CHAPTER 19

Brief Overview of Chimeric Antigen Receptor–Mediated Immunotherapy for Glioblastoma Multiforme

Celine-Lea Halioua-Haubold, David A. Brindley
Healthcare Translation Research Group, Department of Paediatrics, University of Oxford, Oxford, United Kingdom

Under the broad class of immunotherapy falls cancer vaccines, checkpoint inhibitors, oncolytic viral therapy and chimeric antigen receptor (CAR)–functionalised immune cell therapy. We provide in this brief chapter a bird's eye view of cell-based immune therapeutics in development for GBM with focus on CAR-T cells.

GLIOBLASTOMA MULTIFORME IMMUNOSUPPRESSION

GBM is a highly immunosuppressive tumour (Zagzag et al., 2005). Functions of GBM cell–secreted factors include the suppression of T cell activation and proliferation and the induction of T cell apoptosis. GBM cells are also without the costimulatory molecules necessary to activate naïve T cells (Driessens et al., 2009). Notably, regulatory T cells (Tregs) are attracted to the tumour site via these GBM cell–secreted factors, myeloid/microglial cells are M2-polarised and there is a paucity of natural killer (NK) cells, all these factors combine to further exacerbate immune suppression in the tumour microenvironment (TME) (Crane et al., 2012; Komohara et al., 2008). GBM cells have decreased major histocompatibility complex (MHC) expression, thus limiting their antigen presentation; the immunosuppressive microenvironment also compromises the antigen-presenting capabilities of myeloid/microglial cells (Schartner et al., 2005). Like is other cancers, hypoxia plays an important role in the immunosuppressive GBM microenvironment. Particularly, an array of hypoxia-induced genes activate Tregs and upregulate vascular endothelial growth factor (VEGF) to promote the vascularisation of the hypoxic tissues. VEGF itself is immunosuppressive: it inhibits immune cell recruitment, differentiation and function, along with promoting cancer stemness in the tumour cells themselves (Lapeyre-Prost et al., 2017; Li et al., 2016). The hypoxic microenvironment also induces CNS macrophages to transform into tumour-associated macrophages that are M2-polarised – that is an immunosuppressive and tumour-supportive phenotype (Razavi et al., 2016). Another challenge in immune cell recognition of tumour cells is the lower-than-average tumour mutational load (TML) that is typically observed in gliomas,

Second Generation Cell and Gene-based Therapies
ISBN 978-0-12-812034-7
https://doi.org/10.1016/B978-0-12-812034-7.00019-4
© 2020 Elsevier Inc.
All rights reserved.

resulting in fewer neoantigen targets on which to functionalise immune-targeted therapies and lower recognition of the tumour by the immune system (Hodges et al., 2017).

GBM rarely metastasises extracranially, perhaps due to the rapid terminality of the disease after onset or the sensitivity of the tumour cells outside of the CNS. However, circulating tumour cells are found in a subset of GBM patients, and transplant organ recipients from GBM patient donors have been shown to develop the disease extracranially. (Schweitzer et al.; Müller et al., 2014). Although the disease is largely restricted to the CNS, patients show systemic immunosuppression (Gustafson et al., 2010; Bloch et al., 2013). Of note, the current standard of care for GBM is also immunosuppressive, and it is likely that patients undergoing exploratory treatments, that modulate the immune system, will likely have already received one or multiple of these standard treatments (Harris et al., 1976).

CHIMERIC ANTIGEN RECEPTOR–FUNCTIONALISED CELL THERAPY IN GLIOBLASTOMA

Advances in the treatment of acute lymphocytic leukemia (ALL) achieved with CAR-T cells that have resulted in outstanding remission rates have demonstrated that this new therapeutic modality has a paradigm-changing potential. It is thus worth exploring how to deploy this novel technology in solid tumour oncology, in spite of the numerous hurdles of treating solid tumours.

Chimeric Antigen Receptors

The CAR consists of an antibody-derived antigen recognition domain for a tumour antigen of choice, commonly in the form of a single chain variable fragment, a CD3-ζ domain for T cell activation in the absence of a primary immune response and intracellular costimulatory domains (Bridgeman et al., 2014; Fesnak et al., 2016; Sadelain et al., 2013). Importantly, the CAR needs not target an MHC-presented antigen and, in theory, can be functionalised for any cell surface molecule including lipids and carbohydrates – an important distinction considering the MHC downregulation that is typically observed in GBM cells. Although no standard has yet been established, CAR-T interventions in GBM can be delivered intracranially after tumour resection and systemically as T cells are able to cross the blood–brain barrier (Bagley et al., 2018); however, it is worth noting that this treatment has also been administered into the cerebrospinal fluid.

The especially immunosuppressive microenvironment characteristic of GBM will naturally impede the efficacy of CAR-based therapies; cotreatment with agents inhibiting the primary mechanisms of GBM immunosuppression will likely be necessary to reach the degree of efficacy attained in CAR-Ts in liquid cancers. On the whole, immunotherapy of any strategy for GBM has had limited results in the clinic to this date, suggesting that immune-mediated treatments will need to increase in complexity to match that of the metabolism and immune escape exhibited by GBM tumour cells (Lim et al., 2018).

Chimeric Antigen Receptor–Functionalised T Cells for Glioblastoma Multiforme

As highlighted earlier, CAR-T therapy recently scored two big wins in the clinic, with the approval Yescarta (axicabtagene ciloleucel, Kite Pharma Inc.) and Kymriah (tisagenlecleucel, Novartis), both receiving their first FDA market approval in 2017 for subtypes of large B-cell lymphoma and acute lymphoblastic leukaemia, respectively (FDA, 2017a,b). Here, patient-derived (i.e., autologous) T cells are extracted from the patient, genetically modified to express CARs of interest to specifically target the cancer cell and then are reintroduced into the patient.

Although clinical efficacy has on average been mixed, CAR-T cells have been shown to infiltrate the tumour and have shown impressive efficacy in some case studies (Table 19.1) (Brown et al., 2016). Because of the heterogeneous nature of GBM, as referenced in its name – *multiforme*, CAR-T targeting a single antigen may not be enough on its own to ablate sufficient tumour cells to counteract the rapid proliferation of the remaining cells. In line with this thought, the especially rapid proliferation of GBM tumour cells provides ample opportunity for the tumour to undergo microcellular evolution resulting in the selection of cells that do not express the particular antigen or otherwise downregulate the offending antigen ('antigen escape') (Jackson and Brentjens, 2015). To counteract this phenomenon, bi- and trivalent CAR-Ts are being developed to overcome the antigenic heterogeneity of GBM (Bielamowicz et al., 2018). Indeed, CAR-T has thus far shown its most impressive efficacy in lymphomas and leukaemia, which are highly clonal cancers. The clinical results of three CAR targets for GBM have been published: interleukin receptor-13Rα2 (IL13Rα2) (Brown et al., 2015, 2016), human epidermal growth factor receptor 2 (HER2) (Ahmed et al., 2017) and epidermal growth factor receptor variant III (EGFRvIII) (O'Rourke et al., 2017).

IL13Rα2 is present in over 60% of GBMs, has limited expression on healthy tissue and is a prognostic of poor patient survival, making it an attractive target for CAR-T therapy (Brown et al., 2018; Jarboe et al., 2007). This was the first CAR target to be used in the clinic for GBM. CAR-Ts targeting IL13Rα2 have shown encouraging albeit preliminary responses in small sample size studies (Brown et al., 2015). A case study of IL13Rα2 CAR-T in a 50-year-old man with recurrent GBM resistant to standard of care showed tumour regression (Brown et al., 2016). The patient received 6 intracranial and 10 cycles of intravenous infusions. The regression continued for 7.5 months posttherapy.

HER2 is another candidate target for CAR-T, with expression in up to 80% of GBMs (Zhang et al., 2008; Ahmed et al., 2010). Of note, safety concerns were raised regarding CAR-Ts targeting HER2 on the death of a patient with refractory metastatic colon cancer (Morgan et al., 2010). Results of a recent Phase I study of HER2 cytomegalovirus (CMV) bispecific CAR-T in 17 GBM patients showed preliminary safety and efficacy. Briefly, patients received one to seven intravenous doses of the therapy. CMV bispecificity functions to nonspecifically increase the activity of the CAR-T.

Table 19.1 Selection of Clinical Trials deploying Chimeric Antigen Receptor–Based Therapeutics in Glioblastoma Multiforme.

Clinical Trial Identifier	Target	Phase	Primary Sponsor	References and Notes
NCT02664363, NCT03283631	EGFRvIII	Phase I	Duke University Medical Center	Two trials: ExCeL and INTERCEPT
NCT01454596	EGFRvIII	Phase I/II	National Cancer Institute	
NCT02209376	EGFRvIII	Phase I	University of Pennsylvania, University of California San Francisco	Results in first 10 patients (O'Rourke et al., 2017)
NCT02442297	HER2	Phase I	Baylor College of Medicine	
NCT01109095	HER2/CMV	Phase I	Baylor College of Medicine	Results in 16 patients (Ahmed et al., 2017) Earlier oral presentation of results (Ahmed et al., 2015)
N/A	HER2/GD2	Phase IIa/III	Aurora BioPharma	(AU105) Newly diagnosed GBM
N/A	HER2/GD2	Phase Ib/IIa	Aurora BioPharma	(AU101) Recurrent GBM
NCT02208362, NCT03389230	IL13Rα2	Phase I	Mustang Bio	Case study (Brown et al., 2016) (MB-101)
NCT00730613	IL13Rα2	Phase I	City of Hope, National Cancer Institute	Results in three patients (Brown et al., 2015)

CMV, Cytomegalovirus; *EGFRvIII*, Epidermal growth factor receptor variant III; *HER2*, Human epidermal growth factor receptor 2; *IL13Rα2*, Interleukin receptor-13Rα2.

Three patients were still stable with no disease progression after 24–29 months of follow-up on conclusion of the study (Ahmed et al., 2017).

Similarly, ten patients were infused with a single intravenous dose of EGFRvIII CAR-T cells in a first-in-human study (O'Rourke et al., 2017). EGFRvIII is the most common variant of tyrosine receptor kinase EGFR, leading to constitutive receptor activation and promotion of cellular proliferation, with expression in 30% of GBM tumours (Padfield et al., 2015). Of these, seven patients had their tumour resected postinfusion. Analysis of tumour tissue not only found trafficking of the

CAR-Ts to the active regions of the tumour but also demonstrated the highly immunosuppressive character of GBM: particularly increased expression of inhibitory molecules and Tregs were reported. What is more, a decrease of EGFRvIII expression in GBM tumour tissue relative to EGFR occurred in five of these seven patients, an indication of on-target effects. The cells seemed to engraft as assessed by detection of the CAR-T cells in peripheral blood.

Chimeric Antigen Receptor–Functionalised Natural Killer Cell Therapy

A significant bottleneck in the widespread clinical use of CAR-T therapeutics is the present necessity of the modified T cells being autologous instead of allogeneic, albeit several off-the-shelf so-called "universal" cell lines being developed by inactivation of the genes underlying the risk of graft versus host disease, such that one cell line could be used used in multiple patients. However, natural killer (NK) cells can eliminate tumours directly and do not cause graft versus host disease and therefore may offer an attractive allogenic CAR therapeutic strategy for oncology indications, including GBM.

Multiple CAR-NKs are in preclinical development for GBM, largely targeting the same antigens as the above CAR-T therapeutics, including EGFR/EGFRvIII (Han et al., 2015; Müller et al., 2015) and HER2 (Zhang et al., 2016), showing efficacy in mouse models of GBM. Multispecific antigen approaches have also been explored with NK cells, due to the aforementioned heterogeneity of GBM (Genßler et al., 2015). At least one CAR-NK is being explored in Phase I clinical trials for GBM, targeting HER2 (Hodson, 2018) (Table 19.1).

PERSPECTIVES

GBM is an oncology indication that represents a pressing unmet medical need. The lack of therapeutic success to this date with conventional therapeutic approaches strongly suggests that paradigm-changing innovation is required to provide treatments that offer improved remission outcomes to patients suffering from this cancer with otherwise an overall very poor prognosis. Genetically engineered live drugs such as CAR-T cells and CAR-NK cells represent novel therapeutic options that have shown preliminary signals of efficacy. However, to translate this hope into practical clinical benefits for patients who need them, tailored molecular CAR architectures reflecting the large heterogeneity of GBM are still required, for example, multivalent CARs simultaneously addressing various markers of GBM or with improved tumour infiltration properties, as well as innovative combination therapies regimens to fight the strong immunosuppressive effects of the GBM tumour microenvironment and other tumour proliferation and defence mechanisms.

REFERENCES

Ahmed N, et al. HER2-specific T cells target primary glioblastoma stem cells and induce regression of autologous experimental tumors. Clin Cancer Res 2010;16:474–85.

Ahmed N, et al. Autologous HER2 CMV bispecific CAR T cells are safe and demonstrate clinical benefit for glioblastoma in a phase I trial. J. Immunother Cancer 2015;3:O11.

Ahmed N, et al. HER2-specific chimeric antigen receptor–modified virus-specific T cells for progressive glioblastoma: a phase 1 dose-escalation trial. JAMA Oncol 2017;3:1094.

Bagley SJ, Desai AS, Linette GP, June CH, O'Rourke DM. CAR T-cell therapy for glioblastoma: recent clinical advances and future challenges. Neuro Oncol 2018. https://doi.org/10.1093/neuonc/noy032.

Bielamowicz K, et al. Trivalent CAR T cells overcome interpatient antigenic variability in glioblastoma. Neuro Oncol 2018;20:506–18.

Bloch O, et al. Gliomas promote immunosuppression through induction of B7-H1 expression in tumor-associated macrophages. Clin Cancer Res 2013;19:3165–75.

Bridgeman JS, et al. CD3ζ-based chimeric antigen receptors mediate T cell activation via cis- and trans-signalling mechanisms: implications for optimization of receptor structure for adoptive cell therapy. Clin Exp Immunol 2014;175:258–67.

Brown CE, et al. Bioactivity and safety of IL13R 2-redirected chimeric antigen receptor CD8+ T cells in patients with recurrent glioblastoma. Clin Cancer Res 2015;21:4062–72.

Brown CE, et al. Regression of glioblastoma after chimeric antigen receptor T-cell therapy. N Engl J Med 2016;375:2561–9.

Brown CE, et al. Optimization of IL13Rα2-targeted chimeric antigen receptor T cells for improved anti-tumor efficacy against glioblastoma. Mol Ther 2018;26:31–44.

Crane CA, Ahn BJ, Han SJ, Parsa AT. Soluble factors secreted by glioblastoma cell lines facilitate recruitment, survival, and expansion of regulatory T cells: implications for immunotherapy. Neuro Oncol 2012;14:584–95.

Driessens G, Kline J, Gajewski TF. Costimulatory and coinhibitory receptors in anti-tumor immunity. Immunol Rev 2009;229:126–44.

Commissioner, O. of the. Press announcements - FDA approves CAR-T cell therapy to treat adults with certain types of large B-cell lymphoma. 2017a. Available at: https://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm581216.htm.

Commissioner, O. of the. Press announcements - FDA approval brings first gene therapy to the United States. 2017b. Available at: https://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm574058.htm.

Fesnak AD, Levine BL, June CH. Engineered T cells: the promise and challenges of cancer immunotherapy. Nat Rev Cancer 2016;16:566–81.

Genßler S, et al. Dual targeting of glioblastoma with chimeric antigen receptor-engineered natural killer cells overcomes heterogeneity of target antigen expression and enhances antitumor activity and survival. OncoImmunology 2015;5.

Gustafson MP, et al. Systemic immune suppression in glioblastoma: the interplay between CD14+HLA-DRlo/neg monocytes, tumor factors, and dexamethasone. Neuro Oncol 2010;12:631–44.

Han J, et al. CAR-engineered NK cells targeting wild-type EGFR and EGFRvIII enhance killing of glioblastoma and patient-derived glioblastoma stem cells. Sci Rep 2015;5.

Harris J, Sengar D, Stewart T, Hyslop D. The effect of immunosuppressive chemotherapy on immune function in patients with malignant disease. Cancer 1976;37:1058–69.

Hodges TR, et al. Mutational burden, immune checkpoint expression, and mismatch repair in glioma: implications for immune checkpoint immunotherapy. Neuro Oncol 2017;19:1047–57.

Hodson J. NantKwest and Frankfurt University Hospital Announce first in human dosing of Off-the-Shelf HER2.taNK (CAR natural killer) cell therapy in glioblastoma. NantKwest; 2018.

Jackson HJ, Brentjens RJ. Overcoming antigen escape with CART-cell therapy. Cancer Discov 2015;5:1238–40.

Jarboe JS, Johnson KR, Choi Y, Lonser RR, Park JK. Expression of interleukin-13 receptor α2 in glioblastoma multiforme: implications for targeted therapies. Cancer Res 2007;67:7983–6.

Komohara Y, Ohnishi K, Kuratsu J, Takeya M. Possible involvement of the M2 anti-inflammatory macrophage phenotype in growth of human gliomas. J Pathol 2008;216:15–24.
Lapeyre-Prost A, et al. Immunomodulatory activity of VEGF in cancer. Int Rev Cell Mol Biol 2017;330:295–342.
Li Y-L, Zhao H, Ren X-B. Relationship of VEGF/VEGFR with immune and cancer cells: staggering or forward? Cancer Biol Med 2016;13:206–14.
Lim M, Xia Y, Bettegowda C, Weller M. Current state of immunotherapy for glioblastoma. Nat Rev Clin Oncol 2018;15:422–42.
Morgan RA, et al. Case report of a serious adverse event following the administration of T cells transduced with a chimeric antigen receptor recognizing ERBB2. Mol Ther 2010;18:843–51.
Müller C, et al. Hematogenous dissemination of glioblastoma multiforme. Sci Transl Med 2014;6. 247ra101.
Müller N, et al. Engineering NK cells modified with an EGFRvIII-specific chimeric antigen receptor to overexpress CXCR4 improves immunotherapy of CXCL12/SDF-1α-secreting glioblastoma. J Immunother Hagerst Md 1997 2015;38:197–210.
O'Rourke DM, et al. A single dose of peripherally infused EGFRvIII-directed CAR T cells mediates antigen loss and induces adaptive resistance in patients with recurrent glioblastoma. Sci Transl Med 2017;9.
Padfield E, Ellis HP, Kurian KM. Current therapeutic advances targeting EGFR and EGFRvIII in glioblastoma. Front Oncol 2015;5.
Razavi S-M, et al. Immune evasion strategies of glioblastoma. Front Surg 2016;3.
Sadelain M, Brentjens R, Riviere I. The basic principles of chimeric antigen receptor (CAR) design. Cancer Discov 2013;3:388–98.
Schartner JM, et al. Impaired capacity for upregulation of MHC class II in tumor-associated microglia. Glia 2005;51:279–85.
Schweitzer, T., Vince, G.H., Herbold, C., Roosen, K. Extraneural metastases of primary brain tumors, vol. 8.
Zagzag D, et al. Downregulation of major histocompatibility complex antigens in invading glioma cells: stealth invasion of the brain. Lab Investig 2005;85:328–41.
Zhang JG, et al. Tumor antigen precursor protein profiles of adult and pediatric brain tumors identify potential targets for immunotherapy. J Neuro Oncol 2008;88:65–76.
Zhang C, et al. ErbB2/HER2-Specific NK cells for targeted therapy of glioblastoma. J Natl Cancer Inst 2016;108.