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Contents

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REVIEW

138 Immunotherapy in glioblastoma treatment: Current state and future prospects

Rocha Pinheiro SL, Lemos FFB, Marques HS, Silva Luz M, de Oliveira Silva LG, Faria Souza Mendes dos Santos C, da Costa Evangelista K, Calmon MS, Sande Loureiro M, Freire de Melo F

MINIREVIEWS

160 Integration of molecular testing for the personalized management of patients with diffuse large B-cell lymphoma and follicular lymphoma

Stuckey R, Luzardo Henríquez H, de la Nuez Melian H, Rivero Vera JC, Bilbao-Sieyro C, Gómez-Casares MT

171 Current progress on the endoscopic features of colorectal sessile serrated lesions

Wang RG, Wei L, Jiang B

ORIGINAL ARTICLE

Retrospective Cohort Study

179 Interaction between age and gender on survival outcomes in extramedullary multiple myeloma over the past two decades

Bangolo AI, Fwelo P, Trivedi C, Sagireddy S, Aljanaahi H, Auda A, Mohamed M, Onyeka S, Fisher M, Thapa J, Tabucanon EJ, Georgiev L, Wishart A, Kumari S, Erikson C, Bangura M, Paddy O, Madhukar R, Gomez EL, Rathod J, Naria M, Hajal B, Awadhalla M, Siegel D, Parmar H, Biran N, Vesole DH, Phull P, Weissman S

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Contents

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REVIEW

Immunotherapy in glioblastoma treatment: Current state and future prospects

Samuel Luca Rocha Pinheiro, Fabian Fellipe Bueno Lemos, Hanna Santos Marques, Marcel Silva Luz, Luís Guilherme de Oliveira Silva, Clara Faria Souza Mendes dos Santos, Karolaine da Costa Evangelista, Mariana Santos Calmon, Matheus Sande Loureiro, Fabrício Freire de Melo

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Abstract

Glioblastoma remains as the most common and aggressive malignant brain tumor, standing with a poor prognosis and treatment prospective. Despite the aggressive standard care, such as surgical resection and chemoradiation, median survival rates are low. In this regard, immunotherapeutic strategies aim to become more attractive for glioblastoma, considering its recent advances and approaches. In this review, we provide an overview of the current status and progress in immunotherapy for glioblastoma, going through the fundamental knowledge on immune targeting to promising strategies, such as Chimeric antigen receptor T-Cell therapy, immune checkpoint inhibitors, cytokine-based treatment, oncolytic virus and vaccine-based techniques. At last, it is discussed innovative methods to overcome diverse challenges, and future perspectives in this area.

Key Words: Glioblastoma; Immunotherapy; Tumor microenvironment; Chimeric antigen receptor T cell; Oncolytic viruses; Immune-checkpoint inhibitors; Brain cancer

Core Tip: This study aims to review the ongoing status and improvement made in immunotherapy for glioblastoma, a malignant brain tumor. Thus, this review goes through the general concepts of the tumor microenvironment, standard treatment and its limitations and immune targeting promising methods, such as Chimeric antigen receptor T-Cell therapy, immune checkpoint inhibitors, cytokine-based treatment, oncolytic virus and vaccine-based techniques. Finally, it is explained some methods to surpass the various challenges, and future prospects in this field.

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INTRODUCTION

Glioblastomas (GBM) are the most common type of malignant tumor affecting the central nervous system. It is more common among men and its incidence is significantly related to age, being rare among young people and more common among the elderly, especially those aged between 74 and 85 years. It has a very poor prognosis, with survival of 12 to 15 mo after diagnosis, and, when untreated, of only 3 mo[1].

Regarding clinical manifestations, the symptoms are quite diverse and common to other types of brain tumors and include manifestations associated with intracranial hypertension such as intense headache, which can be accompanied by nausea and vomiting, focal neurological deficits, memory and personality changes, and seizures[2].

GBMs are tumors that originate from glial cells and are classified according to their histological characteristics as high-grade gliomas by the WHO, and the characteristics that define this denomination include hypercellularity, nuclear atypia and dysregulation of mitotic activity, besides microvascular proliferation and tumor necrosis[3]. So, they are classified as primary if there is no pre-existing involvement or secondary if they have progressed from low-grade astrocytomas; primary GBMs represent the majority of cases and secondary GBMs correspond to only 5 to 10% and usually affect young people[4].

In addition to histopathological analysis, molecular markers are essential for the understanding of the disease, since different genetic alterations can originate this type of tumor and determine subtypes that behave differently in terms of evolution and response to treatments used, which makes the identification of these factors essential for the establishment of therapeutic strategies. In this sense, GBMs can be grouped into 4 subtypes according to their molecular characteristics: classic, neural, pro-neural and mesenchymal[3,4].

Among the mutations related to the pathogenesis of GBM, we can cite 3 main pathways: receptor tyrosine kinase signaling, inhibition of the p53 pathway, and RB, and in most cases all three types of alterations are present. These mutations are associated with activation of oncogenes that act mainly in neoplastic proliferation, apoptosis disturbances, and cell cycle checkpoint failures that promote tumor cell survival[5]. Moreover, when compared to a normal brain, GBMs present a higher expression of genes related to immune cell infiltration, especially macrophages, and angiogenesis, noticing that hypoxia, which is characteristic of necrotic tumor regions, induces a higher expression of vascular endothelial growth factor (VEGF) and, consequently, a higher vascular proliferation[6].

Due to the characteristics of its pathogenesis, there is a diversity of cells that are found in the analysis of these tumors, including non-neoplastic components of the immune system. This is related to the tumor microenvironment of glioblastoma, since it has an inflammatory and pro-angiogenic characteristic that affects the permeability of the blood-brain barrier and allows the infiltration of defense cells, especially tumor-associated macrophages (TAM). The immune system in the early stages of the disease is responsible for controlling the development of the cancer, however, as proliferation progresses, the tumor cells become able to escape this surveillance and the defense cells not only become unable to perform this control, but start contributing to the growth of the tumor[7].

The available treatment is complex and usually requires a combination of different approaches and is dependent on a number of factors. Although there are other options and studies for the development of new treatments, the therapeutic strategies are still controversial and the prognosis is unsatisfactory with a high recurrence rate[8].

In this review, we provide an analysis of the ongoing status and progress in immunotherapy for glioblastoma, going through the general information about the tumor microenvironment, fundamental knowledge on immune targeting to promising strategies like Chimeric antigen receptor (CAR) T-Cell therapy, cytokine-based treatment, oncolytic virus and vaccine-based approaches. Finally, we discuss

contemporary methods to prevail distinct challenges, and future perspectives in this field.

CURRENT STANDARD CARE LIMITATIONS

The treatment of primary brain tumors such as GBM is still quite limited and, therefore, a major challenge in oncology. Although the treatment is difficult, expensive and subject to therapeutic failure, management protocols for patients with GBM consider multimodal therapeutic strategies that act in synergy in order to destroy the tumor. For this, such strategies must be individualized based on each patient according to their functional status, imaging exam, speed of disease progression, quality of life and clinical diagnosis. However, for new methods to be developed and current ones to be improved, it is necessary to think about the limitations of existing treatments. The Figure 1 synthesizes the current GBM treatment strategies and its advantages and limitations.

Surgical method

The surgical method is based on the maximum safe resection of the tumor and currently comprises the backbone of therapy for GBM[9], as in addition to reducing the volume of the neoplastic mass and the symptoms associated with parenchymal compression, the histological diagnosis and genetic study of the tumor are also possible by surgical intervention[10]. The aim of surgical treatment is to achieve a gross total resection as completely and safely as possible without risking the patient's functional status. Complete resection has been associated with a greater chance of survival and no progression than partial resection or biopsy. In this sense, some tools were developed to maximize the surgical procedure and alleviate as much as possible the neurological deficits that may be associated with the method. Among these tools, monitoring using fluorescence of tumor tissue with 5-aminolevulinic acid in conjunction with functional magnetic resonance imaging shows beneficial results[10,11].

However, GBMs are not cured with surgery alone, as almost all are recurrent and the biological pleomorphism of each tumor influences the degree of resectability of the cancer, with less malignant brain tumors being the most resectable[12]. Furthermore, the surgical method is extremely complex, delicate and expensive, because it demands a qualified neurosurgeon and sophisticated imaging equipment, in addition to the fact that the patient has the possibility of developing a neurological deficit as a result of the intervention, which may even prevent the following steps of the standard treatment, such as radiotherapy and chemotherapy[13]. Thus, it is necessary to accurately weigh the risks and benefits of the surgical technique.

Radiotherapy

Radiotherapy (RT) became popular in the 1970s and 1980s and is currently a therapeutic strategy based on the use of radiation volumes focused on specific regions. This method has become standard for GBMs since 2005, as it was in that year that a phase III clinical trial solidified the role of radiotherapy and adjuvant chemotherapy in the postoperative period of GBM[14]. After the surgical diagnosis, the patient is submitted to doses of 2 Gy for 6 wk until reaching a dose of 60 Gy[13]. It is an effective method that increases patient survival in different types of doses provided, especially hypofractionated doses, which make this method viable in elderly people (over 65 years old) with glioblastoma[9].

The combination of radiotherapy for 6 wk and chemotherapy with adjuvant Temozolomide 75 mg/m² for 6 wk and 150-200 mg/m² every 28 d for 6 mo is the gold standard treatment for young patients with glioblastoma. This combination of strategies significantly improved the survival of younger patients between 2 and 5 years[14].

RT has an important limitation in the sense that its use does not have much favorable evidence in recurrent gliomas, although it is extremely useful as a palliative therapy for small recurrent tumors[15]. In addition, it is necessary to be wise in the use of radiation, since the treatment protocol requires the patient's history of previous radiation, as well as the location of the tumor and the maximum dose for the structure in which it is allocated[16]. Finally, the therapeutic algorithm assesses the speed of disease progression and the patient's functional status. Thus, the use of chemoradiotherapy is not indicated for individuals over 70 years of age who do not have a good functional status, which is measured by the Functional Status Score for the Intensive Care Unit scale[15].

Chemotherapy

Temozolomide: Temozolomide (TMZ) is an alkylating agent that is cell cycle independent and is the most effective chemotherapy for GBM to current date. This efficiency is due to the ability to cross the blood-brain barrier and transportable cytosolic transformation to the cell nucleus[17]. The current standard of care in newly diagnosed GBM includes administration of 75 mg/m² of TMZ daily during the 6 wk of radiotherapy. Then, 150-200 mg/m² are maintained for 5 d at each 28-d cycle with 6 cycles of the drug[13].

However, this therapeutic strategy is variable based on the age of the patient, performance status according to the Karnofsky performance score, the promoter methylation status of the repair enzyme O(6)-Methylguanine-DNA-methyltransferase (MGMT) and the tumor recurrence[14], since TMZ does

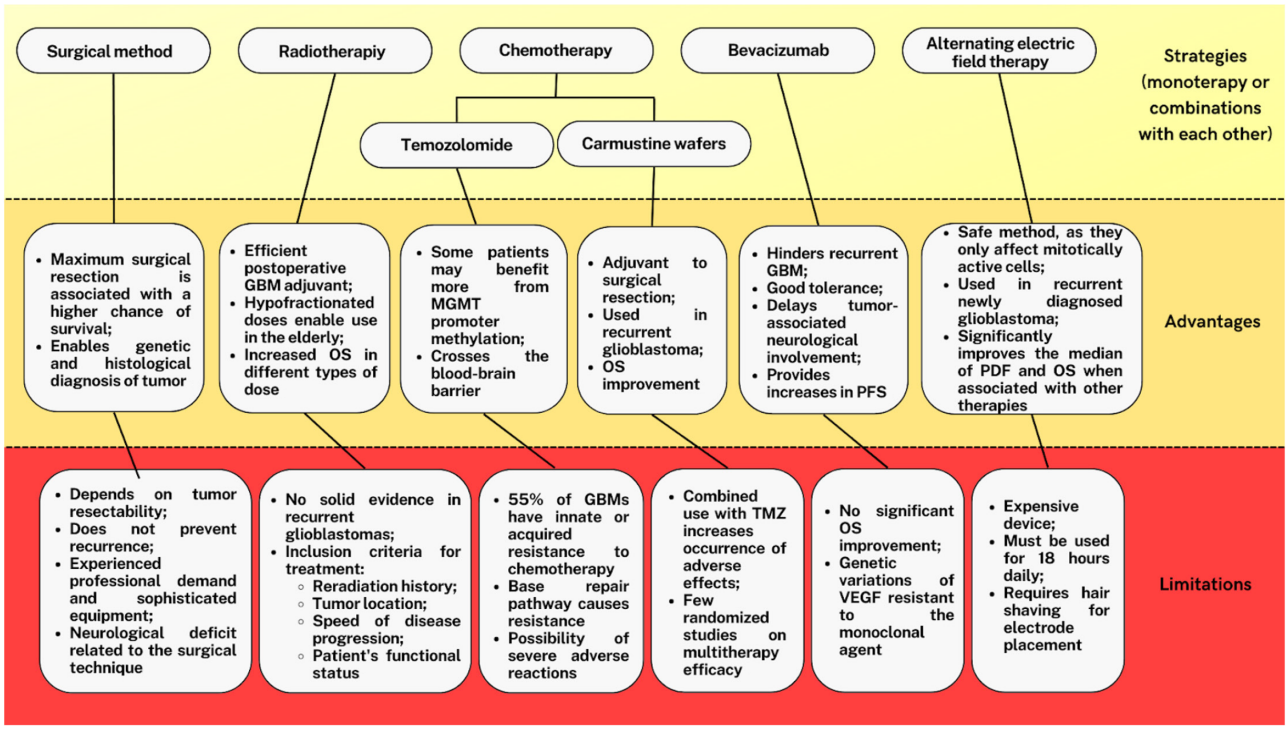

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Figure 1 Scheme about current glioblastomas treatment strategies and its advantages and limitations. GBM: Glioblastomas; OS: Overall survival; PFS: Progression-free survival; TMZ: Temozolomide; VEGF: Vascular endothelial growth factor;

not prevent this event. This enzyme can cause patient resistance to TMZ, and some patients who have MGMT gene promoter methylation in the tumor may benefit from reduced drug resistance.

About 55% GBMs[12] have innate or acquired resistance to chemotherapy due to non-methylation of the MGMT promoter. In this way, the alkyl groups are removed from the O6 position of the guanine, reducing the pharmacological efficacy of the alkylating agents[18]. Another important mechanism of resistance to chemotherapy is the reduction of TMZ cytotoxicity by the base excision repair pathway. This pathway, mainly composed of poly (ADP-ribose) polymerase-1, is capable of repairing the bases methylated by the alkylating agent in the DNA and, therefore, reducing the occurrence of apoptotic events in tumor cells[19,20]. Thus, the use of iniparib and velparib is promising, either alone or in combination with TMZ, to reduce drug resistance[20,21].

It is noteworthy that the MGMT promoter methylation status is not routinely evaluated for all patients with the discussed disease and, if evaluated, the result may not be taken into account for TMZ treatment decision making in some clinics, as there may be lower availability of treatment agents, presence of severe adverse reactions to chemotherapy, associated comorbidities and preference for treatment by the patient.

Carmustine wafers: Carmustine wafers are biodegradable chemotherapy intratumoral implants[22] used as an adjunct to surgical resection since 1995 in patients with recurrent GBM, since there is an improvement in overall survival (OS) of 7.2 mo in the carmustine group *vs* 5.4 mo in the placebo group [23]. However, its combined use with TMZ still divides authors, since some scientists believe that concomitant use is associated with an increase in the occurrence of adverse effects[24]. Therefore, it is necessary to have a randomized controlled clinical trial to support or refute the safety and efficacy of simultaneous use of carmustine wafer with TMZ.

Biological agent: Bevacizumab, a drug containing antiangiogenic monoclonal antibodies that has been in use since 2009 against the progressive form of the disease, binds to the VEGF making it difficult for recurrent GBM and rapid neurological involvement associated with the tumor, being a well-tolerated drug and capable of reducing cerebral edema, which allows a reduction in the use of corticosteroids and associated adverse effects[25].

The aforementioned drug is recommended as monotherapy or in association with other chemotherapy drugs, such as irinotecan, carmustine, lomustine, carboplatin or temozolomide[26,27], in newly diagnosed or recurrent glioblastoma. Several clinical trials over the past decade in patients with newly diagnosed GBM have shown improvements in progression-free survival (PFS), although they have not shown significant improvement in overall survival (OS). A recent study evaluated the combination of lomustine and bevacizumab in recurrent GBM and concluded with a survival of 5.1 mo [28].

However, there are genetic variations of VEGF that can determine the success or failure of bevacizumab therapy, requiring great care in the administration of this biological agent. Moreover, as the anti-VEGF method did not convincingly show improvement in OS as a monotherapy, it is necessary to evaluate the combination of this type of drug with other known therapeutic options used in neuro oncology.

Alternating electric field therapy

Tumor treatment fields (TTFs) are a therapeutic method that uses alternating currents of low intensity (1-2 V/cm) and intermediate frequency through electrodes placed on the skin around the region of a malignant tumor to stop growth and to induce apoptosis of mitotically active cells[29,30], which is considered a safe method, as it does not affect non-dividing cells.

A 2015 study revealed that the combination of TTFs and TMZ significantly improves median PFS and OS compared to TMZ monotherapy during maintenance therapy with less occurrence of electrical device-related adverse effects[31]. Current treatment guidelines incorporate TFT into the therapeutic regimen of patients with newly diagnosed and recurrent GBM[13].

However, the device is expensive, must be used at least 18 h a day and requires hair shaving of users for proper application of electrodes[32]. This can affect the patient's self-esteem and quality of life, in addition to causing a possible low adherence to treatment.

PIVOTAL ROLE OF THE TUMOR MICROENVIRONMENT

The central nervous system as an immune-distinct site

The role of the tumor microenvironment in the modulation of antitumor immune responses is becoming clearer[33]. The central nervous system (CNS) is usually described as an immune-privileged site, which means that it shows attenuated responses to alloantigen challenges[34]. Classically, the property of CNS immune privilege has been attributed to two mechanisms: (1) The blood-brain barrier (BBB); and (2) the absence of classical lymphatic drainage of CNS antigens[35]. The BBB is a semi-permeable cellular barrier composed of specialized endo-thelial cells (non-fenestrated, firmly attached by tight junctions), astrocyte end-feet, and pericytes. Its main function is to tightly regulate the movement of ions, molecules, and cells (*e.g.*, immune cells) between the blood and the brain[36,37]. The ability to block the entry of possibly neurotoxic molecules, primarily through ATP-binding cassette transporter-mediated efflux, is one of the main challenges posed to immunotherapy[38]. On the other hand, the lack of professional antigen-presenting cells in the CNS parenchyma, low expression of MHC class I and II, and the first apparent absence of classic CNS lym-phatic drainage also limit the ability of an immune response to CNS-derived antigens[39,40]. Given that efficient anti-tumor responses require not only that cancer-specific T cells be generated, but also that these T cells come into direct contact with the tumor cells, it becomes evident that the CNS provides an immune-privileged microenvironment for tumor growth and proliferation.

Fortunately, increasing evidence has pointed to the CNS, not as an immune-privileged site, but rather as an immune-distinct site that remains accessible to the onset of antitumor immune responses and immunotherapy[35]. Recent studies suggest the existence of a functional meningeal lymphatic system that drains cerebrospinal fluid (CSF), macromolecules, and immune cells from the CNS into the deep cervical lymph nodes[41]. Investigating these antigenic presentation routes will be an important step in understanding the immune-distinct properties of the GBM microenvironment.

Immunosuppressive mechanisms in GBM

Although revolutionary in the treatment of cancer patients, immunotherapy is critically dependent on the availability of preexisting anti-tumor immunity[42,43]. GBM is widely recognized to induce local and systemic immunosuppression, which is a hindrance to the use of immune-modulating therapies [44].

GBM cells can evade immune surveillance through the release of various soluble mediators that exert a variety of immunosuppressive effects[45]. The best-characterized GBM-derived immunomodulatory factors are the transforming growth factor β (TGF- β), interleukin 10 (IL-10), and prostaglandin E2 (PGE-2)[45-48]. In the presence of TGF-β, CD4+ T cells upregulate FoxP3 and differentiate into Treg cells with potent immunosuppressive potential. These converted suppressor cells not only do not respond to TCR stimulation and produce neither Th-1 nor Th-2 cytokines, but also express TGF-β and inhibit normal T cell proliferation *in vitro*[49,50]. It has also been shown that this cytokine inhibits the expression of five cytolytic gene products - specifically, perforin, granzyme A, granzyme B, Fas ligand, and interferon (IFN)-γ - which are co-responsible for CD8+ T cell-mediated tumor cytotoxicity[51]. Additionally, there is a TGF-β1-mediated downregulation of activating receptor NKG2D on the surface of CD8+ T cells and natural killer (NK) cells, thereby precluding cytotoxicity against GBM cells[52]. On the other hand, TGF-β2 can prevent neoantigen presentation and facilitate immune escape from T lymphocytes through the down-regulation of HLA-DR antigen expression on tumor cells[53]. Altogether, these immunosuppressive stimuli of T or NK cell activity prevent the effective immune-mediated clearance of tumor cells

[54,55].

IL-10 also plays a pivotal role in modulating the activity of resident and infiltrating immune cells and tumor cells in GBM, predominantly inducing an immunosuppressive phenotype[47]. Upon activation by GBM cell-derived IL-10, tumor-microglia and macrophages are then elicited to produce most of the IL-10 in the tumor microenvironment[56]. Increased secretion of IL-10 was associated with enhanced expression of other anti-inflammatory cytokines, such as IL-4, CCL2, and TGF-β[57]. In the presence of IL-10, TAMs downregulate the expression of antigen-presenting molecules, thereby impairing CD4+ T cell activation[58]. Along with TGF-β, IL-10 is also able to exert FOXP3-expressing naive T cells differentiation into Treg cells, hence leading to Treg-driven immunosuppression[59-61]. Conversely, recent data have shown that a subset of IL-10-releasing HMOX1+ myeloid cells, spatially localizing to mesenchymal-like tumor regions, also in-duce T-cell exhaustion and thus contribute to the tumor microenvironment[62].

In turn, PGE-2 has been shown as a key mediator of immunosuppressive activity through the expansion of myeloid-derived suppressor cells (MDSCs)[48,63]. VEGF, on the other hand, is the most important mediator of angiogenesis in glioblastoma, which has made it one of the main therapeutic targets in GBM treatment[64]. Finally, through the activation of hypoxia-inducible factor 1- α, hypoxia regulates the expression levels of the cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), programmed death-ligand 1 (PDL-1), and other immunomodulatory surface ligands, which hinder effective anti-tumor immune responses[65].

GBM cells can attenuate anti-tumor responses through the expression of a plethora of cell surface immunosuppressive factors, including the so-called immune Checkpoint molecules (ICs). Coupled with programmed cell death-1 (PD-1) located on the surface of activated T-cells, GBM and immunosuppressive (*e.g.*, Treg) cells membrane-bound PDL-1 can exert T-cell exhaustion and anergy[66,67]. Hence, PDL-1 upregulation in the tumor microenvironment propitiates resistance against T cell-mediated killing, in a protective process termed a "molecular shield"[68]. Conversely, the expression of the CD95 (Fas) ligand by GBM cells can also attenuate immune attack through the induction of CD95-Dependent apoptosis in infiltrating lymphocytes[69]. In turn, CTLA-4 is also an important ICs due to its capacity to compete with CD28 for binding to costimulatory molecules (CD80 and CD86) on antigen-presenting cells, thereby precluding the activation of T cells[67,68,70,71]. Lastly, indoleamine 2,3-dioxygenase 1 (IDO) and Lectin-like transcript-1 (LLT-1), are known to increase intratumoral Treg and myeloid-derived suppressor cells, and to repress NK cell activity, respectively[72,73].

Increasing evidence has reaffirmed the pivotal role of immunosuppressive monocytes, including MDSCs, and tumor-derived extracellular vesicles (EVs) in GBM-induced local and systemic immunosuppression[74]. EVs are defined as biologically active particles that carry both GBM-derived soluble factors and membrane-bound receptors that can be functionally delivered to target cells[74]. In combination with the tumor milieu, these particles can induce the conversion of monocytes to an immunosuppressive phenotype[75]. The role of EVs in direct T-cell inhibition has also been demonstrated. Ricklefs *et al*[76] recently showed that glioblastoma EVs block T cell activation and proliferation in response to T cell receptor stimulation. This mechanism of immunosuppression and its local and systemic effects have great potential for exploration in the context of immunotherapy. The Figure 2 synthesizes the GBM-induced immunosuppressive microenvironment.

CYTOKINE THERAPY

Cytokine therapy in the treatment of GBM is based on the use of pro-inflammatory cytokines, in order to promote reversal of the immunosuppressive microenvironment triggered by this tumor and subsequent activation of the immune response[76,77]. Mainly, IFN-α, TNF-α and IL-12 have been assessed as possible therapeutic options for glioblastoma[78,79]. In this sense, IFN-α is related to increased activity and reduced exhaustion of T cells and macrophages, besides inhibiting tumor angiogenesis and immune suppression-related gene expression[79]. On the other hand, TNF-α promotes dendritic cells maturation and, consequently, T cell stimulation, while IL-12 is related to enhanced CAR-T cell efficacy, increased infiltration of CD4+ T cells and decreased frequency of T-regulatory cells in the tumor microenvironment[80,81]. Nevertheless, the therapy with IFN-α presents high toxic systemic potential and low efficiency in maximum tolerated doses[82]. The possibility of collateral effects implies a damage to the user, clinical trials reveal hyperthermia, shivering, headaches, gastrointestinal symptoms, decline in systolic and diastolic blood pressure and associated orthostatic hypotension[83]. This means that the therapy is a resource with limited use at least at this moment. It is expected that, in the future, this route will be used in conjunction with other therapeutic forms, such as inhibitors of anti-apoptotic proteins, to increase efficacy and tolerability[84]. In another perspective, glioma cells infected by a vector capable of transducing TNF-α decreased tumor growth rate in a mouse animal model, which constitutes a different therapeutic strategy for the treatment[82]. Additionally, the administration of TNF-α is also a problem to solve because the intravenous administration is known for the capacity to induce toxicities for the patients[76]. Recently, the discover of a interleukin-7 agonist had shown the ability to repair the lymphopenia caused by the standard treatment for GBM and also improved the

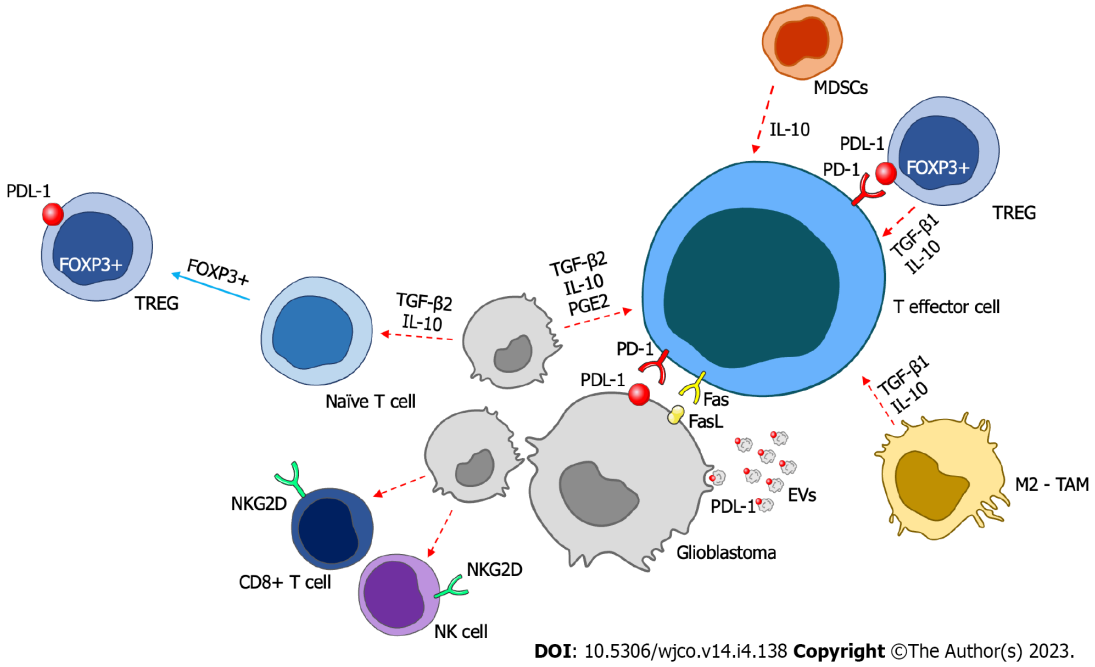

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Figure 2 Simplified scheme of glioblastomas-induced immunosuppressive microenvironment. MDSCs: myeloid-derived suppressor cells; NK: Natural killer. The Figure was partly generated using Servier Medical Art, provided by Servier, licensed under a Creative Commons Attribution 3.0 unported license.

immune system by elevating the CD8 serial lymphocytes in murine models, but this discover needs more studies to be apply for patients with this primary glioma[85].

IMMUNE CHECKPOINT INHIBITORS

Immune checkpoints are molecular receptors that perform an inhibitory function in order to control exacerbated immune activity and prevent uncontrolled activity of this system[86]. These receptors are found on T cells (CD4 and CD8), dendritic cells (DC), NK cells and B cells[87].

Cancer cells have some mechanisms that allow them to reduce the effectiveness of the immune system during the attack on mutated cells[88]. One of these mechanisms is the expression of molecules that interact directly with the immune checkpoint receptors resulting in reduced immune activity from the inhibition of essential cells of the protection system. Thus, immune checkpoint inhibitors have emerged as a therapeutic alternative, in order to prevent the occurrence of inhibition of immune cells from the interaction of receptors of these cells and molecules produced by glioblastoma cancer cells[87].

In this regard, studies have identified the main receptors of immune checkpoints and that have physiological importance in glioblastoma. PD-1, T cell immunoglobulin and mucin domain 3 (TIM3), CTLA4, lymphocyte activation gene 3 (LAG3), T-cell immunoglobulin and ITIM domain (TIGIT) and CD96 are inhibitory receptors expressed on immune system cells, such as lymphocytes (T and B) and NK, and have corresponding ligands produced by cancer cells[87].

Thus, studies aimed at blocking the immune checkpoint in glioblastoma have been initiated[89,90]. A study conducted in murines, associated anti-PD-1 and temozolomide (chemotherapeutic agent used in the treatment of GBM) in the treatment of glioblastoma and obtained a good antitumor efficacy[89]. However, the response in humans did not show the same efficacy, as evidenced by the randomized phase III clinical trial of 369 patients diagnosed with GBM who were treated with nivolumab (anti-PD-1) and did not show improved survival compared to the control group[90]. However, the preclinical trials are promising and the therapeutic model is still recent. This means that therapy based on blocking ICIs may yet yield an important efficiency in the lives of patients diagnosed with GBM. In Figure 3, there is a representation of immune checkpoint inhibition targets: TIM-3/Galactin 9 (GAL-9), PD-1/PDL-1, and CTL-4/CD80 or CD86.

PD-1/PD-L1

The PD-1 receptor is expressed on T cells, B cells, TAMs, MDSCs and NK cells[91]. For inhibition of these cells to occur the PD-1 receptor interacts with PD-L1, which is expressed on GBM tumor cells. This interaction results in T-cell apoptosis, inhibition of T-cell cytotoxicity, and blockage of inflammatory mediator production. Thus, immunotherapy aims to target the PD-1/PD-L1 pathway and generate an antitumor response[87].

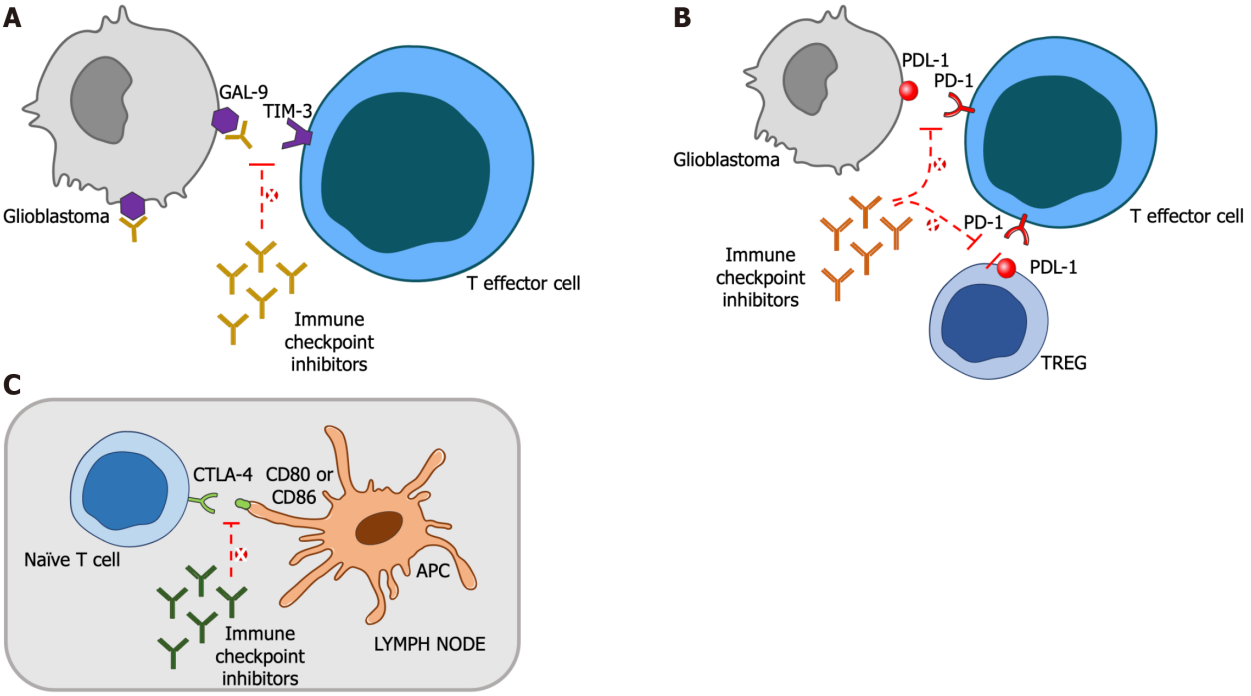

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Figure 3 Immune checkpoint inhibition targets: T cell immunoglobulin and mucin domain 3/ Galactin 9, programmed cell death-1/programmed death-ligand 1, and cytotoxic T-lymphocyte-associated protein 4 /CD80 or CD86. A: T cell immunoglobulin and mucin domain 3/ Galactin 9; B: programmed cell death-1/programmed death-ligand 1; C: cytotoxic T-lymphocyte-associated protein 4/CD80 or CD86. TIM-3: T cell immunoglobulin and mucin domain 3; GAL-9: Galactin 9; PD-1: Programmed cell death-1; PDL-1: Programmed death-ligand 1; CTLA-4: Cytotoxic T-lymphocyte-associated protein 4. The Figure was partly generated using Servier Medical Art, provided by Servier, licensed under a Creative Commons Attribution 3.0 unported license.

The anti-PD-1/PD-L1 class is a category that includes pembrolizumab, nivolumab, durvalumab and atezo-lizumab[92]. These ICIs have shown good results in some types of cancer, such as melanoma and non-small cell lung cancer[93,94], but for GBM, the overall efficacy is not yet optimal, especially in monotherapy, since GBM is a disease with unique peculiarities. However, studies using combination therapy with other ICIs are ongoing and have brought positive preliminary results, despite difficulties that still need to be overcome[92]. One of these challenges is the need for these ICIs to cross the blood brain barrier, which is very peculiar to brain tumors and makes chemical therapy of this type of cancer difficult[95].

TIM3/GAL9

TIM3 is a membrane protein, normally found on CD4+ and CD8+ T lymphocytes, and is also an inhibitory receptor for antitumor T cell activity[11]. GAL9 is a binding protein to TIM3. This binding results in the activation of the TIM3/GAL9 pathway, which induces T cell apoptosis, a fact that directly impacts antitumor immune activity[96,97].

The expression of GAL9 is higher in tissues from glioma patients and the TIM3/GAL9 interaction is involved with a higher malignancy of this type of CNS tumor. Thus, TIM3 has also become a potential target of immune checkpoint inhibitors in an attempt to boost immune activity against tumor invasion and result in a better prognosis for the patient[97].

CTLA4

CTLA4 is an inhibitory receptor expressed on T cells and has relevance when dealing with GBM and a worse prognosis of this disease from the activation of this receptor[70]. The process is based on the interaction of T cells with antigen-presenting cells in the peripheral lymphatic tissue through co-stimulatory and coinhibitory receptors, such as CTLA4[98]. CTLA4 binds to CD80/CD86 receptors on antigen-presenting cells. Thus, this receptor is involved with the initial process (antigen presentation) of immune activity and its activation reduces the activation and proliferation of antigen-specific T cells that will act directly on the CNS and tumor cells[87].

CTLA4 has a higher expression in more serious gliomas and is related to a worse disease prognosis, as it is related to reduced antitumor immune activity[71].

Based on this, in 2011, the Food and Drug Administration approved the use of ipilimumab in the therapy of some tumors. Ipilimumab is a monoclonal antibody that binds to CTLA4 receptors and blocks the inhibition of T cells that occurs through this molecule[87].

LAG3

LAG3 is a regulatory protein expressed on the membrane of T cells and when activated by specific ligands, it generates an inhibitory effect on immunity. It is believed that one of these ligands is FGL1 and that it is expressed by cancer cells and induces a decrease in antitumor activity, but this mechanism is still not well known, especially in relation to gliomas[99].

In addition, it is possible that LAG3 generates immunosuppression by acting in conjunction with other immune checkpoints, such as PD-1[99]. A process that has already been reported in breast cancer studies, which identified a co-expression of LAG3 and PD-1 in the tumor process, generating T-cell inhibition[100].

TIGIT/CD96

TIGIT and CD96 are co-inhibitory receptors[87]. TIGIT is expressed on various immune cells such as T cells, regulatory T cells (Tregs) and natural killer (NK) cells[101]. CD96, on the other hand, has been found mainly on conventional T cells, NK cells and NKT cells[87].

High expression of PD-1 and TIGIT was found in CNS infiltrating lymphocytes, acting at the site of GBM[101]. Thus, a combined blockade therapy for PD-1 and TIGIT has shown improved efficacy and survival for patients with GBM[101].

CD96 is directly linked to the inflammatory response in GBM and additionally, a direct and synergistic correlation of this receptor with other immune checkpoints such as PD-1, CTLA-4, TIGIT and TIM-3 has been described[102]. With this, it was found that a simultaneous blockade of CD96 and other ICIs results in enhanced antitumor immunity and better prognosis[102].

CAR T-CELL THERAPY

Chimeric antigen receptors are synthetic receptors capable of redirecting the immune functions of T lymphocytes to a specific target antigen and thus, T cells exert short and long-term effects by triggering complex antitumor responses[103]. CAR-Ts have an extracellular domain with a tumor binding site as the single-chain variable fragment (scFv), a flexible hinge, a transmembrane region, and an intracellular signaling domain of T cells. In addition, CARs can be subdivided, according to the amount of CD3ζ stimulatory domains, into first, second and third generation, and the most modern CARs have two costimulatory domains linked to CD3ζ in order to potentiate its ability of signaling activation[104]. Since CAR-Ts has been used effectively against hematological tumors, the objective is to adapt the method for solid tumors such as GBM so that the activation of T cells in the tumor microenvironment promotes targeted immunological mechanisms of cell death to specific targets in the tumor, achieving the same success as the treatment in non-solid tumors, regardless of the presentation of the peptide by histocompatibility complexes[105]. The most promising studies addressing T cell therapy against GBM have explored CAR-T cells targeting human epidermal growth factor receptor 2 (HER2), variant epidermal growth factor receptor III (EGFRvIII) and alpha receptor 2 of IL-13 (IL-13 Rα2) mainly, as well as evaluating the different forms of therapy administration (local or systemic)[106-108].

EGFRvIII consists of an oncogenic mutation pattern existing in human tumors that allows the identification of specific tumor antigens by the immune system. EGFRvIII is relatively common, especially when it comes to GBM, in which the mutation is present in approximately 30% of scenarios[109]. EGFRvIII expression in patients with GBM is considered a marker of poor prognosis probably because the receptor enhances tumor oncogenic signaling[110]. In this sense, the first clinical study that investigated CAR-Ts therapy directed at EGFRvIII was conducted by O'Rourke *et al*[107] and evaluated 10 patients with recurrent EGFRvIII + GBM. The results demonstrated that the administration of CAR-Ts Cells by infusion is a safe route to be used, as there was no evidence of toxicity outside the tumor microenvironment or cytokine release syndrome. Although the study did not have the objective of evaluating the effectiveness of the therapy, it was observed that no patient had GBM regression and one patient remained in stable disease for more than 18 mo. Therefore, the assay also revealed a consistent response with immunological checkpoints and immunosuppressive molecules such as IDO 1, PD-L1, TGF-β and IL-10 and this indicated that EGFRvIII+ led to an antitumor response[107]. Complementarily, a recent study evaluated apheresis and infusion products from the previous study to explore EGFRvIII as a therapeutic target for GBM and concluded that PD1 is a predictive marker of peripheral graft and progression-free survival in transduction products of patients with targeted CAR-Ts to EGFRvIII. Furthermore, it was also observed that PD1 was expressed concomitantly with ICIs (CTLA4, TIM3, LAG3) and activation markers (GRZB, HLA-DR) suggesting that PD1 is the protagonist of these correlations with the clinical response surrogates in the study. However, the aforementioned correlations were not present before the generation of CAR-Ts. Therefore, it has been proposed that the PD1 marker may predict better response to therapy against recurrent GBM and that the preparation of the infusion product is responsible for the differences in therapeutic results found in the study[111].

HER2 is also a tumor-associated antigen that is expressed by about 80% of GBM, however, the receptor is also expressed in physiological host cells and this gives HER2 the potential to generate autoimmunity when used as a specific target antigen[112]. An early trial involving HER2 CAR T Cells in

cancer patients did not produce positive effects. The study was associated with acute toxicity with fatal outcome in one patient[113]. However, a subsequent preclinical study yielded a more favorable outcome as CD28-costimulated HER2-CER T cells were tolerated by 17 patients with GBM without dose-associated toxic effects. Trial findings showed that one patient had a partial response to therapy for 9 mo, 7 remained with stable disease for 8 wk to 29 mo, and 8 had tumor progression. Additionally, patients had an overall survival of about 11 mo from T cell infusion (95%CI: 4.1–27.2 mo) and HER2 CAR T cells were present in blood at up to one year of follow-up[106]. IL-13Rα2 is another tumor-associated antigen that is expressed in up to 50% of GBM and despite being expressed in normal tissue, it is not expressed at significant levels in normal brain tissue[114,115]. Interestingly, the first trial that evaluated the safety and feasibility of CAR-T-s targeting IL-13Rα2 for the treatment of recurrent GBM was done by Brown *et al*[116] and included three patients with the malignancy. Among the three patients included, one had reduced global expression of IL-13Rα2 in the tumor after treatment and another patient showed an increase in the necrotic portion of the tumor where IL-13-zetacin + T cells had been administered. Despite the small sample, the findings of the work were favorable and were fundamental for the advancement in knowledge about the therapeutic method[116]. In this regard, new initial studies, albeit promising, have emerged with the aim of improving the CAR-Ts. Some works, for example, such as that of Muhammad *et al*[117], validated a new TanCAR [IL-13 (4MS) and EphA2 scFv] that proved effective in destroying GBM cancer cells recognizing IL-13Rα2 or EphA2 receptors and did not damage normal IL-13Rα1/ IL-4Rα. Therefore, it proved to be an option with the potential to remedy difficulties in current therapy by preventing antigen escape and reducing extra tumor toxicity[117]. In addition, another initial work constructed an IL-13Rα2 directed to humanized third-generation CAR and evaluated its efficacy against GBM *in vitro* and reported that the receptor achieved satisfactory results that support its use in clinical trials[118].

Therefore, CAR-T-s therapy targeting specific antigens is very promising and has the potential to become a therapeutic option for solid malignancies with poor prognosis such as GBM. However, the evidence is still limited, which creates a series of challenges to be overcome by the therapeutic method. The main obstacles to a safe and effective CAR-Ts therapy are the access of immune cells to the CNS and the heterogeneity of the tumor microenvironment. The first is mainly due to the existence of the endothelial blood-brain barrier and the epithelial blood-brain barrier[119]. The second occurs because GBM is characterized by a complex and active tumor microenvironment capable of evading the functionality of CAR-T-s, as well as hindering the recognition of a single specific target antigen[120]. In this regard, one way to improve access to the CNS would be to add property to CAR-T cells through gene editing. The development of innovative CAR-Ts that can target different tumor-associated antigens or program different CAR-Ts to recognize a single tumor-associated antigen is a possible solution to immune escape or target antigen escape. A recent study targeted 3 antigens using a single universal tricistronic (U) transgene product of CAR-T-s specific for HER2, IL-13Rα2 and EphA2 showing an effective alternative to the interpatient variability that is one of the obstacles to therapy. The *in vitro* test of the study showed an improvement in the survival of the animals, corroborating the initial hypothesis [121]. The work by Muhammad *et al*[117], cited above, starts from the same premise that the new TanCAR destroyed tumor cells by recognizing both IL-13Rα2 and EphA2 alone or together, also corroborating for a more effective therapy by avoiding immune escape and recognition of non-target antigens. Another possibility to deal with difficulties in therapy with CAR-Ts cells is the remodeling of immune cells in the tumor microenvironment. This technique is based on the use of CAR-T cells with the objective of recruiting pro-inflammatory cytokines, mainly OL-7, IL-8 and IL-12, enhancing the death of GBM cells[122-124]. In addition, the blocking of immune suppression signals through chimeric decoy and switch receptors has also been explored. For example, Liu *et al*[125] added genetically modified switch receptors including the extracellular domain of PD1 and the transmembrane and cytoplasmic signaling domains of CD28 in order to stimulate the performance of CAR-T cells in solid tumors and the study data revealed a strategy potentially efficient therapy. Finally, the expansion of the use of bispecific T cell couplers (BiTE) in combination with CAR-T cells as a new artifice for the recognition of multiple antigens has also been discussed[126]. Bearing in mind that EGFRvIII-specific CAR-T cells may not be satisfactorily efficient in view of the heterogeneity of the GBM tumor microenvironment, Choi *et al*[127] proposed the use of CARBiTE cells capable of secreting wild-type EGFR-specific BiTEs. The results of the initial study were positive and showed that BiTE cells annihilated heterogeneous GBM tumors in mice and did not promote toxicity against human skin grafts *in vivo*.

ONCOLYTIC VIRUSES

Over the last few years, oncolytic viruses (OVs) have gained prominence in tumor treatment, including GBM. OVs are particularly suitable for GBM therapy due to its privileges, such as lack of distant metastasis and tumor's limitations, allowing the use of viruses at this site as a promising form of immunotherapy[128]. They are administered intravenously or intratumorally to achieve its neutralizing effects.

OVs can be defined as weakly pathogenic viruses that can selectively infect, replicate in, and kill cancer cells without damaging normal cells and leading to tumor cells apoptosis[129]. This occurs through antitumor reactions of tumor-specific cell killing and the induction of the host's systemic antitumor and/or antiviral immunity. Thus, OVs activate the innate immune system *via* pattern recognition receptors and pathogen-associated molecular patterns, leading to a physiological response of immune cells recruitment, such as neutrophils, natural killer cells, macrophages, Th1 cells and its associated cytokines that promotes cell lysis[128,130]. Moreover, this response induces an adaptive immune reaction to new cancer antigens and may possibly develop a long-term immunotherapy repercussion[131]. Besides this, OVs can also be used as non-replicating viral vectors to deliver therapeutic genes, serving as vehicles to efficiently achieve tumor cells[104]. In Figure 4, there is a graphical representation of how OV therapy for GBM works.

Currently, OVs are being tested for their effectiveness against GBM in leading clinical trials using over 20 distinct viral strains like herpes simplex virus[132], adenovirus[133], measles virus[134], parvovirus[135], Newcastle disease virus[136], reovirus[137], poliovirus[138] and zika virus[139]. In Table 1, the clinical trials using virotherapy for GBM are summarized.

As aforementioned, the cooperation of the innate and adaptive immune systems is crucial in oncolytic virotherapy response, and matching it with other immunotherapy strategies such as checkpoint inhibitors increases the immunological response and tumor regression[140-142].

VACCINE-BASED THERAPY

In recent years, it has been discussed the great possibility of combating and stabilizing oncological conditions through immunotherapy, and the proposal of vaccine therapies is a remarkable point. In this sense, when thinking about GBM, the proposal of an alternative therapy that generates a more positive prognosis for patients, through vaccination, is a matter of much research and debate.

Many vaccines with a variety of immunological bases have been developed and tested in the treatment of GBM. There are four commonly used approaches to base GBM vaccines on: Peptide and DNA vaccines, which use genetic information from the tumor itself, and are more specific in their use. Cellular vaccines, based on dendritic cells prepared also with tumor antigens, and mRNA-based ones, with viral vectors[143]. In general, the principle behind this bet is on the immune response, thinking about the ability of the tumor to evade the individual immune response.

Thus, one of the ways found to "combat" this disease is to use the immune system itself, more specifically, a response coordinated by T lymphocytes capable of recognizing tumor antigens and reacting against them. In this sense, the initial proposal aims to use specific tumor antigens (TSAs) to obtain an immune response, having as a basis for this process peptides based on the tumor characteristics that trigger an anti-tumor immune response by mimicking neoantigens in glioblastoma cells[144,145].

Personalized neoantigen vaccines are a different approach to anti-tumor vaccine development, with trials already showing increased survival in patients with a recent diagnosis of GBM, demonstrating a potential to alter the immune environment in GBM[85].

However, there are some points of conflict within this vaccine therapy, since the tumor heterogeneity, with factors expressed differently among individuals, which would generate a high specificity in the manufacture of the vaccine, a need for customization, not being extremely effective on a large scale, hindering the inclusion of patients[146]. This treatment also has a limitation, generated by antigenic escape in the face of tumors that do not express this antigen. In addition, the collection of peptides for the vaccine base, meets a barrier, since the association of a disparate tumor profile, with possible formations of nonspecific epitopes - a tumor formation not from mutations, but from exacerbated expressions of factors that are expressed in normal tissues - raises a predisposition to responses beyond the tumor affection, such as autoimmune responses and inflammatory processes in other regions[146].

Another point of study that has been gaining prominence are DC vaccines, being considered one of the most promising at the moment. This is due to the role they play in immune regulation and in the GBM picture. Thus, they are extremely important for the induction of acquired immunity, also influencing the lymphocytic response, its differentiation, and antigen presentation. With this in mind, within GBM pictures, DCs are found with reduced function, being in an inhibited or immature state, which can be related to the severe tumor microenvironment, DCs are kept with low function due to the inhibitory effect of the immune microenvironment, and this status is problematic for body function, but reversed by DC vaccines[147]. This is due to the fact that the advantages of DCs vaccines are based on *in vitro* matured dendritic cells, usually from the affected individual himself, which can activate previously inhibited Ts lymphocytes, increasing the patient's adaptive response, increasing the expression of MHCs, cytokines and chemokines, and promoting an intense migration of immune cells to the immunosuppressive microenvironment found in GBM[147].

Currently, some studies have shown that DC vaccines can improve the picture of GBM, with some age-related factors seeing a better prognosis in younger patients. Another study, in phase II clinical trial, showed that the use of the vaccine after tumor resection, obtained a median overall survival of 23.4 mo,

Table 1 Ongoing and completed clinical trials of oncolytic virus therapy in glioblastoma

NCT Number	Title	Status	Enrolled patients	Interventions	Country	Phase
NCT03714334	DNX-2440 Oncolytic Adenovirus for Recurrent Glioblastoma	Unknown status	24	Drug: DNX-2440 injection	Spain	Phase 1
NCT03294486	Safety and Efficacy of the oncolytic virus Armed for Local Chemotherapy, TG6002/5- FC, in Recurrent Glioblastoma Patients	Unknown status	78	Drug: Combination of TG6002 and 5-flucytosine (5-FC, Ancotil®)	France	Phase 1 and 2
NCT02197169	DNX-2401 With Interferon Gamma (IFN-#) for Recurrent Glioblastoma or Gliosarcoma Brain Tumors	Completed	37	Drug: Single intratumoral injection of DNX-2401; Drug: Interferon-gamma	United States	Phase 1
NCT01956734	Virus DNX2401 and Temozolomide in Recurrent Glioblastoma	Completed	31	Procedure: DNX2401 and Temozo-lomide	Spain	Phase 1
NCT05095441	A Clinical Study of Intratumoral MVR-C5252 (C5252) in Patients With Recurrent or Progressive Glioblastoma	Not yet recruiting	51	Biological: C5252	United States	Phase 1
NCT01174537	New Castle Disease Virus (NDV) in Glioblastoma Multiforme (GBM), Sarcoma and Neuroblastoma	Withdrawn	0	Biological: New castle disease virus	Israel	Phase 1 and 2
NCT01491893	PVSRIPO for Recurrent Glioblastoma (GBM)	Completed	61	Biological: Recombinant nonpathogenic polio-rhinovirus chimera (PVSRIPO)	United States	Phase 1
NCT00028158	Safety and Effectiveness Study of G207, a Tumor-Killing Virus, in Patients With Recurrent Brain Cancer	Completed	65	Drug: G207, an oncolytic virus	Not provided	Phase 1 and 2
NCT03896568	MSC-DNX-2401 in Treating Patients With Recurrent High Grade Glioma	Recruiting	36	Biological: Oncolytic Adenovirus Ad5- DNX-2401; Procedure: Therapeutic conventional surgery	United States	Phase 1
NCT01582516	Safety Study of Replication competent Adenovirus (Delta-24-rgd) in Patients With Recurrent Glioblastoma	Completed	20	Biological: Delta-24- RGD adenovirus	Netherlands	Phase 1 and 2
NCT03072134	Neural Stem Cell Based Virotherapy of Newly Diagnosed Malignant Glioma	Completed	13	Biological: Neural stem cells loaded with an oncolytic adenovirus	United States	Phase 1
NCT01301430	0 Parvovirus H-1 (ParvOryx) in Patients With Progressive Primary or Recurrent Glioblastoma Multiforme.	Completed	18	Drug: H-1PV	Germany	Phase 1 and 2
NCT05084430	Study of Pembrolizumab and M032 (NSC 733972)	Active, not recruiting	28	Drug: M032; Drug: Pembrolizumab	United States	Phase 1 and 2
NCT02031965	Oncolytic HSV-1716 in Treating Younger Patients With Refractory or Recurrent High Grade Glioma That Can Be Removed By Surgery	Terminated	2	Biological: Oncolytic HSV-1716; Drug: Dexamethasone; Procedure: Therapeutic conventional surgery	United States	Phase 1
NCT02798406	Combination Adenovirus + Pembrol-izumab to Trigger Immune Virus Effects	Completed	49	Biological: DNX-2401; Biological: Pembrolizumab	United States	Phase 2
NCT03657576	Trial of C134 in Patients With Recurrent GBM	Active, not recruiting	24	Biological: C134	United States	Phase 1
NCT03152318	A Study of the Treatment of Recurrent Malignant Glioma With rQNestin34.5v.2	Recruiting	62	Drug: rQNestin; Drug: Cyclophos-phamide Procedure: Stereotactic biopsy	United States	Phase 1
NCT03043391	Phase 1b Study PVSRIPO for Recurrent Malignant Glioma in Children	Active, not recruiting	12	Biological: Polio/ Rhinovirus Recombinant (PVSRIPO)	United States	Phase 1
NCT05139056	Multiple Doses of Neural Stem Cell Virotherapy (NSC-CRAdS-pk7) for the Treatment of Recurrent High-Grade Gliomas	Withdrawn	0	Biological: Neural Stem Cells expressing CRAdS-pk7; Procedure: Resection	Not provided	Phase 1
NCT02062827	Genetically Engineered HSV-1 Phase 1 Study for the Treatment of Recurrent Malignant Glioma	Active, not recruiting	24	Biological: M032 (NSC 733972)	United States	Phase 1
NCT04482933	HSV G207 With a Single Radiation Dose in Children With Recurrent High-Grade	Not yet recruiting	40	Drug: Biological G207	United States	Phase 2

	Glioma					
NCT02986178	PVSRIPO in Recurrent Malignant Glioma	Active, not recruiting	122	Biological: PVSRIPO	United States	Phase 2
NCT03911388	HSV G207 in Children With Recurrent or Refractory Cerebellar Brain Tumors	Recruiting	15	Biological: G207	United States	Phase 1
NCT02457845	HSV G207 Alone or With a Single Radiation Dose in Children With Progressive or Recurrent Supratentorial Brain Tumors	Active, not recruiting	13	Biological: G207	United States	Phase 1
NCT00528684	Safety and Efficacy Study of REOLYSIN® in the Treatment of Recurrent Malignant Gliomas	Completed	18	Biological: REOLYSIN®	United States	Phase 1
NCT03973879	Combination of PVSRIPO and Atezolizumab for Adults With Recurrent Malignant Glioma	Withdrawn	0	Biological: PVSRIPO; Drug: Atezolizumab	Not provided	Phase 1 and 2
NCT00314925	Safety Study of Seneca Valley Virus in Patients With Solid Tumors With Neuroendocrine Features	Unknown status	60	Drug: Seneca Valley virus (biological agent)	United States	Phase 1

Most data were obtained from findings from www.clinicaltrials.gov using the search terms "glioblastoma" and "oncolytic" filter.

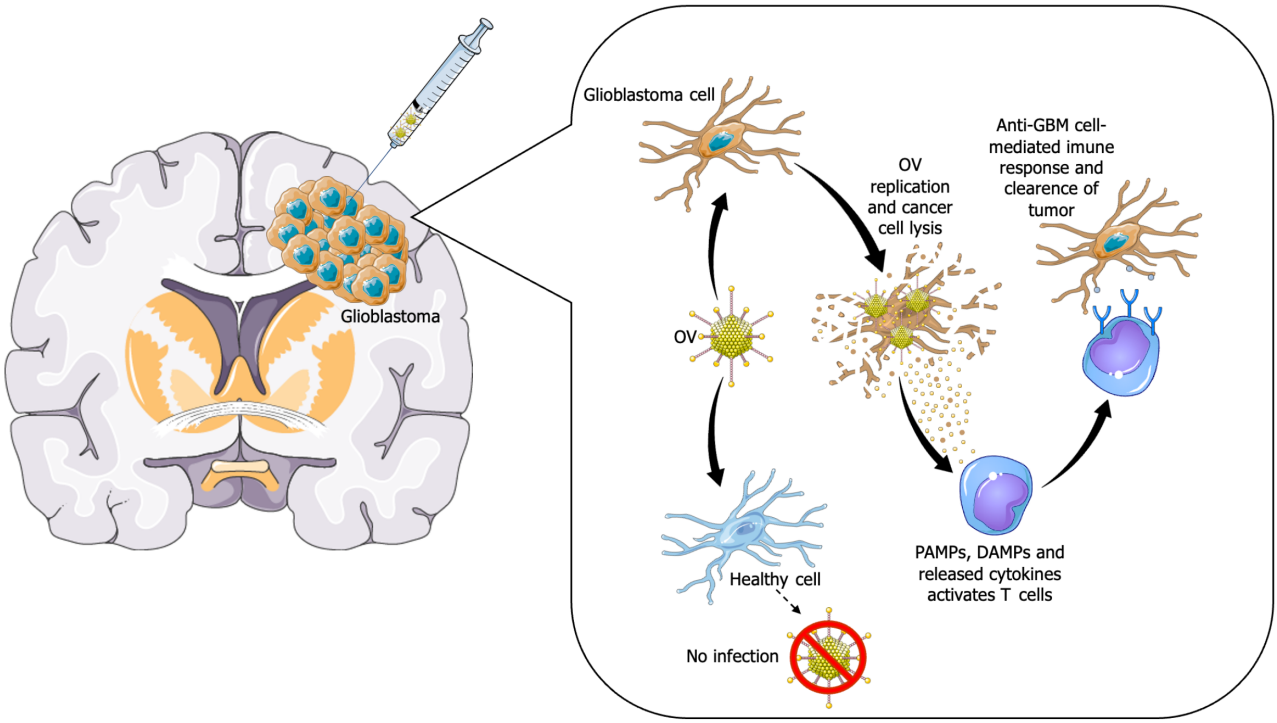

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Figure 4 Simplified scheme of oncolytic virotherapy for glioblastomas. GBM: Glioblastoma; OV: Oncolytic virus; PAMPs: Pathogen-associated molecular patterns; DAMPs: Damage-associated molecular patterns. The Figure was partly generated using Servier Medical Art, provided by Servier, licensed under a Creative Commons Attribution 3.0 unported license.

among some patients[85]. However, a meta-analysis of randomized controlled trials on the efficacy of DC vaccines demonstrated that the use of the vaccine in newly diagnosed glioblastoma patients did not show a substantial effect on overall patient survival[148]. Thus, it is still an area that needs more studies and trials with more advanced phases, and the ability to inhibit glioma is still a point to be better tested in future studies.

Some other vaccine ideas have already been proposed, such as using isocitrate dehydrogenase as the basis for the vaccine, since mutation in this enzyme occurs purely in tumor cells, making it an interesting tumor-specific antigen to use[146]. In addition, vaccines that inactivate tumors are also an attraction for research, given their success in other pathologies, not only in treatment but also in prevention, but there is still a low efficiency for the treatment of neoplasms, requiring more research for the development and application in GBM. More advanced research is needed for the use of these other

vaccine approaches.

Another alternative attempt for the treatment of GBM, are oncolytic virotherapies, using previously known viruses, which would be injected intratumorally, enabling an inflammatory reaction and an immune response against the tumor-virus unit. Many researches and vaccines have already been approved with this type of technology, and it is a promising therapy that acts both by selectively infecting tumor cells, replicating and leading to tumor death, and by being used to transport factors for gene therapy, through viruses with alterations in their replication[104]. Regarding GBM, some vaccines, such as DNX-2401, have already gone through initial testing phases and showed positive results. However, updates of the studies are needed to better understand the spectrum and efficiency of the action of this vaccine. In addition, other vaccines are under study such as ParvOryx, Toca 511, Reovirus, and HSV type 1, being tested in patients with GBM, but still in early stages of testing[86].

Furthermore, vaccination focused on eliminating EGFRvIII is also an important resource against GBM, as it is an important TSA in this pathology[146]. Thus, the EGFRvIII anti-tumor vaccine is another interesting therapy. Some late stage studies were able to observe a good humoral induction and cytotoxic T response with the use of this TSA, after good conduct in animal studies. However, the results were not as significant as expected in survival and remission rate, in human trials[146]. Besides that many adverse effects have been found such as seizures, edema, thrombocytopenia and pulmonary embolism, and these complications when coupled with the fact that not all GBM patients express EGFRvIII, become a limitation for this therapy, since not all patients could use this vaccine[140].

The benefits of vaccination are already found in some studies, demonstrating an increase in patient survival when compared to other measures used, including the surgical approach, demonstrating the advances in this research[148,149]. However, only 3 vaccination agents have reached phase III clinical trial: Rindopepimut, DCvax and PPV[143].

Thus, the key point for vaccine therapy is the choice of the appropriate immune target with a reduction of vaccine toxicity. The search for TSA and possible alternatives must take into account the immune alterations caused by the tumor microenvironment, the immune status of the affected individual and possible adverse effects, which need to be reduced to their maximum. Moreover, there is a very important factor, even with the momentary trend towards personalized vaccines, the questioning of how to make this new reality feasible, generates a need to search for a combination of antigens of greater spectrum, having in mind also, how the vaccine process will reverberate in the organism, thinking about a long-term immune response, and what are the predictions for the future, which makes the development of studies with more solid results indispensable[143]. In addition, the possibility of combining vaccines with other immunotherapies has shown considerable benefit when compared to the use of some vaccines alone, and needs to be further investigated as an approach to be considered in patient management[86,104].

IMMUNOTHERAPY LIMITATIONS AND CHALLENGES

Immunotherapy options currently available for the treatment of GBM are vast. These include vaccines, oncolytic viruses, immune checkpoint inhibitors, and genetically modified T cells[85]. In this sense, the various ongoing studies and clinical trials may provide favorable outcomes in expanding the use of these therapies in the near future, and, given the potential to manipulate or enhance the immune system apparatus to attack and kill tumor cells, immunotherapy has enlightened and generated a lot of excitement in the treatment of GBM. However, so far, there are some limiting factors that hinder the applicability of immunotherapy in the treatment of glioblastoma, whether related to individual anatomical and immunological factors or to routes of administration and adverse effects[140-142].

The blood-brain barrier is one of the major limitations to GBM immunotherapy. These specialized endothelial cells attached to astrocytes and pericytes hinder drug delivery, leading to inefficient therapeutic action[104,150]. Also, GBM is able to induce alterations in the BBB, forming a structurally different barrier (i.e., brain tumor barrier) that also contributes to poor penetration of therapeutic agents [77]. Furthermore, intratumoral heterogeneity plays a pivotal role in immunotherapy resistance, given the rapid growth of resistant clones after the selective destruction of susceptible ones[151]. The immunosuppressive microenvironment of this tumor also poses a challenge in the immunotherapeutic approach[152]. Treg cell upregulation leads to inhibition of effector T cells, thus impairing the use of CAR-T cells[145]. Regarding cytokine therapy, despite its ability to modulate the microenvironment of GBM, leading to increased DC cells maturation, T cell infiltration and reduced exhaustion[81], its systemic use presents severe toxicity and poor absorption, which greatly hampers the use of this therapy[78]. In this regard, future studies on the topic might provide further options for these limitations to be overcome in the near future.

In order to increase the therapeutic effectiveness of the current immunotherapy approaches, various strategies have been developed to increase drug penetration and decrease the occurrence of adverse effects. Of note, we highlight (1) the use of combined therapies, for synergistic action[153]; (2) targeted drug delivery, which increases pharmacokinetic properties and reduces toxicity[79]; and (3) intrathecal administration, to overcome the blood-brain barrier[140-142].

Furthermore, given the intrinsic heterogeneous nature of GBM and its ability to evade and resist single treatments, it is crucial that future interventions should explore the combination of biological (immunomodulators and cell based delivery systems), physical (ultrasound, 3D printed implants, heat) and chemical (delivery technologies, radiation, chemotherapy) approaches to not only treat GBM more adequately but also improve the patient's prognosis, selecting ideal combination strategies to overcome the limiting barriers. In this regard, techniques using anti-PD-1/PD-L1 antibodies combined with antibodies targeting CTLA-4, TIM-3, LAG-3, 4-1BB, or OX-40 are under study[154]. Furthermore, anti-PD-1/PD-L1 therapy combined with tumor-specific peptide vaccination or CAR-T cell therapy is also worth exploring, and can provide a harmonious combination approach to surpass the obstacles[155, 156].

Finally, exploring effective predictive biomarkers of clinical efficacy, combined with other therapeutic strategies, is a critical issue to avoid treatment delay and early mortality[157,158]. In this sense, there is a demanding need to incorporate the status of known biomarkers into daily clinical practice, which may assist not only in patient selection, but also in the adjustment of treatment schedule based on the patient-specific diagnosis.

With various ongoing clinical trials for new molecular targeted therapies, cancer vaccines and immune-modulators, it can be expected that in the near future more compelling interventions against GBM will become available.

CONCLUSION

In this way, it is possible to see that the treatment for GBM is advancing and discoveries are being made. However, the immunosuppressive nature of this primary glioma and the pleomorphism presented by the constitutional cells represents important challenges to implant a successful therapy with less harm for the patient. The need for resolutions to prevent the collateral damage caused by the current standard treatment and for the alternative immunotherapies, which are being developed, demonstrates potential to be the next stage in this field alongside the increase of searching for other approaches. The main objective is to better manage this aggressive malignant brain tumor to modify the current prognostic perspective. This review shows an overview of this reality and it is stated that, based on particular pathogenesis of GBM, it is necessary an individualized treatment according to the tumor progress follow-up.

The potential of the immunotherapy presented by previous and current clinical trials reveals a hopeful perspective for patients with GBM. It is expected that a combination of therapies would be used to avoid collateral damages and improve the recovery. Risks and costs of the surgical method, radiotherapy and chemotherapy suggest several issues that alternative approaches do not have and it is more favorable as a palliative therapy than as a healing mechanism, and still usage problems must be solved for them to be applied. Biological agents and Tumor treatment fields also have benefits, even though they are, respectively, susceptible to genetic variabilities and need expensive devices to put into practice as the Figure 1 illustrates. The intervention with cytokine therapy and agonists are a recently explored field and demonstrates the ability to use different inflammatory cytokines to remodel the immune response, nevertheless there are also problems with the form of administration and the doses due to systemic toxicity. Immune checkpoints inhibitors reveal the ability to curb the immunosuppressive strategies of GBM, but the response in humans has not shown yet the same efficacy demonstrated in animal models. Chimeric antigen receptor T cell therapy is also a hopeful route of treatment due to its potential to redirect the immune response for specific targets, however the difficult to transpass the BBB and the microenvironment possessed by the active tumor, which enables evasion and difficult to recognize, are also challenges to be solved for highly functional deployment. Vaccine-based therapy is also being developed and four approaches are more currently discussed. In summary, the immunotherapy options display advantages and limitations. Thus, more advancements in ways to prevent toxic activity or/and ineffectiveness of the hopeful new recently discovered immunotherapies are fundamental to increase life expectancy and reduce suffering for the patients.

FOOTNOTES

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REFERENCES

1 **Thakkar JP**, Dolecek TA, Horbinski C, Ostrom QT, Lightner DD, Barnholtz-Sloan JS, Villano JL. Epidemiologic and molecular prognostic review of glioblastoma. *Cancer Epidemiol Biomarkers Prev* 2014; **23**: 1985-1996 [PMID: 25053711 DOI: 10.1158/1055-9965.EPI-14-0275]

2 **Wen PY**, Kesari S. Malignant gliomas in adults. *N Engl J Med* 2008; **359**: 492-507 [PMID: 18669428 DOI: 10.1056/NEJMra0708126]

3 **Aldape K**, Zadeh G, Mansouri S, Reifenberger G, von Deimling A. Glioblastoma: pathology, molecular mechanisms and markers. *Acta Neuropathol* 2015; **129**: 829-848 [PMID: 25943888 DOI: 10.1007/s00401-015-1432-1]

4 **Soomro SH**, Ting LR, Qing YY, Ren M. Molecular biology of glioblastoma: Classification and mutational locations. *J Pak Med Assoc* 2017; **67**: 1410-1414 [PMID: 28924284]

5 **Cancer Genome Atlas Research Network**. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. *Nature* 2008; **455**: 1061-1068 [PMID: 18772890 DOI: 10.1038/nature07385]

6 **Liang Y**, Diehn M, Watson N, Bollen AW, Aldape KD, Nicholas MK, Lamborn KR, Berger MS, Botstein D, Brown PO, Israel MA. Gene expression profiling reveals molecularly and clinically distinct subtypes of glioblastoma multiforme. *Proc Natl Acad Sci U S A* 2005; **102**: 5814-5819 [PMID: 15827123 DOI: 10.1073/pnas.0402870102]

7 **Chen Z**, Hambardzumyan D. Immune Microenvironment in Glioblastoma Subtypes. *Front Immunol* 2018; **9**: 1004 [PMID: 29867979 DOI: 10.3389/fimmu.2018.01004]

8 **Lowe S**, Bhat KP, Olar A. Current clinical management of patients with glioblastoma. *Cancer Rep (Hoboken)* 2019; **2**: e1216 [PMID: 32721125 DOI: 10.1002/cnr2.1216]

9 **Kelly PJ**, Daumas-Duport C, Scheithauer BW, Kall BA, Kispert DB. Stereotactic histologic correlations of computed tomography- and magnetic resonance imaging-defined abnormalities in patients with glial neoplasms. *Mayo Clin Proc* 1987; **62**: 450-459 [PMID: 3553757 DOI: 10.1016/s0025-6196(12)65470-6]

10 **Bush NA**, Chang SM, Berger MS. Current and future strategies for treatment of glioma. *Neurosurg Rev* 2017; **40**: 1-14 [PMID: 27085859 DOI: 10.1007/s10143-016-0709-8]

11 **Stummer W**, Suero Molina E. Fluorescence Imaging/Agents in Tumor Resection. *Neurosurg Clin N Am* 2017; **28**: 569-583 [PMID: 28917285 DOI: 10.1016/j.nec.2017.05.009]

12 **Rodríguez-Camacho A**, Flores-Vázquez JG, Moscardini-Martelli J, Torres-Ríos JA, Olmos-Guzmán A, Ortiz-Arce CS, Cid-Sánchez DR, Pérez SR, Macías-González MDS, Hernández-Sánchez LC, Heredia-Gutiérrez JC, Contreras-Palafox GA, Suárez-Campos JJE, Celis-López MÁ, Gutiérrez-Aceves GA, Moreno-Jiménez S. Glioblastoma Treatment: State-of-the-Art and Future Perspectives. *Int J Mol Sci* 2022; **23** [PMID: 35806212 DOI: 10.3390/ijms23137207]

13 **Nabors LB**, Portnow J, Ahluwalia M, Baehring J, Brem H, Brem S, Butowski N, Campian JL, Clark SW, Fabiano AJ, Forsyth P, Hattangadi-Gluth J, Holdhoff M, Horbinski C, Junck L, Kaley T, Kumthekar P, Loeffler JS, Mrugala MM, Nagpal S, Pandey M, Parney I, Peters K, Puduvalli VK, Robins I, Rockhill J, Rusthoven C, Shonka N, Shrieve DC, Swinnen LJ, Weiss S, Wen PY, Willmarth NE, Bergman MA, Darlow SD. Central Nervous System Cancers, Version 3.2020, NCCN Clinical Practice Guidelines in Oncology. *J Natl Compr Canc Netw* 2020; **18**: 1537-1570 [PMID: 33152694 DOI: 10.6004/jnccn.2020.0052]

14 **Stupp R**, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJ, Belanger K, Brandes AA, Marosi C, Bogdahn U, Curschmann J, Janzer RC, Ludwin SK, Gorlia T, Allgeier A, Lacombe D, Cairncross JG, Eisenhauer E, Mirimanoff RO; European Organisation for Research and Treatment of Cancer Brain Tumor and Radiotherapy Groups; National Cancer Institute of Canada Clinical Trials Group. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med* 2005; **352**: 987-996 [PMID: 15758009 DOI: 10.1056/NEJMoa043330]

15 **Batash R**, Asna N, Schaffer P, Francis N, Schaffer M. Glioblastoma Multiforme, Diagnosis and Treatment; Recent Literature Review. *Curr Med Chem* 2017; **24**: 3002-3009 [PMID: 28521700 DOI: 10.2174/0929867324666170516123206]

16 **Patel M**, Siddiqui F, Jin JY, Mikkelsen T, Rosenblum M, Movsas B, Ryu S. Salvage reirradiation for recurrent glioblastoma with radiosurgery: radiographic response and improved survival. *J Neurooncol* 2009; **92**: 185-191 [PMID: 19066727 DOI: 10.1007/s11060-008-9752-9]

17 **Wesolowski JR**, Rajdev P, Mukherji SK. Temozolomide (Temodar). *AJNR Am J Neuroradiol* 2010; **31**: 1383-1384 [PMID: 20538821 DOI: 10.3174/ajnr.A2170]

18 **Hegi ME**, Diserens AC, Gorlia T, Hamou MF, de Tribolet N, Weller M, Kros JM, Hainfellner JA, Mason W, Mariani L, Bromberg JE, Hau P, Mirimanoff RO, Cairncross JG, Janzer RC, Stupp R. MGMT gene silencing and benefit from temozolomide in glioblastoma. *N Engl J Med* 2005; **352**: 997-1003 [PMID: 15758010 DOI: 10.1056/NEJMoa043331]

19 **Denny BJ**, Wheelhouse RT, Stevens MF, Tsang LL, Slack JA. NMR and molecular modeling investigation of the mechanism of activation of the antitumor drug temozolomide and its interaction with DNA. *Biochemistry* 1994; **33**: 9045-9051 [PMID: 8049205 DOI: 10.1021/bi00197a003]

20 **Zhang J**, Stevens MF, Bradshaw TD. Temozolomide: mechanisms of action, repair and resistance. *Curr Mol Pharmacol* 2012; **5**: 102-114 [PMID: 22122467 DOI: 10.2174/1874467211205010102]

21 **Xu J**, Keenan TE, Overmoyer B, Tung NM, Gelman RS, Habin K, Garber JE, Ellisen LW, Winer EP, Goss PE, Yeap BY, Chabner BA, Isakoff SJ. Phase II trial of veliparib and temozolomide in metastatic breast cancer patients with and without BRCA1/2 mutations. *Breast Cancer Res Treat* 2021; **189**: 641-651 [PMID: 34417675 DOI: 10.1007/s10549-021-06292-7]

22 **Fukai J**, Nishibayashi H, Uematsu Y, Kanemura Y, Fujita K, Nakao N. Rapid regression of glioblastoma following carmustine wafer implantation: A case report. *Mol Clin Oncol* 2016; **5**: 153-157 [PMID: 27330789 DOI: 10.3892/mco.2016.894]

23 **Brem H**, Piantadosi S, Burger PC, Walker M, Selker R, Vick NA, Black K, Sisti M, Brem S, Mohr G. Placebo-controlled trial of safety and efficacy of intraoperative controlled delivery by biodegradable polymers of chemotherapy for recurrent gliomas. The Polymer-brain Tumor Treatment Group. *Lancet* 1995; **345**: 1008-1012 [PMID: 7723496 DOI: 10.1016/s0140-6736(95)90755-6]

24 **Gutenberg A**, Lumenta CB, Braunsdorf WE, Sabel M, Mehdorn HM, Westphal M, Giese A. The combination of carmustine wafers and temozolomide for the treatment of malignant gliomas. A comprehensive review of the rationale and clinical experience. *J Neurooncol* 2013; **113**: 163-174 [PMID: 23535992 DOI: 10.1007/s11060-013-1110-x]

25 **Diaz RJ**, Ali S, Qadir MG, De La Fuente MI, Ivan ME, Komotar RJ. The role of bevacizumab in the treatment of glioblastoma. *J Neurooncol* 2017; **133**: 455-467 [PMID: 28527008 DOI: 10.1007/s11060-017-2477-x]

26 **Norden AD**, Young GS, Setayesh K, Muzikansky A, Klufas R, Ross GL, Ciampa AS, Ebbeling LG, Levy B, Drappatz J, Kesari S, Wen PY. Bevacizumab for recurrent malignant gliomas: efficacy, toxicity, and patterns of recurrence. *Neurology* 2008; **70**: 779-787 [PMID: 18316689 DOI: 10.1212/01.wnl.0000304121.57857.38]

27 **Mrugala MM**, Crew LK, Fink JR, Spence AM. Carboplatin and bevacizumab for recurrent malignant glioma. *Oncol Lett* 2012; **4**: 1082-1086 [PMID: 23162656 DOI: 10.3892/ol.2012.839]

28 **Tonder M**, Weller M, Eisele G, Roth P. Carboplatin and Etoposide in Heavily Pretreated Patients with Progressive High-Grade Glioma. *Chemotherapy* 2014; **60**: 375-378 [PMID: 26496463 DOI: 10.1159/000440678]

29 **Pless M**, Weinberg U. Tumor treating fields: concept, evidence and future. *Expert Opin Investig Drugs* 2011; **20**: 1099-1106 [PMID: 21548832 DOI: 10.1517/13543784.2011.583236]

30 **Burri SH**, Gondi V, Brown PD, Mehta MP. The Evolving Role of Tumor Treating Fields in Managing Glioblastoma: Guide for Oncologists. *Am J Clin Oncol* 2018; **41**: 191-196 [PMID: 28832384 DOI: 10.1097/COC.0000000000000395]

31 **Hottinger AF**, Pacheco P, Stupp R. Tumor treating fields: a novel treatment modality and its use in brain tumors. *Neuro Oncol* 2016; **18**: 1338-1349 [PMID: 27664860 DOI: 10.1093/neuonc/now182]

32 **Stupp R**, Taillibert S, Kanner AA, Kesari S, Steinberg DM, Toms SA, Taylor LP, Lieberman F, Silvani A, Fink KL, Barnett GH, Zhu JJ, Henson JW, Engelhard HH, Chen TC, Tran DD, Sroubek J, Tran ND, Hottinger AF, Landolfi J, Desai R, Caroli M, Kew Y, Honnorat J, Idbaih A, Kirson ED, Weinberg U, Palti Y, Hegi ME, Ram Z. Maintenance Therapy With Tumor-Treating Fields Plus Temozolomide vs Temozolomide Alone for Glioblastoma: A Randomized Clinical Trial. *JAMA* 2015; **314**: 2535-2543 [PMID: 26670971 DOI: 10.1001/jama.2015.16669]

33 **Labani-Motlagh A**, Ashja-Mahdavi M, Loskog A. The Tumor Microenvironment: A Milieu Hindering and Obstructing Antitumor Immune Responses. *Front Immunol* 2020; **11**: 940 [PMID: 32499786 DOI: 10.3389/fimmu.2020.00940]

34 **Forrester JV**, McMenamin PG, Dando SJ. CNS infection and immune privilege. *Nat Rev Neurosci* 2018; **19**: 655-671 [PMID: 30310148 DOI: 10.1038/s41583-018-0070-8]

35 **Louveau A**, Harris TH, Kipnis J. Revisiting the Mechanisms of CNS Immune Privilege. *Trends Immunol* 2015; **36**: 569-577 [PMID: 26431936 DOI: 10.1016/j.it.2015.08.006]

36 **Ballabh P**, Braun A, Nedergaard M. The blood-brain barrier: an overview: structure, regulation, and clinical implications. *Neurobiol Dis* 2004; **16**: 1-13 [PMID: 15207256 DOI: 10.1016/j.nbd.2003.12.016]

37 **Daneman R**, Prat A. The blood-brain barrier. *Cold Spring Harb Perspect Biol* 2015; **7**: a020412 [PMID: 25561720 DOI: 10.1101/cshperspect.a020412]

38 **Pardridge WM**. The blood-brain barrier: bottleneck in brain drug development. *NeuroRx* 2005; **2**: 3-14 [PMID: 15717053 DOI: 10.1602/neurorx.2.1.3]

39 **Weller RO**, Djuanda E, Yow HY, Carare RO. Lymphatic drainage of the brain and the pathophysiology of neurological disease. *Acta Neuropathol* 2009; **117**: 1-14 [PMID: 19002474 DOI: 10.1007/s00401-008-0457-0]

40 **Carson MJ**, Doose JM, Melchior B, Schmid CD, Ploix CC. CNS immune privilege: hiding in plain sight. *Immunol Rev* 2006; **213**: 48-65 [PMID: 16972896 DOI: 10.1111/j.1600-065X.2006.00441.x]

41 **Louveau A**, Smirnov I, Keyes TJ, Eccles JD, Rouhani SJ, Peske JD, Derecki NC, Castle D, Mandell JW, Lee KS, Harris TH, Kipnis J. Structural and functional features of central nervous system lymphatic vessels. *Nature* 2015; **523**: 337-341 [PMID: 26030524 DOI: 10.1038/nature14432]

42 **Waldman AD**, Fritz JM, Lenardo MJ. A guide to cancer immunotherapy: from T cell basic science to clinical practice. *Nat Rev Immunol* 2020; **20**: 651-668 [PMID: 32433532 DOI: 10.1038/s41577-020-0306-5]

43 **Crittenden MR**, Zebertavage L, Kramer G, Bambina S, Friedman D, Troesch V, Blair T, Baird JR, Alice A, Gough MJ.

Tumor cure by radiation therapy and checkpoint inhibitors depends on pre-existing immunity. *Sci Rep* 2018; **8**: 7012 [PMID: 29725089 DOI: 10.1038/s41598-018-25482-w]

44 **Pombo Antunes AR**, Scheyltjens I, Duerinck J, Neyns B, Movahedi K, Van Ginderachter JA. Understanding the glioblastoma immune microenvironment as basis for the development of new immunotherapeutic strategies. *Elife* 2020; **9** [PMID: 32014107 DOI: 10.7554/eLife.52176]

45 **Mangani D**, Weller M, Roth P. The network of immunosuppressive pathways in glioblastoma. *Biochem Pharmacol* 2017; **130**: 1-9 [PMID: 28017775 DOI: 10.1016/j.bcp.2016.12.011]

46 **Han J**, Alvarez-Breckenridge CA, Wang QE, Yu J. TGF-β signaling and its targeting for glioma treatment. *Am J Cancer Res* 2015; **5**: 945-955 [PMID: 26045979]

47 **Widodo SS**, Dinevska M, Furst LM, Stylli SS, Mantamadiotis T. IL-10 in glioma. *Br J Cancer* 2021; **125**: 1466-1476 [PMID: 34349251 DOI: 10.1038/s41416-021-01515-6]

48 **Jiang J**, Qiu J, Li Q, Shi Z. Prostaglandin E2 Signaling: Alternative Target for Glioblastoma? *Trends Cancer* 2017; **3**: 75-78 [PMID: 28718447 DOI: 10.1016/j.trecan.2016.12.002]

49 **Chen W**, Jin W, Hardegen N, Lei KJ, Li L, Marinos N, McGrady G, Wahl SM. Conversion of peripheral CD4+CD25- naive T cells to CD4+CD25+ regulatory T cells by TGF-beta induction of transcription factor Foxp3. *J Exp Med* 2003; **198**: 1875-1886 [PMID: 14676299 DOI: 10.1084/jem.20030152]

50 **Guo XY**, Zhang GH, Wang ZN, Duan H, Xie T, Liang L, Cui R, Hu HR, Wu Y, Dong JJ, He ZQ, Mou YG. A novel Foxp3-related immune prognostic signature for glioblastoma multiforme based on immunogenomic profiling. *Aging (Albany NY)* 2021; **13**: 3501-3517 [PMID: 33429364 DOI: 10.18632/aging.202282]

51 **Thomas DA**, Massagué J. TGF-beta directly targets cytotoxic T cell functions during tumor evasion of immune surveillance. *Cancer Cell* 2005; **8**: 369-380 [PMID: 16286245 DOI: 10.1016/j.ccr.2005.10.012]

52 **Crane CA**, Han SJ, Barry JJ, Ahn BJ, Lanier LL, Parsa AT. TGF-beta downregulates the activating receptor NKG2D on NK cells and CD8+ T cells in glioma patients. *Neuro Oncol* 2010; **12**: 7-13 [PMID: 20150362 DOI: 10.1093/neuonc/nop009]

53 **Zuber P**, Kuppner MC, De Tribolet N. Transforming growth factor-beta 2 down-regulates HLA-DR antigen expression on human malignant glioma cells. *Eur J Immunol* 1988; **18**: 1623-1626 [PMID: 3142781 DOI: 10.1002/eji.1830181023]

54 **Barcia C Jr**, Gómez A, Gallego-Sanchez JM, Perez-Vallés A, Castro MG, Lowenstein PR, Barcia C Sr, Herrero MT. Infiltrating CTLs in human glioblastoma establish immunological synapses with tumorigenic cells. *Am J Pathol* 2009; **175**: 786-798 [PMID: 19628762 DOI: 10.2353/ajpath.2009.081034]

55 **Castriconi R**, Daga A, Dondero A, Zona G, Poliani PL, Melotti A, Griffero F, Marubbi D, Spaziante R, Bellora F, Moretta L, Moretta A, Corte G, Bottino C. NK cells recognize and kill human glioblastoma cells with stem cell-like properties. *J Immunol* 2009; **182**: 3530-3539 [PMID: 19265131 DOI: 10.4049/jimmunol.0802845]

56 **Wu A**, Wei J, Kong LY, Wang Y, Priebe W, Qiao W, Sawaya R, Heimberger AB. Glioma cancer stem cells induce immunosuppressive macrophages/microglia. *Neuro Oncol* 2010; **12**: 1113-1125 [PMID: 20667896 DOI: 10.1093/neuonc/noq082]

57 **Li Z**, Zhang J, Zheng H, Li C, Xiong J, Wang W, Bao H, Jin H, Liang P. Modulating lncRNA SNHG15/CDK6/miR-627 circuit by palbociclib, overcomes temozolomide resistance and reduces M2-polarization of glioma associated microglia in glioblastoma multiforme. *J Exp Clin Cancer Res* 2019; **38**: 380 [PMID: 31462285 DOI: 10.1186/s13046-019-1371-0]

58 **Mittal SK**, Cho KJ, Ishido S, Roche PA. Interleukin 10 (IL-10)-mediated Immunosuppression: March-I Induction Regulates Antigen Presentation by Macrophages but Not Dendritic Cells. *J Biol Chem* 2015; **290**: 27158-27167 [PMID: 26408197 DOI: 10.1074/jbc.M115.682708]

59 **Li Z**, Liu X, Guo R, Wang P. CD4(+)Foxp3(-) type 1 regulatory T cells in glioblastoma multiforme suppress T cell responses through multiple pathways and are regulated by tumor-associated macrophages. *Int J Biochem Cell Biol* 2016; **81**: 1-9 [PMID: 27644153 DOI: 10.1016/j.biocel.2016.09.013]

60 **Sawant DV**, Yano H, Chikina M, Zhang Q, Liao M, Liu C, Callahan DJ, Sun Z, Sun T, Tabib T, Pennathur A, Corry DB, Luketich JD, Lafyatis R, Chen W, Poholek AC, Bruno TC, Workman CJ, Vignali DAA. Adaptive plasticity of IL-10(+) and IL-35(+) T(reg) cells cooperatively promotes tumor T cell exhaustion. *Nat Immunol* 2019; **20**: 724-735 [PMID: 30936494 DOI: 10.1038/s41590-019-0346-9]

61 **Fecci PE**, Mitchell DA, Whitesides JF, Xie W, Friedman AH, Archer GE, Herndon JE 2nd, Bigner DD, Dranoff G, Sampson JH. Increased regulatory T-cell fraction amidst a diminished CD4 compartment explains cellular immune defects in patients with malignant glioma. *Cancer Res* 2006; **66**: 3294-3302 [PMID: 16540683 DOI: 10.1158/0008-5472.CAN-05-3773]

62 **Ravi VM**, Neidert N, Will P, Joseph K, Maier JP, Kückelhaus J, Vollmer L, Goeldner JM, Behringer SP, Scherer F, Boerries M, Follo M, Weiss T, Delev D, Kernbach J, Franco P, Schallner N, Dierks C, Carro MS, Hofmann UG, Fung C, Sankowski R, Prinz M, Beck J, Salié H, Bengsch B, Schnell O, Heiland DH. T-cell dysfunction in the glioblastoma microenvironment is mediated by myeloid cells releasing interleukin-10. *Nat Commun* 2022; **13**: 925 [PMID: 35177622 DOI: 10.1038/s41467-022-28523-1]

63 **Fujita M**, Kohanbash G, Fellows-Mayle W, Hamilton RL, Komohara Y, Decker SA, Ohlfest JR, Okada H. COX-2 blockade suppresses gliomagenesis by inhibiting myeloid-derived suppressor cells. *Cancer Res* 2011; **71**: 2664-2674 [PMID: 21324923 DOI: 10.1158/0008-5472.CAN-10-3055]

64 **Weathers SP**, de Groot J. VEGF Manipulation in Glioblastoma. *Oncology (Williston Park)* 2015; **29**: 720-727 [PMID: 26470893]

65 **Hu M**, Li Y, Lu Y, Wang M, Wang C, Li Q, Zhao H. The regulation of immune checkpoints by the hypoxic tumor microenvironment. *PeerJ* 2021; **9**: e11306 [PMID: 34012727 DOI: 10.7717/peerj.11306]

66 **Nduom EK**, Wei J, Yaghi NK, Huang N, Kong LY, Gabrusiewicz K, Ling X, Zhou S, Ivan C, Chen JQ, Burks JK, Fuller GN, Calin GA, Conrad CA, Creasy C, Ritthipichai K, Radvanyi L, Heimberger AB. PD-L1 expression and prognostic impact in glioblastoma. *Neuro Oncol* 2016; **18**: 195-205 [PMID: 26323609 DOI: 10.1093/neuonc/nov172]

67 **Davidson TB**, Lee A, Hsu M, Sedighim S, Orpilla J, Treger J, Mastall M, Roesch S, Rapp C, Galvez M, Mochizuki A, Antonios J, Garcia A, Kotecha N, Bayless N, Nathanson D, Wang A, Everson R, Yong WH, Cloughesy TF, Liau LM,

Herold-Mende C, Prins RM. Expression of PD-1 by T Cells in Malignant Glioma Patients Reflects Exhaustion and Activation. *Clin Cancer Res* 2019; **25**: 1913-1922 [PMID: 30498094 DOI: 10.1158/1078-0432.CCR-18-1176]

68 **Azuma T**, Yao S, Zhu G, Flies AS, Flies SJ, Chen L. B7-H1 is a ubiquitous antiapoptotic receptor on cancer cells. *Blood* 2008; **111**: 3635-3643 [PMID: 18223165 DOI: 10.1182/blood-2007-11-123141]

69 **Gratas C**, Tohma Y, Van Meir EG, Klein M, Tenan M, Ishii N, Tachibana O, Kleihues P, Ohgaki H. Fas ligand expression in glioblastoma cell lines and primary astrocytic brain tumors. *Brain Pathol* 1997; **7**: 863-869 [PMID: 9217971 DOI: 10.1111/j.1750-3639.1997.tb00889.x]

70 **Guan X**, Wang Y, Sun Y, Zhang C, Ma S, Zhang D, Li D, Jia W. CTLA4-Mediated Immunosuppression in Glioblastoma is Associated with the Infiltration of Macrophages in the Tumor Microenvironment. *J Inflamm Res* 2021; **14**: 7315-7329 [PMID: 34992419 DOI: 10.2147/JIR.S341981]

71 **Liu F**, Huang J, Liu X, Cheng Q, Luo C, Liu Z. CTLA-4 correlates with immune and clinical characteristics of glioma. *Cancer Cell Int* 2020; **20**: 7 [PMID: 31911758 DOI: 10.1186/s12935-019-1085-6]

72 **Zhai L**, Bell A, Ladomersky E, Lauing KL, Bollu L, Nguyen B, Genet M, Kim M, Chen P, Mi X, Wu JD, Schipma MJ, Wray B, Griffiths J, Unwin RD, Clark SJ, Acharya R, Bao R, Horbinski C, Lukas RV, Schiltz GE, Wainwright DA. Tumor Cell IDO Enhances Immune Suppression and Decreases Survival Independent of Tryptophan Metabolism in Glioblastoma. *Clin Cancer Res* 2021; **27**: 6514-6528 [PMID: 34479957 DOI: 10.1158/1078-0432.CCR-21-1392]

73 **Roth P**, Mittelbronn M, Wick W, Meyermann R, Tatagiba M, Weller M. Malignant glioma cells counteract antitumor immune responses through expression of lectin-like transcript-1. *Cancer Res* 2007; **67**: 3540-3544 [PMID: 17440061 DOI: 10.1158/0008-5472.CAN-06-4783]

74 **Himes BT**, Geiger PA, Ayasoufi K, Bhargav AG, Brown DA, Parney IF. Immunosuppression in Glioblastoma: Current Understanding and Therapeutic Implications. *Front Oncol* 2021; **11**: 770561 [PMID: 34778089 DOI: 10.3389/fonc.2021.770561]

75 **Himes BT**, Peterson TE, de Mooij T, Garcia LMC, Jung MY, Uhm S, Yan D, Tyson J, Jin-Lee HJ, Parney D, Abukhadra Y, Gustafson MP, Dietz AB, Johnson AJ, Dong H, Maus RL, Markovic S, Lucien F, Parney IF. The role of extracellular vesicles and PD-L1 in glioblastoma-mediated immunosuppressive monocyte induction. *Neuro Oncol* 2020; **22**: 967-978 [PMID: 32080744 DOI: 10.1093/neuonc/noaa029]

76 **Ricklefs FL**, Alayo Q, Krenzlin H, Mahmoud AB, Speranza MC, Nakashima H, Hayes JL, Lee K, Balaj L, Passaro C, Rooj AK, Krasemann S, Carter BS, Chen CC, Steed T, Treiber J, Rodig S, Yang K, Nakano I, Lee H, Weissleder R, Breakefield XO, Godlewski J, Westphal M, Lamszus K, Freeman GJ, Bronisz A, Lawler SE, Chiocca EA. Immune evasion mediated by PD-L1 on glioblastoma-derived extracellular vesicles. *Sci Adv* 2018; **4**: eaar2766 [PMID: 29532035 DOI: 10.1126/sciadv.aar2766]

77 **Janjua TI**, Rewatkar P, Ahmed-Cox A, Saeed I, Mansfeld FM, Kulshreshtha R, Kumeria T, Ziegler DS, Kavallaris M, Mazzieri R, Popat A. Frontiers in the treatment of glioblastoma: Past, present and emerging. *Adv Drug Deliv Rev* 2021; **171**: 108-138 [PMID: 33486006 DOI: 10.1016/j.addr.2021.01.012]

78 **Han SJ**, Zygourakis C, Lim M, Parsa AT. Immunotherapy for glioma: promises and challenges. *Neurosurg Clin N Am* 2012; **23**: 357-370 [PMID: 22748649 DOI: 10.1016/j.nec.2012.05.001]

79 **Weiss T**, Puca E, Silginer M, Hemmerle T, Pazahr S, Bink A, Weller M, Neri D, Roth P. Immunocytokines are a promising immunotherapeutic approach against glioblastoma. *Sci Transl Med* 2020; **12** [PMID: 33028706 DOI: 10.1126/scitranslmed.abb2311]

80 **Enderlin M**, Kleinmann EV, Struyf S, Buracchi C, Vecchi A, Kinscherf R, Kiessling F, Paschek S, Sozzani S, Rommelaere J, Cornelis JJ, Van Damme J, Dinsart C. TNF-alpha and the IFN-gamma-inducible protein 10 (IP-10/CXCL-10) delivered by parvoviral vectors act in synergy to induce antitumor effects in mouse glioblastoma. *Cancer Gene Ther* 2009; **16**: 149-160 [PMID: 18670452 DOI: 10.1038/cgt.2008.62]

81 **Agliardi G**, Liuzzi AR, Hotblack A, De Feo D, Núñez N, Stowe CL, Friebel E, Nannini F, Rindlisbacher L, Roberts TA, Ramasawmy R, Williams IP, Siow BM, Lythgoe MF, Kalber TL, Quezada SA, Pule MA, Tugues S, Straathof K, Becher B. Intratumoral IL-12 delivery empowers CAR-T cell immunotherapy in a pre-clinical model of glioblastoma. *Nat Commun* 2021; **12**: 444 [PMID: 33469002 DOI: 10.1038/s41467-020-20599-x]

82 **Birocchi F**, Cusimano M, Rossari F, Beretta S, Rancoita PMV, Ranghetti A, Colombo S, Costa B, Angel P, Sanvito F, Callea M, Norata R, Chaabane L, Canu T, Spinelli A, Genua M, Ostuni R, Merelli I, Coltella N, Naldini L. Targeted inducible delivery of immunoactivating cytokines reprograms glioblastoma microenvironment and inhibits growth in mouse models. *Sci Transl Med* 2022; **14**: eabl4106 [PMID: 35857642 DOI: 10.1126/scitranslmed.abl4106]

83 **Feinberg B**, Kurzrock R, Talpaz M, Blick M, Saks S, Gutterman JU. A phase I trial of intravenously-administered recombinant tumor necrosis factor-alpha in cancer patients. *J Clin Oncol* 1988; **6**: 1328-1334 [PMID: 3411344]

84 **Kiladjian JJ**, Mesa RA, Hoffman R. The renaissance of interferon therapy for the treatment of myeloid malignancies. *Blood* 2011; **117**: 4706-4715 [PMID: 21389325]

85 **Sener U**, Ruff MW, Campian JL. Immunotherapy in Glioblastoma: Current Approaches and Future Perspectives. *Int J Mol Sci* 2022; **23** [PMID: 35806051 DOI: 10.3390/ijms23137046]

86 **Huang B**, Li X, Li Y, Zhang J, Zong Z, Zhang H. Current Immunotherapies for Glioblastoma Multiforme. *Front Immunol* 2020; **11**: 603911 [PMID: 33767690 DOI: 10.3389/fimmu.2020.603911]

87 **Ghouzlani A**, Kandoussi S, Tall M, Reddy KP, Rafii S, Badou A. Immune Checkpoint Inhibitors in Human Glioma Microenvironment. *Front Immunol* 2021; **12**: 679425 [PMID: 34305910 DOI: 10.3389/fimmu.2021.679425]

88 **Drake CG**, Jaffee E, Pardoll DM. Mechanisms of immune evasion by tumors. *Adv Immunol* 2006; **90**: 51-81 [PMID: 16730261 DOI: 10.1016/S0065-2776(06)90002-9]

89 **Park J**, Kim CG, Shim JK, Kim JH, Lee H, Lee JE, Kim MH, Haam K, Jung I, Park SH, Chang JH, Shin EC, Kang SG. Effect of combined anti-PD-1 and temozolomide therapy in glioblastoma. *Oncoimmunology* 2019; **8**: e1525243 [PMID: 30546966 DOI: 10.1080/2162402X.2018.1525243]

90 **Reardon DA**, Brandes AA, Omuro A, Mulholland P, Lim M, Wick A, Baehring J, Ahluwalia MS, Roth P, Bähr O, Phuphanich S, Sepulveda JM, De Souza P, Sahebjam S, Carleton M, Tatsuoka K, Taitt C, Zwirtes R, Sampson J, Weller M. Effect of Nivolumab vs Bevacizumab in Patients With Recurrent Glioblastoma: The CheckMate 143 Phase 3

Randomized Clinical Trial. *JAMA Oncol* 2020; **6**: 1003-1010 [PMID: 32437507 DOI: 10.1001/jamaoncol.2020.1024]

91 **Shu C**, Li Q. Current advances in PD-1/PD-L1 axis-related tumour-infiltrating immune cells and therapeutic regimens in glioblastoma. *Crit Rev Oncol Hematol* 2020; **151**: 102965 [PMID: 32442903 DOI: 10.1016/j.critrevonc.2020.102965]

92 **Yang T**, Kong Z, Ma W. PD-1/PD-L1 immune checkpoint inhibitors in glioblastoma: clinical studies, challenges and potential. *Hum Vaccin Immunother* 2021; **17**: 546-553 [PMID: 32643507 DOI: 10.1080/21645515.2020.1782692]

93 **Robert C**, Schachter J, Long GV, Arance A, Grob JJ, Mortier L, Daud A, Carlino MS, McNeil C, Lotem M, Larkin J, Lorigan P, Neyns B, Blank CU, Hamid O, Mateus C, Shapira-Frommer R, Kosh M, Zhou H, Ibrahim N, Ebbinghaus S, Ribas A; KEYNOTE-006 investigators. Pembrolizumab versus Ipilimumab in Advanced Melanoma. *N Engl J Med* 2015; **372**: 2521-2532 [PMID: 25891173 DOI: 10.1056/NEJMoa1503093]

94 **Herbst RS**, Baas P, Kim DW, Felip E, Pérez-Gracia JL, Han JY, Molina J, Kim JH, Arvis CD, Ahn MJ, Majem M, Fidler MJ, de Castro G Jr, Garrido M, Lubiniecki GM, Shentu Y, Im E, Dolled-Filhart M, Garon EB. Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial. *Lancet* 2016; **387**: 1540-1550 [PMID: 26712084 DOI: 10.1016/S0140-6736(15)01281-7]

95 **van Tellingen O**, Yetkin-Arik B, de Gooijer MC, Wesseling P, Wurdinger T, de Vries HE. Overcoming the blood-brain tumor barrier for effective glioblastoma treatment. *Drug Resist Updat* 2015; **19**: 1-12 [PMID: 25791797 DOI: 10.1016/j.drup.2015.02.002]

96 **Kandel S**, Adhikary P, Li G, Cheng K. The TIM3/Gal9 signaling pathway: An emerging target for cancer immunotherapy. *Cancer Lett* 2021; **510**: 67-78 [PMID: 33895262 DOI: 10.1016/j.canlet.2021.04.011]

97 **Liu Z**, Han H, He X, Li S, Wu C, Yu C, Wang S. Expression of the galectin-9-Tim-3 pathway in glioma tissues is associated with the clinical manifestations of glioma. *Oncol Lett* 2016; **11**: 1829-1834 [PMID: 26998085 DOI: 10.3892/ol.2016.4142]

98 **Huang J**, Liu F, Liu Z, Tang H, Wu H, Gong Q, Chen J. Immune Checkpoint in Glioblastoma: Promising and Challenging. *Front Pharmacol* 2017; **8**: 242 [PMID: 28536525 DOI: 10.3389/fphar.2017.00242]

99 **Shi AP**, Tang XY, Xiong YL, Zheng KF, Liu YJ, Shi XG, Lv Y, Jiang T, Ma N, Zhao JB. Immune Checkpoint LAG3 and Its Ligand FGL1 in Cancer. *Front Immunol* 2021; **12**: 785091 [PMID: 35111155 DOI: 10.3389/fimmu.2021.785091]

100 **Du H**, Yi Z, Wang L, Li Z, Niu B, Ren G. The co-expression characteristics of LAG3 and PD-1 on the T cells of patients with breast cancer reveal a new therapeutic strategy. *Int Immunopharmacol* 2020; **78**: 106113 [PMID: 31841754 DOI: 10.1016/j.intimp.2019.106113]

101 **Hung AL**, Maxwell R, Theodros D, Belcaid Z, Mathios D, Luksik AS, Kim E, Wu A, Xia Y, Garzon-Muvdi T, Jackson C, Ye X, Tyler B, Selby M, Korman A, Barnhart B, Park SM, Youn JI, Chowdhury T, Park CK, Brem H, Pardoll DM, Lim M. TIGIT and PD-1 dual checkpoint blockade enhances antitumor immunity and survival in GBM. *Oncoimmunology* 2018; **7**: e1466769 [PMID: 30221069 DOI: 10.1080/2162402X.2018.1466769]

102 **Zhang Q**, Zhong H, Fan Y, Liu Q, Song J, Yao S, Cao F. Immune and Clinical Features of CD96 Expression in Glioma by in silico Analysis. *Front Bioeng Biotechnol* 2020; **8**: 592 [PMID: 32695752 DOI: 10.3389/fbioe.2020.00592]

103 **Sadelain M**, Brentjens R, Rivière I. The basic principles of chimeric antigen receptor design. *Cancer Discov* 2013; **3**: 388-398 [PMID: 23550147 DOI: 10.1158/2159-8290.CD-12-0548]

104 **Rong L**, Li N, Zhang Z. Emerging therapies for glioblastoma: current state and future directions. *J Exp Clin Cancer Res* 2022; **41**: 142 [PMID: 35428347 DOI: 10.1186/s13046-022-02349-7]

105 **Akhavan D**, Alizadeh D, Wang D, Weist MR, Shepphird JK, Brown CE. CAR T cells for brain tumors: Lessons learned and road ahead. *Immunol Rev* 2019; **290**: 60-84 [PMID: 31355493 DOI: 10.1111/imr.12773]

106 **Ahmed N**, Brawley V, Hegde M, Bielamowicz K, Kalra M, Landi D, Robertson C, Gray TL, Diouf O, Wakefield A, Ghazi A, Gerken C, Yi Z, Ashoori A, Wu MF, Liu H, Rooney C, Dotti G, Gee A, Su J, Kew Y, Baskin D, Zhang YJ, New P, Grilley B, Stojakovic M, Hicks J, Powell SZ, Brenner MK, Heslop HE, Grossman R, Wels WS, Gottschalk S. HER2-Specific Chimeric Antigen Receptor-Modified Virus-Specific T Cells for Progressive Glioblastoma: A Phase 1 Dose-Escalation Trial. *JAMA Oncol* 2017; **3**: 1094-1101 [PMID: 28426845 DOI: 10.1001/jamaoncol.2017.0184]

107 **O'Rourke DM**, Nasrallah MP, Desai A, Melenhorst JJ, Mansfield K, Morrissette JJD, Martinez-Lage M, Brem S, Maloney E, Shen A, Isaacs R, Mohan S, Plesa G, Lacey SF, Navenot JM, Zheng Z, Levine BL, Okada H, June CH, Brogdon JL, Maus MV. A single dose of peripherally infused EGFRvIII-directed CAR T cells mediates antigen loss and induces adaptive resistance in patients with recurrent glioblastoma. *Sci Transl Med* 2017; **9** [PMID: 28724573 DOI: 10.1126/scitranslmed.aaa0984]

108 **Brown CE**, Alizadeh D, Starr R, Weng L, Wagner JR, Naranjo A, Ostberg JR, Blanchard MS, Kilpatrick J, Simpson J, Kurien A, Priceman SJ, Wang X, Harshbarger TL, D'Apuzzo M, Ressler JA, Jensen MC, Barish ME, Chen M, Portnow J, Forman SJ, Badie B. Regression of Glioblastoma after Chimeric Antigen Receptor T-Cell Therapy. *N Engl J Med* 2016; **375**: 2561-2569 [PMID: 28029927 DOI: 10.1056/NEJMoa1610497]

109 **Li G**, Wong AJ. EGF receptor variant III as a target antigen for tumor immunotherapy. *Expert Rev Vaccines* 2008; **7**: 977-985 [PMID: 18767947 DOI: 10.1586/14760584.7.7.977]

110 **Heimberger AB**, Suki D, Yang D, Shi W, Aldape K. The natural history of EGFR and EGFRvIII in glioblastoma patients. *J Transl Med* 2005; **3**: 38 [PMID: 16236164 DOI: 10.1186/1479-5876-3-38]

111 **Tang OY**, Tian L, Yoder T, Xu R, Kulikovskaya I, Gupta M, Melenhorst JJ, Lacey SF, O'Rourke DM, Binder ZA. PD1 Expression in EGFRvIII-Directed CAR T Cell Infusion Product for Glioblastoma Is Associated with Clinical Response. *Front Immunol* 2022; **13**: 872756 [PMID: 35603165 DOI: 10.3389/fimmu.2022.872756]

112 **Sampson JH**, Choi BD, Sanchez-Perez L, Suryadevara CM, Snyder DJ, Flores CT, Schmittling RJ, Nair SK, Reap EA, Norberg PK, Herndon JE 2nd, Kuan CT, Morgan RA, Rosenberg SA, Johnson LA. EGFRvIII mCAR-modified T-cell therapy cures mice with established intracerebral glioma and generates host immunity against tumor-antigen loss. *Clin Cancer Res* 2014; **20**: 972-984 [PMID: 24352643 DOI: 10.1158/1078-0432.CCR-13-0709]

113 **Morgan RA**, Yang JC, Kitano M, Dudley ME, Laurencot CM, Rosenberg SA. Case report of a serious adverse event following the administration of T cells transduced with a chimeric antigen receptor recognizing ERBB2. *Mol Ther* 2010; **18**: 843-851 [PMID: 20179677 DOI: 10.1038/mt.2010.24]

114 **Jarboe JS**, Johnson KR, Choi Y, Lonser RR, Park JK. Expression of interleukin-13 receptor alpha2 in glioblastoma

multiforme: implications for targeted therapies. *Cancer Res* 2007; **67**: 7983-7986 [PMID: 17804706 DOI: 10.1158/0008-5472.CAN-07-1493]

115 **Mintz A**, Gibo DM, Slagle-Webb B, Christensen ND, Debinski W. IL-13Ralpha2 is a glioma-restricted receptor for interleukin-13. *Neoplasia* 2002; **4**: 388-399 [PMID: 12192597 DOI: 10.1038/sj.neo.7900234]

116 **Brown CE**, Badie B, Barish ME, Weng L, Ostberg JR, Chang WC, Naranjo A, Starr R, Wagner J, Wright C, Zhai Y, Bading JR, Ressler JA, Portnow J, D'Apuzzo M, Forman SJ, Jensen MC. Bioactivity and Safety of IL13Rα2-Redirected Chimeric Antigen Receptor CD8+ T Cells in Patients with Recurrent Glioblastoma. *Clin Cancer Res* 2015; **21**: 4062-4072 [PMID: 26059190 DOI: 10.1158/1078-0432.CCR-15-0428]

117 **Muhammad N**, Wang R, Li W, Zhang Z, Chang Y, Hu Y, Zhao J, Zheng X, Mao Q, Xia H. A novel TanCAR targeting IL13Rα2 and EphA2 for enhanced glioblastoma therapy. *Mol Ther Oncolytics* 2022; **24**: 729-741 [PMID: 35317513 DOI: 10.1016/j.omto.2022.02.012]

118 **Xu C**, Bai Y, An Z, Hu Y, Zhang C, Zhong X. IL-13Rα2 humanized scFv-based CAR-T cells exhibit therapeutic activity against glioblastoma. *Mol Ther Oncolytics* 2022; **24**: 443-451 [PMID: 35141400 DOI: 10.1016/j.omto.2022.01.002]

119 **Engelhardt B**, Ransohoff RM. Capture, crawl, cross: the T cell code to breach the blood-brain barriers. *Trends Immunol* 2012; **33**: 579-589 [PMID: 22926201 DOI: 10.1016/j.it.2012.07.004]

120 **Bagley SJ**, O'Rourke DM. Clinical investigation of CAR T cells for solid tumors: Lessons learned and future directions. *Pharmacol Ther* 2020; **205**: 107419 [PMID: 31629009 DOI: 10.1016/j.pharmthera.2019.107419]

121 **Bielamowicz K**, Fousek K, Byrd TT, Samaha H, Mukherjee M, Aware N, Wu MF, Orange JS, Sumazin P, Man TK, Joseph SK, Hegde M, Ahmed N. Trivalent CAR T cells overcome interpatient antigenic variability in glioblastoma. *Neuro Oncol* 2018; **20**: 506-518 [PMID: 29016929 DOI: 10.1093/neuonc/nox182]

122 **Luo H**, Su J, Sun R, Sun Y, Wang Y, Dong Y, Shi B, Jiang H, Li Z. Coexpression of IL7 and CCL21 Increases Efficacy of CAR-T Cells in Solid Tumors without Requiring Preconditioned Lymphodepletion. *Clin Cancer Res* 2020; **26**: 5494-5505 [PMID: 32816947 DOI: 10.1158/1078-0432.CCR-20-0777]

123 **Chmielewski M**, Abken H. CAR T Cells Releasing IL-18 Convert to T-Bet(high) FoxO1(low) Effectors that Exhibit Augmented Activity against Advanced Solid Tumors. *Cell Rep* 2017; **21**: 3205-3219 [PMID: 29241547 DOI: 10.1016/j.celrep.2017.11.063]

124 **Liu Y**, Di S, Shi B, Zhang H, Wang Y, Wu X, Luo H, Wang H, Li Z, Jiang H. Armored Inducible Expression of IL-12 Enhances Antitumor Activity of Glypican-3-Targeted Chimeric Antigen Receptor-Engineered T Cells in Hepatocellular Carcinoma. *J Immunol* 2019; **203**: 198-207 [PMID: 31142602 DOI: 10.4049/jimmunol.1800033]

125 **Liu X**, Ranganathan R, Jiang S, Fang C, Sun J, Kim S, Newick K, Lo A, June CH, Zhao Y, Moon EK. A Chimeric Switch-Receptor Targeting PD1 Augments the Efficacy of Second-Generation CAR T Cells in Advanced Solid Tumors. *Cancer Res* 2016; **76**: 1578-1590 [PMID: 26979791 DOI: 10.1158/0008-5472.CAN-15-2524]

126 **Goebeler ME**, Bargou RC. T cell-engaging therapies - BiTEs and beyond. *Nat Rev Clin Oncol* 2020; **17**: 418-434 [PMID: 32242094 DOI: 10.1038/s41571-020-0347-5]

127 **Choi BD**, Yu X, Castano AP, Bouffard AA, Schmidts A, Larson RC, Bailey SR, Boroughs AC, Frigault MJ, Leick MB, Scarfò I, Cetrulo CL, Demehri S, Nahed BV, Cahill DP, Wakimoto H, Curry WT, Carter BS, Maus MV. CAR-T cells secreting BiTEs circumvent antigen escape without detectable toxicity. *Nat Biotechnol* 2019; **37**: 1049-1058 [PMID: 31332324 DOI: 10.1038/s41587-019-0192-1]

128 **Wollmann G**, Ozduman K, van den Pol AN. Oncolytic virus therapy for glioblastoma multiforme: concepts and candidates. *Cancer J* 2012; **18**: 69-81 [PMID: 22290260 DOI: 10.1097/PPO.0b013e31824671c9]

129 **Mahmoud AB**, Ajina R, Aref S, Darwish M, Alsayb M, Taher M, AlSharif SA, Hashem AM, Alkayyal AA. Advances in immunotherapy for glioblastoma multiforme. *Front Immunol* 2022; **13**: 944452 [PMID: 36311781 DOI: 10.3389/fimmu.2022.944452]

130 **Foreman PM**, Friedman GK, Cassady KA, Markert JM. Oncolytic Virotherapy for the Treatment of Malignant Glioma. *Neurotherapeutics* 2017; **14**: 333-344 [PMID: 28265902 DOI: 10.1007/s13311-017-0516-0]

131 **Peruzzi P**, Chiocca EA. Viruses in cancer therapy - from benchwarmers to quarterbacks. *Nat Rev Clin Oncol* 2018; **15**: 657-658 [PMID: 30057404 DOI: 10.1038/s41571-018-0077-0]

132 **Alessandrini F**, Menotti L, Avitabile E, Appolloni I, Ceresa D, Marubbi D, Campadelli-Fiume G, Malatesta P. Eradication of glioblastoma by immuno-virotherapy with a retargeted oncolytic HSV in a preclinical model. *Oncogene* 2019; **38**: 4467-4479 [PMID: 30755732 DOI: 10.1038/s41388-019-0737-2]

133 **Kiyokawa J**, Wakimoto H. Preclinical And Clinical Development Of Oncolytic Adenovirus For The Treatment Of Malignant Glioma. *Oncolytic Virother* 2019; **8**: 27-37 [PMID: 31750274 DOI: 10.2147/OV.S196403]

134 **Rajaraman S**, Canjuga D, Ghosh M, Codrea MC, Sieger R, Wedekink F, Tatagiba M, Koch M, Lauer UM, Nahnsen S, Rammensee HG, Mühlebach MD, Stevanovic S, Tabatabai G. Measles Virus-Based Treatments Trigger a Pro-inflammatory Cascade and a Distinctive Immunopeptidome in Glioblastoma. *Mol Ther Oncolytics* 2019; **12**: 147-161 [PMID: 30775418 DOI: 10.1016/j.omto.2018.12.010]

135 **Geletneky K**, Hajda J, Angelova AL, Leuchs B, Capper D, Bartsch AJ, Neumann JO, Schöning T, Hüsing J, Beelte B, Kiprianova I, Roscher M, Bhat R, von Deimling A, Brück W, Just A, Frehtman V, Löbhard S, Terletskaia-Ladwig E, Fry J, Jochims K, Daniel V, Krebs O, Dahm M, Huber B, Unterberg A, Rommelaere J. Oncolytic H-1 Parvovirus Shows Safety and Signs of Immunogenic Activity in a First Phase I/IIa Glioblastoma Trial. *Mol Ther* 2017; **25**: 2620-2634 [PMID: 28967558 DOI: 10.1016/j.ymthe.2017.08.016]

136 **Freeman AI**, Zakay-Rones Z, Gomori JM, Linetsky E, Rasooly L, Greenbaum E, Rozenman-Yair S, Panet A, Libson E, Irving CS, Galun E, Siegal T. Phase I/II trial of intravenous NDV-HUJ oncolytic virus in recurrent glioblastoma multiforme. *Mol Ther* 2006; **13**: 221-228 [PMID: 16257582 DOI: 10.1016/j.ymthe.2005.08.016]

137 **Kicielinski KP**, Chiocca EA, Yu JS, Gill GM, Coffey M, Markert JM. Phase 1 clinical trial of intratumoral reovirus infusion for the treatment of recurrent malignant gliomas in adults. *Mol Ther* 2014; **22**: 1056-1062 [PMID: 24553100 DOI: 10.1038/mt.2014.21]

138 **Gromeier M**, Lachmann S, Rosenfeld MR, Gutin PH, Wimmer E. Intergeneric poliovirus recombinants for the treatment of malignant glioma. *Proc Natl Acad Sci U S A* 2000; **97**: 6803-6808 [PMID: 10841575 DOI: 10.1073/pnas.97.12.6803]

139 **Zhu Z**, Mesci P, Bernatchez JA, Gimple RC, Wang X, Schafer ST, Wettersten HI, Beck S, Clark AE, Wu Q, Prager BC, Kim LJY, Dhanwani R, Sharma S, Garancher A, Weis SM, Mack SC, Negraes PD, Trujillo CA, Penalva LO, Feng J, Lan Z, Zhang R, Wessel AW, Dhawan S, Diamond MS, Chen CC, Wechsler-Reya RJ, Gage FH, Hu H, Siqueira-Neto JL, Muotri AR, Cheresh DA, Rich JN. Zika Virus Targets Glioblastoma Stem Cells through a SOX2-Integrin α(v)β(5) Axis. *Cell Stem Cell* 2020; **26**: 187-204.e10 [PMID: 31956038 DOI: 10.1016/j.stem.2019.11.016]

140 **Chowdhury S**, Bappy MH, Clocchiatti-Tuozzo S, Cheeti S, Chowdhury S, Patel V. Current Advances in Immunotherapy for Glioblastoma Multiforme and Future Prospects. *Cureus* 2021; **13**: e20604 [PMID: 35103180 DOI: 10.7759/cureus.20604]

141 **Lu D**, Gao Y. Immune Checkpoint Inhibitor-related Endocrinopathies. *J Transl Int Med* 2022; **10**: 9-14 [PMID: 35702188 DOI: 10.2478/jtim-2022-0009]

142 **Alok A**, Seok K, Wesolow J. A Case of Abdominal Pain and Diarrhea Post Immunotherapy: Hypophysitis Associated with Immune Checkpoint Inhibitors. *J Transl Int Med* 2022; **10**: 178-180 [PMID: 35959448 DOI: 10.2478/jtim-2022-0030]

143 **Yuan B**, Wang G, Tang X, Tong A, Zhou L. Immunotherapy of glioblastoma: Recent advances and future prospects. *Hum Vaccin Immunother* 2022; **18**: 2055417 [PMID: 35344682 DOI: 10.1080/21645515.2022.2055417]

144 **Apavaloaei A**, Hardy MP, Thibault P, Perreault C. The Origin and Immune Recognition of Tumor-Specific Antigens. *Cancers (Basel)* 2020; **12** [PMID: 32932620 DOI: 10.3390/cancers12092607]

145 **Majc B**, Novak M, Kopitar-Jerala N, Jewett A, Breznik B. Immunotherapy of Glioblastoma: Current Strategies and Challenges in Tumor Model Development. *Cells* 2021; **10** [PMID: 33572835 DOI: 10.3390/cells10020265]

146 **Zhao T**, Li C, Ge H, Lin Y, Kang D. Glioblastoma vaccine tumor therapy research progress. *Chin Neurosurg J* 2022; **8**: 2 [PMID: 35045874 DOI: 10.1186/s41016-021-00269-7]

147 **Zhou J**, Li L, Jia M, Liao Q, Peng G, Luo G, Zhou Y. Dendritic cell vaccines improve the glioma microenvironment: Influence, challenges, and future directions. *Cancer Med* 2023; **12**: 7207-7221 [PMID: 36464889 DOI: 10.1002/cam4.5511]

148 **Tan L**, Peng J, Liu P, Wu Q. The Efficacy of Dendritic Cell Vaccine for Newly Diagnosed Glioblastoma: A Meta-analysis of Randomized Controlled Studies. *Clin Neuropharmacol* 2021; **44**: 216-221 [PMID: 34767325 DOI: 10.1097/WNF.0000000000000452]

149 **Liau LM**, Ashkan K, Brem S, Campian JL, Trusheim JE, Iwamoto FM, Tran DD, Ansstas G, Cobbs CS, Heth JA, Salacz ME, D'Andre S, Aiken RD, Moshel YA, Nam JY, Pillainayagam CP, Wagner SA, Walter KA, Chaudhary R, Goldlust SA, Lee IY, Bota DA, Elinzano H, Grewal J, Lillehei K, Mikkelsen T, Walbert T, Abram S, Brenner AJ, Ewend MG, Khagi S, Lovick DS, Portnow J, Kim L, Loudon WG, Martinez NL, Thompson RC, Avigan DE, Fink KL, Geoffroy FJ, Giglio P, Gligich O, Krex D, Lindhorst SM, Lutzky J, Meisel HJ, Nadji-Ohl M, Sanchin L, Sloan A, Taylor LP, Wu JK, Dunbar EM, Etame AB, Kesari S, Mathieu D, Piccioni DE, Baskin DS, Lacroix M, May SA, New PZ, Pluard TJ, Toms SA, Tse V, Peak S, Villano JL, Battiste JD, Mulholland PJ, Pearlman ML, Petrecca K, Schulder M, Prins RM, Boynton AL, Bosch ML. Association of Autologous Tumor Lysate-Loaded Dendritic Cell Vaccination With Extension of Survival Among Patients With Newly Diagnosed and Recurrent Glioblastoma: A Phase 3 Prospective Externally Controlled Cohort Trial. *JAMA Oncol* 2023; **9**: 112-121 [PMID: 36394838 DOI: 10.1001/jamaoncol.2022.5370]

150 **Alahmari A**. Blood-Brain Barrier Overview: Structural and Functional Correlation. *Neural Plast* 2021; **2021**: 6564585 [PMID: 34912450 DOI: 10.1155/2021/6564585]

151 **Jackson CM**, Choi J, Lim M. Mechanisms of immunotherapy resistance: lessons from glioblastoma. *Nat Immunol* 2019; **20**: 1100-1109 [PMID: 31358997 DOI: 10.1038/s41590-019-0433-y]

152 **Medikonda R**, Dunn G, Rahman M, Fecci P, Lim M. A review of glioblastoma immunotherapy. *J Neurooncol* 2021; **151**: 41-53 [PMID: 32253714 DOI: 10.1007/s11060-020-03448-1]

153 **Reits EA**, Hodge JW, Herberts CA, Groothuis TA, Chakraborty M, Wansley EK, Camphausen K, Luiten RM, de Ru AH, Neijssen J, Griekspoor A, Mesman E, Verreck FA, Spits H, Schlom J, van Veelen P, Neefjes JJ. Radiation modulates the peptide repertoire, enhances MHC class I expression, and induces successful antitumor immunotherapy. *J Exp Med* 2006; **203**: 1259-1271 [PMID: 16636135 DOI: 10.1084/jem.20052494]

154 **Omuro A**, Vlahovic G, Lim M, Sahebjam S, Baehring J, Cloughesy T, Voloschin A, Ramkissoon SH, Ligon KL, Latek R, Zwirtes R, Strauss L, Paliwal P, Harbison CT, Reardon DA, Sampson JH. Nivolumab with or without ipilimumab in patients with recurrent glioblastoma: results from exploratory phase I cohorts of CheckMate 143. *Neuro Oncol* 2018; **20**: 674-686 [PMID: 29106665 DOI: 10.1093/neuonc/nox208]

155 **Schuessler A**, Smith C, Beagley L, Boyle GM, Rehan S, Matthews K, Jones L, Crough T, Dasari V, Klein K, Smalley A, Alexander H, Walker DG, Khanna R. Autologous T-cell therapy for cytomegalovirus as a consolidative treatment for recurrent glioblastoma. *Cancer Res* 2014; **74**: 3466-3476 [PMID: 24795429 DOI: 10.1158/0008-5472.CAN-14-0296]

156 **Nehama D**, Di Ianni N, Musio S, Du H, Patané M, Pollo B, Finocchiaro G, Park JJH, Dunn DE, Edwards DS, Damrauer JS, Hudson H, Floyd SR, Ferrone S, Savoldo B, Pellegatta S, Dotti G. B7-H3-redirected chimeric antigen receptor T cells target glioblastoma and neurospheres. *EBioMedicine* 2019; **47**: 33-43 [PMID: 31466914 DOI: 10.1016/j.ebiom.2019.08.030]

157 **Viscardi G**, Tralongo AC, Massari F, Lambertini M, Mollica V, Rizzo A, Comito F, Di Liello R, Alfieri S, Imbimbo M, Della Corte CM, Morgillo F, Simeon V, Lo Russo G, Proto C, Prelaj A, De Toma A, Galli G, Signorelli D, Ciardiello F, Remon J, Chaput N, Besse B, de Braud F, Garassino MC, Torri V, Cinquini M, Ferrara R. Comparative assessment of early mortality risk upon immune checkpoint inhibitors alone or in combination with other agents across solid malignancies: a systematic review and meta-analysis. *Eur J Cancer* 2022; **177**: 175-185 [PMID: 36368251 DOI: 10.1016/j.ejca.2022.09.031]

158 **Pan S**, Sun S, Liu B, Hou Y. Pan-cancer Landscape of the RUNX Protein Family Reveals their Potential as Carcinogenic Biomarkers and the Mechanisms Underlying their Action. *J Transl Int Med* 2022; **10**: 156-174 [PMID: 35959452 DOI: 10.2478/jtim-2022-0013]

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MINIREVIEWS

Integration of molecular testing for the personalized management of patients with diffuse large B-cell lymphoma and follicular lymphoma

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Abstract

Diffuse large B-cell lymphoma (DLBCL) and follicular lymphoma (FL) are the most common forms of aggressive and indolent lymphoma, respectively. The majority of patients are cured by standard R-CHOP immunochemotherapy, but 30%–40% of DLBCL and 20% of FL patients relapse or are refractory (R/R). DLBCL and FL are phenotypically and genetically hereterogenous B-cell neoplasms. To date, the diagnosis of DLBCL and FL has been based on morphology, immunophenotyping and cytogenetics. However, next-generation sequencing (NGS) is widening our understanding of the genetic basis of the B-cell lymphomas. In this review we will discuss how integrating the NGS-based characterization of somatic gene mutations with diagnostic or prognostic value in DLBCL and FL could help refine B-cell lymphoma classification as part of a multidisciplinary pathology work-up. We will also discuss how molecular testing can identify candidates for clinical trials with targeted therapies and help predict therapeutic outcome to currently available treatments, including chimeric antigen receptor T-cell, as well as explore the application of circulating cell-free DNA, a non-invasive method for patient monitoring. We conclude that molecular analyses can drive improvements in patient outcomes due to an increased understanding

of the different pathogenic pathways affected by each DLBCL subtype and indolent FL *vs* R/R FL.

Key Words: Next-generation sequencing; Prognosis; Molecular analysis; Targeted therapy; Chimeric antigen receptor T-cell therapy; Personalized medicine

Core Tip: Molecular studies in the past decade have improved our understanding of the biological heterogeneity of B-cell lymphomas such as diffuse large B-cell lymphoma (DLBCL) and follicular lymphoma. Next-generation sequencing studies are helping to reveal the different pathogenic pathways affected by each DLBCL subtype and identify new targets for directed therapy. Molecular analysis can also help predict therapeutic outcome to currently available treatments, including chimeric antigen receptor T-cell therapy, and identify candidates for clinical trials with targeted therapies, ultimately leading to improvements in patient outcomes. As such, the incorporation of precision medicine *via* the integration of molecular analyses in clinical practice can improve clinical outcomes in patients and thus contribute to a new standard of care for patients with B-cell lymphomas.

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INTRODUCTION

B-cell lymphomas are classified into over 19 distinct entities, as defined by the 2022 World Health Organization (WHO) classification[1]. Diffuse large B-cell lymphoma (DLBCL) is the most common form of non-Hodgkin lymphoma (NHL), representing approximately 30% of lymphomas of mature B-cells[2], while follicular lymphoma (FL) is the second most common NHL. However, both DLBCL and FL are phenotypically and genetically hereterogenous B-cell neoplasms. For example, the majority of DLBCL patients are cured by standard rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone (R-CHOP) immunochemotherapy, but 30%–40% relapse or are refractory (R/R), while for FL, approximately 20% of patients treated with chemoimmunotherapy will progress within the first two years of diagnosis (POD24)[3]. Thus, improvements in patient outcomes will rely on an increased understanding of the different pathogenic DLBCL and FL pathways that lead to treatment failure and/or progression.

Next-generation sequencing (NGS) studies together with copy-number analysis are determining genes with recurrent alterations in DLBCL and FL, some of which can refine diagnosis and prognostic stratification. In this minireview, we will describe how molecular analyses are revealing differences in somatic mutations according to disease subtypes, helping with differential diagnosis, as well as determining new targets for the development of directed therapies. We will also explore the application of circulating cell-free DNA, a non-invasive method for patient monitoring. Finally, we will discuss how the incorporation of precision medicine can identify candidates for clinical trials with targeted therapies and help predict therapeutic outcome to currently available treatments in a drive towards a more personalized treatment approach.

We aim to convince the reader that the incorporation of molecular testing for somatic gene mutations can improve the diagnosis and prognosis of patients with DLBCL and FL as part of a multidisciplinary pathology work-up.

CONVENTIONAL CLASSIFICATION OF DLBCL

Currently, using immunohistochemistry (IHC), fluorescence in situ hybridization (FISH) and flow cytometry techniques, patients with DLBCL are divided into three subtypes depending on the stage of differentiation and maturation of the B cells of origin: germinal center B-cell like (GCB) or activated B-cell like (ABC), with the remaining 10% to 20% "unclassified" or not otherwise specified (NOS). For example, IHC analysis of *CD10*, *BCL6* and *MUM1* markers helps determine the GCB and ABC subtypes according to the Hans algorithm[4]. Nevertheless, the Hans algorithm doesn't distinguish the NOS DLBCL subtype and gives an incorrect classification in approximately 20% of cases[4].

DLBCL patients show distinct clinical outcomes according to the subtype: patients with the GCB phenotype have a more favorable outcome than ABC in terms of survival when treated with standard chemotherapy, 60% at 5 years *vs* 35% in ABC[5]. In addition, IHC of *MYC* and *BCL2/BCL6* can identify tumors as double- or triple-expressor, associated with worse prognosis[6]. Studies involving FISH analysis of *MYC* rearrangements have shown that *MYC* rearranged with an immunoglobulin (IG) gene has worse prognosis compared to *MYC* with a non-IG partner, with *MYC*/IG double hits associated with an even poorer prognosis[7]. Thus, genomic tests used in routine clinical practice are already adding prognostic value. Even so, the diagnostic work-up and treatment are practically identical for all DLBCL patients despite the high genetic heterogeneity.

In terms of treatment, R-CHOP has been the standard of care for over two decades, and is still administered to the majority of DLBCL patients[8]. To improve treatment response, and predict which patients are likely to be R/R, elucidation of the molecular determinants related to treatment response will be fundamental. One advancement in this area is the observation that high *EZH2* expression (> 70%, detected by IHC) is associated with superior survival of DLBCL patients following R-CHOP[9].

CONVENTIONAL CLASSIFICATION OF FL

FL is characterized by the t(14;18)(q32;q21) translocation, present in 90% of FL patients, resulting in overexpression of *BCL2* under the *IGH* promoter. In cases lacking t(14;18), BCL6 and CD10 expression patterns confirm FL diagnosis[10]. Rearrangement of *BCL6* (3q27) may also be found in grade 3 FL, with or without t(14;18)[11].

Prognostic biological factors include age > 60 years and hemoglobin < 12 g/dL, as well as other biomarkers, such as LDH or β2-microglobin above normal, according to the FLIPI and FLIPI-2 scores, respectively[12,13].

Several first-lines of immunochemotherapy exist, including bendamustine + rituximab, rituximab alone, or R-CHOP, with choice largely down to the clinician's preference. Treatment improvements are a necessity, given that POD24 is a predictor of overall survival (OS), with rates of just 50% for patients with POD24 *vs* 90% in those with no POD24 following R-CHOP treatment[3]. As highlighted in the recent editorial by Leonard[14], there is currently "no reliable way" to determine at diagnosis whether a patient with FL is likely to respond optimally to immunochemotherapy. The hope is that molecular analyses could help identify a subgroup of at-risk patients who would benefit from upfront treatment with a specific targeted therapy.

NGS APPLICATION IN LYMPHOMAS

According to the 2022 WHO and ICC classifications and the European Society for Medical Oncology's 2021 clinical guidelines, no molecular analyses are currently recommended at diagnosis for DLBCL or FL[1,15,16]. To date, only a few entities of lymphoid neoplasms are defined by genomic criteria. This is in stark contrast to other hematological malignancies, in particular myeloid neoplasms, where the use of NGS is well-established in diagnosis and risk-stratification[1,16,17]. For example, for acute myeloid leukemia a complete genomic evaluation, including NGS panel, is obligatory at diagnosis to define disease subtypes and to direct therapies[1,16,17]. Nevertheless, both international consortiums acknowledge that molecular analyses in B-cell lymphomas have identified genomic alterations "with diagnostic, prognostic, and predictive impact in different entities"[18] and explicitly state that it is highly probable that more entities will be defined by genomic criteria in the near future[1,16,18].

MOLECULAR ANALYSES IN DLBCL

In recent years, advances in next-generation sequencing (NGS) techniques are redefining our understanding of the genetic basis of lymphomas. Molecular studies are revealing recurrent genetic events and thus are helping to identify the key pathways that are important in DLBCL pathogenicity and evolution, and may even have prognostic impact[19].

Mutations in the genes *MYD88, CARD11, EZH2* and *CD79A/CD79B* have been identified in approximately 40% of DLBCL and are considered drivers of lymphomagenesis[20]. Moreover, NGS studies have revealed that GCB and ABC have a distinct profile of somatic mutations. For instance, mutations in *GNA13* are found in GCB but are rare in other B-cell lymphoma subtypes[21], whereas the MYD88 L265P mutation is found in ABC but is rarely identified in GCB DLBCL[21]. Thus, mutational information can assist in providing an accurate diagnosis, for example for the differential diagnosis of DLBCL from primary mediastinal large B-cell lymphoma (PMBCL, a relatively rare NHL with large B-cell morphology)[22], mantle cell lymphoma, or grade 3 FL. In addition, relapse has been associated with mutations in certain genes, such as the *B2M* and *CD58* immune surveillance genes[23].

Recent findings suggest that tumor genotype also influences treatment response. For example, whole genome sequencing analysis of 20 patients with high-risk GCB DLBCL revealed that those with cryptic rearrangements of *MYC* or *BCL2* (not detectable by FISH) had worse outcomes to R-CHOP[24]. Moreover, ABC tumors that harboured both a mutation in *CD79B* and the *MYD88* L265P mutation were more sensitive to the BTK inhibitor ibrutinib, whereas NOS subtype tumors with the *MYD88* L265P mutation and *CD79B* wild-type showed a poor response to ibrutinib[25].

Although NGS of DLBCL is not currently recommended in routine clinical practice[1,16], huge efforts are underway to characterize the prognostic value and thus functional impact of driver mutations, for instance *via* the whole-exome sequencing of 1001 DLBCL samples[26].

Such large-scale studies using NGS techniques together with copy-number analysis, to identify genes with recurrent alterations with prognostic value, have led to the proposition of new DLBCL classifications. After studying 574 DLBCL biopsy samples Schmitz *et al*[27] proposed four genetic subtypes termed MCD (based on the co-occurrence of *MYD88* and *CD79B* mutations), BN2 (based on *BCL6* fusions and *NOTCH2* mutations), N1 (based on *NOTCH1* mutations), and EZB (based on *EZH2* mutations and *BCL2* translocations). These subtypes differed in their responses to immunochemotherapy, with favorable survival in the BN2 and EZB subtypes and inferior outcomes in the MCD and N1 subtypes[27], whereas MCD and N1 subtypes responded well to R-CHOP with ibrutinib[28]. Importantly, data on the BN2 subtype, with overlap with the NOS subgroup, revealed that patients are likely to be responsive to antagonists of B-cell receptor signaling such as the BTK inhibitors. Similarly, Chapuy *et al*[29] studied 304 DLBCL biopsies and identified six genetic subgroups. Of note, mutations in *CD79B* were associated with relapse independently of the subtype or International Prognostic Index (IPI) risk group[30].

MOLECULAR ANALYSES IN FL

Mutations in genes encoding epigenetic modifiers (and the resultant pattern of aberrant DNA methylation) are a molecular hallmark of FL (Table 1)[31,32]. Moreover, such mutations are likely to be early driver events[33].

NGS studies have revealed that the acquisition of additional mutations contributes to disease progression and the risk of transformation of FL to DLBCL. For example, *TP53* mutations have been associated with shorter progression-free survival and OS[34,35], while the gain of mutations in genes such as *EBF1*, *MYD88* and *TNFAIP3* are associated with progression to a more aggressive disease[32]. Additionally, expression studies have revealed chromosome regions, such as 1p36 and 6q21 deletion associated with transformation[36]. Thus, genetic analyses can improve the prognostication of patients with FL[37].

In the case of FL, the mutational status of seven genes (*EZH2*, *ARID1A*, *MEF2B*, *EP300*, *FOXO1*, *CREBBP* and *CARD11*) was added to the preexisting Eastern Cooperative Oncology Group (ECOG) performance status, FL International Prognostic Index (ECOG PS and FLIPI) risk stratification algorithms to develop the m7-FLIPI risk score[38]. Application of the m7-FLIPI risk score defined a high-risk group with a significantly shorter failure-free survival after receiving first-line R-CHOP.

Specifically, mutations in *EZH2* were associated with the low-risk m7-FLIPI group and with higher OS[38]. As such, the presence or absence of the *EZH2* Y646 point mutation can help decide the chemotherapy regime in a patient-specific manner, since patients with such a mutation were shown to respond well to R-CHOP[38] (higher OS and lower relapse rate) while patients without this mutation responded better to bendamustine[39].

Although the m7-FLIPI was not prognostic for FL patients who received rituximab, patients with *EZH2* mutations had longer time to treatment failure while *EP300* mutations were associated with shorter time to treatment failure[40]. Therefore, it remains to be determined if the m7-FLIPI risk score is prognostic for FL patients treated with other chemotherapy regimes other than R-CHOP. Furthermore, the use of such risk scores in the routine clinical practice is not common, partly due to lack of availability of mutational studies in some centers[41].

TARGETED THERAPIES IN DLBCL

It is clear that improvements in DLBCL outcomes will rely on an increased understanding of the different pathogenic pathways affected by each DLBCL subtype. Indeed, an *in silico* drug discovery analysis showed that 46% of cases harbored at least one genomic alteration considered to be a potential drug response target (according to early clinical trials or preclinical assays in DLBCL or other B-cell lymphomas)[30].

But, to date, only one targeted therapy against a molecular driver has been approved for DLBCL – selinexor – although many others are in development. Selinexor is a specific inhibitor of the XPO1 nuclear export transporter protein that was approved by the FDA in June 2020 for the treatment of adults with R/R DLBCL NOS, including DLBCL progressed from FL, after at least two previous lines of

Table 1 Frequent mutations detected in follicular lymphoma. Adapted from[32,37]

Gene	Frequency in FL, %
KMT2D	82
CREBBP	65-70
HIST1H1C and/or HIST1H1E	28
EZH2	20-25
EP300	14
STAT6	12
CARD11	11
TNFAIP3	11
SOCS1	8
TP53	6

FL: Follicular lymphoma.

therapy[42].

Besides targeted agents, many immunotherapy strategies are in development, with the aim to promote the immune recognition and cytotoxic attack of T cells or macrophages. Some have achieved approval, such as rituximab, a monoclonal antibody against the common surface antigen CD20, and polatuzumab vedotin (Pola; Polivy™), an antibody-drug conjugate. Pola includes an anti-CD79B monoclonal antibody for cell targeting, which upon binding allows the antineoplastic agent monomethyl auristatin E to enter the cell and inhibit microtubule assembly, preventing cell mitosis and ultimately causing apoptosis[43]. Pola was approved by the FDA in June 2019 in combination with rituximab and bendamustine for the treatment of adults with R/R DLBCL after at least two previous lines of therapy. Such strategies have the advantage that the surface antigens they target are universally expressed on all DLBCL subtypes.

TARGETED THERAPIES IN FL

Genetic studies have identified epigenetic mechanisms in the pathogenesis of both DLBCL and FL, such as acetylation/deacetylation affected by *CREBBP* and *EP300* mutations, or histone methylation changes affected by *EZH2* mutations. Indeed, Tazemetostat (Tazverik™), an EZH2 inhibitor, was the first directed therapy to be approved by the FDA (in June 2020) for the treatment of R/R FL after two lines of previous therapy[44]. The *EZH2* mutation is predictive of Tazemetostat response but, interestingly, this targeted agent was also shown to improve the outcome of patients without an *EZH2* mutation[44].

Other FDA-approved agents for R/R FL include four PI3K signaling inhibitors: Idelalisib (July 2014), copanlisib (September 2017), duvelisib (September 2018), and umbralisib (February 2021)[45-48]. Further information on FL therapies in development can be found in this recent review[37].

CAR-T

Besides targeted therapies, an improved understanding of the genetic and immune biology of DLBCL and FL has led to the development of chimeric antigen receptor T-cell (CAR-T) therapies, considered a major scientific breakthrough and offering an alternative treatment option for patients with R/R B-cell lymphomas[49,50].

In 2020, our center obtained the license to provide the European Medicine Agency-approved anti-CD19 CAR-T axicabtagene ciloleucel (Yescarta™) and Tisagenlecleucel (Kymriah™) for the treatment of adult patients with R/R DLBCL or PMBCL after two or more previous lines of treatment. As of February 2021, the third CAR-T lisocabtagene maraleucel (Breyanzi™) also obtained FDA approval for the treatment of R/R DLBCL[51].

CAR-T is also an option for the treatment of adult R/R FL patients after two or more previous lines of treatment[52,53], following the FDA approval of axicabtagene ciloleucel in March 2021.

The proliferation and persistence of CAR-T cells in the body is an important factor influencing therapy durability, with the loss of a CAR-T signal associated with progression of the disease[54]. A quantitative TaqMan PCR (qPCR) assay can be used to monitor the number of CAR-T cells circulating in

peripheral blood *via* detection of the quimeric CD19 recognition domain (FMC63)[55]. Flow cytometry is an alternative method for CAR-T cell monitoring, but has the disadvantage that it needs to be carried out on fresh samples and has lower sensitivity. Future studies are required to explore the correlation between the expansion/persistence of CAR-T cells and clinical outcomes including treatment efficacy and clinical symptoms.

CIRCULATING CELL-FREE TUMOR DNA

Surgical excision biopsies are the gold-standard technique used in the diagnosis and follow-up of patients with lymphomas, although core needle biopsies are a useful and viable alternative under certain conditions[56]. However, both surgical excision and core needle biopsies are resource intensive, can be painful, and impact negatively on patients, and surgical excision biopsies, in particular, have an associated risk of morbidity due to bleeding and infection. Additionally, some lymphomas may not be easily accessible which can limit the availability of tissue for genomic studies. Moreover, the extraction of genomic DNA from formalin-fixed, paraffin-embedded biopsies for downstream NGS applications is not ideal since chemicals used in the fixation can degrade nucleic acids, thus decreasing NGS sensitivity.

Liquid biopsy techniques are currently being explored as non-invasive methods for tumor diagnosis and disease monitoring[57]. Circulating cell-free DNA (ctDNA), consisting of highly fragmented DNA in plasma that is released by normal or tumor cells that undergo apoptosis or necrosis[58], may better reflect intratumoral heterogeneity than can be obtained from a single tissue biopsy. Indeed, in comparison with the sequencing of genomic DNA extracted from the diagnostic tissue biopsy, the sequencing of ctDNA can identify somatic mutations with a similar accuracy and identified additional clinically relevant mutations that were not detected in the diagnostic tissue biopsy[59]. Moreover, the analysis of ctDNA could overcome some other limitations of biopsies. For example, in the case of a biopsy at an extranodal site, it is not uncommon for the paraffin block to also contain other non-tumoral tissue.

Due to their easy accessibility through non-invasive procedures (such as a simple peripheral blood draw), ctDNA analyses can be repeated regularly to track lymphomas over time, such as to monitor treatment response. Indeed, studies have shown that changes in ctDNA quantification correlated with positive responses to chemotherapy and could even detect relapse, months earlier than conventional CT scan monitoring[60]. Thus they may also be useful as "surveillance" methods in patients who have completed treatment but may be at risk of relapse, *e.g.* those with mutations in *CD79B* or those with a high pretreatment ctDNA quantitative burden for early relapse detection[32,59].

Future studies are required to optimize the application of ctDNA analyses in the management of patients with B-cell lymphomas. Nevertheless, ctDNA is currently used in the clinic in some fields of oncology, such as in the molecular profiling of patients with non-squamous non-small cell lung cancer at diagnosis, as recommended by the National Comprehensive Cancer Network[61].

IMPLEMENTATION OF MOLECULAR TESTING IN CLINICAL PRACTICE

The application of NGS, together with other molecular techniques, is key to the integration of personalized medicine approaches into healthcare services. The use of NGS targeted panels, which focus on a limited and relevant set of genes or gene regions that have known associations with a particular pathology, produce large quantities of genetic information with diagnostic, prognostic and theranostic value with a high sensitivity. The simultaneous analysis of an elevated number of genes (15-200) is more resource efficient as it drastically reduces the cost and time required to obtain such genetic information enabling a more precise diagnosis and prognosis. Furthermore, the use of NGS permits the detection of emerging clones which can help inform disease follow-up and may be associated with treatment resistance, thus providing data that can help guide individualized patient therapeutic plans.

In 2016 our team implemented NGS into the routine diagnosis and prognosis of patients with acute myeloid leukemia[62]. Since then, the use of NGS has expanded to include a targeted myeloid panel for the diagnosis of patients with myeloproliferative neoplasms and myelodysplastic syndromes, a chronic lymphocytic leukemia-specific panel, and a panel for the detection of germline hematologic malignancies. However, the molecular analysis of B-cell lymphoma samples in our center is currently limited to the qPCR-based analysis of several individual genes with prognostic value (including *MYD88, TP53,* and *EZH2*) to complement the conventional cytometry, IHC and FISH tests used in routine clinical practice.

Several commercial gene panels are currently available on the market for the detection of mutations with diagnostic, prognostic or theranostic value in DLBCL and FL, given the considerable overlap of genetic alterations between GCB DLBCL and FL[32], including Oncomine™ Lymphoma (ThermoFisher), FusionPlex® Lymphoma (Archer) and Lymphoma Solution® (SOPHiA).

Incorporating a comprehensive NGS-based characterization of somatic gene mutations as a precision medicine strategy for B-cell lymphomas would assist in the daily practice by refining DLBCL and FL classification and prognosis. Importantly, it would also facilitate individualized therapeutic decision-making for patients and increase treatment opportunities by identifying candidates for clinical trials with targeted therapies. However, feasibility studies would be required to determine the clinical utility and added value of incorporating an NGS panel in the multidisciplinary diagnostic work-up, since "while many stakeholders believe that personalized medicine can provide benefits to patients and the healthcare system, payer and providers are often reluctant to change policies and practices without convincing evidence of clinical and economic value"[63].

It is also important to consider the limitations of introducing such molecular analyses for B-cell lymphomas into routine hematology laboratories. Difficulties arise in interpretation of the results generated by extensive NGS panels due to the data's complexity and uncertainty about the biological relevance as not all molecular variants are clinically actionable. For this reason, it is essential to have highly trained staff with experience in the interpretation of the clinical impact of tumor variants. Other limitations include the economic cost of molecular analyses and the turnaround time, which has a large impact on the applicability of genomic tests to clinical decision-making. The potential to multiplex lymphoma samples with other targeted panels in the same sequencing run would help optimize the resources dedicated to library preparation and sequencing, and minimize the time required to analyze patient samples and report results to guide clinical decision-making. This is essential for aggressive B-cell lymphomas where immediate treatment is frequently required.

CONCLUSION

The incorporation of molecular testing into the routine clinical management of patients with B-cell lymphomas *via* the implementation of a targeted NGS panel would help improve disease subtype classification, allow the prediction of therapeutic outcome to currently available treatments, and identify patients for personalized treatment. Moreover, the optimization of non-invasive ctDNA analysis could allow for closer patient monitoring and earlier relapse detection.

FOOTNOTES

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REFERENCES

1 **Alaggio R**, Amador C, Anagnostopoulos I, Attygalle AD, Araujo IBO, Berti E, Bhagat G, Borges AM, Boyer D, Calaminici M, Chadburn A, Chan JKC, Cheuk W, Chng WJ, Choi JK, Chuang SS, Coupland SE, Czader M, Dave SS, de Jong D, Du MQ, Elenitoba-Johnson KS, Ferry J, Geyer J, Gratzinger D, Guitart J, Gujral S, Harris M, Harrison CJ, Hartmann S, Hochhaus A, Jansen PM, Karube K, Kempf W, Khoury J, Kimura H, Klapper W, Kovach AE, Kumar S, Lazar AJ, Lazzi S, Leoncini L, Leung N, Leventaki V, Li XQ, Lim MS, Liu WP, Louissaint A Jr, Marcogliese A, Medeiros LJ, Michal M, Miranda RN, Mitteldorf C, Montes-Moreno S, Morice W, Nardi V, Naresh KN, Natkunam Y, Ng SB, Oschlies I, Ott G, Parrens M, Pulitzer M, Rajkumar SV, Rawstron AC, Rech K, Rosenwald A, Said J, Sarkozy C, Sayed S, Saygin C, Schuh A, Sewell W, Siebert R, Sohani AR, Tooze R, Traverse-Glehen A, Vega F, Vergier B,

Wechalekar AD, Wood B, Xerri L, Xiao W. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Lymphoid Neoplasms. *Leukemia* 2022; **36**: 1720-1748 [PMID: 35732829 DOI: 10.1038/s41375-022-01620-2]

2 **Bastos-Oreiro M**, Muntañola A, Panizo C, Gonzalez-Barca E, de Villambrosia SG, Córdoba R, López JLB, González-Sierra P, Terol MJ, Gutierrez A, Grande C, Ramirez MJ, Iserte L, Perez E, Navarro B, Gomez P, Salar A, Luzardo H, López A, Del Campo R, García-Belmonte D, Vida MJ, Infante M, Queizan-Hernandez JA, Novelli S, Moreno M, Penarrubia M, Gómez J, Domingo A, Donato E, Viguria MC, López F, Rodriguez MJ, Pardal E, Noriega V, Andreu R, Peñalver J, Martín A, Caballero D, López-Guillermo A. RELINF: prospective epidemiological registry of lymphoid neoplasms in Spain. A project from the GELTAMO group. *Ann Hematol* 2020; **99**: 799-808 [PMID: 32076827 DOI: 10.1007/s00277-020-03918-6]

3 **Casulo C**, Byrtek M, Dawson KL, Zhou X, Farber CM, Flowers CR, Hainsworth JD, Maurer MJ, Cerhan JR, Link BK, Zelenetz AD, Friedberg JW. Early Relapse of Follicular Lymphoma After Rituximab Plus Cyclophosphamide, Doxorubicin, Vincristine, and Prednisone Defines Patients at High Risk for Death: An Analysis From the National LymphoCare Study. *J Clin Oncol* 2015; **33**: 2516-2522 [PMID: 26124482 DOI: 10.1200/JCO.2014.59.7534]

4 **Hans CP**, Weisenburger DD, Greiner TC, Gascoyne RD, Delabie J, Ott G, Müller-Hermelink HK, Campo E, Braziel RM, Jaffe ES, Pan Z, Farinha P, Smith LM, Falini B, Banham AH, Rosenwald A, Staudt LM, Connors JM, Armitage JO, Chan WC. Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. *Blood* 2004; **103**: 275-282 [PMID: 14504078 DOI: 10.1182/blood-2003-05-1545]

5 **Rosenwald A**, Wright G, Chan WC, Connors JM, Campo E, Fisher RI, Gascoyne RD, Muller-Hermelink HK, Smeland EB, Giltnane JM, Hurt EM, Zhao H, Averett L, Yang L, Wilson WH, Jaffe ES, Simon R, Klausner RD, Powell J, Duffey PL, Longo DL, Greiner TC, Weisenburger DD, Sanger WG, Dave BJ, Lynch JC, Vose J, Armitage JO, Montserrat E, López-Guillermo A, Grogan TM, Miller TP, LeBlanc M, Ott G, Kvaloy S, Delabie J, Holte H, Krajci P, Stokke T, Staudt LM; Lymphoma/Leukemia Molecular Profiling Project. The use of molecular profiling to predict survival after chemotherapy for diffuse large-B-cell lymphoma. *N Engl J Med* 2002; **346**: 1937-1947 [PMID: 12075054 DOI: 10.1056/NEJMoa012914]

6 **Friedberg JW**. How I treat double-hit lymphoma. *Blood* 2017; **130**: 590-596 [PMID: 28600333 DOI: 10.1182/blood-2017-04-737320]

7 **Pedersen MØ**, Gang AO, Poulsen TS, Knudsen H, Lauritzen AF, Nielsen SL, Klausen TW, Nørgaard P. MYC translocation partner gene determines survival of patients with large B-cell lymphoma with MYC- or double-hit MYC/BCL2 translocations. *Eur J Haematol* 2014; **92**: 42-48 [PMID: 24118498 DOI: 10.1111/ejh.12212]

8 **Roschewski M**, Staudt LM, Wilson WH. Diffuse large B-cell lymphoma-treatment approaches in the molecular era. *Nat Rev Clin Oncol* 2014; **11**: 12-23 [PMID: 24217204 DOI: 10.1038/nrclinonc.2013.197]

9 **Lee HJ**, Shin DH, Kim KB, Shin N, Park WY, Lee JH, Choi KU, Kim JY, Lee CH, Sol MY. Polycomb protein EZH2 expression in diffuse large B-cell lymphoma is associated with better prognosis in patients treated with rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone. *Leuk Lymphoma* 2014; **55**: 2056-2063 [PMID: 24304372 DOI: 10.3109/10428194.2013.858816]

10 **Leich E**, Salaverria I, Bea S, Zettl A, Wright G, Moreno V, Gascoyne RD, Chan WC, Braziel RM, Rimsza LM, Weisenburger DD, Delabie J, Jaffe ES, Lister A, Fitzgibbon J, Staudt LM, Hartmann EM, Mueller-Hermelink HK, Campo E, Ott G, Rosenwald A. Follicular lymphomas with and without translocation t(14;18) differ in gene expression profiles and genetic alterations. *Blood* 2009; **114**: 826-834 [PMID: 19471018 DOI: 10.1182/blood-2009-01-198580]

11 **Nann D**, Ramis-Zaldivar JE, Müller I, Gonzalez-Farre B, Schmidt J, Egan C, Salmeron-Villalobos J, Clot G, Mattern S, Otto F, Mankel B, Colomer D, Balagué O, Szablewski V, Lome-Maldonado C, Leoncini L, Dojcinov S, Chott A, Copie-Bergman C, Bonzheim I, Fend F, Jaffe ES, Campo E, Salaverria I, Quintanilla-Martinez L. Follicular lymphoma t(14;18)-negative is genetically a heterogeneous disease. *Blood Adv* 2020; **4**: 5652-5665 [PMID: 33211828 DOI: 10.1182/bloodadvances.2020002944]

12 **Solal-Céligny P**, Roy P, Colombat P, White J, Armitage JO, Arranz-Saez R, Au WY, Bellei M, Brice P, Caballero D, Coiffier B, Conde-Garcia E, Doyen C, Federico M, Fisher RI, Garcia-Conde JF, Guglielmi C, Hagenbeek A, Haïoun C, LeBlanc M, Lister AT, Lopez-Guillermo A, McLaughlin P, Milpied N, Morel P, Mounier N, Proctor SJ, Rohatiner A, Smith P, Soubeyran P, Tilly H, Vitolo U, Zinzani PL, Zucca E, Montserrat E. Follicular lymphoma international prognostic index. *Blood* 2004; **104**: 1258-1265 [PMID: 15126323 DOI: 10.1182/blood-2003-12-4434]

13 **Federico M**, Bellei M, Marcheselli L, Luminari S, Lopez-Guillermo A, Vitolo U, Pro B, Pileri S, Pulsoni A, Soubeyran P, Cortelazzo S, Martinelli G, Martelli M, Rigacci L, Arcaini L, Di Raimondo F, Merli F, Sabattini E, McLaughlin P, Solal-Céligny P. Follicular lymphoma international prognostic index 2: a new prognostic index for follicular lymphoma developed by the international follicular lymphoma prognostic factor project. *J Clin Oncol* 2009; **27**: 4555-4562 [PMID: 19652063 DOI: 10.1200/JCO.2008.21.3991]

14 **Leonard JP**. POD24 in follicular lymphoma: time to be "wise". *Blood* 2022; **139**: 1609-1610 [PMID: 35298602 DOI: 10.1182/blood.2021013437]

15 **Dreyling M**, Ghielmini M, Rule S, Salles G, Vitolo U, Ladetto M; ESMO Guidelines Committee. Newly diagnosed and relapsed follicular lymphoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2016; **27**: v83-v90 [PMID: 27664263 DOI: 10.1093/annonc/mdw400]

16 **Campo E**, Jaffe ES, Cook JR, Quintanilla-Martinez L, Swerdlow SH, Anderson KC, Brousset P, Cerroni L, de Leval L, Dirnhofer S, Dogan A, Feldman AL, Fend F, Friedberg JW, Gaulard P, Ghia P, Horwitz SM, King RL, Salles G, San-Miguel J, Seymour JF, Treon SP, Vose JM, Zucca E, Advani R, Ansell S, Au WY, Barrionuevo C, Bergsagel L, Chan WC, Cohen JI, d'Amore F, Davies A, Falini B, Ghobrial IM, Goodlad JR, Gribben JG, Hsi ED, Kahl BS, Kim WS, Kumar S, LaCasce AS, Laurent C, Lenz G, Leonard JP, Link MP, Lopez-Guillermo A, Mateos MV, Macintyre E, Melnick AM, Morschhauser F, Nakamura S, Narbaitz M, Pavlovsky A, Pileri SA, Piris M, Pro B, Rajkumar V, Rosen ST, Sander B, Sehn L, Shipp MA, Smith SM, Staudt LM, Thieblemont C, Tousseyn T, Wilson WH, Yoshino T, Zinzani PL, Dreyling M, Scott DW, Winter JN, Zelenetz AD. The International Consensus Classification of Mature Lymphoid Neoplasms: a report from the Clinical Advisory Committee. *Blood* 2022; **140**: 1229-1253 [PMID: 35653592 DOI: 10.1182/blood.2022015851]

17 **Duncavage EJ**, Bagg A, Hasserjian RP, DiNardo CD, Godley LA, Iacobucci I, Jaiswal S, Malcovati L, Vannucchi AM, Patel KP, Arber DA, Arcila ME, Bejar R, Berliner N, Borowitz MJ, Branford S, Brown AL, Cargo CA, Döhner H, Falini B, Garcia-Manero G, Haferlach T, Hellström-Lindberg E, Kim AS, Klco JM, Komrokji R, Lee-Cheun Loh M, Loghavi S, Mullighan CG, Ogawa S, Orazi A, Papaemmanuil E, Reiter A, Ross DM, Savona M, Shimamura A, Skoda RC, Solé F, Stone RM, Tefferi A, Walter MJ, Wu D, Ebert BL, Cazzola M. Genomic profiling for clinical decision making in myeloid neoplasms and acute leukemia. *Blood* 2022; **140**: 2228-2247 [PMID: 36130297 DOI: 10.1182/blood.2022015853]

18 **de Leval L**, Alizadeh AA, Bergsagel PL, Campo E, Davies A, Dogan A, Fitzgibbon J, Horwitz SM, Melnick AM, Morice WG, Morin RD, Nadel B, Pileri SA, Rosenquist R, Rossi D, Salaverria I, Steidl C, Treon SP, Zelenetz AD, Advani RH, Allen CE, Ansell SM, Chan WC, Cook JR, Cook LB, d'Amore F, Dirnhofer S, Dreyling M, Dunleavy K, Feldman AL, Fend F, Gaulard P, Ghia P, Gribben JG, Hermine O, Hodson DJ, Hsi ED, Inghirami G, Jaffe ES, Karube K, Kataoka K, Klapper W, Kim WS, King RL, Ko YH, LaCasce AS, Lenz G, Martin-Subero JI, Piris MA, Pittaluga S, Pasqualucci L, Quintanilla-Martinez L, Rodig SJ, Rosenwald A, Salles GA, San-Miguel J, Savage KJ, Sehn LH, Semenzato G, Staudt LM, Swerdlow SH, Tam CS, Trotman J, Vose JM, Weigert O, Wilson WH, Winter JN, Wu CJ, Zinzani PL, Zucca E, Bagg A, Scott DW. Genomic profiling for clinical decision making in lymphoid neoplasms. *Blood* 2022; **140**: 2193-2227 [PMID: 36001803 DOI: 10.1182/blood.2022015854]

19 **Rosenquist R**, Beà S, Du MQ, Nadel B, Pan-Hammarström Q. Genetic landscape and deregulated pathways in B-cell lymphoid malignancies. *J Intern Med* 2017; **282**: 371-394 [PMID: 28631441 DOI: 10.1111/joim.12633]

20 **Bohers E**, Mareschal S, Bouzelfen A, Marchand V, Ruminy P, Maingonnat C, Ménard AL, Etancelin P, Bertrand P, Dubois S, Alcantara M, Bastard C, Tilly H, Jardin F. Targetable activating mutations are very frequent in GCB and ABC diffuse large B-cell lymphoma. *Genes Chromosomes Cancer* 2014; **53**: 144-153 [PMID: 24327543 DOI: 10.1002/gcc.22126]

21 **Ngo VN**, Young RM, Schmitz R, Jhavar S, Xiao W, Lim KH, Kohlhammer H, Xu W, Yang Y, Zhao H, Shaffer AL, Romesser P, Wright G, Powell J, Rosenwald A, Muller-Hermelink HK, Ott G, Gascoyne RD, Connors JM, Rimsza LM, Campo E, Jaffe ES, Delabie J, Smeland EB, Fisher RI, Braziel RM, Tubbs RR, Cook JR, Weisenburger DD, Chan WC, Staudt LM. Oncogenically active MYD88 mutations in human lymphoma. *Nature* 2011; **470**: 115-119 [PMID: 21179087 DOI: 10.1038/nature09671]

22 **Mottok A**, Wright G, Rosenwald A, Ott G, Ramsower C, Campo E, Braziel RM, Delabie J, Weisenburger DD, Song JY, Chan WC, Cook JR, Fu K, Greiner T, Smeland E, Holte H, Savage KJ, Glinsmann-Gibson BJ, Gascoyne RD, Staudt LM, Jaffe ES, Connors JM, Scott DW, Steidl C, Rimsza LM. Molecular classification of primary mediastinal large B-cell lymphoma using routinely available tissue specimens. *Blood* 2018; **132**: 2401-2405 [PMID: 30257882 DOI: 10.1182/blood-2018-05-851154]

23 **Jiang Y**, Redmond D, Nie K, Eng KW, Clozel T, Martin P, Tan LH, Melnick AM, Tam W, Elemento O. Deep sequencing reveals clonal evolution patterns and mutation events associated with relapse in B-cell lymphomas. *Genome Biol* 2014; **15**: 432 [PMID: 25123191 DOI: 10.1186/s13059-014-0432-0]

24 **Hilton LK**, Tang J, Ben-Neriah S, Alcaide M, Jiang A, Grande BM, Rushton CK, Boyle M, Meissner B, Scott DW, Morin RD. The double-hit signature identifies double-hit diffuse large B-cell lymphoma with genetic events cryptic to FISH. *Blood* 2019; **134**: 1528-1532 [PMID: 31527075 DOI: 10.1182/blood.2019002600]

25 **Wilson WH**, Young RM, Schmitz R, Yang Y, Pittaluga S, Wright G, Lih CJ, Williams PM, Shaffer AL, Gerecitano J, de Vos S, Goy A, Kenkre VP, Barr PM, Blum KA, Shustov A, Advani R, Fowler NH, Vose JM, Elstrom RL, Habermann TM, Barrientos JC, McGreivy J, Fardis M, Chang BY, Clow F, Munneke B, Moussa D, Beaupre DM, Staudt LM. Targeting B cell receptor signaling with ibrutinib in diffuse large B cell lymphoma. *Nat Med* 2015; **21**: 922-926 [PMID: 26193343 DOI: 10.1038/nm.3884]

26 **Reddy A**, Zhang J, Davis NS, Moffitt AB, Love CL, Waldrop A, Leppa S, Pasanen A, Meriranta L, Karjalainen-Lindsberg ML, Nørgaard P, Pedersen M, Gang AO, Høgdall E, Heavican TB, Lone W, Iqbal J, Qin Q, Li G, Kim SY, Healy J, Richards KL, Fedoriw Y, Bernal-Mizrachi L, Koff JL, Staton AD, Flowers CR, Paltiel O, Goldschmidt N, Calaminici M, Clear A, Gribben J, Nguyen E, Czader MB, Ondrejka SL, Collie A, Hsi ED, Tse E, Au-Yeung RKH, Kwong YL, Srivastava G, Choi WWL, Evens AM, Pilichowska M, Sengar M, Reddy N, Li S, Chadburn A, Gordon LI, Jaffe ES, Levy S, Rempel R, Tzeng T, Happ LE, Dave T, Rajagopalan D, Datta J, Dunson DB, Dave SS. Genetic and Functional Drivers of Diffuse Large B Cell Lymphoma. *Cell* 2017; **171**: 481-494.e15 [PMID: 28985567 DOI: 10.1016/j.cell.2017.09.027]

27 **Schmitz R**, Wright GW, Huang DW, Johnson CA, Phelan JD, Wang JQ, Roulland S, Kasbekar M, Young RM, Shaffer AL, Hodson DJ, Xiao W, Yu X, Yang Y, Zhao H, Xu W, Liu X, Zhou B, Du W, Chan WC, Jaffe ES, Gascoyne RD, Connors JM, Campo E, Lopez-Guillermo A, Rosenwald A, Ott G, Delabie J, Rimsza LM, Tay Kuang Wei K, Zelenetz AD, Leonard JP, Bartlett NL, Tran B, Shetty J, Zhao Y, Soppet DR, Pittaluga S, Wilson WH, Staudt LM. Genetics and Pathogenesis of Diffuse Large B-Cell Lymphoma. *N Engl J Med* 2018; **378**: 1396-1407 [PMID: 29641966 DOI: 10.1056/NEJMoa1801445]

28 **Wilson WH**, Wright GW, Huang DW, Hodkinson B, Balasubramanian S, Fan Y, Vermeulen J, Shreeve M, Staudt LM. Effect of ibrutinib with R-CHOP chemotherapy in genetic subtypes of DLBCL. *Cancer Cell* 2021; **39**: 1643-1653.e3 [PMID: 34739844 DOI: 10.1016/j.ccell.2021.10.006]

29 **Chapuy B**, Stewart C, Dunford AJ, Kim J, Kamburov A, Redd RA, Lawrence MS, Roemer MGM, Li AJ, Ziepert M, Staiger AM, Wala JA, Ducar MD, Leshchiner I, Rheinbay E, Taylor-Weiner A, Coughlin CA, Hess JM, Pedamallu CS, Livitz D, Rosebrock D, Rosenberg M, Tracy AA, Horn H, van Hummelen P, Feldman AL, Link BK, Novak AJ, Cerhan JR, Habermann TM, Siebert R, Rosenwald A, Thorner AR, Meyerson ML, Golub TR, Beroukhim R, Wulf GG, Ott G, Rodig SJ, Monti S, Neuberg DS, Loeffler M, Pfreundschuh M, Trümper L, Getz G, Shipp MA. Molecular subtypes of diffuse large B cell lymphoma are associated with distinct pathogenic mechanisms and outcomes. *Nat Med* 2018; **24**: 679-690 [PMID: 29713087 DOI: 10.1038/s41591-018-0016-8]

30 **Karube K**, Enjuanes A, Dlouhy I, Jares P, Martin-Garcia D, Nadeu F, Ordóñez GR, Rovira J, Clot G, Royo C, Navarro A, Gonzalez-Farre B, Vaghefi A, Castellano G, Rubio-Perez C, Tamborero D, Briones J, Salar A, Sancho JM, Mercadal S, Gonzalez-Barca E, Escoda L, Miyoshi H, Ohshima K, Miyawaki K, Kato K, Akashi K, Mozos A, Colomo L, Alcoceba M, Valera A, Carrió A, Costa D, Lopez-Bigas N, Schmitz R, Staudt LM, Salaverria I, López-Guillermo A, Campo E.

Integrating genomic alterations in diffuse large B-cell lymphoma identifies new relevant pathways and potential therapeutic targets. *Leukemia* 2018; **32**: 675-684 [PMID: 28804123 DOI: 10.1038/leu.2017.251]

31 **Pasqualucci L**, Dominguez-Sola D, Chiarenza A, Fabbri G, Grunn A, Trifonov V, Kasper LH, Lerach S, Tang H, Ma J, Rossi D, Chadburn A, Murty VV, Mullighan CG, Gaidano G, Rabadan R, Brindle PK, Dalla-Favera R. Inactivating mutations of acetyltransferase genes in B-cell lymphoma. *Nature* 2011; **471**: 189-195 [PMID: 21390126 DOI: 10.1038/nature09730]

32 **Okosun J**, Bödör C, Wang J, Araf S, Yang CY, Pan C, Boller S, Cittaro D, Bozek M, Iqbal S, Matthews J, Wrench D, Marzec J, Tawana K, Popov N, O'Riain C, O'Shea D, Carlotti E, Davies A, Lawrie CH, Matolcsy A, Calaminici M, Norton A, Byers RJ, Mein C, Stupka E, Lister TA, Lenz G, Montoto S, Gribben JG, Fan Y, Grosschedl R, Chelala C, Fitzgibbon J. Integrated genomic analysis identifies recurrent mutations and evolution patterns driving the initiation and progression of follicular lymphoma. *Nat Genet* 2014; **46**: 176-181 [PMID: 24362818 DOI: 10.1038/ng.2856]

33 **Pasqualucci L**, Khiabanian H, Fangazio M, Vasishtha M, Messina M, Holmes AB, Ouillette P, Trifonov V, Rossi D, Tabbò F, Ponzoni M, Chadburn A, Murty VV, Bhagat G, Gaidano G, Inghirami G, Malek SN, Rabadan R, Dalla-Favera R. Genetics of follicular lymphoma transformation. *Cell Rep* 2014; **6**: 130-140 [PMID: 24388756 DOI: 10.1016/j.celrep.2013.12.027]

34 **Sander CA**, Yano T, Clark HM, Harris C, Longo DL, Jaffe ES, Raffeld M. p53 mutation is associated with progression in follicular lymphomas. *Blood* 1993; **82**: 1994-2004 [PMID: 8400252 DOI: 10.1182/blood.V82.7.1994.1994]

35 **O'Shea D**, O'Riain C, Taylor C, Waters R, Carlotti E, Macdougall F, Gribben J, Rosenwald A, Ott G, Rimsza LM, Smeland EB, Johnson N, Campo E, Greiner TC, Chan WC, Gascoyne RD, Wright G, Staudt LM, Lister TA, Fitzgibbon J. The presence of TP53 mutation at diagnosis of follicular lymphoma identifies a high-risk group of patients with shortened time to disease progression and poorer overall survival. *Blood* 2008; **112**: 3126-3129 [PMID: 18628487 DOI: 10.1182/blood-2008-05-154013]

36 **Cheung KJ**, Shah SP, Steidl C, Johnson N, Relander T, Telenius A, Lai B, Murphy KP, Lam W, Al-Tourah AJ, Connors JM, Ng RT, Gascoyne RD, Horsman DE. Genome-wide profiling of follicular lymphoma by array comparative genomic hybridization reveals prognostically significant DNA copy number imbalances. *Blood* 2009; **113**: 137-148 [PMID: 18703704 DOI: 10.1182/blood-2008-02-140616]

37 **Cahill KE**, Smith SM. Follicular Lymphoma: a Focus on Current and Emerging Therapies. *Oncology (Williston Park)* 2022; **36**: 97-106 [PMID: 35180337 DOI: 10.46883/2022.25920946]

38 **Pastore A**, Jurinovic V, Kridel R, Hoster E, Staiger AM, Szczepanowski M, Pott C, Kopp N, Murakami M, Horn H, Leich E, Moccia AA, Mottok A, Sunkavalli A, Van Hummelen P, Ducar M, Ennishi D, Shulha HP, Hother C, Connors JM, Sehn LH, Dreyling M, Neuberg D, Möller P, Feller AC, Hansmann ML, Stein H, Rosenwald A, Ott G, Klapper W, Unterhalt M, Hiddemann W, Gascoyne RD, Weinstock DM, Weigert O. Integration of gene mutations in risk prognostication for patients receiving first-line immunochemotherapy for follicular lymphoma: a retrospective analysis of a prospective clinical trial and validation in a population-based registry. *Lancet Oncol* 2015; **16**: 1111-1122 [PMID: 26256760 DOI: 10.1016/S1470-2045(15)00169-2]

39 **Martínez-Laperche C**, Sanz-Villanueva L, Díaz Crespo FJ, Muñiz P, Martín Rojas R, Carbonell D, Chicano M, Suárez-González J, Menárguez J, Kwon M, Diez Martín JL, Buño I, Bastos Oreiro M. EZH2 mutations at diagnosis in follicular lymphoma: a promising biomarker to guide frontline treatment. *BMC Cancer* 2022; **22**: 982 [PMID: 36104682 DOI: 10.1186/s12885-022-10070-z]

40 **Lockmer S**, Ren W, Brodtkorb M, Østenstad B, Wahlin BE, Pan-Hammarström Q, Kimby E. M7-FLIPI is not prognostic in follicular lymphoma patients with first-line rituximab chemo-free therapy. *Br J Haematol* 2020; **188**: 259-267 [PMID: 31423576 DOI: 10.1111/bjh.16159]

41 **Cartron G**, Trotman J. Time for an individualized approach to first-line management of follicular lymphoma. *Haematologica* 2022; **107**: 7-18 [PMID: 34985230 DOI: 10.3324/haematol.2021.278766]

42 **Kalakonda N**, Maerevoet M, Cavallo F, Follows G, Goy A, Vermaat JSP, Casasnovas O, Hamad N, Zijlstra JM, Bakhshi S, Bouabdallah R, Choquet S, Gurion R, Hill B, Jaeger U, Sancho JM, Schuster M, Thieblemont C, De la Cruz F, Egyed M, Mishra S, Offner F, Vassilakopoulos TP, Warzocha K, McCarthy D, Ma X, Corona K, Saint-Martin JR, Chang H, Landesman Y, Joshi A, Wang H, Shah J, Shacham S, Kauffman M, Van Den Neste E, Canales MA. Selinexor in patients with relapsed or refractory diffuse large B-cell lymphoma (SADAL): a single-arm, multinational, multicentre, open-label, phase 2 trial. *Lancet Haematol* 2020; **7**: e511-e522 [PMID: 32589977 DOI: 10.1016/S2352-3026(20)30120-4]

43 **Sehn LH**, Herrera AF, Flowers CR, Kamdar MK, McMillan A, Hertzberg M, Assouline S, Kim TM, Kim WS, Ozcan M, Hirata J, Penuel E, Paulson JN, Cheng J, Ku G, Matasar MJ. Polatuzumab Vedotin in Relapsed or Refractory Diffuse Large B-Cell Lymphoma. *J Clin Oncol* 2020; **38**: 155-165 [PMID: 31693429 DOI: 10.1200/JCO.19.00172]

44 **Morschhauser F**, Tilly H, Chaidos A, McKay P, Phillips T, Assouline S, Batlevi CL, Campbell P, Ribrag V, Damaj GL, Dickinson M, Jurczak W, Kazmierczak M, Opat S, Radford J, Schmitt A, Yang J, Whalen J, Agarwal S, Adib D, Salles G. Tazemetostat for patients with relapsed or refractory follicular lymphoma: an open-label, single-arm, multicentre, phase 2 trial. *Lancet Oncol* 2020; **21**: 1433-1442 [PMID: 33035457 DOI: 10.1016/S1470-2045(20)30441-1]

45 **Gopal AK**, Kahl BS, de Vos S, Wagner-Johnston ND, Schuster SJ, Jurczak WJ, Flinn IW, Flowers CR, Martin P, Viardot A, Blum KA, Goy AH, Davies AJ, Zinzani PL, Dreyling M, Johnson D, Miller LL, Holes L, Li D, Dansey RD, Godfrey WR, Salles GA. PI3Kδ inhibition by idelalisib in patients with relapsed indolent lymphoma. *N Engl J Med* 2014; **370**: 1008-1018 [PMID: 24450858 DOI: 10.1056/NEJMoa1314583]

46 **Dreyling M**, Santoro A, Mollica L, Leppä S, Follows GA, Lenz G, Kim WS, Nagler A, Panayiotidis P, Demeter J, Özcan M, Kosinova M, Bouabdallah K, Morschhauser F, Stevens DA, Trevarthen D, Giurescu M, Cupit L, Liu L, Köchert K, Seidel H, Peña C, Yin S, Hiemeyer F, Garcia-Vargas J, Childs BH, Zinzani PL. Phosphatidylinositol 3-Kinase Inhibition by Copanlisib in Relapsed or Refractory Indolent Lymphoma. *J Clin Oncol* 2017; **35**: 3898-3905 [PMID: 28976790 DOI: 10.1200/JCO.2017.75.4648]

47 **Flinn IW**, Miller CB, Ardeshna KM, Tetreault S, Assouline SE, Mayer J, Merli M, Lunin SD, Pettitt AR, Nagy Z, Tournilhac O, Abou-Nassar KE, Crump M, Jacobsen ED, de Vos S, Kelly VM, Shi W, Steelman L, Le N, Weaver DT, Lustgarten S, Wagner-Johnston ND, Zinzani PL. DYNAMO: A Phase II Study of Duvelisib (IPI-145) in Patients With

Refractory Indolent Non-Hodgkin Lymphoma. *J Clin Oncol* 2019; **37**: 912-922 [PMID: 30742566 DOI: 10.1200/JCO.18.00915]

48 **Fowler NH**, Samaniego F, Jurczak W, Ghosh N, Derenzini E, Reeves JA, Knopińska-Posłuszny W, Cheah CY, Phillips T, Lech-Maranda E, Cheson BD, Caimi PF, Grosicki S, Leslie LA, Chavez JC, Fonseca G, Babu S, Hodson DJ, Shao SH, Burke JM, Sharman JP, Law JY, Pagel JM, Miskin HP, Sportelli P, O'Connor OA, Weiss MS, Zinzani PL. Umbralisib, a Dual PI3Kδ/CK1ε Inhibitor in Patients With Relapsed or Refractory Indolent Lymphoma. *J Clin Oncol* 2021; **39**: 1609-1618 [PMID: 33683917 DOI: 10.1200/JCO.20.03433]

49 **Ramos CA**, Heslop HE, Brenner MK. CAR-T Cell Therapy for Lymphoma. *Annu Rev Med* 2016; **67**: 165-183 [PMID: 26332003 DOI: 10.1146/annurev-med-051914-021702]

50 **Sadelain M**. CAR therapy: the CD19 paradigm. *J Clin Invest* 2015; **125**: 3392-3400 [PMID: 26325036 DOI: 10.1172/JCI80010]

51 **Abramson JS**, Palomba ML, Gordon LI, Lunning MA, Wang M, Arnason J, Mehta A, Purev E, Maloney DG, Andreadis C, Sehgal A, Solomon SR, Ghosh N, Albertson TM, Garcia J, Kostic A, Mallaney M, Ogasawara K, Newhall K, Kim Y, Li D, Siddiqi T. Lisocabtagene maraleucel for patients with relapsed or refractory large B-cell lymphomas (TRANSCEND NHL 001): a multicentre seamless design study. *Lancet* 2020; **396**: 839-852 [PMID: 32888407 DOI: 10.1016/S0140-6736(20)31366-0]

52 **Fowler NH**, Dickinson M, Dreyling M, Martinez-Lopez J, Kolstad A, Butler J, Ghosh M, Popplewell L, Chavez JC, Bachy E, Kato K, Harigae H, Kersten MJ, Andreadis C, Riedell PA, Ho PJ, Pérez-Simón JA, Chen AI, Nastoupil LJ, von Tresckow B, Ferreri AJM, Teshima T, Patten PEM, McGuirk JP, Petzer AL, Offner F, Viardot A, Zinzani PL, Malladi R, Zia A, Awasthi R, Masood A, Anak O, Schuster SJ, Thieblemont C. Tisagenlecleucel in adult relapsed or refractory follicular lymphoma: the phase 2 ELARA trial. *Nat Med* 2022; **28**: 325-332 [PMID: 34921238 DOI: 10.1038/s41591-021-01622-0]

53 **Locke FL**, Miklos DB, Jacobson CA, Perales MA, Kersten MJ, Oluwole OO, Ghobadi A, Rapoport AP, McGuirk J, Pagel JM, Muñoz J, Farooq U, van Meerten T, Reagan PM, Sureda A, Flinn IW, Vandenberghe P, Song KW, Dickinson M, Minnema MC, Riedell PA, Leslie LA, Chaganti S, Yang Y, Filosto S, Shah J, Schupp M, To C, Cheng P, Gordon LI, Westin JR; All ZUMA-7 Investigators and Contributing Kite Members. Axicabtagene Ciloleucel as Second-Line Therapy for Large B-Cell Lymphoma. *N Engl J Med* 2022; **386**: 640-654 [PMID: 34891224 DOI: 10.1056/NEJMoa2116133]

54 **Roschewski M**, Dunleavy K, Pittaluga S, Moorhead M, Pepin F, Kong K, Shovlin M, Jaffe ES, Staudt LM, Lai C, Steinberg SM, Chen CC, Zheng J, Willis TD, Faham M, Wilson WH. Circulating tumour DNA and CT monitoring in patients with untreated diffuse large B-cell lymphoma: a correlative biomarker study. *Lancet Oncol* 2015; **16**: 541-549 [PMID: 25842160 DOI: 10.1016/S1470-2045(15)70106-3]

55 **Wang H**, Du X, Chen WH, Lou J, Xiao HL, Pan YM, Chen H, An N, Zhang QX. Establishment of a Quantitative Polymerase Chain Reaction Assay for Monitoring Chimeric Antigen Receptor T Cells in Peripheral Blood. *Transplant Proc* 2018; **50**: 104-109 [PMID: 29407291 DOI: 10.1016/j.transproceed.2017.11.028]

56 **Gonçalves MC**, de Oliveira CRGCM, Sandes AF, Rodrigues CA, Novis Y, Viana PCC, Serra MMP, Zerbini MCN. Core Needle Biopsy in Lymphoma Diagnosis: The Diagnostic Performance and the Role of the Multidisciplinary Approach in the Optimization of Results. *Am J Surg Pathol* 2023; **47**: 111-123 [PMID: 36395467 DOI: 10.1097/PAS.0000000000001991]

57 **Kurtz DM**, Scherer F, Jin MC, Soo J, Craig AFM, Esfahani MS, Chabon JJ, Stehr H, Liu CL, Tibshirani R, Maeda LS, Gupta NK, Khodadoust MS, Advani RH, Levy R, Newman AM, Dührsen U, Hüttmann A, Meignan M, Casasnovas RO, Westin JR, Roschewski M, Wilson WH, Gaidano G, Rossi D, Diehn M, Alizadeh AA. Circulating Tumor DNA Measurements As Early Outcome Predictors in Diffuse Large B-Cell Lymphoma. *J Clin Oncol* 2018; **36**: 2845-2853 [PMID: 30125215 DOI: 10.1200/JCO.2018.78.5246]

58 **Gormally E**, Caboux E, Vineis P, Hainaut P. Circulating free DNA in plasma or serum as biomarker of carcinogenesis: practical aspects and biological significance. *Mutat Res* 2007; **635**: 105-117 [PMID: 17257890 DOI: 10.1016/j.mrrev.2006.11.002]

59 **Scherer F**, Kurtz DM, Newman AM, Stehr H, Craig AF, Esfahani MS, Lovejoy AF, Chabon JJ, Klass DM, Liu CL, Zhou L, Glover C, Visser BC, Poultsides GA, Advani RH, Maeda LS, Gupta NK, Levy R, Ohgami RS, Kunder CA, Diehn M, Alizadeh AA. Distinct biological subtypes and patterns of genome evolution in lymphoma revealed by circulating tumor DNA. *Sci Transl Med* 2016; **8**: 364ra155 [PMID: 27831904 DOI: 10.1126/scitranslmed.aai8545]

60 **Heitzer E**, Haque IS, Roberts CES, Speicher MR. Current and future perspectives of liquid biopsies in genomics-driven oncology. *Nat Rev Genet* 2019; **20**: 71-88 [PMID: 30410101 DOI: 10.1038/s41576-018-0071-5]

61 **NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines)**. Non-small cell lung cancer. Version 6.2020. Published 2017. Accessed July 20, 2020. Available from: http://www.nccn.org

62 **Stuckey R**, Bilbao-Sieyro C, Gómez-Casares MT. A summary of the molecular testing recommended in acute myeloid leukemia. *Ann Mol Genet Med* 2020; **4**: 012-017 [DOI: 10.17352/amgm.000007]

63 **Novartis Oncology**. The precision oncology annual trend report: perspectives from payers, oncologists, and pathologists. Published 2016. Accessed July 21, 2020. Available from: http://www.hcp.novartis.com/care-management

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MINIREVIEWS

Current progress on the endoscopic features of colorectal sessile serrated lesions

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Abstract

Along with the discovery and refinement of serrated pathways, the World Health Organization amended the classification of digestive system tumors in 2019, recommending the renaming of sessile serrated adenomas/polyps to sessile serrated lesions (SSLs). Given the particularity of the endoscopic appearance of SSLs, it could easily be overlooked and missed in colonoscopy screening, which is crucial for the occurrence of interval colorectal cancer. Existing literature has found that adequate bowel preparation, reasonable withdrawal time, and awareness of colorectal SSLs have improved the quality and accuracy of detection. More particularly, with the continuous advancement and development of endoscopy technology, equipment, and accessories, a potent auxiliary tool is provided for accurate observation and immediate diagnosis of SSLs. High-definition white light endoscopy, chromoendoscopy, and magnifying endoscopy have distinct roles in the detection of colorectal SSLs and are valuable in identifying the size, shape, character, risk degree, and potential malignant tendency. This article delves into the relevant factors influencing the detection rate of colorectal SSLs, reviews its characteristics under various endoscopic techniques, and expects to attract the attention of colonoscopists.

Key Words: Colorectal cancer; Sessile serrated lesions; Endoscopic features

Core Tip: Because of its unique endoscopic patterns and behavior, sessile serrated lesions (SSLs) are easily missing during colonoscopy. SSL is a critical cause of interval colorectal cancer, so it is necessary to summarize the endoscopic features of the sessile serrated lesion to help endoscopists make a better identification and diagnosis.

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INTRODUCTION

Colorectal cancer (CRC) is a common gastrointestinal malignancy with the third-highest incidence and the second-highest mortality rate. In China, more than 550000 new cases were diagnosed and 280000 deaths took place in 2020[1], severely threatening people's lives and health. With the improvements in awareness concerning colonoscopic screening, the occurrence of interval CRC has garnered significant attention. Interval CRC refers to the CRC that is not detected during colorectal screening but discovered prior to the next recommended screening date[2]. The incidence of interval CRC is a vital indicator in assessing the quality of colonoscopic screening. Interval CRC detection in the proximal colon goes beyond being merely a test of the patient's bowel preparation since the colonoscopist's experience is also highly relevant.

Colorectal sessile serrated lesions (SSLs) are poorly defined and pale, covered with mucus, and hardly distinguishable from the surrounding mucosa. For endoscopists who lack awareness of the features of colorectal SSLs, missing or overlooking them becomes inevitable, resulting in the incidence of interval CRCs[3]. Related research observed that the occurrence of interval CRCs three years after colonoscopy was 3.4%-9.0%[2,4].

Colorectal SSLs may proceed to CRCs through the pathways of BRAF mutation, microsatellite instability, CpG island methylation, and the deletion gene of DNA damage repair[5]. It has been exhibited that a 15%-30% incidence of CRCs occurs *via* the serrated pathway, which is known as the vital cancer pathway[5,6].

UPDATE ON THE PATHOLOGICAL CLASSIFICATION OF SERRATED LESIONS

During the previous decade, related studies have generated a more accurate description of the pathogenesis of intestinal adenocarcinoma. Population-based screening for CRCs has led to a comprehensive understanding of precancerous lesions and established a foundation for investigating the molecular pathways and biological behaviors of cancerous lesions. Accordingly, WHO renamed the sessile serrated adenoma/polyp as SSLs, as these may be flat rather than polypoid, and the association with BRAF or KRAS mutation delineates two separate neoplastic pathways.

Currently, the categorization of gastrointestinal tumors classifies serrated lesions as Hyperplastic Polyps (HP), SSL, SSL with dysplasia (SSL-D), Traditional serrated adenomas (TSA), and serrated tubular villous adenoma (STVA)[7-9].

HP is a benign lesion, and the pathological features are primarily epithelial hyperplasia in the upper 2/3 of the saphenous fossa, forming small papillae protruding into the lumen of the saphenous fossa, which then gives the luminal surface a serrated shape. Based on the cell composition and molecular genetic alterations, the two types of HP are the Microvesicular type of Hyperplastic Polyp and the Goblet Cell-rich type of Hyperplastic Polyp. Generally, the TSA is pedicled and has villous structures but is potentially malignant[10-12]. In contrast to the conventional tubulovillous adenoma, STVA usually presents histological changes in advanced adenomas, and the glands are frequently serrated when high-grade dysplasia and invasive carcinoma appear.

The histological diagnosis of SSLs necessitates the detection of at least one abnormal crypt. By way of illustration, the entire saphenous fossa is serrated and grows horizontally along the mucosal muscular layer, the basal expansion, abnormal maturation, and asymmetric proliferation, in which asymmetric proliferation causes structural changes in the entire saphenous fossa. This is the fundamental difference from the HP[8]. Moreover, SSL-D is histologically heterogeneous. Its abnormal crypt structures—being its core feature—differ from the surrounding glands like the appearance of villous structures, which are longer and more crowded, complex branching, sieve-shaped crypt, and increased or decreased serration compared with the background SSL. The morphology of SSL-Ds is often tanglesome and mixed with different subtypes, making it challenging to distinguish the degree of heterogeneous hyperplasia.

COLORECTAL SSL-RELATED RISKS

It has been widely recognized that colorectal SSLs essentially differ from HPs, both with regard to morphological and pathological characteristics analysis, and instead behave comparably to neoplastic lesions with malignant potential.

Meta-analyses have demonstrated that SSLs are associated with an increased risk of concurrent progressive tumors. Patients with larger proximal colorectal serrated lesions are at significant risk and may require closer monitoring and further completion of a colon examination[13,14]. In light of this, a population-based, case-control study from Danish revealed that having an SSL was associated with 3-fold increased odds for CRC, while having SSL-D was associated with a nearly 5-fold increased odds for CRC[15,16].

Reports have confirmed that the risk of developing CRCs in cases with SSL-D is 4.4% within a decade, which is higher than that of conventional adenoma (2.3%). This highlights a significantly increased long-term risk of CRC in patients with SSL[16]. Similarly, correlative studies have found that SSLs have a mean duration of 7-15 years before developing SSL-Ds. Then, 3.03%-12.5% of SSLs develop into CRCs 5-7 years after follow-up[17,18]. However, SSL-Ds progress to CRCs at a much faster rate, and there are reports of SSL-Ds rapidly aggravating submucosal invasive carcinomas within one or two years[19-21].

FACTORS AFFECTING THE DETECTION OF COLORECTAL SSLS

As a critical influencing factor in the occurrence of interval CRCs, the detection rate of SSLs can effectively evaluate the quality of colonoscopy and assess the level of colonoscopists. A retrospective study that included more than 10000 colonoscopies found that bowel preparation, exit time, polyp diameter, and adenoma detection rate were linked to the SSL detection rate. Equally important, a multivariate analysis underlined that adenoma detection rate was an independent predictor of SSL detection rate, implying that patients who developed colorectal adenomas were at higher risk of complicating SSL[22].

Additionally, a colonoscopist's professional experience is vital to the timely and accurate detection of colorectal SSLs. Li *et al*[23] noted that different colonoscopists are independent risk factors for the detection rate of proximal colonic serrated lesions. It underscores that inexperienced colonoscopists detected serrated lesions at only 16%-83% compared with their experienced counterparts. They also found that proximal serrated polyps are more common in men over 50 years old.

ENDOSCOPIC FEATURES OF COLORECTAL SSLS

The development of an endoscopic technique delivers a reliable tool for detecting colorectal SSLs, which are not easily distinguishable from the background mucosa. To effectively prevent the incidence of interval CRCs, an early diagnosis and treatment of SSLs are crucial, thereby improving the quality of life and disease prognosis of patients.

Colorectal SSL characteristics under white light endoscopy

Colorectal SSL and SSL-D are prevalent in the proximal colon, usually > 5 mm in size, accounting for approximately 20%-25% of all serrated lesions. Additionally, colorectal SSLs often present with faint borders and a pale surface under white light endoscopy. Consequently, distinguishing them from the surrounding mucosa is difficult, making them prone to adverse events like missed or delayed diagnoses and incomplete resections. Most colorectal SSLs are accompanied by a mucus cap (Figure 1A), which, when flushed is not easily differentiated from HP. A further study also uncovered that inconspicuous borders and cloud-like surfaces are two independent diagnostic features of colorectal SSL in white light endoscopy[24-26]. Meanwhile, colorectal SSL-Ds are often associated with pedicled, bimodal appearance, central depression, and reddish color (Figure 1B), which can differentiate SSLs from SSL-Ds, with one of such features having a sensitivity of 97.7% and a specificity of 85.3% for the diagnosis of SSL-Ds[27].

Colorectal SSL characteristics under chromoendoscopy

Both HPs and SSLs are generally challenging and complex to identify when small (< 5 mm). To address this issue, the chromoendoscopy technique is adopted.

During endoscopy, chemical dyes (indigo carmine, crystalline violet, acetic acid, among several others) spray on the surface of the lesions, so the particles of the stains are deposited within the folds of the colorectal SSL lesion and surrounding mucosa. Then, the outlining of the lesion border and surface microstructure facilitates the assessment of SSL size and character.

It is important to note that acetic acid spray plays an important role in showing the borders and diameter of colorectal SSLs (Figure 2A). The surface morphology of the SSL is clearer and more easily

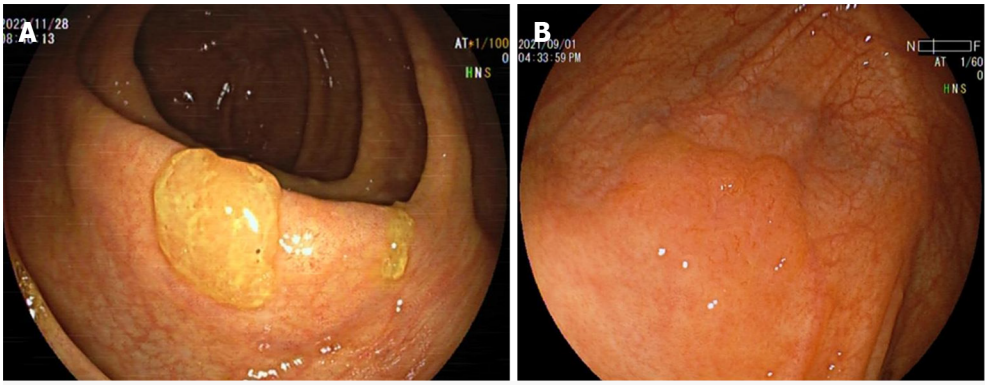

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Figure 1 White light endoscopic features of colorectal sessile serrated lesion cases. A: The sessile serrated lesions (SSLs) case with mucus cap under white light endoscopy; B: The borders of SSLs are not clearly distinguishable from the surrounding mucosa, and the morphology are cloud-like surface under white light. The above figure shows a case of SSL-D which has a reddish surface and a central depression.

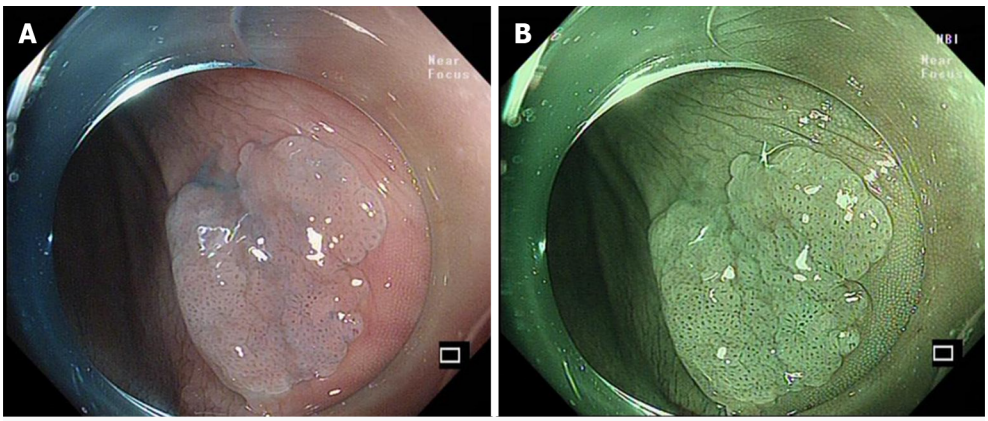

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Figure 2 Endoscopic features of the colorectal sessile serrated lesion case after acetic acid spray. A: The border of the sessile serrated lesions (SSLs) is clearly revealed under acetic acid spray; B: The combined application of acetic acid spray and narrow band imaging can clearly show the borders and surface microstructure of SSLs, which is more conducive to endoscopic treatment.

described after acetic acid spray, and its useful in better delineation of the recurrent colorectal SSL[28-30]. In addition, it has been demonstrated that acetic acid spray can help endoscopists perform cold resection of colorectal SSLs more accurately[31] (Figure 2B).

One study classified the chromoendoscopy images of the surface glands of more than 300 SSLs and indicated that open Type II (Pit II-O) structures, compared with the conventional Pit II type glands opening pattern, were endoscopic characteristics in colorectal SSLs[24]. Moreover, the opening pattern of the Pit II-O gland is similar to that of Pit II, and the former is typically surrounded by the latter, but the former features an expanded and more rounded shape, reflecting the expansion of the SSL crypt (Figure 3).

The image enhanced endoscopy (IEE) is the most common mode of electronic staining used in colonoscopy. Narrow band imaging (NBI) is a widely used IEE, which utilizes a filter to screen the broadband spectrum of the red, blue, and green light emitted by the light source, leaving only the narrowband spectrum for the diagnosis of various digestive disorders. Linked color imaging and blue laser imaging (BLI) are the next-generation IEEs, considering that their imaging principle is founded on light absorption and reflection by the mucosa of the digestive tract. Then, the lesions appear in a different color from the surrounding tissues, yielding a clear distinction between the superficial mucosal microvasculature and microstructure. It is also worth noting that the IEE has a brighter and higher resolution and is known as the "electron chromatography" technique given that the image observed by the IEE resembles a dye-stained image.

Furthermore, the NBI pattern enhances the visibility of colorectal SSLs with a mucus cap and gives it a concentrated red color that contrasts more prominently with the background mucosa[32] (Figure 4A). Also, both the NBI and BLI generally feature small black spots within the glandular opening of SSLs (Figure 4B), which is a critical histological feature within dependent diagnostic value that aids the endoscopist to differentiate SSLs from HPs during colonoscopy[25,33]. It has been confirmed that

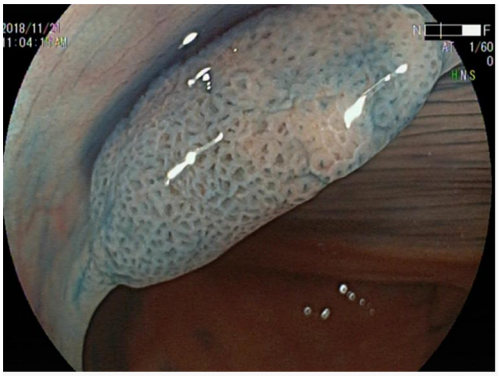

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Figure 3 Endoscopic features of the colorectal sessile serrated lesion case after indigo carmine spray. The opening pattern of the Pit II-O gland, features an expanded and more rounded shape, dilation of the colorectal sessile serrated lesion surface crypt.

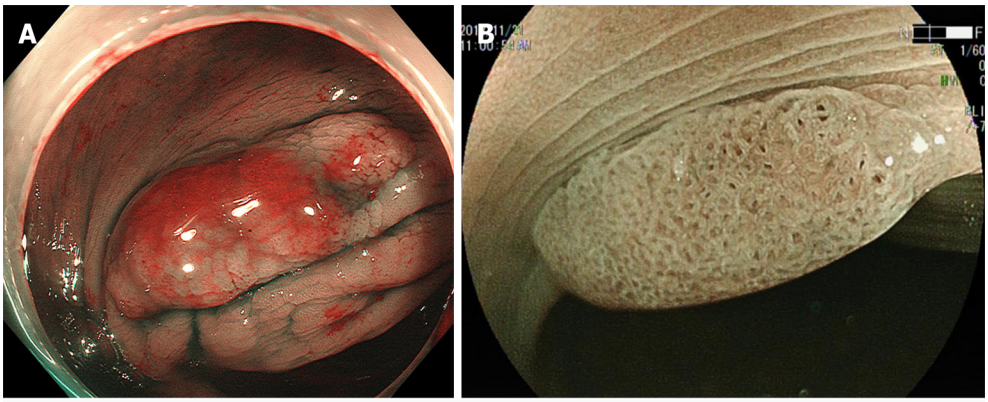

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Figure 4 Endoscopic features of colorectal sessile serrated lesion cases under narrow band imaging mode. A: The mucus cap of sessile serrated lesions (SSLs) shows a brick-red appearance under narrow band imaging (NBI); B: The expansion of the surface crypt in the SSLs shows a black spot under NBI.

dilated and branching vessels in NBI endoscopy differs from the vascular surrounding superficial mucosal glands, and irregular capillaries may be observed at sites of colorectal SSL that show dysplasia [3,34,35].

Additional research showed that the multivariate analysis of the location (proximal colon), size (≥ 10 mm), glandular opening, and microvascular morphology of the serrated lesions exhibited more than 90% positive diagnosis of the SSL, which was 2.3 times more advantageous than its single factor diagnosis[36,37].

Colorectal SSL characteristics under magnified endoscopy

In identifying neoplastic and non-neoplastic lesions, the value of magnified endoscopy combined with chromoscopy has been extensively evident. Close observation of the surface pattern of lesions with a specific combination can effectively predict its pathological characteristics and even the depth of invasion. Relevant literature has demonstrated that Type II-O glands can be used as an indicator to differentiate between SSLs and HPs. The Pit II-O glands also suggest histological variation in the morphology of colorectal SSL glands, significantly boosting the accuracy of diagnosing SSLs[38]. For large colorectal SSLs, magnified endoscopic findings of not only Type II-O glands but also those possibly mixed with Types IIIL, IV, Vi, and Vn glands at the same time often prompt SSL-Ds or cancers [24,27] (Figure 5A).

Magnified endoscopy combined with IEE can further develop the visualization of microvessels (Figure 5B). The varicose microvessels, running through the deep layer of mucosa, on the lesion surface of colorectal SSL, differ from those around the mucosal glands[37]. A similar study in China pointed out a statistical difference between magnified endoscopy and chromic endoscopy for varicose microvessels in predicting colorectal SSLs and HPs[35].

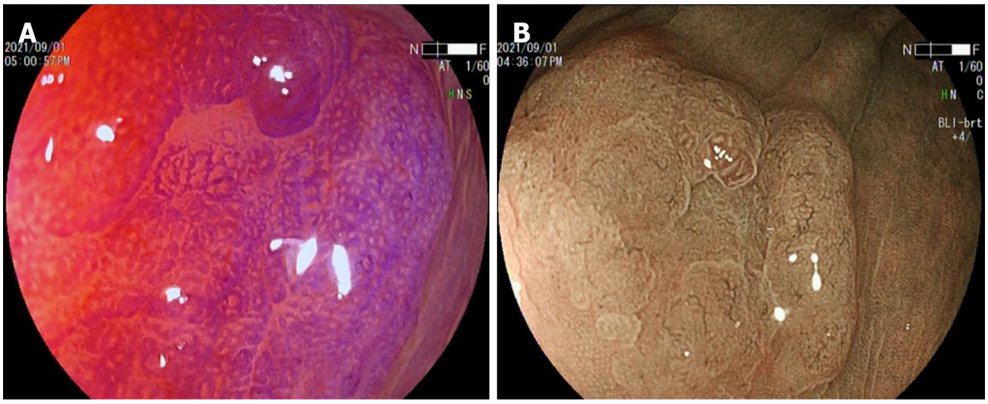

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Figure 5 Endoscopic features of the colorectal sessile serrated lesion case under chromoscopy combined with magnified endoscopy. A: Crystalline violet spray makes the surface glandular structure of colorectal sessile serrated lesions (SSLs) more visible, and combined with magnified endoscopic observation is useful for inferring the pathological characteristics of the lesion; B: Blue light imaging combined with magnified endoscopic observation of the microstructure of the SSL surface revealed that the SSL-D case have a Pit III and/or Pit IV type of glandular duct opening pattern based on Pit II-O, and varicose microvessels on the surface of the lesion are found.

CONCLUSION

Colorectal SSL is potentially malignant and has a higher risk of malignancy than conventional tubular adenomas, thereby making an immediate diagnosis or early detection in colonoscopic screening especially important. However, the current diagnosis of SSLs in screening colonoscopy is undeniably insufficiently high and often depends on the histopathological diagnosis post-biopsy or resection. With advances in endoscopy equipment and imaging techniques, we have witnessed the role of cytoendoscopy in diagnosing gastrointestinal tract tumors[39]. In the future, we hope to discover a more objective and accurate factor in order to characterize the endoscopic presentation of colorectal SSLs, which can swiftly and efficiently identify lesions, reduce missed or delayed diagnoses, and effectively decrease the incidence of interval CRCs.

For colorectal SSLs, good bowel preparation is the foundation, and the endoscopist's knowledge and experience play an essential role. Ultimately, combining all the predictive factors in colonoscopy screening to generate an immediate diagnosis can improve the detection rate.

FOOTNOTES

Author contributions: Wang RG reviewed the literature, wrote and revised the review; Wei L guided the literature search and review writing; Jiang B guided the topic selection, reviewed and suggested revisions.

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REFERENCES

1 **Sung H**, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin* 2021;

71: 209-249 [PMID: 33538338 DOI: 10.3322/caac.21660]
2 **Sanduleanu S**, le Clercq CM, Dekker E, Meijer GA, Rabeneck L, Rutter MD, Valori R, Young GP, Schoen RE; Expert Working Group on 'Right-sided lesions and interval cancers', Colorectal Cancer Screening Committee, World Endoscopy Organization. Definition and taxonomy of interval colorectal cancers: a proposal for standardising nomenclature. *Gut* 2015; **64**: 1257-1267 [PMID: 25193802 DOI: 10.1136/gutjnl-2014-307992]
3 **Murakami T**, Sakamoto N, Fukushima H, Shibuya T, Yao T, Nagahara A. Usefulness of the Japan narrow-band imaging expert team classification system for the diagnosis of sessile serrated lesion with dysplasia/carcinoma. *Surg Endosc* 2021; **35**: 4528-4538 [PMID: 32909209 DOI: 10.1007/s00464-020-07967-w]
4 **Burr NE**, Derbyshire E, Taylor J, Whalley S, Subramanian V, Finan PJ, Rutter MD, Valori R, Morris EJA. Variation in post-colonoscopy colorectal cancer across colonoscopy providers in English National Health Service: population based cohort study. *BMJ* 2019; **367**: l6090 [PMID: 31722875 DOI: 10.1136/bmj.l6090]
5 **Dekker E**, Tanis PJ, Vleugels JLA, Kasi PM, Wallace MB. Colorectal cancer. *Lancet* 2019; **394**: 1467-1480 [PMID: 31631858 DOI: 10.1016/S0140-6736(19)32319-0]
6 **East JE**, Atkin WS, Bateman AC, Clark SK, Dolwani S, Ket SN, Leedham SJ, Phull PS, Rutter MD, Shepherd NA, Tomlinson I, Rees CJ. British Society of Gastroenterology position statement on serrated polyps in the colon and rectum. *Gut* 2017; **66**: 1181-1196 [PMID: 28450390 DOI: 10.1136/gutjnl-2017-314005]
7 **Ahadi M**, Sokolova A, Brown I, Chou A, Gill AJ. The 2019 World Health Organization Classification of appendiceal, colorectal and anal canal tumours: an update and critical assessment. *Pathology* 2021; **53**: 454-461 [PMID: 33461799 DOI: 10.1016/j.pathol.2020.10.010]
8 **Nagtegaal ID**, Odze RD, Klimstra D, Paradis V, Rugge M, Schirmacher P, Washington KM, Carneiro F, Cree IA; WHO Classification of Tumours Editorial Board. The 2019 WHO classification of tumours of the digestive system. *Histopathology* 2020; **76**: 182-188 [PMID: 31433515 DOI: 10.1111/his.13975]
9 **Li J**, Teng XD, Lai MD. [Colorectal non-invasive epithelial lesions: an update on the pathology of colorectal serrated lesions and polyps]. *Zhonghua Bing Li Xue Za Zhi* 2020; **49**: 1339-1344 [PMID: 33287530 DOI: 10.3760/cma.j.cn112151-20200330-00272]
10 **Ng SC**, Ching JY, Chan VC, Wong MC, Tang R, Wong S, Luk AK, Lam TY, Gao Q, Chan AW, Wu JC, Chan FK, Lau JY, Sung JJ. Association between serrated polyps and the risk of synchronous advanced colorectal neoplasia in average-risk individuals. *Aliment Pharmacol Ther* 2015; **41**: 108-115 [PMID: 25339583 DOI: 10.1111/apt.13003]
11 **Crockett SD**, Nagtegaal ID. Terminology, Molecular Features, Epidemiology, and Management of Serrated Colorectal Neoplasia. *Gastroenterology* 2019; **157**: 949-966.e4 [PMID: 31323292 DOI: 10.1053/j.gastro.2019.06.041]
12 **Song YR**, Song SZ, Gong AX. [Advancement in the study of carcinogenesis mechanism and endoscopic diagnosis of colorectal serrated lesions]. *Chinese Journal of Digestive Endoscopy* 2021; 38(5): 412-415 [DOI: 10.3760/cma.j.cn321463-20200420-00093]
13 **Jung YS**, Park JH, Park CH. Serrated Polyps and the Risk of Metachronous Colorectal Advanced Neoplasia: A Systematic Review and Meta-Analysis. *Clin Gastroenterol Hepatol* 2022; **20**: 31-43.e1 [PMID: 33007512 DOI: 10.1016/j.cgh.2020.09.051]
14 **Gao Q**, Tsoi KK, Hirai HW, Wong MC, Chan FK, Wu JC, Lau JY, Sung JJ, Ng SC. Serrated polyps and the risk of synchronous colorectal advanced neoplasia: a systematic review and meta-analysis. *Am J Gastroenterol* 2015; **110**: 501-9; quiz 510 [PMID: 25756237 DOI: 10.1038/ajg.2015.49]
15 **Gupta S**, Lieberman D, Anderson JC, Burke CA, Dominitz JA, Kaltenbach T, Robertson DJ, Shaukat A, Syngal S, Rex DK. Recommendations for Follow-Up After Colonoscopy and Polypectomy: A Consensus Update by the US Multi-Society Task Force on Colorectal Cancer. *Gastroenterology* 2020; **158**: 1131-1153.e5 [PMID: 32044092 DOI: 10.1053/j.gastro.2019.10.026]
16 **Erichsen R**, Baron JA, Hamilton-Dutoit SJ, Snover DC, Torlakovic EE, Pedersen L, Frøslev T, Vyberg M, Hamilton SR, Sørensen HT. Increased Risk of Colorectal Cancer Development Among Patients With Serrated Polyps. *Gastroenterology* 2016; **150**: 895-902.e5 [PMID: 26677986 DOI: 10.1053/j.gastro.2015.11.046]
17 **IJspeert JE**, Vermeulen L, Meijer GA, Dekker E. Serrated neoplasia-role in colorectal carcinogenesis and clinical implications. *Nat Rev Gastroenterol Hepatol* 2015; **12**: 401-409 [PMID: 25963511 DOI: 10.1038/nrgastro.2015.73]
18 **Deng JW**, Fang JW. [Pathway and clinical characteristics of serrated colorectal cancer]. *National Medical Journal of China* 2020; 100 (34): 2716-2720 [DOI: 10.3760/cma.j.cn112137-20191230-02854]
19 **Omori K**, Yoshida K, Tamiya S, Daa T, Kan M. Endoscopic Observation of the Growth Process of a Right-Side Sessile Serrated Adenoma/Polyp with Cytological Dysplasia to an Invasive Submucosal Adenocarcinoma. *Case Rep Gastrointest Med* 2016; **2016**: 6576351 [PMID: 27437153 DOI: 10.1155/2016/6576351]
20 **Kinoshita S**, Nishizawa T, Uraoka T. Progression to invasive cancer from sessile serrated adenoma/polyp. *Dig Endosc* 2018; **30**: 266 [PMID: 29168233 DOI: 10.1111/den.12988]
21 **Amemori S**, Yamano HO, Tanaka Y, Yoshikawa K, Matsushita HO, Takagi R, Harada E, Yoshida Y, Tsuda K, Kato B, Tamura E, Eizuka M, Sugai T, Adachi Y, Yamamoto E, Suzuki H, Nakase H. Sessile serrated adenoma/polyp showed rapid malignant transformation in the final 13 months. *Dig Endosc* 2020; **32**: 979-983 [PMID: 31677187 DOI: 10.1111/den.13572]
22 **Yu-hu L**, Lei-lei Z, Gui-quan C, Dong P, Jin C, Shi-hao, Z. The analysis of detection rate and risk factors of colorectal sessile serrated adenoma. *Journal of Tropical Medicine* 2019; 19 (1): 65-67 [DOI: 10.3969/j.issn.1672-3619.2019.01.016]
23 **Li QY**, Xiao P, Ling TS, Sun YY, Luo LJ, Liang R, Deng ZJ, Situ WJ. [Detection of proximal serrated polyps:a single-center retrospective analysis]. *Chinese Journal of Digestive Endoscopy* 2019; **36** (2): 86-90 [DOI: 10.3760/cma.j.issn.1007-5232.2019.02.002]
24 **Murakami T**, Sakamoto N, Ritsuno H, Shibuya T, Osada T, Mitomi H, Yao T, Watanabe S. Distinct endoscopic characteristics of sessile serrated adenoma/polyp with and without dysplasia/carcinoma. *Gastrointest Endosc* 2017; **85**: 590-600 [PMID: 27663716 DOI: 10.1016/j.gie.2016.09.018]
25 **Hazewinkel Y**, López-Cerón M, East JE, Rastogi A, Pellisé M, Nakajima T, van Eeden S, Tytgat KM, Fockens P, Dekker E. Endoscopic features of sessile serrated adenomas: validation by international experts using high-resolution white-light

endoscopy and narrow-band imaging. *Gastrointest Endosc* 2013; **77**: 916-924 [PMID: 23433877 DOI: 10.1016/j.gie.2012.12.018]

26 **Cassese G**, Amendola A, Maione F, Giglio MC, Pagano G, Milone M, Aprea G, Luglio G, De Palma GD. Serrated Lesions of the Colon-Rectum: A Focus on New Diagnostic Tools and Current Management. *Gastroenterol Res Pract* 2019; **2019**: 9179718 [PMID: 30774654 DOI: 10.1155/2019/9179718]

27 **Murakami T**, Sakamoto N, Nagahara A. Endoscopic diagnosis of sessile serrated adenoma/polyp with and without dysplasia/carcinoma. *World J Gastroenterol* 2018; **24**: 3250-3259 [PMID: 30090005 DOI: 10.3748/wjg.v24.i29.3250]

28 **Yamamoto S**, Varkey J, Hedenström P. Acetic acid spray for better delineation of recurrent sessile serrated adenoma in the colon. *VideoGIE* 2019; **4**: 547-548 [PMID: 31840127 DOI: 10.1016/j.vgie.2019.08.001]

29 **Tribonias G**, Theodoropoulou A, Stylianou K, Giotis I, Mpitouli A, Moschovis D, Komeda Y, Manola ME, Paspatis G, Tzouvala M. Irrigating Acetic Acid Solution During Colonoscopy for the Detection of Sessile Serrated Neoplasia: A Randomized Controlled Trial. *Dig Dis Sci* 2022; **67**: 282-292 [PMID: 33515378 DOI: 10.1007/s10620-021-06858-x]

30 **Kono Y**, Higashi R, Mizushima H, Shimizu D, Katayama T, Kosaka M, Hirata I, Hirata T, Gotoda T, Miyahara K, Moritou Y, Kunihiro M, Nakagawa M, Ichimura K, Okada H. Usefulness of Acetic Acid Spray with Narrow-Band Imaging for Identifying the Margin of Sessile Serrated Lesions. *Dig Dis Sci* 2023 [PMID: 36609732 DOI: 10.1007/s10620-022-07816-x]

31 **Suzuki Y**, Ohata K, Matsuhashi N. Delineating sessile serrated adenomas/polyps with acetic acid spray for a more accurate piecemeal cold snare polypectomy. *VideoGIE* 2020; **5**: 519-521 [PMID: 33204905 DOI: 10.1016/j.vgie.2020.05.030]

32 **Tadepalli US**, Feihel D, Miller KM, Itzkowitz SH, Freedman JS, Kornacki S, Cohen LB, Bamji ND, Bodian CA, Aisenberg J. A morphologic analysis of sessile serrated polyps observed during routine colonoscopy (with video). *Gastrointest Endosc* 2011; **74**: 1360-1368 [PMID: 22018553 DOI: 10.1016/j.gie.2011.08.008]

33 **Yamashina T**, Takeuchi Y, Uedo N, Aoi K, Matsuura N, Nagai K, Matsui F, Ito T, Fujii M, Yamamoto S, Hanaoka N, Higashino K, Ishihara R, Tomita Y, Iishi H. Diagnostic features of sessile serrated adenoma/polyps on magnifying narrow band imaging: a prospective study of diagnostic accuracy. *J Gastroenterol Hepatol* 2015; **30**: 117-123 [PMID: 25088839 DOI: 10.1111/jgh.12688]

34 **Saito S**, Tajiri H, Ikegami M. Serrated polyps of the colon and rectum: Endoscopic features including image enhanced endoscopy. *World J Gastrointest Endosc* 2015; **7**: 860-871 [PMID: 26240687 DOI: 10.4253/wjge.v7.i9.860]

35 **Wang Y**, Ren YB, Yang XS, Huang YH, Zhang L, Li X, Bai P, Wang L, Fan X, Ding YM, Li HL, Lin XC. [Comparison of endoscopic features between colorectal sessile serrated adenoma/polyp with or without cytologic dysplasia and hyperplastic polyp]. *Zhonghua Yi Xue Za Zhi* 2019; **99**: 2214-2220 [PMID: 31434395 DOI: 10.3760/cma.j.issn.0376-2491.2019.28.012]

36 **Yamada M**, Sakamoto T, Otake Y, Nakajima T, Kuchiba A, Taniguchi H, Sekine S, Kushima R, Ramberan H, Parra-Blanco A, Fujii T, Matsuda T, Saito Y. Investigating endoscopic features of sessile serrated adenomas/polyps by using narrow-band imaging with optical magnification. *Gastrointest Endosc* 2015; **82**: 108-117 [PMID: 25840928 DOI: 10.1016/j.gie.2014.12.037]

37 **Uraoka T**, Higashi R, Horii J, Harada K, Hori K, Okada H, Mizuno M, Tomoda J, Ohara N, Tanaka T, Chiu HM, Yahagi N, Yamamoto K. Prospective evaluation of endoscopic criteria characteristic of sessile serrated adenomas/polyps. *J Gastroenterol* 2015; **50**: 555-563 [PMID: 25270966 DOI: 10.1007/s00535-014-0999-y]

38 **Kimura T**, Yamamoto E, Yamano HO, Suzuki H, Kamimae S, Nojima M, Sawada T, Ashida M, Yoshikawa K, Takagi R, Kato R, Harada T, Suzuki R, Maruyama R, Kai M, Imai K, Shinomura Y, Sugai T, Toyota M. A novel pit pattern identifies the precursor of colorectal cancer derived from sessile serrated adenoma. *Am J Gastroenterol* 2012; **107**: 460-469 [PMID: 22233696 DOI: 10.1038/ajg.2011.457]

39 **Takamaru H**, Wu SYS, Saito Y. Endocytoscopy: technology and clinical application in the lower GI tract. *Transl Gastroenterol Hepatol* 2020; **5**: 40 [PMID: 32632391 DOI: 10.21037/tgh.2019.12.04]

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ORIGINAL ARTICLE

Retrospective Cohort Study

Interaction between age and gender on survival outcomes in extramedullary multiple myeloma over the past two decades

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Abstract

BACKGROUND

Extramedullary multiple myeloma (MM) (EMM) is a rare and aggressive sub-entity of MM that can be present at diagnosis or develop anytime during the disease course. There is a paucity of data on the clinical characteristics and overall epidemiology of EMM. Furthermore, there is a scarcity of data on how the interaction of age and gender influences the survival of EMM.

AIM

To evaluate the clinical characteristics of patients with EMM over the past 2 decades and to identify epidemiologic characteristics that may impact overall prognosis.

METHODS

A total of 858 patients diagnosed with EMM, between 2000 and 2017, were ultimately enrolled in our study by retrieving the Surveillance, Epidemiology, and

End Results database. We analyzed demographics, clinical characteristics, and overall mortality (OM) as well as cancer-specific mortality (CSM) of EMM. Variables with a *P* value < 0.1 in the univariate Cox regression were incorporated into the multivariate Cox model to determine the independent prognostic factors, with a hazard ratio (HR) of greater than 1 representing adverse prognostic factors.

RESULTS

From a sample of 858 EMM, the male gender (63.25%), age range 60-79 years (51.05%), and non-Hispanic whites (66.78%) were the most represented. Central Nervous System and the vertebral column was the most affected site (33.10%). Crude analysis revealed higher OM in the age group 80+ [HR = 6.951, 95% confidence interval (95%CI): 3.299-14.647, *P* = 0], Non-Hispanic Black population (HR = 1.339, 95%CI: 1.02-1.759, *P* = 0.036), Bones not otherwise specified (NOS) (HR = 1.74, 95%CI: 1.043-2.902, *P* = 0.034), and widowed individuals (HR = 2.107, 95%CI: 1.511-2.938, *P* = 0). Skin involvement (HR = 0.241, 95%CI: 0.06-0.974, *P* = 0.046) and a yearly income of $75000+ (HR = 0.259, 95%CI: 0.125-0.538, *P* = 0) had the lowest OM in the crude analysis. Crude analysis revealed higher CSM in the age group 80+, Non-Hispanic Black, Bones NOS, and widowed. Multivariate cox proportional hazard regression analyses only revealed higher OM in the age group 80+ (HR = 9.792, 95%CI: 4.403-21.774, *P* = 0) and widowed individuals (HR = 1.609, 95%CI: 1.101-2.35, *P* = 0.014). Multivariate cox proportional hazard regression analyses of CSM also revealed higher mortality of the same groups. Eyes, mouth, and ENT involvement had the lowest CSM in the multivariate analysis. There was no interaction between age and gender in the adjusted analysis for OM and CSM.

CONCLUSION

EMM is a rare entity. To our knowledge, there is a scarcity of data on the clinical characteristics and prognosis factors of patients with extramedullary multiple myeloma. In this retrospective cohort, using a United States-based population, we found that age, marital status, and tumor site were independent prognostic factors. Furthermore, we found that age and gender did not interact to influence the mortality of patients with EMM.

Key Words: Multiple myeloma; Age; Gender; Mortality; Plasmacytoma

Core Tip: Very little is known about extramedullary multiple myeloma (EMM), owing to its rarity and scarcity of data on the subject. So far it was found that advanced age was the single most important prognostic value for poor outcome in EMM. However, how age interacts with gender to affect mortality in EMM remains unknown. We found that age did not interact with gender to affect mortality in EMM.

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INTRODUCTION

Multiple myeloma (MM) is a rare cancer with the hallmark of monoclonal plasma cell proliferation in the bone marrow[1]. MM accounts for approximately 1%-2% of all cancers. A subclone can thrive and grow independent of the bone marrow microenvironment resulting in extramedullary MM (EMM) which is an aggressive subentity of MM[2]. Affecting up to 30% of patients with MM, EMM can be present either at diagnosis or anytime during the disease process[1,2].

EMM is frequently associated with high-risk cytogenetics. As evidenced by a pilot study, which revealed an association of chromosome 1 abnormalities in bone marrow myeloma cells with extramedullary progression. Optical mapping showed the potential for refining the complex genomic architecture in MM and its phenotypes[3]. Only few studies in the literature have addressed the clinical characteristics of patients with EMM[4-8]. Age at the diagnosis of MM and the site of extramedullary

disease have been shown to be independent prognostic factors[4,9]. Furthermore, there is some data associating the male gender with MM[5]. However, to the best of our knowledge, there is a lack of studies addressing the interaction between age and gender in EMM, which makes our study the first of its kind.

To fill in the gaps in the literature, we conducted a retrospective cohort study amongst patients with EMM using the Surveillance, Epidemiology, and End Results (SEER) database, to evaluate the interaction of age and gender in regard to mortality of EMM as well as independent prognostic factors of patients with EMM over the past 2 decades.

MATERIALS AND METHODS

Study design

A population-based retrospective cohort study of patients with EMM was conducted using the SEER research. In addition, 18 registries in the November 2020 submission database were also utilized (http://www.seer.cancer.gov). The SEER Program is one of the largest and most authoritative sources of the cancer-related dataset in the United States, which is sponsored by the United States National Cancer Institute. The SEER 18 database collects cancer incidence, patients' clinicopathological features, and survival data from 18 population-based cancer registries and covers nearly 28% of the United States population[9]. This dataset is de-identified and publicly available, thus, the study is exempt from an Institutional Review Board's review. A detailed description of the database and data collection can be found elsewhere[10].

Patient selection

Inclusion criteria: All patients with EMM diagnosed from 2000 to 2017 were identified following criteria from previous studies[11]. We used site and morphology ICD-O-3 histology/behavior, malignant variables codes 9731/3 (*i.e.*, solitary plasmacytoma of bone) and 9734/3 (*i.e.*, extraosseous plasmacytoma) to identify patients with EMM. We also restricted our cohort to patients with 2 tumors and diagnostic confirmation through positive histology, immunotherapy, or genetic studies. Thus, increasing the accuracy of our findings and eliminating possible false-positive diagnoses.

Exclusion criteria: We excluded patients with unknown age at diagnosis, tumor stage, tumor site, or race. Lastly, we excluded patients diagnosed through autopsy.

Study variables

Main exposures: Gender (male and female), age (0-39, 40-59, 60-79, and 80+), and their interaction were the main exposures of interest.

Sociodemographic and tumor characteristics: Gender, year of diagnosis, extramedullary site of the tumor, location, annual salary, Civil status, year of diagnosis, surgical resection, as well as chemotherapy, were assessed for the purpose of the study.

Statistical analysis

We performed a crude and adjusted Cox proportional hazard regression to investigate the impact of the interaction between age and gender on EMM mortality. Variables with a value < 0.1 in the univariate Cox regression model were incorporated into the multivariate Cox proportional analysis to determine the independent prognostic factors associated with overall mortality (OM) and cancer-specific mortality (CSM), with a hazard ratio (HR) > 1 representing adverse prognostic factors. All tests were two-sided, with a confidence interval set as 95% and P value < 0.05 deemed statistically significant. All statistical tests were performed by using Software STATA16.1.

RESULTS

We enrolled 858 patients with EMM in our study. The baseline characteristics of our study are summarized in Table 1. The male gender (63.25%), age range 60-79 at diagnosis (51.05%), Non-Hispanic Whites (66.78 %), and married patients (66.32%) were the most represented groups. The Central Nervous System and vertebral column were the most affected location (33.10%). Most patients were living in metropolitan areas with a population of at least 1 million people (56.06%). Most patients did not receive chemotherapy (81.47%).

A crude analysis of factors associated with all-cause mortality and EMM-related mortality among United States patients between 2000 and 2017 is demonstrated in Table 2. Crude analysis revealed higher OM in the age group 80+ [HR = 6.951, 95% confidence interval (95%CI): 3.299-14.647, P = 0], Non-Hispanic Black population (HR = 1.339, 95%CI: 1.02-1.759, P = 0.036), other bones (HR = 1.74,

Table 1 Demographic and Clinicopathologic characteristics of United States patients with extramedullary multiple myeloma between 2000 and 2017

Characteristics	*n*	%
Total	858	100
Gender		
Female	311	36.25
Male	547	63.25
Age at diagnosis, yr		
0-39	41	4.78
40-59	309	36.01
60-79	438	51.05
80+	70	8.16
Race		
Non-Hispanic white	573	66.78
Non-Hispanic black	133	15.50
Hispanic	110	12.82
Other	42	4.90
Extramedullary site		
CNS and vertebral column	284	33.10
Bones, subcutaneous tissues, connective tissues, and soft tissues of the trunk	108	12.59
Bones, soft tissues, subcutaneous tissues, and connective tissues of the pelvis and sacrum	97	11.31
Bones, soft tissues, subcutaneous tissues, connective tissues, and lymph nodes of the upper extremities	64	7.46
Bones, soft tissues, subcutaneous tissues, and connective tissues of the lower extremities	45	5.24
Bones, soft tissues, subcutaneous tissues, connective tissues, and lymph nodes of the face and skull	60	6.99
Other bones, NOS	37	4.31
Eyes, mouth, and ENT	101	11.77
Lung, breast, and mediastinum	26	3.03
Gastrointestinal tract	18	2.10
Skin	12	1.40
Kidney, suprarenal glands, and retroperitoneum	6	0.70
Living area		
Counties in metropolitan areas of 1 million persons	481	56.06
Counties in metropolitan areas of 250000 to 1 million persons	173	20.16
Counties in metropolitan areas of 250000 persons	76	8.86
Nonmetropolitan counties adjacent to a metropolitan area	77	8.97
Nonmetropolitan counties not adjacent to a metropolitan area	51	5.94
Income per year		
< $35000	12	1.40
$35000-44999	74	8.62
$45000-54999	154	17.95
$55000-64999	232	27.04
$65000-74999	183	21.33
$75000+	203	23.66

Marital Status		
Married	569	66.32
Single	106	12.35
Divorced/separated	79	9.21
Widowed	62	7.23
Unknown	42	4.90

CNS: Central Nervous System; NOS: Not otherwise specified.

95%CI: 1.043-2.902, P = 0.034), and widowed individuals (HR = 2.107, 95%CI: 1.511-2.938, P = 0). Skin involvement (HR = 0.241, 95%CI: 0.06-0.974, P = 0.046) and a yearly income of $75000+ (HR = 0.259, 95%CI: 0.125-0.538, P = 0) had the lowest OM in the crude analysis. Crude analysis revealed higher CSM in age group 80+ (HR = 10.111, 95%CI: 3.083-33.159, P = 0), Non-Hispanic Black (HR = 1.446, 95%CI: 1.017-2.055, P = 0.04), other bones (HR = 1.887, 95%CI: 1.044-3.411, P = 0.035) and widowed individuals (HR = 2.463, 95%CI: 1.612-3.765, P = 0).

Multivariate cox proportional hazard regression analyses of factors affecting all-cause mortality and EMM-related mortality among United States patients between 2000 and 2017 are demonstrated in Table 3. Multivariate cox proportional hazard regression analyses only revealed higher OM in the age group 80+ (HR = 9.792, 95%CI: 4.403-21.774, P = 0) and widowed individuals (HR = 1.609, 95%CI: 1.101-2.35, P = 0.014). Multivariate cox proportional hazard regression analyses of CSM showed similar findings revealing higher mortality in the age group 80+ (HR = 13.672, 95%CI: 3.915-47.746, P = 0) and widowed individuals (HR = 2.085, 95%CI: 1.275-3.409, P = 0.003). Involvement of eyes, mouth and ENT sites (HR = 0.425, 95%CI: 0.235-0.768, P = 0.005) had the lowest CSM in the multivariate analysis. Importantly, the study also revealed that the interaction between age and gender was not a statistically significant predictor of mortality in patients with EMM as shown in Table 4.

DISCUSSION

In this large SEER data-based retrospective cohort study, we demonstrated that EMM was associated with a higher OM and CSM in patients greater than 80 years of age and those patients who had been widowed. However, interestingly, the interaction between age and gender was not found to be statistically significant in predicting mortality in EMM patients.

EMM is a highly aggressive entity of MM, with clinical behavior distinct from marrow-restricted myeloma[12]. EMM is historically known to bear a worse prognosis compared to marrow-restricted myeloma[13]. Several studies have been carried out to investigate clinical characteristics and prognostic factors of EMM[4-8,12]. However, there is a paucity of data investigating the interaction of age and gender in regard to the mortality of EMM.

The interaction between gender and race and its influence on survival disparities in head and neck cancers has been well-documented[13]. Furthermore, gender was found to be the most important predictor with young and middle-aged females having the most favorable prognosis in non-smokers with oral squamous cell carcinoma[14]. However, no study has evaluated the impact of these interactions in the EMM population subgroup.

Our study did not reveal any interaction between age, gender, and race in regard to adjusted mortality in patients with EMM. Age was found to be the single most important prognostic factor for OM and CSM. Age was also found to be an important prognostic factor for the survival of EMM in a study by Li *et al*[5]. Gender and race were not of prognostic value in our cohort reaffirming the similar results found in the Li series[5].

Several retrospective studies have found marital status to be an independent prognostic factor in the survival of oncologic patients[15-19]. Patients that were married had better survival compared to their nonmarried counterparts[20-24]. This was also true in our study, where widowed patients had the highest OM and CSM, followed by single and divorced patients. This is perhaps due to the lack of psychological and emotional support as well as the increased incidence of depression and other mood disorders amongst these individuals, which could directly, or indirectly influence the treatment and regular oncology follow-up.

We hope that the results of this study will shed some light on the clinical presentation of this rare and aggressive manifestation of MM. In better understanding EMM, we hope to inspire larger prospective studies on the management of this subset of patients, which is particularly important in the era of novel agents including immunomodulatory agents, proteasome inhibitors, monoclonal antibodies, and, more recently, the advent of chimeric antigen receptor T-cell therapy and bispecific agents. This can be especially important with the new emergence of microRNAs that help prevent drug resistance when

Table 2 Crude analysis of factors associated with all-cause mortality and extramedullary multiple myeloma; related mortality among United States patients between 2000 and 2017

Characteristics	Overall mortality	EMD MM mortality
	Crude proportional-hazard ratio (95% confidence interval)	
Gender		
Female	1 (reference)	1 (reference)
Male	1.02 (0.826-1.259)	0.804 (0.611-1.056)
Age at diagnosis, yr		
0-39	1 (reference)	1 (reference)
40-59	1.683 (0.82-3.452)	2.409 (0.754-7.696)
60-79	3.271 (1.615-6.627)[c]	4.918 (1.565-15.461)[c]
80+	6.951 (3.299-14.647)[c]	10.111 (3.083-33.159)[c]
Race		
Non-Hispanic white	1 (reference)	1 (reference)
Non-Hispanic black	1.339 (1.02-1.759)[b]	1.446 (1.017-2.055)[b]
Hispanic	0.991 (0.719-1.365)	0.791 (0.495-1.263)
Other	1.016 (0.63-1.639)	1.209 (0.67-2.179)
Extramedullary site		
CNS and vertebral column	1 (reference)	1 (reference)
Bones, subcutaneous tissues, connective tissues, and soft tissues of the trunk	1.53 (1.113-2.102)[c]	1.187 (0.774-1.82)
Bones, soft tissues, subcutaneous tissues, and connective tissues of the pelvis and sacrum	1.027 (0.716-1.475)	1.15 (0.746-1.772)
Bones, soft tissues, subcutaneous tissues, connective tissues, and lymph nodes of the upper extremities	1.036 (0.682-1.573)	0.718 (0.391-1.32)
Bones, soft tissues, subcutaneous tissues, and connective tissues of the lower extremities	1.668 (1.058-2.63)[b]	1.466 (0.814-2.642)
Bones, soft tissues, subcutaneous tissues, connective tissues, and lymph nodes of the face and skull	1.135 (0.756-1.702)	0.919 (0.529-1.598)
Other bones, NOS	1.74 (1.043-2.902)[b]	1.887 (1.044-3.411)[b]
Eyes, mouth, and ENT	0.929 (0.668-1.293)	0.451 (0.259-0.783)[c]
Lung, breast, and mediastinum	1.588 (0.895-2.816)	1.278 (0.589-2.773)
Gastrointestinal tract	0.787 (0.367-1.688)	0.55 (0.174-1.745)
Skin	0.241 (0.06-0.974)[b]	0.195 (0.027-1.403)
Kidney, suprarenal glands, and retroperitoneum	1.861 (0.591-5.86)	0.907 (0.126-6.531)
Living area		
Counties in metropolitan areas of 1 million persons	1 (reference)	1 (reference)
Counties in metropolitan areas of 250000 to 1 million persons	1.021 (0.789-1.322)	0.803 (0.556-1.158)
Counties in metropolitan areas of 250000 persons	0.794 (0.538-1.17)	0.8 (0.482-1.327)
Nonmetropolitan counties adjacent to a metropolitan area	1.099 (0.775-1.559)	0.937 (0.578-1.518)
Nonmetropolitan counties not adjacent to a metropolitan area	1.191 (0.798-1.779)	1.105 (0.647-1.888)
Income per year		
< $35000	1 (reference)	1 (reference)
$35000-44999	0.457 (0.213-0.984)[b]	0.412 (0.167-1.014)[a]
$45000-54999	0.356 (0.17-0.745)[c]	0.33 (0.139-0.782)[b]
$55000-64999	0.358 (0.174-0.737)[c]	0.236 (0.101-0.554)[c]

$65000-74999	0.328 (0.158-0.681)[c]	0.292 (0.124-0.685)[c]
$75000+	0.259 (0.125-0.538)[c]	0.24 (0.102-0.563)[c]
Marital status		
Married	1 (reference)	1 (reference)
Single	1.305 (0.967-1.763)[a]	1.515 (1.027-2.236)[b]
Divorced/separated	1.531 (1.094-2.142)[b]	1.681 (1.084-2.605)[b]
Widowed	2.107 (1.511-2.938)[c]	2.463 (1.612-3.765)[c]

[a]$P < 0.1$.
[b]$P < 0.05$.
[c]$P < 0.01$.
EMD: Extramedullary disease; MM: Multiple myeloma; CNS: Central Nervous System; NOS: Not otherwise specified.

Table 3 Multivariate cox proportional hazard regression analyses of factors affecting all-cause mortality and extramedullary disease multiple myeloma related mortality among United States patients between 2000 and 2017

Characteristics	Overall mortality	EMD MM mortality
	Adjusted proportional hazard ratio (95% confidence interval)	
Gender		
Female	1 (reference)	1 (reference)
Male	1.256 (0.989-1.594)[a]	1.022 (0.748-1.397)
Age at diagnosis, yr		
0-39	1 (reference)	1 (reference)
40-59	2.206 (1.047-4.647)[b]	3.154 (0.957-10.395)[a]
60-79	4.129 (1.974-8.635)[c]	5.667 (1.738-18.48)[c]
80+	9.792 (4.403-21.774)[c]	13.672 (3.915-47.746)[c]
Race		
Non-Hispanic white	1 (reference)	1 (reference)
Non-Hispanic black	1.315 (0.96-1.802)[a]	1.34 (0.884-2.03)
Hispanic	1.034 (0.734-1.457)	0.833 (0.506-1.371)
Other	1.25 (0.743-2.104)	1.741 (0.916-3.308)[a]
Extramedullary site		
CNS and vertebral column	1 (reference)	1 (reference)
Bones, subcutaneous tissues, connective tissues, and soft tissues of the trunk	1.401 (0.996-1.972)[a]	1.046 (0.664-1.649)
Bones, soft tissues, subcutaneous tissues, and connective tissues of the pelvis and sacrum	0.98 (0.671-1.432)	1.091 (0.689-1.729)
Bones, soft tissues, subcutaneous tissues, connective tissues, and lymph nodes of the upper extremities	1.024 (0.661-1.586)	0.672 (0.353-1.279)
Bones, soft tissues, subcutaneous tissues, and connective tissues of the lower extremities	1.488 (0.909-2.436)	1.382 (0.733-2.605)
Bones, soft tissues, subcutaneous tissues, connective tissues, and lymph nodes of the face and skull	0.99 (0.641-1.53)	0.76 (0.414-1.394)
Other bones, NOS	1.195 (0.694-2.058)	1.199 (0.629-2.284)
Eyes, mouth, and ENT	0.902 (0.631-1.29)	0.425 (0.235-0.768)[c]
Lung, breast, and mediastinum	1.187 (0.628-2.246)	0.959 (0.392-2.346)
Gastrointestinal tract	0.677 (0.303-1.512)	0.383 (0.114-1.283)
Skin	0.327 (0.08-1.34)	0.325 (0.044-2.394)

Kidney, suprarenal glands, and retroperitoneum	3.055 (0.881-10.601)[a]	0.865 (0.108-6.901)
Living area		
Counties in metropolitan areas of 1 million persons	1 (reference)	1 (reference)
Counties in metropolitan areas of 250000 to 1 million persons	0.957 (0.715-1.282)	0.778 (0.512-1.181)
Counties in metropolitan areas of 250000 persons	0.819 (0.523-1.282)	0.85 (0.47-1.539)
Nonmetropolitan counties adjacent to a metropolitan area	0.997 (0.653-1.522)	0.836 (0.461-1.516)
Nonmetropolitan counties not adjacent to a metropolitan area	0.877 (0.533-1.442)	0.757 (0.379-1.512)
Income per year		
< $35000	1 (reference)	1 (reference)
$35000-44999	0.48 (0.21-1.095)[a]	0.381 (0.14-1.036)[a]
$45000-54999	0.452 (0.196-1.04)[a]	0.375 (0.135-1.044)[a]
$55000-64,999	0.446 (0.192-1.036)[a]	0.275 (0.096-0.788)[b]
$65000-74999	0.413 (0.173-0.983)[b]	0.381 (0.129-1.121)[a]
$75000+	0.324 (0.134-0.783)[b]	0.29 (0.095-0.878)[b]
Marital status		
Married	1 (reference)	1 (reference)
Single	1.5 (1.079-2.086)[b]	1.668 (1.089-2.556)[b]
Divorced/separated	1.49 (1.037-2.139)[b]	1.463 (0.908-2.355)
Widowed	1.609 (1.101-2.35)[b]	2.085 (1.275-3.409)[c]

[a]$P < 0.1$.
[b]$P < 0.05$.
[c]$P < 0.01$.
EMD: Extramedullary disease; MM: Multiple myeloma; CNS: Central Nervous System; NOS: Not otherwise specified.

Table 4 Joint test analysis of the predictors of extramedullary multiple myeloma and overall mortality among United States extramedullary multiple myeloma patients, 2000-2017

Variables	MM mortality			Overall mortality	
	DF	χ^2	*P* value	χ^2	*P* value
Race/ethnicity	3	5.7436	0.1248	3.2403	0.3560
Age at diagnosis	3	21.2193	< 0.0001	49.2869	< 0.0001
Gender	1	0.5044	0.4776	0.5168	0.4722
Extramedullary Site	11	16.6070	0.1200	15.6578	0.1543
Living area	4	2.1023	0.7169	0.8175	0.9361
Income	5	7.3539	0.1956	7.4157	0.1915
Marital status	3	10.8183	0.0128	11.7967	0.0081
chemotherapy	1	2.3104	0.1285	1.4536	0.2280
Year of diagnosis	17	25.3142	0.0879	16.1848	0.5108
Interaction between age and gender	3	2.1285	0.5462	1.0296	0.7941

DF: Degree of freedom; MM: Multiple myeloma.

combined with anti-MM drug regimens and improve the patient's management[25].

Our study has several strengths. Firstly, the database used is the largest cancer database in the United States. The sample size of the study is non-negligible. Also, owing to the stringent inclusion criteria and the fact that we used patients with only confirmed EMM for our diagnosis, we eliminated false positive results which increase the accuracy of our study findings. However, a few limitations should be

considered in our study. Information could not be obtained on radiotherapy and Hematopoietic Stem Cell Transplant. The information on chemotherapy was unfulfilled. Furthermore, the SEER database publicly available lacks information on comorbidities, which could lead to missing data on potential confounders owing to the retrospective nature of the study.

CONCLUSION

EMM is a rare entity of MM that can be present at diagnosis or develop during the disease course. In this large retrospective SEER database-based study, we found that age and gender do not interact to influence the mortality of patients with EMM. Age was the single most important prognostic factor. We hope that the results of this study will shed light on this important non-significant interaction between age and gender in regard to mortality amongst EMM patients and perhaps inspire larger prospective studies on this subject.

ARTICLE HIGHLIGHTS

Research background

Age has been established as the single most important prognostic factor of extramedullary multiple myeloma (EMM). However, the interaction between age and gender in the mortality of EMM has yet to be studied.

Research motivation

The main motivation of this study was to identify independent predictors of outcomes, as well as how age and gender interact to affect mortality in EMM.

Research objectives

This study has the objective to establish the overall epidemiology of EMM, as well as the interaction between age and gender on mortality.

Research methods

This is a retrospective study involving 858 patients diagnosed with EMM, between 2000 and 2017 using the Surveillance, Epidemiology, and End Results database.

Research results

Patients older than 80 years and widowed had higher overall mortality (OM) and cancer-specific mortality (CSM). Eyes, mouth, and ENT involvement were protective factors regarding CSM. There was no interaction between age and gender in the adjusted analysis for OM and CSM.

Research conclusions

Although age is the single most important prognostic value of mortality in EMM, it does not interact with gender to affect mortality in patients with EMM.

Research perspectives

Future prospective studies are needed to better understand the impact of newer agents in the management of this aggressive subset of MM.

FOOTNOTES

Author contributions: Bangolo AI searched the literature, wrote, and revised the manuscript; Fwelo P extracted and analysed the data, revised, and edited the manuscript; Trivedi C, Sagireddy S, Aljanaahi H, Auda A, Mohamed M, Onyeka S, Fisher M, Thapa J, Tabucanon EJ, Georgiev L, Wishart A, Kumari S, Erikson C, Bangura M, Paddy O, Madhukar R, Gomez EL, Rathod J, Naria M, Hajal B, Awadhalla M, Siegel D, Parmar H, Biran N, and Vesole DH revised and edited the manuscript; Phull P and Weissman S revised and approved the final version and are the article's guarantors; All authors certify that they contributed sufficiently to the intellectual content and data analysis; Each author has reviewed the final version of the manuscript and approved it for publication.

Institutional review board statement: The study protocol was reviewed by the Ethics Committee at Palisades Medical Center and the need for IRB approval was waived as the SEER database is a public-use dataset.

Informed consent statement: The Surveillance, Epidemiology, and End Results (SEER) database was a public-use

dataset, of which the informed consent was waived.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

Data sharing statement: The data used and/or analyzed in this study are available in the Surveillance, Epidemiology, and End Results (SEER) Database of the National Cancer Institute (http://seer.cancer.gov).

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REFERENCES

1 **Siegel RL**, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. *CA Cancer J Clin* 2022; **72**: 7-33 [PMID: 35020204 DOI: 10.3322/caac.21708]
2 **Bhutani M**, Foureau DM, Atrash S, Voorhees PM, Usmani SZ. Extramedullary multiple myeloma. *Leukemia* 2020; **34**: 1-20 [PMID: 31776467 DOI: 10.1038/s41375-019-0660-0]
3 **Kriegova E**, Fillerova R, Minarik J, Savara J, Manakova J, Petrackova A, Dihel M, Balcarkova J, Krhovska P, Pika T, Gajdos P, Behalek M, Vasinek M, Papajik T. Whole-genome optical mapping of bone-marrow myeloma cells reveals association of extramedullary multiple myeloma with chromosome 1 abnormalities. *Sci Rep* 2021; **11**: 14671 [PMID: 34282158 DOI: 10.1038/s41598-021-93835-z]
4 **Mohan M**, Yarlagadda N, Szabo A, Singh A, Pina Oviedo S, Schinke C. Clinical characteristics of testicular extramedullary involvement in multiple myeloma. *Am J Hematol* 2021; **96**: E77-E81 [PMID: 33338289 DOI: 10.1002/ajh.26072]
5 **Li G**, Song YP, Lv Y, Li ZZ, Zheng YH. Clinical Characteristics and Prognostic Analysis of Multiple Myeloma with Extramedullary Disease: A SEER-Based Study. *J Oncol* 2021; **2021**: 6681521 [PMID: 33603785 DOI: 10.1155/2021/6681521]
6 **Tian C**, Wang L, Wu L, Zhu L, Xu W, Ye Z, Zhao Z, Wang Y, Zhang Y. Clinical characteristics and prognosis of multiple myeloma with bone-related extramedullary disease at diagnosis. *Biosci Rep* 2018; **38** [PMID: 29685954 DOI: 10.1042/BSR20171697]
7 **Gao S**, Li Q, Dong F, Yang P, Chen Y, Wang J, Wang Y, Jing H. Clinical characteristics and survival outcomes of newly diagnosed multiple myeloma patients presenting with extramedullary disease: A retrospective study. *Leuk Res* 2022; **115**: 106793 [PMID: 35248783 DOI: 10.1016/j.leukres.2022.106793]
8 **Janjetovic S**, Lohneis P, Nogai A, Balci D, Rasche L, Jähne D, Bokemeyer C, Schilling G, Blau IW, Schmidt-Hieber M. Clinical and Biological Characteristics of Medullary and Extramedullary Plasma Cell Dyscrasias. *Biology (Basel)* 2021; **10** [PMID: 34356484 DOI: 10.3390/biology10070629]
9 **Wu D**, Wang WJ, Zhang M. [Bone Marrow Morphology of Multiple Myeloma with Non-bone-related Extramedullary Disease]. *Zhongguo Shi Yan Xue Ye Xue Za Zhi* 2018; **26**: 807-811 [PMID: 29950224 DOI: 10.7534/j.issn.1009-2137.2018.03.029]
10 **Duggan MA**, Anderson WF, Altekruse S, Penberthy L, Sherman ME. The Surveillance, Epidemiology, and End Results (SEER) Program and Pathology: Toward Strengthening the Critical Relationship. *Am J Surg Pathol* 2016; **40**: e94-e102 [PMID: 27740970 DOI: 10.1097/PAS.0000000000000749]
11 **Jagosky MH**, Usmani SZ. Extramedullary Disease in Multiple Myeloma. *Curr Hematol Malig Rep* 2020; **15**: 62-71 [PMID: 32198576 DOI: 10.1007/s11899-020-00568-3]
12 **Weinstock M**, Ghobrial IM. Extramedullary multiple myeloma. *Leuk Lymphoma* 2013; **54**: 1135-1141 [PMID: 23210572 DOI: 10.3109/10428194.2012.740562]
13 **Mazul AL**, Naik AN, Zhan KY, Stepan KO, Old MO, Kang SY, Nakken ER, Puram SV. Gender and race interact to influence survival disparities in head and neck cancer. *Oral Oncol* 2021; **112**: 105093 [PMID: 33232879 DOI: 10.1016/j.oraloncology.2020.105093]
14 **Oh LJ**, Asher R, Veness M, Smee R, Goldstein D, Gopalakrishna Iyer N, Balasubramanian D, Low TH, Palme CE, Gupta R, Clark J. Effect of age and gender in non-smokers with oral squamous cell carcinoma: Multi-institutional study. *Oral Oncol* 2021; **116**: 105210 [PMID: 33618102 DOI: 10.1016/j.oraloncology.2021.105210]
15 **Tang L**, Pan Z, Zhang X. The effect of marital status on the survival of patients with multiple myeloma. *Hematology*

2022; **27**: 187-197 [PMID: 35068385 DOI: 10.1080/16078454.2022.2026027]

16 **Wang S**, Chen L, Chen D, Chao J, Shao Y, Tang K, Chen W. Effect of Marital Status on the Survival of Patients With Adenocarcinoma of the Esophagogastric Junction: A Population-Based, Propensity-Matched Study. *Cancer Control* 2021; **28**: 10732748211066309 [PMID: 34910613 DOI: 10.1177/10732748211066309]

17 **Zhou C**, Zhang Y, Hu X, Fang M, Xiao S. The effect of marital and insurance status on the survival of elderly patients with stage M1b colon cancer: a SEER-based study. *BMC Cancer* 2021; **21**: 891 [PMID: 34353300 DOI: 10.1186/s12885-021-08627-5]

18 **Alyabsi M**, Ramadan M, Algarni M, Alshammari K, Jazieh AR. The effect of marital status on stage at diagnosis and survival in Saudis diagnosed with colorectal cancer: cancer registry analysis. *Sci Rep* 2021; **11**: 8603 [PMID: 33883627 DOI: 10.1038/s41598-021-88042-9]

19 **Xiao K**, Zhao Y, Cai Y, Chen P, Chen J, Ye R, Yuan B, Liu X. The effect of marital status on the survival of patients with colorectal neuroendocrine neoplasms: an analysis of the SEER database. *Rev Esp Enferm Dig* 2020; **112**: 109-117 [PMID: 31830797 DOI: 10.17235/reed.2019.6183/2019]

20 **Dong J**, Dai Q, Zhang F. The effect of marital status on endometrial cancer-related diagnosis and prognosis: a Surveillance Epidemiology and End Results database analysis. *Future Oncol* 2019; **15**: 3963-3976 [PMID: 31746633 DOI: 10.2217/fon-2019-0241]

21 **Hinyard L**, Wirth LS, Clancy JM, Schwartz T. The effect of marital status on breast cancer-related outcomes in women under 65: A SEER database analysis. *Breast* 2017; **32**: 13-17 [PMID: 28012410 DOI: 10.1016/j.breast.2016.12.008]

22 **Xie JC**, Yang S, Liu XY, Zhao YX. Effect of marital status on survival in glioblastoma multiforme by demographics, education, economic factors, and insurance status. *Cancer Med* 2018; **7**: 3722-3742 [PMID: 30009575 DOI: 10.1002/cam4.1688]

23 **Liang Y**, Wu X, Lu C, Xiao F. Impact of marital status on the prognosis of liver cancer patients without surgery and the critical window. *Ann Palliat Med* 2021; **10**: 2990-2999 [PMID: 33615813 DOI: 10.21037/apm-20-1885]

24 **Feng Y**, Dai W, Li Y, Mo S, Li Q, Cai S. The effect of marital status by age on patients with colorectal cancer over the past decades: a SEER-based analysis. *Int J Colorectal Dis* 2018; **33**: 1001-1010 [PMID: 29546559 DOI: 10.1007/s00384-018-3017-7]

25 **Desantis V**, Saltarella I, Lamanuzzi A, Melaccio A, Solimando AG, Mariggiò MA, Racanelli V, Paradiso A, Vacca A, Frassanito MA. MicroRNAs-Based Nano-Strategies as New Therapeutic Approach in Multiple Myeloma to Overcome Disease Progression and Drug Resistance. *Int J Mol Sci* 2020; **21** [PMID: 32349317 DOI: 10.3390/ijms21093084]

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