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Applied Cancer Immunogenomics: Leveraging Neoantigen Discovery in Glioblastoma

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Abstract

Glioblastoma (GBM) remains a significant cause of cancer-related mortality in pediatric and adult patients with limited treatment options. Immunotherapy represents a promising new therapeutic approach in many solid and hematologic malignancies, including GBM, though only a subset of patients respond clinically. Thus, current efforts are focused on identifying patients most likely to benefit from immune-based therapies. The cancer immunogenomics approach identifies candidate neoantigens from genomics information and represents a potentially exciting new space in precision neuro-oncology. In this review, we discuss the role of neoantigens in GBM as both predictive biomarkers and as targets of immunotherapy.

Keywords

glioblastoma; neoantigen; biomarker; personalized vaccine; immunogenomics; hypermutated

The Clinical Problem of Glioblastoma

Glioblastoma continues to represent an extremely challenging cancer to treat. There are approximately 13,000 new cases of glioblastoma (GBM) diagnosed each year with a median survival of less than 15 months, making GBM the most common and fatal primary central nervous system (CNS) malignancy in adults^{1,2}. Treatment involves a multimodality

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approach incorporating maximal surgical resection followed by adjuvant radiotherapy and temozolomide chemotherapy². Presently, the addition of temozolomide represents the only adjuvant treatment to improve outcomes in patients with GBM over standard radiotherapy². Moreover, once GBM recurs within 6-9 months, there is no gold standard of treatment demonstrated to prolong survival. Thus, there is an urgent and unmet need for more effective therapies for both newly-diagnosed and recurrent GBM.

In Search of Genomics-based Precision Neuro-Oncology

The multi-platform profiling of human cancer genomes has generated high-resolution pictures of the genomic alterations that underlie all cancers and revolutionized our understanding of cancer biology and clinical oncology. From a precision medicine perspective, the discovery of recurrent “driver” mutations using these approaches has guided targeted therapeutic approaches in a number of cancer types³. Unfortunately, this “mutation-to-drug target” paradigm has not yet been successfully applied to GBM⁴. This failure is not due to a lack of understanding of the genomic landscape of GBM; on the contrary, it was the first TCGA study published⁵, and to date there has been extensive characterization of these cancers⁶. It is likely that several features of this tumor type pose significant challenges to targeted therapeutic approaches. GBM is incredibly heterogeneous⁷ and, in fact, there are likely several different diseases within each patient's tumor. Broadly speaking, GBM exhibits both cellular and molecular heterogeneity⁸⁻¹⁰ such that it is likely difficult to identify the critical tumor-intrinsic biological dependencies that can be disrupted therapeutically⁷. Furthermore, many compounds cannot cross the blood-brain barrier. Thus, further work is needed to apply classical precision medicine-based approaches to GBM.

Cancer Immunogenomics: the “Mutation-to-Antigen” Paradigm in Glioblastoma

Cancer immunogenomics represents a complementary approach to the application of genomics to precision neuro-oncology. In this methodology, candidate tumor-specific neoantigens are identified from expressed exome alterations predicted to bind with high affinity to a patient's specific HLA molecules¹¹. Thus, rather than classifying alterations into “drivers” and “passengers” by inferred biological function, mutational hierarchy is defined by predicted antigenicity, thus establishing a “mutation-to-antigen” paradigm. Although the details of this approach are reviewed extensively elsewhere¹², we will focus on its applications to malignant glioma. In the case of brain cancer, this perspective creates new opportunities in leveraging genomic information in developing immune-based therapeutics. Most pragmatically, the time is right: in the absence of other effective treatments, there has been growing enthusiasm for immunotherapy for patients with glioblastoma; it is clear that the central nervous system is not hermetically immunoprivileged as previously thought¹³, and there are an increasing number of exciting clinical trials in this disease area¹⁴.

Within the expanding portfolio of brain tumor immunotherapy, it is likely that cancer immunogenomics will play an increasingly important role. In the setting of GBM, we will review two neoantigen-based classifications that could guide distinct immune-based treatments (Figure 1). Specifically, a number of studies have suggested that there exists a

threshold number of neoantigens to which an immune response may be augmented by checkpoint blockade immunotherapy. In this setting, the neoantigen burden may serve as a predictive *biomarker* of response to such therapy. Alternatively, a number of clinical trials are designed around therapeutically targeting individual neoantigens in personalized vaccines (Table 1). In this setting, individual neoantigens themselves represent distinct *targets* for immunotherapy. We will explore the concept of neoantigens as biomarkers and targets in GBM in the following sections.

Cancer Immunogenomics Application #1: Neoantigens as Biomarkers

Neoantigens as Biomarkers in Cancer

Checkpoint blockade immunotherapy has demonstrated significant clinical efficacy in a broad range of solid and hematologic malignancies¹⁵. However, because not all patients derive benefit from this therapy, the search for predictive biomarkers to identify those patients most likely to benefit from checkpoint blockade therapy is an intense area of investigation. To date, the most widely used predictive biomarker of response to PD-1:PD-L1-targeted therapy remains the expression level of PD-L1 on tumor cells^{16,17}. While higher expression levels of PD-L1 have correlated with higher overall response rates in multiple tumor types, the observation that patients with PD-L1 negative tumors also can exhibit some clinical responses suggests that PD-L1 status alone is insufficient to stratify patients.

In order to develop biomarkers that more closely predict clinical response and also may be useful in checkpoint blockade treatments outside of the anti-PD-1/PD-L1 classes, it is important to consider the cellular and molecular immunobiology of the anti-tumor immune response as a “pathway”¹⁸ in which multiple components should be integrated. Specifically, the pivotal endpoint in the development of the anti-tumor immune response is the recognition of MHC-presented antigen by the T cell receptor, and it is therefore understandable that parameters of this nexus are emerging as critical components of biomarker discovery in the checkpoint treatment setting to complement PD-L1 characterization^{19,20,21}.

In this perspective, several groups have demonstrated that the total burden of mutations—and, by inference, the likely suite of potential neoantigens recognized by T cells—correlates strongly with response to checkpoint blockade. In a landmark paper by Le and colleagues²², patients with hypermutated colorectal cancers due to underlying defects in DNA mismatch repair (MMR) genes demonstrated remarkable responses to Pembrolizumab. In this small subset of patients, the objective response rate was 40% with a disease control rate of 90% compared to 0% and 11%, respectively, in MMR-proficient patients. Importantly, a similarly high objective response rate (71%) was seen in non-colorectal MMR-deficient tumors, suggesting that hypermutation may be predictive across cancer types. Although this study did not predict candidate neoantigens *per se*, it was clear that the engine for neoantigenesis was present: MMR-deficient tumors harbored a mean of 1,782 nonsynonymous mutations per tumor while the MMR-proficient tumors averaged only 73 mutations. Several additional important studies have also demonstrated a similarly strong association between increased mutational burden and response to checkpoint blockade therapy in NSCLC²³, melanoma²⁴⁻²⁶, and bladder cancer²⁷. In two of these studies, specific predicted neoantigen

burden correlated with response as well. Thus, increasing evidence suggests that mutational burden can correlate with clinical response to checkpoint blockade.

Neoantigens as Biomarkers in Glioblastoma

In the case of glioblastoma, there are at least two clinical settings in which patients' tumors harbor high mutational burdens that may predict improved responses to checkpoint blockade. Although neoantigens were not predicted in every study, we continue to use high mutational burden as a proxy for this analysis. In the newly-diagnosed setting, GBMs typically carry a modest mutational burden of approximately 70-100 exome-wide mutations per tumor^{6,28,29}. However, a small subset of glioblastoma patients with germline or somatic mutations in the polymerase ϵ gene (*POLE*) exhibit an ultramutated genotype, and recent clinical vignettes suggest that these patients may also benefit from checkpoint blockade. Erson-Omay and colleagues identified 2 of 91 adult patients harboring 10,024 and 6,174 DNA exome mutations, respectively³⁰, along with 2 pediatric patients that carried 4,813 and 4,918 mutations, respectively. Interestingly, 3 of these 4 patients were also found to carry germline *MSH6* mutations, suggesting that somatic *POLE* alterations resulted from antecedent DNA MMR lesions.

Given the study of Erson-Omay³⁰, the central applied immunogenomics translational question remains: if we can identify glioblastoma patients with exceptionally high mutational burdens that may yield a rich repository of neoantigens, will they respond to immunotherapy? Two recent studies provided compelling data to suggest that the answer to this question may be "yes". In the adult setting, we recently described the case of a 31 year old man with a GBM harboring over 10,000 DNA exome mutations in the setting of a *POLE* L212V germline mutation³¹. We applied our internal cancer immunogenomics pipeline^{12,32} and identified 1,245 expressed predicted neoantigens within the founder clone alone. When the patient's tumor recurred as a spinal drop metastasis, he was treated with Pembrolizumab. The pseudoprogression of another spinal metastasis prompted surgical resection, and analysis of pre- and post-Pembrolizumab tissue revealed that checkpoint blockade induced a brisk lymphocyte infiltrate associated with a robust IFN- γ -induced gene expression signature. In a similar study by Bouffet *et al.*, the genomic landscape of pediatric GBM associated with germline biallelic MMR deficiency was explored. Interestingly, tumors arising in this setting harbor a hypermutated genotype and, in turn, extremely high neoantigen loads. Consistent with the prior study by Erson-Omay, two siblings carrying biallelic germline *PMS2* deficiency with an acquired *POLE* alteration developed tumors harboring over 20,000 mutations. Strikingly, both patients in this study were treated with Nivolumab at recurrence and demonstrated durable clinical responses. Thus, these early studies together suggest that appropriately identifying patients with high mutational loads represent a subset of patients that may benefit from checkpoint blockade immunotherapy. While these patients are rare, some geographical regions may harbor higher incidences of MMR deficiency³³. Thus, every effort should be made to identify this unique patient subset in order to maximize treatment options, potentially by even moving immunotherapy into the first-line setting if these initial observations can be validated in larger trial settings.

The second clinical scenario in which glioblastoma patients may benefit from checkpoint blockade immunotherapy is at recurrence following temozolomide therapy. Although GBMs with *de novo* deficiencies in DNA repair mechanisms are comparatively rare, recent studies have demonstrated that an estimated 20-30% of recurrent GBMs exhibit the hypermutated genotype^{5,34-37}. Most commonly, these tumors display mutations in the MMR pathway—such as MSH6, MSH2, PMS2, etc—either through acquisition during treatment or by the selection of pre-existing MMR-deficient subclones^{38,39}. Thus, those patients with resulting hypermutated recurrent tumors may represent cohorts that are highly suitable for checkpoint blockade immunotherapy. The pursuit of this possibility will change management for the majority of patients with recurrent glioblastoma because most patients do not undergo tissue sampling of their recurrence unless a redo-craniotomy or biopsy at recurrence is performed. It may be time to consider placing more importance on sampling recurrent disease to search for those patients who are hypermutated and may benefit from checkpoint blockade instead of exposing them to potentially futile second line alkylating agents such as CCNU, as MMR-deficient tumors would be completely resistant. Importantly, whether the clonal architecture of this abundant neoantigen landscape in the hypermutated setting will influence responses to checkpoint blockade in brain tumors remains an open question⁴⁰.

Several recent studies have identified pragmatic methodologies with which to identify the hypermutated genotype that are already part of the clinical workflow. These approaches are based on the hypothesis that the hypermutated genotype is distributed well enough across the exome such that focused cancer gene panel sequencing approaches, rather than more extensive and costly whole exome sequencing methods, may capture this genomically disrupted state. To this end, Stadler *et al.* clearly demonstrated that hypermutated tumors correlated with 20 mutations identified by a 341-gene panel, while non-hypermutated tumors harbored <20 alterations⁴¹. Johnson *et al.* extended these findings to show that melanoma patients with a higher mutation per megabase rate as captured on the commercial FoundationOne cancer gene panel exhibited improved responses to anti-PD-1/PD-L1 immunotherapy²⁶. Thus, these studies show that the neoantigen biomarker state may be captured by focused gene panel sequencing approaches already in clinical use across many institutions.

Cancer Immunogenomics Application #2: Neoantigens as Targets in Glioblastoma

Targeting neoantigens individually using personalized vaccine strategies is a second exciting application of cancer immunogenomics in GBM. Aside from the clinical settings of hypermutation as described, most GBMs harbor a modest number of somatic mutations⁶ when compared to cancers with non-hypermutated but elevated mutational loads such as NSCLC, melanoma, and bladder, in which single agent checkpoint blockade immunotherapy confers durable clinical responses. Rooney *et al.* described a cohort of GBM tumors in the TCGA in which tumors harbored approximately 70 nonsynonymous mutations (range 2 to 258) that were predicted to generate roughly 10 neoantigens (range 0 to 51)²⁹. Thus, while it is likely that this group of patients may not be the most suitable for checkpoint blockade monotherapy, there is considerable enthusiasm behind the possibility that these neoantigens

can serve as specific targets against which patients can be treated using personalized vaccine strategies.

EGFRvIII as a Neoantigen Target

Vaccination against an intragenic deletion product of the epidermal growth factor receptor (EGFR) antigen represents one recent and well-studied example of a neoantigen target vaccine in GBM. *EGFR* is amplified in over 50% of GBM, and approximately 50% of those tumors express the EGFRvIII variant^{6,42}. This mutant variant of EGFR is generated by an in-frame deletion of 267 amino acids of the extracellular domain of the wild type EGFR. The resulting EGFRvIII mutant receptor acquires constitutive signaling capacity and also may promote tumor growth in a paracrine manner⁴³. Importantly, the junctional sequence spanning the in-frame deletion represents a *bona fide* neoantigen and was the focus of the Rindopepimut vaccine that was licensed by Celldex. This vaccine conjugates KLH to a 13-mer peptide spanning the junctional region of EGFRvIII. Although Rindopepimut treatment resulted in significant improvement in overall survival for patients with EGFRvIII⁺ GBM in early phase studies when given concurrently with adjuvant temozolomide⁴⁴, the recent ACT IV randomized phase III trial failed to show a survival benefit.

It is important to highlight several issues that stem from the Rindopepimut experience because it is not the end of the neoantigen targeting effort in GBM—rather, it is the beginning. First, there was a better-than-expected survival in the control group relative to historical controls, which may suggest a small but significant benefit of immunostimulatory adjuvants used such as KLH and/or GM-CSF. In addition, it is possible that single neoantigen targets are insufficient in vaccine approaches. EGFRvIII is known to be heterogeneously expressed by a subset of tumor cells⁴⁵, and prior work has shown that Rindopepimut vaccination can lead to the emergence of EGFRvIII-negative escape variants⁴⁶. Thus, it may be necessary to select vaccine targets that are not only clonal but also exhibit reasonable levels of expression. In the case of EGFRvIII, it is also not clear if a specific HLA haplotype would benefit more from this peptide vaccination, especially as immune monitoring largely demonstrates a predominantly humoral response⁴⁷. Thus, it will likely be critical to design polyvalent vaccines that align with HLA binding predictions to optimize vaccine efficacy by targeting broadly expressed neoantigens and inducing cellular immunity. Nevertheless, the experience with EGFRvIII was certainly not a failure and, on the contrary, will surely represent the opening salvo in the quest to bring neoantigen-based vaccines to patients.

Mutant IDH1 as a Neoantigen Target

The finding of recurrent mutations in the isocitrate dehydrogenase 1 (*IDH1*) gene represents one of the most important discoveries in brain tumors over the last decade⁴⁸. The vast majority of mutations occur at position R132, with over 90% being R132H substitutions⁴⁹. This hemizygous mutation is found in the majority of grades II-III gliomas and a smaller percentage of primary GBM, although “secondary” GBM harbor a higher percentage. Clinically, *IDH1* mutant tumors occur in younger patients who exhibit an improved overall survival⁵⁰. Our understanding of mutant IDH1 function stems largely from the landmark work of Dang *et al*⁵¹. Although IDH1 typically catalyzes the NADP⁺-dependent production

of alpha-ketoglutarate (α -KG) from isocitrate, mutant IDH1 proteins perform a neomorphic function by catalyzing the NADPH-dependent reduction of α -KG to produce the D stereoisomer (or “R”-enantiomer), 2-hydroxyglutamate (2-HG), which is undetectable in the absence of the mutation. *IDH1* mutations are thought to be acquired early in transformation and are therefore observed to be clonal.

Taken together, mutant IDH1 is a compelling neoantigen vaccine target as it is both clonal and broadly expressed in all tumor cells. Moreover, the development of non-invasive magnetic resonance spectroscopy has enabled the prospect of using imaging as a proxy for the presence or absence of the functional 2-HG neoantigen itself⁵². To this end, Schumacher *et al.* demonstrated both cellular and humoral evidence of spontaneous immune responses to an R132H-containing long peptide in patients with *IDH1*-mutant gliomas⁵³. *In silico* analysis suggested that R132H-containing peptides may bind with high affinity to MHC class II HLA-DRB molecules, and this possibility was supported by evidence of CD4⁺ T cell responses generated to mutant IDH1 R132H vaccines in humanized mouse models. The same group then used an elegant proximity ligation assay to show that IDH1 R132H-derived MHC class II peptides are presented by HLA-DR molecules *in situ*⁵⁴. These data not only provide support for pursuing the mutant IDH1 R132H neoantigen as a vaccine target (Table 1) but also highlight the potential importance of incorporating MHC class II neoantigens in vaccine design to prime functional CD4⁺ T cell responses.

Polyvalent Neoantigen Vaccines

Ultimately, neoantigens for vaccine development in GBM will focus on clonality as well as polyvalency. For example, in the case of heterogeneous *IDH1* wild-type GBM where clonal neoantigens are not common, likely targets will be pooled from high-incident subclonal mutations. In preclinical models, the feasibility and efficacy of targeting neoantigens with bivalent vaccines was demonstrated by Gubin *et al.*⁵⁶. As proof-of-principle for this concept in GBM, we applied a cancer immunogenomics approach to two preclinical murine models, the carcinogen-induced GL261 and spontaneously-derived SMA-560 tumors. This approach identified a large number of potential high affinity neoantigen candidates, some of which we credentialed as immunogenic and able to elicit spontaneous neoantigen-specific immune responses detectable in the brain⁵⁵. Notably, not all predicted antigens were spontaneously immunogenic, although this observation does not preclude these particular candidates as therapeutic vaccine targets.

Using the applied cancer immunogenomics pipeline, clinical trials are currently underway in GBM and other malignancies to design personalized neoantigen-targeted vaccines [Table 1]. At Washington University, this approach has already been shown to be feasible in patients, as Carreno *et al.* employed personalized neoantigen vaccines in melanoma patients demonstrating induction of tumor-specific immune responses⁵⁷. Likewise, we have applied this same approach to a patient with newly diagnosed GBM, and were able to identify 9 and 3 predicted HLA class I- and II-restricted patient-specific neoantigens, respectively. Collectively, these studies will generalize the feasibility of personalized vaccine approaches, and correlative studies will be critical to determine how effective these vaccines are at inducing specific immune responses and, most importantly, controlling disease.

Conclusions and Future Directions: Validating Applied Cancer Immunogenomics in GBM

It is an exciting time in oncoimmunology, and the cancer immunogenomics concept will surely influence the immunotherapy space in the coming years. In the management of GBM, we have reviewed two broad approaches to neoantigen discovery and application—as biomarkers and as targets (Figure 1). As with any broad classification scheme, there will be settings in which the distinctions are more fluid. Nevertheless, leveraging neoantigen discovery in GBM represents an active and growing area of investigation in precision neuro-oncology. It would be prudent to characterize the neoantigen burden in both newly diagnosed as well as recurrent GBM patients in order to capture the albeit minority of patients who may harbor highly elevated mutational loads so that checkpoint-based trials may be designed to exploit this landscape. It will also be critical to perform rigorous immune correlative work in those patients undergoing personalized vaccination in order to determine how best to generate effect neoantigen-specific immune responses in patients using these approaches. At the same time, we must be mindful that CNS-specific immunologic deficits have been described⁵⁸ in the pre-⁵⁹ and post-treatment⁶⁰ settings, and therefore considering these phenomena when developing effective anti-tumor immunity in the CNS is essential. Together, these efforts will hopefully lead not only to new and urgently needed personalized immune-based therapies but also to a deeper understanding of fundamental CNS immunobiology.

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Figure 1.

Proposed schema of workflow to incorporate neoantigen burden into treatment decision for patients with GBM. From left to right, tumor tissue following resection will undergo DNA exome and RNA sequencing to quantify the number of expressed, nonsynonymous mutations. Patients with tumors containing high mutational burdens, and thus high neoantigenic potential, would preferentially be treated with checkpoint blockade therapy. Conversely, tumors with reduced mutational load would be targeted with a personalized vaccine with or without checkpoint modulating agents against patient-specific high affinity neoantigen candidates identified using the cancer immunogenomics pipeline.

Table 1

Summary of current clinical trials using either the cancer immunogenomics pipeline to target patient-specific neoantigens or the shared neoantigen, IDH1 R132H, in a personalized vaccine strategy. Abbreviations: GU = genitourinary; HNSCC = head and neck squamous cell carcinoma; NSCLC = non-small cell lung cancer; TNBC = triple-negative breast cancer.

Institution/Sponsor	Phase	Vaccine Platform	Disease Group(s)	NCT #
PERSONALIZED NEOANTIGEN-BASED VACCINE CLINICAL TRIALS				
Washington University School of Medicine	I	Peptide	• Newly diagnosed GBM	NCT02510950
Washington University School of Medicine	I	DNA Peptide	• Newly diagnosed TNBC	NCT02348320 NCT02427581
Neon Therapeutics, Inc Bristol-Meyers Squibb	I	Peptide + Nivolumab	• Recurrent metastatic NSCLC • Recurrent metastatic melanoma • Recurrent metastatic GU carcinoma	NCT02897765
Dana-Farber Cancer Institute	I	Peptide	• Newly diagnosed GBM (unmethylated MGMT) • Newly diagnosed metastatic melanoma	NCT02287428 NCT01970358
Dana-Farber Cancer Institute Bristol-Meyers Squibb Oncovir, Inc	I	Peptide + Ipilimumab	• Newly diagnosed renal cell carcinoma	NCT02950766
MD Anderson	I	Peptide	• Metastatic pancreatic adenocarcinoma or colorectal carcinoma	NCT02600949
Immatics Biotechnologies GmbH	I	Peptide	• Newly diagnosed GBM	NCT02149225
Icahn School of Medicine at Mount Sinai	I	Peptide	Newly diagnosed: • HNSCC • NSCLC • Breast carcinoma • Gynecologic carcinoma • GU carcinoma • Cutaneous SCC	NCT02721043
Biontech RNA Pharmaceuticals GmbH	I	RNA	• Advanced melanoma • Newly diagnosed, resected, TNBC	NCT02035956 NCT02316457
Sichuan University	I	Adoptive T cells	• Recurrent NSCLC	NCT02956551
Uconn Health	I	Peptide	• Newly diagnosed stage III-IV Ovarian carcinoma	NCT02933073
MUTANT IDH1-TARGETED VACCINE CLINICAL TRIALS				

Institution/Sponsor	Phase	Vaccine Platform	Disease Group(s)	NCT #
National Center for Tumor Diseases, Heidelberg	I	Peptide	• Newly diagnosed WHO grade III-IV IDH1-mutated glioma	NCT02454634
Duke University	I	Peptide	• Recurrent WHO grade II IDH1-mutated glioma	NCT02193347
Hebei Tanda Hospital, Beijing	I	Peptide + Nivolumab	• IDH1-mutated glioma	NCT02771301

Abbreviations: GU = genitourinary; HNSCC = head and neck squamous cell carcinoma; NSCLC = non-small cell lung cancer; TNBC = triple-negative breast cancer