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REVIEW ARTICLE

Emerging trends in gene-modified-based chimeric antigen receptor–engineered T-cellular therapy for malignant tumors: The lesson from leukemia to pediatric brain tumors

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Abstract: In 2017 and 2018, Food and Drug Administration has approved YESCARTA (axicabtagene ciloleucel) and KYMRIAH (tisagenlecleucel), two chimeric antigen receptor (CAR)-engineered T-cell products, for B-cell malignancies. It also marked a watershed moment in the development of immunotherapies for cancer. Despite the successes in adults, it remains clinically applicable only in B-cell acute lymphoblastic leukemia in pediatrics. Notably, multiple clinical trials and recent case reports about childhood central nervous system (CNS) tumors, the leading cause of deaths in children, have emerged and granted promising results. With the growing consideration of the biological responses in the interaction of human immunity, the major technical obstacles such as on-target off-tumor toxicity in widespread solid tumors, antigenic heterogeneity, adaptive resistance, difficult T-cell (CD4/CD8) trafficking, and immunosuppressive environments in CNS are gradually approached and ameliorated. The new spotlights of this review are focusing on current development, and emerging treatments for pediatric CNS tumors integrating molecular research with the mainstream of CAR-T therapeutic strategies to sketch a main axis and pathway forward in the improvement of novel gene-modified–based cellular platform.

Keywords: Immunotherapy; Nervous system; Precursor cell lymphoblastic leukemia-lymphoma

1. INTRODUCTION

With the growing knowledge of the importance of the immune system in tumor regulation, multiple effective and durable immunotherapies for patients with previously incurable malignancies have emerged. Among them, chimeric antigen receptor (CAR)-T cells and immune checkpoint inhibition therapy seem to be the two most impactful immunotherapies nowadays, whereas therapeutic vaccines and virotherapy are still going through the ordeal of clinical trials.[1,2] However, although immune checkpoint inhibitors, with the representative drugs of anti-PD1/PDL1 and anti-CTLA4 monoclonal antibodies blocking pathways crucial for immune self-tolerance, have mediated substantial benefit in adult melanoma and renal cell carcinoma refractory to traditional therapies,[3,4] no widespread benefit in childhood cancers have been demonstrated. The dilemma might owe to the lack of neoantigens in pediatric cancers.[5] In contrast, CAR-T cells, which combine the specificity of a monoclonal antibody with the cytolytic power and capacity for immune surveillance of a T cell,[6] have revealed highly potent effects in childhood B-cell acute lymphoblastic leukemia (B-ALL).[7,8] In this framework, we will briefly discuss the current management, challenges, and future applications of gene-modified-based cellular therapeutics, especially focus on using the potential CAR-T remedy for treating pediatric lymphoid cancers and brain tumors.

2. CAR-T THERAPY

CARs are genetically engineered T cells with artificial fusion proteins that incorporate an extracellular antigen-recognition domain, a transmembrane domain, and an intracellular domain-containing signaling elements.[9,10] The antigen-targeting moiety from monoclonal antibody projected out to the extracellular

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space is responsible for triggering T-cell activation once bound to tumor antigens and leads to cytokine release, cytolytic degranulation, and T-cell proliferation.[11] On the other hand, the intracellular domain affects the quality and strength of a T-cell response to antigen.[12] The first generation of CARs contains only the CD3-ζ endodomain and is known to have limited T-cell expansion and persistence.[13–15] The second generation then added intracellularly costimulatory domain, such as CD28[16,17] or 4-1BB,[18] which shows high response rates in patients with B-cell malignancies. Further generation combines both CD28 and 4-1BB in the hope of higher potency.[19,20] Currently, most CAR-T therapy used autologous T cells for transduction (Fig. 1): collect the patient's T cells, then activate with antibodies or antibody-coated beads artificially, and then transduce, using plasmid transfection, mRNA, or most commonly, with a lentivirus or retrovirus, to express the CAR molecule. The modified CAR-T cells are then expanded in vitro to abundant numbers to infuse back into the patient. The modified T cells can be infused via a peripheral intravascular line, intrathecal injection, or intraventricular catheter, with the peripheral intravascular line as the most widely used. Before T-cell infusion, lymphodepleting such as chemotherapy is usually applied to ensure the expansion of CAR-T cells.

2.1. CAR-T in pediatric hematologic malignancies

In pediatric lymphoid cancer, B-ALL has been demonstrated to be highly susceptible to CD19-CAR therapy, with 60% to 93% minimal residual disease (MRD)-negative complete remission (CR) rates across several studies.[21–24] Likely, a CD22-CAR in pediatric B-ALL has achieved a 73% CR rate.[7] This exhilarating high response may result from high and homogeneous expression of CD19 and CD22 target antigens in B cells.[25]

2.2. Costimulatory domains affect the persistence of CAR-T therapy

Aside from the antigen targets, the aforementioned different costimulatory domains incorporated in CAR-T cells may also play an important role in treatment behaviors. A CD28 endodomain, while incorporated in CAR-T cells, results in a more rapid and higher peak expansion manner, which rarely persists over 1 to 2 months.[23,26] In contrast, those incorporating a 4-1BB endodomain show slower and lower peak expansion and often endure for months or even years.[21,22,24] The choice of endodomain in CAR-T therapy manipulation then becomes important for the perseverance of CAR-T cells has a great influence on cure in children and young adults with B-ALL treated with CD19-CARs.[21–24,27] In trials of patients with B-ALL treated with CD19.28.z-CAR, without proceeding to allo-hematopoietic stem cell transplantations (HSCTs), rapid B-cell recovery and disease relapse in complete responders were noted.[23,26–28] Also, in children with B-ALL treated with a CD19.BB.z CAR and achieved an MRD-negative remission but had <3 months of B-cell aplasia (BCA) and in the absence of post-CAR allo-HSCT, relapse was noted.[29] This need for CAR persistence in pediatric and young-adult B-ALL in distinction to other adult lymphoid cancer such as large B-cell lymphoma (LBCL) might relate to the underlying disease biology: multiple years was required for effective chemotherapeutic treatment of pediatric and young-adult with B-ALL, whereas approximately 6 months of effective chemoimmunotherapy for LBCL is sufficient.[30] The association between limited CAR-T-cell persistence and diminished durable responses, therefore, drives treatment preference towards 4-1BB CARs in pediatric and young-adult B-ALL when post-CAR allo-HSCT is contraindicated or undesired.[21,22,31]

3. IMMUNOTHERAPY FOR PEDIATRIC CENTRAL NERVOUS SYSTEM TUMORS

Compared with hematologic malignancies, treating solid tumors with CAR-T cells have been proven more difficult due to the risk of on-target, off-tumor toxicity,[32] which would be discussed later in this article. Among the field of childhood solid tumors, central nervous system (CNS) tumors remain the most common solid tumor and are the leading cause of childhood cancer-related death.[33] In children with high-grade glioma (HGG), including diffuse midline glioma (DMG), and glioblastoma (GBM), the 5-year-overall survival rate is even <20%.[34,35] This dismal survival rate occurs despite recently increased knowledge of the genomics of pediatric HGG.[36] Furthermore, current conventional treatments usually involve irradiation of the brain, resulting in devastating endocrine disease, neurologic and

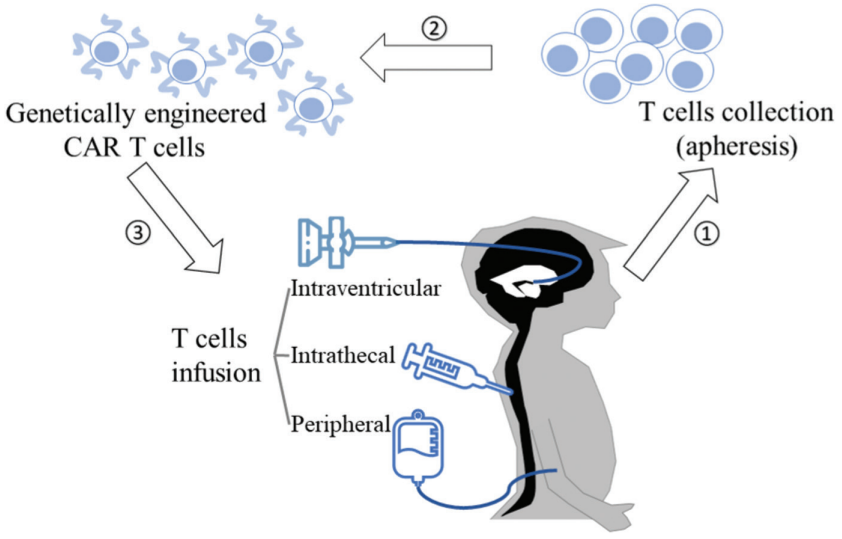

Fig. 1 CAR-T therapy approach. The first arrow indicates T cell collected from patient's peripheral blood. The second arrow shows artificial antigen and costimulatory domains incorporated into T cells and amounts of T cells are then amplified. The third step then demonstrates three different ways to put T cells back into patient's body: through intraventricular catheter into tumor cavity, intrathecal injection, or intravascular infusion.

neurocognitive morbidity, as well as a high incidence of secondary tumors.[37,38] Under this condition, immunotherapy which leverages the high specificity of the immune system to target and eliminates cancer cells while leaving healthy cells undamaged becomes momentous.

3.1. CAR-T therapy in pediatric CNS tumor

In comparison with adults, pediatric groups often exhibit lower mutation rates and usually contain chromosomal rearrangement or aberrant transcription of genes included in growth development.[39] Fewer neoantigens are identified in pediatrics which cause them less responsive than an adult to pure adoptive immunotherapy. Besides, in children, little is known about resident immune cells and their development, plasticity, and interactions as the child grow and develops. Nevertheless, multiple preclinical studies have demonstrated promising results in pediatric CNS tumors. HER2-BBz-CAR T cells were shown to have excellent efficacy both in vitro and in mouse medulloblastoma models, and their intraventricular delivery is feasible and safe in nonhuman primates.[40] Simultaneously targeting HER2 and IL13Rα2 also demonstrated improved antitumor efficacy in preclinical models.[41,42] Mount et al[25] also reported the efficacy of GD2-directed CAR-T-cell therapy for H3-K27M-mutant DMGs, whose locations are usually unresectable due to the structures they infiltrate, with excellent cytotoxicity both in vitro and in vivo. Recently, B7-H3 was also discovered to be a putative target for CAR-T-cell therapy of pediatric solid tumors and brain tumors[43,44] and also exhibits minimal binding to healthy tissues.[45] Another ongoing study also showed intrathecal CAR therapy combined with azacitidine significantly increases survival rates in group 3 medulloblastoma and progression-free survival for posterior fossa ependymoma.[46] Other ongoing CAR-T-cell trials for pediatric patients include those direct against EGFR806, HER2, and IL13Rα2 individually (Table 1).

3.2. Adverse effects of CAR-T therapy in the CNS

Recently, BrainChild-01, compared two methods of locoregional infusion—into the tumor cavity versus into ventricular system—of HER2 CAR-T cells for children and young adults with HER2-positive progressive or recurrent CNS tumors. The cutting edge result revealed that Subject 001, a 19-year-old female with a parietal lobe anaplastic astrocytoma (WHO grade III), after two courses of infusions directly into the tumor cavity, showed acute local inflammation, elevated CRP level, and brain/spine MRI reported increased enhancement and T2/FLAIR hyperintensity surrounding the tumor cavity with mildly augment mass effect, indeterminate for treatment-related inflammatory changes/pseudo-progression versus tumor progression.[47] Similarly, notably, peritumoral edema resulting in murine deaths were also noted in preclinical trials,[25] likely due to tumor infiltration by T cell and consequent hydrocephalus, increased intracranial pressure, and/or brain herniation. This highlights the need for close monitor for CAR-T therapy in future clinical trials. In addition to CNS complications, other general major toxicities include cytokine release syndrome (CRS) and neurotoxicity. CRS ranges from isolated fever to refractory hypotension and consumptive coagulopathy[48,49] and can be treated with the anti-interleukin (IL)-6 receptor antibody tocilizumab. On the other hand, neurotoxicity, also known as immune effector cell–associated neurotoxicity syndrome (ICANS) or CAR-T-cell-related encephalopathy syndrome (CRES) contains symptoms such as headache, confusion, expressive aphasia, apraxia, and myoclonus and can progress to severe encephalopathy, including seizures, obtundation, and even rarely cerebral edema.[50] Encouragingly, a recent case report demonstrated three patients with previous notable neurologic morbidity, bridging from conventional chemotherapy, tolerated CAR-T-cell therapy safely.[51]

3.3. Limitations of CAR-T therapy in CNS tumors

For solid tumors, a major concern is the risk of on-target, off-tumor toxicity. In opposition to lymphoid cancer, where the antigens are restricted to B-cell linage and most patients could live without healthy B cell,[52] solid tumors have rare tumor-specific cell-surface antigens, and the same antigen might be expressed on vital tissues as well. This disadvantage, therefore, restricted the development of the CAR-T trials launched on solid tumors. However, multiple trials have observed an absence of both toxicity and efficacy, but most with limited expansion of CAR-T cells.[30] How to escalate T-cell expansion without increasing on-target, off-tumor toxicity remains the next hurdle. Taking CNS tumors into considerations, a couple more limitations need to be overcome when applying CAR-T-cell therapy. First, limited T-cell expansion and persistence were found with unclear mechanisms about whether it was due to suppression from the tumor microenvironment (TME) or inherent limitations from the CAR construct itself.[35,53] In GBM, baseline immunosuppression within the TME has been demonstrated in several studies.[54] The further acquisition of tissue post-CAR-T treatment will continue to provide critical insight into comprehensive TME responses to adoptive cell therapy. Second, the blood-brain barrier remains a natural impediment in CAR-T-cell trafficking to the CNS system. Local delivery of CAR-T into ventricular systems or tumor cavity might be of use but their safety remains concerned.[55] Also, identifying BBB integrity in a given brain tumor subtype might provide insights towards increasing immunotherapeutic efficacy. Finally, the relapse specimen with low IL13Rα2 expression in Brown et al echoes the antigen-loss relapses

Table 1

Current CAR-T clinical trials including pediatric CNS tumors

Clinical trial and institution	Target	Phase	N	Age	Tumor type
NCT04185038 Seattle Children's Hospital	B7-H3	1	70[a]	1-26 y	Diffuse intrinsic pontine glioma/diffuse midline glioma and recurrent or refractory pediatric CNS tumors
NCT03638167 Seattle Children's Hospital	EGFR806	1	36[a]	1-26 y	EGFR-positive recurrent or refractory pediatric CNS tumors
NCT03500991 Seattle Children's Hospital	HER2	1	48[a]	1-26 y	HER2-positive recurrent/refractory pediatric CNS tumors
NCT02442297 Baylor College of Medicine	HER2	1	28[a]	3 y and older	T cells expressing HER2-specific CAR for patients with HER2-positive CNS tumors
NCT04099797 Baylor College of Medicine	GD2	1	34[a]	12 mo to 18 y	GD2-expressing brain tumors (GAIL-B)
NCT02208362 City of Hope Medical Center	IL13Rα2	1	92[a]	12 to 75 y	Recurrent or refractory malignant glioma

CAR-T = chimeric antigen receptors; CNS= central nervous system; EGFR = epidermal growth factor receptor.
[a]Estimated recruiting numbers.

Table 2

Preclinical trials of CAR antigen target on CNS tumors

Antigen	Expression on brain tumors	Expression on normal tissues
B7-H3[43]	HGGs (high expression) Other brain tumors	Breast, lung, liver, bladder, prostate, testis, placenta, and lymphoid organs
EGFRvIII[59,60,a]	GBM (most common EGFR mutation, around 30%)	Restricted expression
HER2[61-62,a]	GBM (moderate expression) Other solid tumors that metastasize to the brain (high expression)	Skin, muscle, epithelial tissues
GD2[25,a]	DIPGs (uniform) HGGs (low expression)	Central nervous system, peripheral nerves, and skin melanocytes
IL13Rα2[63-64,a]	GBM (majority) HGGs	Testis
CD133[65]	Glioma tumor initiating cancer stem cells	Hematopoietic stem cells, neuronal stem cells, endothelial progenitor cells
CSPG4[66]	GBM (uniform, 67% high expression)	Chondroblasts, cardiomyocytes, pericytes
EphA2[67-68]	HGGs (uniform, various levels)	Epithelial tissues

CAR = chimeric antigen receptors; CNS = central nervous system; DIPGs = diffuse intrinsic pontine gliomas; GBM = glioblastoma; HGGs = high-grade gliomas.
[a]With pediatric CAR-T clinical trials going on.

which have been observed with CAR-T-cell therapy in leukemia as well.[24,56] The tumor intrinsic resistance might be related to the genetic mechanism, antigen density, or antigen masking,[53] which all required further biological experiments and serve as obstacles for the application of CAR-T-cell therapy in CNS tumors.

4. PERSPECTIVE

In broad strokes, adoptive T-cell therapy has come a long way over the past three decades, with significant translation from the bench to the bedside. Giving the encouraging results in pediatric lymphoid malignancies, pediatric brain tumors are now picking up. However, it will be important to thoroughly investigate and vet all new tumor-specific targets and evaluate the outcomes from unforeseen on-target off-tumor binding. Besides, antigenic heterogeneity, adaptive resistance, T-cell trafficking hindered by BBB and the nature of pediatric tumor as immunologically quiescent remain challenges for the future CAR-T therapy in pediatric brain tumors. Recently, a bispecific small-molecule ligand EC17, fluorescein isothiocyanate (FITC) conjugated with folic acid, can be used to treat folate receptor (FR)-positive tumors with good penetration and high affinity, whereas unbound form could be cleared from the blood rapidly in preclinical trial.[57] This may broaden the technique level of CAR-T therapy. Besides, another chapter of combining treatment for lymphoid cancer may be opened up as a case with HIV-1 infection and ALL, transplanted with CCR5-ablated hematopoietic stem and progenitor cells (HSPCs) showed CR.[58] Herein our reviewing works have demonstrated the potential of the novel gene-modified-based cellular therapeutics, including CAR-T- and CAR-T-based immune-specific targeting therapies. In the future, personalized medicine analyzing each patient's tumor antigens specifically and create individualized CAR-T cells as well as a combination with other traditional cancer therapy or immune checkpoint inhibitor might become the next avenue of cancer treatment (Table 2).

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