Personalizing Oncolytic Virotherapy for Glioblastoma

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Oncolytic virus (OV) treatment may offer a new treatment option for the aggressive brain tumor glioblastoma. Clinical trials testing oncolytic viruses in this patient group have shown promising results, with patients achieving impressive long-term clinical responses. However, the number of responders to each OV remains low. This is thought to arise from the large heterogeneity of these tumors, both in terms of molecular make-up and their immune-suppressive microenvironment, leading to variability in responses. An approach that may improve response rates is the personalized utilization of oncolytic viruses against glioblastoma (GBM), based on specific tumor- or patient-related characteristics. In this review, we discuss potential biomarkers for response to different OVs as well as emerging *ex vivo* assays that in the future may enable selection of optimal OV for a specific patient and design of stratified clinical OV trials for GBM.

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1. Introduction

Oncolytic viral therapy or virotherapy is a form of immunotherapy showing promising results for cancers with poor prognosis [1]. In this approach, oncolytic viruses (OVs) are employed to kill tumor cells, while in parallel stimulating an anti-tumor immune response [2]. OVs exhibit either natural tropism to malignant cells or their genome is altered to confer them higher specificity for malignant cells [3]. Viruses from ten different families (Adenoviridae, Herpesviridae, Paramyxoviridae, Reoviridae, Retroviridae, Picornaviridae, Parvoviridae, Poxviridae, Rhabdoviridae, Alphaviruses) have thus far been utilized as oncolytic virus platforms in clinical trials for various cancer types [2].

One deadly type of cancer is glioblastoma multiforme (GBM), the most common and aggressive primary brain tumor [4]. The standard treatment consists of maximal safe surgical resection followed by radiotherapy plus concomitant and adjuvant temozolomide chemotherapy. However, the median overall survival among all GBM patients is less than one year, and only 15 months in patients receiving complete standard treatment with 3-year survival being less than 10% [5][6]. In the past decades, numerous therapeutic approaches have been tested in clinical trials, with disappointing outcomes. The main obstacles in treating GBM include its infiltrative growth, its intrinsic resistance to chemo- and radiotherapy, its notorious intratumoral heterogeneity with dynamic changes in subclones facilitating treatment escape, its protected location behind the blood-brain-barrier and the immunological

'cold' microenvironment of these tumors. These hurdles to more conventional therapies, as well as the dismal prognosis of GBM patients, have encouraged scientists and clinicians to develop and evaluate the local application of various types of oncolytic viruses in this patient group. Table 1 summarizes the most commonly applies OVs in GBM trials. The OVs differ in their primary attachment molecules to host receptors as well as in the source of their tumor selectivity, which may be derived from a natural tropism to cancer cells or by genetic engineering.

Table 1. Characteristics of the most commonly used Oncolytic viruses (OVs) in glioblastoma multiforme (GBM) clinical trials**.**

Family	Genome	OV Examples	Genetic Engineering	Entry Receptor	Tumor Specificity
Herpesvirus	dsDNA	HSV1716	ICP34.5-deleted	HVEM, 3-O-sulfated heparin sulfate and nectin-2	Defects in the p16/Rb, PKR or interferon pathways [7]
		G207	ICP34.5 and ICP6 -deleted mutant oHSV	HVEM, 3-O-sulfated heparin sulfate and nectin-2	Defects in the p16/Rb, PKR or interferon pathways [8]
		G47Δ	ICP34.5, ICP6 and α47 -deleted mutant oHSV	HVEM, 3-O-sulfated heparin sulfate and nectin-2	Defects in the p16/Rb, PKR or interferon pathways [9]
		rQnestinHSV-1	ICP34.5-deleted mutant oHSV, in which γ134.5 gene was reinserted	HVEM, 3-O-sulfated heparin sulfate and	Expression of nestin [10]

			under control of nestin promoter	nectin-2	
Adenovirus	dsDNA	Onyx-015	E1B-55k and E3B - deleted mutant group C adenovirus	CAR	Defects in p53 pathway, defects in cell cycle, late viral RNA export [11]
		Delta24-RGD	24-base pair deletion in the E1A gene and insertion of an RGD sequence in the viral knob	CAR, αvβ3 and αvβ5 integrins	Defects in Rb pathway [12]
Paramyxoviridae	(–) ssRNA	MV-CEA	Edmonston (MV-Edm) vaccine strain with insertion of the human carcinoembryonic antigen gene	CD46, nectin-4, SLAM	Overexpression of CD46, defects in the interferon pathway [13]
		NDV	Natural tropism	Sialic acids	Defects in the interferon pathway [14]
Reovirus	dsRNA	R124	Natural tropism	JAM-A, Nogo Receptor NgR1	Defects in the Ras signaling pathway [15]
Picornaviridae	(+) ssRNA	PVSRIPO	Poliovirus type 1 (Sabin) vaccine with replacement of the internal ribosomal	CD155	Overexpression of CD155 [16][17]

			entry site (IRES) with the human rhinovirus type 2 IRES		
Parvovirus H1	ssDNA	Parvovirus H-1PV	Natural tropism	Sialic acids	Defects in interferon pathway, defects in cell proliferation pathways [18]

In a recent review, Chiocca et al. summarized the findings [19] from all the recent GBM oncolytic virotherapy trials and illustrated that a subgroup of GBM patients responds exceptionally well to OV treatments, with survivors at 36-months, and with some patients exhibiting long term remission [20][21]. This phenomenon has also been observed in OV trials for other cancer types. For instance, a phase II clinical trial employing an oncolytic herpes simplex virus 1 for stage IIIC or IV melanoma showed 26% overall response [22].

These observations raise the question: would the responding patients have been the same individuals if they had been treated with any other OV, or are we looking at responders to a specific OV? In other words, is the elicited immune response a generalized one for all types of OVs, or does each OV elicit a specific anti-tumor immune response? The latter would suggest that response rates may be significantly increased if we are able to define which OV is best suited for a particular patient. Identification of robust predictive biomarkers for OV response would allow future design of stratified clinical trials employing multiple OV strains. The replication efficiency of the virus is thought to be of importance for generation of the subsequent inflammatory and anti-tumor responses. Moreover, host immune status is also expected to contribute to the efficacy of OV treatment. This review, therefore, focuses on tumor and host resistance mechanisms to viral infection, replication and oncolysis and discusses potential biomarkers that have previously been reported in relation to sensitivity or resistance to the most frequently employed OVs in preclinical and clinical GBM research.

2. Glioblastoma

2.1. Heterogeneity, Stem Cells and Therapy Resistance

Common molecular abnormalities involved in the evolution of glioblastomas include aberrations in the oncogenes (EGFR, PDGF and its receptors) and tumor suppressor genes (p16INK4a, p14ARF, PTEN, RB1, and TP53), which are often observed in other human cancers as well [23]. GBM is also characterized by inter-tumoral heterogeneity, which is highlighted by the classification of GBMs into three subgroups: proneural, classical and mesenchymal [24] [25]. Each subtype is characterized by specific gene expression patterns and molecular abnormalities, resulting in

different clinical treatment outcomes [25][26]. Proneural subtype has the most favorable prognosis among the three subtypes; aberrations in the isocitrate dehydrogenase 1 (IDH1) gene and the platelet-derived growth factor receptor A (PDGFRA) define this subgroup. The classical subgroup is characterized by the amplification of EGFR, lack of TP53 mutations and often with homozygous CDKN2A deletions [26]. Lastly, the mesenchymal subtype is the most aggressive and it is characterized by aberrations in the neurofibromin 1 (NF1) and PTEN genes [23]. It is also characterized by a pro-inflammatory environment compared with the other subtypes. It was hypothesized that one underlying cause for this was the higher incidence of tumor-associated antigens (TAAs), however this could not be proven, as specific tumor antigens are expressed in each subtype [27]. Nevertheless, this classification has not led to altered or adapted treatment approaches [28][29].

Apart from intertumoral heterogeneity, intra-tumoral heterogeneity poses another therapeutic obstacle in treatment of GBM, allowing escape of subclones from (targeted) therapies and driving treatment resistance. This heterogeneity was captured by genome-wide and single cell RNA studies, which showed tumor cells with different transcriptional profiles within the same tumor [30][31]. In addition, it was shown that within the same tumor, different subtypes can coexist, highlighting the heterogeneity that characterizes GBM [31]. In another study, paired primary and recurrent tumor tissue samples were analyzed to determine the persistence of possible drug targets. The results showed that the molecular targets between primary and recurrent tumors changed by 90% [32]. This may explain the failure of drugs that target specific molecular mutations in GBM, such as the EGFR [33].

Eventually, most of the patients experience tumor relapse due to therapeutic resistance. This therapeutic resistance is mainly attributed to glioblastoma stem cells (GSCs), which activate DNA repair mechanisms to promote survival after chemo- and radiotherapy [34]. Additionally, outgrowth of resistant subclones and downregulation of targeted molecules contribute to drug resistance. Furthermore, the highly infiltrative nature of GSCs makes total surgical resection of the tumor impossible [35]. The remaining and/or treatment-resistant clones will eventually generate functional vessels for the nutrient transport and develop tumor recurrence.

2.2. GBM Microenvironment: Local Immunosuppressive Mechanisms

Glioblastoma arises in the central nervous system (CNS) [36], which is an immunologically distinct site. In the past, the CNS was considered an immune privileged site, due to its unique properties. For instance, the blood brain barrier, which tightly regulates the transportation of the immune cells from the periphery to the CNS; the lack of antigen presenting cells in a non-inflamed state; and more importantly the lack of a classic lymphatic system [37][38][39]. The concept of CNS being immune privileged has now been revised. Recent studies have shown that antigens derived from the CNS can efficiently elicit an immune response [40]. More importantly, Louveau et al. [41] discovered a functional lymphatic system, parallel to the dural sinuses, a possible route of transportation of antigen-presenting cells to the deep cervical lymph nodes, where they can present CNS-derived antigens and prime T cells. These recent studies have provided evidence that CNS-derived antigens can mount a vigorous immune response, offering ground to investigate immunotherapy approaches for GBM.

The GBM environment is characterized by the high influx of tumor-associated macrophages (TAMs). In a non-inflamed state, the myeloid composition of the CNS consists of the tissue-resident macrophages that arise from the yolk sac, the microglia [42]. However, in GBM, the microenvironment is comprised mainly of a mixture of microglia and infiltrating monocytes from the periphery. Glioma cells produce a milieu of monocyte chemoattractant proteins along with other factors, leading to disruption of the blood-brain barrier and facilitating recruitment of monocytes from the periphery [43]. When monocytes arrive at the tumor site, glioma cells drive their polarization to an immunosuppressive M2 phenotype [44][45]. These M2-like TAMs promote tumor growth and migration as well as the immune invasion by hampering the adaptive immunity [46][47]. TAMs are the most abundant immune cell population in GBM and can consist up to 50% of the GBM tumor mass. Their importance in tumor growth is highlighted by the correlation between increased TAM numbers and worse prognosis in GBM patients; furthermore, TAM infiltration has been associated with the mesenchymal subtype of GBM, being the most aggressive one [48][49].

Another feature that facilitates the local immune suppression in GBM is T cell dysfunction. Severe T cell exhaustion is observed in GBM, which is characterized by upregulation of expression of co-inhibitory molecules like PD-1, LAG-3 and TIM-3 [50]. Furthermore, an increase in numbers of the regulatory T cells (Tregs), which can suppress the antigen-specific T cells, was found in high grade gliomas compared to low grade gliomas [51]. The recruitment of Tregs at the tumor site is mainly facilitated by the production of the attractant indoleamine 2,3 dioxygenase (IDO) by gliomas [52]. Another facet that contributes to the ‘‘cold’’ tumor microenvironment is the relatively low mutational burden of GBM cells, associated with limited expression of neoantigens [53][54]. Taken together, GBM has all the characteristics of a tumor with low immunogenicity. The M2-like macrophages that are abundant at the tumor site, the dysfunctional T cells and the low neoantigen expression are some of the barriers that we need to overcome to design successful immunotherapies.

Considering all of the above, a therapeutic strategy that is not hindered by specificity for a single molecular target or differentiation state of tumor cells, that is delivered locally in a single surgical intervention, hence bypassing the BBB, that is self-perpetuating in its anti-tumor activity, and which can overcome the immune-suppressive tumor microenvironment, may offer opportunities for achieving therapeutic responses in glioblastoma patients. Oncolytic viruses offer such a treatment strategy.

References

1. Zhang, S.; Rabkin S.D. The discovery and development of oncolytic viruses: are they the future of cancer immunotherapy? Expert. Opin. Drug. Discov. 2020. doi:10.1080/17460441.2021.1850689.
2. Harrington, K.; Freeman, D.J.; Kelly, B.; Harper, J.; Soria, J.C. Optimizing oncolytic virotherapy in cancer treatment. Nat. Rev. Drug Discov. 2019, 18, 689–706, doi:10.1038/s41573-019-0029-0.
3. Kaufman, H.L.; Kohlhapp, F.J.; Zloza, A. Oncolytic viruses: A new class of immunotherapy drugs. Nat. Rev. Drug Discov. 2015, 14, 642–662, doi:10.1038/nrd4663.

4. Taylor, O.G.; Brzozowski, J.S.; Skelding, K.A. Glioblastoma multiforme: An overview of emerging therapeutic targets. Front. Oncol. 2019, 9, 963, doi:10.3389/fonc.2019.00963.
5. Stupp, R.; Mason, W.P.; van den Bent, M.J.; Weller, M.; Fisher, B.; Taphoorn, M.J.; Belanger, K.; Brandes, A.A.; Marosi, C.; Bogdahn, U.; et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N. Engl. J. Med. 2005. doi:10.1056/NEJMoa043330.
6. De Witt Hamer, P.C.; Ho, V.K.Y.; Zwinderman, A.H.; Zwinderman, A.H.; Ackermans, L.; Ardon, H.; Boomstra, S.; Bouwknegt, W.; van den Brink, W.A.; Dirven, C.M.; et al. Between-hospital variation in mortality and survival after glioblastoma surgery in the Dutch Quality Registry for Neuro Surgery. J. Neurooncol. 2019, 144, 313–323, doi:10.1007/s11060-019-03229-5.
7. Papanastassiou, V.; Rampling, R.; Fraser, M.; Petty, R.; Hadley, D.; Nicoll, J.; Harland, J.; Mabbs, R.; Brown, M. The potential for efficacy of the modified (ICP 34.5) herpes simplex virus HSV1716 following intratumoural injection into human malignant glioma: A proof of principle study. Gene Ther. 2002, 9, 398-406, doi:10.1038/sj/gt/3301664.
8. Markert, J.M.; Liechty, P.G.; Wang, W.; Gaston, S.; Braz, E.; Karrasch, M.; Nabors, L.B.; Markiewicz, M.; Lakeman, A.D.; Palmer, C.A.; et al. Phase Ib trial of mutant herpes simplex virus G207 inoculated pre-and post-tumor resection for recurrent GBM. Mol. Ther. 2009, 17, 199–207, doi:10.1038/mt.2008.228.
9. Ning, J.; Wakimoto, H. Oncolytic herpes simplex virus-based strategies: Toward a breakthrough in glioblastoma therapy. Front. Microbiol. 2014, 5, 303, doi:10.3389/fmicb.2014.00303.
10. Kambara, H.; Okano, H.; Chiocca, E.A.; Saeki, Y. An Oncolytic HSV-1 Mutant Expressing ICP34. 5 under Control of a Nestin Promoter Increases Survival of Animals even when Symptomatic from a Brain Tumor. Cancer Res. 2005, 67, 8980–8984.
11. Cheng, P.H.; Wechman, S.L.; McMasters, K.M.; Zhou, H.S. Oncolytic replication of E1b-deleted adenoviruses. Viruses 2015, 7, 5767–5779, doi:10.3390/v7112905.
12. Suzuki, K.; Fueyo, J.; Krasnykh, V.; Reynolds, P.N.; Curiel, D.T.; Alemany, R. A conditionally replicative adenovirus with enhanced infectivity shows improved oncolytic potency. Clin. Cancer Res. 2001, 7, 120–126.
13. Allen, C.; Opyrchal, M.; Aderca, I.; Schroeder, M.A.; Sarkaria, J.N.; Domingo, E.; Federspiel, M.J.; Galanis, E. Oncolytic measles virus strains have significant antitumor activity against glioma stem cells. Gene Ther. 2013, 20, 444–449, doi:10.1038/gt.2012.62.
14. Schirrmacher, V.; van Gool, S.; Stuecker, W. Breaking therapy resistance: An update on oncolytic newcastle disease virus for improvements of cancer therapy. Biomedicines 2019, 7, 66, doi:10.3390/BIOMEDICINES7030066.
15. Gong, J.; Mita, M.M. Activated Ras signaling pathways and reovirus oncolysis: An update on the mechanism of preferential reovirus replication in cancer cells. Front. Oncol. 2014, 4, 167,

doi:10.3389/fonc.2014.00167.

16. Gromeier, M.; Lachmann, S.; Rosenfeld, M.R.; Gutin, P.H.; Wimmer, E. Intergeneric poliovirus recombinants for the treatment of malignant glioma. Proc. Natl. Acad. Sci. USA. 2000. doi:10.1073/pnas.97.12.6803.
17. Dobrikova, E.Y.; Broadt, T.; Poiley-Nelson, J.; Yang, X.; Soman, G.; Giardina, S.; Harris, R.; Gromeier, M. Recombinant oncolytic poliovirus eliminates glioma in vivo without genetic adaptation to a pathogenic phenotype. Mol. Ther. 2008. doi:10.1038/mt.2008.184. Epub 2008 Sep 2.
18. Nüesch, J.P.F.; Lacroix, J.; Marchini, A.; Rommelaere, J. Molecular pathways: Rodent parvoviruses—Mechanisms of oncolysis and prospects for clinical cancer treatment. Clin. Cancer Res. 2012, 18, 3516–3523, doi:10.1158/1078-0432.CCR-11-2325.
19. Chiocca, E.A.; Nassiri, F.; Wang, J.; Peruzzi, P.; Zadeh, G. Viral and oTher. therapies for recurrent glioblastoma: Is a 24-month durable response unusual? Neuro Oncol. 2019, 21, 14–25, doi:10.1093/neuonc/noy170.
20. Lang, F.F.; Conrad, C.; Gomez-Manzano, C.; Alfred Yung, W.K.; Sawaya, R.; Weinberg, J.S.; Prabhu, S.S.; Rao, G.; Fuller, G.N.; Aldape, K.D.; et al. Phase I study of DNX-2401 (delta-24-RGD) oncolytic adenovirus: Replication and immunotherapeutic effects in recurrent malignant glioma. J. Clin. Oncol. 2018, 36, 1419–1427, doi:10.1200/JCO.2017.75.8219.
21. Cloughesy, T.F.; Landolfi, J.; Vogelbaum, M.A.; Ostertag, D.; Elder, J.B.; Bloomfield, S.; Carter, B.; Chen, C.C.; Kalkanis, S.N.; Kesari, S.; et al. Durable complete responses in some recurrent high-grade glioma patients treated with Toca 511 + Toca FC. Neuro Oncol. 2018, 20, 1383–1392, doi:10.1093/neuonc/noy075.
22. Senzer, N.N.; Kaufman, H.L.; Amatruda, T.; Nemunaitis, M.; Reid, T.; Daniels, G.; Gonzalez, R.; Glaspy, J.; Whitman, E.; Harrington, K.; et al. Phase II Clinical Trial of a Granulocyte-Macrophage Colony-Stimulating Factor—Encoding, Second-Generation Oncolytic Herpesvirus in Patients With Unresectable Metastatic Melanoma. J. Clin. Oncol. 2009, 27, 5763–5767, doi:10.1200/JCO.2009.24.3675.
23. Crespo, I.; Vital, A.L.; Gonzalez-Tablas, M.; Patino, M.D.C.; Otero, A.; Lopes, M.C.; De Oliveira, C.; Domingues, P.; Orfao, A.; Tabernero, M.D. Molecular and Genomic Alterations in Glioblastoma Multiforme. Am. J. Pathol. 2015, 185, 1820–1833, doi:10.1016/j.ajpath.2015.02.023.
24. Wang, Q.; Hu, X.; Muller, F.; Kim, H.; Squatrito, M.; Mikkelsen, T.; Scarpace, L.; Barthel, F.; Lin, Y.-H.; Satani, N.; et al. Tumor Evolution of Glioma Intrinsic Gene Expression Subtype Associates With Immunological Changes in the Microenvironment. Neuro Oncol. 2016, 18, vi202–vi202, doi:10.1093/neuonc/now212.854.

25. Gill, B.J.; Pisapia, D.J.; Malone, H.R.; Goldstein, H.; Lei, L.; Sonabend, A.; Yun, J.; Samanamud, J.; Sims, J.S.; Banu, M.; et al. MRI-localized biopsies reveal subtype-specific differences in molecular and cellular composition at the margins of glioblastoma. Proc. Natl. Acad. Sci. USA 2014, 111, 12550–12555, doi:10.1073/pnas.1405839111.

26. Verhaak, R.G.; Hoadley, K.A.; Purdom, E.; Wang, V.; Qi, Y.; Wilkerson, M.D.; Miller, C.R.; Ding, L.; Golub, T.; Mesirov, J.P.; et al. Integrated Genomic Analysis Identifies Clinically Relevant Subtypes of Glioblastoma Characterized by Abnormalities in PDGFRA, IDH1, EGFR, and NF1. Cancer Cell. 2010, 17, 98–110, doi:10.1016/j.ccr.2009.12.020.

27. Doucette, T.; Rao, G.; Rao, A.; Shen, L.; Aldape, K.; Wei, J.; Dziurzynski, K.; Gilbert, M.;, Heimberger, A.B. Immune heterogeneity of glioblastoma subtypes: extrapolation from the cancer genome atlas. Cancer Immunol. Res. 2013. doi:10.1158/2326-6066.

28. Weller, M.; Cloughesy, T.; Perry, J.R.; Wick, W. Standards of care for treatment of recurrent glioblastoma-are we there yet? Neuro Oncol. 2013, 15, 4–27, doi:10.1093/neuonc/nos273.

29. Weller, M.; van den Bent, M.; Preusser, M.; Le Rhun, E.; Tonn, J.C.; Minniti, G.; Bendszus, M.; Balana, C.; Chinot, O.; Dirven, L.; et al. EANO guidelines on the diagnosis and treatment of diffuse gliomas of adulthood. Nat. Rev. Clin. Oncol. 2020, doi:10.1038/s41571-020-00447-z.

30. Patel, A.P.; Tirosh, I.; Trombetta, J.J.; Shalek, A.K.; Gillespie, S.M.; Wakimoto, H.; Cahill, D.P.; Nahed, B.V.; Curry, W.T.; Martuza, R.L.; et al. Single-cell RNA-seq highlights intratumoral heterogeneity in primary glioblastoma. Science 2014, 344, 1396–1402.

31. Sottoriva, A.; Spiteri, I.; Piccirillo, S.G.M.; Touloumis, A.; Collins, V.P.; Marioni, J.C.; Curtis, C.; Watts, C.; Tavaré, S.; et al. Intratumor heterogeneity in human glioblastoma reflects cancer evolutionary dynamics. Proc. Natl. Acad. Sci. USA 2013, 110, 4009–4014, doi:10.1073/pnas.1219747110.

32. Schäfer, N.; Gielen, G.H.; Rauschenbach, L.; Kebir, S.; Till, A.; Reinartz, R.; Simon, M.; Niehusmann, P.; Kleinschnitz, C.; Herrlinger, U.; et al. Longitudinal heterogeneity in glioblastoma: Moving targets in recurrent versus primary tumors. J. Transl. Med. 2019, 17, 1–9, doi:10.1186/s12967-019-1846-y.

33. Lee, A.; Arasaratnam, M.; Chan, D.L.H.; Khasraw, M.; Howell, V.M.; Wheeler, H. Anti-epidermal growth factor receptor therapy for glioblastoma in adults. Cochrane Database Syst. Rev. 2020. doi:10.1002/14651858.CD013238.pub2.

34. Cheng, L.; Huang, Z.; Zhou, W.; Wu, Q.; Donnola, S.; James, K.; Fang, X.; Sloan, A.E.; Mao, Y.; Lathia, J.D.; et al. Glioblastoma Stem Cells Generate Vascular Pericytes to Support Vessel Function and Tumor Growth. Cell 2013, 153, 139–152, doi:10.1016/j.cell.2013.02.021.Glioblastoma.

35. Darmanis, S.; Sloan, S.A.; Croote, D.; Mignardi, M.; Chernikova, S.; Samghababi, P.; Zhang, Y.; Neff, N.; Kowarsky, M.; Caneda, C.; et al. Single-Cell RNA-Seq Analysis of Infiltrating Neoplastic Cells at the Migrating Front. of Human Glioblastoma. Cell Rep. 2017, 21, 1399–1410, doi:10.1016/j.celrep.2017.10.030.

36. Alcantara Llaguno, S.R.; Parada, L.F. Cell of origin of glioma: biological and clinical implications. Br. J. Cancer. 2016. doi:10.1038/bjc.2016.354. Epub 2016 Nov 10.

37. Engelhardt, B.; Carare, R.O.; Bechmann, I.; Flügel, A.; Laman, J.D.; Weller, R.O.; Vascular, glial, and lymphatic immune gateways of the central nervous system. Acta. Neuropathol. 2016. doi:10.1007/s00401-016-1606-5. Epub 2016 Aug 13.

38. Ransohoff, R.M.; Engelhardt, B. The anatomical and cellular basis of immune surveillance in the central nervous system. Nat. Publ Gr. 2012;12, doi:10.1038/nri3265.

39. Ludewig, P.; Gallizioli, M.; Urra, X.; Behr, S.; Brait, V.H.; Gelderblom, M.; Magnus, T.; Planas, A.M. Biochimica et Biophysica Acta Dendritic cells in brain diseases. BBA Mol. Basis Dis. 2016, 1862, 352–367, doi:10.1016/j.bbadis.2015.11.003.

40. Miller, S.D.; Mahon, E.J.M.C.; Schreiner, B.; Bailey, S.L. Antigen Presentation in the CNS by Myeloid Dendritic Cells Drives Progression of Relapsing Experimental Autoimmune Encephalomyelitis. Ann. N. Y. Acad. Sci. 2007, 191, 179–191, doi:10.1196/annals.1394.023.

41. Louveau, A.; Smirnov, I.; Keyes, T.J.; Behr, S.; Brait, V.H.; Gelderblom, M.; Magnus, T.; Planas, A.M.; Schwaninger, M.; et al. Structural and functional features of central nervous system lymphatic vessels. Nature 2015, 523, 337–341, doi:10.1038/nature14432.

42. Sevenich, L. Brain-Resident Microglia and Blood-Borne Macrophages Orchestrate Central Nervous System inflammation in Neurodegenerative Disorders and Brain Cancer. Front. Immunol. 2018, 9, 697, doi:10.3389/fimmu.2018.00697.

43. Hambardzumyan, D.; Gutmann, D.H.; Kettenmann, H. The role of microglia and macrophages in glioma maintenance and progression. Nat. Neurosci. 2016, 19, 20–27, doi:10.1038/nn.4185.

44. Takenaka, M.C.; Gabriely, G.; Rothhammer, V.; Mascanfroni, I.D.; Wheeler, M.A.; Chao, C.; Gutiérrez-vázquez, C.; Kenison, J.; Tjon, E.C.; Barroso, A.; et al. Nat. Neurosci. 2019, 22, 729–740, doi:10.1038/s41593-019-0370-y.

45. Zhou, W.; Ke, S.Q.; Huang, Z.; Flavahan, W.; Fang, X.; Paul, J.; Wu, L.; Sloan, A.E.; Mclendon, R.E.; Li, X.; et al. Periostin secreted by glioblastoma stem cells recruits M2 tumour-associated macrophages and promotes malignant growth. Nat. Cell Biol. 2015, 17, 170–182, doi:10.1038/ncb3090.

46. Mantovani, A.; Marchesi, F.; Malesci, A.; Laghi, L.; Allavena, P. Tumour-associated macrophages as treatment targets in oncology. Nat. Rev. Clin. Oncol. 2017, 14, 399–416 doi:10.1038/nrclinonc.2016.217.

47. Shi, Y.; Ping, Y.; Zhou, W.; He, Z.; Chen, C.; Bian, B.; Zhang, L.; Chen, L.; Lan, X.; Zhang, X.; et al. Tumour-associated macrophages secrete pleiotrophin to promote PTPRZ1 signalling in glioblastoma stem cells for tumour growth. Nat. Commun. 2017, 8, 1–17, doi:10.1038/ncomms15080.

48. Sørensen, M.D. Tumour-associated microglia / macrophages predict poor prognosis in high-grade gliomas and correlate with an aggressive tumour subtype. Neuropathol. Appl. Neurobiol. 2018, 44, 185–206, doi:10.1111/nan.12428.

49. Lu-emerson, C.; Snuderl, M.; Kirkpatrick, N.D.; Goveia, J.; Davidson, C.; Huang, Y.; Riedemann, L.; Taylor, J.; Ivy, P.; Duda, G.; et al. Increase in tumor-associated macrophages after antiangiogenic therapy is associated with poor survival among patients with recurrent glioblastoma. Neuro Oncol. 2013, 15, 1079–1087.

50. Woroniecka, K.; Chongsathidkiet, P.; Rhodin, K.; Kemeny, H.; Dechant, C.; Farber, S.H.; Elsamadicy, A.A.; Cui, X.; Koyama, S.; Jackson, C.; et al. T-Cell Exhaustion Signatures Vary with Tumor Type and Are Severe in Glioblastoma. Clin. Cancer Res. 2018, 44, 4175-4187, doi:10.1158/1078-0432.CCR-17-1846.

51. Heimberger, A.B.; Abou-ghazal, M.; Reina-ortiz, C.; Yang, D.S.; Sun, W.; Qiao, W.; Hiraoka, N.; Fuller, G.N. Incidence and Prognostic Impact of FoxP3 + Regulatory T Cells in Human Gliomas. Clin. Cancer Res. 2008, 14, 5166–5173, doi:10.1158/1078-0432.CCR-08-0320.

52. Martinez-lage, M.; Lynch, T.M.; Bi, Y.; Cocito, C.; Way, G.P.; Pal, S.; Haller, J.; Yan, R.E.; Ziober, A.; Nguyen, A.; et al. Immune landscapes associated with different glioblastoma molecular subtypes. Acta Neuropathol. Commun. 2019, 6, 1–12.

53. Büttner, R.; Longshore, J.W.; López-Ríos, F.; Vandergrift, I.I.I.; WA.; Patel, S.J.; Cachia, D.; Bartee, E. Implementing TMB measurement in clinical practice: Considerations on assay requirements. ESMO Open. 2019, 4, e00044, doi:10.1136/esmoopen-2018-000442.

54. Alexandrov, L.B.; Nik-Zainal, S.; Wedge, D.C.; Aparicio, S.A.; Behjati, S.; Biankin, A.V.; Bignell, G.R.; Bolli, N.; Borg, A.; Børresen-Dale, A.L.; et al. Signatures of mutational processes in human cancer. Nature 2013, 500, 415–421, doi:10.1038/nature12477.Signatures.