 180 patients, excluding patients with known MSI-H status, was presented[21]. Despite similar ORR and PFS, OS was improved [4.1 *vs* 6.6 mo, hazard ratio: 0.72, 90% confidence interval: 0.54-0.97], reawakening the interest in this combination in pMMR/MSS MCRC.

COMBINATION STRATEGIES TO ENHANCE IMMUNOGENICITY IN PMMR/MSS OR UNSELECTED MCRC PATIENTS

As indicated by the results of the above-mentioned clinical studies, response to checkpoint inhibition is restricted to dMMR and MSI-H tumor patients. Unfortunately, this subset of patients only accounts for approximately 5% of MCRC cases. Because of the infiltration and activation of T cells, the recognition of neoantigens or tumor associated antigens has led the way to effective immunotherapy of solid tumors. Different combinatorial studies have been conducted or are still ongoing with the ultimate goal to enhance immunogenicity of CRC.

Checkpoint inhibition and local ablation

The abscopal effect was first described by Mole in 1953[22] as a phenomenon observed by local radiation of immunogenic tumors (renal cell carcinoma, melanoma or hepatocellular carcinoma) that led to shrinkage of distant tumors through the activation of immune effector cells[23]. It is unknown whether non-immunogenic tumors like CRC respond in a similar fashion. However, local ablation or radiotherapy may lead to cell death and the release of antigens and type I interferon, which induces maturation of dendritic cells and activation of $CD8^+$ T cells[24].

A small phase II clinical study used radiotherapy or radiofrequency ablation in addition to pembrolizumab in heavily pre-treated MCRC patients. Unfortunately, the ORR was as low as 5%. Similarly, an approach using a PD-L2-Fc fusion protein in combination with radiotherapy did not result in a relevant response[25]. Still, the dual checkpoint inhibition with durvalumab (PD-L1) and tremelimumab (CTLA–4) combined with local ablation is currently being evaluated in the EORTC ILOC phase II study (NCT03101475).

Checkpoint inhibition with chemotherapy +/– VEGF-inhibitor (bevacizumab) or EGFR-antibody (cetuximab)

The induction of immunogenic cell death by oxaliplatin or changes in the immune contexture by 5-fluouracil showed synergistic effects with checkpoint inhibition in mice models of CRC[26]. Further, the addition of the EGFR antibody, cetuximab may lead to antibody dependent cellular cytotoxicity[27], and anti-angiogenic treatment with bevacizumab may lead to favorable changes in the microenvironment[28]. A combination of pembrolizumab (PD-1) with chemotherapy FOLFOX in 30 MCRC patients (including 3 MSI-H patients)[29] resulted in a 43% ORR and 16.9 mo PFS. Further, FOLFOX and VEGF-inhibitor bevacizumab in combination with atezolizumab (PD-L1) led to a 52% ORR and 14.1 mo PFS in 23 patients[30].

However, the addition of atezolizumab to maintenance therapy with fluoro-

pyrimidines and bevacizumab after 3-4 mo induction treatment with FOLFOX and bevacizumab did not result in an improvement of PFS [7.2 mo in the experimental arm *vs* 7.4 mo in the control arm (hazard ratio: 0.96, 95% confidence interval: 0.77-1.20), measured from randomization] (MODUL study, NCT02291289)[31] after median follow up of 18.7 mo. In total 445 MCRC patients (BRAF wildtype) were included and randomized (2:1 for atezolizumab treatment) in the largest randomized trial on immunotherapy in MCRC. Notably, OS curves split late after a similar median of 22.1 *versus* 21.9 mo resulting in a hazard ratio of 0.86 (95% confidence interval: 0.66-1.13).

Interesting results came from a single arm trial in the first line treatment of MCRC of applying an upfront combination of avelumab (PD-L1) with FOLFOX and the EGFR antibody cetuximab. An interim ORR of 75% in the first 20 patients[32] has been reported. Further clinical trials will evaluate the combination of avelumab and cetuximab in first line treatment setting or in the advanced disease setting with 5-fluorouracil, folinic acid and irinotecan (planned FIRE 6 study)[33].

Checkpoint inhibitors with tyrosine kinase inhibitors

In preclinical studies, enhanced T cell infiltration, upregulation of major histocompatibility complex and activation of antigen presenting cells was seen by combining MEK-inhibitors with PD-1/PD-L1 inhibitors[34,35]. In line with these results, a phase Ib study showed meaningful results using the combination of cobimetinib (MEK inhibitor) and atezolizumab (PD-L1) in 20 pretreated KRAS mutated MCRC patients with an ORR of 20% (4/20)[36]. However, the consecutive phase II clinical trial (IMblaze 370) did not show any difference in comparison to regorafenib (tyrosine kinase inhibitor) alone[37]. Although results from the maintenance treatment with cobimetinib and atezolizumab in comparison to 5-fluouracil and bevacizumab after 3-4 mo of FOLFOX treatment (MODUL study-NCT02291289) are still pending, the combination of MEK inhibitors with checkpoint inhibition is not expected to enter clinical use due to results from the IMblaze 370 trial.

TLR agonists

The innate immune system and especially dendritic cells are critical to mount proper immune responses under immune checkpoint inhibition[38]. TLR agonists stimulate the maturation of dendritic cells and account for the production of pro-inflammatory cytokines like IFN- This stimulates the adaptive immune system[39]. The IMPALA study (NCT02077868) evaluated maintenance treatment with MGN1703 (TLR-9 agonist) in comparison to investigator choice after at least stable disease following first line standard induction. Despite promising preliminary results from a single arm phase II study[40], results from the confirmatory IMPALA study are still pending.

Checkpoint inhibition and oncolytic viruses

A variety of viruses termed oncolytic viruses are used in clinical trials to specifically lyse tumor cells and stimulate the anti-cancer immune reaction, thereby acting as an in situ tumor vaccine[41]. Heavily pretreated CRC patients were treated with the oncolytic vaccinia virus (engineered to express GM-CSF, a hematopoietic growth factor that increases dendritic cell differentiation, maturation and function and induced tumor reactive T cells[42] and β-galactosidase) and reached stable disease in 67% (n = 10) of patients. The biweekly injection did not lead to dose-limiting toxicities in this phase Ib study alone[43] or in a phase I/II study in combination with checkpoint inhibitors tremelimumab (CTLA–4) and durvalumab (PD-L1)[44]. As seen in melanoma[45], the combination with checkpoint inhibitors further promises an increase in effectiveness. However, the first results using the combination of the vaccinia virus with checkpoint inhibitors are still pending (NCT03206073).

Adoptive cell therapy

In 2017, the FDA licensing of two chimeric antigen receptor (CAR) T cell products targeting CD19 for the treatment of acute refractory leukemia in children and B cell lymphoma in adults opened the field of adoptive cell therapy in clinical use. However, treatment of solid tumors is much more challenging due to limited trafficking and persistence of T cells into the tumor and an immunosuppressive environment[46,47]. Evidence for the impact of adoptive cell therapy in CRC comes from isolation, *ex vivo* expansion and re-infusion of tumor infiltrating lymphocytes[48]. Despite this elaborate work some quite encouraging results have been obtained. For example, after re-infusion of tumor reactive lymphocytes from tumor draining lymph nodes of MCRC patients an increase in OS from 14 mo (control, n = 16) to 28 mo (n = 9) was observed, although statistical significance was not reached[49]. More specifically, Tran *et al*[50] isolated, expanded and reinfused polyclonal CD8+ T cells from metastatic lung lesions of a MCRC patient reactive against mutant KRAS G12D. Subsequently six out of seven lung metastasis were eradicated with one remaining that lost the

chromosome 6 haplotype to escape reactive T cells.

Another approach transferring *ex vivo* expanded natural killer cells after treatment with IgG1 antibodies trastuzumab (HER2) or cetuximab (EGFR) and chemotherapy was well-tolerated, showed anti-tumor immune induction, and preliminary anti-tumor activity. Stable disease was observed in 67% (n = 6) of patients with advanced gastric or CRC[51].

CAR T cells in CRC are limited to the target antigen because tumor specific neoantigens are promising but not conserved between different patients. Tumor-associated antigens like CEA, EGFR or MUC1 are rather unspecific and could lead to AEs as seen by one death induced by ERBB2-specific CAR T cells[52] or respiratory toxicity by CEACAM5-specific CAR T cells[53]. However, the ERBB2-related death may have been due to the use of an excessive number of CAR T cells because ERBB2-specific CAR T cells were recently proven safe in sarcoma patients[54]. These potential toxicities in addition to the complex production of CAR T cell products further limit the breakthrough in CRC (despite the clinical potential that was validated for CEA-specific CAR T cells after percutaneous intra-artery infusion in a phase I study with an average decrease in CEA levels of 37% in 3 patients with high hepatic metastatic burden)[55]. In essence, CAR T cells, transfer of tumor infiltrating lymphocytes or natural killer cells are not yet ready for clinical use, but phase I or II studies may open up new avenues for future developments and are reviewed elsewhere[48].

FUTURE DEVELOPMENT

Future treatment strategies using immunotherapy to treat CRC will integrate the ever-improving knowledge about the molecular mechanisms that exclude or dampen the immune response in MCRC. Immune signatures revealed that TGF-β signaling is key in the development of CMS4 CRC and led to enhanced tumor metastatic capacities[13]. Preclinical models could show that targeting TGF-β can reset this immune excluding phenotype and may restore susceptibility to checkpoint inhibition[56]. Therefore, it presents an interesting target in future immune oncology of CRC.

Other promising strategies to target the "cold" lymphocyte excluded tumor microenvironment of pMMR/MSS CRC like chemotherapy, targeted therapy, oncolytic viruses, local ablation or TLR agonists already show some promise in early clinical or pre-clinical studies in combination with checkpoint inhibitors but are not yet ready for clinical use. Novel checkpoints like LAG-3[57] may further add to the arsenal of immune oncology in MCRC.

Together, targeting the immune exclusive microenvironment and the quality of tumor reactive T cells of pMMR/MSS CRC is promising, but most approaches still have to find their way from pre-clinical to clinical use. Therefore, approaches that combine already licensed targeted treatments, *e.g.*, EGFR-antibodies or VEGF-antibodies, and chemotherapy with checkpoint inhibitors might enter clinical use earlier if results can be confirmed.

REFERENCES

1 **McCarthy EF**. The toxins of William B. Coley and the treatment of bone and soft-tissue sarcomas. *Iowa Orthop J* 2006; **26**: 154-158 [PMID: 16789469]

2 **Brahmer JR**, Tykodi SS, Chow LQ, Hwu WJ, Topalian SL, Hwu P, Drake CG, Camacho LH, Kauh J, Odunsi K, Pitot HC, Hamid O, Bhatia S, Martins R, Eaton K, Chen S, Salay TM, Alaparthy S, Grosso JF, Korman AJ, Parker SM, Agrawal S, Goldberg SM, Pardoll DM, Gupta A, Wigginton JM. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. *N Engl J Med* 2012; **366**: 2455-2465 [PMID: 22658128 DOI: 10.1056/NEJMoa1200694]

3 **Giannakis M**, Mu XJ, Shukla SA, Qian ZR, Cohen O, Nishihara R, Bahl S, Cao Y, Amin-Mansour A, Yamauchi M, Sukawa Y, Stewart C, Rosenberg M, Mima K, Inamura K, Nosho K, Nowak JA, Lawrence MS, Giovannucci EL, Chan AT, Ng K, Meyerhardt JA, Van Allen EM, Getz G, Gabriel SB, Lander ES, Wu CJ, Fuchs CS, Ogino S, Garraway LA. Genomic Correlates of Immune-Cell Infiltrates in Colorectal Carcinoma. *Cell Rep* 2016; **15**: 857-865 [PMID: 27149842 DOI: 10.1016/j.celrep.2016.03.075]

4 **Llosa NJ**, Cruise M, Tam A, Wicks EC, Hechenbleikner EM, Taube JM, Blosser RL, Fan H, Wang H, Luber BS, Zhang M, Papadopoulos N, Kinzler KW, Vogelstein B, Sears CL, Anders RA, Pardoll DM, Housseau F. The vigorous immune microenvironment of microsatellite instable colon cancer is balanced by multiple counter-inhibitory checkpoints. *Cancer Discov* 2015; **5**: 43-51 [PMID: 25358689 DOI: 10.1158/2159-8290.CD-14-0863]

5 **Le DT**, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, Skora AD, Luber BS, Azad NS, Laheru D, Biedrzycki B, Donehower RC, Zaheer A, Fisher GA, Crocenzi TS, Lee JJ, Duffy SM, Goldberg RM, de la Chapelle A, Koshiji M, Bhaijee F, Huebner T, Hruban RH, Wood LD, Cuka N, Pardoll DM, Papadopoulos N, Kinzler KW, Zhou S, Cornish TC, Taube JM, Anders RA, Eshleman JR, Vogelstein B, Diaz LA. PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. *N Engl J Med* 2015; **372**: 2509-2520 [PMID: 26028255 DOI: 10.1056/NEJMoa1500596]

6 **Tran E**, Ahmadzadeh M, Lu YC, Gros A, Turcotte S, Robbins PF, Gartner JJ, Zheng Z, Li YF, Ray S,

Wunderlich JR, Somerville RP, Rosenberg SA. Immunogenicity of somatic mutations in human gastrointestinal cancers. *Science* 2015; **350**: 1387-1390 [PMID: 26516200 DOI: 10.1126/science.aad1253]

7 **Robbins PF**, Lu YC, El-Gamil M, Li YF, Gross C, Gartner J, Lin JC, Teer JK, Cliften P, Tycksen E, Samuels Y, Rosenberg SA. Mining exomic sequencing data to identify mutated antigens recognized by adoptively transferred tumor-reactive T cells. *Nat Med* 2013; **19**: 747-752 [PMID: 23644516 DOI: 10.1038/nm.3161]

8 **Gubin MM**, Zhang X, Schuster H, Caron E, Ward JP, Noguchi T, Ivanova Y, Hundal J, Arthur CD, Krebber WJ, Mulder GE, Toebes M, Vesely MD, Lam SS, Korman AJ, Allison JP, Freeman GJ, Sharpe AH, Pearce EL, Schumacher TN, Aebersold R, Rammensee HG, Melief CJ, Mardis ER, Gillanders WE, Artyomov MN, Schreiber RD. Checkpoint blockade cancer immunotherapy targets tumour-specific mutant antigens. *Nature* 2014; **515**: 577-581 [PMID: 25428507 DOI: 10.1038/nature13988]

9 **Turajlic S**, Litchfield K, Xu H, Rosenthal R, McGranahan N, Reading JL, Wong YNS, Rowan A, Kanu N, Al Bakir M, Chambers T, Salgado R, Savas P, Loi S, Birkbak NJ, Sansregret L, Gore M, Larkin J, Quezada SA, Swanton C. Insertion-and-deletion-derived tumour-specific neoantigens and the immunogenic phenotype: A pan-cancer analysis. *Lancet Oncol* 2017; **18**: 1009-1021 [PMID: 28694034 DOI: 10.1016/S1470-2045(17)30516-8]

10 **Guinney J**, Dienstmann R, Wang X, de Reyniès A, Schlicker A, Soneson C, Marisa L, Roepman P, Nyamundanda G, Angelino P, Bot BM, Morris JS, Simon IM, Gerster S, Fessler E, De Sousa E Melo F, Missiaglia E, Ramay H, Barras D, Homicsko K, Maru D, Manyam GC, Broom B, Boige V, Perez-Villamil B, Laderas T, Salazar R, Gray JW, Hanahan D, Tabernero J, Bernards R, Friend SH, Laurent-Puig P, Medema JP, Sadanandam A, Wessels L, Delorenzi M, Kopetz S, Vermeulen L, Tejpar S. The consensus molecular subtypes of colorectal cancer. *Nat Med* 2015; **21**: 1350-1356 [PMID: 26457759 DOI: 10.1038/nm.3967]

11 **Gong J**, Wang C, Lee PP, Chu P, Fakih M. Response to PD-1 Blockade in Microsatellite Stable Metastatic Colorectal Cancer Harboring a POLE Mutation. *J Natl Compr Canc Netw* 2017; **15**: 142-147 [PMID: 28188185 DOI: 10.6004/jnccn.2017.0016]

12 **Bourdais R**, Rousseau B, Pujals A, Boussion H, Joly C, Guillemin A, Baumgaertner I, Neuzillet C, Tournigand C. Polymerase proofreading domain mutations: New opportunities for immunotherapy in hypermutated colorectal cancer beyond MMR deficiency. *Crit Rev Oncol Hematol* 2017; **113**: 242-248 [PMID: 28427513 DOI: 10.1016/j.critrevonc.2017.03.027]

13 **Becht E**, de Reyniès A, Giraldo NA, Pilati C, Buttard B, Lacroix L, Selves J, Sautès-Fridman C, Laurent-Puig P, Fridman WH. Immune and Stromal Classification of Colorectal Cancer Is Associated with Molecular Subtypes and Relevant for Precision Immunotherapy. *Clin Cancer Res* 2016; **22**: 4057-4066 [PMID: 26994146 DOI: 10.1158/1078-0432.CCR-15-2879]

14 **Le DT**, Durham JN, Smith KN, Wang H, Bartlett BR, Aulakh LK, Lu S, Kemberling H, Wilt C, Luber BS, Wong F, Azad NS, Rucki AA, Laheru D, Donehower R, Zaheer A, Fisher GA, Crocenzi TS, Lee JJ, Greten TF, Duffy AG, Ciombor KK, Eyring AD, Lam BH, Joe A, Kang SP, Holdhoff M, Danilova L, Cope L, Meyer C, Zhou S, Goldberg RM, Armstrong DK, Bever KM, Fader AN, Taube J, Housseau F, Spetzler D, Xiao N, Pardoll DM, Papadopoulos N, Kinzler KW, Eshleman JR, Vogelstein B, Anders RA, Diaz LA. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. *Science* 2017; **357**: 409-413 [PMID: 28596308 DOI: 10.1126/science.aan6733]

15 **Overman MJ**, McDermott R, Leach JL, Lonardi S, Lenz HJ, Morse MA, Desai J, Hill A, Axelson M, Moss RA, Goldberg MV, Cao ZA, Ledeine JM, Maglinte GA, Kopetz S, André T. Nivolumab in patients with metastatic DNA mismatch repair-deficient or microsatellite instability-high colorectal cancer (CheckMate 142): An open-label, multicentre, phase 2 study. *Lancet Oncol* 2017; **18**: 1182-1191 [PMID: 28734759 DOI: 10.1016/S1470-2045(17)30422-9]

16 **Diaz LA**, Marabelle A, Delord J-P, Shapira-Frommer R, Geva R, Peled N, Kim TW, Andre T, Van Cutsem E, Guimbaud R, Jaeger D, Elez E, Yoshino T, Joe AK, Lam B, Gause CK, Pruitt SK, Kang SP, Le DT. Pembrolizumab therapy for microsatellite instability high (MSI-H) colorectal cancer (CRC) and non-CRC. *J Clin Oncol* 2017; **35**: 3071 [10.1200/JCO.2017.35.15_suppl.3071]

17 **Lenz HJJ**, Cardin D, Dragovich T, Shah U, Atasoy A, Postema R, Boyd Z, Ledeine J-M, Overman M, Lonardi S, Van Cutsem E, Limon ML, Wong KY, Hendlisz A, Aglietta M, Garcia-Alfonso P, Neyns B, Luppi G. LBA18_PR_Durable clinical benefit with nivolumab (NIVO) plus low-dose ipilimumab (IPI) as first-line therapy in microsatellite instability-high/mismatch repair deficient (MSI-H/dMMR) metastatic colorectal cancer (mCRC). *Ann Oncol* 2018; 29 [

18 **Grootscholten C**, Voest EE, Chalabi M, Kuiper M, Van Leerdam ME, Fanchi LF, Schumacher TN, Van den Berg JG, Snaebjornsson P, Aalbers AG, Beets GL, Nuijten E, Lopez-Yurda M, Maas M, Mertz M, Haanen JB, Kok M. LBA37_PR_Neoadjuvant ipilimumab plus nivolumab in early stage colon cancer. *Ann Oncol* 2018; 29 [DOI: 10.1093/annonc/mdy424.047]

19 **Hochster HS**, Bendell JC, Cleary JM, Foster P, Zhang W, He X, Hernandez G, Iizuka K, Eckhardt SG. Efficacy and safety of atezolizumab (atezo) and bevacizumab (bev) in a phase Ib study of microsatellite instability (MSI)-high metastatic colorectal cancer (mCRC). *J Clin Oncol* 2017; **35**: 673 [DOI: 10.1200/JCO.2017.35.4_suppl.673]

20 **Callahan MK**, Odunsi K, Sznol M, Nemunaitis JJ, Ott PA, Dillon PM, Park AJ, Schwarzenberger P, Ricciardi T, Macri MJ, Ryan A, Venhaus RR, Wolchok JD. Phase 1 study to evaluate the safety and tolerability of MEDI4736 (durvalumab, DUR) + tremelimumab (TRE) in patients with advanced solid tumors. In: Journal of Clinical Oncology. *J Clin Oncol* 2017; **35**: 3069 [DOI: 10.1200/JCO.2017.35.15_suppl.3069]

21 **Chen EX**, Jonker DJ, Kennecke HF, Berry SR, Couture F, Ahmad CE, Goffin JR, Kavan P, Harb M, Colwell B, Samimi S, Samson B, Abbas T, Aucoin N, Aubin F, Koski SL, Wei AC, Magoski NM, Tu D, O'Callaghan CJ. CCTG CO.26 trial: A phase II randomized study of durvalumab (D) plus tremelimumab (T) and best supportive care (BSC) versus BSC alone in patients (pts) with advanced refractory colorectal carcinoma (rCRC). *J Clin Oncol* 2019; **37**: 481 [DOI: 10.1200/JCO.2019.37.4_suppl.481]

22 **Mole RH**. Whole body irradiation; radiobiology or medicine? *Br J Radiol* 1953; 234-241 [PMID: 13042090 DOI: 10.1259/0007-1285-26-305-234]

23 **Reynders K**, Illidge T, Siva S, Chang JY, De Ruysscher D. The abscopal effect of local radiotherapy: Using immunotherapy to make a rare event clinically relevant. *Cancer Treat Rev* 2015; **41**: 503-510 [PMID: 25872878 DOI: 10.1016/j.ctrv.2015.03.011]

24 **Spiotto M**, Fu YX, Weichselbaum RR. The intersection of radiotherapy and immunotherapy: Mechanisms and clinical implications. *Sci Immunol* 2016; **1**: 28018989 [PMID: 28018989 DOI: 10.1126/sciimmunol.aag1266]

25 **Duffy AG**, Makarova-Rusher O V, Pratt D, Kleiner DE, Fioravanti S, Walker M, Carey S, Figg WD, Steinberg SM, Anderson V, Levy E, Krishnasamy V, Wood BJ, Jones J, Citrin DE, Greten TF. A pilot study of AMP-224, a PD-L2 Fc fusion protein, in combination with stereotactic body radiation therapy (SBRT) in patients with metastatic colorectal cancer. *J Clin Oncol* 2016; **34**: 560 [DOI: 10.1200/jco.2016.34.4_suppl.560]

26 **Dosset M**, Vargas TR, Lagrange A, Boidot R, Végran F, Roussey A, Chalmin F, Dondaine L, Paul C, Lauret Marie-Joseph E, Martin F, Ryffel B, Borg C, Adotévi O, Ghiringhelli F, Apetoh L. PD-1/PD-L1 pathway: An adaptive immune resistance mechanism to immunogenic chemotherapy in colorectal cancer. *Oncoimmunology* 2018; **7**: e1433981 [PMID: 29872568 DOI: 10.1080/2162402X.2018.1433981]

27 **Holubec L**, Polivka J, Safanda M, Karas M, Liska V. The Role of Cetuximab in the Induction of Anticancer Immune Response in Colorectal Cancer Treatment. *Anticancer Res* 2016; **36**: 4421-4426 [PMID: 27630277 DOI: 10.21873/anticanres.10985]

28 **Ott PA**, Hodi FS, Buchbinder EI. Inhibition of Immune Checkpoints and Vascular Endothelial Growth Factor as Combination Therapy for Metastatic Melanoma: An Overview of Rationale, Preclinical Evidence, and Initial Clinical Data. *Front Oncol* 2015; **5**: 202 [PMID: 26442214 DOI: 10.3389/fonc.2015.00202]

29 **Shahda S**, Noonan AM, Bekaii-Saab TS, O'Neil BH, Sehdev A, Shaib WL, Helft PR, Loehrer PJ, Tong Y, Liu Z, El-Rayes BF. A phase II study of pembrolizumab in combination with mFOLFOX6 for patients with advanced colorectal cancer. *J Clin Oncol* 2017; **35**: 3541 [DOI: 10.1200/JCO.2017.35.15_suppl.3541]

30 **Bendell JC**, Powderly JD, Lieu CH, Eckhardt SG, Hurwitz H, Hochster HS, Murphy JE, Funke RP, Rossi C, Wallin J, Waterkamp D, Pishvaian MJ. Safety and efficacy of MPDL3280A (anti-PDL1) in combination with bevacizumab (bev) and/or FOLFOX in patients (pts) with metastatic colorectal cancer (mCRC). *J Clin Oncol* 2015; **33**: 704 [DOI: 10.1200/jco.2015.33.3_suppl.704]

31 **Grothey A**, Tabernero J, Arnold D, De Gramont A, Ducreux MP, O'Dwyer PJ, Van Cutsem E, Bosanac I, Srock S, Mancao C, Gilberg F, Winter J, Schmoll H-J. LBA19_Fluoropyrimidine (FP) + bevacizumab (BEV) + atezolizumab vs FP/BEV in BRAFwt metastatic colorectal cancer (mCRC): Findings from Cohort 2 of MODUL – a multicentre, randomized trial of biomarker-driven maintenance treatment following first-line induct. *Ann Oncol* 2018; 29 [DOI: 10.1093/annonc/mdy424.020]

32 **Stein A**, Binder M, Al-Batran S-E, Hinke A, Waberer L, Goekkurt E, Meyer T, Statovci D, Depenbusch R, Riera-Knorrenschild J, Lorenzen S, Ettrich TJ, Doerfel S, Bokemeyer C, Hegewisch-Becker S. Avelumab and cetuximab in combination with FOLFOX in patients with previously untreated metastatic colorectal cancer (MCRC): Results of the safety run-in phase of the phase II AVETUX trial (AIO-KRK-0216). *J Clin Oncol* 2018; **36**: 3561 [DOI: 10.1200/JCO.2018.36.15_suppl.3561]

33 **Troiani T**, Martinelli E, Ciardiello D, Zanaletti N, Cardone C, Borrelli C, Avallone A, Falcone A, Maiello E, Bordonaro R, Santini D, Garufi C, G. De Braud F, Pinto C, Gridelli C, Ciardiello F. Phase II study of avelumab in combination with cetuximab in pre-treated RAS wild-type metastatic colorectal cancer patients: CAVE (cetuximab-avelumab) Colon. *J Clin Oncol* 2019; **37**: TPS731 [DOI: 10.1200/JCO.2019.37.4_suppl.TPS731]

34 **Ebert PJR**, Cheung J, Yang Y, McNamara E, Hong R, Moskalenko M, Gould SE, Maecker H, Irving BA, Kim JM, Belvin M, Mellman I. MAP Kinase Inhibition Promotes T Cell and Anti-tumor Activity in Combination with PD-L1 Checkpoint Blockade. *Immunity* 2016; **44**: 609-621 [PMID: 26944201 DOI: 10.1016/j.immuni.2016.01.024]

35 **Liu L**, Mayes PA, Eastman S, Shi H, Yadavilli S, Zhang T, Yang J, Seestaller-Wehr L, Zhang SY, Hopson C, Tsvetkov L, Jing J, Zhang S, Smothers J, Hoos A. The BRAF and MEK Inhibitors Dabrafenib and Trametinib: Effects on Immune Function and in Combination with Immunomodulatory Antibodies Targeting PD-1, PD-L1, and CTLA-4. *Clin Cancer Res* 2015; **21**: 1639-1651 [PMID: 25589619 DOI: 10.1158/1078-0432.CCR-14-2339]

36 **Bendell JC**, Kim TW, Goh BC, Wallin J, Oh D-Y, Han S-W, Lee CB, Hellmann MD, Desai J, Lewin JH, Solomon BJ, Chow LQM, Miller WH, Gainor JF, Flaherty K, Infante JR, Das-Thakur M, Foster P, Cha E, Bang Y-J. Clinical activity and safety of cobimetinib (cobi) and atezolizumab in colorectal cancer (CRC). *J Clin Oncol* 2016; **34**: 3502 [DOI: 10.1200/JCO.2016.34.15_suppl.3502]

37 **Bendell J**, Ciardiello F, Tabernero J, Tebbutt N, Eng C, Di Bartolomeo M, Falcone A, Fakih M, Kozloff M, Segal N, Sobrero A, Shi Y, Roberts L, Yan Y, Chang I, Uyei A, Kim T. LBA-004 Efficacy and safety results from IMblaze370, a randomised Phase III study comparing atezolizumab+cobimetinib and atezolizumab monotherapy vs regorafenib in chemotherapy-refractory metastatic colorectal cancer. *Ann Oncol* 2018; 29 [DOI: 10.1093/annonc/mdy208.003]

38 **Garris CS**, Arlauckas SP, Kohler RH, Trefny MP, Garren S, Piot C, Engblom C, Pfirschke C, Siwicki M, Gungabeesoon J, Freeman GJ, Warren SE, Ong S, Browning E, Twitty CG, Pierce RH, Le MH, Algazi AP, Daud AI, Pai SI, Zippelius A, Weissleder R, Pittet MJ. Successful Anti-PD-1 Cancer Immunotherapy Requires T Cell-Dendritic Cell Crosstalk Involving the Cytokines IFN-γ and IL-12. *Immunity* 2018; **49**: 1148-1161.e7 [PMID: 30552023 DOI: 10.1016/j.immuni.2018.09.024]

39 **Iwasaki A**, Medzhitov R. Regulation of adaptive immunity by the innate immune system. *Science* 2010; **327**: 291-295 [PMID: 20075244 DOI: 10.1126/science.1183021]

40 **Schmoll HJ**, Wittig B, Arnold D, Riera-Knorrenschild J, Nitsche D, Kroening H, Mayer F, Andel J, Ziebermayr R, Scheithauer W. Maintenance treatment with the immunomodulator MGN1703, a Toll-like receptor 9 (TLR9) agonist, in patients with metastatic colorectal carcinoma and disease control after chemotherapy: A randomised, double-blind, placebo-controlled trial. *J Cancer Res Clin Oncol* 2014; **140**: 1615-1624 [PMID: 24816725 DOI: 10.1007/s00432-014-1682-7]

41 **Lawler SE**, Speranza MC, Cho CF, Chiocca EA. Oncolytic Viruses in Cancer Treatment: A Review. *JAMA Oncol* 2017; **3**: 841-849 [PMID: 27441411 DOI: 10.1001/jamaoncol.2016.2064]

42 **Mach N**, Gillessen S, Wilson SB, Sheehan C, Mihm M, Dranoff G. Differences in dendritic cells stimulated in vivo by tumors engineered to secrete granulocyte-macrophage colony-stimulating factor or Flt3-ligand. *Cancer Res* 2000; **60**: 3239-3246 [PMID: 10866317 DOI: 10.1146/annurev.iy.09.040191.001415]

43 **Park SH**, Breitbach CJ, Lee J, Park JO, Lim HY, Kang WK, Moon A, Mun JH, Sommermann EM, Maruri Avidal L, Patt R, Pelusio A, Burke J, Hwang TH, Kirn D, Park YS. Phase 1b Trial of Biweekly Intravenous Pexa-Vec (JX-594), an Oncolytic and Immunotherapeutic Vaccinia Virus in Colorectal Cancer. *Mol Ther* 2015; **23**: 1532-1540 [PMID: 26073886 DOI: 10.1038/mt.2015.109]

44 **Morelli MP**, Xie C, Brar G, Floudas CS, Fioravanti S, Walker M, Mabry-Hrones D, Greten TF. A phase I/II study of pexa-vec oncolytic virus in combination with immune checkpoint inhibition in refractory

colorectal cancer: Safety report. *J Clin Oncol* 2019; **37**: 646 [DOI: 10.1200/JCO.2019.37.4_suppl.646]
45 **Chesney J**, Collichio F, Andtbacka RHI, Puzanov I, Glaspy J, Milhem M, Hamid O, Cranmer L, Saenger Y, Ross M, Chen L, Kim JJ, Kaufman HL. Interim safety and efficacy of a randomized (1:1), open-label phase 2 study of talimogene laherparepvec (T) and ipilimumab (I) vs I alone in unresected, stage IIIB-IV melanoma. *Ann Oncol* 2016; 27 [DOI: 10.1093/annonc/mdw379.04]
46 **Newick K**, Moon E, Albelda SM. Chimeric antigen receptor T-cell therapy for solid tumors. *Mol Ther Oncolytics* 2016; **3**: 16006 [PMID: 27162934 DOI: 10.1038/mto.2016.6]
47 **Abken H**. Adoptive therapy with CAR redirected T cells: The challenges in targeting solid tumors. *Immunotherapy* 2015; **7**: 535-544 [PMID: 26065478 DOI: 10.2217/imt.15.15]
48 **Fan J**, Shang D, Han B, Song J, Chen H, Yang JM. Adoptive Cell Transfer: Is it a Promising Immunotherapy for Colorectal Cancer? *Theranostics* 2018; **8**: 5784-5800 [PMID: 30555581 DOI: 10.7150/thno.29035]
49 **Zhen YH**, Liu XH, Yang Y, Li B, Tang JL, Zeng QX, Hu J, Zeng XN, Zhang L, Wang ZJ, Li XY, Ge HX, Winqvist O, Hu PS, Xiu J. Phase I/II study of adjuvant immunotherapy with sentinel lymph node T lymphocytes in patients with colorectal cancer. *Cancer Immunol Immunother* 2015; **64**: 1083-1093 [PMID: 25990075 DOI: 10.1007/s00262-015-1715-3]
50 **Tran E**, Robbins PF, Lu YC, Prickett TD, Gartner JJ, Jia L, Pasetto A, Zheng Z, Ray S, Groh EM, Kriley IR, Rosenberg SA. T-Cell Transfer Therapy Targeting Mutant KRAS in Cancer. *N Engl J Med* 2016; **375**: 2255-2262 [PMID: 27959684 DOI: 10.1056/NEJMoa1609279]
51 **Ishikawa T**, Okayama T, Sakamoto N, Ideno M, Oka K, Enoki T, Mineno J, Yoshida N, Katada K, Kamada K, Uchiyama K, Handa O, Takagi T, Konishi H, Kokura S, Uno K, Naito Y, Itoh Y. Phase I clinical trial of adoptive transfer of expanded natural killer cells in combination with IgG1 antibody in patients with gastric or colorectal cancer. *Int J Cancer* 2018; **142**: 2599-2609 [PMID: 29388200 DOI: 10.1002/ijc.31285]
52 **Sadelain M**, Brentjens R, Rivière I. The basic principles of chimeric antigen receptor design. *Cancer Discov* 2013; **3**: 388-398 [PMID: 23550147 DOI: 10.1158/2159-8290.CD-12-0548]
53 **Thistlethwaite FC**, Gilham DE, Guest RD, Rothwell DG, Pillai M, Burt DJ, Byatte AJ, Kirillova N, Valle JW, Sharma SK, Chester KA, Westwood NB, Halford SER, Nabarro S, Wan S, Austin E, Hawkins RE. The clinical efficacy of first-generation carcinoembryonic antigen (CEACAM5)-specific CAR T cells is limited by poor persistence and transient pre-conditioning-dependent respiratory toxicity. *Cancer Immunol Immunother* 2017; **66**: 1425-1436 [PMID: 28660319 DOI: 10.1007/s00262-017-2034-7]
54 **Hegde M**, DeRenzo CC, Zhang H, Mata M, Gerken C, Shree A, Yi Z, Brawley V, Dakhova O, Wu M-F, Liu H, Hicks J, Grilley B, Gee AP, Rooney CM, Brenner MK, Heslop HE, Wels W, Gottschalk S, Ahmed NM. Expansion of HER2-CAR T cells after lymphodepletion and clinical responses in patients with advanced sarcoma. *J Clin Oncol* 2017; **35**: 10508 [DOI: 10.1200/JCO.2017.35.15_suppl.10508]
55 **Katz SC**, Burga RA, McCormack E, Wang LJ, Mooring W, Point GR, Khare PD, Thorn M, Ma Q, Stainken BF, Assanah EO, Davies R, Espat NJ, Junghans RP. Phase I Hepatic Immunotherapy for Metastases Study of Intra-Arterial Chimeric Antigen Receptor-Modified T-cell Therapy for CEA+ Liver Metastases. *Clin Cancer Res* 2015; **21**: 3149-3159 [PMID: 25850950 DOI: 10.1158/1078-0432.CCR-14-1421]
56 **Tauriello DVF**, Palomo-Ponce S, Stork D, Berenguer-Llergo A, Badia-Ramentol J, Iglesias M, Sevillano M, Ibiza S, Cañellas A, Hernando-Momblona X, Byrom D, Matarin JA, Calon A, Rivas EI, Nebreda AR, Riera A, Attolini CS, Batlle E. TGFβ drives immune evasion in genetically reconstituted colon cancer metastasis. *Nature* 2018; **554**: 538-543 [PMID: 29443964 DOI: 10.1038/nature25492]
57 **Lichtenegger FS**, Rothe M, Schnorfeil FM, Deiser K, Krupka C, Augsberger C, Schlüter M, Neitz J, Subklewe M. Targeting LAG-3 and PD-1 to Enhance T Cell Activation by Antigen-Presenting Cells. *Front Immunol* 2018; **9**: 385 [PMID: 29535740 DOI: 10.3389/fimmu.2018.00385]

World Journal of Gastroenterology

Submit a Manuscript: https://www.f6publishing.com

World J Gastroenterol 2019 August 7; 25(29): 3929-3940

DOI: 10.3748/wjg.v25.i29.3929

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

MINIREVIEWS

Hepatocellular carcinoma in the post-hepatitis C virus era: Should we change the paradigm?

Hadar Meringer, Oren Shibolet, Liat Deutsch

ORCID number: Hadar Meringer (0000-0002-2624-2505); Oren Shibolet (0000-0003-6111-5067); Liat Deutsch (0000-0001-5022-4318).

Author contributions: All authors equally contributed to this paper with conception and design of the review, drafting, critical revision, editing, and approval of the final version.

Conflict-of-interest statement: No potential conflicts of interest. No financial support.

Manuscript source: Invited manuscript

Received: March 27, 2019
Peer-review started: March 28, 2019
First decision: April 16, 2019
Revised: May 29, 2019
Accepted: July 2, 2019
Article in press: July 3, 2019
Published online: August 7, 2019

P-Reviewer: Aravinthan AD, Barone M, Bartosch B, Jin C, Wang

Hadar Meringer, Oren Shibolet, Liat Deutsch, Department of Gastroenterology and Hepatology, Tel Aviv Sourasky Medical Center and the Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv 6423906, Israel

Corresponding author: Liat Deutsch, MD, Doctor, Department of Gastroenterology and Hepatology, Tel Aviv Sourasky Medical Center and the Sackler Faculty of Medicine, Tel Aviv University, 6 Weizmann St., Tel-Aviv 64239, Israel. liatml@tlvmc.gov.il
Telephone: +972-3-6974119
Fax: +972-3-6973422

Abstract

Hepatocellular carcinoma (HCC) is a common and deadly malignancy. The disease usually develops on a background of chronic liver disease. Until recently, the most common etiology was infection with the hepatitis C virus (HCV). The advent of direct-acting antiviral (DAA) therapies has been a major breakthrough in HCV treatment. Sustained virologic response can now be achieved in almost all treated patients, even in patients with a high risk for the development of HCC, such as the elderly or those with significant fibrosis. Early reports raised concerns of a high risk for HCC occurrence after DAA therapy both in patients with previous resection of tumors and those without previous tumors. As the World Health Organization's goals for eradication of HCV are being endorsed worldwide, the elimination of HCV seems feasible. Simultaneous to the decrease in the burden of cirrhosis from HCV, non-alcoholic fatty liver disease (NAFLD) incidence has been increasing dramatically including significant increased incidence of cirrhosis and HCC in these patients. Surprisingly, a substantial proportion of patients with NAFLD were shown to develop HCC even in the absence of cirrhosis. Furthermore, HCC treatment and potential complications are known to be influenced by liver steatosis. These changes in etiology and epidemiology of HCC suggest the beginning of a new era: The post–HCV era. Changes may eventually undermine current practices of early detection, surveillance and management of HCC. We focused on the risk of HCC occurrence and recurrence in the post–HCV era, the surveillance needed after DAA therapy and current studies in HCC patients with NAFLD.

Key words: Hepatocellular carcinoma; Hepatitis C virus; Direct-acting antivirals; Non-alcoholic fatty liver disease; Non-alcoholic steatohepatitis

L
S-Editor: Yan JP
L-Editor: A
E-Editor: Ma YJ

Core tip: Hepatocellular carcinoma (HCC) is a common and deadly malignancy. One of the leading risk factors for HCC occurrence is liver cirrhosis secondary to hepatitis C virus (HCV) infection. Direct-acting antiviral therapy has revolutionized HCV eradication due to high sustained virologic response rates. However, early reports argued an increased risk of HCC occurrence and recurrence. Recently, non-alcoholic fatty liver disease has become the most common liver disorder in Western countries and a major cause of HCC. We aimed to review the changes in HCC management in the face of the changing epidemiology in the post-HCV era.

Citation: Meringer H, Shibolet O, Deutsch L. Hepatocellular carcinoma in the post-hepatitis C virus era: Should we change the paradigm? *World J Gastroenterol* 2019; 25(29): 3929-3940
URL: https://www.wjgnet.com/1007-9327/full/v25/i29/3929.htm
DOI: https://dx.doi.org/10.3748/wjg.v25.i29.3929

INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cancer and the second most frequent cause of cancer-related death globally[1]. The incidence of HCC in all populations increases progressively with age, reaching a peak in the eight's decade[2]. Cirrhosis is a risk factor for tumor development regardless of its etiology[3]. One of the most common risk factors for HCC worldwide is cirrhosis secondary to chronic infection with either hepatitis C virus (HCV) or hepatitis B virus (HBV)[1]. The incidence of HCC shows high geographical divergence as most cases in Asia and Africa are attributable to HBV while HCV represents a major risk factor in western countries. The annual risk of HCC is as high as 3% in patients with cirrhosis and active HCV infection[4]. Direct-acting antiviral (DAA) therapy has revolutionized the treatment of HCV infection, because of its high efficacy and an excellent safety profile which enabled its use even in patients with decompensated liver disease, in whom interferon (IFN)-based regimens were not recommended[5]. The introduction of DAA agents has improved sustained virologic response (SVR) rates to more than 95% in all HCV genotypes and shortened treatment duration[6]. DAAs have shown high efficacy and safety even in special populations, as patients with human immunodeficiency virus coinfection, dialysis patients and patients with recurrent HCV infection after liver transplantation (LT)[5,7]. SVR reduces patients' risk of developing liver cirrhosis and was shown to cause regression of fibrosis[8,9]. In patients with decompensated cirrhosis that achieved SVR, reduction in Model for End-Stage Liver Disease scores and hepatic venous pressure gradient were observed[10-12]. Recent data has also shown that SVR reduces liver specific and all-cause mortality[13]. The WHO's goal to eradicate HCV might induce HCC risk reduction by preventing advancement of cirrhosis, allowing fibrosis regression and avoiding the carcinogenic effect of the virus[14,15]. However, early reports argued an increased risk of HCC occurrence and recurrence in patients achieving SVR[16-18]. In addition, financial resources needed for both simultaneously scaling up coverage of testing services and costs of therapy are major limitations, especially in resource limited countries[19].

Since 2014, the use of DAAs has decreased the burden of chronic HCV. Nonetheless, this decrease has been countered by a marked increase in the prevalence of nonalcoholic fatty liver disease (NAFLD)[20]. It is currently the second leading cause for LT and waitlist registration in males and females[21]. We aimed to review the changes in HCC management in the face of the changing epidemiology brought about by the advances in HCV therapy and the rise in incidence of NAFLD.

THE EFFECT OF DIRECT-ACTING ANTIVIRALS ON HCC

The introduction of DAA therapy led to short and long-term clinical benefits as a result of HCV elimination[14]. Previous prospective studies with IFN-based therapy concluded that treatment was strongly associated with a reduction in HCC risk[22-24]. A meta-analysis of 12 studies quantitatively evaluated the presumed benefit and showed that achieving an SVR with IFN was associated with a 76% reduction of HCC risk[22]. However, early studies of DAAs raised concerns that DAA-induced SVR didn't reduce occurrence of HCC and suggested a high risk of short-term recurrence in patients previously treated for HCC[16-18]. Conti *et al*[17] followed 344 consecutive

cirrhotic patients, without HCC, who were treated with DAA for 24 wk and reported HCC occurrence in 9/285 patients (3.2%) and HCC recurrence in 17/59 patients (28.8%) previously treated for HCC. Child-Pugh class and a history of HCC were independently associated with HCC development but neither HCV genotype nor therapeutic DAA regimen correlated to HCC occurrence. Additional reports suggested an alarmingly high rates of HCC occurrence with de-novo HCC diagnosis in 6/66 patients (9%) within 6 months of DAA therapy[16] and 4/54 patients (7.4%) after a median follow-up of 12 mo[18]. These studies were small, single-centered, uncontrolled, retrospective cohorts without long term follow up period which precluded definite conclusions. In contrast, multiple large cohort studies have since demonstrated that DAA-induced SVR is associated with reduced risk of HCC occurrence[25-27]. Among 22500 patients treated with DAA in the national Veterans Health Administration system, there were 271 new cases of HCC which developed after DAA treatment, including 183 in patients with SVR[25]. Additional 79/22579 (0.34%) cases developed during the course of DAA treatment and were excluded from primary analysis. The risk for HCC was higher in patients with cirrhosis than non-cirrhotics [adjusted hazard ratio (HR) = 4.73; 95% confidence interval (CI), 3.34-6.68] and SVR was associated with a 76% reduction in the risk of HCC compared with those who did not achieve SVR. Moreover, HCCs that were diagnosed during treatment were not more aggressive than those that occurred after the end of treatment. In a retrospective study by Ioannou *et al*. more than 60000 United States veterans with HCV that were treated with antiviral therapy between 1999-2015 including therapy with DAAs, IFN-based regimens or combined regimens were assessed[27]. HCC cases diagnosed within 6 months of treatment initiation were excluded. After adjustment to baseline characteristics, patients with DAA-induced SVR showed a 71% reduction in the risk for HCC compared to DAA-treatment failures. Furthermore, the reduction in HCC risk associated with SVR was similar irrespective of whether SVR was achieved by DAA-only, IFN alone or combined regimens, suggesting that eradication of HCV reduces the risk of HCC regardless of the antiviral regimen. A systematic meta-analysis of observational studies including 26 studies on HCC occurrence (IFN = 17, DAA = 9) reported higher HCC incidence in patients with DAA induced SVR than after IFN induced SVR (2.96/100 patient years and 1.14/100 patient years, respectively) but patients treated with DAAs were older and had a shorter follow-up[28]. In a meta-regression, after adjustment for study follow-up and age, DAA therapy was not associated with higher HCC occurrence compared to IFN. Additionally, a large national cohort of 17836 HCV-infected United States veterans (ERCHIVES database), compared DAA treated patients to IFN treated patients and untreated patients[26]. DAA-treated patients had a significantly higher HCC incidence rate than IFN treated patients but they also had a significantly higher rate of known risk factors for HCC, including cirrhosis, older age, and higher baseline Alfa-Feto protein level. A sub-analysis in cirrhotic patients (baseline FIB-4 score > 3.5) who achieved SVR, showed no significant difference in HCC incidence rate between the DAAs and IFN-treated groups (22.8 *vs* 21.2 cases per 1000 person-years, $P = 0.7$). Moreover, untreated cirrhotics had a twofold higher incidence rate than both treatment groups (45.31 cases per 1000 person-years, $P = 0.03$). Mariño *et al*[29] reported a 3.73% per 1000 person-years risk of developing HCC in 1123 cirrhotic patients treated with DAA during a median clinical follow-up of 19.6 mo. In agreement with results from the veterans' cohorts, the risk was higher in patients without SVR than those achieving SVR and with more severe disease (Child B or C, high liver stiffness measurement, the presence of clinically significant portal hypertension or decompensation). Moreover, Mariño *et al*[29] reported increased HCC risk (up to 3 times) with the presence of non-characterized nodules before DAAs treatment than in patients without or with well-defined benign nodules and concluded that a time-association to therapy is possible. It seems that the most important determinant of a lower HCC risk is HCV eradication, with a similar risk reduction irrespective of whether it is achieved by DAAs or IFN. However, greater absolute numbers of HCCs might be observed after DAA-based therapy because more patients are treated, and higher proportion are older with more advanced liver disease[14].

More controversial is whether there is a higher risk of tumor recurrence in patients with HCC treated with curative intent (either with resection or radio-frequency ablation) after achieving SVR. Unexpectedly high rates of HCC recurrence were reported in patients with complete radiologic response following DAA therapy[16,17]. HCC recurrence was detected in 17/59 (28.8%) patients in an Italian study[17] and in 16/58 (27.6%) patients in a Spanish population[16], during a median follow-up of 6 months. However, the small cohort size, lack of an untreated control arm, and short median duration of follow-up limited any definitive conclusions regarding the "pro"-malignant potential of anti-HCV treatment and the risk factors for recurrence[16,17]. Furthermore, the Spanish study also included patients treated with non-curative

therapies such as chemoembolization, characterized by high early recurrence rates[16].

Two large controlled studies as well as one propensity-score–adjusted analysis reported no increase in HCC recurrence in patients with adequately treated HCC who received DAAs compared to untreated patients[30-32]. In the French CUPILT cohort (Compassionate use of Protease Inhibitors in viral C Liver Transplantation), 314 HCC-liver transplant recipients were treated with DAAs[30]. The mean time between LT and the initiation of DAA was 67 ± 60 mo. HCC recurrence was observed in only seven patients (2.2%). Most of these patients (5/7) had factors predictive of a recurrence based on histologic criteria in the native liver. Moreover, two patients experienced recurrence after LT but before the introduction of DAA. Hence, incomplete treatment or mistaken initial staging of tumor burden might induce interpretation biases in retrospective studies. This may lead to an erroneous attribution of DAAs being responsible for HCC recurrence[16,30].

In order to further assess the risk of HCC recurrence after DAA it was compared with the risk after IFN treatment[28,33,34]. The same meta-analysis and meta-regression of studies comparing HCC incidence evaluated 17 studies on HCC recurrence after DAA and IFN therapy[28], there was no difference in HCC recurrence after adjusting for study follow-up and age. Furthermore, a Propensity score analysis from Japan also showed no significant difference in HCC recurrence rates between patients treated with IFN-based regimens or DAAs[33]. Cumulative incidence of HCC recurrence in patients who achieved an SVR was significantly lower than in patients without an SVR in both arms of treatment. Another study from Japan reported recurrence rate after DAA therapy of 39% and 61% at 1 and 2 years, respectively, without significant difference from IFN based therapy including the patterns of recurrence between groups[34]. Achievement of an SVR was not significantly associated with the risk of early HCC recurrence in a multivariate analysis but tumor factors such as a history of multiple HCC treatments or short recurrence-free period were found as independent risk factors for recurrence after antiviral therapy.

More evidence against an association of DAA therapy with HCC recurrence arises from a propensity-score weighted analysis of 149 LT candidates with HCV and HCC with initial complete response to loco-regional therapies[35]. DAA use was not associated with increased risk of HCC recurrence but rather was associated with reduced risk of waitlist dropout due to tumor progression or death. In addition, DAA use was not associated with decreased probability of LT or overall survival. Thus, the data suggests a significant net-benefit ratio for DAA use even in this special population.

POST-SVR HCC SURVEILLANCE

Nowadays, most patients with known chronic HCV have either received antiviral treatment or are expected to receive DAAs in the near future. Successful antiviral therapy leading to SVR in chronic HCV, decreases, but does not eliminate the risk of HCC[36]. Surveillance for HCC must therefore be continued following SVR for all HCV patients with advanced fibrosis (F3) and cirrhosis (F4)[37,38]. Despite this recommendation a cost-effectiveness analysis suggested that HCC surveillance is very unlikely to be cost-effective after achieving SVR in patients with advanced fibrosis, whereas both annual and biannual modalities were likely to be cost-effective for patients with cirrhosis[39]. Whether fibrosis regression translates into a reduced HCC risk beyond the benefit of achieving SVR is still unknown and further long term studies are needed to determine if patients who are proven to have marked reduction in fibrosis could discontinue surveillance[40]. D'Ambrosio *et al*[41] followed a small group of HCV patients treated with IFN-based regimens for almost 8 years after SVR but failed to prove any benefit of fibrosis regression on HCC occurrence. Furthermore, surveillance recommendations in HCV infected patients are currently based on a survival benefit for patients whose predicted HCC incidence exceed 1.5% per year but are based on older studies. With the advances in both antiviral therapy and current therapies of HCC, survival benefit may be seen with a lower threshold[36]. Recent studies tried to identify risk factors for HCC incidence after DAA therapy[26,42,43]. Lack of SVR was repeatedly found as the strongest predictor of HCC incidence after DAA therapy[26,36,42,43]. In a single center, longitudinal 3-years follow-up study, which included 565 cirrhotic patients, male gender, diabetes mellitus, and liver stiffness or FIB-4 score > 9 were found to be independent predictors of de-novo HCC. Nevertheless, diabetes mellitus was the only independent predictor of HCC recurrence[43]. Data from almost 2000 patients with 1-year follow-up suggested that age (> 50 years) and the presence of esophageal varices may predict HCC occurrence[44]. In contrast, patients within the "Extended Baveno Criteria" (Platelets >

110000/ μL and Liver Stiffness Measurement < 25 kPa), had a very low probability of developing HCC and could be candidates to a different surveillance program. Multivariate Cox regression analysis based on prospectively collected data from Italy showed that albumin level < 3.5 mg/dL and platelet count < 120 × 10^3/dL as well as absence of an SVR were independently associated with higher risk of HCC development[42]. Apparently, patients with a substantial risk for HCC after DAA induced SVR have other risk factors for HCC occurrence such as age, male sex or features of a severe liver disease[45]. Additionally, the metabolic syndrome showed an additive risk effect in patients with chronic viral hepatitis[38,46,47]. Patients with obesity, diabetes mellitus or the metabolic syndrome are probably still at risk for HCC, in spite of HCV eradication. Thus, it is important to estimate the risk of HCC occurrence in order to establish a proper and cost-effective screening strategy (Figure 1). Ioannou *et al*. developed and internally validated models for prediction of the risk for HCC by using baseline characteristics prior to antiviral treatment[36]. They identified four separate subgroups by cirrhosis and SVR status. HCC incidence was highest in the cirrhosis/no SVR subgroup and lowest in patients with no cirrhosis/SVR. Age, platelet count, aspartate aminotransferase/alanine aminotransferase ratio and albumin accounted for most of the prediction while other characteristics as sex, ethnicity, HCV genotype, body mass index (BMI), hemoglobin and INR had a smaller contribution. The risk model-based screening strategy showed superior net benefit than screening all cirrhotic patients or screening none of the non-cirrhotics. There is an intensive effort to validate sensitive and specific HCC blood-based biomarkers[48,49]. Potentially, these markers may be efficient in early HCC detection and may stratify patients according to their HCC risk. Thus, the strategy of one surveillance program fits all is being challenged as a result of the HCV revolution and stratifying patients according to risk factors seems reasonable but needs to be further validated. Figure 2 illustrates our suggested algorithms for HCC surveillance in HCV patients after DAA therapy according to HCC occurrence (Figure 2A) or recurrence (Figure 2B).

THE RISING INCIDENCE OF NAFLD INDUCED HCC

The global incidence of obesity has markedly increased in the last decades and so has the prevalence and incidence of NAFLD. It is estimated that in the United States, over 64 million people will be diagnosed with NAFLD, with annual direct medical costs of over $100 billion as a result of the high prevalence of the metabolic syndrome and its complications[50]. The definition of NAFLD is based on the evidence of hepatic steatosis (HS) and the absence of other known risk factors for hepatic fat accumulation (*i.e*., daily alcohol consumption, steatogenic medication usage, *etc*.)[38,51]. The liver histology differentiates between nonalcoholic fatty liver (NAFL) (less than 5% steatosis and no evidence of injury to hepatocytes) and nonalcoholic steatohepatitis (NASH) (steatosis is present in more than 5%, and so does hepatocellular injury, such as ballooning)[38,51]. Thus, the definitive diagnosis of NASH requires a liver biopsy.

NAFLD is the most common liver disorder in Western countries; Its prevalence is constantly rising from 15% in 2005 to 25% nowadays[52,53]. In 2016, a meta-analysis of 729 studies (a sample size of over 8 million subjects from 22 countries) estimated that the global prevalence of NAFLD is 25.24% (95%CI: 22.10-28.65) with the highest prevalence in the Middle East (31.79%, 95%CI: 13.48-58.23) and South America (30.45%, 95%CI: 22.74-39.440) while the lowest prevalence was reported from Africa (13.48%, 95%CI: 5.69-28.69)[54]. The prevalence of biopsy confirmed NASH among NAFLD patients ranged between 6.67%-29.85% in random biopsies to 60.64%-69.25% among patients with indicated biopsies. NAFLD is commonly referred to as the hepatic manifestation of the metabolic syndrome. It is associated with metabolic comorbidities such as obesity, diabetes mellitus type 2, and dyslipidemia[54,55]. Current recommendations by the European association for the study of the liver (EASL)[51] state that all individuals with steatosis should be screened for features of the metabolic syndrome and all individuals with metabolic features and persistently abnormal liver enzymes should be screened for NAFLD, because NAFLD is the main reason for unexpectedly elevated liver enzymes. Those metabolic comorbidities also correspond with the liver disease severity. In a Veterans Health Administration study of almost 400 patients, type 2 diabetes mellitus and BMI were the most significant predictors of advanced NAFLD [odds ratio (OR) 11.8, $P < 0.001$ and OR 1.4, $P < 0.001$, respectively][55]. The western diet is also a significant risk factor for NAFLD due to its high-calorie content, excess saturated fats, refined carbohydrates, sugar-sweetened beverages and high fructose intake[56,57]. Another risk factor is sedentary lifestyle which is more prevalent among NAFLD patients[58]. Several disease modifying genes have been investigated but only patatin like phospholipase domain containing 3

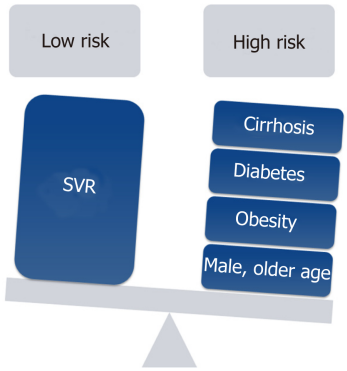


Figure 1 Risk factors and their association with hepatocellular carcinoma in patients with chronic hepatitis C virus infection. SVR: Sustained virologic response.

(PNPLA3) I148M variant at rs738409 was confirmed in multiple cohorts. It was initially identified from genome-wide association studies and was later correlated with disease severity, level of fibrosis and HCC development in patients with histologically proven NAFLD[59,60]. Recently, the transmembrane 6 superfamily member 2 (TM6SF2) gene has been reported as another disease modifier and the E167K variant was suggested to have clinical implication on progression to cirrhosis and HCC[61] and a possible protective effect regarding cardiovascular morbidity[62]. Moreover, a study by Koo *et al*[63] found that PNPLA3 and TM6SF2 risk variants have an additive effect on the risk for NASH (OR per risk allele, 2.03, 95%CI: 1.50-2.73, P < 0.001) and significant fibrosis (1.61, 95%CI: 1.19-2.17, P = 0.002) even when the model was adjusted for age, sex, CRP and insulin resistance. However, there are no current recommendations regarding HCC surveillance for carriers of these variants and genotyping in general is not yet recommended routinely[51].

Approximately 40% of NASH patients experience fibrosis progression[54]. According to a meta-analysis of 11 cohort and 411 patients with biopsy-proven NAFLD the fibrosis advancement rate is twofold higher in NASH compared to NAFL, corresponding to one fibrosis stage every 14.3 years in NAFL (95%CI: 9.1-50.0) and one every 7.1 years in NASH (95%CI: 4.8-14.3)[64]. In a study by Angulo *et al*[65] the stage of liver fibrosis and not the histologic features of steatosis was the determinant of overall mortality and liver-transplantation free-survival in patients with NAFLD. HCC occurrence significantly correlates with the degree of steatosis and stage of fibrosis[65]. In a recent large retrospective cohort study of nearly 600000 patients, the risk of HCC was 7-fold higher in patients with NAFLD than in matched controls (Adjusted HR 7.62 (5.76-10.09), P < 0.0001)[66]. In non-cirrhotic NAFLD patients HCC incidence rate per 1,000 PYs was 0.04 if FIB-4 was low (95%CI: 0.04-0.05) and 0.39 when FIB-4 was high (95%CI: 0.31-0.47). The presence of cirrhosis significantly increased the risk to 4.82 if FIB-4 was low (95%CI: 3.52-6.46), and 13.55 if FIB-4 was high (95%CI: 11.93-15.33)[66]. In a meta-analysis by Younossi *et al*[54] HCC incidence was 0.44 per 1000 person-years (95%CI: 0.29-0.66) in NAFLD patients and more than 12-fold higher in patients with NASH [5.29 per 1000 person-years (95%CI: 0.75-37.56)]. Although this incidence is significantly lower than that of chronic HBV or HCV[67], due to the high absolute number of patients with NAFLD and NASH worldwide this will obviously result in meaningful implications. Moreover, the incidence of NAFLD-related HCC has increased by 9% annually[68]. The incidence of HCC in patients with NAFLD is increased by associated features of the metabolic syndrome[69-71]. In terms of HCC-related mortality, in a large retrospective cohort study of the Surveillance, Epidemiology and End Results registries (2004-2009) which included approximately 5000 patients with HCC and 15000 matched-controls, NAFLD-HCC patients were older at diagnosis with shorter survival time than patients with viral hepatitis-associated HCC (1-year mortality: NAFLD-61.2%, HCV-51.3%, HBV-43.7%). NAFLD-HCC was found to be an independent risk factor for 1-year mortality with an OR of 1.21 (95%CI: 1.01-1.45)[68]. Some studies estimated that almost half of the cases of NASH-induced HCC arise in non-cirrhotic patients[72,73]. In a study by Mittal *et al*[74], around 13% of HCC reported in veterans did not have cirrhosis. Among other factors, having NAFLD was independently associated with HCC in the absence of cirrhosis. Nevertheless, according to the American association for the study of liver disease (AASLD) recommendations the risk of HCC is significantly lower in patients with NAFLD but without cirrhosis compared to NAFLD with cirrhosis, and surveillance is currently not recommended for these patients[67].

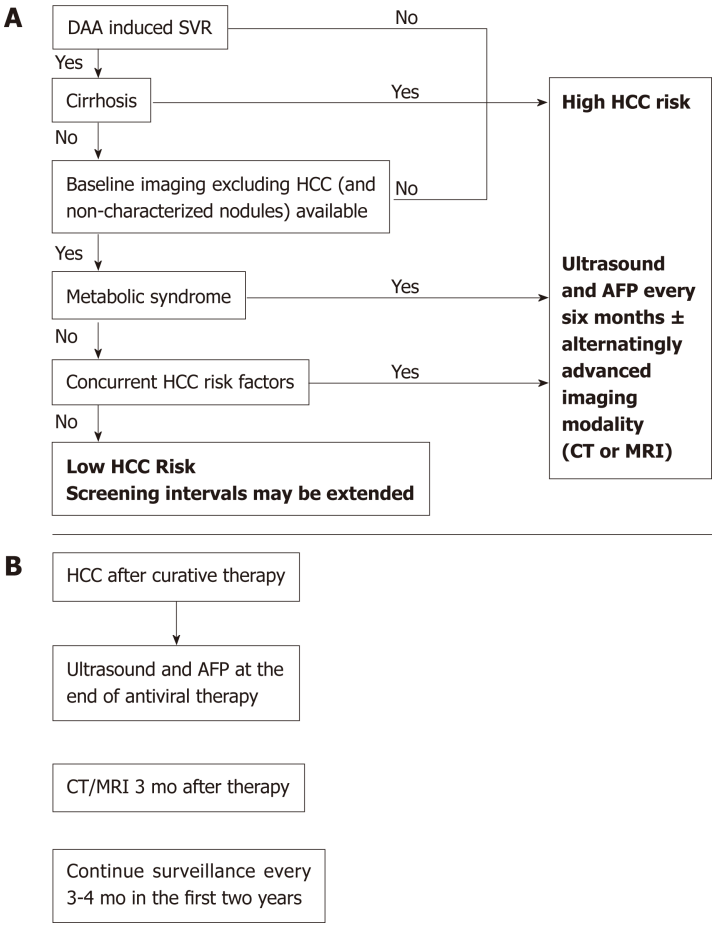


Figure 2 Our suggested algorithm for hepatitis C virus patients after antiviral therapy. A: Suggested algorithm for hepatitis C virus patients [without previous hepatocellular carcinoma (HCC)] after treatment with direct-acting antiviral according to their risk to develop HCC and recommended surveillance strategy; B: Our surveillance strategy for HCC recurrence after DAA therapy. SVR: Sustained virologic response; AFP: Alpha-fetoprotein; CT: Computed tomography; MRI: Magnetic resonance imaging; HCC: Hepatocellular carcinoma.

Abdominal ultrasound is the first-line diagnostic procedure for HCC due to its relatively low-cost and absence of radiation exposure. The ultrasound sensitivity for HCC detection is 58%-89% and the specificity exceeds 90% in the general cirrhotic population[75]. However, its diagnostic ability is frequently limited in NAFLD patients due to excess weight and HS. The increased BMI leads to attenuation of the ultrasound beam by subcutaneous fat and the HS might attenuate the ultrasound pulse and reduce deep hepatic structures visualization[76]. In a study of 941 cirrhotic patients who underwent abdominal ultrasound, 20% of the ultrasound studies were considered inadequate for HCC exclusion[77]. NASH related cirrhosis (OR 2.87, 95%CI: 1.71-4.80), and BMI category (OR 1.67, 95%CI: 1.45-1.93), were found to be independent risk factors for an inadequate study. Other radiological methods, such as computed tomography scans or magnetic resonance imaging, can be utilized for HCC screening but since the population at risk is so large, they are not cost-effective and there is no current evidence to support the use of these modalities for initial HCC screening.

Once HCC is diagnosed, treatment options and potential complications are influenced by the liver steatosis as well. The risk profile of liver resection with curative intent in NAFLD patients with metabolic syndrome and no advanced fibrosis is similar to cirrhotic patients[78]. Co-morbidities such as dyslipidemia, hypertension, diabetes mellitus, obesity, heart and lung chronic dysfunction are commonly observed in these patients and play a significant and negative prognostic role. NASH is also the second leading etiology of HCC-related LT. Between 2002 to 2012, the prevalence of NASH related HCC as an indication for LT increased by nearly 4-fold, while the prevalence of LT due to HCV related HCC increased only by 2-fold[20].

These worrisome findings raise some questions regarding proper HCC screening in the NAFLD population and screening for NAFLD in the general population. As mentioned, in the western countries the average prevalence of NAFLD is reaching a

quarter of the adult population, while our screening tools and appropriate treatment strategy are still inadequate. The EASL recommendations 2016[51] endorse screening for NAFLD in high-risk groups such as patients in diabetes mellitus or obesity clinics by liver enzymes and/or ultrasound as part of a routine work-up. On the other hand, the AASLD[38] recommendations recommend against routine screening for NAFLD because of the uncertain diagnostic accuracy and the limited treatment options alongside lack of cost-effectiveness of screening. A Markov model analysis suggested that screening for NASH in diabetic patients is currently not cost-effective due to a lack of an established effective treatment[79]. These data calls for establishment of a specific high-risk cohort within the NASH population which should undergo HCC surveillance.

CONCLUSION

DAA therapy is efficacious for HCV eradication with few side effects. The absolute risk of HCC occurrence or recurrence is mainly attributed to the more severe liver disease and older age of patients which can now be treated. There is no evidence that HCC occurrence or recurrence is different between patients treated with DAA or IFN therapy and the reduced risk is mainly associated with SVR. HCC surveillance is currently recommended after DAA therapy in all patients with cirrhosis albeit the risk might be reduced. Stratifying patients according to risk factors seems reasonable but needs further validation. In the last decades, NAFLD is becoming a major etiology of HCC in developed regions. The risk of HCC occurrence is increased by other features of the metabolic syndrome, evidence of NASH or advanced fibrosis. Moreover, NASH is an independent risk factor and can promote HCC development in non-cirrhotic patients. NAFLD associated fat depositions and inflammation can hinder HCC detection and treatment effectiveness. The HCC screening and surveillance protocols in the NAFLD population should be re-evaluated in this post-HCV era.

REFERENCES

1 **Global Burden of Disease Liver Cancer Collaboration**. Akinyemiju T, Abera S, Ahmed M, Alam N, Alemayohu MA, Allen C, Al-Raddadi R, Alvis-Guzman N, Amoako Y, Artaman A, Ayele TA, Barac A, Bensenor I, Berhane A, Bhutta Z, Castillo-Rivas J, Chitheer A, Choi JY, Cowie B, Dandona L, Dandona R, Dey S, Dicker D, Phuc H, Ekwueme DU, Zaki MS, Fischer F, Fürst T, Hancock J, Hay SI, Hotez P, Jee SH, Kasaeian A, Khader Y, Khang YH, Kumar A, Kutz M, Larson H, Lopez A, Lunevicius R, Malekzadeh R, McAlinden C, Meier T, Mendoza W, Mokdad A, Moradi-Lakeh M, Nagel G, Nguyen Q, Nguyen G, Ogbo F, Patton G, Pereira DM, Pourmalek F, Qorbani M, Radfar A, Roshandel G, Salomon JA, Sanabria J, Sartorius B, Satpathy M, Sawhney M, Sepanlou S, Shackelford K, Shore H, Sun J, Mengistu DT, Topór-Mądry R, Tran B, Ukwaja KN, Vlassov V, Vollset SE, Vos T, Wakayo T, Weiderpass E, Werdecker A, Yonemoto N, Younis M, Yu C, Zaidi Z, Zhu L, Murray CJL, Naghavi M, Fitzmaurice C. The Burden of Primary Liver Cancer and Underlying Etiologies From 1990 to 2015 at the Global, Regional, and National Level: Results From the Global Burden of Disease Study 2015. *JAMA Oncol* 2017; **3**: 1683-1691 [PMID: 28983565 DOI: 10.1001/jamaoncol.2017.3055]

2 **White DL**, Thrift AP, Kanwal F, Davila J, El-Serag HB. Incidence of Hepatocellular Carcinoma in All 50 United States, From 2000 Through 2012. *Gastroenterology* 2017; **152**: 812-820.e5 [PMID: 27889576 DOI: 10.1053/j.gastro.2016.11.020]

3 **West J**, Card TR, Aithal GP, Fleming KM. Risk of hepatocellular carcinoma among individuals with different aetiologies of cirrhosis: A population-based cohort study. *Aliment Pharmacol Ther* 2017; **45**: 983-990 [PMID: 28144999 DOI: 10.1111/apt.13961]

4 **El-Serag HB**. Hepatocellular carcinoma. *N Engl J Med* 2011; **365**: 1118-1127 [PMID: 21992124 DOI: 10.1056/NEJMra1001683]

5 **European Association for the Study of the Liver**. EASL Recommendations on Treatment of Hepatitis C 2018. *J Hepatol* 2018; **69**: 461-511 [PMID: 29650333 DOI: 10.1016/j.jhep.2018.03.026]

6 **Baumert TF**, Berg T, Lim JK, Nelson DR. Status of Direct-Acting Antiviral Therapy for Hepatitis C Virus Infection and Remaining Challenges. *Gastroenterology* 2019; **156**: 431-445 [PMID: 30342035 DOI: 10.1053/j.gastro.2018.10.024]

7 **AASLD-IDSA HCV Guidance Panel**. Hepatitis C Guidance 2018 Update: AASLD-IDSA Recommendations for Testing, Managing, and Treating Hepatitis C Virus Infection. *Clin Infect Dis* 2018; **67**: 1477-1492 [PMID: 30215672 DOI: 10.1093/cid/ciy585]

8 **Bachofner JA**, Valli PV, Kröger A, Bergamin I, Künzler P, Baserga A, Braun D, Seifert B, Moncsek A, Fehr J, Semela D, Magenta L, Müllhaupt B, Terziroli Beretta-Piccoli B, Mertens JC. Direct antiviral agent treatment of chronic hepatitis C results in rapid regression of transient elastography and fibrosis markers fibrosis-4 score and aspartate aminotransferase-platelet ratio index. *Liver Int* 2017; **37**: 369-376 [PMID: 27678216 DOI: 10.1111/liv.13256]

9 **Sporea I**, Lupușoru R, Mare R, Popescu A, Gheorghe L, Iacob S, Șirli R. Dynamics of liver stiffness values by means of transient elastography in patients with HCV liver cirrhosis undergoing interferon free treatment. *J Gastrointestin Liver Dis* 2017; **26**: 145-150 [PMID: 28617884 DOI: 10.15403/jgld.2014.1121.262.dyn]

10 **Mandorfer M**, Kozbial K, Schwabl P, Freissmuth C, Schwarzer R, Stern R, Chromy D, Stättermayer AF, Reiberger T, Beinhardt S, Sieghart W, Trauner M, Hofer H, Ferlitsch A, Ferenci P, Peck-Radosavljevic M. Sustained virologic response to interferon-free therapies ameliorates HCV-induced portal hypertension. *J*

Hepatol 2016; **65**: 692-699 [PMID: 27242316 DOI: 10.1016/j.jhep.2016.05.027]
11 **Foster GR**, Irving WL, Cheung MC, Walker AJ, Hudson BE, Verma S, McLauchlan J, Mutimer DJ, Brown A, Gelson WT, MacDonald DC, Agarwal K; HCV Research, UK. Impact of direct acting antiviral therapy in patients with chronic hepatitis C and decompensated cirrhosis. *J Hepatol* 2016; **64**: 1224-1231 [PMID: 26829205 DOI: 10.1016/j.jhep.2016.01.029]
12 **Lens S**, Alvarado-Tapias E, Mariño Z, Londoño MC, LLop E, Martinez J, Fortea JI, Ibañez L, Ariza X, Baiges A, Gallego A, Bañares R, Puente A, Albillos A, Calleja JL, Torras X, Hernández-Gea V, Bosch J, Villanueva C, Forns X, García-Pagán JC. Effects of All-Oral Anti-Viral Therapy on HVPG and Systemic Hemodynamics in Patients With Hepatitis C Virus-Associated Cirrhosis. *Gastroenterology* 2017; **153**: 1273-1283.e1 [PMID: 28734831 DOI: 10.1053/j.gastro.2017.07.016]
13 **Backus LI**, Belperio PS, Shahoumian TA, Mole LA. Impact of Sustained Virologic Response with Direct-Acting Antiviral Treatment on Mortality in Patients with Advanced Liver Disease. *Hepatology* 2019; **69**: 487-497 [PMID: 28749564 DOI: 10.1002/hep.29408]
14 **Ioannou GN**, Feld JJ. What Are the Benefits of a Sustained Virologic Response to Direct-Acting Antiviral Therapy for Hepatitis C Virus Infection? *Gastroenterology* 2019; **156**: 446-460.e2 [PMID: 30367836 DOI: 10.1053/j.gastro.2018.10.033]
15 **Lazarus JV**, Wiktor S, Colombo M, Thursz M; EASL International Liver Foundation. Micro-elimination - A path to global elimination of hepatitis C. *J Hepatol* 2017; **67**: 665-666 [PMID: 28760329 DOI: 10.1016/j.jhep.2017.06.033]
16 **Reig M**, Mariño Z, Perelló C, Iñarrairaegui M, Ribeiro A, Lens S, Díaz A, Vilana R, Darnell A, Varela M, Sangro B, Calleja JL, Forns X, Bruix J. Unexpected high rate of early tumor recurrence in patients with HCV-related HCC undergoing interferon-free therapy. *J Hepatol* 2016; **65**: 719-726 [PMID: 27084592 DOI: 10.1016/j.jhep.2016.04.008]
17 **Conti F**, Buonfiglioli F, Scuteri A, Crespi C, Bolondi L, Caraceni P, Foschi FG, Lenzi M, Mazzella G, Verucchi G, Andreone P, Brillanti S. Early occurrence and recurrence of hepatocellular carcinoma in HCV-related cirrhosis treated with direct-acting antivirals. *J Hepatol* 2016; **65**: 727-733 [PMID: 27349488 DOI: 10.1016/j.jhep.2016.06.015]
18 **Cardoso H**, Vale AM, Rodrigues S, Gonçalves R, Albuquerque A, Pereira P, Lopes S, Silva M, Andrade P, Morais R, Coelho R, Macedo G. High incidence of hepatocellular carcinoma following successful interferon-free antiviral therapy for hepatitis C associated cirrhosis. *J Hepatol* 2016; **65**: 1070-1071 [PMID: 27476768 DOI: 10.1016/j.jhep.2016.07.027]
19 **Ward JW**, Hinman AR. What Is Needed to Eliminate Hepatitis B Virus and Hepatitis C Virus as Global Health Threats. *Gastroenterology* 2019; **156**: 297-310 [PMID: 30391470 DOI: 10.1053/j.gastro.2018.10.048]
20 **Wong RJ**, Cheung R, Ahmed A. Nonalcoholic steatohepatitis is the most rapidly growing indication for liver transplantation in patients with hepatocellular carcinoma in the U.S. *Hepatology* 2014; **59**: 2188-2195 [PMID: 24375711 DOI: 10.1002/hep.26986]
21 **Noureddin M**, Vipani A, Bresee C, Todo T, Kim IK, Alkhouri N, Setiawan VW, Tran T, Ayoub WS, Lu SC, Klein AS, Sundaram V, Nissen NN. NASH Leading Cause of Liver Transplant in Women: Updated Analysis of Indications For Liver Transplant and Ethnic and Gender Variances. *Am J Gastroenterol* 2018; **113**: 1649-1659 [PMID: 29880964 DOI: 10.1038/s41395-018-0088-6]
22 **Morgan RL**, Baack B, Smith BD, Yartel A, Pitasi M, Falck-Ytter Y. Eradication of hepatitis C virus infection and the development of hepatocellular carcinoma: A meta-analysis of observational studies. *Ann Intern Med* 2013; **158**: 329-337 [PMID: 23460056 DOI: 10.7326/0003-4819-158-5-201303050-00005]
23 **Di Bisceglie AM**, Shiffman ML, Everson GT, Lindsay KL, Everhart JE, Wright EC, Lee WM, Lok AS, Bonkovsky HL, Morgan TR, Ghany MG, Morishima C, Snow KK, Dienstag JL; HALT-C Trial Investigators. Prolonged therapy of advanced chronic hepatitis C with low-dose peginterferon. *N Engl J Med* 2008; **359**: 2429-2441 [PMID: 19052125 DOI: 10.1056/NEJMoa0707615]
24 **Bruix J**, Poynard T, Colombo M, Schiff E, Burak K, Heathcote EJ, Berg T, Poo JL, Mello CB, Guenther R, Niederau C, Terg R, Bedossa P, Boparai N, Griffel LH, Burroughs M, Brass CA, Albrecht JK; EPIC3 Study Group. Maintenance therapy with peginterferon alfa-2b does not prevent hepatocellular carcinoma in cirrhotic patients with chronic hepatitis C. *Gastroenterology* 2011; **140**: 1990-1999 [PMID: 21419770 DOI: 10.1053/j.gastro.2011.03.010]
25 **Kanwal F**, Kramer J, Asch SM, Chayanupatkul M, Cao Y, El-Serag HB. Risk of Hepatocellular Cancer in HCV Patients Treated With Direct-Acting Antiviral Agents. *Gastroenterology* 2017; **153**: 996-1005.e1 [PMID: 28642197 DOI: 10.1053/j.gastro.2017.06.012]
26 **Li DK**, Ren Y, Fierer DS, Rutledge S, Shaikh OS, Lo Re V, Simon T, Abou-Samra AB, Chung RT, Butt AA. The short-term incidence of hepatocellular carcinoma is not increased after hepatitis C treatment with direct-acting antivirals: An ERCHIVES study. *Hepatology* 2018; **67**: 2244-2253 [PMID: 29205416 DOI: 10.1002/hep.29707]
27 **Ioannou GN**, Green PK, Berry K. HCV eradication induced by direct-acting antiviral agents reduces the risk of hepatocellular carcinoma. *J Hepatol* 2017; **pii**: S0168-8278(17)32273-0 [PMID: 28887168 DOI: 10.1016/j.jhep.2017.08.030]
28 **Waziry R**, Hajarizadeh B, Grebely J, Amin J, Law M, Danta M, George J, Dore GJ. Hepatocellular carcinoma risk following direct-acting antiviral HCV therapy: A systematic review, meta-analyses, and meta-regression. *J Hepatol* 2017; **67**: 1204-1212 [PMID: 28802876 DOI: 10.1016/j.jhep.2017.07.025]
29 **Mariño Z**, Darnell A, Lens S, Sapena V, Díaz A, Belmonte E, Perelló C, Calleja JL, Varela M, Rodriguez M, Rodriguez de Lope C, Llerena S, Torras X, Gallego A, Sala M, Morillas RM, Minguez B, Llaneras J, Coll S, Carrion JA, Iñarrairaegui M, Sangro B, Vilana R, Sole M, Ayuso C, Ríos J, Forns X, Bruix J, Reig M. Time association between hepatitis C therapy and hepatocellular carcinoma emergence in cirrhosis: Relevance of non-characterized nodules. *J Hepatol* 2019; **70**: 874-884 [PMID: 30684506 DOI: 10.1016/j.jhep.2019.01.005]
30 **ANRS collaborative study group on hepatocellular carcinoma (ANRS CO22 HEPATHER, CO12 CirVir and CO23 CUPILT cohorts)**. Lack of evidence of an effect of direct-acting antivirals on the recurrence of hepatocellular carcinoma: Data from three ANRS cohorts. *J Hepatol* 2016; **65**: 734-740 [PMID: 27288051 DOI: 10.1016/j.jhep.2016.05.045]
31 **Cheung MCM**, Walker AJ, Hudson BE, Verma S, McLauchlan J, Mutimer DJ, Brown A, Gelson WTH, MacDonald DC, Agarwal K, Foster GR, Irving WL, HCV Research UK. Outcomes after successful direct-acting antiviral therapy for patients with chronic hepatitis C and decompensated cirrhosis. *J Hepatol* 2016; **65**: 741-747 [PMID: 27388925 DOI: 10.1016/j.jhep.2016.06.019]
32 **Singal AG**, Rich NE, Mehta N, Branch A, Pillai A, Hoteit M, Volk M, Odewole M, Scaglione S, Guy J,

Said A, Feld JJ, John BV, Frenette C, Mantry P, Rangnekar AS, Oloruntoba O, Leise M, Jou JH, Bhamidimarri KR, Kulik L, Tran T, Samant H, Dhanasekaran R, Duarte-Rojo A, Salgia R, Eswaran S, Jalal P, Flores A, Satapathy SK, Wong R, Huang A, Misra S, Schwartz M, Mitrani R, Nakka S, Noureddine W, Ho C, Konjeti VR, Dao A, Nelson K, Delarosa K, Rahim U, Mavuram M, Xie JJ, Murphy CC, Parikh ND. Direct-Acting Antiviral Therapy Not Associated With Recurrence of Hepatocellular Carcinoma in a Multicenter North American Cohort Study. *Gastroenterology* 2019; **156**: 1683-1692.e1 [PMID: 30660729 DOI: 10.1053/j.gastro.2019.01.027]

33 **Nagata H**, Nakagawa M, Asahina Y, Sato A, Asano Y, Tsunoda T, Miyoshi M, Kaneko S, Otani S, Kawai-Kitahata F, Murakawa M, Nitta S, Itsui Y, Azuma S, Kakinuma S, Nouchi T, Sakai H, Tomita M, Watanabe M; Ochanomizu Liver Conference Study Group. Effect of interferon-based and -free therapy on early occurrence and recurrence of hepatocellular carcinoma in chronic hepatitis C. *J Hepatol* 2017; **67**: 933-939 [PMID: 28627363 DOI: 10.1016/j.jhep.2017.05.028]

34 **Nishibatake Kinoshita M**, Minami T, Tateishi R, Wake T, Nakagomi R, Fujiwara N, Sato M, Uchino K, Enooku K, Nakagawa H, Asaoka Y, Shiina S, Koike K. Impact of direct-acting antivirals on early recurrence of HCV-related HCC: Comparison with interferon-based therapy. *J Hepatol* 2019; **70**: 78-86 [PMID: 30336183 DOI: 10.1016/j.jhep.2018.09.029]

35 **Huang AC**, Mehta N, Dodge JL, Yao FY, Terrault NA. Direct-acting antivirals do not increase the risk of hepatocellular carcinoma recurrence after local-regional therapy or liver transplant waitlist dropout. *Hepatology* 2018; **68**: 449-461 [PMID: 29476694 DOI: 10.1002/hep.29855]

36 **Ioannou GN**, Green PK, Beste LA, Mun EJ, Kerr KF, Berry K. Development of models estimating the risk of hepatocellular carcinoma after antiviral treatment for hepatitis C. *J Hepatol* 2018; **69**: 1088-1098 [PMID: 30138686 DOI: 10.1016/j.jhep.2018.07.024]

37 **Heimbach JK**, Kulik LM, Finn RS, Sirlin CB, Abecassis MM, Roberts LR, Zhu AX, Murad MH, Marrero JA. AASLD guidelines for the treatment of hepatocellular carcinoma. *Hepatology* 2018; **67**: 358-380 [PMID: 28130846 DOI: 10.1002/hep.29086]

38 **Chalasani N**, Younossi Z, Lavine JE, Charlton M, Cusi K, Rinella M, Harrison SA, Brunt EM, Sanyal AJ. The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from the American Association for the Study of Liver Diseases. *Hepatology* 2018; **67**: 328-357 [PMID: 28714183 DOI: 10.1002/hep.29367]

39 **Farhang Zangneh H**, Wong WWL, Sander B, Bell CM, Mumtaz K, Kowgier M, van der Meer AJ, Cleary SP, Janssen HLA, Chan KKW, Feld JJ. Cost Effectiveness of Hepatocellular Carcinoma Surveillance After a Sustained Virologic Response to Therapy in Patients With Hepatitis C Virus Infection and Advanced Fibrosis. *Clin Gastroenterol Hepatol* 2018; **pii**: S1542-3565(18)31394-6 [PMID: 30580095 DOI: 10.1016/j.cgh.2018.12.018]

40 **D'Ambrosio R**, Colombo M. Should surveillance for liver cancer be modified in hepatitis C patients after treatment-related cirrhosis regression? *Liver Int* 2016; **36**: 783-790 [PMID: 26936383 DOI: 10.1111/liv.13106]

41 **D'Ambrosio R**, Aghemo A, Rumi MG, Degasperi E, Sangiovanni A, Maggioni M, Fraquelli M, Perbellini R, Rosenberg W, Bedossa P, Colombo M, Lampertico P. Persistence of hepatocellular carcinoma risk in hepatitis C patients with a response to IFN and cirrhosis regression. *Liver Int* 2018; **38**: 1459-1467 [PMID: 29377616 DOI: 10.1111/liv.13707]

42 **Calvaruso V**, Cabibbo G, Cacciola I, Petta S, Madonia S, Bellia A, Tinè F, Distefano M, Licata A, Giannitrapani L, Prestileo T, Mazzola G, Di Rosolini MA, Larocca L, Bertino G, Digiacomo A, Benanti F, Guarneri L, Averna A, Iacobello C, Magro A, Scalisi I, Cartabellotta F, Savalli F, Barbara M, Davì A, Russello M, Scifo G, Squadrito G, Cammà C, Raimondo G, Craxì A, Di Marco V; Rete Sicilia Selezione Terapia–HCV (RESIST-HCV). Incidence of Hepatocellular Carcinoma in Patients With HCV-Associated Cirrhosis Treated With Direct-Acting Antiviral Agents. *Gastroenterology* 2018; **155**: 411-421.e4 [PMID: 29655836 DOI: 10.1053/j.gastro.2018.04.008]

43 **Degasperi E**, D'Ambrosio R, Iavarone M, Sangiovanni A, Aghemo A, Soffredini R, Borghi M, Lunghi G, Colombo M, Lampertico P. Factors Associated With Increased Risk of De Novo or Recurrent Hepatocellular Carcinoma in Patients With Cirrhosis Treated With Direct-Acting Antivirals for HCV Infection. *Clin Gastroenterol Hepatol* 2019; **17**: 1183-1191.e7 [PMID: 30613002 DOI: 10.1016/j.cgh.2018.10.038]

44 **Lleo A**, Aglitti A, Aghemo A, Maisonneuve P, Bruno S, Persico M; collaborators. Predictors of hepatocellular carcinoma in HCV cirrhotic patients treated with direct acting antivirals. *Dig Liver Dis* 2019; **51**: 310-317 [PMID: 30473220 DOI: 10.1016/j.dld.2018.10.014]

45 **van der Meer AJ**, Feld JJ, Hofer H, Almasio PL, Calvaruso V, Fernández-Rodríguez CM, Aleman S, Ganne-Carrié N, D'Ambrosio R, Pol S, Trapero-Marugan M, Maan R, Moreno-Otero R, Mallet V, Hultcrantz R, Weiland O, Rutter K, Di Marco V, Alonso S, Bruno S, Colombo M, de Knegt RJ, Veldt BJ, Hansen BE, Janssen HLA. Risk of cirrhosis-related complications in patients with advanced fibrosis following hepatitis C virus eradication. *J Hepatol* 2017; **66**: 485-493 [PMID: 27780714 DOI: 10.1016/j.jhep.2016.10.017]

46 **Chen CL**, Yang HI, Yang WS, Liu CJ, Chen PJ, You SL, Wang LY, Sun CA, Lu SN, Chen DS, Chen CJ. Metabolic factors and risk of hepatocellular carcinoma by chronic hepatitis B/C infection: A follow-up study in Taiwan. *Gastroenterology* 2008; **135**: 111-121 [PMID: 18505690 DOI: 10.1053/j.gastro.2008.03.073]

47 **Yu MW**, Lin CL, Liu CJ, Yang SH, Tseng YL, Wu CF. Influence of Metabolic Risk Factors on Risk of Hepatocellular Carcinoma and Liver-Related Death in Men With Chronic Hepatitis B: A Large Cohort Study. *Gastroenterology* 2017; **153**: 1006-1017.e5 [PMID: 28711626 DOI: 10.1053/j.gastro.2017.07.001]

48 **Kisiel JB**, Dukek BA, V S R Kanipakam R, Ghoz HM, Yab TC, Berger CK, Taylor WR, Foote PH, Giama NH, Onyirioha K, Abdallah MA, Burger KN, Slettedahl SW, Mahoney DW, Smyrk TC, Lewis JT, Giakoumopoulos M, Allawi HT, Lidgard GP, Roberts LR, Ahlquist DA. Hepatocellular Carcinoma Detection by Plasma Methylated DNA: Discovery, Phase I Pilot, and Phase II Clinical Validation. *Hepatology* 2019; **69**: 1180-1192 [PMID: 30168613 DOI: 10.1002/hep.30244]

49 **El-Emshaty HM**, Entsar A, Gouida MS, ELSHAHAWY ZRJB. Associations between CD133, CK19 and G2/M in cirrhotic HCV (genotype-4) patients with or without accompanying tumor. *Biocell* 2018; **42**: 55-60

50 **Younossi ZM**, Blissett D, Blissett R, Henry L, Stepanova M, Younossi Y, Racila A, Hunt S, Beckerman R. The economic and clinical burden of nonalcoholic fatty liver disease in the United States and Europe. *Hepatology* 2016; **64**: 1577-1586 [PMID: 27543837 DOI: 10.1002/hep.28785]

51 **European Association for the Study of the Liver (EASL)**. European Association for the Study of

Diabetes (EASD); European Association for the Study of Obesity (EASO). EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. *J Hepatol* 2016; **64**: 1388-1402 [PMID: 27062661 DOI: 10.1016/j.jhep.2015.11.004]

52 **Vernon G**, Baranova A, Younossi ZM. Systematic review: The epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. *Aliment Pharmacol Ther* 2011; **34**: 274-285 [PMID: 21623852 DOI: 10.1111/j.1365-2036.2011.04724.x]

53 **Younossi ZM**. Non-alcoholic fatty liver disease - A global public health perspective. *J Hepatol* 2019; **70**: 531-544 [PMID: 30414863 DOI: 10.1016/j.jhep.2018.10.033]

54 **Younossi ZM**, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology* 2016; **64**: 73-84 [PMID: 26707365 DOI: 10.1002/hep.28431]

55 **Patel YA**, Gifford EJ, Glass LM, McNeil R, Turner MJ, Han B, Provenzale D, Choi SS, Moylan CA, Hunt CM. Risk factors for biopsy-proven advanced non-alcoholic fatty liver disease in the Veterans Health Administration. *Aliment Pharmacol Ther* 2018; **47**: 268-278 [PMID: 29115682 DOI: 10.1111/apt.14411]

56 **Barrera F**, George J. The role of diet and nutritional intervention for the management of patients with NAFLD. *Clin Liver Dis* 2014; **18**: 91-112 [PMID: 24274867 DOI: 10.1016/j.cld.2013.09.009]

57 **Chiu S**, Sievenpiper JL, de Souza RJ, Cozma AI, Mirrahimi A, Carleton AJ, Ha V, Di Buono M, Jenkins AL, Leiter LA, Wolever TM, Don-Wauchope AC, Beyene J, Kendall CW, Jenkins DJ. Effect of fructose on markers of non-alcoholic fatty liver disease (NAFLD): A systematic review and meta-analysis of controlled feeding trials. *Eur J Clin Nutr* 2014; **68**: 416-423 [PMID: 24569542 DOI: 10.1038/ejcn.2014.8]

58 **Gerber L**, Otgonsuren M, Mishra A, Escheik C, Birerdinc A, Stepanova M, Younossi ZM. Non-alcoholic fatty liver disease (NAFLD) is associated with low level of physical activity: A population-based study. *Aliment Pharmacol Ther* 2012; **36**: 772-781 [PMID: 22958053 DOI: 10.1111/apt.12038]

59 **Valenti L**, Al-Serri A, Daly AK, Galmozzi E, Rametta R, Dongiovanni P, Nobili V, Mozzi E, Roviaro G, Vanni E, Bugianesi E, Maggioni M, Fracanzani AL, Fargion S, Day CP. Homozygosity for the patatin-like phospholipase-3/adiponutrin I148M polymorphism influences liver fibrosis in patients with nonalcoholic fatty liver disease. *Hepatology* 2010; **51**: 1209-1217 [PMID: 20373368 DOI: 10.1002/hep.23622]

60 **Liu YL**, Patman GL, Leathart JB, Piguet AC, Burt AD, Dufour JF, Day CP, Daly AK, Reeves HL, Anstee QM. Carriage of the PNPLA3 rs738409 C >G polymorphism confers an increased risk of non-alcoholic fatty liver disease associated hepatocellular carcinoma. *J Hepatol* 2014; **61**: 75-81 [PMID: 24607626 DOI: 10.1016/j.jhep.2014.02.030]

61 **Liu YL**, Reeves HL, Burt AD, Tiniakos D, McPherson S, Leathart JB, Allison ME, Alexander GJ, Piguet AC, Anty R, Donaldson P, Aithal GP, Francque S, Van Gaal L, Clement K, Ratziu V, Dufour JF, Day CP, Daly AK, Anstee QM. TM6SF2 rs58542926 influences hepatic fibrosis progression in patients with non-alcoholic fatty liver disease. *Nat Commun* 2014; **5**: 4309 [PMID: 24978903 DOI: 10.1038/ncomms5309]

62 **Dongiovanni P**, Petta S, Maglio C, Fracanzani AL, Pipitone R, Mozzi E, Motta BM, Kaminska D, Rametta R, Grimaudo S, Pelusi S, Montalcini T, Alisi A, Maggioni M, Kärjä V, Borén J, Käkelä P, Di Marco V, Xing C, Nobili V, Dallapiccola B, Craxi A, Pihlajamäki J, Fargion S, Sjöström L, Carlsson LM, Romeo S, Valenti L. Transmembrane 6 superfamily member 2 gene variant disentangles nonalcoholic steatohepatitis from cardiovascular disease. *Hepatology* 2015; **61**: 506-514 [PMID: 25251399 DOI: 10.1002/hep.27490]

63 **Koo BK**, Joo SK, Kim D, Bae JM, Park JH, Kim JH, Kim W. Additive effects of PNPLA3 and TM6SF2 on the histological severity of non-alcoholic fatty liver disease. *J Gastroenterol Hepatol* 2018; **33**: 1277-1285 [PMID: 29193269 DOI: 10.1111/jgh.14056]

64 **Singh S**, Allen AM, Wang Z, Prokop LJ, Murad MH, Loomba R. Fibrosis progression in nonalcoholic fatty liver vs nonalcoholic steatohepatitis: A systematic review and meta-analysis of paired-biopsy studies. *Clin Gastroenterol Hepatol* 2015; **13**: 643-654.e1-9; quiz e39-40 [PMID: 24768810 DOI: 10.1016/j.cgh.2014.04.014]

65 **Angulo P**, Kleiner DE, Dam-Larsen S, Adams LA, Bjornsson ES, Charatcharoenwitthaya P, Mills PR, Keach JC, Lafferty HD, Stahler A, Haflidadottir S, Bendtsen F. Liver Fibrosis, but No Other Histologic Features, Is Associated With Long-term Outcomes of Patients With Nonalcoholic Fatty Liver Disease. *Gastroenterology* 2015; **149**: 389-397.e10 [PMID: 25935633 DOI: 10.1053/j.gastro.2015.04.043]

66 **Kanwal F**, Kramer JR, Mapakshi S, Natarajan Y, Chayanupatkul M, Richardson PA, Li L, Desiderio R, Thrift AP, Asch SM, Chu J, El-Serag HB. Risk of Hepatocellular Cancer in Patients With Non-Alcoholic Fatty Liver Disease. *Gastroenterology* 2018; **155**: 1828-1837.e2 [PMID: 30144434 DOI: 10.1053/j.gastro.2018.08.024]

67 **Marrero JA**, Kulik LM, Sirlin CB, Zhu AX, Finn RS, Abecassis MM, Roberts LR, Heimbach JK. Diagnosis, Staging, and Management of Hepatocellular Carcinoma: 2018 Practice Guidance by the American Association for the Study of Liver Diseases. *Hepatology* 2018; **68**: 723-750 [PMID: 29624699 DOI: 10.1002/hep.29913]

68 **Younossi ZM**, Otgonsuren M, Henry L, Venkatesan C, Mishra A, Erario M, Hunt S. Association of nonalcoholic fatty liver disease (NAFLD) with hepatocellular carcinoma (HCC) in the United States from 2004 to 2009. *Hepatology* 2015; **62**: 1723-1730 [PMID: 26274335 DOI: 10.1002/hep.28123]

69 **Schlesinger S**, Aleksandrova K, Pischon T, Jenab M, Fedirko V, Trepo E, Overvad K, Roswall N, Tjønneland A, Boutron-Ruault MC, Fagherazzi G, Racine A, Kaaks R, Grote VA, Boeing H, Trichopoulou A, Pantzalis M, Kritikou M, Mattiello A, Sieri S, Sacerdote C, Palli D, Tumino R, Peeters PH, Bueno-de-Mesquita HB, Weiderpass E, Quirós JR, Zamora-Ros R, Sánchez MJ, Arriola L, Ardanaz E, Tormo MJ, Nilsson P, Lindkvist B, Sund M, Rolandsson O, Khaw KT, Wareham N, Travis RC, Riboli E, Nöthlings U. Diabetes mellitus, insulin treatment, diabetes duration, and risk of biliary tract cancer and hepatocellular carcinoma in a European cohort. *Ann Oncol* 2013; **24**: 2449-2455 [PMID: 23720454 DOI: 10.1093/annonc/mdt204]

70 **Tsilidis KK**, Kasimis JC, Lopez DS, Ntzani EE, Ioannidis JP. Type 2 diabetes and cancer: Umbrella review of meta-analyses of observational studies. *BMJ* 2015; **350**: g7607 [PMID: 25555821 DOI: 10.1136/bmj.g7607]

71 **Dyson J**, Jaques B, Chattopadyhay D, Lochan R, Graham J, Das D, Aslam T, Patanwala I, Gaggar S, Cole M, Sumpter K, Stewart S, Rose J, Hudson M, Manas D, Reeves HL. Hepatocellular cancer: The impact of obesity, type 2 diabetes and a multidisciplinary team. *J Hepatol* 2014; **60**: 110-117 [PMID: 23978719 DOI: 10.1016/j.jhep.2013.08.011]

72 **Kodama K**, Kawaguchi T, Hyogo H, Nakajima T, Ono M, Seike M, Takahashi H, Nozaki Y, Kawanaka M, Tanaka S, Imajo K, Sumida Y, Kamada Y, Fujii H, Seko Y, Takehara T, Itoh Y, Nakajima A, Masaki N, Torimura T, Saibara T, Karino Y, Chayama K, Tokushige K. Clinical features of hepatocellular

carcinoma in nonalcoholic fatty liver disease patients without advanced fibrosis. *J Gastroenterol Hepatol* 2019 [PMID: 30668889 DOI: 10.1111/jgh.14608]

73 **Mohamad B**, Shah V, Onyshchenko M, Elshamy M, Aucejo F, Lopez R, Hanouneh IA, Alhaddad R, Alkhouri N. Characterization of hepatocellular carcinoma (HCC) in non-alcoholic fatty liver disease (NAFLD) patients without cirrhosis. *Hepatol Int* 2016; **10**: 632-639 [PMID: 26558795 DOI: 10.1007/s12072-015-9679-0]

74 **Mittal S**, El-Serag HB, Sada YH, Kanwal F, Duan Z, Temple S, May SB, Kramer JR, Richardson PA, Davila JA. Hepatocellular Carcinoma in the Absence of Cirrhosis in United States Veterans is Associated With Nonalcoholic Fatty Liver Disease. *Clin Gastroenterol Hepatol* 2016; **14**: 124-131.e1 [PMID: 26196445 DOI: 10.1016/j.cgh.2015.07.019]

75 **Bolondi L**. Screening for hepatocellular carcinoma in cirrhosis. *J Hepatol* 2003; **39**: 1076-1084 [PMID: 14642630 DOI: 10.1016/S0168-8278(03)00349-0]

76 **Uppot RN**, Sahani DV, Hahn PF, Kalra MK, Saini SS, Mueller PR. Effect of obesity on image quality: Fifteen-year longitudinal study for evaluation of dictated radiology reports. *Radiology* 2006; **240**: 435-439 [PMID: 16801372 DOI: 10.1148/radiol.2402051110]

77 **Simmons O**, Fetzer DT, Yokoo T, Marrero JA, Yopp A, Kono Y, Parikh ND, Browning T, Singal AG. Predictors of adequate ultrasound quality for hepatocellular carcinoma surveillance in patients with cirrhosis. *Aliment Pharmacol Ther* 2017; **45**: 169-177 [PMID: 27862091 DOI: 10.1111/apt.13841]

78 **European Association for the Study of the Liver**. EASL Clinical Practice Guidelines: Management of hepatocellular carcinoma. *J Hepatol* 2018; **69**: 182-236 [PMID: 29628281 DOI: 10.1016/j.jhep.2018.03.019]

79 **Corey KE**, Klebanoff MJ, Tramontano AC, Chung RT, Hur C. Screening for Nonalcoholic Steatohepatitis in Individuals with Type 2 Diabetes: A Cost-Effectiveness Analysis. *Dig Dis Sci* 2016; **61**: 2108-2117 [PMID: 26825843 DOI: 10.1007/s10620-016-4044-2]

World Journal of Gastroenterology

Submit a Manuscript: https://www.f6publishing.com

World J Gastroenterol 2019 August 7; 25(29): 3941-3955

DOI: 10.3748/wjg.v25.i29.3941

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Basic Study

Honokiol-enhanced cytotoxic T lymphocyte activity against cholangiocarcinoma cells mediated by dendritic cells pulsed with damage-associated molecular patterns

Arunya Jiraviriyakul, Worawat Songjang, Pongsathorn Kaewthet, Phachsita Tanawatkitichai, Punyapat Bayan, Sutatip Pongcharoen

ORCID number: Arunya Jiraviriyakul (0000-0003-0122-5984); Worawat Songjang (0000-0002-7799-2947); Pongsathorn Kaewthet (0000-0003-1338-4288); Phachsita Tanawatkitichai (0000-0002-4228-5302); Punyapat Bayan (0000-0001-9040-5017); Sutatip Pongcharoen (0000-0002-2415-9468).

Author contributions: Jiraviriyakul A and Pongcharoen S conceived the study; Jiraviriyakul A, Songjang W, Kaewthet P, Tanawatkitichai P and Bayan P performed experiments; Jiraviriyakul A, Songjang W and Pongcharoen S wrote the manuscript.

Supported by the grant from the Thailand Research Fund, No. BRG6180010; and Naresuan University Research Grant, No. R2561B001.

Institutional review board statement: The study was reviewed and approved by the Naresuan University Institutional Review Board.

Conflict-of-interest statement: All the Authors have no conflict of interest related to the manuscript.

Arunya Jiraviriyakul, Worawat Songjang, Sutatip Pongcharoen, Biomedical Science Graduate School, Faculty of Allied Health Sciences, Naresuan University, Phitsanulok 65000, Thailand

Arunya Jiraviriyakul, Worawat Songjang, Pongsathorn Kaewthet, Phachsita Tanawatkitichai, Punyapat Bayan, Department of Medical technology, Faculty of Allied Health Sciences, Naresuan University, Phitsanulok 65000, Thailand

Sutatip Pongcharoen, Division of Immunology, Department of Medicine, Faculty of Medicine, Naresuan University, Phitsanulok 65000, Thailand

Sutatip Pongcharoen, Research Centre of Academic Excellence in Petroleum, Petrochemical, and Advanced Materials, Faculty of Science, Naresuan University, Phitsanulok 65000, Thailand

Sutatip Pongcharoen, Centre of Excellence in Medical Biotechnology, Faculty of Medical Science, Naresuan University, Phitsanulok 65000, Thailand

Corresponding author: Sutatip Pongcharoen, MD, PhD, Division of Immunology, Department of Medicine, Faculty of Medicine, Naresuan University, Tapho District, Muang, Phitsanulok 65000, Thailand. sutatipp@nu.ac.th
Telephone: +66-89-5628060

Abstract

BACKGROUND

Cholangiocarcinoma or biliary tract cancer has a high mortality rate resulting from late presentation and ineffective treatment strategy. Since immunotherapy by dendritic cells (DC) may be beneficial for cholangiocarcinoma treatment but their efficacy against cholangiocarcinoma was low. We suggest how such anti-tumor activity can be increased using cell lysates derived from an honokiol-treated cholangiocarcinoma cell line (KKU-213L5).

AIM

To increase antitumour activity of DCs pulsed with cell lysates derived from honokiol-treated cholangiocarcinoma cell line (KKU-213L5).

METHODS

The effect of honokiol, a phenolic compound isolated from *Magnolia officinalis*, on choangiocarcinoma cells was investigated in terms of the cytotoxicity and the

Manuscript source: Unsolicited manuscript

Received: March 29, 2019
Peer-review started: March 29, 2019
First decision: June 10, 2019
Revised: June 21, 2019
Accepted: July 5, 2019
Article in press: July 5, 2019
Published online: August 7, 2019

P-Reviewer: Guo ZS, Hibberd AD, Ju SQ, Rajcani J, Uhlmann D
S-Editor: Yan JP
L-Editor: A
E-Editor: Ma YJ

expression of damage-associated molecular patterns (DAMPs). DCs were loaded with tumour cell lysates derived from honokiol-treated cholangiocarcinoma cells their efficacy including induction of T lymphocyte proliferation, proinflammatory cytokine production and cytotoxicity effect on target cholangiocarcinoma cells were evaluated.

RESULTS
Honokiol can effectively activate cholangiocarcinoma apoptosis and increase the release of damage-associated molecular patterns. DCs loaded with cell lysates derived from honokiol-treated tumour cells enhanced priming and stimulated T lymphocyte proliferation and type I cytokine production. T lymphocytes stimulated with DCs pulsed with cell lysates of honokiol-treated tumour cells significantly increased specific killing of human cholangiocarcinoma cells compared to those associated with DCs pulsed with cell lysates of untreated cholangiocarcinoma cells.

CONCLUSION
The present findings suggested that honokiol was able to enhance the immunogenicity of cholangiocarcinoma cells associated with increased effectiveness of DC-based vaccine formulation. Treatment of tumour cells with honokiol offers a promising approach as an *ex vivo* DC-based anticancer vaccine.

Key words: Cholangiocarcinoma; Dendritic cells; Honokiol; Damage-associated molecular patterns; Tumor cell lysates

Core tip: We constructed dendritic cells (DCs) loaded with cell lysates derived from honokiol-treated cholangiocarcinoma cells, with the aim of eliciting apoptosis in tumour cells and creating a broad array of tumour associated antigents in the form of dead and dying cells. Our data demonstrated that DCs primed with tumour cell lysates derived from honokiol-treated cholangiocarcinoma cells could improve the fuction of effector T lymphocytes in killing of the cancer cells. This suggested that honokiol enhanced the immunogenicity of cholangiocarcinoma antigens with increased effectiveness of DC-based vaccine formulation.

Citation: Jiraviriyakul A, Songjang W, Kaewthet P, Tanawatkitichai P, Bayan P, Pongcharoen S. Honokiol-enhanced cytotoxic T lymphocyte activity against cholangiocarcinoma cells mediated by dendritic cells pulsed with damage-associated molecular patterns. *World J Gastroenterol* 2019; 25(29): 3941-3955
URL: https://www.wjgnet.com/1007-9327/full/v25/i29/3941.htm
DOI: https://dx.doi.org/10.3748/wjg.v25.i29.3941

INTRODUCTION

Cholangiocarcinoma (CCA) is the most common biliary tract tumour and second commonest primary hepatic malignancy[1]. High incidence of CCA in Southeast Asia is strongly associated with liver fluke *Opisthorchis viverrini* infection, while numbers of cases in Europe and North America have significantly increased in recent decades. CCA has poor prognosis with high mortality rates since patients with early stages of cancer are often asymptomatic and no specific biomarkers for clinical diagnosis currently exist[2,3]. Unfortunately, surgical resection is also limited by advanced cancer metastasis and chemotherapeutic drugs have shown unsatisfactory outcome for survival in inoperable patients. Therefore, a new therapeutic strategy for CCA treatment and prevention should be urgently addressed.

Dendritic cells (DCs) are potent inducers of antitumour responses and they are often used as tumour antigen delivery vehicles in cancer therapy. DC cancer vaccines are aimed to stimulate anticancer immunity in patients through their capacity to activate tumour-specific T cells[4]. Incubating DCs with whole tumour lysates or killed cancer cells generates a broad array of tumour-associated antigens (TAAs) on DCs. Previous preclinical and clinical studies indicated that DCs loaded with tumour cell

lysates exhibit antitumour activity and can induce tumour regression in various cancers such as colon cancer[5], breast cancer[6], hepatocellular carcinoma[7] and CCA[8]. The efficacy of DCs loaded with whole CCA cell lysates has been argued in terms of tumour antigen properties and antitumour treatment[8]. Therefore, an improvement of tumour preparation protocol to enhance CCA immunogenicity for a putative DC cancer vaccine approach is urgently required.

Honokiol is a bioactive, biophenolic phytochemical compound extracted from *Magnolia officinalis* that has shown multiple pharmacological anti-inflammatory, anti-oxidant, anti-anxiety, anti-depressant, anti-stress and anti-tumour effects[9]. Previous studies have shown that honokiol can inhibit tumour growth both *in vitro* and in animal models by induction of cell apoptosis in many types of colon, breast, glioblastoma and liver cancers[9]. Interestingly, one recent study demonstrated that herbal-derived compounds can enhance the antitumour response of DCs loaded with tumour cell lysates by induction of cancer cell apoptosis and expression of damage-associated molecular patterns (DAMPs)[10]. Pulsing of DCs with DAMP components results in full activation of MyD88 signaling of DCs and activation of CD8+ lymphocytes leading to subsequent antitumour immune response[11]. Moreover, honokiol potentially suppresses the immunoresistant ability of glioblastoma without disrupting T lymphocyte function and may be recommended for combined immunotherapy[12].

Taken together, the efficacy of DC cancer vaccines against CCA requires improvement but untill now there have been no reports on the effect of pulsing DCs with tumour antigen generated by honokiol. Hence, here, we constructed DCs loaded with cell lysates derived from honokiol-treated CCA tumour cells, with the aim of eliciting apoptosis in tumour cells and creating a broad array of TAAs in the form of dead and dying cells. Effects of honokiol on the CCA cell line associated with *Opisthorchis viverrini*, the Southeast Asian liver fluke, were studied in terms of cell cytotoxicity and apoptosis inducer. Furthermore, CCA cell lysates were used as tumour antigens for loading into DCs grown *ex vivo* and the DCs were then characterised for their phenotypic features. Moreover, the efficacy of DCs pulsed with tumour cell lysates derived from honokiol-treated CCA cells was investigated in terms of stimulating T lymphocyte proliferation, type I cytokine production and cytotoxic activity. Our model improved cancer vaccine efficacy against CCA based on DCs and demonstrated the use of honokiol as a herbal-derived compound in combination with tumour antigen pulsed DCs to stimulate cytotoxic antitumour T lymphocytes.

MATERIALS AND METHODS

Cell lines

Well differentiated human CCA cell line, KKU-213L5 was obtained from the Japanese Collection of Research Bioresources Cell Bank (Osaka, Japan). The immortalized cholangiocyte, MMNK1 cell line was a gift from Prof. Naoya Kobayashi. The cell lines were maintained in Dulbecco's modified Eagle's medium (Gibco, Thermo Fisher Scientific, MA, United States), supplemented with 5% fetal bovine serum, 100 units/mL of penicillin, 100 μg/mL of streptomycin, and 0.25 μg/mL of amphotericin B. Cell grown in a humidified incubator at 37 °C with 5% CO_2.

Cell cytotoxicity

CCA cell line was seeded at a density of 5 × 10^3 cells/well in 96-well plate. After cultivation for 12 h, 0-100 μM honokiol were added at different concentrations. The cells were then further incubated for 24 and 48 h. Subsequently, 0.5 mg/mL of MTT reagent was added and incubated for another 4 h. After that, the formazan product was dissolved by DMSO and the light absorbance was read at 540 nm using microplate spectrophotometer (PerkinElmer, MA, United States). The percentage of cell viability was calculated following the formula [(honokiol treated Abs540)/(control Abs540)] × 100 (%).

Apoptosis analysis

Cell apoptosis was determined using the Muse™ Cell Analyzer from Millipore (MA, United States) following manufacturer's instruction. Briefly, honokiol treated cells were washed with phosphate buffered saline (PBS) and resuspended using the Annexin V and Dead Cell Reagent (7-AAD, Millipore, MA, United States). This was incubated for 20 min before assessment. The results were presented as the percentage of live cell, apoptotic cell and dead cell.

Western blot analysis

KKU-213L5 cells were incubated with honokiol at indicated concentrations for 20 h. For the analysis of intracellular proteins, treated cells were washed with ice-cold PBS before cell lysis using RIPA lysis buffer plus protease inhibitor cocktail (AMRESCO, OH, United States). Then, protein lysates were collected by centrifugation and the total protein concentration was qualified by using Bradford assay. In addition, the secreted protein was collected from conditioned medium, which was concentrated using Amicon® Ultra-2 Centrifugal Filter units (Millipore, MA, United States) following the manufacturer's instruction for 20× final concentration. The protein was then separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred onto the polyvinylidene fluoride membranes. After that, the non-specific binding was blocked with 5% skim milk buffer for 1 h before washing with TBST buffer. The membranes were then incubated with each primary antibody, anti-caspase 3 (Cell Signaling, MA, United States), anti-HMGB1 (ELabScience, TX, United States) and anti-HSP90 (Merck, Darmstadt, Germany) antibodies with gentle shaking at 4 °C overnight. Then, membranes were washed with TBST and incubated with horseradish peroxidase-linked anti-rabbit antibody (Cell Signaling, MA, United States) for 1 h at room temperature, and washed again before incubated with detection reagent. The image was developed by Chimidoc™ XRS (Bio-rad, CA, United States) and analyzed by Image Lab (Bio-rad, CA, United States).

Generation of human monocyte-derived DCs

Peripheral blood monocytes were isolated from healthy donors by gradient centrifugation using Ficoll-Hypaque and Percoll (GE Healthcare, Freiburg, Germany). The use of human blood with informed consent was approved by the ethics committee of Naresuan University (protocol No.0846/60). The monocyte fraction was resuspended in RPMI 1640 medium containing 10% foetal bovine serum (FBS) and 2 mM L-glutamine (Gibco, Thermo Fisher Scientific, MA, United States) in cell culture flask for 2 h. The non-adherent cells were gently removed before washing with PBS for 3 times. The adherent cells were then cultured in RPMI 1640 complete medium supplemented with 100 ng/mL of human recombinant granulocyte-macrophage colony-stimulating factor (GM-CSF, Miltenyi Biotec, Bergisch Gladbach, Germany) and 50 ng/mL of human recombinant interleukin-4 (IL-4, Miltenyi Biotec, Bergisch Gladbach, Germany) for 6 d. The medium was replaced every 3 d with fresh medium containing the same concentration of GM-CSF and IL-4.

Flow cytometric analysis

Flow cytometric analysis was carried out using the following antibodies: antihuman CD11c antibody-PE (eBioscience, CA, United States) and antihuman CD14 antibody-FITC (Abcam, Cambridge, United Kindom). Monocytes and DCs were harvested and washed with PBS containing 3% FBS before staining with fluorescent-conjugated antibodies for 45 min. After that cells were washed with PBS containing 3% FBS and suspended in FACs buffer (PBS containing 10% FBS). Stained cells were analyzed on Cytomics FC 500 using CXP software (Beckman Coulter, IN, United States).

Preparation of honokiol-derived tumor cell lysates

The CCA cell line were stained with CellTracker™ Red CMPTX (Thermo Fisher Scientific, MA, United States). Briefly, 6×10^6 cells were washed with PBS solution before incubation with fluorescent dye for 15 min. After that, the stained cells were washed twice with PBS and seeded at 6×10^6 cells per 100 mm dish. Then, 50 μM honokiol was add into the culture and incubated for 24 h. The honokiol treated cells were harvested and resuspended with 500 μL RPMI1640 serum free medium. Cell suspension was frozen in liquid nitrogen for 1.5 min and thawed in 37 °C water-bath for 5 min. This step of freezing and thawing was repeated for 3 times. Then, cell debris was removed by centrifugation at 5000 rpm for 10 min before collecting cell supernatant. In addition, the conditioned medium of honokiol treated tumor cells was collected and concentrated using Amicon® Ultra-2 Centrifugal Filter units (Millipore, MA, United States) for 20X final concentration. The protein concentration of tumor cell lysates and conditioned medium were measured using Bradford assay. These protein preparations were then used as honokiol derived-tumor cell lysates plus secreted protein.

Preparation of DCs pulsed with honokiol-derived tumor cell lysates

Immature DCs were harvested from induction medium and stained with CellTracker™ Green CMFDA (Thermo Fisher Scientific, MA, United States) following the protocol described above. After that, DCs were suspended in RPMI1640 complete medium, supplemented with tumor cell lysates derived from honokiol treated tumor cells at the amount of 2×10^5 DCs per 100 μg of tumor cell lysate and 20 μg of secreted protein. The DCs alone and DCs cocultured with tumor cell lysates were studied as

control and comparative groups, respectively. After 24 h, the DCs were maturated by adding 50 ng/mL of tumor necrosis factor alpha and 50 ng/mL of interferon gamma (IFN-γ) (ImmunoTool, Friesoythe, Germany) for another 24 h.

Fluorescence microscopic analysis

DCs-loaded with tumor cell lysates derived from honokiol-treated tumor cells were washed twice with PBS before they were seeded onto chamber slide and incubated for 12 h to allow cell adhesion. Then, adherent cells were fixed with 2% formadehyde for 20 min and mounted with prolong gold antifade reagent with DAPI (Invitrogen, CA, United States). Co-expression of green and red fluorescent was observed using EVOS fluorescent system (Invitrogen, CA, United States).

Effector T lymphocyte activation

To activate the effector cell T lymphocytes, autologous T lymphocytes were isolated using Ficoll-Hypaque and Percoll gradient centrifugation as described above. For T lymphocyte enrichment, lymphocyte fraction was resuspended in RMPI1640 medium and incubated with nylon wool column for 1 h. Non-adhered cells were collected by gently eluting with RMPI1640 medium. The samples of autologous T lymphocytes with CD3-positive cells of more than 70% as analyzed by flow cytometry were used in T lymphocyte activation study.

After loading of tumor cell lysates into DCs, different groups of DCs (unpulsed, pulsed with tumor cell lysates and pulsed with honokiol derived tumor cell lysates) were harvested as stimulator cells. The stimulator cells were then cocultured with autologous T lymphocytes in a 96 well culture plate at a ratio of 1:10. They were continually cultured for 5 d. The lymphocyte culture alone was set as a control. The proliferation of activated of T lymphocytes was measured using direct counting by trypan blue exclusion and MTT assays. The absorption (A) at 540 nm was used to calculate relative T lymphocyte proliferation rate as: A experiment/A control.

Cytokine analysis

During stimulation of effector T lymphocytes, the conditioned medium of different DCs (unpulsed, pulsed with tumor cell lysates and pulsed with honokiol derived tumor cell lysates) was collected at day 1, 3 and 5 for measurement of cytokines. IFN-γ and IL-12 concentrations in supernatants were measured by specific sandwich ELISA (PeproTech, NJ, United States) according to the manufacturer's instruction.

Cytotoxicity assay

DCs (unpulsed, pulsed with tumor cell lysates and pulsed with honokiol derived tumor cell lysates) were harvested as stimulator cells and cocultured with autologous T lymphocytes at a ratio of 1:10 for 5 d. Then, differently treated effector T cells were added to the target KKU-213L5 and MMNK-1 cells at ratios ranking from 1:10 to 1:20 and they were cultured for 24 and 48 h. The unbound cells were washed with PBS and the cells were photographed under microscopy. The viability of target cell was measured using MTT assay. The absorbance at 540 nm of effector cells only was set as control, and the absorption of different groups relative to control was calculated as the percentage of cell viability.

RESULTS

Honokiol induced CCA cell apoptosis and caused of DAMPs secretion

Results showed that honokiol significantly caused cell death in a dose- and time-dependent manner (Figure 1A and B). The IC_{50} of this compound at 24 and 48 h was 49.99 and 26.31 μM respectively with the underlying mechanism of cell death investigated using annexin V/PI staining. Treatment of honokiol for 24 h induced apoptosis of KKU-213L5 cells with significant increase in apoptotic cells in a dose-dependent manner (control = 3.93%, 50 μM honokiol = 30.4% and 70 μM honokiol = 52%) (Figure 1C). Increased apoptosis was confirmed by decrease of intact caspase-3, whereas cleaved caspase-3 increased (Figure 1D). Results suggested that honokiol was capable of inducing CCA death *via* cell apoptosis.

We investigated both intracellular and secreted protein expression of two DAMPs as the high mobility group box 1 (HMGB1) and heat shock protein90 (HSP90) molecules[13]. Results showed that treatment with honokiol at 50 μM concentration induced release of HMGB1 and HSP90 proteins in the conditioned medium. However, levels of intracellular HMGB1 and HSP90 did not change (Figure 1D). Data suggested that honokiol was able to induce CCA apoptosis with secretion of DAMPs.

DCs morphology and immunophenotype

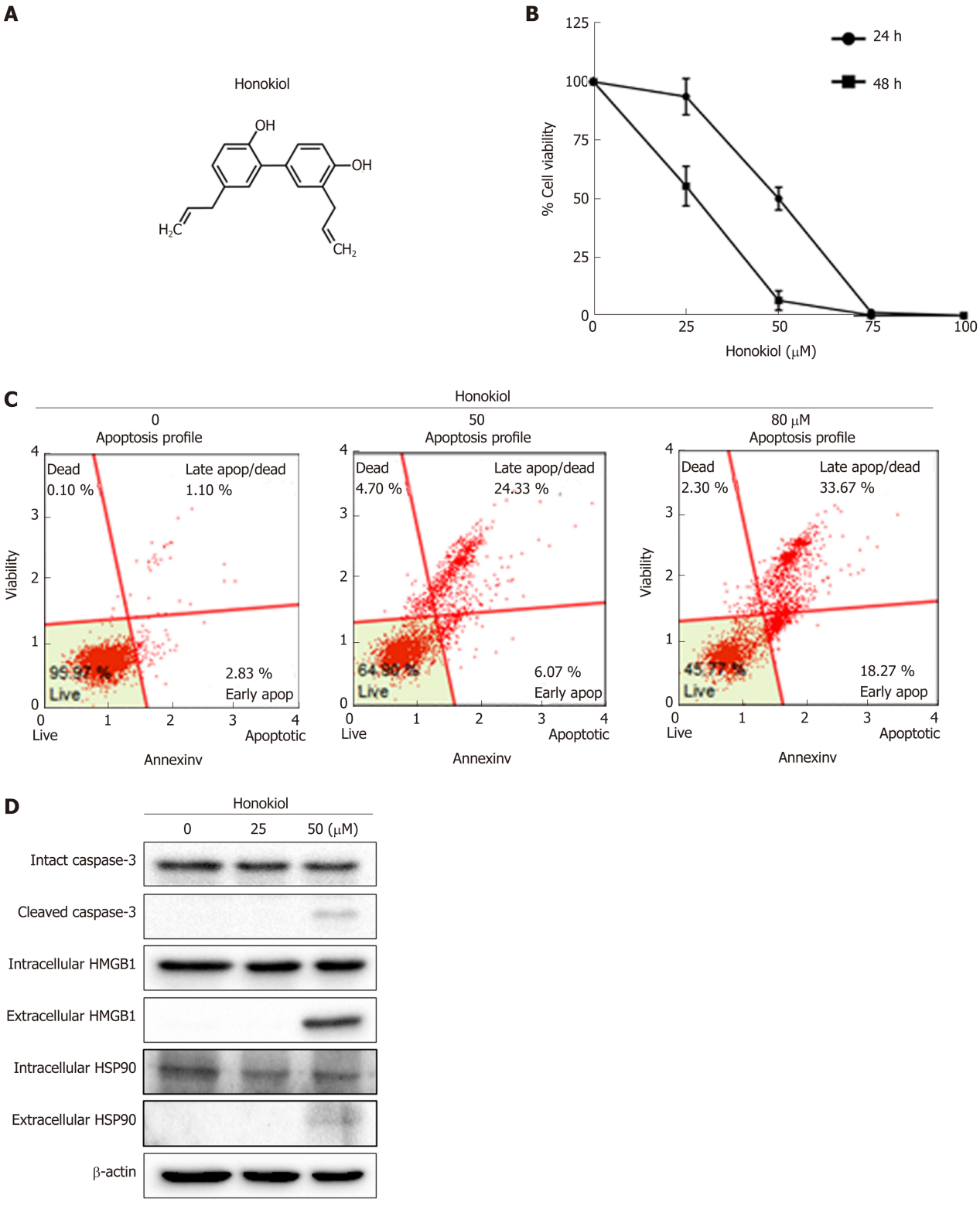


Figure 1 Honokiol induced cholangiocarcinoma cell apoptosis and cause of Damage- associated molecular pattern secretion. A: Chemical structure of honokiol; B: KKU-213L5 cells were treated with various concentrations of honokiol. After 24 and 48 h, cell viability was accessed using MTT assay. Negative control was cells treated with dimethyl sulfoxide and all groups were normalised with the control group. Results were presented as mean ± SD of three independent experiments; C: Treated KKU-213L5 cells were analyzed using Muse™ Cell Analyser with annexin V/PI staining. Annexin V versus propidium iodide from the gated cells revealed cell populations as live, early apoptotic, late apoptotic/dead and dead; D: Protein samples and conditioned medium were collected and analysed using Western blot. HMGB1: High mobility group box1; HSP: Heat shock protein.

This study used monocyte-derived DCs as a model to construct DCs loaded with tumour cell lysates. Peripheral blood monocytes were induced to become mature DCs and the differentiation was indicated by changing cell morphology from spherical to large dendritic shape and a more expanded shape of mature DCs (Figure 2A). The

cellular phenotype was confirmed by the expression of DC marker CD11c that dramatically increased from day 0 to day 6, whereas expression of the monocyte marker CD14 markedly decreased (Figure 2B). These results indicated that mature DCs were successfully generated *in vitro* from human peripheral blood.

Fluorescent microscopy evaluation of DCs loading with CCA tumor cell lysates

The ability of DCs to uptake honokiol-derived CCA tumour cell lysates was confirmed using fluorescence microscopy. Both immature DCs and tumour cell lysates were fluorescently labelled and cocultured for 24 h. Image analysis revealed co-localisation of green and red fluorescence in DCs pulsed with honokiol-derived tumour cell lysates (Figure 3), whereas green and red fluorescence appeared separately in DCs and honokiol-derived CCA tumour cell lysates only groups (Figure S1). Localisation of tumour antigen was indicated by 100× magnification which showed cytoplasmic localisation of tumour antigen on DCs, suggesting that generated immature DCs had phagocytic activity and were able to uptake honokiol-derived CCA tumour cell lysates.

DCs loaded with honokiol-derived tumor cell lysates induced T lymphocyte proliferation

To determine the effect of DCs loaded with honokiol-derived CCA tumour cell lysates on T lymphocyte proliferation, autologous T lymphocytes were cocultured with various types of DCs including unpulsed DCs, DCs pulsed with tumour cell lysates and DCs pulsed with honokiol-derived tumour cell lysates. After 5 d, lymphocyte number was reflected. Results showed that DCs loaded with honokiol-derived tumour cell lysates tended to increase T lymphocyte number compared with unpulsed DCs and DCs loaded with tumour cell lysates (Figure 4A). As well as relative lymphocyte proliferation (Figure 4B), DCs pulsed with honokiol-derived tumour cell lysates induced significantly higher T lymphocyte proliferation than untreated CCA antigen, suggesting that tumour cell lysates derived from honokiol-treated CCA cells may differentially activate DCs and mediate T lymphocyte proliferation.

DCs pulsed with tumor cell lysates derived from honokiol-treated CCA cells induced cytokines production

IFN-γ and IL-12 production were measured in supernatants collected from a culture system containing autologous T lymphocytes and DCs unloaded or loaded with tumour cell lysates and or tumour cell lysates derived from honokiol-treated CCA cells. Figure 5 shows that production of IFN-γ by DCs loaded with tumour cell lysates derived from honokiol-treated CCA cells significantly increased at day 1, 3 and 5 compared with control and unloaded DC groups (Figure 5A). Moreover, IL-12 production in similar conditions also significantly increased at day 3 and 5 (Figure 5B). Interestingly, between DCs-loaded with tumour cell lysates groups, production of both cytokines by DCs loaded with tumour cell lysates derived from honokiol-treated CCA cells was significantly higher than DCs loaded with tumour cell lysates. Results indicated that tumour cell lysates derived from honokiol-treated CCA cells enhanced cytokine production by DCs and activated T lymphocytes.

Tumor cell lysates derived from honokiol-treated CCA cells enhanced T lymphocyte killing of CCA cells

We further investigated the effect of DCs loaded with tumour cell lysates derived from honokiol-treated CCA cells on specific T lymphocyte killing effect of CCA cells. Autologous T lymphocytes were stimulated with different groups of DCs before collecting as effector cells and continually cocultured with KKU-213L5 at indicated ratios. After coculture, numbers of remaining target cells in the honokiol-treated group markedly decreased compared to unloaded DCs and DCs loaded with tumour cell lysates (Figure 6A). Moreover, results of specific killing effect measured using MTT assay showed that DCs primed with honokiol-derived tumour cell lysates and tumour cell lysates gave significantly more enhanced killing activity on target cells than naïve T lymphocytes, unloaded DCs and DCs loaded with tumour cell lysates (Figure 6B and C). Moreover, this specific killing of CCA cells by effector T lymphocytes activated by DCs loaded with CCA KKU-213L5 cell lysates was confirmed because coculturing of these T cells with the human cholangiocyte cell line (MMNK1) for 48 h did not significantly increase MMNK-1 cell death (Figure 6D). These findings suggested that DCs primed with cell lysates from honokiol-treated CCA cells specifically enhanced cytotoxic activity of effector T lymphocytes.

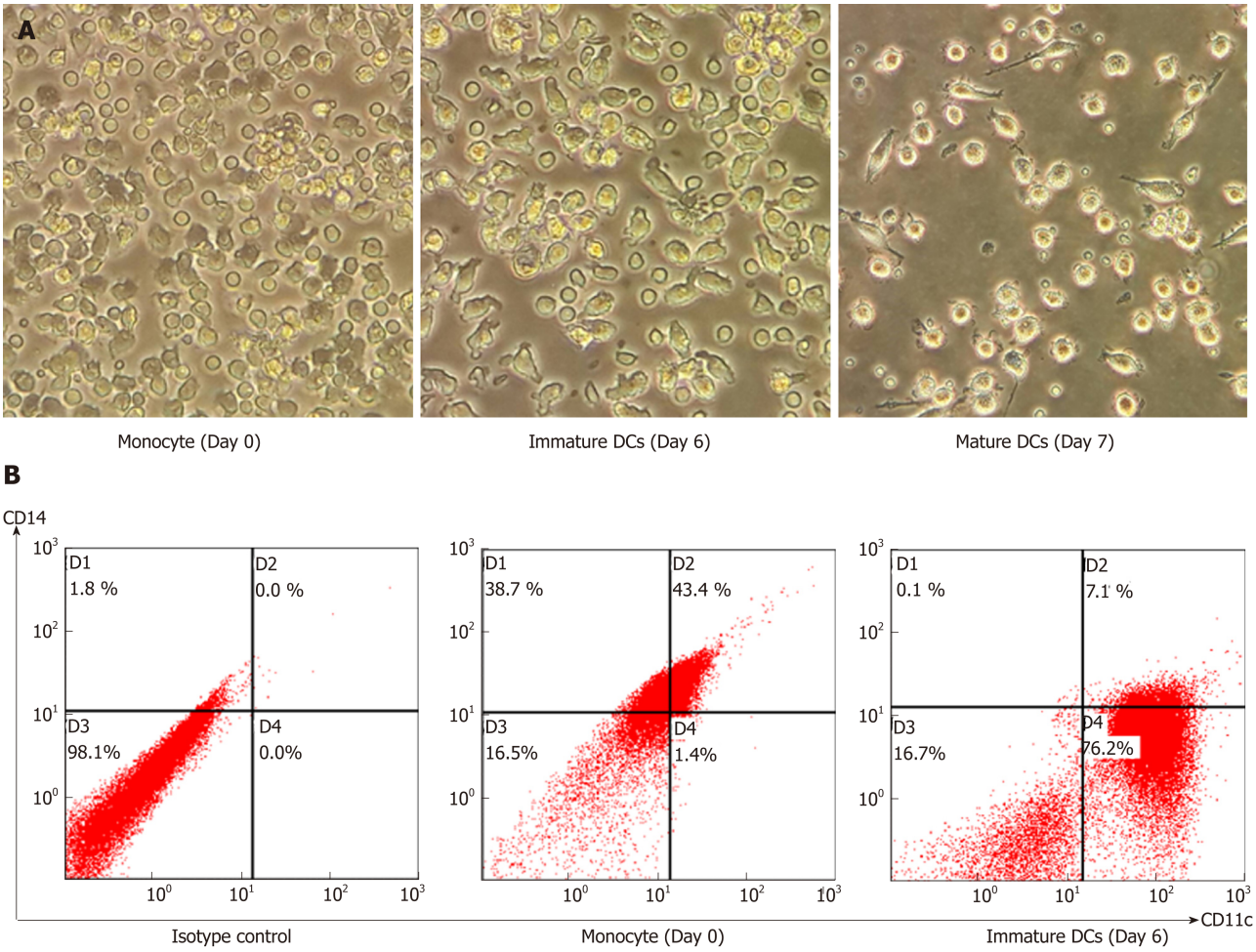


Figure 2 Morphology and immunophenotype of human monocyte-derived dendritic cells. Human monocytes were cultured in RPMI1640 medium supplemented with granulocyte-macrophage colony-stimulating factor and interleukin-4 for 6 d. Immature dendritic cells were then stimulated to become mature by adding tumor necrosis factor-α and interferon-γ for 1 d. A: Cell morphology of monocytes. Immature and mature dendritic cells were observed under inverted microscopy at 40×; B: Expressions of CD14 and CD11c human monocytes and mature DCs were presented as percentage of gated cells using flow cytometry CXP software. DCs: Dendritic cells.

DISCUSSION

In the last decade, incidence of CCA has globally increased. Advanced metastatic stages of CCA cannot be treated by surgery. Moreover, palliative treatment by chemotherapy is generally unsuccessful because extreme chemoresistance leads to poor prognosis and high mortality rates[3]. Immunotherapy is used in cancer clinical trials. For CCA, DC cancer vaccines have been studied in non-*Opisthorchis viverrini*-associated CCA, including loading of DCs with synthetic peptide antigens[14] and tumour lysate-pulsed DCs plus *ex vivo* adoptive transfer T cells[15]. Recently, DCs loaded with pooled mRNA and tumour cell lysates of *Opisthorchis viverrini*-associated CCA were shown to effectively kill human CCA *in vitro*[8]. DCs loaded with whole tumour antigen could probably activate polyclonal effector immune cells since the broad array of tumour antigens would effectively eliminate the heterogeneous tumour. In particular, use of tumour cell lysates would be most feasible because the preparation process is easy to manipulate and inexpensive compared with other procedures. However, the efficacy of DCs loaded with tumour cell lysates is limited by antigen processing and presentation, mostly mediated by MHC class II to CD4+ T cells[16]. Here, using tumour cell lysates from honokiol-treated CCA cells to prime DCs, we demonstrated specific T lymphocyte killing enhancement of CCA cells mediated by primed DCs. Honokiol is known to have diverse pharmacological effects, including antitumour activities[9]. It also has immunoadjuvant activity. One previous study reported that honokiol activates cancer cell apoptosis either by receptor- or mitochondria-mediated mechanisms[17]. Moreover, honokiol could avtivate cancer cells death by other mechanism such as autophagy and necrosis[18,19]. We demonstrated that honokiol exhibited a cytotoxic effect on CCA that is likely to be mediated by activation of caspase-3. Moreover, the IC_{50} of honokiol on cholangiocyte

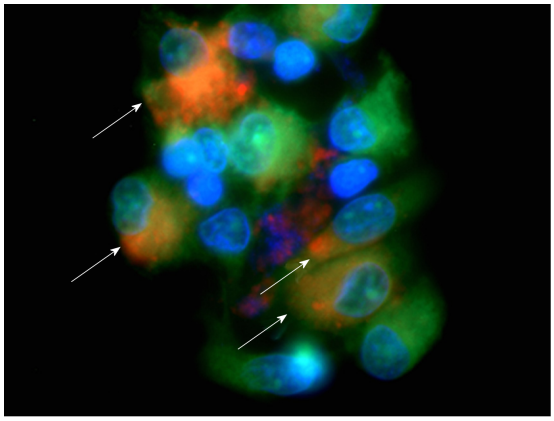


Figure 3 Investigation of tumour antigen on dendritic cells by fluorescence microscopy. Dendritic cells were stained with CellTracker™ Green CMFDA before loading with honokiol-derived tumour cell lysates pre-stained with CellTracker™ Red CMPTX. Antigen load was verified by visualisation of tumour antigen (red) within dendritic cells (green). Arrowheads indicate co-expression of green and red fluorescence. DCs: Dendritic cells.

and human derived-macrophage is higher than KKU-213L5, meaning that this compound shows less cytotoxicity on normal cells compared with cancer cells (manuscript in preparation). Immunogenic cell death is mainly mediated by expression of DAMPs which release or expose molecules of injured, damaged and apoptotic cells[13]. We examined the expression of two members of DAMPs as secreted HMGB1 and HSP90. The results indicated that both molecules were expressed when CCA cells were treated with honokiol at sub IC_{50}, the same concentration that caused cell apoptosis. Therefore, we concluded that honokiol exhibited cytotoxicity against CCA cells by induction of cell apoptosis and caused DAMP expression in these cells. HMGB1 is a non-histone nuclear protein that responds to damage signals by translocation from the nucleus to extracellular space which then activates the immune system[20]. HMGB1 proficiently interacts with pattern recognition receptors including advanced glycosylation end product-specific receptor and toll-like receptor 4 (TLR4)[21]. Binding of extracellular HMGB1 with TLR4 on the surface of DCs can stimulate the MYD88-dependent signalling pathway that leads to optimal antigen processing[22]. Moreover, loading of tumour cell lysates plus immunogenic cell death molecules including HMGB1 can enhance DC maturation and antitumour activities in DC-based anticancer vaccine[10]. HSP90 is a molecular chaperone and an important driver for the posttranslational modification process. High expression of HSP90 is associated with poor prognosis in CCA patients[23]. Recently, a DC vaccine based on immunogenic cell death molecules including HMGB1 and HSP90 was shown to elicit danger signals and T cell activation, resulting in rejection of high-grade glioma in an animal model[11]. These data suggest that DAMPs plus tumour cell lysates may provide maximal efficacy of DC-based cancer vaccines.

To study the significance of honokiol-derived tumour cell lysates, DCs were pulsed with tumour cell lysates from KKU-213L5 cell line derived from *Opisthorchis viverrini*-associated CCA of a Thai patient. Peptide loading procedure is an important parameter for DC-based cancer vaccines and coculturing with tumour cell lysates or peptide antigens is the most commonly used strategy in clinical trials[24]. In coculture systems, a tumour antigen is recognised by phagocytic receptors, resulting in phagocytosis and subsequent processing and presentation on the MHC molecule[25]. Here, we demonstrated the localisation of tumour cell lysates in the cytoplasmic area of DCs. KKU-213L5 cell lysates might be engulfed by DCs; we were successful in constructing DCs loaded with honokiol-derived tumour cell lysates. Although we focus only on one CCA cell line, KKU-213L5, this cell is the representative of highly metastasis CCA cells that mimic the characteristic of lung metastatic CCA cells in CCA patients[26].

Presentation of tumour antigen either on class I or class II MHC molecules triggers the activation of T lymphocyte receptors and co-stimulatory molecules. DAMPs-associated tumour cell lysates can enhance effector T cell activation that mediates fully mature DCs loaded with tumour cell lysates[27]. We showed that autologous T lymphocytes were efficiently activated after coculture with DCs loaded with honokiol-derived tumour cell lysates. These activated T lymphocytes increased proliferation and production of type I cytokines. Interestingly, DCs loaded with tumour cell lysates from honokiol-treated CCA cells activate T lymphocytes better

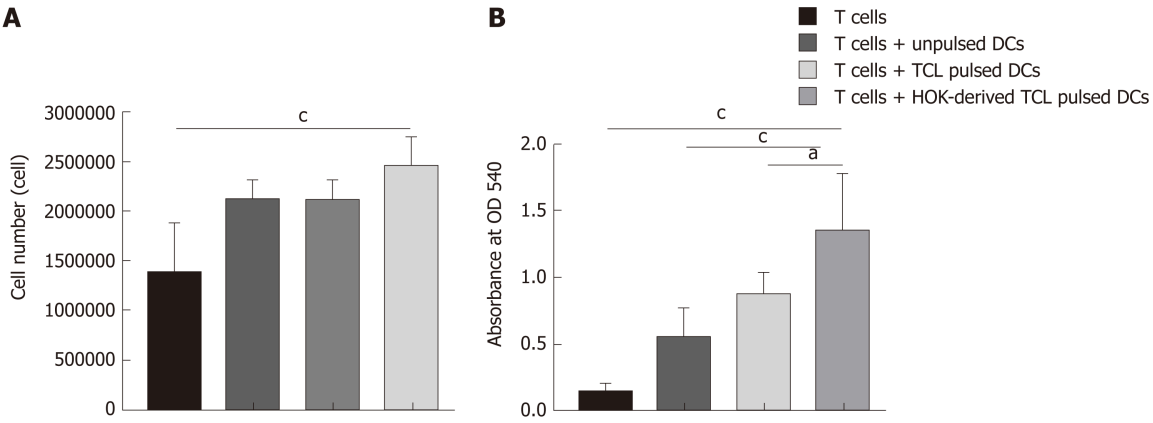


Figure 4 Honokiol-derived tumour cell lysates pulsed dendritic cells significantly stimulated T lymphocyte proliferation. KKU-213L5 cells were pretreated with honokiol or left untreated before they were lysed and loaded into dendritic cells (DCs). T lymphocytes were stimulated with different types of DCs (unpulsed, pulsed with tumour cell lysates and pulsed with honokiol-derived tumour cell lysates) at 1:10 ratios for 5 d. Lymphocyte number was reflected by direct counting (A) and relative lymphocyte proliferation (B). T lymphocytes cultured alone were set as control and all experiments were performed in triplicate. Results are shown as mean ± SD, A P value < 0.05 was considered significant; $^{a}P < 0.05$, $^{b}P < 0.01$ and $^{c}P < 0.001$. DCs: Dendritic cells; HOK: Honokiol; TCL: Tumour cell lysate.

than DCs loaded with tumour cell lysates from untreated cells. Triggering DCs with HMGB1 through TLR-4 leads to full expression of co-stimulatory receptor molecules including CD80 and CD86 and increased production of proinflammatory cytokines[10,20]. In this study, we demonstrated that honokiol-derived tumour cell lysates enhanced IFN-γ and IL-12 secretion from DCs and effector T cells. Secretion of IL-12 from stimulated DCs preferentially drives Th1 effector T cell development, leading to high IFN-γ production[28]. Moreover, IL-12 is a key cytokine for activation of CD8+ T cells and crucial for the change of Th17 to Th1-like phenotype that can be armed to destroy cancer cells[29,30]. Therefore, we concluded that cell lysates from honokiol-treated CCA cells may modulate maturation of DCs that were then able to effectively activate T lymphocytes to differentiate and become effector Th1 and CD8+ T cells.

When the antitumour activity of effector immune cells was examined, T cells stimulated by honokiol-derived tumour cell lysate-primed DCs showed greater efficiency in killing KKU-213L5 cells compared with those stimulated by DCs primed with tumour cell lysates of untreated cells. Moreover, cytotoxicity against human cholangiocytes was not significantly changed, indicating this antitumour cytotoxicity as specific on CCA cells. Although the HLA typing was not performed in this study, KKU213L5 cell line was established from Thai CCA patients and the immune cells were also separated from Thai healthy donors, of which the chance for their compatibility would be high as being HLA-A2[8]. Patients with multiple myeloma have an impaired DC function when loaded with tumour cell lysates. This may be associated with abnormality of STAT3 and the NF-kappaB signalling pathway[31]. On the other hand, DAMPs function to trigger DC maturation *via* TLR4/2, which involves p38 MAPK and NF-kappaB downstream signalling pathways[32]. Moreover, honokiol could down-regulate the expression of CRT mediated by ER-stress and inhibit gastics tumour growth through reduction of epithelial-to-mesenchymal transition mechanisms[33]. This would explain why the antitumour response of effector T cells mediated by DCs loaded with honokiol-derived tumour cell lysates is superior to those mediated by DCs loaded with tumour cell lysates from cells not exposed to honokiol.

Although we focused only on HMGB1 and HSP90 molecules, several other proteins responsible for damage or danger signals include calreticulin, adenosine triphosphate and other members of the heat shock protein family. Interestingly, DAMPs could activate innate immune cells *via* many types of receptors as either membrane bound (*e.g.*, TLR4) or intracellular (*e.g.*, TLR3, TLR79, all NOD-like receptors and RIG-I-like receptors)[34]. For example, previous studies reported that the HSP family could activate the TLR-4 signalling pathway leading to facilitation of optimal tumour antigen processing that subsequently elicits antitumour immune response[35,36]. Moreover, treatment with anthracyclines on some cancer cell lines including prostate cancer, ovarian cancer and acute lymphoblastic leukemia cells could induce nuclear translocation of calreticulin, HSP70 and HSP90 as well as the release of HMGB1, causing maturation of DCs. These DCs could then stimulate tumour-specific IFN-γ-producing T cells[37]. Antitumour activity facilitated by the function of DCs loaded with honokiol-derived tumour cell lysates may, however, not involve only HMGB1

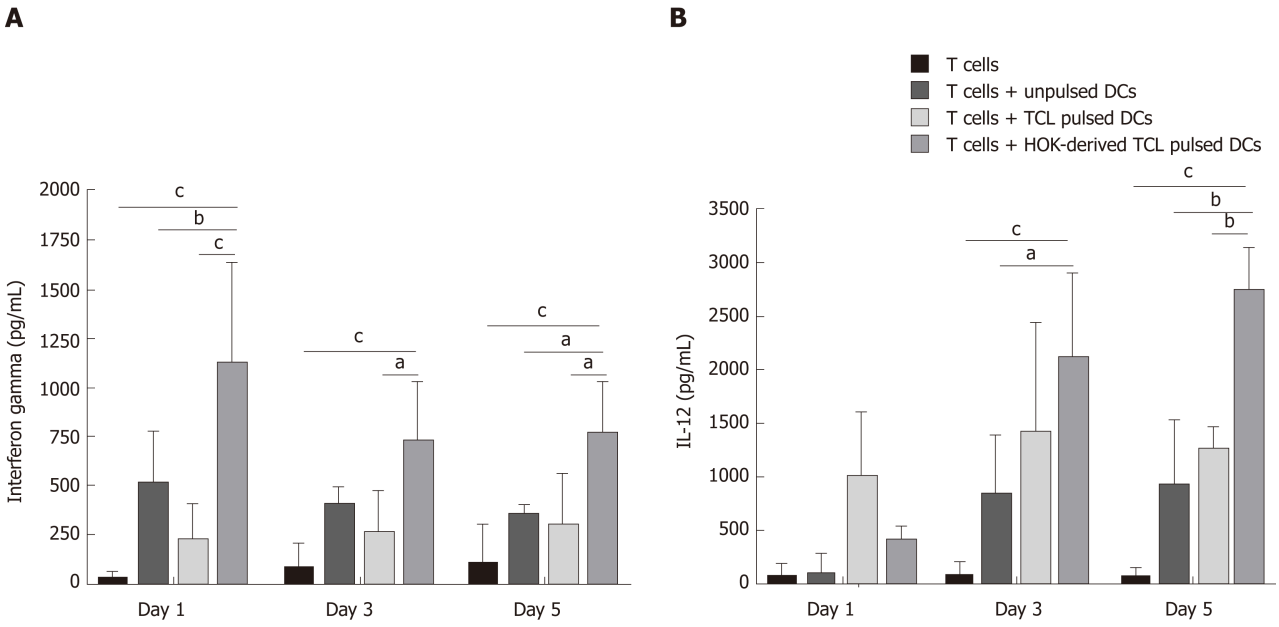


Figure 5 Type I cytokines interferon-γ and interleukin-12 production. Autologous T lymphocytes were cocultured either with unloaded or loaded tumour cell lysates or tumour cell lysates derived from honokiol-treated CCA cells for 1-5 days before measurement of cytokines. Levels of interferon-γ (A) and interleukin-12 (B) in conditioned medium were analysed by ELISA. Results are shown as mean ± SD of three independent experiments. A *P* value < 0.05 was considered significant; [a]*P* < 0.05, [b]*P* < 0.01 and [c]*P* < 0.001. DCs: Dendritic cells; HOK: Honokiol; TCL: Tumour cell lysate.

and HSP90 and roles of other molecules cannot be excluded. DAMPs may contribute to cancer progression and promote resistance to anticancer treatments[38]. Further *in vivo* study is required to ensure the effectiveness of this treatment and to differentiate the double-edged sword potential of DAMPs.

In summary, we demonstrated the immunoadjuvant effect of honokiol-derived CCA tumour antigens on a DC-based cancer vaccine approach, which enhanced tumour specific T lymphocyte responses including cell proliferation, cytokine production and cytotoxicity against human CCA cells. Antitumour T cell immunity might be mediated by induced expression of DAMPs in honokiol-treated KKU-213L5 cells. Therefore, *in vitro* and *in vivo* studies are urgently needed to assess for the use of honokiol in tumour antigen preparation as one promising approach to discover an effective DC-based vaccine against CCA.

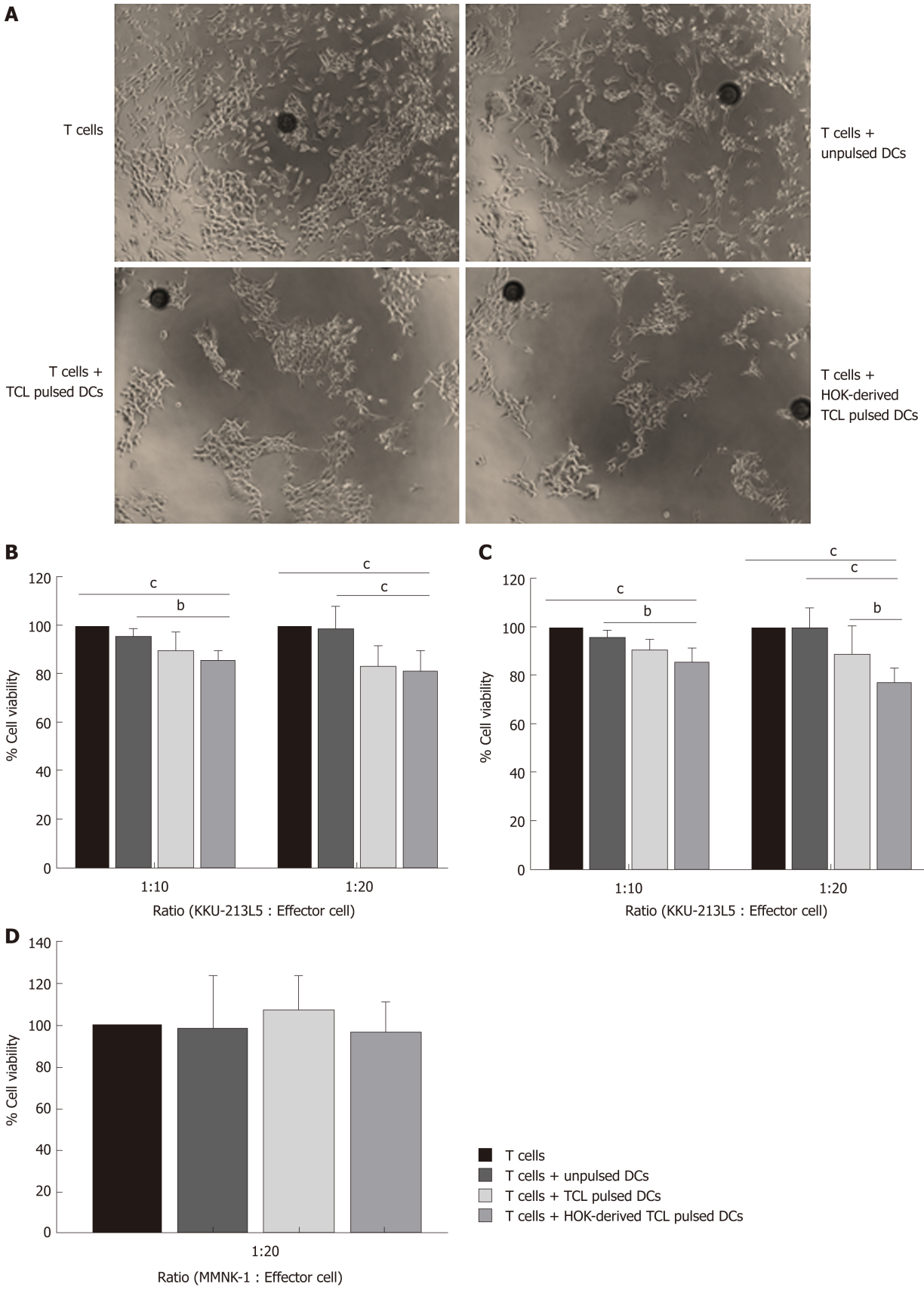


Figure 6 Dendritic cells pulsed with tumour cell lysates derived from honokiol-treated cholangiocarcinoma cells significantly enhanced T lymphocyte killing of cholangiocarcinoma cells. Differently primed effector T lymphocytes were cocultured with target KKU-213L5 cell line at ratios of 1:10 and 1:20. Killing capacity was evaluated using the MTT assay. A: Remaining target cells after coculture with effector cells for 48 h at 1:20 ratio photographed at 100×. Cytotoxic effect of T lymphocytes on KKU-213L5 is shown as % viability of target cells at (B) 24 h, (C) 48 h and for MMNK1 (D) at 48 h. Results are presented as mean ± SD of three independent experiments. A P value < 0.05 was considered significant; $^{a}P < 0.05$, $^{b}P < 0.01$ and $^{c}P < 0.001$. DCs: Dendritic cells; HOK: Honokiol; TCL: Tumour cell lysate.

ARTICLE HIGHLIGHTS

Research background

Cholangiocarcinoma (CCA) is a biliary tract malignancy. As no specific biomarkers are available, CCA patients frequently present with a disseminated tumour too late for curative treatment. Honokiol is a hydroxylated biphenyl compound isolated from *Magnolia offinalis*. Many studies have reported that honokiol has anti-tumour properties on various types of cancer by induction of cell apoptosis. A dendritic cell (DC)-based cancer vaccine is a vaccine that aims to stimulate anticancer immunity in patients through the capacity to activate tumour-specific T cells. However, pulsing DCs with whole tumour cell lysates have shown low efficacy against CCA cells *in vitro*.

Research motivation

Evidence suggests that the efficacy of DC-based cancer vaccines on CCA is low, especially DCs loaded with tumour cell lysates strategy. In addition, the anti-tumour activity of honokiol could be due to the induction of cancer cell apoptosis. This effect may be associated with the release of damage-associated molecular patterns (DAMPs) from cancer cells, which increases the immunogenicity of tumour antigens. Therefore, the authors of this study were interested in the construction of DCs loaded with cell lysates derived from honokiol-treated CCA tumour cells with the aim of eliciting apoptosis in tumour cells as well as creating a broad array of TTAs in the form of dead and dying cells.

Research objectives

The aim of this study was to maximise the anti-tumour activities of DCs loaded with cell lysates from honokiol-treated CCA cells.

Research methods

Anti-tumour activity of honokiol was studied, including the cytotoxicity and cell apoptosis assay. The effects of honokiol on DAMPs expression from CCA cells were also investigated. Then, CCA cells with or without honokiol treatments were derived to obtain tumour cell lysates used to pulse the DC cells, after which the latter were used to further stimulate T cells. Finally, the stimulated T cells were exposed to CCA cells and the killing of CCA cells by T cells was determined.

Research results

The data showed that honokiol was cytotoxic to human CCA cells KKU-213L5 *via* intrinsic or extrinsic apoptotic pathways. Interestingly, the induction of cell apoptosis by honokiol was associated with DAMPs release, including HMGB1 and HSP90. DCs loaded with tumour lysates derived from honokiol-treated KKU-213L5 cells enhanced priming and stimulated T lymphocyte proliferation as well as type I cytokine production. Importantly, T lymphocytes stimulated with DCs pulsed with cell lysates of honokiol-treated tumour cells, which significantly increased the specific killing of human CCA cells compared to those associated with DCs pulsed with cell lysates of untreated CCA cells.

Research conclusions

These findings provide new evidence that honokiol may have anticancer properties against CCA cells. Further, honokiol may possess the potential to enhance DC-based cancer vaccines, most probably by enhancing the immunogenicity of CCA, which further promotes DCs and T cell stimulation.

Research perspectives

Our model showed the improvement of cancer vaccine efficacy against CCA based on DCs and demonstrated the use of honokiol as a herbal-derived compound in combination with tumour antigen pulsed DCs to maximise the antitumour response of cytotoxic antitumour T lymphocytes.

ACKNOWLEDGEMENTS

We are grateful to Prof. Sopit Wongkham, Liver Fluke and Cholangiocarcinoma Research Center, Khon Kaen University, for kindly providing us with the CCA cell lines used in this study. Thank also goes to Asst. Prof. Sarawut Kumphune and Assoc. Prof. Kanchana Usuwanthim for their suggestion and for providing T lymphocyte isolation protocol.

REFERENCES

1 **Global Burden of Disease Cancer Collaboration**. Fitzmaurice C, Dicker D, Pain A, Hamavid H, Moradi-Lakeh M, MacIntyre MF, Allen C, Hansen G, Woodbrook R, Wolfe C, Hamadeh RR, Moore A, Werdecker A, Gessner BD, Te Ao B, McMahon B, Karimkhani C, Yu C, Cooke GS, Schwebel DC, Carpenter DO, Pereira DM, Nash D, Kazi DS, De Leo D, Plass D, Ukwaja KN, Thurston GD, Yun Jin K, Simard EP, Mills E, Park EK, Catalá-López F, deVeber G, Gotay C, Khan G, Hosgood HD 3rd, Santos IS,

Leasher JL, Singh J, Leigh J, Jonas JB, Sanabria J, Beardsley J, Jacobsen KH, Takahashi K, Franklin RC, Ronfani L, Montico M, Naldi L, Tonelli M, Geleijnse J, Petzold M, Shrime MG, Younis M, Yonemoto N, Breitborde N, Yip P, Pourmalek F, Lotufo PA, Esteghamati A, Hankey GJ, Ali R, Lunevicius R, Malekzadeh R, Dellavalle R, Weintraub R, Lucas R, Hay R, Rojas-Rueda D, Westerman R, Sepanlou SG, Nolte S, Patten S, Weichenthal S, Abera SF, Fereshtehnejad SM, Shiue I, Driscoll T, Vasankari T, Alsharif U, Rahimi-Movaghar V, Vlassov VV, Marcenes WS, Mekonnen W, Melaku YA, Yano Y, Artaman A, Campos I, MacLachlan J, Mueller U, Kim D, Trillini M, Eshrati B, Williams HC, Shibuya K, Dandona R, Murthy K, Cowie B, Amare AT, Antonio CA, Castañeda-Orjuela C, van Gool CH, Violante F, Oh IH, Deribe K, Soreide K, Knibbs L, Kereselidze M, Green M, Cardenas R, Roy N, Tillmann T, Li Y, Krueger H, Monasta L, Dey S, Sheikhbahaei S, Hafezi-Nejad N, Kumar GA, Sreeramareddy CT, Dandona L, Wang H, Vollset SE, Mokdad A, Salomon JA, Lozano R, Vos T, Forouzanfar M, Lopez A, Murray C, Naghavi M. The Global Burden of Cancer 2013. *JAMA Oncol* 2015; **1**: 505-527 [PMID: 26181261 DOI: 10.1001/jamaoncol.2015.0735]

2 **Blechacz B**, Gores GJ. Cholangiocarcinoma: Advances in pathogenesis, diagnosis, and treatment. *Hepatology* 2008; **48**: 308-321 [PMID: 18536057 DOI: 10.1002/hep.22310]

3 **Banales JM**, Cardinale V, Carpino G, Marzioni M, Andersen JB, Invernizzi P, Lind GE, Folseraas T, Forbes SJ, Fouassier L, Geier A, Calvisi DF, Mertens JC, Trauner M, Benedetti A, Maroni L, Vaquero J, Macias RI, Raggi C, Perugorria MJ, Gaudio E, Boberg KM, Marin JJ, Alvaro D. Expert consensus document: Cholangiocarcinoma: current knowledge and future perspectives consensus statement from the European Network for the Study of Cholangiocarcinoma (ENS-CCA). *Nat Rev Gastroenterol Hepatol* 2016; **13**: 261-280 [PMID: 27095655 DOI: 10.1038/nrgastro.2016.51]

4 **Saxena M**, Bhardwaj N. Re-Emergence of Dendritic Cell Vaccines for Cancer Treatment. *Trends Cancer* 2018; **4**: 119-137 [PMID: 29458962 DOI: 10.1016/j.trecan.2017.12.007]

5 **Wu YG**, Wu GZ, Wang L, Zhang YY, Li Z, Li DC. Tumor cell lysate-pulsed dendritic cells induce a T cell response against colon cancer in vitro and in vivo. *Med Oncol* 2010; **27**: 736-742 [PMID: 19669608 DOI: 10.1007/s12032-009-9277-x]

6 **Lin M**, Liang S, Jiang F, Xu J, Zhu W, Qian W, Hu Y, Zhou Z, Chen J, Niu L, Xu K, Lv Y. 2003-2013, a valuable study: Autologous tumor lysate-pulsed dendritic cell immunotherapy with cytokine-induced killer cells improves survival in stage IV breast cancer. *Immunol Lett* 2017; **183**: 37-43 [PMID: 28143792 DOI: 10.1016/j.imlet.2017.01.014]

7 **Wang Q**, Luan W, Warren L, Kadri H, Kim KW, Goz V, Blank S, Isabel Fiel M, Hiotis SP. Autologous Tumor Cell Lysate-Loaded Dendritic Cell Vaccine Inhibited Tumor Progression in an Orthotopic Murine Model for Hepatocellular Carcinoma. *Ann Surg Oncol* 2016; **23**: 574-582 [PMID: 26786094 DOI: 10.1245/s10434-015-5035-9]

8 **Junking M**, Grainok J, Thepmalee C, Wongkham S, Yenchitsomanus PT. Enhanced cytotoxic activity of effector T-cells against cholangiocarcinoma by dendritic cells pulsed with pooled mRNA. *Tumour Biol* 2017; **39**: 1010428317733367 [PMID: 29034817 DOI: 10.1177/1010428317733367]

9 **Lee YJ**, Lee YM, Lee CK, Jung JK, Han SB, Hong JT. Therapeutic applications of compounds in the Magnolia family. *Pharmacol Ther* 2011; **130**: 157-176 [PMID: 21277893 DOI: 10.1016/j.pharmthera.2011.01.010]

10 **Chen HM**, Wang PH, Chen SS, Wen CC, Chen YH, Yang WC, Yang NS. Shikonin induces immunogenic cell death in tumor cells and enhances dendritic cell-based cancer vaccine. *Cancer Immunol Immunother* 2012; **61**: 1989-2002 [PMID: 22527248 DOI: 10.1007/s00262-012-1258-9]

11 **Garg AD**, Vandenberk L, Koks C, Verschuere T, Boon L, Van Gool SW, Agostinis P. Dendritic cell vaccines based on immunogenic cell death elicit danger signals and T cell-driven rejection of high-grade glioma. *Sci Transl Med* 2016; **8**: 328ra27 [PMID: 26936504 DOI: 10.1126/scitranslmed.aae0105]

12 **Crane CA**, Panner A, Murray JC, Wilson SP, Xu H, Chen L, Simko JP, Waldman FM, Pieper RO, Parsa AT. PI(3) kinase is associated with a mechanism of immunoresistance in breast and prostate cancer. *Oncogene* 2009; **28**: 306-312 [PMID: 18850006 DOI: 10.1038/onc.2008.384]

13 **Krysko DV**, Garg AD, Kaczmarek A, Krysko O, Agostinis P, Vandenabeele P. Immunogenic cell death and DAMPs in cancer therapy. *Nat Rev Cancer* 2012; **12**: 860-875 [PMID: 23151605 DOI: 10.1038/nrc3380]

14 **Kobayashi M**, Sakabe T, Abe H, Tanii M, Takahashi H, Chiba A, Yanagida E, Shibamoto Y, Ogasawara M, Tsujitani S, Koido S, Nagai K, Shimodaira S, Okamoto M, Yonemitsu Y, Suzuki N, Nagaya M; DC-vaccine study group at the Japan Society of Innovative Cell Therapy (J-SICT). Dendritic cell-based immunotherapy targeting synthesized peptides for advanced biliary tract cancer. *J Gastrointest Surg* 2013; **17**: 1609-1617 [PMID: 23877328 DOI: 10.1007/s11605-013-2286-2]

15 **Shimizu K**, Kotera Y, Aruga A, Takeshita N, Takasaki K, Yamamoto M. Clinical utilization of postoperative dendritic cell vaccine plus activated T-cell transfer in patients with intrahepatic cholangiocarcinoma. *J Hepatobiliary Pancreat Sci* 2012; **19**: 171-178 [PMID: 21874278 DOI: 10.1007/s00534-011-0437-y]

16 **Gilboa E**. DC-based cancer vaccines. *J Clin Invest* 2007; **117**: 1195-1203 [PMID: 17476349 DOI: 10.1172/JCI31205]

17 **Lu CH**, Chen SH, Chang YS, Liu YW, Wu JY, Lim YP, Yu HI, Lee YR. Honokiol, a potential therapeutic agent, induces cell cycle arrest and program cell death in vitro and in vivo in human thyroid cancer cells. *Pharmacol Res* 2017; **115**: 288-298 [PMID: 27940017 DOI: 10.1016/j.phrs.2016.11.038]

18 **Lin CJ**, Chen TL, Tseng YY, Wu GJ, Hsieh MH, Lin YW, Chen RM. Honokiol induces autophagic cell death in malignant glioma through reactive oxygen species-mediated regulation of the p53/PI3K/Akt/mTOR signaling pathway. *Toxicol Appl Pharmacol* 2016; **304**: 59-69 [PMID: 27236003 DOI: 10.1016/j.taap.2016.05.018]

19 **Li L**, Han W, Gu Y, Qiu S, Lu Q, Jin J, Luo J, Hu X. Honokiol induces a necrotic cell death through the mitochondrial permeability transition pore. *Cancer Res* 2007; **67**: 4894-4903 [PMID: 17510419 DOI: 10.1158/0008-5472.CAN-06-3818]

20 **VanPatten S**, Al-Abed Y. High Mobility Group Box-1 (HMGb1): Current Wisdom and Advancement as a Potential Drug Target. *J Med Chem* 2018; **61**: 5093-5107 [PMID: 29268019 DOI: 10.1021/acs.jmedchem.7b01136]

21 **Bianchi ME**. HMGB1 loves company. *J Leukoc Biol* 2009; **86**: 573-576 [PMID: 19414536 DOI: 10.1189/jlb.1008585]

22 **Apetoh L**, Ghiringhelli F, Tesniere A, Obeid M, Ortiz C, Criollo A, Mignot G, Maiuri MC, Ullrich E, Saulnier P, Yang H, Amigorena S, Ryffel B, Barrat FJ, Saftig P, Levi F, Lidereau R, Nogues C, Mira JP, Chompret A, Joulin V, Clavel-Chapelon F, Bourhis J, André F, Delaloge S, Tursz T, Kroemer G, Zitvogel

L. Toll-like receptor 4-dependent contribution of the immune system to anticancer chemotherapy and radiotherapy. *Nat Med* 2007; **13**: 1050-1059 [PMID: 17704786 DOI: 10.1038/nm1622]
23 **Shirota T**, Ojima H, Hiraoka N, Shimada K, Rokutan H, Arai Y, Kanai Y, Miyagawa S, Shibata T. Heat Shock Protein 90 Is a Potential Therapeutic Target in Cholangiocarcinoma. *Mol Cancer Ther* 2015; **14**: 1985-1993 [PMID: 26141945 DOI: 10.1158/1535-7163.MCT-15-0069]
24 **Constantino J**, Gomes C, Falcão A, Cruz MT, Neves BM. Antitumor dendritic cell-based vaccines: Lessons from 20 years of clinical trials and future perspectives. *Transl Res* 2016; **168**: 74-95 [PMID: 26297944 DOI: 10.1016/j.trsl.2015.07.008]
25 **Banchereau J**, Briere F, Caux C, Davoust J, Lebecque S, Liu YJ, Pulendran B, Palucka K. Immunobiology of dendritic cells. *Annu Rev Immunol* 2000; **18**: 767-811 [PMID: 10837075 DOI: 10.1146/annurev.immunol.18.1.767]
26 **Uthaisar K**, Vaeteewoottacharn K, Seubwai W, Talabnin C, Sawanyawisuth K, Obchoei S, Kraiklang R, Okada S, Wongkham S. Establishment and characterization of a novel human cholangiocarcinoma cell line with high metastatic activity. *Oncol Rep* 2016; **36**: 1435-1446 [PMID: 27461717 DOI: 10.3892/or.2016.4974]
27 **Lin TJ**, Lin HT, Chang WT, Mitapalli S P, Hsiao PW, Yin SY, Yang NS. Shikonin-enhanced cell immunogenicity of tumor vaccine is mediated by the differential effects of DAMP components. *Mol Cancer* 2015; **14**: 174 [PMID: 26403780 DOI: 10.1186/s12943-015-0435-9]
28 **Watford WT**, Hissong BD, Bream JH, Kanno Y, Muul L, O'Shea JJ. Signaling by IL-12 and IL-23 and the immunoregulatory roles of STAT4. *Immunol Rev* 2004; **202**: 139-156 [PMID: 15546391 DOI: 10.1111/j.0105-2896.2004.00211.x]
29 **Henry CJ**, Ornelles DA, Mitchell LM, Brzoza-Lewis KL, Hiltbold EM. IL-12 produced by dendritic cells augments CD8+ T cell activation through the production of the chemokines CCL1 and CCL17. *J Immunol* 2008; **181**: 8576-8584 [PMID: 19050277 DOI: 10.4049/jimmunol.181.12.8576]
30 **Lee YK**, Turner H, Maynard CL, Oliver JR, Chen D, Elson CO, Weaver CT. Late developmental plasticity in the T helper 17 lineage. *Immunity* 2009; **30**: 92-107 [PMID: 19119024 DOI: 10.1016/j.immuni.2008.11.005]
31 **Yang DH**, Park JS, Jin CJ, Kang HK, Nam JH, Rhee JH, Kim YK, Chung SY, Choi SJ, Kim HJ, Chung IJ, Lee JJ. The dysfunction and abnormal signaling pathway of dendritic cells loaded by tumor antigen can be overcome by neutralizing VEGF in multiple myeloma. *Leuk Res* 2009; **33**: 665-670 [PMID: 18922577 DOI: 10.1016/j.leukres.2008.09.006]
32 **Saenz R**, Futalan D, Leutenez L, Eekhout F, Fecteau JF, Sundelius S, Sundqvist S, Larsson M, Hayashi T, Minev B, Carson D, Esener S, Messmer B, Messmer D. TLR4-dependent activation of dendritic cells by an HMGB1-derived peptide adjuvant. *J Transl Med* 2014; **12**: 211 [PMID: 25123824 DOI: 10.1186/1479-5876-12-211]
33 **Liu SH**, Lee WJ, Lai DW, Wu SM, Liu CY, Tien HR, Chiu CS, Peng YC, Jan YJ, Chao TH, Pan HC, Sheu ML. Honokiol confers immunogenicity by dictating calreticulin exposure, activating ER stress and inhibiting epithelial-to-mesenchymal transition. *Mol Oncol* 2015; **9**: 834-849 [PMID: 25619450 DOI: 10.1016/j.molonc.2014.12.009]
34 **Rubartelli A**, Lotze MT. Inside, outside, upside down: Damage-associated molecular-pattern molecules (DAMPs) and redox. *Trends Immunol* 2007; **28**: 429-436 [PMID: 17845865 DOI: 10.1016/j.it.2007.08.004]
35 **Asea A**, Kraeft SK, Kurt-Jones EA, Stevenson MA, Chen LB, Finberg RW, Koo GC, Calderwood SK. HSP70 stimulates cytokine production through a CD14-dependant pathway, demonstrating its dual role as a chaperone and cytokine. *Nat Med* 2000; **6**: 435-442 [PMID: 10742151 DOI: 10.1038/74697]
36 **Tsan MF**, Gao B. Heat shock proteins and immune system. *J Leukoc Biol* 2009; **85**: 905-910 [PMID: 19276179 DOI: 10.1189/jlb.0109005]
37 **Fucikova J**, Kralikova P, Fialova A, Brtnicky T, Rob L, Bartunkova J, Spísek R. Human tumor cells killed by anthracyclines induce a tumor-specific immune response. *Cancer Res* 2011; **71**: 4821-4833 [PMID: 21602432 DOI: 10.1158/0008-5472.CAN-11-0950]
38 **Krysko O**, Løve Aaes T, Bachert C, Vandenabeele P, Krysko DV. Many faces of DAMPs in cancer therapy. *Cell Death Dis* 2013; **4**: e631 [PMID: 23681226 DOI: 10.1038/cddis.2013.156]

World Journal of Gastroenterology

Submit a Manuscript: https://www.f6publishing.com

World J Gastroenterol 2019 August 7; 25(29): 3956-3971

DOI: 10.3748/wjg.v25.i29.3956

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Basic Study

Berberine prevents stress-induced gut inflammation and visceral hypersensitivity and reduces intestinal motility in rats

Zhi-Chao Yu, Yong-Xin Cen, Ben-Hua Wu, Cheng Wei, Feng Xiong, De-Feng Li, Ting-Ting Liu, Ming-Han Luo, Li-Liangzi Guo, Ying-Xue Li, Li-Sheng Wang, Jian-Yao Wang, Jun Yao

ORCID number: Zhi-Chao Yu (0000-0001-5123-9191); Yong-Xin Cen (0000-0003-0793-7261); Ben-Hua Wu (0000-0002-9392-9470); Cheng Wei (0000-0002-6373-1826); Feng Xiong (0000-0002-4021-0817); De-Feng Li (0000-0003-3118-6840); Ting-Ting Liu (0000-0002-1533-5220); Ming-Han Luo (0000-0002-5056-3421); Li-Liangzi Guo (0000-0003-1521-6245); Ying-Xue Li (0000-0001-6350-612X); Li-Sheng Wang (0000-0002-7418-6114); Jian-Yao Wang (0000-0003-1568-4479); Jun Yao (0000-0002-3472-1602).

Author contributions: Yu ZC designed and wrote the manuscript; Cen YX and Wu BH reviewed the manuscript for its intellectual content and revised the entire work; Wei C, Xiong F, Li DF, Liu TT, Luo MH, Guo LL, and Li YX performed the histological assessments and evaluations; Wang LS, Wang JY, and Yao J reviewed the manuscript for its intellectual content; all authors have read and approved the final manuscript.

Supported by: the National Natural Science Foundation of China, No. 81800489; Shenzhen Health and Family Planning System Research Project, No. SZXJ2017030; and Technical Research and Development Project of Shenzhen, Nos. JCYJ20170307100538697 and JCYJ20170307100911479.

Institutional review board statement: This study was reviewed and approved by the Institutional Review Board of

Zhi-Chao Yu, Ben-Hua Wu, Cheng Wei, Feng Xiong, De-Feng Li, Ting-Ting Liu, Ming-Han Luo, Li-Liangzi Guo, Ying-Xue Li, Li-Sheng Wang, Jun Yao, Department of Gastroenterology, Jinan University of Second Clinical Medical Sciences, Shenzhen Municipal People's Hospital, Shenzhen 518020, Guangdong Province, China

Yong-Xin Cen, Department of Gastroenterology, Foshan Gaoming Affiliated Hospital of Guangdong Medical University, Foshan 528500, Guangdong Province, China

Jian-Yao Wang, Department of General Surgery, Shenzhen Children's Hospital, Shenzhen 518026, Guangdong Province, China

Corresponding author: Jun Yao, MD, PhD, Doctor, Department of Gastroenterology, Jinan University of Second Clinical Medical Sciences, Shenzhen Municipal People's Hospital, 1017 East Gate Road, Shenzhen 518020, Guangdong Province, China. yj_1108@126.com
Telephone: +86-755-25533497
Fax: +86-755-25533497

Abstract

BACKGROUND

Irritable bowel syndrome (IBS) is a common chronic non-organic disease of the digestive system. Berberine (BBR) has been used to treat patients with IBS, but the underlying therapeutic mechanism is little understood. We believe that BBR achieves its therapeutic effect on IBS by preventing stress intestinal inflammation and visceral hypersensitivity and reducing bowel motility.

AIM

To test the hypothesis that BBR achieves its therapeutic effect on IBS by preventing subclinical inflammation of the intestinal mucosa and reducing visceral hypersensitivity and intestinal motility.

METHODS

IBS was induced in rats *via* water avoidance stress (WAS). qRT-PCR and histological analyses were used to evaluate the levels of cytokines and mucosal inflammation, respectively. Modified ELISA and qRT-PCR were used to evaluate the nuclear factor kappa-B (NF-κB) signal transduction pathway. Colorectal distention test, gastrointestinal transit measurement, Western blot, and qRT-PCR were used to analyze visceral sensitivity, intestinal motility, the expression of C-kit (marker of Cajal mesenchymal cells), and the expression of brain derived neurotrophic factor (BDNF) and its receptor TrkB.

Shenzhen Municipal People's Hospital.

Institutional animal care and use committee statement: This study was reviewed and approved by the Animal Ethical and Welfare Committee of Shenzhen University on January 12, 2016.

Conflict-of-interest statement: The authors of this manuscript have no conflicts of interest to disclose.

ARRIVE guidelines statement: The authors have read the ARRIVE guidelines, and the manuscript was prepared and revised according to the ARRIVE guidelines.

Manuscript source: Unsolicited manuscript

Received: February 27, 2019
Peer-review started: February 27, 2019
First decision: April 11, 2019
Revised: June 26, 2019
Accepted: July 5, 2019
Article in press: July 5, 2019
Published online: August 7, 2019

P-Reviewer: Bourgoin SG, Kamiya T
S-Editor: Yan JP
L-Editor: Wang TQ
E-Editor: Ma YJ

RESULTS

WAS led to mucosal inflammation, visceral hyperalgesia, and high intestinal motility. Oral administration of BBR inhibited the NF-κB signal transduction pathway, reduced the expression of pro-inflammatory cytokines [interleukin (IL)-1β, IL-6, interferon-γ, and tumor necrosis factor-α], promoted the expression of anti-inflammatory cytokines (IL-10 and transforming growth factor-β), and improved the terminal ileum tissue inflammation. BBR inhibited the expression of BDNF, TrkB, and C-kit in IBS rats, leading to the reduction of intestinal motility and visceral hypersensitivity. The therapeutic effect of BBR at a high dose (100 mg/kg) was superior to than that of the low-dose (25 mg/kg) group.

CONCLUSION

BBR reduces intestinal mucosal inflammation by inhibiting the intestinal NF-κB signal pathway in the IBS rats. BBR reduces the expression of BDNF, its receptor TrkB, and C-kit. BBR also reduces intestinal motility and visceral sensitivity to achieve its therapeutic effect on IBS.

Key words: Irritable bowel syndrome; Visceral hypersensitivity; Berberine; Rifampicin; Nuclear factor kappa-B; Brain-derived neurotrophic factor; Cajal mesenchymal cells; C-kit

Core tip: Irritable bowel syndrome (IBS) is a common chronic non-organic disease of the digestive system and the pathophysiology of IBS is still not completely understood. Berberine has been used to treat patients with IBS, but little is known regarding to its therapeutic mechanism. This study aimed to determine the therapeutic effect of berberine on IBS and its underlying mechanisms. The results demonstrated that the therapeutic efficacy of berberine was dose-dependent and may be associated with the inhibition of the intestinal nuclear factor kappa-B signal pathway, the expression of brain derived neurotrophic factor and its receptor TrkB, and the expression of C-kit to reduce intestinal motility and visceral sensitivity.

Citation: Yu ZC, Cen YX, Wu BH, Wei C, Xiong F, Li DF, Liu TT, Luo MH, Guo LL, Li YX, Wang LS, Wang JY, Yao J. Berberine prevents stress-induced gut inflammation and visceral hypersensitivity and reduces intestinal motility in rats. *World J Gastroenterol* 2019; 25(29): 3956-3971
URL: https://www.wjgnet.com/1007-9327/full/v25/i29/3956.htm
DOI: https://dx.doi.org/10.3748/wjg.v25.i29.3956

INTRODUCTION

Berberine (BBR) is an isoquinoline alkaloid extracted from various Chinese medicinal herbs, *e.g.*, Huanglian and scutellaria. BBR has abundant medicinal value, such as biological effects on the central nervous system, anti-tumor, anti-inflammatory, and anti-Alzheimer's disease effects, and reducing blood fat. A large number of basic and clinical studies have also shown the efficacy of BBR in the treatment of irritable bowel syndrome (IBS). The common dosage of BBR for treating diarrhea in adults is 100-300 mg, three times a day[1-4].

The pathophysiology of IBS is still not completely understood. In the past decade, there has been increasing focuses on the possible connection of IBS with increased intestinal mucosal permeability, inflammation, intestinal bacterial overgrowth, dysfunction of the cerebral intestinal axis, and visceral hypersensitivity[5]. At present, the pathogenesis of IBS is explained by the mechanism of environment-psycho-neuroendocrine-immunity[6,7].

The immunologic disorder of the intestinal tract is closely associated with the pathogenesis of IBS, and the nuclear factor kappa-B (NF-κB) signal pathway plays a very important role in the immune response[8,9]. NF-κB can regulate the transcription of the genes related to inflammation and pain, activate the transcription of inflammatory factors, affect intestinal inflammation, and lead to abdominal pain[10].

Cytokines are an essential part of intestinal immune regulation. According to the performance of cytokines in the immune response, they can be divided into two categories: (1) Th1 cell-secreted pro-inflammatory cytokines, including interleukin-1β (IL-1β), IL-6, tumor necrosis factor-α (TNF-α), and interferon γ (IFN-γ); and (2) Th2 cell-secreted anti-inflammatory cytokines, including IL-10 and transforming growth factor-β (TGF-β). The immune response in IBS is not just limited to the intestine. Systemic immune activation characterized by the elevation of pro-inflammatory cytokines and the decrease of anti-inflammatory cytokines is also involved in IBS[11-14].

BBR can reduce visceral hypersensitivity in IBS rats and regulate intestinal motility, but the underlying mechanism is not yet fully understood[1]. Brain derived neurotrophic factor (BDNF) plays an important role in the visceral hypersensitivity and intestinal hyperdynamics of IBS through the brain-gut axis. The increased expression of BDNF in the colonic mucosa and central nervous system may contribute to the visceral hyperalgesia in IBS[15,16]. Higher expression of BDNF in colonic mucosa and central nervous system would indicate the greater degree of abdominal pain in patients[15].

Cajal mesenchymal cells (ICC) can affect the intestinal motility and visceral sensitivity in IBS patients through the brain-gut axis. The corresponding receptors of many neurotransmitters are expressed on ICC, which are an important intermediary for the central nervous system to regulate visceral sensitivity and intestinal dynamics[17]. At the same time, some studies suggest that abnormalities in the structure and number of ICC in the intestinal tract of IBS patients can lead to abnormal electrophysiological activity in the intestinal tract[18]. C-Kit signaling plays a vital role in the development and maintenance of ICC. Thus, C-kit has also been used as a cell marker of ICC[19,20].

Rifaximin has achieved a good therapeutic effect for IBS patients without constipation[21]. It has been shown that rifaximin relieves symptoms of IBS by reducing visceral hypersensitivity in rats[22]. However, rifaximin has disadvantages, *e.g.*, high prices. Furthermore, long-term oral administration may lead to cross-resistance to similar antibiotics such as rifampicin and rifabutin[23,24]. This study aimed to explore the possible mechanism of BBR in the treatment of IBS at the level of brain and intestinal axis through the study of the high visceral sensitivity and intestinal dynamic mechanism in rats with experimental IBS.

MATERIALS AND METHODS

Animals

All experiments were performed on adult male Wistar rats weighing 200-225 g. Rats were obtained from the Guangdong experimental animal center. Animals were housed in plastic cages, with three rats per cage at room temperature (22 ± 1 °C) and 65%-70% humidity. Animals were maintained on a 12 h light/12 h dark cycle, with free access to water and feed. External oblique muscle electrode implantation was implemented after 5-7 d. Surgical preparations involved anesthetization with a xylazine/ketamine mixture. The period of postoperative recovery of rats was 4-6 d. Experimental animals were maintained in accordance with internationally accepted principles for laboratory animal use.

Chronic water avoidance stress protocol

Chronic exposure of adult rats to water avoidance stress (WAS) was conducted as described previously[25]. Briefly, animals were placed on a block in the middle of a Plexiglas tank filled with sterile water (25 °C; 1 cm below the platform height). They were maintained on the block for 1 h daily for 10 consecutive days (Figure 1). Control rats were placed similarly in a tank without water for 1 h daily for 10 d. In separate studies, rats were treated by oral gavage of 3 mL rifaximin suspension (150 mg/kg, twice daily 6 h apart), 3 mL water once a day, 3 mL low dose BBR suspension (25 mg/kg, once a day), or 3 mL high dose BBR suspension (100 mg/kg, once a day) for 10 consecutive days. The rats were then submitted to daily sessions of WAS or sham WAS 3 h after each AM gavage for 10 d. Specific rifaximin and BBR doses were based on previous studies[22,26,27].

Visceromotor response to colorectal distention

The protocol for measuring electromyogram (EMG) in response to colorectal distention (CRD) has been previously described[28]. Briefly, rats with a surgery had 5 d for recovery, and were fasted for 24h before intracolonic infusion, CRD, and EMG. EMG of rats were detected on day 0 and day 11 under different pressures. The baseline was the average of EMG amplitudes measured in the control group at the 0-

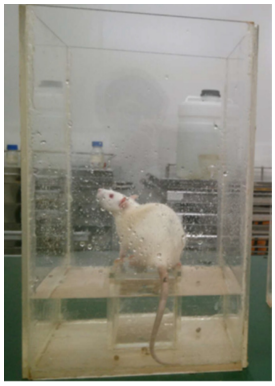


Figure 1 A rat model of irritable bowel syndrome was established by using water avoidance stress.

day CRD pressure of 60 mmHg. The amplitudes of EMG under different CRD pressures were compared with the baseline (% of control). We took the average EMG amplitude measured on day 11 subtracted by the average EMG amplitude detected on day 0, and express it as EMG.

Determination of gastrointestinal motility

The protocol for detecting the gastrointestinal transit has been previously described[29]. For total gastrointestinal transit, after the animals were fasted overnight, activated carbon ink was orally administered at a dose of 10 mL/kg. The time that animals first defecated black feces was recorded. For small intestinal transit, after an overnight fast, activated carbon ink was orally administered at a dose of 10 mL/kg to each animal. After 30 min, the rats were sacrificed by cervical dislocation. The small intestine was immediately excised carefully without stretching and the distance traveled by ink was measured as well as the total length of the small intestine. Data are expressed as the proportion (%) of the distance traveled by the ink along the entire length of the small intestine.

Evaluation of intestinal inflammatory response in rats

Hematoxylin and eosin (HE) staining was carried out in rat distal ileum tissue, and the inflammatory changes were observed under a microscope.

Enzyme-linked immunosorbent assay

Distal ileum tissue (50 mg) was homogenized in a glass homogenizer containing 2 mL cold saline. The homogenates were centrifuged at low temperature for 20 min at 3000 rpm. The protein concentration in the supernatant was quantified on a Nanodrop 2000 (Thermo Fisher Scientific, Waltham, MA, United States). The concentrations of IL-1β, IL-6, TNF-α, IFN-γ, IL-10, and TGF-β were determined according to the manufacturer's instructions.

Modified enzyme-linked immunosorbent assay

Cytoplasmic protein and nuclear protein were extracted from the rat ileum. Expression of NF-κB (P65) DNA-binding protein was measured with a commercially available modified ELISA kit [Cayman NF-κB (P65) Transcription Factor Assay Kit].

Western blot analysis

Cytoplasmic protein and nuclear protein were extracted and quantified. Protein (20 μg) was separated on an SDS-PAGE gel and then electro-transferred onto a nitrocellulose membrane (0.2μm pore; WHATMAN, England). The membrane was incubated with primary antibodies overnight at 4°C, followed by incubation with secondary antibodies labeled with horseradish peroxidase. Signals were quantified using ImageJ software. Antibodies used included anti-NF-kappa B P65 antibody (mouse, 1:1000, Santa Cruz, United States), anti-C-kit antibody (mouse, 1:1000, Santa Cruz, United States), anti-Trkb monoclonal antibody (rabbit 1:1000, Cell signaling Technology, United States), anti-GAPDH antibody (1:1000, Proteintech, United States), and horseradish peroxidase-conjugated anti-rabbit/mouse secondary antibodies (1:10000, Zhongshan Gold Bridge, Beijing, China).

Quantitative real-time polymerase chain reaction

Total RNA was extracted from distal ileum samples using TRIzol reagent (Thermo Scientific, United States). cDNA was synthesized using a reverse transcription kit (Thermo Scientific, United States). Quantitative PCR for inflammatory cytokines and

C-kit mRNA was performed with an iCycler IQ real-time detection system (Bio-Rad Laboratories, Hercules, CA, United States) and detected with SYBR Green in a fluorescence thermocycler (LightCycler; Roche Diagnostics, Mannheim, Germany). Primer sequences used for PCR are listed in Table 1. The double standard curve method was used to calculate the results.

Statistical analysis

Changes of EMG under different CRD pressures before and after experiments were analyzed using SPSS13.0. The statistical data are represented by the mean with standard deviation. We conducted normal test and variance homogeneity test for each group of experimental data. If the variance is homogeneous, the one-factor ANOVA was adopted, and the Bonferroni test was used to compare the two groups for the overall difference. If the variance was not homogenous, Kruskal Wallis *H* test was adopted and the Mann Whitney *U* test was used to compare each group for the overall difference. $P < 0.05$ was considered statistically significant.

RESULTS

WAS induces intestinal inflammation in rats

Compared with the control group, the WAS group showed a low inflammatory response in the intestinal tract (Figure 2). The expression levels of P65 DNA binding protein and NF-κB (P65) were significantly increased in the WAS group ($P < 0.05$) (Figure 3). The levels of pro-inflammatory cytokines IL-1β, IL-6, TNF-α, and IFN-γ were increased, while the levels of anti-inflammatory cytokines IL-10 and TGF-β were decreased ($P < 0.05$) in the WAS group compared with the control group (Figures 4 and 5).

Chronic WAS induces visceral hyperalgesia and intestinal hyperdynamics

After 10 d of WAS or sham WAS (control group), the rats showed a pressure-dependent increase in EMG amplitude in response to CRD. On day 11, chronic WAS induced a greater increase in EMG amplitude in response to CRD compared to sham WAS. Such increase was significantly different at 40 mmHg (ΔEMG response after WAS over baseline: 46.14 ± 11.1 *vs* 3.8 ± 13.8 after sham WAS over baseline, $P < 0.05$), and 60 mmHg (ΔEMG response after WAS over baseline: 59.58 ± 17.8 *vs* 0.45 ± 9.6 after sham WAS over baseline, $P < 0.05$) (Figure 6). Gastrointestinal motility assay showed that the time to the first black feces in the WAS group was significantly shorter than that in the control group (457.47 ± 25.99 min *vs* 580.40 ± 40.44 min, $P < 0.05$). The proportion (%) of the distance traveled by the ink along the entire length of the small intestine in the WAS group was significantly lower than that in the control group (63.77 ± 2.77% *vs* 49.03 ± 4.60%, $P < 0.05$) (Figure 7). Compared with the control group, the expression of BDNF and its receptor Trkb was significantly increased in the WAS group ($P < 0.05$) (Figure 8). C-kit expression in the WAS group was also significantly increased in the WAS group compared to the control group ($P < 0.05$) (Figure 9).

BBR prevents mucosal inflammation

Compared with the WAS group, after oral administration of rifaximin, 25 mg/kg BBR, or 100 mg/kg BBR, the tissues were intact, and there were no significant neutrophils at the distal ileum (Figure 2). In the treatment group, the expression level of DNA-binding protein and NF-κB (P65) in distal ileum tissues was significantly reduced compared to the WAS group ($P < 0.05$). The therapeutic effect of oral administration of rifaximin or 100 mg/kg BBR was superior to that of 25 mg/kg BBR ($P < 0.05$) (Figure 3). The expression levels of pro-inflammatory cytokines in the treatment groups were lower than those in the WAS group ($P < 0.05$), and the therapeutic effect of rifaximin or 100 mg/kg BBR was superior to that of 25 mg/kg BBR ($P < 0.05$). The expression levels of anti-inflammatory cytokines IL-10 and TGF-β in the treatment groups were higher than those in the WAS group ($P < 0.05$) (Figures 4 and 5).

BBR regulates intestinal motility and visceral hypersensitivity

On day 11, rifaximin, 25 mg/kg BBR, or 100 mg/kg BBR treatment reduced the increased level of visceromotor response to CRD induced by both forms of stress at 40 and 60 mmHg ($P < 0.05$). Furthermore, rifaximin or 100 mg/kg BBR resulted in smaller EMG compared to 25 mg/kg BBR ($P < 0.05$) (Figure 6). The time to the first black feces was significantly shorter in the rifaximin and 100 mg/kg BBR groups than that in the WAS and 25 mg/kg BBR groups ($P < 0.05$). There was no significant difference between the rifaximin group and 100 mg/kg BBR groups. The proportion

Table 1 Sequences of primers used for qRT-PCR

Gene	Forward sequence	Reverse sequence
IL-1β	AGTCTGCACAGTTCCCCAAC	TTAGGAAGACACGGGTTCCA
IL-6	CCAACTTCCAATGCTCTCCT	GGTTTGCCGAGTAGACCTCA
IL-10TGF-βIFN-γTNF-aBDNFTrkbC-kitRat B-actin	GACTGCTATCTTGCCTGCTCTTAC ATTCCTGGCGTTACCTTGG TCTGTGGGTTGTTCACCTCG CCTCCTCTCTGCCATCAACA CTTGGAGAAGGAAACCGCCT AGAGCTTCCCTGTCCCTCAG AATCCGACAACCAAAGCAAC TGTCACCAACTGGGACGATA	GGGTCTGGCTGACTGGGAAG AGCCCTGTATTCCGTCTCCT TATGGAAGGAAAGAGCCTCC GCAATGACTCCAAAGTAGACCTG GTCCACACAAAGCTCTCGGA TTGGGAAGGTAACCAGATCG TGTCACGGAAGCACTGACAT GGGGTGTTGAAGGTCTCAAA

(%) of the distance traveled by the ink along the entire length of the small intestine in the rifaximin and 100 mg/kg BBR groups was significantly lower than that in the WAS group and 25 mg/kg BBR groups ($P < 0.05$). There was no significant difference between the rifaximin and 100 mg/kg BBR groups (Figure 7).

Compared with the WAS group, the expression levels of BDNF and its receptor Trkb showed no significant difference in the 25 mg/kg BBR treatment group ($P > 0.05$). Compared with the WAS group, the expression levels of BDNF and its receptor Trkb were significantly decreased in the rifaximin group and 100 mg/kg BBR treatment group ($P < 0.05$). Compared with the 25 mg/kg BBR group, the expression levels of BDNF and its receptor Trkb were significantly reduced in the rifaximin group and 100 mg/kg BBR group ($P < 0.05$). The expression levels of BDNF and its receptor Trkb were not significantly different between the rifaximin group and 100 mg/kg BBR group ($P > 0.05$) (Figure 8).

Compared with the WAS group, C-kit expression was not significantly different in the 25 mg/kg BBR group ($P > 0.05$). Compared with the WAS group, the expression levels of C-kit were significantly decreased in the rifaximin and 100 mg/kg BBR groups ($P < 0.05$). Compared with the 25 mg/kg BBR group, the expression levels of C-kit were significantly decreased in the rifaximin and 100 mg/kg BBR groups ($P < 0.05$). There was no significant difference in C-kit expression between the rifaximin group and 100 mg/kg BBR group ($P > 0.05$) (Figure 9).

DISCUSSION

We exposed adult rats to chronic WAS to study the effects of BBR on mucosal inflammation, visceral hypersensitivity, and intestinal motility. Similar to previous studies, chronic exposure of adult rats to WAS induced mucosal inflammation, and the amplitude of EMG induced by CRD in rats of the WAS group was significantly enhanced, suggesting that the visceral sensitivity was enhanced. Further analysis also showed that intestinal motility was enhanced[22,30,31].

Our experiments showed that the mucosal inflammation occurred in the distal ileum in WAS group rats. Previous studies have shown that there is positive feedback regulation between the NF-κB signaling pathway and inflammatory cytokines. On one hand, activated NF-κB can promote the expression of pro-inflammatory cytokines. On the other hand, the released pro-inflammatory cytokines react to the activation state of the NF-κB, leading to a cascade of inflammatory response in the intestinal tract, and pro-inflammatory cytokines are further released[10,32-34]. Thus, it is reasonable to consider that the activation of the NF-κB signaling pathway and the activation of inflammatory cytokines in IBS rats lead to low mucous mucosal inflammation in the distal ileum. Our experiments showed that oral administration of high-dose BBR or rifaximin can decrease the expression levels of NF-κB (P65) DNA-binding protein and NF-κB (P65) in IBS rats, which suggests that BBR or rifaximin may restrain the NF-κB signaling pathway, inhibit the activation of pro-inflammatory cytokines, increase the expression of anti-inflammatory cytokines, and reduce the inflammatory response in the distal ileum of rats. As mentioned above, high-dose BBR effectively inhibited mucosal inflammation by interdicting the positive feedback between the NF-κB signal pathway and inflammatory factors in IBS rats (Figure 10). Studies have shown that mucosal inflammation can lead to visceral hypersensitivity[35]. Therefore, oral high-dose BBR reduced visceral sensitivity possibly by controlling mucosal inflammation in the intestine.

The expression levels of BDNF and its receptor TrkB were increased in WAS group rats. Previous studies have shown that BDNF plays an important regulatory role in

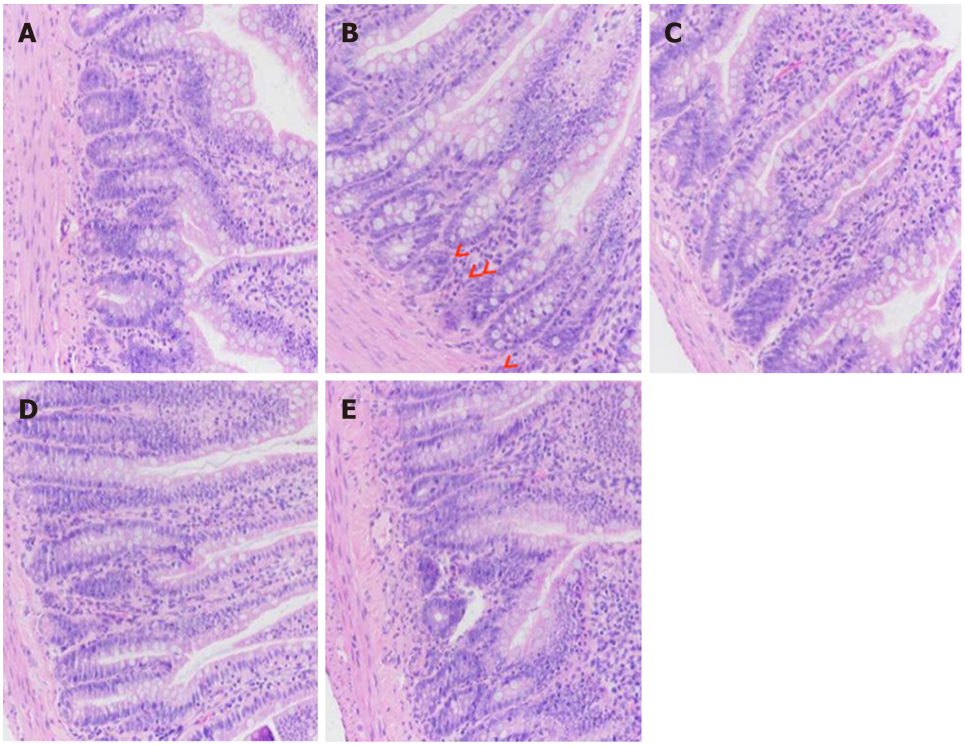


Figure 2 Effects of berberine on histological score of the distal intestine in rats (×200). A: Control group; B: Water avoidance stress group; C: Rifaximin group; D: 25 mg/kg berberine (BBR) group; E: 100 mg/kg BBR group (the labeled cells are neutrophils). Compared with the control group (A), rats in the water avoidance stress (WAS) group (B) showed low-grade intestinal inflammatory reaction. Microscopically, the tissues of the terminal ileum were intact, the mucosal structure and epithelium were intact, and the crypt was intact. Compared with the WAS group, after treatment with rifaximin (C), 25 mg/kg BBR (D), or 100 mg/kg BBR (E), the tissues of the terminal ileum of rats were intact, the mucosal structure and epithelium were intact, the crypt was intact, and no obvious neutrophil infiltration was observed. WAS: Water avoidance stress; BBR: Berberine.

IBS patients' visceral sensitivity and intestinal motility through the braingut axis. BDNF activates the signal transduction pathways of CaMK and MAPK after binding the high affinity receptor protein Trkb[36,37]. High-dose BBR or rifaximin can reduce the visceral hypersensitivity and intestinal motility of IBS rats and decrease the expression levels of BDNF mRNA, Trkb mRNA, and Trkb protein in the distal ileum. BBR reduces visceral hypersensitivity and intestinal dynamics by reducing BDNF and its receptor Trkb expression perhaps *via* the following mechanisms: (1) The increased expression of BDNF may damage the ultrastructure of intestinal nerve fibers, and result in a higher density of nerve fibers in the intestinal tract and the release of excitatory neurotransmitters, leading to the allergy of intestinal neurons[38]. BBR inhibits the expression of BDNF in the IBS rats to maintain the normal form of the enteric nervous system, keeps normal operation of the enteric nervous system, and eventually prevents the visceral hypersensitivity; (2) BDNF promotes the release of pain mediators in the intestinal tract[38], and BBR alleviates the pain of the IBS rats by reducing the expression of BDNF; and (3) BBR reduces the activity of serine protease by reducing the expression of BDNF in the intestinal tract, thereby improving the symptoms of diarrhea in IBS patients, as well as the severity and frequency of abdominal pain.

C-kit mRNA and protein expression levels increased in the distal ileum of WAS group rats, suggesting that the C-kit signaling pathway in rats was activated. C-kit activation may affect the regulatory effect of ICC cells on intestinal sensitivity and intestinal motility in rats. ICC can spontaneously produce rhythmic slow wave, transmit the intestinal nerve signals to the smooth muscle cells to ensure the normal movement of the intestinal tract, and regulate the intestinal motility and visceral sensitivity of IBS patients through the brain-gut axis[20,39,40]. The C-kit signal pathway plays an important role in the proliferation and development of ICC cells. Thus, C-kit protein expression is an important marker for ICC cells[41,42]. Stem cell factor (SCF), an important ligand of C-kit, is one of the important cytokines that induce IBS[19,43]. High-dose BBR or rifaximin can reduce the visceral hypersensitivity and intestinal motility of IBS rats, and decrease the expression levels of C-kit in the distal ileum. BBR reduces visceral hypersensitivity and intestinal dynamics by reducing C-kit expression, which may be associated with the following mechanisms: (1) Through both SCF and C-kit, the JAK/STAT signaling pathway is activated, and STAT translocates into the

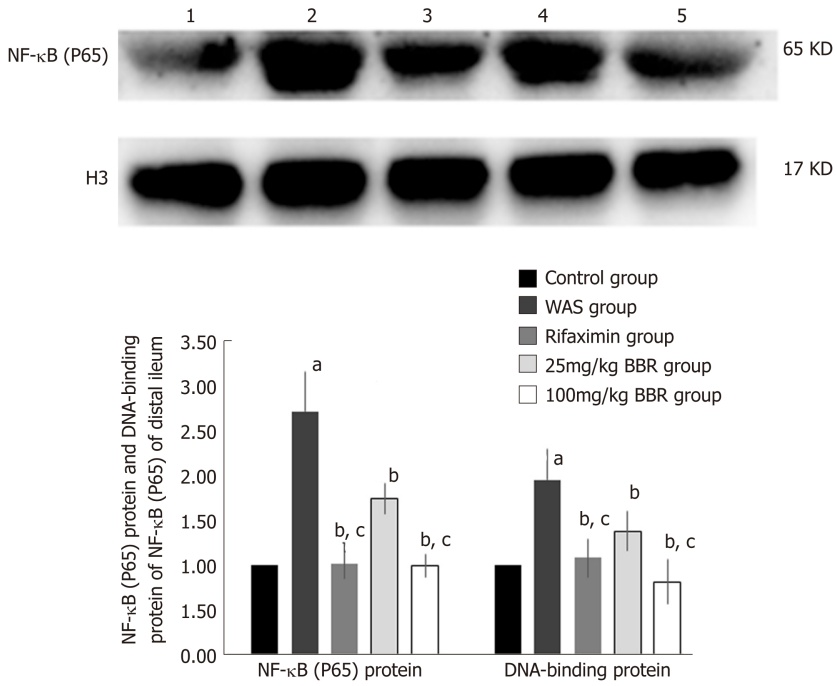


Figure 3 Effects of berberine on NF-κB (P65) protein and NF-κB (P65) DNA-binding protein expression. Expression of NF-κB (P65) protein and DNA-binding protein of NF-κB in 1: Control group; 2: Water avoidance stress (WAS) group; 3: Rifaximin group; 4: 25 mg/kg berberine (BBR) group; 5: 100 mg/kg BBR group. Letters a, b, and c: $P < 0.05$ compared with those in the control group, WAS group, and 25 mg/kg BBR group, respectively. WAS: Water avoidance stress; BBR: Berberine.

nucleus, leading to the proliferation of ICC cells. Pathological changes occur in the new ICC, leading to abnormal intestinal electrophysiological pacing. BBR may inhibit the activation of the JAK/STAT signaling pathway by decreasing the expression of C-kit, reducing the abnormal regeneration of ICC, inhibiting the generation of abnormal electrical signals, and ultimately reducing the intestinal motility; (2) SCF and C-kit activate the P13K-AKT signaling pathway, which increases the expression of gap junction proteins in the intestinal smooth muscle cells. The electrical signals generated by the ICC are more likely to be transmitted to each other, which increases intestinal movement. Reduction in the expression level of C-kit can inhibit the activity of the P13K-AKT signaling pathway, leading to a decline of the expression levels of gap junction proteins in the intestinal smooth muscle cells and transmission efficiency of abnormal electrical signals being reduced, which eventually causes reduction of the intestinal motility; and (3) Activation of the RAS-ERK signaling by SCF and C-kit increases the sensitivity of the neurotransmitter receptors on the ICC surface, making them easier to bind the neurotransmitter secreted by the central nervous system and the enteric nervous system. Thus, intestinal sensitivity and intestinal motility are increased. BBR inhibits the RAS-ERK signaling pathway by reducing the expression of C-kit, decreases the sensitivity of neuron receptors on the surface of ICC cells, and ultimately reduces visceral sensitivity and intestinal motility[6,44,45].

Our results showed that BBR can reduce inflammation of the distal ileum in IBS rats by inhibiting NF-κB signal pathways, and regulate visceral sensitivity and intestinal motility in the treatment of IBS by reducing the expression of BDNF, its receptor TrkB, and C-kit. Recent studies have shown that when patients experience the colon expansion test (CRD), IBS patients not only show increased abdominal withdrawal reflex, but also increased range of brain activity reflex. These results suggest that IBS patients not only have visceral hypersensitivity, but also have increased sensitivity of the central system. This interaction may be achieved through the axis of the brain. First, hormones and neurotransmitters secreted by the neuroendocrine system act on immune cells and mast cells in the intestinal mucosa. Mast cells release SCF and inflammatory media. Subsequently, inflammation reduces intestinal sensitivity and intestinal motivation *via* influencing the intestinal smooth muscle cells and neurons[46-49]. Second, in the stress state, the primary afferent neurons in the gastrointestinal tract are activated and the spinal cord is sensitized[50], which increases the permeability of the intestine[51,52]. The increase of intestinal permeability leads to the defect of mucosal barrier, and enhances bacterial adhesion and infiltration into the gastrointestinal mucosa[32,48]. DNA of these bacteria can interact with Toll-like

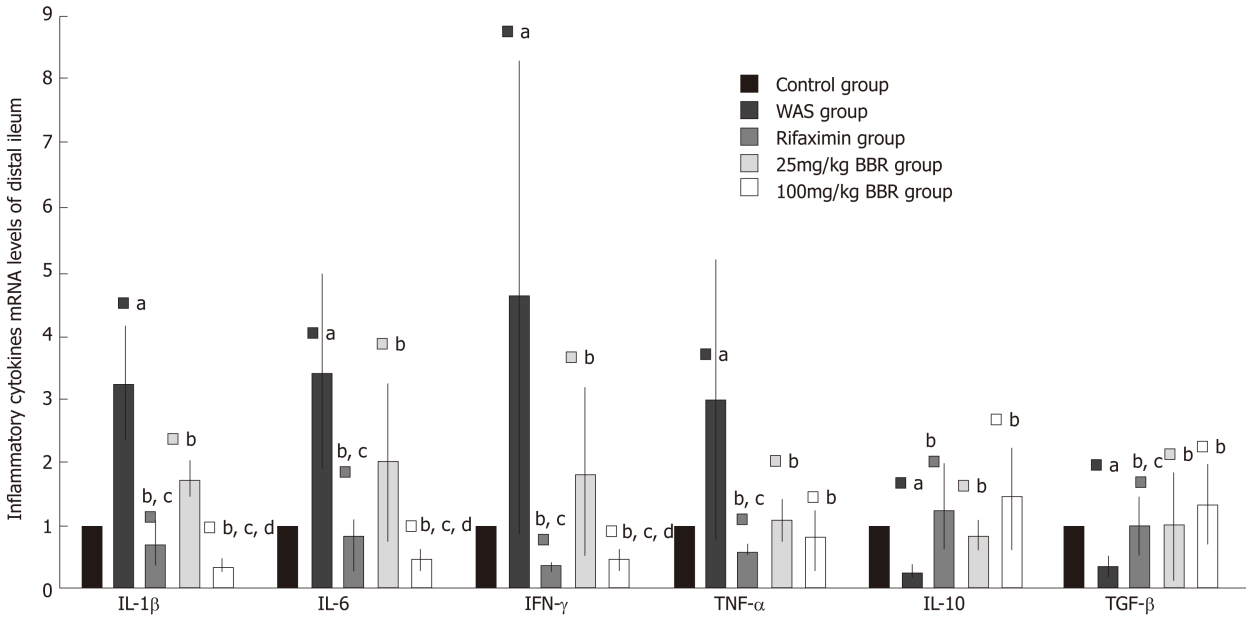


Figure 4 Effects of berberine on cytokine mRNA levels. Cytokine mRNA levels in the control group, water avoidance stress (WAS) group, rifaximin group, 25 mg/kg berberine (BBR) group, and 100 mg/kg BBR group are shown. Letters a, b, c, and d: $P < 0.05$ compared with those in the control group, WAS group, 25 mg/kg BBR group, and rifaximin group, respectively. WAS: Water avoidance stress; BBR: Berberine; IL: Interleukin; IFN: Interferon; TNF-α: Tumor necrosis factor-α; TGF-β: Transforming growth factor-β.

increases the permeability of the intestine[51,52]. The increase of intestinal permeability leads to the defect of mucosal barrier, and enhances bacterial adhesion and infiltration into the gastrointestinal mucosa[32,48]. DNA of these bacteria can interact with Toll-like receptors[53] to regulate cytokines such as TNF and IFN[54] and activate intestinal mucosal immune response[55]. Third, anatomically, the gut immune cells are closely linked to the axons of the gut neurons. The inflammatory factors may change the structure of the nerve and increase the visceral sensation through the distal end of the afferent nerve and the activated spinal dorsal horn[56,57]. Ultimately, the intestinal motility is enhanced[58]. The three pathways studied in this study were also connected through the brain-gut axis. The immune activation of IBS is not only limited to the intestinal wall, but also the whole body, which is manifested by the increase of the pro-inflammatory cytokines and the reduction of anti-inflammatory cytokines mediated by the NF-κB signal pathway[13,14]. Cytokines are involved in the interaction of the brain-gut axis, and these inflammatory factors can act on smooth muscle cells and neurons in the gut, leading to the changes in intestinal motility and visceral sensitivity[13,59]. Stress stimulates intestinal smooth muscle cells to release SCF. Binding of SCF and C-kit activates C-kit kinase and promotes the secretion of mast cells to release a series of inflammatory mediators and inflammatory factors, leading to low inflammatory response in the intestinal tract. In the central nervous system, BDNF promotes the release of pro-inflammatory cytokines by activating nerve cells such as astrocytes, and the inflammatory response also causes BDNF to increase in dorsal root ganglia. Therefore, BDNF interacts not only with the intestinal nervous system, but also with the intestinal immune system, which can affect the visceral sensitivity and intestinal dynamics of IBS rats[60,61]. These pathways reinforce each other through the brain-gut axis, resulting in intestinal inflammation, visceral hypersensitivity, and increased intestinal motility in IBS patients. BBR can treat IBS patients by regulating these three pathways and blocking the interaction among them.

In conclusion, BBR inhibits the mucosal inflammation of the intestinal tract by inhibiting the intestinal NF-κB signal pathway in the IBS rats. BBR reduces the expression of BDNF and its receptor TrkB, and the expression of C-kit to reduce intestinal motility and visceral sensitivity and produce a therapeutic effect on IBS. The therapeutic effect of 100 mg/kg BBR is superior to that of 25 mg/kg BBR.

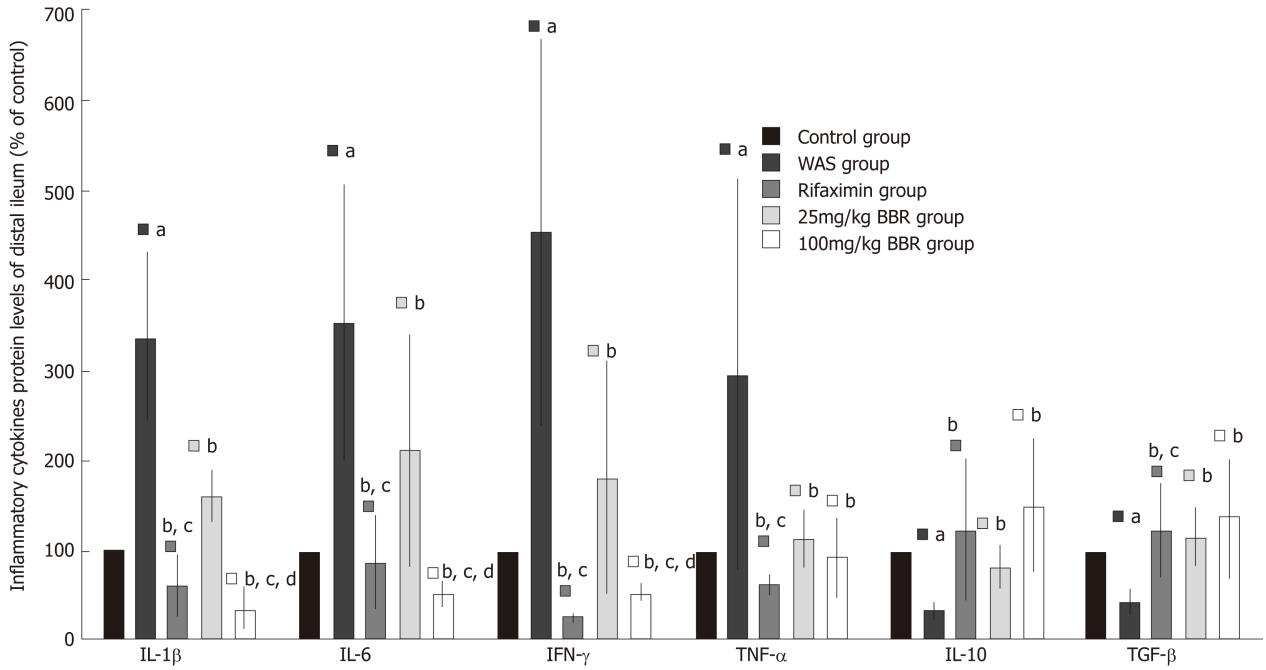


Figure 5 Effects of berberine on cytokine protein levels. Cytokine protein levels in the control group, water avoidance stress (WAS) group, rifaximin group, 25 mg/kg berberine (BBR) group, and 100 mg/kg BBR group are shown. Letters a, b, c, and d; $P < 0.05$ compared with those in the control group, WAS group, 25 mg/kg BBR group, and rifaximin group, respectively. WAS: Water avoidance stress; BBR: Berberine; IL: Interleukin; IFN: Interferon; TNF-α: Tumor necrosis factor-α; TGF-β: Transforming growth factor-β.

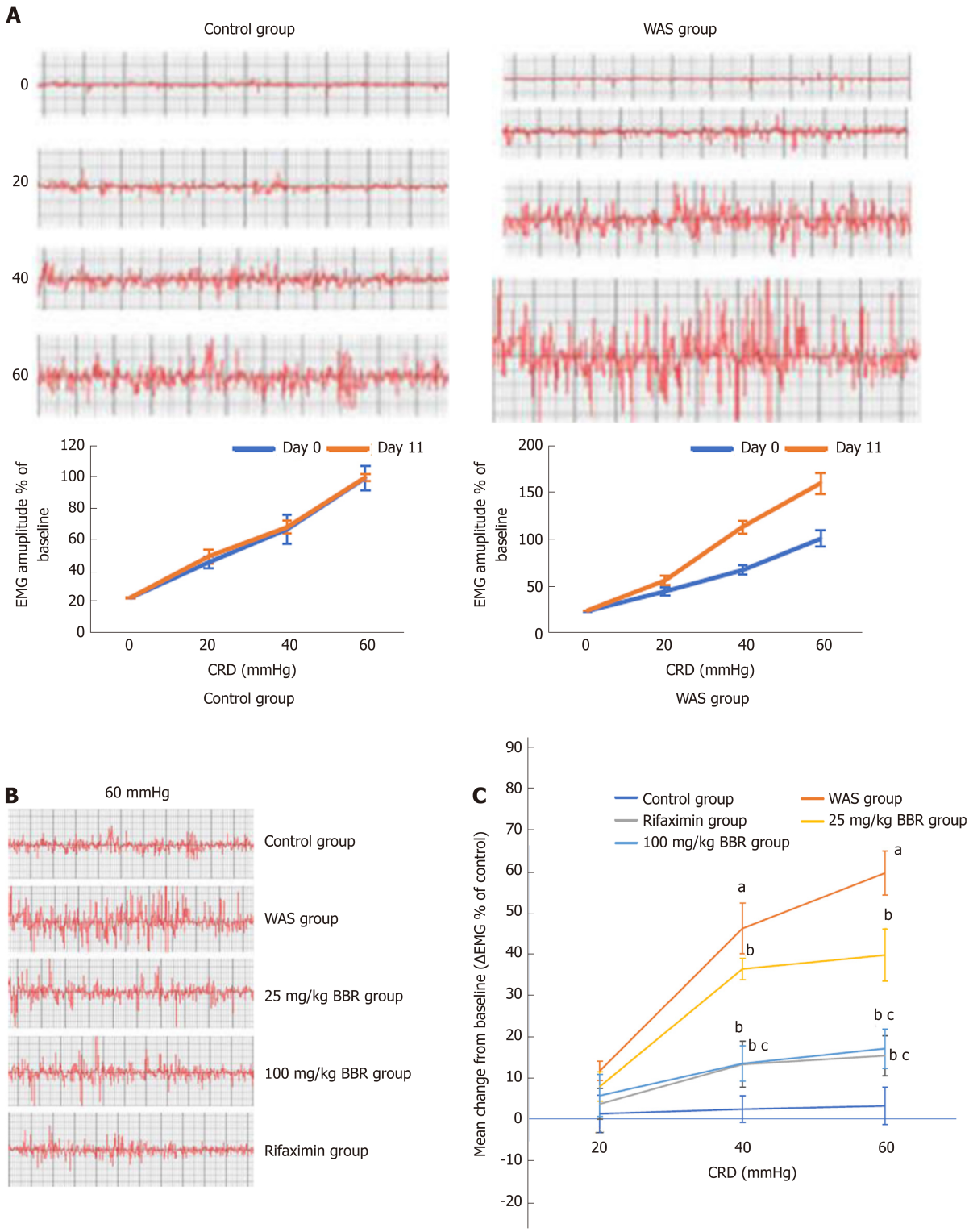


Figure 6 Effects of berberine on visceromotor response to colorectal distention in rats. We took the average EMG amplitude measured on day 11 subtracted by the average EMG amplitude detected on day 0, and express it as EMG. A: The amplitude of electromyogram (EMG) was changed in the control group and water avoidance stress (WAS) group under different pressures of colorectal distention (CRD); B: The amplitude of EMG in the control group, WAS group, rifaximin group, 25 mg/kg berberine (BBR) group, and 100 mg/kg BBR group under 60 mmHg of CRD on day 11; C: EMG of different group rats under different pressures of CRD. Letters a, b, and c: $P < 0.05$ compared with those in the control group, WAS group, and 25 mg/kg BBR group, respectively. WAS: Water avoidance stress; BBR: Berberine; CRD: Colorectal distention; EMG: Electromyogram.

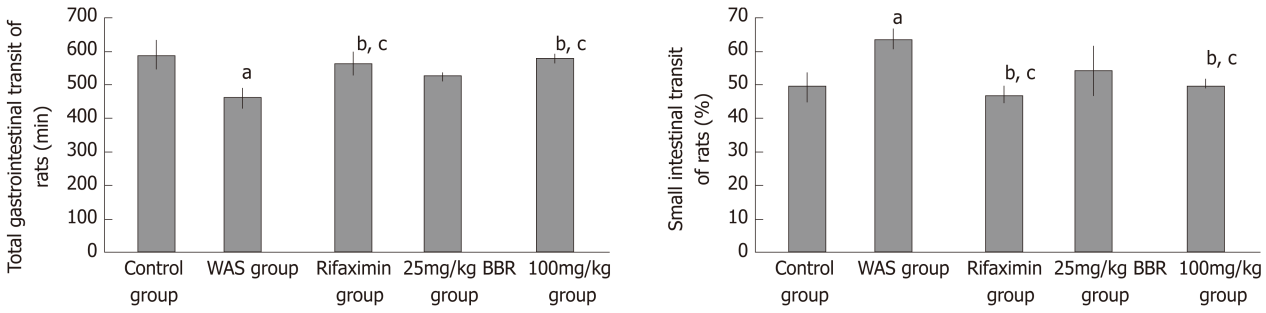


Figure 7 Effects of berberine on total gastrointestinal transit and small intestinal transit. Total gastrointestinal transit and small intestinal transit in the control group, water avoidance stress (WAS) group, rifaximin group, 25 mg/kg berberine (BBR) group, and 100 mg/kg BBR group are shown. Letters a, b, and c: $P < 0.05$ compared with those in the control group, WAS group, and 25 mg/kg BBR group, respectively. WAS: Water avoidance stress; BBR: Berberine.

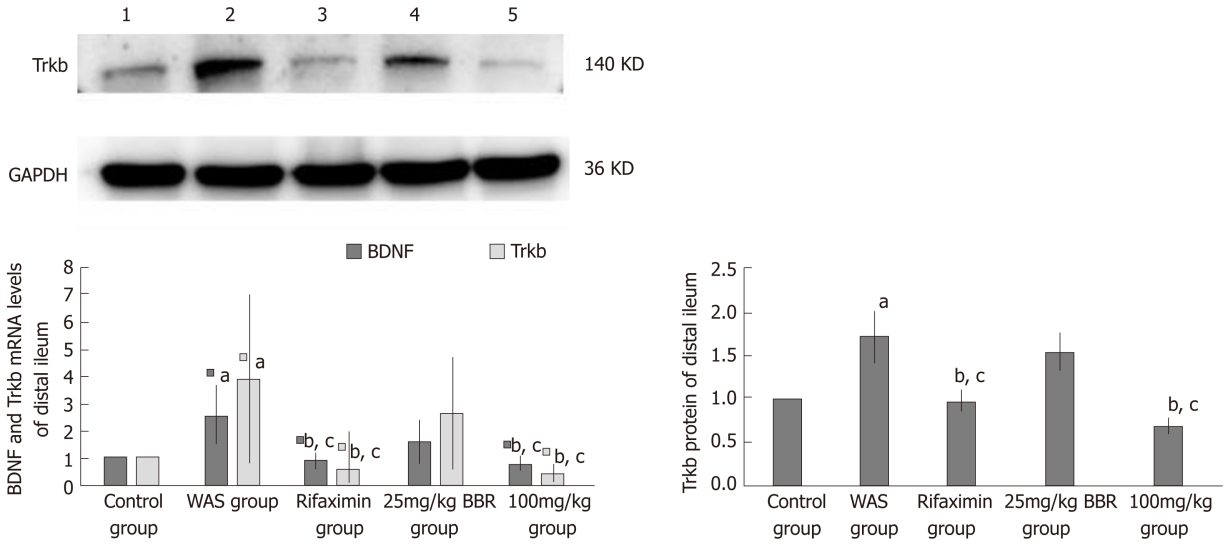


Figure 8 Effects of berberine on brain derived neurotrophic factor and Trkb mRNA and protein expression. Expression of brain derived neurotrophic factor and Trkb mRNA and protein in 1: control group; 2: water avoidance stress (WAS) group; 3: rifaximin group; 4: 25 mg/kg berberine (BBR) group; and 5: 100 mg/kg BBR group. Letters a, b, and c: $P < 0.05$ compared with those in the control group, WAS group, and 25 mg/kg BBR group, respectively. BDNF: Brain derived neurotrophic factor; WAS: Water avoidance stress; BBR: Berberine.

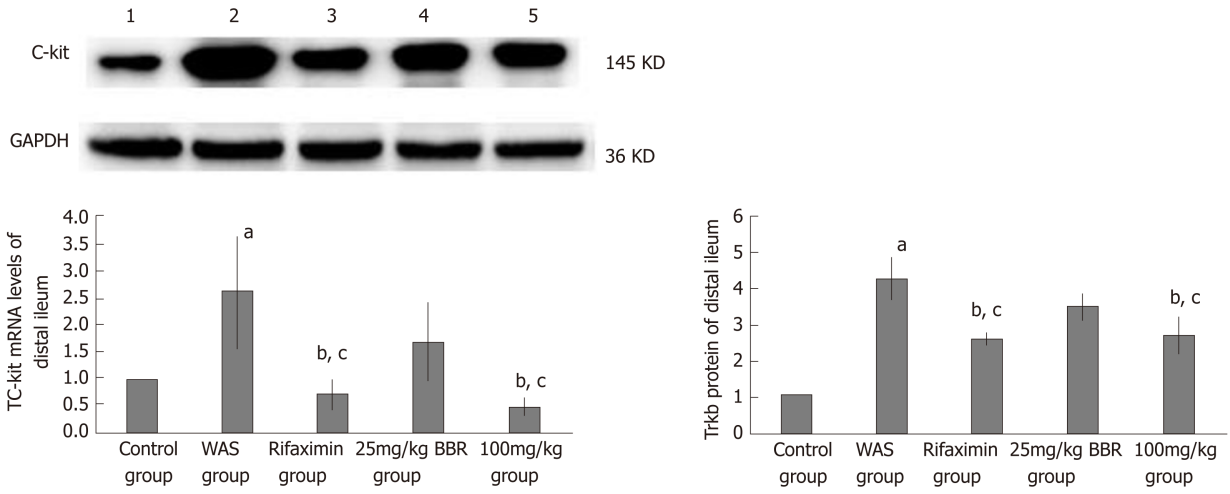


Figure 9 Effects of berberine on C-kit mRNA and protein expression. Expression of C-kit mRNA and protein in 1: control group; 2: water avoidance stress (WAS) group; 3: rifaximin group; 4: 25 mg/kg berberine (BBR) group; and 5: 100 mg/kg BBR group. Letters a, b, and c: $P < 0.05$ compared with those in the control group, WAS group, and 25 mg/kg BBR group, respectively. WAS: Water avoidance stress; BBR: Berberine.

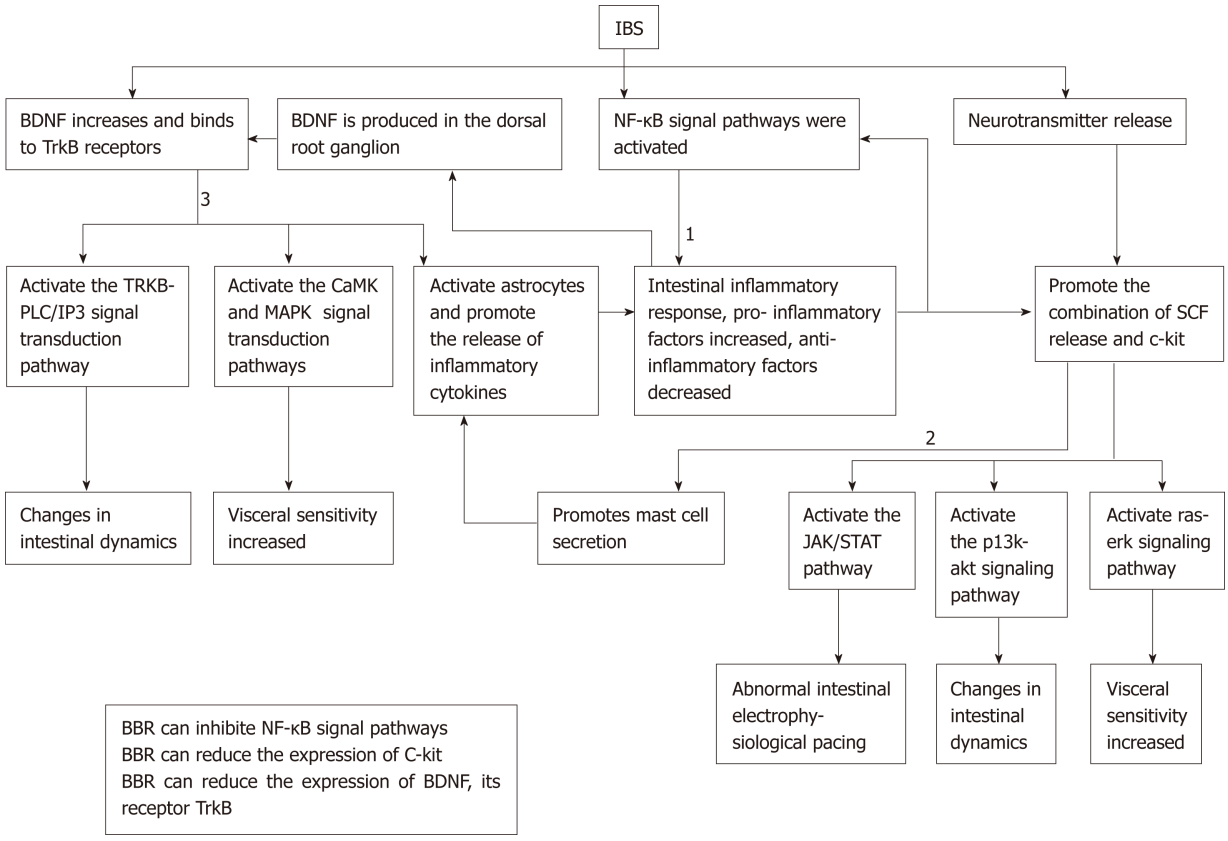


Figure 10 Relationship among NF-kB signaling, brain derived neurotrophic factor, and C-kit and the underlying role of berberine. IBS: Irritable bowel syndrome; BDNF: Brain derived neurotrophic factor; SCF: Stem cell factor.

ARTICLE HIGHLIGHTS

Research background

Irritable bowel syndrome (IBS) is a common chronic non-organic disease of the digestive system. Rifaximin has been used in clinical treatment of patients with IBS and has achieved good efficacy. However, rifaximin is expensive, and long-term oral administration may lead to cross-resistance to rifabutin and rifampicin. A large number of basic and clinical studies have also shown the efficacy of berberine in the treatment of IBS.

Research motivation

Many studies have demonstrated that the pathogenesis of IBS may be related to the disorder of brain-gut axis, visceral hypersensitivity, intestinal immune abnormality, intestinal motility change, increased intestinal mucosal permeability, and intestinal flora disorder. The treatment mechanism of berberine for IBS is still unclear. In this study, we tried to investigate the effect of berberine on intestinal inflammation, intestinal motility, and intestinal sensitivity in rats with IBS and explore the therapeutic mechanism of berberine for IBS.

Research objectives

The purpose of this study was to investigate the effect of berberine on the NF-κB signaling pathway in rats with IBS, which may improve intestinal inflammation in rats with IBS. And we studied the influence of berberine on the expression levels of brain-derived neurotrophic factor (BDNF) and C-kit in the intestinal tract of rats, which may affect the intestinal motility and visceral sensitivity of rats.

Research methods

Water avoidance stress (WAS) was used to establish an IBS rat model, and the rats were divided into a control group, a WAS group, a rifaximin group, a 25 mg/kg BBR group, and a 100 mg/kg BBR group. We evaluated the histopathological changes of the terminal ileum in rats by hematoxylin and eosin (HE) staining. We measured the expression levels of NF-Kb (P65) DNA binding protein in the terminal ileum tissues of rats of each group by modified ELISA and Western blot. The mRNA expression levels of IL-1β, IL-6, IFN-γ, TNF-α, IL-10, and TGF-β were determined by qRT-PCR. ELISA was used to detect the inflammatory cytokines. Intestinal motility of rats in each group was detected by total gastrointestinal and small intestinal transit functions. The mRNA expression levels of BDNF and its receptor TrkB were detected by qRT-PCR. Western blot was used to detect the expression level of TrkB protein in the terminal ileum tissues in each group of rats. The mRNA expression levels of C-kit in ileum terminal tissues of

rats in each group were detected by qRT-PCR. The expression level of C-kit protein in ileum terminal tissues of each group of rats was detected by Western blot.

Research results

We successfully applied the WAS model to induce visceral hypersensitivity in rats, changes in intestinal inflammation, and intestinal motility, which are consistent with the characteristics of IBS. Berberine can effectively improve the inflammation of in terminal tissues. Berberine can inhibit the activated NF-κB signal pathway in the intestinal tract of IBS rats, significantly reduce the expression of inflammatory IL-1β, IL-6, IFN-γ, and TNF-α in the terminal ileum tissues of IBS rats, and significantly increase the expression levels of anti-inflammatory cytokines IL-10 and TGF-β. Berberine can effectively regulate the intestinal motility of IBS rats and inhibit the expression of BDNF and its receptor TrkB as well as C-kit in ileum terminal tissues of IBS rats. The treatment effect of large dose (100 mg/kg) berberine on IBS rats was significantly better than that of small dose (25 mg/kg) berberine.

Research conclusions

Berberine can inhibit the mucosal inflammation of the intestinal tract by inhibiting the intestinal NF-κB signal pathway in IBS rats. Berberine reduces the expression of BDNF and its receptor TrkB as well as C-kit to reduce intestinal motility and visceral sensitivity and produce a therapeutic effect on IBS. The therapeutic effect of 100 mg/kg berberine is superior to that of 25 mg/kg berberine.

Research perspectives

This study confirms the exact therapeutic effect of berberine on IBS at the level of animal experiments, discusses the possible mechanism of its therapeutic effect, and provides a theoretical basis for the clinical application of berberine in the treatment of IBS. However, the optimal dosage of berberine in the clinical treatment of IBS still needs further pharmacological and toxicological studies

REFERENCES

1 **Chen C**, Lu M, Pan Q, Fichna J, Zheng L, Wang K, Yu Z, Li Y, Li K, Song A, Liu Z, Song Z, Kreis M. Berberine Improves Intestinal Motility and Visceral Pain in the Mouse Models Mimicking Diarrhea-Predominant Irritable Bowel Syndrome (IBS-D) Symptoms in an Opioid-Receptor Dependent Manner. *PLoS One* 2015; **10**: e0145556 [PMID: 26700862 DOI: 10.1371/journal.pone.0145556]

2 **Chen FL**, Yang ZH, Liu Y, Li LX, Liang WC, Wang XC, Zhou WB, Yang YH, Hu RM. Berberine inhibits the expression of TNFalpha, MCP-1, and IL-6 in AcLDL-stimulated macrophages through PPARgamma pathway. *Endocrine* 2008; **33**: 331-337 [PMID: 19034703 DOI: 10.1007/s12020-008-9089-3]

3 **Kim S**, Kim Y, Kim JE, Cho KH, Chung JH. Berberine inhibits TPA-induced MMP-9 and IL-6 expression in normal human keratinocytes. *Phytomedicine* 2008; **15**: 340-347 [PMID: 17951041 DOI: 10.1016/j.phymed.2007.09.011]

4 **Tjong Y**, Ip S, Lao L, Fong HH, Sung JJ, Berman B, Che C. Analgesic effect of Coptis chinensis rhizomes (Coptidis Rhizoma) extract on rat model of irritable bowel syndrome. *J Ethnopharmacol* 2011; **135**: 754-761 [PMID: 21511022 DOI: 10.1016/j.jep.2011.04.007]

5 **Camilleri M**, Lasch K, Zhou W. Irritable bowel syndrome: Methods, mechanisms, and pathophysiology. The confluence of increased permeability, inflammation, and pain in irritable bowel syndrome. *Am J Physiol Gastrointest Liver Physiol* 2012; **303**: G775-G785 [PMID: 22837345 DOI: 10.1152/ajpgi.00155.2012]

6 **Ohman L**, Simrén M. Pathogenesis of IBS: Role of inflammation, immunity and neuroimmune interactions. *Nat Rev Gastroenterol Hepatol* 2010; **7**: 163-173 [PMID: 20101257 DOI: 10.1038/nrgastro.2010.4]

7 **Langhorst J**, Choi KE. The role of human defensins in gastrointestinal diseases. *Expert Rev Clin Immunol* 2011; **7**: 779-787 [PMID: 22014019 DOI: 10.1586/eci.11.62]

8 **Bradesi S**, McRoberts JA, Anton PA, Mayer EA. Inflammatory bowel disease and irritable bowel syndrome: Separate or unified? *Curr Opin Gastroenterol* 2003; **19**: 336-342 [PMID: 15703574 DOI: 10.1097/00001574-200307000-00003]

9 **Spiller RC**. Neuropathology of IBS? *Gastroenterology* 2002; **123**: 2144-2147 [PMID: 12454870 DOI: 10.1053/gast.2002.37287]

10 **Hartung JE**, Eskew O, Wong T, Tchivileva IE, Oladosu FA, O'Buckley SC, Nackley AG. Nuclear factor-kappa B regulates pain and COMT expression in a rodent model of inflammation. *Brain Behav Immun* 2015; **50**: 196-202 [PMID: 26187567 DOI: 10.1016/j.bbi.2015.07.014]

11 **Binshtok AM**, Wang H, Zimmermann K, Amaya F, Vardeh D, Shi L, Brenner GJ, Ji RR, Bean BP, Woolf CJ, Samad TA. Nociceptors are interleukin-1beta sensors. *J Neurosci* 2008; **28**: 14062-14073 [PMID: 19109489 DOI: 10.1523/JNEUROSCI.3795-08.2008]

12 **Gudes S**, Barkai O, Caspi Y, Katz B, Lev S, Binshtok AM. The role of slow and persistent TTX-resistant sodium currents in acute tumor necrosis factor-α-mediated increase in nociceptors excitability. *J Neurophysiol* 2015; **113**: 601-619 [PMID: 25355965 DOI: 10.1152/jn.00652.2014]

13 **Kindt S**, Van Oudenhove L, Broekaert D, Kasran A, Ceuppens JL, Bossuyt X, Fischler B, Tack J. Immune dysfunction in patients with functional gastrointestinal disorders. *Neurogastroenterol Motil* 2009; **21**: 389-398 [PMID: 19126184 DOI: 10.1111/j.1365-2982.2008.01220.x]

14 **Macsharry J**, O'Mahony L, Fanning A, Bairead E, Sherlock G, Tiesman J, Fulmer A, Kiely B, Dinan TG, Shanahan F, Quigley EM. Mucosal cytokine imbalance in irritable bowel syndrome. *Scand J Gastroenterol* 2008; **43**: 1467-1476 [PMID: 18752146 DOI: 10.1080/00365520802276127]

15 **Yu YB**, Zuo XL, Zhao QJ, Chen FX, Yang J, Dong YY, Wang P, Li YQ. Brain-derived neurotrophic factor contributes to abdominal pain in irritable bowel syndrome. *Gut* 2012; **61**: 685-694 [PMID: 21997550 DOI: 10.1136/gutjnl-2011-300265]

16 **O'Sullivan E**, Barrett E, Grenham S, Fitzgerald P, Stanton C, Ross RP, Quigley EM, Cryan JF, Dinan TG. BDNF expression in the hippocampus of maternally separated rats: Does Bifidobacterium breve 6330 alter BDNF levels? *Benef Microbes* 2011; **2**: 199-207 [PMID: 21986359 DOI: 10.3920/BM2011.0015]

17 **Koh SD**, Kim TW, Jun JY, Glasgow NJ, Ward SM, Sanders KM. Regulation of pacemaker currents in interstitial cells of Cajal from murine small intestine by cyclic nucleotides. *J Physiol* 2000; **527** Pt 1: 149-162 [PMID: 10944178 DOI: 10.1111/j.1469-7793.2000.00149.x]

18 **Wang XY**, Vannucchi MG, Nieuwmeyer F, Ye J, Faussone-Pellegrini MS, Huizinga JD. Changes in interstitial cells of Cajal at the deep muscular plexus are associated with loss of distention-induced burst-type muscle activity in mice infected by Trichinella spiralis. *Am J Pathol* 2005; **167**: 437-453 [PMID: 16049330 DOI: 10.1016/S0002-9440(10)62988-4]

19 **Rich A**, Gordon S, Brown C, Gibbons SJ, Schaefer K, Hennig G, Farrugia G. Kit signaling is required for development of coordinated motility patterns in zebrafish gastrointestinal tract. *Zebrafish* 2013; **10**: 154-160 [PMID: 23297728 DOI: 10.1089/zeb.2012.0766]

20 **Eshraghian A**, Eshraghian H. Interstitial cells of Cajal: A novel hypothesis for the pathophysiology of irritable bowel syndrome. *Can J Gastroenterol* 2011; **25**: 277-279 [PMID: 21647464 DOI: 10.1155/2011/478370]

21 **Pimentel M**, Lembo A, Chey WD, Zakko S, Ringel Y, Yu J, Mareya SM, Shaw AL, Bortey E, Forbes WP; TARGET Study Group. Rifaximin therapy for patients with irritable bowel syndrome without constipation. *N Engl J Med* 2011; **364**: 22-32 [PMID: 21208106 DOI: 10.1056/NEJMoa1004409]

22 **Xu D**, Gao J, Gillilland M, Wu X, Song I, Kao JY, Owyang C. Rifaximin alters intestinal bacteria and prevents stress-induced gut inflammation and visceral hyperalgesia in rats. *Gastroenterology* 2014; **146**: 484-96.e4 [PMID: 24161699 DOI: 10.1053/j.gastro.2013.10.026]

23 **Farrell DJ**. Rifaximin in the treatment of irritable bowel syndrome: Is there a high risk for development of antimicrobial resistance? *J Clin Gastroenterol* 2013; **47**: 205-211 [PMID: 23340064 DOI: 10.1097/MCG.0b013e31827559a3]

24 **Senol G**, Erbaycu A, Ozsöz A. Incidence of cross resistance between rifampicin and rifabutin in Mycobacterium tuberculosis strains in Izmir, Turkey. *J Chemother* 2005; **17**: 380-384 [PMID: 16167516 DOI: 10.1179/joc.2005.17.4.380]

25 **Bradesi S**, Schwetz I, Ennes HS, Lamy CM, Ohning G, Fanselow M, Pothoulakis C, McRoberts JA, Mayer EA. Repeated exposure to water avoidance stress in rats: A new model for sustained visceral hyperalgesia. *Am J Physiol Gastrointest Liver Physiol* 2005; **289**: G42-G53 [PMID: 15746211 DOI: 10.1152/ajpgi.00500.2004]

26 **Gong Z**, Chen Y, Zhang R, Yang Q, Wang Y, Guo Y, Zhou B, Weng X, Liu X, Li Y, Zhu X, Dong Y. Pharmacokinetic difference of berberine between normal and chronic visceral hypersensitivity irritable bowel syndrome rats and its mechanism. *Arch Pharm Res* 2015; **38**: 1888-1896 [PMID: 25716428 DOI: 10.1007/s12272-015-0568-9]

27 **Tang QL**, Lai ML, Zhong YF, Wang AM, Su JK, Zhang MQ. Antinociceptive effect of berberine on visceral hypersensitivity in rats. *World J Gastroenterol* 2013; **19**: 4582-4589 [PMID: 23901236 DOI: 10.3748/wjg.v19.i28.4582]

28 **Wang P**, Chen FX, Du C, Li CQ, Yu YB, Zuo XL, Li YQ. Increased production of BDNF in colonic epithelial cells induced by fecal supernatants from diarrheic IBS patients. *Sci Rep* 2015; **5**: 10121 [PMID: 25998025 DOI: 10.1038/srep10121]

29 **Xie W**, Xing D, Zhao Y, Su H, Meng Z, Chen Y, Du L. A new tactic to treat postprandial hyperlipidemia in diabetic rats with gastroparesis by improving gastrointestinal transit. *Eur J Pharmacol* 2005; **510**: 113-120 [PMID: 15740731 DOI: 10.1016/j.ejphar.2005.01.019]

30 **Morteau O**, Hachet T, Caussette M, Bueno L. Experimental colitis alters visceromotor response to colorectal distension in awake rats. *Dig Dis Sci* 1994; **39**: 1239-1248 [PMID: 8200256 DOI: 10.1007/BF02093789]

31 **Davey KJ**, O'Mahony SM, Schellekens H, O'Sullivan O, Bienenstock J, Cotter PD, Dinan TG, Cryan JF. Gender-dependent consequences of chronic olanzapine in the rat: Effects on body weight, inflammatory, metabolic and microbiota parameters. *Psychopharmacology (Berl)* 2012; **221**: 155-169 [PMID: 22234378 DOI: 10.1007/s00213-011-2555-2]

32 **Gareau MG**, Jury J, Yang PC, MacQueen G, Perdue MH. Neonatal maternal separation causes colonic dysfunction in rat pups including impaired host resistance. *Pediatr Res* 2006; **59**: 83-88 [PMID: 16326990 DOI: 10.1203/01.pdr.0000190577.62426.45]

33 **Ogata H**, Hibi T. Cytokine and anti-cytokine therapies for inflammatory bowel disease. *Curr Pharm Des* 2003; **9**: 1107-1113 [PMID: 12769750 DOI: 10.2174/1381612033455035]

34 **Christianson JA**, Gebhart GF. Assessment of colon sensitivity by luminal distension in mice. *Nat Protoc* 2007; **2**: 2624-2631 [PMID: 17948005 DOI: 10.1038/nprot.2007.392]

35 **Dal Sasso M**, Culici M, Guffanti EE, Bianchi T, Fonti E, Braga PC. A combination of budesonide and the SH-metabolite I of erdosteine acts synergistically in reducing chemiluminescence during human neutrophil respiratory burst. *Pharmacology* 2005; **74**: 127-134 [PMID: 15753624 DOI: 10.1159/000084295]

36 **Wang P**, Du C, Chen FX, Li CQ, Yu YB, Han T, Akhtar S, Zuo XL, Tan XD, Li YQ. BDNF contributes to IBS-like colonic hypersensitivity via activating the enteroglia-nerve unit. *Sci Rep* 2016; **6**: 20320 [PMID: 26837784 DOI: 10.1038/srep20320]

37 **Andero R**, Choi DC, Ressler KJ. BDNF-TrkB receptor regulation of distributed adult neural plasticity, memory formation, and psychiatric disorders. *Prog Mol Biol Transl Sci* 2014; **122**: 169-192 [PMID: 24484701 DOI: 10.1016/B978-0-12-420170-5.00006-4]

38 **Boesmans W**, Gomes P, Janssens J, Tack J, Vanden Berghe P. Brain-derived neurotrophic factor amplifies neurotransmitter responses and promotes synaptic communication in the enteric nervous system. *Gut* 2008; **57**: 314-322 [PMID: 17965066 DOI: 10.1136/gut.2007.131839]

39 **Deng J**, He P, Zhong X, Wang Q, Li L, Song B. Identification of T-type calcium channels in the interstitial cells of Cajal in rat bladder. *Urology* 2012; **80**: 1389.e1-1389.e7 [PMID: 22995572 DOI: 10.1016/j.urology.2012.07.031]

40 **Jee SR**, Morales W, Low K, Chang C, Zhu A, Pokkunuri V, Chatterjee S, Soffer E, Conklin JL, Pimentel M. ICC density predicts bacterial overgrowth in a rat model of post-infectious IBS. *World J Gastroenterol* 2010; **16**: 3680-3686 [PMID: 20677340 DOI: 10.3748/wjg.v16.i29.3680]

41 **Abramovic M**, Radenkovic G, Velickov A. Appearance of interstitial cells of Cajal in the human midgut. *Cell Tissue Res* 2014; **356**: 9-14 [PMID: 24414177 DOI: 10.1007/s00441-013-1772-x]

42 **Li Z**, Zheng H, Li GB, Zhi H, Yuan WT. [Alterations of Cajal cells in the colon of slow transit constipation rats]. *Zhonghua Wei Chang Wai Ke Za Zhi* 2013; **16**: 777-779 [PMID: 23980052]

43 **Ghaith O**, El-Halabi M, Hashash JG, Sharara AI. Investigational agents for the irritable bowel syndrome. *Expert Opin Investig Drugs* 2010; **19**: 1161-1178 [PMID: 20836617 DOI: 10.1517/13543784.2010.513380]

44 **DiNitto JP**, Deshmukh GD, Zhang Y, Jacques SL, Coli R, Worrall JW, Diehl W, English JM, Wu JC. Function of activation loop tyrosine phosphorylation in the mechanism of c-Kit auto-activation and its implication in sunitinib resistance. *J Biochem* 2010; **147**: 601-609 [PMID: 20147452 DOI: 10.1093/jb/mvq015]

45 **Midrio P**, Vannucchi MG, Pieri L, Alaggio R, Faussone-Pellegrini MS. Delayed development of interstitial cells of Cajal in the ileum of a human case of gastroschisis. *J Cell Mol Med* 2008; **12**: 471-478 [PMID: 18266958 DOI: 10.1111/j.1582-4934.2008.00277.x]

46 **Mazzon E**, Sturniolo GC, Puzzolo D, Frisina N, Fries W. Effect of stress on the paracellular barrier in the rat ileum. *Gut* 2002; **51**: 507-513 [PMID: 12235072 DOI: 10.1136/gut.51.4.507]

47 **Barreau F**, Cartier C, Leveque M, Ferrier L, Moriez R, Laroute V, Rosztoczy A, Fioramonti J, Bueno L. Pathways involved in gut mucosal barrier dysfunction induced in adult rats by maternal deprivation: Corticotrophin-releasing factor and nerve growth factor interplay. *J Physiol* 2007; **580**: 347-356 [PMID: 17234701 DOI: 10.1113/jphysiol.2006.120907]

48 **Zareie M**, Johnson-Henry K, Jury J, Yang PC, Ngan BY, McKay DM, Soderholm JD, Perdue MH, Sherman PM. Probiotics prevent bacterial translocation and improve intestinal barrier function in rats following chronic psychological stress. *Gut* 2006; **55**: 1553-1560 [PMID: 16638791 DOI: 10.1136/gut.2005.080739]

49 **Róka R**, Ait-Belgnaoui A, Salvador-Cartier C, Garcia-Villar R, Fioramonti J, Eutamène H, Bueno L. Dexamethasone prevents visceral hyperalgesia but not colonic permeability increase induced by luminal protease-activated receptor-2 agonist in rats. *Gut* 2007; **56**: 1072-1078 [PMID: 17309885 DOI: 10.1136/gut.2006.115352]

50 **Seybold VS**. The role of peptides in central sensitization. *Handb Exp Pharmacol* 2009; 451-491 [PMID: 19655115 DOI: 10.1007/978-3-540-79090-7_13]

51 **Dunlop SP**, Hebden J, Campbell E, Naesdal J, Olbe L, Perkins AC, Spiller RC. Abnormal intestinal permeability in subgroups of diarrhea-predominant irritable bowel syndromes. *Am J Gastroenterol* 2006; **101**: 1288-1294 [PMID: 16771951 DOI: 10.1111/j.1572-0241.2006.00672.x]

52 **Barau E**, Dupont C. Modifications of intestinal permeability during food provocation procedures in pediatric irritable bowel syndrome. *J Pediatr Gastroenterol Nutr* 1990; **11**: 72-77 [PMID: 2117653 DOI: 10.1097/00005176-199007000-00015]

53 **Rachmilewitz D**, Katakura K, Karmeli F, Hayashi T, Reinus C, Rudensky B, Akira S, Takeda K, Lee J, Takabayashi K, Raz E. Toll-like receptor 9 signaling mediates the anti-inflammatory effects of probiotics in murine experimental colitis. *Gastroenterology* 2004; **126**: 520-528 [PMID: 14762789 DOI: 10.1053/j.gastro.2004.05.052]

54 **Jijon H**, Backer J, Diaz H, Yeung H, Thiel D, McKaigney C, De Simone C, Madsen K. DNA from probiotic bacteria modulates murine and human epithelial and immune function. *Gastroenterology* 2004; **126**: 1358-1373 [PMID: 15131797 DOI: 10.1053/j.gastro.2004.02.003]

55 **Barbara G**. Mucosal barrier defects in irritable bowel syndrome. Who left the door open? *Am J Gastroenterol* 2006; **101**: 1295-1298 [PMID: 16771952 DOI: 10.1111/j.1572-0241.2006.00667.x]

56 **Cenac N**, Andrews CN, Holzhausen M, Chapman K, Cottrell G, Andrade-Gordon P, Steinhoff M, Barbara G, Beck P, Bunnett NW, Sharkey KA, Ferraz JG, Shaffer E, Vergnolle N. Role for protease activity in visceral pain in irritable bowel syndrome. *J Clin Invest* 2007; **117**: 636-647 [PMID: 17304351 DOI: 10.1172/jci29255]

57 **Bueno L**, Fioramonti J, Delvaux M, Frexinos J. Mediators and pharmacology of visceral sensitivity: From basic to clinical investigations. *Gastroenterology* 1997; **112**: 1714-1743 [PMID: 9136853 DOI: 10.1016/S0016-5085(97)70056-8]

58 **Drossman DA**, Camilleri M, Mayer EA, Whitehead WE. AGA technical review on irritable bowel syndrome. *Gastroenterology* 2002; **123**: 2108-2131 [PMID: 12454866 DOI: 10.1053/gast.2002.37095]

59 **Wouters MM**, Boeckxstaens GE. Neuroimmune mechanisms in functional bowel disorders. *Neth J Med* 2011; **69**: 55-61 [PMID: 21411840 DOI: 10.4065/mcp.2011.0031]

60 **Kleinschmidt S**, Nolte I, Hewicker-Trautwein M. Structural and functional changes of neuronal and glial components of the feline enteric nervous system in cats with chronic inflammatory and non-inflammatory diseases of the gastrointestinal tract. *Res Vet Sci* 2011; **91**: e129-e135 [PMID: 21349562 DOI: 10.1016/j.rvsc.2011.01.026]

61 **Jiang W**, Kirkup AJ, Grundy D. Mast cells drive mesenteric afferent signalling during acute intestinal ischaemia. *J Physiol* 2011; **589**: 3867-3882 [PMID: 21669977 DOI: 10.1113/jphysiol.2011.209478]

World Journal of Gastroenterology

Submit a Manuscript: https://www.f6publishing.com

World J Gastroenterol 2019 August 7; 25(29): 3972-3984

DOI: 10.3748/wjg.v25.i29.3972

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Basic Study

LncRNA MEG3 acts a biomarker and regulates cell functions by targeting ADAR1 in colorectal cancer

Wei Wang, Ying Xie, Fei Chen, Xu Liu, Li-Li Zhong, Hai-Qiang Wang, Qing-Chang Li

ORCID number: Wei Wang (0000-0003-3416-3919); Ying Xie (0000-0003-4288-1435); Fei Chen (0000-0003-2936-5030); Xu Liu (0000-0002-7860-3544); Li-Li Zhong (0000-0001-7834-4629); Hai-Qiang Wang (0000-0002-1183-0283); Qing-Chang Li (0000-0002-4869-625X).

Author contributions: Li QC designed the research; Wang W, Xie Y, Chen F, Liu X, Zhong LL and Wang HQ performed the research; Wang W and Xie Y analyzed the data; Wang W and Li QC wrote the paper.

Institutional review board statement: This study was reviewed and approved by the Institutional Review Board Committee of the First Affiliated Hospital of China Medical University.

Conflict-of-interest statement: We declare no conflicts of interest.

Manuscript source: Unsolicited manuscript

Wei Wang, Qing-Chang Li, College of Basic Medical Sciences, China medical University and Department of Pathology, the First Affiliated Hospital of China Medical University, Shenyang 110001, Liaoning Province, China

Wei Wang, Teaching and Research Department of Pathology, Basic Medical College, Heilongjiang University of Traditional Chinese Medicine, Harbin 150040, Heilongjiang Province, China

Ying Xie, Fei Chen, Department of Synopsis of The Golden Chamber, School of Basic Medical Sciences, Heilongjiang University of Traditional Chinese Medicine, Harbin 150040, Heilongjiang Province, China

Xu Liu, Experiment and Training Center, Heilongjiang University of Traditional Chinese Medicine, Harbin 150040, Heilongjiang Province, China

Li-Li Zhong, Department of Pathology, the First Clinical Medical College, Heilongjiang University of Traditional Chinese Medicine, Harbin 150040, Heilongjiang Province, China

Hai-Qiang Wang, Department of Gastroenterology, the First Clinical Medical College, Heilongjiang University of Traditional Chinese Medicine, Harbin 150040, Heilongjiang Province, China

Corresponding author: Qing-Chang Li, PhD, Doctor, College of Basic Medical Sciences, China medical University and Department of Pathology, the First Affiliated Hospital of China Medical University, No. 77 Puhe Road, Shenyang North New Area, Shenyang 110001, Liaoning Province, China. sci18846185819@126.com
Telephone: +86-24-62255001

Abstract

BACKGROUND

Colorectal cancer (CRC) is the third most prevalent malignancy and has the fourth highest global cancer mortality rate. Early diagnosis and prompt medical attention can improve quality of life and the prognosis of CRC patients. Accumulating evidence reveals that long non-coding RNAs (lncRNAs) function as oncogenes or anti-oncogenes, as well as biomarkers in various cancers.

AIM

To investigate the levels and molecular mechanism of the lncRNA maternally expressed gene 3 (MEG3) in CRC.

METHODS

The levels of lncRNA MEG3 in CRC tissue, serum and cell line samples were

Received: April 28, 2019
Peer-review started: April 28, 2019
First decision: May 24, 2019
Revised: June 7, 2019
Accepted: June 25, 2019
Article in press: June 26, 2019
Published online: August 7, 2019

P-Reviewer: Katuchova J, Tanabe S
S-Editor: Yan JP
L-Editor: Filipodia
E-Editor: Ma YJ

explored *via* qRT-PCR. The relationship between MEG3 levels and clinicopathological features in CRC was investigated. The diagnostic and prognostic values of serum MEG3 levels were analyzed with ROC curves and KaplanMeier survival curves, respectively.

RESULTS
Significant decreased levels of MEG3 existed in CRC tissue, cell lines and serum. CRC patients with down-regulated serum MEG3 levels had larger tumor sizes, and advanced clinical stages. The sensitivity and specificity of serum MEG3 levels in CRC detection was 0.667 and 0.875, respectively. Tumor size, T stages, and serum MEG3 levels are indie factors that produce an effect on CRC patients' prognosis. KaplanMeier survival curves suggested that CRC patients with high levels of MEG3 had a remarkably better overall survival rate.

CONCLUSION
LncRNA MEG3 is down-regulated in CRC, and regulates cell functions by targeting adenosine deaminase's effect on RNA 1 in CRC.

Key words: LncRNA; Maternally expressed gene 3; Biomarker; Colorectal cancer; Adenosine deaminase acting on RNA 1

Core tip: Long non-coding RNA (LncRNA) maternally expressed gene 3 (MEG3) is down-regulated in tissue, cell lines and serum. Colorectal cancer (CRC) patients with down-regulated serum MEG3 levels were had larger tumor sizes, and advanced clinical stages. LncRNA MEG3 functions as a diagnostic and prognostic marker in CRC. LncRNA MEG3 promotes cell proliferation and induced apoptosis in CRC. The effect of adenosine deaminase on RNA 1 may be the target of lncRNA MEG3 in CRC.

Citation: Wang W, Xie Y, Chen F, Liu X, Zhong LL, Wang HQ, Li QC. LncRNA MEG3 acts a biomarker and regulates cell functions by targeting ADAR1 in colorectal cancer. *World J Gastroenterol* 2019; 25(29): 3972-3984
URL: https://www.wjgnet.com/1007-9327/full/v25/i29/3972.htm
DOI: https://dx.doi.org/10.3748/wjg.v25.i29.3972

INTRODUCTION

Colorectal cancer (CRC), which is the third most prevalent malignancy and causes the fourth highest cancer mortality rate globally, is a severe disease and significant threat to human health[1]. Worldwide, there are an estimated 1.2 million new cases and 0.6 million deaths from CRC each year[2]. Nevertheless, the clinical methods for screening, diagnosing, and treating CRC are limited. For example, early course screening of CRC, such as fecal occult blood tests and carcinoembryonic antigens, are often affected by other health disorders and factors, resulting in low specificity and sensitivity, as well as inaccurate clinical diagnoses. At malignancy stages, the tumor node metastasis (TNM) staging system can be used to describe the stage of malignancy and assess patient prognosis. However, TNM suffers from difficulties in invasion and specimen collection, limiting the application of this method for the prediction and prognosis of CRC. Previous studies have highlighted the need for better clinical methods, revealing that nearly 90% of patients with early-stage CRC were alive five years following prognosis, while 14% of patients with advanced-stage CRC were alive five years later. However, only 39.6% of CRC cases are diagnosed at early stages[3,4], thus indicating a need for clinical markers with high sensitivity and specificity that can be used in the early detection and prognosis of CRC.

Typically consisting of more than 200 nucleotides, long non-coding RNAs (lncRNAs) are referred to as endogenous cellular RNAs[5]. LncRNAs lack classically-defined open reading frames, and thus have limited or no protein-coding potential[6-8], yet a large number of aberrant lncRNAs are known to be involved in carcinogenesis, dissemination, and metastasis[9,10]. Moreover, investigations into the roles of lncRNAs as oncogenes or anti-oncogene factors, and their potential as serum biomarkers for the

detection of various cancers including CRC, have increasingly garnered the attention of experts[11-14]. For example, CRC tissues up-regulate the lncRNA SPINT1-AS1, which is associated with partial clinical features (*e.g.*, regional lymph node metastasis, distant metastasis, and shorter relapse-free survival time), suggesting that SPINT1-AS1 is a prognostic marker for CRC[3].

One of the best-studied lncRNAs called maternally expressed gene 3 (MEG3) was reported to be aberrantly expressed in multiple types of malignancies, such as hemangioma, glioma, cervical cancer, and bladder cancer[15-18]. In addition, MEG3 was recently found to act as an anti-oncogene in CRC, specifically by targeting the clusterin in CRC cells to inhibit cell proliferation and migration[19]. Another study revealed that down-regulation of MEG3 in CRC cells activates sphingosine kinase 1, accelerating cell proliferation and suppressing transforming growth factor β1-mediated apoptosis[11]. However, the potential biomarker applications and the mechanisms underlying the roles of MEG3 in CRC require further investigation. Here, we aimed to determine the levels of MEG3 in CRC tissue, cell lines, and serum, further exploring the roles of MEG3 in cellular processes. We uncovered the diagnostic and prognostic value of MEG3 in CRC.

MATERIALS AND METHODS

Tissue and serum specimens

Forty-two CRC tissue specimens and corresponding normal tissues were collected from the First Affiliated Hospital of China Medical University. Among the 42 patients in this study, none of them received any therapy pre-operation. All patients diagnosed with CRC had been verified *via* pathological methods, and patients with other tumors or diseases were excluded from our study. Fresh surgical specimens were processed within half an hour, and then submerged in RNAlater reagent (Qiagen) for half an hour. After that, CRC tissue specimens were stored in liquid nitrogen until RNA extraction.

Serum samples were obtained from 126 CRC patients, as well as 48 healthy control individuals. Serum from 35 paired pre- and post-operative CRC individuals was also collected. All venous blood was disposed within 1 h after extraction. Briefly, serum samples were isolated by centrifugation (1200 × g, 10 min) followed by another centrifugation (10000 × g, 10 min) to discard residual cellular debris. All centrifugations were performed at 4 °C. Similarly, serum samples were stored in liquid nitrogen until RNA extraction.

Our research was managed under the Ethics Committee of the First Affiliated Hospital of China Medical University. Every participant in our study provided full consent. Table 1 shows the clinical characteristics of the CRC patients.

Cell culture and cell transfection

Human CRC cell lines (HCT-116 and HT29) and normal colorectal mucosa epithelial cells (NCM460) were obtained from the American Type Culture Collection. All cells were cultivated with DMEM (Gibco; Thermo Fisher Scientific) supplemented with 10% fetal bovine serum (Gibco; Thermo Fisher Scientific), 5% antibiotics (penicillin and streptomycin sulfates), and 20mM glutamine. HCT-116, HT29, and NCM460 cells were maintained in an incubator (37 °C, 5% CO_2). For cell transfection, a pCDNA3.1 vector containing the MEG3 sequence was purchased from Invitrogen. HCT-116 and HT29 cells were pre-seeded in 6-well plates and cultivated until they reached 50-60% confluency. After that, HCT-116 and HT29 cells were transfected with pCDNA-MEG3 or empty vector using the X-tremeGENE HP DNA transfection reagent (Roche).

RNA isolation

For RNA isolation of tissue specimens, TRIzol reagent (Invitrogen) was used according to the manufacturer's procedures. For RNA isolation of serum samples, the miRNeasy Serum/Plasma Kit (Qiagen) was used. The quantity of RNA in all samples was measured with a NanoDrop 2000c (Thermo Fisher Scientific), and any RNA samples that exhibited an optical density ratio (260/280) of less than 1.8 or over 2.0 were excluded from further experiments. RNA samples were either stored in liquid nitrogen or subsequently used for cDNA synthesis.

Reverse transcription and quantitative real-time polymerase chain reaction (RT-qPCR)

cDNA was synthesized *via* the PrimeScript RT Master Mix (Takara Biotechnology) with 0.1 μg of sample-derived RNA, then used for RT-qPCR to detect and quantify the levels of lncRNA MEG3 in tissues and serum. This assay was conducted on the Roche

Table 1 Correlation of maternally expressed gene 3 levels and clinicopathological features in colorectal cancer

Clinical features	Cases	Serum MEG3 expression	*P* value
Age in yr			0. 956
≥ 60	54	0.678 ± 0.013	
< 60	72	0.679± 0.043	
Gender			0.400
Male	85	0.665 ± 0.032	
Female	41	0.696 ± 0.038	
Tumor site			0.843
Colon	58	0.671 ± 0.044	
Rectum	68	0.676 ± 0.032	
Tumor size in cm			**0.001**
≥ 5	43	0.748 ± 0.031	
< 5	83	0.567 ± 0.039	
pT, TNM			0.098
T1+T2	93	0.617 ± 0.025	
T3+T4	33	0.693 ± 0.019	
Lymph node metastasis			0.236
Positive	71	0.717 ± 0.046	
Negative	55	0.662 ± 0.034	
Clinical stage, TNM			
III	72	0.867 ± 0.049	**0.001**
I+II	54	0.562 ± 0.018	
Pathological differentiation			
Well/moderate	59	0.665 ± 0.050	0.322
Poor	67	0.710 ± 0.032	

TNM: Tumor node metastasis; MEG3: Maternally expressed gene 3.

Lightcycler 480 Real-Time PCR system (Roche Diagnostics) with SYBR-Green PCR master mix (Roche). After normalization to GADPH, changes in lncRNA MEG3 expression were calculated using the $2^{-\Delta Ct}$ method. The primers used were as follows: *MEG3* forward, 5′-CTGCCCATCTACACCTCACG -3′ and reverse, 5′-CTCTCCGCCGTCTGCGCTAGGGGCT- 3′; *GAPDH* forward, 5′-AGCCACAT CGCTCAGACAC-3′ and reverse, 5′-GCCCAATACGACCAAATCC-3′.

Cell proliferation assays

A CCK-8 kit (US Everbright, lnc.) was used for evaluating cell proliferative capacity. After transfection, we plated CRC cells (HCT-116 and HT29) in a 96-well plate, and measured the optical density (OD) every 24 h according to the manufacturer's protocol. Before each detection, the CCK-8 kit (10 μL) was added to each well, and an enzyme immunoassay instrument (Bio Rad Laboratories) was used for value readings.

Flow cytometry assay

Annexin V (Invitrogen; Thermo Fisher Scientific, Inc.) was used to assess the apoptosis rate of HCT-116 and HT29 cells. After transfection with pCDNA-MEG3 or empty vector, CRC cells (HCT-116 and HT29) were grown to 100% confluency, then collected *via* centrifugation and washed with phosphate buffered saline. After resuspending in binding buffer, the cells were stained with 5 μL Annexin V. Fifteen min later, apoptotic cells were detected by flow cytometry (EPICS XL 4; Beckman Coulter, Inc.).

Protein extraction and western blotting

After rinsing with pre-cooled phosphate buffered saline, total protein in CRC (HCT-116 and HT29) cells was extracted with RIPA buffer (Thermo Fisher Scientific) and a protease/phosphatase inhibitor cocktail (Roche). Protein concentration was evaluated with a Bio-Rad BCA assay system, and equal amounts of protein were loaded into each lane of a polyacrylamide gel. Proteins were separated *via* SDS-PAGE, transferred

to polyvinylidene difluoride membranes (Millipore, Billerica), and blocked with 5% skim milk. Membranes were stained with primary antibodies [anti-adenosine deaminase acting on RNA 1 (ADAR1) 1:500, Proteintech; anti-GAPDH 1:1000, Proteintech] for 16 h, then rinsed with TBST (3 × 10 min), and stained with secondary antibody (HRP conjugated goat anti-rabbit 1:1000, Sigma) at room temperature for 60 min. An enhanced chemiluminescence kit (Merck Millipore) and Kodak film (Kodak) was used to detect the blots. ADAR1 protein abundance was normalized to GAPDH.

Statistical methods

All reported statistics were visualized with SPSS 21.0 and Graphpad Prism 7 software. Significance values were determined by student's *t*-test. Associations between MEG3 levels and clinicopathological features were analyzed with a Chi-square test and Fisher's exact test, and receiver operating characteristic (ROC) curves were drawn to evaluate the value of serum MEG3 for CRC detection. The Kaplan-Meier method was applied for assessing the value of serum MEG3 in the prognosis of CRC. A P value < 0.05 is indicated as a significant difference.

RESULTS

Decreased levels of lncRNA MEG3 in CRC

MEG3 levels in CRC tissue, serum, and cell lines were first detected by qRT-PCR. Neither age nor gender appeared to affect MEG3 expression when comparing between the CRC group and healthy group (Table 2). As expected, lncRNA MEG3 expression was significantly decreased in CRC tissues *versus* corresponding colorectal tissue (P < 0.01; Figure 1A). Results from cell lines agreed with these findings, and revealed significant down-regulation of MEG3 in CRC cell lines compared to NCM460 cells (P < 0.01 for HCT-116, P < 0.05 for HT29; Figure 1B). Moreover, significant down-regulation of MEG3 existed in serum samples of CRC patients *versus* the NC (P < 0.05; Figure 1C).

Association between lncRNA MEG3 levels and clinic pathological features

We next investigated the relationship between MEG3 levels and clinical pathological features of CRC patients. In our study, the 126 CRC patients were divided into two equal-sized groups of high and low MEG3 expression, with a cut-off point at the median MEG3 level (63 high serum MEG3 level, and 63 low serum MEG3 level). The comparisons displayed in Table 1 reveal that no statistically significant associations were uncovered between the serum MEG3 concentration and age (P = 0.956), gender (P = 0.400), tumor site (P = 0.843), T stage (P = 0.098), lymph node metastasis (P = 0.236) or pathological differentiation (P = 0.322). However, serum MEG3 levels clearly correlated with tumor size (P = 0.001) and clinical stage (P = 0.001). Thus, our results demonstrate that CRC patients with down-regulated serum MEG3 levels are more prone to developing larger tumors and reaching advanced clinical stages.

Diagnostic value of serum lncRNA MEG3 in CRC

In order to assess the value of measuring MEG3 expression in diagnosing CRC, the ROC method was applied. As shown in Figure 2A, no significant difference was observed between the serum MEG3 levels in samples 24 h post-operation and serum MEG3 levels in the preoperative samples (P > 0.05). The opposite result was obtained in the samples 1 mo after surgery, which revealed that the serum MEG3 levels were significantly elevated compared to the preoperative samples (P < 0.01). A ROC curve was applied to the results, which demonstrated that the sensitivity and specificity of serum MEG3 levels in CRC detection was 0.667 and 0.875, respectively [area under the curve (AUC) 0.798; 95% confidence interval (CI) 0.730-0.866, P < 0.001; Figure 2B]. Overall, lncRNA MEG3 is a reliable marker for CRC diagnosis.

Prognostic value of lncRNA MEG3 in CRC

KaplanMeier survival curves and Cox proportional hazard regression analyses were applied to assess the prognostic value of lncRNA MEG3 in CRC. Data from univariate analysis showed that tumor size [hazard ratio (HR) = 0.576, 95%CI = 0.342-0.968, P = 0.037], T stage (HR = 0.572, 95%CI = 0.122-0.414, P = 0.044), clinical stage (HR = 0.225, 95%CI = 0.122-0.414, P = 0.001), pathological differentiation (HR = 0.440, 95%CI = 0.258-0.752, P = 0.003) and serum MEG3 levels (HR = 2.789, 95%CI = 1.615-4.816, P = 0.001) are indeed factors that affect a CRC patient's prognosis. In multivariate analysis, tumor size (HR = 0.436, 95%CI = 0.236-0.806, P = 0.008), T stage (HR = 0.039, 95%CI = 0.012-0.124, P = 0.001), and serum MEG3 levels (HR = 0.173, 95%CI = 0.063-0.480, P = 0.002) were identified as factors affecting CRC patient prognosis (Table 3). KaplanMeier survival curves (Figure 3) were plotted, and suggested that CRC

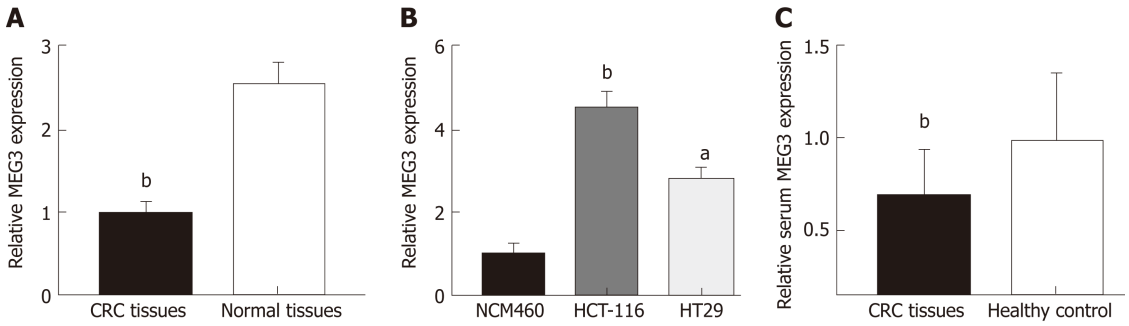


Figure 1 Long non-coding RNA MEG3 in tissue, serum and cell lines. A: Relative levels of MEG3 in CRC tissues and normal tissues; B: Relative levels of MEG3 in cell lines; C: Relative levels of serum MEG3 in CRC patients and healthy controls. All data were repeated three times; $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$. CRC: Colorectal cancer; MEG3: Maternally expressed gene 3.

patients with high levels of MEG3 had remarkably better overall survival (OS) rates ($P < 0.001$). These data demonstrate that lncRNA MEG3 is a reliable marker for CRC prognosis.

Cell transfection efficiency

Changes in lncRNA MEG3 expression in two different CRC cell lines (HCT-116 and HT29) after transfection with pcDNA MEG3 were measured with RT-qPCR. The amount of lncRNA MEG3 present in transfected HCT-116 cells was 9.87 times higher than the control group ($P < 0.01$; Figure 4A), and MEG3 expression in similarly transfected HT-29 cells was increased 7.32 times compared to cells transfected with an empty vector ($P < 0.001$; Figure 4B).

MEG3 promotes cell proliferation and induces apoptosis in CRC

In our study, CCK-8 and flow cytometry assays were conducted to evaluate cell proliferation and apoptosis, respectively. As expected, the CCK-8 assay revealed that up-regulation of MEG3 could suppress cell proliferation both in HCT-116 ($P < 0.05$; Figure 5A) and HT29 ($P < 0.01$; Figure 5B) cell lines compared with the control group. Flow cytometry showed that elevated expression of MEG3 induced a significant amount of cell apoptosis compared with the control group [(HCT-116, $P < 0.01$; Figure 5C) (HT-29, $P < 0.05$; Figure 5D)].

MEG3-regulated ADAR1 expression in CRC cells (HCT-116 and HT29)

Western blotting was conducted on cells that were transfected to overexpress MEG3. From these blots, we were able to elucidate that ADAR1 protein expression in both HCT-116 ($P < 0.01$; Figure 5E) and HT29 ($P < 0.01$; Figure 5F) cells was elevated when MEG3 was expressed at higher levels. MEG3 levels were up-regulated in HCT-116 cells.

DISCUSSION

The carcinogenesis and progression of CRC is an extremely complex process involving multiple steps of cellular reprogramming. In recent years, despite many efforts to improve clinical treatments, the prognosis of CRC patients has not significantly improved. Early detection of CRC is one of the main prerequisites for satisfactory therapy, and can help significantly improve a patient's chances for survival. Thus, it is an issue of particular significance and urgency to explore and understand the mechanism behind the carcinogenesis and progression of CRC, so as to unearth relevant biomarkers for diagnosis and prognosis, as well as enable the accurate monitoring of CRC progression.

Previous studies have revealed that lncRNAs exert significant effects on numerous biological processes, such as neoplastic angiopoiesis, cell migration, and drug resistance[20,21]. An estimated 1×10^5 lncRNAs have been identified thus far, but it is difficult to determine the precise number due to many factors, such as varying tissues and stages[22]. Despite extensive research conducted on the role of lncRNAs, fewer than 2% of lncRNA species have been ascribed to a particular biological role[23]. Moreover, how lncRNAs exert their functions at the molecular level, and how lncRNAs are targeted to specific genomic sites remains elusive[24]. In addition to the important functions of lncRNA in tissues and cells, the function of lncRNAs in serum has also interested many researchers, who agree that lncRNAs in serum have great

Table 2 Clinicopathological characteristics in our study

	Tissue samples, *n* = 42	Serum samples	
		Colorectal cancer, *n* = 126	Healthy controls, *n* = 48
Age			
mean ± SD	56.8 ± 6.5	56.9 ± 5.9	56.3 ± 6.4
Median (range)	57 (44-73)	58 (38-74)	58 (38-73)
Gender			
Male	27	85	33
Female	15	41	15
Tumor site			
Colon	17	58	
Rectum	25	68	
Tumor size in cm			
≥ 5	12	43	
< 5	30	83	
pT, TNM			
T1+T2	33	93	
T3+T4	9	33	
Lymph node metastasis			
Positive	18	71	
Negative	22	55	
Clinical stage, TNM			
III	13	72	
I+II	29	54	
Pathological differentiation	26	59	
Well/moderate Poor	16	67	
high *vs* low			
Lymph node metastasis, positive *vs* negative	0.988		
	0.225		
Clinical stage of	0.440		
TNM, III *vs* I + II	2.789		
Pathological differentiation, well/moderate *vs* poor			
Serum lncRNA			
MEG3 expression,			

TNM: Tumor node metastasis.

potential for clinical use in diagnosing tumors and predicting patient prognosis.

Previous studies have demonstrated that dysregulated lncRNA MEG3 was widespread in malignancies. It was found that MEG3 levels were reduced in the tissues of prostate cancer patients, and that the MEG3 inhibitory role in various cellular functions (invasion, proliferation and migration) relied on regulating the miR-9-5p/QKI-5 axis[25]. Decreased levels of MEG3 were found in bladder cancer tissues, and increased levels of MEG3 could hinder the ability of BC cell migration and invasion. Furthermore, bladder cancer cells with up-regulated MEG3 were sensitized to cisplatin, which is a drug used for bladder cancer chemotherapy[26]. Likewise, MEG3 levels were significantly reduced in liver cancer tissues, and hepatoma cell proliferation and invasion could be promoted by down-regulation of MEG3[27]. In gastric cancer, MEG3 was lowly expressed in tumor tissue, and up-regulation of MEG3 suppressed cancer cell proliferation, metastasis, and p53 levels[28]. MEG3 also plays a fatal role in kidney cancer, chronic myeloid leukemia, thyroid carcinoma, and endometrial carcinoma[29-32]. In our study, MEG3 was significantly reduced in CRC tumor tissue, serum, and cell lines. Up-regulation of MEG3 by transfection inhibited CRC cellular proliferation and induced apoptosis.

ADAR1, a significant member of the ADAR protein family, has been reported to participate in multiple biological functions, such as cell proliferation and apoptosis[33,34]. There are two major isoforms of ADAR1: An interferon-inducible

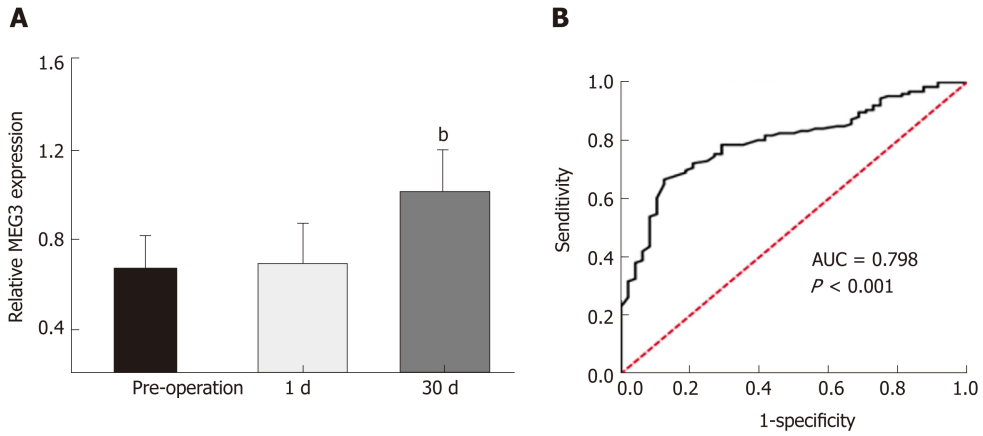


Figure 2 Results of MEG3 in the diagnosis of colorectal cancer. A: Serum maternally expressed gene 3 levels in the sample of 1 d and 1 mo after surgery; B: Receiver-operator characteristic curve for colorectal cancer detection. All data were repeated three times; $^{b}P < 0.01$. 1 d: The 1st d after surgical removal of the tumor; 1 mo: The 30th d after surgical removal of the tumor; ROC: Receiver-operating characteristic curve; AUC: Area under curve; MEG3: Maternally expressed gene 3.

ADAR1 p150 that contains both the Za and Zb Z-DNA-binding domains and a constitutive ADAR1 p110 that lacks the N-terminal Za Z-DNA-binding domain[35]. ADAR1 has emerged as a biomarker in numerous solid tumors, including gastric cancer and esophageal cancer[36,37]. In our study, StarBase 3.0 (http://starbase.sysu.edu.cn/index.php) was used, and ADAR1 was found to be co-expressed with MEG3 in CRC. Thus, we assume that ADAR1 is a potential target regulated by MEG3 in CRC cells. Western blotting was then performed to test our hypothesis. As expected, the cells overexpressing MEG3 exhibited increased ADAR1 expression, thus implicating ADAR1 as a target of MEG3 in CRC cells. These results agree with the StarBase data.

Previous studies demonstrated that MEG3 acts as a diagnostic biomarker in malignancies. In bladder cancer, the AUC of the three lncRNA panel (MEG3, SNHG16 and MALAT1) in detecting bladder cancer was 0.828, and the diagnostic performances of the lncRNA panel for Ta, T1, and T2-T4 were 0.778, 0.805, and 0.880, respectively. This thus identified a three lncRNA panel for use in diagnosing bladder cancer[38]. In our study, MEG3 could discriminate between CRC patients and healthy controls with a sensitivity and specificity of 0.667 and 0.875, respectively, thus demonstrating that lncRNA MEG3 was a reliable marker for CRC diagnosis.

Previous studies demonstrated that lncRNA MEG3 acts as a prognostic biomarker in malignancies. Bioinformatics analysis revealed that high MEG3 levels were a suitable prognostic factor for patients with lung cancer, especially in younger patients (≤ 60 years old), indicating MEG3 as a promising prognostic factor in lung cancer[39]. In breast cancer, MEG3 levels are closely related to the TNM stage differentiation grade, and lymph node metastasis. MEG3 levels were an insusceptible undesirable factor of prognosis [5-year OS and 5-year progression-free survival (PFS)] in BC patients. Breast cancer patients that had MEG3 levels would experience a poor prognosis (poor OS and PFS)[40]. In osteosarcoma, MEG3 levels were particularly lower in tumor tissues, and correlated with both clinical stage and metastasis. The results of Kaplan-Meier analysis suggested that patients with high MEG3 levels generally live longer. These data therefore demonstrate that MEG3 acts as a prognostic biomarker in osteosarcoma[38]. In our study, we found that MEG3 expression was indeed a factor affecting the prognosis of CRC patients, and that patients with high levels of MEG3 had a remarkably higher OS rate, which demonstrated that lncRNA MEG3 was a good marker for the prognosis of CRC. In conclusion, lncRNA MEG3 is down-regulated in CRC, and regulates cell function by targeting ADAR1 in CRC.

Table 3 Univariate and multivariate survival analysis

Variables	Univariate analysis			Multivariate analysis		
	HR	95%CI	*P* value	HR	95%CI	*P* value
Age in yr, ≥ 60 *vs* < 60	0.806	0.481-1.352	0.414	---	---	---
Gender, male *vs* female	1.265	0.757-2.178	0.396	---	---	---
Tumor site, colon *vs* rectum	0.681	0.405-1.143	0.146	---	---	---
Tumor size in cm, ≥ 5 *vs* < 5	0.576	0.342-0.968	0.037	0.436	0.236-0.806	0.008
pT of TNM, T1 + T2 *vs* T3 + T4	0.572	0.332-0.986	0.044	0.942	0.477-1.861	0.863
Lymph node metastasis, positive *vs* negative	0.988	0.591-1.651	0.964	---	---	---
Clinical stage of TNM, III *vs* I + II	0.225	0.122-0.414	0.001	0.039	0.012-0.124	0.001
Pathological differentiation, well/moderate *vs* poor	0.440	0.258-0.752	0.003	0.641	0.324-1.267	0.201
Serum lncRNA MEG3 expression, high *vs* low	2.789	1.615-4.816	0.001	0.173	0.063-0.480	0.002

TNM: Tumor node metastasis; lncRNA: Long non-coding RNAs; MEG3: Maternally expressed gene 3; CI: Confidence interval; HR: Hazard ratio.

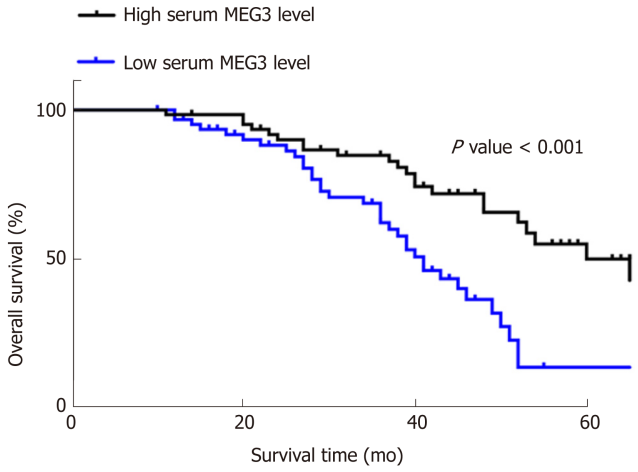


Figure 3 Results of MEG3 in the prognosis of CRC in KaplanMeier survival curves. All data were repeated three times. CRC: Colorectal cancer; MEG3: Maternally expressed gene 3.

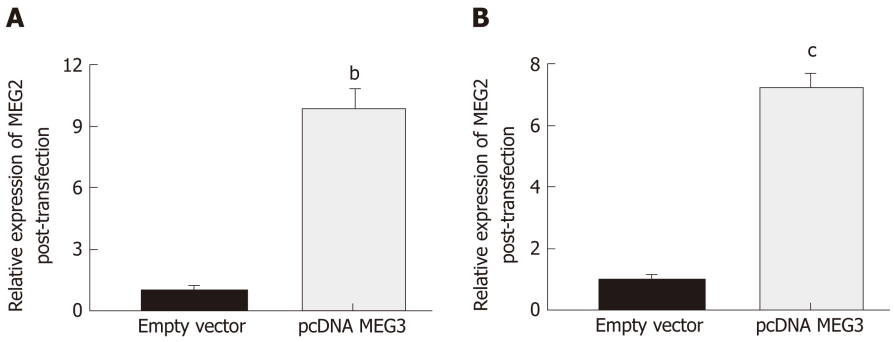


Figure 4 Result of transfection efficacies. A: Transfection efficacies of pcDNA MEG3 in HCT-116 cells; B: Transfection efficacies of pcDNA MEG3 in HT29 cells. All data were repeated three times; $^{b}P < 0.01$, $^{c}P < 0.001$. MEG3: Maternally expressed gene 3.

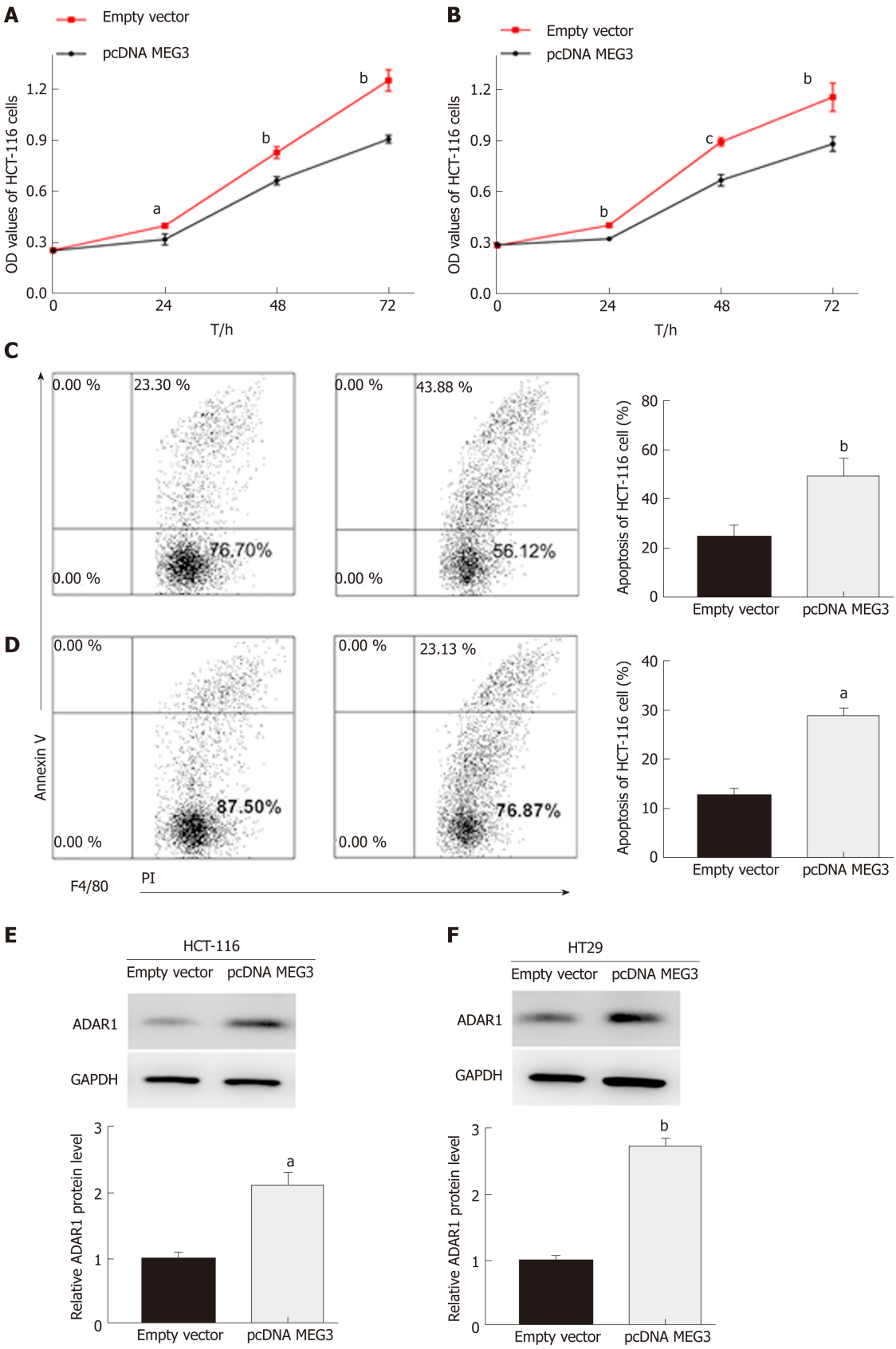


Figure 5 The role of MEG3 in cell function. A: Cell proliferation in HCT-116 cells after overexpression of MEG3; B: Cell proliferation in HT29 cells after overexpression of MEG3; C: Apoptosis in HCT-116 cells after overexpression of MEG; D: Apoptosis in HT29 cells after overexpression of MEG; E: Protein expression of ADAR1 after overexpression of MEG3 in HCT-116 cells; F: Protein expression of ADAR1 after overexpression of MEG3 in HT29 cells. All data were repeated three times; $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$. OD: Optical density; ADAR1: Adenosine deaminase acting on RNA 1; MEG3: Maternally expressed gene 3.

ARTICLE HIGHLIGHTS

Research background

Among common types of gastrointestinal malignancies, colorectal cancer (CRC) has seen a dramatic increase in annual global incidence rate. Many recent studies have demonstrated the molecular mechanisms involved in the transcriptional regulation in CRC, and shown that long non-coding RNAs (lncRNAs) play an irreplaceable role in the initiation and progression of CRC, such as maintaining cell growth, evasion of apoptosis, promotion of invasion and metastasis, stemness maintenance and EMT.

Research motivation

To identify more biomarkers for the diagnosis and treatment of CRC.

Research objectives

To investigate the underlying mechanisms of lncRNA maternally expressed gene 3 (MEG3) in CRC.

Research methods

LncRNA MEG3 expression was observed by qRT-PCR assays on CRC tissue, cell lines and serum. Clinicopathological characteristics were collected, arranged and combined with expression analysis of CRC to evaluate the functions of lncRNA MEG3. Cell function assays were performed to explore the functions of MEG3 in CRC cell lines. Moreover, western blots were performed to explore the targeted regulation by MEG3 in CRC cell lines.

Research results

We found that levels of LncRNA MEG3 decreased in CRC tissues, cell lines and serum, and exhibited a significant negative relation with tumor size, TNM stage, and lymph node metastasis. Cell experiments showed that MEG3 levels declined during CR cell line proliferation and invasion, and that ADAR1 may be the target regulated by lncRNA MEG3 in CRC cells. Importantly, CRC patients with higher lncRNA MEG3 levels have a better overall survival rate.

Research conclusions

Our study demonstrated that lncRNA MEG3 can significantly inhibit cell growth, migration and invasion of gastric cancer. Furthermore, it can work through ADAR1. Therefore, our study provides some molecular mechanism and two new biomarkers for CRC.

Research perspectives

In the future, research may reveal the important role of lncRNA MEG3 that enhances the sensitivity of CRC detection, and further develop its application for anti-cancer treatments. The identification of the lncRNA MEG3/ADAR1 molecular axis may further explain the underlying mechanism.

REFERENCES

1 **Liu HN**, Liu TT, Wu H, Chen YJ, Tseng YJ, Yao C, Weng SQ, Dong L, Shen XZ. Serum microRNA signatures and metabolomics have high diagnostic value in colorectal cancer using two novel methods. *Cancer Sci* 2018; **109**: 1185-1194 [PMID: 29363233 DOI: 10.1111/cas.13514]

2 **Conev NV**, Donev IS, Konsoulova-Kirova AA, Chervenkov TG, Kashlov JK, Ivanov KD. Serum expression levels of miR-17, miR-21, and miR-92 as potential biomarkers for recurrence after adjuvant chemotherapy in colon cancer patients. *Biosci Trends* 2015; **9**: 393-401 [PMID: 26781797 DOI: 10.5582/bst.2015.01170]

3 **Liao G**, Zhang X, Li Z, Lan S, Huang M, Huang W. Renal-type clear cell carcinoma of prostate: A case report and review of literature. *Indian J Pathol Microbiol* 2018; **61**: 431-433 [PMID: 30004074 DOI: 10.4103/IJPM.IJPM_396_17]

4 **Nikolaou S**, Qiu S, Fiorentino F, Rasheed S, Tekkis P, Kontovounisios C. Systematic review of blood diagnostic markers in colorectal cancer. *Tech Coloproctol* 2018; **22**: 481-498 [PMID: 30022330 DOI: 10.1007/s10151-018-1820-3]

5 **Zhao W**, Song M, Zhang J, Kuerban M, Wang H. Combined identification of long non-coding RNA CCAT1 and HOTAIR in serum as an effective screening for colorectal carcinoma. *Int J Clin Exp Pathol* 2015; **8**: 14131-14140 [PMID: 26823726]

6 **Tong YS**, Wang XW, Zhou XL, Liu ZH, Yang TX, Shi WH, Xie HW, Lv J, Wu QQ, Cao XF. Identification of the long non-coding RNA POU3F3 in plasma as a novel biomarker for diagnosis of esophageal squamous cell carcinoma. *Mol Cancer* 2015; **14**: 3 [PMID: 25608466 DOI: 10.1186/1476-4598-14-3]

7 **van Werven FJ**, Neuert G, Hendrick N, Lardenois A, Buratowski S, van Oudenaarden A, Primig M, Amon A. Transcription of two long noncoding RNAs mediates mating-type control of gametogenesis in budding yeast. *Cell* 2012; **150**: 1170-1181 [PMID: 22959267 DOI: 10.1016/j.cell.2012.06.049]

8 **Ponting CP**, Oliver PL, Reik W. Evolution and functions of long noncoding RNAs. *Cell* 2009; **136**: 629-641 [PMID: 19239885 DOI: 10.1016/j.cell.2009.02.006]

9 **Zhang L**, Zhou Y, Huang T, Cheng AS, Yu J, Kang W, To KF. The Interplay of LncRNA-H19 and Its Binding Partners in Physiological Process and Gastric Carcinogenesis. *Int J Mol Sci* 2017; **18**: pii: E450 [PMID: 28230721 DOI: 10.3390/ijms18020450]

10 **Li J**, Meng H, Bai Y, Wang K. Regulation of lncRNA and Its Role in Cancer Metastasis. *Oncol Res* 2016; **23**: 205-217 [PMID: 27098144 DOI: 10.3727/096504016X14549667334007]

11 **Kong H**, Wu Y, Zhu M, Zhai C, Qian J, Gao X, Wang S, Hou Y, Lu S, Zhu H. Long non-coding RNAs:

Novel prognostic biomarkers for liver metastases in patients with early stage colorectal cancer. *Oncotarget* 2016; **7**: 50428-50436 [PMID: 27391432 DOI: 10.18632/oncotarget.10416]
12 **Chen S**, Wu DD, Sang XB, Wang LL, Zong ZH, Sun KX, Liu BL, Zhao Y. The lncRNA HULC functions as an oncogene by targeting ATG7 and ITGB1 in epithelial ovarian carcinoma. *Cell Death Dis* 2017; **8**: e3118 [PMID: 29022892 DOI: 10.1038/cddis.2017.486]
13 **Wang J**, Yang K, Yuan W, Gao Z. Determination of Serum Exosomal H19 as a Noninvasive Biomarker for Bladder Cancer Diagnosis and Prognosis. *Med Sci Monit* 2018; **24**: 9307-9316 [PMID: 30576305 DOI: 10.12659/MSM.912018]
14 **Zhang S**, Du L, Wang L, Jiang X, Zhan Y, Li J, Yan K, Duan W, Zhao Y, Wang L, Wang Y, Shi Y, Wang C. Evaluation of serum exosomal LncRNA-based biomarker panel for diagnosis and recurrence prediction of bladder cancer. *J Cell Mol Med* 2019; **23**: 1396-1405 [PMID: 30467945 DOI: 10.1111/jcmm.14042]
15 **Chen X**, Qu J. Long non-coding RNA MEG3 suppresses survival, migration, and invasion of cervical cancer. *Onco Targets Ther* 2018; **11**: 4999-5007 [PMID: 30174437 DOI: 10.2147/OTT.S167053]
16 **Dai Y**, Wan Y, Qiu M, Wang S, Pan C, Wang Y, Ou J. lncRNA MEG3 Suppresses the Tumorigenesis of Hemangioma by Sponging miR-494 and Regulating PTEN/ PI3K/AKT Pathway. *Cell Physiol Biochem* 2018; **51**: 2872-2886 [PMID: 30562741 DOI: 10.1159/000496040]
17 **Gong X**, Huang M. Long non-coding RNA MEG3 promotes the proliferation of glioma cells through targeting Wnt/β-catenin signal pathway. *Cancer Gene Ther* 2017; **24**: 381-385 [PMID: 29027534 DOI: 10.1038/cgt.2017.32]
18 **Liu G**, Zhao X, Zhou J, Cheng X, Ye Z, Ji Z. Long non-coding RNA MEG3 suppresses the development of bladder urothelial carcinoma by regulating miR-96 and TPM1. *Cancer Biol Ther* 2018; 1-18 [PMID: 30461333 DOI: 10.1080/15384047.2018.1480279]
19 **Zhu Y**, Chen P, Gao Y, Ta N, Zhang Y, Cai J, Zhao Y, Liu S, Zheng J. MEG3 Activated by Vitamin D Inhibits Colorectal Cancer Cells Proliferation and Migration via Regulating Clusterin. *EBioMedicine* 2018; **30**: 148-157 [PMID: 29628342 DOI: 10.1016/j.ebiom.2018.03.032]
20 **Liu Z**, Wu C, Xie N, Wang P. Long non-coding RNA MEG3 inhibits the proliferation and metastasis of oral squamous cell carcinoma by regulating the WNT/β-catenin signaling pathway. *Oncol Lett* 2017; **14**: 4053-4058 [PMID: 28959364 DOI: 10.3892/ol.2017.6682]
21 **Xu DH**, Chi GN, Zhao CH, Li DY. Long noncoding RNA MEG3 inhibits proliferation and migration but induces autophagy by regulation of Sirt7 and PI3K/AKT/mTOR pathway in glioma cells. *J Cell Biochem* 2018 [PMID: 30417553 DOI: 10.1002/jcb.28026]
22 **Sherpa C**, Rausch JW, Le Grice SF. Structural characterization of maternally expressed gene 3 RNA reveals conserved motifs and potential sites of interaction with polycomb repressive complex 2. *Nucleic Acids Res* 2018; **46**: 10432-10447 [PMID: 30102382 DOI: 10.1093/nar/gky722]
23 **Hon CC**, Ramilowski JA, Harshbarger J, Bertin N, Rackham OJ, Gough J, Denisenko E, Schmeier S, Poulsen TM, Severin J, Lizio M, Kawaji H, Kasukawa T, Itoh M, Burroughs AM, Noma S, Djebali S, Alam T, Medvedeva YA, Testa AC, Lipovich L, Yip CW, Abugessaisa I, Mendez M, Hasegawa A, Tang D, Lassmann T, Heutink P, Babina M, Wells CA, Kojima S, Nakamura Y, Suzuki H, Daub CO, de Hoon MJ, Arner E, Hayashizaki Y, Carninci P, Forrest AR. An atlas of human long non-coding RNAs with accurate 5' ends. *Nature* 2017; **543**: 199-204 [PMID: 28241135 DOI: 10.1038/nature21374]
24 **Blank-Giwojna A**, Postepska-Igielska A, Grummt I. lncRNA KHPS1 Activates a Poised Enhancer by Triplex-Dependent Recruitment of Epigenomic Regulators. *Cell Rep* 2019; **26**: 2904-2915.e4 [PMID: 30865882 DOI: 10.1016/j.celrep.2019.02.059]
25 **Wu M**, Huang Y, Chen T, Wang W, Yang S, Ye Z, Xi X. LncRNA MEG3 inhibits the progression of prostate cancer by modulating miR-9-5p/QKI-5 axis. *J Cell Mol Med* 2019; **23**: 29-38 [PMID: 30565858 DOI: 10.1111/jcmm.13658]
26 **Feng SQ**, Zhang XY, Fan HT, Sun QJ, Zhang M. Up-regulation of LncRNA MEG3 inhibits cell migration and invasion and enhances cisplatin chemosensitivity in bladder cancer cells. *Neoplasma* 2018; **65**: 925-932 [PMID: 29940769 DOI: 10.4149/neo_2018_180125N55]
27 **Sun Y**, Cao FL, Qu LL, Wang ZM, Liu XY. MEG3 promotes liver cancer by activating PI3K/AKT pathway through regulating AP1G1. *Eur Rev Med Pharmacol Sci* 2019; **23**: 1459-1467 [PMID: 30840267 DOI: 10.26355/eurrev_201902_17103]
28 **Wei GH**, Wang X. lncRNA MEG3 inhibit proliferation and metastasis of gastric cancer via p53 signaling pathway. *Eur Rev Med Pharmacol Sci* 2017; **21**: 3850-3856 [PMID: 28975980]
29 **He H**, Dai J, Zhuo R, Zhao J, Wang H, Sun F, Zhu Y, Xu D. Study on the mechanism behind lncRNA MEG3 affecting clear cell renal cell carcinoma by regulating miR-7/RASL11B signaling. *J Cell Physiol* 2018; **233**: 9503-9515 [PMID: 29968912 DOI: 10.1002/jcp.26849]
30 **Li Z**, Yang L, Liu X, Nie Z, Luo J. Long noncoding RNA MEG3 inhibits proliferation of chronic myeloid leukemia cells by sponging microRNA21. *Biomed Pharmacother* 2018; **104**: 181-192 [PMID: 29772439 DOI: 10.1016/j.biopha.2018.05.047]
31 **Liu Y**, Yue P, Zhou T, Zhang F, Wang H, Chen X. LncRNA MEG3 enhances 131I sensitivity in thyroid carcinoma via sponging miR-182. *Biomed Pharmacother* 2018; **105**: 1232-1239 [PMID: 30021359 DOI: 10.1016/j.biopha.2018.06.087]
32 **Sun KX**, Wu DD, Chen S, Zhao Y, Zong ZH. LncRNA MEG3 inhibit endometrial carcinoma tumorigenesis and progression through PI3K pathway. *Apoptosis* 2017; **22**: 1543-1552 [PMID: 29094270 DOI: 10.1007/s10495-017-1426-7]
33 **Nemlich Y**, Greenberg E, Ortenberg R, Besser MJ, Barshack I, Jacob-Hirsch J, Jacoby E, Eyal E, Rivkin L, Prieto VG, Chakravarti N, Duncan LM, Kallenberg DM, Galun E, Bennett DC, Amariglio N, Bar-Eli M, Schachter J, Rechavi G, Markel G. MicroRNA-mediated loss of ADAR1 in metastatic melanoma promotes tumor growth. *J Clin Invest* 2013; **123**: 2703-2718 [PMID: 23728176 DOI: 10.1172/jci62980]
34 **Nakano M**, Fukami T, Gotoh S, Nakajima M. A-to-I RNA Editing Up-regulates Human Dihydrofolate Reductase in Breast Cancer. *J Biol Chem* 2017; **292**: 4873-4884 [PMID: 28188287 DOI: 10.1074/jbc.M117.775684]
35 **Shevchenko G**, Morris KV. All I's on the RADAR: Role of ADAR in gene regulation. *FEBS Lett* 2018; **592**: 2860-2873 [PMID: 29770436 DOI: 10.1002/1873-3468.13093]
36 **Takeda S**, Shigeyasu K, Okugawa Y, Yoshida K, Mori Y, Yano S, Noma K, Umeda Y, Kondo Y, Kishimoto H, Teraishi F, Nagasaka T, Tazawa H, Kagawa S, Fujiwara T, Goel A. Activation of AZIN1 RNA editing is a novel mechanism that promotes invasive potential of cancer-associated fibroblasts in colorectal cancer. *Cancer Lett* 2019; **444**: 127-135 [PMID: 30583079 DOI: 10.1016/j.canlet.2018.12.009]
37 **Qiao JJ**, Chan TH, Qin YR, Chen L. ADAR1: A promising new biomarker for esophageal squamous cell carcinoma? *Expert Rev Anticancer Ther* 2014; **14**: 865-868 [PMID: 24928581 DOI:

10.1586/14737140.2014.928595]
38 **Tian ZZ**, Guo XJ, Zhao YM, Fang Y. Decreased expression of long non-coding RNA MEG3 acts as a potential predictor biomarker in progression and poor prognosis of osteosarcoma. *Int J Clin Exp Pathol* 2015; **8**: 15138-15142 [PMID: 26823857 DOI: 10.1380/ejssnt.2008.171]
39 **Zhang Z**, Liu T, Wang K, Qu X, Pang Z, Liu S, Liu Q, Du J. Down-regulation of long non-coding RNA MEG3 indicates an unfavorable prognosis in non-small cell lung cancer: Evidence from the GEO database. *Gene* 2017; **630**: 49-58 [PMID: 28782577 DOI: 10.1016/j.gene.2017.08.001]
40 **Zhang JJ**, Guo SH, Jia BQ. Down-regulation of long non-coding RNA MEG3 serves as an unfavorable risk factor for survival of patients with breast cancer. *Eur Rev Med Pharmacol Sci* 2016; **20**: 5143-5147 [PMID: 28051255]

World Journal of Gastroenterology

Submit a Manuscript: https://www.f6publishing.com

World J Gastroenterol 2019 August 7; 25(29): 3985-3995

DOI: 10.3748/wjg.v25.i29.3985

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Retrospective Cohort Study

Liquid biopsy for non-invasive assessment of liver injury in hepatitis B patients

Wen-Ying Xia, Li Gao, Er-Hei Dai, Dan Chen, Er-Fu Xie, Li Yang, Shi-Chang Zhang, Bing-Feng Zhang, Jian Xu, Shi-Yang Pan

ORCID number: Wen-Ying Xia (0000-0003-2359-1379); Li Gao (0000-0002-9762-448X); Er-Hei Dai (0000-0001-8835-6199); Dan Chen (0000-0003-1141-3890); Er-Fu Xie (0000-0002-6787-7646); Li Yang (0000-0002-8762-2923); Shi-Chang Zhang (0000-0002-6587-2518); Bing-Feng Zhang (0000-0003-0240-3447); Jian Xu (0000-0003-2032-251X); Shi-Yang Pan (0000-0001-9320-6988).

Author contributions: Xia WY, Gao L, and Dai EH contributed equally to this work as co-first authors and finished the major experiments; Pan SY designed the research; Chen D, Xie EF, and Yang L collected and analyzed the data; Gao L and Zhang SC wrote the paper; Zhang BF and Xu J critically revised the manuscript for important content.

Supported by the National Natural Science Foundation of China, No. 81672100 and No. 81371894; the Key Laboratory for Laboratory Medicine of Jiangsu Province of China, No. ZDXKB2016005; and Jiangsu Provincial Commission of Health and Family Planning, No. H201609.

Institutional review board statement: This study was approved for publication by our Institutional Reviewer.

Informed consent statement: All study participants or their legal guardian provided informed written consent for personal and medical data collection prior to study enrolment.

Wen-Ying Xia, Li Gao, Dan Chen, Er-Fu Xie, Shi-Chang Zhang, Bing-Feng Zhang, Jian Xu, Shi-Yang Pan, Department of Laboratory Medicine, the First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, Jiangsu Province, China

Er-Hei Dai, Li Yang, Department of Laboratory Medicine, the Fifth Hospital of Shijiazhuang, Shijiazhuang 050021, Hebei Province, China

Corresponding author: Shi-Yang Pan, MD, Professor, Department of Laboratory Medicine, the First Affiliated Hospital of Nanjing Medical University, No. 300, Guangzhou Road, Nanjing 210029, Jiangsu Province, China. sypan@njmu.edu.cn
Telephone: +86-25-68303452

Abstract

BACKGROUND

Hepatitis B is a major public health problem in China. Accurate liver injury assessment is essential for clinical evidence-based treatment. Liver biopsy is considered the gold standard method to stage liver disease, but it is not widely used in resource-limited settings. Therefore, non-invasive liquid biopsy tests are needed.

AIM

To assess liver injury in hepatitis B patients using quantified cell free DNA combined with other serum biomarker as a liquid biopsy-based method.

METHODS

A cohort of 663 subjects including 313 hepatitis B patients and 350 healthy controls were enrolled. Ultrasound-guided liver biopsies followed by histopathological assessments were performed for the 263 chronic hepatitis B patients to determine the degree of liver injury. Cell-free DNA was quantified using a novel duplex real-time polymerase chain reaction assay.

RESULTS

Compared with healthy controls, patients with hepatitis B virus (HBV) infection had significantly higher plasma DNA, serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin, and HBV DNA levels ($P < 0.01$). Serum ALT, AST, bilirubin, and plasma DNA levels of patients with marked-severe inflammation were significantly higher than those with mild-moderate inflammation ($P < 0.01$). There was a statistically significant correlation between hepatocyte inflammation severity and serum bilirubin ($R^2 = 0.673$, $P < 0.01$) or

Conflict-of-interest statement: All authors have no conflict of interest related to the manuscript.

Data sharing statement: The original anonymous dataset is available on request from the corresponding author at sypan@njmu.edu.cn.

STROBE statement: The authors have read the STROBE Statement-checklist of items, and the manuscript was prepared and revised according to STROBE Statement-checklist of items.

Manuscript source: Unsolicited manuscript

Received: March 26, 2019
Peer-review started: March 26, 2019
First decision: May 24, 2019
Revised: June 13, 2019
Accepted: July 5, 2019
Article in press: July 3, 2019
Published online: August 7, 2019

P-Reviewer: Demonacos C, Muench MO, Zapater P
S-Editor: Yan JP
L-Editor: Wang TQ
E-Editor: Ma YJ

plasma DNA (R^2 = 0.597, P < 0.01) levels. The areas under the curves of serum ALT, bilirubin, plasma DNA, and their combination to distinguish between patients with mild–moderate and marked-severe inflammation were 0.8059, 0.7910, 0.7921, and 0.9564, respectively.

CONCLUSION
The combination of plasma DNA, serum ALT, and bilirubin could be a candidate liquid biopsy for non-invasive assessment of liver injury in hepatitis B patients.

Key words: Liquid biopsy; plasma DNA; Hepatitis B; Alanine aminotransferase; Duplex real-time quantitative polymerase chain reaction

Core tip: Our study used quantified cell free DNA combined with other serum biomarker as a liquid biopsy-based method to assess liver injury in hepatitis B patients. A cohort of 663 subjects including 313 hepatitis B patients and 350 healthy controls were enrolled. Ultrasound-guided liver biopsies followed by histopathological assessments were performed for the 263 chronic hepatitis B patients to determine the degree of liver injury. Cell-free DNA was quantified using a novel duplex real-time polymerase chain reaction assay. Our results demonstrated that the combination of plasma DNA, serum alanine aminotransferase, and bilirubin could be a candidate liquid biopsy for non-invasive assessment of liver injury in hepatitis B patients.

Citation: Xia WY, Gao L, Dai EH, Chen D, Xie EF, Yang L, Zhang SC, Zhang BF, Xu J, Pan SY. Liquid biopsy for non-invasive assessment of liver injury in hepatitis B patients. *World J Gastroenterol* 2019; 25(29): 3985-3995
URL: https://www.wjgnet.com/1007-9327/full/v25/i29/3985.htm
DOI: https://dx.doi.org/10.3748/wjg.v25.i29.3985

INTRODUCTION

The liver, playing an important role in many bodily functions from protein production and blood clotting to cholesterol, glucose, and iron metabolism, is the most important detoxification organ in human body[1]. Because of this, the liver becomes the vulnerable organ to be injured in various kinds of diseases, including pathogen infection, inherited metabolic disease, alcoholic hepatitis, drug-induced liver disease, autoimmune liver disease, and fatty liver disease. In clinical practice, accurate liver injury assessment is necessary for the evidence-based treatment.

Liver biopsy, which is thought to be the gold standard method for the assessment of liver injury severity, has been used to ascertain the degree of necroinflammation and fibrosis[1]. Unfortunately, liver biopsy is not always an available option for many patients with liver disease. It is also impractical to monitor the disease by frequent biopsy. Blood biomarkers, such as liver aminotransferases and bilirubin, are the routine assessments as liquid biopsy to monitor patients and to guide clinical treatment[1]. However, it is widely known that the degree of liver injury is quite different in various types of liver diseases. It is also thought that serum alanine aminotransferase (ALT) or aspartate aminotransferase (AST) concentrations are not necessarily a reliable index of hepatocyte necrosis, and this is especially seen in massive hepatic necrosis[2]. To assess liver injury more accurately, more reliable biomarkers for liquid biopsy are required.

In this study, we focused on hepatitis B caused by the hepatitis B virus (HBV) which is a DNA virus that infects the liver. There are approximately two billion people exposed to HBV and more than 350 million chronically infected people worldwide[3,4]. Hepatitis B is a major public health problem worldwide, especially in China[5]. HBV infection can be either asymptomatic or symptomatic depending on the severity of liver injury, which is important for patients' outcome and to help guide the choice of clinical therapy. The aim of the present study was to quantify cell released DNA, a sensitive biomarker for cell death assessment, in hepatitis B patients using the duplex real-time quantitative polymerase chain reaction (PCR) to explore its potential use as a liquid biopsy-based biomarker combined with other liver function tests for

assessment of liver injury.

MATERIALS AND METHODS

Patients and clinicopathological characteristics

This study included a total of 313 HBV-infected patients (median age, 36 years; range, 12-78 years; 89 females) with chronic (n = 263) or acute (n = 50) infection. Chronic HBV infection was defined as a continuous positive hepatitis B surface antigen (HBsAg) test result for more than 6 months, while acute HBV infection was a positive HBsAg test result for less than 6 months. Clinical information including demographic characteristics was obtained from the patients' medical records. Healthy volunteers (n = 350; median age, 36 years; range, 18-72 years; 100 females) who attended the First Affiliated Hospital of Nanjing Medical University for an annual health check-up were recruited as a control group.

Liver biopsy

Ultrasound-guided liver biopsies followed by histopathological assessment were performed for the 263 chronic HBV infected patients to determine the degrees of liver injury including inflammation (grade) and fibrosis (stage) according to the Grading and Staging System in China[6], which was established based on the Ishak system[7]. In this study, mild or moderate portal area inflammation and spotty or piecemeal necrosis without bridging and multi-acinar necrosis were categorized as mild–moderate inflammation, while marked portal area inflammation, marked spotty or piecemeal necrosis, and/or bridging and multi-acinar necrosis were categorized marked-severe inflammation. The fibrosis stages range from 0 to 4, where Stage 0 is for no fibrosis, Stage 1 for portal fibrosis without septa, Stage 2 for portal fibrosis with few septa, Stage 3 for numerous septa without cirrhosis, and Stage 4 for cirrhosis.

Blood sample collection and processing

After informed consent was obtained and the research protocol was approved by the Ethics Committee of Nanjing Medical University, a 2-mL peripheral blood sample was collected into an ethylenediamine tetraacetic acid-containing tube from each participant at the time of preliminary diagnosis. All of the blood samples were centrifuged (1600 g, 10 min) within 2 h after collection and plasma was carefully transferred into EP tubes. After an additional centrifugation at 16000 g for 10 min, 200 μL of plasma supernatant without blood cells was collected and 5×10^4 copies of recombinant internal standard plasmid DNA were added as described previously[8]. The mixed samples were stored at -80 °C until further processing. Serum samples were recovered from serum-separator tubes following centrifugation of whole blood at 3000 g for 10 min at room temperature.

Plasma DNA extraction and quantification

DNA was extracted from the 200 μL plasma samples containing the internal standard using the BILATEST DNA/RNA kit (BILATEC, Viernheim, Germany), following the manufacturer's recommendations. After DNA extraction, plasma DNA was detected with plasma cell-free DNA quantitative detection kit (Code Biotech, Jiangsu, China) using duplex real-time PCR, which was performed for both the human β–actin gene and the internal control recombinant plasmid DNA in the same tube. Duplex amplification curves were analyzed with Sequence Detection System Software (Ver. 1.4, Applied Biosystems). Specific probes and primers were described in our previous report[8].

Detection of other serum biomarkers

The routine clinical chemistry panel, including ALT, AST, bilirubin, and albumin was measured on an automatic biochemistry analyzer (AU5800, Beckman-Coulter, United States). Serum HBV DNA was quantified by real-time PCR assay (ABI7500, Applied Biosystem, United States). ALT and AST activities were measured using the recommended IFCC method. Bilirubin was measured using the vanadic acid oxidation method. Albumin was measured using the bromocresol green colorimetry method. Serum HBV DNA was extracted using an HBV nucleic acid quantitative detection kit (KHB, China) and quantified by real-time PCR assay (ABI7500, Applied Biosystem, United States).

Statistical analysis

Results are presented as the median and interquartile range. Data were analyzed using the Kruskal–Wallis rank test and Mann–Whitney U test. Spearman's rank correlation was performed to estimate the correlation between the degree of liver

injury and plasma DNA, serum ALT, or bilirubin, respectively. The area under receiver operator characteristic (ROC) curve (AUC) was calculated to evaluate the diagnostic efficacy. All of these data were analyzed using Stata 9.2 software (Stata Corporation, College Station, TX, United States). A *P*-value < 0.05 was considered statistically significant.

RESULTS

Serum ALT, AST, bilirubin, albumin, plasma DNA, and HBV DNA levels in healthy controls and HBV-infected patients

The concentrations of serum ALT, AST, bilirubin, albumin, total plasma DNA, and HBV DNA in healthy controls and patients with HBV infection are shown in Table 1. There were statistically significant differences in serum ALT, AST, and plasma DNA levels between healthy males and females ($P < 0.01$). Healthy controls with a history of drinking alcohol had higher serum ALT, AST, bilirubin, and plasma DNA levels than those who rarely drank alcohol ($P < 0.01$).

Compared with healthy controls, patients with HBV infection had significantly higher plasma DNA, serum ALT, AST, bilirubin, HBV DNA levels, and lower serum albumin levels ($P < 0.01$). There were no statistically significant differences in the six blood-based biomarkers between patients with different sex or drinking history ($P > 0.05$). Although there were significant differences in serum ALT and HBV DNA levels between patients younger and older than 36 years, the Spearman's rank correlation analysis showed that there were no statistical correlations between patient age and the blood-based biomarkers. Serum HBV DNA levels in HBeAg positive patients were significantly higher than those with negative HBeAg results ($P < 0.01$).

Quantification of serum ALT, AST, bilirubin, albumin, total plasma DNA, and HBV DNA for assessment of liver injury in patients with chronic HBV infection

As shown in Table 2, serum ALT, AST, bilirubin, and plasma DNA levels of patients with marked-severe inflammation were significantly higher than those of patients with mild–moderate inflammation ($P < 0.01$). However, serum ALT or AST levels of 42 patients with severe liver injury were lower than those of patients with marked inflammation. There was no statistically significant difference in serum HBV DNA or albumin levels between patients with mild–moderate and marked-severe inflammation ($P > 0.05$). The positive correlation between serum ALT and inflammation severity was statistically significant but weak ($R^2 = 0.214$, $P < 0.01$; Figure 1A) because the severe patients had low ALT levels. Inflammation severity determined by liver biopsy was correlated with serum bilirubin ($R^2 = 0.673$, $P < 0.01$; Figure 1B) and plasma DNA ($R^2 = 0.597$, $P < 0.01$; Figure 1C), but had no statistically significant correlation with HBV DNA ($R^2 = 0.004$, $P = 0.281$; Figure 1D).

As shown in Figure 2, the AUC of using plasma DNA to distinguish between patients with mild–moderate and marked-severe inflammation was 0.7921, which was similar to the AUCs of using serum ALT and bilirubin (0.8059 and 0.7910). The AUCs of using serum AST, albumin, and HBV DNA to distinguish between patients with mild-moderate and marked-severe inflammation were 0.6530, 0.4877, and 0.4952, respectively. After the combination of serum ALT, bilirubin, and plasma DNA (blue), there was a statistically significant increase of AUC (0.9564). There was also a significant difference in plasma DNA levels or bilirubin between patients with and without cirrhosis ($P < 0.01$), but there was no difference in serum ALT, AST, albumin, or HBV DNA ($P > 0.05$; Table 2).

As shown in Figure 3A, there was no statistically significant correlation between plasma DNA and serum ALT ($R^2 = 0.012$, $P = 0.08$). Most of the patients with high serum ALT levels (> 100.0 U/L) had marked-severe liver injury (red), suggesting a high specificity (89.3%) and positive predictive value (84.0%). However, there were still 33.5% of patients with low serum ALT levels (≤ 100.0 U/L) who had marked-severe liver injury, which suggests a low sensitivity (55.3%) of serum ALT. For example, patient 85 in Figure 3A was a 20-year-old man with chronic hepatitis B (CHB). Laboratory studies showed an HBV DNA level of 2.04×10^7 IU/mL and an ALT level of only 40 U/L. However, a needle biopsy of the liver showed severe piecemeal necrosis (marked portal inflammation, Grade 3; Figure 3B). These false negative patients could be further distinguished from patients with mild–moderate inflammation by plasma DNA with a sensitivity of 97.5% and specificity of 68.6% at the cutoff value of 95 ng/mL, as shown in Figure 3A. After the combination of serum ALT, bilirubin, and plasma DNA, there was a statistically significant increase in AUC (0.9564), with a maximum sensitivity and specificity of 88.64% and 80.15%, respectively (the blue line in Figure 2).

Table 1 Correlation between clinicopathological characteristics and serum alanine aminotransferase, alanine aminotransferase, bilirubin, albumin, plasma DNA, or hepatitis B virus DNA levels in the 350 healthy controls and 313 patients

Group	Number of cases	Serum ALT (U/L)	*P*	Bilirubin (μM)	*P*	Plasma DNA (ng/mL)	*P*	HBV DNA (IU/mL)	*P*	Serum AST (U/L)	*P*	Albumin (g/L)	*P*
Healthy controls	350	19.1 (15.1)				25.5 (17.6)		0.0 (0.0)		16.4 (14.3)		46.0 (22.2)	
Sex			< 0.01		0.865		< 0.01		---		< 0.01		0.062
Male	250	22.3 (14.1)		10.6 (5.8)		30.0 (16.1)		0.0 (0.0)		19.6 (14.1)		46.4 (20.8)	
Female	100	12.6 (7.6)		10.2 (5.5)		16.7 (8.1)		0.0 (0.0)		10.1 (5.5)		45.5 (20.1)	
Age (yr)			0.819		0.833		0.056		---		0.916		0.066
< 36	175	18.9 (17.9)		9.7 (5.4)		23.6 (16.4)		0.0 (0.0)		16.0 (15.9)		47.6 (20.3)	
≥ 36	175	19.8 (13.7)		10.5 (5.7)		26.8 (19.3)		0.0 (0.0)		16.7 (12.2)		45.8 (23.4)	
HBsAb			0.263		0.439		0.060		---		0.549		0.884
Negative	166	18.7 (15.1)		10.3 (5.6)		27.3 (19.1)		0.0 (0.0)		15.8 (15.1)		45.7 (22.5)	
Positive	184	19.1 (15.0)		10.3 (5.6)		24.2 (16.0)		0.0 (0.0)		16.5 (15.2)		46.2 (21.6)	
Drinking history			< 0.01		0.002		0.000		---		0.000		0.711
No	278	18.6 (12.1)		10.4 (5.7)		22.8 (15.5)		0.0 (0.0)		15.8 (11.1)		46.1 (20.9)	
Yes	72	34.1 (29.2)		21.0 (19.2)		38.3 (19.0)		0.0 (0.0)		33.5 (27.4)		45.4 (23.7)	
Hepatitis B patients	313	103.3 (118.5)	< 0.01[1]	85.6 (77.2)	0.000[1]	132.9 (253.7)	< 0.01[1]	6.1 (2.9)	< 0.01[1]	97.0 (101.6)	< 0.01[1]	42.7 (31.2)	< 0.01[1]
Sex			0.972		0.606		0.772		0.149		0.656		0.761
Male	224	101.4 (114.4)		88.3 (91.6)		134.2 (260.2)		6.2 (2.7)		95.3 (99.5)		42.4 (32.7)	
Female	89	113.5 (143.8)		82.9 (89.1)		132.2 (170.7)		5.6 (2.8)		101.7 (116.6)		43.0 (33.2)	
Age (yr)			0.011		0.221		0.312		< 0.01		0.831		0.591
< 36	155	89.2 (120.0)		86.3 (90.4)		135.9 (256.6)		6.7 (3.0)		96.5 (98.3)		42.2 (30.4)	
≥ 36	158	115.9 (124.9)		83.9 (91.5)		115.8 (244.5)		5.6 (2.6)		97.8 (103.2)		43.1 (29.7)	
HBeAg			0.236		0.173		0.833		< 0.01		0.742		0.070
Negative	159	109.7 (126.6)		84.4 (89.4)		126.3 (294.0)		5.4 (3.1)		97.7 (101.8)		43.3 (32.5)	
Positive	154	101.4 (117.0)		86.1 (90.7)		135.7 (228.9)		6.5 (2.3)		97.4 (99.6)		40.6 (34.6)	
Drinking history			0.487		0.577		0.574		0.200		0.433		0.654
No	187	107.0 (122.8)		85.0 (87.3)		126.3 (255.4)		6.2 (2.7)		95.8 (98.5)		42.0 (30.8)	
Yes	126	100.6 (118.0)		86.9 (90.3)		142.8 (243.6)		6.0 (3.0)		98.3 (100.1)		43.2 (30.7)	

[1]Compared with the healthy controls. All continuous data are expressed as medians (interquartile range). ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; HBV: hepatitis B virus; HBsAb: Hepatitis B surface antibody; HBeAg: Hepatitis B E-antigen.

Comparison of serum ALT, AST, bilirubin, albumin, total plasma DNA, and HBV DNA levels in patients with chronic or acute HBV infection

Serum ALT, AST, bilirubin, albumin, total plasma DNA, and HBV DNA levels in patients with chronic or acute HBV infection are shown in Table 2. There was no

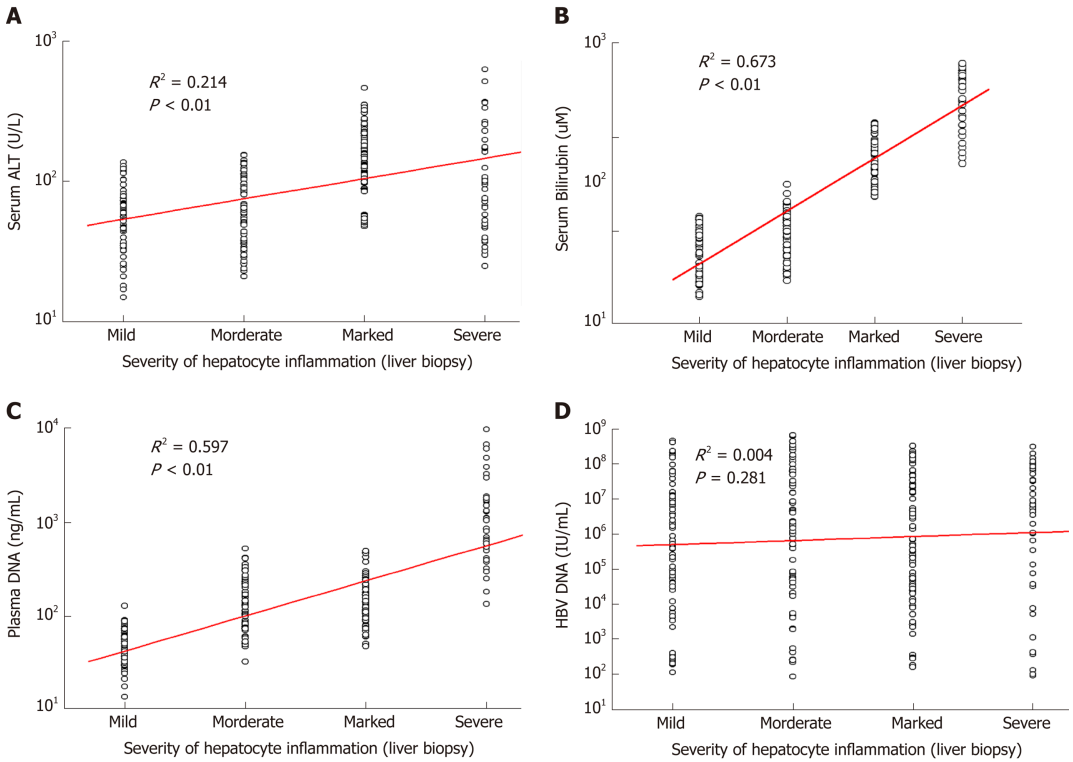


Figure 1 Correlations of blood biomarkers with severity of hepatocyte inflammation in 263 chronic hepatitis B patients. The scatter plots show that there are statistically significant correlations between the severity of hepatocyte inflammation and levels of serum biomarkers. A: Alanine aminotransferase; B: Bilirubin; C: Plasma DNA; D: Hepatitis B virus DNA. ALT: Alanine aminotransferase; HBV: Hepatitis B virus.

statistically significant difference in HBV DNA levels between patients with chronic and acute HBV infection ($P > 0.05$). However, total plasma DNA, bilirubin, serum ALT, AST, and albumin levels in patients with acute HBV infection were significantly higher than those of patients with chronic HBV infection ($P < 0.01$). ROC curve analysis showed that the AUC of using serum ALT levels to distinguish between patients with chronic and acute HBV infection was 0.8702, which was significantly higher than that of using plasma DNA (0.7800; $P = 0.01$).

DISCUSSION

Hepatitis B is a viral infectious disease caused by the HBV which primarily interferes with the functions of the liver by replicating in hepatocytes[5]. During HBV infection, the host immune response causes both viral clearance and hepatocellular damage[9]. In clinical practice, hepatitis B patients usually need to undergo a liver biopsy, which is the current gold standard method for liver injury assessment by direct cell morphological observation[1,10]. However, biopsy results are too dependent on the representation of the punctured sample and show significant variability which can lead to a wrong diagnosis[11]. Besides, liver biopsy requires a skilled expert and well-equipped hospital, and still has the risk of potentially lethal complications, such as pneumothorax and bleeding[12]. Therefore, liver biopsy is not always the best option for monitoring disease and some other more reliable techniques to assess liver injury, such as liquid biopsy focusing on biomarkers in body fluids, are required.

HBV infection can stimulate the body to produce various kinds of cell and humoral immunity responses to virus antigens and lead to persistent or massive hepatocellular apoptosis and necrosis[9,13,14]. As a result of cell damage, the components of liver cells including proteins and nucleic acids are released into the peripheral blood stream. This could increase the reference values and the quantitative detection of these substances released from damaged liver cells into body fluids may serve as a noninvasive liquid biopsy to evaluate and monitor hepatitis-related liver damage[15].

Liver-specific enzymes such as ALT and AST are the most common serum biomarkers for liver function assessment[16]. However, the point of using ALT or AST for liver cell injury assessment remains controversial. Desmet *et al*[17] found that the serum ALT level rose in almost all chronic liver diseases, yet this marker could not reliably reflect the degree of inflammatory injury. Kew suggested that serum ALT was

Table 2 Serum alanine aminotransferase, alanine aminotransferase, bilirubin, albumin, plasma DNA, and serum hepatitis B virus DNA levels in different patient groups

Group	Number of cases	Serum ALT (U/L)	*P*	Bilirubin (μM)	*P*	Plasma DNA (ng/mL)	*P*	HBV DNA (IU/mL)	*P*	Serum AST (U/L)	*P*	Albumin (g/L)	*P*
Healthy controls	350	19.1 (15.1)				25.5 (17.6)		0.0 (0.0)		16.4 (14.3)		46.0 (22.2)	
Acute HBV infection	50	213.8 (355.9)		121.6 (207.0)		336.8 (179.5)		5.5 (2.4)		197.6 (312.5)		45.4 (24.6)	
Chronic HBV infection	263	88.3 (98.0)	< 0.01[1]	73.9 (88.3)	< 0.01[1]	106.4 (174.1)	< 0.01[1]	6.2 (2.9)	0.059*	90.6 (93.7)	< 0.01[1]	40.2 (41.3)	0.004[1]
Inflam-mation			< 0.01		< 0.01		< 0.01		0.893		< 0.01		0.444
Mild	68	59.9 (41.5)		30.6 (31.4)		46.9 (29.5)		5.7 (2.5)		51.2 (50.6)		41.7 (28.6)	
Modera-te	70	62.8 (68.0)		45.4 (50.4)		106.1 (127.2)		6.4 (3.1)		61.3 (72.9)		40.9 (30.4)	
Marked	83	152.8 (114.7)		130 (125.5)		141.1 (77.4)		5.8 (2.8)		153.2 (160.8)		39.5 (32.6)	
Severe	42	99.9 (148.0)		228 (254.9)		862.5 (1213.6)		6.8 (2.8)		91.7 (135.1)		39.8 (33.2)	
Fibrosis			0.552		0.015		< 0.01		0.060		0.121		0.606
No cirrhosis	212	88.0 (106.4)		65.2 (58.2)		131.0 (235.8)		6.3 (3.1)		87.0 (93.0)		40.8 (38.6)	
Stage 0	59	83.5 (98.6)		71.9 (70.3)		111.8 (477.0)		6.6 (3.0)		87.7 (91.6)		41.6 (38.5)	
Stage 1	72	96.6 (81.2)		60.2 (66.7)		179.6 (235.3)		6.5 (3.0)		88.4 (90.3)		40.1 (38.3)	
Stage 2	46	87.4 (89.0)		64.8 (79.6)		98.8 (90.1)		6.0 (2.7)		79.1 (82.5)		40.1 (38.7)	
Stage 3	35	91.7 (110.3)		77.7 (72.4)		87.2 (77.7)		6.0 (2.7)		84.6 (88.0)		40.2 (39.9)	
Cirrho-sis (Stage 4)	51	91.4 (73.5)		92.0 (99.4)		73.1 (44.8)		5.7 (2.1)		91.9 (94.5)		39.9 (43.8)	

[1]Compared with the acute hepatitis B virus infection patients. All continuous data are expressed as medians (interquartile range). ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; HBV: Hepatitis B virus.

not a reliable indicator in extensive hepatic necrosis in severe hepatitis, when decreasing serum ALT concentrations might signify a paucity of hepatocytes from which the enzymes could leak, rather than recovery[2]. Our current findings also demonstrate that serum ALT and AST levels are not always correlated with hepatocyte inflammation, especially in the patients with severe hepatocyte injury (Figure 1). The sensitivity of serum ALT or AST is less than 60%, which will lead to missed diagnosis and delay in clinical treatment.

Circulating plasma DNA, a kind of cell-free extracellular nucleic acid present in normal healthy individuals at low concentrations, is believed to derive primarily from apoptosis of normal cells[18]. The short half-life of plasma DNA in the circulation suggests a model of continuous release from apoptotic cells and rapid clearance[19]. In the context of various disease states characterized by abnormal cell death, such as cancer, trauma, and transplant rejection, a large amount of nucleic acids are released from necrotic cells into blood stream and significantly increase the level of plasma DNA[20-26]. Although plasma DNA quantification was proved to be a potential marker for cell damage, various preanalytical factors and lack of accurate and precise quantitative methods have become a considerable pitfall, hampering its application for liver injury assessment in clinical laboratories[30,31,32]. In our previous study, a duplex real-time PCR assay with a novel internal standard was developed for plasma DNA quantification and proved to be able to eliminate variations and allow for more sensitive, repeatable, accurate, and stable quantitative measurements of plasma

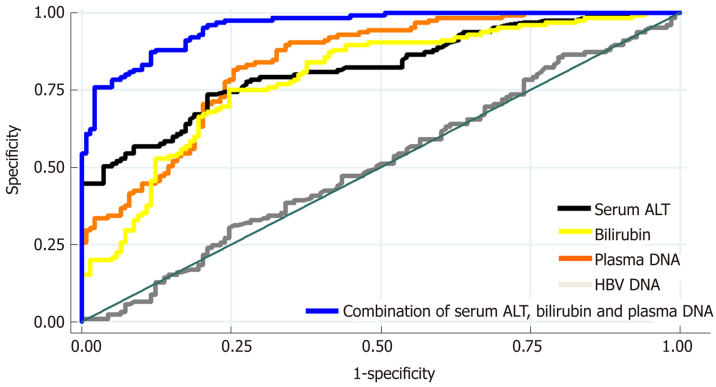


Figure 2 The receiver operator characteristic curves of using four blood biomarkers to assess hepatocellular injury severity in 263 chronic hepatitis B patients. The areas under the curves (AUCs) of using serum alanine aminotransferase (ALT, black), bilirubin (yellow), plasma DNA (orange), serum aspartate aminotransferase (green), albumin (purple), and hepatitis B virus DNA (gray) to distinguish between patients with mild-moderate and marked-severe inflammation were 0.8059, 0.7910, 0.7921, 0.6530, 0.4877, and 0.4952, respectively. After the combination of serum ALT, bilirubin, and plasma DNA (blue), there was a statistically significant increase of AUC (0.9564). ALT: Alanine aminotransferase; HBV: Hepatitis B virus; AST: Aspartate aminotransferase; AUC: Area under the curve.

DNA[8].

In the current study, we quantified total plasma DNA in 350 healthy controls and 313 HBV infected patients by using our novel assay, combined with several other serum biomarkers, to develop a novel liquid biopsy for non-invasive assessment of liver injury. Among healthy controls, higher serum ALT, AST, and plasma DNA levels were found in males and people with a history of drinking alcohol. While males have a higher basal metabolic rate than females, a higher activity rate of liver cells can lead to more ALT, AST, and genomic DNA released from apoptotic liver cells into the blood[27]. Drinking alcohol causes mild hepatocellular damage, which can lead to hepatocyte necrosis followed by ALT, AST, and genomic DNA release. We also demonstrated that HBV infected patients had statistically significantly higher serum ALT, AST, and total plasma DNA levels than healthy controls.

In 263 CHB patients with varying degrees of liver injury, we demonstrated statistically significantly higher serum ALT, AST, bilirubin, albumin, total plasma DNA, and HBV DNA levels than those of healthy controls. We then compared these blood-based biomarker levels in patients with different degrees of liver injury according to liver biopsy. While HBV is a noncytopathic virus and its replication does not directly damage the liver cells, the degree of hepatocyte injury has no direct correlation with the number of HBV DNA copies[28]. In this study, the HBV DNA level did not reflect the severity of liver injury in hepatitis B patients (Table 2). As to serum ALT, it had a high specificity (89.3%) but low sensitivity (55.3%) to discriminate between mild–moderate and marked-severe inflammation. Similar results were found for serum AST. This suggests that nearly half of patients, which were diagnosed with severe liver injury based solely on serum ALT or AST levels, may be misdiagnosed (*e.g.*, patient 85 in Figure 3). By using our novel duplex real-time PCR assay with internal standard, it was demonstrated that plasma DNA concentration was more correlated with the severity of hepatocyte injury than serum ALT levels (Figure 1) and more sensitive to assess the severity of liver injury in patients with low serum ALT (≤ 100.0 U/L; Figure 3A). Cell-free plasma DNA, a superior indicator of cell death, was shown to be a good complement to serum aminotransferases to improve sensitivity. It has been estimated that when 1% of liver cells are damaged, enzymes such as ALT are released into the peripheral blood and this could increase the reference value about one-fold. Considering that 1% of genomic DNA out of the total 2500000000 liver cells is released into plasma, the concentration of plasma DNA would increase by approximate 66 ng/mL, which is 2.6-fold higher than the median value of healthy people. Here, we suggest that this might be an explanation for the effectiveness of plasma DNA in assessing the severity of liver injury in patients with low serum ALT. However, because of the low specificity (68.6%), plasma DNA alone is not sufficient to evaluate hepatic cell injury. After the combination of plasma DNA, serum ALT, and bilirubin, there was a significant improvement in AUC (Figure 2).

Because of the invasiveness and risk of complications, there are many limitations for liver biopsy in clinical practice. Certain conditions, including thrombocytopenia, bleeding diathesis, cirrhosis, ascites, and amyloidosis, are recognized relative or absolute contraindications to biopsy[29]. Therefore, noninvasive liquid biopsy is the

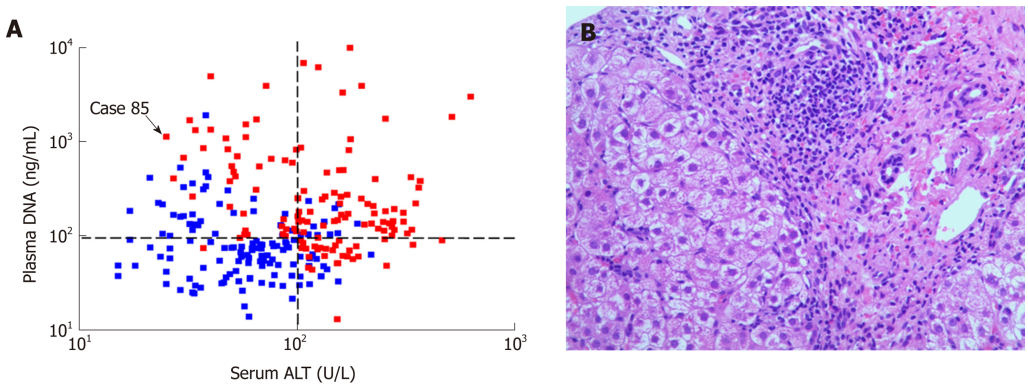


Figure 3 Quantitative analysis of serum alanine aminotransferase and plasma DNA of 263 chronic hepatitis B patients to assess hepatocellular injury severity. A: The scatter plot of serum alanine aminotransferase (ALT) and plasma DNA levels of chronic hepatitis B patients with mild-moderate (blue dots) or marked-severe (red dots) inflammation. Most (84.0%) of the patients with high serum ALT levels (> 100.0 U/L) had marked-severe hepatocyte injury, while the other patients with marked-severe hepatocyte injury and low serum ALT levels (≤ 100.0 U/L) can be distinguished from patients with mild-moderate hepatocyte injury by plasma DNA quantification (*e.g.*, Case 85); B: The hematoxylin and eosin stained microscopic image (× 400) of hepatic tissue of the case 85 showed severe piecemeal necrosis (marked portal inflammation) (Grade 3). ALT: alanine aminotransferase.

necessary and useful substitution for patients who are not suitable to undertake liver biopsy. According to the WHO's guidelines[1], there are several non-invasive tests (NITs) based on blood or serum now available and increasingly used for evaluating and staging liver fibrosis. However, except for serum ALT, AST, and bilirubin, few new NITs has been developed for assessment of liver injury, which can reduce the need for liver biopsy in persons with hepatitis B. In this study, by quantifying serum ALT and plasma DNA, clinicians can assess the severity of liver injury and evaluate the patient's condition to determine the best course of treatment. For example, as shown in Figure 4, low levels of serum ALT, bilirubin, and plasma DNA indicate that there is no significant liver injury, while persistent high level of plasma DNA combined with elevated bilirubin can indicate the severity of hepatocellular injury in the case of severe liver cell damage with the "enzyme bilirubin separate" phenomenon. Furthermore, liquid biopsy can be repeated and present a dynamic change throughout the clinical treatment. But as to liver biopsy, that is impossible. This liquid biopsy for non-invasive assessment of liver injury in hepatitis B patients may be an important supplement be written into the guideline in the future. It is also believed that this kind of non-invasive liquid biopsy can be applied for liver injury assessment in many other liver diseases besides hepatitis B.

This is believed to be the first study about combination of plasma DNA, serum ALT, and bilirubin as a sensitive and unique liquid biopsy for the noninvasive assessment of degree of liver injury. This novel liquid biopsy technique is expected to assist in making more precise diagnoses for hepatitis B patients.

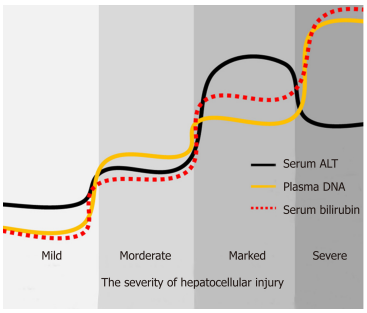


Figure 4 Pattern diagram of liquid biopsy with serum alanine aminotransferase, bilirubin, and plasma DNA for assessment of hepatocellular injury in hepatitis B patients. Serum alanine aminotransferase levels in patients with severe hepatocellular injury may not be very high and plasma DNA combined with serum bilirubin may be a good complementary biomarker for these patients. ALT: Alanine aminotransferase.

ARTICLE HIGHLIGHTS

Research background

Hepatitis B is a major public health problem in China. It is important that the severity of liver injury is evaluated accurately for clinical treatment. Liver biopsy is considered the gold standard method to stage liver disease. However, it is not widely used in resource-limited settings. Therefore, the methods of non-invasive liquid biopsy need to be explored for assessment of liver injury.

Research motivation

Plasma DNA quantification was proved to be a potential marker for cell damage, which may be a non-invasive method for evaluating the severity of liver injury. However, the application of plasma DNA quantification still needs to be investigated in patients with hepatitis B.

Research objectives

The aim of this study was to evaluate liver injury in hepatitis B patients using quantified cell free DNA combined with other serum biomarker as a liquid biopsy-based method.

Research methods

A cohort of 663 subjects including 313 hepatitis B patients and 350 healthy controls were enrolled. Ultrasound-guided liver biopsies followed by histopathological assessments were performed for the 263 chronic hepatitis B patients to determine the degree of liver injury. Cell-free DNA was quantified using a novel duplex real-time polymerase chain reaction assay.

Research results

Compared with healthy controls, patients with hepatitis B virus (HBV) infection had significantly higher plasma DNA, serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin, and HBV DNA levels ($P < 0.01$). Serum ALT, AST, bilirubin, and plasma DNA levels of patients with marked-severe inflammation were significantly higher than those of patients with mild-moderate inflammation ($P < 0.01$). There was a statistically significant correlation between hepatocyte inflammation severity and serum bilirubin ($R^2 = 0.673$, $P < 0.01$) or plasma DNA ($R^2 = 0.597$, $P < 0.01$) levels. The area under the curves of serum ALT, bilirubin, plasma DNA, and their combination to distinguish between patients with mild–moderate and marked-severe inflammation were 0.8059, 0.7910, 0.7921, and 0.9564, respectively.

Research conclusions

The combination of plasma DNA, serum ALT, and bilirubin could be a candidate liquid biopsy for non-invasive assessment of liver injury in hepatitis B patients.

Research perspectives

The combination of plasma DNA, serum ALT, and bilirubin as a novel liquid biopsy technique is expected to assist in making more precise diagnoses for hepatitis B patients, which will be validated in multiple clinical centers in the future.

REFERENCES

1 Geneva: World Health Organization. 2015 [PMID: 26225396]
2 **Kew MC**. Serum aminotransferase concentration as evidence of hepatocellular damage. *Lancet* 2000; **355**: 591-592 [PMID: 10696975 DOI: 10.1016/S0140-6736(99)00219-6]
3 **Schweitzer A**, Horn J, Mikolajczyk RT, Krause G, Ott JJ. Estimations of worldwide prevalence of chronic hepatitis B virus infection: A systematic review of data published between 1965 and 2013. *Lancet* 2015; **386**: 1546-1555 [PMID: 26231459 DOI: 10.1016/S0140-6736(15)61412-X]

4 **Ott JJ**, Horn J, Krause G, Mikolajczyk RT. Time trends of chronic HBV infection over prior decades - A global analysis. *J Hepatol* 2017; **66**: 48-54 [PMID: 27592304 DOI: 10.1016/j.jhep.2016.08.013]

5 **Lao TT**, Sahota DS, Law LW, Cheng YK, Leung TY. Age-specific prevalence of hepatitis B virus infection in young pregnant women, Hong Kong Special Administrative Region of China. *Bull World Health Organ* 2014; **92**: 782-789 [PMID: 25378739 DOI: 10.2471/BLT.13.133413]

6 **Chinese Society of Hepatology and Chinese Society of Infectious Diseases, Chinese Medical Association**. [The guideline of prevention and treatment for chronic hepatitis B (2010 version)]. *Zhonghua Liu Xing Bing Xue Za Zhi* 2011; **32**: 405-415 [PMID: 21569677 DOI: 10.3760/cma.j.issn.0254-6450.2011.04.019]

7 **Ishak K**, Baptista A, Bianchi L, Callea F, De Groote J, Gudat F, Denk H, Desmet V, Korb G, MacSween RN. Histological grading and staging of chronic hepatitis. *J Hepatol* 1995; **22**: 696-699 [PMID: 7560864 DOI: 10.1016/0168-8278(95)80226-6]

8 **Chen D**, Pan S, Xie E, Gao L, Xu H, Xia W, Xu T, Huang P. Development and Evaluation of a Duplex Real-Time PCR Assay With a Novel Internal Standard for Precise Quantification of Plasma DNA. *Ann Lab Med* 2017; **37**: 18-27 [PMID: 27834061 DOI: 10.3343/alm.2017.37.1.18]

9 **Shin EC**, Sung PS, Park SH. Immune responses and immunopathology in acute and chronic viral hepatitis. *Nat Rev Immunol* 2016; **16**: 509-523 [PMID: 27374637 DOI: 10.1038/nri.2016.69]

10 **Mani H**, Kleiner DE. Liver biopsy findings in chronic hepatitis B. *Hepatology* 2009; **49**: S61-S71 [PMID: 19399798 DOI: 10.1002/hep.22930]

11 **Regev A**, Berho M, Jeffers LJ, Milikowski C, Molina EG, Pyrsopoulos NT, Feng ZZ, Reddy KR, Schiff ER. Sampling error and intraobserver variation in liver biopsy in patients with chronic HCV infection. *Am J Gastroenterol* 2002; **97**: 2614-2618 [PMID: 12385448 DOI: 10.1111/j.1572-0241.2002.06038.x]

12 **Lidbury JA**. Getting the Most Out of Liver Biopsy. *Vet Clin North Am Small Anim Pract* 2017; **47**: 569-583 [PMID: 28081862 DOI: 10.1016/j.cvsm.2016.11.007]

13 **Ganem D**. Persistent infection of humans with hepatitis B virus: Mechanisms and consequences. *Rev Infect Dis* 1982; **4**: 1026-1047 [PMID: 6293033 DOI: 10.1093/clinids/4.5.1026]

14 **Ganem D**, Prince AM. Hepatitis B virus infection--natural history and clinical consequences. *N Engl J Med* 2004; **350**: 1118-1129 [PMID: 15014185 DOI: 10.1056/NEJMra031087]

15 **Schütz E**, Fischer A, Beck J, Harden M, Koch M, Wuensch T, Stockmann M, Nashan B, Kollmar O, Matthaei J, Kanzow P, Walson PD, Brockmöller J, Oellerich M. Graft-derived cell-free DNA, a noninvasive early rejection and graft damage marker in liver transplantation: A prospective, observational, multicenter cohort study. *PLoS Med* 2017; **14**: e1002286 [PMID: 28441386 DOI: 10.1371/journal.pmed.1002286]

16 **Giannini EG**, Testa R, Savarino V. Liver enzyme alteration: A guide for clinicians. *CMAJ* 2005; **172**: 367-379 [PMID: 15684121 DOI: 10.1503/cmaj.1040752]

17 **Desmet VJ**, Gerber M, Hoofnagle JH, Manns M, Scheuer PJ. Classification of chronic hepatitis: Diagnosis, grading and staging. *Hepatology* 1994; **19**: 1513-1520 [PMID: 8188183 DOI: 10.1002/hep.1840190629]

18 **Chan AK**, Chiu RW, Lo YM; Clinical Sciences Reviews Committee of the Association of Clinical Biochemists. Cell-free nucleic acids in plasma, serum and urine: A new tool in molecular diagnosis. *Ann Clin Biochem* 2003; **40**: 122-130 [PMID: 12662399 DOI: 10.1258/000456303763046030]

19 **Lo YM**, Zhang J, Leung TN, Lau TK, Chang AM, Hjelm NM. Rapid clearance of fetal DNA from maternal plasma. *Am J Hum Genet* 1999; **64**: 218-224 [PMID: 9915961 DOI: 10.1086/302205]

20 **Sozzi G**, Conte D, Leon M, Ciricione R, Roz L, Ratcliffe C, Roz E, Cirenei N, Bellomi M, Pelosi G, Pierotti MA, Pastorino U. Quantification of free circulating DNA as a diagnostic marker in lung cancer. *J Clin Oncol* 2003; **21**: 3902-3908 [PMID: 14507943 DOI: 10.1200/JCO.2003.02.006]

21 **Jiang T**, Zhai C, Su C, Ren S, Zhou C. The diagnostic value of circulating cell free DNA quantification in non-small cell lung cancer: A systematic review with meta-analysis. *Lung Cancer* 2016; **100**: 63-70 [PMID: 27597282 DOI: 10.1016/j.lungcan.2016.06.013]

22 **Bedin C**, Enzo MV, Del Bianco P, Pucciarelli S, Nitti D, Agostini M. Diagnostic and prognostic role of cell-free DNA testing for colorectal cancer patients. *Int J Cancer* 2017; **140**: 1888-1898 [PMID: 27943272 DOI: 10.1002/ijc.30565]

23 **Lo YM**, Rainer TH, Chan LY, Hjelm NM, Cocks RA. Plasma DNA as a prognostic marker in trauma patients. *Clin Chem* 2000; **46**: 319-323 [PMID: 10702517 DOI: 10.1016/0049-3848(78)90297-9]

24 **Lam NY**, Rainer TH, Chan LY, Joynt GM, Lo YM. Time course of early and late changes in plasma DNA in trauma patients. *Clin Chem* 2003; **49**: 1286-1291 [PMID: 12881444 DOI: 10.1373/49.8.1286]

25 **Snyder TM**, Khush KK, Valantine HA, Quake SR. Universal noninvasive detection of solid organ transplant rejection. *Proc Natl Acad Sci U S A* 2011; **108**: 6229-6234 [PMID: 21444804 DOI: 10.1073/pnas.1013924108]

26 **Lui YY**, Chik KW, Chiu RW, Ho CY, Lam CW, Lo YM. Predominant hematopoietic origin of cell-free DNA in plasma and serum after sex-mismatched bone marrow transplantation. *Clin Chem* 2002; **48**: 421-427 [PMID: 11861434 DOI: 10.1016/S0009-9120(02)00282-5]

27 **Dufour DR**, Lott JA, Nolte FS, Gretch DR, Koff RS, Seeff LB. Diagnosis and monitoring of hepatic injury. I. Performance characteristics of laboratory tests. *Clin Chem* 2000; **46**: 2027-2049 [PMID: 11106349 DOI: 10.1016/S0009-8981(00)00329-6]

28 **Liu X**, Chen JM, Lou JL, Huang YX, Yan Y, Sun GZ, Li N. Correlation between hepatitis B virus DNA levels and diagnostic tests for HBsAg, HBeAg, and PreS1-Ag in chronic hepatitis B. *Genet Mol Res* 2016; 15 [PMID: 27421011 DOI: 10.4238/gmr.15028282]

29 **Grant A**, Neuberger J. Guidelines on the use of liver biopsy in clinical practice. British Society of Gastroenterology. *Gut* 1999; **45**: IV1-IV11 [PMID: 10485854 DOI: 10.1136/gut.45.2008.iv1]

30 **Wang H**. Zhang B, Chen D, Xia W, Zhang J, Wang F, Xu J, Zhang Y, Zhang M, Zhang L, Lu Y, Geng Y, Huang P, Huang P, Wang H, Pan S. Real-time monitoring efficiency and toxicity of chemotherapy in patients with advanced lung cancer. *Clin Epigenetics* 2015; **31**: 119 [PMID: 26550041 DOI: 10.1136/gut.45.2008.iv1]

31 **Gao L**, Xie E, Yu T, Chen D, Zhang L, Zhang B, Wang F. Methylated APC and RASSF1A in multiple specimens contribute to the differential diagnosis of patients with undetermined solitary pulmonary nodules. *J Thorac Dis* 2015; **7**: 422-432

32 **Pan S**, Xia W, Ding Q, Shu Y, Xu T, Geng Y, Lu Y. Can plasma DNA monitoring be employed in personalized chemotherapy for patients with advanced lung cancer? *Biomed Pharmacother* 2012; **66**: 131-137

World Journal of Gastroenterology

Submit a Manuscript: https://www.f6publishing.com

World J Gastroenterol 2019 August 7; 25(29): 3996-4006

DOI: 10.3748/wjg.v25.i29.3996

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Retrospective Study

Additional laparoscopic gastrectomy after noncurative endoscopic submucosal dissection for early gastric cancer: A single-center experience

Yan-Tao Tian, Fu-Hai Ma, Gui-Qi Wang, Yue-Ming Zhang, Li-Zhou Dou, Yi-Bin Xie, Yu-Xin Zhong, Ying-Tai Chen, Quan Xu, Dong-Bing Zhao

ORCID number: Yan-Tao Tian (0000-0001-6479-7547); Fu-Hai Ma (0000-0003-2437-6881); Gui-Qi Wang (0000-0001-7767-1564); Yue-Ming Zhang (0000-0002-8406-5877); Dou-Li Zhou (0000-0003-1455-4701); Yi-Bin Xie (0000-0002-7887-1389); Yu-Xin Zhong (0000-0002-8865-3297); Ying-Tai Chen (0000-0003-4980-6315); Quan Xu (0000-0001-9246-3253); Dong-Bing Zhao (0000-0001-8339-687X).

Author contributions: Tian YT and Ma FH contributed equally to this work and they were involved in study concept, data acquisition, analysis, and interpretation, and production of tables, wrote the first draft, and revised it critically in light of comments from other authors; Zhao DB was involved in study conception and design, data interpretation, manuscript revision, and discussion; Wang GQ, Zhang YM, Dou LZ, and Xu Q were involved in data acquisition and literature review; Xie YB, Zhong YX, and Chen YT were involved in the manuscript revision and discussion; all authors approved the final version submitted.

Supported by the National Natural Science Foundation of China, No. 81772642; Beijing Municipal Science and Technology Commission, No. Z161100000116045; and Capital's Funds for Health Improvement and Research, No. CFH 2018-2-4022.

Yan-Tao Tian, Fu-Hai Ma, Yi-Bin Xie, Yu-Xin Zhong, Ying-Tai Chen, Quan Xu, Dong-Bing Zhao, Department of Pancreatic and Gastric Surgery, National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100021, China

Gui-Qi Wang, Yue-Ming Zhang, Li-Zhou Dou, Department of Endoscopy, National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100021, China

Corresponding author: Dong-Bing Zhao, MD, Doctor, Professor, Surgeon, Department of Pancreatic and Gastric Surgery, National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, No. 17, Panjiayuan Nanli, Chaoyang District, Beijing 100021, China.
dbzhao2003@sina.com
Telephone: +86-10-87787120
Fax: +86-10-87787120

Abstract

BACKGROUND

The necessity of additional gastrectomy for early gastric cancer (EGC) patients who do not meet curative criteria after endoscopic submucosal dissection (ESD) is controversial.

AIM

To examine the clinicopathologic characteristics of patients who underwent additional laparoscopic gastrectomy after ESD and to determine the appropriate strategy for treating those after noncurative ESD.

METHODS

We retrospectively studied 45 patients with EGC who underwent additional laparoscopic gastrectomy after noncurative ESD from January 2013 to January 2019 at the Cancer Hospital of the Chinese Academy of Medical Sciences. We analyzed the patients' clinicopathological data and identified the predictors of residual cancer (RC) and lymph node metastasis (LNM).

RESULTS

Surgical specimens showed RC in ten (22.2%) patients and LNM in five (11.1%).

Institutional review board statement: This study was approved by the Institutional Review Board of the National Cancer Center Hospital.

Informed consent statement: The need for informed consent was waived due to the retrospective nature of the study, and the data were anonymously analyzed.

Conflict-of-interest statement: The authors declare that they have no conflicts of interest.

Data sharing statement: No additional data are available.

Manuscript source: Unsolicited manuscript

Received: May 5, 2019
Peer-review started: May 6, 2019
First decision: June 10, 2019
Revised: June 28, 2019
Accepted: July 5, 2019
Article in press: July 5, 2019
Published online: August 7, 2019

P-Reviewer: Afzal M, Herbella FAM
S-Editor: Yan JP
L-Editor: Wang TQ
E-Editor: Ma YJ

Multivariate analysis revealed that positive horizontal margin [odds ratio (OR) = 13.393, 95% confidence interval (CI): 1.435-125, P = 0.023] and neural invasion (OR = 14.714, 95%CI: 1.087-199, P = 0.043) were independent risk factors for RC. Undifferentiated type was an independent risk factor for LNM (OR = 12.000, 95%CI: 1.197-120, P = 0.035). Tumors in all patients with LNM showed submucosal invasion more than 500 μm. Postoperative complications after additional laparoscopic gastrectomy occurred in five (11.1%) patients, and no deaths occurred among patients with complications.

CONCLUSION
Gastrectomy is necessary not only for patients who have a positive margin after ESD, but also for cases with neural invasion, undifferentiated type, and submucosal invasion more than 500 μm. Laparoscopic gastrectomy is a safe, minimally invasive, and feasible procedure for additional surgery after noncurative ESD. However, further studies are needed to apply these results to clinical practice.

Key words: Early gastric cancer; Endoscopic submucosal dissection; Laparoscopic gastrectomy; Residual cancer; Lymph node metastasis

Core tip: It is controversial whether additional gastrectomy is necessary for all patients who do not meet the curative criteria after endoscopic submucosal dissection (ESD). Therefore, it would be valuable to determine which factors could increase the risk of residual cancer or lymph node metastasis in patients after noncurative ESD in order to avoid unnecessary surgery. We found that gastrectomy was necessary not only for patients who had a positive margin in ESD, but also for cases with neural invasion, undifferentiated type, and submucosal invasion more than 500 μm. Laparoscopic gastrectomy is a safe, minimally invasive, and feasible procedure for additional surgery after noncurative ESD.

Citation: Tian YT, Ma FH, Wang GQ, Zhang YM, Dou LZ, Xie YB, Zhong YX, Chen YT, Xu Q, Zhao DB. Additional laparoscopic gastrectomy after noncurative endoscopic submucosal dissection for early gastric cancer: A single-center experience. *World J Gastroenterol* 2019; 25(29): 3996-4006
URL: https://www.wjgnet.com/1007-9327/full/v25/i29/3996.htm
DOI: https://dx.doi.org/10.3748/wjg.v25.i29.3996

INTRODUCTION

Gastric cancer is the fourth most common cancer and the second leading cause of cancer-related death in the world[1]. Early gastric cancer (EGC) is defined as a tumor confined to the mucosa or submucosa, regardless of the regional lymph node metastasis (LNM)[2]. The detection rates of EGC have been improved with the increase in cancer surveillance and widespread endoscopic examinations[3]. Endoscopic submucosal dissection (ESD) as a treatment for EGC has been rapidly spreading due to the advantages of this technique including reduced postoperative complications, decreased medical cost, fast recovery, and improved quality of life[4]. As ESD is now performed more frequently, noncurative ESD is also becoming more and more frequent, thus warranting appropriate treatment[3].

For patients who have undergone noncurative ESD, some reports[5-9] recommend additional surgery to prevent residual cancer (RC) or LMN. However, high morbidity, poor quality of life, and medical cost of gastrectomy for these patients cannot be neglected, and it is controversial whether additional gastrectomy is necessary for all patients who do not meet the curative criteria after ESD[10,11]. Therefore, it would be valuable to determine which factors could increase the risk of RC or LNM in patients after noncurative ESD in EGC patients in order to avoid unnecessary surgery.

Laparoscopic gastrectomy (LG) has been accepted as a standard procedure for the treatment of EGC because it is minimally invasive, results in decreased postoperative pain, and has a shorter recovery time than other procedures[12,13]. ESD-induced

inflammation causes edema, fibrosis, and intraabdominal adhesions, which might increase the difficulties and the risk of complications during subsequent LG[3,14]. However, relatively few data are available on the influence of previous ESD on LG[15-17].

In the present study, we aimed to examine the predictive factors for LNM and RC as well as to explore the appropriate strategy for treating these patients after noncurative ESD. We also aimed to assess the feasibility and safety of LG as additional surgery after ESD.

MATERIALS AND METHODS

In this retrospective cohort study, the clinical data of consecutive EGC patients who underwent additional gastrectomy after ESD at the Cancer Hospital of the Chinese Academy of Medical Sciences, Chinese National Cancer Center between January 2013 and January 2019 were reviewed. The rate of LNM or RC was investigated. The associations between various clinicopathological factors and RC or LNM were examined by univariable and multivariable analyses. This retrospective study was approved by the Institutional Review Board at the Cancer Hospital of the Chinese Academy of Medical Sciences. The need for informed consent was waived due to the retrospective nature of the study, and the data were anonymously analyzed. The datasets in the current study are available from the corresponding author on reasonable request.

Indications and procedures for ESD

Depth of tumor invasion and tumor stage were assessed initially before ESD by endoscopic ultrasonography and contrast-enhanced computed tomography of the abdomen and pelvis. The extended indications for ESD were as follows: (1) Differentiated mucosal cancer without ulceration regardless of lesion size; (2) Differentiated mucosal cancer, with ulceration, < 3 cm in diameter; (3) Differentiated minimally invasive submucosal cancer < 3 cm in diameter; and (4) Undifferentiated mucosal cancer ≤ 2 cm in size.

ESD was performed by one experienced gastrointestinal endoscopist in our hospital. An incision line were made at about 5 mm lateral to the margin of the cancerous lesion using a needle. Hypertonic saline mixed with epinephrine (1:10000) and sodium hyaluronate were injected into the submucosal layer to lift the lesion. A circumferential mucosal incision surrounding the marking dots was performed. The submucosa beneath the target lesion was dissected and the entire lesion was completely removed with a surgical electronic knife.

Histopathological evaluation

After being fixed in 10% formalin, resected specimens were sectioned perpendicularly at 2-mm intervals. The histological evaluation was based on the World Health Organization classification of gastric cancer. Gross types were categorized into elevated, flat, or depressed type. Well or moderately differentiated tubular adenocarcinoma and papillary adenocarcinoma were classified as differentiated adenocarcinoma type, while poorly differentiated adenocarcinoma, signet ring cell carcinoma, and mucinous adenocarcinoma were classified as undifferentiated type. Tumor involvement in the lateral or vertical resection margin, tumor size, lymphovascular invasion, neural invasion, and the depth of tumor invasion were evaluated. The depth of tumor invasion was measured and quantified and was classified as M (mucosal invasion), SM1 (submucosal invasion < 500 μm of the lower margin of the muscularis mucosae), and SM2 (tumor invasion into submucosa > 500 μm from the muscularis mucosa).

Criteria for noncurative resection of ESD

The lesions that were considered not to meet the noncurative criteria for ESD were defined as lesions that met at least one of the following criteria based on histopathologic findings of the ESD specimens: (1) Positive horizontal margin; (2) Positive vertical margin; (3) Presence of lymphovascular involvement; (4) SM2 or deeper invasion; (5) Differentiated mucosal cancer with ulceration and size ≥ 30 mm; (6) Differentiated SM1 cancer ≥ 30 mm; and (7) undifferentiated cancer accompanied by submucosal invasion, size > 20 mm, or ulceration.

Statistical analysis

Univariate analyses by the χ^2 test or Fisher's exact test were performed to explore the clinicopathological differences between the RC and non-RC groups, and between the LNM and non-LNM groups. Furthermore, multivariate logistic regression analysis

was used to identify independent risk factors for RC and LNM, including those factors with $P < 0.3$ in univariate analysis. A P-value < 0.05 was considered significant. All analyses were performed with SPSS for Windows version 22.0.

RESULTS

Demographics and clinicopathological characteristics of the patients

A total of 640 ESDs were performed, and 45 (7.0%) noncurative ESDs were found during the study period. The demographics and clinicopathological characteristics of the patients who received additional gastrectomy because of noncurative ESD are summarized in Table 1. The reasons for additional gastrectomy consisted of positive horizontal margin (7 cases), positive vertical margin (29 cases), SM2 (31 cases), lymphovascular invasion (19 cases), and undifferentiated type (14 cases). And two cases were suspected recurrence on esophagogastroduodenoscopy at the 3-month follow-up after ESD. Of the 45 patients, 34 (75.6%) were male and 11 (24.4%) were female. The mean age was 58.2 ± 9.3 years. The median interval between ESD and additional gastrectomy was 47 ± 26 d. The final depth of tumor invasion was M in 9 patients, SM1 in 5, SM2 in 26, muscularis propria in 2, and subserosa in 3.

Associations between clinicopathological characteristics and RC

RC was found in 10 (22.2%) of the 45 patients. The patients who did and did not have RC were compared in terms of their clinicopathological characteristics, as shown in Table 2. Univariate analyses determined that horizontal margin ($P = 0.034$) and neural invasion ($P = 0.007$) were significant factors for RC. In contrast, tumor location, macroscopic type, tumor size, histological differentiation, Lauren type, vertical margin, depth of invasion, and lymphovascular invasion did not show significant correlations. Multivariate analysis showed that horizontal margin [(odds ratio OR) = 13.393, 95% confidence interval (CI): 1.435-125, $P = 0.023$] and neural invasion (OR = 18.495, 95%CI: 1.585-215, $P = 0.020$) were associated with a higher incidence of RC within specimens after surgery (Table 3).

Associations between clinicopathological characteristics and LNM

LNM was detected in 5 (11.1%) out of 45 cases. Relationships between clinicopathological characteristics and LNM are summarized in Table 4. Undifferentiated type was the only significant factor for LNM ($P = 0.027$). Macroscopic type and depth of tumor invasion had weak relationships. Multivariate analysis revealed that undifferentiated type (OR = 12.000, 95%CI: 1.197-120, $P = 0.035$) was associated with a higher incidence of LNM within specimens after surgery. All five patients showed tumor depth of more than SM1 in the specimen from the initial endoscopic resection. Of the five patients with LNM, four previously exhibited undifferentiated type post-ESD treatment.

Operative data and postoperative outcomes

Details of the intraoperative course and postoperative course are shown in Table 5. The type of LG was determined based on the tumor location. Proximal gastrectomy was performed in 15 (33.3%) cases and distal gastrectomy in 23 (51.1%). Total gastrectomy was performed in five (11.1%) cases and partial gastrectomy in two (4.4%). The mean number of harvested lymph nodes was 29.7 ± 13.7. The mean operative time and mean estimated blood loss were 180 ± 47 min and 107 ± 69 mL, respectively. The time to first flatus was 3.4 ± 0.8 d, the time to recommencement of oral intake was 5.3 ± 1.4 d, and the length of hospital stay was 9.9 ± 2.9 d. Postoperative complications occurred five (11.1%) patients. Two patients developed leakage from the anastomotic site, and one each developed wound infection, hemorrhage, and abdominal infection. These complications were conservatively treated and consequently improved. None of these patients died.

DISCUSSION

The rate of RC in our series (22.2%) was similar to those in the previous reports (5.2%-28.6%)[3,4,18-24]. LNM was detected in 5 (11.1%) out of 45 cases. The majority of these cases harbored neither RC nor LNM, indicating that additional surgery may be unnecessary. Therefore, it is important to identify which patients will benefit the most from additional gastrectomy after noncurative ESD for EGC. However, the studies of predictive factors for RC and LNM in additional surgery gastrectomy specimens after ESD have been very limited. Our study revealed that positive horizontal and neural

Table 1 Demographic characteristics of the patients

Characteristic	All patients (*n* = 45)	
	Number	Percent
Age (yr)	58.24 ± 9.3	
Gender		
Male	34	75.6
Female	11	24.4
Abdominal operation history		
Yes	8	17.8
No	37	82.2
ASA score		
I-II	34	75.6
III-IV	11	24.4
Comorbidity		
Any comorbidity	15	33.3
Hypertension	10	22.2
Diabetes	5	10.5
Coronary artery disease	2	11.1
Others	4	8.9
Surgical indication		
Vertical margin positive	29	64.4
SM2	31	68.9
Horizontal margin positive	7	15.6
Lymphovascular invasion	19	42.2
Undifferentiated type	14	31.1
Suspected recurrence 3 mo after ESD	2	4.4
Interval (d)	47 ± 26	
RC		
Yes	10	22.2
No	35	77.8
LNM		
Yes	5	11.1
No	40	88.9
Depth of invasion		
T1a	9	20.0
T1b SM1	5	11.1
T1b SM2	26	57.8
T2	2	4.4
T3	3	6.7

RC: Residual cancer; LNM: Lymph node metastasis; ASA: American Society of Anesthesiologists; ESD: Endoscopic submucosal dissection.

invasion were independent risk factors for RC. Undifferentiated type was an independent risk factor for LNM.

Regarding RC, positive vertical margin and positive horizontal margin were independent predictors in some previous studies[18], while many authors also reported only positive horizontal margin as a risk factor for RC, as found in our study[4,21,22]. Hyuk *et al* thought that the possibility of the tumor cells in the corresponding area opposite an involved resection margin being completely removed by the cautery effect was much lower in the horizontal rather than in the vertical direction[4,5]. The feasibility of secondary ESD for local control in positive horizontal margin cases has been reported; however, the management of these patients is debated[25]. If there is an additional noncurative factor combined with the positive horizontal margin, additional surgery should be considered. Neural invasion is a way of cancer spreading and is related to advanced stage, higher risk of recurrence, and poor long-

Table 2 Characteristics of cases with and without residual cancer, *n* (%)

Characteristic	Residual cancer		*P*-value
	Yes (*n* = 10)	No (*n* = 35)	
Location			1.000
Upper third	4 (23.5)	13 (76.5)	
Middle third	2 (18.2)	9 (81.8)	
Lower third	4 (23.5)	13 (76.5)	
Macroscopic appearance			0.694
Elevated type	1 (25.0)	3 (75.0)	
Surface type	9 (23.1)	30 (76.9)	
Depressed type	0 (0)	2 (100)	
Tumor size			0.720
< 3 cm	5 (19.2)	21 (80.8)	
≥ 3 cm	5 (26.3)	14 (73.7)	
Differentiation			1.000
Differentiated	7 (22.6)	24 (77.4)	
Undifferentiated	3 (21.4)	11 (78.6)	
Lauren type			0.722
Intestinal	4 (18.2)	18 (81.8)	
Diffused/Mixed	6 (26.1)	17 (73.9)	
Depth of invasion			0.469
Mucosal invasion/SM1	2 (14.3)	12 (85.7)	
> SM1 invasion	8 (25.8)	23 (74.2)	
Horizontal margin			0.034
Positive	4 (57.1)	3 (42.9)	
Negative	6 (15.8)	32 (84.2)	
Vertical margin			0.292
Positive	8 (27.6)	21 (72.4)	
Negative	2 (12.5)	14 (87.5)	
Lymphovascular invasion			1.000
Yes	4 (21.1)	15 (78.9)	
No	6 (23.1)	20 (76.9)	
Neural invasion			0.007
Yes	6 (54.5)	5 (45.5)	
No	4 (11.8)	30 (88.2)	

term survival in gastric cancer[26,27]. In the stomach, the nerve plexuses are concentrated in the Meissner's plexus in the submucosa and Auerbach's plexus between the circular and longitudinal fibers of the muscularis propria[28]. Thus, neural invasion is observed more frequently in advanced gastric cancer. Interestingly, neural invasion has not been established as a predictor of RC after noncurative ESD, while our study confirmed that neural invasion was an independent risk factor for RC. Although the number of cases was limited, it is a reminder that RC might be detected for those patients with neural invasion and additional gastrectomy may be needed.

In previous studies of patients who underwent additional surgery following noncurative ESD of EGC, the LNM rates ranged from 5.1% to 9.8%[4,18,19,21,23,24,29,30], which are similar to the present finding of 11.1%. Previous reports have indicated that lymphovascular invasion, SM2 invasion, lesion size > 3 cm, and positive vertical margin were associated with a greater risk of LNM in patients with EGC[31-33]. Lymphovascular invasion has been proven to be an independent risk factor for LNM in those patients who underwent noncurative ESD[18,21,34,35]. However, lymphovascular invasion was not correlated with LNM in the present study and two patients without lymphovascular invasion were found to have LMN. Previous studies have demonstrated that the rate of LNM was higher in patients with differentiated EGC with undifferentiated components than in those with EGC without undifferentiated components [4,36]. Lee *et al*[37] reported that the rate of LNM increased with the increase in undifferentiated components in differentiated type mucosal cancers. Kim *et al*[38]

Table 3 Multivariate analysis of the risk factors for residual cancer

Risk factor	OR	95%CI	*P*-value
Vertical margin positive	0.670	0.065-6.909	0.737
Depth of invasion: > SM1	0.637	0.075-5.423	0.680
Horizontal margin positive	13.393	1.435-125	0.023
Neural invasion positive	18.495	1.585-215	0.020

OR: Odds ratio; CI: Confidence interval.

and Abdelfatah *et al*[39] demonstrated that undifferentiated histology was an important risk factor for LNM. In the present series, undifferentiated histology was a major risk factor for LNM. SM2 invasion was another factor reported to be associated with a greater risk for LNM in patients with EGC[30,40]. This was thought to be due to the presence of larger diameter lymphatic vessels in the deeper third of the lamina propria, and the progressive increase in diameter as these vessels go deeper into the submucosal layer, where the lymphatic network is richer[39]. In our study, tumors in five lymph node-positive patients showed invasion deeper than SM1 in the surgical pathology specimen. Therefore, cases with submucosal invasion deeper than SM1 require additional gastrectomy and lymphadenectomy.

ESD in EGC causes an artificial gastric ulceration, local inflammation, subsequent fibrosis, and even adhesions in the outer gastric wall, which has a negative intraprocedural impact on additional LG in patients who have undergone non-curative ESD[14]. Previous studies have demonstrated that ESD is not associated with postoperative complications during or after an additional LG in patients who underwent noncurative ESD[15-17]. Our study found that LG can achieve good short-term surgical outcomes for gastric cancer after noncurative ESD.

This study had several limitations. First, it was a retrospective study conducted in a single center and the sample size was relatively small. Such limitations may lead to issues of selection bias and heterogeneous patient group. Second, we did not report long-term outcomes of patients with noncurative ESD because the mean follow-up period was too short.

In conclusion, gastrectomy is necessary not only for patients who have a positive margin in ESD, but also for cases with neural invasion, undifferentiated type, and submucosal invasion more than 500 μm due to the risk of RC or LMN. In terms of short-term surgical outcomes, LG is a safe, minimally invasive, and feasible procedure for additional surgery after noncurative ESD. However, further studies are needed to apply these results to clinical practice.

Table 4 Characteristics of patients with and without lymph node metastasis in surgical specimens, *n* (%)

Characteristic	LNM		*P*-value
	Yes (*n* = 5)	No (*n* = 40)	
Location			0.417
Upper third	3 (17.6)	14 (86.7)	
Middle third	0 (0)	11 (100)	
Lower third	2 (11.8)	15 (88.2)	
Macroscopic appearance			0.125
Elevated type	1 (25)	3 (75)	
Surface type	3 (7.7)	36 (92.3)	
Depressed type	1 (50)	1 (50)	
Tumor size			1.000
< 3 cm	3 (11.5)	23 (89.5)	
≥ 3 cm	2 (10.5)	17 (89.5)	
Differentiation			0.027
Differentiated	1 (3.2)	30 (96.8)	
Undifferentiated	4 (28.6)	10 (71.4)	
Lauren type			0.665
Intestinal	3 (13.6)	19 (86.4)	
Diffused/Mixed	2 (8.7)	21 (91.3)	
Depth of invasion			0.305
Mucosal invasion/SM1	0 (0)	14 (100)	
> SM1 invasion	5 (16.1)	26 (83.9)	
Horizontal margin			0.577
Positive	0 (0)	7 (100)	
Negative	5 (13.2)	33 (86.8)	
Vertical margin			1.000
Positive	3 (10.3)	26 (89.7)	
Negative	2 (12.5)	14 (87.5)	
Lymphovascular invasion			0.636
Yes	3 (15.8)	16 (84.2)	
No	2 (7.7)	24 (92.3)	
Neural invasion			1.000
Yes	1 (9.1)	10 (90.9)	
No	4 (11.8)	30 (88.2)	

LNM: Lymph node metastasis.

Table 5 Operative data and postoperative outcomes

Variable	*n* (%)
Type of gastrectomy	
Proximal	15 (33.3)
Distal	23 (51.1)
Total	5 (11.1)
Partial	2 (4.4)
Retrieved lymph node	29.7 ± 13.7
Complications	
Any	5 (11.1)
Wound infection	1 (2.2)
Postoperative bleeding	1 (2.2)
Anastomotic leakage	2 (4.4)
Abdominal infection	1 (2.2)
30-day mortality	0
Estimated blood loss (mL)	107 ± 69
Operation time (min)	180 ± 47
Time to resume soft diet (d)	5.3 ± 1.4
Time until the first flatus (d)	3.4 ± 0.8
Postoperative hospital stay (d)	9.9 ± 2.9

ARTICLE HIGHLIGHTS

Research background

Endoscopic submucosal dissection (ESD) as a treatment for early gastric cancer (EGC) has been rapidly spreading. As ESD is now performed more frequently, noncurative resection after ESD is also becoming more frequent. It is controversial whether additional gastrectomy is necessary for all patients who do not meet the curative criteria after ESD.

Research motivation

It would be valuable to determine which factors could increase the risk of residual cancer (RC) or lymph node metastasis (LNM) in patients after noncurative ESD of EGC in order to avoid unnecessary surgery.

Research objectives

The objectives of this study were to identify the predictive factors for LNM and RC as well as to explore the appropriate strategy for treating those after non-curative ESD. We also aimed to assess the feasibility and safety of LG as additional surgery after ESD.

Research methods

We analyzed the patients' clinicopathological data and identified the predictors of RC and LNM.

Research results

Surgical specimens showed RC in ten patients and LNM in five. Multivariate analysis revealed that positive horizontal margin and neural invasion were independent risk factors for RC. Undifferentiated type was an independent risk factor for LNM. Tumors in all patients with LNM showed submucosal invasion more than 500 μm. Postoperative complications after additional laparoscopic gastrectomy occurred in five patients, and no deaths occurred among patients with complications.

Research conclusions

Our study revealed that positive horizontal and neural invasion are independent risk factors for RC. Undifferentiated type is an independent risk factor for LNM. Laparoscopic gastrectomy is a safe, minimally invasive, and feasible procedure for additional surgery after noncurative ESD. Gastrectomy is necessary not only for patients who have a positive margin in ESD, but also for cases with neural invasion, undifferentiated type, and submucosal invasion more than 500 μm due to the risk of RC or LMN. Laparoscopic gastrectomy is a safe, minimally invasive, and feasible procedure for additional surgery after noncurative ESD.

Research perspectives

A study of larger sample size is needed. Long-term outcomes of patients with noncurative ESD need to be investigated in a prospective multicenter trial.

REFERENCES

1 **Torre LA**, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. *CA Cancer J Clin* 2015; **65**: 87-108 [PMID: 25651787 DOI: 10.3322/caac.21262]
2 **Japanese Gastric Cancer Association**. Japanese classification of gastric carcinoma: 3rd English edition. *Gastric Cancer* 2011; **14**: 101-112 [PMID: 21573743 DOI: 10.1007/s10120-011-0041-5]
3 **Noh GY**, Ku HR, Kim YJ, Park SC, Kim J, Han CJ, Kim YC, Yang KY. Clinical outcomes of early gastric cancer with lymphovascular invasion or positive vertical resection margin after endoscopic submucosal dissection. *Surg Endosc* 2015; **29**: 2583-2589 [PMID: 25480609 DOI: 10.1007/s00464-014-3973-0]
4 **Toyokawa T**, Ohira M, Tanaka H, Minamino H, Sakurai K, Nagami Y, Kubo N, Yamamoto A, Sano K, Muguruma K, Tominaga K, Nebiki H, Yamashita Y, Arakawa T, Hirakawa K. Optimal management for patients not meeting the inclusion criteria after endoscopic submucosal dissection for gastric cancer. *Surg Endosc* 2016; **30**: 2404-2414 [PMID: 26463497 DOI: 10.1007/s00464-015-4491-4]
5 **Yoon H**, Kim SG, Choi J, Im JP, Kim JS, Kim WH, Jung HC. Risk factors of residual or recurrent tumor in patients with a tumor-positive resection margin after endoscopic resection of early gastric cancer. *Surg Endosc* 2013; **27**: 1561-1568 [PMID: 23263643 DOI: 10.1007/s00464-012-2627-3]
6 **Kusano C**, Iwasaki M, Kaltenbach T, Conlin A, Oda I, Gotoda T. Should elderly patients undergo additional surgery after non-curative endoscopic resection for early gastric cancer? Long-term comparative outcomes. *Am J Gastroenterol* 2011; **106**: 1064-1069 [PMID: 21407189 DOI: 10.1038/ajg.2011.49]
7 **Lee HJ**, Jang YJ, Kim JH, Park SS, Park SH, Park JJ, Kim SJ, Kim CS, Mok YJ. Clinical Outcomes of Gastrectomy after Incomplete EMR/ESD. *J Gastric Cancer* 2011; **11**: 162-166 [PMID: 22076221 DOI: 10.5230/jgc.2011.11.3.162]
8 **Eom BW**, Kim YI, Kim KH, Yoon HM, Cho SJ, Lee JY, Kim CG, Kook MC, Kim YW, Nam BH, Ryu KW, Choi IJ. Survival benefit of additional surgery after noncurative endoscopic resection in patients with early gastric cancer. *Gastrointest Endosc* 2017; **85**: 155-163.e3 [PMID: 27460389 DOI: 10.1016/j.gie.2016.07.036]
9 **Suzuki S**, Gotoda T, Hatta W, Oyama T, Kawata N, Takahashi A, Yoshifuku Y, Hoteya S, Nakagawa M, Hirano M, Esaki M, Matsuda M, Ohnita K, Yamanouchi K, Yoshida M, Dohi O, Takada J, Tanaka K, Yamada S, Tsuji T, Ito H, Hayashi Y, Shimosegawa T. Survival Benefit of Additional Surgery After Non-curative Endoscopic Submucosal Dissection for Early Gastric Cancer: A Propensity Score Matching Analysis. *Ann Surg Oncol* 2017; **24**: 3353-3360 [PMID: 28795364 DOI: 10.1245/s10434-017-6039-4]
10 **Hatta W**, Gotoda T, Oyama T, Kawata N, Takahashi A, Yoshifuku Y, Hoteya S, Nakagawa M, Hirano M, Esaki M, Matsuda M, Ohnita K, Yamanouchi K, Yoshida M, Dohi O, Takada J, Tanaka K, Yamada S, Tsuji T, Ito H, Hayashi Y, Nakamura T, Nakaya N, Shimosegawa T. Is the eCura system useful for selecting patients who require radical surgery after noncurative endoscopic submucosal dissection for early gastric cancer? A comparative study. *Gastric Cancer* 2018; **21**: 481-489 [PMID: 28983696 DOI: 10.1007/s10120-017-0769-7]
11 **Heo J**, Jeon SW. The clinical significance and management of noncurative endoscopic resection in early gastric cancer. *Clin Endosc* 2013; **46**: 235-238 [PMID: 23767032 DOI: 10.5946/ce.2013.46.3.235]
12 **Kim W**, Kim HH, Han SU, Kim MC, Hyung WJ, Ryu SW, Cho GS, Kim CY, Yang HK, Park DJ, Song KY, Lee SI, Ryu SY, Lee JH, Lee HJ; Korean Laparo-endoscopic Gastrointestinal Surgery Study (KLASS) Group. Decreased Morbidity of Laparoscopic Distal Gastrectomy Compared With Open Distal Gastrectomy for Stage I Gastric Cancer: Short-term Outcomes From a Multicenter Randomized Controlled Trial (KLASS-01). *Ann Surg* 2016; **263**: 28-35 [PMID: 26352529 DOI: 10.1097/SLA.0000000000001346]
13 **Aoyama T**, Sato T, Hayashi T, Yamada T, Cho H, Ogata T, Oba K, Yoshikawa T. Does a laparoscopic approach attenuate the body weight loss and lean body mass loss observed in open distal gastrectomy for gastric cancer? a single-institution exploratory analysis of the JCOG 0912 phase III trial. *Gastric Cancer* 2018; **21**: 345-352 [PMID: 28623524 DOI: 10.1007/s10120-017-0735-4]
14 **Akagi T**, Shiraishi N, Hiroishi K, Etoh T, Yasuda K, Kitano S. Case series of intra-abdominal adhesions induced by artificial ulceration after endoscopic submucosal dissection before additional laparoscopic gastrectomy. *Gastrointest Endosc* 2010; **72**: 438-443 [PMID: 20541190 DOI: 10.1016/j.gie.2010.03.1066]
15 **Jiang X**, Hiki N, Yoshiba H, Nunobe S, Kumagai K, Sano T, Yamaguchi T. Laparoscopy-assisted gastrectomy in patients with previous endoscopic resection for early gastric cancer. *Br J Surg* 2011; **98**: 385-390 [PMID: 21254013 DOI: 10.1002/bjs.7358]
16 **Tsujimoto H**, Yaguchi Y, Kumano I, Takahata R, Matsumoto Y, Yoshida K, Horiguchi H, Aosasa S, Ono S, Yamamoto J, Hase K. Laparoscopic gastrectomy after incomplete endoscopic resection for early gastric cancer. *Oncol Rep* 2012; **28**: 2205-2210 [PMID: 22993111 DOI: 10.3892/or.2012.2046]
17 **Ebihara Y**, Okushiba S, Kurashima Y, Noji T, Nakamura T, Murakami S, Tamoto E, Tsuchikawa T, Okamura K, Shichinohe T, Hirano S. Totally laparoscopic gastrectomy for gastric cancer after endoscopic submucosal dissection: A propensity score matching analysis. *Langenbecks Arch Surg* 2015; **400**: 967-972 [PMID: 26476630 DOI: 10.1007/s00423-015-1349-0]
18 **Sunagawa H**, Kinoshita T, Kaito A, Shibasaki H, Kaneko K, Ochiai A, Ohtsu A, Nishida T. Additional surgery for non-curative resection after endoscopic submucosal dissection for gastric cancer: A retrospective analysis of 200 cases. *Surg Today* 2017; **47**: 202-209 [PMID: 27194020 DOI: 10.1007/s00595-016-1353-1]
19 **Ishida R**, Kanaji S, Maehara R, Hasegawa H, Yamamoto M, Matsuda Y, Yamashita K, Matsuda T, Oshikiri T, Sumi Y, Nakamura T, Suzuki S, Kakeji Y. Significance of Additional Gastrectomy Including Endoscopic Submucosal Dissection Scar for Gastric Cancer. *Anticancer Res* 2018; **38**: 5289-5294 [PMID: 30194180 DOI: 10.21873/anticanres.12855]
20 **Katsube T**, Murayama M, Yamaguchi K, Usuda A, Shimazaki A, Asaka S, Konnno S, Miyaki A, Usui T, Yokomizo H, Shiozawa S, Yoshimatsu K, Shimakawa T, Naritaka Y. Additional Surgery After Non-curative Resection of ESD for Early Gastric Cancer. *Anticancer Res* 2015; **35**: 2969-2974 [PMID: 25964583]
21 **Ito H**, Inoue H, Ikeda H, Odaka N, Yoshida A, Satodate H, Onimaru M, Takayanagi D, Santi EG, Kudo SE. Surgical outcomes and clinicopathological characteristics of patients who underwent potentially noncurative endoscopic resection for gastric cancer: A report of a single-center experience. *Gastroenterol Res Pract* 2013; **2013**: 427405 [PMID: 23762035 DOI: 10.1155/2013/427405]
22 **Jung H**, Bae JM, Choi MG, Noh JH, Sohn TS, Kim S. Surgical outcome after incomplete endoscopic submucosal dissection of gastric cancer. *Br J Surg* 2011; **98**: 73-78 [PMID: 21136563 DOI: 10.1002/bjs.7274]

23 **Kim ER**, Lee H, Min BH, Lee JH, Rhee PL, Kim JJ, Kim KM, Kim S. Effect of rescue surgery after non-curative endoscopic resection of early gastric cancer. *Br J Surg* 2015; **102**: 1394-1401 [PMID: 26313295 DOI: 10.1002/bjs.9873]

24 **Lee JH**, Kim JH, Kim DH, Jeon TY, Kim DH, Kim GH, Park DY. Is Surgical Treatment Necessary after Non-curative Endoscopic Resection for Early Gastric Cancer? *J Gastric Cancer* 2010; **10**: 182-187 [PMID: 22076184 DOI: 10.5230/jgc.2010.10.4.182]

25 **Bae SY**, Jang TH, Min BH, Lee JH, Rhee PL, Rhee JC, Kim JJ. Early additional endoscopic submucosal dissection in patients with positive lateral resection margins after initial endoscopic submucosal dissection for early gastric cancer. *Gastrointest Endosc* 2012; **75**: 432-436 [PMID: 22248614 DOI: 10.1016/j.gie.2011.09.044]

26 **De Franco L**, Marrelli D, Voglino C, Vindigni C, Ferrara F, Di Mare G, Iudici L, Marini M, Roviello F. Prognostic Value of Perineural Invasion in Resected Gastric Cancer Patients According to Lauren Histotype. *Pathol Oncol Res* 2018; **24**: 393-400 [PMID: 28555306 DOI: 10.1007/s12253-017-0257-8]

27 **Liebl F**, Demir IE, Mayer K, Schuster T, D'Haese JG, Becker K, Langer R, Bergmann F, Wang K, Rosenberg R, Novotny AR, Feith M, Reim D, Friess H, Ceyhan GO. The impact of neural invasion severity in gastrointestinal malignancies: A clinicopathological study. *Ann Surg* 2014; **260**: 900-7; discussion 907-8 [PMID: 25379860 DOI: 10.1097/SLA.0000000000000968]

28 **Kim YB**, Han SU, Lee D. Prominent neural invasion of mucosal gastric cancer into the muscularis propria. *Histopathology* 2017; **71**: 661-662 [PMID: 28524618 DOI: 10.1111/his.13259]

29 **Nakata B**, Tendo M, Okuyama M, Nakahara K, Ishizu H, Masuda G, Lee T, Hori T, Ohsawa M, Sato H, Ishikawa T. Additional surgical resection after endoscopic mucosal dissection for early gastric cancer: A medium-sized hospital's experience. *Int J Surg* 2016; **36**: 335-341 [PMID: 27871804 DOI: 10.1016/j.ijsu.2016.11.084]

30 **Yang HJ**, Kim SG, Lim JH, Choi J, Im JP, Kim JS, Kim WH, Jung HC. Predictors of lymph node metastasis in patients with non-curative endoscopic resection of early gastric cancer. *Surg Endosc* 2015; **29**: 1145-1155 [PMID: 25171882 DOI: 10.1007/s00464-014-3780-7]

31 **Hatta W**, Gotoda T, Oyama T, Kawata N, Takahashi A, Yoshifuku Y, Hoteya S, Nakagawa M, Hirano M, Esaki M, Matsuda M, Ohnita K, Yamanouchi K, Yoshida M, Dohi O, Takada J, Tanaka K, Yamada S, Tsuji T, Ito H, Hayashi Y, Nakaya N, Nakamura T, Shimosegawa T. A Scoring System to Stratify Curability after Endoscopic Submucosal Dissection for Early Gastric Cancer: "eCura system". *Am J Gastroenterol* 2017; **112**: 874-881 [PMID: 28397873 DOI: 10.1038/ajg.2017.95]

32 **Li H**, Huo ZB, Kong FT, He QQ, Gao YH, Liang WQ, Liu DX. Predictive factors for lymph node metastasis and defining a subgroup treatable for laparoscopic lymph node dissection after endoscopic submucosal dissection in poorly differentiated early gastric cancer. *World J Gastrointest Oncol* 2018; **10**: 360-366 [PMID: 30364712 DOI: 10.4251/wjgo.v10.i10.360]

33 **Zhao B**, Zhang J, Zhang J, Luo R, Wang Z, Xu H, Huang B. Risk Factors Associated with Lymph Node Metastasis for Early Gastric Cancer Patients Who Underwent Non-curative Endoscopic Resection: A Systematic Review and Meta-analysis. *J Gastrointest Surg* 2019; **23**: 1318-1328 [PMID: 30187319 DOI: 10.1007/s11605-018-3924-5]

34 **Suzuki H**, Oda I, Abe S, Sekiguchi M, Nonaka S, Yoshinaga S, Saito Y, Fukagawa T, Katai H. Clinical outcomes of early gastric cancer patients after noncurative endoscopic submucosal dissection in a large consecutive patient series. *Gastric Cancer* 2017; **20**: 679-689 [PMID: 27722825 DOI: 10.1007/s10120-016-0651-z]

35 **Kawata N**, Kakushima N, Takizawa K, Tanaka M, Makuuchi R, Tokunaga M, Tanizawa Y, Bando E, Kawamura T, Sugino T, Kusafuka K, Shimoda T, Nakajima T, Terashima M, Ono H. Risk factors for lymph node metastasis and long-term outcomes of patients with early gastric cancer after non-curative endoscopic submucosal dissection. *Surg Endosc* 2017; **31**: 1607-1616 [PMID: 27495338 DOI: 10.1007/s00464-016-5148-7]

36 **Takizawa K**, Ono H, Kakushima N, Tanaka M, Hasuike N, Matsubayashi H, Yamagichi Y, Bando E, Terashima M, Kusafuka K, Nakajima T. Risk of lymph node metastases from intramucosal gastric cancer in relation to histological types: How to manage the mixed histological type for endoscopic submucosal dissection. *Gastric Cancer* 2013; **16**: 531-536 [PMID: 23192620 DOI: 10.1007/s10120-012-0220-z]

37 **Lee JH**, Choi IJ, Han HS, Kim YW, Ryu KW, Yoon HM, Eom BW, Kim CG, Lee JY, Cho SJ, Kim YI, Nam BH, Kook MC. Risk of lymph node metastasis in differentiated type mucosal early gastric cancer mixed with minor undifferentiated type histology. *Ann Surg Oncol* 2015; **22**: 1813-1819 [PMID: 25344305 DOI: 10.1245/s10434-014-4167-7]

38 **Kim H**, Kim JH, Park JC, Lee YC, Noh SH, Kim H. Lymphovascular invasion is an important predictor of lymph node metastasis in endoscopically resected early gastric cancers. *Oncol Rep* 2011; **25**: 1589-1595 [PMID: 21455589 DOI: 10.3892/or.2011.1242]

39 **Abdelfatah MM**, Barakat M, Othman MO, Grimm IS, Uedo N. The incidence of lymph node metastasis in submucosal early gastric cancer according to the expanded criteria: A systematic review. *Surg Endosc* 2019; **33**: 26-32 [PMID: 30298447 DOI: 10.1007/s00464-018-6451-2]

40 **Miyahara K**, Hatta W, Nakagawa M, Oyama T, Kawata N, Takahashi A, Yoshifuku Y, Hoteya S, Hirano M, Esaki M, Matsuda M, Ohnita K, Shimoda R, Yoshida M, Dohi O, Takada J, Tanaka K, Yamada S, Tsuji T, Ito H, Aoyagi H, Shimosegawa T. The Role of an Undifferentiated Component in Submucosal Invasion and Submucosal Invasion Depth After Endoscopic Submucosal Dissection for Early Gastric Cancer. *Digestion* 2018; **98**: 161-168 [PMID: 29870985 DOI: 10.1159/000488529]

World Journal of Gastroenterology

Submit a Manuscript: https://www.f6publishing.com

World J Gastroenterol 2019 August 7; 25(29): 4007-4018

DOI: 10.3748/wjg.v25.i29.4007

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Observational Study

Management of skin toxicities during panitumumab treatment in metastatic colorectal cancer

Olivier Bouché, Meher Ben Abdelghani, Jean-Luc Labourey, Simon Triby, René-Jean Bensadoun, Thomas Jouary, Gaétan Des Guetz

ORCID number: Olivier Bouche (0000-0002-8284-3743); Meher Ben Abdelghani (0000-0003-2512-4272); Jean-Luc Labourey (0000-0002-7453-1352); Simon TRIBY (0000-0002-2980-178X); René-Jean Bensadoun (0000-0003-1290-7497); Thomas Jouary (0000-0002-9080-3661); Gaétan Des Guetz (0000-0001-96436877).

Author contributions: Bouché O, Ben Abdelghani M, Labourey JL, Bensadoun RJ, Jouary T, and Des Guetz G participated to the design of the study; Bouché O, Ben Abdelghani M, Labourey JL, Bensadoun RJ, Jouary T, Des Guetz G, and Triby S participated to the collection and interpretation of data, and revised the manuscript.

Supported by Amgen 20090656 POPEC.

Institutional review board statement: Study protocol, information sheet and all other relevant documents received the approval of CCTIRS (French consultative committee for the processing of information in the field of health research).

Informed consent statement: The study was non-interventional and patients were informed both orally and in writing on the objectives of the study. The study was conducted according to the current revision of the 1964 Helsinki declaration and with the French laws and regulations.

Conflict-of-interest statement:

Olivier Bouché, Department of Gastroenterology and Digestive Oncology, Hôpital Robert Debré, CHU Reims, Reims 51000, France

Meher Ben Abdelghani, Oncology Department, Centre Paul Strauss, Strasbourg 67000, France

Jean-Luc Labourey, Oncology Department, Centre Hospitalier, Carcassonne 11000, France

Simon Triby, Medical Department, AMGEN France, Boulogne-Billancourt 92100, France

René-Jean Bensadoun, Radiation Oncology, Centre de Haute Energie, Nice 06000, France

Thomas Jouary, Dermatology Department, Hôpital Saint-André, CHU de Bordeaux, Bordeaux 33000, France

Gaétan Des Guetz, Oncology Department, Avicenne Hospital, Bobigny 93000, France

Corresponding author: Olivier Bouché, MD, Professor, Department of Gastroenterology and Digestive Oncology, Hôpital Robert Debré, CHU Reims, Avenue du Général Koenig, Reims 51092, France. obouche@chu-reims.fr
Telephone: +33-326-783113
Fax: +33-326-788836

Abstract

BACKGROUND

Anti-epidermal growth factor receptor therapy is associated with skin adverse events not previously reported with conventional chemotherapy. Prophylactic actions are recommended, but routine clinical management of these toxicities and their impact on quality of life remain unknown.

AIM

To assess the dermatological toxicities reported after panitumumab initiation, their impact on the quality of life and the clinical practices for their management.

METHODS

Patients included in this prospective multicenter observational study were over 18 years of age and began treatment with panitumumab for wild-type KRAS metastatic colorectal cancer. The incidence of dermatological toxicities, clinical practices for their management and impact on quality of life were recorded during a 6-mo follow-up.

RESULTS

Bouché O: Amgen, Roche, Merck Sereno, Bayer, Pierre Fabre, Servier; Ben Abdelghani M: Amgen, Sanofi, Bayer, Roche, Servier; Triby S: Amgen employee; Labourey JL, Bensadoun RJ, Jouary T, and Des Guetz G: no conflict of interest.

Data sharing statement: No additional data are available.

STROBE statement: The authors have read the "STROBE Statement—checklist of items" and the manuscript was prepared and revised according to the "STROBE Statement-checklist of items".

Manuscript source: Unsolicited manuscript

Received: January 17, 2019
Peer-review started: January 18, 2019
First decision: January 30, 2019
Revised: February 7, 2019
Accepted: February 22, 2019
Article in press: February 23, 2019
Published online: August 7, 2019

P-Reviewer: Augustin G, Chan AT, Luglio G
S-Editor: Yan JP
L-Editor: A
E-Editor: Ma YJ

Overall, 229 patients (males, 57.6%; mean age, 66.2 years) were included. At day 15, 59.3% of patients had dermatological toxicity; the rate peaked at month 2 (74.7%) and decreased at month 6 (46.5%). The most frequent dermatological toxicities were rash/acneiform rash, xerosis and skin cracks. At least one preventive treatment was administered to 65.9% of patients (oral antibiotics, 84.1%; emollients, 75.5%; both, 62.9%). The rates of patients who received at least one curative treatment peaked at month 2 (63.4%) and decreased at month 6 (44.8%). The impact of the dermatological toxicities on quality of life was limited as assessed with Dermatology Life Quality Index scores and inconvenience visual analogic scale score. The rates of topical corticosteroids administration and visits to specialists were low.

CONCLUSION

The rates of the different skin toxicities peaked at various times and were improved at the end of follow-up. Nevertheless, their clinical management could be optimized with a better adherence to current recommendations. The impact of skin toxicities on patient's quality of life appeared to be limited.

Key words: Metastatic colorectal cancer; Epidermal growth factor receptor inhibitors; Panitumumab; Skin toxicity; Quality of life

Core tip: Anti-epidermal growth factor receptor therapy is associated with skin adverse events not previously reported with conventional chemotherapy. Prophylactic actions are recommended, but routine clinical management of these toxicities and their impact on quality of life remain unknown. This observational study describes a cohort of patients who began treatment with panitumumab for metastatic colorectal cancer. The rates of the different skin toxicities peaked at various times and were improved at the end of the follow-up. Nevertheless, their clinical management could be optimized with a better adherence to current recommendations. The impact of skin toxicities on patient's quality of life appeared to be limited.

Citation: Bouché O, Ben Abdelghani M, Labourey JL, Triby S, Bensadoun RJ, Jouary T, Des Guetz G. Management of skin toxicities during panitumumab treatment in metastatic colorectal cancer. *World J Gastroenterol* 2019; 25(29): 4007-4018
URL: https://www.wjgnet.com/1007-9327/full/v25/i29/4007.htm
DOI: https://dx.doi.org/10.3748/wjg.v25.i29.4007

INTRODUCTION

Colorectal cancer is the second most common cancer disease in women and the third most common in men in France. In 2015, the number of new cases was 19500 in women and 23500 in men (8500 and 9300 deaths, respectively)[1]. The treatment of metastatic colorectal cancer is based on chemotherapy protocols. The arsenal of anti-cancer treatments has been expanded by new targeted biotherapies such as epidermal growth factor receptor (EGFR) inhibitors. Thus, panitumumab is an EGFR inhibitor which demonstrated its efficacy in wild-type KRAS metastatic colorectal cancer[2-7]. Unlike conventional chemotherapy, EGFR inhibitors are associated with low hematotoxicity and have a more targeted and specific action on tumor cells than conventional cytotoxic chemotherapy drugs. Nevertheless, new adverse reactions have been reported that include cutaneous effects which are observed in two thirds of patients[8]. These adverse events are not unexpected since EGFR is involved in the physiology of epidermidis. Thus, acneiform papulo-pustular reactions are observed in 50% to 80% of cases and generally occur after the first or second infusion of the drug[8-11]. These reactions always regress when treatment is stopped. Other rarer but also incapacitating skin reactions have been reported, such as eczematiform rashes or paronychia[8-11]. Excessive sun exposure, concomitant radiotherapy and inadequate skin hydration are exacerbating factors for dermatological toxicities associated with EGFR inhibitors.

The dermatological toxicity can have a significant impact on the quality of life of

patients, especially in inflammatory and extensive forms affecting the face and leading to poor treatment compliance and need for dosage reduction or even treatment discontinuation[12,13]. At present, no real standards or official recommendations exist concerning the management of these skin reactions. Therefore, the management of skin lesions remains empirical and varies according to personal experience. Nevertheless, some recommendations resulting from meetings with oncology and dermatology experts have been published[8,13-16]. A therapeutic algorithm has been proposed by a French interdisciplinary committee[17].

The diagnostic and symptomatic management of these skin toxicities still needs to be improved in order to limit dosage reductions or treatment discontinuations. Another goal is to reduce the impact on quality of life in patients treated for long periods. It is therefore important to describe accurately the skin symptoms and to identify appropriate dermatological treatments, in order to guarantee both the physical and psychological well-being of patients as well as optimum cancer treatment conditions. The purpose of the present study was to assess the dermatological toxicities reported after panitumumab initiation, their impact on the quality of life and the clinical practices for their management.

MATERIALS AND METHODS

Study design and patients

This was a national, multicenter, descriptive, observational study (POPEC study). Gastroenterologists and oncologists treating colorectal cancer patients were selected and received individual scientific training. A glossary defining precisely the dermatological toxicity was created by the dermatologist of the Scientific Committee and given to the physicians. Physicians saw patients within the context of routine visits, without any special visits being organized for the purposes of the study. The decision to prescribe treatments was freely taken by the clinician prior to the study. The physician-patient relationship and patient follow-up were not modified.

The primary objective was to assess, in patients treated with panitumumab, the incidence, grade and management of the following dermatological toxicities reported at Day 15 after panitumumab (Vectibix®) initiation and at each monthly visit over the 6-month follow-up period: Rash/acneifom rash, skin cracks, paronychia/perionyxis, xerosis, mucositis, hypertrichosis or other. The secondary objective was to assess the impact of dermatological toxicities on quality of life with the 6 dimensions of the Dermatology Life Quality Index (DLQI) scores and with the inconvenience visual analogic scale (VAS) score.

The investigating physicians included all consecutive patients seen in consultation who met the following criteria: patients over 18 years of age, beginning treatment or treated for less than two weeks with panitumumab (Vectibix®) in monotherapy for wild-type KRAS metastatic colorectal cancer, after failure of fluoropyrimidine-, oxaliplatin-, and irinotecan-containing chemotherapy regimens or in combination with chemotherapy as follows: In first line in combination with FOLFOX (folinic acid, fluorouracil and oxaliplatin); in second line in combination with FOLFIRI (folinic acid, fluorouracil and irinotecan) for patients who have received first-line fluoropyrimidine-based chemotherapy (excluding irinotecan). The patients were followed up for a maximum period of 6 mo. The patients were informed both orally and in writing on the objectives of the study. This study was conducted according to the current revision of the 1964 Helsinki declaration and with the French laws and regulations.

Data collection

All data collected were obtained from the medical records of the patients. Data with dates before inclusion of the patient (demographic data, medical history, cancer characteristics, performance status, previous chemotherapies and radiotherapy, previous dermatological history and concomitant skin conditions) were collected retrospectively. Prospective data were collected as part of routine patient follow-up: Cancer treatment, performance status, toxicities and management, DLQI questionnaire and inconvenience VAS. A glossary defining precisely the dermatological toxicity created by the dermatologist of the Scientific Committee was given to the physicians.

The DLQI questionnaire included 10 questions scored from 0 (not at all, not relevant, not answered) to 3 (very much). The DLQI score was calculated by summing the scores of each question resulting in a minimum of 0 and a maximum of 30. Higher scores indicate more quality-of-life impairment. DLQI sub-scale scores were: Symptoms and feelings, daily activities, leisure, work and school, personal

relationships and treatment. All scores were calculated as recommended by the author, including handling of missing answers for score computation[18]. The VAS reflected, on a 10-cm horizontal line, the inconvenience of skin disorders on patient's life. Scores ranged from 0 to 10 cm with 0 meaning "no inconvenience at all" and 10 meaning "a great deal of inconvenience".

Statistical analysis

Due to the observational nature of the study, the statistical analyses were only descriptive. The primary endpoint was the proportion of dermatological toxicities observed during the study. The secondary endpoints were the DLQI scores and the inconvenience VAS score.

In previous studies, dermatological reactions were reported in almost all patients (around 90%) treated with panitumumab or other EGFR inhibitors. The rates of the different types of dermatological effects induced by anti-EGFR monoclonal antibodies ranged from 2%-3% to 60%-80%. It was calculated that a population of 300 patients guaranteed a precision [half-length of 95% confidence interval (CI)] of 2.5% for proportions in the region of 5% and 4.5% in the region of 20%; it did not exceed 6% for higher proportions. In addition, a sample size of 300 patients provided a precision of 3.4% for the 95%CI (86.6%-93.4%) of a proportion of 90%, which corresponds to the proportion of any dermatological reaction observed in patients treated with panitumumab.

The analyses were conducted on all patients enrolled into the study who respected inclusion and exclusion criteria (primary analysis set) and on sub-groups of patients according to age and gender. Age groups were decided by the Scientific Committee and defined during the statistical analysis based on the number of patients observed by age class. The statistical analyses were performed with SAS software (SAS Institute, Cary, NC, United States).

RESULTS

Disposition of patients

Thirty-nine centers in France included a total of 231 patients from June 2011 to February 2013. Two patients did not meet inclusion criteria: Patients not beginning treatment or treated for more than 2 wk with panitumumab in monotherapy or in combination with chemotherapy for wild-type KRAS metastatic colorectal cancer as required, n = 1 (0.4%); patients not presenting the wild type KRAS gene, n = 1 (0.4%). Therefore, the primary analysis set included 229 patients. During the 6-mo follow-up, 142 patients (62.0%) discontinued the study. The reasons for discontinuation were death (n = 78), disease progression (n = 46), lost to follow-up (n = 4) or others (n = 14).

Characteristics of patients

The primary analysis set included 97 women (42.4%) and 132 men (57.6%) with a mean age of 66.2 years; 29.7% had an age ≥ 75 years (Table 1). The mean duration between inclusion and diagnosis of colorectal cancer was 2.9 years and was 2.0 years for metastatic diagnosis. The most frequent metastatic sites were liver (74.2%) and lung (40.2%). Serine/threonine-protein kinase B-Raf (BRAF) genotyping was performed in 31.1% of patients; when performed, mutated BRAF was evidenced in 7.1% of patients. Previous radiotherapy treatment had been received by 27.3% of patients and previous adjuvant chemotherapy by 44.5%. In the context of the metastatic disease, 90.4% received chemotherapy and 13.1% radiotherapy (Table 1). A history of skin disorders was reported for 17.0% of patients and 5.7% of patients had skin disorders at inclusion.

Dermatological toxicities during the follow-up

The rates of patients with at least one dermatological toxicity during the 6-mo follow-up are described in Table 2. At day 15, more than half of patients had dermatological toxicity (59.3%); the rate peaked at month 2 (74.7%) and decreased at Month 6 (46.5%). Among patients with dermatological toxicity, those with rash/acneiform rash were the most frequent (at least 3/4 of patients with skin toxicity at each visit) (Figure 1). Patients with xerosis were also frequent: 21.3% at day 15, 41.1% at month 3 and 27.5% at month 6. The rate of patients with skin cracks steadily increased from 3.9% at day 15 to 42.5% at month 6.

Other dermatological toxicities (paronychia/perionyxis, mucositis, hypertrichosis, other) involved lower numbers of patients (Table 2). Most skin toxicities were grade 1-2. Factors associated with dermatological toxicities have been analyzed: Sex, age, duration since primary disease, duration metastatic disease, metastatic sites, previous adjuvant chemotherapy, previous chemotherapy for metastatic disease, previous

Table 1 Demographic data (primary analysis set, *n* = 229)

	n	Results
Male gender, *n* (%)	229	132 (57.6)
Age (yr)	229	
Mean (SD)	229	66.2 (11.5)
≥ 75, *n* (%)	229	68 (29.7)
Cancer other than metastatic colorectal cancer, *n* (%)	228	20 (8.8)
Duration since diagnosis of primary disease (yr), mean (SD)	226	2.9 (2.3)
Duration since diagnosis of metastatic disease (yr), mean (SD)	227	2.0 (1.5)
Metastatic sites, *n* (%)		
Liver	229	170 (74.2)
Lung	229	92 (40.2)
Peritoneum	229	38 (16.6)
Lymph nodes	229	59 (25.8)
Bone	229	10 (4.4)
Other	229	29 (12.7)
BRAF genotyping performed, *n* (%)	225	70 (31.1)
If performed, BRAF genotyping		
Non-mutated BRAF	70	62 (88.6)
Mutated BRAF	70	5 (7.1)
BRAF not assessable	70	3 (4.3)
Previous radiotherapy treatment (any cancer), *n* (%)	227	62 (27.3)
Previous adjuvant chemotherapy[a], *n* (%)	227	101 (44.5)
Previous chemotherapy for metastatic disease[b], *n* (%)	229	207 (90.4)
Total treatment duration, weeks, mean (SD)		
Line 1	207	26.3 (21.9)
Line 2	165	23.0 (20.5)
Line 3	97	19.9 (17.1)
Line 4	24	19.9 (17.6)
Previous radiotherapy for metastatic disease, *n* (%)		30 (13.1)
Abdominal lymph nodes	218	5 (26.3)
Pelvic	221	10 (45.5)
Other	221	18 (81.8)

[a]Most frequent chemotherapy protocols (*n* = 99): LV5FU2/oxaliplatin FOLFOX (*n* = 42, 42.4%). FOLFIRI/bevacizumab (*n* = 9, 9.1%), LV5FU2 (*n* = 6, 6.1%), FOLFOX/bevacizumab (*n* = 5, 5.1%);
[b]Most frequent chemotherapy protocols for lines 1 to 4, respectively: LV5FU2/oxaliplatin FOLFOX (22.2%, 13.3%, 8.2%, 16.7%), FOLFIRI/bevacizumab (28.0%, 18.2%, 23.7%, 0%); for lines 5 to 8 (*n* = 11, 6, 3,1): LV5FU2/oxaliplatin (27.3%, 0%, 33.3%, 0%). BRAF: Serine/threonine-protein kinase B-Raf; FOLFIRI: Folinic acid, fluorouracil and irinotecan; FOLFOX: Folinic acid, fluorouracil and oxaliplatin.

radiotherapy for metastatic disease, history of skin conditions, and preventive treatment for dermatological toxicities. No factor appeared to be more frequently associated with dermatological toxicities.

Doses of panitumumab and treatment discontinuations

The mean dosage of panitumumab dosage per injection at baseline was 6.0 mg/kg (recommended dose every two weeks) and it did not significantly change during the 6-mo follow-up. Panitumumab treatment was discontinued in 68.2% (150/220) of patients during the 6-mo follow-up. For patients with treatment discontinuation, the mean (SD) duration of treatment before discontinuation was 80.4 (50.8) d. The main reason for discontinuation was related to disease progression (68.7%, 103/150) and toxicity (18.7%, 28/150). There was no discontinuation related to allergic episode. Skin toxicity accounted for 46% (13/28) of all cases of discontinuations related to toxicity. Doses delayed and/or dose adjustment (decrease) in patients with skin toxicities occurred mainly from month 3 including grade 1-2 toxicities. Thus, for rash/acneiform rash grade 1-2, 25.9% (21/81) of patients had delayed dose at month 3 and 25.5% (12/47) had dose adjustment at month 4 (Table 3).

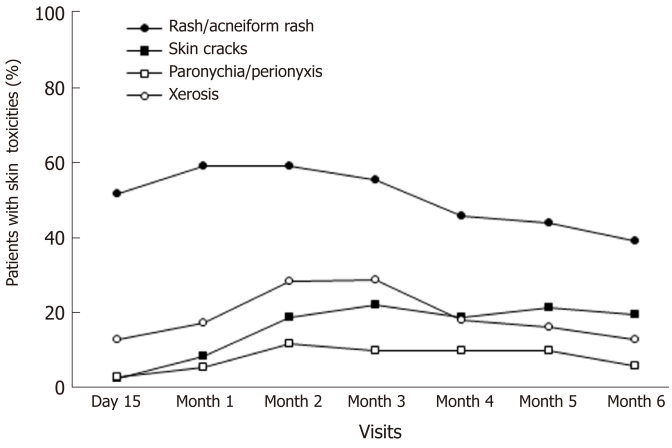


Figure 1 Rates of the main skin toxicities during the 6-mo follow-up.

Preventive and curative treatments of dermatological toxicities

Study patients frequently received preventive treatment (at least one treatment for 65.9% of them) (Table 4). When preventive treatment was administered, the most frequent were oral antibiotics (84.1%), emollients (75.5%) or emollients plus oral antibiotics (62.9%). Topical corticosteroids were administered as preventive treatment in 9.3% of patients.

The curative treatments of dermatological toxicities are described in Table 5. At least one curative treatment was administered to a majority of patients during the 6-mo follow-up. This rate was maximal at month 2 (63.4%). Antibiotics plus emollients were administered to a majority of patients (51.7% at month 2) who received curative treatment (treatment duration was about one mo). Topical corticosteroids were administered to about one patient out of five who received curative treatment (treatment duration ranged from 2 wk to one month).

Specialized consultations for dermatological toxicities

A small proportion of patients required at least one specialized consultation for dermatological toxicities (about 8% for the first two mo and about 5% for the next months). The specialists consulted by these patients were mainly dermatologists (70%), psychologists (22%) and oncology estheticians (14%). Patients with dermatological toxicities consulted more frequently specialists. From 0% to 6.0% at each monthly visit in the absence of toxicity and from 6.5% to 11.0% in the presence of toxicity.

Impact of dermatological toxicities on quality of life

A slight increase of mean DLQI total score from baseline was observed for the entire population with a peak at month 3: From 0.9 at baseline to 3.7 at month 3 (n = 149) for a maximum score equal to 30. The same analysis was performed only in patients with dermatological toxicities and comparable results were observed: From 1.0 at baseline to 4.0 at month 3 (n = 91). The mean inconvenience VAS score increased from 1.5 at baseline (n = 184) to 3.2 at month 3 (n = 95) and decreased to 2.4 at month 6 (n = 50) for the entire population, thus indicating a moderate inconvenience during the 6-mo follow-up. Comparable results were obtained in the sub-group of patients with dermatological toxicities (1.6 at baseline, n = 140; 3.2 at month 3, n = 87; and 2.5 at month 6, n = 48).

DISCUSSION

This observational study included 229 patients with wild-type KRAS colorectal cancer with a mean age of 66.2 years. The inclusion criteria fitted the indications of panitumumab; demographic data and patient characteristics were representative of the population of patients treated with panitumumab.

One of the strengths of this study is the assessment of the kinetics of skin toxicities during a 6-mo follow-up. The rate of patients with dermatological toxicity peaked at month 2 (74.7%). The most frequent dermatological toxicities were rash/acneiform rash (at least 3 out 4 patients at each monthly visit). Patients with xerosis were also frequent. The rate of patients with skin cracks steadily increased from during the follow-up. These findings confirm previous reports on time-course of the most

Table 2 Primary endpoint: dermatological toxicities (primary analysis set, *n* = 229)

	Day 15 (*n* = 214)	Month 1 (*n* = 208)	Month 2 (*n* = 186)	Month 3 (*n* = 153)	Month 4 (*n* = 122)	Month 5 (*n* = 93)	Month 6 (*n* = 87)
At least one dermatological toxicity, *n* (%)	127 (59.3)	141 (67.8)	139 (74.7)	107 (69.9)	76 (63.3)	52 (57.1)	40 (46.5)
Dermatological toxicities							
Rash/acneiform rash, *n* (%)	111 (51.9)	123 (59.1)	110 (59.1)	85 (55.6)	56 (45.9)	41 (44.1)	34 (39.1)
Grade 1-2	101 (47.2)	115 (55.3)	99 (53.2)	82 (53.6)	51 (41.8)	39 (41.9)	33 (37.9)
Grade 3-4	10 (4.7)	7 (3.4)	8 (4.3)	2 (1.1)	4 (3.2)	1 (1.1)	1 (1.1)
Grade missing	0	1	3	1	1	1	0
Skin cracks, *n* (%)	5 (2.3)	17 (8.2)	35 (18.8)	34 (22.2)	23 (18.9)	20 (21.5)	17 (19.5)
Grade 1-2	4 (1.9)	16 (7.7)	32 (17.2)	33 (21.6)	22 (18.0)	19 (20.4)	17 (19.8)
Grade 3-4	1 (0.5)	1 (0.5)	3 (1.6)	1 (0.6)	1 (0.8)	0	0
Grade missing	0	0	0	0	0	1	0
Paronychia/Perionyxis, *n* (%)	6 (2.8)	11 (5.3)	22 (11.8)	15 (9.8)	12 (9.8)	9 (9.7)	5 (5.7)
Grade 1-2	6 (2.8)	10 (4.8)	21 (11.3)	15 (9.8)	12 (9.8)	8 (8.6)	5 (5.7)
Grade 3-4	0	1 (0.5)	1 (0.5)	0	0	1 (1.1)	0
Xerosis, *n* (%)	27 (12.6)	36 (17.3)	53 (28.5)	44 (28.8)	22 (18.0)	15 (16.1)	11 (12.6)
Grade 1-2	27 (12.6)	34 (16.3)	51 (27.4)	43 (28.1)	21 (17.2)	15 (16.1)	11 (12.6)
Grade 3-4	0	2 (1.0)	2 (1.1)	0	1 (0.8)	0	0
Grade missing	0	0	0	1	0	0	0
Mucositis, *n* (%)	12 (5.6)	15 (7.2)	19 (10.2)	8 (5.2)	3 (2.5)	3 (3.2)	2 (2.3)
Grade 1-2	12 (5.6)	15 (7.2)	18 (9.7)	8 (5.2)	3 (2.5)	3 (3.2)	2 (2.3)
Grade 3-4	0	0	1 (0.5)	0	0	0	0
Hypertrichosis, *n* (%)	1 (0.5)	4 (1.9)	5 (2.7)	7 (4.6)	7 (5.7)	2 (2.2)	2 (2.3)
Grade 1-2	1 (0.5)	4 (1.9)	5 (2.7)	7 (4.6)	7 (5.7)	2 (2.2)	2 (2.3)
Other, *n* (%)	5 (2.3)	5 (2.4)	4 (2.2)	4 (2.6)	3 (2.5)	0	1 (1.1)
Grade 1-2	5 (2.3)	5 (2.4)	4 (2.2)	4 (2.6)	3 (2.5)	0	1 (1.1)

common skin adverse events associated with panitumumab[19]. Thus, the earliest and most common skin adverse events are rashes/acneiform rashes[19]. These rashes differ from true acne since no cystic lesions or comedones are associated.

When the study was performed, there was no clinical and patient guidance for these frequent skin lesions and their management remained empirical. Preemptive treatments are currently the preferred approach[20]. Emollients and antihistamines are often used[21]. For acneiform rashes, class II or III topical corticosteroids are proposed[9,22]. Systemic treatments such as doxycycline have also been proposed. For widespread eczematiform rashes, treatment is primarily preventive, based on avoidance of sun exposure and the use of sunscreens with very high protection[9]. Due to possible spontaneous improvement, it is difficult to assess the efficacy of these dermatological treatments. However, the STEPP study showed that preemptive treatment of dermatological toxicities compared with reactive treatment led to more than 50% reduction skin toxicities with grade ≥ 2 and was associated with an improvement of the quality of life and no change in response rates[23]. Preemptive treatment consisted of skin moisturizer, sunscreen, topical steroid and doxycycline 100 mg twice per day[23]. These results were confirmed in a similar study (J-STEPP) performed in 95 Japanese patients with metastatic colorectal cancer with a 6-wk follow-up[24]. The cumulative incidence of skin toxicities with grade ≥ 2 were lower for preemptive treatment compared to reactive treatment (21.3% *vs* 62.5%; risk ratio: 0.34; $P < 0.001$). In a meta-analysis, the rate of skin rash due to anti-EGFR treatment was significantly decreased in patients with solid tumors who received prophylactic treatment with antibiotics (odds-ratio, 0.53; 95%CI 0.39-0.72; $P < 0.01$)[25].

In our study, only 65.9% of patients received at least one preventive treatment; the most frequent were oral antibiotics (84.1%), emollients (75.5%) or emollients plus oral antibiotics (62.9%). Only 9.3% of patients were administered topical corticosteroids as

Table 3 Doses delayed and dose adjustment (decrease) in patients with rash/acneiform rash according to the toxicity grades (Primary analysis set, *n* = 229)

	Day 15 (*n* = 214)	Month 1 (*n* = 208)	Month 2 (*n* = 186)	Month 3 (*n* = 153)	Month 4 (*n* = 122)	Month 5 (*n* = 93)	Month 6 (*n* = 87)
Rash/acneiform rash, grade 1-2, *n*	101	115	99	82	51	39	33
Doses delayed, *n* (%)	2 (2.0)	4 (3.5)	13 (13.1)	21 (25.9)	4 (7.8)	4 (10.3)	9 (27.3)
MD	0	0	0	1	0	0	0
Dose adjustment, *n* (%)	0	7 (6.3)	3 (3.3)	13 (16.9)	12 (25.5)	8 (20.5)	6 (18.2)
MD	1	3	8	5	4	0	3
Rash/acneiform rash, grade 3-4, *n*	10	7	8	2	4	1	1
Doses delayed, *n* (%)	1 (10.0)	1 (14.3)	4 (50.0)	2 (100)	1 (25.0)	0	0
MD	0	0	0	0	0	0	0
Dose adjustment, *n* (%)	2 (25.0)	0	2 (33.3)	2 (100)	1 (25.0)	0	0
MD	2	2	2	0	0	0	1

MD: Missing data.

preventive treatment. Indeed, local treatments with corticosteroids are not recommended in French guidelines[17]. At least one curative treatment was administered to a majority of patients during the entire 6-month follow-up. Among patients with curative treatment, antibiotics and emollients were administered to a majority of them at each visit and corticosteroids were administered to very few patients (about one patient out of five). Overall, these results indicate that the rate of preventive treatments, although recommended, was relatively low with two patients out three; emollient and oral antibiotics were preventively administered together to 62.9% of patients.

The summary of product characteristics of panitumumab recommends the suspension of treatment for 1 or 2 doses and a possible continuation at a lower dose only for adverse events grade ≥ 3. In our study, the rates of doses delayed and dose adjustments were relatively high even in patients with low grade skin toxicity. Thus, in patients with rash/acneiform rash grade 1-2, the dose was delayed for 25.9% of them at month 3 and the dose was adjusted for 25.5% at month 4; these rates remained high for the next months. It remains unclear whether these high rates of doses delayed/adjustments were related to patient willingness and/or physician decision. One possibility is that some skin toxicities classified as low grade are nevertheless unbearable for a number of patients. In contrast, in the STEPP study, the doses of panitumumab were adjusted in only 1% of patients in patients with skin toxicities of grade ≥ 2 in the preemptive treatment group and 6% in the curative treatment group[23].

Previous surveys in Germany, United States and France have been performed in practitioners treating colorectal cancer patients with EGFR inhibitors[26-28]. Overall these surveys reported a disparity in terms of grade assessment and management of skin toxicities. As observed in the present study, consultations to dermatologists were not frequent; in the French survey of Peuvrel *et al*[28], visits to dermatologists were planned for persisting or worsening lesions beyond two weeks, but never at the initiation of treatment.

The impact of the dermatological toxicities on the quality of life in our study was limited as assessed with DLQI scores and inconvenience VAS score. No differences according to age or gender were observed for dermatological toxicities, management and impact on patient's quality of life. These results are consistent with the recent study of Koukakis *et al*[29] that summarized data from three clinical trials with panitumumab in patients with metastatic colorectal cancer. No significant difference was observed between panitumumab and comparator groups for the score of quality of life and overall health.

Table 4 Preventive treatments for dermatological toxicities (Primary analysis set, *n* = 229)

	***n* = 229**
At least one preventive treatment, *n* (%)	151/229 (65.9)
Emollients	114/151 (75.5)
Oral antibiotics	127/151 (84.1)
Emollients and oral antibiotics	95/151 (62.9)
Sunscreen	9/150 (6.0)
Topical corticosteroids	14/151 (9.3)
Other (including topical antibiotics)	33/151 (21.9)
Topical antibiotics	30/33 (90.9)

The limitations of this study are common to any observational study. It was planned to enroll 300 patients and only 231 were included. In addition, the rate of study discontinuation was high (62.0%); the main reasons for discontinuations were death and disease progression. However, the development of cutaneous side effects during treatment with EGFR inhibitors appears to have a major prognostic significance. In initial phase II trials, it was shown that patients who developed skin lesions lived longer than those who did not. In addition, higher response rates and longer survival times were observed as a function of the severity of the skin rash[9,10,14]. Although this notion remains controversial, it is possible that the rates of dermatological toxicities were overestimated for the late time points of the study due to a possible selection of patients with improved outcome over time. When the study was performed, there was no guidelines for skin toxicities in this setting[20]. Therefore, the management of patients with dermatological toxicities could have benefited from their inclusion in the study. Physicians who included patients received information that could have modify their habits for the management of these dermatological toxicities.

In conclusion, the rates of the different skin toxicities peaked at various times and were improved at the end of the follow-up. Nevertheless, their clinical management could be optimized with a better adherence to current recommendations. The impact of skin toxicities on patient's quality of life appeared to be limited.

Table 5 Curative treatments for dermatological toxicities (Primary analysis set, *n* = 229)

	Day 15 (*n* = 214)	Month 1 (*n* = 208)	Month 2 (*n* = 186)	Month 3 (*n* = 153)	Month 4 (*n* = 122)	Month 5 (*n* = 93)	Month 6 (*n* = 87)
At least one curative treatment, *n* (%)	117/214 (54.7)	124/208 (59.6)	118/186 (63.4)	92/153 (60.1)	63/120 (52.5)	47/92 (51.1)	39/87 (44.8)
Emollients	74 (63.2)	82 (66.1)	84 (71.2)	69 (75.0)	45 (71.4)	29 (61.7)	24 (61.5)
Oral antibiotics	88 (75.2)	90 (72.6)	88 (74.6)	65 (70.7)	43 (68.3)	31 (66.0)	28 (71.8)
Emollients and oral antibiotics	60 (51.3)	61 (49.2)	61 (51.7)	47 (51.1)	28 (44.4)	17 (36.2)	16 (41.0)
Antiseptics	9 (7.8)	7 (5.6)	11 (9.3)	7 (7.6)	2 (3.2)	1 (2.1)	0 (0.0)
Antihistamines	12 (10.3)	10 (8.1)	11 (9.4)	10 (10.9)	6 (9.5)	5 (10.6)	2 (5.1)
Corticosteroids	19 (16.2)	26 (21.0)	23 (19.5)	19 (20.7)	12 (19.0)	6 (12.8)	9 (23.1)
Other (including topical antibiotics)	51 (44.0)	47 (37.9)	35 (29.7)	29 (31.9)	20 (31.7)	16 (34.0)	13 (33.3)
Topical antibiotics	48 (94.1)	43 (91.5)	30 (85.7)	25 (86.2)	18 (90.0)	16 (100)	13 (100)

ARTICLE HIGHLIGHTS

Research background

Skin adverse events not previously reported with conventional chemotherapy are associated with anti-epidermal growth factor receptor therapy.

Research motivation

Although prophylactic actions are recommended to prevent these skin toxicities, routine clinical management and impact on quality of life remain unknown.

Research objective

The present study aimed to assess the dermatological toxicities reported after panitumumab initiation, their impact on the quality of life and the clinical practices for their management.

Research methods

We performed a prospective multicenter observational study in 229 adult patients who began treatment with panitumumab for wild-type KRAS metastatic colorectal cancer. The incidence of dermatological toxicities, clinical practices for their management and impact on quality of life were recorded during a 6-mo follow-up.

Research results

More than half of patients had dermatological toxicity; this rate peaked at month 2. The most frequent dermatological toxicities were rash/acneiform rash, xerosis and skin cracks. At least one preventive treatment was administered to two thirds of patients (oral antibiotics, emollients or both). The impact of the dermatological toxicities on quality of life was limited.

Research conclusions

The rates of the different skin toxicities peaked at various times and were improved at the end of follow-up. The impact of skin toxicities on patient's quality of life appeared to be limited.

Research perspectives

The management of the skin toxicities could be optimized with a better adherence to current recommendations.

ACKNOWLEDGEMENTS

Francis Beauvais (Scientific and Medical Writing, Sèvres) provided writing services, which were funded by Amgen.

REFERENCES

1 **Leone N**, Voirin N, Roche L, Binder-Foucard F, Woronoff A, Delafosse P, Remontet L, Bossard N, Uhry Z. *Projection de l'incidence et de la mortalité par cancer en France métropolitaine en 2015. Rapport technique*. Saint-Maurice (Fra): Institut de veille sanitaire 2015; 62 Available from: http://invs.santepub-

liquefrance.fr/Publications-et-outils/Rapports-et-syntheses/Maladies-chroniques-et-traumatismes/2015/Projection-de-l-incidence-et-de-la-mortalite-par-cancer-en-France-metropolitaine-en-2015
2 **Douillard JY**, Oliner KS, Siena S, Tabernero J, Burkes R, Barugel M, Humblet Y, Bodoky G, Cunningham D, Jassem J, Rivera F, Kocákova I, Ruff P, Błasińska-Morawiec M, Šmakal M, Canon JL, Rother M, Williams R, Rong A, Wiezorek J, Sidhu R, Patterson SD. Panitumumab-FOLFOX4 treatment and RAS mutations in colorectal cancer. *N Engl J Med* 2013; **369**: 1023-1034 [PMID: 24024839 DOI: 10.1056/NEJMoa1305275]
3 **Hocking CM**, Price TJ. Panitumumab in the management of patients with KRAS wild-type metastatic colorectal cancer. *Therap Adv Gastroenterol* 2014; **7**: 20-37 [PMID: 24381645 DOI: 10.1177/1756283X13498660]
4 **Nishi T**, Hamamoto Y, Nagase M, Denda T, Yamaguchi K, Amagai K, Miyata Y, Yamanaka Y, Yanai K, Ishikawa T, Kuroki Y, Fujii H. Phase II trial of panitumumab with irinotecan as salvage therapy for patients with advanced or recurrent colorectal cancer (TOPIC study). *Oncol Lett* 2016; **11**: 4049-4054 [PMID: 27313739 DOI: 10.3892/ol.2016.4532]
5 **Zaniboni A**, Formica V. The Best. First. Anti-EGFR before anti-VEGF, in the first-line treatment of RAS wild-type metastatic colorectal cancer: From bench to bedside. *Cancer Chemother Pharmacol* 2016; **78**: 233-244 [PMID: 27091467 DOI: 10.1007/s00280-016-3032-8]
6 **Yamaguchi T**, Iwasa S, Nagashima K, Ikezawa N, Hamaguchi T, Shoji H, Honma Y, Takashima A, Okita N, Kato K, Yamada Y, Shimada Y. Comparison of Panitumumab Plus Irinotecan and Cetuximab Plus Irinotecan for KRAS Wild-type Metastatic Colorectal Cancer. *Anticancer Res* 2016; **36**: 3531-3536 [PMID: 27354619]
7 **Takaoka T**, Kimura T, Shimoyama R, Kawamoto S, Sakamoto K, Goda F, Miyamoto H, Negoro Y, Tsuji A, Yoshizaki K, Goji T, Kitamura S, Yano H, Okamoto K, Kimura M, Okahisa T, Muguruma N, Niitsu Y, Takayama T. Panitumumab in combination with irinotecan plus S-1 (IRIS) as second-line therapy for metastatic colorectal cancer. *Cancer Chemother Pharmacol* 2016; **78**: 397-403 [PMID: 27342247 DOI: 10.1007/s00280-016-3096-5]
8 **Lynch TJ**, Kim ES, Eaby B, Garey J, West DP, Lacouture ME. Epidermal growth factor receptor inhibitor-associated cutaneous toxicities: An evolving paradigm in clinical management. *Oncologist* 2007; **12**: 610-621 [PMID: 17522250 DOI: 10.1634/theoncologist.12-5-610]
9 **Guillot B**, Bessis D. Aspects cliniques et prise en charge des effets secondaires cutanés des inhibiteurs du récepteur à l'EGF. *Ann Dermatol Venereol* 2006; **133**: 1017-1020 [DOI: 10.1016/S0151-9638(06)71093-6]
10 **Peréz-Soler R**, Saltz L. Cutaneous adverse effects with HER1/EGFR-targeted agents: Is there a silver lining? *J Clin Oncol* 2005; **23**: 5235-5246 [PMID: 16051966 DOI: 10.1200/JCO.2005.00.6916]
11 **Segaert S**, Van Cutsem E. Clinical signs, pathophysiology and management of skin toxicity during therapy with epidermal growth factor receptor inhibitors. *Ann Oncol* 2005; **16**: 1425-1433 [PMID: 16012181 DOI: 10.1093/annonc/mdi279]
12 **Wagner LI**, Lacouture ME. Dermatologic toxicities associated with EGFR inhibitors: The clinical psychologist's perspective. Impact on health-related quality of life and implications for clinical management of psychological sequelae. *Oncology (Williston Park)* 2007; **21**: 34-36 [PMID: 18154217]
13 **Bouché O**, Scaglia E, Reguiai Z, Singha V, Brixi-Benmansour H, Lagarde S. [Targeted biotherapies in digestive oncology: Management of adverse effects]. *Gastroenterol Clin Biol* 2009; **33**: 306-322 [PMID: 19345533 DOI: 10.1016/j.gcb.2009.02.008]
14 **Pérez-Soler R**, Delord JP, Halpern A, Kelly K, Krueger J, Sureda BM, von Pawel J, Temel J, Siena S, Soulières D, Saltz L, Leyden J. HER1/EGFR inhibitor-associated rash: Future directions for management and investigation outcomes from the HER1/EGFR inhibitor rash management forum. *Oncologist* 2005; **10**: 345-356 [PMID: 15851793 DOI: 10.1634/theoncologist.10-5-345]
15 **Melosky B**, Burkes R, Rayson D, Alcindor T, Shear N, Lacouture M. Management of skin rash during EGFR-targeted monoclonal antibody treatment for gastrointestinal malignancies: Canadian recommendations. *Curr Oncol* 2009; **16**: 16-26 [PMID: 19229368 DOI: 10.3747/co.v16i1.361]
16 **Potthoff K**, Hofheinz R, Hassel JC, Volkenandt M, Lordick F, Hartmann JT, Karthaus M, Riess H, Lipp HP, Hauschild A, Trarbach T, Wollenberg A. Interdisciplinary management of EGFR-inhibitor-induced skin reactions: A German expert opinion. *Ann Oncol* 2011; **22**: 524-535 [PMID: 20709812 DOI: 10.1093/annonc/mdq387]
17 **Reguiai Z**, Bachet JB, Bachmeyer C, Peuvrel L, Beylot-Barry M, Bezier M, Boucher E, Chevelle C, Colin P, Guimbaud R, Mineur L, Richard MA, Artru P, Dufour P, Gornet JM, Samalin E, Bensadoun RJ, Ychou M, André T, Dreno B, Bouché O. Management of cutaneous adverse events induced by anti-EGFR (epidermal growth factor receptor): A French interdisciplinary therapeutic algorithm. *Support Care Cancer* 2012; **20**: 1395-1404 [PMID: 22539049 DOI: 10.1007/s00520-012-1451-6]
18 **Finlay AY**, Khan GK. Dermatology Life Quality Index (DLQI)--a simple practical measure for routine clinical use. *Clin Exp Dermatol* 1994; **19**: 210-216 [PMID: 8033378 DOI: 10.1111/j.1365-2230.1994.tb01167.x]
19 **Hofheinz RD**, Segaert S, Safont MJ, Demonty G, Prenen H. Management of adverse events during treatment of gastrointestinal cancers with epidermal growth factor inhibitors. *Crit Rev Oncol Hematol* 2017; **114**: 102-113 [PMID: 28477738 DOI: 10.1016/j.critrevonc.2017.03.032]
20 **Hofheinz RD**, Deplanque G, Komatsu Y, Kobayashi Y, Ocvirk J, Racca P, Guenther S, Zhang J, Lacouture ME, Jatoi A. Recommendations for the Prophylactic Management of Skin Reactions Induced by Epidermal Growth Factor Receptor Inhibitors in Patients With Solid Tumors. *Oncologist* 2016; **21**: 1483-1491 [PMID: 27449521 DOI: 10.1634/theoncologist.2016-0051]
21 **Molinari E**, De Quatrebarbes J, André T, Aractingi S. Cetuximab-induced acne. *Dermatology* 2005; **211**: 330-333 [PMID: 16286741 DOI: 10.1159/000088502]
22 **Jacot W**, Bessis D, Jorda E, Ychou M, Fabbro M, Pujol JL, Guillot B. Acneiform eruption induced by epidermal growth factor receptor inhibitors in patients with solid tumours. *Br J Dermatol* 2004; **151**: 238-241 [PMID: 15270903 DOI: 10.1111/j.1365-2133.2004.06026.x]
23 **Lacouture ME**, Mitchell EP, Piperdi B, Pillai MV, Shearer H, Iannotti N, Xu F, Yassine M. Skin toxicity evaluation protocol with panitumumab (STEPP), a phase II, open-label, randomized trial evaluating the impact of a pre-Emptive Skin treatment regimen on skin toxicities and quality of life in patients with metastatic colorectal cancer. *J Clin Oncol* 2010; **28**: 1351-1357 [PMID: 20142600 DOI: 10.1200/JCO.2008.21.7828]
24 **Kobayashi Y**, Komatsu Y, Yuki S, Fukushima H, Sasaki T, Iwanaga I, Uebayashi M, Okuda H, Kusumi T, Miyagishima T, Sogabe S, Tateyama M, Hatanaka K, Tsuji Y, Nakamura M, Konno J, Yamamoto F,

Onodera M, Iwai K, Sakata Y, Abe R, Oba K, Sakamoto N. Randomized controlled trial on the skin toxicity of panitumumab in Japanese patients with metastatic colorectal cancer: HGCSG1001 study; J-STEPP. *Future Oncol* 2015; **11**: 617-627 [PMID: 25686117 DOI: 10.2217/fon.14.251]

25 **Petrelli F**, Borgonovo K, Cabiddu M, Coinu A, Ghilardi M, Lonati V, Barni S. Antibiotic prophylaxis for skin toxicity induced by antiepidermal growth factor receptor agents: A systematic review and meta-analysis. *Br J Dermatol* 2016; **175**: 1166-1174 [PMID: 27214209 DOI: 10.1111/bjd.14756]

26 **Hassel JC**, Kripp M, Al-Batran S, Hofheinz RD. Treatment of epidermal growth factor receptor antagonist-induced skin rash: Results of a survey among German oncologists. *Onkologie* 2010; **33**: 94-98 [PMID: 20215799 DOI: 10.1159/000277656]

27 **Boone SL**, Rademaker A, Liu D, Pfeiffer C, Mauro DJ, Lacouture ME. Impact and management of skin toxicity associated with anti-epidermal growth factor receptor therapy: Survey results. *Oncology* 2007; **72**: 152-159 [PMID: 18160805 DOI: 10.1159/000112795]

28 **Peuvrel L**, Bachmeyer C, Reguiai Z, Bachet JB, André, T, Bensadoun RJ, Bouché, O, Ychou M, Dré, no B, Regional expert groups PROCUR. Survey on the management of skin toxicity associated with EGFR inhibitors amongst French physicians. *J Eur Acad Dermatol Venereol* 2013; **27**: 419-429 [PMID: 22211860 DOI: 10.1111/j.1468-3083.2011.04421.x]

29 **Koukakis R**, Gatta F, Hechmati G, Siena S. Skin toxicity and quality of life during treatment with panitumumab for RAS wild-type metastatic colorectal carcinoma: Results from three randomised clinical trials. *Qual Life Res* 2016; **25**: 2645-2656 [PMID: 27083443 DOI: 10.1007/s11136-016-1288-4]

World Journal of Gastroenterology

Submit a Manuscript: https://www.f6publishing.com

World J Gastroenterol 2019 August 7; 25(29): 4019-4042

DOI: 10.3748/wjg.v25.i29.4019

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

SYSTEMATIC REVIEW

Post-endoscopic retrograde cholangiopancreatography pancreatitis: A systematic review for prevention and treatment

Murat Pekgöz

ORCID number: Murat Pekgoz (0000-0002-1985-2567).

Author contributions: Pekgöz M solely contributed to this manuscript.

Manuscript source: Invited manuscript

Received: March 15, 2019
Peer-review started: March 15, 2019
First decision: May 14, 2019
Revised: May 19, 2019
Accepted: July 1, 2019
Article in press: July 3, 2019
Published online: August 7, 2019

P-Reviewer: Farhat S, Huan C, Manenti A, Yu XJ, Zhang ZH
S-Editor: Gong ZM
L-Editor: A
E-Editor: Ma YJ

Murat Pekgöz, Department of Gastroenterology, VM Medical Park Bursa Hospital, Bursa 16022, Turkey

Corresponding author: Murat Pekgöz MD, Assistant Professor, Department of Gastroenterology, VM Medical Park Bursa Hospital, Fevzi Cakmak Caddesi, Bursa 16022, Turkey. mesculape@hotmail.com
Telephone: +90-532-6015967
Fax:+90-224-2235571

Abstract

BACKGROUND

Post endoscopic retrograde cholangiopancreatography (ERCP) is comparatively complex application. Researchers has been investigated prevention of post-ERCP pancreatitis (PEP), since it has been considered to be the most common complication of ERCP. Although ERCP can lead various complications, it can also be avoided.

AIM

To study the published evidence and systematically review the literature on the prevention and treatment for PEP.

METHODS

A systematic literature review on the prevention of PEP was conducted using the electronic databases of ISI Web of Science, PubMed and Cochrane Library for relevant articles. The electronic search for the review was performed by using the search terms "Post endoscopic retrograde cholangiopancreatography pancreatitis" AND "prevention" through different criteria. The search was restricted to randomized controlled trials (RCTs) performed between January 2009 and February 2019. Duplicate studies were detected by using EndNote and deleted by the author. PRISMA checklist and flow diagram were adopted for evaluation and reporting. The reference lists of the selected papers were also scanned to find other relevant studies.

RESULTS

726 studies meeting the search criteria and 4 relevant articles found in the edited books about ERCP were identified. Duplicates and irrelevant studies were excluded by screening titles and abstracts and assessing full texts. 54 studies were evaluated for full text review. Prevention methods were categorized into three groups as (1) assessment of patient related factors; (2) pharmacoprevention; and (3) procedural techniques for prevention. Most of studies in the literature showed

that young age, female gender, absence of chronic pancreatitis, suspected Sphincter of Oddi dysfunction, recurrent pancreatitis and history of previous PEP played a crucial role in posing high risks for PEP. 37 studies designed to assess the impact of 24 different pharmacologic agents to reduce the development of PEP delivered through various administration methods were reviewed. Nonsteroidal anti-inflammatory drugs are widely used to reduce risks for PEP. Rectal administration of indomethacin immediately prior to or after ERCP in all patients is recommended by European Society for Gastrointestinal Endoscopy guidelines to prevent the development of PEP. The majority of the studies reviewed revealed that rectally administered indomethacin had efficacy to prevent PEP. Results of the other studies on the other pharmacological interventions had both controversial and promising results. Thirteen studies conducted to evaluate the efficacy of 4 distinct procedural techniques to prevent the development of PEP were reviewed. Pancreatic Stent Placement has been frequently used in this sense and has potent and promising benefits in the prevention of PEP. Studies on the other procedural techniques have had inconsistent results.

CONCLUSION

Prevention of PEP involves multifactorial aspects, including assessment of patients with high risk factors for alternative therapeutic and diagnostic techniques, administration of pharmacological agents and procedural techniques with highly precise results in the literature.

Key words: Endoscopic retrograde cholangiopancreatography; Pancreatitis; Prevention; Treatment; Indomethacin; Stent replacement; Prophylaxis

Core tip: This study systematically reviewed the literature on the prevention and treatment for post Endoscopic retrograde cholangiopancreatography (ERCP) pancreatitis. PRISMA checklist and flow diagram were adopted for the evaluation and the reporting. Prevention methods were categorized in three groups as (1) assessment of patient related factors; (2) pharmacoprevention; and (3) procedural techniques for prevention. Patients with high risk factors should be carefully assessed, and alternative therapeutic and diagnostic techniques may be preferable for them instead of ERCP.

Citation: Pekgöz M. Post-endoscopic retrograde cholangiopancreatography pancreatitis: A systematic review for prevention and treatment. *World J Gastroenterol* 2019; 25(29): 4019-4042
URL: https://www.wjgnet.com/1007-9327/full/v25/i29/4019.htm
DOI: https://dx.doi.org/10.3748/wjg.v25.i29.4019

INTRODUCTION

Endoscopic retrograde cholangiopancreatography (ERCP) has been a prominent technological innovation that has advanced in the field of gastrointestinal endoscopy[1] since its inception in 1968[2]. ERCP, a comparatively more complicated integral therapeutic modality among endoscopic techniques, is clinically the most common and specialized procedure used for the diagnosis and treatment of pancreatic and biliary system disorders[3-8]. Although it is superior to the traditional operation due to limited trauma, simplicity of the operation, and short recovery time in the treatment and diagnosis of duodenal and pancreatobiliary disorders[5,6], diagnostic and therapeutic ERCP can cause various complications such as pancreatitis, cholangitis, perforation, hemorrhage (especially postsphincterotomy), cholecystitis, cardiopulmonary depression, asymptomatic hyperamylasemia, aspiration, hypoxia, bleeding, sepsis, adverse medication reactions, and death[9-16].

PEP is the most common complication of ERCP,[9,17-19] and itis a crucial factor in morbidity and mortality[20-25]. Chemical, mechanical, enzymatic, hydrostatic and thermal causes are considered as the pathophysiology of the PEP[22]. Although its

determinants are unclear, development of PEP is thought to be based on a pro-inflammatory cascade caused by pancreatic acinar cell injury that induces to systemic cytokine release[3].

The incidence rates of PEP have been reported vary from less than 1% up to 40%, because of its dependence on patient factors, procedures, study definitions and methodology[9,23,26-30]. Incidence rates of the severe pancreatitis after ERCP changes between 0.1% and 0.5%[10,27,31-34].

The economic and the social impacts of PEP have been reported to be substantial[35]. The estimated annual cost of PEP in the USA is assessed to be around 200 million USD[36], while the overall mortality rate of PEP is found to be0,7%[37,38]. Furthermore, PEP has a crucial impact on endoscopist stress[39] and is considered as the most common determinant of malpractice lawsuits involving ERCP[40].

The standardized consensus definitions for PEP in the literature[3,9,28,41-43] were introduced by Cotton, Lehman[44] and Banks, Bollen[45]. The standard definition proposed by Cotton, Lehman[44] is as follows: "Pancreatitis after ERCP is a clinical illness with typical pain, associated with at least a threefold increase in serum amylase (or lipase) at 24 h, with symptoms impressive enough to require admission to hospital for treatment (or extension of an existing or planned admission)."The Atlanta criteria-based definition of PEP proposed by Banks, Bollen[45] is as follows: "The diagnosis of acute pancreatitis requires two of the following three features: (1) Abdominal pain consistent with acute pancreatitis (acute onset of a persistent, severe, epigastric pain often radiating to the back); (2) Serum lipase activity (or amylase activity) at least three times greater than the upper limit of normal; and (3) Characteristic findings of acute pancreatitis on contrast-enhanced computed tomography (CECT) and less commonly magnetic resonance imaging (MRI) or transabdominal ultrasonography"

PEP was viewed as an inevitable complication, with uncertain outcomes, and with no practicable strategy for its prevention in the past[35]. Research on the prevention of PEP has identified various approaches to reduce the occurrence and probability of PEP. Based on this research, three different strategies for prevention of the PEP including patient related, procedure related and pharmacological approaches were developed[3,9,30,35,46].

Development and improvement of efficient, safe, and cost-effective techniques for the prevention of PEP area crucial focus of endoscopic research[46] and will be reviewed and assessed in this study. In this context, risk factors and preventative measures extracted from the literature are identified and categorized to evaluate recent developments and approaches for the prevention of PEP.

MATERIALS AND METHODS

Literature search strategy

The relevant studies in the literature were searched by the author using the databases of PubMed, ISI Web of was Science and Cochrane Library. The review was restricted covering the period between January 2009 and February 2019 in order to focus on the updates and the recent developments in the relevant field. The search terms for all databases consisted of the words ["Post endoscopic retrograde cholangio-pancreatography pancreatitis" [All Fields] AND "prevention" (All Fields)] OR "treatment" [(All Fields),] "post-erpc pancreatitis" [(All Fields) AND "prevention" (All Fields) OR "treatment" (All Fields)], ("Post endoscopic retrograde cholangiopancreatography" (All Fields) AND "pancreatitis" (All Fields) AND "prevention" (All Fields) OR "treatment" (All Fields), ("post-erpc" [(All Fields)] AND "pancreatitis" (All Fields) AND "prevention" (All Fields) OR "treatment" [(All Fields)].

Inclusion and exclusion criteria

The relevance of the studies was determined by using the hierarchical approach of the PRISMA 2009 Statement. The assessment of the studies was based on title, abstract, and the full manuscript of the studies. The references of the selected studies were also scanned to find out further relevant studies. The inclusion criteria of the studies assessed in these reviews are as follows: (1) RCTs conducted to analyze prevention of PEP; (2) Publication in English; (3) Availability of the full text; and (4) Publication date between 2009 and February 2019.

Exclusion criteria of this review were determined as follows: (1) The article type as reviews, editorial letters, commentaries, clinical study protocols, retrospective studies and case reports; (2) Studies with insufficient information and descriptions; and (3) Duplicate studies in all databases were found by EndNote and excluded manually.

RESULTS

The stages of the literature review adopted from PRISMA 2009 are presented in Figure 1. The literature search through databases of PubMed, ISI Web of was Science and Cochrane Library identified 726 studies that met the search criteria. Additionally, 4 relevant articles found in the edited books on ERCP were included in the review. Search results were put together in EndNote to check for duplicate studies. 257 studies were found to be duplicates and these studies were removed from the list of search results. The eligibility of the 473 studies was evaluated by screening the titles and abstracts to see if they met the inclusion criteria. In this stage, the author only included RCTs and excluded all other publication types such as reviews, editorial letters, commentaries, clinical study protocols, retrospective studies and case reports. 381 studies were excluded due to not meeting the inclusion criteria. The full-text of the 92 remaining studies was reviewed. 38 of these studies were found to be irrelevant and excluded. The remaining 54 studies were included and assessed in this literature review.

The literature on the prevention of the PEP has mainly focused on the specific procedural techniques and pharmacological interventions to reduce the risk for PEP. Since the identification of risk factors increasing the probability of PEP is crucial for the prevention of PEP, the review has also focused on the risk factors related to patients. Therefore, the reviewed studies are categorized in these main topics.

Assessment of patient related factors

Careful patient selection is considered to be the most significant and primary strategy for the prevention of PEP[26]. Alternative methods providing highly precise pancreaticobiliary imaging such as endoscopic ultrasound and magnetic resonance cholangiopancreatography can be preferred to prevent PEP for patients with high risk factors, particularly for the identification and exclusion of choledocholithiasis[47-49]. Therefore, identification of the patient-related risk factors is one of the most important aspects of prevention for PEP.

The patient related risk factors for the development of the PEP in the literatureare summarized in the Table 1. Patient-related factors for developing the PEP found to be significant in the relevant studies include young age[23,50-53], female gender[23,51], suspected Sphincter of Oddi dysfunction (SOD)[50], history of previous PEP[51,52] and recurrent pancreatitis[51,52]. Although female gender has been found to have high risk for the PEP in the studies, it is not easy to distinguish the impact of SOD, mostly suspected in women with post-cholecystectomy abdominal pain[54]. On the contrary, PEP is less likely to occur in patients with chronic pancreatitis[9,30] indicating a partical loss of sensitivity to PEP stimulation[54], probably because of atrophy and decreased enzymatic activity[27].

History of ERCP with sphincterotomy is also considered to decrease the risk of developing PEP, since prior sphincterotomy mostly separates the common bile from the main pancreatic duct, therefore decreasing the probability of pancreatic duct cannulation or injection, and enabling comparatively uncomplicated and efficient cannulation of the common bile duct (CBD)[26]. Regarding gland atrophy and calcification, chronic pancreatitis is also considered to reduce the risk of developing PEP[27].

While previous studies indicated that small CBD may be a risk factor for PEP, recent studies[23,51,52,55] found that it has no independent impact on the risk for PEP. Periampullary diverticulum, pancreas divisum and allergy to contrast medium are among the factors which have been found to have no risk on PEP[9,41]. Yet a recent study[23] analyzed data obtained from 3178 procedures administered on 2691 patients and concluded that periampullary diverticulum was one of the significant patient-related risk factors.

DiMagno *et al*[50] also found that chronic liver disease and smoking were among the predictors of prophylaxis for PEP.

Pharmacoprevention

More than 35 pharmacologic agents have been analyzed in terms of prevention for PEP in the literature[56]. These studies focused on the intervention of one or more hypothesized structures of injury within the framework of the main six fields as below (adapted from Cheon[57]): (1) The prevention of the inflammatory cascade; (2) The facilitation of cannulation; (3) The relief of a sphincter of Oddi spasm; (4) The inhibition of intra-acinartrypsinogen activation; and (5) The decrease of pancreatic enzyme secretion.

The reviewed articles studied the impact of the rectal indomethacin on the prevention of PEP are summarized in Table 2. The studies on other pharmacologic agents are summarized in Table 3.

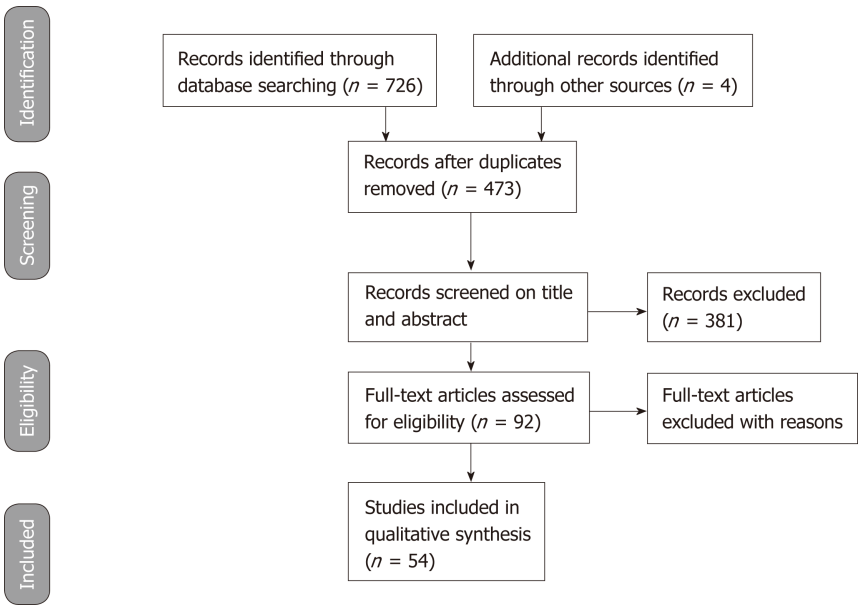


Figure 1 PRISMA 2009 Flow diagram describing the selection of the studies reporting prevention for post-endoscopic retrograde cholangiopancreatography pancreatitis in our review.

The prevention of the inflammatory cascade

Nonsteroidal anti-inflammatory drugs: Nonsteroidal anti-inflammatory drugs (NSAIDs) are inexpensive, easily administered and very effective inhibitors of phospholipase A2, cyclooxygenase and neutrophil–endothelial interactions and are considered to have a significant impact on the pathogenesis of acute pancreatitis[56]. Given the findings of clinical trials in the literature, rectal indomethacin, an NSAID, is administered to patients with high risk factors undergoing ERCP to reduce risk for PEP[58]. Administration of rectal indomethacin right before and after ERCP has been recommended by European Society for Gastrointestinal Endoscopy guidelines for all patients without contraindication to prevent the development of PEP[59]. Only two of eight studies in this review concluded no supporting findings for indomethacin to prevent PEP (Table 2).

Andrade-Davila *et al*[56] conducted a controlled RCT between 2012 and 2013 in Mexico by comparing the administration of 100 mg of rectal indomethacin on 82 patients versus 2.6 g suppository of glycerin on the placebo group of 84 patients without placement of a pancreatic stent. Patients had at least one major and/or two minor risk factors for PEP. The PEP rate for the experimental group was 4.87% (4/82) and was 20.23% (17/84) for the placebo group (P = 0.01). Rectal indomethacin administered immediately after ERPC decreased the incidence of PEP among patients with high risk factors.

Elmunzer *et al*[60] investigated the impact of rectal indomethacin on 602 patients at high risk for PEP in a multicenter, randomized, placebo-controlled, double-blind RCT in United States. The rate of PEP was 9.2% among patients who received indomethacin and was 16.9% among patients who received placebo (P = 0.005). Rectal indomethacin decreased the development of PEP among patients with high risk factors.

In their placebo-controlled, prospective RCT, Patai *et al*[61] also found positive impact of indomethacin on the prevention of PEP. Their study showed that rectally administered 100 mg indomethacin reduced development of PEP, especially in cases with patient and procedure-related risk factors and with difficult cannulation.

The administration timing of indomethacin and characteristics of patients can be significant impact on the clinical applications. Luo *et al*[62] compared impact of pre-procedural administration of 100 mg rectal indomethacin in 1297 patients (universal group) within 30 min before ERCP versus post-procedural administration of 100 mg rectal indomethacin in 1303 patients with high-risk factors (risk-stratified group) immediately after ERCP to prevent PEP. The rate of PEP was 4% in universal group and was 8% in the risk stratified group (P < 0.0001). Results showed that administration of rectal indomethacin prior to ERCP in universal group decreased PEP development in comparison of risk stratified group.

Hosseini *et al*[63] assessed rectal indomethacin with and without intravenous perfusion of normal saline to prevent PEP. In this RCT, 406 patients underwent ERCP

Table 1 Patient-related risk factors

Definite	Possible	No risk
Young age	Absence of CBD stone	Normal/small CBD diameter
Female gender	Normal serum bilirubin	Pancreas divisum
Suspected SOD	Periampullary diverticulum	Allergy to contrast medium
Recurrent pancreatitis		
Absence of chronic pancreatitis		
History of previous PEP		

Adapted from Guda *et al*[9], Cotton *et al*[30], Cotton[41], and Srinivasan *et al*[54]. CBD: Common bile duct; SOD: Sphincter of Oddi dysfunction; PEP: Post-endoscopic retrograde cholangiopancreatography pancreatitis.

and were randomized into four groups with different interventions. Interventions of (1) rectal indomethacin (100 mg); (2) intravenous (IV) saline perfusion; (3) both rectal indomethacin and IV saline; and (4) rectal glycerin were administered to groups before ERCP. The results indicated that intervention of rectal indomethacin and intravenous normal saline together before ERCP significantly reduced incidence rate of PEP.

Mok *et al*[64] performed a randomized, double-blinded, placebo-controlled RCT to analyze the effectiveness of indomethacin with or without bolus lactated Ringer's solution (LR) in patients with high risk factors. Patients were randomized into four groups and received different interventions, including normal saline solution (NS) + placebo, LR + placebo, LR + IND NS + IND. Compared with NS + placebo, LR + IND decreased development of PEP and readmission rates.

There are also contradictory findings in the literature about the impact of indomethacin on the prevention of PEP. Döbrönte *et al*[65] conducted a prospective, randomized, placebo-controlled and multicentred study between 2012 and 2013 in order to compare 100 mg of rectally administered indomethacin on 347 patients *vs* an inert placebo on 318 patients, 10-15 min before ERCP. They found that rectally administered 100 mg of indomethacin prior to ERCP had no efficacy in preventing the development of PEP.

A prospective, double-blind, placebo-controlled RCT on the consecutive patients performed by Levenick *et al*[58] also found contradictory results about the impact of indomethacin on the prevention of PEP. 449 consecutive patients undergoing ERCP between 2013 and 2014 in the United States. 223 patients received a single dose of 100 mg dose of rectal indomethacin and 226 patients were received a placebo suppository during the ERCP. The incidence rate of PEP for these groups were 7.2% and 4.9%, respectively. The study revealed that rectally administered indomethacin did not have positive impact on the prevention of PEP.

The majority of clinical trials investigating impact of NSAIDs on the prevention of the PEP have been rectally administered[66]. Diclofenac is another NSAID and is often parenterally administrate because of its faster effect[67]. Park *et al*[66] administered either 90 mg of diclofenac or placebo to randomized 343 patients by intramuscular injection immediately after ERCP. PEP rate was 12.7% for the group that received diclofenac and 11.8% for the placebo group (P = 0.87). The results of the multivariate regression analysis also failed to demonstrate the prevention impact of diclofenac on the development of PEP. On the other hand, in their prospective, multicenter, controlled and RCT Otsuka *et al*[68] found contradictory results. Patients underwent ERCP were randomized into two groups and administered either 50 mg of rectal diclofenac with a saline infusion or only saline infusion 30 min before ERCP. The incidence of PEP was 3.9% (2/51) and 18.9% (10/53) (p = 0.017), respectively. They concluded that low-dose rectal diclofenac may have preventative impact on the development of PEP.

Aproinflammatory cascade with a little favorable circumstance for intervention will be induced after the injury of pancreatic acinar cell[69]. Cyclo-oxygenase (COX) enzymes are considered to have a crucial proinflammatory function in pancreatitis[70]. It was reported that the severity of experimental acute pancreatitis was alleviated when COX-2 was pharmacologically inhibited[71]. Bhatia *et al*[70] investigated the benefits of valdecoxib, a COX-2 inhibitor, and Glyceryltrinitrate (GTN) transdermal patch on PEP. 121 patients were administered 20 mg intravenous valdecoxib, 124 patients were administered GTN patch (10 mg/h) at the beginning of ERCP and 126 patients were assigned as control group. No significant difference was found in the frequency of PEP, indicating that valdecoxib and GTN had no beneficial impact on prophylaxis of PEP.

Table 2 Brief contents of reviewed articles on rectal indomethacin

Authors	Year	Country	*n*	Intervention	Design	Incidence of PEP[1]		*P* value
						Study group	Control (compared)	
Elmunzer *et al*[60]	2012	United States	602	2 × 50-mg indomethacin or 2 × placebo right after ERCP	Prospective, multicenter, placebo-controlled, double-blind	27/295 (9.2%) [IND]	52/307 (16.9%) [Placebo]	0.005
Döbrönte *et al*[65]	2014	Hungary	665	100 mg indomethacin or an placebo 10-15 min prior to ERCP	Prospective, multicenter, placebo-controlled	20/347 (5.76%) [IND]	22/318 (6.92%) [Placebo]	0.541
Patai *et al*[61]	2015	Hungary	539	100 mg indomethacin or placebo 1 h prior to ERCP	Prospective, single center, placebo-controlled, double-blind	18/270 (6.7%) [IND]	37/269 (13.8%) [Placebo]	0.406
Andrade-Davila *et al*[56]	2015	Mexico	166	100 mg of indomethacin or 2.6 g suppository of glycerin right after ERCP	Prospective, single center, placebo-controlled	4/82 (4.87%) [IND]	17/84 (20.23%) [GS]	0.01
Luo *et al*[62]	2016	China	2600	100 mg indomethacin for unselected patients within 30 min prior to ERCP or 100 mg indomethacin just after ERCP for patients with high risks	Prospective, multicenter, single-blind	47/1297 (4%) [Universal IND]	100/1303 (8%) [Risk-stratified IND]	< 0.001
Levenick *et al*[58]	2016	United States	449	100 mg indomethacin or placebo during ERCP	Prospective, single center, double-blind, placebo-controlled	16/223 (7.2%) [IND]	11/226 (4.9%) [Placebo]	0.33
Hosseini *et al*[63]	2016	Iran	406	100 mg indomethacin two hours before the ERCP or 1 L of ISP within 2 h before ERCP and 2 L within 16 h after ERCP or indomethacin and ISP or 2 g of glycerin in suppositories	Prospective, single center, blinded subject data	11/100 (11%) [IND] 10/100 (10%) [ISP]	0/101 (0) [IND+ISP] 17/105 (16%) [RG]	-

Mok *et al*[64]	2017	United States	192	LR + IND, NS + IND, LR + placebo or NS + placebo	Prospective, single center, double-blind, placebo-controlled	3/48 (6%) [LR+IND]	6/48 (13%) [NS+IND]	9/48 (19%) [LR+Placebo]	10/48 (21%) [NS+Placebo]	0.04

[1]The fractional ratios are "Number of PEP incidences/number of patients in the group". Rate of PEP incidences are given in the parenthesis. Definitions of the procedures applied to groups are given in the brackets. n: Number of patients (sample size); IND: Indomethacin; ISP: Intravenous (IV) saline perfusion; RG: Rectal glycerin; LR: Lactated ringer's solution; NS: Standard normal saline solution; GS: Glycerin suppository.

Ketoprofen, an effective NSAID, is an inhibitor of both COX1 and COX2, and can reach serum peak in minutes when received intravenously, while NSAIDS such as diclofenacor indomethacin can reach serum apex within 2–3 h when received rectally or orally[72]. Because of these advantages, Onofrio *et al*[73] tested intravenously administration of ketoprofen on consecutive patients with naïve papilla. Patients were randomly assigned to receive saline infusion with or without ketoprofenjust prior to ERCP. PEP rates were 2.2% in the ketoprofen group and 2 % in the control group (P = 1), indicating intravenously received ketoprofen just before ERCP did not reduced PEP incidence.

Prophylactic impact of rectal NSAIDs in PEP is considered to occur by inhibiting cyclooxygenase (COX) and phospholipase A2 enzymes, which are considered significant part of the primary inflammatory cascade of acute pancreatitis through regulation of proinflammatory mediators, *i.e.*, platelet-activating factors and arachidonic acid products[74-76]. Kato *et al*[77] conducted a prospective, single center, controlled RCT to assess the prophylactic potential of celecoxib, a cyclooxygenase-2 inhibitor, on PEP. 85 patients received oral 400-mg celecoxib tablets 1 h prior to ERCP and saline infusion and another 85 patients received only saline infusion. The incidence of PEP for two groups was 15.3% (13/85) and 11.7% (10/85), respectively (P = 0.65). The difference between the frequency of PEP of groups was insignificant and demonstrated that orally administered of celecoxib did not reduced the rate of PEP.

Hydration: The basis of treatment for acute pancreatitis depends on hydration[78]. Animal studies concluded that pancreatic microvascular hypoperfusion developed necrosis[79]. Clinical researches on patients with acute pancreatitis testing fluid resuscitation indicated that hemoconcentration and reduced systemic perfusion can develop risk of pancreas necrosis and adverse results[80]. Wu *et al*[81] suggested that hydration with lactated Ringer's solution (LRS) may reduce the risk for systemic inflammatory response syndrome. Trypsinogen activation and incidence of pancreatitis can be triggered by an acidic environment[82]. Buxbaum *et al*[83] performed a prospective, multicenter and controlled RCT to determine whether aggressive peri-procedural hydration with LRS diminish the incidence of PEP.

Thirty-nine patients received aggressive hydration with LRS (3 cc/kg/h during the ERCP, a 20 cc/kg bolus after the ERCP, and 3 cc/kg/h for 8 h after ERCP) and 23 patients received standard hydration with the same solution (1.5 cc/kg/hr during and for 8 h after ERCP). There was no PEP incidence in the first group and 17% of patients in the second group developed PEP (P = 0.016). Aggressive intravenous hydration with LRS was found to be effective in decreasing risk of PEP. Their findings also suggested that LRS is less risky than saline to lead metabolic acidosis, indicating protective impact of LRS.

The justification for hydration depends on the requirement for resolution of the hypovolemia[84] . Vigorous intravenous fluid resuscitation (IVFR) with LRS may lead a better acid-base balance and may induce an anti-inflammatory reaction, when compared with other crystalloid preparations[85,86]. In a prospective, multicenter, double-blind RCT Choi *et al*[87] tested the impact of periprocedural vigorous IVFR on the prevention of PEP. 510 patients with native papilla in Korea were randomized into two groups in a1:1 ratio. The first group received vigorous IVFR (LRS in an initial bolus of 10 mL/kg before the ERCP, 3 mL/kg/h during the ERCP, for 8 h after the ERCP, and a post-ERCPbolus of 10 mL/kg) and the second group received a standard IVFR (LRS at 1.5 mL/kg/h during and for 8 h after the ERCP). The incidence rate of PEP was 4.3% in the first group and 9.8% in the latter one (P = 0.016). The findings indicated that IVFR with LRS had preventative effect on PEP and reduced severity of PEP in both high-risk and average-risk cases.

Cytokines and mediators: Regardless of the trigger of pancreatitis, early intracellular events are followed by initial local and systemic inflammatory reactions which are increased by proinflammatory cytokines and chemokines. These are considered to

Table 3 Brief contents of reviewed articles on pharmacological agents

Agent	Authors	Year	Country	*n*	Design	Incidence of PEP[1]		*P* value
						Study group	Control (or compared)	
Intraduodenal Acetic Acid (IAA)	Fang *et al*[97]	2018	China	210	Prospective, single center, double-blind	8/105 (7.6%) [IAA]	11 /105 (10.5%) [Saline]	0.47
Celecoxib	Kato *et al*[77]	2017	Japan	170	Prospective, single center	10/85 (11.7%) [Celecoxib]	13/85 (15.3%) [Saline]	0.65
Raw Rhubarb Solution (RRS)	Wang *et al*[133]	2017	China	500	Prospective, single center	5/250 (2%) [RRS]	19/250 (7.6%) [Water]	0.003
Nitroglycerin + Glucagon	Katsinelos *et al*[106]	2017	Greece	455	Prospective, single center, double–blind	7/227 (3.08%) [Nitroglycerin + glucagon]	17/228 (7.46%) [HBW]	0.037
Ketoprofen	Onofrio *et al*[73]	2017	Brazil	477	Prospective, single center, double-blind, placebo-controlled	5/224 (2.2 %) [Ketoprofen]	5/253 (2 %) [Placebo]	1.0
Vigorous IVFR	Choi *et al*[87]	2017	South Korea	510	Prospective, multi center, double-blind	11/255 (4.3%) [Vigorous IVFR]	25/255 (9.8%) [StandartIVFR]	0.016
Aggressive Hydration with Lactated Ringer's Solution (AHLRS)	Buxbaum *et al*[83]	2014	United States	71	Prospective, multicenter, controlled	0/39 (0%) [AHLRS]	4/23 (17%) [SHLRS]	0.016
Udenafil+Ac-eclofenac	Lee *et al*[109]	2015	South Korea	216	Prospective, multicenter, double-blind, placebo-controlled	17/107 (15.8%) [Udenafil+Aceclofenac]	18/109 (16.5%) [Placebo]	0.901
Somatostatin	Bai *et al*[127]	2015	China	900	Prospective, multicenter, open-label	18/445 (4 %) [Somatostatin]	34/455 (7.5 %) [No Somatostatin]	0.03
	Concepcion-Martin *et al*[129]	2014	Spain	510	Prospective, single-center, placebo-controlled, double-blind	19/255 (7.5 %) [Somatostatin]	17/255 (6.7 %) [Placebo]	0.73
	Wang *et al*[130]	2013	China	124	Prospective, single-center, placebo-controlled	6/36 (16.7%) [Pre-ERCP somatostatin] 5/47 (10.6%) [Post-ERCP somatostatin]	6/41 (14.6%) [Placebo]	0.715
Intramuscular Diclofenac	Park *et al*[66]	2015	South Korea	343	Prospective, single center, double-blind, placebo-controlled	22/173 (12.7 %) [Intramuscular Diclofenac]	20/170 (11.8 %) [Placebo]	0.87
Rectal Diclofenac	Otsuka *et al*[68]	2012	Japan	104	Prospective, multicenter, controlled	2/51 (3.9%) [Diclofenac with Saline]	10/53 (18.9%) [Saline]	0.017
Nafamostat Mesilate (NM)	Kim *et al*[118]	2016	South Korea	382	Prospective, single center, comparative	5/179 (2.8%) [NM - 24 hour infusion]	4/192 (2.1%) [NM - 6 hour infusion]	0.744
	Ohuchida *et al*[114]	2015	Japan	809	Prospective, single center, double-blind	14/405 (3.5) [NM]	27/404 (6.7) [Glucose Solution]	0.035
	Park *et al*[115]	2011	South Korea	595	Prospective, single-center, controlled	8/198 (4%) [20 mg of NM] 10/197 (5.1%) [50 mg of NM]	26/200 (13%) [Dextrose]	< 0.0001

	Yoo *et al*[117]	2011	South Korea	286	Prospective, single-center, double-blind, placebo controlled	4/143 (2.8%) [NM]		13/143 (9.1%) [Dextrose Solution]	0.03
	Choi *et al*[116]	2009	South Korea	704	Prospective, single-center, double-blind, controlled	12/354 (3.3%) [NM]		26/350 (7.4%) [Dextrose Solution]	0.018
Ulinastatin + Nafamostat	Park *et al*[121]	2014	South Korea	159	Prospective, single center, placebo-controlled	1/53 (1.9%) [Ulinastatin]	2/53 (3.8%) [Nafamotat]	7/53 (13.2) [Placebo]	0.037
Risperidone + Ulinastatin	Tsujino *et al*[125]	2013	Japan	226	Prospective, multicenter, placebo-controlled	6/113 (5.3%) [Risperidone + Ulinastatin]		10/113 (8.8 %,) [Ulinastatin]	0.438
Udenafil	Oh *et al*[107]	2011	South Korea	278	Prospective, multicenter, double-blind, placebo-controlled	11/137 (8.0%) [Udenafil]		11/141 (7.8%) [Placebo]	944
Allopurinol	Abbasinazari *et al*[135]	2011	Iran	74	Prospective, single-center, double-blind, placebo-controlled	3/29 (10,4%) [Allopurinol]		5/45 (11,1%) [Placebo]	0.97
Neurokinin-1 receptor antagonist (Aprepitant)	Shah *et al*[136]	2012	United States	73	Prospective, single-center, double-blind, placebo-controlled	7/34 (20.6%) [Aprepitant]		7/39 (17.9%) [Placebo]	1.0
Secretin	Jowell *et al*[96]	2011	United States	869	Prospective, single-center, double-blind, placebo controlled	36/413 (8.7%) [Secretin]		65/431 (15.1%) [Placebo]	0.004
Epinephrine	Xu *et al*[143]	2011	China	941	Prospective, single-center, placebo controlled	9/461 (1.95%) [Epinephrine]		31/480 (6.45%) [Saline]	0.0086
Valdecoxib and GlycerylTrinitrate (GTN)	Bhatia *et al*[70]	2011	India	371	Prospective, single-center, controlled	12/121 (9.9%) [Valdecoxib]	12/124 (9.7%) [GTN]	13/126 (10.3%) [No intervention]	0.986
Glyceryl Nitrate	Nøjgaard *et al*[100]	2009	Multi Country	806	Prospective, multicenter, double-blind, placebo-controlled	18/401 (4.5%) [Glyceryl Nitrate]		29/405 (7.1%) [Placebo]	0.11
Platelet Activating Factor (PAF)	Sherman *et al*[91]	2009	United States	600	Prospective, multicenter, double-blind, placebo-controlled	35/200 (17.5%) [PAF 1 mg/kg]	32/201 (15.9%) [PAF 5 mg/kg]	39/199 (19.6%) [Placebo]	0.59
Interleukin-10 (IL-10)	Sherman *et al*[88]	2009	Multi Country	305	Prospective, multicenter, double-blind, placebo-controlled	14/91 (15.4%) [IL-10 8 μg/kg]	24/109 (22%) [IL-10 20 μg/kg]	15/105 (14.3%) [Placebo]	0.83 0.14

[1]The fractional ratios are "Number of PEP incidences/number of patients in the group". Rate of PEP incidences are given in the parenthesis. Definitions of the procedures applied to groups are given in the brackets. HBW: Hyoscine n-butyl plus sterile water; IVFR: Intravenous fluid resuscitation; SHLRS: Standart hydration with lactated ringer's solution.

contribute in the progress of pancreatic necrosis[88]. The administration of endogenous Interleukin-10 (IL-10), a potent inhibitor of cytokines, in animal models of pancreatitis with cerulein reduced the severity of acute pancreatitis[89], principally through inhibition of the development of acinar necrosis[90]. Sherman *et al*[88] investigated impact of IL-10 on PEP in patients with high risks. 91 patients received 8 μg/kg and 101 patients received 20 μg/kg of IL-10 and 105 patients received placebo through a

single intravenous injection 15-30 min before ERCP. PEP incidences were 15%, 22%, and 14% in research groups respectively (P = 0.83). The study showed administration IL-10 failed to prevent PEP.

Platelet-activating factor (PAF), potent proinflammatory mediator, was reported to be related to acute pancreatitis, since its degradation or production is considered to be dysregulated, leading to inflammation *via* effector mechanisms that stimulate systemic or local tissue injury[91]. The release of amylase from isolated pancreatic acini was observed to increase due to the administration of exogenous PAF[92]. rPAF-AH, developed to prevent adverse implications of dysregulated PAF activity[91], alleviated pancreatic injury, cut down the lipase and amylase increment, and reduced pancreatitis-associated acute lung injury in an animal model of acute pancreatitis[93]. In their randomized, multicenter, double-blind, placebo-controlled RCT Sherman *et al*[91] analyzed prophylactic rPAF-AH administration in reduction of PEP incidence in high-risk patients. 200 patients received 1 mg/kg of rPAF-AH, 201 patients received 5 mg/kg ofrPAF-AH and another 199 patients received placebo intravenously. They concluded that rPAF-AH had no preventative impact on PEP.

Facilitation of cannulation

Difficulties in cannulation of the CBD can cause papillary trauma and, thereby increasing the incidence of PEP[28,55]. Facilitation of cannulation may decrease risk for complications of ERCP, because difficult cannulation is reported to be a significant procedure-related risk factor of PEP[23,94]. Secretin, a gastrointestinal peptide endocrine hormone, can stimulate pancreatic bicarbonate excretion, thereby facilitating the cannulation[95]. Jowell *et al*[96] conducted a single center, prospective, double-blind, placebo-controlled RCT to evaluate effects of synthetic secretin in preventions of PEP. 426 patients were received secretin (16 μg) and 443 patients received placebo before ERCP. The incidence of PEP in the two groups were 8.7% and 15.1%, respectively (P = 0.004). Results showed that synthetic secretin was effective in prevention of PEP.

The limitations of secretin, such as its high price and limited availability, makes its use complicated in clinics[97]. Alternatively, intraduodenal acid infusion (ACI) was used in clinical trials, since it can physiologically stimulate secretin release in the body[98,99]. Fang *et al*[97] conducted a single center, double-blind RCT between 2016 and 2017 in China to investigate the impact of ACI on pancreatic duct cannulation during ERCP. Consecutive patients were randomized into two groups (105 in each group) and received 50 mL ACI infusion or 50 mL saline. The incidence rate of PEP for two groups was 7.6% and 10.5%, respectively (P = 0.470). Despite the statistically insignificant difference in the incidence of PEP in the two groups, ACI infusion significantly facilitated pancreatic duct cannulation and reduced radiation exposure.

The relief of a sphincter of Oddi spasm

Promoting efficient drainage of the pancreatic duct at the end of ERCP by administrating pharmacologic agents, instead of procedural techniques such as pancreatic stent placement, may be effective in ameliorating the adverse impacts of temporal blockage outflow of pancreatic juice induced by papillary edema and/or sphincter of Oddi spasm triggered by manipulation during ERCP and papillary trauma[70]. Glyceryltrinitrate (GTN), a nitric oxide donor, may prevent papillary edema through facilitating primary cannulation and may support pancreatic duct drainage after ERCP, ultimately leading relaxation of the sphincter of Oddi[70]. Glyceryl nitrate (GN), a nitrogen oxide donor, may stimulate dilation of the microvascular vessels and periampullar sphincter relief, therefore enhancing nutrition and circulation[100]. However, the results of RCTs conducted by *et al*[70] and Nøjgaard *et al*[100] showed that GTN and GN were not effective for the prevention of PEP.

Nitric oxide (NO) donor is another pharmacologic agent thought to facilitate CBD cannulation by decreasing the amplitude and baseline pressure caused by the sphincter of Oddi[101-103]. Additionally, intravenous glucagon, applied throughout the ERCP for prevention of duodenal motility, can be beneficial for relaxing the sphincter of Oddi[104,105], and therefore, can improve CBD cannulation[106]. The impact of the combination of sublingual nitroglycerin and intravenous glucagon administration on PEP was investigated by Katsinelos *et al*[106] between 2012 and 2015 in Greece through a prospective, single center, double–blind RCT study. 227 patients intravenously received 6 puffs (2.4 mg) sublingual nitroglycerin and glucagon 1mg and another 228 patients intravenously received 6 puffs sterile water and 20mg hyoscine-n-butyl bromide. PEP rates were significantly lower in the first group than the latter one. Administration of combined nitroglycerin and glucagon contributed a high selective CBD cannulation rates, thereby reducing of PEP incidence.

Phosphodiesterase type 5 (PDE-5) inhibitor, smooth-muscle relaxant, is considered to diminish basal sphincter of Oddi pressure[107]. It can reduce sphincter of Oddi tone, contribute easy cannulation and eventually decrease risks for PEP[108]. Oh *et al*[107]

investigated administration of prophylactic udenafil, a phosphodiesterase-5 inhibitor, for the prevention of PEP. 280 patients were randomized in a 1:1 ratio and received udenafil (100 mg) or placebo. They found no significant difference between rates of PEP incidence of two groups, indicating udenafil had no prophylactic impact on PEP. Lee *et al*[109] performed a prospective, randomized, double-blind, placebo-controlled, multicenter RCT to investigate the efficacy of a combination of a high dose of udenafil (PDE-5 inhibitor) and aceclofenac (NSAID) on development of PEP in high-risk patients. Their rationale for this study depended on the potential of the combination to decrease the pressure of the sphincter of Oddi and inflammation in acute pancreatitis through modulation of the cytokine cascade. 216 patients were assigned into two groups in a 1:1 ratio and orally administered either PDE-5 inhibitor udenafil (200 mg.) and aceclofenac (100 mg.) or placebo. The incidence rate of PEP for two groups were 15.8% and 16.5%, respectively (P = 0.901). The statistically insignificant results indicated that administration of combined udenafil and aceclofenac had no impact to on the prevention of PEP.

The inhibition of intra-acinar trypsinogen activation

The possible contribution of proteolytic enzymes on the development of PEP have made protease inhibitors (nafamostatmesilate, ulinastatin, gabexate) the focus of clinical trials[110]. Nafamostatmesilate (NM), a strong synthetic serine protease inhibitor, was developed by Fujii *et al*[111]. NM powerfully inhibits trypsin, a proteolytic enzyme which is thought to have a crucial role in triggering acute pancreatitis, as well as kallikrein, plasmin, and the complement components C1s and C1r[111-113]. Only one of five studies in our reviews showed no contribution of NM on the prevention of the PEP.

In a prospective, single center and double-blind RCT Ohuchida *et al*[114] administered either 20 mg of NM dissolved in 500 mL of 5% glucose solution to 405 patients or 500 mL of 5% glucose solution to 404 patients, over 2 h from the beginning of ERCP. The incidence of PEP was found to be 3.5% and 6.7% in the groups, respectively (P = 0.0349). The findings revealed that 20 mg NM administered in the short run can prevent PEP.

Park *et al*[115] conducted a prospective, single-center and controlled RCT to assess the administration of 50 mg NM for prevention of PEP. Enrolled patients underwent ERCP were assigned into three groups and intravenously administered 500 mL of 5% dextrose solution alone or with 20 mg or 50 mg of NM. Incidence of PEP was found 13.0%, 4.0% and 5.1%, respectively (P < 0.0001). They concluded that NM (20 or 50 mg) may effectively prevent PEP.

Choi *et al*[116] and Yoo *et al*[117] also found supportive evidence, indicating that prophylactic intravenous NM may decrease the risk for PEP. However, Kim *et al*[118] found contradictory results in their prospective, single center, comparative RCT investigating the impact of 24 and 6 h intravenous infusions of 20 mg NM. They randomized 382 patients undergoing ERCP into two groups and administered NM (20 mg) infusion prior to ERCP and continued for either 6 or 24. The incidence of PEP were 2.8% (5/179) and 2.1% (4/192), respectively (P = 0.744). They found that NM infusion had no benefit on the prevention of PEP, regardless of the duration.

Ulinastatin, another protease inhibitor, is obtained by purifying healthy human urine[118]. It can prevent the onset and development of pancreatitis through inhibition of the pancreatic enzyme activation pathway[119,120]. In a prospective, single center, placebo-controlled RCT Park *et al*[121] compared the impact of ulinastatin and nafamostat on the prevention of PEP. They assigned 159 patients into three groups and administered 150000 units of ulinastatin, 20 mg of nafamostat or placebo for a 2-4 h prior to ERCP to 6-8 h after ERCP. The incidence of PEP was 1.9%, 3.8% and 13.2, respectively (P = 0.037), indicating that both pharmacologic agents reduced the incidence of PEP.

Serotonin [5-hydroxytryptamine (5-HA)], a monoamine neurotransmitter found in the platelets, central nervous system and intestinal mucosa and can induce 11 subtypes of the 5-HA receptor which is considered to be related to acute pancreatitis[122,123]. Some research has concluded that 5-HA2A antagonists may have amendatory effect on acute pancreatitis[124]. Risperidone, a potent 5-HA2A antagonist, is considered to prevent or decrease the primary events of acute pancreatitis[125]. It was reported that risperidone mitigated the increase of pancreatic enzymes and cellular infiltration into the pancreatic interstitial tissues in caerulein-induced acute pancreatitis[126]. Tsujino *et al*[125] performed a prospective, multicenter, placebo-controlled RCT in Japan to investigate the prophylaxis of risperidone combined with ulinastatin for PEP in high-risk patients.

Patients were randomized to receive either ulinastatin with or without risperidone. An oral risperidone tablet was administered 30–60 min prior to ERCP and ulinastatin was intravenously administered for 10 min just prior to ERCP. The incidence of PEP

in these groups was 5.3% and 8.8 %, respectively (P = 0.438). They concluded that combination of oral risperidone with ulinastatindid did not decrease the rate of PEP in patients at high-risk.

The decrease of pancreatic enzyme secretion

Somatostatin is considered as a potent inhibitor of pancreatic enzyme secretion[127]. Somatostatin and its synthetic analog, octreotide, can influence exocrine function directly through decreasing the secretion of digestive enzymes, and indirectly through causing inhibition of secretin and cholecystokinin production[57]. In addition, somatostatin and octreotide may adjust the cytokine cascade and may have a cytoprotective impact on pancreatic cells, while the mechanisms of their cytoprotective effect are still unclear[128]. However, research investigating the prophylactic effect of somastatin on PEP have shown inconsistent results[127,129,130]. Only one of three studies reviewed showed that somatostatin was effective and beneficial for the prevention of PEP. Bai *et al*[127] conducted a multicenter, open-label RCT in China. 908 patients underwent ERCP were randomly assigned to administrate somatostatin 250 μg bolus injection before ERCP and 250 μg/h intravenous infusion for 11 h after ERCP or no somatostatin treatments. The results of this study showed that incidence of PEP for these groups were 7.5% and 4.0%, respectively (P = 0.03). Significant results indicated that somatostatin was effective in preventing PEP. The other two studies assessed the impact of somatostatin on the prevention of PEP conducted by Concepcion-Martin *et al*[129] and Wang *et al*[130] found contradictory results.

Concepcion-Martin *et al*[129] administered either an intravenous bolus of somatostatin followed by a 4-hourcontinuous infusion or a similar placebo to patients undergoing ERCP. Wang *et al*[130] administered 0.5 mg/h of somatostatin for 24 h starting from 1 h before ERCP to 36 patients in the first group, 0.5 mg/h of somatostatin for 24 h starting from 1 h after ERCP to 47 patients in the second group and saline for 24 hours starting from 1 hour before ERCP to 41 patients in the third group. Both of these studies did not find any supportive evidence of the preventive effect of somastation on PEP.

Other prophylaxis agents

Raw rhubarb is a traditional Chinese medicine and considered to adequately relieve clinical symptoms, prevent the production of inflammatory mediators and cytokines and bacterial translocation, and mitigate abdominal compartment syndrome[131,132]. In a prospective, single center RCT Wang *et al*[133] assessed the efficiency of raw rhubarb for prevention of PEP. High risk patients were randomized into two groups. 250 patients drank a raw rhubarb soak solution per 3 h until defecation after ERCP and another 250 patients drank water after ERCP. PEP incidence was 2% (5/250) and 7.6% (19/250), respectively (P < 0.01). The results suggested that raw rhubarb solution is efficient for prevention of PEP in high-risk patients.

Oxygen derived free radicals may damage epithelial cells causing to capillary permeability and initiation of pancreatitis[134]. Allopurinol, one of inhibitors of xanthine oxidase, is thought to prevent or mitigate the initial complications caused by the cascade causing PEP by these agents[135]. In this context, Abbasinazari *et al*[135] performed a prospective, single-center, double-blind, placebo-controlled RCT to assess impact of allopurinol on prevention of PEP. Patients were divided in two groups and received 2 tablets of allopurinol (300 mg) or 2 tablets of placebo. One of the tablets was administered 3 h before ERCP and the other one just before the ERCP. PEP was developed in 3 of 29 patients (10.4%) in the allopurinol group and 5 of 45 patients in the control group (11.1%) (P = 0.97). The results of the study indicated that allopurinol was not effective in preventing PEP.

Neurogenic inflammation (pathologic activation of sensory neurons) is considered to contribute to the pathogenesis of acute pancreatitis[136]. Release of substance P, related to pancreatic vasodilation, edema, and cellular infiltration, can be stimulated by initiation of the capsaicin receptor (TRPV1) on sensory C and Aδ fibers[137,138]. Complications of neurogenic inflammation, such as pancreatitis, may be initiated through the attachment of Substance P to the neurokinin-1 receptor in the pancreas[139,140]. It was reported that intra-ductal administration of a neurokin1 antagonist diminished the severity of inflammation in a rat experiment of PEP[141]. Shah *et al*[136] conducted a prospective, single-center, double-blind, placebo-controlled RCT to evaluate the efficacy of aprepitant, a selective neurokinin-1 receptor antagonist, on the prevention of PEP in high risk patients. 39 patients received placebo and 34 patients received 125 mg oral aprepitant 4 h before ERCP, 80 mg 24 h after the first dose, and 80 mg 24 h after the second dose. 7 patients in each group developed PEP (P = 0.772). It was concluded that aprepitant was not efficient to reduce the incidence of PEP.

Papillary edema, triggered by manipulations during endoscopic treatment or cannulation, may temporarily prevent outflow of pancreatic juice[142], thereby raising ductal pressure, ultimately causing pancreatitis[143]. It was reported that the administration of epinephrine on the papilla may decrease papillary edema[144] and prevent acute pancreatitis after endoscopic balloon sphincteroplasty[142]. Application of epinephrine on the papilla is considered to mitigate edema, contribute the vascular permeability, relieve the muscles in the sphincter of Oddiand muscular layer of the duodenum, preventing increased pressure in the pancreatic duct by stopping the activation of pancreatic enzymes and the drainage of the pancreatic fluid[143]. In a hospital-based, prospective, controlled RCT, Xu *et al*[143] assessed to the impact of epinephrine sprayed on the papilla on reducing the development of PEP. 941 patients underwent ERCP were randomized to administer 20 mL of either 0.02% epinephrine or saline sprayed on the papilla after diagnostic ERCP. PEP occurred in 31 of 480 (6.45%) patients in the control and in 9 of 461 (1.95%) patients in the epinephrine group (P = 0.0086). They concluded that epinephrine administration on the papilla reduced the development of PEP.

Procedural techniques for prevention: The reviewed articles investigated the impact of the procedural techniques on the prevention of PEP are summarized on the Table 4.

Endoscopic nasobiliary drainage

Endoscopic nasobiliary drainage (ENBD) placement ensures dependable biliary drainage and perfusion, as well as cholangiography[145]. ENBD decreases the necessity of instrumental stone extraction and repetitive endoscopy and cholangiography to evaluate whether the stones have been completely removed by transnasalcholangiograms[146]. It was reported that endoscopic sphincterotomy (EST) or endoscopic papillary balloon dilatation(EPBD) pursued by ENBD diminished development of PEP, especially in patients with infected bile, lasting stones, or blood clots in the biliary tree[147-149].

Huang *et al*[150] assessed whether the placement of an ENBD had any benefits on the prevention of PEP after endoscopic papillary large balloon dilation together with endoscopic biliary sphincterotomy. 155 patients with bile duct stones were randomized to an ENBD group or no-ENBD group. PEP incidences were 1.28% and 10.4%, respectively (P = 0.018). Results showed that administration of ENBD reduced and was safe for PEP.

Another study conducted by Xu *et al*[145], evaluated the efficacy of ENBD catheter placement after clearance of CBD stones, also showed that ENBD was beneficial for the prevention of PEP only with an accompanying EPBD procedure.

Pancreatic stent placement

It is postulated that pancreatic stent placement (PSP) across the pancreatic sphincter may maintain flow of pancreatic secretions, which can be interrupted through papillary edema, and thereby contributing reduction of the PEP[9]. Five studies[151-155] in our review investigated efficacy of PSP in the prevention of PEP through a control group administered through non-stent procedure showed that PSP can reduce incidence rate of PEP.

The study with the largest number of participating patients in the reviewed articles was conducted by Sofuni *et al*[152] at 37 endoscopic units in Japan. They performed a prospective, multicenter and controlled RCT to investigate efficacy of a temporary-type PSP for the prevention of PEP through analyzing data obtained from 426 patients who underwent ERCP. 213 patients received stents and another 213 patients did not. PEP incidence was 7.9% and 15.2%, respectively (P = 0.021). The study concluded that PSP reduced the incidence of PEP.

Fujisawa *et al*[156] compared the impact of PSP between 3 cm and 5 cm pancreatic stents on prevention of PEP. 240 patients were randomized in a 1:1 ratio and underwent prophylactic insertion with 5-Fr unflanged 3 or 5-cm pancreatic stent. Per-protocol analysis showed that 3-cm stents are superior than 5-cm stents for prevention of PEP

In a prospective, multicenter, blinded RCT Conigliaro *et al*[157] compared the efficacy of duration of PSP in prevention of PEP using data obtained from patients receiving immediate 5-Fr unflanged pigtail pancreatic duct stenting after accidental wire-guided pancreatic duct cannulation during ERCP. After the ERCP process, stents were removed in 21 patients and were left in another 19 patients. PEP incidence was 29% in the first group and 0% in the second group (P = 0.021). They demonstrated that leaving pancreatic stents in place until spontaneous dislodgment occurs might reduce the development of PEP.

Wire-guided biliary cannulation

Table 4 Brief contents of reviewed articles on procedural techniques

Procedure	Authors	Year	Country	*n*	Design	Incidence of PEP[1]						*P* value
						Study group			Control (or compared)			
Endoscopic Nasobiliary Drainage (ENBD)	Huang *et al*[150]	2018	China	155	Prospective, single center	1/78 (1.28%) [ENBD]			8/77 (10.4%) [No ENBD]			0.018
	Xu *et al*[145]	2015	China	218	Prospective, single center	0/41 (0%) [ENBD +EPBD]	2/34 (5.9%) [ENBD +EST]	5/38 (13.2%) [Only ENBD]	6/36(16.7 %) [EPBD]	3/39 (7.7%) [EST]	2/30(6.7%) [Neither]	-
Wire-Guided Biliary Cannulation (WGC)	Kobayashi *et al*[158]	2013	Japan	322	Prospective, multicenter, controlled	10/163 (6.1%) [WGC]			10/159 (6.3%) [CC]			0.95
	Lee *et al*[161]	2009	South Korea	300	Prospective, single center	3/150 (2%) [WGC]			17/150 (11.3%)) [CC]			0.001
	Bassan *et al*[162]	2018	Asia Pacific Region	707	Prospective, multicenter, single-blinded	NA/355 (9.3%) [0.035-inch wire]			NA/357 (7.8%) [0.025-inch wire]			0.51
Pancreatic Stent Placement (PSP)	Fujisawa *et al*[156]	2016	Japan	200	Prospective, single center	2/98 (2%) [PSP 3 cm]			9/102 (8.8%) [PSP 5 cm]			0.035
	Conigliaro *et al*[157]	2013	Italy	40	Prospective, multicenter, blinded	6/21 (29 %) [Immediate stent removal]			0/19 [leaving the stent]			0.021
	Lee *et al*[151]	2012	South Korea	101	Prospective, multicenter,	6/50 (12%) [3F PSP]			15/51 (29.4) [Nonstent]			0.031
	Sofuni *et al*[152]	2011	Japan	407	Prospective, multicenter	16/203 (7.9%) (PSP)			31/204 (15.2%) [Nonstent]			0.021
	Pan *et al*[153]	2011	China	40	Prospective, single center	4/20 (20%) [PSP]			14/20 (70%) [Nonstent]			< 0.01
	Ito *et al*[154]	2010	Japan	70	Prospective, single center	0/35 (0%) [PSP]			9/35 (24%) [Nonstent]			< 0.01
	Kawaguchi *et al*[155]	2012	Japan	120	Prospective, single center	1/60 (1.7%) [PSP]			8/60 (13.3%) [Nonstent]			0.0322
Needle Knife Sphincter-otomy (NKS)	Swan *et al*[164]	2013	Australia	73	Prospective, single center, single blind	8/39 (20.5%) [NKS]			6/34 (17.6%) [CSC]			1.0

[1]The fractional ratios indicates "Number of PEP incidences/number of patients in the group". Rate of PEP incidences are given in the parenthesis. Definitions of the procedures applied to groups are given in the brackets. n: Number of patients (sample size); EPBD: Endoscopic papillary balloon dilatation; CC: Conventional cannulation; EST: Endoscopic sphincterotomy; CSC: Continued standart cannulation.

Wire-guided biliary cannulation (WGC) technique has been recommended for the reduction of PEP development and the facilitation of bile duct cannulation through using a radiopaque guidewire pierced the tip of a sphincterotome or a catheter[158].

Accession to bile duct using a guidewire is considered to decrease traumatic injury to the papilla and the pancreatic duct or prevent from hydrostatic pressure related to contrast injection, thereby contributing the prevention of PEP[159,160].

Kobayashi *et al*[158] and Lee *et al*[161] compared the effect of the WGC procedure versus conventional cannulation (CC) procedure on the prevention of PEP. Their findings were inconsistent. While Lee *et al*[161] found that WGC may be beneficial for the prevention of PEP, Kobayashi *et al*[158] concluded that the WGC technique did not decrease the risk of PEP.

In a single blinded, prospective, multicenter RCT, Bassan *et al*[162] compared 0.025-inch versus 0.035-inch guidewire on prevention of ERCP adverse events. 710 patients, with a healthy papilla and conventional anatomy, were randomized to either a 0.025-inch or 0.035-inch guidewire administration. The difference between the rate of PEP in these groups was found to be insignificant.

Needle knife sphincterotomy

Needle knife sphincterotomy (NKS) is an advanced therapeutic measure used to facilitate deep cannulation in cases when traditional deep cannulation is insufficient[163]. Therefore, NKS is related to PEP, since it is often administered as a last resort after multiple and repeated failed cannulation attempts[164].

Swan *et al*[164] conducted a prospective, single center, single blind RCT to assess efficacy of early application of NKS during difficult cannulation on the prevention of PEP. 73 patients with an intact papilla underwent ERCP with difficult biliary cannulation were randomized to groups that administered either NKS or continued standard cannulation. The difference in rate of PEP between these groups was insignificant, revealing that early application of NKS during difficult cannulation was not effective in preventing the development of PEP.

DISCUSSION

PEP remains an important complication of ERCP and may have adverse impacts on the quality of patient life, morbidity, and mortality[165]. Its pathophysiology is still unclear and considered to be multifactorial. Clinical trials have analyzed different approaches for the prevention of PEP. Studies that investigate the prevention of PEP may be categorized into (1) assessment of patient related risk factors; (2) pharmacoprevention; and (3) procedural techniques for prevention.

Determination of patients with high risk factors for PEP is one of the most important aspects for the prevention of PEP. Patients with high risk factors should be carefully assessed, and alternative therapeutic and diagnostic techniques may be preferable for them instead of ERCP. EUS, MRCP and the other non-invasive techniques including percutaneous drain fluid analysis and radionucleotide-labeled scan, providing very accurate results in diagnosing pancreaticobiliary disorders and meet the need for diagnostic ERCP[26], can be preferable alternatives to reduce risks of PEP for these patients.

Pharmacological agents with highly precise results in the literature, such as NSAIDs, can be beneficial to attenuate development of PEP. Although many pharmacologic agents has been analyzed through data obtained from patients undergoing ERCP, NSAIDs (indomethacin and diclofenac) are in widespread use and the most promising option for the prevention of PEP[57]. NSAIDs should be rectally administered to all patients with high-risks and considered for patients with average-risks[3]. Other pharmacological agents, found consistently to have impact on the prevention of PEP in various studies, can be alternatively considered for the prevention of PEP (Table 3). Further studies are required for other pharmacological agents to identify their impacts more accurately.

Among the reviewed studies focused on the procedural techniques, PSP and ENBD are considered to have most efficacy in preventing PEP (Table 4). PSP and ENBD should be performed for to all patients with high-risks and considered for patients with average-risks. These techniques can facilitate the difficult and failed cannulation cases.

Due to the multifactorial mechanism of the introduction of PEP[57], prevention of PEP can fail through targeting only one causative factor[35]. Combination of multiple interventions may be more effective through proper patient selection, administration of prophylaxis pharmacologic agents and procedural techniques. However, further studies are needed to consolidate prophylaxis impacts of each of these interventional approaches on the prevention of PEP. Further researches should focus on performing meta-analysis to get pool effect and overcome heterogeneity, imprecision, and risk of publication bias. Thereby the assessment of the evidence quality obtained through the

studies in the literature can be enhanced.

ARTICLE HIGHLIGHTS

Research background

Endoscopic retrograde cholangiopancreatography (ERCP) has been used in the field of gastrointestinal endoscopy since its introduction as an important technological innovation. It is comparatively complex and can lead various complications. Post-ERCP pancreatitis (PEP) has been on the focus of the researches to investigate its prevention, since it has been considered to be the most common complication of ERCP.

Research motivation

Clinical trials have investigated different methods for the prevention of PEP. Each of these studies focused on a specific method. Our review gathered all preventative approaches for PEP investigated in the last ten years. Due to the conflicting data in the literature, advances in the reviewed field needed to be updated and supporting evidence needed review.

Research objectives

The objective of this study was to systematically review the literature on prevention of PEP with different preventive approaches.

Research methods

We conducted an electronic search through databases of PubMed, ISI Web of Science and Cochrane Library for relevant articles performed *via* RCTs covering the time span of January 2009 and February 2019. The search was performed through terms "Post endoscopic retrograde cholangio-pancreatography pancreatitis" AND "prevention". The reference lists of the identified papers were also scanned to find out further relevant studies.

Research results

54 studies were finally identified for full text review. The studies were categorized regarding prevention methods as (1) assessment of patient related factors, (2) pharmacoprevention and (3) procedural techniques for prevention. Female gender, young age, suspected Sphincter of Oddi dysfunction, absence of chronic pancreatitis, recurrent pancreatitis and history of previous PEP were the most common high risk factors for the patients to develop PEP. Rectally administered NSAIDs has been highly recommended for the prevention of PEP among the pharmacologic agents, while others had conflicting results and needed further research. Of the procedural techniques, Pancreatic Stent Placement and Endoscopic Nasobiliary Drainage can be beneficial in preventing PEP.

Research conclusions

PEP is the most common complication in ERCP procedure and can be risky in patients with high risk factors. The pathophysiology of PEP is still in dispute. Due to its multifactorial pathophysiology, prevention of PEP should be assessed in multi aspects through evaluation of patient related risk factors, prophylaxis pharmacological agents and procedural techniques.

Research perspectives

The multifactorial nature of PEP requires prophylaxis measures in multi facets. Due to its relation to a combination of various factors, multifactorial approach should be taken into account to prevent PEP through assessment of patient related risks and prophylaxis preventions of pharmacologic agents and procedural techniques.

REFERENCES

1 **Baron TH**, Kozarek RA, Carr-Locke DL. ERCP. Philadelphia: Elsevier 2019.
2 **McCune WS**, Shorb PE, Moscovitz H. Endoscopic cannulation of the ampulla of vater: a preliminary report. *Ann Surg* 1968; **167**: 752-756 [PMID: 5646296 DOI: 10.1097/00000658-196805000-00013]
3 **Morales SJ**, Sampath K, Gardner TB. A Review of Prevention of Post-ERCP Pancreatitis. *Gastroenterol Hepatol (N Y)* 2018; **14**: 286-292 [PMID: 29991936]
4 **Inamdar S**, Han D, Passi M, Sejpal DV, Trindade AJ. Rectal indomethacin is protective against post-ERCP pancreatitis in high-risk patients but not average-risk patients: a systematic review and meta-analysis. *Gastrointest Endosc* 2017; **85**: 67-75 [PMID: 27612923 DOI: 10.1016/j.gie.2016.08.034]
5 **Matsushita M**, Koyabu M, Nishio A, Seki T, Okazaki K. Techniques of ERCP with a conventional endoscope in pancreatoduodenectomy anatomy. *Gastrointest Endosc* 2017; **86**: 747-748 [PMID: 28917352 DOI: 10.1016/j.gie.2017.05.007]
6 **Li DP**, Zhang QY, Cheng J, Li JZ, Wang L. The effect of somatostatin retained enema in the treatment of pancreatic ileus. *Eur Rev Med Pharmacol Sci* 2017; **21**: 3476-3481 [PMID: 28829514]
7 **Tierney J**, Bhutiani N, Stamp B, Richey JS, Bahr MH, Vitale GC. Predictive risk factors associated with cholangitis following ERCP. *Surg Endosc* 2018; **32**: 799-804 [PMID: 28733732 DOI: 10.1007/s00464-017-5746-z]
8 **Rodrigues-Pinto E**, Macedo G, Baron TH. ERCP competence assessment: Miles to go before standardization. *Endosc Int Open* 2017; **5**: E718-E721 [PMID: 28791318 DOI: 10.1055/s-0043-107780]
9 **Guda NM**, Freeman ML. Overview of ERCP Complications: Prevention and Management. *Lee LS, editor*

ERCP and EUS: A Case-Based Approach. New York: Springer 2015; 37-56 [DOI: 10.1007/978-1-4939-2320-5]
10 **Freeman ML**, Nelson DB, Sherman S, Haber GB, Herman ME, Dorsher PJ, Moore JP, Fennerty MB, Ryan ME, Shaw MJ, Lande JD, Pheley AM. Complications of endoscopic biliary sphincterotomy. *N Engl J Med* 1996; **335**: 909-918 [PMID: 8782497 DOI: 10.1056/nejm199609263351301]
11 **Kahaleh M**, Freeman M. Prevention and management of post-endoscopic retrograde cholangiopancreatography complications. *Clin Endosc* 2012; **45**: 305-312 [PMID: 22977824 DOI: 10.5946/ce.2012.45.3.305]
12 **Zimmon DS**, Falkenstein DB, Riccobono C, Aaron B. Complications of endoscopic retrograde cholangiopancreatography. Analysis of 300 consecutive cases. *Gastroenterology* 1975; **69**: 303-309 [PMID: 1097296 DOI: 10.1016/S0016-5085(19)32569-7]
13 **Bilbao MK**, Dotter CT, Lee TG, Katon RM. Complications of endoscopic retrograde cholangiopancreatography (ERCP). A study of 10,000 cases. *Gastroenterology* 1976; **70**: 314-320 [PMID: 1248697 DOI: 10.1016/S0016-5085(76)80139-4]
14 **Skude G**, Wehlin L, Maruyama T, Ariyama J. Hyperamylasaemia after duodenoscopy and retrograde cholangiopancreatography. *Gut* 1976; **17**: 127-132 [PMID: 1261883 DOI: 10.1136/gut.17.2.127]
15 **Deschamps JP**, Allemand H, Janin Magnificat R, Camelot G, Gillet M, Carayon P. Acute pancreatitis following gastrointestinal endoscopy without ampullary cannulation. *Endoscopy* 1982; **14**: 105-106 [PMID: 7075561 DOI: 10.1055/s-2007-1021593]
16 **Odes HS**, Novis BN, Barbezat GO, Bank S. Effect of calcitonin on the serum amylase levels after endoscopic retrograde cholangiopancreatography. *Digestion* 1977; **16**: 180-184 [PMID: 355022 DOI: 10.1159/000198070]
17 **Mine T**, Morizane T, Kawaguchi Y, Akashi R, Hanada K, Ito T, Kanno A, Kida M, Miyagawa H, Yamaguchi T, Mayumi T, Takeyama Y, Shimosegawa T. Clinical practice guideline for post-ERCP pancreatitis. *J Gastroenterol* 2017; **52**: 1013-1022 [PMID: 28653082 DOI: 10.1007/s00535-017-1359-5]
18 **Wang AY**. Medications and Methods for the Prevention of Post-ERCP Pancreatitis. *Gastroenterol Hepatol (N Y)* 2017; **13**: 188-191 [PMID: 28539848]
19 **Testoni PA**, Mariani A, Aabakken L, Arvanitakis M, Bories E, Costamagna G, Devière J, Dinis-Ribeiro M, Dumonceau JM, Giovannini M, Gyokeres T, Hafner M, Halttunen J, Hassan C, Lopes L, Papanikolaou IS, Tham TC, Tringali A, van Hooft J, Williams EJ. Papillary cannulation and sphincterotomy techniques at ERCP: European Society of Gastrointestinal Endoscopy (ESGE) Clinical Guideline. *Endoscopy* 2016; **48**: 657-683 [PMID: 27299638 DOI: 10.1055/s-0042-108641]
20 **Fogel EL**, Eversman D, Jamidar P, Sherman S, Lehman GA. Sphincter of Oddi dysfunction: pancreaticobiliary sphincterotomy with pancreatic stent placement has a lower rate of pancreatitis than biliary sphincterotomy alone. *Endoscopy* 2002; **34**: 280-285 [PMID: 11932782 DOI: 10.1055/s-2002-23629]
21 **Tarnasky PR**, Palesch YY, Cunningham JT, Mauldin PD, Cotton PB, Hawes RH. Pancreatic stenting prevents pancreatitis after biliary sphincterotomy in patients with sphincter of Oddi dysfunction. *Gastroenterology* 1998; **115**: 1518-1524 [PMID: 9834280 DOI: 10.1016/S0016-5085(98)70031-9]
22 **Silviera ML**, Seamon MJ, Porshinsky B, Prosciak MP, Doraiswamy VA, Wang CF, Lorenzo M, Truitt M, Biboa J, Jarvis AM, Narula VK, Steinberg SM, Stawicki SP. Complications related to endoscopic retrograde cholangiopancreatography: a comprehensive clinical review. *J Gastrointestin Liver Dis* 2009; **18**: 73-82 [PMID: 19337638]
23 **Wang P**, Li ZS, Liu F, Ren X, Lu NH, Fan ZN, Huang Q, Zhang X, He LP, Sun WS, Zhao Q, Shi RH, Tian ZB, Li YQ, Li W, Zhi FC. Risk factors for ERCP-related complications: a prospective multicenter study. *Am J Gastroenterol* 2009; **104**: 31-40 [PMID: 19098846 DOI: 10.1038/ajg.2008.5]
24 **Cohen S**. Bacon BR, Berlin JA, Fleischer D, Hecht GA, Loehrer PJ Sr, McNair AE Jr, Mulholland M, Norton NJ, Rabeneck L, Ransohoff DF, Sonnenberg A, Vannier MW. National Institutes of Health State-of-the-Science Conference Statement: ERCP for diagnosis and therapy, January 14-16, 2002. *Gastrointest Endosc* 2002; **56**: 803-809 [PMID: 12447289 DOI: 10.1067/mge.2002.129875]
25 **Brugge WR**, Van Dam J. Pancreatic and biliary endoscopy. *N Engl J Med* 1999; **341**: 1808-1816 [PMID: 10588967 DOI: 10.1056/nejm199912093412406]
26 **Elmunzer BJ**. Reducing the risk of post-endoscopic retrograde cholangiopancreatography pancreatitis. *Dig Endosc* 2017; **29**: 749-757 [PMID: 28636774 DOI: 10.1111/den.12908]
27 **Freeman ML**, DiSario JA, Nelson DB, Fennerty MB, Lee JG, Bjorkman DJ, Overby CS, Aas J, Ryan ME, Bochna GS, Shaw MJ, Snady HW, Erickson RV, Moore JP, Roel JP. Risk factors for post-ERCP pancreatitis: a prospective, multicenter study. *Gastrointest Endosc* 2001; **54**: 425-434 [PMID: 11577302 DOI: 10.1067/mge.2001.117550]
28 **Cheng CL**, Sherman S, Watkins JL, Barnett J, Freeman M, Geenen J, Ryan M, Parker H, Frakes JT, Fogel EL, Silverman WB, Dua KS, Aliperti G, Yakshe P, Uzer M, Jones W, Goff J, Lazzell-Pannell L, Rashdan A, Temkit M, Lehman GA. Risk factors for post-ERCP pancreatitis: a prospective multicenter study. *Am J Gastroenterol* 2006; **101**: 139-147 [PMID: 16405547 DOI: 10.1111/j.1572-0241.2006.00380.x]
29 **Zhou W**, Li Y, Zhang Q, Li X, Meng W, Zhang L, Zhang H, Zhu K, Zhu X. Risk factors for postendoscopic retrograde cholangiopancreatography pancreatitis: a retrospective analysis of 7,168 cases. *Pancreatology* 2011; **11**: 399-405 [PMID: 21894057 DOI: 10.1016/S1424-3903(11)80094-3]
30 **Cotton PB**, Yaghoobi M, Cotton PB, Leung J. Adverse events: definitions, avoidance, and management. Cotton PB, Leung J. *ERCP: The Fundamentals*. Oxford: John Wiley Sons 2015; 371-396
31 **Freeman ML**. Pancreatic stents for prevention of post-endoscopic retrograde cholangiopancreatography pancreatitis. *Clin Gastroenterol Hepatol* 2007; **5**: 1354-1365 [PMID: 17981248 DOI: 10.1016/j.cgh.2007.09.007]
32 **Freeman ML**. Adverse outcomes of ERCP. *Gastrointest Endosc* 2002; **56**: S273-S282 [PMID: 12447281 DOI: 10.1067/mge.2002.129028]
33 **Vandervoort J**, Soetikno RM, Tham TC, Wong RC, Ferrari AP, Montes H, Roston AD, Slivka A, Lichtenstein DR, Ruymann FW, Van Dam J, Hughes M, Carr-Locke DL. Risk factors for complications after performance of ERCP. *Gastrointest Endosc* 2002; **56**: 652-656 [PMID: 12397271 DOI: 10.1067/mge.2002.129086]
34 **Chen YK**, Foliente RL, Santoro MJ, Walter MH, Collen MJ. Endoscopic sphincterotomy-induced pancreatitis: increased risk associated with nondilated bile ducts and sphincter of Oddi dysfunction. *Am J Gastroenterol* 1994; **89**: 327-333 [PMID: 8122639]
35 **Freeman ML**, Guda NM. Prevention of post-ERCP pancreatitis: a comprehensive review. *Gastrointest Endosc* 2004; **59**: 845-864 [PMID: 15173799 DOI: 10.1016/S0016-5107(04)00353-0]

36 **Elmunzer BJ**. *Prevention of ERCP-induced Pancreatitis*. The Pancreapedia: Exocrine Pancreas Knowledge Base 2015; [DOI: 10.3998/panc.2015.19]

37 **Elmunzer BJ**, Waljee AK, Elta GH, Taylor JR, Fehmi SM, Higgins PD. A meta-analysis of rectal NSAIDs in the prevention of post-ERCP pancreatitis. *Gut* 2008; **57**: 1262-1267 [PMID: 18375470 DOI: 10.1136/gut.2007.140756]

38 **Kochar B**, Akshintala VS, Afghani E, Elmunzer BJ, Kim KJ, Lennon AM, Khashab MA, Kalloo AN, Singh VK. Incidence, severity, and mortality of post-ERCP pancreatitis: a systematic review by using randomized, controlled trials. *Gastrointest Endosc* 2015; **81**: 143-149.e9 [PMID: 25088919 DOI: 10.1016/j.gie.2014.06.045]

39 **Keswani RN**, Taft TH, Coté GA, Keefer L. Increased levels of stress and burnout are related to decreased physician experience and to interventional gastroenterology career choice: findings from a US survey of endoscopists. *Am J Gastroenterol* 2011; **106**: 1734-1740 [PMID: 21979198 DOI: 10.1038/ajg.2011.148]

40 **Cotton PB**. Analysis of 59 ERCP lawsuits; mainly about indications. *Gastrointest Endosc* 2006; **63**: 378-82; quiz 464 [PMID: 16500382 DOI: 10.1016/j.gie.2005.06.046]

41 **Cotton PB**, Cotton PB, Leung J. ERCP: Risks, Prevention, and Management. Cotton PB, Leung J. *Advances Digestive Endoscopy: ERCP*. Massachusetts: Blackwell Publishing 2005; 339-403 [DOI: 10.1002/9780470987490]

42 **Parekh PJ**, Majithia R, Sikka SK, Baron TH. The "Scope" of Post-ERCP Pancreatitis. *Mayo Clin Proc* 2017; **92**: 434-448 [PMID: 28160947 DOI: 10.1016/j.mayocp.2016.10.028]

43 **Dumonceau JM**, Andriulli A, Deviere J, Mariani A, Rigaux J, Baron TH, Testoni PA; European Society of Gastrointestinal Endoscopy. European Society of Gastrointestinal Endoscopy (ESGE) Guideline: prophylaxis of post-ERCP pancreatitis. *Endoscopy* 2010; **42**: 503-515 [PMID: 20506068 DOI: 10.1055/s-0029-1244208]

44 **Cotton PB**, Lehman G, Vennes J, Geenen JE, Russell RC, Meyers WC, Liguory C, Nickl N. Endoscopic sphincterotomy complications and their management: an attempt at consensus. *Gastrointest Endosc* 1991; **37**: 383-393 [PMID: 2070995 DOI: 10.1016/S0016-5107(91)70740-2]

45 **Banks PA**, Bollen TL, Dervenis C, Gooszen HG, Johnson CD, Sarr MG, Tsiotos GG, Vege SS; Acute Pancreatitis Classification Working Group. Classification of acute pancreatitis--2012: revision of the Atlanta classification and definitions by international consensus. *Gut* 2013; **62**: 102-111 [PMID: 23100216 DOI: 10.1136/gutjnl-2012-302779]

46 **Zhang H**, Cho J, Buxbaum J. Update on the Prevention of Post-ERCP Pancreatitis. *Curr Treat Options Gastroenterol* 2018; **16**: 428-440 [PMID: 30334143 DOI: 10.1007/s11938-018-0194-y]

47 **Tse F**, Liu L, Barkun AN, Armstrong D, Moayyedi P. EUS: a meta-analysis of test performance in suspected choledocholithiasis. *Gastrointest Endosc* 2008; **67**: 235-244 [PMID: 18226685 DOI: 10.1016/j.gie.2007.09.047]

48 **Romagnuolo J**, Bardou M, Rahme E, Joseph L, Reinhold C, Barkun AN. Magnetic resonance cholangiopancreatography: a meta-analysis of test performance in suspected biliary disease. *Ann Intern Med* 2003; **139**: 547-557 [PMID: 14530225 DOI: 10.7326/0003-4819-139-7-200310070-00006]

49 **Giljaca V**, Gurusamy KS, Takwoingi Y, Higgie D, Poropat G, Štimac D, Davidson BR. Endoscopic ultrasound versus magnetic resonance cholangiopancreatography for common bile duct stones. *Cochrane Database Syst Rev* 2015; CD011549 [PMID: 25719224 DOI: 10.1002/14651858.CD011549]

50 **DiMagno MJ**, Spaete JP, Ballard DD, Wamsteker EJ, Saini SD. Risk models for post-endoscopic retrograde cholangiopancreatography pancreatitis (PEP): smoking and chronic liver disease are predictors of protection against PEP. *Pancreas* 2013; **42**: 996-1003 [PMID: 23532001 DOI: 10.1097/MPA.0b013e31827e95e9]

51 **Omar M**, Ahmed A, Said O, El-Amin H. Risk factors for post-ERCP pancreatitis: a prospective multicenter study in upper Egypt. *Egypt J Surg* 2015; **34**: 1-10 [DOI: 10.4103/1110-1121.153364]

52 **Testoni PA**, Mariani A, Giussani A, Vailati C, Masci E, Macarri G, Ghezzo L, Familiari L, Giardullo N, Mutignani M, Lombardi G, Talamini G, Spadaccini A, Briglia R, Piazzi L; SEIFRED Group. Risk factors for post-ERCP pancreatitis in high- and low-volume centers and among expert and non-expert operators: a prospective multicenter study. *Am J Gastroenterol* 2010; **105**: 1753-1761 [PMID: 20372116 DOI: 10.1038/ajg.2010.136]

53 **Lin Y**, Liu X, Cao DQ, Tang JH, Wen JJ, Li TF, Liu PF, Xia TS. Analysis of risk factors and prevention strategies of post-ERCP pancreatitis. *Eur Rev Med Pharmacol Sci* 2017; **21**: 5185-5190 [PMID: 29228432 DOI: 10.26355/eurrev_201711_13838]

54 **Srinivasan I**, Freeman ML, Baron TH, Kozarek RA, Carr-Locke DL. Adverse Events of ERCP: Prediction, Prevention, and Management. Baron TH, Kozarek RA, Carr-Locke DL. *ERCP*. China: Elsevier 2019; 59-67

55 **Cotton PB**, Garrow DA, Gallagher J, Romagnuolo J. Risk factors for complications after ERCP: a multivariate analysis of 11,497 procedures over 12 years. *Gastrointest Endosc* 2009; **70**: 80-88 [PMID: 19286178 DOI: 10.1016/j.gie.2008.10.039]

56 **Andrade-Dávila VF**, Chávez-Tostado M, Dávalos-Cobián C, García-Correa J, Montaño-Loza A, Fuentes-Orozco C, Macías-Amezcua MD, García-Rentería J, Rendón-Félix J, Cortés-Lares JA, Ambriz-González G, Cortés-Flores AO, Alvarez-Villaseñor Adel S, González-Ojeda A. Rectal indomethacin versus placebo to reduce the incidence of pancreatitis after endoscopic retrograde cholangiopancreatography: results of a controlled clinical trial. *BMC Gastroenterol* 2015; **15**: 85 [PMID: 26195123 DOI: 10.1186/s12876-015-0314-2]

57 **Cheon YK**. Can postendoscopic retrograde cholangiopancreatography pancreatitis be prevented by a pharmacological approach? *Korean J Intern Med* 2013; **28**: 141-148 [PMID: 23525264 DOI: 10.3904/kjim.2013.28.2.141]

58 **Levenick JM**, Gordon SR, Fadden LL, Levy LC, Rockacy MJ, Hyder SM, Lacy BE, Bensen SP, Parr DD, Gardner TB. Rectal Indomethacin Does Not Prevent Post-ERCP Pancreatitis in Consecutive Patients. *Gastroenterology* 2016; **150**: 911-7; quiz e19 [PMID: 26775631 DOI: 10.1053/j.gastro.2015.12.040]

59 **Dumonceau JM**, Andriulli A, Elmunzer BJ, Mariani A, Meister T, Deviere J, Marek T, Baron TH, Hassan C, Testoni PA, Kapral C; European Society of Gastrointestinal Endoscopy. Prophylaxis of post-ERCP pancreatitis: European Society of Gastrointestinal Endoscopy (ESGE) Guideline - updated June 2014. *Endoscopy* 2014; **46**: 799-815 [PMID: 25148137 DOI: 10.1055/s-0034-1377875]

60 **Elmunzer BJ**, Scheiman JM, Lehman GA, Chak A, Mosler P, Higgins PD, Hayward RA, Romagnuolo J, Elta GH, Sherman S, Waljee AK, Repaka A, Atkinson MR, Cote GA, Kwon RS, McHenry L, Piraka CR, Wamsteker EJ, Watkins JL, Korsnes SJ, Schmidt SE, Turner SM, Nicholson S, Fogel EL; U. S. Cooperative for Outcomes Research in Endoscopy (USCORE). A randomized trial of rectal indomethacin

to prevent post-ERCP pancreatitis. *N Engl J Med* 2012; **366**: 1414-1422 [PMID: 22494121 DOI: 10.1056/NEJMoa1111103]
61 **Patai Á**. Solymosi N, Patai ÁV. Effect of rectal indomethacin for preventing post-ERCP pancreatitis depends on difficulties of cannulation: results from a randomized study with sequential biliary intubation. *J Clin Gastroenterol* 2015; **49**: 429-437 [PMID: 25790233 DOI: 10.1097/MCG.0000000000000168]
62 **Luo H**, Zhao L, Leung J, Zhang R, Liu Z, Wang X, Wang B, Nie Z, Lei T, Li X, Zhou W, Zhang L, Wang Q, Li M, Zhou Y, Liu Q, Sun H, Wang Z, Liang S, Guo X, Tao Q, Wu K, Pan Y, Guo X, Fan D. Routine pre-procedural rectal indometacin versus selective post-procedural rectal indometacin to prevent pancreatitis in patients undergoing endoscopic retrograde cholangiopancreatography: a multicentre, single-blinded, randomised controlled trial. *Lancet* 2016; **387**: 2293-2301 [PMID: 27133971 DOI: 10.1016/S0140-6736(16)30310-5]
63 **Hosseini M**, Shalchiantabrizi P, Yektaroudy K, Dadgarmoghaddam M, Salari M. Prophylactic Effect of Rectal Indomethacin Administration, with and without Intravenous Hydration, on Development of Endoscopic Retrograde Cholangiopancreatography Pancreatitis Episodes: A Randomized Clinical Trial. *Arch Iran Med* 2016; **19**: 538-543 [PMID: 27544361 DOI: 0161908/AIM.004]
64 **Mok SRS**, Ho HC, Shah P, Patel M, Gaughan JP, Elfant AB. Lactated Ringer's solution in combination with rectal indomethacin for prevention of post-ERCP pancreatitis and readmission: a prospective randomized, double-blinded, placebo-controlled trial. *Gastrointest Endosc* 2017; **85**: 1005-1013 [PMID: 27816497 DOI: 10.1016/j.gie.2016.10.033]
65 **Döbrönte Z**, Szepes Z, Izbéki F, Gervain J, Lakatos L, Pécsi G, Ihász M, Lakner L, Toldy E, Czakó L. Is rectal indomethacin effective in preventing of post-endoscopic retrograde cholangiopancreatography pancreatitis? *World J Gastroenterol* 2014; **20**: 10151-10157 [PMID: 25110443 DOI: 10.3748/wjg.v20.i29.10151]
66 **Park SW**, Chung MJ, Oh TG, Park JY, Bang S, Park SW, Song SY. Intramuscular diclofenac for the prevention of post-ERCP pancreatitis: a randomized trial. *Endoscopy* 2015; **47**: 33-39 [PMID: 25409167 DOI: 10.1055/s-0034-1390743]
67 **Zeitlinger M**, Rusca A, Oraha AZ, Gugliotta B, Müller M, Ducharme MP. Pharmacokinetics of a new diclofenac sodium formulation developed for subcutaneous and intramuscular administration. *Int J Clin Pharmacol Ther* 2012; **50**: 383-390 [PMID: 22677302 DOI: 10.5414/CP201600]
68 **Otsuka T**, Kawazoe S, Nakashita S, Kamachi S, Oeda S, Sumida C, Akiyama T, Ario K, Fujimoto M, Tabuchi M, Noda T. Low-dose rectal diclofenac for prevention of post-endoscopic retrograde cholangiopancreatography pancreatitis: a randomized controlled trial. *J Gastroenterol* 2012; **47**: 912-917 [PMID: 22350703 DOI: 10.1007/s00535-012-0554-7]
69 **Demols A**, Deviere J. New frontiers in the pharmacological prevention of post-ERCP pancreatitis: the cytokines. *JOP* 2003; **4**: 49-57 [PMID: 12555016]
70 **Bhatia V**, Ahuja V, Acharya SK, Garg PK. A randomized controlled trial of valdecoxib and glyceryl trinitrate for the prevention of post-ERCP pancreatitis. *J Clin Gastroenterol* 2011; **45**: 170-176 [PMID: 20717044 DOI: 10.1097/MCG.0b013e3181eb600e]
71 **Ethridge RT**, Chung DH, Slogoff M, Ehlers RA, Hellmich MR, Rajaraman S, Saito H, Uchida T, Evers BM. Cyclooxygenase-2 gene disruption attenuates the severity of acute pancreatitis and pancreatitis-associated lung injury. *Gastroenterology* 2002; **123**: 1311-1322 [PMID: 12360491 DOI: 10.1053/gast.2002.35951]
72 **Insel P**, Hardman J, Limbird L. Analgesic-antipyretic and antiinflammatory agents and drugs employed in the treatment of gout. Hardman J, Limbird L. *The pharmacological basis of therapeutics*. New York: Mc-graw hill 1996; 617–657
73 **de Quadros Onófrio F**, Lima JCP, Watte G, Lehmen RL, Oba D, Camargo G, Dos Santos CEO. Prophylaxis of pancreatitis with intravenous ketoprofen in a consecutive population of ERCP patients: a randomized double-blind placebo-controlled trial. *Surg Endosc* 2017; **31**: 2317-2324 [PMID: 27651353 DOI: 10.1007/s00464-016-5234-x]
74 **Gross V**, Leser HG, Heinisch A, Schölmerich J. Inflammatory mediators and cytokines--new aspects of the pathophysiology and assessment of severity of acute pancreatitis? *Hepatogastroenterology* 1993; **40**: 522-530 [PMID: 7509768]
75 **Whitcomb DC**. Acute pancreatitis: molecular biology update. *J Gastrointest Surg* 2003; **7**: 940-942 [PMID: 14675701 DOI: 10.1016/j.gassur.2003.10.001]
76 **Mäkelä A**, Kuusi T, Schröder T. Inhibition of serum phospholipase-A2 in acute pancreatitis by pharmacological agents in vitro. *Scand J Clin Lab Invest* 1997; **57**: 401-407 [PMID: 9279965 DOI: 10.3109/00365519709084587]
77 **Kato K**, Shiba M, Kakiya Y, Maruyama H, Ominami M, Fukunaga S, Sugimori S, Nagami Y, Watanabe T, Tominaga K, Fujiwara Y. Celecoxib Oral Administration for Prevention of Post-Endoscopic Retrograde Cholangiopancreatography Pancreatitis: A Randomized Prospective Trial. *Pancreas* 2017; **46**: 880-886 [PMID: 28697127 DOI: 10.1097/MPA.0000000000000852]
78 **Banks PA**, Freeman ML; Practice Parameters Committee of the American College of Gastroenterology. Practice guidelines in acute pancreatitis. *Am J Gastroenterol* 2006; **101**: 2379-2400 [PMID: 17032204 DOI: 10.1111/j.1572-0241.2006.00856.x]
79 **Kusterer K**, Enghofer M, Zendler S, Blöchle C, Usadel KH. Microcirculatory changes in sodium taurocholate-induced pancreatitis in rats. *Am J Physiol* 1991; **260**: G346-G351 [PMID: 1996652 DOI: 10.1152/ajpgi.1991.260.2.G346]
80 **Muddana V**, Whitcomb DC, Khalid A, Slivka A, Papachristou GI. Elevated serum creatinine as a marker of pancreatic necrosis in acute pancreatitis. *Am J Gastroenterol* 2009; **104**: 164-170 [PMID: 19098865 DOI: 10.1038/ajg.2008.66]
81 **Wu BU**, Hwang JQ, Gardner TH, Repas K, Delee R, Yu S, Smith B, Banks PA, Conwell DL. Lactated Ringer's solution reduces systemic inflammation compared with saline in patients with acute pancreatitis. *Clin Gastroenterol Hepatol* 2011; **9**: 710-717.e1 [PMID: 21645639 DOI: 10.1016/j.cgh.2011.04.026]
82 **Seyama Y**, Otani T, Matsukura A, Makuuchi M. The pH modulator chloroquine blocks trypsinogen activation peptide generation in cerulein-induced pancreatitis. *Pancreas* 2003; **26**: 15-17 [PMID: 12499911 DOI: 10.1097/00006676-200301000-00003]
83 **Buxbaum J**, Yan A, Yeh K, Lane C, Nguyen N, Laine L. Aggressive hydration with lactated Ringer's solution reduces pancreatitis after endoscopic retrograde cholangiopancreatography. *Clin Gastroenterol Hepatol* 2014; **12**: 303-7.e1 [PMID: 23920031 DOI: 10.1016/j.cgh.2013.07.026]
84 **Gardner TB**, Vege SS, Chari ST, Petersen BT, Topazian MD, Clain JE, Pearson RK, Levy MJ, Sarr MG. Faster rate of initial fluid resuscitation in severe acute pancreatitis diminishes in-hospital mortality.

Pancreatology 2009; **9**: 770-776 [PMID: 20110744 DOI: 10.1159/000210022]
85 **Noble MD**, Romac J, Vigna SR, Liddle RA. A pH-sensitive, neurogenic pathway mediates disease severity in a model of post-ERCP pancreatitis. *Gut* 2008; **57**: 1566-1571 [PMID: 18625695 DOI: 10.1136/gut.2008.148551]
86 **Bhoomagoud M**, Jung T, Atladottir J, Kolodecik TR, Shugrue C, Chaudhuri A, Thrower EC, Gorelick FS. Reducing extracellular pH sensitizes the acinar cell to secretagogue-induced pancreatitis responses in rats. *Gastroenterology* 2009; **137**: 1083-1092 [PMID: 19454288 DOI: 10.1053/j.gastro.2009.05.041]
87 **Choi JH**, Kim HJ, Lee BU, Kim TH, Song IH. Vigorous Periprocedural Hydration With Lactated Ringer's Solution Reduces the Risk of Pancreatitis After Retrograde Cholangiopancreatography in Hospitalized Patients. *Clin Gastroenterol Hepatol* 2017; **15**: 86-92.e1 [PMID: 27311618 DOI: 10.1016/j.cgh.2016.06.007]
88 **Sherman S**, Cheng CL, Costamagna G, Binmoeller KF, Puespoek A, Aithal GP, Kozarek RA, Chen YK, Van Steenbergen W, Tenner S, Freeman M, Monroe P, Geffner M, Deviere J; Interleukin-10 ERCP Study Group. Efficacy of recombinant human interleukin-10 in prevention of post-endoscopic retrograde cholangiopancreatography pancreatitis in subjects with increased risk. *Pancreas* 2009; **38**: 267-274 [PMID: 19214137 DOI: 10.1097/MPA.0b013e31819777d5]
89 **Rongione AJ**, Kusske AM, Kwan K, Ashley SW, Reber HA, McFadden DW. Interleukin 10 reduces the severity of acute pancreatitis in rats. *Gastroenterology* 1997; **112**: 960-967 [PMID: 9041259 DOI: 10.1053/gast.1997.v112.pm9041259]
90 **Van Laethem JL**, Marchant A, Delvaux A, Goldman M, Robberecht P, Velu T, Devière J. Interleukin 10 prevents necrosis in murine experimental acute pancreatitis. *Gastroenterology* 1995; **108**: 1917-1922 [PMID: 7539389 DOI: 10.1016/0016-5085(95)90158-2]
91 **Sherman S**, Alazmi WM, Lehman GA, Geenen JE, Chuttani R, Kozarek RA, Welch WD, Souza S, Pribble J; rPAF-AH ERCP Study Group. Evaluation of recombinant platelet-activating factor acetylhydrolase for reducing the incidence and severity of post-ERCP acute pancreatitis. *Gastrointest Endosc* 2009; **69**: 462-472 [PMID: 19231487 DOI: 10.1016/j.gie.2008.07.040]
92 **Jaworek J**, Konturek SJ. Effects of prostaglandin of E, F and I series, leukotriene C and platelet activating factor on amylase release from isolated rat pancreatic acini. *J Physiol Pharmacol* 1993; **44**: 365-381 [PMID: 7510150]
93 **Hofbauer B**, Saluja AK, Bhatia M, Frossard JL, Lee HS, Bhagat L, Steer ML. Effect of recombinant platelet-activating factor acetylhydrolase on two models of experimental acute pancreatitis. *Gastroenterology* 1998; **115**: 1238-1247 [PMID: 9797380 DOI: 10.1016/S0016-5085(98)70096-4]
94 **Mariani A**, Giussani A, Di Leo M, Testoni S, Testoni PA. Guidewire biliary cannulation does not reduce post-ERCP pancreatitis compared with the contrast injection technique in low-risk and high-risk patients. *Gastrointest Endosc* 2012; **75**: 339-346 [PMID: 22075192 DOI: 10.1016/j.gie.2011.09.002]
95 **Devereaux BM**, Fein S, Purich E, Trout JR, Lehman GA, Fogel EL, Phillips S, Etemad R, Jowell P, Toskes PP, Sherman S. A new synthetic porcine secretin for facilitation of cannulation of the dorsal pancreatic duct at ERCP in patients with pancreas divisum: a multicenter, randomized, double-blind comparative study. *Gastrointest Endosc* 2003; **57**: 643-647 [PMID: 12709690 DOI: 10.1067/mge.2003.195]
96 **Jowell PS**, Branch MS, Fein SH, Purich ED, Kilaru R, Robuck G, d'Almada P, Baillie J. Intravenous synthetic secretin reduces the incidence of pancreatitis induced by endoscopic retrograde cholangiopancreatography. *Pancreas* 2011; **40**: 533-539 [PMID: 21499206 DOI: 10.1097/MPA.0b013e3182152eb6]
97 **Fang J**, Wang SL, Zhao SB, Hu LH, Yao J, Shen Z, Gu L, Xia T, Cai Q, Li ZS, Bai Y. Impact of intraduodenal acetic acid infusion on pancreatic duct cannulation during endoscopic retrograde cholangiopancreatography: A double-blind, randomized controlled trial. *J Gastroenterol Hepatol* 2018; **33**: 1804-1810 [PMID: 29633339 DOI: 10.1111/jgh.14148]
98 **Brodows RG**, Chey WY. Physiological doses of secretin do not stimulate acute insulin release. *J Clin Endocrinol Metab* 1980; **50**: 603-606 [PMID: 6987259 DOI: 10.1210/jcem-50-3-603]
99 **Kilander AF**, Hanssen LE, Gillberg RE. Secretin release in coeliac disease. Plasma secretin concentration and bicarbonate output to the duodenum after intraduodenal acid infusion in coeliac patients before and after treatment. *Scand J Gastroenterol* 1983; **18**: 765-769 [PMID: 6669941 DOI: 10.3109/00365528309182092]
100 **Nøjgaard C**, Hornum M, Elkjaer M, Hjalmarsson C, Heyries L, Hauge T, Bakkevold K, Andersen PK, Matzen P; European Post-ERCP Pancreatitis Preventing Study Group. Does glyceryl nitrate prevent post-ERCP pancreatitis? A prospective, randomized, double-blind, placebo-controlled multicenter trial. *Gastrointest Endosc* 2009; **69**: e31-e37 [PMID: 19410035 DOI: 10.1016/j.gie.2008.11.042]
101 **Staritz M**, Poralla T, Ewe K, Meyer zum Büschenfelde KH. Effect of glyceryl trinitrate on the sphincter of Oddi motility and baseline pressure. *Gut* 1985; **26**: 194-197 [PMID: 3917965 DOI: 10.1136/gut.26.2.194]
102 **Kaufman HS**, Shermak MA, May CA, Pitt HA, Lillemoe KD. Nitric oxide inhibits resting sphincter of Oddi activity. *Am J Surg* 1993; **165**: 74-80 [PMID: 7678190 DOI: 10.1016/S0002-9610(05)80407-6]
103 **Luman W**, Pryde A, Heading RC, Palmer KR. Topical glyceryl trinitrate relaxes the sphincter of Oddi. *Gut* 1997; **40**: 541-543 [PMID: 9176086 DOI: 10.1136/gut.40.4.541]
104 **Carr-Locke DL**, Gregg JA, Aoki TT. Effects of exogenous glucagon on pancreatic and biliary ductal and sphincteric pressures in man demonstrated by endoscopic manometry and correlation with plasma glucagon. *Dig Dis Sci* 1983; **28**: 312-320 [PMID: 6831995 DOI: 10.1007/BF01324947]
105 **Lahoti S**, Catalano MF, Geenen JE, Hogan WJ. A prospective, double-blind trial of L-hyoscyamine versus glucagon for the inhibition of small intestinal motility during ERCP. *Gastrointest Endosc* 1997; **46**: 139-142 [PMID: 9283863 DOI: 10.1016/S0016-5107(97)70061-0]
106 **Katsinelos P**, Lazaraki G, Chatzimavroudis G, Katsinelos T, Georgakis N, Anastasiadou K, Gatopoulou A, Zeglinas C, Psarras K, Kountouras J. Impact of nitroglycerin and glucagon administration on selective common bile duct cannulation and prevention of post-ERCP pancreatitis. *Scand J Gastroenterol* 2017; **52**: 50-55 [PMID: 27690682 DOI: 10.1080/00365521.2016.1228117]
107 **Oh HC**, Cheon YK, Cho YD, Do JH. Use of udenafil is not associated with a reduction in post-ERCP pancreatitis: results of a randomized, placebo-controlled, multicenter trial. *Gastrointest Endosc* 2011; **74**: 556-562 [PMID: 21802079 DOI: 10.1016/j.gie.2011.04.047]
108 **Cheon YK**, Cho YD, Moon JH, Im HH, Jung Y, Lee JS, Lee MS, Shim CS. Effects of vardenafil, a phosphodiesterase type-5 inhibitor, on sphincter of Oddi motility in patients with suspected biliary sphincter of Oddi dysfunction. *Gastrointest Endosc* 2009; **69**: 1111-1116 [PMID: 19243765 DOI: 10.1016/j.gie.2008.07.014]

109 **Lee TY**, Choi JS, Oh HC, Song TJ, Do JH, Cheon YK. Oral udenafil and aceclofenac for the prevention of post-endoscopic retrograde cholangiopancreatography pancreatitis in high-risk patients: a randomized multicenter study. *Korean J Intern Med* 2015; **30**: 602-609 [PMID: 26354054 DOI: 10.3904/kjim.2015.30.5.602]

110 **Kubiliun NM**, Adams MA, Akshintala VS, Conte ML, Cote GA, Cotton PB, Dumonceau JM, Elta GH, Fogel EL, Freeman ML, Lehman GA, Naveed M, Romagnuolo J, Scheiman JM, Sherman S, Singh VK, Elmunzer BJ; United States Cooperative for Outcomes Research in Endoscopy (USCORE). Evaluation of Pharmacologic Prevention of Pancreatitis After Endoscopic Retrograde Cholangiopancreatography: A Systematic Review. *Clin Gastroenterol Hepatol* 2015; **13**: 1231-9; quiz e70-1 [PMID: 25579870 DOI: 10.1016/j.cgh.2014.11.038]

111 **Fujii S**, Hitomi Y. New synthetic inhibitors of C1r, C1 esterase, thrombin, plasmin, kallikrein and trypsin. *Biochim Biophys Acta* 1981; **661**: 342-345 [PMID: 6271224]

112 **Keck T**, Balcom JH, Antoniu BA, Lewandrowski K, Warshaw AL, Fernández-del Castillo CF. Regional effects of nafamostat, a novel potent protease and complement inhibitor, on severe necrotizing pancreatitis. *Surgery* 2001; **130**: 175-181 [PMID: 11490346 DOI: 10.1067/msy.2001.115827]

113 **Masci E**, Cavallini G, Mariani A, Frulloni L, Testoni PA, Curioni S, Tittobello A, Uomo G, Costamagna G, Zambelli S, Macarri G, Innocenti P, Dragonetti C, Gabexate in Digestive Endoscopy-Italian Group II. Comparison of two dosing regimens of gabexate in the prophylaxis of post-ERCP pancreatitis. *Am J Gastroenterol* 2003; **98**: 2182-2186 [PMID: 14572565 DOI: 10.1111/j.1572-0241.2003.07698.x]

114 **Ohuchida J**, Chijiiwa K, Imamura N, Nagano M, Hiyoshi M. Randomized controlled trial for efficacy of nafamostat mesilate in preventing post-endoscopic retrograde cholangiopancreatography pancreatitis. *Pancreas* 2015; **44**: 415-421 [PMID: 25479585 DOI: 10.1097/MPA.0000000000000278]

115 **Park KT**, Kang DH, Choi CW, Cho M, Park SB, Kim HW, Kim DU, Chung CW, Yoon KT. Is high-dose nafamostat mesilate effective for the prevention of post-ERCP pancreatitis, especially in high-risk patients? *Pancreas* 2011; **40**: 1215-1219 [PMID: 21775918 DOI: 10.1097/MPA.0b013e31822116d5]

116 **Choi CW**, Kang DH, Kim GH, Eum JS, Lee SM, Song GA, Kim DU, Kim ID, Cho M. Nafamostat mesylate in the prevention of post-ERCP pancreatitis and risk factors for post-ERCP pancreatitis. *Gastrointest Endosc* 2009; **69**: e11-e18 [PMID: 19327467 DOI: 10.1016/j.gie.2008.10.046]

117 **Yoo KS**, Huh KR, Kim YJ, Kim KO, Park CH, Hahn T, Park SH, Kim JH, Park CK, Kwon YJ, Lehman GA. Nafamostat mesilate for prevention of post-endoscopic retrograde cholangiopancreatography pancreatitis: a prospective, randomized, double-blind, controlled trial. *Pancreas* 2011; **40**: 181-186 [PMID: 21206331 DOI: 10.1097/MPA.0b013e3181f94d46]

118 **Kim SJ**, Kang DH, Kim HW, Choi CW, Park SB, Song BJ, Nam HS. A Randomized Comparative Study of 24- and 6-Hour Infusion of Nafamostat Mesilate for the Prevention of Post-Endoscopic Retrograde Cholangiopancreatography Pancreatitis: A Prospective Randomized Comparison Trial. *Pancreas* 2016; **45**: 1179-1183 [PMID: 27253231 DOI: 10.1097/MPA.0000000000000625]

119 **Yoo JW**, Ryu JK, Lee SH, Woo SM, Park JK, Yoon WJ, Lee JK, Lee KH, Hwang JH, Kim YT, Yoon YB. Preventive effects of ulinastatin on post-endoscopic retrograde cholangiopancreatography pancreatitis in high-risk patients: a prospective, randomized, placebo-controlled trial. *Pancreas* 2008; **37**: 366-370 [PMID: 18953247 DOI: 10.1097/MPA.0b013e31817f528f]

120 **Chen CC**, Wang SS, Lee FY. Action of antiproteases on the inflammatory response in acute pancreatitis. *JOP* 2007; **8**: 488-494 [PMID: 17625305]

121 **Park JY**, Jeon TJ, Hwang MW, Sinn DH, Oh TH, Shin WC, Choi WC. Comparison between ulinastatin and nafamostat for prevention of post-endoscopic retrograde cholangiopancreatography complications: a prospective, randomized trial. *Pancreatology* 2014; **14**: 263-267 [PMID: 25062874 DOI: 10.1016/j.pan.2014.03.022]

122 **Oguchi H**, Terashima M, Koiwai T, Kawa S, Furuta S, Kobayashi M, Homma T. Effects of the S2-serotonergic receptor antagonist, ketanserin, on cerulein-induced pancreatitis in the rat. *Life Sci* 1992; **50**: 733-737 [PMID: 1371180 DOI: 10.1016/0024-3205(92)90476-6]

123 **Yoshino T**, Yamaguchi I. Possible involvement of 5-HT2 receptor activation in aggravation of diet-induced acute pancreatitis in mice. *J Pharmacol Exp Ther* 1997; **283**: 1495-1502 [PMID: 9400026]

124 **Ogawa T**, Sugidachi A, Tanaka N, Fujimoto K, Fukushige J, Tani Y, Asai F. Effects of R-102444 and its active metabolite R-96544, selective 5-HT2A receptor antagonists, on experimental acute and chronic pancreatitis: Additional evidence for possible involvement of 5-HT2A receptors in the development of experimental pancreatitis. *Eur J Pharmacol* 2005; **521**: 156-163 [PMID: 16183055 DOI: 10.1016/j.ejphar.2005.08.033]

125 **Tsujino T**, Isayama H, Nakai Y, Ito Y, Togawa O, Toda N, Arizumi T, Kogure H, Yamamoto K, Mizuno S, Yashima Y, Yagioka H, Sasaki T, Matsubara S, Yamamoto N, Hirano K, Sasahira N, Tada M, Koike K. The results of the Tokyo trial of prevention of post-ERCP pancreatitis with risperidone (Tokyo P3R): a multicenter, randomized, phase II, non-placebo-controlled trial. *J Gastroenterol* 2013; **48**: 982-988 [PMID: 23090004 DOI: 10.1007/s00535-012-0698-5]

126 **Hamada K**, Yoshida M, Isayama H, Yagi Y, Kanazashi S, Kashihara Y, Takeuchi K, Yamaguchi I. Possible involvement of endogenous 5-HT in aggravation of cerulein-induced acute pancreatitis in mice. *J Pharmacol Sci* 2007; **105**: 240-250 [PMID: 17965538 DOI: 10.1254/jphs.FP0071049]

127 **Bai Y**, Ren X, Zhang XF, Lv NH, Guo XG, Wan XJ, Nie ZG, Han ST, Bie P, Tian DA, Ji M, Li ZS. Prophylactic somatostatin can reduce incidence of post-ERCP pancreatitis: multicenter randomized controlled trial. *Endoscopy* 2015; **47**: 415-420 [PMID: 25590178 DOI: 10.1055/s-0034-1391227]

128 **Abdel Aziz AM**, Lehman GA. Pancreatitis after endoscopic retrograde cholangio-pancreatography. *World J Gastroenterol* 2007; **13**: 2655-2668 [PMID: 17569133 DOI: 10.3748/wjg.v13.i19.2655]

129 **Concepción-Martín M**, Gómez-Oliva C, Juanes A, Díez X, Prieto-Alhambra D, Torras X, Sainz S, Villanueva C, Farre A, Guarner-Argente C, Guarner C. Somatostatin for prevention of post-ERCP pancreatitis: a randomized, double-blind trial. *Endoscopy* 2014; **46**: 851-856 [PMID: 24977398 DOI: 10.1055/s-0034-1377306]

130 **Wang ZK**, Yang YS, Cai FC, Wang YH, Shi XL, Ding C, Li W. Is prophylactic somatostatin effective to prevent post-endoscopic retrograde cholangiopancreatography pancreatitis or hyperamylasemia? A randomized, placebo-controlled pilot trial. *Chin Med J (Engl)* 2013; **126**: 2403-2408 [PMID: 23823808]

131 **Li ZF**, Xia XM, Huang C, Zhang S, Zhang J, Zhang AJ. Emodin and baicalein inhibit pancreatic stromal derived factor-1 expression in rats with acute pancreatitis. *Hepatobiliary Pancreat Dis Int* 2009; **8**: 201-208 [PMID: 19357036]

132 **Xia XM**, Wang FY, Wang ZK, Wan HJ, Xu WA, Lu H. Emodin enhances alveolar epithelial barrier function in rats with experimental acute pancreatitis. *World J Gastroenterol* 2010; **16**: 2994-3001 [PMID:

20572302 DOI: 10.3748/wjg.v16.i24.2994]
133 **Wang C**, Li Q, Ye P, Zeng S, Li GH, Chen YX, Zhou XJ, Lv NH. Value of Raw Rhubarb Solution in the Precaution of Post-endoscopic Retrograde Cholangiopancreatography Pancreatitis in Patients with High-Risk Factors: A Predictive Random Compared Research in One Center. *Dig Dis Sci* 2017; **62**: 1043-1050 [PMID: 28194668]
134 **Nordback IH**, Cameron JL. The mechanism of conversion of xanthine dehydrogenase to xanthine oxidase in acute pancreatitis in the canine isolated pancreas preparation. *Surgery* 1993; **113**: 90-97 [PMID: 8417495]
135 **Abbasinazari M**, Mohammad Alizadeh AH, Moshiri K, Pourhoseingholi MA, Zali MR. Does allopurinol prevent post endoscopic retrograde cholangio- pancreatography pancreatitis? A randomized double blind trial. *Acta Med Iran* 2011; **49**: 579-583 [PMID: 22052140]
136 **Shah TU**, Liddle R, Branch MS, Jowell P, Obando J, Poleski M. Pilot study of aprepitant for prevention of post-ERCP pancreatitis in high risk patients: a phase II randomized, double-blind placebo controlled trial. *JOP* 2012; **13**: 514-518 [PMID: 22964958 DOI: 10.6092/1590-8577/855]
137 **Nathan JD**, Patel AA, McVey DC, Thomas JE, Prpic V, Vigna SR, Liddle RA. Capsaicin vanilloid receptor-1 mediates substance P release in experimental pancreatitis. *Am J Physiol Gastrointest Liver Physiol* 2001; **281**: G1322-G1328 [PMID: 11668042 DOI: 10.1152/ajpgi.2001.281.5.G1322]
138 **Nathan JD**, Peng RY, Wang Y, McVey DC, Vigna SR, Liddle RA. Primary sensory neurons: a common final pathway for inflammation in experimental pancreatitis in rats. *Am J Physiol Gastrointest Liver Physiol* 2002; **283**: G938-G946 [PMID: 12223354 DOI: 10.1152/ajpgi.00105.2002]
139 **Bhatia M**, Saluja AK, Hofbauer B, Frossard JL, Lee HS, Castagliuolo I, Wang CC, Gerard N, Pothoulakis C, Steer ML. Role of substance P and the neurokinin 1 receptor in acute pancreatitis and pancreatitis-associated lung injury. *Proc Natl Acad Sci U S A* 1998; **95**: 4760-4765 [PMID: 9539812 DOI: 10.1073/pnas.95.8.4760]
140 **Baluk P**, Bertrand C, Geppetti P, McDonald DM, Nadel JA. NK1 receptors mediate leukocyte adhesion in neurogenic inflammation in the rat trachea. *Am J Physiol* 1995; **268**: L263-L269 [PMID: 7864147 DOI: 10.1152/ajplung.1995.268.2.L263]
141 **He ZJ**, Winston JH, Yusuf TE, Micci MA, Elfert A, Xiao SY, Pasricha PJ. Intraductal administration of an NK1 receptor antagonist attenuates the inflammatory response to retrograde infusion of radiological contrast in rats: implications for the pathogenesis and prevention of ERCP-induced pancreatitis. *Pancreas* 2003; **27**: e13-e17 [PMID: 12826913 DOI: 10.1097/00006676-200307000-00018]
142 **Ohashi A**, Tamada K, Tomiyama T, Wada S, Higashizawa T, Gotoh Y, Satoh Y, Miyata T, Tano S, Ido K, Sugano K. Epinephrine irrigation for the prevention of pancreatic damage after endoscopic balloon sphincteroplasty. *J Gastroenterol Hepatol* 2001; **16**: 568-571 [PMID: 11350556 DOI: 10.1046/j.1440-1746.2001.02483.x]
143 **Xu LH**, Qian JB, Gu LG, Qiu JW, Ge ZM, Lu F, Wang YM, Li YM, Lu HS. Prevention of post-endoscopic retrograde cholangiopancreatography pancreatitis by epinephrine sprayed on the papilla. *J Gastroenterol Hepatol* 2011; **26**: 1139-1144 [PMID: 21392105 DOI: 10.1111/j.1440-1746.2011.06718.x]
144 **Ohno T**, Katori M, Nishiyama K, Saigenji K. Direct observation of microcirculation of the basal region of rat gastric mucosa. *J Gastroenterol* 1995; **30**: 557-564 [PMID: 8574325 DOI: 10.1007/BF02367779]
145 **Xu XD**, Dai JJ, Qian JQ, Wang WJ. Nasobiliary drainage after endoscopic papillary balloon dilatation may prevent postoperative pancreatitis. *World J Gastroenterol* 2015; **21**: 2443-2449 [PMID: 25741153 DOI: 10.3748/wjg.v21.i8.2443]
146 **Ricci E**, Conigliaro R, Bertoni G, Mortilla MG, Bedogni G, Contini S. Nasobiliary drainage following endoscopic sphincterotomy. A useful method of preventing and treating early complications. *Surg Endosc* 1987; **1**: 147-150 [PMID: 3503371 DOI: 10.1007/BF00590920]
147 **Lee DW**, Chan AC, Lam YH, Ng EK, Lau JY, Law BK, Lai CW, Sung JJ, Chung SC. Biliary decompression by nasobiliary catheter or biliary stent in acute suppurative cholangitis: a prospective randomized trial. *Gastrointest Endosc* 2002; **56**: 361-365 [PMID: 12196773 DOI: 10.1016/S0016-5107(02)70039-4]
148 **Sharma BC**, Kumar R, Agarwal N, Sarin SK. Endoscopic biliary drainage by nasobiliary drain or by stent placement in patients with acute cholangitis. *Endoscopy* 2005; **37**: 439-443 [PMID: 15844022 DOI: 10.1055/s-2005-861054]
149 **Park SY**, Park CH, Cho SB, Yoon KW, Lee WS, Kim HS, Choi SK, Rew JS. The safety and effectiveness of endoscopic biliary decompression by plastic stent placement in acute suppurative cholangitis compared with nasobiliary drainage. *Gastrointest Endosc* 2008; **68**: 1076-1080 [PMID: 18635173 DOI: 10.1016/j.gie.2008.04.025]
150 **Huang Q**, Shao F, Wang C, Qi W, Qiu LJ, Liu Z. Nasobiliary drainage can reduce the incidence of post-ERCP pancreatitis after papillary large balloon dilation plus endoscopic biliary sphincterotomy: a randomized controlled trial. *Scand J Gastroenterol* 2018; **53**: 114-119 [PMID: 29043861 DOI: 10.1080/00365521.2017.1391329]
151 **Lee TH**, Moon JH, Choi HJ, Han SH, Cheon YK, Cho YD, Park SH, Kim SJ. Prophylactic temporary 3F pancreatic duct stent to prevent post-ERCP pancreatitis in patients with a difficult biliary cannulation: a multicenter, prospective, randomized study. *Gastrointest Endosc* 2012; **76**: 578-585 [PMID: 22771100 DOI: 10.1016/j.gie.2012.05.001]
152 **Sofuni A**, Maguchi H, Mukai T, Kawakami H, Irisawa A, Kubota K, Okaniwa S, Kikuyama M, Kutsumi H, Hanada K, Ueki T, Itoi T. Endoscopic pancreatic duct stents reduce the incidence of post-endoscopic retrograde cholangiopancreatography pancreatitis in high-risk patients. *Clin Gastroenterol Hepatol* 2011; **9**: 851-8; quiz e110 [PMID: 21749851 DOI: 10.1016/j.cgh.2011.06.033]
153 **Pan XP**, Dang T, Meng XM, Xue KC, Chang ZH, Zhang YP. Clinical study on the prevention of post-ERCP pancreatitis by pancreatic duct stenting. *Cell Biochem Biophys* 2011; **61**: 473-479 [PMID: 21739262 DOI: 10.1007/s12013-011-9230-4]
154 **Ito K**, Fujita N, Noda Y, Kobayashi G, Obana T, Horaguchi J, Takasawa O, Koshita S, Kanno Y, Ogawa T. Can pancreatic duct stenting prevent post-ERCP pancreatitis in patients who undergo pancreatic duct guidewire placement for achieving selective biliary cannulation? A prospective randomized controlled trial. *J Gastroenterol* 2010; **45**: 1183-1191 [PMID: 20607310 DOI: 10.1007/s00535-010-0268-7]
155 **Kawaguchi Y**, Ogawa M, Omata F, Ito H, Shimosegawa T, Mine T. Randomized controlled trial of pancreatic stenting to prevent pancreatitis after endoscopic retrograde cholangiopancreatography. *World J Gastroenterol* 2012; **18**: 1635-1641 [PMID: 22529693 DOI: 10.3748/wjg.v18.i14.1635]
156 **Fujisawa T**, Kagawa K, Ochiai K, Hisatomi K, Kubota K, Sato H, Nakajima A, Matsuhashi N. Prophylactic Efficacy of 3- or 5-cm Pancreatic Stents for Preventing Post-ERCP Pancreatitis: A

Prospective, Randomized Trial. *J Clin Gastroenterol* 2016; **50**: e30-e34 [PMID: 26280707 DOI: 10.1097/MCG.0000000000000397]
157 **Conigliaro R**, Manta R, Bertani H, Manno M, Barbera C, Caruso A, Olivetti G, Melotti G, Frazzoni M. Pancreatic duct stenting for the duration of ERCP only does not prevent pancreatitis after accidental pancreatic duct cannulation: a prospective randomized trial. *Surg Endosc* 2013; **27**: 569-574 [PMID: 22926890 DOI: 10.1007/s00464-012-2487-x]
158 **Kobayashi G**, Fujita N, Imaizumi K, Irisawa A, Suzuki M, Murakami A, Oana S, Makino N, Komatsuda T, Yoneyama K. Wire-guided biliary cannulation technique does not reduce the risk of post-ERCP pancreatitis: multicenter randomized controlled trial. *Dig Endosc* 2013; **25**: 295-302 [PMID: 23368891 DOI: 10.1111/j.1443-1661.2012.01372.x]
159 **Lella F**, Bagnolo F, Colombo E, Bonassi U. A simple way of avoiding post-ERCP pancreatitis. *Gastrointest Endosc* 2004; **59**: 830-834 [PMID: 15173796 DOI: 10.1016/S0016-5107(04)00363-3]
160 **Artifon EL**, Sakai P, Cunha JE, Halwan B, Ishioka S, Kumar A. Guidewire cannulation reduces risk of post-ERCP pancreatitis and facilitates bile duct cannulation. *Am J Gastroenterol* 2007; **102**: 2147-2153 [PMID: 17581267 DOI: 10.1111/j.1572-0241.2007.01378.x]
161 **Lee TH**, Park DH, Park JY, Kim EO, Lee YS, Park JH, Lee SH, Chung IK, Kim HS, Park SH, Kim SJ. Can wire-guided cannulation prevent post-ERCP pancreatitis? A prospective randomized trial. *Gastrointest Endosc* 2009; **69**: 444-449 [PMID: 19007927 DOI: 10.1016/j.gie.2008.04.064]
162 **Bassan MS**, Sundaralingam P, Fanning SB, Lau J, Menon J, Ong E, Rerknimitr R, Seo DW, Teo EK, Wang HP, Reddy DN, Goh KL, Bourke MJ. The impact of wire caliber on ERCP outcomes: a multicenter randomized controlled trial of 0.025-inch and 0.035-inch guidewires. *Gastrointest Endosc* 2018; **87**: 1454-1460 [PMID: 29317269 DOI: 10.1016/j.gie.2017.11.037]
163 **Lee A**, Lee LS. Training in Endoscopic Retrograde Cholangiopancreatography. *Lee LS, editor ERCP and EUS: A Case-Based Approach*. New York: Springer 2015; 3-18
164 **Swan MP**, Alexander S, Moss A, Williams SJ, Ruppin D, Hope R, Bourke MJ. Needle knife sphincterotomy does not increase the risk of pancreatitis in patients with difficult biliary cannulation. *Clin Gastroenterol Hepatol* 2013; **11**: 430-436.e1 [PMID: 23313840 DOI: 10.1016/j.cgh.2012.12.017]
165 **Ilone S**, Fauzi A. Diagnostic and Prevention Approach in Post Endoscopic Retrograde Cholangiopancreatography Pancreatitis. *Indones J Gastroenterol Hepatol Digest Endosc* 2016; **17**: 188-193 [DOI: 10.24871/1732016188-193]

Published By Baishideng Publishing Group Inc
7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: bpgoffice@wjgnet.com
Help Desk:http://www.f6publishing.com/helpdesk
http://www.wjgnet.com