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The State of Cellular Adoptive Immunotherapy for Neuroblastoma and Other Pediatric Solid Tumors

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Research on adult cancer immunotherapy is proceeding at a rapid pace resulting in an impressive success rate exemplified by a few high profile cases. However, this momentum is not readily extended to pediatric immunotherapy, and it is not for lack of trying. Though reasons for the slower advance are not apparent, some issues can be raised. Pediatric cancer patients represent a distinct demographic group whose immune system is inherently different from that of mature adults. Treating pediatric patients with immunotherapy designed for adults may not yield objective clinical responses. Here, we will present an update on adoptive T-cell and natural killer-cell therapies for neuroblastoma and other childhood solid tumors. Additionally, we will delineate key differences between human fetal/neonatal and adult immune systems. We hope this will generate interests leading to the discussion of potential future directions for improving adoptive cancer immunotherapy for children.

Keywords: pediatric solid tumors, recurrent/refractory/relapsed neuroblastoma, adoptive T-cell therapy, immune cell-based therapy, natural killer cells, *Cbx3*/HP1γ, CD4$^+$ regulatory T cells, effector CD8$^+$ T cells

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INTRODUCTION

In the past few decades, pivotal studies have yielded invaluable information on pediatric oncology leading to the formulation of standard therapies still being performed today (1–9). However, it is now evident that the majority of resistant, metastatic, recurrent/refractory tumors are non-responsive to conventional therapies (10–20). In addition, current approaches often rely on non-specific, cytotoxic chemotherapy and/or radiotherapy that result in long lasting, debilitating toxicities, and in some instance morbidity (21–26). Therefore, there is a need to explore new avenues to eradicate pediatric cancers.

Two seminal reports, published by the surgeon and cancer researcher William Bradley Coley in the late 19th century, show sarcoma tumor regression in patients repeatedly immunized with live or killed streptococcus bacteria (27, 28). His observations suggest an active function for the immune system to control tumor growth, thus laying the foundation for modern cancer immunotherapy. Today, harnessing the immune system to control cancer is proven effective and garnering momentum. Currently, immunotherapy is largely classified into two functional treatment groups: (1) those that amplify/reactivate host existing innate and adaptive tumor immunity including check point inhibitors, dendritic cell (DC) vaccines and cytokines; (2) those that involve the adoptive transfer of genetically manipulated immune cells to target tumor cells *in vivo* such as chimeric antigen receptor (CAR) T cells, genetically enhanced effector T cells and natural killer (NK) cells. We will focus primarily on T- and NK-cell adoptive immunotherapy in this perspective.

ADOPTIVE T-CELL THERAPY (ACT)

Adoptive T-cell therapy represents an attractive viable option for the control of solid tumor growth for the following reasons: T cells can systemically home to tumor sites through the entire body and can cross the blood–brain barrier (BBB). By contrast, antibodies such as checkpoint inhibitors cannot effectively cross the BBB and are not consistently or adequately distributed deep inside solid tumors. To date, ACT with CAR T cells is the prevalent type of immunotherapy to treat solid tumors.

Neuroblastoma (NB)

Neuroblastoma is the most common extracranial solid tumor of childhood and the third most common cause of pediatric cancer death (29–32). Despite conventional multimodal therapy, patients with high-risk NB have a poor prognosis due to high relapse rate (33). Since 1999, tremendous efforts and funds have been dedicated to discovering and testing various forms of immunotherapy to control refractory/recurrent NB (**Table 1**). To date the most studied NB-associated antigen (NBAA) identified is the ganglioside GD2 expressed on a subset of NB tumor cells (34). This discovery heralds in the era of GD2-based immunotherapy to treat human NB. The current standard of care for refractory/recurrent NB in human is anti-GD2 therapy, which recognizes and binds GD2 (34–39). However, because GD2 is also expressed on pain fibers, this therapy has side effects that include severe pain requiring continuous opiate infusions (34). Because of the paucity of identified NBAAs, all CAR clinical trials are GD2-based or variations thereof (38, 40–45) (**Table 1**). It is still early to know whether these GD2-dependent CAR trials will yield objective clinical responses.

The antigen 4Ig-B7-H3 (CD276), a member of the B7 family of immune regulators such as CD80, CD86, PD-L1, PD-L2 and ICOSL, is expressed in a subset of NB tumors (46–49). In mouse and human, 4Ig-B7-H3 is expressed on many normal tissues such as spleen, lymph nodes, thymus and fetal liver as well as other tumors. However, its expression is induced on DCs and macrophages by inflammatory cytokines. 4Ig-B7-H3 binds to a yet to be identified cognate receptor induced on activated T and NK cells, and blockade of this interaction results in reduced interferon γ production and loss of cytotoxic activity of these cells. In mice, deficiency or blockade of B7-H3 leads to improved antitumor immunity suggesting that B7-H3 checkpoint may serve as a novel target for immunotherapy against cancers (50). Indeed, a mouse mAb anti-B7-H3 conjugated to iodine 131 (131I-burtomab) has been designed to bind and directly kill NB cells. 131I-burtomab is recently designated a breakthrough drug to treat metastatic NB by the Food and Drug Administration and a clinical trial has been filed (NCT03275402). The immune status of NB tumors treated with 131I-burtomab has not been published. It would be interesting

TABLE 1 | Present and past adoptive immunotherapy trials for refractory/recurrent/relapsed neuroblastoma.

Approach	Status	NCT#	Sponsor	Year
Adoptive therapy of donor lymphocytes	Completed/no results	00003887	Fred Hutchinson Cancer Research Center	1999–2011
Adoptive therapy of autologous lymphocytes	Completed/no results	00006480	Fred Hutchinson Cancer Research Center	2000–2010
Adoptive therapy of autologous lymphocytes with EBV-lymphoblastoid vaccine	Unknown/no results	00101309	Milton S. Hershey Medical Center	2005–2007
Adoptive therapy of donor-derived tri-virus specific cytotoxic T cells	Completed/no results	01460901	Children's Mercy Hospital Kansas City	2011–2015
Adoptive therapy of activated autologous chimeric GD2-iCas9 CAR T cells	Ongoing/no results	01822652	Baylor College of Medicine	2013–present
Adoptive therapy of CAR T cells expressing anti-GD2	Completed/no results	02107963	National Cancer Institute	2014–2017
Adoptive therapy of CAR T cells expressing anti-CD171	Recruiting	02311621	Seattle children's Hospital	2014–present
Adoptive therapy of activated bispecific GD2 CAR T cells	Recruiting	02173093	Barbara Ann Karmanos Cancer Institute	2014–present
Infusion of haploidentical NK cells	Terminated/no results	00698009	MD Anderson Cancer Center	2008–2012
Infusion of allogeneic NK cells, humanized anti-GD2, and standard chemotherapy	Ongoing/no results	00877110	Memorial Sloan Kettering Cancer Center	2009–present
Infusion of allogeneic NK cells, humanized anti-GD2, and standard chemotherapy	Ongoing/no results	01576692	St. Jude Children's Research Hospital	2012–present
Infusion of *in vitro*-activated/expanded NK cells	Completed/no results	01875601	National Cancer Institute (NCI)	2013–2016
Infusion of allogeneic NK cells from an acceptable parent	Recruiting	01857934	St. Jude Children's Research Hospital	2013–present
Infusion of donor NK cells following haploidentical hematopoietic cell transplant	Recruiting	02100891	Monica Thakar Medical College of Wisconsin	2014–present
Infusion of $CD133^+$ autologous stem cells followed by haploidentical NK cells	Recruiting	02130869	St. Jude Children's Research Hospital	2014–present
Infusion of autologous expanded NK cells and anti-GD2	Not yet recruiting	02573896	New Approaches to Neuroblastoma Therapy Consortium	2015–present
Infusion of allogeneic NK cells and humanized anti-GD2	Recruiting	02650648	Memorial Sloan Kettering Cancer Center	2016–present
Infusion of *in vitro*-activated/expanded haploidentical NK cells and anti-GD2-IL2	Not yet recruiting	03209869	University of Wisconsin, Madison	2017

EBV, Epstein–Barr virus; CAR, chimeric antigen receptor; GD2, glanglioside GD2; NK, natural killer; iCas9, inducible caspase 9.

to know whether the antitumor activity of burtomab results from direct killing of tumor cells or activated DCs thus reactivating existing anti-NB immunity. Although no published data are available to show the feasibility of B7-H3 CAR T cells for the treatment of human NB, two patents have been filed for such an invention (US Application No. 14/779,586; US Application No. 61/805,001; PCT Application No. PCT/US2014/031543 and PCT Application No. PCT/US2016/050887; US Application No. 62/216,447). It remains to be determined whether B7-H3 CAR T cells can inhibit NB tumor growth and whether the potential antitumor activity of B7-H3 CAR T cells is due to killing of intratumoral suppressive DCs/macrophages or tumor cells or both.

The anaplastic lymphoma kinase (ALK or CD246) is a receptor protein tyrosine kinase predominantly expressed in the central nervous system (CNS) and peripheral nervous system in mouse and human suggesting its role in normal brain development and function (51). A series of studies show that *Alk* is frequently mutated (mainly ALK^{R1275Q} and ALK^{F1174L}) and duplicated in high-risk NB tumors (52–58). The ALK^{R1275Q} mutation results in a constitutively active kinase suggesting a role for ALK in NB development. However, mice harboring human ALK^{R1275Q} or ALK^{F1174L} alone do not develop aggressive NB irrespective of genetic background (53, 57). In the contrary, animals having both MYCN amplification and ALK^{R1275Q} or ALK^{F1174L} mutation succumb to NB at a higher rate (53, 57). These findings suggest that mutations in *Alk* are necessary but not sufficient to drive aggressive NB development. Because ALK is a cell surface kinase, developing CAR T cells targeting ALK has been suggested. Indeed, in a xenogeneic NSG mouse model for NB, human ALK CAR T cells can eradicate ALK-positive tumors; both tumor antigen and receptor density governs the efficacy of these CAR T cells (59). Clinical trials have not been initiated.

Although CAR T-cell therapy is being propelled to the forefront, problems exist that need further investigation. Production of CAR T cells requires the identification of tumor-associated antigen (TAA), generation of an antibody or T-cell receptor (TCR) capable of recognizing the TAA, cloning of genes encoding the antibody or TCR to be introduced into isolated tumor-infiltrating lymphocytes or haploidentical T cells. For most pediatric solid tumors, the identity of TAAs is still unknown and neoantigen load is low thus limiting the use of CAR T cells for this group. In patients with solid tumors for which TAAs have been identified, the use of CAR T cells has proven less effective than in patients with fluid tumors. Recent data are showing a previously unpredicted phenomenon observed in patients treated with CAR T cells: the emergence of tumor cells that have lost expression of the TAA targeted by CAR T cells, undoubtedly due to negative selection imposed by CAR therapy (60–62). New evidence demonstrates that CAR T cells once in the tumor microenvironment (TME) may suffer from exhaustion caused by suppressor cells including myeloid-derived suppressor cells or CD4^{+} regulatory T (Treg) cells present in the TME (63–65). Perhaps a more dangerous issue arisen is the development of cytokine release syndrome (CRS) (66, 67) and neurologic toxicity observed in patients undergoing CAR therapy (68).

To circumvent problems posed by CAR T cells, we propose that perhaps the most effective strategy to control solid tumor growth is one that does not require identifying TAAs and corresponding tumor-reactive CD8^{+} T cells, can enhance effector activity of CD8^{+} T cells, and can simultaneously eliminate immune suppression within the TME. Our recent studies suggest that such an approach is attainable. We show that by targeting the histone reader *Cbx3*/HP1γ, we can enhance the tumor killing capacity of effector CD8^{+} T cells (69, 70). As a result, adoptive transfer of *Cbx3*/HP1γ-deficient CD8^{+} effector T cells alone into wild type (wt) tumor-bearing mice greatly reduces NB growth. Within the NB TME of *Cbx3*/HP1γ-deficient mice or wt mice treated with *Cbx3*/HP1γ-deficient CD8^{+} T cells, we detect an increase of *Klrk1*/NKG2D^{+} infiltrating CD8^{+} effector T cells and a decrease in CD4^{+} Treg cells. PD-1 and *Pdl1* expression is not altered in CD8^{+} T cells or NB tumors, respectively. Moreover, *Cbx3*/HP1γ-deficient CD8^{+} T cells appear to have overcome exhaustion. These findings suggest that targeting *Cbx3*/HP1γ can represent an alternative and rational therapeutic approach to control NB as well as other solid tumors.

Other Pediatric Solid Tumors (CNS Tumors, Sarcomas, and Nasopharyngeal Sarcomas)

As for NB, there is a dearth of identified TAAs available for the formulation of CAR therapy to treat most pediatric solid tumors (71–73). Tumor immunity against pediatric solid tumors is not completely understood. The human epidermal growth factor receptor 2 (HER2) is expressed on pediatric as well as adult glioblastoma, glioma, and medulloblastoma tumors, and overexpression of HER2 has been associated with poorer prognosis. In animal models, HER2 CAR T cells efficiently cause the regression of CNS tumors. These preclinical studies have paved the way for a few HER2-based CAR clinical trials (74–77) (**Table 2**); results of these trials are not yet available. It would be crucial to determine whether HER2-targeted CAR therapy will induce the emergence of HER2-negative tumors as has been shown in animal models and in patients receiving CD19 targeted CAR therapy (60, 61) or will cause CRS as in adults (67).

Pediatric nasopharyngeal carcinoma patients with local-regional bulky and metastatic disease have a poor prognosis (78). It is a rare tumor that is almost always associated with Epstein–Barr virus (EBV) (78, 79), and EBV-specific cytotoxic T lymphocytes (CTLs) can be found in individuals infected with this ubiquitous virus. These findings lead to the design of EBV-based T-cell therapy. In adults, ACT with EBV-specific CTLs is more effective in patients with low disease burden while results for pediatric trials are not available (80–83) (**Table 2**).

Prognosis for pediatric patients with recurrent/refractory sarcomas is poor, the survival rate ranges from 10 to 30% (73). For this group of children, few immunotherapy clinical trials are being tested, and past trials using autologous T cells (NCT00001566 and NCT 00001564) have not yielded much information to advance the field (**Table 2**).

TABLE 2 | Present and past adoptive immunotherapy for pediatric central nervous system tumors, sarcomas, and nasopharyngeal carcinomas.

Approach	Status	NCT#	Sponsor	Year
Adoptive therapy with autologous T cells following tumor vaccine	Completed (Ref)	00001566	National Cancer Institute (NCI)	1999–2012
Adoptive therapy with autologous T cells followed by DC vaccine	Completed/no results	00001564	National Cancer Institute (NCI)	1999–2014
Adoptive therapy with allogeneic EBV-specific T cells	Recruiting	00002663	Atara Biotherapeutics	1999–present
Adoptive therapy with autologous EBV-specific CTLs	Completed/no results	00516087	Baylor College of Medicine	2007–2017
Adoptive therapy with EBV-specific CTLs expressing HER2 CAR	Ongoing	00889954	Baylor College of Medicine	2009–present
Adoptive therapy with HER2/CD28 CAR T cells	Recruiting	00902044	Baylor College of Medicine	2009–present
Adoptive therapy with autologous EBV-specific CTLs	Completed/no results	00953420	Baylor College of Medicine	2009–2017
Adoptive therapy with CMV-specific CTLs expressing HER2 CAR	Ongoing	01109095	Baylor College of Medicine	2010–present
Adoptive therapy with haploidentical EBV-specific CTLs	Ongoing	01447056	Baylor College of Medicine	2011–present
Adoptive therapy with CD22 CAR T cells	Recruiting	02315612	National Cancer Institute (NCI)	2014–present
Adoptive therapy with glycan 3-specific autologous CAR T cells	Not yet recruiting	02932956	Baylor College of Medicine	2016–present
Adoptive therapy with NY-ESO-1 TCR transduced PBMC and NY-ESO-1 DC vaccine	Recruiting	02775292	Jonsson Comprehensive Cancer Center	2016–present
Infusion of autologous NK cells following peripheral blood stem cell transplant	Ongoing/no results	01287104	National Cancer Institute (NCI)	2011–present
Infusion of *in vitro*-activated/expanded NK cells	Completed/no results	01875601	National Cancer Institute (NCI)	2013–2016
Infusion of *in vitro*-activated/expanded NK cells	Completed/no results	01875601	National Cancer Institute (NCI)	2013–2016
Infusion of CD133+ autologous stem cells followed by haploidentical NK cells	Recruiting	02130869	St. Jude Children's Research Hospital	2014–present
Infusion of donor NK cells following haploidentical hematopoietic cell transplant	Recruiting	02100891	Monica Thakar Medical College of Wisconsin	2014–2016

EBV, Epstein–Barr virus; HER2, human epidermal growth factor receptor 2; CMV, cytomegalovirus.

Some studies have shown that the cancer-testes antigen NY-ESO-1 is expressed on a subset of pediatric tumors, which lead to a trial using T cells engineered to express NY-ESO-1-specific TCR (NCT02775292) (84) (**Table 2**). HER2 CAR therapy has also been proposed to treat sarcomas (85). CAR T cells targeting glypican-3, a proteoglycan expressed on a small number of solid tumors (86), are being tested in clinical trials to treat pediatric solid tumors (87) (NCT02932956). Data are not yet available for these trials.

The lack of available immunotherapy for pediatric solid tumors may be due to the paucity of identified TAAs, and few basic studies designed to understand tumor immunity during development from infancy to young adulthood in either mouse or human. As a result, most of these trials are based on those that have been designed to treat adult solid tumors.

ADOPTIVE NK- AND NATURAL KILLER T (NKT)-CELL THERAPY

Natural killer cells and NKT cells have been shown to play crucial roles in antitumor immunity by directly killing tumor cells or indirectly through antibody-dependent cellular cytotoxicity. Based on results from preclinical studies (88–90), several clinical trials have been initiated to test the ability of *in vitro*-activated/expanded or engineered NK cells and NKT cells to control pediatric solid tumors including NB (91, 92) (**Tables 1** and **2**). Results of these trials are not yet available to indicate whether NK- or NKT-cell therapy would be a viable option.

Nonetheless, clinical data have demonstrated that despite the large number of NK cells infused, the antitumor effects of these cells have been modest in adults. NK and NKT cells express a number of inhibitory receptors that bind to MHC class I and other molecules. Additionally, NK and NKT cells are sensitive to various inhibitory molecules within the TME (93). Moreover, we show that the frequency of NK and NKT cells in NB tumors is low, and no differences are detected in tumors from wt or *Cbx3*/HP1γ-deficient mice yet NB tumor growth is greatly abrogated in *Cbx3*/HP1γ-deficient mice (70). Our findings imply that NK or NKT cells may not play an important role in controlling NB tumor growth.

DISCUSSION AND FUTURE DIRECTIONS

In the past 40–50 years, pediatric oncologists have made significant, basic advances toward our understanding of molecular pathways driving the development of tumors in children. This achievement hinges on the belief that cancer pediatric patients represent a distinct demographic group, and the biology of their tumors is fundamentally different from that of adults.

Adult cancer immunotherapy is experiencing a renaissance while that of children is still at its infancy. The momentum that drives adult cancer immunotherapy is built upon decades of basic research designed to understand how an adult immune system responses to tumors developing within an adult host. Thus, there is a need to recognize that pediatric cancer patients represent a distinct demographic group whose immune system is fundamentally different than that of mature adults.

Fetal and adult T cells are distinct populations that arise from different hematopoietic stem cell populations present at different developmental stages (94), and human NK cells follow similar developmental evolution (95, 96). Notably, fetal CD4$^+$ T cells are poised to differentiate into CD4$^+$ Treg cells upon allogeneic stimulation. Indeed, human fetus and cord blood (CB) contains an abundance of phenotypically naïve CD25$^+$CD4$^+$ Treg cells, but functionally mature, capable of suppressing T- and NK-cell proliferation and function (97–101). Similar suppressive mechanism is observed in the mouse fetus (102). Thus, T- and NK-cell lineages in the developing human or mouse are biased toward immune tolerance mediated by active suppression of early immunity; in some instances, this suppression persists at least until early adulthood (101). The tolerogenic tendency of fetal/neonatal immune system can be attributed to marked differences in response to alloantigens between human fetal and adult DCs. Fetal DCs strongly promote Treg-cell induction and inhibit T-cell tumor-necrosis factor-α production when cultured with alloantigens (103). This may explain why CAR T cells once in the NB TME often suffer from exhaustion. In addition to functional disparities, neonatal and adult immune systems differ quantitatively. Overall, there is a greater number of circulating CD4$^+$ T cells and a lower number of CD8$^+$ T cells in neonates compared to adults (104). Consequently, the ratio of CD4:CD8 is higher in neonates than adults. Together these results suggest that for some children, the persistence of fetal immune suppression, mediated by fetal CD4$^+$ Treg cells, and the lower number of CD8$^+$ T cells may render their immune system incapable of surveilling and eradicating tumor. Therefore in the future, it might be essential to study the effects of persistent fetal immune suppression on tumor development and growth. If the persistence of fetal immune suppression does influence antitumor immunity in pediatric patients, it would be crucial to determine the developmental age at which intervention can be mounted to prevent tumorigenesis and growth without breaking tolerance. Clinically, it might be important to collect data on the immune status of children bearing solid tumors in addition to dissecting their tumor immune environment. Results from these studies might help direct the design of T- and NK-cell therapies that can circumvent suppression and prevent exhaustion induction.

In adult tumors, mutation load appears to correlate with tumor immunity. However, the number of somatic mutations in pediatric solid tumors is low. In the future, it would be necessary to determine mechanisms controlling tumor immunity independent of somatic mutation loads in pediatric patients.

For this demographic group, perhaps the most effective strategy to control solid tumor growth is one that does not require identifying TAAs or neoantigens and their corresponding reactive CD8$^+$ T cells, can enhance effector activity of CD8$^+$ T cells, and can simultaneously eliminate immune suppression within the TME.

We believe these are crucial issues that need to be addressed in order to move the field of pediatric adoptive cancer immunotherapy to the fore. Until there is a will to allow for the funding of such studies and those that are outside traditional belief, children with cancers will continue to be treated as adults, and may not benefit from the cancer immunotherapy renaissance.

AUTHOR CONTRIBUTIONS

TPL and THT conceived and cowrote this manuscript.

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