ISSN 1948-5182 (online)

World Journal of *Hepatology*

World J Hepatol 2016 July 28; 8(21): 863-914

Published by Baishideng Publishing Group Inc

World Journal of Hepatology

A peer-reviewed, online, open-access journal of hepatology

Editorial Board

2014-2017

The *World Journal of Hepatology* Editorial Board consists of 474 members, representing a team of worldwide experts in hepatology. They are from 52 countries, including Algeria (1), Argentina (6), Armenia (1), Australia (2), Austria (4), Bangladesh (2), Belgium (3), Botswana (2), Brazil (13), Bulgaria (2), Canada (3), Chile (1), China (97), Czech Repoublic (1), Denmark (2), Egypt (12), France (6), Germany (20), Greece (11), Hungary (5), India (15), Indonesia (3), Iran (4), Israel (1), Italy (54), Japan (35), Jordan (1), Malaysia (2), Mexico (3), Moldova (1), Netherlands (3), Nigeria (1), Pakistan (1), Philippines (2), Poland (1), Portugal (2), Qatar (1), Romania (6), Russia (2), Saudi Arabia (4), Singapore (1), South Korea (12), Spain (20), Sri Lanka (1), Sudan (1), Sweden (1), Switzerland (1), Thailand (4), Turkey (21), Ukraine (3), United Kingdom (18), and United States (55).

Chile

Luis A Videla, *Santiago*

China

Guang-Wen Cao, *Shanghai*
En-Qiang Chen, *Chengdu*
Gong-Ying Chen, *Hangzhou*
Jin-lian Chen, *Shanghai*
Jun Chen, *Changsha*
Alfred Cheng, *Hong Kong*
Chun-Ping Cui, *Beijing*
Shuang-Suo Dang, *Xi'an*
Ming-Xing Ding, *Jinhua*
Zhi-Jun Duang, *Dalian*
He-Bin Fan, *Wuhan*
Xiao-Ming Fan, *Shanghai*
James Yan Yue Fung, *Hong Kong*
Yi Gao, *Guangzhou*
Zuo-Jiong Gong, *Wuhan*
Zhi-Yong Guo, *Guangzhou*
Shao-Liang Han, *Wenzhou*
Tao Han, *Tianjin*
Jin-Yang He, *Guangzhou*
Ming-Liang He, *Hong Kong*
Can-Hua Huang, *Chengdu*
Bo Jin, *Beijing*
Shan Jin, *Hohhot*
Hui-Qing Jiang, *Shijiazhuang*
Wan-Yee Joseph Lau, *Hong Kong*
Guo-Lin Li, *Changsha*
Jin-Jun Li, *Shanghai*
Qiang Li, *Jinan*
Sheng Li, *Jinan*
Zong-Fang Li, *Xi'an*
Xu Li, *Guangzhou*
Xue-Song Liang, *Shanghai*
En-Qi Liu, *Xi'an*
Pei Liu, *Shenyang*
Zhong-Hui Liu, *Changchun*
Guang-Hua Luo, *Changzhou*
Yi Lv, *Xi'an*
Guang-Dong Pan, *Liuzhou*
Wen-Sheng Pan, *Hangzhou*
Jian-Min Qin, *Shanghai*
Wai-Kay Seto, *Hong Kong*
Hong Shen, *Changsha*
Xiao Su, *Shanghai*
Li-Ping Sun, *Beijing*
Wei-Hao Sun, *Nanjing*
Xue-Ying Sun, *Harbin*
Hua Tang, *Tianjin*
Ling Tian, *Shanghai*
Eric Tse, *Hong Kong*
Guo-Ying Wang, *Changzhou*
Yue Wang, *Beijing*
Shu-Qiang Wang, *Chengdu*
Mary MY Waye, *Hong Kong*
Hong-Shan Wei, *Beijing*
Danny Ka-Ho Wong, *Hong Kong*
Grace Lai-Hung Wong, *Hong Kong*
Bang-Fu Wu, *Dongguan*
Xiong-Zhi Wu, *Tianjin*
Chun-Fang Xu, *Suzhou*
Rui-An Xu, *Quanzhou*
Rui-Yun Xu, *Guangzhou*
Wei-Li Xu, *Shijiazhuang*
Shi-Ying Xuan, *Qingdao*
Ming-Xian Yan, *Jinan*
Lv-Nan Yan, *Chengdu*
Jin Yang, *Hangzhou*
Ji-Hong Yao, *Dalian*
Winnie Yeo, *Hong Kong*
Zheng Zeng, *Beijing*
Qi Zhang, *Hangzhou*
Shi-Jun Zhang, *Guangzhou*
Xiao-Lan Zhang, *Shijiazhuang*
Xiao-Yong Zhang, *Guangzhou*
Yong Zhang, *Xi'an*
Hong-Chuan Zhao, *Hefei*
Ming-Hua Zheng, *Wenzhou*
Yu-Bao Zheng, *Guangzhou*
Ren-Qian Zhong, *Shanghai*
Fan Zhu, *Wuhan*
Xiao Zhu, *Dongguan*

Czech Repoublic

Kamil Vyslouzil, *Olomouc*

Denmark

Henning Gronbaek, *Aarhus*
Christian Mortensen, *Hvidovre*

Egypt

Ihab T Abdel-Raheem, *Damanhour*
NGB G Bader EL Din, *Cairo*
Hatem Elalfy, *Mansoura*
Mahmoud M El-Bendary, *Mansoura*
Mona El SH El-Raziky, *Cairo*
Mohammad El-Sayed, *Cairo*
Yasser M Fouad, *Minia*
Mohamed AA Metwally, *Benha*
Hany Shehab, *Cairo*
Mostafa M Sira, *Shebin El-koom*
Ashraf Taye, *Minia*
MA Ali Wahab, *Mansoura*

France

Laurent Alric, *Toulouse*
Sophie Conchon, *Nantes*
Daniel J Felmlee, *Strasbourg*
Herve Lerat, *Creteil*
Dominique Salmon, *Paris*
Jean-Pierre Vartanian, *Paris*

Germany

Laura E Buitrago-Molina, *Hannover*
Enrico N De Toni, *Munich*
Oliver Ebert, *Muenchen*
Rolf Gebhardt, *Leipzig*
Janine V Hartl, *Regensburg*
Sebastian Hinz, *Kiel*
Benjamin Juntermanns, *Essen*
Roland Kaufmann, *Jena*
Viola Knop, *Frankfurt*
Veronika Lukacs-Kornek, *Homburg*
Benjamin Maasoumy, *Hannover*
Jochen Mattner, *Erlangen*
Nadja M Meindl-Beinker, *Mannheim*
Ulf P Neumann, *Aachen*
Margarete Odenthal, *Cologne*
Yoshiaki Sunami, *Munich*
Christoph Roderburg, *Aachen*
Frank Tacke, *Aachen*
Yuchen Xia, *Munich*

Greece

Alex P Betrosian, *Athens*
George N Dalekos, *Larissa*
Ioanna K Delladetsima, *Athens*
Nikolaos K Gatselis, *Larissa*
Stavros Gourgiotis, *Athens*
Christos G Savopoulos, *Thessaloniki*
Tania Siahanidou, *Athens*
Emmanouil Sinakos, *Thessaloniki*
Nikolaos G Symeonidi, *Thessaloniki*
Konstantinos C Thomopoulos, *Larissa*
Konstantinos Tziomalos, *Thessaloniki*

Hungary

Gabor Banhegyi, *Budapest*
Peter L Lakatos, *Budapest*
Maria Papp, *Debrecen*
Ferenc Sipos, *Budapest*
Zsolt J Tulassay, *Budapest*

India

Deepak N Amarapurkar, *Mumbai*
Girish M Bhopale, *Pune*
Sibnarayan Datta, *Tezpur*
Nutan D Desai, *Mumbai*
Sorabh Kapoor, *Mumbai*
Jaswinder S Maras, *New Delhi*
Nabeen C Nayak, *New Delhi*
C Ganesh Pai, *Manipal*
Amit Pal, *Chandigarh*
K Rajeshwari, *New Delhi*
Anup Ramachandran, *Vellore*
D Nageshwar Reddy, *Hyderabad*
Shivaram P Singh, *Cuttack*
Ajith TA, *Thrissur*
Balasubramaniyan Vairappan, *Pondicherry*

Indonesia

Pratika Yuhyi Hernanda, *Surabaya*
Cosmas RA Lesmana, *Jakarta*
Neneng Ratnasari, *Yogyakarta*

Iran

Seyed M Jazayeri, *Tehran*
Sedigheh Kafi-Abad, *Tehran*
Iradj Maleki, *Sari*
Fakhraddin Naghibalhossaini, *Shiraz*

Israel

Stephen DH Malnick, *Rehovot*

Italy

Francesco Angelico, *Rome*
Alfonso W Avolio, *Rome*
Francesco Bellanti, *Foggia*
Marcello Bianchini, *Modena*
Guglielmo Borgia, *Naples*
Mauro Borzio, *Milano*
Enrico Brunetti, *Pavia*
Valeria Cento, *Roma*
Beatrice Conti, *Rome*
Francesco D'Amico, *Padova*
Samuele De Minicis, *Fermo*
Fabrizio De Ponti, *Bologna*
Giovan Giuseppe Di Costanzo, *Napoli*
Luca Fabris, *Padova*
Giovanna Ferraioli, *Pavia*
Matteo Garcovich, *Rome*
Edoardo G Giannini, *Genova*
Rossano Girometti, *Udine*
Alessandro Granito, *Bologna*
Alberto Grassi, *Rimini*
Alessandro Grasso, *Savona*
Francesca Guerrieri, *Rome*
Quirino Lai, *Aquila*
Andrea Lisotti, *Bologna*
Marcello F Maida, *Palermo*
Lucia Malaguarnera, *Catania*
Andrea Mancuso, *Palermo*
Luca Maroni, *Ancona*
Francesco Marotta, *Milano*
Pierluigi Marzuillo, *Naples*
Sara Montagnese, *Padova*
Giuseppe Nigri, *Rome*
Claudia Piccoli, *Foggia*
Camillo Porta, *Pavia*
Chiara Raggi, *Rozzano (MI)*
Maria Rendina, *Bari*
Maria Ripoli, *San Giovanni Rotondo*
Kryssia I Rodriguez-Castro, *Padua*
Raffaella Romeo, *Milan*
Amedeo Sciarra, *Milano*
Antonio Solinas, *Sassari*
Aurelio Sonzogni, *Bergamo*
Giovanni Squadrito, *Messina*
Salvatore Sutti, *Novara*
Valentina Svicher, *Rome*
Luca Toti, *Rome*
Elvira Verduci, *Milan*
Umberto Vespasiani-Gentilucci, *Rome*
Maria A Zocco, *Rome*

Japan

Yasuhiro Asahina, *Tokyo*
Nabil AS Eid, *Takatsuki*
Kenichi Ikejima, *Tokyo*
Shoji Ikuo, *Kobe*
Yoshihiro Ikura, *Takatsuki*
Shinichi Ikuta, *Nishinomiya*
Kazuaki Inoue, *Yokohama*
Toshiya Kamiyama, *Sapporo*
Takanobu Kato, *Tokyo*
Saiho Ko, *Nara*
Haruki Komatsu, *Sakura*
Masanori Matsuda, *Chuo-city*
Yasunobu Matsuda, *Niigata*
Yoshifumi Nakayama, *Kitakyushu*
Taichiro Nishikawa, *Kyoto*
Satoshi Oeda, *Saga*
Kenji Okumura, *Urayasu*
Michitaka Ozaki, *Sapporo*
Takahiro Sato, *Sapporo*
Junichi Shindoh, *Tokyo*
Ryo Sudo, *Yokohama*
Atsushi Suetsugu, *Gifu*
Haruhiko Sugimura, *Hamamatsu*
Reiji Sugita, *Sendai*
Koichi Takaguchi, *Takamatsu*
Shinji Takai, *Takatsuki*
Akinobu Takaki, *Okayama*
Yasuhito Tanaka, *Nagoya*
Takuji Tanaka, *Gifu City*
Atsunori Tsuchiya, *Niigata*
Koichi Watashi, *Tokyo*
Hiroshi Yagi, *Tokyo*
Taro Yamashita, *Kanazawa*
Shuhei Yoshida, *Chiba*
Hitoshi Yoshiji, *Kashihara*

Jordan

Kamal E Bani-Hani, *Zarqa*

Malaysia

Peng Soon Koh, *Kuala Lumpur*
Yeong Yeh Lee, *Kota Bahru*

Mexico

Francisco J Bosques-Padilla, *Monterrey*
María de F Higuera-de la Tijera, *Mexico City*
José A Morales-Gonzalez, *México City*

Moldova

Angela Peltec, *Chishinev*

Netherlands

Wybrich R Cnossen, *Nijmegen*
Frank G Schaap, *Maastricht*
Fareeba Sheedfar, *Groningen*

Nigeria

CA Asabamaka Onyekwere, *Lagos*

Pakistan

Bikha Ram Devrajani, *Jamshoro*

Philippines

Janus P Ong, *Pasig*
JD Decena Sollano, *Manila*

Poland

Jacek Zielinski, *Gdansk*

Portugal

Rui T Marinho, *Lisboa*
Joao B Soares, *Braga*

Qatar

Reem Al Olaby, *Doha*

Romania

Bogdan Dorobantu, *Bucharest*
Liana Gheorghe, *Bucharest*
George S Gherlan, *Bucharest*
Romeo G Mihaila, *Sibiu*
Bogdan Procopet, *Cluj-Napoca*
Streba T Streba, *Craiova*

Russia

Anisa Gumerova, *Kazan*
Pavel G Tarazov, *St.Petersburg*

Saudi Arabia

Abdulrahman A Aljumah, *Riyadh*
Ihab MH Mahmoud, *Riyadh*
Ibrahim Masoodi, *Riyadh*
Mhoammad K Parvez, *Riyadh*

Singapore

Ser Yee Lee, *Singapore*

South Korea

Young-Hwa Chung, *Seoul*
Jeong Heo, *Busan*
Dae-Won Jun, *Seoul*
Bum-Joon Kim, *Seoul*
Do Young Kim, *Seoul*
Ji Won Kim, *Seoul*
Moon Young Kim, *Wonu*
Mi-Kyung Lee, *Suncheon*
Kwan-Kyu Park, *Daegu*
Young Nyun Park, *Seoul*
Jae-Hong Ryoo, *Seoul*
Jong Won Yun, *Kyungsan*

Spain

Ivan G Marina, *Madrid*

Juan G Acevedo, *Barcelona*
Javier Ampuero, *Sevilla*
Jaime Arias, *Madrid*
Andres Cardenas, *Barcelona*
Agustin Castiella, *Mendaro*
Israel Fernandez-Pineda, *Sevilla*
Rocio Gallego-Duran, *Sevilla*
Rita Garcia-Martinez, *Barcelona*
José M González-Navajas, *Alicante*
Juan C Laguna, *Barcelona*
Elba Llop, *Madrid*
Laura Ochoa-Callejero, *La Rioja*
Albert Pares, *Barcelona*
Sonia Ramos, *Madrid*
Francisco Rodriguez-Frias, *Córdoba*
Manuel L Rodriguez-Peralvarez, *Córdoba*
Marta R Romero, *Salamanca*
Carlos J Romero, *Madrid*
Maria Trapero-Marugan, *Madrid*

Sri Lanka

Niranga M Devanarayana, *Ragama*

Sudan

Hatim MY Mudawi, *Khartoum*

Sweden

Evangelos Kalaitzakis, *Lund*

Switzerland

Christoph A Maurer, *Liestal*

Thailand

Taned Chitapanarux, *Chiang mai*
Temduang Limpaiboon, *Khon Kaen*
Sith Phongkitkarun, *Bangkok*
Yong Poovorawan, *Bangkok*

Turkey

Osman Abbasoglu, *Ankara*
Mesut Akarsu, *Izmir*
Umit Akyuz, *Istanbul*
Hakan Alagozlu, *Sivas*
Yasemin H Balaban, *Istanbul*
Bulent Baran, *Van*
Mehmet Celikbilek, *Yozgat*
Levent Doganay, *Istanbul*
Fatih Eren, *Istanbul*
Abdurrahman Kadayifci, *Gaziantep*
Ahmet Karaman, *Kayseri*
Muhsin Kaya, *Diyarbakir*
Ozgur Kemik, *Van*
Serdar Moralioglu, *Uskudar*
A Melih Ozel, *Gebze - Kocaeli*
Seren Ozenirler, *Ankara*
Ali Sazci, *Kocaeli*
Goktug Sirin, *Kocaeli*
Mustafa Sunbul, *Samsun*
Nazan Tuna, *Sakarya*
Ozlem Yonem, *Sivas*

Ukraine

Rostyslav V Bubnov, *Kyiv*
Nazarii K Kobyliak, *Kyiv*
Igor N Skrypnyk, *Poltava*

United Kingdom

Safa Al-Shamma, *Bournemouth*
Jayantha Arnold, *Southall*
Marco Carbone, *Cambridge*
Rajeev Desai, *Birmingham*
Ashwin Dhanda, *Bristol*
Matthew Hoare, *Cambridge*
Stefan G Hubscher, *Birmingham*
Nikolaos Karidis, *London*
Lemonica J Koumbi, *London*
Patricia Lalor, *Birmingham*
Ji-Liang Li, *Oxford*
Evaggelia Liaskou, *Birmingham*
Rodrigo Liberal, *London*
Wei-Yu Lu, *Edinburgh*
Richie G Madden, *Truro*
Christian P Selinger, *Leeds*
Esther Una Cidon, *Bournemouth*
Feng Wu, *Oxford*

United States

Naim Alkhouri, *Cleveland*
Robert A Anders, *Baltimore*
Mohammed Sawkat Anwer, *North Grafton*
Kalyan Ram Bhamidimarri, *Miami*
Brian B Borg, *Jackson*
Ronald W Busuttil, *Los Angeles*
Andres F Carrion, *Miami*
Saurabh Chatterjee, *Columbia*
Disaya Chavalitdhamrong, *Gainesville*
Mark J Czaja, *Bronx*
Jonathan M Fenkel, *Philadelphia*
Catherine Frenette, *La Jolla*
Lorenzo Gallon, *Chicago*
Kalpana Ghoshal, *Columbus*
Hie-Won L Hann, *Philadelphia*
Shuang-Teng He, *Kansas City*
Wendong Huang, *Duarte*
Rachel Hudacko, *Suffern*
Lu-Yu Hwang, *Houston*
Ijaz S Jamall, *Sacramento*
Neil L Julie, *Bethesda*
Hetal Karsan, *Atlanta*
Ahmed O Kaseb, *Houston*
Zeid Kayali, *Pasadena*
Timothy R Koch, *Washington*
Gursimran S Kochhar, *Cleveland*
Steven J Kovacs, *East Hanover*
Mary C Kuhns, *Abbott Park*
Jiang Liu, *Silver Spring*
Li Ma, *Stanford*
Francisco Igor Macedo, *Southfield*
Sandeep Mukherjee, *Omaha*
Natalia A Osna, *Omaha*
Jen-Jung Pan, *Houston*
Christine Pocha, *Minneapolis*
Yury Popov, *Boston*
Davide Povero, *La Jolla*
Phillip Ruiz, *Miami*
Takao Sakai, *Cleveland*
Nicola Santoro, *New Haven*
Eva Schmelzer, *Pittsburgh*
Zhongjie Shi, *Philadelphia*
Nathan J Shores, *New Orleans*
Siddharth Singh, *Rochester*
Shailendra Singh, *Pittsburgh*
Veysel Tahan, *Iowa City*
Mehlika Toy, *Boston*
Hani M Wadei, *Jacksonville*
Gulam Waris, *North Chicago*
Ruliang Xu, *New York*
Jun Xu, *Los Angeles*
Matthew M Yeh, *Seattle*
Xuchen Zhang, *West Haven*
Lixin Zhu, *Buffalo*
Sasa Zivkovic, *Pittsburgh*

World Journal of Hepatology

Contents

Three issues per month Volume 8 Number 21 July 28, 2016

NAME OF JOURNAL
World Journal of Hepatology

ISSN
ISSN 1948-5182 (online)

LAUNCH DATE
October 31, 2009

FREQUENCY
36 Issues/Year (8th, 18th, and 28th of each month)

EDITORIAL OFFICE
Jin-Lei Wang, Director

Xiu-Xia Song, Vice Director
World Journal of Hepatology
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-59080039
Fax: +86-10-85381893
E-mail: editorialoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com

PUBLISHER
Baishideng Publishing Group Inc
8226 Regency Drive,
Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com

PUBLICATION DATE
July 28, 2016

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

ONLINE SUBMISSION
http://www.wjgnet.com/esps/

World Journal of Hepatology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4254/wjh.v8.i21.863

World J Hepatol 2016 July 28; 8(21): 863-873
ISSN 1948-5182 (online)

EDITORIAL

New horizon for radical cure of chronic hepatitis B virus infection

Kazuto Tajiri, Yukihiro Shimizu

Kazuto Tajiri, the Third Department of Internal Medicine, Toyama University Hospital, Toyama 930-0194, Japan

Yukihiro Shimizu, Gastroenterology Center, Nanto Municipal Hospital, Toyama 932-0211, Japan

Author contributions: Tajiri K and Shimizu Y wrote this paper; Shimizu Y conducted this work.

Conflict-of-interest statement: Tajiri K and Shimizu Y declare there is no conflict of interest related to this publication.

Manuscript source: Invited manuscript

Correspondence to: Yukihiro Shimizu, MD, PhD, Gastroenterology Center, Nanto Municipal Hospital, 938 Inami, Toyama 932-0211, Japan. rsf14240@nifty.com
Telephone: +81-76-3821475
Fax: +81-76-3821853

Received: March 27, 2016
Peer-review started: March 28, 2016
First decision: May 17, 2016
Revised: May 28, 2016
Accepted: June 27, 2016
Article in press: June 29, 2016
Published online: July 28, 2016

Abstract

About 250 to 350 million people worldwide are chronically infected with hepatitis B virus (HBV), and about 700000 patients per year die of HBV-related cirrhosis or hepatocellular carcinoma (HCC). Several anti-viral agents, such as interferon and nucleos(t)ide analogues (NAs), have been used to treat this disease. NAs especially have been shown to strongly suppress HBV replication, slowing the progression to cirrhosis and the development of HCC. However, reactivation of HBV replication often occurs after cessation of treatment, because NAs alone cannot completely remove covalently-closed circular DNA (cccDNA), the template of HBV replication, from the nuclei of hepatocytes. Anti-HBV immune responses, in conjunction with interferon-γ and tumor necrosis factor-α, were found to eliminate cccDNA, but complete eradication of cccDNA by immune response alone is difficult, as shown in patients who recover from acute HBV infection but often show long-term persistence of small amounts of HBV-DNA in the blood. Several new drugs interfering with the life cycle of HBV in hepatocytes have been developed, with drugs targeting cccDNA theoretically the most effective for radical cure of chronic HBV infection. However, the safety of these drugs should be extensively examined before application to patients, and combinations of several approaches may be necessary for radical cure of chronic HBV infection.

Key words: Covalently-closed circular DNA; Genome editing technology; Immune response; Immunotherapy; Program death-1; Interferon-γ; Tumor necrosis factor-α

Core tip: Among the agents used to treat chronic hepatitis B virus (HBV) infection are nucleos(t)ide analogues, which have been shown to strongly suppress HBV replication. HBV replication, however, may be reactivated after cessation of treatment, because complete removal of covalently-closed circular DNA (cccDNA) from hepatocyte nuclei is extremely difficult. Immune responses have been shown to destroy cccDNA, but immune response alone is insufficient for complete eradication of template DNA. Several drugs were

recently developed to block the HBV life cycle in hepatocytes, with drugs targeting cccDNA being, at least theoretically, the most effective for radical cure of chronic HBV infection. The safety of these agents should be extensively examined before their use in patients. Combinations of two or more classes of agent may be necessary for radical cure of chronic HBV infection.

Tajiri K, Shimizu Y. New horizon for radical cure of chronic hepatitis B virus infection. *World J Hepatol* 2016; 8(21): 863-873 Available from: URL: http://www.wjgnet.com/1948-5182/full/v8/i21/863.htm DOI: http://dx.doi.org/10.4254/wjh.v8.i21.863

INTRODUCTION

About 250 to 350 million people worldwide are chronically infected with hepatitis B virus (HBV)[1,2], with about 700000 patients per year dying from HBV-related cirrhosis or hepatocellular carcinoma (HCC)[3]. Several anti-viral agents, including interferons and nucleos(t)ide analogues (NAs), have been shown effective, with NA-based treatment strongly suppressing the replication of HBV-DNA and normalizing serum alanine aminotransferase activity, resulting in little or no progression of liver disease[4-6]. NAs target the viral reverse transcriptase, effectively reducing serum HBV-DNA concentrations. However, intrahepatic HBV-DNA, such as converted covalently closed circular DNA (cccDNA), is not a direct target of NAs. cccDNA is a template for all viral RNAs and HBV-DNA replication can be induced to start from residual cccDNA after cessation of treatment with NAs[7]. Small amounts of HBV-DNA can be found in serum long after patients recover from acute HBV infection, suggesting that cccDNA may persist for decades[8]. Thus, cccDNA is difficult to eradicate once infection is established, and should be the main target for the complete eradication of HBV infection. However, measuring intrahepatic cccDNA concentrations is difficult in a clinical setting[9]. The cccDNA levels in HBV-infected human hepatocytes are low, ranging from 1 to 50 copies per hepatocyte[10]. Real-time polymerase chain reaction (PCR) amplification with specific primers for cccDNA or Southern blotting can be used for the detection. However, PCR amplification may be hampered by other co-extracted viral DNA and Southern blotting needs much time and effort. Moreover, the form of cccDNA may be changed during the DNA extraction procedure. Therefore, further investigation should be required to establish the precise evaluation of intrahepatic cccDNA. As an alternative, the reduction in HBV surface antigen (HBsAg) concentration has been reported to partly reflect the decrease in intrahepatic cccDNA, with the goal of treatment for chronic HBV infection being the complete disappearance of HBsAg[11]. Fewer than 10% of patients receiving interferon-based therapy[4-6], and few patients treated with NAs[12,13], achieve complete loss of HBsAg. Various trials have tested agents targeting the life cycle of HBV in hepatocytes, including the elimination of cccDNA. This review summarizes and discusses the radical cure (Table 1) of chronic HBV infection, mainly focusing on the elimination of cccDNA.

HBV REPLICATION CYCLE AND THE PRODUCTION OF HBV-RELATED PROTEINS

HBV replication cycle

HBV is a DNA virus that belongs to the family *Hepadnaviridae*, with a 3.2 kb-long partially double-stranded relaxed circular DNA (rcDNA) genome[14]. The life cycle of HBV is shown in Figure 1. HBV virions are thought to enter hepatocytes through a high-affinity interaction between the myristoylated preS1 region of HBV and the surface structures of hepatocytes, including sodium taurocholate cotransporting polypeptide (NTCP)[15-17]. After entry into hepatocytes, uncoated rcDNA is released into the cytoplasm and then enters the nucleus, where it is converted to cccDNA. The cccDNA remains for a long time in the nucleus, where it serves as a template for the transcription of viral mRNA[17,18]. All viral RNAs, pregenomic RNAs (pgRNA) and RNAs encoding the surface proteins, precore and HBx of HBV, are transcribed from cccDNA, with efficient transcription regulated by liver-specific transcription factors[19] and the HBx protein itself[20]. Epigenetic control of cccDNA transcriptional activity, such as acetylation, methylation or phosphorylation, appears to occur[21]. Cytoplasmic pgRNA and polymerase protein are subsequently packaged into envelope proteins, with rcDNA produced from the reverse transcription of pgRNA. Nucleocapsids packaging rcDNA are encapsulated by HBsAg as the envelope protein and released from hepatocytes as virions. The precise understanding of these processes is important for the development of new strategies for the radical cure of chronic HBV infection.

HBV-related proteins and their roles in hepatocarcinogenesis

The HBV-related proteins translated from cccDNA consist not only of the envelope, core and polymerase proteins of HBV, but may play a role in hepatocarcinogenesis itself.

Studies analyzing the role of HBx proteins in hepatocellular transformation and HCC progression have found that low levels of HBx protein are present in non-tumor tissues of HBV-infected liver, whereas high levels of HBx protein are present in HCCs arising in HBV infected individuals, suggesting that this protein has an oncogenic function[22,23]. Moreover, HBx transgenic mice often develop liver cancer[24,25], and HBx protein has been found to accumulate in hepatocytes, affecting the expression of genes associated with signal transduction, cell cycle control, transcription, and immune response[23,26]. Expression of genes on the X-chromosome is regulated epigenetically, including by DNA and histone methyltransferases[27,28], and by microRNAs[29,30].

Table 1 Cure status of hepatitis B virus infection

	Serum HBV-DNA	Serum HBsAg	Intraheptic cccDNA	HBV-DNA-intergrated hepatocytes
Functional cure (clinical cure)	Low	(-)-(++)	(+)	(-)-(+)
Radical cure (virological cure)	(-)	(-)	(-)	(-)

HBV: Hepatitis B virus; cccDNA: Covalently-closed circular DNA; HBsAg: Hepatitis B virus surface antigen.

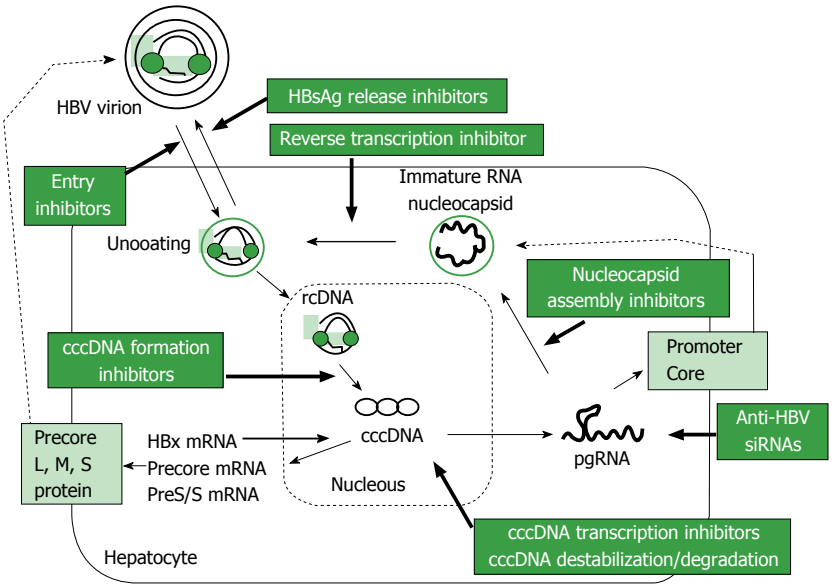

Figure 1 Simplified schema of the hepatitis B virus life cycle and possible targets of therapy. HBV: Hepatitis B virus; cccDNA: Covalently-closed circular DNA; HBsAg: Hepatitis B virus surface antigen; rcDNA: Relaxed circular DNA; siRNAs: Small interfering RNAs; pgRNA: Pregenomic RNAs.

HBx is not only involved in carcinogenesis but in the progression of HCC. HBx has been shown to increase beta-catenin signaling through epigenetic control or microRNA[31,32] and to be an independent predictor of survival after HCC resection[33].

HBsAg is also involved in hepatocarcinogenesis. The ground glass appearance of hepatocytes was shown to be a typical histological finding in HBV-infected livers, with this ground glass appearance resulting from the accumulation of HBsAg with preS mutations[34-36]. PreS-mutated HBsAg, especially large HBsAg, was found to accumulate in cytoplasm, leading to the induction of ER stress and oxidative DNA damage[35-37]. Furthermore preS mutations upregulated intracellular signaling *via* hepatocyte proliferation[35,38]. High serum HBsAg levels showed a definite correlation with HCC development in patients with controlled HBV-DNA[39-41]. Like HBV-related proteins, spliced HBV proteins were found to activate intracellular signaling *via* hepatocyte proliferation[42,43]. These findings suggest that not only HBV replication, but the production of HBV-related proteins, should be suppressed to efficiently prevent hepatocarcinogenesis.

IMMUNE RESPONSE AGAINST HBV INFECTION

Immune responses against HBV are involved in both the pathogenesis and control of HBV infection[44-47]. Therefore, understanding the immune response against HBV may result in better control of HBV infection.

Acute infection

Analysis of immune responses that occur during acute HBV infection may provide valuable information on strategies by which immune responses control HBV infection.

A mouse model of acute viral hepatitis B was established by injecting HBsAg-specific T-cell clones into HBV transgenic mice[48]. Although HBsAg-specific T-cells were found to kill small numbers of HBV-replicating hepatocytes, these T cell clones destroyed intracellular HBV-RNA and HBV-DNA in most infected hepatocytes without killing these cells. This effect was found to be due to interferon (IFN)-γ and tumor necrosis factor (TNF)-α[40,49-51]. Because HBV transgenic mice do not have cccDNA[52], the effects of these cytokines on cccDNA were unclear. In cccDNA-expressing cultured cells, however, IFN-γ and TNF-α inhibited HBV replication and reduced cccDNA in an additive manner[53]. Moreover, the decay of cccDNA was found to require activation of APOBEC3 deaminases[53], which are expressed in liver tissues of individuals with acute, but not chronic, HBV infection. These observations indicate that HBV-specific T-cell activation followed by treatment with anti-viral cytokines, such as IFN-γ and TNF-α, could eradicate HBV without cytolysis.

In a chimpanzee model, cccDNA was found to dis-

appear during the course of acute hepatitis B, and HBV-DNA was found to be susceptible to noncytolytic control by cytokines[54]. Moreover, HBV-DNA titers in these livers were reduced before T-cell influx, suggesting that non-T-cells, possibly natural killer cells, may have an important role in the noncytolytic destruction of HBV-DNA in liver during early phases of acute HBV infection[54].

Broad and vigorous $CD4^+$ and $CD8^+$ T-cell responses have been reported in patients with acute hepatitis B[55]. Moreover, HBV-specific T-cell responses were observed during the incubation period of acute hepatitis, with HBV-DNA reduced before alanine aminotransferase concentration peaked, indicating that noncytolytic eradication of HBV also occurs in acute hepatitis B in humans[56]. However, recovery from acute hepatitis B does not imply complete eradication of HBV, as small amounts of HBV-DNA can be detected in the blood for a long time after resolution of acute hepatitis B[8]. T-cell responses are therefore not sufficient to completely eradicate cccDNA from infected livers, even in acute hepatitis B.

Chronic infection

Immune responses in patients chronically infected with HBV were found to consist of four phases: The immuno-tolerant, immune-active, inactive carrier, and reactivation phases[57]. Although the exact mechanism by which HBV induces immune tolerance is unclear, it may arise from central deletion or peripheral non-recognition of HBV-specific T-cells[58]. Immune tolerance may be broken after several decades by as yet undetermined mechanisms, but these may involve the maturation of dendritic cell (DC) function[59]. Breaking immune tolerance to HBV can lead to the immune-active phase, resulting in some degree of hepatitis. During this phase, suppression of HBV replication is observed in 85% to 90% of patients, leading to an inactive carrier state. Most patients in an inactive carrier state do not need antiviral treatments, but cccDNA may be present in their livers. The cccDNA persisting in inactive carriers may be a template for reactivation of HBV replication. The 10% to 15% of patients who remain in the immune-active phase continue to experience liver inflammation with active replication of HBV, and may be at high risk for progression to liver cirrhosis and the development of HCC. The number of HBV-specific $CD8^+$ T-cells was found to be the same in livers with low HBV replication and little hepatitis and in livers with high HBV replication and severe hepatitis[60]. These findings suggest that HBV replication is suppressed by immune surveillance of HBV-specific T-cells in the liver and that these T-cells are important in controlling HBV replication in a noncytolytic manner in inactive carriers. In contrast, HBV-specific immune responses are thought to be dysregulated in livers with active hepatitis, and several possible mechanisms have been proposed.

Impairment of innate immune response

Innate immune system such as pattern recognition receptors, macrophages, DCs, natural killer cells or natural killer T cells are involved in the pathogenesis of HBV infection especially at an early stage of infection[61,62]. HBV has been shown to alter the function of macrophages by modulating the secretion of cytokines[63,64] or type-1 IFN gene expression[64]. Hepatitis B e antigen was shown to directly suppress toll-like receptor (TLR) signaling *via* interaction with Toll/IL-1 receptor-containing proteins such as TRAM and Mal[65]. HBV has been shown to downregulate TLR-2 expression in patients with chronic HBV infection[66]. Thus, innate immunity alteration plays a role, at least in part, in the pathogenesis of chronic HBV infection and TLR-7 agonists have been applied as immune-modulatory components[67,68]. On the other hand, the effect of IFN-α on intrahepatic cccDNA has been recently explored[69], and IFN-α in addition to lymphotoxin-β receptor (LTβR) activation has been shown to induce cccDNA degradation through upregulation of nuclear APOBEC3 deaminases[70]. APOBEC3 can deaminate double-stranded DNA cytidines to uridines[71] and induce cccDNA degradation. IFN-γ and TNF-α produced form T-cells can induce deamination of cccDNA without cytolysis, supporting the essential role of APOBEC3 in reduction of cccDNA[53]. Collectively, type-1 IFN-mediated effects, especially APOBEC3 upregulation, will be a key subject for development of new therapeutics.

Dysfunction of dendritic cells

DCs are the most potent antigen-presenting cells, stimulating both T- and B-cells. In patients with chronic hepatitis, the cytokine-induced maturation of circulating myeloid DCs is impaired, possibly by exposure to high amounts of HBV or HBsAg[72,73]. Dysfunctional DCs may act as tolerogenic antigen-presenting cells, resulting in a failure to induce HBV-specific immune responses.

Alteration of the hierarchy of epitope-specific $CD8^+$ T-cell responses

In acute hepatitis B, the $CD8^+$ T-cell response to the immunogenic epitope HBc18-27 (HLA-A2 restricted epitope) is dominant. In contrast, HBc18-27-specific $CD8^+$ T-cell responses are low and $CD8^+$ T-cell responses against less immunogenic envelope (183-191) are dominant in chronic hepatitis B[74]. Although the mechanisms underlying changes in the major epitope to $CD8^+$ T-cell response are not yet known, they may account, at least in part, for the different $CD8^+$ T-cell responses observed in patients with acute and chronic hepatitis.

Regulatory T-cells

Regulatory T-cells (Tregs) expressing the forkhead family transcription factor, Foxp3, are specialized cells that have a major role in the maintenance of immunological self-tolerance by suppressing self-reactive cells[75]. Tregs express CD25 [interleukin (IL)-2 receptor α-chain] and/or cytotoxic T-lymphocyte antigen-4 (CTLA-4), which are excellent inhibitors of IL-2 production or downregulation of CD80 and CD86 on DCs by a CTLA-4-dependent mechanism[76].

The numbers of $CD4^+CD25^+FoxP3^+$ Tregs were higher in the livers of patients with chronic hepatitis B,

suggesting that these cells suppress intrahepatic HBV-specific T-cell responses, leading to insufficient immune control of HBV replication in the liver[77].

Inhibitory receptors

Program death (PD)-1 is a surface receptor critical for the regulation of T-cell function[78,79]. Binding of the ligand PD-L1 to PD-1 on T-cells results in the antigen-specific inhibition of T-cell proliferation, with a molecule related to T-cell exhaustion found in the livers of patients with chronic hepatitis B. T-cell exhaustion is characterized by poor cytotoxic activity and cytokine production, as well as by the expression of inhibitory receptors, including not only PD-1 but lymphocyte activation gene-3, CTLA-4, T-cell immunoglobulin domain and mucin domain-3, and CD244[66]. These inhibitory receptors are thought to be induced by persistent exposure of intrahepatic T-cells to HBV or HBV-related proteins[80]. Exhaustion of T-cells could also account for impaired T-cell responses in the livers of patients with chronic hepatitis B, and blockade of these receptors could be therapeutic.

Patients with high serum HBV-DNA concentration have been reported likely to progress to cirrhosis and eventually HCC[81]. Transition of immune-active patients to an inactive state with low HBV-DNA replication by the direct stimulation of HBV-specific T-cells or removal of immunosuppressive factors, may be sufficient to inhibit progression to cirrhosis or HCC. Inactive HBV carriers may not require specific treatment, because spontaneous HBsAg develops at a rate of 1% to 1.9%/year in these patients, making the development of HCC rare[82]. Therefore, an inactive HBV carrier may be regarded as in a state of functional cure (Table 1). However, HBV replication may be reactivated, either spontaneously or during treatment with an immunosuppressive or anticancer agent, resulting in a higher risk of hepatocarcinogenesis than in the general population[83]. The rate of HCC development was recently reported to be greater in patients with high than with low serum HBsAg concentrations, even in inactive HBV carriers with low serum HBV-DNA concentrations[36,37].

Collectively, these results suggest that induction of immune control against HBV infection may result in functional cure of HBV infection. Functional cure, however, may be an unstable condition, allowing progression to cirrhosis or HCC under various conditions. Although radical cure (Table 1) is desirable, it is problematic because of the difficulty in eliminating HBV cccDNA from the liver.

THERAPEUTIC STRATEGIES FOR HBV INFECTION

Immunotherapy

Radical cure of HBV infection could be achieved by both the elimination of cccDNA in the liver and the destruction of HBV-DNA-integrated hepatocytes. The primary goals of immunotherapy in HBV-infected individuals include the induction or stimulation of HBV-specific immune responses, leading to the killing of infected cells or the degradation of HBV-RNA and HBV-DNA in a noncytolytic manner, inhibiting progression to liver cirrhosis and hepatocarcinogenesis. Although immune responses involving cytokines such as IFN-γ and TNF-α can eliminate cccDNA[50,53], cccDNA is not completely eliminated even after resolution of acute hepatitis B[8], suggesting that immune responses alone may be insufficient to achieve radical cure of HBV infection.

Induction or stimulation of HBV-specific immune responses

Efforts to stimulate HBV-specific T-cells have included immunizations with HBV-peptides, viral proteins, DCs, and DNA, as well as treatment with cytokines[84]. Because HBV-specific T-cells in patients with chronic hepatitis B are exhausted by long-term exposure to high levels of HBV-related antigens, activation of those cells by immunization would be ineffective without functional restoration of the cells by blocking the inhibitory signals responsible for T-cell exhaustion. Blockade of PD-1, CTLA-4 or Tim-3 has been shown to restore exhausted HBV-specific T-cells[80], suggesting that the combination of immunization and blockade of inhibitory signals would be effective in activating HBV-specific T-cells.

Other immunotherapeutic approaches to HBV infection include administration of cytokines, such as IFN-γ, IL-6, IL-1β, LTβR-agonists and/or TLR-7 agonist, as well as IFN-γ and TNF-α which were shown to cause silencing or degradation of cccDNA[67]. This strategy may be more effective in the complete eradication of HBV infection than strategies involving the activation of HBV-specific cells, suggesting that only cytokine administration results in the elimination of cccDNA.

Elimination of HBV-infected hepatocytes by a novel approach

A novel approach to eliminate HBV-core containing hepatocytes[85] was based on findings showing that elimination of HBV is impaired by cellular inhibitor of apoptosis proteins (cIAPs), which inhibit the TNF-α-mediated death of HBV-infected cells[86]. This led to testing the effects of inhibitors of cIAPs, including birinapant and other Smac mimetics, on HBV-infected hepatocytes. These inhibitors of cIAPs resulted in the rapid reduction in serum HBV-DNA and HBsAg concentrations, possibly by eliminating HBV-core containing hepatocytes. However, the effects of those drugs on cccDNA are unclear.

Immunotherapeutic strategies for HBV-DNA-integrated hepatocytes

Three main mechanisms are responsible for hepatocarcinogenesis: (1) the oncogenic potential of the HBV-related proteins, HBsAg and HBx; (2) HBV-DNA integration into the host genome, dysregulating the cell cycle by the introduction of deletions, cis/trans-activations, and/or translocations, and/or inducing generalized genomic instability; and (3) persistent inflammation in the

liver causing rapid turnover of hepatocyte regeneration, enhancing the instability and/or mutagenesis of host genomes.

Therefore, if future advances in therapeutic modalities result in the complete elimination of cccDNA, hepatocarcinogenesis resulting from HBV-DNA integration into the host genome should be addressed. HBV-DNA integration into the hepatocyte genome has been observed in 86.4% of HBV-related HCCs and in 30.7% of adjacent liver tissue[87]. Integration of HBV-DNA into areas of the host genome encoding genes that regulate cellular proliferation, such as telomerase or proliferation signal transduction genes, may lead to cis-/trans-activation, inducing malignant transformation[88]. Furthermore, integration of HBV-DNA may induce genetic instability by altering the expression of oncogenes, tumor suppressor genes and microRNAs[87,89]. In addition, a viral-human chimeric transcript was reported to function as a noncoding RNA and promote hepatocarcinogenesis[90]. Integration of HBV-DNA into the host hepatocyte genome of transiently infected individuals has been reported to be a rare event, occurring in 0.01%-0.1% of hepatocytes[91]. Further investigations are needed to determine the mechanism by which HBV-DNA integration into the host genome induces carcinogenesis. The immune cytolysis of cells expressing HBV-related peptides may be the only strategy that effectively eliminates HBV-DNA-integrated hepatocytes. However, if non-immunogenic regions of HBV-DNA are integrated, elimination of those cells by immune attack would be impossible.

Taken together, these findings indicate that immunotherapy against HBV can control viral replication and reduce cccDNA, but may not be sufficient to completely eradicate HBV-infected or -integrated hepatocytes.

Inhibition of HBV replication

Currently available NAs can efficiently reduce viremia but cannot eliminate intracellular cccDNA. However, complete suppression of HBV polymerase can result in the complete elimination of cccDNA through the death of cccDNA-containing hepatocytes after one natural lifespan of these cells[92]. Among the agents being tested are prodrugs of HBV polymerase inhibitors[93]. These include prodrugs of tenofovir, such as AGX1009 (Agenix) and TAF (GS-7340, Gilead Sciences), which have been evaluated in phase 3 trials[93,94], and CMX157, a lipid conjugate of tenofovir, which has been evaluated in phase 1/2 trials[93,95]. RNase H inhibitors are also being tested, based on the specificity of HBV replication, which depends on the RNase H activity of HBV polymerase to degrade pgRNA[10]. Evaluations of selective inhibitors of HBV polymerase RNase H activity[96] suggest that they might be more effective when combined with NAs[93].

Destruction of cccDNA

Eradication of cccDNA in hepatocytes is essential to achieve radical cure of established HBV infection. Several trials have targeted cccDNA. For example, gene silencing techniques, such as small interfering RNAs (siRNAs) or antisense oligonucleotides (ASOs), have been evaluated for their ability to reduce viremia and cccDNA. Although siRNAs may have promising activity, methods to effectively deliver them to hepatocytes have not been determined[97]. RNAi can inhibit all steps of HBV replication, and ARC-520 has been tested in a phase 2 trial in patients with chronic hepatitis B[95]. In contrast, a single injection of ASO, consisting of liver-targeted peptides, into a mouse model of chronic HBV infection was shown to reduce HBV-RNA, proteins and HBV-DNA for a long time, suggesting that ASO may become a promising treatment in patients with chronic HBV[98]. Furthermore, disubstituted sulfonamide was shown to selectively inhibit the formation of cccDNA[99].

In addition, several genome editing technologies have been developed to silence sequence-specific cleavage of cccDNA. These include zinc finger nucleases (ZFNs)[100,101], transcription activator-like effector nucleases (TALENs)[102,103], and the clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR associated system (Cas). These sequence-specific genome editing technologies could induce double-stranded breaks at certain DNA sites. ZFNs consist of a zinc finger domain, which contains a sequence-specific binding site, and a *Fok I* nuclease domain. ZFNs form heterodimers and induce double-stranded breaks at targeted sites. These breaks are subsequently repaired by homology-directed repair or non-homologous end joining. The specificity of ZFNs may be context-dependent, resulting from interactions between DNA binding domains and neighboring zinc fingers[104]. TALENs have transcription activator-like effector specific DNA binding activity, with DNA-binding sites more specific than those of ZFNs[105]. However, both ZFNs and TALENs require pairs of site-specific nucleases for each target to produce customized proteins[9]. In contrast, CRISPR/Cas technology is a novel genome-editing method, which is more useful than ZFNs or TALENs[106]. CRISPR/Cas loci encode RNA guided endonucleases, which are induced by immune responses against foreign genetic elements such as bacteriophages and plasmids[107]. The type 2 CRISPR/Cas system from *Streptococcus pyogenes* is a chimeric single-guide RNA with Cas9 protein[108]. The CRISPR/Cas9 system was shown to suppress HBV replication in cultured cells and in mouse models[109-117], reducing both HBsAg[109,110,112-116] and cccDNA[110,113-115,117]. These findings suggest that genome editing technology, such as a CRISPR/Cas system, may be a potential therapeutic option for the complete eradication of HBV infection in future. However, cleavage of cccDNA and subsequent DNA repair may introduce mutations into the host genome. These mutations may be harmful to the host, resulting in the possible development of malignancy[9,118,119], suggesting the need for further improvements in efficacy and safety prior to the therapeutic use of these systems.

Future perspectives on radical cure of chronic HBV infection

Various trials have assessed agents that can terminate

Table 2 Tgerapectic agents against hepatitis B virus currently in clinical development

Mode of actions	Target	Stage of development	Ref.
Entry inhibitions	NTCP	Myrcludex in phage 2	[14,123]
cccDNA			
Formation inhibitions	DSS	Preclinical	[99]
Transcription inhibitions	ASO	IONIS-HBVRx in phase 1	[98]
Destabilization/degradation	ZFN	Preclinical	[100,101]
	TALEN	Preclinical	[102,103]
	CRISPR/Cas9	Preclinical	[109-117]
SiRNA	PgRNA	ARC-520 in phase 2	[95]
Nucleocapsid assembly inhibitions	Capsid formation	BAY4109 in phase 1	[93,95]
		NV1221 in phase 1	[93,95]
Reverse transcription inhibitions	Polymerase	TAF in phase 3	[93,94]
		Cmx157 in phase 1/2	[93,95]
HBsAg release inhibitions	HBsAg secretion	Preclinical	[109]
	HBsAg secretion	Rep2139 in phase 1/2	[110]
Immune modulating	TLR-7 agonist	GS-9620 in phase 2	[67,68]
	HBV-specific	Preclinical	[84]
	cIAPS	Preclinical	[86]

cccDNA: Covalently-closed circular DNA; ZFN: Zinc finger nuclease; TALEN: Transcription activator-like effector nuclease; CRISPR: Clustered regularly interspaced short palindromic repeat; Cas: CRISPR associated system; pgRNA: Pregenomic RNAs; HBsAg: Hepatitis B virus surface antigen; TLR: Toll-like receptor; HBV: Hepatitis B virus; cIAPS: Cellular inhibitor of apoptosis proteins; DSS: Disubstituted sulfonamide; ASO: Antisense oligonucleotides; TAF: Tenofovir Alafenamide; NTCP: Na^+/taurocholate cotransporting polypeptide.

the HBV life cycle in hepatocytes, including inhibitors of HBV-DNA polymerase, virus entry, core assembly and HBsAg secretion (Table 2)[93,95,120,121]. Especially Myrcludex B, a synthetic lipopeptide that targets NTCP, has been shown to efficiently prevent viral spread and has been applied in clinical trials[15,17,122,123]. These agents, including Myrcludex, are not themselves sufficient to eliminate HBV from chronically infected hepatocytes, as shown by the remaining cccDNA in the nuclei and HBV-DNA-integrated hepatocytes. Immunotherapy may potentially eliminate both cccDNA and HBV-DNA-integrated hepatocytes, but its effects would be limited. Although drugs targeting cccDNA in hepatocytes are theoretically ideal for complete eradication of HBV, no single drug or strategy, whether currently available or under development, has shown the ability to completely eliminate HBV with established safety and efficacy. Future trials, testing combination of different agents or strategies, will be necessary.

REFERENCES

1 **Liaw YF**, Chu CM. Hepatitis B virus infection. *Lancet* 2009; **373**: 582-592 [PMID: 19217993 DOI: 10.1016/S0140-6736(09)60207-5]

2 **Ott JJ**, Stevens GA, Groeger J, Wiersma ST. Global epidemiology of hepatitis B virus infection: new estimates of age-specific HBsAg seroprevalence and endemicity. *Vaccine* 2012; **30**: 2212-2219 [PMID: 22273662 DOI: 10.1016/j.vaccine.2011.12.116]

3 **Lozano R**, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, Abraham J, Adair T, Aggarwal R, Ahn SY, Alvarado M, Anderson HR, Anderson LM, Andrews KG, Atkinson C, Baddour LM, Barker-Collo S, Bartels DH, Bell ML, Benjamin EJ, Bennett D, Bhalla K, Bikbov B, Bin Abdulhak A, Birbeck G, Blyth F, Bolliger I, Boufous S, Bucello C, Burch M, Burney P, Carapetis J, Chen H, Chou D, Chugh SS, Coffeng LE, Colan SD, Colquhoun S, Colson KE, Condon J, Connor MD, Cooper LT, Corriere M, Cortinovis M, de Vaccaro KC, Couser W, Cowie BC, Criqui MH, Cross M, Dabhadkar KC, Dahodwala N, De Leo D, Degenhardt L, Delossantos A, Denenberg J, Des Jarlais DC, Dharmaratne SD, Dorsey ER, Driscoll T, Duber H, Ebel B, Erwin PJ, Espindola P, Ezzati M, Feigin V, Flaxman AD, Forouzanfar MH, Fowkes FG, Franklin R, Fransen M, Freeman MK, Gabriel SE, Gakidou E, Gaspari F, Gillum RF, Gonzalez-Medina D, Halasa YA, Haring D, Harrison JE, Havmoeller R, Hay RJ, Hoen B, Hotez PJ, Hoy D, Jacobsen KH, James SL, Jasrasaria R, Jayaraman S, Johns N, Karthikeyan G, Kassebaum N, Keren A, Khoo JP, Knowlton LM, Kobusingye O, Koranteng A, Krishnamurthi R, Lipnick M, Lipshultz SE, Ohno SL, Mabweijano J, MacIntyre MF, Mallinger L, March L, Marks GB, Marks R, Matsumori A, Matzopoulos R, Mayosi BM, McAnulty JH, McDermott MM, McGrath J, Mensah GA, Merriman TR, Michaud C, Miller M, Miller TR, Mock C, Mocumbi AO, Mokdad AA, Moran A, Mulholland K, Nair MN, Naldi L, Narayan KM, Nasseri K, Norman P, O'Donnell M, Omer SB, Ortblad K, Osborne R, Ozgediz D, Pahari B, Pandian JD, Rivero AP, Padilla RP, Perez-Ruiz F, Perico N, Phillips D, Pierce K, Pope CA, Porrini E, Pourmalek F, Raju M, Ranganathan D, Rehm JT, Rein DB, Remuzzi G, Rivara FP, Roberts T, De León FR, Rosenfeld LC, Rushton L, Sacco RL, Salomon JA, Sampson U, Sanman E, Schwebel DC, Segui-Gomez M, Shepard DS, Singh D, Singleton J, Sliwa K, Smith E, Steer A, Taylor JA, Thomas B, Tleyjeh IM, Towbin JA, Truelsen T, Undurraga EA, Venketasubramanian N, Vijayakumar L, Vos T, Wagner GR, Wang M, Wang W, Watt K, Weinstock MA, Weintraub R, Wilkinson JD, Woolf AD, Wulf S, Yeh PH, Yip P, Zabetian A, Zheng ZJ, Lopez AD, Murray CJ, AlMazroa MA, Memish ZA. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet* 2012; **380**: 2095-2128 [PMID: 23245604 DOI: 10.1016/S0140-6736(12)61728-0]

4 **Trépo C**, Chan HL, Lok A. Hepatitis B virus infection. *Lancet* 2014; **384**: 2053-2063 [PMID: 24954675 DOI: 10.1016/S0140-6736(14)60220-8]

5 **Lau GK**, Piratvisuth T, Luo KX, Marcellin P, Thongsawat S, Cooksley G, Gane E, Fried MW, Chow WC, Paik SW, Chang WY, Berg T, Flisiak R, McCloud P, Pluck N. Peginterferon Alfa-2a, lamivudine, and the combination for HBeAg-positive chronic hepatitis B. *N Engl J Med* 2005; **352**: 2682-2695 [PMID: 15987917 DOI: 10.1056/NEJMoa043470]

6 **Janssen HL**, van Zonneveld M, Senturk H, Zeuzem S, Akarca US, Cakaloglu Y, Simon C, So TM, Gerken G, de Man RA, Niesters

HG, Zondervan P, Hansen B, Schalm SW. Pegylated interferon alfa-2b alone or in combination with lamivudine for HBeAg-positive chronic hepatitis B: a randomised trial. *Lancet* 2005; **365**: 123-129 [PMID: 15639293 DOI: 10.1016/S0140-6736(05)17701-0]
7 **Tuttleman JS**, Pourcel C, Summers J. Formation of the pool of covalently closed circular viral DNA in hepadnavirus-infected cells. *Cell* 1986; **47**: 451-460 [PMID: 3768961]
8 **Rehermann B**, Ferrari C, Pasquinelli C, Chisari FV. The hepatitis B virus persists for decades after patients' recovery from acute viral hepatitis despite active maintenance of a cytotoxic T-lymphocyte response. *Nat Med* 1996; **2**: 1104-1108 [PMID: 8837608]
9 **Ohno M**, Otsuka M, Kishikawa T, Yoshikawa T, Takata A, Koike K. Novel therapeutic approaches for hepatitis B virus covalently closed circular DNA. *World J Gastroenterol* 2015; **21**: 7084-7088 [PMID: 26109795 DOI: 10.3748/wjg.v21.i23.7084]
10 **Seeger C**, Mason WS. Hepatitis B virus biology. *Microbiol Mol Biol Rev* 2000; **64**: 51-68 [PMID: 10704474]
11 **Lampertico P**, Maini M, Papatheodoridis G. Optimal management of hepatitis B virus infection - EASL Special Conference. *J Hepatol* 2015; **63**: 1238-1253 [PMID: 26150256 DOI: 10.1016/j.jhep.2015.06.026]
12 **Lai CL**, Gane E, Liaw YF, Hsu CW, Thongsawat S, Wang Y, Chen Y, Heathcote EJ, Rasenack J, Bzowej N, Naoumov NV, Di Bisceglie AM, Zeuzem S, Moon YM, Goodman Z, Chao G, Constance BF, Brown NA. Telbivudine versus lamivudine in patients with chronic hepatitis B. *N Engl J Med* 2007; **357**: 2576-2588 [PMID: 18094378 DOI: 10.1056/NEJMoa066422]
13 **Chang TT**, Gish RG, de Man R, Gadano A, Sollano J, Chao YC, Lok AS, Han KH, Goodman Z, Zhu J, Cross A, DeHertogh D, Wilber R, Colonno R, Apelian D. A comparison of entecavir and lamivudine for HBeAg-positive chronic hepatitis B. *N Engl J Med* 2006; **354**: 1001-1010 [PMID: 16525137 DOI: 10.1056/NEJMoa051285]
14 **Summers J**, O'Connell A, Millman I. Genome of hepatitis B virus: restriction enzyme cleavage and structure of DNA extracted from Dane particles. *Proc Natl Acad Sci USA* 1975; **72**: 4597-4601 [PMID: 1060140]
15 **Gripon P**, Cannie I, Urban S. Efficient inhibition of hepatitis B virus infection by acylated peptides derived from the large viral surface protein. *J Virol* 2005; **79**: 1613-1622 [PMID: 15650187 DOI: 10.1128/JVI.79.3.1613-1622.2005]
16 **Ni Y**, Lempp FA, Mehrle S, Nkongolo S, Kaufman C, Fälth M, Stindt J, Königer C, Nassal M, Kubitz R, Sültmann H, Urban S. Hepatitis B and D viruses exploit sodium taurocholate co-transporting polypeptide for species-specific entry into hepatocytes. *Gastroenterology* 2014; **146**: 1070-1083 [PMID: 24361467 DOI: 10.1053/j.gastro.2013.12.024]
17 **Brahmania M**, Feld J, Arif A, Janssen HL. New therapeutic agents for chronic hepatitis B. *Lancet Infect Dis* 2016; **16**: e10-e21 [PMID: 26795693 DOI: 10.1016/S1473-3099(15)00436-3]
18 **Summers J**, Mason WS. Replication of the genome of a hepatitis B--like virus by reverse transcription of an RNA intermediate. *Cell* 1982; **29**: 403-415 [PMID: 6180831]
19 **Tang H**, McLachlan A. Transcriptional regulation of hepatitis B virus by nuclear hormone receptors is a critical determinant of viral tropism. *Proc Natl Acad Sci USA* 2001; **98**: 1841-1846 [PMID: 11172038 DOI: 10.1073/pnas.041479698]
20 **Lucifora J**, Arzberger S, Durantel D, Belloni L, Strubin M, Levrero M, Zoulim F, Hantz O, Protzer U. Hepatitis B virus X protein is essential to initiate and maintain virus replication after infection. *J Hepatol* 2011; **55**: 996-1003 [PMID: 21376091 DOI: 10.1016/j.jhep.2011.02.015]
21 **Nassal M**. HBV cccDNA: viral persistence reservoir and key obstacle for a cure of chronic hepatitis B. *Gut* 2015; **64**: 1972-1984 [PMID: 26048673 DOI: 10.1136/gutjnl-2015-309809]
22 **Paterlini P**, Poussin K, Kew M, Franco D, Brechot C. Selective accumulation of the X transcript of hepatitis B virus in patients negative for hepatitis B surface antigen with hepatocellular carcinoma. *Hepatology* 1995; **21**: 313-321 [PMID: 7843699]
23 **Zhang XD**, Wang Y, Ye LH. Hepatitis B virus X protein accelerates the development of hepatoma. *Cancer Biol Med* 2014; **11**: 182-190 [PMID: 25364579 DOI: 10.7497/j.issn.2095-3941.2014.03.004]
24 **Kim CM**, Koike K, Saito I, Miyamura T, Jay G. HBx gene of hepatitis B virus induces liver cancer in transgenic mice. *Nature* 1991; **351**: 317-320 [PMID: 2034275 DOI: 10.1038/351317a0]
25 **Yu DY**, Moon HB, Son JK, Jeong S, Yu SL, Yoon H, Han YM, Lee CS, Park JS, Lee CH, Hyun BH, Murakami S, Lee KK. Incidence of hepatocellular carcinoma in transgenic mice expressing the hepatitis B virus X-protein. *J Hepatol* 1999; **31**: 123-132 [PMID: 10424292]
26 **Qiu X**, Dong S, Qiao F, Lu S, Song Y, Lao Y, Li Y, Zeng T, Hu J, Zhang L, Zhang L, Fan H. HBx-mediated miR-21 upregulation represses tumor-suppressor function of PDCD4 in hepatocellular carcinoma. *Oncogene* 2013; **32**: 3296-3305 [PMID: 23604124 DOI: 10.1038/onc.2013.150]
27 **Jung JK**, Arora P, Pagano JS, Jang KL. Expression of DNA methyltransferase 1 is activated by hepatitis B virus X protein via a regulatory circuit involving the p16INK4a-cyclin D1-CDK 4/6-pRb-E2F1 pathway. *Cancer Res* 2007; **67**: 5771-5778 [PMID: 17575144 DOI: 10.1158/0008-5472.CAN-07-0529]
28 **Rivière L**, Gerossier L, Ducroux A, Dion S, Deng Q, Michel ML, Buendia MA, Hantz O, Neuveut C. HBx relieves chromatin-mediated transcriptional repression of hepatitis B viral cccDNA involving SETDB1 histone methyltransferase. *J Hepatol* 2015; **63**: 1093-1102 [PMID: 26143443 DOI: 10.1016/j.jhep.2015.06.023]
29 **Arzumanyan A**, Reis HM, Feitelson MA. Pathogenic mechanisms in HBV- and HCV-associated hepatocellular carcinoma. *Nat Rev Cancer* 2013; **13**: 123-135 [PMID: 23344543 DOI: 10.1038/nrc3449]
30 **Yuan K**, Lian Z, Sun B, Clayton MM, Ng IO, Feitelson MA. Role of miR-148a in hepatitis B associated hepatocellular carcinoma. *PLoS One* 2012; **7**: e35331 [PMID: 22496917 DOI: 10.1371/journal.pone.0035331]
31 **Lee JO**, Kwun HJ, Jung JK, Choi KH, Min DS, Jang KL. Hepatitis B virus X protein represses E-cadherin expression via activation of DNA methyltransferase 1. *Oncogene* 2005; **24**: 6617-6625 [PMID: 16007161 DOI: 10.1038/sj.onc.1208827]
32 **Arzumanyan A**, Friedman T, Kotei E, Ng IO, Lian Z, Feitelson MA. Epigenetic repression of E-cadherin expression by hepatitis B virus x antigen in liver cancer. *Oncogene* 2012; **31**: 563-572 [PMID: 21706058 DOI: 10.1038/onc.2011.255]
33 **Xie Y**, Liu S, Zhao Y, Guo Z, Xu J. X protein mutations in hepatitis B virus DNA predict postoperative survival in hepatocellular carcinoma. *Tumour Biol* 2014; **35**: 10325-10331 [PMID: 25034530 DOI: 10.1007/s13277-014-2331-0]
34 **Su IJ**, Wang HC, Wu HC, Huang WY. Ground glass hepatocytes contain pre-S mutants and represent preneoplastic lesions in chronic hepatitis B virus infection. *J Gastroenterol Hepatol* 2008; **23**: 1169-1174 [PMID: 18505413 DOI: 10.1111/j.1440-1746.2008.05348.x]
35 **Pollicino T**, Cacciola I, Saffioti F, Raimondo G. Hepatitis B virus PreS/S gene variants: pathobiology and clinical implications. *J Hepatol* 2014; **61**: 408-417 [PMID: 24801416 DOI: 10.1016/j.jhep.2014.04.041]
36 **Wu HC**, Tsai HW, Teng CF, Hsieh WC, Lin YJ, Wang LH, Yuan Q, Su IJ. Ground-glass hepatocytes co-expressing hepatitis B virus X protein and surface antigens exhibit enhanced oncogenic effects and tumorigenesis. *Hum Pathol* 2014; **45**: 1294-1301 [PMID: 24767856 DOI: 10.1016/j.humpath.2013.10.039]
37 **Na B**, Huang Z, Wang Q, Qi Z, Tian Y, Lu CC, Yu J, Hanes MA, Kakar S, Huang EJ, Ou JH, Liu L, Yen TS. Transgenic expression of entire hepatitis B virus in mice induces hepatocarcinogenesis independent of chronic liver injury. *PLoS One* 2011; **6**: e26240 [PMID: 22022578 DOI: 10.1371/journal.pone.0026240]
38 **Hildt E**, Munz B, Saher G, Reifenberg K, Hofschneider PH. The PreS2 activator MHBs(t) of hepatitis B virus activates c-raf-1/Erk2 signaling in transgenic mice. *EMBO J* 2002; **21**: 525-535 [PMID: 11847101]
39 **Kawanaka M**, Nishino K, Nakamura J, Oka T, Urata N, Goto D, Suehiro M, Kawamoto H, Kudo M, Yamada G. Quantitative Levels of Hepatitis B Virus DNA and Surface Antigen and the Risk of Hepatocellular Carcinoma in Patients with Hepatitis B Receiving

Long-Term Nucleos(t)ide Analogue Therapy. *Liver Cancer* 2014; **3**: 41-52 [PMID: 24804176 DOI: 10.1159/000343857]
40 **Tseng TC**, Liu CJ, Yang HC, Su TH, Wang CC, Chen CL, Hsu CA, Kuo SF, Liu CH, Chen PJ, Chen DS, Kao JH. Serum hepatitis B surface antigen levels help predict disease progression in patients with low hepatitis B virus loads. *Hepatology* 2013; **57**: 441-450 [PMID: 22941922 DOI: 10.1002/hep.26041]
41 **Liu J**, Yang HI, Lee MH, Lu SN, Jen CL, Batrla-Utermann R, Wang LY, You SL, Hsiao CK, Chen PJ, Chen CJ. Spontaneous seroclearance of hepatitis B seromarkers and subsequent risk of hepatocellular carcinoma. *Gut* 2014; **63**: 1648-1657 [PMID: 24225939 DOI: 10.1136/gutjnl-2013-305785]
42 **Voehringer D**, Blaser C, Grawitz AB, Chisari FV, Buerki K, Pircher H. Break of T cell ignorance to a viral antigen in the liver induces hepatitis. *J Immunol* 2000; **165**: 2415-2422 [PMID: 10946266]
43 **Chen WN**, Chen JY, Jiao BY, Lin WS, Wu YL, Liu LL, Lin X. Interaction of the hepatitis B spliced protein with cathepsin B promotes hepatoma cell migration and invasion. *J Virol* 2012; **86**: 13533-13541 [PMID: 23035214 DOI: 10.1128/JVI.02095-12]
44 **Tan AT**, Koh S, Goh V, Bertoletti A. Understanding the immunopathogenesis of chronic hepatitis B virus: an Asian prospective. *J Gastroenterol Hepatol* 2008; **23**: 833-843 [PMID: 18565018 DOI: 10.1111/j.1440-1746.2008.05385.x]
45 **Chisari FV**, Isogawa M, Wieland SF. Pathogenesis of hepatitis B virus infection. *Pathol Biol* (Paris) 2010; **58**: 258-266 [PMID: 20116937 DOI: 10.1016/j.patbio.2009.11.001]
46 **Bertoletti A**, Wang FS. Overview of the special issue on HBV immunity. *Cell Mol Immunol* 2015; **12**: 253-254 [PMID: 25864914 DOI: 10.1038/cmi.2015.24]
47 **Guidotti LG**, Isogawa M, Chisari FV. Host-virus interactions in hepatitis B virus infection. *Curr Opin Immunol* 2015; **36**: 61-66 [PMID: 26186123 DOI: 10.1016/j.coi.2015.06.016]
48 **Moriyama T**, Guilhot S, Klopchin K, Moss B, Pinkert CA, Palmiter RD, Brinster RL, Kanagawa O, Chisari FV. Immunobiology and pathogenesis of hepatocellular injury in hepatitis B virus transgenic mice. *Science* 1990; **248**: 361-364 [PMID: 1691527]
49 **Chisari FV**. Cytotoxic T cells and viral hepatitis. *J Clin Invest* 1997; **99**: 1472-1477 [PMID: 9119989 DOI: 10.1172/JCI119308]
50 **Guidotti LG**, Ishikawa T, Hobbs MV, Matzke B, Schreiber R, Chisari FV. Intracellular inactivation of the hepatitis B virus by cytotoxic T lymphocytes. *Immunity* 1996; **4**: 25-36 [PMID: 8574849]
51 **Guidotti LG**, Chisari FV. Noncytolytic control of viral infections by the innate and adaptive immune response. *Annu Rev Immunol* 2001; **19**: 65-91 [PMID: 11244031 DOI: 10.1146/annurev.immunol.19.1.65]
52 **Guidotti LG**, Matzke B, Schaller H, Chisari FV. High-level hepatitis B virus replication in transgenic mice. *J Virol* 1995; **69**: 6158-6169 [PMID: 7666518]
53 **Xia Y**, Stadler D, Lucifora J, Reisinger F, Webb D, Hösel M, Michler T, Wisskirchen K, Cheng X, Zhang K, Chou WM, Wettengel JM, Malo A, Bohne F, Hoffmann D, Eyer F, Thimme R, Falk CS, Thasler WE, Heikenwalder M, Protzer U. Interferon-γ and Tumor Necrosis Factor-α Produced by T Cells Reduce the HBV Persistence Form, cccDNA, Without Cytolysis. *Gastroenterology* 2016; **150**: 194-205 [PMID: 26416327 DOI: 10.1053/j.gastro.2015.09.026]
54 **Guidotti LG**, Rochford R, Chung J, Shapiro M, Purcell R, Chisari FV. Viral clearance without destruction of infected cells during acute HBV infection. *Science* 1999; **284**: 825-829 [PMID: 10221919]
55 **Rehermann B**, Fowler P, Sidney J, Person J, Redeker A, Brown M, Moss B, Sette A, Chisari FV. The cytotoxic T lymphocyte response to multiple hepatitis B virus polymerase epitopes during and after acute viral hepatitis. *J Exp Med* 1995; **181**: 1047-1058 [PMID: 7532675]
56 **Webster GJ**, Reignat S, Maini MK, Whalley SA, Ogg GS, King A, Brown D, Amlot PL, Williams R, Vergani D, Dusheiko GM, Bertoletti A. Incubation phase of acute hepatitis B in man: dynamic of cellular immune mechanisms. *Hepatology* 2000; **32**: 1117-1124 [PMID: 11050064 DOI: 10.1053/jhep.2000.19324]
57 **Wu JF**, Chang MH. Natural history of chronic hepatitis B virus infection from infancy to adult life - the mechanism of inflammation triggering and long-term impacts. *J Biomed Sci* 2015; **22**: 92 [PMID: 26487087 DOI: 10.1186/s12929-015-0199-y]
58 **Mamun-Al-Mahtab SM**, Uddin H, Khan SI, Rahman S. Early termination of immune tolerance state of hepatitis B virus infection explains liver damage. *World J Hepatol* 2014; **6**: 621-625 [PMID: 25232455 DOI: 10.4254/wjh.v6.i8.621]
59 **Shimizu Y**, Guidotti LG, Fowler P, Chisari FV. Dendritic cell immunization breaks cytotoxic T lymphocyte tolerance in hepatitis B virus transgenic mice. *J Immunol* 1998; **161**: 4520-4529 [PMID: 9794377]
60 **Maini MK**, Boni C, Lee CK, Larrubia JR, Reignat S, Ogg GS, King AS, Herberg J, Gilson R, Alisa A, Williams R, Vergani D, Naoumov NV, Ferrari C, Bertoletti A. The role of virus-specific CD8(+) cells in liver damage and viral control during persistent hepatitis B virus infection. *J Exp Med* 2000; **191**: 1269-1280 [PMID: 10770795]
61 **Fisicaro P**, Valdatta C, Boni C, Massari M, Mori C, Zerbini A, Orlandini A, Sacchelli L, Missale G, Ferrari C. Early kinetics of innate and adaptive immune responses during hepatitis B virus infection. *Gut* 2009; **58**: 974-982 [PMID: 19201769 DOI: 10.1136/gut.2008.163600]
62 **Busca A**, Kumar A. Innate immune responses in hepatitis B virus (HBV) infection. *Virol J* 2014; **11**: 22 [PMID: 24507433 DOI: 10.1186/1743-422X-11-22]
63 **Xu L**, Yin W, Sun R, Wei H, Tian Z. Kupffer cell-derived IL-10 plays a key role in maintaining humoral immune tolerance in hepatitis B virus-persistent mice. *Hepatology* 2014; **59**: 443-452 [PMID: 23929689 DOI: 10.1002/hep.26668]
64 **Zeisel MB**, Lucifora J, Mason WS, Sureau C, Beck J, Levrero M, Kann M, Knolle PA, Benkirane M, Durantel D, Michel ML, Autran B, Cosset FL, Strick-Marchand H, Trépo C, Kao JH, Carrat F, Lacombe K, Schinazi RF, Barré-Sinoussi F, Delfraissy JF, Zoulim F. Towards an HBV cure: state-of-the-art and unresolved questions--report of the ANRS workshop on HBV cure. *Gut* 2015; **64**: 1314-1326 [PMID: 25670809 DOI: 10.1136/gutjnl-2014-308943]
65 **Lang T**, Lo C, Skinner N, Locarnini S, Visvanathan K, Mansell A. The hepatitis B e antigen (HBeAg) targets and suppresses activation of the toll-like receptor signaling pathway. *J Hepatol* 2011; **55**: 762-769 [PMID: 21334391 DOI: 10.1016/j.jhep.2010.12.042]
66 **Visvanathan K**, Skinner NA, Thompson AJ, Riordan SM, Sozzi V, Edwards R, Rodgers S, Kurtovic J, Chang J, Lewin S, Desmond P, Locarnini S. Regulation of Toll-like receptor-2 expression in chronic hepatitis B by the precore protein. *Hepatology* 2007; **45**: 102-110 [PMID: 17187404 DOI: 10.1002/hep.21482]
67 **Isorce N**, Lucifora J, Zoulim F, Durantel D. Immune-modulators to combat hepatitis B virus infection: From IFN-α to novel investigational immunotherapeutic strategies. *Antiviral Res* 2015; **122**: 69-81 [PMID: 26275801 DOI: 10.1016/j.antiviral.2015.08.008]
68 **Gane EJ**, Lim YS, Gordon SC, Visvanathan K, Sicard E, Fedorak RN, Roberts S, Massetto B, Ye Z, Pflanz S, Garrison KL, Gaggar A, Mani Subramanian G, McHutchison JG, Kottilil S, Freilich B, Coffin CS, Cheng W, Kim YJ. The oral toll-like receptor-7 agonist GS-9620 in patients with chronic hepatitis B virus infection. *J Hepatol* 2015; **63**: 320-328 [PMID: 25733157 DOI: 10.1016/j.jhep.2015.02.037]
69 **Perrillo R**. Benefits and risks of interferon therapy for hepatitis B. *Hepatology* 2009; **49**: S103-S111 [PMID: 19399806 DOI: 10.1002/hep.22956]
70 **Lucifora J**, Xia Y, Reisinger F, Zhang K, Stadler D, Cheng X, Sprinzl MF, Koppensteiner H, Makowska Z, Volz T, Remouchamps C, Chou WM, Thasler WE, Hüser N, Durantel D, Liang TJ, Münk C, Heim MH, Browning JL, Dejardin E, Dandri M, Schindler M, Heikenwalder M, Protzer U. Specific and nonhepatotoxic degradation of nuclear hepatitis B virus cccDNA. *Science* 2014; **343**: 1221-1228 [PMID: 24557838 DOI: 10.1126/science.1243462]
71 **Stenglein MD**, Burns MB, Li M, Lengyel J, Harris RS. APOBEC3 proteins mediate the clearance of foreign DNA from human cells. *Nat Struct Mol Biol* 2010; **17**: 222-229 [PMID: 20062055 DOI: 10.1038/nsmb.1744]
72 **Wang FS**, Xing LH, Liu MX, Zhu CL, Liu HG, Wang HF, Lei ZY.

Dysfunction of peripheral blood dendritic cells from patients with chronic hepatitis B virus infection. *World J Gastroenterol* 2001; **7**: 537-541 [PMID: 11819824]

73 **Op den Brouw ML**, Binda RS, van Roosmalen MH, Protzer U, Janssen HL, van der Molen RG, Woltman AM. Hepatitis B virus surface antigen impairs myeloid dendritic cell function: a possible immune escape mechanism of hepatitis B virus. *Immunology* 2009; **126**: 280-289 [PMID: 18624732 DOI: 10.1111/j.1365-2567.2008.02896.x]

74 **Webster G**, Bertoletti A. Quantity and quality of virus-specific CD8 cell response: relevance to the design of a therapeutic vaccine for chronic HBV infection. *Mol Immunol* 2001; **38**: 467-473 [PMID: 11741696]

75 **Miyara M**, Sakaguchi S. Human FoxP3(+)CD4(+) regulatory T cells: their knowns and unknowns. *Immunol Cell Biol* 2011; **89**: 346-351 [PMID: 21301480 DOI: 10.1038/icb.2010.137]

76 **Sakaguchi S**, Yamaguchi T, Nomura T, Ono M. Regulatory T cells and immune tolerance. *Cell* 2008; **133**: 775-787 [PMID: 18510923 DOI: 10.1016/j.cell.2008.05.009]

77 **Xu D**, Fu J, Jin L, Zhang H, Zhou C, Zou Z, Zhao JM, Zhang B, Shi M, Ding X, Tang Z, Fu YX, Wang FS. Circulating and liver resident CD4+CD25+ regulatory T cells actively influence the antiviral immune response and disease progression in patients with hepatitis B. *J Immunol* 2006; **177**: 739-747 [PMID: 16785573]

78 **Francisco LM**, Sage PT, Sharpe AH. The PD-1 pathway in tolerance and autoimmunity. *Immunol Rev* 2010; **236**: 219-242 [PMID: 20636820 DOI: 10.1111/j.1600-065X.2010.00923.x]

79 **Fife BT**, Pauken KE. The role of the PD-1 pathway in autoimmunity and peripheral tolerance. *Ann N Y Acad Sci* 2011; **1217**: 45-59 [PMID: 21276005 DOI: 10.1111/j.1749-6632.2010.05919.x]

80 **Ye B**, Liu X, Li X, Kong H, Tian L, Chen Y. T-cell exhaustion in chronic hepatitis B infection: current knowledge and clinical significance. *Cell Death Dis* 2015; **6**: e1694 [PMID: 25789969 DOI: 10.1038/cddis.2015.42]

81 **Chen CJ**, Yang HI, Iloeje UH. Hepatitis B virus DNA levels and outcomes in chronic hepatitis B. *Hepatology* 2009; **49**: S72-S84 [PMID: 19399801 DOI: 10.1002/hep.22884]

82 **Invernizzi F**, Viganò M, Grossi G, Lampertico P. The prognosis and management of inactive HBV carriers. *Liver Int* 2016; **36** Suppl 1: 100-104 [PMID: 26725905 DOI: 10.1111/liv.13006]

83 **Tong MJ**, Trieu J. Hepatitis B inactive carriers: clinical course and outcomes. *J Dig Dis* 2013; **14**: 311-317 [PMID: 23433008 DOI: 10.1111/1751-2980.12051]

84 **Shimizu Y**. T cell immunopathogenesis and immunotherapeutic strategies for chronic hepatitis B virus infection. *World J Gastroenterol* 2012; **18**: 2443-2451 [PMID: 22654441 DOI: 10.3748/wjg.v18.i20.2443]

85 **Ebert G**, Allison C, Preston S, Cooney J, Toe JG, Stutz MD, Ojaimi S, Baschuk N, Nachbur U, Torresi J, Silke J, Begley CG, Pellegrini M. Eliminating hepatitis B by antagonizing cellular inhibitors of apoptosis. *Proc Natl Acad Sci USA* 2015; **112**: 5803-5808 [PMID: 25902530 DOI: 10.1073/pnas.1502400112]

86 **Ebert G**, Preston S, Allison C, Cooney J, Toe JG, Stutz MD, Ojaimi S, Scott HW, Baschuk N, Nachbur U, Torresi J, Chin R, Colledge D, Li X, Warner N, Revill P, Bowden S, Silke J, Begley CG, Pellegrini M. Cellular inhibitor of apoptosis proteins prevent clearance of hepatitis B virus. *Proc Natl Acad Sci USA* 2015; **112**: 5797-5802 [PMID: 25902529 DOI: 10.1073/pnas.1502390112]

87 **Sung WK**, Zheng H, Li S, Chen R, Liu X, Li Y, Lee NP, Lee WH, Ariyaratne PN, Tennakoon C, Mulawadi FH, Wong KF, Liu AM, Poon RT, Fan ST, Chan KL, Gong Z, Hu Y, Lin Z, Wang G, Zhang Q, Barber TD, Chou WC, Aggarwal A, Hao K, Zhou W, Zhang C, Hardwick J, Buser C, Xu J, Kan Z, Dai H, Mao M, Reinhard C, Wang J, Luk JM. Genome-wide survey of recurrent HBV integration in hepatocellular carcinoma. *Nat Genet* 2012; **44**: 765-769 [PMID: 22634754 DOI: 10.1038/ng.2295]

88 **Paterlini-Bréchot P**, Saigo K, Murakami Y, Chami M, Gozuacik D, Mugnier C, Lagorce D, Bréchot C. Hepatitis B virus-related insertional mutagenesis occurs frequently in human liver cancers and recurrently targets human telomerase gene. *Oncogene* 2003; **22**: 3911-3916 [PMID: 12813464 DOI: 10.1038/sj.onc.1206492]

89 **Feitelson MA**, Lee J. Hepatitis B virus integration, fragile sites, and hepatocarcinogenesis. *Cancer Lett* 2007; **252**: 157-170 [PMID: 17188425 DOI: 10.1016/j.canlet.2006.11.010]

90 **Lau CC**, Sun T, Ching AK, He M, Li JW, Wong AM, Co NN, Chan AW, Li PS, Lung RW, Tong JH, Lai PB, Chan HL, To KF, Chan TF, Wong N. Viral-human chimeric transcript predisposes risk to liver cancer development and progression. *Cancer Cell* 2014; **25**: 335-349 [PMID: 24582836 DOI: 10.1016/j.ccr.2014.01.030]

91 **Summers J**, Jilbert AR, Yang W, Aldrich CE, Saputelli J, Litwin S, Toll E, Mason WS. Hepatocyte turnover during resolution of a transient hepadnaviral infection. *Proc Natl Acad Sci USA* 2003; **100**: 11652-11659 [PMID: 14500915 DOI: 10.1073/pnas.1635109100]

92 **Block TM**, Gish R, Guo H, Mehta A, Cuconati A, Thomas London W, Guo JT. Chronic hepatitis B: what should be the goal for new therapies? *Antiviral Res* 2013; **98**: 27-34 [PMID: 23391846 DOI: 10.1016/j.antiviral.2013.01.006]

93 **Block TM**, Rawat S, Brosgart CL. Chronic hepatitis B: A wave of new therapies on the horizon. *Antiviral Res* 2015; **121**: 69-81 [PMID: 26112647 DOI: 10.1016/j.antiviral.2015.06.014]

94 **Menéndez-Arias L**, Álvarez M, Pacheco B. Nucleoside/nucleotide analog inhibitors of hepatitis B virus polymerase: mechanism of action and resistance. *Curr Opin Virol* 2014; **8**: 1-9 [PMID: 24814823 DOI: 10.1016/j.coviro.2014.04.005]

95 **Liang TJ**, Block TM, McMahon BJ, Ghany MG, Urban S, Guo JT, Locarnini S, Zoulim F, Chang KM, Lok AS. Present and future therapies of hepatitis B: From discovery to cure. *Hepatology* 2015; **62**: 1893-1908 [PMID: 26239691 DOI: 10.1002/hep.28025]

96 **Tavis JE**, Lomonosova E. The hepatitis B virus ribonuclease H as a drug target. *Antiviral Res* 2015; **118**: 132-138 [PMID: 25862291 DOI: 10.1016/j.antiviral.2015.04.002]

97 **Wooddell CI**, Rozema DB, Hossbach M, John M, Hamilton HL, Chu Q, Hegge JO, Klein JJ, Wakefield DH, Oropeza CE, Deckert J, Roehl I, Jahn-Hofmann K, Hadwiger P, Vornlocher HP, McLachlan A, Lewis DL. Hepatocyte-targeted RNAi therapeutics for the treatment of chronic hepatitis B virus infection. *Mol Ther* 2013; **21**: 973-985 [PMID: 23439496 DOI: 10.1038/mt.2013.31]

98 **Billioud G**, Kruse RL, Carrillo M, Whitten-Bauer C, Gao D, Kim A, Chen L, McCaleb ML, Crosby JR, Hamatake R, Hong Z, Garaigorta U, Swayze E, Bissig KD, Wieland S. In vivo reduction of hepatitis B virus antigenemia and viremia by antisense oligonucleotides. *J Hepatol* 2016; **64**: 781-789 [PMID: 26658683 DOI: 10.1016/j.jhep.2015.11.032]

99 **Cai D**, Mills C, Yu W, Yan R, Aldrich CE, Saputelli JR, Mason WS, Xu X, Guo JT, Block TM, Cuconati A, Guo H. Identification of disubstituted sulfonamide compounds as specific inhibitors of hepatitis B virus covalently closed circular DNA formation. *Antimicrob Agents Chemother* 2012; **56**: 4277-4288 [PMID: 22644022 DOI: 10.1128/AAC.00473-12]

100 **Hoeksema KA**, Tyrrell DL. Inhibition of viral transcription using designed zinc finger proteins. *Methods Mol Biol* 2010; **649**: 97-116 [PMID: 20680830 DOI: 10.1007/978-1-60761-753-2_6]

101 **Zimmerman KA**, Fischer KP, Joyce MA, Tyrrell DL. Zinc finger proteins designed to specifically target duck hepatitis B virus covalently closed circular DNA inhibit viral transcription in tissue culture. *J Virol* 2008; **82**: 8013-8021 [PMID: 18524822 DOI: 10.1128/JVI.00366-08]

102 **Bloom K**, Ely A, Mussolino C, Cathomen T, Arbuthnot P. Inactivation of hepatitis B virus replication in cultured cells and in vivo with engineered transcription activator-like effector nucleases. *Mol Ther* 2013; **21**: 1889-1897 [PMID: 23883864 DOI: 10.1038/mt.2013.170]

103 **Chen J**, Zhang W, Lin J, Wang F, Wu M, Chen C, Zheng Y, Peng X, Li J, Yuan Z. An efficient antiviral strategy for targeting hepatitis B virus genome using transcription activator-like effector nucleases. *Mol Ther* 2014; **22**: 303-311 [PMID: 24025750 DOI: 10.1038/mt.2013.212]

104 **Maeder ML**, Thibodeau-Beganny S, Osiak A, Wright DA, Anthony RM, Eichtinger M, Jiang T, Foley JE, Winfrey RJ, Townsend JA, Unger-Wallace E, Sander JD, Müller-Lerch F, Fu F, Pearlberg J,

Göbel C, Dassie JP, Pruett-Miller SM, Porteus MH, Sgroi DC, Iafrate AJ, Dobbs D, McCray PB, Cathomen T, Voytas DF, Joung JK. Rapid "open-source" engineering of customized zinc-finger nucleases for highly efficient gene modification. *Mol Cell* 2008; **31**: 294-301 [PMID: 18657511 DOI: 10.1016/j.molcel.2008.06.016]

105 **Holkers M**, Maggio I, Liu J, Janssen JM, Miselli F, Mussolino C, Recchia A, Cathomen T, Gonçalves MA. Differential integrity of TALE nuclease genes following adenoviral and lentiviral vector gene transfer into human cells. *Nucleic Acids Res* 2013; **41**: e63 [PMID: 23275534 DOI: 10.1093/nar/gks1446]

106 **Sander JD**, Joung JK. CRISPR-Cas systems for editing, regulating and targeting genomes. *Nat Biotechnol* 2014; **32**: 347-355 [PMID: 24584096 DOI: 10.1038/nbt.2842]

107 **Barrangou R**, Fremaux C, Deveau H, Richards M, Boyaval P, Moineau S, Romero DA, Horvath P. CRISPR provides acquired resistance against viruses in prokaryotes. *Science* 2007; **315**: 1709-1712 [PMID: 17379808 DOI: 10.1126/science.1138140]

108 **Jinek M**, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science* 2012; **337**: 816-821 [PMID: 22745249 DOI: 10.1126/science.1225829]

109 **Lin SR**, Yang HC, Kuo YT, Liu CJ, Yang TY, Sung KC, Lin YY, Wang HY, Wang CC, Shen YC, Wu FY, Kao JH, Chen DS, Chen PJ. The CRISPR/Cas9 System Facilitates Clearance of the Intrahepatic HBV Templates In Vivo. *Mol Ther Nucleic Acids* 2014; **3**: e186 [PMID: 25137139 DOI: 10.1038/mtna.2014.38]

110 **Kennedy EM**, Bassit LC, Mueller H, Kornepati AV, Bogerd HP, Nie T, Chatterjee P, Javanbakht H, Schinazi RF, Cullen BR. Suppression of hepatitis B virus DNA accumulation in chronically infected cells using a bacterial CRISPR/Cas RNA-guided DNA endonuclease. *Virology* 2015; **476**: 196-205 [PMID: 25553515 DOI: 10.1016/j.virol.2014.12.001]

111 **Seeger C**, Sohn JA. Targeting Hepatitis B Virus With CRISPR/Cas9. *Mol Ther Nucleic Acids* 2014; **3**: e216 [PMID: 25514649 DOI: 10.1038/mtna.2014.68]

112 **Liu X**, Hao R, Chen S, Guo D, Chen Y. Inhibition of hepatitis B virus by the CRISPR/Cas9 system via targeting the conserved regions of the viral genome. *J Gen Virol* 2015; **96**: 2252-2261 [PMID: 25904148 DOI: 10.1099/vir.0.000159]

113 **Zhen S**, Hua L, Liu YH, Gao LC, Fu J, Wan DY, Dong LH, Song HF, Gao X. Harnessing the clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated Cas9 system to disrupt the hepatitis B virus. *Gene Ther* 2015; **22**: 404-412 [PMID: 25652100 DOI: 10.1038/gt.2015.2]

114 **Dong C**, Qu L, Wang H, Wei L, Dong Y, Xiong S. Targeting hepatitis B virus cccDNA by CRISPR/Cas9 nuclease efficiently inhibits viral replication. *Antiviral Res* 2015; **118**: 110-117 [PMID: 25843425 DOI: 10.1016/j.antiviral.2015.03.015]

115 **Ramanan V**, Shlomai A, Cox DB, Schwartz RE, Michailidis E, Bhatta A, Scott DA, Zhang F, Rice CM, Bhatia SN. CRISPR/Cas9 cleavage of viral DNA efficiently suppresses hepatitis B virus. *Sci Rep* 2015; **5**: 10833 [PMID: 26035283 DOI: 10.1038/srep10833]

116 **Karimova M**, Beschorner N, Dammermann W, Chemnitz J, Indenbirken D, Bockmann JH, Grundhoff A, Lüth S, Buchholz F, Schulze zur Wiesch J, Hauber J. CRISPR/Cas9 nickase-mediated disruption of hepatitis B virus open reading frame S and X. *Sci Rep* 2015; **5**: 13734 [PMID: 26334116 DOI: 10.1038/srep13734]

117 **Wang J**, Xu ZW, Liu S, Zhang RY, Ding SL, Xie XM, Long L, Chen XM, Zhuang H, Lu FM. Dual gRNAs guided CRISPR/Cas9 system inhibits hepatitis B virus replication. *World J Gastroenterol* 2015; **21**: 9554-9565 [PMID: 26327763 DOI: 10.3748/wjg.v21.i32.9554]

118 **Liu S**, Zhang H, Gu C, Yin J, He Y, Xie J, Cao G. Associations between hepatitis B virus mutations and the risk of hepatocellular carcinoma: a meta-analysis. *J Natl Cancer Inst* 2009; **101**: 1066-1082 [PMID: 19574418 DOI: 10.1093/jnci/djp180]

119 **Wu Y**, Gan Y, Gao F, Zhao Z, Jin Y, Zhu Y, Sun Z, Wu H, Chen T, Wang J, Sun Y, Fan C, Xiang Y, Qian G, Groopman JD, Gu J, Tu H. Novel natural mutations in the hepatitis B virus reverse transcriptase domain associated with hepatocellular carcinoma. *PLoS One* 2014; **9**: e94864 [PMID: 24788140 DOI: 10.1371/journal.pone.0094864]

120 **Yu W**, Goddard C, Clearfield E, Mills C, Xiao T, Guo H, Morrey JD, Motter NE, Zhao K, Block TM, Cuconati A, Xu X. Design, synthesis, and biological evaluation of triazolo-pyrimidine derivatives as novel inhibitors of hepatitis B virus surface antigen (HBsAg) secretion. *J Med Chem* 2011; **54**: 5660-5670 [PMID: 21786803 DOI: 10.1021/jm200696v]

121 **Noordeen F**, Vaillant A, Jilbert AR. Nucleic acid polymers inhibit duck hepatitis B virus infection in vitro. *Antimicrob Agents Chemother* 2013; **57**: 5291-5298 [PMID: 23939902 DOI: 10.1128/AAC.01003-13]

122 **Wang YJ**, Yang L, Zuo JP. Recent developments in antivirals against hepatitis B virus. *Virus Res* 2016; **213**: 205-213 [PMID: 26732483 DOI: 10.1016/j.virusres.2015.12.014]

123 **Urban S**, Bartenschlager R, Kubitz R, Zoulim F. Strategies to inhibit entry of HBV and HDV into hepatocytes. *Gastroenterology* 2014; **147**: 48-64 [PMID: 24768844 DOI: 10.1053/j.gastro.2014.04.030]

P- Reviewer: Bowden S, Gong ZJ, Jin DY
S- Editor: Qi Y **L- Editor**: A **E- Editor**: Li D

World Journal of Hepatology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4254/wjh.v8.i21.874

World J Hepatol 2016 July 28; 8(21): 874-880
ISSN 1948-5182 (online)

Hepatocellular carcinoma beyond Milan criteria: Management and transplant selection criteria

Mohammed Elshamy, Federico Aucejo, KV Narayanan Menon, Bijan Eghtesad

Mohammed Elshamy, Federico Aucejo, Bijan Eghtesad, Hepato-biliary and Transplant Surgery, Digestive Disease and Surgery Institute, Cleveland Clinic Foundation, Cleveland, OH 44195, United States

KV Narayanan Menon, Gastroenterology and Hepatology, Digestive Disease and Surgery Institute, Cleveland Clinic Foundation, Cleveland, OH 44195, United States

Author contributions: This manuscript was written completely by the stated authors.

Conflict-of-interest statement: None.

Manuscript source: Invited manuscript

Correspondence to: Bijan Eghtesad, MD, Hepato-biliary and Transplant Surgery, Digestive Disease and Surgery Institute, Cleveland Clinic Foundation, 9500 Euclid Avenue/A100, Cleveland, OH 44195, United States. eghtesb@ccf.org
Telephone: +1-216-4445914
Fax: +1-216-4449375

Received: March 30, 2016
Peer-review started: April 6, 2016
First decision: June 7, 2016
Revised: June 17, 2016
Accepted: July 11, 2016
Article in press: July 13, 2016
Published online: July 28, 2016

Abstract

Liver transplantation (LT) for hepatocellular carcinoma (HCC) has been established as a standard treatment in selected patients for the last two and a half decades. After initially dismal outcomes, the Milan criteria (MC) (single HCC $\leqslant$ 5 cm or up to 3 HCCs $\leqslant$ 3 cm) have been adopted worldwide to select HCC patients for LT, however cumulative experience has shown that MC can be too strict. This has led to the development of numerous expanded criteria worldwide. Morphometric expansions on MC as well as various criteria which incorporate biomarkers as surrogates of tumor biology have been described. HCC that presents beyond MC initially can be downstaged with locoregional therapy (LRT). Post-LRT monitoring aims to identify candidates with favorable tumor behavior. Similarly, tumor marker levels as response to LRT has been utilized as surrogate of tumor biology. Molecular signatures of HCC have also been correlated to outcomes; these have yet to be incorporated into HCC-LT selection criteria formally. The ongoing discrepancy between organ demand and supply makes patient selection the most challenging element of organ allocation. Further validation of extended HCC-LT criteria models and pre-LT treatment strategies are required.

Key words: Hepatocellular carcinoma; Milan criteria; Liver transplantation; Expanded criteria; Locoregional therapy; Down staging

Core tip: Numerous expanded selection criteria for hepatocellular carcinoma (HCC)-liver transplantation (LT) have been proposed worldwide. Surrogates of favorable tumor biology such as Post-locoregional therapy strategies which observe tumor behavior, and the addition of HCC biomarkers to selection criteria have been explored. Further investigation is encouraged to identify patients beyond MC with the most favorable tumor biology for LT.

Elshamy M, Aucejo F, Menon KVN, Eghtesad B. Hepatocellular carcinoma beyond Milan criteria: Management and transplant

selection criteria. *World J Hepatol* 2016; 8(21): 874-880 Available from: URL: http://www.wjgnet.com/1948-5182/full/v8/i21/874.htm DOI: http://dx.doi.org/10.4254/wjh.v8.i21.874

INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common primary malignancy of the liver, with over 700000 new cases diagnosed yearly worldwide[1]. HCC continues to be a global health problem due to insufficient screening and surveillance and poorly controlled risk factors[2]. HCC arises most frequently in patients with chronic liver disease from diverse etiologies, and liver transplantation (LT) has been established as a standard treatment in selected patients for the last two and a half decades[3]. However, an ongoing conundrum is the discrepancy between organ demand and supply, making patient selection the most challenging piece of the puzzle to prevent organ misutilization[4].

Poor patient selection (excessive tumor burden, unknown tumor biology) made initial results of LT for HCC quite dismal[5]. It wasn't until 1996, when Mazzaferro *et al*[6] defined tumor criteria for patient selection (single lesion $\leqslant$ 5 cm, or up to 3 lesions $\leqslant$ 3 cm each in the absence of tumor vascular invasion or evidence of extra-hepatic metastases) associated with comparable outcome to patients undergoing LT without HCC. The study revealed 4 year post-LT survival > 75% and post-LT recurrence rate in the order of 8%. These criteria have since been known as the Milan criteria (MC), and have been adopted worldwide to select HCC patients for LT[7].

Patients who present with HCC beyond MC can be down-staged *via* loco-regional therapy (LRT). LRT are trans-catheter, needle based or radiation treatments which target the tumor and induce selective tumor necrosis[8]. The efficacy of these treatments is gauged radiologically by the modified Response Evaluation Criteria in Solid Tumors[9]. Tumor response to LRT, post LRT observation before LT, and HCC biomarkers have been described for selecting the most favorable tumor biology in patients presenting with HCC beyond MC[9-11].

Although strict adherence to MC can produce outcomes comparable to LT for non-HCC, cumulative experience over the last two decades have shown that MC can be too strict, and that select patients beyond MC may benefit from LT with adequate survival[12]. This has led to the development of numerous HCC expanded criteria worldwide, applied for both cadaveric and live donor liver transplantation.

Herein, we review various expanded HCC criteria and outcomes, impact of tumor response to LRT in post-LT outcome and emerging HCC molecular signatures that may be incorporated into patient selection criteria in the near future.

EXTENDED LT-HCC CRITERIA

In 2001, Yao *et al*[13] published one of the most popular expanded LT-HCC criteria. The University of California, San Francisco (UCSF) criteria considered a single lesion $\leqslant$ 6.5 cm, or 2-3 lesions $\leqslant$ 4.5 cm each, with total tumor diameter $\leqslant$ 8 cm.

Tumor recurrence was 11.4% and 5 years post-LT survival was in the order of 72.4%[13]. The original UCSF criteria were developed based on explant histopathological analysis, but subsequently have been validated utilizing pre-LT imaging. In 2007, Yao *et al*[14] published a prospective study utilizing the UCSF criteria revealing 80% 5 years post-LT recurrence free survival (RFS). Alongside MC, UCSF criteria have been the most widely recognized transplant criteria for HCC, and can expand 5%-20% the indication of LT for HCC patients[14]. Currently, some worldwide transplant centers utilize UCSF as the standard selection LT criteria for HCC[15].

The Navarro extended criteria described by Herrero *et al*[16] in 2001 can expand the MC by considering LT for a single lesion $\leqslant$ 6 cm, or 2-3 lesions $\leqslant$ 5 cm each. In their analysis, 12.7% of the cohort experienced tumor recurrence. Post-LT 5 years overall survival and RFS was 79% and 70% respectively.

Silva *et al*[17] published the Valencia criteria in 2008. These would consider LT in HCC patients with 1-3 lesions $\leqslant$ 5 cm each, and total tumor $\leqslant$ 10 cm. Two hundred and fifty-seven patients undergoing LT for HCC were analyzed, however only 10% were beyond MC based on pre-LT imaging. Patients who fell within the Valencia criteria demonstrated post-LT 5 year survival comparable to patients within MC. The Valencia criteria expands LT to a higher maximum tumor burden compared to both MC and UCSF criteria, without detriment to patient survival, however similar to the Navarro criteria, due to the small number of patients in this cohort, these criteria require further validation.

Correlation of tumor size and number according to explant pathology and post-LT survival in 1206 patients from the International Registry of Hepatic Tumors, led to the recommendation of LT for a single lesion $\leqslant$ 6 cm, or 2-4 lesions $\leqslant$ 5 cm each by Onaca *et al*[18] in 2007. Survival in patients exceeding MC but meeting these criteria were not significantly lower than for patients meeting MC. Five years post-LT RFS with a single lesion 5.1-6.0 cm in diameter, or with 2-4 lesions (largest 3.1-5.0 cm) were 63.9%, and 64.6% respectively, compared to 5 years post-LT RFS of 61.8% if MC were met[18].

Other proposed extended criteria do not put a limit to number of tumors recommended for LT. Roayaie *et al*[19] in 2002, demonstrated 55% 5 years post- LT RFS for patients with lesions 5-7 cm in diameter. In 2004, Kneteman *et al*[20] reported the outcomes of LT utilizing extended criteria described as a singles lesion < 7.5 cm, or multiple lesions < 5 cm each. Four year post-LT survival was 82.9% *vs* 87.4% in the MC group.

One of the more recently proposed extended criteria is the Up-to-7 criteria proposed by Mazzaferro *et al*[21] in 2009. A cohort of 1556 patients undergoing cadaveric LT and LDLT for HCC from 36 transplant centers was analyzed, 71.5% of the cohort had HCC exceeding MC. The Up-to-7 criteria are defined as the sum of the

size of the largest tumor in cm and the total number of tumors in the absence of tumor microvascular invasion. Five years post-LT survival for patients within the Up-to-7 criteria compared to MC were 71.2% *vs* 73.3%[21]. The major limitation of these criteria is the lack of pre-LT information about microvascular invasion. Currently, this can only be partially projected *via* assessment of alpha-fetoprotein (AFP) level.

Extended LT-HCC criteria using living donors

Outcomes in HCC patients undergoing living donor liver transplantation (LDLT) were shown to be equivalent to cadaveric liver transplantation[22]. Soejima *et al*[23] reported that tumor diameter > 5 cm was associated with worse prognosis; however the number of tumors was not. In the cohort of 60 patients who underwent LDLT for HCC, 67% were beyond MC based on pre-LT imaging. Three years post-LT survival of 68.6% was reported for patients beyond MC[23].

Jonas *et al*[24] also described their extended criteria based on a cohort of 21 patients undergoing LDLT for HCC. Three year survival rates for patients not meeting MC or USCF criteria were 62% and 53% respectively. Sugawara *et al*[25] proposed an expansion of selection criteria to include up to 5 HCC lesions, ⩽ 5 cm each. In their cohort of 78 patients, post-LT RFS at 3 years was 94%.

Table 1 demonstrates an overview of proposed morphometric based expanded selection criteria.

INCORPORATION OF SURROGATES OF TUMOR BIOLOGY TO SELECTION CRITERIA

Tumor markers

Post-LT outcomes in patients with HCC are in part a consequence of tumor biology. As a result of the impossibility to unveil this feature solely through morphometric imaging characteristics, multiple studies have attempted to include other indicators of tumor behavior as selection criteria. AFP and des-γ-carboxyprothrombin (DCP) both have established correlations with post treatment prognosis[26,27]. A pre-LT AFP level > 1000 ng/mL has been demonstrated as a significant predictor of HCC recurrence post-LT[26]. A large scale analysis of United Network for Organ Sharing (UNOS) data has demonstrated that patients transplanted beyond MC with an AFP level of 0 to 15 ng/mL (normal range) had improved survival[28].

One of the most popular HCC-LT extended criteria including biomarkers as surrogates of tumor biology are the Hangzhou criteria (absence of macrovascular invasion and total tumor diameter ⩽ 8 cm. If the tumor burden is > 8 cm, histopathology *via* tumor biopsy should be non-poorly differentiated HCC and AFP level should be ⩽ 400 ng/mL[29].

In the original cohort of 195 patients, fulfilling Hangzhou criteria led to a 5 year survival of 70.7% and DFS: 62.4%. On the other hand, patients beyond Hangzhou criteria had a 5 year survival of 18.9% and DFS: 4.7%[29]. A large scale comparative study of multiple extended criteria confirmed post LT survival associated with LT beyond MC but meeting Hangzhou at 1-, 3-, 5- and 10-years was 89.5%, 70.8%, 62.4% and 52.9% respectively. Additionally, 1-, 3-, 5- and 10-year RFS was 81.6%, 64.3%, 56.5%, and 37.2% respectively. Compared to MC, expanded criteria expanded transplantable patients by 12.4% for Valencia, 16.3% for UCSF, 19.6% for Navarro, and 51.5% for Hangzhou. RFS rates were comparable to MC[30].

In 2012, Lai *et al*[31] also suggested that the combination of total tumor diameter > 8 cm and an AFP level ⩽ 400 ng/mL would result in favorable survival outcomes. The 5 year DFS rate was 74.4%. It was also noted that patients with increased AFP values in response to LRT had higher recurrence rates[31]. Duvoux *et al*[32] have suggested a predictive scoring model that combines the AFP level at listing with MC. In their model, an AFP level ⩽ 100 ng/mL in the setting of patients beyond MC (1-3 lesions with a maximum tumor diameter of 6 cm) demonstrated 5- year survival near 70%[32].

Similar criteria have been applied to LDLT as well. In a multicenter study from Japan, Todo *et al*[33] suggested that the combination of an AFP cut of level ⩾ 200 ng/mL and protein induced by vitamin K absence or antagonism factor Ⅱ (PIVKA Ⅱ) ⩾ 100 mAU/mL are significant predictors for poor post LT survival. These combined were described as the A-P level. Five year DFS for beyond MC HCC patients and within the A-P cutoff level was similar to those within MC at 78.7% and 90.4% respectively.

Kwon *et al*[34] demonstrated their outcomes incorporating an AFP level ⩽ 400 ng/mL as a selection criteria along with any number of lesions ⩽ 5 cm each. In a cohort of 139 patients, 5 year survival was noted at 79.9%, without a significant difference between patients within or beyond MC[34]. More recently in 2015, Toso *et al*[35] in a prospective study suggested extended LT criteria described as a combination of a total tumor volume ⩽ 115 cm^3 and an AFP level ⩽ 400 ng/mL. Four year post LT survival was similar between the extended criteria group and the MC group at 78.7% and 74.6% respectively[35].

A lower AFP cut off rate of < 100 ng/mL as a critera for HCC-LT was recommended by Grąt *et al*[36]. A retrospective analysis of a 121 patients demonstrated significant prediction of recurrence in patients transplanted within UCSF and Up-to-7 criteria who surpassed this limit. Five year RFS for patients meeting UCSF and within the AFP cut off was superior to those meeting USCF but beyond the cut off limit at 100% *vs* 69% respectively. Similarly, when applied to the Up-to-7 criteria, 5 year RFS for those meeting both the criteria and cut off limit was noted at 100% *vs* 71.9% for beyond the cut off limit[36].

DCP, often utilized as a tumor marker for HCC in Japan, has been incorporated into the Kyoto criteria published by Fujiki *et al*[37] in 2009: A DCP level of ⩽ 400 mAU/mL in addition to morphometric criteria of up

Table 1 Expanded morphometric criteria for hepatocellular carcinoma-liver transplantation

Ref.	Year	Description	Donor type	*n*	Survival
Yao *et al*[13]	2001	1 lesion ≤ 6.5 cm, or 2-3 lesions ≤ 4.5 cm each. Total tumor diameter ≤ 8 cm	Cadaveric	70	5 yr OS: 72.4%
Herrero *et al*[16]	2001	1 lesion ≤ 6 cm, or 2-3 lesions ≤ 5 cm each	Cadaveric	47	5 yr OS: 79%
Roayaie *et al*[19]	2002	Any number of lesions, 5-7 cm each	Cadaveric	43	5 yr RFS: 55%
Keneteman *et al*[20]	2004	1 lesion < 7.5 cm, or multiple lesions < 5 cm each	Cadaveric	40	4 yr OS: 82.9% 4 yr RFS: 76.8%
Onaca *et al*[18]	2007	1 lesion ≤ 6 cm, or 2-4 lesions ≤ 5 cm each	Cadaveric	1206	5 yr RFS: 1 lesion ≤ 6 cm: 63.9%/or 2-4 lesions 3.1 cm-5 cm each: 64.6%
Soejima *et al*[23]	2007	Any number lesions ≤ 5 cm each	Living	67	3 yr OS: 68.6%
Jonas *et al*[24]	2007	Single lesion and diameter, or any number of lesions ≤ 6 cm each. Total tumor diameter ≤ 15 cm	Living	21	3 yr OS: 53%
Sugawara *et al*[25]	2007	Up to 5 lesions ≤ 5 cm each	Living	78	3 yr RFS: 94%
Silva *et al*[17]	2008	1-3 lesions ≤ 5 cm each. Total tumor diameter ≤ 10 cm	Cadaveric	257	5 yr OS: 67%
Mazzaferro *et al*[21]	2009	The sum of the size and number of tumors not exceeding 7 in the absence of microvascular invasion	Both	1556	5 yr OS: 71.2%

RFS: Recurrence free survival; OS: Overall survival.

to 10 nodules ≤ 5 cm each. Five year recurrence was similar for patients within MC, and patients beyond MC but meeting Kyoto criteria at 7% and 4% respectively. Five year survival for patients meeting Kyoto criteria was 89%[37]. Takada *et al*[38] also propose similar selection criteria. In their cohort of 136 patients, those who met the proposed selection criteria demonstrated a 5 year survival rate of 87%.

Lee *et al*[39] proposes the incorporation of 18F-Fluoro-deoxyglucose positron emission tomography (PET) to HCC-LT selection criteria. Retrospective analysis of 2806 patients demonstrated that patients with PET negative scans preoperatively in combination with a total tumor diameter ≤ 10 cm demonstrated 5 year overall survival and DFS rates of 73.4% and 80.4% respectively, which was not significantly different from those within MC[39].

Table 2 demonstrates an overview of proposed expanded selection criteria which incorporate biomarkers to morphometric tumor measurements.

Downstaging and response to LRT

LRT in HCC-LT candidates is considered an element of two approaches: For patients listed/to be listed within MC, LRT is applied neo-adjuvently as bridging therapy to halt tumor progression[40]. Patients who present initially beyond MC are downstaged to reduce tumor size to meet MC[41]. Both strategies provide the opportunity to evaluate radiological and laboratory surrogates of tumor response, which could unveil more aggressive tumors with less favorable biology in order to be excluded from LT.

Since tumor behavior over time is a surrogate of tumor biology, LRT followed by a required waiting time before LT can help to unveil tumor biology and has been coined as the "ablate and wait" strategy[10].

A systematic review and pooled analysis of 13 studies revealed the success rate of downstaging raging between 11%-77%. There was no significant difference in utilizing Transarterial Chemoembolization or Transarterial Radioemobilzation. Post LT recurrence rates were noted to be as high as 16%, however survival outcomes could not be calculated due to heterogeneity of the data which prevented adequate analysis. Further investigation is required to determine the effect of heterogeneous downstaging protocols in term of LRT modality, frequency, and waiting period pre- LT[42].

The correlation between the AFP expression in response to LRT and post LT survival has also been investigated. A multicentric study which included 422 patients who underwent LRT before LT for HCC (306 within MC, 116 beyond MC) demonstrated an increased risk for HCC recurrence and death with an AFP slope > 15 ng/mL per month[43].

Future directions: Molecular signatures

Genetic molecular signatures have been explored for their potential as biomarkers for HCC[44]. Dvorchik *et al*[45] assessed fractional allelic imbalance rates in a panel of 9 tumor suppressor genes. A higher rate of tumor suppressor gene mutation correlated with worse post-LT outcome independently of tumor vascular invasion or tumor burden[45].

MicroRNA (miRNA) signatures detected in serum exosomes have also been described as potential biomarkers for HCC. In a cohort of 6 HCC patients miR-718 was described as significantly linked to HCC; and this was further validated in a cohort of 59 LDLT HCC cases. In the validation cohort, miR-718 expression levels were significantly lower in patients beyond MC, and with poorer histological differentiation. However, due to the small incidence of recurrence in this cohort, no direct association could be linked to miR-718[46].

Another study analyzed paraffin embedded tissue from 69 HCC LT patients (which included 40 post LT recurrences) for miRNA expression. The biomarker proposed by this study consisted of 67 miRNAs, this biomarker had significantly identified the HCC recurrent cases, and it also displayed significance when applied to patients within and beyond MC[47].

A predictive scoring system was recently published combining MC with miRNA markers to identify the risk of

Table 2 Expanded criteria that incorporate tumor biomarkers for hepatocellular carcinoma-liver transplantation

Ref.	Year	Morphometric criteria	Biomarker criteria	Donor type	*n*	Survival
Kwon *et al*[34]	2007	Any number of lesions ≤ 5 cm each	AFP ≤ 400 ng/mL	Living	139	5 yr OS: 79.9%
Takada *et al*[38]	2007	Up to 10 lesions ≤ 5 cm each	PIVKA-II ≤ 400 mAU/mL	Living	136	5 yr OS: 87%
Zheng *et al*[29]	2008	Total tumor diameter ≤ 8 cm or total tumor diameter > 8 cm with histopathologic grade Ⅰ or Ⅱ	If total tumor diameter > 8 cm: AFP ≤ 400 ng/mL	Cadaveric	195	5 yr OS: 70.7%, 5 yr DFS: 62.4%
Fujiki *et al*[37]	2009	Up to 10 lesions ≤ 5 cm each	DCP ≤ 400 mAU/mL	Living	144	5 yr OS: 89%
Lai *et al*[31]	2012	Total tumor diameter ≤ 8 cm	AFP ≤ 400 ng/mL	Cadaveric	158	5 yr DFS: 74.4%
Grąt *et al*[36]	2014	UCSF or Up-to-7 criteria	AFP < 100 ng/mL	Cadaveric	121	5 yr OS: 100%
Toso *et al*[35]	2015	Total tumor volume ≤ 115 cm^3	AFP ≤ 400 ng/mL	Cadaveric	166	4 yr OS: 74.6%
Lee *et al*[39]	2015	Total tumor diameter ≤ 10 cm	PET/CT negative uptake	Living	280	5 yr OS: 73.4%, 5 yr DFS: 80.4%

AFP: Alpha fetal protein; UCSF: University of California, San Francisco; DFS: Disease free survival; PIVKA-Ⅱ: Protein induced by vitamin K absence or antagonism factor Ⅱ; OS: Overall survival.

HCC recurrence post- LT. Two miRNA markers significant of tumor recurrence (miR-214, miR-3187) were identified *via* microarray analysis of paraffin explant samples of 40 patients. In another validation cohort of 22 patients, high expression of miR-214 and low expression of miR-3187 were significantly associated with HCC recurrence. A predictive score including levels of these miRNAs and MC status was successful in identifying patients with a lower risk for tumor recurrence and death[48].

CONCLUSION

Although there remains a large discrepancy between cadaveric organ availability and demand, numerous selection criteria for HCC exceeding the well-established MC have been proposed worldwide. Only a few of these criteria have been validated by multiple independent studies. The current direction of incorporating biomarkers and other surrogates of tumor biology to morphometric criteria is highly encouraged, however this is not without challenge. The most commonly used HCC biomarker AFP, is not a reliable indicator for HCC. AFP levels are not elevated in up to 40% of cases [49,50], furthermore AFP is challenged by its poor sensitivity and specificity[51]. Pre-LT tumor biopsy is somehow discouraged, due in part to tumor heterogeneity when multifocal HCC is present, as well as the risk of needle-tract seeding[52].

In light of the current organ shortage, hepatic resection followed by salvage LT has also been suggested as a treatment strategy for HCC. A systematic review by Chan *et al*[53] demonstrated median overall survival at 1-, 3- and 5-years post LT was 89%, 80%, and 62% respectively. Additionally, tissue specimens obtained from a pre-LT resection can assist in selection of tumors with a favorable histopathological profile for LT[53].

Monitoring radiologic and laboratory (tumor markers) tumor response post-LRT has been utilized to identify tumors with favorable biology; and in line with this current UNOS guidelines for organ allocation in the United States require listing HCC patients for 6 mo before qualification for HCC exception points[54].

miRNAs are stable in blood and resistant to RNAases, which makes them promising HCC biomarkers[46]. Further validation of extended HCC-LT criteria models that incorporate predictors of tumor biology are needed to optimize organ utilization in an ongoing era of organ shortage.

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

3 **Freeman RB Jr**, Steffick DE, Guidinger MK, Farmer DG, Berg CL, Merion RM. Liver and intestine transplantation in the United States, 1997-2006. *Am J Transplant* 2008; **8**: 958-976 [PMID: 18336699 DOI: 10.1111/j.1600-6143.2008.02174.x]

4 **Martin P**, DiMartini A, Feng S, Brown R Jr, Fallon M. Evaluation for liver transplantation in adults: 2013 practice guideline by the American Association for the Study of Liver Diseases and the American Society of Transplantation. *Hepatology* 2014; **59**: 1144-1165 [PMID: 24716201 DOI: 10.1002/hep.26972]

5 **Iwatsuki S**, Gordon RD, Shaw BW Jr, Starzl TE. Role of liver transplantation in cancer therapy. *Ann Surg* 1985; **202**: 401-407 [PMID: 2996449 DOI: 10.1097/00000658-198510000-00001]

6 **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]

7 **Mazzaferro V**, Bhoori S, Sposito C, Bongini M, Langer M, Miceli R, Mariani L. Milan criteria in liver transplantation for hepatocellular carcinoma: an evidence-based analysis of 15 years of experience. *Liver Transpl* 2011; **17** Suppl 2: S44-S57 [PMID: 21695773 DOI: 10.1002/lt.22365]

8 **Cescon M**, Cucchetti A, Ravaioli M, Pinna AD. Hepatocellular carcinoma locoregional therapies for patients in the waiting list. Impact on transplantability and recurrence rate. *J Hepatol* 2013; **58**: 609-618 [PMID: 23041304 DOI: 10.1016/j.jhep.2012.09.021]

9 **Lencioni R**, Llovet JM. Modified RECIST (mRECIST) assessment for hepatocellular carcinoma. *Semin Liver Dis* 2010; **30**: 52-60 [PMID: 20175033 DOI: 10.1055/s-0030-1247132]

10 **Roberts JP**, Venook A, Kerlan R, Yao F. Hepatocellular carcinoma: Ablate and wait versus rapid transplantation. *Liver Transpl* 2010; **16**: 925-929 [PMID: 20658555 DOI: 10.1002/lt.22103]

11 **Merani S**, Majno P, Kneteman NM, Berney T, Morel P, Mentha G, Toso C. The impact of waiting list alpha-fetoprotein changes

on the outcome of liver transplant for hepatocellular carcinoma. *J Hepatol* 2011; **55**: 814-819 [PMID: 21334400 DOI: 10.1016/j.jhep.2010.12.040]

12 **Yao FY**, Ferrell L, Bass NM, Bacchetti P, Ascher NL, Roberts JP. Liver transplantation for hepatocellular carcinoma: comparison of the proposed UCSF criteria with the Milan criteria and the Pittsburgh modified TNM criteria. *Liver Transpl* 2002; **8**: 765-774 [PMID: 12200775 DOI: 10.1053/jlts.2002.34892]

13 **Yao FY**, Ferrell L, Bass NM, Watson JJ, Bacchetti P, Venook A, Ascher NL, Roberts JP. Liver transplantation for hepatocellular carcinoma: expansion of the tumor size limits does not adversely impact survival. *Hepatology* 2001; **33**: 1394-1403 [PMID: 11391528 DOI: 10.1053/jhep.2001.24563]

14 **Yao FY**, Xiao L, Bass NM, Kerlan R, Ascher NL, Roberts JP. Liver transplantation for hepatocellular carcinoma: validation of the UCSF-expanded criteria based on preoperative imaging. *Am J Transplant* 2007; **7**: 2587-2596 [PMID: 17868066 DOI: 10.1111/j.1600-6143.2007.01965.x]

15 The Transplantation Society Of Australia and New Zealand Organ Transplantation from Deceased Donors: Consensus Statement on Eligibility Criteria and Allocation Protocols. [updated 2015 Apr 15; accessed 2016 Mar 28]. Available from: URL: http://www.tsanz.com.au/organallocationprotocols/documents/CSVs1.4_V4_Final.pdf

16 **Herrero JI**, Sangro B, Quiroga J, Pardo F, Herraiz M, Cienfuegos JA, Prieto J. Influence of tumor characteristics on the outcome of liver transplantation among patients with liver cirrhosis and hepatocellular carcinoma. *Liver Transpl* 2001; **7**: 631-636 [PMID: 11460231 DOI: 10.1053/jlts.2001.25458]

17 **Silva M**, Moya A, Berenguer M, Sanjuan F, López-Andujar R, Pareja E, Torres-Quevedo R, Aguilera V, Montalva E, De Juan M, Mattos A, Prieto M, Mir J. Expanded criteria for liver transplantation in patients with cirrhosis and hepatocellular carcinoma. *Liver Transpl* 2008; **14**: 1449-1460 [PMID: 18825681 DOI: 10.1002/lt.21576]

18 **Onaca N**, Davis GL, Goldstein RM, Jennings LW, Klintmalm GB. Expanded criteria for liver transplantation in patients with hepatocellular carcinoma: a report from the International Registry of Hepatic Tumors in Liver Transplantation. *Liver Transpl* 2007; **13**: 391-399 [PMID: 17318865 DOI: 10.1002/lt.21095]

19 **Roayaie S**, Frischer JS, Emre SH, Fishbein TM, Sheiner PA, Sung M, Miller CM, Schwartz ME. Long-term results with multimodal adjuvant therapy and liver transplantation for the treatment of hepatocellular carcinomas larger than 5 centimeters. *Ann Surg* 2002; **235**: 533-539 [PMID: 11923610 DOI: 10.1097/00000658-200204000-00012]

20 **Kneteman NM**, Oberholzer J, Al Saghier M, Meeberg GA, Blitz M, Ma MM, Wong WW, Gutfreund K, Mason AL, Jewell LD, Shapiro AM, Bain VG, Bigam DL. Sirolimus-based immunosuppression for liver transplantation in the presence of extended criteria for hepatocellular carcinoma. *Liver Transpl* 2004; **10**: 1301-1311 [PMID: 15376305 DOI: 10.1002/lt.20237]

21 **Mazzaferro V**, Llovet JM, Miceli R, Bhoori S, Schiavo M, Mariani L, Camerini T, Roayaie S, Schwartz ME, Grazi GL, Adam R, Neuhaus P, Salizzoni M, Bruix J, Forner A, De Carlis L, Cillo U, Burroughs AK, Troisi R, Rossi M, Gerunda GE, Lerut J, Belghiti J, Boin I, Gugenheim J, Rochling F, Van Hoek B, Majno P; Metroticket Investigator Study Group. Predicting survival after liver transplantation in patients with hepatocellular carcinoma beyond the Milan criteria: a retrospective, exploratory analysis. *Lancet Oncol* 2009; **10**: 35-43 [PMID: 19058754 DOI: 10.1016/S1470-2045[08]70284-5]

22 **Bhangui P**, Vibert E, Majno P, Salloum C, Andreani P, Zocrato J, Ichai P, Saliba F, Adam R, Castaing D, Azoulay D. Intention-to-treat analysis of liver transplantation for hepatocellular carcinoma: living versus deceased donor transplantation. *Hepatology* 2011; **53**: 1570-1579 [PMID: 21520172 DOI: 10.1002/hep.24231]

23 **Soejima Y**, Taketomi A, Yoshizumi T, Uchiyama H, Aishima S, Terashi T, Shimada M, Maehara Y. Extended indication for living donor liver transplantation in patients with hepatocellular carcinoma. *Transplantation* 2007; **83**: 893-899 [PMID: 17460559 DOI: 10.1097/01.tp.0000259015.46798.ec]

24 **Jonas S**, Mittler J, Pascher A, Schumacher G, Theruvath T, Benckert C, Rudolph B, Neuhaus P. Living donor liver transplantation of the right lobe for hepatocellular carcinoma in cirrhosis in a European center. *Liver Transpl* 2007; **13**: 896-903 [PMID: 17538994 DOI: 10.1002/lt.21189]

25 **Sugawara Y**, Tamura S, Makuuchi M. Living donor liver transplantation for hepatocellular carcinoma: Tokyo University series. *Dig Dis* 2007; **25**: 310-312 [PMID: 17960065 DOI: 10.1159/000106910]

26 **Hameed B**, Mehta N, Sapisochin G, Roberts JP, Yao FY. Alpha-fetoprotein level & gt; 1000 ng/mL as an exclusion criterion for liver transplantation in patients with hepatocellular carcinoma meeting the Milan criteria. *Liver Transpl* 2014; **20**: 945-951 [PMID: 24797281 DOI: 10.1002/lt.23904]

27 **Hakamada K**, Kimura N, Miura T, Morohashi H, Ishido K, Nara M, Toyoki Y, Narumi S, Sasaki M. Des-gamma-carboxy prothrombin as an important prognostic indicator in patients with small hepatocellular carcinoma. *World J Gastroenterol* 2008; **14**: 1370-1377 [PMID: 18322950 DOI: 10.3748/wjg.14.1370]

28 **Berry K**, Ioannou GN. Serum alpha-fetoprotein level independently predicts posttransplant survival in patients with hepatocellular carcinoma. *Liver Transpl* 2013; **19**: 634-645 [PMID: 23536495 DOI: 10.1002/lt.23652]

29 **Zheng SS**, Xu X, Wu J, Chen J, Wang WL, Zhang M, Liang TB, Wu LM. Liver transplantation for hepatocellular carcinoma: Hangzhou experiences. *Transplantation* 2008; **85**: 1726-1732 [PMID: 18580463 DOI: 10.1097/TP.0b013e31816b67e4]

30 **Xu X**, Lu D, Ling Q, Wei X, Wu J, Zhou L, Yan S, Wu L, Geng L, Ke Q, Gao F, Tu Z, Wang W, Zhang M, Shen Y, Xie H, Jiang W, Wang H, Zheng S. Liver transplantation for hepatocellular carcinoma beyond the Milan criteria. *Gut* 2016; **65**: 1035-1041 [PMID: 25804634 DOI: 10.1136/gutjnl-2014-308513]

31 **Lai Q**, Avolio AW, Manzia TM, Sorge R, Agnes S, Tisone G, Berloco PB, Rossi M. Combination of biological and morphological parameters for the selection of patients with hepatocellular carcinoma waiting for liver transplantation. *Clin Transplant* 2012; **26**: E125-E131 [PMID: 22192083 DOI: 10.1111/j.1399-0012.2011.01572.x]

32 **Duvoux C**, Roudot-Thoraval F, Decaens T, Pessione F, Badran H, Piardi T, Francoz C, Compagnon P, Vanlemmens C, Dumortier J, Dharancy S, Gugenheim J, Bernard PH, Adam R, Radenne S, Muscari F, Conti F, Hardwigsen J, Pageaux GP, Chazouillères O, Salame E, Hilleret MN, Lebray P, Abergel A, Debette-Gratien M, Kluger MD, Mallat A, Azoulay D, Cherqui D; Liver Transplantation French Study Group. Liver transplantation for hepatocellular carcinoma: a model including α-fetoprotein improves the performance of Milan criteria. *Gastroenterology* 2012; **143**: 986-994.e3; quiz e14-e15 [PMID: 22750200 DOI: 10.1053/j.gastro.2012.05.052]

33 **Todo S**, Furukawa H, Tada M; Japanese Liver Transplantation Study Group. Extending indication: role of living donor liver transplantation for hepatocellular carcinoma. *Liver Transpl* 2007; **13**: S48-S54 [PMID: 17969069 DOI: 10.1002/lt.21334]

34 **Kwon CH**, Kim DJ, Han YS, Park JB, Choi GS, Kim SJ, Joh JW, Lee SK. HCC in living donor liver transplantation: can we expand the Milan criteria? *Dig Dis* 2007; **25**: 313-319 [PMID: 17960066 DOI: 10.1159/000106911]

35 **Toso C**, Meeberg G, Hernandez-Alejandro R, Dufour JF, Marotta P, Majno P, Kneteman NM. Total tumor volume and alpha-fetoprotein for selection of transplant candidates with hepatocellular carcinoma: A prospective validation. *Hepatology* 2015; **62**: 158-165 [PMID: 25777590 DOI: 10.1002/hep.27787]

36 **Grąt M**, Kornasiewicz O, Lewandowski Z, Hołówko W, Grąt K, Kobryń K, Patkowski W, Zieniewicz K, Krawczyk M. Combination of morphologic criteria and α-fetoprotein in selection of patients with hepatocellular carcinoma for liver transplantation minimizes the problem of posttransplant tumor recurrence. *World J Surg* 2014; **38**: 2698-2707 [PMID: 24858191 DOI: 10.1007/

s00268-014-2647-3]
37 **Fujiki M**, Takada Y, Ogura Y, Oike F, Kaido T, Teramukai S, Uemoto S. Significance of des-gamma-carboxy prothrombin in selection criteria for living donor liver transplantation for hepatocellular carcinoma. *Am J Transplant* 2009; **9**: 2362-2371 [PMID: 19656125 DOI: 10.1111/j.1600-6143.2009.02783.x]
38 **Takada Y**, Ito T, Ueda M, Sakamoto S, Haga H, Maetani Y, Ogawa K, Ogura Y, Oike F, Egawa H, Uemoto S. Living donor liver transplantation for patients with HCC exceeding the Milan criteria: a proposal of expanded criteria. *Dig Dis* 2007; **25**: 299-302 [PMID: 17960063]
39 **Lee SD**, Kim SH, Kim SK, Kim YK, Park SJ. Clinical Impact of 18F-Fluorodeoxyglucose Positron Emission Tomography/Computed Tomography in Living Donor Liver Transplantation for Advanced Hepatocellular Carcinoma. *Transplantation* 2015; **99**: 2142-2149 [PMID: 25905981 DOI: 10.1097/TP.0000000000000719]
40 **Otto G**, Herber S, Heise M, Lohse AW, Mönch C, Bittinger F, Hoppe-Lotichius M, Schuchmann M, Victor A, Pitton M. Response to transarterial chemoembolization as a biological selection criterion for liver transplantation in hepatocellular carcinoma. *Liver Transpl* 2006; **12**: 1260-1267 [PMID: 16826556 DOI: 10.1002/lt.20837]
41 **Yao FY**, Kerlan RK, Hirose R, Davern TJ, Bass NM, Feng S, Peters M, Terrault N, Freise CE, Ascher NL, Roberts JP. Excellent outcome following down-staging of hepatocellular carcinoma prior to liver transplantation: an intention-to-treat analysis. *Hepatology* 2008; **48**: 819-827 [PMID: 18688876 DOI: 10.1002/hep.22412]
42 **Parikh ND**, Waljee AK, Singal AG. Downstaging hepatocellular carcinoma: A systematic review and pooled analysis. *Liver Transpl* 2015; **21**: 1142-1152 [PMID: 25981135 DOI: 10.1002/lt.24169]
43 **Lai Q**, Avolio AW, Graziadei I, Otto G, Rossi M, Tisone G, Goffette P, Vogel W, Pitton MB, Lerut J; European Hepatocellular Cancer Liver Transplant Study Group. Alpha-fetoprotein and modified response evaluation criteria in solid tumors progression after locoregional therapy as predictors of hepatocellular cancer recurrence and death after transplantation. *Liver Transpl* 2013; **19**: 1108-1118 [PMID: 23873764 DOI: 10.1002/lt.23706]
44 **Woo HG**, Park ES, Thorgeirsson SS, Kim YJ. Exploring genomic profiles of hepatocellular carcinoma. *Mol Carcinog* 2011; **50**: 235-243 [PMID: 21465573 DOI: 10.1002/mc.20691]
45 **Dvorchik I**, Schwartz M, Fiel MI, Finkelstein SD, Marsh JW. Fractional allelic imbalance could allow for the development of an equitable transplant selection policy for patients with hepatocellular carcinoma. *Liver Transpl* 2008; **14**: 443-450 [PMID: 18266211 DOI: 10.1002/lt.21393]
46 **Sugimachi K**, Matsumura T, Hirata H, Uchi R, Ueda M, Ueo H, Shinden Y, Iguchi T, Eguchi H, Shirabe K, Ochiya T, Maehara Y, Mimori K. Identification of a bona fide microRNA biomarker in serum exosomes that predicts hepatocellular carcinoma recurrence after liver transplantation. *Br J Cancer* 2015; **112**: 532-538 [PMID: 25584485 DOI: 10.1038/bjc.2014.621]
47 **Barry CT**, D'Souza M, McCall M, Safadjou S, Ryan C, Kashyap R, Marroquin C, Orloff M, Almudevar A, Godfrey TE. Micro RNA expression profiles as adjunctive data to assess the risk of hepatocellular carcinoma recurrence after liver transplantation. *Am J Transplant* 2012; **12**: 428-437 [PMID: 22008552 DOI: 10.1111/j.1600-6143.2011.03788.x]
48 **Liese J**, Peveling-Oberhag J, Doering C, Schnitzbauer AA, Herrmann E, Zangos S, Hansmann ML, Moench C, Welker MW, Zeuzem S, Bechstein WO, Ulrich F. A possible role of microRNAs as predictive markers for the recurrence of hepatocellular carcinoma after liver transplantation. *Transpl Int* 2016; **29**: 369-380 [PMID: 26697811 DOI: 10.1111/tri.12733]
49 **Chen DS**, Sung JL, Sheu JC, Lai MY, How SW, Hsu HC, Lee CS, Wei TC. Serum alpha-fetoprotein in the early stage of human hepatocellular carcinoma. *Gastroenterology* 1984; **86**: 1404-1409 [PMID: 6201411]
50 **Sherman M**. Alphafetoprotein: an obituary. *J Hepatol* 2001; **34**: 603-605 [PMID: 11394662]
51 **Waghray A**, Murali AR, Menon KN. Hepatocellular carcinoma: From diagnosis to treatment. *World J Hepatol* 2015; **7**: 1020-1029 [PMID: 26052391 DOI: 10.4254/wjh.v7.i8.1020]
52 **Durand F**, Belghiti J, Paradis V. Liver transplantation for hepatocellular carcinoma: role of biopsy. *Liver Transpl* 2007; **13**: S17-S23 [PMID: 17969095 DOI: 10.1002/lt.21326]
53 **Chan DL**, Alzahrani NA, Morris DL, Chua TC. Systematic review of efficacy and outcomes of salvage liver transplantation after primary hepatic resection for hepatocellular carcinoma. *J Gastroenterol Hepatol* 2014; **29**: 31-41 [PMID: 24117517 DOI: 10.1111/jgh.12399]
54 **Wedd JP**, Nordstrom E, Nydam T, Durham J, Zimmerman M, Johnson T, Thomas Purcell W, Biggins SW. Hepatocellular carcinoma in patients listed for liver transplantation: Current and future allocation policy and management strategies for the individual patient. *Liver Transpl* 2015; **21**: 1543-1552 [PMID: 26457885 DOI: 10.1002/lt.24356]

P- Reviewer: Bouras AF, Dondossola D
S- Editor: Gong ZM **L- Editor**: A **E- Editor**: Li D

World Journal of Hepatology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4254/wjh.v8.i21.881

World J Hepatol 2016 July 28; 8(21): 881-890
ISSN 1948-5182 (online)

TOPIC HIGHLIGHT

2016 Hepatocellular Carcinoma: Global view

Contribution of alpha-fetoprotein in liver transplantation for hepatocellular carcinoma

Bérénice Charrière, Charlotte Maulat, Bertrand Suc, Fabrice Muscari

Bérénice Charrière, Charlotte Maulat, Bertrand Suc, Fabrice Muscari, Department of Visceral Surgery, Toulouse-Rangueil University Hospital, 31059 Toulouse, France

Fabrice Muscari, Service de Chirurgie Digestive, CHU Toulouse Rangueil, 31059 Toulouse, France

Author contributions: Charrière B, Maulat C and Muscari F performed the research and wrote the paper; Suc B revised the manuscript for important intellectual content.

Conflict-of-interest statement: No potential conflicts of interest relevant to this article were reported.

Manuscript source: Invited manuscript

Correspondence to: Fabrice Muscari, Professor, Service de Chirurgie Digestive, CHU Toulouse Rangueil, 1 Avenue du Pr. Jean Poulhès, Cedex 9, 31059 Toulouse, France. muscari.f@chu-toulouse.fr
Telephone: +33-561-322088
Fax: +33-561-322936

Received: March 27, 2016
Peer-review started: March 28, 2016
First decision: May 17, 2016
Revised: May 30, 2016
Accepted: June 27, 2016
Article in press: June 29, 2016
Published online: July 28, 2016

Abstract

Alpha-fetoprotein (AFP) is the main tumor biomarker available for the management of hepatocellular carcinoma (HCC). Although it is neither a good screening test nor an accurate diagnostic tool for HCC, it seems to be a possible prognostic marker. However, its contribution in liver transplantation for HCC has not been fully determined, although its use to predict recurrence after liver transplantation has been underlined by international societies. In an era of organ shortages, it could also have a key role in the selection of patients eligible for liver transplantation. Yet unanswered questions remain. First, the cut-off value of serum AFP above which liver transplantation should not be performed is still a subject of debate. We show that a concentration of 1000 ng/mL could be an exclusion criterion, whereas values of < 15 ng/mL indicate patients with an excellent prognosis whatever the size and number of tumors. Monitoring the dynamics of AFP could also prove useful. However, evidence is lacking regarding the values that should be used. Today, the real input of AFP seems to be its integration into new criteria to select patients eligible for a liver transplantation. These recent tools have associated AFP values with morphological criteria, thus refining pre-existing criteria, such as Milan, University of California, San Francisco, or "up-to-seven". We provide a review of the different criteria submitted within the past years. Finally, AFP can be used to monitor recurrence after transplantation, although there is little evidence to support this claim. Future challenges will be to draft new international guidelines to implement the use of AFP as a selection tool, and to determine a clear cut-off value above which liver transplantation should not be performed.

Key words: Hepatocellular carcinoma; Downstaging; Alpha-fetoprotein; Liver transplantation; Selection criteria

Core tip: Alpha-fetoprotein (AFP) is the main biomarker available for the management of hepatocellular carcinoma (HCC). Yet, its contribution in liver transplantation for HCC has not been fully determined. We discuss the interest of AFP as a prognostic factor to predict tumor recurrence after liver transplantation, and as a selection tool to assess the best candidates to receive a graft. We also provide an overview of the different ways that AFP could be included in decisional algorithms before liver transplantation, through its static and dynamic values.

Charrière B, Maulat C, Suc B, Muscari F. Contribution of alpha-fetoprotein in liver transplantation for hepatocellular carcinoma. *World J Hepatol* 2016; 8(21): 881-890 Available from: URL: http://www.wjgnet.com/1948-5182/full/v8/i21/881.htm DOI: http://dx.doi.org/10.4254/wjh.v8.i21.881

INTRODUCTION

Hepatocellular carcinoma (HCC) is the second most common cause of death from cancer worldwide. It is estimated to have caused nearly 745000 deaths in 2012[1]. It represents a frequent indication for liver transplantation (LT). Good results are now achieved by accurate selection of patients. The Milan criteria (MC) are considered as the reference by health systems worldwide and are currently used by the United Network for Organ Sharing[2-4]. The overall survival rates after LT for HCC range from 65% to 80% at 5 years for patients fulfilling these criteria[5-7]. As the incidence of HCC is currently rising, several teams have attempted to extend the selection criteria in order to treat more patients: *i.e.*, University of California, San Francisco (UCSF), "up-to-seven", or "5/5" criteria[8-11]. These criteria are all based on the number and the size of nodules, but other features can influence recurrence rate after LT. Among these, histopathologic findings, poor differentiation, and microvascular invasion are negative prognostic factors[12-14]. However, data on these are difficult to obtain before transplantation. Therefore, we need preoperative prognostic elements to help improve the selection of patients eligible for LT. Today, alpha-fetoprotein (AFP) is the main tumor biomarker available to manage HCC[15]. It has many advantages, as it is simple to use, relatively inexpensive, and is widely available. In this article, we discuss the contribution of AFP in LT in HCC. First we assess its value as a screening and diagnosing tool, then we focus on its prognostic relevance, and finally we analyze its interest for the selection of the best candidates to receive a graft.

AFP: WHAT IS IT?

AFP is a 67-kDa glycoprotein that is produced in early fetal life by the liver and by a variety of tumors including HCC, hepatoblastoma, and non-seminomatous germ-cell tumors of the ovary and testis (*e.g.,* yolk sac and embryonal carcinoma). Tumor cells synthesize fetal proteins because of the "de-differentiation" of adult hepatocytes[16]. During fetal life, AFP is synthesized at first by the yolk sac, then by the liver. By the end of the first trimester, the fetal liver produces nearly all of the AFP. Although synthesis is reduced markedly shortly after birth, small amounts of AFP continue to be produced during adulthood[17]. Normal concentrations of AFP in adult serum are $\leqslant$ 20 ng/mL. AFP can increase temporarily in cases of liver injury or regeneration, particularly after liver resection, during fulminant viral hepatitis, or chronic viral hepatitis[18,19]. Patients with chronic hepatitis or cirrhosis and persistently elevated AFP levels are at higher risk of developing HCC[20-22]. More than the AFP rate at a given time, it is the increased expression of AFP that suggests the presence of HCC[23].

Up to 20% of cases of HCC do not produce AFP[24]. For others, AFP can raise from normal to $\geqslant$ 100000 μg/L[25]. AFP concentrations do not differ if HCC is developed on a cirrhotic liver or not. Serum AFP levels increase by 20%-80% in patients with HCC and are strongly related to tumor aggressiveness[26-28]. Its concentrations are correlated with tumor size, microvascular invasion and poorly differentiated HCC[15,20,29,30]. However, the utility of AFP is restricted by the existence of non-AFP-secreting tumors[24].

AFP: A POOR MARKER FOR SCREENING AND DIAGNOSING HCC AMONG PATIENTS ON A LT-WAITING LIST

Use of AFP for HCC screening

Literature has shown that serum AFP (> 15 or 20 ng/mL) as a screening test for HCC had a sensitivity of between 39% and 64%, and a specificity of between 76% and 91%. The positive predictive value is estimated at between 9% and 33%[20,31-33].

The association of AFP with ultrasonography only improved the sensitivity by 6%-7% and the specificity by 2% compared to ultrasonography alone[31,34], while also increasing the cost of HCC screening[35].

These results clearly show that AFP is not a useful screening tool for HCC[36]. The first reason is that fluctuating levels of AFP in patients with cirrhosis can reflect flare-ups of HBV or HCV infection, or exacerbation of an underlying liver disease other than HCC development[7,37]. In addition, only a small proportion of tumors at an early stage (10%-20%) present with abnormal AFP serum levels[7].

Current guidelines from the American Association for the Study of Liver Disease and the European Association for the Study of the Liver (EASL) have stopped recommending the use of AFP anymore to screen for HCC in cirrhotic patients. Only ultrasonography must be performed every 6 mo[7,38].

AFP for the diagnosis of HCC

In a case-control study of 340 cirrhotic patients, Trevisani *et al*[39] have shown that AFP levels of > 20 ng/mL had a sensitivity of 60% and a specificity of 91% to diagnose HCC. At this threshold, 40% of all cases of HCC would be missed. An increase in this cut-off value would result in a lower rate of HCC detection whereas a lower cut-off value would increase the false-positive rate. These results demonstrate that AFP should not be used to diagnose HCC. Thus, AFP is no longer part of the diagnostic algorithm for HCC[7,38].

AFP: A PREDICTOR OF RECURRENCE AFTER LT

Although AFP is no longer used to diagnose HCC, several teams have shown that it could be a very interesting tool for prognosis[40,41].

Thus, it could prove useful when discussing LT. Shetty *et al*[42] in 2004, were among the first to suggest the potential prognostic usefulness of AFP when used specifically for patients who have received a liver graft. In their study, they have shown that elevated serum levels of AFP before LT were significantly associated with poorer recurrence-free survival and overall survival. In the following years, multiple studies have confirmed the prognostic role of AFP to predict outcomes after LT. Most of them are based on small cohorts of patients[28,43-47] and their main drawbacks are their retrospective designs. Yet all of them display the same tendency: Elevated AFP at the time of LT is associated with a worse prognosis after LT. Between 2008 and 2011, three large cohort studies that included thousands of patients, also showed the same pattern[48-50]. As a result, the EASL-EORTC advises on the prognostic relevance of AFP in their Clinical Practice Guidelines for the management of HCC[7]. Nevertheless, AFP alone is not sufficient to predict recurrence. Its interpretation must be associated with other demonstrated prognostic factors such as histopathologic findings, tumor differentiation, and microvascular invasion[12-14].

USE OF AFP TO SELECT LT CANDIDATES

Although the prognostic value of AFP seems well established today, one issue remains: How can we use AFP to improve the selection of LT candidates and ensure acceptable outcomes?

This question raises other issues: What cut-off value must we use to define an "elevated" level of AFP? Is it important to consider the evolution of AFP over time? Can AFP be included in an algorithm to help assess the best candidates for LT?

Defining a cut-off value for AFP

To this day, there is no clear consensus regarding the level of AFP above which a patient should not be a candidate for LT. The international consensus report regarding liver transplantation, published in 2012, mentions that "AFP concentration adds prognostic information in HCC patients and may be used for making decisions regarding transplantation"[4], but with a weak level of evidence. According to these recommendations, whatever the level of AFP, LT can be considered as long as a patient fits within the Milan, UCSF, "up-to-seven" or "5/5" criteria[2,8,11,51].

More than 20 studies have tried to define a cut-off value for pre-LT AFP, above which the prognosis would be too impaired to propose a LT. The main studies are reported Table 1. Several values have been studied, ranging from 15 ng/mL[52,53] to 1000 ng/mL[30,45,54-57]. Three reviews have also focused on the static values of AFP in an attempt to synthesize these various findings[58-60], but none have been designed as a meta-analysis and thus no clear conclusion could be drawn.

However, three values appear repeatedly in the different studies: 15 ng/mL, 400 ng/mL and 1000 ng/mL.

The value of 15 ng/mL is interesting because it could indicate a population with a very good prognosis, even for patients with HCC graded beyond the MC. Lai *et al*[52] and Berry *et al*[53] report almost identical conclusions regarding this 15 ng/mL cut-off point: Patients outside the MC but with AFP < 15 ng/mL and no other adverse prognostic factors have excellent outcomes after a LT. This suggests that, in some cases, AFP could be used to select people with excellent outcomes and who would have been unfairly excluded from receiving a LT because they exceeded the MC.

The value of 1000 ng/mL appears as a value that should exclude patients from receiving a LT, at least in the absence of downstaging. Yao *et al*[8], when defining UCSF criteria in 2001, had already pointed out that an AFP of > 1000 ng/mL was related to a worse outcome, but only in univariate analyses. Later, the same team published a study concluding that AFP > 1000 ng/mL was an independent predictor of vascular invasion and should be an exclusion criterion for LT[30]. According to their study, using this cut-off value could have led to the exclusion of 4.7% of patients from receiving a LT, while decreasing tumor recurrence by 20%. Other publications observed that an AFP > 1000 ng/mL was a predictor of recurrence after a LT[45,55,61]. In 2012, Duvoux *et al*[57] proposed a score that integrated AFP for the selection of patients eligible for LT. The value of 1000 ng/mL automatically led to the exclusion of these patients. In France, Duvoux's algorithm is currently in use and an AFP value of 1000 ng/mL is recognized as a limit over which a LT should not be performed. The UCSF team now applies a similar policy[62].

What about the values in between 15 and 1000 ng/mL? Several cut-off values have been studied over the last few years. The endpoints differ between studies: Some teams have studied the relationships between AFP and recurrence, whereas other have focused on the relationships between AFP and microvascular

Table 1 Main studies suggesting a cut-off value for α-fetoprotein when selecting candidates for liver transplantation

Ref.	Year	No. of patients	Country	Study design	AFP cut-off value	Endpoint
Yamashiki *et al*[43]	2004	93	United States	Prospective	100 ng/mL	Drop-out from list
Shetty *et al*[42]	2004	109	United States	Retrospective	300 ng/mL	Recurrence, death
Todo *et al*[54]	2007	653	Japan	Retrospective	200 ng/mL 1000 ng/mL	Recurrence
Parfitt *et al*[61]	2007	75	Canada	Retrospective	1000 ng/mL	Recurrence
Pérez-Saborido *et al*[44]	2007	95	Spain	Retrospective	200 ng/mL	Recurrence
Onaca *et al*[10]	2007	902	United States	Retrospective	200 ng/mL	Recurrence
Adler *et al*[86]	2008	226	Belgium	Retrospective	100 ng/mL	Recurrence
Zou *et al*[45]	2008	303	China	Retrospective	1000 ng/mL	Fatal recurrence
Ioannou *et al*[50]	2008	5028	United States	Retrospective	455 ng/mL	Death
Xu *et al*[46]	2009	97	China	Retrospective	400 ng/mL	Recurrence
Toso *et al*[49]	2009	6478	Canada	Retrospective	400 ng/mL	Death
Lao *et al*[55]	2009	124	United States	Prospective	1000 ng/mL	Recurrence
Xiao *et al*[87]	2009	224	China	Retrospective	800 ng/mL	Death
McHugh *et al*[47]	2010	101	United States	Retrospective	100 ng/mL	Recurrence, death
Levi *et al*[88]	2010	244	United States	Retrospective	100 ng/mL	Recurrence
Merani *et al*[66]	2011	6817	United States	Retrospective	400 ng/mL	Death
Lai *et al*[89]	2011	153	Italy	Retrospective	210 ng/mL	Recurrence
Mailey *et al*[48]	2011	2253	United States	Retrospective	400 ng/mL	Death
Muscari *et al*[28]	2012	122	France	Retrospective	500 ng/mL	Recurrence, death
Ciccarelli *et al*[65]	2012	137	Belgium	Retrospective	400 ng/mL	Recurrence
Wong *et al*[59]	2013	211	United States	Retrospective	400 ng/mL	Recurrence
Harimoto *et al*[90]	2013	167	Japan	Retrospective	300 ng/mL	Recurrence
Abdel-Wahab *et al*[68]	2013	170	Egypt	Retrospective	200 ng/mL	Recurrence, death
Grąt *et al*[67]	2014	121	Poland	Retrospective	100 ng/mL	Recurrence
Hameed *et al*[30]	2014	211	United States	Retrospective	1000 ng/mL	Microvascular invasion
Lee *et al*[91]	2014	69	South Korea	Retrospective	200 ng/mL	Recurrence
Grąt *et al*[92]	2016	146	Poland	Retrospective	100 ng/mL	Recurrence

AFP: Alpha-fetoprotein.

invasion, or AFP and drop-out rates from waiting lists. The most frequent cut-off value reported in the literature is 400 ng/mL. This has been reported by authors from various countries in Asia[63], Europe[64,65] and the United States[49,59,66]. It appears to be linked to recurrence but also to the risk of dropout while on a waiting list. However, it seems difficult to use the cut-off value of 400 ng/mL to directly exclude patients from a waiting list, because this value has been mostly studied as part of algorithms that include tumor volume, tumor size, the MC, and/or the UCSF-criteria. Moreover, many other cut-off values have been suggested, such as 100 ng/mL[47,57,67] and 200 ng/mL[10,68]. The level of evidence to define an optimal value is very weak and thus calls for further studies.

As to which AFP value should be considered, Merani *et al*[66] showed that only the last pre-transplant value of AFP independently predicted survival, unlike the AFP at the time of listing. Most of the studies cited above also used the last pre-transplant value of AFP to perform their analyses.

Evolution of AFP over time: A critical marker

Studies have tried to assess the impact of the dynamic behavior of AFP. They are presented Table 2. The first team to address this issue was Han *et al*[69] in 2007. Although focusing on only 47 patients, this Canadian study found out that the preoperative AFP slope was an independent prognostic factor for recurrence, with a cut-off at 50 ng per month. Later, Vibert *et al*[70] studied the outcomes of 153 patients in a monocentric French cohort, and concluded that a progression of AFP of > 15 ng per month was associated with decreased overall survival. Lai *et al*[52] in 2013, in a multicentric European study, obtained the same results. A fourth study proposed the cut-off value of 0.1 ng per day[71]. The main drawback of these four studies was the small number of data points used to determine the slope of AFP: Only two values were used by Vibert *et al*[70] (lowest and highest) and by Lai *et al*[52] (time of listing and time of LT). Han *et al*[69] used a median of 4 values (ranging from 2 to 11).

Other studies have focused on AFP dynamics, but with a different goal. They have evaluated the prognostic value of AFP evolution after loco-regional therapy. One of the first teams to address this question was Riaz *et al*[72] in 2009. They showed that a drop in AFP following loco-regional therapy was associated with better outcomes after LT. Bhat *et al*[73] used a logistic regression model to show that a decrease in AFP value after trans-arterial chemoembolization was significantly associated with better overall survival[73]. Wong *et al*[59] also obtained similar results. These studies enabled AFP to be part of the definition of a successful downstaging, along with radiological features. In fact, Yao *et al*[62] in California require that patients with an initial AFP > 1000 ng/mL have AFP decreased to < 500 ng/mL after loco-regional therapy, before undergoing LT. Similarly, in

Table 2 Studies focusing on dynamic values of α-fetoprotein before liver transplantation

Ref.	Year	No. of patients	AFP slope
Han *et al*[69]	2007	47	50 ng/mo
Vibert *et al*[70]	2010	153	15 ng/mo
Lai *et al*[52]	2013	422	15 ng/mo
Dumitra *et al*[71]	2013	92	0.1 ng/d

AFP: Alpha-fetoprotein.

France, the use of the Duvoux algorithm enables a patient with an AFP of > 1000 ng/mL to be back on the waiting list if AFP drops below this value[57]. Yet, to this day, the international recommendations only mention the number and size of viable tumors as criteria for successful downstaging[4]. The AFP concentrations before and after downstaging are just considered as giving "additional information" because evidence is not strong enough to enforce the wider use of AFP dynamics in the management of LT candidates. These recommendations date back from 2012 and they may evolve based on the recent studies mentioned above.

Designing new scores that integrate AFP: The end of the MC?

If AFP can be used to obtain additional information to select LT candidates, then it appears logical to integrate it into an algorithm, along with other prognostic factors. Since Mazzaferro's study in 1996[2], attempts have been made to improve the MC. Including AFP to create a new selection tool could be a key.

This idea arose as early as 2007, when a Korean team designed a score based on tumor size, number of tumors, and value of AFP in order to select the best candidates for living donor LT[56]. For each feature, the patient was awarded between 1 and 4 points. In this small study (n = 63), the different values of AFP used were < 20 ng/mL, 200 ng/mL, and 1000 ng/mL. According to the authors, this score allowed a slight expansion of the MC with comparable outcomes. Five years later, Duvoux *et al*[57] developed a very similar score. Their multicentric French study was based on a much larger cohort of patients (n = 492), and used the same three characteristics for the selection of patients: *i.e.*, tumor size, number, and AFP. However, the number of points awarded for each feature was different; as were the cut-off values for AFP: *i.e.,* 100 ng/mL and 1000 ng/mL. It is interesting to note that in this latter score, an AFP > 1000 ng/mL provided enough points for patients to be excluded directly from LT, whatever the size and number of tumors. This means that, according to this score, AFP overpowers the MC. In France, Duvoux *et al*[51]'s study precluded to a radical change in the allocation policy for LT: This score is now used to select candidates for LT. Patients exceeding the criteria are classed as having a temporary contra-indication as long as a downstaging is not successfully performed. A recent study by Varona *et al*[74] has confirmed the accuracy of this model for the prediction of recurrence and survival after a LT.

Other teams have come up with different scoring systems that include AFP when selecting LT candidates. The main ones are presented in Table 3. In 2008, a Chinese team designed the Hangzhou criteria[63], based on total tumor diameter, AFP, and histopathologic grade. The main issue with this score was the necessity for histopathologic evaluation prior to LT, which is not easy to obtain and may be inaccurate as it is based on a biopsy. Nevertheless, this work raised the idea of total tumor size, rather than maximum size of tumor, or number of tumors. Lai's team simplified the Hangzhou score and suggested using a score that featured only AFP and total tumor diameter (TTD), with a cut-off value at 400 ng/mL for AFP and 8 cm for TTD[64]. Various teams have developed slightly different scores, still using an AFP cut-off value of 400 ng/mL but replacing TTD with total tumor volume[49,75] or actual tumor volume[76]. More recently, a Korean team suggested that a combination of AFP and F-FDG PET data could be a very interesting selection tool[77]: A positive PET (cut-off at 1.10) and an AFP of > 200 ng/mL defined a group of patients with a high risk of recurrence and who should not be selected for LT. The main drawback of this study is the cost of F-FDG PET, but the authors point out the usefulness of PET to predict tumor aggressiveness, rather than sheer size and number.

Despite a few discrepancies, these studies share many common points: All of them agree that an AFP value of > 1000 ng/mL should lead to exclusion of these patients from receiving a LT; most suggest an association between AFP and morphological characteristics (size, number, and/or volume of tumors); and a few of these studies suggest the probable need for another marker for aggressiveness, such as histopathologic findings or PET.

MONITORING AFP AFTER LIVER TRANSPLANTATION: A WISE POLICY OR A WASTE OF TIME (AND MONEY)?

In the absence of HCC recurrence, AFP levels decrease to < 20 ng/mL within 2 mo post-transplantation[78]. Hepatocellular carcinoma recurs in 10%-20% of transplant recipients, despite careful patient selection[2,7,78-80]. There is no evidence-based recommendation to be applied after transplantation in order to promptly detect and treat HCC recurrence.

Because few recurrences after LT can benefit from curative treatment, this raises questions about the usefulness of active surveillance after LT[81,82]. Roberts[82] suggest that screening all patients for HCC recurrence after transplantation, using both imaging and serum biomarkers, is probably not cost effective. However, AFP monitoring, in itself, is not very costly and may be appropriate at regular intervals[83]. Yamashiki *et al*[78] proposed to measure AFP at monthly periods for the first two years after LT, to detect any HCC recurrence. When a cut-off level of 20 ng/mL was used, the sensitivity and

Table 3 Suggestions for new selection criteria for liver transplantation that integrate α-fetoprotein

Ref.	Year	No. of patients	Study design	Criteria	AFP cut-off values
Yang *et al*[56]	2007	63	Retrospective	AFP Tumor size Number of tumors	20 ng/mL, 200 ng/mL, 1000 ng/mL
Zheng *et al*[63]	2008	195	Retrospective	AFP Total tumor diameter Histopathologic grade	400 ng/mL
Lai *et al*[64]	2012	158	Retrospective	AFP Total tumor diameter	400 ng/mL
Duvoux *et al*[57]	2012	435	Prospective	AFP Tumor size Number of tumors	100 ng/mL, 1000 ng/mL
Kashkoush *et al*[76]	2014	115	Retrospective	AFP Actual tumor volume	400 ng/mL
Toso *et al*[75]	2015	233	Prospective	AFP Total tumor volume	400 ng/mL
Hong *et al*[77]	2015	123	Retrospective	AFP F-FDG PET positivity	200 ng/mL

AFP: Alpha-fetoprotein; F-FDG PET: F-fluorodeoxyglucose positron emission tomography.

specificity of AFP to detect HCC recurrence after liver transplantation were 67% and 100%, respectively[78]. Several other studies suggest that active surveillance with AFP should be performed, but the optimal frequency is not clear[83-85]. Since 2010, international guidelines state that post-transplant monitoring may be performed every 6 to 12 mo, using contrast-enhanced computed tomography or magnetic resonance imaging imaging in addition to AFP measurements[4].

CONCLUSION

Today, AFP is a key element to consider in the management of patients with HCC and who are eligible for LT. Although it does not contribute to screening or obtaining a diagnosis of HCC among patients on a LT waiting list, it can help predict the aggressiveness of the tumor and its risk of recurrence after LT.

The main usefulness of AFP regarding LT for HCC is its ability to assess the best LT candidates. It can be considered as an excellent selection criterion in association with the size and number of HCC nodules. This enables a reasonable enlargement of the MC while also guaranteeing satisfactory outcomes. Integrating an upper limit of 1000 ng/mL to the selection criteria would also allow exclusion of the few patients within the MC but who have a high risk of recurrence after LT. Furthermore, AFP can be used to monitor the evolution of HCC while on a waiting list, particularly in cases where there is downstaging.

Future challenges lie in the drafting of new international guidelines to implement the use of AFP as a selection tool, and to clarify the exact values that must be considered when using this biomarker in LT for HCC.

REFERENCES

1 **Ferlay J**, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* 2015; **136**: E359-E386 [PMID: 25220842 DOI: 10.1002/ijc.29210]

2 **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]

3 **Duffy JP**, Vardanian A, Benjamin E, Watson M, Farmer DG, Ghobrial RM, Lipshutz G, Yersiz H, Lu DS, Lassman C, Tong MJ, Hiatt JR, Busuttil RW. Liver transplantation criteria for hepatocellular carcinoma should be expanded: a 22-year experience with 467 patients at UCLA. *Ann Surg* 2007; **246**: 502-509; discussion 509-511 [PMID: 17717454 DOI: 10.1097/SLA.0b013e318148c704]

4 **Clavien PA**, Lesurtel M, Bossuyt PM, Gores GJ, Langer B, Perrier A. Recommendations for liver transplantation for hepatocellular carcinoma: an international consensus conference report. *Lancet Oncol* 2012; **13**: e11-e22 [PMID: 22047762 DOI: 10.1016/S1470-2045(11)70175-9]

5 **Mazzaferro V**, Bhoori S, Sposito C, Bongini M, Langer M, Miceli R, Mariani L. Milan criteria in liver transplantation for hepatocellular carcinoma: an evidence-based analysis of 15 years of experience. *Liver Transpl* 2011; **17** Suppl 2: S44-S57 [PMID: 21695773 DOI: 10.1002/lt.22365]

6 **Yoo HY**, Patt CH, Geschwind JF, Thuluvath PJ. The outcome of liver transplantation in patients with hepatocellular carcinoma in the United States between 1988 and 2001: 5-year survival has improved significantly with time. *J Clin Oncol* 2003; **21**: 4329-4335 [PMID: 14581446 DOI: 10.1200/JCO.2003.11.137]

7 **European Association for Study of Liver; European Organisation for Research and Treatment of Cancer.** EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. *Eur J Cancer* 2012; **48**: 599-641 [PMID: 22424278 DOI: 10.1016/j.ejca.2011.12.021]

8 **Yao FY**, Ferrell L, Bass NM, Watson JJ, Bacchetti P, Venook A, Ascher NL, Roberts JP. Liver transplantation for hepatocellular carcinoma: expansion of the tumor size limits does not adversely impact survival. *Hepatology* 2001; **33**: 1394-1403 [PMID: 11391528 DOI: 10.1053/jhep.2001.24563]

9 **Roayaie S**, Frischer JS, Emre SH, Fishbein TM, Sheiner PA, Sung M, Miller CM, Schwartz ME. Long-term results with multimodal adjuvant therapy and liver transplantation for the treatment of hepatocellular carcinomas larger than 5 centimeters. *Ann Surg*

2002; **235**: 533-539 [PMID: 11923610]
10 **Onaca N**, Davis GL, Goldstein RM, Jennings LW, Klintmalm GB. Expanded criteria for liver transplantation in patients with hepatocellular carcinoma: a report from the International Registry of Hepatic Tumors in Liver Transplantation. *Liver Transpl* 2007; **13**: 391-399 [PMID: 17318865 DOI: 10.1002/lt.21095]
11 **Muscari F**, Foppa B, Kamar N, Peron JM, Selves J, Suc B. Liberal selection criteria for liver transplantation for hepatocellular carcinoma. *Br J Surg* 2009; **96**: 785-791 [PMID: 19526621 DOI: 10.1002/bjs.6619]
12 **Cha C**, Fong Y, Jarnagin WR, Blumgart LH, DeMatteo RP. Predictors and patterns of recurrence after resection of hepatocellular carcinoma. *J Am Coll Surg* 2003; **197**: 753-758 [PMID: 14585409 DOI: 10.1016/j.jamcollsurg.2003.07.003]
13 **Scatton O**, Zalinski S, Terris B, Lefevre JH, Casali A, Massault PP, Conti F, Calmus Y, Soubrane O. Hepatocellular carcinoma developed on compensated cirrhosis: resection as a selection tool for liver transplantation. *Liver Transpl* 2008; **14**: 779-788 [PMID: 18508370 DOI: 10.1002/lt.21431]
14 **Jonas S**, Bechstein WO, Steinmüller T, Herrmann M, Radke C, Berg T, Settmacher U, Neuhaus P. Vascular invasion and histopathologic grading determine outcome after liver transplantation for hepatocellular carcinoma in cirrhosis. *Hepatology* 2001; **33**: 1080-1086 [PMID: 11343235 DOI: 10.1053/jhep.2001.23561]
15 **Liu C**, Xiao GQ, Yan LN, Li B, Jiang L, Wen TF, Wang WT, Xu MQ, Yang JY. Value of α-fetoprotein in association with clinicopathological features of hepatocellular carcinoma. *World J Gastroenterol* 2013; **19**: 1811-1819 [PMID: 23555170 DOI: 10.3748/wjg.v19.i11.1811]
16 **Sell S**. Alpha-fetoprotein, stem cells and cancer: how study of the production of alpha-fetoprotein during chemical hepatocarcinogenesis led to reaffirmation of the stem cell theory of cancer. *Tumour Biol* 2008; **29**: 161-180 [PMID: 18612221 DOI: 10.1159/000143402]
17 **Tomasi TB**. Structure and function of alpha-fetoprotein. *Annu Rev Med* 1977; **28**: 453-465 [PMID: 67821 DOI: 10.1146/annurev.me.28.020177.002321]
18 **Behne T**, Copur MS. Biomarkers for hepatocellular carcinoma. *Int J Hepatol* 2012; **2012**: 859076 [PMID: 22655201 DOI: 10.1155/2012/859076]
19 **Pastore G**, Dentico P, Angarano G, Zanetti AR, Ferroni P, Frappampina V, Schiraldi O, Roggendorf M, Frösner G. Hepatitis B virus markers, alpha-fetoprotein and survival in fulminant viral hepatitis. *J Med Virol* 1981; **7**: 97-103 [PMID: 6167671]
20 **Tsukuma H**, Hiyama T, Tanaka S, Nakao M, Yabuuchi T, Kitamura T, Nakanishi K, Fujimoto I, Inoue A, Yamazaki H. Risk factors for hepatocellular carcinoma among patients with chronic liver disease. *N Engl J Med* 1993; **328**: 1797-1801 [PMID: 7684822 DOI: 10.1056/NEJM199306243282501]
21 **Oka H**, Tamori A, Kuroki T, Kobayashi K, Yamamoto S. Prospective study of alpha-fetoprotein in cirrhotic patients monitored for development of hepatocellular carcinoma. *Hepatology* 1994; **19**: 61-66 [PMID: 7506227]
22 **Lok AS**, Everhart JE, Wright EC, Di Bisceglie AM, Kim HY, Sterling RK, Everson GT, Lindsay KL, Lee WM, Bonkovsky HL, Dienstag JL, Ghany MG, Morishima C, Morgan TR. Maintenance peginterferon therapy and other factors associated with hepatocellular carcinoma in patients with advanced hepatitis C. *Gastroenterology* 2011; **140**: 840-889; quiz e12 [PMID: 21129375 DOI: 10.1053/j.gastro.2010.11.050]
23 **Arrieta O**, Cacho B, Morales-Espinosa D, Ruelas-Villavicencio A, Flores-Estrada D, Hernández-Pedro N. The progressive elevation of alpha fetoprotein for the diagnosis of hepatocellular carcinoma in patients with liver cirrhosis. *BMC Cancer* 2007; **7**: 28 [PMID: 17288606 DOI: 10.1186/1471-2407-7-28]
24 **Ryder SD**. Guidelines for the diagnosis and treatment of hepatocellular carcinoma (HCC) in adults. *Gut* 2003; **52** Suppl 3: iii1-iii8 [PMID: 12692148 DOI: 10.1136/gut.52.suppl_3.iii1]
25 **Koteish A**, Thuluvath PJ. Screening for hepatocellular carcinoma. *J Vasc Interv Radiol* 2002; **13**: S185-S190 [PMID: 12354835]
26 **Fujioka M**, Nakashima Y, Nakashima O, Kojiro M. Immunohistologic study on the expressions of alpha-fetoprotein and protein induced by vitamin K absence or antagonist II in surgically resected small hepatocellular carcinoma. *Hepatology* 2001; **34**: 1128-1134 [PMID: 11732002 DOI: 10.1053/jhep.2001.29202]
27 **Sherman M**. The resurrection of alphafetoprotein. *J Hepatol* 2010; **52**: 939-940 [PMID: 20395007 DOI: 10.1016/j.jhep.2010.02.006]
28 **Muscari F**, Guinard JP, Kamar N, Peron JM, Otal P, Suc B. Impact of preoperative α-fetoprotein level on disease-free survival after liver transplantation for hepatocellular carcinoma. *World J Surg* 2012; **36**: 1824-1831 [PMID: 22532309 DOI: 10.1007/s00268-012-1587-z]
29 **Toro A**, Ardiri A, Mannino M, Arcerito MC, Mannino G, Palermo F, Bertino G, Di Carlo I. Effect of pre- and post-treatment α-fetoprotein levels and tumor size on survival of patients with hepatocellular carcinoma treated by resection, transarterial chemoembolization or radiofrequency ablation: a retrospective study. *BMC Surg* 2014; **14**: 40 [PMID: 24993566 DOI: 10.1186/1471-2482-14-40]
30 **Hameed B**, Mehta N, Sapisochin G, Roberts JP, Yao FY. Alpha-fetoprotein level & gt; 1000 ng/mL as an exclusion criterion for liver transplantation in patients with hepatocellular carcinoma meeting the Milan criteria. *Liver Transpl* 2014; **20**: 945-951 [PMID: 24797281 DOI: 10.1002/lt.23904]
31 **Sherman M**, Peltekian KM, Lee C. Screening for hepatocellular carcinoma in chronic carriers of hepatitis B virus: incidence and prevalence of hepatocellular carcinoma in a North American urban population. *Hepatology* 1995; **22**: 432-438 [PMID: 7543434]
32 **Chen JG**, Parkin DM, Chen QG, Lu JH, Shen QJ, Zhang BC, Zhu YR. Screening for liver cancer: results of a randomised controlled trial in Qidong, China. *J Med Screen* 2003; **10**: 204-209 [PMID: 14738659 DOI: 10.1258/096914103771773320]
33 **Pateron D**, Ganne N, Trinchet JC, Aurousseau MH, Mal F, Meicler C, Coderc E, Reboullet P, Beaugrand M. Prospective study of screening for hepatocellular carcinoma in Caucasian patients with cirrhosis. *J Hepatol* 1994; **20**: 65-71 [PMID: 7515408]
34 **Singal A**, Volk ML, Waljee A, Salgia R, Higgins P, Rogers MA, Marrero JA. Meta-analysis: surveillance with ultrasound for early-stage hepatocellular carcinoma in patients with cirrhosis. *Aliment Pharmacol Ther* 2009; **30**: 37-47 [PMID: 19392863 DOI: 10.1111/j.1365-2036.2009.04014.x]
35 **Zhang B**, Yang B. Combined alpha fetoprotein testing and ultrasonography as a screening test for primary liver cancer. *J Med Screen* 1999; **6**: 108-110 [PMID: 10444731]
36 **Gupta S**, Bent S, Kohlwes J. Test characteristics of alpha-fetoprotein for detecting hepatocellular carcinoma in patients with hepatitis C. A systematic review and critical analysis. *Ann Intern Med* 2003; **139**: 46-50 [PMID: 12834318]
37 **Di Bisceglie AM**, Sterling RK, Chung RT, Everhart JE, Dienstag JL, Bonkovsky HL, Wright EC, Everson GT, Lindsay KL, Lok AS, Lee WM, Morgan TR, Ghany MG, Gretch DR. Serum alpha-fetoprotein levels in patients with advanced hepatitis C: results from the HALT-C Trial. *J Hepatol* 2005; **43**: 434-441 [PMID: 16136646]
38 **Bruix J**, Sherman M. Management of hepatocellular carcinoma: an update. *Hepatology* 2011; **53**: 1020-1022 [PMID: 21374666 DOI: 10.1002/hep.24199]
39 **Trevisani F**, D'Intino PE, Morselli-Labate AM, Mazzella G, Accogli E, Caraceni P, Domenicali M, De Notariis S, Roda E, Bernardi M. Serum alpha-fetoprotein for diagnosis of hepatocellular carcinoma in patients with chronic liver disease: influence of HBsAg and anti-HCV status. *J Hepatol* 2001; **34**: 570-575 [PMID: 11394657]
40 **Vora SR**, Zheng H, Stadler ZK, Fuchs CS, Zhu AX. Serum alpha-fetoprotein response as a surrogate for clinical outcome in patients receiving systemic therapy for advanced hepatocellular carcinoma. *Oncologist* 2009; **14**: 717-725 [PMID: 19581525 DOI: 10.1634/

theoncologist.2009-0038]
41 **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]
42 **Shetty K**, Timmins K, Brensinger C, Furth EE, Rattan S, Sun W, Rosen M, Soulen M, Shaked A, Reddy KR, Olthoff KM. Liver transplantation for hepatocellular carcinoma validation of present selection criteria in predicting outcome. *Liver Transpl* 2004; **10**: 911-918 [PMID: 15237377 DOI: 10.1002/lt.20140]
43 **Yamashiki N**, Gaynor JJ, Kato T, Reddy KR, Sobhonslidsuk A, Levi D, Nishida S, Madariaga J, Nery J, Schiff ER, Tzakis AG. Competing risks analysis of predictors of delisting owing to tumor progression in liver transplant candidates with hepatocellular carcinoma. *Am J Transplant* 2004; **4**: 774-781 [PMID: 15084174 DOI: 10.1111/j.1600-6143.2004.00412.x]
44 **Pérez-Saborido B**, de los Galanes SJ, Menéu-Díaz JC, Romero CJ, Elola-Olaso AM, Suárez YF, Valencia VB, Moreno-González E. Tumor recurrence after liver transplantation for hepatocellular carcinoma: recurrence pathway and prognostic factors. *Transplant Proc* 2007; **39**: 2304-2307 [PMID: 17889172 DOI: 10.1016/j.transproceed.2007.06.059]
45 **Zou WL**, Zang YJ, Chen XG, Shen ZY. Risk factors for fatal recurrence of hepatocellular carcinoma and their role in selecting candidates for liver transplantation. *Hepatobiliary Pancreat Dis Int* 2008; **7**: 145-151 [PMID: 18397848]
46 **Xu X**, Ke QH, Shao ZX, Wu J, Chen J, Zhou L, Zheng SS. The value of serum alpha-fetoprotein in predicting tumor recurrence after liver transplantation for hepatocellular carcinoma. *Dig Dis Sci* 2009; **54**: 385-388 [PMID: 18563566 DOI: 10.1007/s10620-008-0349-0]
47 **McHugh PP**, Gilbert J, Vera S, Koch A, Ranjan D, Gedaly R. Alpha-fetoprotein and tumour size are associated with microvascular invasion in explanted livers of patients undergoing transplantation with hepatocellular carcinoma. *HPB* (Oxford) 2010; **12**: 56-61 [PMID: 20495646 DOI: 10.1111/j.1477-2574.2009.00128.x]
48 **Mailey B**, Artinyan A, Khalili J, Denitz J, Sanchez-Luege N, Sun CL, Bhatia S, Nissen N, Colquhoun SD, Kim J. Evaluation of absolute serum α-fetoprotein levels in liver transplant for hepatocellular cancer. *Arch Surg* 2011; **146**: 26-33 [PMID: 21242442 DOI: 10.1001/archsurg.2010.295]
49 **Toso C**, Asthana S, Bigam DL, Shapiro AM, Kneteman NM. Reassessing selection criteria prior to liver transplantation for hepatocellular carcinoma utilizing the Scientific Registry of Transplant Recipients database. *Hepatology* 2009; **49**: 832-838 [PMID: 19152426 DOI: 10.1002/hep.22693]
50 **Ioannou GN**, Perkins JD, Carithers RL. Liver transplantation for hepatocellular carcinoma: impact of the MELD allocation system and predictors of survival. *Gastroenterology* 2008; **134**: 1342-1351 [PMID: 18471511 DOI: 10.1053/j.gastro.2008.02.013]
51 **Mazzaferro V**, Llovet JM, Miceli R, Bhoori S, Schiavo M, Mariani L, Camerini T, Roayaie S, Schwartz ME, Grazi GL, Adam R, Neuhaus P, Salizzoni M, Bruix J, Forner A, De Carlis L, Cillo U, Burroughs AK, Troisi R, Rossi M, Gerunda GE, Lerut J, Belghiti J, Boin I, Gugenheim J, Rochling F, Van Hoek B, Majno P. Predicting survival after liver transplantation in patients with hepatocellular carcinoma beyond the Milan criteria: a retrospective, exploratory analysis. *Lancet Oncol* 2009; **10**: 35-43 [PMID: 19058754 DOI: 10.1016/S1470-2045(08)70284-5]
52 **Lai Q**, Avolio AW, Graziadei I, Otto G, Rossi M, Tisone G, Goffette P, Vogel W, Pitton MB, Lerut J. Alpha-fetoprotein and modified response evaluation criteria in solid tumors progression after locoregional therapy as predictors of hepatocellular cancer recurrence and death after transplantation. *Liver Transpl* 2013; **19**: 1108-1118 [PMID: 23873764 DOI: 10.1002/lt.23706]
53 **Berry K**, Ioannou GN. Serum alpha-fetoprotein level independently predicts posttransplant survival in patients with hepatocellular carcinoma. *Liver Transpl* 2013; **19**: 634-645 [PMID: 23536495 DOI: 10.1002/lt.23652]
54 **Todo S**, Furukawa H, Tada M. Extending indication: role of living donor liver transplantation for hepatocellular carcinoma. *Liver Transpl* 2007; **13**: S48-S54 [PMID: 17969069 DOI: 10.1002/lt.21334]
55 **Lao OB**, Weissman J, Perkins JD. Pre-transplant therapy for hepatocellular carcinoma is associated with a lower recurrence after liver transplantation. *Clin Transplant* 2009; **23**: 874-881 [PMID: 19453644 DOI: 10.1111/j.1399-0012.2009.00993.x]
56 **Yang SH**, Suh KS, Lee HW, Cho EH, Cho JY, Cho YB, Kim IH, Yi NJ, Lee KU. A revised scoring system utilizing serum alphafetoprotein levels to expand candidates for living donor transplantation in hepatocellular carcinoma. *Surgery* 2007; **141**: 598-609 [PMID: 17462459 DOI: 10.1016/j.surg.2006.11.006]
57 **Duvoux C**, Roudot-Thoraval F, Decaens T, Pessione F, Badran H, Piardi T, Francoz C, Compagnon P, Vanlemmens C, Dumortier J, Dharancy S, Gugenheim J, Bernard PH, Adam R, Radenne S, Muscari F, Conti F, Hardwigsen J, Pageaux GP, Chazouillères O, Salame E, Hilleret MN, Lebray P, Abergel A, Debette-Gratien M, Kluger MD, Mallat A, Azoulay D, Cherqui D. Liver transplantation for hepatocellular carcinoma: a model including α-fetoprotein improves the performance of Milan criteria. *Gastroenterology* 2012; **143**: 986-994.e3; quiz e14-15 [PMID: 22750200 DOI: 10.1053/j.gastro.2012.05.052]
58 **Lai Q**, Levi Sandri GB, Lerut J. Selection tool alpha-fetoprotein for patients waiting for liver transplantation: How to easily manage a fractal algorithm. *World J Hepatol* 2015; **7**: 1899-1904 [PMID: 26244064 DOI: 10.4254/wjh.v7.i15.1899]
59 **Wong LL**, Naugler WE, Schwartz J, Scott DL, Bhattacharya R, Reyes J, Orloff SL. Impact of locoregional therapy and alpha-fetoprotein on outcomes in transplantation for liver cancer: a UNOS Region 6 pooled analysis. *Clin Transplant* 2013; **27**: E72-E79 [PMID: 23278701 DOI: 10.1111/ctr.12056]
60 **Hakeem AR**, Young RS, Marangoni G, Lodge JP, Prasad KR. Systematic review: the prognostic role of alpha-fetoprotein following liver transplantation for hepatocellular carcinoma. *Aliment Pharmacol Ther* 2012; **35**: 987-999 [PMID: 22429190 DOI: 10.1111/j.1365-2036.2012.05060.x]
61 **Parfitt JR**, Marotta P, Alghamdi M, Wall W, Khakhar A, Suskin NG, Quan D, McAllister V, Ghent C, Levstik M, McLean C, Chakrabarti S, Garcia B, Driman DK. Recurrent hepatocellular carcinoma after transplantation: use of a pathological score on explanted livers to predict recurrence. *Liver Transpl* 2007; **13**: 543-551 [PMID: 17394152 DOI: 10.1002/lt.21078]
62 **Yao FY**, Hameed B, Mehta N, Roberts JP. Response to letter to the editors. *Liver Transpl* 2014; **20**: 1285 [PMID: 25155484 DOI: 10.1002/lt.23982]
63 **Zheng SS**, Xu X, Wu J, Chen J, Wang WL, Zhang M, Liang TB, Wu LM. Liver transplantation for hepatocellular carcinoma: Hangzhou experiences. *Transplantation* 2008; **85**: 1726-1732 [PMID: 18580463 DOI: 10.1097/TP.0b013e31816b67e4]
64 **Lai Q**, Avolio AW, Manzia TM, Sorge R, Agnes S, Tisone G, Berloco PB, Rossi M. Combination of biological and morphological parameters for the selection of patients with hepatocellular carcinoma waiting for liver transplantation. *Clin Transplant* 2012; **26**: E125-E131 [PMID: 22192083 DOI: 10.1111/j.1399-0012.2011.01572.x]
65 **Ciccarelli O**, Lai Q, Goffette P, Finet P, De Reyck C, Roggen F, Sempoux C, Doffagne E, Reding R, Lerut J. Liver transplantation for hepatocellular cancer: UCL experience in 137 adult cirrhotic patients. Alpha-foetoprotein level and locoregional treatment as refined selection criteria. *Transpl Int* 2012; **25**: 867-875 [PMID: 22716073 DOI: 10.1111/j.1432-2277.2012.01512.x]
66 **Merani S**, Majno P, Kneteman NM, Berney T, Morel P, Mentha G, Toso C. The impact of waiting list alpha-fetoprotein changes on the outcome of liver transplant for hepatocellular carcinoma. *J Hepatol* 2011; **55**: 814-819 [PMID: 21334400 DOI: 10.1016/j.jhep.2010.12.040]

67 **Grąt M**, Kornasiewicz O, Lewandowski Z, Hołówko W, Grąt K, Kobryń K, Patkowski W, Zieniewicz K, Krawczyk M. Combination of morphologic criteria and α-fetoprotein in selection of patients with hepatocellular carcinoma for liver transplantation minimizes the problem of posttransplant tumor recurrence. *World J Surg* 2014; **38**: 2698-2707 [PMID: 24858191 DOI: 10.1007/s00268-014-2647-3]

68 **Abdel-Wahab M**, Sultan AM, Fathy OM, Salah T, Elshobary MM, Elghawalby NA, Yassen AM, Elsarraf WM, Elsaadany MF, Zalatah K. Factors affecting recurrence and survival after living donor liver transplantation for hepatocellular carcinoma. *Hepatogastroenterology* 2013; **60**: 1847-1853 [PMID: 24719918]

69 **Han K**, Tzimas GN, Barkun JS, Metrakos P, Tchervenkov JL, Hilzenrat N, Wong P, Deschênes M. Preoperative alpha-fetoprotein slope is predictive of hepatocellular carcinoma recurrence after liver transplantation. *Can J Gastroenterol* 2007; **21**: 39-45 [PMID: 17225881]

70 **Vibert E**, Azoulay D, Hoti E, Iacopinelli S, Samuel D, Salloum C, Lemoine A, Bismuth H, Castaing D, Adam R. Progression of alphafetoprotein before liver transplantation for hepatocellular carcinoma in cirrhotic patients: a critical factor. *Am J Transplant* 2010; **10**: 129-137 [PMID: 20070666 DOI: 10.1111/j.1600-6143.2009.02750.x]

71 **Dumitra TC**, Dumitra S, Metrakos PP, Barkun JS, Chaudhury P, Deschênes M, Paraskevas S, Hassanain M, Tchervenkov JI. Pretransplantation α-fetoprotein slope and milan criteria: strong predictors of hepatocellular carcinoma recurrence after transplantation. *Transplantation* 2013; **95**: 228-233 [PMID: 23222895 DOI: 10.1097/TP.0b013e31827743d7]

72 **Riaz A**, Ryu RK, Kulik LM, Mulcahy MF, Lewandowski RJ, Minocha J, Ibrahim SM, Sato KT, Baker T, Miller FH, Newman S, Omary R, Abecassis M, Benson AB, Salem R. Alpha-fetoprotein response after locoregional therapy for hepatocellular carcinoma: oncologic marker of radiologic response, progression, and survival. *J Clin Oncol* 2009; **27**: 5734-5742 [PMID: 19805671 DOI: 10.1200/JCO.2009.23.1282]

73 **Bhat M**, Hassanain M, Simoneau E, Tzimas GN, Chaudhury P, Deschenes M, Valenti D, Ghali P, Wong P, Cabrera T, Barkun J, Tchervenkov JI, Metrakos P. Magnitude of change in alpha-fetoprotein in response to transarterial chemoembolization predicts survival in patients undergoing liver transplantation for hepatocellular carcinoma. *Curr Oncol* 2013; **20**: 265-272 [PMID: 24155631 DOI: 10.3747/co.20.1270]

74 **Varona MA**, Soriano A, Aguirre-Jaime A, Garrido S, Oton E, Diaz D, Portero J, Bravo P, Barrera MA, Perera A. Risk factors of hepatocellular carcinoma recurrence after liver transplantation: accuracy of the alpha-fetoprotein model in a single-center experience. *Transplant Proc* 2015; **47**: 84-89 [PMID: 25645778 DOI: 10.1016/j.transproceed.2014.12.013]

75 **Toso C**, Meeberg G, Hernandez-Alejandro R, Dufour JF, Marotta P, Majno P, Kneteman NM. Total tumor volume and alpha-fetoprotein for selection of transplant candidates with hepatocellular carcinoma: A prospective validation. *Hepatology* 2015; **62**: 158-165 [PMID: 25777590 DOI: 10.1002/hep.27787]

76 **Kashkoush S**, El Moghazy W, Kawahara T, Gala-Lopez B, Toso C, Kneteman NM. Three-dimensional tumor volume and serum alpha-fetoprotein are predictors of hepatocellular carcinoma recurrence after liver transplantation: refined selection criteria. *Clin Transplant* 2014; **28**: 728-736 [PMID: 24708263 DOI: 10.1111/ctr.12373]

77 **Hong G**, Suh KS, Suh SW, Yoo T, Kim H, Park MS, Choi Y, Paeng JC, Yi NJ, Lee KW. Alpha-fetoprotein and (18)F-FDG positron emission tomography predict tumor recurrence better than Milan criteria in living donor liver transplantation. *J Hepatol* 2016; **64**: 852-859 [PMID: 26658686 DOI: 10.1016/j.jhep.2015.11.033]

78 **Yamashiki N**, Sugawara Y, Tamura S, Tateishi R, Yoshida H, Kaneko J, Matsui Y, Togashi J, Akahane M, Makuuchi M, Omata M, Kokudo N. Postoperative surveillance with monthly serum tumor markers after living-donor liver transplantation for hepatocellular carcinoma. *Hepatol Res* 2010; **40**: 278-286 [PMID: 20070400 DOI: 10.1111/j.1872-034X.2009.00591.x]

79 **Roayaie S**, Schwartz JD, Sung MW, Emre SH, Miller CM, Gondolesi GE, Krieger NR, Schwartz ME. Recurrence of hepatocellular carcinoma after liver transplant: patterns and prognosis. *Liver Transpl* 2004; **10**: 534-540 [PMID: 15048797 DOI: 10.1002/lt.20128]

80 **Regalia E**, Fassati LR, Valente U, Pulvirenti A, Damilano I, Dardano G, Montalto F, Coppa J, Mazzaferro V. Pattern and management of recurrent hepatocellular carcinoma after liver transplantation. *J Hepatobiliary Pancreat Surg* 1998; **5**: 29-34 [PMID: 9683751]

81 **Kakodkar R**, Soin AS. Liver Transplantation for HCC: A Review. *Indian J Surg* 2012; **74**: 100-117 [PMID: 23372314 DOI: 10.1007/s12262-011-0387-2]

82 **Roberts JP**. Tumor surveillance-what can and should be done? Screening for recurrence of hepatocellular carcinoma after liver transplantation. *Liver Transpl* 2005; **11**: S45- S46 [PMID: 16237702 DOI: 10.1002/lt.20605]

83 **Kneteman N**, Livraghi T, Madoff D, de Santibañez E, Kew M. Tools for monitoring patients with hepatocellular carcinoma on the waiting list and after liver transplantation. *Liver Transpl* 2011; **17** Suppl 2: S117-S127 [PMID: 21584926 DOI: 10.1002/lt.22334]

84 **Sposito C**, Mariani L, Germini A, Flores Reyes M, Bongini M, Grossi G, Bhoori S, Mazzaferro V. Comparative efficacy of sorafenib versus best supportive care in recurrent hepatocellular carcinoma after liver transplantation: a case-control study. *J Hepatol* 2013; **59**: 59-66 [PMID: 23500153 DOI: 10.1016/j.jhep.2013.02.026]

85 **Ortiz J**, Danniel J, Chavez M, Davogustto G. Monitoring for post-transplant hepatocellular carcinoma recurrence. *HPB* (Oxford) 2012; **14**: 351; author reply 352 [PMID: 22487073 DOI: 10.1111/j.1477-2574.2012.00449.x]

86 **Adler M**, De Pauw F, Vereerstraeten P, Fancello A, Lerut J, Starkel P, Van Vlierberghe H, Troisi R, Donckier V, Detry O, Delwaide J, Michielsen P, Chapelle T, Pirenne J, Nevens F. Outcome of patients with hepatocellular carcinoma listed for liver transplantation within the Eurotransplant allocation system. *Liver Transpl* 2008; **14**: 526-533 [PMID: 18383082 DOI: 10.1002/lt.21399]

87 **Xiao L**, Fu ZR, Ding GS, Fu H, Ni ZJ, Wang ZX, Shi XM, Guo WY. Liver transplantation for hepatitis B virus-related hepatocellular carcinoma: one center's experience in China. *Transplant Proc* 2009; **41**: 1717-1721 [PMID: 19545714 DOI: 10.1016/j.transproceed.2009.03.058]

88 **Levi DM**, Tzakis AG, Martin P, Nishida S, Island E, Moon J, Selvaggi G, Tekin A, Madrazo BL, Narayanan G, Garcia MT, Feun LG, Tryphonopoulos P, Skartsis N, Livingstone AS. Liver transplantation for hepatocellular carcinoma in the model for end-stage liver disease era. *J Am Coll Surg* 2010; **210**: 727-734, 735-736 [PMID: 20421039 DOI: 10.1016/j.jamcollsurg.2010.01.007]

89 **Lai Q**, Avolio AW, Manzia TM, Agnes S, Tisone G, Berloco PB, Rossi M. Role of alpha-fetoprotein in selection of patients with hepatocellular carcinoma waiting for liver transplantation: must we reconsider it? *Int J Biol Markers* 2011; **26**: 153-159 [PMID: 21928243 DOI: 10.5301/JBM.2011.8557]

90 **Harimoto N**, Shirabe K, Nakagawara H, Toshima T, Yamashita Y, Ikegami T, Yoshizumi T, Soejima Y, Ikeda T, Maehara Y. Prognostic factors affecting survival at recurrence of hepatocellular carcinoma after living-donor liver transplantation: with special reference to neutrophil/lymphocyte ratio. *Transplantation* 2013; **96**: 1008-1012 [PMID: 24113512 DOI: 10.1097/TP.0b013e3182a53f2b]

91 **Lee S**, Hyuck David Kwon C, Man Kim J, Joh JW, Woon Paik S, Kim BW, Wang HJ, Lee KW, Suh KS, Lee SK. Time of hepatocellular carcinoma recurrence after liver resection and alpha-fetoprotein are important prognostic factors for salvage liver transplantation. *Liver Transpl* 2014; **20**: 1057-1063 [PMID: 24862741 DOI: 10.1002/lt.23919]

92 **Grąt M**, Krasnodębski M, Patkowski W, Wronka KM, Masior Ł, Stypułkowski J, Grąt K, Krawczyk M. Relevance of Pre-Transplant α-fetoprotein Dynamics in Liver Transplantation for Hepatocellular Cancer. *Ann Transplant* 2016; **21**: 115-124 [PMID: 26887339]

P- Reviewer: Guan YS, Miyoshi E **S- Editor**: Qi Y
L- Editor: A **E- Editor**: Li D

World Journal of Hepatology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4254/wjh.v8.i21.891

World J Hepatol 2016 July 28; 8(21): 891-901
ISSN 1948-5182 (online)

REVIEW

Acute renal injury after partial hepatectomy

Luis Alberto Batista Peres, Luis Cesar Bredt, Raphael Flavio Fachini Cipriani

Luis Alberto Batista Peres, Department of Nephrology, University Hospital of Western Paraná, State University of Western Paraná, Cascavel, Paraná 85819-110, Brazil

Luis Cesar Bredt, Department of Surgical Oncology and Hepatobiliary Surgery, University Hospital of Western Paraná, State University of Western Paraná, Cascavel, Paraná 85819-110, Brazil

Raphael Flavio Fachini Cipriani, Department of General Surgery, University Hospital of Western Paraná, State University of Western Paraná, Cascavel, Paraná 85819-110, Brazil

Author contributions: Peres LAB, Bredt LC and Cipriani RFF contributed equally to this review article; all authors equally contributed to this paper with conception and design of the study, literature review and analysis, drafting and critical revision and editing, and final approval of the final version.

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

Manuscript source: Invited manuscript

Correspondence to: Luis Cesar Bredt, Professor, Department of Surgical Oncology and Hepatobiliary Surgery, University Hospital of Western Paraná, State University of Western Paraná, Dom Pedro II Street, 2099, apto 701, Cascavel, Paraná 85819-110, Brazil. lcbredt@gmail.com
Telephone: +55-45-99369943
Fax: +55-45-33215151

Received: February 25, 2016
Peer-review started: February 27, 2016
First decision: May 13, 2016
Revised: June 2, 2016
Accepted: June 27, 2016
Article in press: June 29, 2016

Published online: July 28, 2016

Abstract

Currently, partial hepatectomy is the treatment of choice for a wide variety of liver and biliary conditions. Among the possible complications of partial hepatectomy, acute kidney injury (AKI) should be considered as an important cause of increased morbidity and postoperative mortality. Difficulties in the data analysis related to postoperative AKI after liver resections are mainly due to the multiplicity of factors to be considered in the surgical patients, moreover, there is no consensus of the exact definition of AKI after liver resection in the literature, which hampers comparison and analysis of the scarce data published on the subject. Despite this multiplicity of risk factors for postoperative AKI after partial hepatectomy, there are main factors that clearly contribute to its occurrence. First factor relates to large blood losses with renal hypoperfusion during the operation, second factor relates to the occurrence of post-hepatectomy liver failure with consequent distributive circulatory changes and hepatorenal syndrome. Eventually, patients can have more than one factor contributing to post-operative AKI, and frequently these combinations of acute insults can be aggravated by sepsis or exposure to nephrotoxic drugs.

Key words: Hepatectomy; Liver resection; Acute renal injury; Hepatorenal syndrome; Kidney

Core tip: In the specific scenario of liver resections, there are limited and heterogeneous data regarding the occurrence of acute kidney injury (AKI) in the post-operative period, and its clinical relevance (mortality, morbidity and hospital stay) were not conclusively explored and clarified. Difficulties in the data analysis related to postoperative AKI after liver resections are mainly due the scarce data published on the subject.

Peres LAB, Bredt LC, Cipriani RFF. Acute renal injury after partial hepatectomy. *World J Hepatol* 2016; 8(21): 891-901 Available from: URL: http://www.wjgnet.com/1948-5182/full/v8/i21/891.htm DOI: http://dx.doi.org/10.4254/wjh.v8.i21.891

INTRODUCTION

Currently, partial hepatectomy is the treatment of choice for a wide variety of primary liver tumors (benign or malignant), tumors of the bile ducts and secondary malignant liver tumors. The partial liver resections may also be necessary in the management of complex cystic liver diseases, benign biliary structures, some cases of hepatic trauma and more recently with living donor liver transplantation[1]. With the refinement of surgical techniques, improved selection of patients to procedure, advances in anesthetic support and perioperative care, this traditionally complex and feared operation has become a routine procedure in the past 20 years, with acceptable mortality rates ranging from 3.1% to 4.5%[2-4].

Among the possible complications of major surgical procedures, including the partial hepatectomy, acute kidney injury (AKI) should be considered as an important cause of increased morbidity and postoperative mortality[5,6], with an incidence ranging from 10% to 30% after major operations[7,8]. Literature data report an incidence of 1% of AKI in the postoperative major non-cardiac surgery without liver resection[6] about 20% after cardiac surgery[9-11] and 50% after liver transplantation[12-18].

In the specific scenario of liver resections, there are limited and heterogeneous data regarding the occurrence of AKI in the postoperative period, with an incidence ranging from 0.9% to 15.1% of the patients[19-23], and its clinical relevance (mortality, morbidity and hospital stay) were not conclusively explored and clarified.

Difficulties in the data analysis related to postoperative AKI after liver resections are mainly due to the multiplicity of factors to be considered in this surgical patients, such as general medical conditions and comorbidities, nutritional disorders, metastatic malignancy with low physiological reserve systems, immunological disorders, chemotherapy treatment, functional capacity and volume of liver parenchyma to be preserved, and the perioperative hemodynamic effects of the different modalities of partial hepatectomy. Moreover, there is no consensus of the exact definition of AKI after liver resection in the literature, which hampers comparison and analysis of the scarce data published on the subject[22].

Despite this multiplicity of risk factors for postoperative AKI after partial hepatectomy, there are main factors that clearly contribute to its occurrence. First factor relates to large blood losses with renal hypoperfusion during the operation[20], that very often can be associated by the deleterious renal effects of red blood cell transfusion[23], and in some occasions this renal hypoperfusion occurs in patients with increased renal susceptibility to ischemia, usually elderly patients with underlying cardiovascular or renal disorders, or eventually it may be drug-induced[21-24]. Second factor relates to the occurrence of post-hepatectomy liver failure (PLF) with consequent distributive circulatory changes and hepatorenal syndrome (HRS)[20]. Eventually, patients can have more than one factor contributing to post-operative AKI, and frequently these combinations of acute insults can be aggravated by sepsis[20-24] or exposure to nephrotoxic drugs, such as aminoglycosides[25].

The aim of this review is to present the definition of postoperative AKI after partial hepatectomy, the different pathophysiological mechanisms for its occurrence and methods for preventing these events.

DEFINITION OF POSTOPERATIVE AKI AFTER PARTIAL HEPATECTOMY

AKI is characterized by the deterioration of kidney function over a period of hours to days, resulting in the failure of the kidney to excrete nitrogenous waste products and to maintain fluid and electrolyte homeostasis[26]. In recent years, several criteria have been proposed for the diagnosis of AKI in general population, particularly the "Risk, Injury, Failure, Loss of Renal Function and End-Stage Renal Disease" (RIFLE) criteria[27], the "Acute Kidney Injury Network" (AKIN) criteria[28] and more recently, the criteria suggested by a panel of experts, which combine the AKIN and the RIFLE criteria, thus proposing a new classification: The "Kidney Disease Improving Global Outcomes" criteria[29] (Table 1).

The first question regarding the definition of postoperative AKI after partial hepatectomy, would be determining which of these proposed AKI criteria is most appropriate for these patients undergoing liver resection. Whereas acute tubular necrosis (ATN), resulting from hypoxic damage to the renal medulla, is considered as a major cause of postoperative AKI[30], different from general population, liver resections are often performed in the presence of functional deficit of the hepatic parenchyma, as in fibrosis, steatosis, cirrhosis, chemotherapy-induced injury and also in biliary obstruction[2]. Moreover, the recent technical improvements in liver surgery have resulted in an expansion and more liberal indications for major hepatectomies in patients with these underlying liver conditions[2,3,31-34], however, the risk of postoperative complications, such as AKI, have remained important concerns[3,31,35].

In the specific case of hepatocellular carcinoma, the tumor generally appears in a cirrhotic liver, which is a contributor to unfavorable postoperative results in large procedures[36], regarding renal dysfunction, AKI is a common and potentially fatal event in patients with cirrhosis[37-39], with a reported prevalence of 14%-50% in patients with cirrhosis[40-45], this wide range in prevalence is likely due to different study populations and varying definitions of renal dysfunction. Studies evaluating survival predictors in cirrhosis, renal dysfunction was a

Table 1 Current diagnostic criteria for acute kidney injury in general population

	RIFLE criteria[27]	AKIN criteria[28]	KDIGO criteria[29]
Diagnostic criteria	Increase in SCr to ⩾ 1.5 times baseline, within 7 d; or GFR decrease > 25%; or urine volume < 0.5 mL/kg per hour for 6 h	Increase in sCr by ⩾ 0.3 mg/dL (26.5 mmol/L) within 48 h; or increase in sCr ⩾ 1.5 times baseline within 48 h; or urine volume < 0.5 mL/kg per hour for 6 h	Increase in sCr by ⩾ 0.3 mg/dL (26.5 mmol/L) within 48 h; or increase in SCr to ⩾ 1.5 times baseline, which is known or presumed to have occurred within the prior 7 d; or urine volume < 0.5 mL/kg per hour for 6 h
	Risk: sCr increase 1.5-1.9 times baseline; or GFR decrease 25%-50%; or urine output < 0.5 mL/kg per hour for 6 h	Stage 1: sCr increase 1.5-1.9 times baseline; or sCr increase ⩾ 0.3 mg/dL (26.5 mmol/L); or urine output < 0.5 mL/kg per hour for 6 h	Stage 1: sCr increase 1.5-1.9 times baseline; or sCr increase ⩾ 0.3 mg/dL (26.5 mmol/L); or urine output < 0.5 mL/kg per hour for 6-12 h
Staging	Injury: sCr increase 2.0-2.9 times baseline; or GFR decrease 50%-75%; or urine output < 0.5 mL/kg per hour for 12 h	Stage 2: sCr increase 2.0-2.9 times baseline; or urine output < 0.5 mL/kg per hour for 12 h	Stage 2: sCr increase 2.0-2.9 times baseline; or urine output < 0.5 mL/kg per hour for ⩾ 12 h
	Failure: sCr increase ⩾ 3.0 times baseline: or GFR decrease 50%-75%; or sCr increase ⩾ 4.0 mg/dL (353.6 mmol/L) with an acute increase of at least 0.5 mg/dL (44 mmol/L); or urine output < 0.3 mL/kg per hour for ⩾ 24 h; or anuria for ⩾ 12 h	Stage 3: sCr increase 3.0 times baseline; or sCr increase ⩾ 4.0 mg/dL (353.6 mmol/L) with an acute increase of at least 0.5 mg/dL (44 mmol/L); or urine output < 0.3 mL/kg per hour for ⩾ 24 h; or anuria for ⩾ 12 h	Stage 3: sCr increase 3.0 times baseline; or sCr increase to ⩾ 4.0 mg/dL (353.6 mmol/L); or initiation of renal replacement therapy; or urine output < 0.3 mL/kg per hour for ⩾ 24 h; or Anuria for ⩾ 12 h

AKIN: Acute Kidney Injury Network; GFR: Glomerular filtration rate; KDIGO: Kidney Disease Improving Global Outcome; RIFLE: Risk, Injury, Failure, Loss, End stage renal disease; sCr: Serum creatinine.

powerful predictor of death, as Child-Pugh score[46-48].

Along with parenchymal dysfunction, the portal hypertension levels and its hemodynamic consequences are directly related to the degree of underlying liver injury[49-51], as it is observed in cirrhosis and others conditions, such as severe steatosis and chemotherapy-induced injury[52]. The types of chemotherapy-induced liver toxicity include steatosis[53], sinusoidal changes[54], steatohepatitis[55], and hemorrhagic central lobular necrosis[52]. Steatosis represents fatty changes in the liver, with the presence of fat droplets within the hepatocytes[56], and it has been shown that steatosis may interfere with circulation through sinusoids and impair regeneration, and in addition the liver's protective mechanism against oxidative stress appear to be impaired[57,58]. The morbidity following liver resection associated with steatosis has been reported by Belghiti *et al*[2], in this study with 747 patients, the mortality rate was higher in patients having steatosis than in those with no steatosis, 22% *vs* 8%, respectively (P = 0.003). Likewise, according to Behrns *et al*[32] in 135 liver resections, morbidity was seen in 29% and 10% of the patients with steatosis and without steatosis, respectively.

Besides the fact that a significant portion of patients eligible for partial hepatectomy have underlying chronic liver disease or were exposed to systemic therapies with liver toxicity, the hemodynamic changes in patients after major liver resections may have similarities with those of patients with cirrhosis or acute liver failure, and depending on the remnant liver volume and functional quality of parenchyma (steatosis/cirrhosis) the clinical effects may be more evident[59].

In 1953, Kowalski and Abelmann[60] reported the results of a study which have demonstrated that cardiac output in cirrhotic patients was significantly higher compared with healthy volunteers. The reason for this so-called hyperdynamic state is that patients with cirrhosis develop portal hypertension with resultant splanchnic vasodilation and pooling of blood secondary to increased resistance to portal flow. This is due to (1) vasodilators such as nitric oxide, carbon monoxide, and endogenous cannabinoids[61,62]; and (2) vasodilation from inflammatory cytokines such as tumor necrosis factor-alpha and interleukin-6 induced by bacterial translocation from the gut[63]. As a result, the concentration of cyclic guanosine monophosphate cyclic is increased, resulting in splanchnic vasodilation, decrease in central and arterial blood volume, low capillary pressure, low central venous pressure (LCVP), low systemic vascular resistance, and reduction of mean arterial pressure[64]. This compensatory increase in cardiac output *via* activation of the sympathetic nervous system by carotid baroreceptors maintains sufficient renal perfusion, however, with decompensation of cirrhosis and increasing severity of portal hypertension, the compensatory increase in cardiac output is inadequate to maintain circulatory blood volume and adequate renal perfusion[65]. Therefore, it would be reasonable that diagnostic and staging AKI criteria that consider this circulatory impairment could be better applied in patients undergoing liver resections, particularly large resections and those with chronic liver disease.

It is extremely important to point out that in the case of patients with chronic liver disease, isolated dosages of serum creatinine (sCr) levels can not reveal the actual renal function of the patient, because: (1) there is decreased creatine formation in the secondary muscles loss of muscle mass[66]; (2) is increased renal tubular secretion of creatinine (Cr)[67]; (3) increasing the circulating volume of distribution in cirrhosis can dilute the sCr[68]; and (4) interference in the measurement of Cr due to elevated bilirubin[69]. As a result, the serum levels of Cr in patients

with cirrhosis overestimate glomerular filtration rate (GFR). Therefore, a dynamic definition referring to the elevation of serum Cr of ⩾ 50% of preoperative levels to a final value ⩾ 1.5 mg/dL (133 mol/L) could be more suitable for these patients, and clinical studies have shown that AKI according to these criteria was a strong predictor of hospital mortality in patients with liver disease[70-72].

Another situation relates to the measurement of urine output of patients with chronic liver disease and ascites, since these patients can often present oliguria with high sodium retention, but they can still maintain a relatively normal GFR[73]. On the other hand, these patients can also have an increased diuresis because of diuretics therapy.

Thus, the current criteria suggested by the "International Ascites Club" for definition of AKI in cirrhotic patients do not include unreal measurements for these patients[68] (Table 2), and apparently would be the most appropriate criteria for the diagnosis and management of AKI after partial hepatectomy, especially in cases of large resections and underlying chronic liver disease.

HEMODYNAMIC INSTABILITY AND RENAL HYPOPERFUSION

Although the extent of liver resection correlates with the magnitude of the procedure, and patients undergoing resection of more than three segments or an additional extrahepatic procedure have an increased risk of complications[74-76], this is not a rigid rule. For example, an isolated resection of segment Ⅰ is technically more demanding than a right hepatectomy, similarly, resection of segments Ⅳ, Ⅴ, Ⅷ or posterior right segments (segments Ⅵ, Ⅶ) may be technically more difficult than the left or right hepatectomy, although the transection area is larger. Therefore, a minor hepatectomy should not be considered as an operation of less magnitude, and most important, the prevention of intraoperative hemorrhage should not be neglected. If excessive blood loss persists and a reduction in oxygen delivery is not corrected, the renal medulla may be susceptible to ischemic ATN[77], and as a result, patients may suffer from AKI. The results of two large studies[3,31] suggest that a blood loss of 1250 mL is the cutoff value for major complications after liver resections, such as AKI. Furthermore, red blood cell transfusion, that can be necessary in the case of haemorrhage, can be an additional risk factor for postoperative AKI[78].

Increased susceptibility to renal hypoperfusion

The kidneys are most vulnerable to moderate hypoperfusion when autoregulation is impaired. Factors increasing susceptibility to renal hypoperfusion may be seen in elderly patients or in patients with atherosclerosis, hypertension, or chronic renal failure, in whom hyalinosis and myointimal hyperplasia cause structural narrowing of the arterioles[79-81]. Increased susceptibility to renal ischemia may also occur in malignant hypertension because of intimal thickening and fibrinoid necrosis of the small arteries and arterioles[82]. In addition, in chronic kidney disease, afferent arterioles in the functioning glomeruli become dilated with impairment of the kidney's ability to autoregulate the glomerular filtration rate in low-perfusion states[83].

Impaired decreasing of afferent arteriolar resistance can occur when a patient is receiving nonsteroidal anti-inflammatory drugs or cyclooxygenase-2 inhibitors, which reduce the synthesis of prostaglandins in the kidneys, as consequence a decreasing in glomerular capillary pressure occurs in occasions of low-perfusion states[82,84-86]. In other situations, calcineurin inhibitors[87], and radiocontrast agents[88] can act through various vasoconstrictor mediators to increase afferent arteriolar resistance, the later may have direct toxic effects on the tubules as well[81,82,88-92]. Decreased renal perfusion may also may have an exaggerated drop in the GFR in low-perfusion states as a consequence of not raising efferent arteriolar resistance by angiotensin Ⅱ in patients who are receiving angiotensin-receptor blockers or angiotensin-converting-enzyme inhibitors.

Red blood cell transfusion and postoperative AKI

Despite the deleterious effect of hemodynamic instability in renal perfusion, red blood cell transfusion, that can be necessary in the case of haemorrhage, can be an additional risk factor for postoperative AKI[78]. Although the exact causal link between red blood cell transfusion and postoperative AKI is not fully elucidated, there are several mechanisms that may be implicated: Deficiency in 2,3-diphosphoglycerate with impaired oxygen unloading from hemoglobin, less deformability of stored red blood cells with obstruction of smaller capillaries[93] stored red blood cells hemolysis with an increase in circulating free iron[94]. Other mechanisms might include loss of the ability to generate nitric oxide, release of procoagulant phospholipids, increased adhesiveness to vascular endothelium, and accumulation of proinflammatory phospholipids[93,95-98].

POSTHEPATECTOMY LIVER FAILURE AND HEPATORENAL SYNDROME

Apart from blood loss, that can leads to ATN because of severe hemodynamic instability, others risk factors for postoperative AKI after partial hepatectomy would be those that favor PLF, characterized by jaundice, coagulopathy, encephalopathy, ascites, and renal and pulmonary failure, all of which may become apparent only 3 to 5 d after surgery[1]. These risk factors for PLF are well described, such as a small volume of remaining liver with marked volume reduction of organ parenchyma[35,99,100] associated to parenchymal cell injury due portal hyperperfusion[59,101], liver cirrhosis or steatosis[102,103], and liver toxicity induced by chemotherapy[104]. In patients with liver cirrhosis, the postoperative liver failure may occur

Table 2 International Club of Ascites new definitions for the diagnosis and management of acute kidney injury in patients with cirrhosis[68]

Baseline sCr	A value of sCr obtained in the previous 3 mo, when available, can be used as baseline sCr. In patients with more than one value within the previous 3 mo, the value closest to the admission time to the hospital should be used. In patients without a previous sCr value, the sCr on admission should be used as baseline
Definition of AKI	Increase in sCr ≥ 0.3 mg/dL (≥ 26.5 mmol/L) within 48 h; or a percentage increase sCr ≥ 50% from baseline which is known, or presumed, to have occurred within the prior 7 d
Staging of AKI	Stage 1: Increase in sCr ≥ 0.3 mg/dL (26.5 mmol/L) or an increase in sCr ≥ 1.5-fold to twofold from baseline Stage 2: Increase in sCr > two to threefold from baseline Stage 3: Increase of sCr > threefold from baseline or sCr ≥ 4.0 mg/dL (353.6 mmol/L) with an acute increase ≥ 0.3 mg/dL (26.5 mmol/L) or initiation of renal replacement therapy
Progression of AKI	Progression: Progression of AKI to a higher stage and/or need for RRT Regression: Regression of AKI to a lower stage
Response to treatment	No response: No regression of AKI Partial response: Regression of AKI stage with a reduction of sCr to ≥ 0.3 mg/dL (26.5 mmol/L) above the baseline value Full response: Return of sCr to a value within 0.3 mg/dL (26.5 mmol/L) of the baseline value

AKI: Acute kidney injury; RRT: Renal replacement therapy; sCr: Serum creatinine.

due the compromised liver microcirculation, with less resistance to ischemia-reperfusion injury[105] and impaired regeneration[106], in addition, portal hypertension, if present, is associated with a poor outcome because of compromised portal flow and the risk of postoperative upper gastrointestinal bleeding[107].

Liver steatosis is usually related to obesity, the presence of metabolic disorders, or the intake of alcohol or drugs, and this liver disorder increases the operative risk of partial hepatectomy[2,53,108]. The extent of liver resection in these patients with steatosis in order to avoid PLF is unclear, but the severity of fatty infiltration must be considered: Mild steatosis (up to 30% of hepatocytes containing fat) represents a minimal additional risk, in moderate steatosis (30% to 60% containing fat) caution is necessary, thus, a conservative resection should be favored, and patients with severe steatosis (more than 60% of hepatocytes containing fat) should undergo only limited resection[108].

Regarding the chemotherapy-induced liver aggression, the rates of complications and death after major liver resection are likely to be increased[55,109]. Oxaliplatin can induce a veno-occlusive syndrome, occasionally associated with nodular regenerative hyperplasia, these vascular obstructions result in a bluish appearance of the liver (blue liver syndrome)[54,110,111], and irinotecan can cause chemotherapy associated steatohepatitis[112], and liver impairment can be amplified after partial hepatectomy in both situations, triggering PLF[113].

A major concern regarding PLF is the onset of HRS. HRS is a reversible functional renal impairment that occurs in patients with advanced liver cirrhosis or hepatic failure. It is characterized by marked decrease in GFR and renal plasma flow in the absence of other cause of renal failure[114] (Table 3). The pathophysiological alterations of SHR consist of intravascular hypovolemia with activation of the renin-angiotensin-aldosterone system and vasoconstrictive sympathetic nervous system, leading to renal vasoconstriction of the afferent vessels and subsequent decrease in GFR[20]. Two subtypes of HRS have been identified: SHR type 1 is characterized by a rapidly progressive renal insufficiency defined as a doubling of the initial serum creatinine to a level greater than 2.5 mg/dL or 220 μmol/L in less than 2 wk, it is associated with very poor prognosis, and SHR Type 2 is characterized by a moderate renal insufficiency (Cr greater than 1.5 mg/dL or 133 μmol/L), follows a steady course or slowly progressive, often associated with refractory ascites[114].

KEYPOINTS FOR PREVENTION OF AKI AFTER PARTIAL HEPATECTOMY

Despite the fact that patients can have more than one factor contributing to post-operative AKI after partial hepatectomy, eventually aggravated by sepsis[20,21-24] or exposure to nephrotoxic drugs[25], there are particular risk factors that must be controlled and specific operative and non-operative procedures that must be undertaken for prevention of post-operative renal injury in these patients (Figure 1).

Vascular control of the liver

For prevention of intraoperative blood loss with consequent hemodynamic instability during the partial hepatectomy, there are intraoperative maneuvers that may be crucial in the moment of parenchymal transaction, such as vascular control of the liver[21].

The vascular control of the liver is an effective method to reduce bleeding during the hepatectomy. While various techniques have been proposed, the two most widely used methods are the vascular inflow occlusion and complete vascular exclusion[115,116]. Occlusion of the hepatic vascular inflow[117] by the application of tourniquet in hepatoduodenal ligament[118] is the oldest and simplest way to reduce blood loss during hepatectomy. The "Pringle maneuver" can be used continuously to normal livers under normothermic conditions for a maximum of 60 min, and for 30 min in cirrhotic or steatotic livers, although longer periods have already been described[119-122].

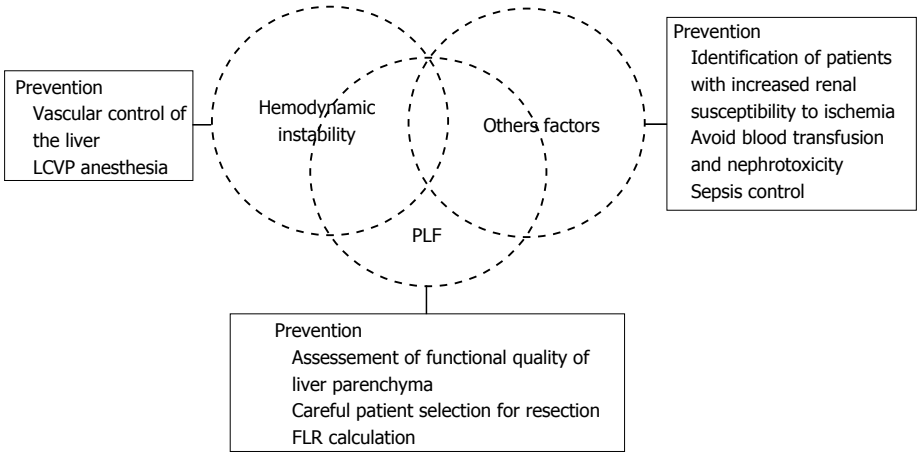

Figure 1 Main risk factors and prevention of acute kidney injury after partial hepatectomy. LCVP: Low central venous pressure; PLF: Posthepatectomy liver failure; FLR: Future liver remnant.

Table 3 Diagnostic criteria of hepatorenal syndrome type of acute kidney injury in patients with cirrhosis[68]

HRS-AKI
Diagnosis of cirrhosis and ascites
Diagnosis of AKI according to ICA-AKI criteria (Table 2)
No response after 2 consecutive days of diuretic withdrawal and plasma volume expansion with albumin 1 g/kg bodyweight
Absence of shock
No current or recent use of nephrotoxic drugs (NSAIDs, aminoglycosides, iodinated contrast media, *etc.*)
No macroscopic signs of structural kidney injury, defined as
Absence of proteinuria (> 500 mg/d)
Absence of microhaematuria (> 50 RBCs per high power field)
Normal findings on renal ultrasonography
Patients who fulfil these criteria may still have structural damage such as tubular damage. Urine biomarkers will become an important element in making a more accurate differential diagnosis between HRS and acute tubular necrosis

HRS: Hepatorenal syndrome; AKI: Acute kidney injury; ICA: International club of ascites; NSAIDs: Non-steroidal anti-inflammatory drugs; RBCs: Red blood cells.

According Belghiti *et al*[123] there is no significant difference in blood loss during surgery using the Pringle maneuver continuously or intermittently (15 min of ischemia for 5 min reperfusion). These concerns about longer periods of hepatic vascular inflow is mainly because that obstruction of the portal blood flow causes venous congestion of the bowel, and in combination with warm ischemic liver injury it results in a flush of anaerobic metabolites and cytokines back into the circulation on the clamp release[124]. In the total vascular exclusion[125], the occlusion of the hepatic vascular inflow is combined to hepatic venous exclusion. The complete hepatic ischemia can be associated to hypothermic perfusion with cooled preservation solution[126] and extracorporeal venovenous bypass, with "*ex situ*" liver resection[127] or "*in situ*" liver resection[128].

LCVP anesthesia

During the parenchymal transaction, a LCVP prevents the back bleeding from hepaticveins[19,129,130], and along with vascular control of the liver, these techniques test the patients cardiovascular reserve[21]. LCVP anesthesia is based on patients being maintained in hypovolaemic state until liver resection has been completed[19,129], this is in contrast to most other major surgical procedures, where patients receive large volumes of crystalloid and colloid during the peri-operative period[21]. Moreover, vasodilators are often used to further reduce central venous pressure (CVP), leading to distributive changes in blood flow[129], and whereas these techniques are applied for haemorrhage control and consequently promoting AKI prevention, a potential consequence of such circulatory changes is ATN, with subsequent renal impairment or failure[20]. The kidneys are at greater risk with abrupt fall in blood pressure, if the mean arterial pressure reaches values below 80 mmHg, there is a significant decrease in GFR[24].

In the study of Wang *et al*[131], the maintenance of CVP $\leqslant$ 4 mmHg has reduced blood loss during partial hepatectomy, and has shortened the length of hospital stay, with no detrimental effects on hepatic or renal function. According to Melendez *et al*[19], in 496 liver resections with an anesthetic protocol of fluid restriction, with the use of nitroglycerin, furosemide, and with the maintenance of a systolic blood pressure of 90 mmHg, the median volume blood loss was 645 mL and the incidence of AKI was 3.1%. A study with 2116 LCVP-assisted hepatectomies reported an estimated mean blood loss of 300 mL (IQR: 200-600 mL), 90-d mortality of 2%, and postoperative AKI of 16% in the whole cohort (13% at risk, 2% at injury and 1% experienced failure)[132]. A study reported a low incidence of AKI requiring renal replacement therapy after liver resection (< 1%), confirming that the routine use of LCVP anaesthesia in combination with intermittent inflow occlusion is safe[21].

Although there are strong evidences that LCVP during partial hepatectomy can minimize blood loss and mortality[19], it is not clear whether it would play a role

in AKI prevention, as renal perfusion pressure can be decreased during relative hypovolemia, thus, further studies are required to prove this hypothesis.

Prevention of post-hepatectomy liver failure

In order to reduce the incidence of PLF, a careful preoperative planning and patient selection is mandatory. In the case of underlying cirrhosis, the best candidates for surgical resection are the exclusive Child-Pugh A patients with normal bilirubin values, the absence of clinical signs of portal hypertension (platelet count, splenomegaly and esophageal varices), only tumor diameter < 5 cm (without vascular invasion), asymptomatic and MELD < 8[107,133,134]. Hyperbilirubinemia, portal hypertension and clinical deterioration criteria are considered signs of poor postoperative course, despite the tumor resectability[135].

Analyzing the issue of remnant liver volume after partial hepatectomy, the functional quality of parenchyma should not be ignored. In obtaining the computed tomography images, it enables the calculation of the future liver remnant (FLR), in patients with normal liver function, it must be greater than 25% of the liver total volume, corresponding to 0.5 of the patient weight. In patients with cirrhosis, prolonged exposure to chemotherapy and biliary obstruction, this value is 40%, corresponding to 0.7 of the patient weight[136]. The occlusion of a branch of the portal vein can be performed in order to minimize the occurrence of hepatic insufficiency after major resections. This procedure makes possible the treatment of tumors previously classified as unresectable, providing contralateral liver hypertrophy, thereby increasing the FLR[137,138]. In some situations resectability only occurs when performing two sequential hepatectomies associated with portal ligation for manipulation of the FLR, the two-stage hepatectomy[139].

FINAL CONSIDERATIONS

In the context of liver resections, the risk assessment of postoperative AKI requires the analysis of multiple variables involved in this complex universe, but probably there are main factors which significantly influence these patients for the occurrence of AKI: The massive blood loss during operation with or without an increased renal susceptibility to ischemia, and the occurrence of PLF. Certainly, the key interventions for preventing postoperative AKI after partial hepatectomy would be an appropriate preoperative work up, careful patient selection for surgery and rigorous perioperative control of the patient hemodynamic status by the surgical team.

REFERENCES

1 **Clavien PA**, Petrowsky H, DeOliveira ML, Graf R. Strategies for safer liver surgery and partial liver transplantation. *N Engl J Med* 2007; **356**: 1545-1559 [PMID: 17429086 DOI: 10.1056/NEJMra065156]

2 **Belghiti J**, Hiramatsu K, Benoist S, Massault P, Sauvanet A, Farges O. Seven hundred forty-seven hepatectomies in the 1990s: an update to evaluate the actual risk of liver resection. *J Am Coll Surg* 2000; **191**: 38-46 [PMID: 10898182 DOI: 10.1016/S1072-7515(00)00261-1]

3 **Jarnagin WR**, Gonen M, Fong Y, DeMatteo RP, Ben-Porat L, Little S, Corvera C, Weber S, Blumgart LH. Improvement in perioperative outcome after hepatic resection: analysis of 1,803 consecutive cases over the past decade. *Ann Surg* 2002; **236**: 397-406; discussion 406-407 [PMID: 12368667 DOI: 10.1097/01.SLA.0000029003.66466.B3]

4 **Poon RT**, Fan ST, Lo CM, Liu CL, Lam CM, Yuen WK, Yeung C, Wong J. Improving perioperative outcome expands the role of hepatectomy in management of benign and malignant hepatobiliary diseases: analysis of 1222 consecutive patients from a prospective database. *Ann Surg* 2004; **240**: 698-708; discussion 708-710 [PMID: 15383797 DOI: 10.1097/01.sla.0000141195.66155.0c]

5 **Chertow GM**, Burdick E, Honour M, Bonventre JV, Bates DW. Acute kidney injury, mortality, length of stay, and costs in hospitalized patients. *J Am Soc Nephrol* 2005; **16**: 3365-3370 [PMID: 16177006 DOI: 10.1681/asn.2004090740]

6 **Kheterpal S**, Tremper KK, Englesbe MJ, O'Reilly M, Shanks AM, Fetterman DM, Rosenberg AL, Swartz RD. Predictors of postoperative acute renal failure after noncardiac surgery in patients with previously normal renal function. *Anesthesiology* 2007; **107**: 892-902 [PMID: 18043057 DOI: 10.1097/01.anes.0000290588.29668.38]

7 **Bihorac A**, Yavas S, Subbiah S, Hobson CE, Schold JD, Gabrielli A, Layon AJ, Segal MS. Long-term risk of mortality and acute kidney injury during hospitalization after major surgery. *Ann Surg* 2009; **249**: 851-858 [PMID: 19387314 DOI: 10.1097/SLA.0b013e3181a40a0b]

8 **Abelha FJ**, Botelho M, Fernandes V, Barros H. Outcome and quality of life of patients with acute kidney injury after major surgery. *Nefrologia* 2009; **29**: 404-414 [PMID: 19820752 DOI: 10.3265/Nefrologia.2009.29.5.5456.en.full]

9 **Andersson LG**, Ekroth R, Bratteby LE, Hallhagen S, Wesslén O. Acute renal failure after coronary surgery--a study of incidence and risk factors in 2009 consecutive patients. *Thorac Cardiovasc Surg* 1993; **41**: 237-241 [PMID: 8211928 DOI: 10.1055/s-2007-1013861]

10 **Bove T**, Calabrò MG, Landoni G, Aletti G, Marino G, Crescenzi G, Rosica C, Zangrillo A. The incidence and risk of acute renal failure after cardiac surgery. *J Cardiothorac Vasc Anesth* 2004; **18**: 442-445 [PMID: 15365924 DOI: 10.1053/j.jvca.2004.05.021]

11 **Landoni G**, Bove T, Crivellari M, Poli D, Fochi O, Marchetti C, Romano A, Marino G, Zangrillo A. Acute renal failure after isolated CABG surgery: six years of experience. *Minerva Anestesiol* 2007; **73**: 559-565 [PMID: 17952028]

12 **Rimola A**, Gavaler JS, Schade RR, el-Lankany S, Starzl TE, Van Thiel DH. Effects of renal impairment on liver transplantation. *Gastroenterology* 1987; **93**: 148-156 [PMID: 3556303]

13 **McCauley J**, Van Thiel DH, Starzl TE, Puschett JB. Acute and chronic renal failure in liver transplantation. *Nephron* 1990; **55**: 121-128 [PMID: 2362625 DOI: 10.1159/000185938]

14 **Ishitani M**, Wilkowski M, Stevenson W, Pruett T. Outcome of patients requiring hemodialysis after liver transplantation. *Transplant Proc* 1993; **25**: 1762-1763 [PMID: 8470156]

15 **Nuño J**, Cuervas-Mons V, Vicente E, Turrión V, Pereira F, Mora NP, Barrios C, Millán I, Ardaiz J. Renal failure after liver transplantation: analysis of risk factors in 139 liver transplant recipients. *Transplant Proc* 1995; **27**: 2319-2320 [PMID: 7652826]

16 **Bilbao I**, Charco R, Balsells J, Lazaro JL, Hidalgo E, Llopart L, Murio E, Margarit C. Risk factors for acute renal failure requiring dialysis after liver transplantation. *Clin Transplant* 1998; **12**: 123-129 [PMID: 9575400]

17 **Cabezuelo JB**, Ramírez P, Ríos A, Acosta F, Torres D, Sansano T, Pons JA, Bru M, Montoya M, Bueno FS, Robles R, Parrilla P. Risk factors of acute renal failure after liver transplantation. *Kidney Int* 2006; **69**: 1073-1080 [PMID: 16528257 DOI: 10.1038/sj.ki.5000216]

18 **Yalavarthy R**, Edelstein CL, Teitelbaum I. Acute renal failure and chronic kidney disease following liver transplantation. *Hemodial Int* 2007; **11** Suppl 3: S7- S12 [PMID: 17897111 DOI: 10.1111/j.1542-4758.2007.00223.x]

19 **Melendez JA**, Arslan V, Fischer ME, Wuest D, Jarnagin WR,

Fong Y, Blumgart LH. Perioperative outcomes of major hepatic resections under low central venous pressure anesthesia: blood loss, blood transfusion, and the risk of postoperative renal dysfunction. *J Am Coll Surg* 1998; **187**: 620-625 [PMID: 9849736 DOI: 10.1016/s1072-7515(98)00240-3]

20 **Saner F**. Kidney failure following liver resection. *Transplant Proc* 2008; **40**: 1221-1224 [PMID: 18555153 DOI: 10.1016/j.transproceed.2008.03.068]

21 **Armstrong T**, Welsh FK, Wells J, Chandrakumaran K, John TG, Rees M. The impact of pre-operative serum creatinine on short-term outcomes after liver resection. *HPB* (Oxford) 2009; **11**: 622-628 [PMID: 20495629 DOI: 10.1111/j.1477-2574.2009.00094.x]

22 **Slankamenac K**, Breitenstein S, Held U, Beck-Schimmer B, Puhan MA, Clavien PA. Development and validation of a prediction score for postoperative acute renal failure following liver resection. *Ann Surg* 2009; **250**: 720-728 [PMID: 19809295 DOI: 10.1097/sla.0b013e3181bdd840]

23 **Tomozawa A**, Ishikawa S, Shiota N, Cholvisudhi P, Makita K. Perioperative risk factors for acute kidney injury after liver resection surgery: an historical cohort study. *Can J Anaesth* 2015; **62**: 753-761 [PMID: 25925634 DOI: 10.1007/s12630-015-0397-9]

24 **Abuelo JG**. Normotensive ischemic acute renal failure. *N Engl J Med* 2007; **357**: 797-805 [PMID: 17715412 DOI: 10.1056/nejmra064398]

25 **Moore RD**, Smith CR, Lipsky JJ, Mellits ED, Lietman PS. Risk factors for nephrotoxicity in patients treated with aminoglycosides. *Ann Intern Med* 1984; **100**: 352-357 [PMID: 6364908 DOI: 10.7326/0003-4819-100-3-352]

26 **Thadhani R**, Pascual M, Bonventre JV. Acute renal failure. *N Engl J Med* 1996; **334**: 1448-1460 [PMID: 8618585 DOI: 10.1056/nejm199605303342207]

27 **Bellomo R**, Ronco C, Kellum JA, Mehta RL, Palevsky P. Acute renal failure - definition, outcome measures, animal models, fluid therapy and information technology needs: the Second International Consensus Conference of the Acute Dialysis Quality Initiative (ADQI) Group. *Crit Care* 2004; **8**: R204-R212 [PMID: 15312219 DOI: 10.1186/cc2872]

28 **Mehta RL**, Kellum JA, Shah SV, Molitoris BA, Ronco C, Warnock DG, Levin A. Acute Kidney Injury Network: report of an initiative to improve outcomes in acute kidney injury. *Crit Care* 2007; **11**: R31 [PMID: 17331245 DOI: 10.1186/cc5713]

29 **KDIGO Board Members**. KDIGO Clinical Practice Guideline for Acute Kidney Injury. *Kidney Int Suppl* 2012; **2**: 1-138 [DOI: 10.1038/kisup.2012.3]

30 **Noor S**, Usmani A. Postoperative renal failure. *Clin Geriatr Med* 2008; **24**: 721-729, ix [PMID: 18984383 DOI: 10.1016/j.cger.2008.07.004]

31 **Imamura H**, Seyama Y, Kokudo N, Maema A, Sugawara Y, Sano K, Takayama T, Makuuchi M. One thousand fifty-six hepatectomies without mortality in 8 years. *Arch Surg* 2003; **138**: 1198-1206; discussion 1206 [PMID: 14609867 DOI: 10.1001/archsurg.138.11.1198]

32 **Behrns KE**, Tsiotos GG, DeSouza NF, Krishna MK, Ludwig J, Nagorney DM. Hepatic steatosis as a potential risk factor for major hepatic resection. *J Gastrointest Surg* 1998; **2**: 292-298 [PMID: 9841987 DOI: 10.1016/S1091-255X(98)80025-5]

33 **Cohnert TU**, Rau HG, Buttler E, Hernandez-Richter T, Sauter G, Reuter C, Schildberg FW. Preoperative risk assessment of hepatic resection for malignant disease. *World J Surg* 1997; **21**: 396-400; discussion 401 [PMID: 9143571 DOI: 10.1007/pl00012260]

34 **Choti MA**, Sitzmann JV, Tiburi MF, Sumetchotimetha W, Rangsin R, Schulick RD, Lillemoe KD, Yeo CJ, Cameron JL. Trends in long-term survival following liver resection for hepatic colorectal metastases. *Ann Surg* 2002; **235**: 759-766 [PMID: 12035031]

35 **Schindl MJ**, Redhead DN, Fearon KC, Garden OJ, Wigmore SJ. The value of residual liver volume as a predictor of hepatic dysfunction and infection after major liver resection. *Gut* 2005; **54**: 289-296 [PMID: 15647196 DOI: 10.1136/gut.2004.046524]

36 **Simons JP**, Hill JS, Ng SC, Shah SA, Zhou Z, Whalen GF, Tseng JF. Perioperative mortality for management of hepatic neoplasm: a simple risk score. *Ann Surg* 2009; **250**: 929-934 [PMID: 19855257 DOI: 10.1097/sla.0b013e3181bc9c2f]

37 **Moreau R**, Lebrec D. Acute renal failure in patients with cirrhosis: perspectives in the age of MELD. *Hepatology* 2003; **37**: 233-243 [PMID: 12540770 DOI: 10.1053/jhep.2003.50084]

38 **Mackelaite L**, Alsauskas ZC, Ranganna K. Renal failure in patients with cirrhosis. *Med Clin North Am* 2009; **93**: 855-869, viii [PMID: 19577118 DOI: 10.1016/j.mcna.2009.03.003]

39 **Salerno F**, Guevara M, Bernardi M, Moreau R, Wong F, Angeli P, Garcia-Tsao G, Lee SS. Refractory ascites: pathogenesis, definition and therapy of a severe complication in patients with cirrhosis. *Liver Int* 2010; **30**: 937-947 [PMID: 20492521 DOI: 10.1111/j.1478-3231.2010.02272.x]

40 **Hampel H**, Bynum GD, Zamora E, El-Serag HB. Risk factors for the development of renal dysfunction in hospitalized patients with cirrhosis. *Am J Gastroenterol* 2001; **96**: 2206-2210 [PMID: 11467654 DOI: 10.1111/j.1572-0241.2001.03958.x]

41 **Péron JM**, Bureau C, Gonzalez L, Garcia-Ricard F, de Soyres O, Dupuis E, Alric L, Pourrat J, Vinel JP. Treatment of hepatorenal syndrome as defined by the international ascites club by albumin and furosemide infusion according to the central venous pressure: a prospective pilot study. *Am J Gastroenterol* 2005; **100**: 2702-2707 [PMID: 16393223 DOI: 10.1111/j.1572-0241.2005.00271.x]

42 **Terra C**, Guevara M, Torre A, Gilabert R, Fernández J, Martín-Llahí M, Baccaro ME, Navasa M, Bru C, Arroyo V, Rodés J, Ginès P. Renal failure in patients with cirrhosis and sepsis unrelated to spontaneous bacterial peritonitis: value of MELD score. *Gastroenterology* 2005; **129**: 1944-1953 [PMID: 16344063 DOI: 10.1053/j.gastro.2005.09.024]

43 **du Cheyron D**, Bouchet B, Parienti JJ, Ramakers M, Charbonneau P. The attributable mortality of acute renal failure in critically ill patients with liver cirrhosis. *Intensive Care Med* 2005; **31**: 1693-1699 [PMID: 16244877 DOI: 10.1007/s00134-005-2842-7]

44 **Wu CC**, Yeung LK, Tsai WS, Tseng CF, Chu P, Huang TY, Lin YF, Lu KC. Incidence and factors predictive of acute renal failure in patients with advanced liver cirrhosis. *Clin Nephrol* 2006; **65**: 28-33 [PMID: 16429839 DOI: 10.5414/CNP65028]

45 **Montoliu S**, Ballesté B, Planas R, Alvarez MA, Rivera M, Miquel M, Masnou H, Cirera I, Morillas RM, Coll S, Sala M, García-Retortillo M, Cañete N, Solà R. Incidence and prognosis of different types of functional renal failure in cirrhotic patients with ascites. *Clin Gastroenterol Hepatol* 2010; **8**: 616-622; quiz e80 [PMID: 20399905 DOI: 10.1016/j.cgh.2010.03.029]

46 **Llach J**, Ginès P, Arroyo V, Rimola A, Titó L, Badalamenti S, Jiménez W, Gaya J, Rivera F, Rodés J. Prognostic value of arterial pressure, endogenous vasoactive systems, and renal function in cirrhotic patients admitted to the hospital for the treatment of ascites. *Gastroenterology* 1988; **94**: 482-487 [PMID: 3335320]

47 **Krag A**, Bendtsen F, Henriksen JH, Møller S. Low cardiac output predicts development of hepatorenal syndrome and survival in patients with cirrhosis and ascites. *Gut* 2010; **59**: 105-110 [PMID: 19837678 DOI: 10.1136/gut.2009.180570]

48 **Lim YS**, Larson TS, Benson JT, Kamath PS, Kremers WK, Therneau TM, Kim WR. Serum sodium, renal function, and survival of patients with end-stage liver disease. *J Hepatol* 2010; **52**: 523-528 [PMID: 20185195 DOI: 10.1016/j.jhep.2010.01.009]

49 **de Franchis R**, Dell'Era A, Primignani M. Diagnosis and monitoring of portal hypertension. *Dig Liver Dis* 2008; **40**: 312-317 [PMID: 18294933 DOI: 10.1016/j.dld.2007.12.007]

50 **Cucchetti A**, Ercolani G, Vivarelli M, Cescon M, Ravaioli M, Ramacciato G, Grazi GL, Pinna AD. Is portal hypertension a contraindication to hepatic resection? *Ann Surg* 2009; **250**: 922-928 [PMID: 19855258 DOI: 10.1097/SLA.0b013e3181b977a5]

51 **Santambrogio R**, Kluger MD, Costa M, Belli A, Barabino M, Laurent A, Opocher E, Azoulay D, Cherqui D. Hepatic resection for hepatocellular carcinoma in patients with Child-Pugh's A cirrhosis: is clinical evidence of portal hypertension a contraindication? *HPB* (Oxford) 2013; **15**: 78-84 [PMID: 23216782 DOI: 10.1111/j.1477-2574.2012.00594.x]

52 **Tisman G**, MacDonald D, Shindell N, Reece E, Patel P, Honda

N, Nishimora EK, Garris J, Shannahan W, Chisti N, McCarthy J, Nasser Moaddeli S, Sargent D, Plant A. Oxaliplatin toxicity masquerading as recurrent colon cancer. *J Clin Oncol* 2004; **22**: 3202-3204 [PMID: 15284280 DOI: 10.1200/JCO.2004.99.100]

53 **Kooby DA**, Fong Y, Suriawinata A, Gonen M, Allen PJ, Klimstra DS, DeMatteo RP, D'Angelica M, Blumgart LH, Jarnagin WR. Impact of steatosis on perioperative outcome following hepatic resection. *J Gastrointest Surg* 2003; **7**: 1034-1044 [PMID: 14675713 DOI: 10.1016/j.gassur.2003.09.012]

54 **Rubbia-Brandt L**, Audard V, Sartoretti P, Roth AD, Brezault C, Le Charpentier M, Dousset B, Morel P, Soubrane O, Chaussade S, Mentha G, Terris B. Severe hepatic sinusoidal obstruction associated with oxaliplatin-based chemotherapy in patients with metastatic colorectal cancer. *Ann Oncol* 2004; **15**: 460-466 [PMID: 14998849 DOI: 10.1093/annonc/mdh095]

55 **Vauthey JN**, Pawlik TM, Ribero D, Wu TT, Zorzi D, Hoff PM, Xiong HQ, Eng C, Lauwers GY, Mino-Kenudson M, Risio M, Muratore A, Capussotti L, Curley SA, Abdalla EK. Chemotherapy regimen predicts steatohepatitis and an increase in 90-day mortality after surgery for hepatic colorectal metastases. *J Clin Oncol* 2006; **24**: 2065-2072 [PMID: 16648507 DOI: 10.1200/JCO.2005.05.3074]

56 **Kemeny N**. Presurgical chemotherapy in patients being considered for liver resection. *Oncologist* 2007; **12**: 825-839 [PMID: 17673614 DOI: 10.1634/theoncologist.12-7-825]

57 **Maroş T**, Seres-Sturm L, Lakatos O, Seres-Sturm M, Mody E, Blazsek V. Data regarding the restorative effects of the partial removal of the liver in advanced stages of toxic cirrhosis. *Morphol Embryol* (Bucur) 1975; **21**: 213-217 [PMID: 129696]

58 **Haney A**, Peacock EE, Madden JW. Liver regeneration and hepatic collagen deposition in rats with dimethjylnitrosamine-induced cirrhosis. *Ann Surg* 1972; **175**: 863-869 [PMID: 5029843]

59 **Golriz M**, Majlesara A, El Sakka S, Ashrafi M, Arwin J, Fard N, Raisi H, Edalatpour A, Mehrabi A. Small for Size and Flow (SFSF) syndrome: An alternative description for posthepatectomy liver failure. *Clin Res Hepatol Gastroenterol* 2016; **40**: 267-275 [PMID: 26516057 DOI: 10.1016/J.Clinre.2015.06.024]

60 **Kowalski HJ**, Abelmann WH. The cardiac output at rest in Laennec's cirrhosis. *J Clin Invest* 1953; **32**: 1025-1033 [PMID: 13096569 DOI: 10.1172/JCI102813]

61 **Martin PY**, Ginès P, Schrier RW. Nitric oxide as a mediator of hemodynamic abnormalities and sodium and water retention in cirrhosis. *N Engl J Med* 1998; **339**: 533-541 [PMID: 9709047 DOI: 10.1056/Nejm199808203390807]

62 **Ros J**, Clària J, To-Figueras J, Planagumà A, Cejudo-Martín P, Fernández-Varo G, Martín-Ruiz R, Arroyo V, Rivera F, Rodés J, Jiménez W. Endogenous cannabinoids: a new system involved in the homeostasis of arterial pressure in experimental cirrhosis in the rat. *Gastroenterology* 2002; **122**: 85-93 [PMID: 11781284 DOI: 10.1053/gast.2002.30305]

63 **Navasa M**, Follo A, Filella X, Jiménez W, Francitorra A, Planas R, Rimola A, Arroyo V, Rodés J. Tumor necrosis factor and interleukin-6 in spontaneous bacterial peritonitis in cirrhosis: relationship with the development of renal impairment and mortality. *Hepatology* 1998; **27**: 1227-1232 [PMID: 9581675 DOI: 10.1002/hep.510270507]

64 **Albillos A**, Rossi I, Cacho G, Martínez MV, Millán I, Abreu L, Barrios C, Escartín P. Enhanced endothelium-dependent vasodilation in patients with cirrhosis. *Am J Physiol* 1995; **268**: G459-G464 [PMID: 7900807]

65 **Ginès P**, Schrier RW. Renal failure in cirrhosis. *N Engl J Med* 2009; **361**: 1279-1290 [PMID: 19776409 DOI: 10.1056/nejmra0809139]

66 **Sherman DS**, Fish DN, Teitelbaum I. Assessing renal function in cirrhotic patients: problems and pitfalls. *Am J Kidney Dis* 2003; **41**: 269-278 [PMID: 12552488 DOI: 10.1053/ajkd.2003.50035]

67 **Caregaro L**, Menon F, Angeli P, Amodio P, Merkel C, Bortoluzzi A, Alberino F, Gatta A. Limitations of serum creatinine level and creatinine clearance as filtration markers in cirrhosis. *Arch Intern Med* 1994; **154**: 201-205 [PMID: 8285815 DOI: 10.1001/archinte.1994.00420020117013]

68 **Angeli P**, Gines P, Wong F, Bernardi M, Boyer TD, Gerbes A, Moreau R, Jalan R, Sarin SK, Piano S, Moore K, Lee SS, Durand F, Salerno F, Caraceni P, Kim WR, Arroyo V, Garcia-Tsao G. Diagnosis and management of acute kidney injury in patients with cirrhosis: revised consensus recommendations of the International Club of Ascites. *Gut* 2015; **64**: 531-537 [PMID: 25631669 DOI: 10.1136/gutjnl-2014-308874]

69 **Spencer K**. Analytical reviews in clinical biochemistry: the estimation of creatinine. *Ann Clin Biochem* 1986; **23** (Pt 1): 1-25 [PMID: 3532908 DOI: 10.1177/000456328602300101]

70 **Ginès A**, Fernández-Esparrach G, Monescillo A, Vila C, Domènech E, Abecasis R, Angeli P, Ruiz-Del-Arbol L, Planas R, Solà R, Ginès P, Terg R, Inglada L, Vaqué P, Salerno F, Vargas V, Clemente G, Quer JC, Jiménez W, Arroyo V, Rodés J. Randomized trial comparing albumin, dextran 70, and polygeline in cirrhotic patients with ascites treated by paracentesis. *Gastroenterology* 1996; **111**: 1002-1010 [PMID: 8831595 DOI: 10.1016/S0016-5085(96)70068-9]

71 **Sort P**, Navasa M, Arroyo V, Aldeguer X, Planas R, Ruiz-del-Arbol L, Castells L, Vargas V, Soriano G, Guevara M, Ginès P, Rodés J. Effect of intravenous albumin on renal impairment and mortality in patients with cirrhosis and spontaneous bacterial peritonitis. *N Engl J Med* 1999; **341**: 403-409 [PMID: 10432325 DOI: 10.1056/nejm199908053410603]

72 **Angeli P**, Fasolato S, Mazza E, Okolicsanyi L, Maresio G, Velo E, Galioto A, Salinas F, D'Aquino M, Sticca A, Gatta A. Combined versus sequential diuretic treatment of ascites in non-azotaemic patients with cirrhosis: results of an open randomised clinical trial. *Gut* 2010; **59**: 98-104 [PMID: 19570764 DOI: 10.1136/gut.2008.176495]

73 **Angeli P**, Gatta A, Caregaro L, Menon F, Sacerdoti D, Merkel C, Rondana M, de Toni R, Ruol A. Tubular site of renal sodium retention in ascitic liver cirrhosis evaluated by lithium clearance. *Eur J Clin Invest* 1990; **20**: 111-117 [PMID: 2108033 DOI: 10.1111/j.1365-2362.1990.tb01800.x]

74 **Breitenstein S**, DeOliveira ML, Raptis DA, Slankamenac K, Kambakamba P, Nerl J, Clavien PA. Novel and simple preoperative score predicting complications after liver resection in noncirrhotic patients. *Ann Surg* 2010; **252**: 726-734 [PMID: 21037427 DOI: 10.1097/SLA.0b013e3181fb8c1a]

75 **Oussoultzoglou E**, Jaeck D, Addeo P, Fuchshuber P, Marzano E, Rosso E, Pessaux P, Bachellier P. Prediction of mortality rate after major hepatectomy in patients without cirrhosis. *Arch Surg* 2010; **145**: 1075-1081 [PMID: 21079096 DOI: 10.1001/archsurg.2010.225]

76 **Andres A**, Toso C, Moldovan B, Schiffer E, Rubbia-Brandt L, Terraz S, Klopfenstein CE, Morel P, Majno P, Mentha G. Complications of elective liver resections in a center with low mortality: a simple score to predict morbidity. *Arch Surg* 2011; **146**: 1246-1252 [PMID: 21768406 DOI: 10.1001/archsurg.2011.175]

77 **Jones DR**, Lee HT. Perioperative renal protection. *Best Pract Res Clin Anaesthesiol* 2008; **22**: 193-208 [PMID: 18494397 DOI: 10.1016/j.bpa.2007.08.005]

78 **Karkouti K**, Wijeysundera DN, Yau TM, Callum JL, Cheng DC, Crowther M, Dupuis JY, Fremes SE, Kent B, Laflamme C, Lamy A, Legare JF, Mazer CD, McCluskey SA, Rubens FD, Sawchuk C, Beattie WS. Acute kidney injury after cardiac surgery: focus on modifiable risk factors. *Circulation* 2009; **119**: 495-502 [PMID: 19153273 DOI: 10.1161/circulationaha.108.786913]

79 **Badr KF**, Ichikawa I. Prerenal failure: a deleterious shift from renal compensation to decompensation. *N Engl J Med* 1988; **319**: 623-629 [PMID: 3045546 DOI: 10.1056/nejm198809083191007]

80 **Brady HR**, Clarkson MR, Lieberthal W. Acute Renal Failure. In: Brenner BM, Ed. Brenner and Rector's The Kidney. 7th ed. Philadelphia: Saunders, 2004: 1215-1292

81 **Palmer BF**. Renal dysfunction complicating the treatment of hypertension. *N Engl J Med* 2002; **347**: 1256-1261 [PMID: 12393824 DOI: 10.1056/NEJMra020676]

82 **Abuelo JG**. Diagnosing vascular causes of renal failure. *Ann Intern Med* 1995; **123**: 601-614 [PMID: 7677302]

83 **Taal MW**, Luyckx VA, Brenner BM. Adaptation to nephron loss. In: Brenner BM, Ed. Brenner And Rector's The Kidney. 7th ed. Philadelphia: Saunders, 2004: 1955-1997

84 **Schlondorff D**. Renal complications of nonsteroidal anti-inflammatory drugs. *Kidney Int* 1993; **44**: 643-653 [PMID: 8231040 DOI: 10.1038/ki.1993.293]

85 **Perazella MA**, Eras J. Are selective COX-2 inhibitors nephrotoxic? *Am J Kidney Dis* 2000; **35**: 937-940 [PMID: 10793030 DOI: 10.1016/S0272-6386(00)70266-6]

86 **Braden GL**, O'Shea MH, Mulhern JG, Germain MJ. Acute renal failure and hyperkalaemia associated with cyclooxygenase-2 inhibitors. *Nephrol Dial Transplant* 2004; **19**: 1149-1153 [PMID: 14993496 DOI: 10.1093/ndt/gfg622]

87 **Klein IH**, Abrahams A, van Ede T, Hené RJ, Koomans HA, Ligtenberg G. Different effects of tacrolimus and cyclosporine on renal hemodynamics and blood pressure in healthy subjects. *Transplantation* 2002; **73**: 732-736 [PMID: 11907418]

88 **Oudemans-van Straaten HM**. Contrast nephropathy, pathophysiology and prevention. *Int J Artif Organs* 2004; **27**: 1054-1065 [PMID: 15645616]

89 **Schor N**. Acute renal failure and the sepsis syndrome. *Kidney Int* 2002; **61**: 764-776 [PMID: 11849423 DOI: 10.1046/j.1523-1755.2002.00178.x]

90 **Lee HY**, Kim CH. Acute oliguric renal failure associated with angiotensin II receptor antagonists. *Am J Med* 2001; **111**: 162-163 [PMID: 11501548 DOI: 10.1016/S0002-9343(01)00784-7]

91 **Johansen TL**, Kjaer A. Reversible renal impairment induced by treatment with the angiotensin II receptor antagonist candesartan in a patient with bilateral renal artery stenosis. *BMC Nephrol* 2001; **2**: 1 [PMID: 11388887 DOI: 10.1186/1471-2369-2-1]

92 **Toto RD**. Renal insufficiency due to angiotensin-converting enzyme inhibitors. *Miner Electrolyte Metab* 1994; **20**: 193-200 [PMID: 7845322]

93 **Tinmouth A**, Fergusson D, Yee IC, Hébert PC. Clinical consequences of red cell storage in the critically ill. *Transfusion* 2006; **46**: 2014-2027 [PMID: 17076859 DOI: 10.1111/j.1537-2995.2006.01026.x]

94 **Karkouti K**, Wijeysundera DN, Yau TM, McCluskey SA, Chan CT, Wong PY, Crowther MA, Hozhabri S, Beattie WS. Advance targeted transfusion in anemic cardiac surgical patients for kidney protection: an unblinded randomized pilot clinical trial. *Anesthesiology* 2012; **116**: 613-621 [PMID: 22354243 DOI: 10.1097/ALN.0b013e3182475e39]

95 **van de Watering L**. Red cell storage and prognosis. *Vox Sang* 2011; **100**: 36-45 [PMID: 21175654 DOI: 10.1111/j.1423-0410.2010.01441.x]

96 **Almac E**, Ince C. The impact of storage on red cell function in blood transfusion. *Best Pract Res Clin Anaesthesiol* 2007; **21**: 195-208 [PMID: 17650772 DOI: 10.1016/j.bpa.2007.01.004]

97 **Comporti M**, Signorini C, Buonocore G, Ciccoli L. Iron release, oxidative stress and erythrocyte ageing. *Free Radic Biol Med* 2002; **32**: 568-576 [PMID: 11909691 DOI: 10.1016/S0891-5849(02)00759-1]

98 **Bennett-Guerrero E**, Veldman TH, Doctor A, Telen MJ, Ortel TL, Reid TS, Mulherin MA, Zhu H, Buck RD, Califf RM, McMahon TJ. Evolution of adverse changes in stored RBCs. *Proc Natl Acad Sci USA* 2007; **104**: 17063-17068 [PMID: 17940021 DOI: 10.1073/pnas.0708160104]

99 **Lo CM**, Fan ST, Liu CL, Chan JK, Lam BK, Lau GK, Wei WI, Wong J. Minimum graft size for successful living donor liver transplantation. *Transplantation* 1999; **68**: 1112-1116 [PMID: 10551638]

100 **Nishizaki T**, Ikegami T, Hiroshige S, Hashimoto K, Uchiyama H, Yoshizumi T, Kishikawa K, Shimada M, Sugimachi K. Small graft for living donor liver transplantation. *Ann Surg* 2001; **233**: 575-580 [PMID: 11303141]

101 **Wang F**, Pan KT, Chu SY, Chan KM, Chou HS, Wu TJ, Lee WC. Preoperative estimation of the liver graft weight in adult right lobe living donor liver transplantation using maximal portal vein diameters. *Liver Transpl* 2011; **17**: 373-380 [PMID: 21445920 DOI: 10.1002/lt.22274]

102 **Shirabe K**, Shimada M, Gion T, Hasegawa H, Takenaka K, Utsunomiya T, Sugimachi K. Postoperative liver failure after major hepatic resection for hepatocellular carcinoma in the modern era with special reference to remnant liver volume. *J Am Coll Surg* 1999; **188**: 304-309 [PMID: 10065820 DOI: 10.1016/S1072-7515(98)00301-9]

103 **Balzan S**, Belghiti J, Farges O, Ogata S, Sauvanet A, Delefosse D, Durand F. The "50-50 criteria" on postoperative day 5: an accurate predictor of liver failure and death after hepatectomy. *Ann Surg* 2005; **242**: 824-828, discussion 824-828 [PMID: 16327492 DOI: 10.1097/01.sla.0000189131.90876.9e]

104 **Fong Y**, Bentrem DJ. CASH (Chemotherapy-Associated Steatohepatitis) costs. *Ann Surg* 2006; **243**: 8-9 [PMID: 16371729 DOI: 10.1097/01.sla.0000193599.57858.9b]

105 **Seifalian AM**, Piasecki C, Agarwal A, Davidson BR. The effect of graded steatosis on flow in the hepatic parenchymal microcirculation. *Transplantation* 1999; **68**: 780-784 [PMID: 10515377]

106 **Yamanaka N**, Okamoto E, Kawamura E, Kato T, Oriyama T, Fujimoto J, Furukawa K, Tanaka T, Tomoda F, Tanaka W. Dynamics of normal and injured human liver regeneration after hepatectomy as assessed on the basis of computed tomography and liver function. *Hepatology* 1993; **18**: 79-85 [PMID: 8392029 DOI: 10.1002/hep.1840180114]

107 **Bruix J**, Castells A, Bosch J, Feu F, Fuster J, Garcia-Pagan JC, Visa J, Bru C, Rodés J. Surgical resection of hepatocellular carcinoma in cirrhotic patients: prognostic value of preoperative portal pressure. *Gastroenterology* 1996; **111**: 1018-1022 [PMID: 8831597 DOI: 10.1016/S0016-5085(96)70070-7]

108 **McCormack L**, Petrowsky H, Jochum W, Furrer K, Clavien PA. Hepatic steatosis is a risk factor for postoperative complications after major hepatectomy: a matched case-control study. *Ann Surg* 2007; **245**: 923-930 [PMID: 17522518 DOI: 10.1097/01.sla.0000251747.80025.b7]

109 **Fernandez FG**, Ritter J, Goodwin JW, Linehan DC, Hawkins WG, Strasberg SM. Effect of steatohepatitis associated with irinotecan or oxaliplatin pretreatment on resectability of hepatic colorectal metastases. *J Am Coll Surg* 2005; **200**: 845-853 [PMID: 15922194 DOI: 10.1016/j.jamcollsurg.2005.01.024]

110 **Bilchik AJ**, Poston G, Curley SA, Strasberg S, Saltz L, Adam R, Nordlinger B, Rougier P, Rosen LS. Neoadjuvant chemotherapy for metastatic colon cancer: a cautionary note. *J Clin Oncol* 2005; **23**: 9073-9078 [PMID: 16361615 DOI: 10.1200/jco.2005.03.2334]

111 **Aloia T**, Sebagh M, Plasse M, Karam V, Lévi F, Giacchetti S, Azoulay D, Bismuth H, Castaing D, Adam R. Liver histology and surgical outcomes after preoperative chemotherapy with fluorouracil plus oxaliplatin in colorectal cancer liver metastases. *J Clin Oncol* 2006; **24**: 4983-4990 [PMID: 17075116 DOI: 10.1200/JCO.2006.05.8156]

112 **Morris-Stiff G**, Tan YM, Vauthey JN. Hepatic complications following preoperative chemotherapy with oxaliplatin or irinotecan for hepatic colorectal metastases. *Eur J Surg Oncol* 2008; **34**: 609-614 [PMID: 17764887 DOI: 10.1016/j.ejso.2007.07.007]

113 **Rahbari NN**, Garden OJ, Padbury R, Brooke-Smith M, Crawford M, Adam R, Koch M, Makuuchi M, Dematteo RP, Christophi C, Banting S, Usatoff V, Nagino M, Maddern G, Hugh TJ, Vauthey JN, Greig P, Rees M, Yokoyama Y, Fan ST, Nimura Y, Figueras J, Capussotti L, Büchler MW, Weitz J. Posthepatectomy liver failure: a definition and grading by the International Study Group of Liver Surgery (ISGLS). *Surgery* 2011; **149**: 713-724 [PMID: 21236455 DOI: 10.1016/j.surg.2010.10.001]

114 **Wadei HM**, Mai ML, Ahsan N, Gonwa TA. Hepatorenal syndrome: pathophysiology and management. *Clin J Am Soc Nephrol* 2006; **1**: 1066-1079 [PMID: 17699328 DOI: 10.2215/cjn.01340406]

115 **Abdalla EK**, Noun R, Belghiti J. Hepatic vascular occlusion: which technique? *Surg Clin North Am* 2004; **84**: 563-585 [PMID: 15062662 DOI: 10.1016/S0039-6109(03)00231-7]

116 **Smyrniotis V**, Farantos C, Kostopanagiotou G, Arkadopoulos N. Vascular control during hepatectomy: review of methods and results. *World J Surg* 2005; **29**: 1384-1396 [PMID: 16222453 DOI:

10.1007/s00268-005-0025-x]
117 **Pringle JH**. V. Notes on the Arrest of Hepatic Hemorrhage Due to Trauma. *Ann Surg* 1908; **48**: 541-549 [PMID: 17862242]
118 **Li AK**, Mok SD. Simplified hepatectomy: the tourniquet method. *Aust N Z J Surg* 1989; **59**: 161-163 [PMID: 2920001 DOI: 10.1111/j.1445-2197.1989.tb01489.x]
119 **Huguet C**, Gavelli A, Chieco PA, Bona S, Harb J, Joseph JM, Jobard J, Gramaglia M, Lasserre M. Liver ischemia for hepatic resection: where is the limit? *Surgery* 1992; **111**: 251-259 [PMID: 1311871]
120 **Hannoun L**, Borie D, Delva E, Jones D, Vaillant JC, Nordlinger B, Parc R. Liver resection with normothermic ischaemia exceeding 1 h. *Br J Surg* 1993; **80**: 1161-1165 [PMID: 8402122 DOI: 10.1002/bjs.1800800933]
121 **Midorikawa Y**, Kubota K, Takayama T, Toyoda H, Ijichi M, Torzilli G, Mori M, Makuuchi M. A comparative study of postoperative complications after hepatectomy in patients with and without chronic liver disease. *Surgery* 1999; **126**: 484-491 [PMID: 10486600 DOI: 10.1016/S0039-6060(99)70089-9]
122 **Smyrniotis VE**, Kostopanagiotou GG, Contis JC, Farantos CI, Voros DC, Kannas DC, Koskinas JS. Selective hepatic vascular exclusion versus Pringle maneuver in major liver resections: prospective study. *World J Surg* 2003; **27**: 765-769 [PMID: 14509502 DOI: 10.1007/s00268-003-6978-8]
123 **Belghiti J**, Noun R, Malafosse R, Jagot P, Sauvanet A, Pierangeli F, Marty J, Farges O. Continuous versus intermittent portal triad clamping for liver resection: a controlled study. *Ann Surg* 1999; **229**: 369-375 [PMID: 10077049]
124 **Choukèr A**, Schachtner T, Schauer R, Dugas M, Löhe F, Martignoni A, Pollwein B, Niklas M, Rau HG, Jauch KW, Peter K, Thiel M. Effects of Pringle manoeuvre and ischaemic preconditioning on haemodynamic stability in patients undergoing elective hepatectomy: a randomized trial. *Br J Anaesth* 2004; **93**: 204-211 [PMID: 15194628 DOI: 10.1093/bja/aeh195]
125 **Delva E**, Nordlinger B, Parc R, Lienhart A, Hannoun L, Huguet C. Hepatic vascular exclusion (HVE) for major liver resections. *Int Surg* 1987; **72**: 78-81 [PMID: 3610538]
126 **Belzer FO**, Southard JH. Principles of solid-organ preservation by cold storage. *Transplantation* 1988; **45**: 673-676 [PMID: 3282347]
127 **Hannoun L**, Balladur P, Delva E, Panis Y, Camus Y, Honiger J, Levy E, Parc R. ["Ex situ-in vivo" surgery of the liver: a new technique in liver surgery. Principles and preliminary results]. *Gastroenterol Clin Biol* 1991; **15**: 758-761 [PMID: 1667768]
128 **Delrivière L**, Hannoun L. In situ and ex situ in vivo procedures for complex major liver resections requiring prolonged hepatic vascular exclusion in normal and diseased livers. *J Am Coll Surg* 1995; **181**: 272-276 [PMID: 7670689]
129 **Rees M**, Plant G, Wells J, Bygrave S. One hundred and fifty hepatic resections: evolution of technique towards bloodless surgery. *Br J Surg* 1996; **83**: 1526-1529 [PMID: 9014666 DOI: 10.1002/bjs.1800831110]
130 **Smyrniotis V**, Kostopanagiotou G, Theodoraki K, Tsantoulas D, Contis JC. The role of central venous pressure and type of vascular control in blood loss during major liver resections. *Am J Surg* 2004; **187**: 398-402 [PMID: 15006570 DOI: 10.1016/j.amjsurg.2003.12.001]
131 **Wang WD**, Liang LJ, Huang XQ, Yin XY. Low central venous pressure reduces blood loss in hepatectomy. *World J Gastroenterol* 2006; **12**: 935-939 [PMID: 16521223 DOI: 10.3748/wjg.v12.i6.935]
132 **Correa-Gallego C**, Berman A, Denis SC, Langdon-Embry L, O'Connor D, Arslan-Carlon V, Kingham TP, D'Angelica MI, Allen PJ, Fong Y, DeMatteo RP, Jarnagin WR, Melendez J, Fischer M. Renal function after low central venous pressure-assisted liver resection: assessment of 2116 cases. *HPB* (Oxford) 2015; **17**: 258-264 [PMID: 25387727 DOI: 10.1111/hpb.12347]
133 **Llovet JM**, Fuster J, Bruix J. Intention-to-treat analysis of surgical treatment for early hepatocellular carcinoma: resection versus transplantation. *Hepatology* 1999; **30**: 1434-1440 [PMID: 10573522 DOI: 10.1002/hep.510300629]
134 **Bellavance EC**, Lumpkins KM, Mentha G, Marques HP, Capussotti L, Pulitano C, Majno P, Mira P, Rubbia-Brandt L, Ferrero A, Aldrighetti L, Cunningham S, Russolillo N, Philosophe B, Barroso E, Pawlik TM. Surgical management of early-stage hepatocellular carcinoma: resection or transplantation? *J Gastrointest Surg* 2008; **12**: 1699-1708 [PMID: 18709418]
135 **Emond JC**, Samstein B, Renz JF. A critical evaluation of hepatic resection in cirrhosis: optimizing patient selection and outcomes. *World J Surg* 2005; **29**: 124-130 [PMID: 15654659 DOI: 10.1007/s00268-004-7633-8]
136 **Liau KH**, Blumgart LH, DeMatteo RP. Segment-oriented approach to liver resection. *Surg Clin North Am* 2004; **84**: 543-561 [PMID: 15062661 DOI: 10.1016/j.suc.2003.12.003]
137 **Malinowski M**, Geisel D, Stary V, Denecke T, Seehofer D, Jara M, Baron A, Pratschke J, Gebauer B, Stockmann M. Portal vein embolization with plug/coils improves hepatectomy outcome. *J Surg Res* 2015; **194**: 202-211 [PMID: 25454977 DOI: 10.1016/j.jss.2014.10.028]
138 **Broering DC**, Hillert C, Krupski G, Fischer L, Mueller L, Achilles EG, Schulte am Esch J, Rogiers X. Portal vein embolization vs. portal vein ligation for induction of hypertrophy of the future liver remnant. *J Gastrointest Surg* 2000; **6**: 905-913; discussion 913 [PMID: 12504230 DOI: 10.1016/S1091-255X(02)00122-1]
139 **Adam R**, Laurent A, Azoulay D, Castaing D, Bismuth H. Two-stage hepatectomy: A planned strategy to treat irresectable liver tumors. *Ann Surg* 2000; **232**: 777-785 [PMID: 11088072]

P- Reviewer: He ST, Peltec A, Qin JM, Sirin G, Wang GY
S- Editor: Qiu S **L- Editor**: A **E- Editor**: Li D

World Journal of Hepatology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4254/wjh.v8.i21.902

World J Hepatol 2016 July 28; 8(21): 902-914
ISSN 1948-5182 (online)

ORIGINAL ARTICLE

Basic Study

Anti-hepatitis C virus potency of a new autophagy inhibitor using human liver slices model

Sylvie Lagaye, Sonia Brun, Jesintha Gaston, Hong Shen, Ruzena Stranska, Claire Camus, Clarisse Dubray, Géraldine Rousseau, Pierre-Philippe Massault, Jerôme Courcambeck, Firas Bassisi, Philippe Halfon, Stanislas Pol

Sylvie Lagaye, Jesintha Gaston, Stanislas Pol, Institut Pasteur, INSERM U1223, 75015 Paris, France

Sylvie Lagaye, Jesintha Gaston, Hong Shen, Institut Cochin, INSERM U1016, CNRS UMR8104, Université Paris Descartes (UMR S1016), 75014 Paris, France

Sonia Brun, Clarisse Dubray, Jerôme Courcambeck, Firas Bassisi, Philippe Halfon, Genoscience Pharma, 13006 Marseille, France

Ruzena Stranska, KU Leuven, Rega Institute, 3000 Leuven, Belgium

Claire Camus, Philippe Halfon, Laboratoire Alphabio, 13006 Marseille, France

Géraldine Rousseau, APHP, Groupe Hospitalier La Pitié Salpétrière, Service de Chirurgie digestive et Hépato bilio pancréatique, 75013 Paris, France

Pierre-Philippe Massault, APHP, Groupe Hospitalier Cochin, Service de Chirurgie digestive, Hépato-biliaire et Endocrinienne, 75014 Paris, France

Stanislas Pol, Université Paris Descartes, 75014 Paris, France

Stanislas Pol, APHP, Groupe Hospitalier Cochin, Unité d' Hépatologie, 75014 Paris, France

Stanislas Pol, Institut Pasteur, Département de Recherche Translationnelle, INSERM UMS20, 75015 Paris, France

Author contributions: Lagaye S wrote the paper; Lagaye S, Brun S, Gaston J, Shen H, Stranska R, Camus C, Dubray C, Rousseau G, Massault PP, Courcambeck J and Bassisi F performed the experiments; Lagaye S, Brun S, Camus C, Halfon P and Pol S analyzed the data; Lagaye S, Halfon P and Pol S conceived and designed the experiments; Halfon P and Pol S contributed equally to the work.

Supported by The Institut National de la Santé et de la Recherche Médicale (INSERM, France); and the personal support of Professor Jean-François Delfraissy as Director of the French Agency, Agence Nationale de Recherches sur le Sida et les hépatites virales (ANRS).

Institutional review board statement: Institutional review board statement is not required for manuscript submission in our Institution.

Institutional animal care and use committee statement: No animal use in the experiments.

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

Data sharing statement: No data sharing.

Manuscript source: Invited manuscript

Correspondence to: Sylvie Lagaye, PhD, DSc, Senior Scientist, Institut Pasteur, INSERM U1223, 25-28 rue du Dr Roux, 75015 Paris, France. sylvie.lagaye@inserm.fr
Telephone: +33-1-40613424
Fax: +33-1-45688548

Received: March 24, 2016
Peer-review started: April 6, 2016
First decision: May 16, 2016
Revised: June 1, 2016
Accepted: June 27, 2016
Article in press: June 29, 2016
Published online: July 28, 2016

Abstract

AIM: To evaluate the antiviral potency of a new anti-hepatitis C virus (HCV) antiviral agent targeting the cellular autophagy machinery.

METHODS: Non-infected liver slices, obtained from human liver resection and cut in 350 μm-thick slices (2.7×10^6 cells per slice) were infected with cell culture-grown HCV Con1b/C3 supernatant (multiplicity of infection = 0.1) cultivated for up to ten days. HCV infected slices were treated at day 4 post-infection with GNS-396 for 6 d at different concentrations. HCV replication was evaluated by strand-specific real-time quantitative reverse transcription - polymerase chain reaction. The infectivity titers of supernatants were evaluated by foci formation upon inoculation into naive Huh-7.5.1 cells. The cytotoxic effect of the drugs was evaluated by lactate dehydrogenase leakage assays.

RESULTS: The antiviral efficacy of a new antiviral drug, GNS-396, an autophagy inhibitor, on HCV infection of adult human liver slices was evidenced in a dose-dependent manner. At day 6 post-treatment, GNS-396 EC50 was 158 nmol/L without cytotoxic effect (compared to hydroxychloroquine EC50 = 1.17 μmol/L).

CONCLUSION: Our results demonstrated that our *ex vivo* model is efficient for evaluation the potency of autophagy inhibitors, in particular a new quinoline derivative GNS-396 as antiviral could inhibit HCV infection in a dose-dependent manner without cytotoxic effect.

Key words: Host antiviral therapy; Hepatitis C virus; Tissue culture; Autophagy; Quinoline derivative

Core tip: Hepatitis C virus (HCV) infection (or spread) is a serious public health challenge counting approximately 170 million people that are chronically infected worldwide. Efficient interferon-free treatments with new direct acting antivirals are expected to cure more than 90% of HCV-infected patients but they are not available in all the countries. Autophagy machinery is required to initiate HCV replication. Host antiviral therapy is an additional option for the treatment of HCV infection. The new autophagy inhibitor GNS-396 demonstrated significant efficacy and additive activity in inhibiting HCV replication and might be an additional option to treat HCV infected individuals.

Lagaye S, Brun S, Gaston J, Shen H, Stranska R, Camus C, Dubray C, Rousseau G, Massault PP, Courcambeck J, Bassisi F, Halfon P, Pol S. Anti-hepatitis C virus potency of a new autophagy inhibitor using human liver slices model. *World J Hepatol* 2016; 8(21): 902-914 Available from: URL: http://www.wjgnet.com/1948-5182/full/v8/i21/902.htm DOI: http://dx.doi.org/10.4254/wjh.v8.i21.902

INTRODUCTION

Approximately 170 million people worldwide are chronically infected with hepatitis C virus (HCV)[1]. Until recently, the most effective treatment against HCV infection was the combination of pegylated interferon-α 2a or b and ribavirin (PR) which achieved sustained virological response (SVR) in about 45% of individuals infected by HCV genotype 1, 65% by HCV genotype 4, 70% by HCV genotype 3 and more than 85% by HCV genotype 2[2,3]. The frequent side effects associated with PR and the rates of non response to PR includes partial or null virologic response and breakthrough or relapse after PR discontinuation. Thus, development of novel and more effective antiviral treatments were essential[4].

Two HCV NS3 protease inhibitors (PI), boceprevir (BOC) and telaprevir (TVR) have been approved and combined with PR, have increased the SVR to about 75% in therapy naïve HCV genotype 1 infected patients[5-9]. Over the past few years, other direct acting antivirals (DAAs) were developed[10-14] as second generation of PI with higher antiviral potency, HCV NS5A replication complex inhibitors and nucleotide analogue HCV NS5B polymerase inhibitors[13] as well as host-targeted indirect antivirals like cyclophilin inhibitors[15] and lambda interferon[15]. Interferon-free treatments with new DAAs are expected to cure more than 90% of HCV-infected patients[16]. But they are not available in all the countries[17]. At the present time, triple therapy combining PR with NS3 PI (TVR or BOC) is going to remain the main treatment for HCV patients[16-21]. That is why it appears important to continue research in limiting virus replication and the autophagy inhibition could be a new additional pathway because of recent evidences obtained regarding to an increased autophagic response in the liver of chronically HCV infected patients[22].

Autophagy is a catabolic process which degrades a cellular own component through the lysosomal machinery[23]. It has been shown that autophagy is activated during virus and bacterial infection[24] and that some viruses can use the autophagy system to facilitate their own replication[25-29]. Previously, several studies evidenced that HCV infection resulted in endoplasmic reticulum stress and autophagy responses, that HCV regulated the autophagy pathway, that the autophagy machinery was required to initiate HCV replication, and finally, that the suppression of autophagy inhibited HCV replication[30-35]. Interestingly, it has been demonstrated that HCV induces autophagosomes *via* a Class Ⅲ PI3K-independent pathway and uses autophagosomal membranes as sites for its RNA replication[36].

The lysosomotropic anti-malarial drugs, chloroquine (CQ) and hydroxychloroquine (HCQ), belonging to the quinoline family, are among the autophagy inhibitors, which act by preventing the acidification of lysosomes, leading to the inhibition of both fusion of autophagosome with lysosome and lysosomal protein degradation[23]. In fact, CQ exerts an inhibitory effect for several RNA viruses including coronaviruses, flaviviruses and human

immunodeficiency virus[37-39]. Recently, it has been shown that a treatment with CQ of HCV infected cells suppressed the replication of the virus in a dose-dependent manner by preventing the autophagic proteolysis[40].

In the present study, we used the established *ex vivo* model of primary human liver slices culture which allows to the *de novo* replication of primary viral isolates and production of high titer infectious HCV particles[41] to evaluate the potential antiviral potency GNS-396[42], a new autophagy inhibitor in comparison with a well-known autophagy inhibitor, HCQ. Presented results might be additional options to treat HCV infected individuals.

MATERIALS AND METHODS

Human liver tissue specimens

Adult human primary liver tissue samples were obtained from HCV and also hepatitis B virus, and human immunodeficiency virus seronegative patients who underwent liver resection surgery, mainly for liver metastasis in the absence of underlying liver disease. Experimental procedures were carried out in accordance with French laws and Regulations.

Liver slices preparation, culture and infection

Slices were prepared and cultured as described[41,43]. Briefly, uninfected human liver slices, obtained from human liver resection, were cut into 350 μm thick slices of (2.7×10^6 cells per slice) with a vibratome (Leica, VTS1200) and transferred to 0.4 μm organotypic culture inserts (Millipore) in 12-wells plates (1 slice/well) containing 2 mL of complete Dulbecco's modified eagles's medium (DMEM) culture media and maintained at 37 ℃ under a constant flow of humidified 95% O_2/5% CO_2 for up to 24 h before viral infection. Cell number for tissue slices was estimated at approximately 2.7×10^6 cells per slice based on a 14-cell thick slice (cell diameter approximately 25 μm)[41]. The complete culture medium consisted of DMEM with 4.5 g/L D-glucose and glutamine (Life Technologies) supplemented with 10% fetal calf serum (Life Technologies, 16000-044), 5% penicillin-streptomycin (Life Technologies, 10378-016), 1% amphotericin (Sigma Aldrich), 5 μg/mL insulin (Life Technologies, 51500-056), 0.4 μg/mL dexamethasone (Sigma Aldrich, D4902), 10 mmol/L HEPES (Life Technologies, 15630080), non-essential amino acids (Life Technologies), 20 mmol/Lsodium pyruvate (Life Technologies) and 50 μg/mL ascorbic acid (Sigma Aldrich). One day post-culture in twelve-transwell plates, human primary liver slices were inoculated with HCV Con1/C3 at a multiplicity of infection equal to 0.1 at 37 ℃ in the same culture conditions as described above, for overnight. The infectious clone Con1/C3 (genotype 1b) (JFH1-derived chimeric viruses whose structural proteins are encoded by the genotype 1b-HCV sequence Con1)[44] could efficiently infect human liver slices which maintain their hepatocyte differentiation and retain normal physiological and biochemical parameters for at least 10 d. The inoculum was then removed; the slices were washed three times with PBS and then supplemented with complete culture medium. Then, liver slices were cultured without medium replacements, as previously described[41].

HCV RNA transfection and virus production

To produce HCVcc, viral RNAs were transcribed *in vitro* and electroporated into Huh-7.5.1 cell line (kindly provided by Professor Francis V Chisari, The Scripps Research Institute, La Jolla, CA), as described previously[45]. The infectious titer of cell culture supernatants was evaluated by classical titration assay[45]. In brief, the HCV infection of Huh-7.5.1 cells was performed with serial 10-fold dilution of viral supernatants. Seventy-two hours later, the formation of infected cells foci were detected by staining with human HCV positive sera or mouse monoclonal antibodies directed against HCV core (Ozyme) and nonstructural (NS5A) (Virostat, clone1877) proteins. Titrations were performed in duplicate.

Quantification of HCV strands RNA by real-time quantitative reverse transcription- polymerase chain reaction

A strand-specific real-time quantitative reverse transcription-polymerase chain reaction technique to quantify the intracellular levels of positive and negative strand HCV RNA was performed as previously described[46-49]. The quantification of 28S rRNA was used as an internal standard to quantify HCV in total liver RNA, as previously described[46], (threshold of detection: 25 copies/reaction). Briefly, reverse transcription was carried out using oligo(dT) primer (Life Technologies) and Moloney murine leukemia virus reverse transcriptase (Promega) as recommended by the manufacturer. Real-time polymerase chain reactions were performed using the Light CyclerR (Roche Applied Science) and Fast Start DNA Master SYBR Green Ⅰ kit (Life Science, Roche) according with the manufacturer's protocol.

Reverse transcription was performed using primers located in the 5' NCR region of HCV genome, tag-RC1 (5'-GGC CGT CAT GGT GGC GAA TAA GTC TAG CCA TGG CGT TAG TA-3')[47] and RC21 (5'-CTC CCG GGG CAC TCG CAA GC-3')[48] for the negative and positive strands, respectively, as described previously[46]. After a denaturation step performed at 70 ℃ for 8 min, the RNA template was incubated at 48 ℃ for 5 min in the presence of 200 ng of tag-RC1 primer and 1.25 mmol/L of each deoxynucleoside triphosphate (dNTP) (Promega) in a total volume of 12 μL. Reverse transcription was carried out for 60 min at 60 ℃ in the presence of 20 U RNaseOutTM (Life Technologies) and 7.5 U ThermoscriptTM reverse transcriptase (Life Technologies), in the buffer recommended by the manufacturer. An additional treatment was applied by adding 1 μL (2U) RNaseH (Life Technologies) for 20 min at 37 ℃. The first round of nested PCR was performed with 2 μL of the cDNA obtained in a total volume of 50 μL, containing 3 U Taq polymerase (Promega), 0.5 mmol/L dNTP Mix (Promega), and 0.5 μmol/L RC1 (5'-GTC TAG CCA TGG CGT TAG TA-3')

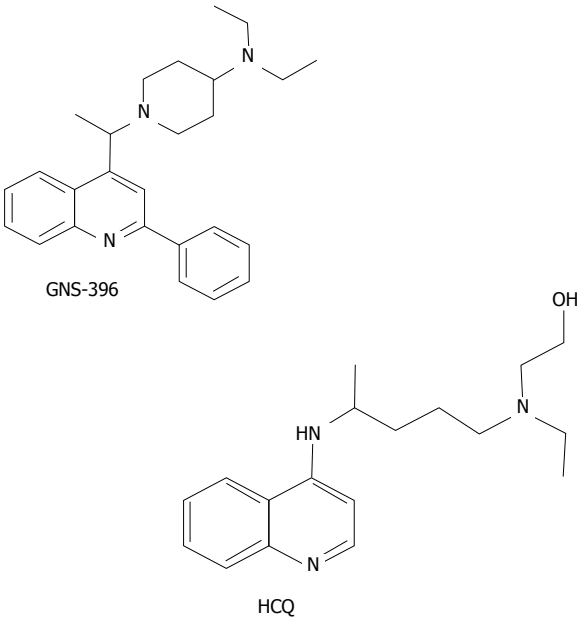

Figure 1 GNS-396 and hydroxychloroquine structures. HCQ: Hydroxychloroquine.

and RC21 primers for positive-strand amplification, or tag (5′-GGC CGT CAT GGTGGC GAA TAA-3′) and RC21 primers for negative strand amplification. The PCR protocol consisted of 18 cycles of denaturation (94 ℃ for 1 min), annealing (55 ℃ for 45 s), and extension (72 ℃ for 2 min). The cDNA obtained was purified using the Quick-clean kit (Qiagen), according to the manufacturer's instructions, and 2 μL of the purified product suspended in 10 μL nuclease free water (Promega) were then subjected to real-time PCR. The reaction was carried out using the DNA Fast Start SYBR Green Kit (Life Science, Roche), with LightcyclerTM instruments and technology (Roche Diagnostics). PCR amplifications were performed in a total volume of 20 μL, containing 3 mmol/L $MgCl_2$, 2 μL DNA Master green (Life Science) and 50 ng of the 197 R (5′-CTTTCGCGACCCAACACTAC-3′) and 104 (5′-AGAGCCATAGTGGTCTGCGG-3′) primers[48,49]. The PCR protocol consisted of one step of initial denaturation for 10 min at 94 ℃, followed by 40 cycles of denaturation (95 ℃ for 15 s), annealing (57 ℃ for 5 s), and extension (72 ℃ for 8 s). After amplification, the specificity of PCR products was checking by a melting curve analysis.

Western blotting

Western blotting was performed as following. Each liver slice was washed 3 times in PBS, incubated in Laemmli buffer[50] at 100 ℃ for 10 min. The lysate was passed through a 26 G needle, 10 times and kept at -80 ℃. Before electrophoresis in pre-cast sodium dodecyl sulfate polyacrylamide gel 4%-12% (Life Technologies), the samples were incubated at 95 ℃ for 5 min. After electrophoresis, proteins were transferred to a 0.22 μm Protran membrane BA83 (Schleicher and Schuell) and HCV proteins were detected by Western blotting using mouse monoclonal antibodies to core (C7-50, 1:10000) (Ozyme), to NS5A (1:2000) (Virostat, clone 1877), to LC3 proteins (Sigma-Aldrich) and to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Abcam), and to β-actin (Pierce biotechnology). Horseradish peroxidase-conjugated anti-mouse IgG (GeHealthCare Life Sciences) at the dilution of 1:50000 were used as secondary antibodies. The reactions were developed using enhanced chemiluminescence detection reagents (GeHealthCare Life Sciences), followed by exposure to X-OMAT film (GeHealthCare Life Sciences). LC3-Ⅱ protein expression analysis was performed with Image J software.

Drugs inhibition of HCVcc Con1/C3 replication and cytotoxicity assays

The HCVcc Con1/C3 inhibition either by pegylated-interferon α-2a (peg-INF) (Roche, Pegasys) or/and ribavirin (RBV) (Schering Plough, Rebetol) or TVR (Janssen-Cilag, Incivo) or BOC (Schering-Plough, Boceprevir) or SOF (Gilead Sciences Intl Ltd, Sofosbuvir) or GNS-396 (Figure 1) (Genoscience Pharma, Marseille, France) or HCQ (Figure 1) (Genoscience Pharma, Marseille, France) or 0.5% dimethylsufoxide (DMSO) (Sigma Aldrich) as a carrier control, and the cytotoxicity assays were performed as following. At day 4 post-infection with HCVcc Con1/C3 the human liver slices were treated by addition of different concentrations of the following drugs: peg-INF or/and RBV or TVR or BOC or SOF or HCQ (0.1, 1, 2.5, 5 μmol/L or a new quinoline derivative, GNS-396 (0.1, 1, 2.5, 5 μmol/L) alone or 0.5% DMSO as a carrier control, to culture medium, twice daily, up to day 10 post-infection. The infectivity (ffu/mL) was measured at day 2, day 4 or day 6 post-treatment depending on the experiment as described[41]. All the experiments were performed in triplicate. The cytoTox 96R Non-Radioactive Cytotoxicity Assay (Promega, G1780) was used to assess the potential cytotoxicity of the drugs. Results of lactate deshydrogenase (LDH) leakage were compared to the carrier control calculated (Figure 2) as described previously[51].

Evaluation of autophagy modulation and inhibition

Autophagy modulation was evaluated on HeLa cells treated with GNS-396, a new quinolone derivative. For tracking different stages of autophagy the tandem fusion of mRFP and EGFP fused to LC3 make a pH-sensitive sensor (mRFP-EGFP-LC3) that is used to monitor autophagy in live cells[52]. The EGFP tag is acid-sensitive while the mRFP tag is not. The double tagged LC3 can be used to label autophagosomes, amphisomes and autolysosomes. In autophagosomes, both tags emit yellow light. However, the fusion of autophagosomes to acidic lysosomes results in acidic autolysosomes where the green fluorescence from GFP is lost. Subsequently, the red fluorescence from mRFP is lost when the double tagged protein is degraded. The autophagic flux inhibition was shown using a SkBr3 mRFP1-EGFP-LC3 stable breast cancer cell line treated with 100 μmol/L GNS-396 or 100 μmol/L HCQ during 6 h. HCQ was used as a positive control of autophagy inhibition. Cell images were obtained using an epifluorescence microscope (Nikon,

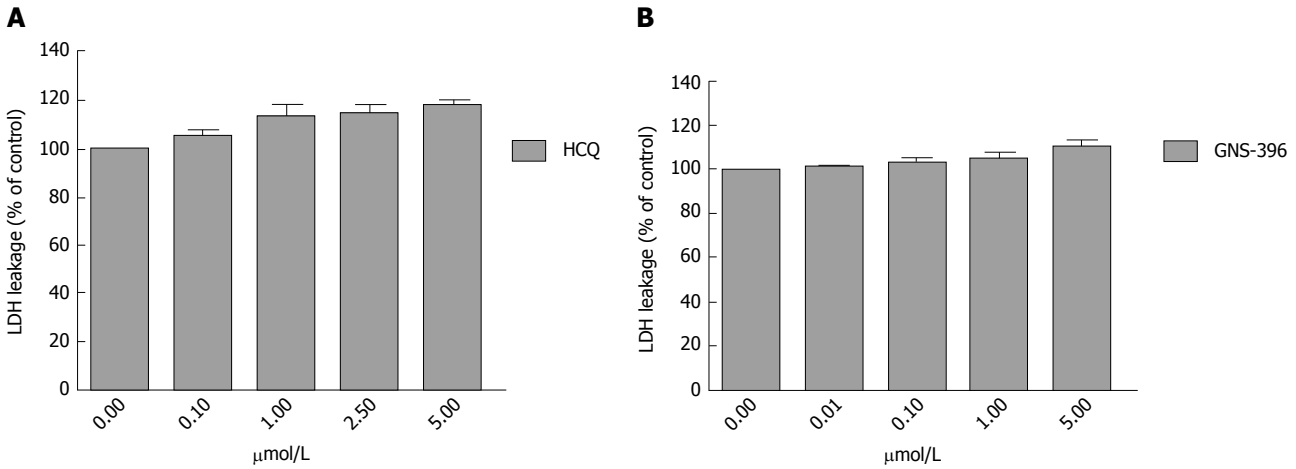

Figure 2 Cytotoxicity assays of the drugs used hydroxychloroquine (A) and GNS-396 (B). Percentages of lactate deshydrogenase (LDH) leakage are relative to DMSO control-treated liver slices. Drugs cytotoxicity is significant at % of control > 120. All experiments were performed on triplicate. Values are expressed as means ± SE, comparisons were performed using the Mann-Whitney rank-sum test ($P < 0.001$). HCQ: Hydroxychloroquine; DMSO: Dimethylsufoxide.

Eclipse Ci).

Autophagy inhibition was evaluated on HeLa cells treated with GNS-396, at different drug concentrations (1, 10, 100 μmol/L) during 4 h or 6 h in the presence or absence of bafilomycine A1 (100 nmol/L) (Sigma-Aldrich) added for the last 2 h. Bafilomycin A1 (BafA1) is an autophagy inhibitor as endosomal acidification inhibitor. It is a known inhibitor of the late phase of autophagy. Bafilomycin A1 prevents maturation of autophagic vacuoles by inhibiting fusion between autophagosomes and lysosomes. Bafilomycin A1 acts by inhibiting vacuolar H+ ATPase[53,54]. HCQ was used as a positive control of autophagy inhibition. The LC3-Ⅱ protein expression in cell lysates was evaluated by Western-blot assay [anti-LC3 antibody (Sigma-Aldrich)] compared to either GAPDH protein expression [anti-GAPDH antibody (Abcam)] or β-actin protein expression [anti-actin antibody (Pierce biotechnology). LC3-Ⅱ protein expression analysis was performed with Image J software.

Statistical analysis

At different days of the kinetics, the results were obtained from the mean of the three slices culture. Statistical tests were performed using the GraphPad Prism 5.0 software (GraphPad Software, La Jolla, CA, United States). Values are expressed as means ± standard errors of the mean. The data were compared using an unpaired two-tailed student's *t*-test or the Mann-Whitney rank-sum test. *P*-value < 0.05 was considered significant.

RESULTS

Validation of GNS-396, a new quinoline derivative, as inhibitor of autophagy

We evaluated the effect of GNS-396 (Figure 1)[42], a new quinoline derivative, on autophagy by treatment of HeLa cells with various concentrations of GNS-396 during 6 h. HCQ was used as a positive control of autophagy inhibition. The microtubule-associated protein 1A/1B-light chain 3 (LC3) is a soluble ubiquitin-like protein with a molecular mass of approximately 17 kDa that exists ubiquitously in mammalian tissues and cultured cells, as an unconjugated form (LC3-Ⅰ) or conjugated to autophagosomes membranes (LC3-Ⅱ: lipidated form). During autophagy, a cytosolic form of LC3 (LC3-Ⅰ) is conjugated to phosphatidylethanolamine to form LC3-phosphatidylethanolamine conjugate (LC3-Ⅱ), which is recruited to autophagosomal membranes allowing for the closure of the autophagic vacuole. Autophagosomes fuse with lysosomes to form autolysosomes, and intra-autophagosomal components are degraded by lysosomal hydrolases. At the same time, LC3-Ⅱ in autolysosomal lumen is degraded. Thus, lysosomal turnover of the autophagosomal marker LC3-Ⅱ reflects autophagic activity. Analysis of LC3 intracellular expression by Western blotting demonstrated an increase of normalized LC3-Ⅱ protein expression when HeLa cells were treated with GNS-396, in a dose-dependent manner (Figure 3A), reflecting the accumulation of autophagosomes in cells, and therefore an effective modulation of the autophagy. Consequently, GNS-396 is a dose-dependent autophagy modulator with a magnitude of normalized LC-3 Ⅱ similar to which achieved with HCQ treatment, a well-known autophagic inhibitor (Figure 3B). Similar results were obtained on Huh7.5.1 cell line (data not shown).

To evaluate if the observed accumulation of autophagosomes after GNS-396 treatment was a consequence of either a stimulated production of new autophagosomes (in this case, GNS-396 would be an autophagy inducer) or a result of autophagosome clearance blockage (in this case, GNS-396 would be an autophagy inhibitor), HeLa cells were treated with different concentrations of GNS-396 in the absence or presence of a lysosomal protease inhibitor, Bafilomycin A1, that increases lysosomal pH and blocks autophagosome-lysosome fusion (Figure 3C) and LC3 protein levels were measured. HCQ was used as a positive control of autophagy inhibition (Figure 3D). After 4 h exposure of HeLa cells to GNS-396 (100 μmol/L),

the accumulation of LC3-Ⅱ was observed (Figure 3C) which was not enhanced in the presence of BafA1, supporting the idea that GNS-396 inhibits autophagic flux as HCQ (Figure 3D). To confirm that GNS-396 is an autophagy inhibitor, the autophagic flux was monitored by fluorescence microscopy, using the mRFP-EGFP-LC3 tandem-tagged fluorescent protein in SkBr3 mRFP-EGFP-LC3 stable cell line (Figure 4). In green/red merged images, yellow dots (*i.e.*, mRFP+EGFP+) indicate autophagosomes or non-acidic autolysosomes, while red dots (*i.e.*, mRFP+EGFP-) indicate autolysosomes. The autophagy flux is increased when both yellow and red punctua (dots) are increased in cells while the autophagic flux is blocked when only yellow punctua (dots) are increased without an accompanying increase of red punctua in cells. SkBr3 mRFP-EGFP-LC3 stable cell line was treated during 6 h with either GNS-396 (Figure 4C) or HCQ, a well-known autophagic inhibitor (Figure 4B) (100 μmol/L). An accumulation of yellow punctua (dots) corresponding to autophagosomes or non-acidic autolysosomes was noticeable (Figure 4B and C), indicating that GNS-396 blocks the autophagic flux, and may act as lysosomotropic agent as HCQ.

Modulation of autophagy and inhibition of HCV infection in human liver slices model by GNS-396 treatment

The level of LC3 and viral proteins expression were analysed by Western blotting after 1, 4, 6 and 10 d post-infection (Figure 5). HCV infection induced autophagy with an increase of protein LC3-Ⅱ expression (Figure 5B) as compared to non-infected liver slices (Figure 5A), along with an increase of intracellular expression of the core and NS5A proteins consistent with the previous reports[22,36]. Intracellular expression of the viral proteins was decreased significantly at day 6 post-treatment with HCQ (1 μmol/L) or GNS-396 (1 μmol/L) (Figure 5D) in comparison with HCVcc infected liver slices not treated (Figure 5B). The HCQ- and GNS-396-treatment induced an accumulation of LC3-Ⅱ protein in HCV infected liver slices treated with 1 μmol/L HCQ or 1 μmol/L GNS-396 (Figure 5D) in comparison either with not infected liver slices treated (Figure 5C) or not (Figure 5A), or with HCV infected liver slices without treatment (Figure 5B). At day 10, the normalized LC3-Ⅱ protein expression increased when liver slices infected (Figure 5D) or not (Figure 5C) were treated either with GNS-396 (1 μmol/L) or HCQ (1 μmol/L). The GNS-396 and HCQ effects were tested on the *de novo* viral production of HCVcc Con1 infected liver slices (Figures 6 and 7). At day 4 post-infection, HCVcc Con1 infected liver slices were treated for 6 d with different concentrations either of GNS-396 or HCQ. From day 1 to day 6 post-treatment, the HCV RNA replication (Figure 6A and B) and the infectivity (Figure 7A and B) were inhibited in a dose-dependent manner. The addition of RBV with the new drug GNS-396 showed no significant difference in the viral inhibition (data not shown).

EC50 analysis of HCV replication with GNS-396 treatment compared to that of validated antiviral drugs

The ability of various concentrations of different antiviral drugs to inhibit HCV replication was measured by detecting the replication of negative strands HCV RNA (Figure 6A and C) (Table 1). The calculated EC50 of different antiviral drugs is listed as Table 1 and compared to GNS-396. In summary, our model confirms that the antiviral activity of triple therapy was higher than that of the dual therapy by PR as extensively reported in clinical trials[5,6]. The new quinoline derivative GNS-396 has about 10-fold lower EC50 than HCQ (0.158 μmol/L as compared to 1.17 μmol/L) (Figure 6B and D). No significant cytotoxic effects were observed when evaluated by the lactate dehydrogenase leakage (LDH) assays (Figure 2A and B). A 50% cytotoxic concentration (CC50 value) of 25 μmol/L was obtained for GNS-396 in the human liver slices culture at day 6 post-treatment. Similar CC50 values were obtained in proliferating Huh-7-5-1 replicon cells (23 μmol/L).

DISCUSSION

Our study evidenced that: (1) the *ex vivo* model of human liver slices HCVcc Con1 infection may be efficiently used for the assay of the antiviral potency of a new inhibitor (GNS-396 compared to HCQ) which interfered with autophagy; and (2) GNS-396 was a potent autophagy inhibitor, acting as "lysosomotropic agent" which was able to inhibit HCV replication in primary human adult HCVcc infected liver slices culture.

The establishment of the *ex vivo* model (feasibility, rapidity, specificity, potency) was already described in detail in 2012[41] with comparison between primary human hepatocytes, Huh-7.5.1 cell line. The Huh-7 cell system has several limitations that includes the inability to study the effects of pharmacologic inhibitors targeting the non-structural proteins of the most prevalent and problematic viral strains (*e.g.*, genotypes 1a and 1b). Moreover, the study of virus/host cell interactions is limited since the permissive cell lines are transformed and poorly differentiated. Firstly, the human liver slices culture maintains the original three-dimensional structure of the liver that allows cell crosstalk: The extra-cellular matrix and Kupffer cells essential for the normal function of the hepatocytes and the lobular structure. Secondly, the gene expression profiles in liver tissue slices were similar to that of the *in vivo* gene expression. Thirdly, primary hepatocytes preparations undergo treatment with collagenase (a treatment might have a negative effect on integrity of the proteins repertoire on the cell surface), but not the liver slices. Noteworthy, using established procedures, the tissue slices remained viable for, at least 10 d as it was shown by the secretion of albumin and urea. Moreover, the Huh-7 cell infection with primary isolates from patients are not very efficient. The infection of adult human liver slices culture allowed to achieve the robust replication of HCVcc genotype 2a, 1a and 1b genome and to obtain infectivity titers above 105 ffu/mL. In addition, we reported robust and productive infection using human primary isolates HCV genotype 1b. Stem cell-derived hepatocytes (hESC-Heps) displayed

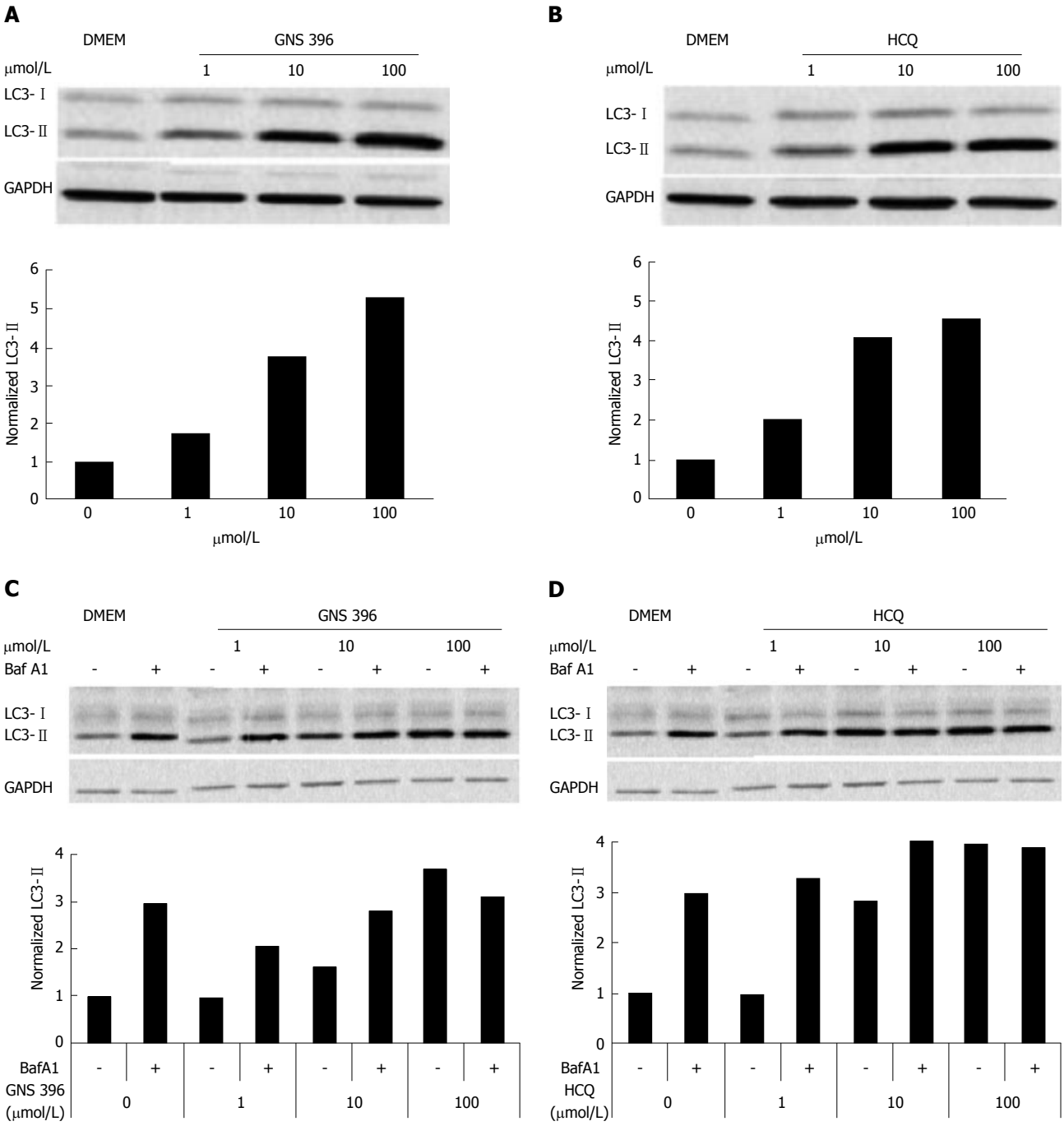

Figure 3 Modulation and inhibition of autophagy by treatment with GNS-396 in HeLa cells. A and B: Autophagy modulation was evaluated using HeLa cells treated either with (A) GNS-396 or (B) HCQ at different concentrations (1, 10 and 100 μmol/L) during 6 h. HCQ was used as a positive control of autophagy modulation. Intracellular expression of proteins LC3 was evaluated by Western-blot assay and normalized for LC3- Ⅱ; C and D: Autophagy inhibition by treatment with GNS-396 in HeLa cells. Autophagy inhibition was evaluated using HeLa cells treated either with (C) GNS-396 or (D) HCQ at different concentrations (1, 10 and 100 μmol/L) during 4 h in the presence or absence of 100 nmol/L bafilomycine A1. HCQ was used as a positive control of autophagy inhibition. LC3- Ⅱ intracellular expression was evaluated by Western-blot assay and normalized. HCQ: Hydroxychloroquine; LC3: Microtubule-associated protein 1A/1B-light chain; DMEM: Dulbecco's modified eagles's medium; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; BafA1: Bafilomycin A1.

equivalence to primary adult hepatocytes. HESC-Heps were capable of supporting the full HCV life cycle (JFH1), including the release of infectious virions. Although supportive, hESC-Hep viral infection levels were not as great as those observed in Huh7 cells. Up to now, the hESC-Heps were not infected with primary isolates[55]. Currently, we are establishing a culture of liver slices for 21 d, which allows us to follow the variation of different parameters and in particular, complete inhibition of viral production (data not shown).

Previous studies have reported that autophagy proteins are required to initiate HCV replication and translation[28,30-36]. Some data demonstrated that the suppression of LC3 protein lipidation, a necessary step for the formation of autophagosomes could also suppress HCV replication[30]. CQ is a well-known autophagic inhibitor

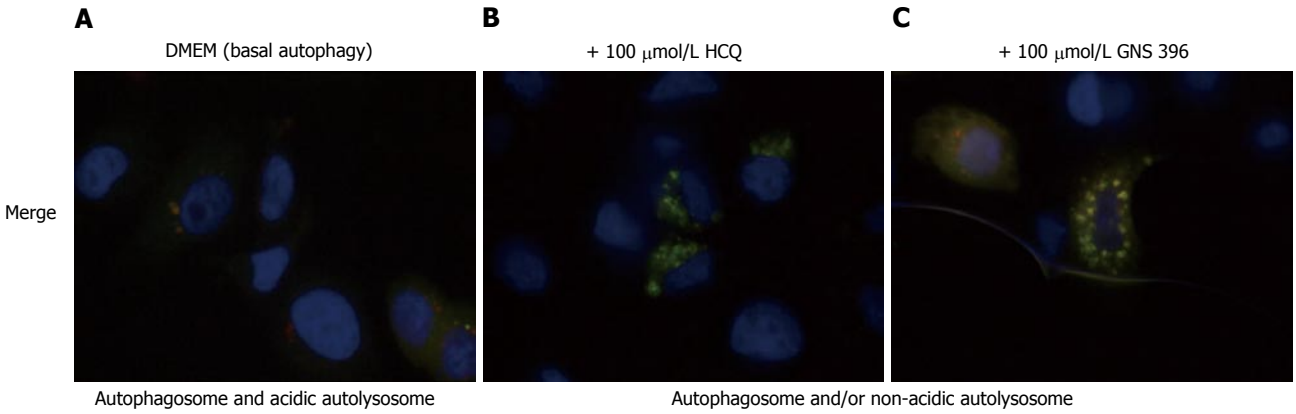

Figure 4 Inhibition of autophagic flux by treatment with GNS-396 in SkBr3 mRFP-EGFP-LC3 stable cell line. Autophagic flux was monitored using the mRFP-EGFP-LC3 tandem-tagged fluorescent protein in SkBr3 mRFP-EGFP-LC3 stable cell line. A: SkBr3 mRFP-EGFP-LC3 stable cell line without any treatment is representative of basal autophagy; SkBr3 mRFP1-EGFP-LC3 stable cell line was treated either with (B) 100 μmol/L HCQ or (C) 100 μmol/L GNS-396 during 6 h. In green/red merged images, yellow punctua (*i.e.*, mRFP+EGFP+) indicate autophagosomes or non-acidic autolysosomes, while red punctua (*i.e.*, mRFP+EGFP) indicate autolysosomes. HCQ is used as a positive control of autophagy inhibition. HCQ: Hydroxychloroquine; DMEM: Dulbecco's modified eagles's medium; LC3: Microtubule-associated protein 1A/1B-light chain.

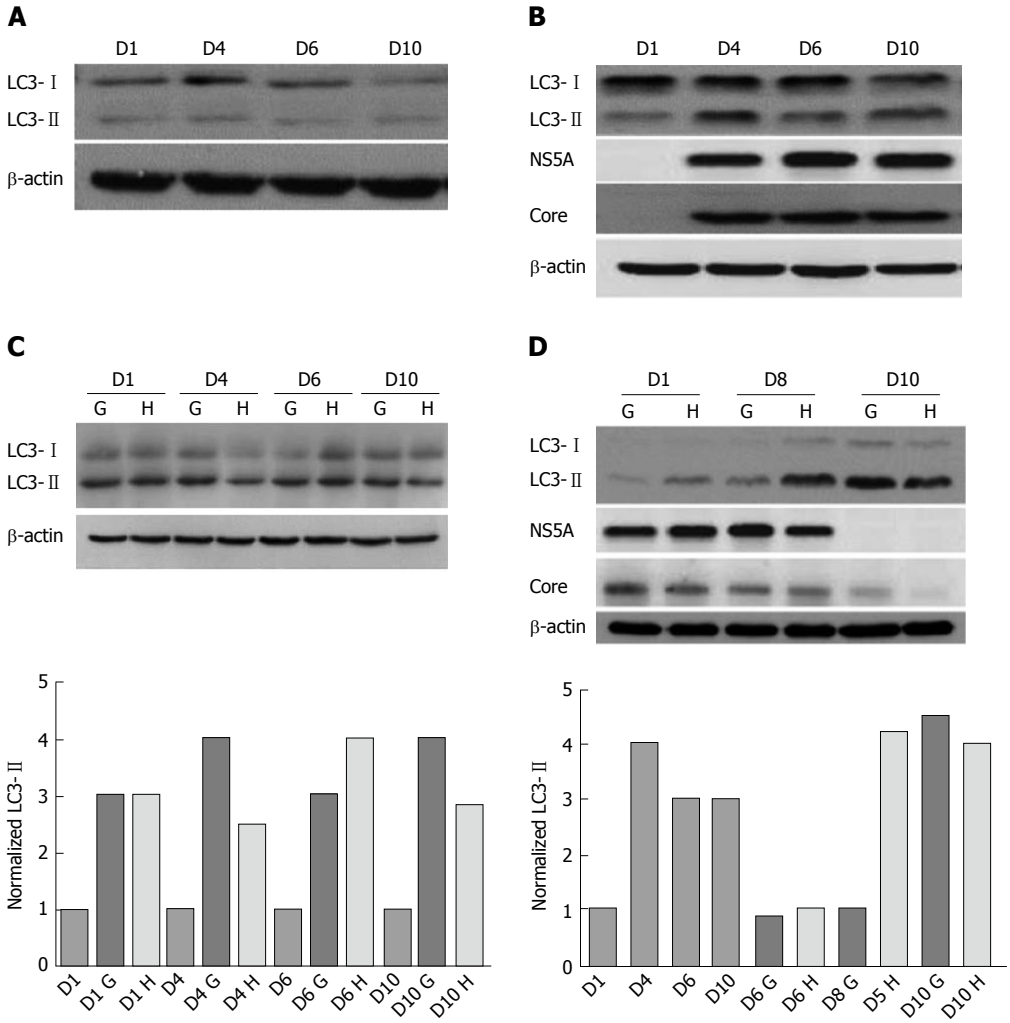

Figure 5 Inhibition of autophagy by treatment with GNS-396 (1 μmol/L) in primary adult human liver slices infected with cell culture-grown hepatitis C virus Con1 (multiplicity of infection = 1). A: Intracellular expression of LC3-Ⅰ/LC3-Ⅱ proteins in non-infected liver slices without treatment; B: Intracellular expression of LC3-Ⅰ/LC3-Ⅱ proteins and the normalization of intracellular protein LC3-Ⅱ expression, for 10 d in non-infected liver slices with treatment either by GNS-396 (1 μmol/L) (G) or HCQ (1 μmol/L) (H) or without treatment (D: day); C: Expression of LC3-Ⅰ/LC3-Ⅱ proteins and HCV core and NS5A proteins in HCVcc Con1 infected liver slices either without treatment or (D) either with treatment by GNS-396 (1 μmol/L) or HCQ (1 μmol/L) and the normalization of Intracellular protein LC3-Ⅱ expression for 10 d in HCVcc Con1 infected liver slices with treatment either by GNS-396 (1 μmol/L) (G) or HCQ (1 μmol/L) (H) or without treatment (D: day). LC3: Microtubule-associated protein 1A/1B-light chain; HCVcc: Cell culture-grown hepatitis C virus; NS5A: HCV nonstructural protein 5A.

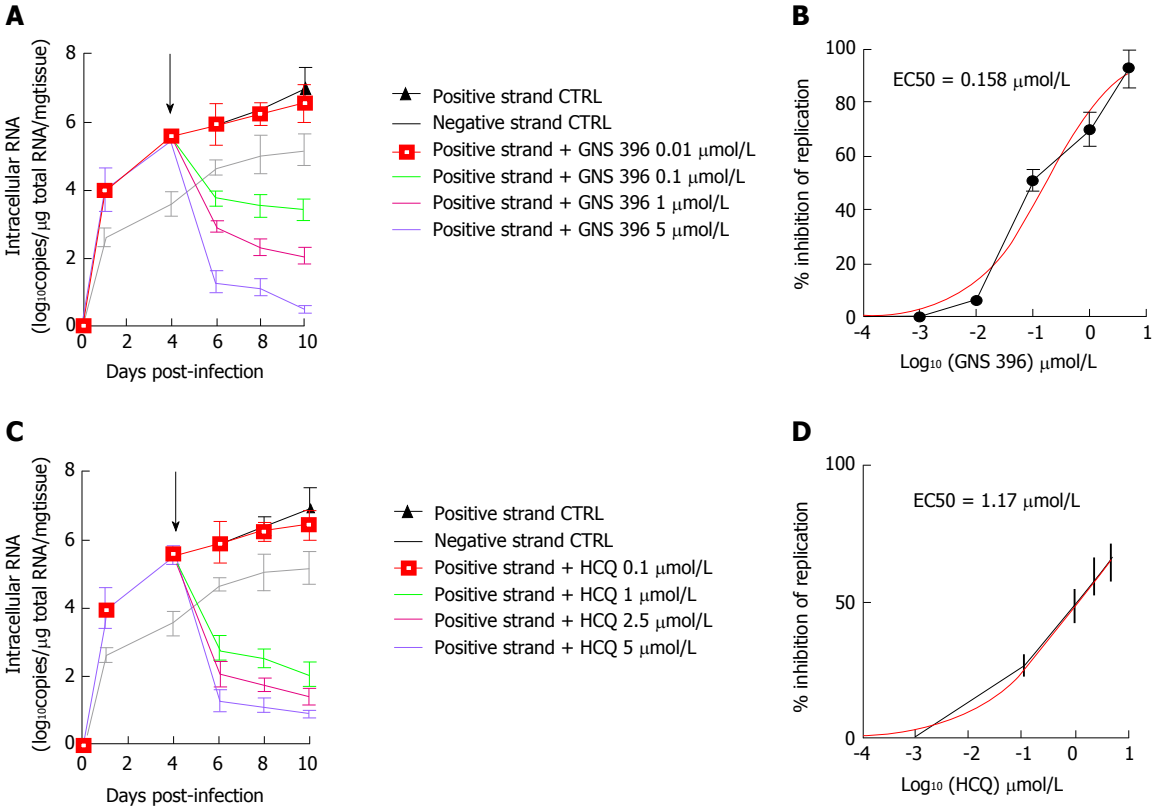

Figure 6 Inhibition of hepatitis C virus RNA replication by treatment either with GNS-396 or hydroxychloroquine in a dose-dependent manner in primary adult human cell culture-grown hepatitis C virus Con1 infected liver slices. Human liver slices were infected overnight with HCVcc Con1 (MOI = 0.1). The supernatant is then removed, the human liver slices washed and cultured. The liver slices and culture supernatants were collected different times post-infection. At day 4 post-infection, the liver slices were treated with increasing concentrations either of GNS-396 (0.01, 0.1, 1, 5 μmol/L) (A, B) or HCQ (C, D) for 6 d (black arrow: Start of the treatment either with GNS-396 or HCQ). Human HCVcc Con1 infected liver slices were lysed to evaluate intracellular levels of positive- and negative-strand HCV RNA by specific strand RT-qPCR at 1, 4, 6, 8, 10 d post-infection. The results were compared using the two-paired Student's test. Values are expressed as means ± standard errors: (A) HCV RNA replication by treatment with GNS-396: Positive strand (black line), $P < 0.03$; negative strand (grey line), $P < 0.013$, GNS-396 0.01 μmol/L (red line), $P < 0.04$; GNS-396 0.1 μmol/L (green line), $P < 0.05$; GNS-396 1 μmol/L (pink line), $P < 0.05$; GNS-396 5 μmol/L (blue line), $P < 0.05$; (C) HCV RNA replication by treatment with HCQ: Positive strand (black line), $P < 0.03$; negative strand (grey line), $P < 0.015$; HCQ 0.1 μmol/L (red line), $P < 0.0001$; HCQ 1 μmol/L (green line), $P < 0.0001$; HCQ 2.5 μmol/L (pink line), $P < 0.01$; HCQ 5 μmol/L (blue line), $P < 0.03$. The detection of negative strand of HCV RNA evidences active replication as well as the increase overtime of both positive and negative strands of HCV RNA; B: Inhibition of HCV replication (%) with GNS-396 treatment $P < 0.0038$; D: Inhibition of HCV replication (%) with HCQ treatment $P < 0.0013$. The replication was significantly inhibited in a dose-dependent manner in presence of increasing concentrations either of GNS-396 (B) or HCQ (D) for 6 d. HCVcc: Cell culture-grown hepatitis C virus; HCQ: Hydroxychloroquine; qRT-PCR: Quantitative technique consisting of reverse transcription followed by real-time polymerase chain reaction; MOI: Multiplicity of infection; CTRL: Control-treated liver slices.

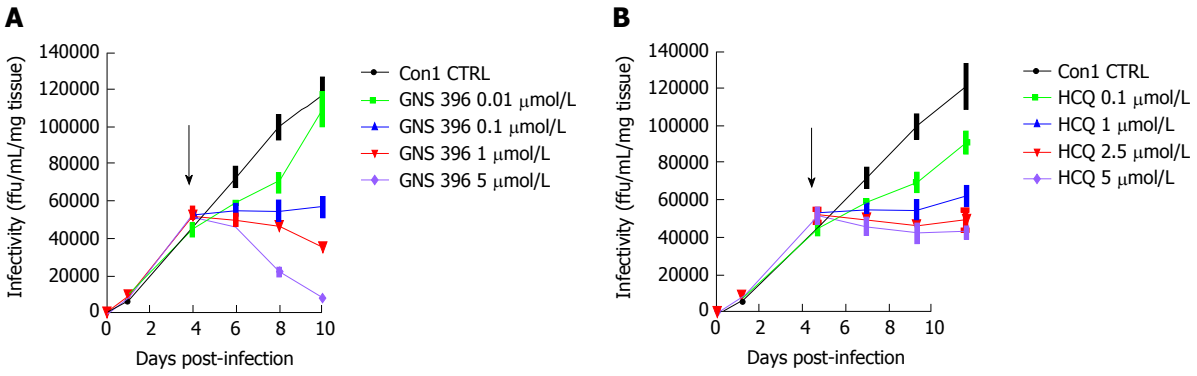

Figure 7 Dose-dependent inhibition of primary-culture-derived virus infectivity in primary adult human cell culture–grown hepatitis C virus Con1 infected liver slices by treatment either with GNS-396 (A) or hydroxychloroquine (B). Kinetics of infectivity of culture supernatants from human liver slices infected by HCV Con1 (MOI = 0.1) and treated either GNS-396 (A) or with HCQ (B) or at day 4 post-infection for 6 d. A: Con1 (black line), $P < 0.0001$; GNS-396 0.01 μmol/L (green line), $P < 0.0003$; GNS-396 0.1 μmol/L (blue line), $P < 0.019$; GNS-396 1 μmol/L (red line), $P < 0.05$; GNS-396 5 μmol/L (purple line), $P < 0.05$; B: Con1 (black line), $P < 0.0001$; HCQ 0.1 μmol/L (red line), $P < 0.0001$; HCQ 1 μmol/L (green line), $P < 0.0001$; HCQ 2.5 μmol/L (red line), $P < 0.0001$; HCQ 5 μmol/L (purple line), $P < 0.0003$. Each curve represented the average of 2 independent infections performed in triplicate from 2 different donors. Values are expressed as means ± SE. The results were compared using the two-paired student's test. HCV: Hepatitis C virus; HCQ: Hydroxychloroquine; MOI: Multiplicity of infection; CTRL: Control-treated liver slices.

Table 1 Inhibition of hepatitis C virus infectivity and 50% effective concentration of hepatitis C virus replication with direct active antivirals and autophagy inhibitors

Drugs[1]	Infectivity inhibition (%)	Average (SD)[2]	Replication inhibition (EC50)[3]	Average (SD)[4]	CC50 (SD)[5]
INF (2.6 to 260 nmol/L)[6]	Up to 95%	5	17 ng/mL	7.2	40 ng/mL (± 4)
RBV (1 to 100 μmol/L)	3% to 37%	3	146 μmol/L	13	400 μmol/L (± 21)
[7]TVR (0.01 to 50 μmol/L)	62% to 89%	4	0.395 μmol/L	0.038	40 μmol/L (± 3)
[7]BOC (0.01 to 50 μmol/L)	61% to 95%	5	0.417 μmol/L	0.024	41 μmol/L (± 5)
[7]SOF (0.01 to 50 μmol/L)	75% to 95%	4	0.147 μmol/L	0.017	23 μmol/L (± 2)
[7]HCQ (0.1 to 50 μmol/L)	25% to 94%	4	1.17 μmol/L	0.023	27 μmol/L (± 2)
[7]GNS-396 (0.01 to 5 μmol/L)	6% to 93%	3	0.158 μmol/L	0.014	25 μmol/L (± 2)
INF (2.6 to 260 nmol/L)/RBV 100 μmol/L	Up to 98%	6	10 ng/mL	3.1	43 ng/mL (± 4)
[7]HCQ (0.1 to 5 μmol/L)/RBV 50 μmol/L	27% to 85%	2	0.456 μmol/L	0.044	31 μmol/L (± 3)
[7]GNS-396 (0.01 to 5 μmol/L)/RBV 100 μmol/L	9% to 94%	2	0.157 μmol/L	0.012	26 μmol/L (± 2)
[7]TVR (0.01 to 50 μmol/L)/RBV 100 μmol/L	Up to 98%	3	0.310 μmol/L	0.029	49 μmol/L (± 3)
[7]BOC (0.01 to 50 μmol/L)/RBV 100 μmol/L	Up to 95%	2	0.370 μmol/L	0.035	48 μmol/L (± 4)
[7]SOF (0.01 to 50 μmol/L)/RBV 100 μmol/L	Up to 100%	2	0.080 μmol/L	0.028	17 μmol/L (± 5)
[7]TVR 1 μmol/L/7BOC 1 μmol/L	Up to 89%	2	0.410 μmol/L	0.039	50 μmol/L (± 3)
[7]TVR 1 μmol/L/INF 26 nmol/L/RBV 100 μmol/L	Up to 99%	3	0.315 μmol/L	0.031	44 μmol/L (± 4)
[7]BOC 1 μmol/L/INF 26 nmol/L/RBV 100 μmol/L	Up to 97%	2	0.350 μmol/L	0.033	47 μmol/L (± 3)
[7]SOF 1 μmol/L/INF 26 nmol/L/RBV 100 μmol/L	Up to 100%	3	0.055 μmol/L	0.029	18 μmol/L (± 3)

[1]Drugs added at day 4 post-infection for 6 d; [2]Average (SD) of infectivity inhibition at day 6 post-treatment; [3]EC50 of the drugs written in bold at day 6 post-treatment; [4]Average (SD) of EC50 at day 6 post-treatment; [5]CC50 (SD): 50% cytotoxic concentration of the drugs written in bold at day 6 post-treatment (standard deviation); [6]INF 26 nmol/L: Peg-INF concentration corresponding to SOC; [7]DAAs and autophagy inhibitors in bold. EC50: 50% effective concentration; BOC: An inhibitor of the HCV-encoded NS3 protein; TVR: An inhibitor of the HCV-encoded NS3/4A hepatitis C protease; SOF: An uridine analogue inhibitor of the HCV NS5B polymerase; HCV: Hepatitis C virus; TVR: Telaprevir; BOC: Boceprevir; SOF: Sofosbuvir; RBV: Ribavirin; SD: Standard deviation; Peg-INF: Pegylated-interferon α-2a; DAAs: Direct acting antivirals; HCQ: Hydroxychloroquine.

which is often used as an anti-malarial agent. HCQ is a "lysosomotropic" weak base that raises the lysosomal pH quickly[37]. Furthermore, many studies have reported the antiviral effect of CQ on other positive strand RNA viruses, such as polioviruses, coxsackieviruses, dengue viruses, coronaviruses (SARS-CoV virus)[24-29], HIV-1[56]. In our study, we demonstrated the antiviral effect of HCQ and the new quinoline derivative GNS-396 on HCVcc replication in a dose - dependent manner. Compared to the treatment with HCQ alone, HCQ inhibition was more pronounced in combination with RBV or with other direct antivirals, suggesting a synergistic effect of the combined drugs on HCVcc infection in human liver slices. This result is consistent with a previous study which demonstrated the antiviral effect of CQ in combination with peg-IFN in HCV infected Huh-7 cell line[33]. Similarly, on Huh-7 cells infected with HCVpp (genotype 1a and 3a), it has been shown that CQ reduced by 50% virus infectivity at 50 μmol/L concentration, when the antiviral effect was tested[57]. Recently, ferroquine (FQ), an antimalarial ferrocenic analog of CQ, has been described as a novel inhibitor of HCV. FQ potently inhibited HCV infection of hepatoma cell lines[58]. Compared to these investigations, our study using the quinoline derivative GNS-396, revealed an inhibition of the virus infectivity up to 93% respectively at day 6 post-treatment with lower drug amounts (EC50 = 0.158 μmol/L). This demonstrates that GNS-396 is a stronger antiviral than HCQ (EC50 = 1.17 μmol/L). EC50 is a measure of the effectiveness of the drug in inhibiting the biochemical function. In our study, we evaluated the EC50 of HCV replication at day 6 post-treatment. The lower EC50 value indicates the greater potency of inhibiting HCV replication. As shown in Table 1, the infectivity inhibition, consistent with the inhibition of HCV replication, demonstrated that the new drug evaluated in the human HCV infected liver slices culture model, had a potent antiviral effect compared to the well-known established antivirals. In combination with the other well established drugs like DAA or inhibitors of other host targets (cyclophilin), quinoline derivatives could be additional therapeutic options for HCV infected patients.

In conclusion, this study demonstrated the relevance of the human HCV infected liver slices culture in preclinical studies of the new anti-viral drugs. New host-targeted therapies inhibiting autophagy (GNS-396, HCQ) have demonstrated significant efficiency and additive activity in inhibiting HCV replication. The *ex vivo* model of culture of human HCV infected liver slices might allow further evaluation of the efficacy of new antiviral drugs in single or in combined therapy and their potential toxicity in particular for patients "difficult to treat". Moreover, the infection of human liver slices culture with primary viral isolates from patients that we succeed to establish[41], should allow highlighting the potential of early emergence of drug resistant viral variants during the anti-viral treatments.

ACKNOWLEDGMENTS

The authors are grateful to Professor Francis V Chisari, for the kind gift of Huh-7.5.1 cells and to Professor Ralf Bartenschlager for kindly providing the chimeric pFK-Con1/C3 plasmid. We are also grateful to the members of the Departments of Digestive Surgery in the Groupe Hospitalier La Pitié Salpétrière and Cochin-Hôtel Dieu

as to the members of the Department of Hepatology in Groupe Hospitalier Cochin-Hôtel Dieu, APHP, Paris, France, for the technical assistance. We are deeply indebted to Doctor Vladimir A. Morozov and Doctor Matthew Albert for the critical reading of the manuscript.

COMMENTS

Background

Hepatitis C virus (HCV) infection (or spread) is a serious public health challenge counting approximately 170 million people that are chronically infected worldwide. Host antiviral therapy is an additional option for the treatment of HCV infection.

Research frontiers

Interferon-free treatments with new direct acting antivirals are expected to cure more than 90% of HCV-infected patients. But they are not available in all the countries. At the present time, triple therapy combining pegylated interferon-α 2a or b and ribavirin with NS3 protease inhibitors (telaprevir or boceprevir) is going to remain the main treatment for HCV patients. That is why it appears important to continue research in limiting virus replication and the autophagy inhibition could be a new additional pathway because of recent evidences obtained regarding to an increased autophagic response in the liver of chronically HCV infected patients.

Innovations and breakthroughs

This is the first study evaluating a new autophagy inhibitor as antiviral that could inhibit HCV infection in a dose-dependent manner without cytotoxic effect using the relevant ex vivo model of the human liver slices culture.

Applications

This study highlight the relevance of the *ex vivo* model of the human HCV infected liver slices culture in preclinical studies of the new anti-viral drugs in single or in combined therapy and their potential toxicity in particular for patients "difficult to treat". Moreover, the infection of human liver slices culture with primary viral isolates from patients that the authors succeed to establish, should allow highlighting the potential of early emergence of drug resistant viral variants during the anti-viral treatments.

Terminology

Autophagy is a catabolic process which degrades a cellular own component through the lysosomal machinery. It has been shown that autophagy is activated during virus and bacterial infection and that some viruses can use the autophagy system to facilitate their own replication.

Peer-review

The manuscript is clear and comprehensive.

REFERENCES

1 **Szabó E**, Lotz G, Páska C, Kiss A, Schaff Z. Viral hepatitis: new data on hepatitis C infection. *Pathol Oncol Res* 2003; **9**: 215-221 [PMID: 14688826 DOI: 10.1007/BF02893380]

2 **Corouge M**, Pol S. New treatments for chronic hepatitis C virus infection. *Med Mal Infect* 2011; **41**: 579-587 [PMID: 21764234 DOI: 10.1016/j.medmal.2011.04.003]

3 **Mallet V**, Gilgenkrantz H, Serpaggi J, Verkarre V, Vallet-Pichard A, Fontaine H, Pol S. Brief communication: the relationship of regression of cirrhosis to outcome in chronic hepatitis C. *Ann Intern Med* 2008; **149**: 399-403 [PMID: 18794559 DOI: 10.7326/0003-4819-149-6-200809160-00006]

4 **Ashfaq UA**, Javed T, Rehman S, Nawaz Z, Riazuddin S. An overview of HCV molecular biology, replication and immune responses. *Virol J* 2011; **8**: 161 [PMID: 21477382 DOI: 10.1186/1743-422X-8-161]

5 **López-Labrador FX**. Hepatitis C Virus NS3/4A Protease Inhibitors. *Recent Pat Antiinfect Drug Discov* 2008; **3**: 157-167 [PMID: 18991798]

6 **Asselah T**, Marcellin P. New direct-acting antivirals' combination for the treatment of chronic hepatitis C. *Liver Int* 2011; **31** Suppl 1: 68-77 [PMID: 21205141 DOI: 10.1111/j.1478-3231.2010.02411.x]

7 **Poordad F**, McCone J, Bacon BR, Bruno S, Manns MP, Sulkowski MS, Jacobson IM, Reddy KR, Goodman ZD, Boparai N, DiNubile MJ, Sniukiene V, Brass CA, Albrecht JK, Bronowicki JP. Boceprevir for untreated chronic HCV genotype 1 infection. *N Engl J Med* 2011; **364**: 1195-1206 [PMID: 21449783 DOI: 10.1056/NEJMoa1010494]

8 **Bacon BR**, Gordon SC, Lawitz E, Marcellin P, Vierling JM, Zeuzem S, Poordad F, Goodman ZD, Sings HL, Boparai N, Burroughs M, Brass CA, Albrecht JK, Esteban R. Boceprevir for previously treated chronic HCV genotype 1 infection. *N Engl J Med* 2011; **364**: 1207-1217 [PMID: 21449784 DOI: 10.1056/NEJMoa1009482]

9 **Jacobson IM**, McHutchison JG, Dusheiko G, Di Bisceglie AM, Reddy KR, Bzowej NH, Marcellin P, Muir AJ, Ferenci P, Flisiak R, George J, Rizzetto M, Shouval D, Sola R, Terg RA, Yoshida EM, Adda N, Bengtsson L, Sankoh AJ, Kieffer TL, George S, Kauffman RS, Zeuzem S. Telaprevir for previously untreated chronic hepatitis C virus infection. *N Engl J Med* 2011; **364**: 2405-2416 [PMID: 21696307 DOI: 10.1056/NEJMoa1012912]

10 **Zeuzem S**, Asselah T, Angus P, Zarski JP, Larrey D, Müllhaupt B, Gane E, Schuchmann M, Lohse A, Pol S, Bronowicki JP, Roberts S, Arasteh K, Zoulim F, Heim M, Stern JO, Kukolj G, Nehmiz G, Haefner C, Boecher WO. Efficacy of the protease inhibitor BI 201335, polymerase inhibitor BI 207127, and ribavirin in patients with chronic HCV infection. *Gastroenterology* 2011; **141**: 2047-2055; quiz e14 [PMID: 21925126 DOI: 10.1053/j.gastro.2011.08.051]

11 **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. 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]

12 **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]

13 **Sulkowski MS**, Gardiner DF, Rodriguez-Torres M, Reddy KR, Hassanein T, Jacobson I, Lawitz E, Lok AS, Hinestrosa F, Thuluvath PJ, Schwartz H, Nelson DR, Everson GT, Eley T, Wind-Rotolo M, Huang SP, Gao M, Hernandez D, McPhee F, Sherman D, Hindes R, Symonds W, Pasquinelli C, Grasela DM. 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]

14 **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]

15 **Hopkins S**, DiMassimo B, Rusnak P, Heuman D, Lalezari J, Sluder A, Scorneaux B, Mosier S, Kowalczyk P, Ribeill Y, Baugh J, Gallay P. The cyclophilin inhibitor SCY-635 suppresses viral replication and induces endogenous interferons in patients with chronic HCV genotype 1 infection. *J Hepatol* 2012; **57**: 47-54 [PMID: 22425702 DOI: 10.1016/j.jhep.2012.02.024]

16 **Schaefer EA**, Chung RT. Anti-hepatitis C virus drugs in development. *Gastroenterology* 2012; **142**: 1340-1350.e1 [PMID: 22537441 DOI: 10.1053/j.gastro.2012.02.015]

17 **Hill A**, Khoo S, Fortunak J, Simmons B, Ford N. Minimum costs for producing hepatitis C direct-acting antivirals for use in large-scale treatment access programs in developing countries. *Clin Infect Dis* 2014; **58**: 928-936 [PMID: 24399087 DOI: 10.1093/cid/ciu012]

18 **Deuffic-Burban S**, Schwarzinger M, Obach D, Mallet V, Pol S, Pageaux GP, Canva V, Deltenre P, Roudot-Thoraval F, Larrey D, Dhumeaux D, Mathurin P, Yazdanpanah Y. Should we await IFN-free regimens to treat HCV genotype 1 treatment-naive patients? A cost-effectiveness analysis (ANRS 95141). *J Hepatol* 2014; **61**: 7-14 [PMID: 24650691 DOI: 10.1016/j.jhep.2014.03.011]

19 **European Association for the Study of the Liver**. EASL Recommendations on Treatment of Hepatitis C 2015. *J Hepatol* 2015; **63**: 199-236 [PMID: 25911336 DOI: 10.1016/j.jhep. 2015.03.025]

20 **AASLD/IDSA HCV Guidance Panel**. Hepatitis C guidance: AASLD-IDSA recommendations for testing, managing, and treating adults infected with hepatitis C virus. *Hepatology* 2015; **62**: 932-954 [PMID: 26111063 DOI: 10.1002/hep.27950]

21 **Zopf S**, Kremer AE, Neurath MF, Siebler J. Advances in hepatitis C therapy: What is the current state - what come's next? *World J Hepatol* 2016; **8**: 139-147 [PMID: 26839638 DOI: 10.4254/wjh.v8.i3.139]

22 **Rautou PE**, Cazals-Hatem D, Feldmann G, Mansouri A, Grodet A, Barge S, Martinot-Peignoux M, Duces A, Bièche I, Lebrec D, Bedossa P, Paradis V, Marcellin P, Valla D, Asselah T, Moreau R. Changes in autophagic response in patients with chronic hepatitis C virus infection. *Am J Pathol* 2011; **178**: 2708-2715 [PMID: 21641393 DOI: 10.1016/j.ajpath.2011.02.021]

23 **Shintani T**, Klionsky DJ. Autophagy in health and disease: a double-edged sword. *Science* 2004; **306**: 990-995 [PMID: 15528435 DOI: 10.1126/science.1099993]

24 **Orvedahl A**, Levine B. Eating the enemy within: autophagy in infectious diseases. *Cell Death Differ* 2009; **16**: 57-69 [PMID: 18772897 DOI: 10.1038/cdd.2008.130]

25 **Jackson WT**, Giddings TH, Taylor MP, Mulinyawe S, Rabinovitch M, Kopito RR, Kirkegaard K. Subversion of cellular autophagosomal machinery by RNA viruses. *PLoS Biol* 2005; **3**: e156 [PMID: 15884975 DOI: 10.1371/journal.pbio.0030156]

26 **Lee YR**, Lei HY, Liu MT, Wang JR, Chen SH, Jiang-Shieh YF, Lin YS, Yeh TM, Liu CC, Liu HS. Autophagic machinery activated by dengue virus enhances virus replication. *Virology* 2008; **374**: 240-248 [PMID: 18353420 DOI: 10.1016/j.virol.2008.02.016]

27 **Wong J**, Zhang J, Si X, Gao G, Mao I, McManus BM, Luo H. Autophagosome supports coxsackievirus B3 replication in host cells. *J Virol* 2008; **82**: 9143-9153 [PMID: 18596087 DOI: 10.1128/JVI.00641-08]

28 **Dreux M**, Chisari FV. Viruses and the autophagy machinery. *Cell Cycle* 2010; **9**: 1295-1307 [PMID: 20305376 DOI: 10.4161/cc.9.7.11109]

29 **Silva LM**, Jung JU. Modulation of the autophagy pathway by human tumor viruses. *Semin Cancer Biol* 2013; **23**: 323-328 [PMID: 23727156 DOI: 10.1016/j.semcancer.2013.05.005]

30 **Sir D**, Chen WL, Choi J, Wakita T, Yen TS, Ou JH. Induction of incomplete autophagic response by hepatitis C virus via the unfolded protein response. *Hepatology* 2008; **48**: 1054-1061 [PMID: 18688877 DOI: 10.1002/hep.22464]

31 **Dreux M**, Gastaminza P, Wieland SF, Chisari FV. The autophagy machinery is required to initiate hepatitis C virus replication. *Proc Natl Acad Sci USA* 2009; **106**: 14046-14051 [PMID: 19666601 DOI: 10.1073/pnas.0907344106]

32 **Dreux M**, Chisari FV. Impact of the autophagy machinery on hepatitis C virus infection. *Viruses* 2011; **3**: 1342-1357 [PMID: 21994783 DOI: 10.3390/v3081342]

33 **Mohl BP**, Tedbury PR, Griffin S, Harris M. Hepatitis C virus-induced autophagy is independent of the unfolded protein response. *J Virol* 2012; **86**: 10724-10732 [PMID: 22837205 DOI: 10.1128/JVI.01667-12]

34 **Shrivastava S**, Bhanja Chowdhury J, Steele R, Ray R, Ray RB. Hepatitis C virus upregulates Beclin1 for induction of autophagy and activates mTOR signaling. *J Virol* 2012; **86**: 8705-8712 [PMID: 22674982 DOI: 10.1128/JVI.00616-12]

35 **Huang H**, Kang R, Wang J, Luo G, Yang W, Zhao Z. Hepatitis C virus inhibits AKT-tuberous sclerosis complex (TSC), the mechanistic target of rapamycin (MTOR) pathway, through endoplasmic reticulum stress to induce autophagy. *Autophagy* 2013; **9**: 175-195 [PMID: 23169238 DOI: 10.4161/auto.22791]

36 **Sir D**, Kuo CF, Tian Y, Liu HM, Huang EJ, Jung JU, Machida K, Ou JH. Replication of hepatitis C virus RNA on autophagosomal membranes. *J Biol Chem* 2012; **287**: 18036-18043 [PMID: 22496373 DOI: 10.1074/jbc.M111.320085]

37 **Savarino A**, Boelaert JR, Cassone A, Majori G, Cauda R. Effects of chloroquine on viral infections: an old drug against today's diseases? *Lancet Infect Dis* 2003; **3**: 722-727 [PMID: 14592603 DOI: 10.1016/S1473-3099(03)00806-5]

38 **Vincent MJ**, Bergeron E, Benjannet S, Erickson BR, Rollin PE, Ksiazek TG, Seidah NG, Nichol ST. Chloroquine is a potent inhibitor of SARS coronavirus infection and spread. *Virol J* 2005; **2**: 69 [PMID: 16115318 DOI: 10.1186/1743-422X-2-69]

39 **Rolain JM**, Colson P, Raoult D. Recycling of chloroquine and its hydroxyl analogue to face bacterial, fungal and viral infections in the 21st century. *Int J Antimicrob Agents* 2007; **30**: 297-308 [PMID: 17629679 DOI: 10.1016/j.ijantimicag.2007.05.015]

40 **Mizui T**, Yamashina S, Tanida I, Takei Y, Ueno T, Sakamoto N, Ikejima K, Kitamura T, Enomoto N, Sakai T, Kominami E, Watanabe S. Inhibition of hepatitis C virus replication by chloroquine targeting virus-associated autophagy. *J Gastroenterol* 2010; **45**: 195-203 [PMID: 19760134 DOI: 10.1007/s00535-009-0132-9]

41 **Lagaye S**, Shen H, Saunier B, Nascimbeni M, Gaston J, Bourdoncle P, Hannoun L, Massault PP, Vallet-Pichard A, Mallet V, Pol S. Efficient replication of primary or culture hepatitis C virus isolates in human liver slices: a relevant ex vivo model of liver infection. *Hepatology* 2012; **56**: 861-872 [PMID: 22454196 DOI: 10.1002/hep.25738]

42 **Halfon P**, Nallet J, Petit SJ, Bouzidi M, Joly F, Camus C, Benech P, Dubuisson, Courcambeck J, Wychowski C. Chloroquine and related compounds are Inhibitors of Hepatitis C Virus RNA-by inhibiting autophagy proteolysis. *Hepatology* 2010; **52**: 807 [DOI: 10.1002/hep.23991]

43 **Olinga P**, Groothuis GM. Use of human tissue slices in drug targeting research. Drug targeting. Organ-specific strategies. Series: Methods and Principles in Medicinal Chemistry. G Molema, DKF Meijer, editors series, Mannhold R, Kubinyi H, Timmerman H, eds Weinheim, Germany: Wiley-VCH, 2001: 309-331 [DOI: 10.1002/352760006x.ch12]

44 **Pietschmann T**, Kaul A, Koutsoudakis G, Shavinskaya A, Kallis S, Steinmann E, Abid K, Negro F, Dreux M, Cosset FL, Bartenschlager R. Construction and characterization of infectious intragenotypic and intergenotypic hepatitis C virus chimeras. *Proc Natl Acad Sci USA* 2006; **103**: 7408-7413 [PMID: 16651538 DOI: 10.1073/pnas.0504877103]

45 **Zhong J**, Gastaminza P, Cheng G, Kapadia S, Kato T, Burton DR, Wieland SF, Uprichard SL, Wakita T, Chisari FV. Robust hepatitis C virus infection in vitro. *Proc Natl Acad Sci USA* 2005; **102**: 9294-9299 [PMID: 15939869 DOI: 10.1073/pnas.0503596102]

46 **Carrière M**, Pène V, Breiman A, Conti F, Chouzenoux S, Meurs E, Andrieu M, Jaffray P, Grira L, Soubrane O, Sogni P, Calmus Y, Chaussade S, Rosenberg AR, Podevin P. A novel, sensitive, and specific RT-PCR technique for quantitation of hepatitis C virus replication. *J Med Virol* 2007; **79**: 155-160 [PMID: 17177304 DOI: 10.1002/jmv.20773]

47 **Komurian-Pradel F**, Perret M, Deiman B, Sodoyer M, Lotteau V, Paranhos-Baccalà G, André P. Strand specific quantitative real-time PCR to study replication of hepatitis C virus genome. *J Virol Methods* 2004; **116**: 103-106 [PMID: 14715313 DOI: 10.1016/j.jviromet.2003.10.004]

48 **Besnard NC**, Andre PM. Automated quantitative determination of hepatitis C virus viremia by reverse transcription-PCR. *J Clin Microbiol* 1994; **32**: 1887-1893 [PMID: 7989537]

49 **Nozaki A**, Kato N. Quantitative method of intracellular hepatitis C virus RNA using LightCycler PCR. *Acta Med Okayama* 2002; **56**:

107-110 [PMID: 12002616]
50 **Laemmli UK**. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970; **227**: 680-685 [PMID: 5432063 DOI: 10.1038/227680a0]
51 **Braydich-Stolle L**, Hussain S, Schlager JJ, Hofmann MC. In vitro cytotoxicity of nanoparticles in mammalian germline stem cells. *Toxicol Sci* 2005; **88**: 412-419 [PMID: 16014736 DOI: 10.1093/toxsci/kfi256]
52 **Kimura M**, Taniguchi M, Mikami Y, Masuda T, Yoshida T, Mishina M, Shimizu T. Identification and characterization of zebrafish semaphorin 6D. *Biochem Biophys Res Commun* 2007; **363**: 762-768 [PMID: 17897628 DOI: 10.1016/j.bbrc.2007.09.038]
53 **Klionsky DJ**, Abeliovich H, Agostinis P, Agrawal DK, Aliev G, Askew DS, Baba M, Baehrecke EH, Bahr BA, Ballabio A, Bamber BA, Bassham DC, Bergamini E, Bi X, Biard-Piechaczyk M, Blum JS, Bredesen DE, Brodsky JL, Brumell JH, Brunk UT, Bursch W, Camougrand N, Cebollero E, Cecconi F, Chen Y, Chin LS, Choi A, Chu CT, Chung J, Clarke PG, Clark RS, Clarke SG, Clavé C, Cleveland JL, Codogno P, Colombo MI, Coto-Montes A, Cregg JM, Cuervo AM, Debnath J, Demarchi F, Dennis PB, Dennis PA, Deretic V, Devenish RJ, Di Sano F, Dice JF, Difiglia M, Dinesh-Kumar S, Distelhorst CW, Djavaheri-Mergny M, Dorsey FC, Dröge W, Dron M, Dunn WA, Duszenko M, Eissa NT, Elazar Z, Esclatine A, Eskelinen EL, Fésüs L, Finley KD, Fuentes JM, Fueyo J, Fujisaki K, Galliot B, Gao FB, Gewirtz DA, Gibson SB, Gohla A, Goldberg AL, Gonzalez R, González-Estévez C, Gorski S, Gottlieb RA, Häussinger D, He YW, Heidenreich K, Hill JA, Høyer-Hansen M, Hu X, Huang WP, Iwasaki A, Jäättelä M, Jackson WT, Jiang X, Jin S, Johansen T, Jung JU, Kadowaki M, Kang C, Kelekar A, Kessel DH, Kiel JA, Kim HP, Kimchi A, Kinsella TJ, Kiselyov K, Kitamoto K, Knecht E, Komatsu M, Kominami E, Kondo S, Kovács AL, Kroemer G, Kuan CY, Kumar R, Kundu M, Landry J, Laporte M, Le W, Lei HY, Lenardo MJ, Levine B, Lieberman A, Lim KL, Lin FC, Liou W, Liu LF, Lopez-Berestein G, López-Otín C, Lu B, Macleod KF, Malorni W, Martinet W, Matsuoka K, Mautner J, Meijer AJ, Meléndez A, Michels P, Miotto G, Mistiaen WP, Mizushima N, Mograbi B, Monastyrska I, Moore MN, Moreira PI, Moriyasu Y, Motyl T, Münz C, Murphy LO, Naqvi NI, Neufeld TP, Nishino I, Nixon RA, Noda T, Nürnberg B, Ogawa M, Oleinick NL, Olsen LJ, Ozpolat B, Paglin S, Palmer GE, Papassideri I, Parkes M, Perlmutter DH, Perry G, Piacentini M, Pinkas-Kramarski R, Prescott M, Proikas-Cezanne T, Raben N, Rami A, Reggiori F, Rohrer B, Rubinsztein DC, Ryan KM, Sadoshima J, Sakagami H, Sakai Y, Sandri M, Sasakawa C, Sass M, Schneider C, Seglen PO, Seleverstov O, Settleman J, Shacka JJ, Shapiro IM, Sibirny A, Silva-Zacarin EC, Simon HU, Simone C, Simonsen A, Smith MA, Spanel-Borowski K, Srinivas V, Steeves M, Stenmark H, Stromhaug PE, Subauste CS, Sugimoto S, Sulzer D, Suzuki T, Swanson MS, Tabas I, Takeshita F, Talbot NJ, Tallóczy Z, Tanaka K, Tanaka K, Tanida I, Taylor GS, Taylor JP, Terman A, Tettamanti G, Thompson CB, Thumm M, Tolkovsky AM, Tooze SA, Truant R, Tumanovska LV, Uchiyama Y, Ueno T, Uzcátegui NL, van der Klei I, Vaquero EC, Vellai T, Vogel MW, Wang HG, Webster P, Wiley JW, Xi Z, Xiao G, Yahalom J, Yang JM, Yap G, Yin XM, Yoshimori T, Yu L, Yue Z, Yuzaki M, Zabirnyk O, Zheng X, Zhu X, Deter RL. Guidelines for the use and interpretation of assays for monitoring autophagy in higher eukaryotes. *Autophagy* 2008; **4**: 151-175 [PMID: 18188003 DOI: 10.4161/auto.5338]
54 **Yamamoto A**, Tagawa Y, Yoshimori T, Moriyama Y, Masaki R, Tashiro Y. Bafilomycin A1 prevents maturation of autophagic vacuoles by inhibiting fusion between autophagosomes and lysosomes in rat hepatoma cell line, H-4-II-E cells. *Cell Struct Funct* 1998; **23**: 33-42 [PMID: 9639028 DOI: 10.1247/csf.23.33]
55 **Zhou X**, Sun P, Lucendo-Villarin B, Angus AG, Szkolnicka D, Cameron K, Farnworth SL, Patel AH, Hay DC. Modulating innate immunity improves hepatitis C virus infection and replication in stem cell-derived hepatocytes. *Stem Cell Reports* 2014; **3**: 204-214 [PMID: 25068132 DOI: 10.1016/j.stemcr.2014.04.018]
56 **Savarino A**, Gennero L, Chen HC, Serrano D, Malavasi F, Boelaert JR, Sperber K. Anti-HIV effects of chloroquine: mechanisms of inhibition and spectrum of activity. *AIDS* 2001; **15**: 2221-2229 [PMID: 11698694 DOI: 10.1097/00002030-200111230-00002]
57 **Ashfaq UA**, Javed T, Rehman S, Nawaz Z, Riazuddin S. Lysosomotropic agents as HCV entry inhibitors. *Virol J* 2011; **8**: 163 [PMID: 21481279 DOI: 10.1186/1743-422X-8-163]
58 **Vausselin T**, Calland N, Belouzard S, Descamps V, Douam F, Helle F, François C, Lavillette D, Duverlie G, Wahid A, Fénéant L, Cocquerel L, Guérardel Y, Wychowski C, Biot C, Dubuisson J. The antimalarial ferroquine is an inhibitor of hepatitis C virus. *Hepatology* 2013; **58**: 86-97 [PMID: 23348596 DOI: 10.1002/hep.26273]

P- Reviewer: Jin B, Irato P **S- Editor**: Ji FF
L- Editor: A **E- Editor**: Li D

Published by **Baishideng Publishing Group Inc**
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com