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ADOPTIVE CELL THERAPY IN TREATING PEDIATRIC SOLID TUMORS

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Abstract

Purpose of Review—This review will discuss the challenges facing adoptive cell techniques in the treatment of solid tumors and examine the therapies that are in development for specifically pediatric solid tumors.

Recent Findings—Targeting solid tumors with adoptive cell therapy has been limited by the inhibitory tumor microenvironment and heterogeneous expression of targetable antigens. Many creative strategies to overcome these limitations are being developed but still need to be tested clinically. Early phase clinical trials in neuroblastoma with GD2 CAR T cells are promising but results need to be validated on a larger scale. Most research in other pediatric solid tumors is still in early stages.

Summary—Adoptive cell therapy represents a useful tool to improve the outcomes of many pediatric solid tumors but significant study is still required. Several clinical trials are ongoing to test therapies that have shown promise in the lab.

Keywords

immunotherapy; adoptive cell therapy; chimeric antigen receptor; pediatric solid tumors; transgenic TCR

INTRODUCTION

Over the past several decades, the prognosis of pediatric cancers has improved dramatically through multi-disciplinary treatment and emphasis on supportive care. However, many patients with metastatic or recurrent disease still face suboptimal outcomes. In addition, pediatric cancer survivors endure a variety of acute and long term toxicities from chemotherapy and radiation therapy. Recent advances in oncology have been focused on targeted and immune-based therapies. Adoptive cell therapy involves harvesting immune cells, expanding them *ex vivo* and re-directing them to target cancer cells. The most successful example has been in the use of chimeric antigen receptor (CAR) T cells to treat adults and children with B-cell leukemias and lymphomas. CAR-T cells targeting the B cell

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antigen CD19 have produced complete remissions in up to 90% of patients with relapsed or refractory disease, with some remissions lasting several years [1–4]. However, efforts to duplicate this success in solid tumors have not achieved the same outcomes, elucidating the unique challenges that this approach faces in solid tumors. This review will focus on the current progress in developing adoptive cell therapies to treat pediatric solid tumors.

Adoptive Cell Therapies and Current Applications

Although adoptive cell therapies have been studied for decades, they have only recently become refined enough to have true clinical impacts. Observations that EBV-specific cytotoxic T cells (CTLs) from seropositive donors were able to control EBV-transformed B cells *in vitro* led to the first antigen specific T cell therapies that were used to treat post-transplant lymphoproliferative disorder (PTLD) [5–7]. As the presence of tumor infiltrating lymphocytes (TILs) was known to confer a positive prognosis in many cancers, early efforts at adoptive cell therapy exploited these cells by extracting them from tumor samples, expanding them and infusing them into patients. However, while feasible for tumors like melanoma, it proved difficult to reliably isolate and expand these cells for many other cancers [8]. Attempts to harness TILs for adoptive transfer in a variety of pediatric solid tumors have been largely unsuccessful with poor cell viability, expansion and tumor killing [9]. These results are likely a consequence of impaired T cell targeting and activation due to the down-regulation of the major histocompatibility complex (MHC) and/or costimulatory molecules, the reduced occurrence of somatic mutations targetable by TILs in pediatric tumors, as well as the production of immunosuppressive factors by tumor cells.

More recent efforts have focused on genetically modifying T cells to recognize tumor cells either via the T cell receptor (TCR) or with the addition of a CAR [Figure 1]. The native TCR recognizes peptide antigens presented by professional antigen presenting cells (APCs) in the context of MHC. TCRs specific for various tumor associated antigens (TAA) have been expressed in T cells, but this approach is limited by MHC restriction and to the targeting of intracellular proteins. Also TCRs targeting self-antigens are usually of low affinity so the most promising results have been with TCRs targeting cancer/germline antigens such as NY-ESO-1 [10]. Some of these limitations are overcome by using CAR-T cells. A CAR contains an antibody derived single chain variable fragment (scFv), conferring target specificity, which is attached to a CD3 ζchain. This structure allows the T cell to recognize antigens including non-peptide targets like glycolipids and carbohydrates, and become activated without MHC presentation [11].

One area of emerging research is exploring the use of γδ-T cells, whose unique TCR recognize unprocessed antigens in an MHC independent manner. Importantly γδT cells do not cause graft versus host disease (GvHD) making them a prime candidate for future “off the shelf” therapy. Although γδ-T cells have been evaluated in clinical trials for adult solid tumors such as breast, prostate and renal cell carcinomas, they have not yet been tested in pediatric malignancies [12, 13]. Difficulty in their *ex vivo* expansion has resulted in the use of only a small subset that can be expanded using aminobiphosphonates. However, other expansion techniques are also being tested [14].

In addition to T cells, the adoptive transfer of Natural Killer (NK) cells is gaining interest in a variety of pediatric solid tumors, which are sensitive to NK cytotoxicity [15]. NK cells kill through “missing-self mechanisms” by attacking cells that decrease expression of MHC I. NK cells express a variety of receptors that modulate their cytotoxic activity based on the balance of activating and suppressive factors. For example, they express killer-cell immunoglobulin like receptors (KIR) which interact with MHC molecules to suppress NK cell cytotoxicity. Thus, allogeneic NK cells that have a mismatch with tumor MHC are not subject to this inhibitory signal [16]. Natural Killer T (NKT) cells are another interesting subset of lymphocytes that recognize through their invariant TCR lipid antigens presented in the context of the CD1d molecule. This allows them to kill CD1d positive tumors and tumor associated macrophages, making them a powerful tool in overcoming the suppressive tumor microenvironment [17, 18].

Challenges in Solid Tumors

With the knowledge gained from the advances in adoptive cell therapy for hematologic malignancies, we are poised to translate these therapies to the treatment of solid tumors. We will review the unique challenges posed by solid tumors and discuss how they may be overcome to produce successful therapies.

Choosing the Right Target—The ideal target of cell therapy is an antigen that is expressed by all of the malignant cells and critical to their survival, but absent on normal human tissues. When the target is recognized on normal tissues, on-target/off-tumor toxicities occur. An emblematic example is the CD19 molecule, which is expressed by the entire B cell lineage. Targeting this antigen induces remission of B cell malignancies but can also cause B cell aplasia often managed with immunoglobulin support. Solid tumors express antigens heterogeneously, making it difficult to find a target that can yield the efficacy seen in the CD19 example. Also, recognition of the antigen on normal tissue can have devastating consequences. In a clinical trial using a MAGE-A3 affinity-enhanced TCR for patients with myeloma or melanoma, two patients died of cardiogenic shock because the engineered T cells cross-reacted with the protein titin found in cardiac muscle [19]. Thus, caution is required when targeting novel molecules of limited explored expression. Techniques designed to mitigate such potential lethal effects include inducible suicide genes or the use of RNA electroporation to transiently express CARs [20, 21].

TAAs that have been explored in pediatric solid tumors include cancer testis antigens, like NY-ESO-1 and PRAME, oncofetal antigens, like EGFRvIII, and tumor-selective antigens expressed at higher levels in malignant cells than on normal tissue such as GD2 and CD171 [22, 10, 23]. However, even if an ideal TAA is found, the tumor may escape immune targeting due to heterogeneous expression of the molecule by the tumor population, or by immune-editing leading to defective antigen processing machinery and/or loss of expression of the targeted antigen. Strategies to prevent this include simultaneously or sequentially targeting multiple tumor antigens, or encouraging epitope spreading by inducing immune responses to non-targeted antigens [24–26].

Trafficking—In contrast to hematologic malignancies, where antigens are accessible through the blood stream, cell therapies directed at solid tumors face the additional hurdle of traveling from the site of infusion to their target. Trafficking is often impaired by a mismatch between the chemokine receptor on T cells and the chemokines secreted by the tumors. Tumors often produce chemokines that attract inhibitory cells to create physical barriers for antigen specific T cells. To combat this problem, TILs and CAR-T cells have been engineered with various chemokine receptors [27–29]. Alternatively, oncolytic viruses injected intra-tumorally or with specific tumor tropisms can be leveraged to secrete chemokines that attract T cells to the tumor sites (8, 16). Local instillation of T cells has also been attempted but can be technically challenging and not feasible for metastatic tumors [30, 31].

Tumor Microenvironment (TME)—Once they arrive at the tumor sites, immune cells must overcome the challenging TME which lacks necessary nutrients and factors for T cells (oxygen and glucose) but contains high levels of toxic metabolites, such as kyneurines or nitric oxide (NO). Furthermore, the TME contains immunosuppressive molecules (like TGFβ, PD1) and cells (like myeloid derived suppressor cells [MDSCs], tumor associated macrophages [TAMs], Tregs), and physical barriers (stroma and fibrotic material) [Figure 2].

Various strategies to overcome immune evasion by the tumor have been employed in clinical trials. For example, tumor cells do not provide the co-stimulatory signals required to prevent T cell anergy but CARs can be designed with various co-stimulatory domains to improve T cell activation and proliferation [32, 33]. Additionally, tumor specific T cells have been engineered to be resistant to TGFβ, resulting in long-term persistence and clinical responses in relapsed Hodgkin patients [34].

Conditioning with lymphodepleting chemotherapy prior to adoptive cell transfer increases the anti-tumor activity of the adoptively transferred T cells by depleting suppressive cells like Tregs. This process also removes other immune cells that serve as cytokine sinks and increases the amount of available cytokines such IL-15 and IL-7 that are important for T cell survival [35–37]. Additionally, lymphodepletion decreases indoleamine 2,3-dioxygenase-1 (IDO) enzyme production by tumor cells. Interestingly, IDO inhibition allowed T cells to proliferate and improve tumor killing in a preclinical model [38].

To improve T cell persistence, concomitant infusion of cytokines like IL-2 have been used [39, 40]. However, the toxicity associated with systemic administration of cytokines is of concern [41]. *Ex vivo* expansion of T cells in the presence of IL-15 and IL-7 is able to preserve the “T-memory stem cells” subset which is associated with greater expansion of CAR-T cells *in vivo* [42]. Lymphocytes can be engineered to secrete cytokines such as IL-12 and IL-15 to augment the function of TILs. Alternatively, TILs can be administered with oncolytic viruses secreting these cytokines [41, 43, 44].

Other techniques are being explored preclincally to counteract the suppressive TME. T cells have been engineered to produce heparanase, which breaks down parts of the extracellular matrix improving the T cells ability to penetrate solid tumors [45]. A CAR targeting both cancer cells and cancer associated fibroblasts (the predominant component of tumor stroma)

in a mouse model showed improved tumor activity and survival of infused T cells [46]. The discovery that effector memory T cells with increased expression of HIF1a and glycolysis proliferate in hypoxic environments indicates that this pathway could be exploited to improve the survival of adoptive cell therapies experiencing hypoxia [47]. Checkpoint inhibitors have been used in pre-clinical and clinical settings to combat T cell exhaustion mediated by interactions of PD-1 on T cells with PD- ligand 1 (PD-L1), which is overexpressed in tumor cells [43, 48, 47]. However, the role of suppressive immune cells in the TME for CAR T cells is for example difficult to study since most *in vivo* models use immunodeficient mice.

Measuring Response—Finally, when these novel therapies are ready for evaluation in clinical trials, determining their efficacy is not straightforward. Historical methods of determining responses such as the RECIST criteria were designed to measure the effects of cytotoxic chemotherapy on tumors. Immune-based therapies however tend to have a delayed response due to the need for expansion and proliferation of immune cells to induce tumor killing [49]. Some patients have a long period of stable disease prior to tumor regression or conditions tend to worsen just after infusions. This particular condition is usually referred as “pseudo-progression”. Developing appropriate biomarkers and monitoring of T cells’ fate are important to differentiate progressing and responding patients, as treatment initiation with other chemotherapy regimens in the case of pseudo-progression would likely ablate an ongoing effective response.

SUMMARY OF CLINICAL TRIALS OF ADOPTIVE CELL THERAPY FOR PEDIATRIC PATIENTS WITH SOLID TUMORS. (TABLE 1)

NEUROBLASTOMA

Neuroblastoma is the most prevalent extra-cranial solid tumor affecting children. Despite intensive treatment (including chemotherapy, stem cell transplant, radiation, and antibody therapy), only 50% of patients with high-risk disease survive long-term [50]. Considerable research efforts are being dedicated to target this aggressive tumor with adoptive cell therapy but significant clinical benefits have not yet been realized.

The most common target of immunotherapy for this disease is the Disialoganglioside (GD2) molecule, which is uniformly expressed in tumor cells [51]. The addition of an anti-GD2 monoclonal antibody (mAb) to standard therapy improved event free survival by 20% but caused significant pain due to antibody binding to GD2 expressed on normal nervous tissues [52].

Since NK cells are the primary effector cells in antibody-dependent cell cytotoxicity, studies have combined adoptively transferred NK cells with anti-GD2 mAb therapy to improve responses [53, 54]. An advantage of using NK cells is that, as mediators of innate immunity, they induce local release of Type-I interferons, which induce MHC upregulation by neuroblastoma cells, thus eliciting recognition of infiltrating CTLs [23, 55]. In a phase 2 clinical trial, relapsed or refractory neuroblastoma patients receiving a combination of chemotherapy, antibody therapy, cytokines and haplo-identical NK cells achieved a response

rate of 61.5% [53]. In another trial, haplo-identical NK cells were administered to some neuroblastoma patients receiving consolidation therapy with autologous stem cell transplant and anti-GD2 mAb and cytokines. Although there was no difference in acute toxicity between patients who received haplo-NK cells and those who did not, the difference in late toxicities and tumor response is yet to be seen [54].

With an available antibody, the natural next generation of therapy for this disease is utilizing CAR-T cells, thus overcoming the limited persistence and biodistribution of the antibody. The first clinical trial with a GD2.CAR lacking costimulatory endodomains attempted to enhance persistence by providing CAR-T cells with costimulation through the physiologic engagement of their native TCR. Specifically, CARs were engrafted on EBV-CTLs. Although CAR-EBV- CTLs initially survived at higher levels compared to polyclonal T cells expressing the same CAR, by six weeks both populations were either undetectable or present at very low levels. However, this trial highlighted the importance of CD4 and central memory cells for the persistence of CAR-T cells in this patient population, which correlated with delays in tumor progression [56, 57]. In pre-clinical and clinical studies, 2nd and 3rd generation CARs with various co-stimulatory domains have been employed in an effort to improve persistence [32, 33, 58].

As lymphodepletion increase persistence of CAR-T cells in clinical trials for hematological cancers, and PD1 checkpoint blockade overcomes T cell exhaustion, a recent clinical trial investigated the role of these factors using 3 cohorts of neuroblastoma patients who received a 3rd generation GD2.CAR alone, with prior lymphodepletion, or with lymphodepletion and anti-PD1 antibody [59, 47, 48]. While lymphodepletion significantly improved expansion of CAR-T cells, surprisingly no added difference was observed after PD1 blockade. PD1 expression on these CAR-T cells was low and may explain the lack of measurable improvement with checkpoint inhibition. Overall survival of patients who received lymphodepletion was greater than those who did not, but the study was not powered to detect statistical significance [48].

Current studies are exploring additional ways to enhance persistence and thus antitumor activity. Preclinical efforts on one side are concentrating on vaccines. For example, investigations using a varicella zoster virus (VZV) vaccine to stimulate GD2.CAR modified VZV-CTLs in vivo showed that these T cells killed neuroblastoma tumor cells on initial exposure but failed on successive encounters. However, CAR function was partially regained after re-stimulation through the TCR or by exposure to APCs, supporting the hypothesis that vaccination can be used to re-stimulate virus-CAR-CTLs that have lost function [60]. The choice of costimulatory domain also influences persistence as GD2-CARs with a CD28 endo-domain have a propensity towards exhaustion which can be ameliorated by the inclusion of 4–1BB instead [33].

Recent work also underlines that CAR molecules can be engrafted onto other cells, like NK or NKT cells. An NK cell line expressing a GD2-CAR demonstrated anti-tumor activity in a drug resistant neuroblastoma mouse model [61]. Another study showed similarly promising anti-tumor activity in mouse models of neuroblastoma with NKT cells expressing a GD2.CAR [62]. These preliminary results need to be verified in clinical trials.

Alternative antigens are also being explored. Among the most advanced is the L1-cell adhesion molecule (L1-CAM or CD171), which is uniformly expressed on neuroblastoma cells at a much higher level than on normal tissues [63, 60]. A first generation CAR targeting the CE7 epitope of CD171 was found to be safe in a clinical trial but had poor persistence and only minimal tumor response [63]. The ineffectiveness of the CAR in this trial may be related to the lack of co-stimulatory domains in the first generation CAR. A phase 1 trial of a CD171 specific 3rd generation CAR is currently underway (NCT02311621). In addition, NY-ESO-1, a protein that is normally expressed only in fetal tissues and in the testes, has been found in a quarter of neuroblastoma tumors [10]. As an intracellular antigen, it can be targeted with *ex vivo* expanded TAA- CTLs or transgenic TCR redirected T cells. Another interesting target to explore is PRAME, a cancer/germline antigen with immunogenic potential that is expressed in 94% of stage 4 neuroblastoma samples [23].

SARCOMAS

Sarcomas are a diverse group of cancers originating in bone and soft tissues that account for 10–14% of pediatric malignancies. The most common pediatric sarcomas include osteosarcoma, Ewing sarcoma, and rhabdomyosarcoma. The majority of children with localized disease can be cured with conventional chemotherapy, radiation, and/or surgery, but the prognosis for patients with recurrent or metastatic disease remains poor [64]. In addition, the toxicity associated with traditional therapy makes immunotherapy an attractive option.

Because of the diversity of sarcomas, identifying optimal TAAs that are widely expressed has been challenging. GD2 is a promising target because it is expressed in almost all osteosarcomas as well as some Ewing sarcomas and rhabdomyosarcomas [65–67]. However, the encouraging results with GD2.CAR-T cell studies in neuroblastoma have not been duplicated in sarcomas. Although a third generation GD2.CAR demonstrated anti-tumor activity in osteosarcoma cell lines, it had no activity in a mouse model. This lack of efficacy was accompanied with expansion in murine MDSCs and was ameliorated with co-administration of all-*trans* retinoic acid (ATRA), indicating that ATRA may be an effective adjuvant therapy to combat the immunosuppressive tumor microenvironment in solid tumors [67]. To improve persistence, VZV-CTLs expressing a GD2.CAR are being tested in sarcoma patients (NCT01953900).

In contrast to neuroblastomas, 80% of synovial sarcomas express NY-ESO-1, making this disease a prototype for transgenic TCR targeting. In a preliminary phase 1 study, four out of six adults with metastatic synovial sarcoma achieved partial responses [68]. These results are being further investigated in an ongoing clinical trial which will include children (NCT01343043). Unfortunately, this antigen is only sporadically expressed in other sarcomas, so that TAA-specific CTLs with broader coverage are likely needed. Currently, the feasibility of using TAA-specific CTLs generated against five antigens (PRAME, SSX2, MAGEA4, NY-ESO1–1 and/or Survivin) is being explored in pediatric and adult patients with solid tumors expressing at least one of these antigens (NCT02239861).

HER2 is expressed in over 70% of osteosarcomas but at levels too low to be effectively targeted by an antibody. HER2 specific CAR-T cells showed promising anti-tumor effect in

xenograft mouse models of osteosarcoma [69]. Safety concerns of “on-target, off tumor” toxicity were raised after the death of an adult colon cancer patient in a clinical trial using HER2.CAR-T cells [70]. However, HER2.CAR-T cells appeared safe in a phase I/II study of osteosarcoma patients, where a dose escalation strategy was used starting with an ultra-low dose. Interestingly, this study found that CAR-T cells persisted in biopsy samples even though they were absent in peripheral blood, suggesting that they traffic to disease sites and expand locally [71].

Other studies have demonstrated positive results in sarcoma cell lines or mouse models with CAR-T cells or TCR-transgenic T cells directed against various targets such as IL11Ra, EWS-FLI1, PAPPA, NKG2D, and fAchR [72–77]. Pre-clinical studies of γδ T cells have also yielded promising outcomes [78, 79]. NK cells are another attractive option since they naturally recognize ligands upregulated by tumors through their activating receptors DNAM-1 and NKG2D [80]. In preclinical studies, the natural cytotoxic activity of NK cells towards sarcoma cells has been augmented by activation with IL-15 and by combination with an anti-EGFR antibody [81, 82]. However, these therapies have not yet been evaluated in clinical trials and seem further away for application in children.

BRAIN TUMORS

Brain malignancies as a group are the most common solid tumors in children. They include malignancies such as glioblastoma (GBM) and diffuse intrinsic pontine glioma (DIPG) which are almost universally fatal despite multimodal treatment. It is clear that new treatment options are needed for these highly aggressive brain tumors.

Historically, the CNS was thought to be an immune privileged site but recent evidence has shown that T cells travel to the CSF via meningeal blood vessels and that lymphatic vessels allow antigens to drain from the brain into cervical lymph nodes [83]. The fact that T cells have access to antigens in the CNS is evidenced by the regression of brain metastases in patients with melanoma after the adoptive transfer of T cells [84]. Neurotoxicity has also been observed when MAGE-A3 targeted TCR-transgenic T cells which cross-reacted with MAGE-A12 expressed in human brains, further illustrating the potential ability of immune cells to traffic and target brain cells [85].

Most brain tumor immunotherapy research has focused on GBMs. However, these tumors are very heterogeneous with many patient-specific neo-antigens, making it difficult to identify a TAA to target with adoptive cell therapy [83]. IL13Ra2 is a particularly attractive antigen because it is over-expressed not only by GBM but many pediatric brain tumors and associated with a poorer prognosis in gliomas [86]. This antigen is also expressed by TAMs and MDSCs but not at significant levels on normal brain tissue [87]. EGFRvIII is a common mutant of the EGFR tyrosine kinase found in GBM and is a promising target of immunotherapy because it is absent in normal tissues [88]. Other antigens, including HER2, EphA2, and CD133, are being targeted but due to heterogeneous expression, immune escape has been observed [87]. To combat this problem, CAR-T cell therapies targeting 2 or 3 of these antigens simultaneously have improved tumor control in pre-clinical studies but have yet to be tested in patients [89–91].

A phase I trial investigating injection of first generation IL13Ra2.CAR-T cells into the tumor cavity following resection of recurrent GBM showed transient tumor responses in 2 out of 3 adult patients. One patient had a recurrence in the resection cavity with significantly diminished IL13Ra2 expression, illustrating the problem of antigen escape. Furthermore, T cell persistence was poor with low levels of the CAR-T cells detectable in the recurrent tumor at 14 weeks after infusion. In hopes of improving T cell persistence and anti-tumor activity, an ongoing clinical trial of pediatric and adult glioma patients is using a 2nd generation IL13Ra2.CAR (NCT02208362). Interestingly, one patient on this trial initially had progression of distant lesions including a new spinal lesion with intra-cavitary administration of T cells but all lesions regressed with intraventricular administration, suggesting that local instillation of T cells may not be adequate to prevent distant recurrence [31].

Systemic administration of CAR-T cells has been shown to have anti-tumor effects in a clinical trial of a 3rd generation HER2.CAR modified virus–CTLs administered to children and adults with progressive HER2 positive GBM. The trial was limited by poor expansion of T cells, which might be improved through lymphodepletion [92]. This was demonstrated in pre-clinical studies of EGFRvIII.CAR-T cells in immunocompetent mice with GBM who had greater survival with lymphodepletion prior to CAR-T cell treatment [93]. An ongoing clinical trial for adults with GBM is testing systemic administration of EGFRvIII.CAR-T cells with prior lymphodepletion (NCT01454596) and this strategy should be explored in future pediatric studies as well.

CONCLUSION

There are many opportunities and challenges for adoptive cell therapy in solid tumors. Despite ongoing research, most of these therapies are still far from being widely applicable clinically. Future efforts should focus on finding TAAs that are expressed widely by tumor cells and not on normal tissue, as well as strategies to combat the immunosuppressive TME. In addition, improvements in safety are needed especially when offering these treatments to children.

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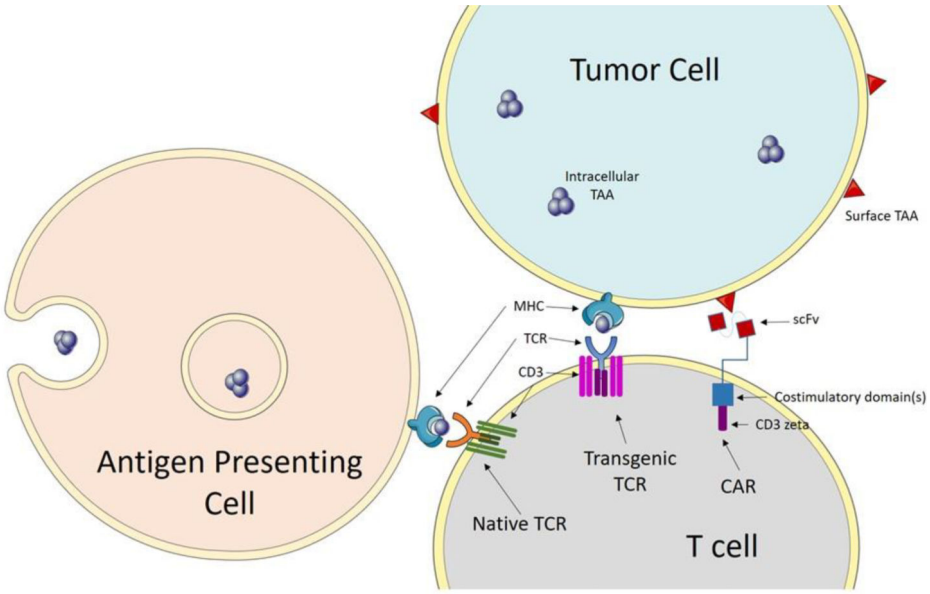

Figure 1:

Intracellular antigens presented by MHC molecules on tumor cells or antigen presenting cells are recognized by the native or transgenic TCR. TCR complex incluces variable alpha beta chains of the TCR AND invariant CD3 chains CAR consists of single chain variable fragment derived from an antibody that recognizes surface antigens and is linked to various co-stimulatory domains (such as 4–1BB, CD28, OX40) and a CD3 zeta chain

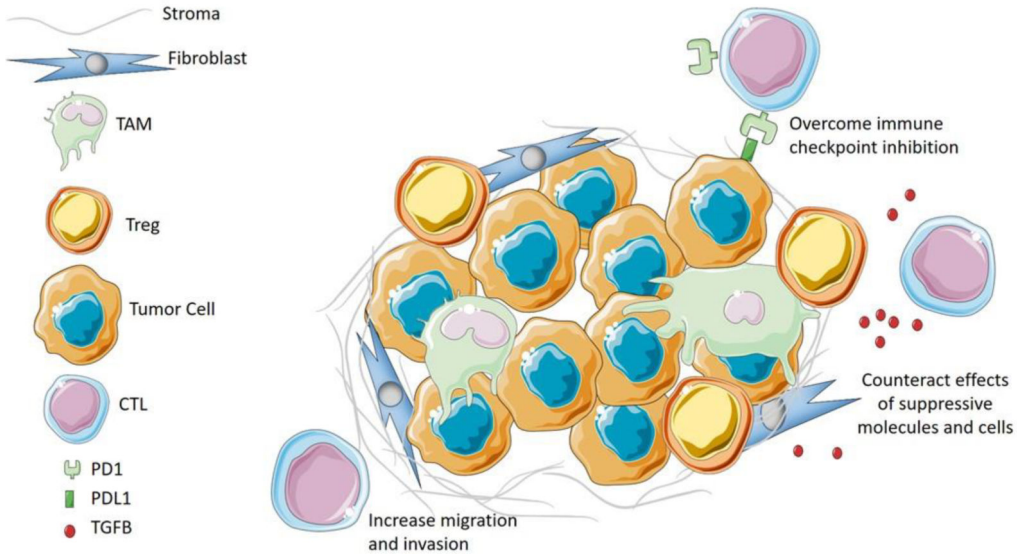

Figure 2:

The tumor microenvironment poses several challenges for adoptive cell therapy including physical barriers such as stroma; suppressive cells such as fibroblasts, TAMs, MDSCs, and Tregs; inhibitory molecules such as TGFb; and immune checkpoints with PD1/PDL1 interaction. Strategies are in developmentto overcome these hurdles in order to design more effective adoptive cell therapy for solid tumors.

TABLE 1.

Clinical trials with immune cells for pediatric solid tumors

Clinical Trial	Patients	Disease	Therapy	Outcomes	Adverse Effects
Heczey et al. [48]	9 children, 2 adults	R/R NBL	3rd generation GD2 CAR (cohort 1: CAR; cohort 2: CAR + LD; cohort 3: CAR + LD + PD-1 inhibitor)	Overall Survival 0/4 (Cohort 1) 6/7 (Cohort 2 & 3)	No dose limiting toxicities Fever: 5/9; FN: 2/9; CRS: 1/9
Pule et al. [56]	11 children	R/R NBL	GD2 CAR VSTs	CR in 1/11; tumor necrosis in 2/11; NED in 4/11; SD in 2/11; PD in 2/11	No severe or dose-limiting toxicities
Louis et al. [57]	18 children, 1 adult	NBL (8 in remission; 11 active disease)	GD2 CAR VSTs	Of patients with active disease: CR in 3/11; PR in 1/11; PD in 4/11; tumor necrosis in 2/11	No severe or dose-limiting toxicities
Park et al. [63]	6 children	R/R NBL	CE7R CAR T cells	CR in 1/6; SD in 2/6; PD in 4/6	Grade 3/4 lymphopenia in 1/6; neutropenia 2/6; anemia 1/6; bacteremia 2/6; pneumonitis 1/6.
Talleur et al. [53]	30 children (21 received haplo-NK)	Newly dx NBL	Chemo, SCT, humanized antiGD2 mAb, GM-CSF, IL-2, +/− haplo-NK cells		VOD in 8/30; Grade 3/4 FN in 20/30; mucositis in 17/30; hypokalemia in 11/30
Federico et al. [54]	13 children	R/R NBL	Haplo- NK cells, GD2 mAb, chemo, cytokines	RR 61.5% (4/11 CR, 1/11 VGPR, 3/11 PR); 5/11 SD	Grade 3/4
					myelosuppression (13/13 patients) and grade 1/2 pain (13/13 patients)
Ahmed et al. [92]	7 children, 10 adults	Recurrent or progressive HER2+ GBM	HER2 CAR VSTs	16 evaluable: PR in 1/16; SD in 7/16; PD in 8/16	Seizures in 2 patients
Ahmed et al. [71]	12 children, 7 adults	HER2- positive tumors (16 OS, one ES, one PNET, and one DSRCT)	HER2 CAR T cells	17 evaluable: PR in 1/17; SD in 4/17; PD in 12/17; tumor removed in 3/17	Fever in 1 patient

CR = complete response; PR = partial response; SD = stable disease; PD = progressive disease; NED = no evidence of disease; SCT = transplant; mab = monoclonal antibody; haplo-NK = haploidentical natural killer cells; R/R = relapsed/refractory; GD2 = disialoganglioside; CAR = chimeric antigen receptor; FN = febrile neutropenia; EBV = Epstein-Barr virus; CTL = Cytotoxic T lymphocytes; VST = virus-specific T cells; OS = osteosarcoma; ES = Ewing's Sarcoma PNET = primitive neuroectodermal tumor; DSRCT = desmoplastic small round cell tumor; LD = lymphodepletion