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Immune check-point in glioblastoma multiforme

F. De Felice[a,*], N. Pranno[b], F. Marampon[a], D. Musio[a], M. Salducci[c], A. Polimeni[b,1], V. Tombolini[a,1]

[a] Department of Radiotherapy, Policlinico Umberto I "Sapienza" University of Rome, Rome, Italy
[b] Department of Oral and Maxillo Facial Sciences, Policlinico Umberto I "Sapienza" University of Rome, Italy
[c] Department of Experimental Medicine, Sapienza University of Rome, Rome, Italy

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ABSTRACT

Glioblastoma multiforme (GBM) represents one of the main frequent and aggressive primary brain neoplasms among adults worldwide. Despite a first-line multimodal treatment, including radical surgery and adjuvant radiation therapy with concomitant temozolomide-based chemotherapy, GBM prognosis continues to be unfavourable. During this decade, different research groups have explored immune check-point inhibitors role in order to improve response to therapy and subsequently prolong survival rate. The aim of this review was to analyze published literature to support immune check-point inhibitors use in the management of patients with GBM diagnosis. The hope was to help physicians for better decision-making.

1. Introduction

Glioblastoma multiforme (GBM) is the most frequent primary malignant brain tumor in adults and accounts approximately 1% of all new cancer cases worldwide (Bray et al., 2018). Despite trimodality therapy, including surgery (S), radiation therapy (RT) and temozolomide-based chemotherapy (CHT) has become a standard convention, its prognosis is still extremely poor, with median overall survival (OS) rates smaller than 15 months (National Comprehensive Cancer Network (NCCN), 2018; Stupp et al., 2005; Pirtoli et al., 2009). Because of these discouraging survival data, development of novel treatment strategies is still a priority in GBM management. In the last years, immunotherapy, especially checkpoint inhibitors, achieved exciting results in different human cancers, like melanoma skin cancer, non-small cell lung cancer, head and neck cancer and gynecological tumors (Hodi et al., 2010; Kazandjian et al., 2016; Ferris et al., 2016; De Felice et al., 2015, 2018).

Here, we reviewed the immune checkpoint mechanisms and emerging clinical evidence for immune checkpoint inhibitors in GBM. We briefly described specific properties of immune system in central nervous system (CNS). The aim was to provide some directions for future researches.

2. Literature search

Papers were identified in three different electronic databases (PubMed, Scopus and ClinicalTrials.gov), as well as using hand searching (both review articles and meeting proceedings, mainly of European Society for Medical Oncology –ESMO–, Society for NeuroOncology –SNO– and World Federation of NeuroOncology Societies –WFNOS–).We conducted a Boolean search strategy using the following combinations of terms: "glioblastoma multiforme", "glioblastoma", "brain tumor", "temozolomide", "immunotherapy", "immune", "checkpoint", "inhibitors", "PD-1", "PD-L1", "CTLA-4", "anti-", "ipilimumab", "nivolumab", "durvalumab", "pembrolizumab", "microenvironment". Search strategy was performed up to October 2018. Only publications written in English were included. Titles and abstracts of literature search results were checked to verify suitability for the document. Reference lists of selected studies and review papers were manually searched for additional relevant publications. Abstract from international meetings were selected only if with proper and adequately powered statistical results.

3. Immune system in central nervous system

CNS is an immunologically privileged site, mainly because of blood brain barrier (BBB) that prevents free diffusion of both molecules and cells and assures a stable microenvironment in the neural parenchyma (Rascher et al., 2002). Recently, the traditional assumption about the absence of lymphatic drainage system has been withdrawn, confirming several historic observations (Louveau et al., 2015; Mascagni, 1787).

* Corresponding author at: Department of Radiotherapy, Policlinico Umberto I "Sapienza" University of Rome, Viale Regina Elena 326, 00161 Rome, Italy.
E-mail address: fradefelice@hotmail.it (F. De Felice).
[1] These authors contributed equally to this work.

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Nowadays it is accepted that functional lymphatic vessels are present in the dural sinuses between the brain surface and the skull. These vessels: i) are directly connected to the deep lymph nodes located in the neck region by Virchow-Robin spaces progressing in the olfactory nerves, passing the cribroid plate and nasal submucosa (Goldmann et al., 2006); ii) possess unique characteristics, such as limited expansion secondary to high cerebrospinal fluid (CSF) pressure (Louveau et al., 2015). Consequently, brain lymphatic vasculature provide an important pathway in both fluid and immune cells circulation from CSF to systemic lymph system, suggesting a role in antigen presentation and immune surveillance of the CNS.

Basically, human immune system consists of two types of cells: i) the innate immunity, includes thenatural killer (NK) cells, the dendritic cells, the macrophages and the neutrophils; ii) the adaptive immunity comprises bothB cells and T cells, such as the cytotoxic cells (CD8 + T or CTL), the helper cells (CD4 + CTh), the regulatory cells (CD4 + Tregs) and the NK T cells (De Felice et al., 2015). Cancer immunoediting is divided in three mainly phases: the elimination phase, the equilibrium phase and the escape phase. During the first phase (elimination), the cells of the innate immunity secrete a plethora of cytokines able to recruit immune cells and therefore the adaptive immune system start to process the “non-self” cells. The aim is to promote tumor cells death. But malignant cells are capable of expressing tumor antigens in order to elude host immune system. These residual tumor cells come into the equilibrium phase, until they start to grow in an active and uncontrolled manner acquiring resistance to immune system and entering into the escape phase (De Felice et al., 2017; Sengupta et al., 2010).

4. Rationale for checkpoint inhibitors in glioblastoma multiforme

GBM knowledge has advanced with a superior comprehension of the tumor microenvironment. Generally, the immuno-tolerance is primarily assured by co-stimulatory pathway, such as the programmed death-1 receptor (PD-1) and its ligand (PD-L) and the cytotoxic T lymphocyte associated antigen 4 (CTLA-4). The main action of PD-1/ PDL pathway is to inhibit the activation and the proliferation of T cells, as well as arrest the production of cytokines. Whereas, the CTLA-4 path causes cell-cycle arrest and apoptosis, in both Tregs and activated T cells (De Felice et al., 2015, 2017).

The immune microenvironment of GBM is distinctively “cold”, due to its capability to decrease major histocompatibility complex (MHC) class II expression, up-regulate checkpoint molecules such as programmed death ligand-1 (PDL-1) and indoleamine 2,3-dioxygenase (IDO), and enroll suppressor immune cells like Tregs that constitutively express CTLA-4 (See et al., 2012). GBM cells adjust the immune system in order to increase their malignant capacity. On the other hand, in GBM tumors, the BBB integrity is altered due to endothelial tight junctions damage reflecting molecular composition changes (Rascher et al., 2002). The BBB breakdown facilitates CD8 + T cells migration to the CNS, as well as innate and adaptive immune responses activation, producing cytokines and chemokines for lymphocyte recruitment and up-regulation of immunomodulatory markers on cells surface (Yang et al., 2010). In the meantime, the exposure to ionizing radiations mainly produces i) a direct and indirect damage to tumor cells causing cell deaths, ii) an alteration of the tumor stromal microenvironment and iii) an activation of CD8 + T cells. Radiation induces activation of sequential biological mechanisms and biochemical events, including stimulator of interferon genes (STING) pathway and up-regulation of transforming growth factor β (TGF-β) signaling, leading to trigger immune responses (Wang et al., 2018).

The target of immune check-point inhibitors characteristically consists of these inhibitory pathways. Therefore stimulatory signals and/or inhibitory signals using agonist and/or antagonist antibodies can be useful to adequately block every single immune check-point (Pardoll, 2012).

5. Checkpoint inhibitor drugs

CTLA-4 and PD-1 pathways operate at distinct stages of an immune response, resulting in negative effects on T-cell activity. CTLA-4 pathway acts at the early stage of T cell activation binding CD28 molecules with B7-1 (CD80) and/or B7-2 (CD86) molecules on the surface of an antigen-presenting cell (APC), in lymph nodes. Whereas PD-1 pathway blocks T cells at the last part of the immune response binding to PD-L1 and programmed death ligand 2 (PD-L2), in peripheral tissues (Buchbinder and Desai, 2016). The blockage of these two pathways resulted in Food and Drug Administration (FDA) approval of an anti-CTLA-4 (ipilimumab) and two anti-PD-1 drugs (pembrolizumab () and nivolumab) in different cancer therapy, including melanoma skin cancer, non-small cell lung cancerand head and neck cancer (Hodi et al., 2010; Kazandjian et al., 2016; Ferris et al., 2016). Toxicity profile of checkpoint inhibitors is associated with potential immunologic etiology secondary to the increased immune activity. The immune-mediated effects mainly include pruritus, dematologic events, gastrointestinal disorders, endocrinopathies, hepatic events, fatigue and injection-site reactions (De Felice et al., 2018). Generally, anti-PD-1 drugs are well tolerated and have a favorable adverse event profile rather than anti-CTLA-4. Preliminary data suggested that a greater toxicity, in term of both frequency and severity rates, could be related to a better therapeutic response (Postow et al., 2015). Additional results are needed to determine whether this hypothesis is validated. Despite a different spectrum of adverse events, a safety and effective management of checkpoint inhibitors toxicity is mainly based on early sign and symptom recognition and primarily includes corticosteroids with delay in checkpoint inhibitor administration till symptoms return to baseline (De Felice et al., 2018). Corticosteroids therapy is recommended in case of mild to severe suspected pulmonary, renal, gastrointestinal and neurological toxicity, as well as severe hepatotoxicity. In case of symptomatic endocrinopathy it should be also necessary to initiate an appropriate hormone therapy. Topic steroids should be prescribed in skin toxicity and in case of severe toxicity they should be switched to iv or oral formulation. A regular monitoring is suggested and patients should be educate to advise and report any change.

Concerning treatment response, immunotherapy can manifest atypical patterns of response compared to systemic chemotherapy. The pseudoprogression phenomena has been described with the use of immune checkpoint inhibitors in different solid tumors (Kurra et al., 2016). It is defined as progression from nadir followed by response from peak (Kurra et al., 2016). Pseudoprogression is related to the immune cells infiltration into tumor site and due to the time required to determine an adaptive immune response it usually become manifest several weeks after treatment. To better evaluate and define immunotherapy response, the response evaluation criteria for solid tumor (RECIST) working group developed a new consensus guidelines for immune-based therapeutics (iRECIST) (Seymour et al., 2017). The iRECIST defined five type of immune response: i) complete response (iCR): total remission of all target and non-target lesions, including the lack of appearance of new lesions; ii) partial response (iPR): a decrease of at least 30% in the sum of diameters of target lesions compared to baseline sum diameters; iii) stable disease (iSD): Neither sufficient shrinkage to qualify for iPR nor sufficient increase to qualify for iCPD; iiii) unconfirmed progressive disease (iUPD): increase the sum of diameters of target lesions of at least 20% compared to nadir; further confirmation at imaging is needed to rule out pseudoprogression; iiiii)

Table 1
Immune checkpoint inhibitors pre-clinical studies in glioblastoma multiforme.

Author	Model mouse	Treatment	Results
Fecci et al. (2007)	spontaneous murine astrocytoma cell line 560 (SMA-560) in VM/Dk mice	anti-CTLA-4 (9H10)	MS: 26 days; 80% long-term (> 100 days) survivors
Kim et al. (2017)	C57BL/6 J wild-type female mice	Treatment arms: (1) control; (2) SRS; (3) anti-PD-1 antibody;(4) anti-TIM-3 antibody; (5) anti-PD-1 + SRS; (6) anti- TIM-3 + SRS; (7) anti-PD-1 + anti-TIM-3; (8) anti-PD- 1 + anti-TIM-3 + SRS	Control group: MS 22 days; OS 0% Anti-TIM-3: MS 20.5 days; OS 0% SRS: MS 27; OS ≃ 10% Anti-PD-1: MS 33 days; OS 27.8% Anti-TIM-3 + SRS: MS 100 days; OS ≃ 50%. Anti-TIM-3 + Anti-PD-1: MS 100 days; OS 57.9% Anti-TIM-3 + Anti-PD-1 + SRS: OS 100%
Reardon et al. (2016a)	Mouse anti-mouse mAbs with specific gene-deficient mice: PD-1 – 332.8H3 (mouse IgG1, K); PD-L1 – 339.6A2 (mouse IgG1, K),PD-L2 – 3.2 (mouse IgG1, K). Mouse anti-mouse CTLA-4 – 9D9 (mouse IgG2b, K) purchased from BioXCell	Therapeutic arms: (1) Isotype murine IgG (control animals); (2) anti–PD-1; (3) anti–PD-L1; (4) anti–PD-L2; (5) anti–CTLA-4; (6) Combination therapy	Isotype control: MS 27 days; LTS° 0%. Anti-CTLA-4: MS 36.5 days; LTS° 16.6% Anti–PD-1: MS 96.5 days; LTS° 50% Anti–PD-L1: MS 32 days; LTS° 20.8% Anti–PD-L2: MS 27.5 days LTS° 0% Anti-CTLA-4 + Anti–PD-1: MS > 146 days ; LTS° 75% Anti-CTLA-4 + Anti–PD-L1: MS 30 days; LTS° 37.5% Anti–PD-1 + Anti–PD-L1: MS 33.5 days; LTS° 37.5% Anti–PD-L1 + Anti–PD-L2: MS 35.5 days; LTS° 12.5%
Wainwright et al. (2014)	C57BL/6 (wild-type; Cat# 000664), IDO / (Cat# 005897), Rag1 / (Cat# 002216), and OT-II (Cat# 004194) mice	Therapeutic arms: (1) 1-methyl-D-tryptophan (D1-MT); (2) 1-methyl-L-tryptophan (L1-MT); (3) Temozolomide; (4) anti-CTLA-4; (5) anti-PD-L1;	Untreated mice (control): MS 24.5 days Temozolomide alone: MS 37.5 days D1-MT + Temozolomide: MS 46 days L1-MT + Temozolomide: 35 days Anti-CTLA-4: LTS* 40% Anti-PD-L1: LTS* 60% Anti-CTLA-4 + anti-PD-L1: LTS* 90% Anti-CTLA-4 + anti-PD-L1 and Anti-CTLA-4 + anti-PD-L1 + 1-MT (2 weeks post intracranial injection): LTS* 78%
Zeng et al. (2013)	Female C57BL/6 J mice (Harlan)	Therapeutic arms: (1) untreated control; (2) Radiation; (3) anti-PD-1; (4) Radiation + anti-PD-1 antibody	Untreated control: MS 26 days; LTS* 0% Radiation; MS 27 days; LTS* 0% Anti-PD-1: MS 30 days; LTS* 0% Radiation + anti-PD-1 antibody: MS 52 days; LTS* 15-40%

MS: median survival; OS: overall survival; LTS: long-term survival (defined as > 100 days° or > 90 days* after implantation).

confirmed progressive disease (iCPD): increase in the sum of diameters of target lesions of at least 20% confirmed in the next assessment (4–8 weeks later). Generally immunotherapy response assessment should be performed every 6–12 weeks using same imaging techniques.

6. Clinical trial results

GBM is one of the biggest unsolved oncologic problem in the modern era. Based on remarkable results in metastatic melanoma skin cancer and non-small cell lung cancer, a growing interest on immune checkpoint has been raised in GBM treatment in order to improve survival outcomes. Currently, definitive clinical data is limited, but preliminary results are promising. We summarized trials characteristics, including study identifier, phase, patient population, sample size, treatment, primary outcome and clinical outcomes, if available, in Table 1 (preclinical studies) and Table 2 (clinical studies).

6.1. Preclinical studies

Preclinical studies in orthotopic glioma murine models showing improved survival with IDO, CTLA-4 and PD-1 pathway blockade, as well as combination of anti-PD-1 plus RT, provide the strong rationale for immune checkpoint inhibition in patient with GBM (Wainwright et al., 2014; Zeng et al., 2013; Kim et al., 2017; Fecci et al., 2007; Reardon et al., 2016a). Details are shown in Table 1.

In addition, several lines of evidence have reported a RT-related bystander effect to doses below 1 Gy. Bystander effect describes the biological damage to unirradiated cells in direct contact with or mediated by stress signals secrete from neighbouring irradiated cells. Different bystander signaling pathways have been found in brain cancer cell models, including p53 activation, tumor necrosis factor- (TNF-) alpha, TNF-beta1 and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) up-regulation and phosphoinositide 3-kinase (PI3K) inhibition (Fernandez-Palomo et al., 2016; Shao et al., 2008). However, to improve GBM treatment, further researches are needed to better define the detailed relationship between radiation dose and bystander effect.

6.2. Clinical trials

Table 2 summarized data of clinical trials (clinicaltrials.gov, 2019a,b; clinicaltrials.gov, 2019c,d; Sahebjam et al., 2017; clinicaltrials.gov, 2019e,f,g,h,i,j,k,l,m,n,o,p,q,r,s,t,u,v,w,x,y,z,aa,ab,ac,ad,ae,af,ag,ah). The main results published or available in meeting abstract form were also extensively discussed below.

The CheckMate 143 study (NCT02017717) is a randomized phase III clinical trial powered to evaluate the OS of nivolumab administered as single agent compared to bevacizumab in recurrent GBM patients (clinicaltrials.gov, 2019a). Actually study design also includes multiple phase I steps aimed to estimate both safety and tolerability of nivolumab gave as single agent or in association with ipilimumab in patients with different lines of GBM therapy (clinicaltrials.gov, 2019a). Phase III trial results were reported in April of 2017 at the WFNOS conference (Reardon et al., 2017a). Patients were randomly assigned (1:1) to receive nivolumab 3 mg/kg intravenously (iv) once every two weeks (n = 184) or bevacizumab 10 mg/kg iv once every 2 weeks (n = 185) until disease progression or undesirable toxicity. At the time of final analyses (Jan 20, 2017), 182 received nivolumab and 165 received bevacizumab (Reardon et al., 2017a). Globally, nivolumab did not show a superior survival rate against bevacizumab and the study did not reach its primary endpoint. Median OS was 9.8 months (95% CI from8.2–11.8 months) with nivolumab and 10.0 months (95% CI from 9.0 to11.8 months) with bevacizumab. The OS rate at one year was 42% in both arms. The median progression-free survival rate was 1.5 months and 3.5 months, respectively. Overall response rate was lower in nivolumab cohort (8% versus 23%), but duration of response was higher compared to bevacizumab treatment (11.1 months versus 5.3 months). Treatment-related toxicity was recorded in 57% (nivolumab) and 58% (bevacizumab) of patients, with severe toxicity rates of 18% and 15% respectively. Of note, nivolumab patients required higher doses of dexamethasone to manage toxicity profile than bevacizumab patients (Simonelli et al., 2018).

Phase I trial results were presented at ESMO in September 2017 (Lim et al., 2017). Exploratory cohorts of CheckMate 143 assessed safety and tolerability of nivolumab concomitant to RT with or without

Table 2
Immune checkpoint inhibitors clinical trials in glioblastoma multiforme.

Trial	Phase	Patient population	N planned	Treatment	Primary outcome	Results	Status	Estimated study completion date
NCT03422094 (clinicaltrials.gov, 2019e)	I	Newly diagnosed histologically confirmed unmethylated GBM. Patients with secondary GBM will not be excluded.	E 30	Cohort A: NeoVax + Nivolumab (start at time of progression)Cohort B: NeoVax + Nivolumab (start with Cycle 2)Cohort C: NeoVax + Nivolumab (start with Cycle 1)Cohort D: NeoVax + Ipilimumab + Nivolumab (start with Cycle 3)Cohort E: NeoVax + Ipilimumab + Nivolumab (day 1&15 each cycle)	1) ≤ 33% DLT rate 2) Feasibility of generating a personalized neoantigen peptide vaccine as measured by the ability to identify candidate tumor specific neoantigens.3) Feasibility of generating a personalized neoantigen peptide vaccine as measured by the the ability to manufacture a neoantigen-based synthetic long peptide vaccine. 4) Feasibility of generating a personalized neoantigen peptide vaccine as measured by the ability to administer the vaccine to a patient at 4 weeks post-completion of RT.		Active, not recruiting	October 2023
NCT03576612 (clinicaltrials.gov, 2019f)	I	Operable high grade glioma based on clinical and radiologic evaluation.	E 36	Cohort 1 (MGMT Unmethylated) AdV-tk injection + Valacyclovir + RT + TMZ + Nivolumab Cohort 2 (MGMT Methylated & undetermined) AdV-tk injection + Valacyclovir + RT + TMZ + Nivolumab	MTD of AdV-tk injection + Valacyclovir + RT + TMZ + Nivolumab.		Active, not recruiting	January 2022
NCT03493932 (clinicaltrials.gov, 2019g)	I	Solitary recurrent GBM amenable to surgical resection.	E 15	Experimental: Nivolumab + BMS-986016	1) Proportion of patients who have a measurable increase of interferon gamma levels in the brain tumor tissue after their first dose of Nivolumab as compared to the pre-treatment baseline. 2) Safety of using brain tumor microdialysis to monitor response to immune modulators. 3) Safety of Nivolumab + BMS-986016.		Recruiting	June 2021
NCT02617589 (clinicaltrials.gov, 2019b)	III	1) Newly-diagnosed of GBM2) Tumor test result shows MGMT unmethylated type	E 550	Experimental: Nivolumab + RT Comparator: TMZ + RT	OS		Recruiting	October 2019
NCT02667587 (clinicaltrials.gov, 2019c)	III	1) Newly-diagnosed of GBM2) Tumor test result shows MGMT methylated or indeterminate tumor subtype	E 693	Experimental: Nivolumab + TMZ + RTPlacebo Comparator: Nivolumab placebo + TMZ + RT	OS		Recruiting	August 2023
NCT02017717 (clinicaltrials.gov, 2019a)	III	1) Histologically confirmed Grade IV malignant glioma2) Previous treatment with RT and TMZ (Cohorts 1, 1b and 2 only)3) First recurrence of GBM (Cohorts 1, 1b and 2 only)4) First diagnosis of GBM with resectable disease (Cohorts 1c Part A only)5) First diagnosis of unmethylated MGMT GBM (Cohort 1d and Cohort 1c Part B only)	A 626	Experimental (Cohort 1, 1c, 1d, 2): Nivolumab Experimental (Cohort 1, 1b): Nivolumab + Ipilimumab Comparator (Cohort 2): Bevacizumab	1) Cohorts 1,1b, 1c and 1d : Safety and tolerability 2) Cohort 2: OS-12.	Better tolerability Nivolumab alone vs Nivolumab + Ipilimumab OS-12: 40% vs 32.5%	Active, not recruiting	October 2018
	II		A 159		OS-12 PFS-6PFS-6 OS-6			

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Table 2 (*continued*)

Trial	Phase	Patient population	N planned	Treatment	Primary outcome	Results	Status	Estimated study completion date
NCT02336165 (clinicaltrials.gov, 2019d)		Newly diagnosed or recurrent GBM		Durvalumab + RT (cohort A: unmethylated MGMT GBM)Durvalumab (cohort B: recurrent GBM)Durvalumab + bevacizumab (cohort B2-B3: bevacizumab-naive subjects with recurrent GBM) Durvalumab + bevacizumab (cohort C: bevacizumab-refractory recurrent GBM)		/PFS-6 20%;OS-6 59%; OS-12 44.4%/OS > 21 w in 36%; PFS > 7 w in 50%	Active, not recruiting	December 2019
NCT02054806 (clinicaltrials.gov, 2019h)	I	Histologically or cytologically documented locally-advanced and/or metastatic solid malignancy failed prior standard therapy or for which standard therapy is not appropriate	A 477	Experimental: Pembrolizumab	Best ORR	mOS 14.4 m; mPFS 2.8 m; severe toxicity 15.4%	Active, not recruiting	August 2019
NCT02313272 (Sahebjam et al., 2017)	I	Histologically confirmed diagnosis Grade III or IV malignant glioma.	A 32	Experimental: HFSRT with Pembrolizumab and Bevacizumab	MTD	OS-12 64%	Active, not recruiting	Apil 2020
NCT02337491 (clinicaltrials.gov, 2019i)	II	Histologically confirmed Grade IV malignant glioma	A 80	Experimental: Cohort A Safety Lead-In: Pembrolizumab (DL 0) + Bevacizumab. Experimental: Cohort A: Pembrolizumab + Bevacizumab. Experimental: Cohort B: Pembrolizumab.	1) MTD of Pembrolizumab 2) DLT of Pembrolizumab 3) PFS-6	1) MTD = 200 mg every 3 weeks2) DLT = 0 Participants 3) PFS-6 = Cohort A 26% (14.6 - 40.3); Cohort B 6.7% (0.9 - 22.1)	Active, not recruiting	December 2018
NCT02829931 (clinicaltrials.gov, 2019j)	I	Histologically confirmed diagnosis Grade III or IV malignant glioma.	E 26	Experimental: HFSRT + Ipilimumab + Nivolumab + Bevacizumab	Treatment-related adverse events		Recruiting	April 2021
NCT02529072 (clinicaltrials.gov, 2019k)	I	First or second recurrence of Grade III or IV glioma or astrocytoma	A 7	Group 1: nivolumab every 2 weeks for 8 weeks followed by surgery. Following resection, nivolumab and DC vaccine will be administered every 2 weeks (± 1) for a total of 3 vaccines, followed by biweekly treatment with nivolumab and monthly DC vaccinations for a total of 5 more vaccines. Group 2: nivolumab and DC vaccine every 2 weeks for a total of 3 vaccines, and then surgery. Subsequent to surgery, the patient will resume biweekly treatment with nivolumab and monthly DC vaccinations for a total of 5 more vaccines.	Unacceptable toxicity		Active, not recruiting	September 2018
NCT02798406 (clinicaltrials.gov, 2019l)	II	Single GBM or gliosarcoma tumor with histopathological confirmation for first or presenting second recurrence.	E 48	Experimental: DNX-2401 + pembrolizumab	ORR		Recruiting	June 2020
NCT03058289 (clinicaltrials.gov, 2019m)	I/II	Refractory cancers	E 60	Experimental (Cohort A superficial tumors): INT230-6 low starting dose, low concentration per tumor. Experimental (Cohort B1 superficial or deep tumors): INT230-6 low starting dose, low concentration per tumor. Experimental (Cohort B2 superficial or deep tumors): INT230-6 medium starting dose, low drug concentration per tumor. Experimental (Cohort B3 superficial or deep tumors): INT230-6 high starting	Safety and tolerability of single and multiple intratumoral doses of INT230-6		Recruiting	August 2020

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Table 2 (*continued*)

Trial	Phase	Patient population	N planned	Treatment	Primary outcome	Results	Status	Estimated study completion date
				dose, low drug concentration per tumor. Experimental (Cohort C1 superficial or deep tumors): INT230-6 low starting dose, high drug concentration per tumor. Experimental (Cohort C2 superficial or deep tumors): INT230-6 medium starting dose, high drug concentration per tumor. Experimental (Cohort C3 superficial or deep tumors): INT230-6 high starting dose, high drug concentration per tumor. Experimental (Cohort D and E superficial or deep tumors): INT230-6 + anti-PD-1 antibodies.			
NCT02335918 (clinicaltrials.gov, 2019n)	I/II	Histologically-diagnosed advanced Non-small Cell Lung Cancer, Melanoma, Colorectal, Head and Neck SCC, Ovarian Cancer, GBM or Renal Cell Carcinoma.	A 175	Experimental: Varlilumab + Nivolumab	1) (Phese I) treatment-related adverse events, DLT, laboratory abnormalities. 2) (Phese II) OS-12		Active, not recruiting	December 2018
NCT02852655 (clinicaltrials.gov, 2019o)	NA	Histologically confirmed Grade IV malignant glioma	A 35	Experimental: Pre-surgery MK-3475 Comparator: No MK-3475 at Pre-Surgery	1) Tumor Infiltrating T Lymphocyte Density 2) Treatment-emergent adverse events		Active, not recruiting	August 2021
NCT02658981 (clinicaltrials.gov, 2019p)	I	Histologically proven GBM or gliosarcoma (progressive or recurrent following RT and TMZ)	E 100	Experimental: (A1) Anti-LAG-3 Experimental: (A2) Anti-CD137 (Urelumab) Experimental: (B1) Anti-LAG3 + Anti-PD-1 (nivolumab) Experimental: (B2) Anti-CD137 + Anti-PD-1 Experimental: (Intratumoral Studies) Patients pre-operatively receive drug from one of the 4 arms	1) MTD of Anti-LAG-3 2) MTD of Urelumab 3) MTD of nivolumab 4) TDM of Anti-CD137 + Anti-PD-1		Recruiting	December 2020
NCT02526017 (clinicaltrials.gov, 2019q)	I	GBM	E 295	Experimental: FPA008 (dose escaletion) Experimental: FPA008 + BMS-936558 (dose escaletion) Experimental: FPA008 + BMS-936558 (dose expansion)	1) Grade ≥ 3 adverse events, DTL, ECG abnormalities 2) Treatment discontinuations, modifications and interruptions 3) ORR, CR, PR		Active, not recruiting	March 2020
NCT03233152 (clinicaltrials.gov, 2019r)	I	Newly diagnosed or recurrent grade IV glioma	E 6	Experimental: ipilimumab + nivolumab	1) PFS 2) OS		Recruiting	November 2019
NCT02968940 (clinicaltrials.gov, 2019s)	II	1) Documentation of IDH1 or IDH2 mutation in any tumor specimen. 2) Grade II or III glioma prior to treatment with TMZ or PCV chemotherapy. 3) Grade IV glioma after treatment with TMZ or PCV chemotherapy	E 43	Experimental: Avelumab + HFRT	1) Adverse events 2) PFS6		Recruiting	February 2020
NCT02327078 (clinicaltrials.gov, 2019t)	I/II	Histologically or cytologically Non-small Cell Lung Cancer, Melanoma, Colorectal, Head and Neck SCC, Ovarian Cancer, recurrent B cell non-Hodgkin Lymphoma or	A 309	Experimental: (Phase 1, Part 1) : Nivolumab + Epacadostat Experimental: (Phase 2): Nivolumab + Epacadostat Experimental: (Phase 1, Part 2): Nivolumab + Epacadostat + Chemotherapy	1) Phase 1, Part 1 DLTs of Nivolumab + Epacadostat 2) Phase 1, Part 2 DLTs of Nivolumab + Epacadostat + Chemotherapy 3) Phase 1, Part 1 and 2 frequency of adverse events, serious adverse events, deaths 4) Phase 2, ORR 5) Phase 2, PFS 6) Phase 2, OS		Active, not recruiting	October 2020

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Table 2 (*continued*)

Trial	Phase	Patient population	N planned	Treatment	Primary outcome	Results	Status	Estimated study completion date
		Hodgkin Lymphoma, or GBM						
NCT02794883 (clinicaltrials.gov, 2019u)	II	Grade III or IV glioma progressed after standard RT and TMZ	E 36	Experimental: Tremelimumab Comparator: Durvalumab Comparator: Tremelimumab + Durvalumab	T-cell (immunologic) changes in blood		Recruiting	June 2020
NCT02311582 (clinicaltrials.gov, 2019v)	I/II	Phase I: Histologically confirmed grade III or IV malignant glioma.Phase II: Histologically confirmed grade IV malignant glioma	E 58	Experimental: Phase I: MK-3475 + MLA Experimental: Phase II: MK-3475 Only (Arm B) Experimental: Phase II: MK-3475 + MLA (Arm A)	1)Phase 1, MTD of MK-3475 + MLA 2) Phase 2, PFS of MK-3475 alone vs. MK-3475 + MLA		Recruiting	June 2021
NCT02937844 (clinicaltrials.gov, 2019w)	I	Recurrent GBM	E 20	Experimental: Anti-PD-L1 CSR T cells	Adverse events related to CSR T cell infusion		Recruiting	July 2019
NCT02866747 (clinicaltrials.gov, 2019x)	I/II	Histopathologic confirmation of GBM	E 62	Experimental: RT + Durvalumab Comparator: RT	1) Phase 1, DLT incidence 2) Phase 2, Rate of patient without local progression of the irradiated tumor		Recruiting	July 2020
NCT03014804 (clinicaltrials.gov, 2019y)	II	Original diagnosis of GBM	E 30	Experimental: DCVax-L Eperimental: DCVax-L + nivolumab	1) Adverse events 2) OS		Not yet recruiting	January 2022
NCT02550249 (clinicaltrials.gov, 2019z)	II	GBM candidates to primary or salvage resection surgery	A 29	Experimental: Nivolumab	Changes in percentage and level of expression of PD-L1 by tumor cells and lymphocytes		Completed	March 2017
NCT02530502 (clinicaltrials.gov, 2019aa)	I/II	Histologically confirmed newly diagnosed GBM	E 50	Experimental: RT + TMZ + pembrolizumab	1) Phase 1, DLT of RT + TMZ + pembrolizumab 2) Phase 2, PFS of RT + TMZ + pembrolizumab		Active, not recruiting	November 2020
NCT03174197 (clinicaltrials.gov, 2019ab)	I/II	Histologically confirmed Grade IV glioma	E 60	Experimental (Concurrent Phase Group): TMZ + Atezolizumab + RT Experimental (Adjuvant Phase Group): TMZ + Atezolizumab	1) DLT of Atezolizumab 2) OS of TMZ + Atezolizumab + RT		Recruiting	June 2021
NCT02311920 (clinicaltrials.gov, 2019ac)	I	Histopathologically proven diagnosis of GBM or gliosarcoma	A 32	Experimental: TMZ + ipilimumab Experimental: TMZ + nivolumab Experimental: TMZ + nivolumab + ipilimumab	1) Immune-related DLT for ipilimumab 2) Immune-related DLT for nivolumab 3) Immune-related DLT for ipilimumab + nivolumab		Active, not recruiting	December 2017
NCT03018288 (clinicaltrials.gov, 2019ad)	II	Newly diagnosed GBM that has not been treated	E 108	Experimental: 2/RT + TMZ + Pembrolizumab+HSPPC-96 Experimental: 1/RT + TMZ + Pembrolizumab Placebo Comparator: 3/RT + TMZ + Pembrolizumab + Placebo	OS in newly diagnosed MGMT unmethylated GBM patients treated with RT + TMZ + Pembrolizumab followed by Pembrolizumab + TMZ +/- HSPPC-96		Recruiting	January 2024
NCT03341806 (clinicaltrials.gov, 2019ae)	I	Histologically proven GBM from the initial resection	E 30	Experimental: Part A - Avelumab Part B - Avelumab + MRI-guided LITT therapy	1) DLT level 2) ORR		Recruiting	February 2020
NCT02658279 (clinicaltrials.gov, 2019af)	NA	Histologically confirmed diagnosis of malignant glioma	E 44	Experimental: Pembrolizumab	1) RR		Recruiting	January 2019

(*continued on next page*)

Table 2 (*continued*)

Trial	Phase	Patient population	N planned	Treatment	Primary outcome	Results	Status	Estimated study completion date
NCT02829723 (clinicaltrials.gov, 2019ag)	I/II	Advanced/metastatic solid tumors	E 151	Experimental: BLZ945 Experimental: BLZ945 + PDR001	1) Number of participants with DLTs 2) PFS		Recruiting	June 2019
NCT02423343 (clinicaltrials.gov, 2019ah)	I/II	1) Phase 1b, advanced refractory solid tumors in any line of therapy 2) Phase 2, recurrent or refractory NSCLC, or HCC with AFP ≥ 200 ng/mL	E 75	Experimental: Galunisertib + Nivolumab (Phase 1b) Experimental: Galunisertib + Nivolumab (NSCLC) (Phase 2) Experimental: Galunisertib + Nivolumab (HCC) (Phase 2)	1) Phase 1b, MTD of Galunisertib + Nivolumab		Recruiting	December 2019

N: number; GBM: glioblastoma multiforme; TMZ: temozolomide; NA: not applicable; E: estimated enrollment; A: actual enrollment; DLT: dose-limiting toxicity; OS: overall survival; OS-6: 6-months overall survival; OS-12: 12-months overall survival; PFS: progression-free survival; PFS-6: 6-month progression-free survival; RR: response rate; ORR: overall response rate; RT: radiotherapy; MRI: magnetic resonance imaging; LITT: laser interstitial thermotherapy; AFP: alpha-fetoprotein; mg: milligram; ng: nanogram; mL: milliliter; MTD: maximum tolerated dose.

temozolomide in newly diagnosed GBM patients. Nivolumab with RT with or without temozolomide was well tolerated. Treatment-related toxicity was comparable to that reported in other GBM studies (Lim et al., 2017). At present, the addition of nivolumab to standard first line treatment in newly diagnosed GBM patients is tested in two ongoing trials, the CheckMate 498 (NCT02617589) and CheckMate 548 (NCT02667587) (clinicaltrials.gov, 2019b, c) (see Table 2).

The ongoing phase II, multicenter, open-label trial (NCT02336165) is designed to assess the clinical efficacy and safety of durvalumab, a PD-L1 inhibitor, in 3 patient population: i) newly diagnosed unmethylated O6-methylguanine-DNA methyltransferase GBM patients submitted to RT, ii) bevacizumab-naïve recurrent GBM patients, iii) recurrent GBM patients that received prior bevacizumab therapy (clinicaltrials.gov, 2019d). An update to the interim analysis of study reveled a durable activity of durvalumab in the subset of bevacizumab-naïve recurrent GBM patients (Reardon et al., 2017b). Durvalumab (10 mg/kg every two weeks up to 12 months) as monotherapy was prescribed to these patients. The primary endpoint was the 6-month progression free survival (PFS-6). As of 16 December 2016, 30 patients were evaluable and the PFS-6 rate was 20.0% (90% CI: 9.7, 33.0) (Reardon et al., 2017b).

Recently preliminary results of the phase Ib KeyNote-028 multicohort study of pembrolizumab alone in GBM patients with PD-L1-positive recurrent disease have been published (Reardon et al., 2016b). Data of the bevacizumab-naive GBM cohort, including 26 patients, showed a manageable safety profile of pembrolizumab (10 mg/kg every two weeks for up to 24 months) monotherapy. There was 1 partial response and an additional 12 patients presented stable disease, with a median period of stability of 39.4 weeks.

Interestingly, preliminary results of a phase I study of hypofractionated stereotactic RT plus concomitant pembrolizumab and bevacizumab in recurrent GBM or anaplastic astrocytoma (NCT02313272) cases demonstrated an tolerable toxicity profile and promising anti-tumor effect (Sahebjam et al., 2017). Patients received hypofractionated stereotactic RT to the GBM recurrence (30 Gy; 6 Gy/fraction) in association with bevacizumab (10 mg/kg, iv every 2 weeks) and pembrolizumab (100 mg or 200 mg iv based on dose level, every 3 weeks). There were neither dose limiting toxicities nor treatment-related neurologic side effects.

This data suggested a synergistic effect, maybe based on RT-induced cancer antigens presentation.

In fact, pembrolizumab, an anti-PD-1 monoclonal antibody, binds to the PD-1 receptor expressed on activated T-cells and blocks its interaction with both PD-L1 and PD-L2 ligands. Mechanistically, giving RT before checkpoint blockade might result in a PD-1 expression increase on T cells, promoting a superior pembrolizumab efficacy. Thus, probably, the RT / immune checkpoint inhibitors sequencing is important to improve clinical outcomes. Probably, depending on the mechanism of action of the single immunotherapy drug utilized, the immune checkpoint inhibitor should be administered before, concurrently or after RT (Crittenden, 2016).

Globally, these trials highlighted the potential use and tolerable toxicity profile of immune checkpoint inhibitors for GBM patients, both in first-line standard treatment or metastatic/recurrent setting. Considering that optimal dose and sequence timing have yet to be well defined, as well as survival benefit still be proven, definitive conclusion cannot be drawn. Identification of biomarkers to immune checkpoint inhibitors could represent a right perspective in GBM patients. At present very few data are available in literature (Passiglia et al., 2018; Zhao et al., 2019). Preliminary experiences indicated that PTEN mutations, mismatch repair deficiency, mutations in the polymerase Ɛ gene (POLE) and MSH6 mutation, but tumor-infiltrating T lymphocytes (TILs), could have a potential role in GBM scenario.

7. Conclusion

Given this immune checkpoints scenario mainly based on manageable safety profile, differences in timing, target and effects suggest that anti-CTLA-4 and anti-PD-1 have the potential for additive and/or synergistic effect in GBM treatment. To produce a robust survival benefit, a well-designed combinatorial clinical trial should be proposed. Attention should be paid to immunosuppressive steroids that otherwise should negatively interfere with immune checkpoints activity, as probably has occurred in CheckMate 143 trial. At present, the association of immune checkpoint inhibitors with RT seems to be the most interestingly strategy in GBM management. RT contributes to BBB damage, as well as phenotypic changes in glioma cells, improving immune checkpoints efficacy. Although the clinical potential of immune checkpoint inhibitors is clear, definitive results and further methodologically robust researches are necessary to confirm preliminary data and define the development of more effective treatment strategy in GBM.

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Conflicts of interest

The authors declare no conflict of interest.

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Dr Francesca De Felice now is a researcher at the Department of Radiological Sciences, Oncology, and Pathology, “Sapienza” University of Rome. She was specialized in Radiation Oncology at the “Sapienza” University of Rome in 2012 and got her PhD at the Department of Radiological Sciences, Oncology, and Pathology, “Sapienza” University of Rome. She is author of several indexed papers, all in the field of clinical and experimental oncology.