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Immunotherapy for neuro-oncology: the critical rationale for combinatorial therapy

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A successful therapeutic paradigm established historically in oncology involves combining agents with potentially complementary mechanisms of antitumor activity into rationally designed regimens. For example, cocktails of cytotoxic agents, which were carefully designed based on mechanisms of action, dose, and scheduling considerations, have led to dramatic improvements in survival including cures for childhood leukemia, Hodgkin's lymphoma, and several other complex cancers. Outcome for glioblastoma, the most common primary malignant CNS cancer, has been more modest, but nonetheless our current standard of care derives from confirmation that combination therapy surpasses single modality therapy. Immunotherapy has recently come of age for medical oncology with exciting therapeutic benefits achieved by several types of agents including vaccines, adoptive T cells, and immune checkpoint inhibitors against several types of cancers. Nonetheless, most benefits are relatively short, while others are durable but are limited to a minority of treated patients. Critical factors limiting efficacy of immunotherapeutics include insufficient immunogenicity and/or inadequate ability to overcome immunosuppressive factors exploited by tumors. The paradigm of rationally designed combinatorial regimens, originally established by cytotoxic therapy for oncology, may also prove relevant for immunotherapy. Realization of the true therapeutic potential of immunotherapy for medical oncology and neuro-oncology patients may require development of combinatorial regimens that optimize immunogenicity and target tumor adaptive immunosuppressive factors.

Keywords: glioblastoma, immunotherapy, immune checkpoint, programmed-death 1, vaccine.

The past few years have seen remarkable outcomes achieved by a variety of immunotherapeutic strategies across a spectrum of cancers. Nonetheless, with the exception of chimeric antigen receptor (CAR) T cell therapy targeting CD19 for B lineage leukemia, where the majority of treated patients have achieved deep and durable antitumor benefit,[1,2] outcomes achieved by single agent immunotherapeutics have been limited to either relatively short duration of benefit or benefit achieved by a relatively small percentage of treated patients. Sipuleucel-T, the first noninfectious, agent-based vaccine approved for cancer therapy, achieved approval for patients with metastatic, advanced prostate cancer based on a 4.1 month median overall survival (OS) improvement over placebo,[3] while the CTLA-4 inhibitor ipilimumab has received approval for metastatic melanoma based on a 3.6 month median survival benefit.[4] The humanized anti-PD-1 monoclonal antibodies nivolumab and pembrolizumab are associated with unprecedented $>$ 5-year survival for metastatic melanoma, a disease previously regarded as treatment refractory and as deadly as glioblastoma, but only 10%–15% of patients achieve this success. These modest, yet encouraging, results provide proof-of-concept that immunotherapy can successfully battle cancer but, as with most important advances, additional questions arise. Are these successes a glimpse of the potential power of the immune system, or are they the best we can hope to achieve? Why are some cancers more responsive to immunotherapy than others? Are there bona fide immunotherapy refractory malignancies? For neuro-oncology, are CNS tumor patients different from other cancer patients? As detailed in a comprehensive, recent review, historical dogma regarding immunoprivilege of the CNS has been steadily replaced by data demonstrating a dynamic and effective

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interaction of the immune system between systemic and CNS compartments.[5]

Nonetheless, the answers to these important questions regarding the anticancer potential of immunotherapy lie in addressing 2 fundamental, yet paramount, considerations: (i) how robust is the tumor-specific immune response generated by a given immunotherapeutic, and (ii) is the antitumor immune response able to sufficiently overcome self-protective, immunosuppressive strategies generated by cancers? As detailed by Weller and Heimberger in this supplement, tumors—especially high-grade gliomas—exploit multiple mechanisms systemically as well as within the tumor microenvironment to abrogate antitumor immune responses. Although single- agent immunotherapy approaches may be able to address either immunogenicity or immunosuppression considerations, carefully designed combinatorial regimens will likely be required to address both issues effectively and ensure that a sufficiently potent antitumor immune response has been achieved and that the immunosuppressive barriers erected by tumors are sufficiently negated to allow ample opportunity for immune attack.

Immunotherapy Combined With Cytotoxic Therapy

Cytotoxic radiotherapy and chemotherapy, established therapeutic cornerstones for many malignancies including high-grade glioma, have historically been considered immunosuppressive and thus appear to be paradoxical partners for immunotherapy. Nonetheless, an intact immune system has been linked with enhanced antitumor activity for both radiation therapy and chemotherapy in preclinical cancer models, suggesting that immune responses contribute to the effectiveness of cytotoxic therapy.[6,7] Furthermore, growing data link several mechanisms by which cytotoxic agents may lead to more robust antitumor immune attack.[8–13] Prominent among these is the induction of immunogenic cell death, which is defined by 3 primary components: (i) translocation of calreticulin from endoplasmic reticulum to the cell surface, where it activates dendritic cells (DCs); (ii) ATP release, which recruits and matures DCs and upregulates CD40, CD80, and CD86 costimulatory molecules; and (iii) extracellular release of high-mobility group protein B1, which binds to pattern recognition receptor toll-like receptor 4 (TLR4) on DCs and enhances antigen cross-presentation and secretion of proinflammatory cytokines.[7,14]

Beyond induction of immunogenic cell death, radiation therapy and chemotherapy can enhance antitumor immune responses by myriad additional mechanisms as summarized in Table 1.[15–40] The abscopal effect, which manifests as a radiation therapy-induced tumor response outside the targeted field, represents a striking example of the ability of cytotoxic therapies to generate systemic antitumor immune responses.[41] Temozolomide, an alkylating agent approved for the treatment of glioblastoma, has been shown to enhance antiglioma immune activity by different mechanisms that appear to be dose/schedule dependent including: (i) depletion of Tregs when administered in a metronomic dosing schedule[22,23]; (ii) generation of increased levels of cytotoxic T cells relative to Tregs during homeostatic proliferation following standard adjuvant dosing[42]; and (iii) marked expansion of antigen-specific CD8+ T cells following myeloablative dosing.[43]

Nonetheless, radiation and chemotherapy can also exert potent immunosuppressive actions including: (i) increased TGF-β activation[44]; (ii) fostering an M2 macrophage phenotype[45]; (iii) increased proportion of Tregs[46]; (iv) increased tumor cell PD-L1 expression induced by INFγ secreted by CD8+ T cells[47]; and (v) profound and sustained levels of lymphopenia.[48–50] Thus, the net effect of cytotoxic therapy on antitumor immune responses will likely reflect an imbalance of immune-enhancing versus immunosuppressive actions. Important variables that may impact this balance warrant further investigation and include dose, schedule and, for chemotherapy, agent of choice.

To date there has been limited preclinical investigation of cytotoxic agents combined with immunotherapeutics for glioblastoma. Temozolomide can enhance vaccine efficacy when administered via low, daily (metronomic), or myeloablative dosing schedules,[43,51] while stereotactic radiation therapy can augment the antitumor activity of PD-1 blockade.[52] Nonetheless, there is substantial effort underway to evaluate immunotherapy combined with cytotoxic therapy in the clinic. Table 2 presents a partial listing of ongoing clinical trials for newly diagnosed glioblastoma patients. Similarly, there is growing interest in evaluating immune checkpoint blockade with radiation therapy administered as either whole brain or stereotactic radiosurgery in patients with brain metastases. Trials to date focus on CTLA-4 blockade with radiotherapy for melanoma metastatic to the brain (NCT02115139, NCT02097732, NCT02107755, NCT01703507, and NCT01950195) while additional trials evaluating other immune checkpoint inhibitors as well as other types of brain metastases are in active planning.

Although many trials are underway, limited data are available from completed trials that evaluated immunotherapy integrated with cytotoxic therapy for glioblastoma. Encouraging outcomes have been reported for single-arm studies evaluating vaccines derived from autologous DCs pulsed with tumor lysate,[53–58] tumor-associated antigens,[55,59] heat-shock protein peptide complex-96 (HSPPC-96),[60] and EGFRvIII peptide.[42,61,62] The only randomized, placebo-controlled clinical trial data to date derive from a preliminary outcome analysis of ICT-107, a vaccine consisting of autologous DCs pulsed with a cocktail of 6 tumor-associated antigens administered with standard radiation and temozolomide for newly diagnosed glioblastoma patients (NCT01280552). Although the impact of ICT-107 administration on OS did not reach statistical significance for the intent-to-treat population in this study, a subset analysis of HLA-A2 positive patients revealed that ICT-107 led to a 4-month improvement in both PFS and OS among *MGMT*-unmethylated patients and an improvement from 8.5 to 24.1 months in PFS for MGMT-methylated patients (HR: 0.259; $P = .005$), while the median survival for *MGMT*-methylated patients has not been reached.[63]

Immunotherapy Combined with Antiangiogenic Therapy

The precise impact of antiangiogenic agents on the pathophysiology of many cancers including glioblastoma is not clear. Nonetheless, growing data support the ability of antiangiogenic

Table 1. Potential mechanisms cytotoxic radiotherapy and chemotherapy can enhance immune system activity

Mechanism	Radiotherapy	Chemotherapy	Reference(s)
Immunogenic cell death	√	√	7,14
Chemokine induction (CXCL9/10/11)	√	√	24,35,40
Proinflammatory cytokine production	√	√	15,33
Increase MHC class I molecule expression	√		17
Increase co-stimulatory molecule expression	√		18,19
Decrease Tregs		√	22,23,28
Increased CD8 + effector cells (homeostatic proliferation)		√	32,40
Decrease inhibitory immune checkpoint molecules		√	36
Decrease myeloid suppressor cells		√	37–39
Recruit/activate dendritic cells		√	25,27

Table 2. Partial listing of ongoing clinical trials evaluating immunotherapy and cytotoxic therapy for newly diagnosed glioblastoma

Immunotherapy/Agent	Phase	# Patients	Accrual Status	Clinicaltrials.gov	Comment
EGFRvIII peptide vaccine/Rindopepimut	3	700	Completed	NCT01480479	Randomized; double-blind; placebo-controlled;
Tumor lysate loaded DCs/DCVax®-L	3	300	Ongoing	NCT00045968	Randomized; double-blind; placebo-controlled;
Tumor lysate-loaded DCs	2	100	Ongoing	NCT01567202	Randomized; double-blind; placebo-controlled;
Tumor-associated antigen vaccine/ICT107	2	124	Completed	NCT01280552	Randomized; double-blind; placebo-controlled
Tumor-associated antigen vaccine/ IMA950	1/2	16	Ongoing	NCT01920191	IMA950
Autologous and allogeneic tumor-specific neoantigens/APVAC	1	30	Ongoing	NCT02149225	
Autologous tumor-specific neoantigens/ NeoVax	1	16	Ongoing	NCT02287428	MGMT-unmethylated patients only; excludes temozolomide
Autologous DCs + allogeneic stem cell lysate	1	40	Ongoing	NCT02010606	
Autologous DCs + allogeneic stem cell lysate	1	10	Ongoing	NCT01957956	
Autologous DCs loaded with CMV pp65-LAMP RNA	1	16	Ongoing	NCT00639639	
Autologous DCs loaded with CMV pp65-LAMP RNA + basliximab	1	18	Ongoing	NCT00626483	
PD-L1 mAb/MEDI4736	2	37	Ongoing	NCT02336165	MGMT-unmethylated patients only; excludes temozolomide

agents to shift the tumor microenvironment phenotype from immunosuppressive to immunosupportive.[64,65] Vascular endothelial growth factor (VEGF), the primary proangiogenic growth factor in many cancers including malignant glioma, contributes significantly to the immunosuppressive ability of tumors.[66–68] Specifically, VEGF can inhibit DC maturation and antigen presentation, induce apoptosis of CD8+ T cells, enhance Treg activity, and diminish infiltration of T cells across tumor endothelium.[69–72] Of note, preclinical studies suggest that immunotherapeutics may be combined with VEGF inhibitors to generate enhanced antitumor benefit.[67–79] The rationale underlying the combination of immunotherapy approaches with antiangiogenic agents is based on the ability of VEGF inhibition to diminish immunosuppressive features of tumors[69–72,76,78,79] and enhance the antitumor activity of immunotherapies.[73,75,76,78,79] Furthermore, preclinical strategies to normalize tumor vasculature, including administration of anti-VEGF therapy, can shift tumor-associated macrophages from an immune-inhibitory M2-like phenotype toward an immune-stimulatory M1-phenotype, increase tumor infiltrating CD8+ T cells, and enhance survival following whole tumor cell vaccination.[74]

Data from a recently published phase 1 study among metastatic melanoma patients revealed that administration of bevacizumab with ipilimumab, an inhibitor of the CTLA-4

immune checkpoint, led to improved OS relative to historical benchmarks of ipilimumab as well as evidence of increased intratumoral immune-cell trafficking.[80] For glioblastoma, data have been reported from only one clinical trial evaluating immunotherapy plus antiangiogenic therapy. In the ReACT study, 70 patients with EGFRvIII-positive glioblastoma at first or second recurrence were randomized to receive bevacizumab plus placebo vaccine versus bevacizumab plus the EGFRvIII peptide vaccine, rindopepimut. In this double-blind, randomized, placebo-controlled study, rindopepimut recipients achieved a significantly longer OS (HR: 0.57; $P = .0386$). Administration of rindopepimut also conveyed a modest, yet not statistically significant, improvement in PFS (HR: 0.79; $P = .3756$) as well as a higher rate of durable (≥6 mo) radiographic responses.[81] Importantly, these data represent the first randomized clinical trial to demonstrate a survival benefit associated with any type of immunotherapy for glioblastoma to date. Although the results of this trial indicate that rindopepimut improved outcome achieved by bevacizumab, it is not clear whether bevacizumab improved the outcome of rindopepimut because the trial lacked a rindopepimut-alone arm. Nonetheless, the overall results of this study support further clinical trials evaluating combinatorial regimens of immunotherapeutics plus antiangiogenic agents for glioblastoma. Currently, ongoing clinical trials evaluating this approach include trials that combine bevacizumab with: (i) PD-1 blockade (NCT02337491); (ii) PD-L1 blockade (NCT02336165); (iii) HSPPC-96 vaccine (NCT01814813); (iv) autologous tumor lysate vaccine (NCT02010606); or (v) a vaccine derived from combined autologous/allogeneic tumor lysates (NCT01903330).

Immunotherapy Plus Immunotherapy Combinatorial Strategies

Among possible combinatorial strategies for immunotherapy, the most exciting involves combining immunotherapeutics with complementary mechanisms of antitumor immune attack. As previously described, the efficacy of immunotherapeutics against cancer is ultimately dependent on 2 factors: (i) immunogenicity (ability to generate an immune response); and (ii) tumor self-protective immunosuppression strategies. A major contributing factor limiting the overall efficacy of most immunotherapeutics to date, which typically reflects single-agent therapy experience, is an inability to adequately address both of these factors.

One factor that may impact the immunogenicity of cancer vaccines is choice of antigen. Many vaccines target tumor-associated antigens. Immunoreactivity induced by these vaccines is predicted to be relatively low because tumor-associated antigens can also be expressed by normal tissues and may therefore evoke immunotolerance. In contrast, vaccines targeting tumor-specific antigens, which by definition are uniquely expressed by tumor cells and are not present on normal tissues, are expected to generate more potent immune responses that are not limited by normal self-tolerance mechanisms.

Another factor likely limiting the efficacy of cancer vaccines is that tumors can escape immunogenic immune responses induced by vaccines by downregulating target antigen expression or by expanding an existing subset of cells that lack target antigen expression. For example, among glioblastoma patients treated with the EGFRvIII-targeting peptide vaccine rindopepimut, expression of EGFRvIII was no longer detectable at the time of confirmed recurrence.[62] This finding suggests that targeting multiple tumor-specific antigens may lessen the likelihood of immune escape and thereby generate more durable antitumor benefit compared with vaccines targeting a single antigen or a small number of antigens.

An insurmountable therapeutic hurdle for glioblastoma to date is the remarkable degree of heterogeneity within individual tumors.[82,83] Given this challenge, it is not surprising that cytotoxic agents achieve modest benefit at best, while targeted molecular agents have essentially failed, even among genetically enriched patient populations.[84,85] Exploiting the mutanome or constellation of tumor-specific mutations within a given tumor, which include both passenger and driver mutations, represents a challenging yet highly exciting opportunity for immunotherapy. Multiple studies point to the critical relationship between immune responses against tumor-specific mutations often referred to as neoantigens and effective tumor control.[86–92] In recent analyses, expression of a panel of tumor-specific neoantigens was demonstrated to be a critical predictor of long-term response following immune checkpoint therapy among patients with advanced melanoma[93] or non–small cell lung cancer.[94] The ability to target a spectrum of tumor-specific mutations, even if heterogeneously expressed within a given tumor, provides immunotherapy a unique opportunity to effectively exploit the challenge posed by intratumoral heterogeneity for therapeutic benefit.

On the other hand, immunosuppressive adaptations exploited by tumors can essentially neutralize antitumor immune responses regardless of the potency, specificity, and breadth. As detailed by Weller and Heimberger in this supplement, glioblastomas invoke a wide array of immunosuppressive strategies that include systemic factors such as lowered levels of T cell responsiveness, immunoglobulins, and monocyte/dendritic function as well as increased Tregs.[95–98] Even more remarkable are the host of immunosuppressive factors generated by tumors that function in the immediate microenvironment to act as a localized shield or cloaking device against antitumor immune attack including: (i) expression of multiple immunoinhibitory molecules including TGF-β, STAT-3, VEGF, IL-10, prostaglandin E2, tryptophan, and lectin-like transcript 1[99–104]; (ii) downregulation of MHC molecule expression[101,105,106]; (iii) enhanced infiltration of Tregs[101,102,107–111]; (iv) polarization of microglia and tumor-associated macrophages toward an M2 phenotype[112,113]; (v) upregulation of Fas ligand[114,115]; (vi) impaired T cell function due to hostile factors including hypoxia[116]; and (vii) increased expression of inhibitory immune checkpoint molecules such as PD-L1, which was recently described to be prominently expressed by 80%–90% of glioblastoma tumors[117] as well as the other B7H family molecule, B7H3[104,118]

Given the plethora of immunosuppressive mechanisms utilized by tumors including glioblastoma, combinatorial regimens will likely require one or more components that block immunosuppressive mechanisms. Certainly, the emergence of humanized mAbs able to block the CTLA-4, PD-1, and PD-L1 immune checkpoints offer much hope for oncology patients. Although the role of these agents for neuro-oncology patients is currently being defined in ongoing clinical trials, preclinical studies against orthotopic, immunocompetent glioblastoma models

demonstrate exciting efficacy including evidence of enhanced benefit with combinatorial regimens.[52,119–121] Combinations of immune checkpoint inhibitors such as PD-1 plus CTLA-4 blockade offer potential synergy to expand effector T cells while decreasing regulatory T cells and myeloid suppressor cells.[122] Furthermore, enhanced therapeutic benefit has been observed with such regimens in preclinical GBM models[119,121] and in patients with advanced melanoma.[123] However, such regimens may also be limited clinically by enhanced immune-mediated toxicity.[123]

Additional therapeutics against immunosuppressive mechanisms invoked by glioblastoma tumors are slowly evolving, but further research is critically needed. Initial results of a phase 1 clinical trial evaluating galunisertib (LY2157299;. an oral inhibitor against TGF-β) are encouraging,[124] although phase 2 results in monotherapy or in combination with lomustine are negative.[125] A clinical trial combining this agent with the PD-1 inhibitor nivolumab will begin in the near future (NCT02423343). Another ongoing study is evaluating the role of Treg targeting and incorporates basiliximab in combination with a vaccine (NCT00626483). In addition, VEGF inhibition plus immunotherapy warrants further investigation, as previously discussed.

Finally, a "ying-yang" combinatorial strategy of high appeal includes immunotherapy regimens integrating an agent capable of generating a robust antitumor immune response (eg, a vaccine against tumor specific antigens) with an agent capable of targeting tumor immunosuppression (eg, a checkpoint inhibitor). Although checkpoint blockade therapy alone may help relieve local immune suppression and overcome T cell exhaustion or anergy, it may be constrained by the size and specificity of the existing T cell population (T cells arising from the normal physiological presentation of the evolving tumor to the host immune system). This consideration may be particularly relevant for tumors that are considered relatively less immunogenic. The lack of coincident, focused immune stimulation may thus be a critical factor that limits maximal efficacy of checkpoint inhibition therapy, a deficit that may be overcome by an effective vaccine. Indeed, in many preclinical animal studies, the antitumor activity of immune-checkpoint blockade was dramatically enhanced when combined with a vaccine.[126–130] Although clinical data are only beginning to emerge, enhanced activity has also been observed among patients treated with combinatorial tumor vaccination plus immune-checkpoint blockade regimens.[131–133]

Conclusion

The ability of immunotherapies to achieve their therapeutic potential for medical and neuro-oncology will likely depend on the development of rationally designed combinatorial regimens capable of optimizing potent antitumor immune responses while effectively negating self-protective immunosuppressive strategies exploited by many tumors. Glioblastoma tumors exhibit a profound repertoire of immunosuppressive adaptations. Nonetheless, multiple opportunities are emerging to incorporate immunotherapies into regimens that may enhance immunogenicity while targeting immunosuppression including combinations with cytotoxic agents, antiangiogenics, and other immunotherapeutics. Critical considerations for these regimens include evaluation of complementary mechanisms of action, dose, and scheduling. In addition, effective measurement of immunogenicity through incorporation of immunocorrelative assays, as well as investigation of changes in immunosuppressive factors, will also be of great value in ascertaining why or why not these agents succeed for our patients.

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