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PD-1/PD-L1 immune-checkpoint inhibitors in glioblastoma: A concise review

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ABSTRACT

Glioblastoma is the most aggressive and most common primary brain tumor in adults, with a very poor prognosis, due to limited therapeutic efficacy of available treatments. The promising data deriving from the use of immune checkpoint inhibitors (ICI) in other cancers have prompted evaluation of its efficacy and possible use in patients with glioblastoma. In this review, we analyzed the available data about these drugs in glioblastoma. Although data are not yet mature and preliminary studies do not show a clear-cut benefit, we are far from excluding the concrete possibility of using ICI as potential treatment in patients with glioblastoma. Moreover, many molecular and immunological aspects of this approach have yet to be clarified.

For this reason, it is essential to identify potential predictive biomarkers for the selection of patients who will benefit most from treatment with ICI.

Additional effort is needed to better understand the mechanisms that will allow us to establish whether ICI have a place in the treatment of patients with glioblastoma.

1. Introduction

Glioblastoma (GBM) is the most common and most aggressive brain tumor diagnosed in adults, showing extremely poor prognosis, with less than 10% of 5-year survival rates after standard treatment (Stupp et al., 2005; Stupp et al., 2009). Despite primary management with maximal surgical resection followed by radiation therapy and chemotherapy (Stupp et al., 2005), nearly all GBM have dismal prognosis, with median survival of about 12–14 months (Stupp et al., 2009). Treatment options following the first recurrence are limited and lack efficacy (Weller et al., 2013). Besides further surgical resection when possible, treatment of recurrent GBM, outside a clinical trial includes an anti-vascular endothelial growth factor (VEGF) agent, bevacizumab, and low-intensity alternating electric fields (TTFields).However, neither of these treatments has shown substantial improvement in survival in the populations analyzed (Stupp et al., 2012; Friedman et al., 2009). Post-bevacizumab progression treatment is often the continuation of treatment with bevacizumab with the addition of a cytotoxic agent; this strategy has not shown significant improvements in terms of survival (Quant et al., 2009; Chamberlain and Johnston, 2010). Other possible treatments in this setting of patients (recurrent GBM) include conventional chemotherapy, such as temozolomide (with different dosing schedules) and nitrosoureas (Chamberlain, 2015).Development of effective treatments for GBM (primary or recurrent) is difficult, due to the intracranial localization of the lesion, molecular heterogeneity and the immunosuppressive mechanisms implemented by the tumor. The blood brain barrier (BBB), a semipermeable membrane of endothelial cells connected by tight junctions, prevents passage of most drugs to the tumor site, thus limiting achievement of therapeutic drug concentrations (Miura et al., 2013; Chen and Liu, 2012). In addition, GBM establishes adaptation strategies that impair antitumor immune response and involve expression of immune-suppressive molecules in the tumor microenvironment (Dix et al., 1999; Wintterle et al., 2003). A programmed cell death (PD) pathway represents just one of the mechanisms implemented by the tumor (including GBM) as negative feedback for T-Cells to inhibit the activity of cytotoxic T lymphocytes (CD8 +) within the tumor (Wintterle et al., 2003; Chen and Han, 2015). PD-1 or PD-L1 inhibition can block the interaction between PD-L1 (ligand) and its receptor (PD1) to overcome T cell inhibition and to promote an immune response against the tumor. Immune checkpoint inhibition with monoclonal antibodies against the receptor PD-1 or its ligand, PD-L1, has produced exciting results in the treatment of several cancers, such as metastatic melanoma (Weber et al., 2015; Larkin et al., 2015), NSCLC (Brahmer et al., 2015; Reck et al., 2016), and renal cell carcinoma (Motzer et al., 2015). The activity of immune-checkpoint inhibitors in untreated brain metastases from melanoma and from NSCLC

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has been analyzed and results are encouraging (Goldberg et al., 2016). Based on the success of using immune-checkpoint inhibitors in other cancers and the increasing focus of research to better understand the interactions between tumor and immune system, the use of PD-1 / PD-L1 inhibitors has been proposed as a possible treatment of gliomas, including glioblastoma.

In the various studies conducted (Berghoff et al., 2015; Nduom et al., 2016), the rate of patients with GBM with any PD-L1 expression in tumor cells were heterogeneous with a 61% in Nduom's work and 88% (newly diagnosed GBM) / 72% (recurrent GBM) in Berghoff's work. The differences reported may derive from different methodologies used in the analyzes using different antibodies and different immune staining protocols. Anti-PD-1 blocking antibodies were shown to cause an increase in CD8 T cell to Treg ratio, which is indicative of a successful outcome (Reardon et al., 2016a; Zeng et al., 2013).

In this concise review, we analyze the evidence in the literature regarding immunotherapeutic treatment with immune-checkpoint inhibitors in patients with glioblastoma; we also want to evaluate ongoing clinical trials that involve the use of ICI (monotherapy or in combination with other drugs) in this setting of patients and determine if any predictive efficacy biomarker has been identified that can be used for a better selection of patients.

2. Nivolumab

Nivolumab is a fully human IgG4 subtype programmed death-1 (PD-1) immune checkpoint inhibitor antibody that binds with high affinity to PD-1 receptors on T-cells and blocks their interaction with PD-1 ligand 1 and 2 (PD-L1/PD-L2), restoring T-cell antitumor function (Topalian et al., 2012; Tumeh et al., 2014; Pardoll, 2012; Reardon et al., 2014). Checkmate 143 (NCT 02017717) was the first large-scale, randomized clinical trial that explored the possibility of inhibiting the PD pathway in patients with glioma. Safety and efficacy of Nivolumab in patients with GBM was assessed in this trial; furthermore, this trial included a study of nivolumab monotherapy as compared to bevecizumab in patients with recurrent GBM.

Based on the safety and efficacy observed in melanoma (Curran et al., 2010), Nivolumab was evaluated with or without Ipilimumab (another immune-checkpoint inhibitor targeting CTLA-4) in a phase I safety cohort study (cohorts 1 e 1b), in patients with recurrent GBM. In Cohort 1, 20 pts were randomized 1:1 to receive Nivolumab 3 mg/kg (N3) every 2 weeks (Q2W) or Nivo 1 mg/kg + Ipilimumab 3 mg/kg every 3 weeks (Q3W; N1 + I3) for 4 doses followed by N3 Q2W. Pts in Cohort 1b (n = 20) received Nivolumab 3 mg/kg + Ipilimumab 1 mg/kg Q3W (N3 + I1) for 4 doses followed by N3 Q2W. At last update (Reardon et al., 2016b), stable disease, or better, was achieved in 6/10, 4/10 and 9/20 patients who were treated with N3, N1 + I3, and N3 + I1, respectively. The OSs at 12 months were 40% (95% CI 12–67), 30% (95% CI 7–58) and 25% (95% CI 8–48) in the N3, N1 + I3 and N3 + I1 groups, respectively. Three pts on N1 + I3, 1 pt on N3 + I1, and 0 pts receiving N3 discontinued treatment for treatment-related serious adverse events (TRAEs) with grade 3–4 TRAEs 0% in N3, 90% in N1 + I3 e 25% in N3 + I1.

In the phase III clinical trial (Reardon et al., 2017a), Checkmate 143, in which Nivolumab was compared to Bevacizumab, 369 patients with first recurrence of GBM, previously treated with concomitant radiation and TMZ were enrolled; patients received either Nivolumab 3 mg/kg or Bevacizumab 10 mg/kg every 2 weeks, until disease progression or unacceptable toxicity. The results, presented in April 2017 at the World Federation of Neuro-Oncology Societies (WFNOS) conference, showed, in both treatment groups, a 12-month OS of 42%; the median OS for the Nivolumab arm was 9.8 months as compared to 10 months for the Bevacizumab arm. Median PFS was 1.5 months and 3.5 months for Nivolumab and Bevacizumab, respectively. Overall response rates were 8% for Nivolumab and 23% for Bevacizumab and median duration of response were 11.1 months and 5.3 months for Nivolumab and Bevacizumab, respectively. TRAEs occurred in 57% (Nivolumab) and 58% (Bevacizumab) of patients with grade 3–4 TRAEs occurred in 18% of patients treated with Nivolumab and in 15% in patients treated with Bevacizumab. In this trial, Nivolumab failed to extend OS in patients with recurrent GBM as compared to Bevacizumab.

In a single institution retrospective study (Chamberlain and Kim, 2017), Nivolumab was evaluated in patients with glioblastoma and previously treated with surgery, concurrent radiotherapy and temozolomide, post-radiotherapy temozolomide and Bevacizumab (with or without lomustine) at first recurrence. Patients received Nivolumab monotherapy (3 mg/kg) once every 2 weeks at second recurrence. One cycle was defined as 2 treatments; 16 patients, aged 52–72 years (median 62 y), were enrolled. The primary objective of this retrospective analysis was to determine whether Nivolumab could delay progression in adult patients with bevacizumab-refractory recurrent GBM. In this retrospective study, Nivolumab salvage therapy demonstrated no survival benefit in patients with bevacizumab-refractory recurrent GBM.

The data from these studies show that the reisstill much to be learned about the use of Nivolumab in the setting of patients with gliobalstoma. Exactly for this reason, several trials are ongoing, exploring, in different settings and in combination with other drugs, the potential role of Nivolumab in patients with glioblastoma. Nivolumabis currently being investigated as a monotherapy, "neoadjuvant" treatment in a phase II trial (NCT 02550249) for patients with primary and recurrent glioblastoma that require surgery. Nivolumab will be continued following surgery until toxicity or progression. Two additional trials of combination Nivolumab and RT with or without temozolomide in patients with newly diagnosed MGMT-unmethylated (CheckMate-498) and MGMT-methylated (CheckMate-548) GBM are ongoing; both are recruiting participants (NCT 02617589, NCT 02667587). Furthermore, an ongoing basket-trial of Nivolumab in combination with galunisertib (a kinase inhibitor of TGFbeta RI) is being conducted in patients with recurrent or refractory GBM (NCT 02423343).

3. Pembrolizumab

Pembrolizumab is a humanized monoclonal IgG4 anti PD-1 antibody consisting of a high-affinity mouse anti-PD-1-derived variable region grafted onto a human IgG4 immunoglobulin molecule with an engineered Fc region for stabilization. Pembrolizumab received FDA approval for treatment of patients with Ipilimumab-treated advanced melanoma (Robert et al., 2014), for first line treatment of patients with NSCLC and PD-L1 > 50% and for second-line treatment for patients with PD-L1 > 1% (Reck et al., 2016; Herbst et al., 2016). In a phase II clinical trial, Pembrolizumab was used as monotherapy in patients with untreated brain metastases from melanoma and NSCLC (10 mg/kg every 2 weeks, until progression). Response of CNS lesions was obtained in 22% of patients with melanoma (95% CI 7–48) and in 33% of patients with NSCLC (95% CI 14–59) (Goldberg et al., 2016).

Data on the efficacy and safety of Pembrolizumab in patients with GBM were obtained from the basket trial, Keynote-028, looking at the PD-1 inhibitor monotherapy, Pembrolizumab, across a number of solid tumor types, including GBM (Reardon et al., 2016c); the GBM basket included patients with any recurrence with two-thirds being treated after their first recurrence. All patients received Pembrolizumab at a standard dosing schedule of 10 mg/kg every 2 weeks; 26 (Reardon et al., 2016c) patients with confirmed diagnosis of GBM were treated. Key inclusion criteria included advanced (unresectable and/or metastatic) bevacizumab-naive GBM, failure of or inability to receive standard therapy, ECOG PS 0–1, and PD-L1 expression in ≥1% of tumor or stroma cells by immunohistochemistry. Response was assessed every 8 weeks for the first 6 months and every 12 weeks thereafter. The primary end point was ORR per RECIST v1.1 (Schwartz et al., 2016) by investigator review. A little more than half (53.8%) had an ECOG PS of 1; all had received prior chemotherapy. There was 1 partial response

(n = 25; ORR, 4.0%, 95% CI, 0.1–20.4); an additional 12 (48.0%) patients experienced stable disease (SD). Median duration of SD was 39.4 weeks (range, 7.1 + -85.9 +). Median PFS was 2.8 months (95% CI, 1.9–9.1); median OS was 14.4 months (95% CI, 10.3-not reached). Treatment-related AEs occurred in 19 (73.1%) patients, most commonly fatigue and rash (n = 6 each, 23.1%). Four (15.4%) patients experienced grade 3–4 treatment-related AEs (lymphopenia, type 2 diabetes mellitus, arthritis, and syncope). No patients died or discontinued pembrolizumab because of a treatment-related AE. Correlative analysis are ongoing, including work to correlate the level of PD-L1 expression or other immune markers with outcome.

In a single-center observational retrospective study performed ad Memorial Sloan Kettering Cancer Center, Pembrolizumab was evaluated in patients with pathologically confirmed high grade malignant glioma (Reiss et al., 2017). The primary objective of this study was to describe overall response rate (ORR) on contrast MRI; secondary objectives included characterizing toxicities as well as describing progression free survival (PFS) and overall survival (OS). Twenty-nine patients received Pembrolizumab; four patients were excluded (3 patients received previous checkpoint inhibitor therapy as part of a clinical trial and 1 patient did not have a high grade glioma). Thirteen patients had pathologically confirmed glioblastoma (52%), 7 anaplastic astrocytoma (28%), 2 anaplastic oligodendroglioma (8%), 2 unspecified high grade glioma (8%) and 1 gliosarcoma (4%). Patients were heavily pre-treated with a median of 4 prior lines of therapy (range 1–9), and 19 patients (76%) previously progressed with bevacizumab treatment; of these 19 patients, 17 (68%) continued bevacizumab with pembrolizumab therapy. Other concurrent treatment with Pembrolizumab included bevacizumab plus temozolomide in 2 patients (8%). Response and toxicity were evaluated in 24 patients; best radiographic response was partial response (n = 2, 8%); stable disease (n = 5, 21%) and progressive disease (n = 17, 71%); both patients with a partial response received concomitant bevacizumab. Median progression free survival (PFS) was 1.4 months (range 0.2–9.4) and median overall survival (OS) was 4 months (range 0.5–13,8). Six-month PFS was 12% and 6 month OS was 28%. The most common adverse events reported were fatigue (grade 3–4: 4%; grade 1-2: 75%), headache (grade 3–4: 4%, grade 1-2: 43%), nausea (grade 3–4: 4%, grade 1–2: 37.5%), diarrhea (grade 3–4: 0%, grade 1-2: 17%), seizures (grade 3–4: 4%, grade 1-2: 17%), vomiting (grade 3–4: 4%, grade 1-2: 17%), myalgias/arthralgia (grade 3–4: 0%, grade 1–2: 13%), and rush (grade 3–4: 0%, grade 1-2: 8%). Laboratory abnormalities (G1-2) were hyperglycemia (79%), thrombocytopenia (50%), leukopenia (37.5%), elevated ALT and AST (33% and 29%), elevated bilirubin (21%), neutropenia (21%), hypothyroidism (17%).

Recently, the results of a phase II study evaluating the use of pembrolizumab (200 mg IV Q3W)with (cohort A) or without (cohort B) bevacizumab (10 mg/kg IV Q2W), in bevacizumab-naïve patients at first or second recurrence, were presented at the ASCO Annual Meeting 2018 (Reardon et al., 2018). The primary end point was PFS at 6 months (PFS-6). With median follow-up of 25.3 months, PFS-6 was 26% (95% CI: 16.3, 41.5) for the cohort A and 6.7% (95% CI: 1.6, 25.4) for cohort B. Median OS was 8.8 months (95%CI: 7.7,14.2) for cohort A and 10.3 months (95% CI: 8.5, 12.5) for cohort B. These data shown that pembrolizumabmono therapy has limited activity for recurrent GBM and the anti-tumor activity of pembrolizumab with standard dose of bevacizumab was comparable to historical bevacizumab monotherapy data (Weller et al., 2013; Friedman et al., 2009; Chamberlain and Johnston, 2010).

At ASCO Annual Meeting 2018 in Chicago, preliminary results of a phase II trial aimed to ascertain tumor immune modulatory properties of pembrolizumab in patients with GBM that requiring surgery at first or second recurrence were presented. The primary objectives were to evaluate immune effect or function in resected GBM tissue after pembrolizumab treatment and to determine progression-free survival at 6 months (PFS6). In this study, 15patients with GBM, at first or second recurrence whore quiredre operation for tumor progression were enrolled and other ten (n = 10) patients with recurrent GBM patients receiving standard of care (SOC) were immunologically analysed as a comparator. The most common adverse event was grade 1 or 2 fatigue in 40% of patients. There were no treatment-related deaths. The Median PFS was 7 months (95% CI: 4–16) and PFS6 was 53% (95% CI: 33%–86%). Median overall survival has not been reached (95% CI: 15 to not reached), with an estimated 1-year overall survival of 72% (95% CI: 52%–99.6%). Analysis with 35 immune markers by mass cytometry revealed that GBM tumors are poorly infiltrated with T cells but are enriched with distinct CD68 + populations. These CD68 + cells are less frequent in pembrolizumab-treated patients (n = 6) relative to those treated with SOC (n = 10; $P = 0.01$); however, GBM-infiltrating Tregs are more frequent in patients treated with pembrolizumab (P = 0.02).This trial concludedthatAlthoughpembrolizumabwaswelltolerated, PFS6 data and immune analysis indicates that anti-PD-1 monotherapy is insufficient for a response in the majority of GBM patients, likely secondary to a marked scarcity of T cells within the tumor microenvironment and a preponderance of CD68 + cells (De Groot et al., 2018).

Another on going trial use Pembrolizumab in newly diagnosed GBM in a phase 1 / 2 study (NCT02530502), in combination with standard temozolomide and radiation treatment to ascertain dose-limiting toxicity and 6-monts PFS.

4. Other immuno-checkpoint inhibitors

4.1. Durvalumab

Durvalumab is a fully human immunoglobulin G1k monoclonal antibody against programmed cell death ligand-1 (PD-L1). Durvalumab blocks PD-L1 binding to both PD-1 and CD80, resulting in enhanced recognition and killing of tumour cells by T-cells (Ibrahim et al., 2015; Stewart et al., 2015). Intravenous durvalumab received US FDA accelerated approval in May 2017 for the treatment of patients with locally advanced or metastatic urothelial carcinoma and disease progression during or following platinum-containing chemotherapy, or within 12 months of neoadjuvant or adjuvant platinum-containing chemotherapy.

A phase III study evaluating durvalumab monotherapy or in combination with tremelimumab is underway in urothelial carcinoma, small cell lung cancer (SCLC) and head and neck squamous cell carcinoma (HNSCC). In the adjuvant treatment of NSCLC stage III, Durvalumab shows a significantly longer progression free survival (PFS) than placebo (Antonia et al., 2017). Durvalumabis also being extensively evaluated in phase I and II clinical trials in a wide range of solid tumours and haematological malignancies.

Durvalumab was evaluated in patients with GBM in a phase II clinical trial where it was administered at 10 mg/kg every 2 weeks for up 12 months (Reardon et al., 2017b); it is a 5-arm study that includes 1 arm of newly diagnosed patients, and 4 arms of recurrent GBM patients (bevacizumab-naive and bevacizumab-refractory). The bevacizumab-naive population is divided into 3 groups based on concomitant therapy: no bevacizumab (n:30), bevacizumab at 10 mg/kg once every 2 weeks (n:32) and bevacizumab at 3 mg/kg every 2 weeks (n:32). Partial outcome and safety data are available only for the patient cohort receiving no bevacizumab (n:30): the 6-month progression free survival (PFS) rate was 20.0% (6 patients;90% CI; 9.7–33.0). The median PFS was 13.9 weeks (95% CI. 8.1–24.0). Among the 6 patients who were progression-free at 6 months, 5 of them remained progression-free for more than 1 year, while 1 patient until 48 weeks. Overall best responses were partial response in 4 patients (13.3%) and stable disease in 14 patients (46.7%) for a disease control rate of 60.0% (18 patients). The median overall survival (OS) was 28.9 weeks (95% CI. 22.9-not estimated). The 6-month OS rate was 59.0% (90% CI. 42.6–72.2) and the 12-month OS rate was 44% (90% CI. 28.9–58.9). Incidence of treatment-related adverse events (TRAEs) were grade 1:

Table 1
Immune-checkpoint inhibitors and glioma.

Study	Drug	Phase	Design	PatientsPopulation	n° of Pts/n° of Ptsevaluated	Best Response (n/%) (Immuno)	mPFS	mOS	ORR
Reardon DA et al. (Reardon et al., 2017a) (ChecMate143)	Nivolumab (anti PD-1)	III	Nivolumab Vs Bevacizumab	Recurrent GBM (first recurrence)	369/369	CR: 2 (1,3) PR: 10 (6,5) SD: 33 (21,6) PD: 107 (69,9) No Treat: 1(0,7)	1.5 m Vs 3,5 m	9.8 m Vs 10m	8% Vs 23%
Chamberlain MC et al (Chamberlain and Kim, 2017)	Nivolumab (anti PD-1)	Single Center Retrospective	Nivolumab single arm	Recurrent GBM (second recurrence, after Bevacizumab)	16/16	CR: 0 (0) PR: 0 (0) SD: 9 (57) PD: 7 (43)	2 months	16.25 months (from initialsurgery)	/
Reardon DA et al (Reardon et al., 2016c) (Keynote 028)	Pembrolizumab (anti PD-1)		Pembrolizumab	Recurrent GBM (anyrecurrence)	26/25	CR: 0 (0) PR: 1 (4) SD: 12 (48) PD: 10 (40) No Evalu: 1 (4) No Assess: 1 (4)	2.8 months	14.4 months	4%
Reiss SN et al (Reiss et al., 2017)	Pembrolizumab (anti PD-1)	Single Center Retrospective	Pembrolizumab	Recurrent (GBM, AA, OligodendrogliomaA, Gliosarcoma, HGG)	29/24	CR: 0 (0) PR: 2 (8%) SD: 5 (21) PD: 17 (71%)	1.4 months	4 months	8%
Reardon DA et a (Reardon et al., 2018) l	Pembrolizumab (anti PD-1)	II	Pembrolizumab + Bevacizumab Vs Pembrolizumab	Recurrent GBM	80		26% Vs 6.7% (PFS-6months)	8.8 m Vs 10.3 m	13.3%
NCT02336165	Durvalumab (anti PD-L1)	II (5-arm study)	Durvalumab +/- Bevacizumab	New Diagnoses and Recurrent	30/24 (single arm)	CR: 0 (0) PR: 4 (13,3) SD: 14 (46,7) PD: 6 (20)	13.9 weeks	28.9 weeks	/
NCT03174197 NCT01375842 NCT02458638	Atezolizumab (anti PD-L1)	I/II I II	Atezolizumab	New Diagnoses	Recruiting	/	/	/	/
NCT01952769	Pidilizumab (anti PD-1)	I/II	Pidilizumab	Diffuse Pontine Glioma and Recurrent GBM	Active butnotrecrutinig	/	/	/	/

mPFS: mediano Progression Free Survival; **mOS**: medianOverallSurvival; **DCR**: Disease Control Rate; **CR**: Complete Response; **PR**: Partialresponse; **SD**: StableDisease; **PD**: Progression of disease**AA**: AnaplasticAstrocytoma; **Oligodendroglioma A**: AnaplasticOligodendroglioma; **HGG**: High Grade Glioma; **GBM**: Glioblastoma; **No Treat**: NotTreated; **No Evalu**: No Evaluable; **No assess**: No assessment.

35.5%; grade 2: 41.9%; grade 3: 9.7%; and grade 4/5: 0%. Most common TRAEs (≥3 pts): fatigue, headache, hemiparesis, gait disturbance, increased AST, and decreased platelets/WBCs (Table 1)

4.2. Atezolizumab

Atezolizumab is a humanized monoclonal antibody immunoglobulin G1 (IgG1) engineered Fc directed against PD-L1: by binding to L1, it prevents interaction with the receptors PD-1 and B7.1. Atezolizumabwas approved as monotherapy for the treatment of adult patients with locally advanced or metastatic urothelial carcinoma (UC) after prior platinum-containing chemotherapy or who are considered cisplatin ineligible (Powles et al., 2018) and for the treatment as monotherapy of adult patients with locally advanced or metastatic non-small cell lung cancer (NSCLC) after prior chemotherapy (Rittmeyer et al., 2017). A phase I/II trial (NCT03174197) is currently recruiting to evaluate the safety and clinical efficacy of Atezolizumab in combination with temozolomide and radiation in patients with newly diagnosed glioblastoma. Other two current open phase I and phase II studies (NCT 01375842 and NCT02458638) are looking at Atezolizumab in solid cancer including GBM, the results of which are awaited.

4.3. Pidilizumab

Pidilizumab is a humanized, immunoglobulin (Ig) G1 monoclonal antibody directed against human inhibitory receptor programmed cell death 1 (PD-1), with potential immune checkpoint inhibitory and antineoplastic activities. Pidilizumab binds to PD-1 and blocks the interaction between PD-1 and its ligands, PD-1 ligand 1 (PD-L1) and PD-1 ligand 2 (PD-L2).Pidilizumab was evaluated in a phase 2 trial in patients with metastatic melanoma and revealed an OS at 12 months of 64.5% (Atkins et al., 2014). A randomized phase I/II study has been planned (active but not recruiting) to test the effect of pidilizumab against diffuse intrinsic pontine glioma and recurrent GBM (NCT01952769).

5. Potential biomarkers of response to immuno-checkpoint inhibitors

Since ICIare used both in clinical practice and in clinical trials, efforts have been made to identify the presence of potential biomarkers that identify patients who could obtain the greatest benefit from this type of treatment.

Initially, the focus was on the expression of programmed cell death protein 1 (PD-1) and PD ligand 1 (PD-L1) as a potential biomarker associated with clinical response (Ansell et al., 2015) It is known that in the literature the role of PD-L1 expression in gliomas has been much studied and how it can be correlated with further histological and molecular features (Berghoff and Preusser, 2016; Garber et al., 2016) in particular, Berghoff et al showed a possible correlation between PD-L1 expression and IDH status (Berghoff et al., 2017); indeed, the authors demonstrated that PD-L1 expression can be higher in IDH wtgliom as compared to IDH mutant gliomas; and so, this group of patients could have more benefit from an immune checkpoint inhibitor treatment. Subsequently, the spectrum of analysis on the possible predictive biomarkers of response to immune-checkpoint inhibitors was expanded by integrating the assessment of Tumor Mutational Load (TML), which would be associated with an increase in neoantigen formation with consequent increase in tumor immunogenicity (Schumacher et al., 2015; Chan et al., 2015; Champiat et al., 2014). However, the techniques and cut-off points for defining TML are not standardized and turn out to be different among cancer types (Chan et al., 2015; Rizvi et al., 2015; Le et al., 2015). Several early stage clinical trials have shown a clinical response to immune checkpoint inhibitors in patients with altered mismatch repair genes (MMR), even in patients with glioblastoma (Le et al., 2017; Brahmer et al., 2010; Bouffet et al., 2016). In fact, a rather recent study has shown significant clinical and radiographic responses with nivolumab in 2 siblings diagnosed with recurrent multifocal biallelic MMR-deficiency GBM; both, in fact, had a higher number of neoantigens and hypermutant profiles compared to sporadic cancers Bouffet et al., 2016).The "hypermutated profiles" are typically characterized by mutations in at least one of the MMR genes, MSH2, MLH1, MSH6 and PMS2(Cancer Genome Atlas Research Network, 2008). This type of alteration to MMR genes may also not be present at the time of the first diagnosis but occurs at the time of relapse of the disease, as a possible effect of the treatment performed. In fact, several studies have indicated a high rate of acquired MMR deficiency, particularly at the MSH6 gene, at recurrence of GBM. (Hunter et al., 2006; Cahill et al., 2007; Yip et al., 2009). In particular, with exome sequencing of initial and recurrent GBM from individual centers and the Cancer Genome Atlas (TCGA), it was found that about 26% of recurrent tumors harbor somatic mutations in MSH6 and, as a result, a higher TML was demonstrated (Hunter et al., 2006; Cahill et al., 2007; Yip et al., 2009).

In a recent study (Hodges et al., 2017), 327 glioma patients (198 with diagnosis of glioblastoma) were analyzed and profiled for TML (calculated with an algorithm that included nonsynonymous mutation counts for tumorwith germline mutations filtered out), PD-1 and PD-L1expression (IHC and NGS), MMR (MLH1, MSH2, MSH6 and PMS2) protein expression and mutations, and DNA plymerase epsilon (POLE), to clarify the relationships among these potential biomarkers to predict treatment response with immune checkpoint inhibitors. In this study, no significant association between TML and intratumoral increase of cytotoxic CD8 + T cells was found, which should be related to better antitumor reactivity. However, an association between high TML and loss of MMR protein expression was found.

An additional feature of tumor cells which is likely to impact on response to ICI is expression of MHC class I molecules. In fact, MHC class I expression is required for proper antigen presentation to cytotoxic T cells and its expression is highly heterogeneous in GBM (Indraccolo S. et al., manuscript under revision). We speculate that – irrespective of the high TML – any MHC class I negative GBM remains a “cold tumor”, in terms of capability of being recognized and killed by the immune system. Along this line, there are indeed several examples in the literature of mutations of the gene encoding β2-microglobulin, a protein required for MHC class I expression, in solid tumors which acquired resistance to pembrolizumab (Le et al., 2017; Zaretsky et al., 2016). Therefore, assessment of MHC class I expression should be considered, along with TML and loss of MMR protein expression, in developing predictions to response to ICI in GBM

In light of such data, there are several candidate biomarkers to predict treatment response with immune checkpoints in patients with GBM but, to date, no strong or significant correlation has been found between the expression of these markers and clinical and radiological response to immunotherapeutic treatment. Further analysis is absolutely necessary within clinical and pre-clinical studies to identify predictive biomarkers for selection of patients who could benefit from treatment with ICI.

6. Conclusion

Treatment of different types of cancer, to date, has been markedly revolutionized by the advent of immunotherapy. In particular, immune checkpoint inhibitors provided a substantial benefit in terms of outcome, which has probably never been achieved thus far in non-oncogene addicted tumors. Glioblastoma remains a devasting and universally lethal cancer and this is why continuous efforts are needed to improve the outcome of patients suffering from this disease, using, in the best way, the newest drugs in the armamentarium of available treatments.

The data available so far regarding the treatment of patients with glioblastoma with ICI are modest and still too immature to classify among the therapeutic standards for this type of patient. The limitations

in the treatment of glioblastoma with immune checkpoint inhibitors are many, including tumor localization, the presence of the blood-brain barrier (BBB), which makes it difficult for molecules such as nivolumab, which has a calculated molecular mass of 146 kDa, to access the tumor (the BBB does not allow the passage of molecules with molecular mass greater than 400–600 Da) (Miura et al., 2013; Banks, 2009), and the state of global immune dysfunction of patients with glioblastoma, with reduced levels of circulating CD4 + and CD8 + lymphocytes as an effect of chemotherapy treatment (Gustafson et al., 2010; Mirzaei et al., 2017). As in other tumors, we are still very far from identifying patient subpopulations which benefit most from this immunotherapeutic approach, in the absence of a clear molecular profile that can be predictive of response to immunotherapeutic treatments.

However, this scenario should not discourage, but rather it must encourage and drive the continuous study and research in the right direction for immunotherapeutic treatment with immune checkpoint inhibitors in patients with glioblastoma, to try to improve their outcomes and to better understand the molecular and immunological structure of this cancer, which remains one of the most complex challenges of international oncology.

Conflict of interest

The authors declare no conflict of interest and no funding was received

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