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Current Perspective

Immunotherapy for brain metastases and primary brain tumors

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Abstract During the V Siena Immuno-Oncology (IO) Think Tank meeting in 2021, conditions were discussed which favor immunotherapy responses in either primary or secondary brain malignancies. Core elements of these discussions have been reinforced by important publications in 2021 and 2022. In primary brain tumors (such as glioblastoma) current immunotherapies have failed to deliver meaningful clinical benefit. By contrast, brain metastases frequently respond to current immunotherapies. The main differences between both conditions seem to be related to intrinsic factors (e.g., type of driver mutations) and more importantly extrinsic factors, such as the blood brain barrier and immune suppressive microenvironment (e.g., T cell counts, functional differences in T cells, myeloid cells). Future

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therapeutic interventions may therefore focus on rebalancing the immune cell population in a way which enables the host to respond to current or future immunotherapies.

1. Introduction

Immunotherapies have delivered practice-changing treatments with significant overall survival (OS) benefit [1]. For patients with primary brain tumors, the OS benefits have been less significant as compared to patients with brain metastases [2,3]. The V Siena IO Think Tank meeting held (Siena, Italy) on 7–8 October 2021, dedicated a session to immunotherapy in brain malignancies of adults. During this session, underlying mechanisms of resistance to current immunotherapies were discussed and future strategies proposed to improve treatments of brain malignancies. The following article summarizes the findings from the session and as with previous Think Tank meetings identified future clinical development initiatives [4,5].

Secondary malignancies of the brain in adults are associated with a wide range of OS, which are mostly determined by the histology of the primary tumor. Because OS in patients with brain metastases varies significantly, diagnosis-specific prognostic tools are being developed, such as the diagnosis-specific Graded Prognostic Assessment (GPA) [6]. In a patient cohort of 6984 patients with brain metastases OS ranged as follows: 7–47 months (non-small cell lung cancer), 3–36 months (breast cancer), 5–34 months (melanoma), 3–17 months (gastrointestinal cancer) and 4–35 months (renal cell carcinoma) [6] (Table 1). Recent treatments with immunotherapy and targeted therapy have improved OS in the aforementioned tumor types, including in patients who developed brain metastases.

In contrast to patients with brain metastases the OS has not significantly improved in the past decade for patients with primary brain tumors. Depending on the type of molecular and pathological stage, the OS may range between 12 and 23 months (e.g., glioblastoma, isocitrate dehydrogenase (IDH)-wildtype) and 6–15 years (e.g., lower-grade glioma, IDH-mutated) [7]. Based on the growing understanding of molecular pathways, a new classification for primary brain tumors was introduced [8,9]. While this updated World Health Organization (WHO) classification from 2021 introduced greater clarity in defining biologically congruous tumor entities and helped estimating prognosis more accurately, the OS has not changed for most brain tumors. While immunotherapies have not shown a convincing clinical benefit in patients with primary brain malignancies, they did have significant clinical benefit in patients with brain metastases.

1.1. The immune-pathologic landscape in primary and secondary brain tumors

Asymptomatic patients with non-small cell lung cancer (NSCLC) and melanoma brain metastases showed intracranial responses to immune checkpoint inhibitors in phase II clinical trials [10,11]. The response was observed regardless of the underlying tumor histology and occurred within the first 2 months of treatment. For patients with metastatic melanoma, intracranial complete response occurred in patients who received the combination of nivolumab and ipilimumab (26%; 24/94) [11]. By contrast, patients with recurrent glioblastoma and receiving nivolumab showed few overall or durable responses and had a comparable OS to treatment with bevacizumab [3]. This lack of clinical benefit was also

Table 1
Main Differences between glioblastoma and brain metastases.

	Brain Malignancies	
	Glioblastoma	Brain metastases (NSCLC, melanoma, RCC, breast cancer and GI cancer)
Overall Survival	Approx. 12 months	3–47 months
Immune Cell Infiltration		
$CD3^+$ Tumor Infiltrating Lymphocyte (TIL)	Low	High
Myeloid Cells	High	Low
Absence of Checkpoints in Tumor Tissue		
PD-L1	Low	High
LAG-3	Low	High
Systemic Immune Markers Inflammation		
sPD-L1	High	Low

observed in patients with glioblastoma and receiving immune checkpoint inhibitors as 1st line treatment for both O6-methylguanine methyltransferase (MGMT) promoter methylated and unmethylated glioblastoma [12,13]. To provide a framework on how to best explain this difference of responses to current immunotherapies, the "cancer immunogram" can be used to determine whether both types of malignancies are characterized by fundamental differences in their immune landscape [14] (Table 1).

First, brain metastases and primary brain malignancies differ in their number of tumor-infiltrating lymphocytes [15–19]. Brain metastases have nearly 50% more $CD3^+$ lymphocyte infiltrations than either glioblastoma or low-grade glioma [16]. This presence of $CD3^+$ immune cells appeared to be associated with the IDH mutational status [16,20,21]. The mechanistical underpinnings of IDH mutation determining the cellular composition of the inflammatory microenvironment are still under investigation. One proposed mechanism is the presence of the oncometabolite 2-hydroxyglutarate which is produced by mutant IDH and inhibits T cell activation [22]. The inflammatory microenvironment of gliomas is mainly characterized by microglial cells, and IDH-mutant tumors harbour a considerable fraction of monocyte-derived macrophages. Furthermore, distinct epigenetic subgroups are known to impact lymphocyte infiltration [23].

Second, the absence of checkpoints may explain why current immunotherapies have failed in glioma. For example, programmed death-ligand 1 (PD-L1) expression is higher in brain metastases (>50% of cases) compared to glioblastoma, where membranous PD-L1 expression is seen in about 37% of samples [17,18]. Lower-grade gliomas have even less PD-L1 expression within the tumor microenvironment [16,24].

Alternative checkpoints such as lymphocyte-activation gene 3 (LAG-3) are nearly absent in patients with glioma except for a small subset of patients with IDH wildtype tumors [25]. In peripheral blood, soluble PD-L1 (sPD-L1) is higher in glioma than in patients with brain metastases, and sPD-L1 levels were observed to be associated with survival [26]. Consistent with the concept of an immunosuppressive environment, a higher neutrophil-to-lymphocyte ratio (NLR) was identified in patients with glioblastoma [27].

Third, tumor mutational burden (TMB) is generally lower in patients with glioblastoma than in patients with melanoma or NSCLC [28]. While there is a correlation of anti-tumor responses and TMB in patients with melanoma and NSCLC receiving immune checkpoint inhibitors, there is no such correlation of responses and TMB in glioblastoma patients [29].

1.2. Gene signatures in glioblastoma multiforme (GBM)

Research has focused on identifying unique driver mutations for which specific inhibitors can be developed and consequently the development of such drugs could result in improved OS [30]. Hence, discovery of molecular pathways in gliomagenesis promises the identification of new targets in either primary or secondary brain malignancies [31]. Among glioblastoma with IDH wildtype, three different subtypes have been identified and each subtype was evaluated for their presence of specific immune cells [32]. To further explore the gene expression based subsets and their relationship to immune signature, single-cell studies have uncovered four main cellular states of glioblastoma that are influenced by the microenvironment compositions and specific genetic events: (a) neural-progenitor-like cells enriched with cyclin-dependent kinase (CDK) amplifications; (b) oligodendrocyte-progenitor-like cell enriched with platelet-derived growth factor receptor alpha (PDGFRA) amplification, (c) astrocyte-like cells showing higher frequency of epidermal growth factor receptor (EGFR) amplification and (d) mesenchymal-like cells characterized by NF1 mutations [33]. When combining all molecular information, four pathway-based types can be defined: (1) glycolytic/plurimetabolic; (2) neuronal; (3) mitochondrial; (4) proliferative/progenitor [34]. This approach has identified new subtype-specific targets pathways, such as Mitochondrial Oxidative Phosphorylation [34].

A second application for molecular defined pathway analyses is the evaluation of temporal changes of glioblastoma. This evaluation can determine whether a target remains stable over time and may help to uncover mechanisms of resistance [35]. This approach shows a high diversity of change over time within the different subgroups, including metabolic changes.

Third, gene expression evaluation can also be used to characterize the immune microenvironment [36]. Transcriptional regulatory network found the genes PPARG, BATF and SPI1 as promoters for attracting tissue-associated macrophages (TAM) [37]. Associated with these TAM is the expression of macrophage receptor with collagenous structure (MARCO) to drive mesenchymal transformation [36,38,39].

1.3. The immune microenvironment of melanoma brain metastases: implications for immunotherapy

Based on the early responses to immunotherapies in patients with melanoma and NSCLC [11,40–42], intratumoral CD8, stromal PD-L1 expression and density of immune cells were associated with responses in patients with brain metastases [43,44]. The localization of the immune cells varies between the tumor types and their brain metastases [17,45]. The larger the size of the metastatic lesion, the more likely a decrease of immune cells numbers will be observed, which in turn may have an impact on response to immunotherapy [17,45]. When comparing brain metastases to other metastatic sites, the composition of myeloid cells differs by anatomical location [46]. Prior therapies, such as radiotherapy, can

influence the microenvironment creating either an immune-enriched or -depleted environment [47]. Drivers of this immune composition are genes associated with metabolic features, such as oxidative phosphorylation (OXPHOS) [48].

1.4. Success, pitfalls, and failures: a clinical perspective

Combination studies were designed to overcome resistance in patients with brain metastases [49,50]. In considering treatment outcomes, disease stabilization of intracranial metastases may be an important driver for OS [50]. The drug fotemustine is a commonly used drug in glioblastoma and thus the combination with an immune checkpoint inhibitor was expected to have a disease stabilizing effect.

Single-agent or combination immune checkpoint inhibitors have been investigated in several studies but showed no OS benefit [3,12,13,51]. Survival benefit was observed if the programmed death protein 1 (PD-1) inhibitor pembrolizumab was given in a neo-adjuvant setting [52]. Also, intracerebral application of either cytotoxic T-lymphocyte antigen 4 (CTLA-4) or PD-1 inhibitor could be an interesting approach, as early-phase data showed promising results [53].

1.5. Immunotherapy for primary brain tumors: future therapeutic strategies

New treatment concepts have been explored in recent years, including antibody–drug conjugates, immunocytokines, chimeric antigen receptor T (CAR-T) cells, novel immune checkpoint inhibitors and vaccines (Table 2). With unique expression of EGFR in glioblastoma, antibody-conjugates targeting EGFR domain II (for example, ABT-414) exploit the overexpression of this protein to deliver chemotherapy locally. Whereas the phase 2 study appeared to deliver the expected improved OS in recurrent glioblastoma, the phase 3 trial in newly diagnosed glioblastoma failed to show a survival benefit [54].

Another intervention is based on delivering immunocytokines which are intended to convert "immune-deserted" (="cold") tumors into "immune-competent" (="hot"). Examples of such immunocytokines are interleukin (IL)-2, IL-12 and Tumor Necrosis Factor alpha (TNF-α) linked to an antibody to the extra-domain B (EDB) of fibronectin [55]. The "Gliomoon Trial" is such an example for evaluating novel immunocytokines in glioblastoma treatment. Patients were treated with L19-TNF (onfekafusp alfa) if they had an IDH wildtype and grade 3 or 4 gliomas. A third approach consists in delivering modified T cells, such as CAR-T cells. Utilizing the overexpression of EGFR, a first-generation CAR-T cell approach was constructed targeting the EGFR variant III [56]. This study in recurrent glioblastoma demonstrated that such chimeric T cells can be generated and are safe. The fourth approach targets reversing immune suppression by targeting specific signalling pathways, such as transforming growth factor beta (TGF-β)/activin receptor-like kinase 5 (ALK5), CTLA-4, tryptophane, signal transducer and activator of transcription 3 (STAT3) [57]. The role of TGF-β/ALK5 in glioblastoma has been recognized for many years but so far trials with TGF-β/ALK5 inhibitors have not shown clinical benefit compared to existing standard therapy [58,59]. Since the prior TGF-β/ALK5 inhibitors were not studied in specifically selected patients or with drugs that may achieve optimal therapeutic levels, novel inhibitors are currently being developed [60,61]. A fifth option is to improve the current therapy with immune therapy. While immune checkpoint inhibitors have not shown OS benefits, subgroup evaluations showed that patients with MGMT promoter methylation in their tumor and no baseline corticosteroid treatment had higher OS than patients treated with bevacizumab [3]. A sixth approach consists in utilizing vaccines. There are vaccines directed against the EGFR variant III (e.g., rindopepimut), dendritic cell-based vaccines (e.g., ICT-107, DCVax), tumor-derived peptides (e.g., HSPPC-96) and personalized multipeptide vaccines (e.g., GAPVAC) [62]. However, the phase 3 study with rindopepimut added to standard of care in the newly diagnosed setting did not show a clinical benefit compared to the control arm in patients after initial resection of their first tumor [54]. While

Table 2
Examples of novel therapeutic approaches in malignant glioma.

General Approach	Example
Combination with Chemotherapy and Immune checkpoint inhibitor	Fotemustine + immune checkpoint inhibitor
Intracranial Delivery	Immune Checkpoint Inhibitors, oncolytic virus
Antibody–Drug Conjugate	Targeting EGFR domain II (ABT-414)
Immunocytokines	Onfekafusp alfa
CAR-T cells	EGFRvIII, IL13R
Reduction of Immune Suppression	• TGF-β/ALK5 small molecule inhibitors • CTLA-4 inhibitors
Immune Checkpoint Inhibitors	Novel Inhibitors, such as LAG-3
Vaccine	• Peptide-based vaccines • Dendritic cell-based

Table 3
Future areas of research.

Influence Factors Limiting Current Immunotherapies	Diagnostics and Biomarkers
Genetic Predispositions • Hypermutation of genes affecting immune responses • Molecular characterization of genetics-epigenetics-transcriptomics in relationship to anti-tumor responses Immune Conditions • Antigen release and presentation • Subset analysis of immune cell activation and recruitment • Rebalance of immune subsets (e.g., increase effector T cells while reducing immune suppressive macrophages) Therapeutic Interventions • Assess current therapies on their immune suppressive effect (e.g., radiation, chemotherapy) • Select therapies with immune enhancing effect	• Develop detection tools to overcome intra-tumor heterogeneity to identify novel therapeutic targets • Use single-cell evaluation to identify therapeutic vulnerabilities • Assess time-dependent changes in tumor cell population to uncover unknown factors leading to resistance to immunotherapy

these trials failed, this remains an area where combination immunotherapy needs to be evaluated. A recent report identified the defect in immunity to brain tumors is the absence of antigen presenting cells that can prime and boost anti-cancer immunity [63]. J Immunother Cancer 2021; 9:e002181. PMID 34083417).

. This is not a problem for melanoma and other brain metastases, as priming and expansion continues in the periphery. Advanced combination immunotherapy trials that take into consideration priming, application of immune agonists and checkpoint blockers, while also addressing suppressive mechanisms represent opportunities to design aggressive therapeutic options for patients with this disease.

2. Discussion

The Think Tank members identified the following main areas for future research (Table 3):

First, factors that limit current immunotherapies and diagnostic tools to identify such factors. Among the factors limiting current immunotherapies, three main contributing conditions may need to be assessed further:

(a) Genetic predisposition: Immunotherapies may have a unique activity in patients with biallelic mismatch repair deficiency (bMMRD) [64]. Furthermore, hypermutations appear to influence responses [65]. The consequences of such mutations on influencing immune response are not known and hence a need for molecular characterization of the genetic/epigenetic/transcriptomic landscape of primary and secondary brain malignancies is desirable.

(b) Immune cell landscape: Current understanding shows a fundamental difference between the immune cell landscape of primary and secondary malignancies, which may affect anti-tumor responses [66]. This difference may result in differences of antigen presentation, immune cell priming, activation, recruitment and consequently, causing an imbalance of the effector T cell population. In pediatric patients, the intra-tumor injection of the oncolytic virus-based therapy DNX-2401 altered the tumor immune environment and was associated with an OS of 17.8 months [67]. Perhaps novel approaches of therapeutic vaccines may offer new avenues for treatment concepts (Table 2).

(c) Impact of current therapies on future immunotherapies: Current standard therapies may have to be adjusted depending on their impact of affecting the immune cell landscape. This may help in designing future studies with the appropriate background therapies.

Second, research should focus on tools and diagnostic measures which may help to uncover unappreciated mechanisms of resistance. These measures should take into consideration the intra-tumor heterogeneity as this may hinder to identification of novel therapeutic targets. Single-cell studies while cumbersome may offer a more detailed look at the complex landscape of brain malignancies. All such diagnostic and biomarker studies may need to include time-course evaluations given the highly dynamic nature of brain malignancies. As in other disease settings, neo-adjuvant study designs may provide such a platform for using broad multi-omics study designs [52,68].

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Author contributions

All authors critically contributed to the final manuscript draft and approved the final version. Michele Maio conceived the review together with key participants. The

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Conflict of interest statement

Anna Maria Di Giacomo has served as a consultant and/or advisor to Incyte, Pierre Fabre, Glaxo Smith Kline, Bristol-Myers Squibb, Merck Sharp Dohme, and Sanofi and has received compensated educational activities from Bristol Myers Squibb, Merck Sharp Dohme, Pierre Fabre and Sanofi.

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Ramy Ibrahim is on the board of directors of Surface Oncology and 2Seventy. He is a consultant to Georgiammune, Harpoon and Altos labs.

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Michael Lahn is employed by iOnctura SA and holds company stocks.

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