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An Update on the Role of Immunotherapy and Vaccine Strategies for Primary Brain Tumors

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Opinion statement

Existing therapies for glioblastoma (GBM), the most common malignant primary brain tumor in adults, have fallen short of improving the dismal patient outcomes, with an average 14–16-month median overall survival. The biological complexity and adaptability of GBM, redundancy of dysregulated signaling pathways, and poor penetration of therapies through the blood–brain barrier contribute to poor therapeutic progress. The current standard of care for newly diagnosed GBM consists of maximal safe resection, followed by fractionated radiotherapy combined with concurrent temozolomide (TMZ) and 6–12 cycles of adjuvant TMZ. At progression, bevacizumab with or without additional chemotherapy is an option for salvage therapy. The recent FDA approval of sipuleucel-T for prostate cancer and ipilumimab, nivolumab, and pembrolizumab for select solid tumors and the ongoing trials showing clinical efficacy and response durability herald a new era of cancer treatment with the potential to change standard-of-care treatment across multiple cancers. The evaluation of various immunotherapeutics is advancing for GBM, putting into question the dogma of the CNS as an immuno-privileged site. While the field is yet young, both active immunotherapy involving vaccine strategies and cellular therapy as well as reversal of GBM-induced global immune-suppression through immune checkpoint blockade are showing promising results and revealing essential immunological insights regarding kinetics of the immune response, immune evasion, and correlative biomarkers.

The future holds exciting promise in establishing new treatment options for GBM that harness the patients' own immune system by activating it with immune checkpoint inhibitors, providing specificity using vaccine therapy, and allowing for modulation and enhancement by combinatorial approaches.

Introduction

In 2015, about 23,189 new primary malignant brain tumors will be diagnosed in the United States with 13,330 predicted deaths [1–3]. Gliomas, the most common malignant primary tumor of the central nervous system (CNS), present with an annual incidence of about 5 in 100,000 with glioblastoma (GBM), the most common and aggressive subtype constituting 54 % of gliomas [2, 3]. With metastases exceedingly rare, GBM is restricted to the CNS, where it is highly invasive, contributing to recurrence [4]. The prognosis for GBM remains grim, with a median survival of 14–16 months and 5-year survival rate of less than 5 % [2, 5, 3], due partly to extensive tumor heterogeneity allowing for treatment resistance, as well as difficulty in delivering therapeutics through the blood–brain barrier (BBB) [6]. For newly diagnosed GBM, the standard-of-care involves maximal safe resection and subsequent 60 Gy fractionated radiotherapy with concurrent daily temozolomide, followed by 6 to 12 monthly cycles of adjuvant temozolomide [7, 5]. For recurrent GBM, bevacizumab, a humanized anti-vascular endothelial growth factor (VEGF) monoclonal antibody, received accelerated approval by the US FDA in 2009 due to durable radiographic response rates compared to historic controls, although the overall survival (OS) remains unchanged [8–10]. In the recurrent setting, adding lomustine to bevacizumab may prolong survival, and the potential benefit of this combination is currently undergoing confirmation in the EORTC 26101 study (Clinicaltrials.gov: NCT01290939) [11].

The current standard of care has improved the dismal prognosis in GBM patients but marginally, highlighting the urgency for effective and durable therapies. Recent FDA approval of the sipuleucel-T (APC8015) prostate cancer vaccine and of the immune checkpoint inhibitors ipilumimab, pembrolizumab, and nivolumab for select solid tumors including melanoma and non-small cell lung cancer and the success in treating refractory leukemias with engineered chimeric antigen receptor (CAR) autologous T cells have opened a new chapter in cancer treatment [12••, 13, 14, 15••, 16]. Early data from accrual of GBM into immunotherapy trials encompassing vaccines, checkpoint inhibitors, and CAR T cells are showing promising results, putting into question the dogma of the CNS as an immunoprivileged site [17, 18]. While immune-tolerance in the CNS is under intense study, the CNS has emerged as an immunologically active environment where antigen is presented and activated T cells can infiltrate through, despite the absence of traditional lymphatics and of resident naïve T cells, as evidenced by auto-immunity in the brain in demyelinating disease and the presence of a rich GBM-associated immune-environment, including tumor-specific T cells [17, 18].

GBM counteracts the anti-tumor immune response in the CNS by causing local and systemic immune-suppression through a myriad of mechanisms. These include tumor camouflage through downregulation of tumor-associated major histocompatibility complex (MHC)-I, expression of immune-checkpoint regulators such as programmed cell death ligand 1 (PD-L1), elaboration of immune-silencing factors such as transforming growth factor-b, vascular endothelial growth factor (VEGF), and interleukin-10, recruitment of immunosuppressive cells including regulatory T cells (Tregs) and M2 tumor-associated macrophages, and outright killing of tumor-specific activated T cells through tumor expression of the Fas ligand [19–28, 18, 29, 30]. Aiding the immune system in elimination of tolerogenic tumors, active immunization with cancer vaccines, cellular therapy with modified, activated T cells, and systemic immune-modulation counteracting GBM-induced immunosuppression hold promise as the new cornerstones of effective and durable treatment against

GBM [17, 18]. This review highlights some of the key preclinical and clinical results that have emerged in the past year evaluating various immunotherapy approaches for neuro-oncology patients.

Treatment

Anti-glioma vaccines

- Vaccines can direct multiple branches of the immune system against target antigens and provide durability of the response through immunological memory. Vaccines activate immune responses, with repeated exposures leading to refinement in quality and speed of the response, termed active immunotherapy. While effective anti-glioma vaccines are in various stages of development, they offer encouraging safety profiles, as well as potential durability of anti-tumor response. GlioVac is a mixture of autologous tumor cells as well as three distinct allogeneic GBM cell populations, inactivated through irradiation. Nine recurrent GBM patients were vaccinated with GlioVac in combination with GM-CSF, preceded by low-dose cyclophosphamide depletion of immune-suppressive Tregs [31]. GlioVac showed minimal toxicity with 77 % 40-week survival in vaccinated patients compared to 10 % in controls [31]. This approach is currently under validation in a phase II trial of recurrent, bevacizumab-naïve GBM patients (Clinicatrials.gov: NCT01903330). Targeted vaccine strategies are designed to elicit specific immune responses to tumor-specific antigens (TSAs) such as EGFRvIII, human cytomegalovirus (CMV)-derived antigens, and IDH-1 (R132H) or tumor-associated antigens (TAA) including IL13Ra2, HER-2, gp100, TRP2, EphA2, survivin, WT1, SOX2, SOX11, MAGE-A1, MAGE-A3, AIM2, and SART1 among many [17, 18, 32, 33].
- Early clinical and preclinical data show the potential of strengthening and refining vaccine-induced anti-glioma immune responses. CMV-antigens are almost universally expressed by gliomas. Preconditioning the vaccination site with tetanus/diphtheria toxoid, a potent recall antigen, enhanced vaccination with CMV phosphoprotein 65 (pp65) RNA-pulsed autologous dendritic cells (DCs) in 12 GBM patients randomized to immunotherapy with or without immune-preconditioning [34•]. Improved progression-free survival (PFS) and OS were associated with preconditioning, and three of the pre-conditioned patients were alive at >36.6 months. This was recapitulated in mice, with recall-antigen preconditioning leading to improved trafficking of professional antigen presenting dendritic cells (DCs) to lymph nodes in a CCL-3-dependent mechanism, suggesting a possible

role for the chemokine CCL-3 in clinical vaccine potentiation [34•].

- Involved in cellular metabolism, the isocitrate dehydrogenase-1 (IDH-1) (R132H) mutation is a promising TSA [35, 36•]. IDH-1 (R132H) is rarely expressed in primary GBM but rather in low-grade glioma and secondary GBM, where the mutation is tumorigenic by inducing a hypermethylated CpG island methylator (CIMP) phenotype leading to tumor suppressor down-modulation [35, 36•]. Preclinical data show anti-tumor efficacy of a 15-mer peptide vaccine mapping to the R132H mutation in a human MHC-II transgenic orthotropic mouse model for glioma [36•]. Notably, a subset of patients with IDH-1 (R132H) expressing gliomas were found to have circulating IDH-1 (R132H)-specific peripheral T cells and antibodies, absent in patients with wild-type tumors, indicating that IDH-1 (R132H) is a natural immuno-dominant epitope in anti-tumor immune-surveillance [36•]. An IDH-1 (R132H) peptide vaccine is about to undergo phase I clinical testing (Clinicaltrials.gov: NCT02454634) [36•].

Dendritic-cell-based vaccines

- DCs are professional antigen-presenting cells (APCs), demonstrating feasibility and efficacy in multiple phase I/II vaccine trials for GBM [17, 18, 32, 33]. DC-based vaccines involve the isolation of patient's peripheral blood monocytes coupled with in vitro differentiation to DCs with GM-CSF and IL-4 [20], followed by antigen pulsing, further maturation, and subsequent intra-dermal vaccination [37–39]. There are a wealth of DC-cell based GBM vaccine trials currently open or completed [20, 51, 45]. Of these, a trial using DCs pulsed with a synthetic TAA peptide cocktail containing HER2, TRP-2, gp100, MAGE-1, IL13Ra2, and AIM-2 showed promising phase I results with a median OS of 38.4 months [40••]. Data from the randomized phase II trial (ICT-107) in 124 patients with newly diagnosed GBM showed the strongest treatment effects in human leukocyte antigen-A2 (HLA-A2) expressing patients.

Peptide vaccines

- Subject to similar limitations including HLA restriction, peptide and protein vaccines seek to resolve the variability and production complexity of cell-based vaccines by pulsing DCs in vivo through administration of peptide antigen in combination with an immune-adjuvant [17, 18, 32, 33]. Rindopepimut (Celldex Therapeutics), a synthetic mutated epidermal growth factor receptor variant III (EGFRvIII) neo-antigen-specific peptide, conjugated to the immune adjuvant keyhole limpet hemocyanin (KLH), and administered with GM-CSF, is the most advanced peptide vaccine having undergone phases I, II, and III clinical trials [41, 42]. EGFRvIII, found in approximately 20–30 % of

GBMs, contains an in-frame deletion of exons 2–7, creating a neoantigenic junction not expressed on normal cells [33, 43, 44] and is an independent negative prognostic factor in GBM [43, 45].

- In phases I and II testing, rindopepimut was well tolerated with adverse effects limited largely to injection site reactions and showed improved mean OS ranging from 22.8 to 26 months, with even higher median OS of 47.7 months in patients with robust anti-EGFRvIII antibody responses [46]. Remarkably, about 85 % of patients developed ≥fourfold increase in EGFRvIII-specific antibodies in an expanded phase II trial of rindopepimut, highlighting the possibility of using anti-EGFRvIII antibody titer as a response predictive biomarker [47••]. Progressive tumor following rindopepimut vaccination no longer expressed EGFRvIII, proving that a targeted vaccine strategy can eradicate its target cell population [48, 46]. ReACT (Clinicaltrials.gov: NCT01498328) is the first phase II study to assess rindopepimut in the recurrent setting. In this trial, GBM patients received bevacizumab in combination with either rindopepimut or placebo vaccine [49, 47••, 43]. Results from ReACT, presented at the American Society for Clinical Oncology meeting in June 2015, were historic in that this is the first randomized immunotherapy trial to demonstrate a survival benefit for glioblastoma patients. Specifically, patients who received rindopepimut had a median OS of 11.6 months compared to 9.3 months of those treated with placebo vaccine (p=0.0386; HR 0.57). In addition, patients on rindopepimut had higher PFS and durable radiographic rates as well as decreased corticosteroid requirement compared to controls. As previously demonstrated, the administration of rindopepimut was associated with good tolerability and no unexpected toxicities were observed when co-administered with bevacizumab. Rindopepimut was also shown to elicit EGFRvIII-specific humoral immune responses both in bevacizumab-naïve and bevacizumab-refractory patients, despite heavy pretreatment. The early generation of EGFRvIII antibodies following rindopepimut administration may serve as a potential biomarker of anti-tumor activity.

Heat shock–peptide complex vaccines

A different vaccine approach relies on the ability of heat shock proteins (HSPs) in their function as intracellular chaperones to couple with nascent proteins and broadly activate both innate and adaptive immune systems, as well as augmenting antigen presentation through MHC-I and MHC-II molecules [50, 17, 18]. The high metabolic rate of tumor cells drives upregulation of HSPs to meet intensified translation demands in the context of an increase in misfolded and aborted proteins [50]. Of the HSPs upregulated in GBM, HSP96 has garnered particular interest in immunotherapy. As a member of the HSP90 class of chaperones, HSP96 has such notable substrates as EGFRvIII, FAK, AKT, hTERT, p53, cdk4, MAPK, and PI3 kinase, which play important roles in tumorigenesis [50]. HSP vaccines are an adaptation of the tumor-lysate vaccine approach, in which patients' tumor cells are lysed, HSP96-peptide complexes isolated, and used for vaccination [50, 17, 18]. In a phase I recurrent GBM trial,

immunogenicity characterized by tumor-specific intra-tumoral immune responses in 11 of 12 tested patients as well as the safety of this approach was validated with a median OS of 47 weeks in responders versus 16 weeks in non-responders [51] leading to a randomized phase II study of HSP96 vaccination with bevacizumab for GBM in the recurrent setting. In this trial, the primary endpoint of OS >6 months was reached in 90.1 % of patients (95 % confidence interval (CI) 75.9–96.8) [52•] with further early phase clinical trials underway (Clinicatrials.gov: NCT02122822, NCT01814813, and NCT00293423). Data presented at the 2015 American Society for Clinical Oncology meeting showed that, when combined with standard of care therapy, an autologous HSP-based vaccine was well tolerated and increased median PFS to 7.8 months (95 % CI 11.3–21.6) and median OS to 23.8 months (95 % CI 19.8–30.2) in 46 patients [53]. This study highlighted PD-L1 expression on circulating monocytes as a possible biomarker in immunotherapy, with PD-L1 expression signifying tumor-induced immune suppression. Median OS for patients with high PD-L1 expression was 18.0 months (95 % CI 10.0–23.3) compared to the median OS of 44.7 months for low PD-L1 expressers (hazard ratio for death 3.35; 95 % CI 1.36–8.23; p=0.003). Together with MGMT methylation status, PD-L1 expression was a significant independent predictor of survival (Clinicatrials.gov: NCT00905060) [53].

Ongoing clinical trials

- CMV proteins are attractive TSAs [17, 18] and almost ubiquitously expressed in glioma in the absence of productive infection, raising the question whether CMV itself drives oncogenes or expression of CMV proteins reflects reactivation in the immune-suppressive tumor environment [54–56]. Clinicaltrials.gov: NCT00639639 is currently assessing the efficacy of CMV pp65-LAMP-pulsed DCs with and without autologous T-cell transfer. To circumvent the difficulty of DC-based vaccine production, a phase 1 trial of a CMV peptide vaccine in currently underway (Clinicatrials.gov: NCT01854099). Rindopepimut is undergoing further validation in ACT IV, a double-blinded phase III trial with randomization to either rindopepimut or control KLH injection enrolling some 374 patients with newly diagnosed, EGFRvIII-positive GBM following gross total resection at over 100 centers worldwide (Clinicaltrials.gov: NCT01480479, expected completion in November 2016) [47••, 49, 43]. In addition, it will also include patients with subtotal resection, which will assess the efficacy of rindopepimut at targeting bulky residual disease [49]. Other peptide vaccine trials in GBM target TAAs including WT1 (Clinicatrials.gov: NCT02078648) [57], as well as a combination of IL13Ra2, survivin, EphA2 (Clinicatrials.gov: NCT02149225, NCT01920191).
- Personalized anti-GBM peptide vaccines take advantage of unique mutations leading to expression of neo-antigens in an individual patient targetable by peptide vaccines [17, 32, 31, 58]. These are identified through next-generation DNA sequencing [59, 60]. The potential peptides are then selected based on binding affinity predictions to patient-

specific, class I HLA molecules [61]. Preclinical data for this approach have been promising, showing both specific immune responses to the selected peptides as well as vaccine efficacy in a melanoma model [62] leading to an ongoing clinical trial in advanced melanoma (Clinicatrials.gov: NCT01970358) as well as initiation of a trial of personalized peptide vaccine (Enova) against GBM (Clinicatrials.gov: NCT01903330).

Checkpoint blockade

- To maintain tolerance and protect from autoimmunity, immune responses require exquisite regulation both at activation and attenuation steps with respect to T cell selection in the thymus, priming in and export from lymphoid tissue, activation in target tissue, subsequent recruitment of accessory cells, and finally a precisely orchestrated response shutdown [17, 43, 18]. This is partly achieved through an elegant interplay between T cell co-stimulatory and inhibitory molecules of the B7/CD28 family, termed immune checkpoints that modulate activity of T cell receptor (TCR) signaling. This mechanism has been subverted by tumors to induce early exhaustion, anergy, and T cell death leading to immune evasion of tumor [17, 43, 18]. Two checkpoint mediators in the spotlight of cancer immunotherapy are cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) and programmed cell death protein 1 (PD-1) [17, 43, 18].
- CTLA-4 and PD-1 differ in timing, cue for upregulation, and location of modulator activity. CTLA-4, expressed on T cells and upregulated by TCR activation, binds to its ligands CD80 and CD86 (B7-1 and B7-2) modulating early priming of T cells in lymphoid tissue. PD-1, expressed on T cells and pro-B cells, is upregulated by cytokines and responds to its ligands PD-L1 and PD-L2. PD-1 generally acts at a later time point during immune activation in target tissue with broader effects including enhancement of Treg and dampening of B and NK cell responses [17, 43, 18]. One mechanism of tumor immune evasion is the expression of PD-L1 on tumor cells directly and in the tumor microenvironment. Blocking the immune checkpoints CTLA-4 and PD-1 can potentiate and refine an active immune response. Thus, while global and non-specific, immune checkpoint blockade has the potential of enabling development of durable and specific responses with long-acting memory [17, 43, 18].
- Blocking antibodies to CTLA-4 (ipilumimab, and tremilimumab) and PD-1 (nivolumab, pembrolizumab, pidilizumab, lambrolizumab, BMS 936559, and MPDL3280A) as well as the ligand PD-L1 (MDX-1105) have revolutionized cancer treatment. The 2010 FDA approval of ipilumimab for the treatment of metastatic melanoma was based on a phase III trial showing safety and significantly improved survival in a subset of ipilumimab-treated patients [13]. In long-term follow-up studies, when robust anti-tumor responses were elicited, they showed

unprecedented durability (median 88 months) [63]. Phase I analysis of BMS-936558 and nivolumab (human anti-PD-1 mABs) showed robust responses in melanoma, renal cell carcinoma (RCC), and non-small cell lung cancer (NSCLC), but no significant responses in pancreatic or colorectal cancer [64]. Improved radiographic response correlated with the level of PD-L1 expression on archival tumor [65]. Immune-related adverse events (irAEs) occur in about 75 % of patients treated with ipilumimab and 15–30 % have >grade 3 irAEs [13, 66, 67]. Generally treatable, possible irAEs follow a well-described chronicity: early rash, followed by colitis and hepatitis, later hypophysitis, less frequently neuropathy, pancreatitis, lymphadenopathy, nephritis, and rare but serious epidermal necrolysis and pneumonitis [67].

- Published clinical experience with checkpoint blockade in treatment of CNS tumors is limited to reassuring safety and response profiles with ipilumimab treatment of melanoma brain metastases [68]. Preclinical data of checkpoint blockade in glioma treatment, however, are encouraging. In immuno-competent mice with established SMA-650 intracranial tumors, CTLA-4 blockade was well tolerated with no evidence of CNS autoimmunity and immune profiles normalized with increased CD4 and decreased Treg counts, leading to 80 % long-term survival in treated mice [69]. Immunological memory blocked tumor formation upon contralateral intracranial re-inoculation of tumor in a mouse model in which intratumoral IL-12 administration had previously been combined with systemic CTLA-4 blockade [70]. Promising preclinical data for PD-1 blockade combined with a 10-Gy radiation dose improved median survival from 26 to 52 days among C57/B6 mice with GL261 intracranial tumors [71]. In a systematic preclinical study testing PD-1, PD-L1, PD-L2, and CTLA-4 blockade (single agent and combinations), combined anti-PD-1 and anti-CTLA-4 therapy showed the most robust survival benefit with 75 % of treated mice alive at 140 days with no evidence of residual tumor. Notably, long-term survivors showed initial tumor growth followed by gradual disappearance of tumors on MRI and blocked tumor growth following re-challenge with tumor cells [72].
- Results from ongoing clinical trials of checkpoint blockade are highly anticipated, as GBM is accruing to numerous immunotherapy trials including the CTLA-4 blocking ipilumimab for both newly diagnosed and recurrent GBM (Clinicaltrials.gov: NCT02017717), the PD-1 blocking mAb nivolumab for recurrent GBM both alone and in combination with ipilumimab (Clinicaltrials.gov: NCT02017717), the PD-1 blocking mAb pidilizumab in diffuse pontine glioma and recurrent GBM (Clinicaltrials.gov: NCT01952769), the PD-1 blocking mAb pembrolizumab with or without bevacizumab in recurrent GBM (Clinicaltrials.gov: NCT02337491), and finally the PD-L1 blocking MEDI4736 (Clinicaltrials.gov: NCT02336165).

Cellular immunotherapy

Adoptive autologous T cell transfer

- Augmentation of an already primed tumor-antigen-specific immune response can be achieved through infusion of ex vivo-expanded immune cells, termed adoptive transfer [17, 18, 73]. One subtype of lymphocytes educated against tumor in vivo are tumor infiltrating lymphocytes (TILs) [17, 18, 73]. Harvesting of autologous TILs from tumor biopsy sample followed by expansion in vitro with IL-2 and reinfusion into patients has led to tumor regression and durable responses in some melanoma patients [74, 75], especially when coupled with co-administration of immune-depleting treatments, as effector T cells expand preferentially in a lymphopenic environment [76]. Experiments using Epstein Barr virus-specific ex-vivo expanded autologous T cells to treat post-transplant lymphoproliferative disorder show efficacy of targeting a specific tumor antigen with cellular immunotherapy [77]. In one study for recurrent GBM, CMV-specific T cells were successfully isolated and expanded from 13 of a total 19 patients, and 11 patients received up to four infusions [78•] of expanded autologous CMV-specific T cells with an encouraging median overall survival in these patient of 403 days [78•]. General drawbacks of cellular immunotherapy are difficulty and cost of large-scale production and limited post-infusion survival of T cells in vivo, the latter which can potentially be improved upon in an immune-ablative context or with concomitant vaccination as shown in preclinical models [79]. Furthermore, dependence on the T cell receptor (TCR) stimulatory apparatus imposes MHC class restriction, preventing off-the-shelf high-throughput production and renders these cells sensitive to tumor-mediated immune evasion including PD-L1 expression and MHC-I down-modulation on tumor cells.

CAR T cells

- An innovative approach to overcome these limitations is the generation of engineered chimeric antigen receptor (CAR) autologous T cells [17, 18]. These are T cells that have the MHC-restricted TCR replaced with an engineered chimeric receptor containing a single antibody variable chain (scFv) TAA/TSA-specific extracellular domain coupled to the intracellular activation domain of the TCR [17, 18, 73]. Designed to be antigen-specific, this elegant construct circumvents both MHC restriction and allows for generation of large numbers of expanded T cells, as they are engineered simply from patients' PBMCs, vastly increasing the yield of primed T cells [17, 18, 73]. CAR T cells have shown efficacy in treatment of refractory lymphoid leukemia patients [12••, 80] as well as in patients with GD2 positive neuroblastoma [81]. Preclinical trials with CARs to target different glioma TAAs show promising results.

Adoptive transfer of EphA2-specific CAR T cells lead to regression of intracranial glioma xenografs in immunocompromised SCID mice with survival benefit in treated mice [82]. In another approach, bi-specific CAR T cells targeting both HER2 and IL-13R were generated to avoid antigen escape and were found to show enhanced anti-tumor activity compared to unispecific CAR T cells. Furthermore, in an orthotropic GBM xenograft model, median survival of control mice was 35 days, with unispecific HER2 and IL-13R CAR T cell treatment improving median survival to 79 and 84 days, respectively, and a median survival of >120 days when bispecifc CAR T cells were used [83]. EGFRvIII has also been targeted by CAR T cells in preclinical models. EGFRvIII-specific CARs administered systemically can home to areas of invasive intracranial tumors in an orthotropic mouse model and suppress growth, leading to survival benefit [84]. Current clinical trials are assessing safety and efficacy of CAR T cells targeting EGFRvIII (Clinicaltrials.gov: NCT01454596) [85], HER2 (Clinicaltrials.gov: NCT01109095), and IL13Rα2 (Clinicaltrials.gov: NCT02208362) [86].

Response monitoring in immunotherapy

- Immunotherapy and standard-of-care treatment differ in kinetics and amount of inflammation expected, calling for a revision of radiographic response criteria. The response assessment in neuro-oncology (RANO) criteria improved on prior radiographic response criteria by acknowledging the problem of radiographically distinguishing pseudo-progression, occurring in 10–20 % of patients, from true progression (PD), by requiring that PD persist for 3 months after completion of chemoradiation [87]. Experience from immunotherapy trials in melanoma highlighted the importance of immune-related response criteria (irRC) to prevent premature treatment withdrawal [88]. The Immunotherapy Response Assessment in Neuro-oncology (iRANO) working group has updated RANO criteria to incorporate immunotherapy kinetics and redefine responses and disease progression (PD) in immunotherapy-treated GBM, low-grade glioma, and CNS metastases [89••]. Appearance of progressive or new lesions alone does not necessarily signify disease progression within 6 months of immunotherapy initiation [89••]. This allows for ongoing immunotherapy in patients tolerating treatment for 3 months after initial "progression" on neuro-imaging [89••]. Should progression be confirmed at 3 months after initial appearance, PD is dated back to its first appearance on MRI; otherwise, treatment with immunotherapy continues as long as tolerated [89••]. These revised response criteria are geared to incorporating our understanding of kinetics and mechanism of immunotherapy with our clinical experience to date and are awaiting validation moving forward.

Emerging therapies and future directions

Combinatorial approaches

- Treatment resistance to single-modality immunotherapy was seen with EGFRvIII peptide vaccination, with recurrent tumor having lost EGFRvIII expression [48, 39]. Combinatorial approaches could be valuable in potentiating an immune response and in targeting non-redundant immunosuppressive pathways. Combining CTLA-1 with PD-1 checkpoint blockade has shown more rapid and durable responses in melanoma treatment [90•]. Moreover, data from the cancer genome atlas shows that GBM subtypes express distinct immunosuppressive genes, indicating that different combinatorial approaches may be warranted based on tumor expression profiling [91]. Preclinical models combining vaccines with checkpoint blockade support combinatorial synergy [92, 93]. Several clinical trials are underway in GBM, for instance, combining CMV peptide antigen vaccination with CMV-specific adoptive T cell transfer (Clinicaltrials.gov: NCT 00693095), augmenting EGFRvIII vaccination efficacy with depletion of Tregs using the anti-IL2 mAb daclizumab (Clinicaltrials.gov: NCT00626015), and combined CTLA-4 and PD-1 blockade for recurrent GBM patients (Clinicaltrials.gov: NCT02017717) [18].
- Immunotherapy appears to synergize with current standard-of-care treatment both for newly diagnosed and recurrent GBM. Regression of metastatic tumor distant from an initial irradiation site is possibly caused by radiation-mediated "in situ" vaccine generation and termed an abscopal effect [94]. This was noted in a patient with advanced melanoma following treatment with targeted radiation-therapy and ipilumimab [95]. In preclinical models, radiation combined with anti-tumor vaccination showed significant survival benefit compared to each modality alone [96], and in another model, radiation significantly increased survival when combined with PD-1 checkpoint blockade compared to either alone [71]. Radiation leads to immune activation by upregulation of MHC-I molecules on irradiated tumor cells [97], enhancement of antigen presentation through danger signals and TAA release [99] following radiation-induced accelerated protein degradation [98], and increased expression of IFN-beta by tumor cells leading to improved DC maturation and antigen presentation [96].
- Clinical studies combining TMZ with rindopepimut surprisingly show augmentation of immune responses rather than the expected response attenuation in the context of cytotoxin-induced lymphopenia [39, 48]. This is postulated to occur through multiple mechanisms including enhanced antigen release in dying cells, refined APC function in the context of danger signals, immuno-stimulatory cytokines, lymphopenia-driven homeostatic T cell proliferation leading to a relative decrease in Tregs, and further selective depletion of Tregs [18]. VEGF blockade and the standard-of-care in recurrent GBM also may enhance anti-tumor immune responses [21]. In addition to driving

tumor vascularization, VEGF restricts T cell migration through the tumor vasculature, enhances Treg activity, inhibits DC maturation, and induces apoptosis of CD8 T cells [18]. Trials combining bevacizumab with either rindopepimut (ReACT) or pembrolizumab are underway (Clinicaltrials.gov: NCT01498328 and NCT02337491)

Biomarkers for response to immunotherapy

- Despite successes, only a subset of patients treated with immunotherapy show clinical responses and the tumor-intrinsic factors conferring immune evasion remain largely unknown, calling for predictive biomarkers of immunotherapy response. Some studies show a correlation between PD-L1 on archival tumor and response to PD-1 checkpoint blockade [65]. EGFRvIII expression on tumor is required for EGFRvIII-specific vaccine efficacy, with circulating anti-EGFRvIII antibody levels serving as a possible biomarker for vaccine response [47••]. A more generalizable immune-activation signature, however, could enable consistent monitoring and cross-study evaluations. High-throughput molecular profiling is defining immune signatures for both activation and specificity of the immune response [99]. Multi-parameter flow cytometry assessing the relative abundance of effector T cells, Tregs, NK cells, and monocytes including immunosuppressive monocytes shows association with survival in GBM, non-Hodgkin's lymphoma, and RCC [100] and has been enhanced with the development of time-of-flight mass cytometry (CyTOF), enabling flow cytometry analysis in 25 dimensions [99]. High-throughput RNAseq transcriptome profiling of tumor samples has been optimized for the clinical setting (NanoString, nCounter platform) to quantify cell types by markers, tumor antigens, and >400 immune genes. Transcriptome interrogation was correlated to OS and PFS in neuroblastoma [101] and risk of recurrence, subtype, and trastuzumab response in breast cancer [102]. A further breakthrough is whole-repertoire T and B receptor amplification with high-throughput sequencing (TCRseq and BCRseq, respectively), allowing for antigen specificity analysis as well as informing about clonal B and T cell population modulation through treatment, response to immunotherapy, and tumor recurrence [103]. As these techniques become standardized and data analysis streamlined, a response to immunotherapy signature may crystallize as a predictive biomarker to couple with radiographic and clinical monitoring to guide clinical decision-making.

Summary and future directions

Experience from immunotherapy treatment of advanced and metastatic cancers such as melanoma, RCC, NSCLC, and hematologic malignancies has shown specific, robust, and durable anti-tumor responses. Immunotherapy for primary brain tumors is feasible, safe, and has shown encouraging results against primary brain tumors in preclinical and early clinical

studies. While our understanding of anti-tumor immune responses in the brain is evolving with ongoing immunotherapy trials, we require new tools in clinical monitoring. The complex interplay between tumor heterogeneity, immune surveillance, activation, and evasion on the one hand, and the power of synergism and risk of incompatibility of combined therapy on the other, calls for treatment guidance beyond radiographic monitoring. Incorporation of response-predictive biomarkers will be essential. In globally recruiting the immune system and overcoming tumor immune-evasion with checkpoint blockade, providing the immune response with specificity using targeted or personalized vaccines and cellular immunotherapy, and finally allowing for refinement, enhancement, and modulation of the anti-tumor immune response with combinatorial approaches, immunotherapy will likely come to the forefront of GBM treatment. High-throughput immune-monitoring to provide activation and specificity signatures will require combination with genetic tumor characterization as different subtypes of GBM (pro-neuronal, neural, classical, and mesenchymal) are distinct pathologic entities with differing immune-suppression phenotypes and antigen profiles [17, 104]. Combining immunophenotyping and molecular tumor phenotyping of patients has the potential to help define the optimal immunotherapy approach for each patient.

Compliance with Ethics Guidelines

Conflict of Interest

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David A. Reardon has served on advisory boards for AbbVie, Amgen, Bristol-Myers Squibb, Cavion, Genentech/Roche, Merck, Midatech, Regeneron, and Stemline Therapeutics and has conducted lectures on behalf of Genentech/Roche and Merck.

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

References and Recommended Reading

Papers of particular interest, published recently, have been highlighted as:

- • Of importance
- •• Of major importance

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