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Biomarkers for glioma immunotherapy: the next generation

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Abstract The term "biomarker" historically refers to a single parameter, such as the expression level of a gene or a radiographic pattern, used to indicate a broader biological state. Molecular indicators have been applied to several aspects of cancer therapy: to describe the genotypic and phenotypic state of neoplastic tissue for prognosis, to predict susceptibility to anti-proliferative agents, to validate the presence of specific drug targets, and to evaluate responsiveness to therapy. For glioblastoma (GBM), immunohistochemical and radiographic biomarkers accessible to the clinical lab have informed traditional regimens, but while immunotherapies have emerged as potentially disruptive weapons against this diffusely infiltrating, heterogeneous tumor, biomarkers with strong predictive power have not been fully established. The cancer immunotherapy field, through the recently accelerated expansion of trials, is currently leveraging this wealth of clinical and biological data to define and revise the use of biomarkers for improving prognostic accuracy, personalization of therapy, and evaluation of responses across the wide variety of tumors. Technological advancements in DNA sequencing, cytometry, and microscopy have facilitated the exploration of more integrated, high-dimensional profiling of the disease system—incorporating both immune and tumor parameters—rather than single metrics, as biomarkers for therapeutic sensitivity. Here we discuss the utility of traditional GBM biomarkers in immunotherapy and how the impending transformation of the biomarker paradigm—from single markers to integrated profiles—may offer the key to bringing predictive, personalized immunotherapy to GBM patients.

Keywords Biomarkers · Glioblastoma · Immunotherapy · Immunology · Targeted therapy · Immunosuppression

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Targeting Immunosuppression in GBM

Glioblastoma (GBM) is a WHO grade IV malignant glioma which invariably results in recurrence and mortality despite the current standard of care—maximal safe surgical resection, fractionated radiation, and systemic temozolamide chemotherapy [1]. Patient treatment failure is attributed to GBM cellular heterogeneity and the aggressive diffuse infiltration observed at the tumor margins, which allows resistance to radiotherapy and the cytotoxic agents to propagate under selective pressure [2–5], especially upon tumor recurrence [6], and is further complicated by local and systemic tumor derived immunosuppression. While not yet fully elucidated, the causal mechanisms that link the physiology of the tumor to the dysfunction of the infiltrating and peripheral immune cells are complex and interdependent. They include: direct cell–cell inhibition, exposure to immunosuppressive cytokines, intermediate cell death signaling [7, 8], persistent or self-antigen mediated tolerance [9], and exhaustion from chronic exposure to tumor antigens [10, 11]. Underexpression of immunostimulatory MHC class I [12] and overexpressesion of suppressive surface proteins (e.g., FasL and PD-1L) and cytokines (e.g., TGF-β, IL-10, and CCL2) fosters the

accumulation of regulatory T cells (Treg) and myeloid-derived suppressor cells (MDSC), while impairing the proliferation and functional activation of the cytotoxic lymphocyte (CTL) population. Meanwhile, accumulation of natural killer T (NKT) cells and Tregs in the peripheral circulation of GBM patients leaves them immunocompromised and leukopenic [13, 14].

Establishing effective, predictive immunotherapy regimens in GBM requires alleviating glioma-associated immune suppression while instigating, targeting, protecting, and resourcing specific responsiveness. Despite the obvious benefits of such a strategy due to difficulty targeting residual tumor cells and accumulating examples of therapeutic success, consistent biomarkers providing guidance in the design and prescription of regimens in GBM have lagged far behind the ability to administer the major classes of immunotherapy: *effector cell* therapy, *antigen-presenting cell* therapy, defined and complex *vaccines*, and *monoclonal antibodies*.

Effector cell therapies utilize adoptive immune function and employ ex vivo activation of potential anti-tumor effector cells—with non-specific lymphokines and/or potential tumor antigens—followed by re-introduction of these cells into the patient either via systemic injection or directly into the tumor site, guaranteeing some degree of cytotoxic function. Early trials using *Lymphokine-activated killer cells* (LAKs) administered peripherally with IL-2 as an adjuvant for recurrent glioma produced anti-tumor responses in a subset of patients, but also dose-limiting systemic and neurological adverse events, such as aseptic meningitis, increased intracranial pressure, and fever following infusion [15–18]. Lillehei et al. delivered LAKs, along with IL-2, directly to the resection cavities of 20 recurrent GBM patients, finding much better tolerance of therapy, but no significant improvement in survival [19]. Subsequently, a small local delivery trial decreased tumor volume in two of four patients [20], while trials by Dillman et al. reported a modest survival benefit (median 20.5 months, 1-year survival rate 75 %) among 36 patients [21, 22]. None of these studies employed biomarkers for enrollment, nor conducted follow-up analysis of molecular correlates of the clinical outcomes.

Autologous CTLs, stimulated with tumor-derived antigens, offer the advantage of glioma-directed cytotoxic function. Following preclinical studies confirming the survival, localization, and persistent anti-tumor specificity of autologous stimulated glioma-infiltrating lymphocytes [23], Holladay et al. tested the ability of autologous monocytes pre-loaded with irradiated autologous tumor cells and stimulated with IL-2 to selectively expand $CD4^+$ and $CD8^+$ T cells, compared to simple vaccination with such autologous irradiated tumor cells and adjuvant, first in a rodent model, then human patients. Although treatment was well tolerated in both arms of the clinical trial, and tumor regression and improved survival were observed among a subset of patients, there was no retrospective analysis of potential immune biomarkers associated with treatment responses [24, 25].

Chimeric Antigen Receptor (CAR) cells are CTLs, generally autologous, engineered ex vivo using recombinant DNA to express tumor antigen-specific proteins, most commonly chimeras of antigen-specific antibodies and T cell receptor (TCR) signaling domains, then reintroduced into the patient. CAR targeting the EGFRvIII mutated protein was first demonstrated in glioma cell culture [26], then by in vivo localization in preclinical murine studies, leading to tumor cell infiltration and increased survival [27, 28]. Currently, a single-group pilot clinical study of an optimized EGFRvIII-CAR [29] in GBM (NCT02209376) includes pre-screening for expression of EGFRvIII (31 % of GBM [30]) as an enrollment criterion, utilizing the artificially-targeted nature of these constructs as a biomarker to ensure targeting potential in light of the severity of off-target, adverse events. The more general tumor antigens HER2 and EphA2 have been successfully targeted by CARs in glioma cell culture and preclinical models [31, 32], leading to a phase I clinical trial of autologous generated HERT-CD28 fusion receptor cells (NCT01109095).

Vaccination strategies rely on multi-step activation of adaptive immune cells to yield a viable population of tumor-specific CTLs, thus igniting fewer adverse events caused due to spurious cytolytic activity than effector cell therapies, but providing less predictable targeting of antigens, especially in immunoprivileged tissues, such as the CNS. The latter concern was alleviated by the success of amyloid-β vaccination in Alzheimers [33], and recombinant peptides, the most accessible and scalable vaccine inoculum, were first proven effective against tumor antigens in melanoma [34]. However, single peptide vaccination may lead to selection for tumor mutations, epitope masking, and ultimately immune escape, and are also limited by peptide degradation, MHC presentation efficiency, and recognition by the endogenous TCR repertoire [35]. Therefore, multi-peptide vaccines in GBM have employed mixtures of several potentially immunogenic, HLA-A02-restricted peptides representing tumor markers: WT-1, HER2, MAGE-A3, and MAGE-A1 or gp100 [36] (NCT02149225). Although this study includes the collection of longitudinal peripheral blood samples for evaluation of overall immunological response and their correlation with tumor progression, and to compare responses to each of the individual vaccine components, results are not available to date.

Vaccination with a non-defined, lysate-derived protein extract utilizing the immunogenicity of heat shock protein peptide complex 96 (HSPPC96) has provided a shift toward a stimulatory peripheral and intratumoral immune response,

Table 1 Single biomarkers in GBM prognosis & therapy

Biomarker	Primary indication	Therapeutic	Function	Detect	References
Tumor					
Chr10	Prognostic - loss of 10q	(None)	PTEN deleted	Cytogeny, PCR	Bigner et al. [176]
IDH-1 (R132H point mutation)	Prognostic metabolic, epigenetic effects, potential vaccine target	R132H-specific vaccine	Conversion of isocitrate to 2-oxoglutarate	IHC	Yan et al. [131]
EGFR (amplification, overexpression)	Prognostic, target	I[131]-conjugated anti-EGFR, EMD55900, erbB family kinase inhibitors (gefitinib, erlotinib)	erbB tyrosine kinase receptor family—cell growth regulation of epithelial tissue	FISH	Schlegel et al. [114], Libermann et al. [172]
EGFRvIII (truncation mutation)	Prognostic, target	PEPvIII-peptide vaccine (rindopepimut, CDX); anti-EGFRvIIImAbs, immunotoxins; EGFRvIII-CAR	Constitutive tyrosine kinase activation of EGFR	PCR	Wong et al. [115], Shinojima et al. [118], Heimberger et al. [119], Sampson et al. [125], Chandramohan et al. [177], Johnson et al. [29]
PDGFRα	Prognostic (Proneural)	(None)	Cell regulation in mesenchymal derived tissue	IHC	Verhaak et al. [109]
gp100	Target	Dendritic cell vaccine target	Transmembrane glycoprotein	RT-PCR, flow	Phuphanich [168], Liu [171]
Ki67	Prognostic nuclear protein, cell proliferation marker	Antiproliferative & neoadjuvants, tamoxifen	Cellular proliferation	IHC	Mastronardi et al. [170]
MGMT (O^6-Methyl-guanine-DNA methyltransferase silencing)	Prognostic TMZ susceptibility phenotype with low expression	Responsiveness to TMZ	Removal of TMZ-induced DNA adducts	PCR	Hegi et al. [128]
HER2 (overexpression)	Prognostic—primary GBM target	HER2-specific chimeric antigen receptors, erbB family kinase inhibitors (gefitinib/erlotinib)	erbB tyrosine kinase receptor family - cell regulation	FISH, IHC	Mineo et al. [169]
PTEN (deletion)	Cell cycle regulation, cell survival signaling	Recurrence, chemotherapy resistance	Tumor suppressor	IHC	Li et al. [107]
p53 (deletion)	Cell cycle checkpoint regulation	Chemotherapy resistance	Cell cycle regulation	IHC	Watanabe [167]
VEGF (overexpression)	Vascular endothelial growth factor	Bevacizumab	Angiogenesis	ELISA, RT-PCR	Kreisl et al. [156], Friedman et al. [157], Folkman et al. [173]
Immune					
IL-13R (overexpression)	Immunoperturbation (Th2 bias)	IL13-PE38QQR	Cytokine receptor	Flow, IHC, IFA	Kunwar et al. [144]
IL-2Rα (CD25) (overexpression)	Immunosuppression (Treg)	Daclizumab; basilixumab, novartis	Cytokine receptor	Flow, IHC	Sampson et al. [146]
PD-1/PD-1L (overexpression)	Immunosuppression (Exhaustion)	Pembrolizumab, nivolumab, BMS; lambrolizumab, MK	Regulation of T-cell activation/tolerance	Flow	Hamid et al. [57]
CTLA-4/B7-1/B7-2 (overexpression)	Immunosuppression (Exhaustion)	Ipilimumab, BMS	Regulation of T-cell activation/tolerance	Flow	Korman et al. [60]

marked by increases in $CD4^+$, $CD8^+$, and $CD56^+$ T cells and IFNγ production. [37] Early phase success has led to a 3-arm phase III clinical trial (NCT01814813) evaluating both HSPPC96 vaccination and its combination with the anti-VEGF agent bevacizumab [37]. Although initial experiments retrospectively associated a decreased peripheral Treg abundance and increased $CD8^+$ T cell functionality with survival benefit, these biomarkers are not used directly in regimen design or assessment.

Ex vivo instruction of antigen presenting cells circumvents the need to achieve sufficient immune activation in vivo, while maintaining the lower risk profile of vaccination. *Autologous dendritic cells (DC)* are harvested from peripheral blood, stimulated with tumor-specific antigens—from tumor lysate, recombinant peptides, or tumor mRNA transfection [38] —and reintroduced, specifically activating endogenous CTLs. In phase I/II studies, DC-treated patients achieved decreased tumor size, increases tumor-infiltrating $CD8^+$ cells, and reversal of prevaccination CD4:CD8 ratios. Significantly, adverse events were limited to transient elevations in liver transaminases with no secondary autoimmune disease [39–43]. In a similar trial with 25 randomized patients, DC vaccination with heat shocked autologous tumor lysate delayed tumor recurrence compared to a control cohort, increased levels of peripheral T cells ($CD3^+$, $CD4^+$, $CD8^+$), and NK cells, and restored CD4:CD8 ratios [44]. Response of peripheral lymphocytes to cytokine stimulation via pSTAT signaling was later identified as a potential predictor of two-year survival and therapeutic efficacy [45].

Monoclonal antibody therapy, stimulatory or inhibitory, targets critical receptors that are markers or regulators of immune cell functional states. Early pre-clinical studies aimed at tumor-mediated immunosuppression, targeted Tregs through peripheral injection of monoclonal antibodies against CD25 (IL2Rα receptor) in a GL261 mouse model, resulting in Treg depletion, increased CTL activity and immunoactivating cytokines [46–48]. The importance of the EGFR pathway in GBM motivated the use of cetuximab (anti-EGFR) blockade, which strongly curbs tumor growth except in the presence of compensatory mutations and expression of alternative receptors (i.e. k-Ras, erbB1/2, [49, 50]), turning such features into contraindicative biomarkers for this antibody therapy.Three antibodies with distinct immune targets showed efficacy in various glioma mouse models: anti-CCL2, targeting suppressive monocytes, provided a survival benefit when paired with temozolamide [51]; anti-PD1, blockading T cell exhaustion, provided survival with localized radiotherapy [51]; and anti-CTLA4 prevented T cell exhaustion when combined with the inflammatory cytokine IL-12 [52]. In human trials, Sampson et al. administered dacluzimab (anti-CD25) to patients receiving the PEPvIII vaccine. Positive response and depletion of circulating Tregs in the dacluzimab-treated patients supported potential therapeutic synergy [53, 54], as well as the use of peripheral Treg as a surrogate marker for response for these and other immunotherapeutic regimens in GBM.

Profiling, prediction, and personalization in immunotherapy

In 2013, over two decades of contributions by innumerable scientists and clinicians toward demonstrating the clinical power of cancer immunotherapy were recognized by Science Magazine (AAAS) as the breakthrough of the year. Durable and curative responses from, most notably, "checkpoint-blockading" antibodies against CTLA-4 [55] and PD-1 [56–60] in several cancers, alone and in combination (for review, see [61]), and genetically engineered chimeric antigen receptors (CAR) cells, in both leukemia and solid tumors [62, 63], have motivated the rapid expansion of clinical trials to new targets, mechanisms, and delivery systems. Yet, the potential to synergize these technologies with each other [64] and with more traditional immune interventions [65] depends heavily on clearing what the World Immunotherapy Council outlined in 2011 as "critical hurdles" [66]. Among these, re-evaluation of the mainly radiographic and survival-based response evaluation criteria in solid tumors (RECIST) [67] was a priority, given their failure to capture molecular and cellular correlates of subclinical outcomes or to adequately stratify responders prospectively or retrospectively.

Presently, the expression of immunotherapeutic target proteins at the tumor site or among the circulating immune cells is commonly monitored by flow cytometry or RNA expression. However, the only ones formally utilized in clinical trials as prospective biomarkers are the T cell "exhaustion marker" PD-1 and its tumor-expressed ligand PD-L1 for predicting efficacy of the anti-PD-1 monoclonal antibody [68] and other antibodies targeting that pathway. Even in this case, the fidelity of PD-1 as an "exhaustion" marker, compared to other T cell surface markers (such as LAG-3, TIM-3, and TIGIT, associated exclusively with specific subsets of exhausted T cells [69, 70], remains disputed, and the biological mechanism by which these antibodies restore functionality of the CTL population (specifically, blockade of exhaustion signaling versus depletion of exhausted cells [71] is not completely resolved). For anti-CTLA-4 therapy, a clinical biomarker has not yet been ratified, though retrospective analysis of clinical data has suggested clusters of diagnostic correlates (for a review see [72]), and subsequent laboratory work on patient samples has validated its dependence on related immune receptors [73]. Meanwhile, the list of single factors with direct

and indirect influence on the evolution of the immune response continues to grow—particularly small molecule metabolites and the pathways regulating them (e.g. indoleamine dioxygenase [74], arginase [75], and α-galactosylceramide [76]), "danger" pathways (e.g., DAMPs, TLRs, and high-mobility group box-1 release [77], for a review see [78]), and innate immune, wound-healing, and inflammatory signals (e.g. STING pathway ligands, inflammasome expression, TGF-β family signaling, and NFκB induction).

Despite the benefits of simple biomarkers, achieving prognostic and therapeutic predictability through any single expression-based marker beyond the targeted pathway has proved an expanding challenge, especially across tumor types. However, this need no longer constitutes a roadblock. Evolving technology in gene expression profiling, flow cytometry, and histopathology has allowed these assay paradigms to achieve depth and accessibility, leveraging "whole profile" metrics with the prognostic potential, rather than singular indicators associated with these underlying states.

Quantitative, high-sensitivity resolution of rare populations and functional markers by flow cytometry has drastically increased the amount data obtained per sample. Multi-parameter profiles, including the relative abundance of T cells ($CD3^+$), B cells ($CD19^+$), NK cells ($CD56^+CD16^+$), Treg ($CD4^+CD25^+CD127^{lo}$), total monocytes ($CD86^+$) and immunosuppressive monocytes ($CD14^+HLA\text{-}DR^{lo/neg}$) have been associated with survival across GBM, non-Hodgkins lymphoma, and renal cell carcinoma, with particular significance in the relationship between $CD4^+$ T cells and immunosuppressive monocytes in circulating blood [79].

Histopathology, meanwhile, has leveraged improvements in high-throughput automated microscopy of tumor infiltrating lymphocytes in situ. In particular, the recently commercialized Immunoscore system utilizes relative abundance of functionally marked T cell populations in the histologically identified tumor core vs. infiltrating margin, achieving survival-significant prognostic resolution beyond standard clinical staging of colorectal cancer [80].

With strong evidence that tumor-associated immunosuppression originates locally, characterization of gene expression patterns in the tumor microenvironment became an early focus. Microarray and later next-generation sequencing (RNAseq) of biopsied tissue have provided simultaneous whole-transcriptome profiling of tumor and immune genes, and given rise to several sub-transcriptomic technologies aimed at making high-content, abbreviated profiling widely accessible to clinical samples. For example, the nanostring [81] nCounter platform (quantifying cell type markers, tumor antigens, and a suite of >400 immune genes) has been applied to both cryopreserved and FFPE tissue from several tumor types, bringing mid-throughput gene expression profiling to archival samples. These profiles were successfully correlated with risk of recurrence (ROR) [82], intrinsic subtype, and trastuzumab responsiveness in breast cancer, OS and PFS neuroblastoma [83], and with subtype in diffuse large B cell lymphoma (DLBCL) [84]. The expression of other smaller-scale panels of genes and miRNA have been correlated with clinical and pathological outcomes in different tumor types [85], including a recent computational study of TCGA samples further reduced prognostic immunoprofiling to a minimal geneset reflecting cytolytic activity [86].

Gene expression profiling, combined with in situ pathology, has given rise to the broad proposition of an inflamed vs. non-inflamed tumor state [87] characterized by the balance and activation of the immune pathways driving them. Importantly, such a model offers a context in which to triage the needs to elicit an anti-tumor response (through vaccines, adjuvants, antigen presenting cell therapies, or effector cell therapies) and to alleviate immunosuppression (by depleting Tregs, MDSCs, and immunosuppressive cytokines, chemokines, and small metabolites, or blockading tolerance/exhaustion pathways), and predict synergistic combinations of therapies depending on that state. However, like the orthogonally informative model of immunoediting [88], this framework is broad and does not yet resolve known confounding factors such as the organ-specificity of baseline and tumor-associated immune activity, and what drives transitions between states.

Two new platforms with great disruptive potential in immunological profiling of cancer and other diseases have emerged in the last five years. Time-of-flight mass cytometry (CyTOF) of cells using antibodies conjugated to heavy metal molecular tags in place of fluorophores allows quantification up to 25+ dimensions [89, 90], allowing unprecedented detection of specific populations, informing the relationship between immune cell development, function, and perturbation in disease [91]. Whole-repertoire amplification and high-throughput sequencing of the antigen-specific receptors of whole lymphocyte populations (TCRseq and BCRseq) provides a link between antigen specificity and function, as well as novel statistical indicators of clonal selection and bias during immunosuppression, treatment response, and residual disease in cancer, infection [92–94], autoimmunity [95], and response to immunotherapy [96, 97]. Both technologies are rapidly expanding into new clinical paradigms, and developing tools for analysis and integration of these "big data" readouts with their corresponding clinical and lower-dimensional phenotypes promises enrich immunological biomarker profiles in both infectious disease [10, 98, 99] and cancer.

GBM immunotherapy and the biomarkers of the future

Motivated in part by the characteristics that make GBM a therapeutic challenge [2, 100–102], glioma research has

already seen the clinical impact of high-dimensional, "big data" profiling of the tumor tissue and its immune components on our interpretation of classical biomarkers (Table 1). For example, the cytologically diagnosable 10q23 deletion [103] as a biomarker of tumor etiology and prognosis [104–107] preceded elucidation of the tumor suppressor functions of PTEN and their cross-talk with glioma-specific growth pathways. In the broadest synthesis to date, comparative sequencing of genomic rearrangements [108] and gene expression profiling through the TCGA collaborative produced a cohesive set of >800 genes [109] by which to classify GBM tissue into subtypes (proneural, classical, and mesenchymal). These subtypes stratified prognoses, drug and radio-sensitivity [110, 111], as well as the expression of many other known biomarkers. EGFR, for which overexpression [112], genomic amplification [113, 114], and truncation [115–117] were each associated with differential prognosis [118, 119] and therapeutic response [6, 120–122], was found to have a pleiotropic role in tumor cell growth and angiogenesis [123], concomitant with these variations [113, 116] [124]. The "classical" subtype, which captures this EGFR-active state, is predictive of sensitivity to PEPvIII-KLH DC vaccination [125] (see above) and therapeutic targeting of the pathway [126]. Hypermethylation of O^6-methylguanine DNA methyltransferase (MGMT) [127] predicted increased sensitivity to temozolamide and radiation [128] before the "hypermethylator" phenotype was well-established in GBM and other tumors [129, 130]. Isocitrate dehydrogenase-1 mutation (IDH1-R132H), was a known biomarker of longer OS in GBM [108, 131] before its association with transcriptome-wide expression alterations characteristic profile of the "proneural" subtype, and functional contribution to genome-wide hypermethylation [132] were appreciated.

Beyond functioning as biomarkers, high-dimensional profiling have rendered new targets and informed diagnostic and therapeutic options directly. Inference of regulatory networks from transcriptomic data has further elucidated these profiles [133–135], motivating clinical and pre-clinical attempts to synergistically target subtype-specific regulatory nodes [134]. Thus, use of the IDH1-R132H peptide vaccine [136] and EGFRvIII-targeting therapies can be guided not only by the single target epitopes but by the broader phenotypes of proneural and classical GBM. Meanwhile, in silico [137, 138] and experimental efforts have begun to capture the diversity of new, potentially immunogenic GBM antigens [139]. A mass spectrometry-based study [140] yielded 11 candidate peptides now included in the IMA950 vaccine trial (NCT01929191). Gene expression profiling of stereotactically localized biopsies, combined with novel computational analyses, has uncovered cell type-specific signatures at the non-contrast enhancing infiltrated margins with the potential to inform post-surgical therapy targeting the residual tissue from which recurrence arises [61, 141].

Fundamental, outstanding questions in experimental GBM treatment and immunotherapy paradigms likely require the combination of gene expression, tissue pathology, and immunological profiles to resolve. The largest randomized Phase III clinical trial in GBM (PRECISE) leverages dysregulated immune signaling (overexpression of IL-4 and IL-13 receptors [142, 143]) to target high local concentrations of the protein toxin-conjugated IL13-PE38QQR (cintredekin besudotox) to the tumor stroma [142, 144, 145]. Despite distinct drug distribution profiles [146], no difference in median OS nor adverse events were observed compared to carmustine wafer delivery of BCNU (Gliadel) in recurrent GBM [144, 147]. Microarray analysis of treated cultured cells [148] confirmed cytotoxicity (tumor cell apoptosis) as well as differential expression of immune-related genes (e.g. IL-8). Yet, confirmation of this phenotype and its relationship to subclinical efficacy in patient samples has not been established. Meanwhile, bevacizumab, a neutralizing antibody against vascular endothelial growth factor A (VEGF-A) [149]—both a biomarker and effector of angiogenesis [150–154, 155]—presents a conflict between reported efficacy by PFS [156, 157] and OS [158], with an apparent negative impact on concurrent or subsequent therapies [159, 160]. Both the similar shortfall of radiographic metrics as progression criteria and known cross-talk between VEGF and immune signaling [161–163] suggest that combined tumor-immune profiling may be key to interpreting these findings. Reconciling such conflicting or occult therapeutic outcomes requires a profile-wide understanding of the role of the targets, the metrics used to evaluate responses, and confounding factors in the tumor microenvironment.

High-dimensional profiling now allows both animal models [164] and experimentally treated tissue [165], to be compared to their human counterpart or source and validated in unprecedented detail, producing higher-fidelity translational platforms for pre-clinical experiments. Perhaps more importantly, the recent technological advances being integrated into the biomarker strategies of the cancer immunotherapy field at large allow unprecedented volumes of molecular and immunological data to be extracted from clinical samples which are relatively straightforward to procure—such as tissue cryopreserved during resection, and reasonable quantities of peripheral blood obtained throughout care—directly from patients in immunotherapy trials. Although currently no single biomarkers adequately stratify responses in GBM, tumorigenesis research and immunotherapy research are converging, and have begun to use high-dimensional profiles to capture tumor and immune states predictive of disease and therapeutic response

[166], promising synergy for these two fields in the development of a new generation of biomarkers for immunotherapy.

[167–176].

Conflict of interest None.

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