CAR T Cell Therapy for Pediatric Brain Tumors

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Chimeric Antigen Receptor (CAR) T cell therapy has recently begun to be used for solid tumors such as glioblastoma multiforme. Many children with pediatric malignant brain tumors develop extensive long-term morbidity of intensive multimodal curative treatment. Others with certain diagnoses and relapsed disease continue to have limited therapies and a dismal prognosis. Novel treatments such as CAR T cells could potentially improve outcomes and ameliorate the toxicity of current treatment. In this review, we discuss the potential of using CAR therapy for pediatric brain tumors. The emerging insights on the molecular subtypes and tumor microenvironment of these tumors provide avenues to devise strategies for CAR T cell therapy. Unique characteristics of these brain tumors, such as location and associated morbid treatment induced neuro-inflammation, are novel challenges not commonly encountered in adult brain tumors. Despite these considerations, CAR T cell therapy has the potential to be integrated into treatment schema for aggressive pediatric malignant brain tumors in the future.

Keywords: chimeric antigen receptor T cell, pediatric brain tumor, medulloblastoma, ependymoma, ATRT, pediatric glioma, immune therapy, adoptive cell therapy

INTRODUCTION

Chimeric antigen receptor (CAR) T cells are a form of adoptive cell therapy used for immunotherapy. CAR T cells were initially FDA approved in 2017 for hematological malignancies, however, many preclinical and clinical studies have shown efficacy of CAR T cells for solid tumors including glioblastoma, medulloblastoma, and ependymoma (1–3). Pediatric central nervous system (CNS) cancers remain the leading cause of pediatric cancer related death, thus there is an urgent need to develop new therapies (4). These new therapies need to be specifically directed to malignant cells and limit off target cytotoxicity inherent in chemotherapeutics while having a strong, sustained cytotoxic effect on cancer cells to minimize recurrence. CAR-T cells have the potential to accomplish these goals. In this review, we discuss the current progress in CAR T cell development for specific pediatric brain tumors as well as future implementation strategies.

Overview of CAR-T Cell Targeting

The concept of engineering chimeric antigen receptors (CAR) has been around for over 25 years, and entails combining a single-chain variable fragment (scFv) of an antibody with the T cell receptor (TCR) signaling domain CD3, thus conferring antibody like antigen recognition to T cell cytolytic activity (5, 6). This ingenuity allows for the recognition of a target antigen without

presentation by major histocompatibility complex (MHC) (5). However, in order for the T cell to carry out its cytolytic activity, proliferate and maintain persistence in the local microenvironment, co-stimulation is still necessary (7). In nature these co-stimulatory signals are provided by the antigen presenting cell, but in engineered CAR T cell constructs multiple co-stimulatory domains can be included to promote T cell functionality (8–10).

For applications of CAR T cells in cancer treatment, the engineered target is ideally only present on the tumor and not on normal cells thus limiting off target therapeutic effects (1). CAR T cell therapy became FDA approved in 2017 for B cell malignancies by targeting CD19 (11). The applications for CAR T cells continues to expand in the clinical setting especially in the treatment of hematological malignancies (12). Multiple clinical trials are in place for evaluating the efficacy of CAR T cell therapy in solid tumors but to date CAR therapy for this indication is not FDA approved. Many obstacles are present that hinder the efficacy of CAR T therapy in solid tumors which includes but are not limited to difficulty in trafficking to the tumor site, presence of an immunosuppressive environment, toxicity, and tumor antigen heterogeneity (13).

Solid tumors in the brain present further difficulties due to the semipermeable properties of the blood brain barrier (BBB) which limits the delivery of many therapeutics (14). The BBB is comprised of specialized endothelial cells that prevent entry of large hydrophophilic molecules and unwanted cells from entering the brain. Brain tumors disrupt the BBB to form the blood- tumor barrier (BTB) which has heterogenous perfusion and permeability throughout the tumor and also hinders the delivery of therapeutics (15). CAR T cell infusion to the brain can include delivery via the blood, cerebral spinal fluid (CSF), or locally in the tumor cavity (**Figure 1**). Brain tumors are currently the most common solid tumor types undergoing clinical trial testing for CAR T cell efficacy and have shown early promise in the treatment of glioblastoma (GBM) (16). In this review, we focus on pediatric brain tumors as novel interventions are needed given the grim prognoses for many patients.

PEDIATRIC BRAIN TUMORS

Medulloblastoma

Medulloblastoma is the most common malignant brain tumor in children (10–20% of all pediatric brain tumors) with

FIGURE 1 | Options for infusion of CAR-T cells. **(A)** Intravenous (IV), **(B)** Intrathecal/Ventricular (IT), **(C)** Intratumor/cavity (IC).

an incidence rate of 6.0 per million in patients 1–9 years old (17). Until recent years, medulloblastoma prognosis and classification was primarily stratified on a histological basis, as well as characteristics such as age and metastatic status (18). With increasing accessibility to advanced molecular genetic techniques, medulloblastoma has been further classified based on its distinct molecular subtypes (WNT, SHH, Group 3, and Group 4), shedding new light on potential therapeutic targets (19). These tumors develop in the cerebellar vermis and thus are exclusively in the posterior fossa (20).

The current standard of care for medulloblastoma remains surgical resection, craniospinal irradiation, and chemotherapy. The expected 5-year survival is 70–75% amongst children greater than 3-years-old (21–26). New risk stratification groups and survival outcomes have been proposed taking into account the molecular subtypes. After review of data from several cohorts of the 4 molecular subtypes, Ramaswamy et al. proposed a four group stratification scheme: low risk (>90% survival), standard risk (75–90% survival), high risk (50–75% survival) and very high risk (<50% survival). The WNT subtype and MYCN-amplified SHH subtype had the best and worst overall survival, respectively (27). The prognostic information provided by molecular subtyping can influence treatment specifically the need for adjuvant radiation given the increased risk of neurocognitive impairment in children (28).

Molecular Subtypes

The WNT subgroup of medulloblastoma, known for its excellent long-term prognosis compared to other subgroups, has mortality outcomes related more to complications of treatment or secondary neoplasms rather than tumor recurrence (19, 29). The classic WNT pathway defects in this subgroup occur through somatic deletions of CTNNB1 on chromosome 6 (encoding b-catenin), or monosomic deletions of chromosome 6, and thus have positive immunohistochemical staining for b-catenin (30). Additionally, germline mutations of WNT pathway inhibitor APC are associated with Turcot syndrome and associated medulloblastoma (31). Current studies in this subgroup aim to improve excellent survival outcomes while decreasing whole brain and spine radiation doses (NCT01878617, NCT02724579).

The SHH (sonic hedgehog) group is characterized by mutations resulting in activation of the SHH pathway. Germline mutations in SHH receptor PTCH (Gorlin syndrome) and SHH inhibitor SUFU predispose to medulloblastoma, especially infantile forms (32–36). This activation occurs primarily through somatic PTCH1/SMO/SUFU mutations, as well as amplifications of GLI1, GLI2, and MYCN (34, 37, 38). Tumors of this group occur in all ages and is the predominant subgroup in children <3 years of age and adults. Outcomes are good in young children with a less favorable outcome in older children and adults, especially with TP53 mutations (27, 39).

Group 3 tumors are characterized primarily by MYC amplification, as opposed to MYCN amplification characteristic of the SHH subgroup (40–42). Amplification of oncogene OTX2 can also be seen in group 3, as well as group 4 tumor (43–45). Furthermore, while the pathogenesis is not yet clear, these tumors frequently overexpress genes involved in retinal development and
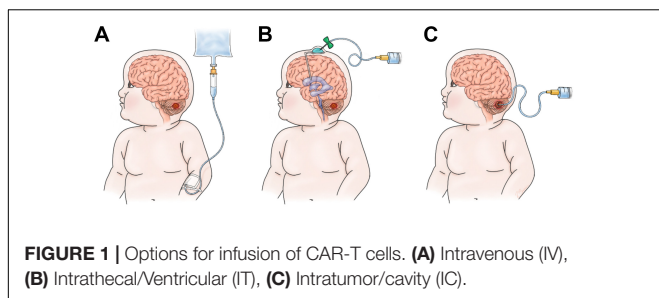
GABAergic pathways (40–42). Overall, these tumors frequently metastasize and thus have the worst outcome of all subgroups (19, 27).

Group 4 tumors remain the least understood of the subgroups, however, they make up >30% of all medulloblastomas (19). Isochromosome 17q is a common feature of group 4 tumors, occurring in 66% of tumors (41, 46). Also notable is the high incidence of cytogenetic loss of the X chromosome in 80% of females with group 4 medulloblastomas (40). These tumors frequently metastasize and can be high risk depending on their genetic features (27). Further research on the etiopathogenesis of medulloblastoma is ongoing.

Antigenic Targets

Receptor tyrosine-protein kinase ERBB2 (HER2) expression is most well-known for its role in a subset of breast cancers; however, HER2 expression is seen in approximately 40% of medulloblastomas (47). Given that ERBB2 protein is not detected in normal brain (48), this makes it an attractive target for CAR T cell therapy. Treatment with monoclonal antibodies targeting HER2 was ineffective in medulloblastoma likely due to lower expression profiles than in breast cancer and lack of HER2 gene amplification (49).

Early application with first generation CAR T cells (i.e., CAR T cells with an intracellular CD3 zeta domain and no co-stimulatory domain) in targeting HER2 showed promise by demonstrating effective targeting and regression of medulloblastomas in an orthotopic xenogenic mouse model (49). Efficacy of this study was likely limited by reliance on use of first generation CD3 constructs. Nellan et al. recently showed improved response and durable regression when using second generation CAR T cells with 4-1BB co-stimulation administered regionally to target HER2 in a preclinical xenograft model (50). These second generation CAR T constructs have shown improved persistence, increased T cell activation and decreased T cell exhaustion (51).

B7-H3 (CD276) overexpression has been found in a variety of human cancers, including lung adenocarcinoma, craniopharyngioma, neuroblastoma, medulloblastoma, glioma, ovarian cancer, pancreatic cancer, and acute myeloid leukemia. This pan-cancer antigen has absent or low in normal tissues making it an ideal CAR T cell target (52). In xenograft models of pediatric osteosarcoma, medulloblastoma, and Ewing sarcoma, B7-H3 CAR T cells demonstrated efficacy against tumors high surface target antigen density (53). Given the heterogeneity of cell surface antigen expression in brain tumors, such as glioblastoma (GBM), multivalent CAR T cells have been designed. These CAR T cells are capable of targeting multiple antigens simultaneously. Trivalent targets to EPHA2, HER2, and IL13Rα2 with CAR T cells has demonstrated efficacy in preclinical models of recurrent medulloblastoma and GBM (3, 54).

Targeting cancer testis antigens is of interest given their limited normal tissue expression on testicular germ cells and placental trophoblasts, thereby decreasing off target effects (55). The cancer testis antigen, PRAME, has been estimated to be expressed in up to 80% of medulloblastomas and could potentially serve as an immunotherapeutic target (47). Many cancer testis antigens remain MHC restricted immune targets making widespread application difficult due to the vide variance of MHC alleles across populations. However, Orlando et al. recently showed some success in orthotopic medulloblastoma models using CAR-T cells specific for the PRAME-derived peptide SLL (56). This peptide, which is presented in context of HLA-A*02, is thought to be present in up to 48.4% in caucasians and 22.6% in black ethnic groups (57).

Tumor Microenvironment

The tumor microenvironment is comprised of tumor-infiltrating immune cells, fibroblasts, and endothelial cells, as well as a dynamic extra-cellular matrix which all can modulate tumor progression and the response to immunotherapy (58). The tumor microenvironment in solid tumors is typically immunosuppressive and impairs the efficacy of immunotherapy including CAR T cell therapy (59). Pediatric brain tumors are less immunosuppressive compared to their adult counterparts (60). The tumor microenvironment is still being characterized in medulloblastoma but there are differences in tumor infiltrating leukocytes between molecular subtypes. SHH group tumors are characterized by higher immune cell infiltration such as tumor associated macrophages (TAMs) and increased expression of inflammation-related genes compared to group 3 and 4 tumors (61, 62). Interestingly, Pham et al. measured immunosuppressive subsets of CTLA-4 or PD-1-expressing T cells in murine Group 3 tumors and found they contained higher percentages of PD-1+ and CD8+ T cells (62). In xenograft models, a significant survival benefit was only present in group 3 medulloblastoma subtypes treated with anti-PD-1 alone or in combination with anti-CTLA-4 (62, 63). The differential response to anti-PD-1 blockade observed in group 3 medulloblastoma suggests that the PD-1/PD-L1 environment is a key immunoregulatory pathway.

In previous small human cohort studies, there was no subgroup specific patterns of tumor infiltrating lymphocytes observed (64, 65). Murata et al. reported that 56% of cases had high expression of PD-L1 and was actually associated with low CD8 + T cell infiltration and poor prognosis (66). However, two other cohort studies reported no significant expression of PD-L1 in any of the studied cases of medulloblastomas (64, 65). A recent, more comprehensive study, by Bockmayr et al. which included 763 medulloblastoma cases did find significant differences in tumor microenvironments between subgroups using gene expression data analysis (67).

Pediatric Ependymoma

Ependymomas comprise 5.2% of pediatric CNS tumors making these tumors the third most common in the pediatric population (68, 69). These tumors arise from cells along the lining of the cerebral ventricles or the spinal cord central canal. For the purposes of this review we will only discuss intracranial ependymomas. Ependymomas are classified by the World Health Organization (WHO) as grades I, II, and III based on their grade of anaplasia (70). Standard therapy includes aggressive gross total resection (GTR) is combination with radiotherapy. Merchant et al. showed that conformational radiation therapy (CRT) significantly improves survival in children and is similar

across pedatric age groups including patients less than 3 years of age (71, 72). Aggressive GTR is critical in ependymoma patients to prevent recurrence and can sometimes be difficult given local infiltration. Recurrent disease can be difficult to manage and overall survival at 5 years is as low as 37% for recurrent tumors (72, 73). While the utility of radiotherapy has clearly been shown in treating ependymomas in children, the benefit with upfront chemotherapy is less understood. However, a recently closed trial exploring this will soon be reporting results (COG trial ACNS0831).

Molecular Supbtypes
With advances in molecular genetics, we now know that ependymomas have four subtypes, two in the posterior fossa and two in the supratentorial space. Posterior fossa ependymomas (PFE) have 2 distinct groups, PFE group A (PFA) and group B (PFB), each of which have distinct demographics, epigenetics, and outcomes (74, 75). PFA tumors are typically only found in infants, while PFB occurs equally in adults and adolesents (76). Patients with PFA have increased risk of recurrence and worse overall survival (75).

Supratentorial ependymoma are divided into two subtypes based on mutational drivers namely, C11orf95-RELA (RELA) fusions and YAP1 (YAP) fusions. These two supratentorial subtypes are genetically and clinically distinct with RELA patients having a poorer prognosis than those with YAP (76).

Antigenic Targets
Studies have demonstrated increased expression of EphA2, IL-13Rα2, HER2 and Survivin in ependymomas (77, 78). Therefore, these antigens may be potentially effective targets in CAR T cell mediated therapy clinically. CAR-T cells with trivalent targets to EPHA2, HER2 and IL13Rα2 did show efficacy in xenograft models of ependymomas (3). While ependymomas may hold favorable outcomes with traditional therapy in some pediatric patients, recurrence is often fatal and further work in novel treatments and immunotherapy is needed.

Tumor Microenvironment
Similar to medulloblastoma, tumor microenvironment characteristics directly correlate with molecular subgroup. PFA tumors have enrichment in inflammatory response genes in comparison to PFB tumors (79). IL6/STAT3 pathway activation and crosstalk between cancer cells and myeloid cells is a potential mechanism underlying the PFA tumor phenotype. This pathway could potentially be used as a therapeutic target (80). RELA ependymomas have higher PD-L1 gene expression in comparison to other subtypes indicating a potential increased immunosuppression in this group (81). Further immune phenotyping of ependymomas with microarrays demonstrated that myeloid cells in the microenvironment have a more M1 or pro-inflammatory phenotype (82). Charactization of primary and matched recurrent ependymoms demonstrate that some tumors did change molecular subtype and inflammatory profiles upon recurrence (83). This indicates that the microenvironmnet can change during a patient's treatment course, a critical consideration when choosing a therapy such as CAR T cells.

Atypical Teratoid/Rhabdoid Tumors
Atypical teratoid rhabdoid tumors (ATRTs) are aggressive embryonal CNS tumors that occur in 0.66 per 100,000 children. These tumors usually occur in children less than 4 years old and can occur in the posterior fossa or the supratentorium. The majority of tumors in children younger than 1 year occurred infratentorially. ATRT dianosis portends a grave diagnosis with a median survival less than 1 year after diagnosis for most patients (84–86). No standard treatment exists for this tumor but surgical resection is indicated. Given the young age of the patient at presentation, radiation treatment can lead to severe neurocognitive deficits. Despite newer therapies such as intensified multimodal therapy with whole craniospinal irradiation (87, 88) or high-dose chemotherapy with stem-cell rescue (89, 90) showing improved survival, they come with significant treatment-related morbidity and mortality (91). With the further classification of ATRTs using integrated (epi)-genomic analysis, use of targeted/biological agents may show further promise for some subtypes of ATRTs (92, 93).

Molecular Subtypes
The hallmark of ATRTs is biallelic mutations of SMARCB1 (INI1/hSNF5/BAF47), or rarely SMARCA4, both of which are involved in the SWItch/Sucrose Non-fermentable chromatin remodeling complex (94). Loss of one copy of the entire chromosome 22 or a deletion or translocation specifically involving chromosome band 22q11.2 has been described (95). Recent epigenetic studies have further subdivided ATRT into three methylation subgroups, ATRT-SHH, ATRT-TYR, ATRT-MYC, each of which has characteristic locations and methylation patterns (96).

Antigenic Targets
To date, no definitive ATRT neoantigens have been described, however, preclinical data with cancer stem cells and tyrosine kinase inhibitors in ATRTs may highlight potential antigens for CAR-T targeting and trafficking. Cancer stem cells (CSCs) are subpopulations of tumors cells thought to be associated with treatment failure, tumor recurrence, and metastasis of many cancers primarily due to their quiescence, migratory ability, resistance to drug therapy, and immune evasion (97). Use of patient-derived xenografts (PDXs) to identify and isolate human (CSCs)/tumor-initiating cells (TICs) demonstrated distinct gene signatures associated with CSCs/TICs in malignant rhabdoid tumors (98). Two of these overexpressed genes, CXCL5/CXCL6, may have potential for adjuvant CAR-T therapeutic targeting.

CXCL5/CXCL6 are both chemokine proteins thought to play a role not only in chemotaxis, but angiogenesis and tumorigenesis in many cancer types (99–101). CXCL5 has recently come to the forefront in research for its involvement in proliferation, migration, invasion, and immune evasion in multiple cancer types. Integrated proteomics data suggests that these chemokines are not normally expressed in nervous system tissues, a key factor in trafficking and minimizing off-tumor toxicity for ATRT therapy (102, 103). While these ligand are not viable targets, their receptors could potentially be used as targets for adoptive cell therapy.

Another potential antigenic target for CAR-T therapy is PTK7, a RTK family member, single-pass transmembrane receptor that lies at the signaling crossroads of WNT, VEGF, and stem cell function (104). Recent studies have shown a significant increase in PTK7 RNA expression levels in ATRT tumors, with subsequent repression of proliferation and viability of ATRT cells by vatalanib, which target multiple tyrosine kinases, and siRNA-mediated PTK7 knockdown, respectively (105). Preclinical data with antigen-drug conjugate, anti-PTK7 (PF-06647020), in breast, ovarian, and non-small cell lung cancer, shows that PTK7 targeting of CSCs and the tumor microenvironment induces direct and indirect anti-tumor effects, by reducing tumor initiating cells and inducing sustained tumor regression (106).

There are many ongoing and completed clinical trials for patients with ATRTs involving combination therapies, biological agents, and enzyme inhibitors, as well as molecular genetics studies. Much work is needed in preclinical studies to further delineate neoantigens for CAR-T cell targeting in this aggressive pediatric brain tumor.

Tumor Microenvironment

ATRT are rare tumors and therefore studies on characterizing the tumor microenvironment are limited. There is critical crosstalk between mesenchymal stromal cells and ATRT cancer cells in the microenvironment that leads to the promotion of cancer cell migration (107). The tumor immune microenvironment in ATRTs appears to have some unique features. Tumor immune phenotyping demontrated that ATRTs have significantly increased CD8 T cells in comparison to GBMs (108). Similar to other pediatric tumors, the immune infiltrate composition corresponds with the molecular subtype. Macrophages predominantly infiltrate the ATRT-SHH and ATRT-MYC subtypes and correlate with poor prognosis and treatment resistance (109). These data again indicate that therapeutic strategies must take into account the subtype classification and its respective immune microenvironment properties in pediatric brain tumors.

Pediatric High Grade Gliomas

Pediatric High-Grade Gliomas (pHGGs) represent less than 20% of tumors in this population, and are comprised primarily of anaplastic astrocytomas (AA, WHO grade III), glioblastoma (GBM, WHO grade IV), and the newly defined Diffuse Midline Glioma (DMG, WHO grade IV) (110–114). Pediatric non-brainstem/midline glioblastomas (pNMGs) are often cortical and thus treated with aggressive combinations of surgery, chemotherapy, and radiation due to poor prognosis (114). Even with gross total resection and chemotherapy, 3-year event free survival is reported to only be 11% in children with high-grade pNMG (115).

The 2016 WHO classification of CNS tumors introduced a new classification known as H3 K27M-mutant diffuse midline gliomas (DMG), which refers to the specific lysine-to-methionine substitution at position 27 in histone 3 (70, 112, 116). These tumors, as their name suggests, arise in midline structures such as the thalamus, brainstem, and spinal cord. They carry a dismal prognosis due to the eloquent location of these tumors making

surgery not feasible. The DMG classification thus includes tumors previously referred to as diffuse intrinsic pontine glioma (DIPG).

No standard therapy currently exists for DMG. Treatments include radiation therapy (117, 118), targeted chemotherapy (119, 120), and several types of adjuvant therapy capable of targeting cellular mechanisms of tumor proliferation, cell cycle progression, angiogenesis and DNA repair (121). Due to the difficult anatomical location of these tumors, surgical resection and even biopsies for tissue characterization, remains uncommon.

Molecular Subtypes

In contrast to adult gliomas, pediatric non-brainstem/midline glioblastomas (pNMGs) in children more often have TP53 mutations, as opposed to EGFR or PTEN alterations (114, 122). Glioblastomas in children however, almost exclusively arise de novo compared to the secondary development in IDH-mutants from low grade gliomas in adults (122). NMGs in children are also associated with genetics syndromes such as Turcot syndrome, Li-Fraumeni syndrome, and Neurofibromatosis type 1, all of which have their own known associated mutations in the context of tumor development.

In pediatric high-grade gliomas, approximately 40% are associated with TP53 mutations, and low p53 expression correlates with improved 5-year progression-free survival (114, 122). Overall, compared to adult high-grade gliomas, pNMGs are less likely to have EGFR gene amplification and less likely to have mutations within the PTEN tumor supresor gene, although correlations with prognosis and clincial significance are limited (123–125).

Due to the mortality of DMG tumors and difficulty of surgical resection, many clinical trials have attempted to target genetic mutations in the tumor. Early trials showed little success as many targeted the genetic mutations known to adult glioblastomas (126). As more genetic studies have been completed, there are clear differences in gene expression that distinguish DMG from both adult glial tumors and supratentorial pediatric gliomas (127, 128). The first of these differences to be uncovered was that nearly 80% of DIPG tumors contained frequent histone 3 mutations (K27M-H3.3 or K27M-H3.1) (111, 112, 116). Later studies further subcatagorized DIPGs into three molecularly distinct subgroups (H3-K27M, Silent, and MYCN) (129).

Antigenic Targets

Antigen targeting potential in high grade gliomas is currently the most robust due to translation of research from adult high-grade glioma counterparts. The main antigen targets in adult gliomas inlude: IL13Rα2, EphA2, EGFRvIII, and HER2 (1). CD70, a member of the tumor necrosis factor receptor family, and podoplanin (PDPN), a type I transmembrane mucin-like glycoprotein, have also been recently identified as a CAR T cell target in glioma (130, 131). **Table 1** summarizes antigen targets for pediatric brain tumors including pHGG. While pediatric clinical trials are in progress that include these antigens, recruitment is more limited due to the difference in antigen expression seen between adult and pediatric high-grade gliomas. However, some activity has been seen in the pediatric population.

TABLE 1 | Primary antigens targeted in CAR-T therapy for pediatric brain tumors.

Antigen	Tumor expression	Function	References
EGFRVIII	Glioblastoma, Ependymoma, Medulloblastoma	Brain development and is associated with tumor development and progression	(186, 187)
HER-2	Glioblastoma, Ependymoma, Medulloblastoma, metastatic tumors	Cell cycle homeostasis	(49, 54, 132)
B7-H3	Diffuse Midline gliomas, medulloblastoma, and most pediatric solid tumors	Potent immune inhibitory functions	(53, 136)
GD2	Primarily Diffuse Midline Gliomas, neuroblastoma, melanoma	Attachment of tumor cells to extracellular matrix proteins	(138, 188)
IL13RA2	High-grade glioma, Ependymoma, Medulloblastoma	Apoptosis escape mechanism	(54, 189, 190)
EPHA2	Gliomas, Ependymoma, Medulloblastoma	Enhances tumorigenesis, tumor cell migration and invasion, angiogenesis, and metastasis	(54, 191, 192)
SURVIVIN	Ependymoma, glioma	Apoptosis inhibitor protein	(142)
PRAME	Medulloblastoma	Inhibits retinoic acid signaling	(47)
CD 70	Glioma	Tumor necrosis factor receptor family	(130)
PDPN	Carcinoma, sarcoma, seminoma, brain tumors	Transmembrane glycoprotein	(151)

A prospective trial including 17 adult and adolescent patients with relapsed or refractory glioblastoma included an adolescent patient with an objective response after systemically administered HER2 CAR T cells (132).

In pediatric diffuse midline gliomas, both B7-H3 and GD2 specific CAR-T cells, which are highly expressed in DMGs, are also currently targets in clinical trials recruiting pediatric patients (NCT04185038, NCT0409979). B7-H3 is a transmembrane protein that belongs to the B7 immune co-stimulatory and co-inhibitory family, has potent immune inhibitory function (133, 134). Studies have shown that it downregulates T-cell activation and INFg, and correlates with fewer tumor-infiltrating lymphocytes, faster cancer progression, and poor clinical outcome in multiple malignancies (135, 136). In one study, it was demonstrated that 100% of DIPG specimens were B7-H3 immunoreactive, and mRNA expression was significantly higher in DIPG samples vs juvenile pilocytic astrocytoma or normal brain (137). Majzner et al. demonstrated that B7-H3 CAR T cells in mice significantly prolonged survival in both medulloblastoma and DIPG xenografts through production of IFNg, TNFa, and IL2 (53).

A recently published study demonstrated the potent anti-tumorgenic capabilities of CAR-T cells targeted to pediatric diffuse midline gliomas (138). In these tumors, the presence of the H3-K27M mutation correlated with high levels of GD2 diganglioside expression. Many immunotherapies targeting GD2 expression are being investigated in both preclinical and clinical trials for a variety of neurologic conditions including neuroblastoma (139–142). To date, CAR-T cell therapies targeting GD2 expression in neuroblastoma have been well tolerated in many of these clinical trials (139–141).

Due to the known heterogenicity of high-grade gliomas and potential for antigen escape, multi-valent immunotherapy could prove to be a superior approach in treating recurrent gliomas in both adult and pediatric populations. Pollack et al. showed feasibility, tolerability, and some evidence of efficacy in using a trivalent peptide vaccine targeting EphA2, IL13Rα2, and survivin in pediatric malignant gliomas (143). Similar preliminary work has also shown potential efficacy for pediatric and adult ependymomas (77). Early CAR-T studies showed the benefit of targeting multiple antigens and decreasing tumor antigen escape (144). Bielamowicz et al. has also demonstrated that trivalent CAR-T cells targeting HER2, IL13Rα2, and EphA2 demonstrated superior antitumor activity in GBM patient derived xenografts compared to patient-specific single valent and bivalent models (54). A multivalent is particularly useful in HGG given the tumor heterogeneity of antigen expression which can change over time following CAR T cell therapy (145).

Challenges in the Microenvironment

pHGGs have been shown to differ from their adult counterparts through the presence of varying somatic histone modifications (110–112). Differential genetic expression between adult and pediatric gliomas result in proteomes with varied biomarker expression with subsequent influences on the tumor microenvironment. Although still controversial, it is thought the pHGGs may arise from neural precursor cells of the oligodendroglial lineage (113, 146, 147). These types of tumors have been shown to possess distinct discrete patterns of spatial and temporal development that correspond with periods of developmental myelination within the pediatric and adolescent brain (110, 113, 148, 149).

In adult gliomas, the heterogenity of both antigen expression and the tumor microenvironment proves to be a main hurdle in using adoptive cell therapies like CAR-T cells (1, 150). Genetic sequencing of spatially different regions of GBMs has shed light on these regional niches within the tumor containing multiple cell types and functions (151, 152). As with adult glioma tumors, infiltrating immune cells play an important role in the makeup of the pediatric tumor microenvironment. Although the knowledge regarding the functional roles of such cells in pediatric gliomas remain incomplete, in mouse models of low-grade pediatric gliomas, tumor associated macrophages have been implicated in promotion of tumor cell proliferation (153, 154). Similarly, in adult gliomas, the amount of infiltrating TAMs that are CD68 + increases with increasing tumor grade (155). This signifies a correlation between immune cell presence within the tumor microenvironment and tumor malignancy and growth.

TABLE 2 | Ongoing clinical trials using chimeric antigen-receptor T-cells (CAR-T) for pediatric brain tumors.

NCT#	Phase	Study Name	Responsible party	Target	Additional agents/therapies	Delivery	Disease
03638167	Phase I	EGFR806-specific CAR T Cell Locoregional Immunotherapy for EGFR-positive Recurrent or Refractory Pediatric Central Nervous System Tumors	Seattle Children's Hospital	EGFR		IT, IC	Pediatric Glioma, Ependymoma, Medulloblastoma, Germ Cell Tumor, ATRT, PNET, Choroid Plexus Carcinoma, Pineoblastoma
03500991	Phase I	HER2-Specific CAR T Cell Locoregional Immunotherapy for HER2 Positive Recurrent/Refractory Pediatric Central Nervous System Tumors	Seattle Children's Hospital	HER2		IT, IC	Pediatric Glioma, Ependymoma, Medulloblastoma, Germ Cell Tumor, ATRT, PNET, Choroid Plexus Carcinoma, Pineoblastoma
04185038	Phase I	B7-H3-Specific CAR T Cell Locoregional Immunotherapy for Diffuse Intrinsic Pontine Glioma/Diffuse Midline Glioma and Recurrent or Refractory Pediatric Central Nervous System Tumors	Seattle Children's Hospital	B7-H3		IT, IC	DIPG/DMG, Ependymoma, Medulloblastoma, Childhood Germ Cell Tumor, ATRT, PNET, Choroid Plexus Carcinoma, Pineoblastoma, Childhood Glioma
02442297	Phase I	Intracranial Injection of T Cells Expressing HER2-specific Chimeric Antigen Receptors (CAR) in Subjects With HER2-Positive Tumors of the Central Nervous System (iCAR)	Texas Children's Hospital	HER2		IT, IC	Brain Tumor, Recurrent Brain Tumor, Refractory Excludes DIPG/DMG
04099797	Phase I	Autologous T Lymphocytes Expressing GD2-specific Chimeric Antigen and Constitutively Active IL-7 Receptors for the Treatment of Patients With GD2-expressing Brain Tumors (GAIL-B)	Texas Children's Hospital	GD2	Lymphodepletion chemotherapy; Cyclophosphamide, Fludarabine	IV	Diffuse Intrinsic Pontine Glioma (DMG), High Grade Glioma

Table data accrued July 19, 2020 from ClinicalTrials.gov.

While little is known about the differences between tumor immune microenvironments in pedatric versus adult gliomas, some studies have shown that pDMGs show unique properties (156). In contrast to pNMGs, pDMGs do not have increased T cell infiltration compared to adjacent non-tumor infiltrated brain and appears to not have an inflammatory cytokine profile (157). Sequencing of macrophages from tissue samples in both pDMGs and adult GBM have shown that both express genetic changes related to extracellular matrix remodeling and angiogenesis. However, pDMG-associated macrophages express less inflammatory factors than adult GBM macrophages (158). Thus, the lack of T-cell invasion and overall low inflammatory environment could potentially make pDMGs excellent candidates for CAR-T cells as the microenvironment is less immunosuppressive relative to adult tumor counterparts (158, 159).

TREATMENT TOXICITY AND RISKS

CAR T cell therapy can lead to treatment associated toxicity. Cytokine release syndrome (CRS) is one of the most common and severe toxicities associated with CAR T cell therapy results from systemic immune activation. While elevation of inflammatory cytokines is expected, and often produces mild flu-like symptoms, multiorgan system failure and death has been reported from CRS (160). The most elevated cytokines typically seen are IL-10, IL-6, and IFN-γ. CRS can be managed with the use of corticosteroids and interleukin-6 blockade (161, 162). Corticosteroids can have a negative impact on T-cell proliferation and therefore clinicians may consider other forms of CRS management. Out of the 3 cytokines involved, regulation of IL-6 should produce both desired control of CRS without downregulation of desired immune function (163). IL-6 blockade

via tocilizumab has shown dramatic reversal of severe CRS in patients treated with CART-19, and thus is actively being studied in other therapies (164, 165). Another method managing severe CRS is engineering suicide genes into the CAR-T cells. With a suicide gene/switch in place, administration of an antibody or small molecule can induce apoptosis of CAR-T cells to prevent further immune stimulation and reverse CRS (166–168).

Despite the availability of multiple treatment modalities, CRS can still be deadly and needs to be recognized early (169). Neurotoxicity is another complication which has recently been termed immune effector cell-associated neurotoxicity syndrome (ICANS). ICANS is the second most common adverse event following CAR T-cell infusion. ICANS has a wide spectrum of symptoms and can range from mild confusion to being in a comatose state (170). Similar to CRS, ICANS can be deadly and therefore any neurological symptom occurring after CAR T cell infusion must be considered potential ICANS until proven otherwise. In a meta-analysis of CAR T cell trials for cancer, 55.3 and 37.2% of all patients experienced CRS and neurotoxicity, respectively (171).

Intrathecal or locoregional infusion of CAR T cells for brain tumor therapy may mitigate these toxicities experienced following intravenous administration (**Figure 1**). Intrathecal therapy is a passive form of delivery and normal CSF flow is required for adequate dissemination throughout the CNS. Furthermore, this modality may not penetrate bulky disease sites (172). These are clinical considerations to account for when choosing a delivery method. One other particular concern in regards to pediatric brain tumors is the prediliction for tumors in the posterior fossa or along the ventricular pathway. In preclinical models, expected treatment related inflammation following CAR T cell therapy can cause brain swelling which can lead to hydrocephalus (137). Hydrocephalus can be morbid and require urgent CSF diversion. These risks may occur more frequently as the clinical experience expands in pediatric brain tumors.

DISCUSSION AND FUTURE DIRECTIONS

Overall, current clinical trials using CAR-T cells for pediatric brain tumors are in early stages (**Table 2**). The antigenic targets of these trials have been discussed in previous sections and are summarized in **Table 1**. Five clinical trials are currently recruiting pediatric patients, however, results will likely be slow to report due to the combined rarity and mortality of these tumors. However, CAR-T therapy in xenograft models continues to provide hope in reaching long-term disease free survival.

The first in-human studies of CAR-T cells for GBM was administered through intravenous delivery. O'Rourke et al. demonstrated that with while all patients had detectable transient expansion of EGFRvIII CAR-T cell in peripheral blood, not all patients had significant signs of trafficking and intra-tumor effects (173). Ahmed et al. showed a dramatic response in an adolescent patient, and stable disease in 7 others after HER2 CAR-T infusion (132). Brown et al. was among the first to show regression of GBM in a patient after intratumor and intraventricular infusion of IL13Rα2 CAR-T cells (145). Route of administration that optimizes efficacy will be a main factor to delineate in future trials.

Assessing response to immunotherapy remains another hurdle to overcome in the context of CNS cancer. The standard of care in monitoring tumor progression and response is magnetic resonance imaging, but, pseudo-progression after immunotherapy can make interpretation of imaging difficult. Pseudo-progression and immune related edema is an expected result of targeting and stimulating the immune system, and more advanced imaging analysis stratagies are underway in helping to differentiate between true progression of disease (174–176). Despite these concerns, multiple clinical trials have shown that patients still achieve meaningful clinical response despite early pseudo-progression findings (174, 177–181).

Currently, trials are targeting cell surface antigens on cancer cells but scFv targeting epitope/HLA complex makes it possible for intracellular proteins to be candidate targets (182). This technology will be a significant game changer allowing for clinicians to target intracellular oncogenic pathways. Besides targeting cancer cells, considerations of how to target the microenvironment to enhance anti-tumor activity is underway (183). In pediatric brain tumors, further work is needed to delinate the tumor microenvironment taking into account subtype variability. With the advent of immunomodulatory agents such as checkpoint inhibition, multimodal immunotherapy can also be considered for patients (184). Lastly, the application of CAR engineering to other immune effector cells such as NK cells opens another avenue of potential innovation (185).

CONCLUSION

There has been critical advancements in the last decade that have allowed for the development of CAR T cells for brain tumors. The prognosis for many pediatric brain tumors remains dismal and there is promise in CAR T cell therapy. Continued preclinical research is needed to address our current gaps such as identifying the best mode of delivery, defining the microenvironment, developing new targets and increasing efficacy. Despite these challenges the future of CAR T cells for pediatric brain tumor management is bright.

AUTHOR CONTRIBUTIONS

JP: methodology, data curation, and writing – original draft, review, and editing. JH: writing – original draft. RB: figure construction. KB: supervision and writing – review and editing. AR: conceptualization, resources, supervision, and writing – review and editing. All authors contributed to the article and approved the submitted version.

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