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Reviews

Will Post-Transplantation Cell Therapies for Pediatric Patients Become Standard of Care?

Arjan C. Lankester [1,*], Franco Locatelli [2], Peter Bader [3], Eva Rettinger [3], Maarten Egeler [4], Satyendra Katewa [4], Michael A. Pulsipher [5], Stefan Nierkens [6], Kirk Schultz [7], Rupert Handgretinger [8], Stephan A. Grupp [9], Jaap Jan Boelens [10], Catherine M. Bollard [11], on behalf of the Westhafen Intercontinental Group

[1] *Division of Stem Cell Transplantation, Department of Pediatrics, Leiden University Medical Center, Leiden, The Netherlands*
[2] *Department of Pediatric Hematology and Oncology, IRCCS Istituto Di Ricovero e Cura a Carattere Scientifico Ospedale Pediatrico, Bambino Gesù, Rome, Italy*
[3] *Department of Stem Cell Transplantation and Immunology, Hospital for Children and Adolescents, Frankfurt am Main, Germany*
[4] *Division of Haematology/Oncology, The Hospital for Sick Children, University of Toronto, Toronto, Ontario, Canada*
[5] *Primary Children's Hospital, Division of Hematology and Hematological Malignancies, Huntsman Cancer Institute/University of Utah School of Medicine, Salt Lake City, Utah*
[6] *Utrecht-Dendritic cells AgaiNst CancEr (U-DANCE), Lab Translational Immunology, Utrecht center for Diagnostic Advances in Immunology Research (U-DAIR), Lab Translational Immunology, University Medical Center Utrecht, Utrecht, The Netherlands*
[7] *BC Children's Hospital, Division of Oncology, University of British Columbia, Vancouver, British Columbia, Canada*
[8] *Department of Pediatric Hematology/Oncology, Children's University Hospital, University of Tübingen, Tübingen, Germany*
[9] *Division of Oncology, The Children's Hospital of Philadelphia and Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania*
[10] *Department of Pediatrics, Blood and Marrow Transplantation Program, Utrecht-Dendritic cells AgaiNst CancEr (U-DANCE), Lab Translational Immunology, University Medical Center Utrecht, Utrecht, The Netherlands*
[11] *Departments of Pediatrics and Microbiology, Immunology and Tropical Medicine, Children's National Health System and George Washington University, Division of Pediatric Hematology-Oncology, Washington, DC*

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A B S T R A C T

Although allogeneic hematopoietic stem cell transplantation (HSCT) is a curative approach for many pediatric patients with hematologic malignancies and some nonmalignant disorders, some critical obstacles remain to be overcome, including relapse, engraftment failure, graft-versus-host disease (GVHD), and infection. Harnessing the immune system to induce a graft-versus-tumor effect or rapidly restore antiviral immunity through the use of donor lymphocyte infusion (DLI) has been remarkably successful in some settings. Unfortunately, however, the responses to DLI can be variable, and GVHD is common. Thus, manipulations to minimize GVHD while restoring antiviral immunity and enhancing the graft-versus-tumor effect are needed to improve outcomes after allogeneic HSCT. Cellular therapies, defined as treatment modalities in which hematopoietic or nonhematopoietic cells are used as therapeutic agents, offer this promise for improving outcomes post-HSCT. This review presents an overview of the field for pediatric cell therapies in the transplant setting and discusses how we can broaden applicability beyond phase I.

INTRODUCTION

Cellular therapies have been developed primarily as therapeutic agents to treat malignancies, infections in immunocompromised hosts, and inflammatory disorders. Minimally manipulated products, such as donor lymphocyte infusion (DLI), involve a manufacturing process that does not alter the original relevant characteristics of the tissue relating to the tissue's utility for reconstruction, repair, or replacement. In contrast, if the biological characteristics of the cells change during the processing, then the cells are more than minimally manipulated. In this review, we focus on complex cellular therapies that require more than minimal manipulation. To date, the clinical experience with novel cell therapeutics to treat pediatric patients after allogeneic hematopoietic stem cell transplantation (HSCT) generally has been restricted to phase I/II pediatric or combined studies (Table 1). This review presents an overview of the field in pediatric cell therapies after HSCT and then discusses how we can move these therapies from investigational status to the standard of care by moving beyond phase I to more definitive clinical trials.

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* Correspondence and reprint requests: Arjan C. Lankester, MD, PhD, Division of Stem Cell Transplantation, Department of Pediatrics, Leiden University Medical Center, Albinusdreef 2, 2300 RC Leiden, The Netherlands.
E-mail address: a.lankester@lumc.nl (A.C. Lankester).

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Table 1
Adoptive Cellular Therapy Studies after HSCT in Pediatric Patients

Cellular Therapy	References	Pediatric or Combined Study	Key Findings
Virus-specific CTLs			
EBV	Doubrinova et al. [8]	Combined	Efficacy of donor EBV CTLs in EBV-LPD
	Comoli et al. [10]	Pediatric	Efficacy of donor EBV CTLs in $CD19^+/CD20^-$ EBV-LPD after rituximab
	Icheva et al. [11]	Combined	Efficacy of EBNA1-specific donor CTLs in EBV-LPD
CMV	Feuchtinger et al. [18]	Pediatric	Safety and efficacy of donor CMV CTLs using pp65 protein stimulation/IFN-γ selection
	Meij et al. [20]	Combined	Safety and efficacy of donor CMV CTLs using pp65 peptide stimulation/IFN-γ selection
AdV	Feuchtinger et al. [26]	Pediatric	Safety and efficacy of AdV-specific CD4/CD8 donor T cells using protein stimulation/IFN-γ selection
Multivirus	Leen et al. [28]	Pediatric	Safety and in vivo persistence of EBV- and AdV-bispecific donor CTLs after HLA-mismatched HSCT
	Gerdemann et al. [19]	Combined	Safety and efficacy of trivirus-specific CTLs generated using DC nucleofection technology
	Papadopoulou et al. [29]	Combined	Safety and efficacy of single-culture virus-specific T cells recognizing 12 immunogenic antigens from AdV, CMV, AdV, BK and HHV6
Third-party	Leen et al. [33]	Combined	Multicenter study demonstrating the feasibility and efficacy of banked third-party virus-specific CTLs
NK cells	Stern et al. [55]	Combined	Feasibility and safety of purified ($CD3^-/CD56^+$) donor NK cell infusions after haploidentical HSCT
	Rubnitz et al. [58]	Pediatric	Safety and engraftment of KIR ligand-mismatched haploidentical purified ($CD3^-/CD56^+$) NK cells in AML
	Brehm et al. [59]	Pediatric	Difference in efficacy of nonstimulated versus IL-2–stimulated purified ($CD3^-/CD56^+$) NK cells after haploidentical HSCT
	Kloess et al. [56]	Pediatric	Efficacy of IL-2–stimulated NK cells after haploidentical HSCT in neuroblastoma and role of soluble MICA
CIK cells	Rettinger et al. [68]	Pediatric	Safety and feasibility of IL-15–stimulated donor CIK cells after haploidentical HSCT
CAR T cells	Grupp et al. [91]	Pediatric	First report on safety and remission induction of autologous CD19.CAR T cells in ALL
	Cruz et al. [93]	Combined	Safety and efficacy of allogeneic virus-specific CD19.CAR T cells
	Grupp et al. [98]	Pediatric	Efficacy and management of CRS after treatment with autologous and allogeneic CD19.CAR T cells
MSCs (acute GVHD)	LeBlanc et al. [124]	Pediatric	First report of successful remission induction by MSC treatment in a child with refractory GVHD
	LeBlanc et al. [125]	Combined	Multicenter study showing feasibility, safety and efficacy of fetal bovine serum expanded MSCs in GVHD
	Lucchini et al. [126]	Pediatric	Safety and efficacy using platelet-lysate expanded MSCs in steroid-refractory GVHD
	Ball et al. [127]	Pediatric	Multicenter study showing feasibility and efficacy of MSCs in steroid-refractory GVHD grade III-IV
	Introna et al. [128]	Combined	Multicenter study reporting feasibility and efficacy in of MSCs in steroid-refractory GVHD grade II-IV
	Prasad et al. [129]	Pediatric	Report on clinical outcome using the Prochymal MSC product in steroid-refractory GVHD grade II-IV

OVERVIEW OF CURRENT CELLULAR THERAPIES

Virus-Specific T Cells

Viral infections are a major cause of morbidity and mortality after HSCT. Given that the recovery of virus-specific T cells is clearly associated with protection from viral infection, adoptive immunotherapy to decrease the time to immune reconstitution or to treat viral reactivations and infections is an attractive approach. The majority of T cell studies in pediatrics have focused on cytomegalovirus (CMV), Epstin-Barr virus (EBV), and adenovirus (AdV) and trials of donor-derived cytotoxic T lymphocytes (CTLs) specific for single viruses [1]. Although methodologies have been developed and clinical trials evaluating T cells specific for other viral pathogens, such as BK virus, human herpesvirus 6, and varicella zoster, have shown promising results, the patient numbers are still relatively small. In contrast, T cell therapies aimed at reconstituting EBV-specific T cell immunity have been used for almost 20 years [2-5], In the first reported studies, unmanipulated DLIs were administered to pediatric HSCT recipients with established disease or falling donor chimerism [6,7]. Although effective in some patients, this approach has limited efficacy, however, and is further limited by the presence of alloreactive T cells in the DLI product and the resultant potential for graft-versus-host disease (GVHD), which led to the development of donor-derived EBV-specific T cells for clinical use [7-9]. Although many of the studies using EBV-specific T cells were conducted during the pre-rituximab era, there are still an appreciable number of pediatric patients with rituximab-resistant disease that is responsive to EBV-specific T cells [10]. The results of these studies confirm that donor-derived EBV-specific CTL therapy is safe and effective when used either as prophylaxis or as treatment for EBV-mediated post-transplantation lymphoproliferative disease after HSCT, and this approach is now focused on rapid manufacturing using sorting strategies with HLA-peptide multimers or IFN-γ capture [11-13].

The administration of CMV-specific T cells after HSCT was first explored by Walter et al. [14] and Riddell et al. [15], who infused donor-derived CMV-specific $CD8^+$ clones to recipients of matched sibling donor grafts. Numerous groups have built on these initial studies of CMV-specific T cell

therapy for pediatric as well as adult patients after HSCT [16-24]. Overall, the response rates in studies of donor-derived CMV-specific CTLs to treat CMV reactivation or disease have been encouraging, ranging from 80% to 100% [1].

Published reports on the use of T cells targeting adenovirus are limited, although a case report documented the successful use of DLI in a patient with hemorrhagic cystitis due to adenovirus infection [25]. Feuchtinger et al. [26] reported a study that selected adenovirus-reactive T cells by stimulation of donor peripheral blood mononuclear cells with adenoviral antigen followed by IFN-γ capture. Because pediatric patients often develop multiple viral infections after HSCT, several groups have explored the use of T cells targeting more than 1 virus (eg, CMV, EBV, and AdV; EBV and AdV; CMV and AdV; AdV, CMV, EBV, BK, HHV6) to administer after HSCT as either treatment or prophylaxis [27,28,29]. In summary, these studies show that virus-specific T cells targeting 1-5 viruses after HSCT has efficacy for reconstituting immunity and clearing viral reactivation and disease. Although the potential for de novo alloreactivity is a concern in all these approaches, the incidence of GVHD does not appear to be increased over what would be expected in this patient population. There was a particular concern in the HLA-mismatched transplant setting, given that in vitro studies have shown that that a majority of virus-specific CTL lines have cross-reactivity against allogeneic HLA molecules [30]; however, such alloreactivity was not observed among 153 recipients who received virus-specific CTLs, including 73 in whom there was an donor–recipient HLA mismatch [31].

Adoptive transfer of virus-specific CTLs is more problematic when the donor lacks viral immunity for the infecting virus or when the recipient has received an umbilical cord blood (UCB) graft. This is of particular concern in the pediatric setting, because these patients are more frequently recipients of grafts from virus-naïve donors (eg, UCB and seronegative sibling donors). As a result, there is considerable interest in modifying culture conditions to reactivate CTLs from virus naive donors [32]. Finally several studies have evaluated whether third-party CTLs sharing HLA antigens with HSCT recipients have activity against viral antigens [1,33].

In summary, the adoptive transfer of virus-specific CTLs can reconstitute viral immunity and treat viral infections that have failed to respond to conventional therapies. The overall response rate in pediatric as well as adult HSCT recipients with active disease is approximately 80% for those receiving donor-derived virus-specific CTLs and approximately 70% for those receiving third-party cells [33]. In most cases, the responses are durable, and thus this strategy has many advantages compared with pharmacologic therapies; however, broader application has been limited by the complexity of some of the CTL manufacturing methodologies and lengthy production time. Over the past few years, several groups have developed more rapid CTL generation protocols that appear to have equivalent activity and may allow definitive testing in late-phase trials [1,19]. Furthermore, strategies using EBV-specific T cells targeting latent membrane proteins are now being used for pediatric and adult patients with EBV-positive Hodgkin lymphoma and non-Hodgkin lymphoma, which develop in immunocompetent hosts [34]. These studies have shown promise both for patients with active disease (50% event-free survival at 2 years) and as adjuvant therapy (>80% event-free survival at 2 years) after autologous and allogeneic HSCT, and multicenter studies are now underway.

Tumor-Reactive Lymphocytes

Naturally occurring tumor-reactive lymphocytes

Tumor-reactive lymphocytes, which may include αβ-T lymphocytes, γδ-T lymphocytes, natural killer (NK) cells, and NK T cells, have been identified in peripheral blood, bone marrow and the tumor microenvironment (ie, tumor-infiltrating lymphocytes [TILs]), in a wide variety of human cancers. Interactions between cancer cells and host immune surveillance in the tumor microenvironment are known to influence tumor growth and clinical outcome [35]. These naturally occurring cellular immune responses may either promote or antagonize tumor growth, depending on their composition and functional properties [36,37]. Although previous studies have been performed primarily in adults, studies in childhood tumors have demonstrated that a proinflammatory, IFN-γ–rich tumor microenvironment is linked to the presence of TILs (particularly $CD8^+$) and is positively correlated with clinical outcome [38,39]. Exploitation of naturally occurring host antitumor immunity has been successfully pioneered in patients with melanoma using ex vivo expanded TILs for adoptive transfer [40]. Similarly, the presence and clinical relevance of leukemia-specific immunity have proven to be of prognostic significance. Post-treatment leukemia cytolytic activity by NK cells, as well as the presence of leukemia-reactive T lymphocytes in pediatric patients with acute myelogenous leukemia (AML) in first remission, are correlated with sustained remission [41,42]. In addition, CTLs directed against leukemia-restricted antigen WT-1 have been demonstrated in pediatric patients with postremission acute lymphoblastic leukemia (ALL) [43].

In the setting of allogeneic HSCT, additional and distinct spontaneous antitumor immune responses may emerge. Polymorphisms in hematopoietic minor histocompatibility antigens (mHAgs) serve as leukemia-specific targets and facilitate T cell–mediated graft-versus-tumor effects [44]. Thus, identification of mHAg-specific T cell reactivity in HSCT recipients has provided the rationale for developing adoptive immunotherapeutic strategies using ex vivo–generated mHAg-specific T cells [45].

NK cells

NK cell function is finely regulated by a large array of receptors transducing either inhibitory or activating signals [46]. In an allogeneic HSCT setting, donor NK cells can kill recipient cells through the mechanism of missing self-recognition, provided that the donor expresses a killer cell immunoglobulin-like receptor (KIR) ligand that is missing in the recipient HLA genotype and expresses the specific KIR, leading to a KIR–KIR ligand mismatch in the graft-versus-host direction [47-49]. According to the concept of missing self-recognition, donor NK cell alloreactivity can be predicted to occur in approximately 50% of patients undergoing HSCT [34,50]. In addition, NK cells are equipped with various triggering receptors that play crucial roles in NK cell activation and help define which tissues the NK cells attack. In this regard, NK cells offer the unique advantage of inducing a graft-versus-leukemia effect without necessarily promoting GVHD. The reasons why NK cells may not cause GVHD are multifactorial and include the fact that activated NK cells can directly lyse GVHD-inducing T cells and host antigen-presenting cells (APCs), and that healthy recipient non-hematopoietic tissues often lack ligands for activating NK cell receptors [34,51,52]. The clinical evidence demonstrating the potential importance of this KIR–KIR ligand mismatch in pediatric cancer has been particularly evident in the setting of haploidentical HSCT for acute leukemias [53].

Along with spontaneously occurring NK cell reactivity, NK cells also can be harnessed for adoptive cellular therapy. The adoptive transfer of unstimulated and ex vivo cytokine-activated NK cells in pediatric patients has been explored both in the haploidentical HSCT context [54-56] and as a strategy for consolidating remission in patients with AML after a lymphodepleting chemotherapy regimen and IL-2 administration [57,58]. From these initial studies, it may be concluded that infusion of purified unstimulated NK cells either immediately after collection or after ex vivo activation with cytokines (eg, IL-2 or IL-15) is feasible and safe. Immunologic efficacy appears to be more pronounced with cytokine-activated NK cells [59]. Nonetheless, although some encouraging responses have been reported, the actual clinical benefits and antitumor efficacy of NK cell adoptive therapy await further evaluation in phase II/III studies.

Cytokine-Induced Killer Cells

Cytokine-induced killer (CIK) cells are in vitro–expanded effector cells that can be generated from peripheral blood mononuclear cells, bone marrow mononuclear cells, or UCB by the timed addition of IFN-γ, activating anti-CD3 antibody, and IL-2 [60-62]. Expanded CIK cells represent a heterogeneous population of mainly $CD3^+$ T cells sharing both $CD3^+$ T cell and $CD56^+$ NK cell phenotypes, as well as low numbers of $CD3^-CD56^+$ NK cells.

CIK cells are known to be capable of eradicating various hematologic malignancies and solid tumors in a non–MHC-restricted manner with no significant alloreactive potential. The molecule that likely plays the most important role in CIK cell–mediated killing is the NKG2D receptor, an activating NK cell receptor [63-65]. The known ligands of this receptor are relatively restricted in tumor cells. Although NKG2D mediates the interaction between CIK cells and malignant cells, the final execution of apoptosis is mediated through perforin and granzyme release.

These cells have been tested in the allogeneic HSCT setting in 3 clinical trials to date. These 3 studies enrolled adult patients with relapsed hematologic malignancy post-HSCT. In all patients, CIK cell infusions showed a good safety profile, with no severe toxicities and a low incidence of acute GVHD and limited chronic GVHD even in the haploidentical donor setting. Between 30% and 50% of the treated patients showed transient clinical responses [61,66,67], suggesting that CIK cells may have antileukemic activity in vivo; however, long-lasting efficacy has not been seen, most likely related to a limited persistence of the infused CIK cells in vivo [68].

Regulatory T Cells

In the last 2 decades, $CD4^+CD25^+$ regulatory T cells (Tregs) have been shown to be potential regulators of the immune response. These cells have proven crucial in the prevention of autoimmune diseases and in the blunting of some pathogen-specific immune responses. $CD4^+$ Tregs are defined according to their site of development and expression of Foxp3. In animal models, the adoptive transfer of Tregs can prevent autoimmune disease, graft rejection, and GVHD [69-72].

Human $CD4^+CD25^+$ Treg cells isolated from peripheral blood can suppress allogeneic responses in mixed lymphocyte reactions and have a role in tolerance induction to alloantigens [73,74]. To date, all of the clinical data have been generated exclusively in adults. The first clinical experience using Tregs in the HSCT setting focused on GVHD prevention and treatment [75]. Because the Tregs were administered with standard GVHD prophylaxis strategies, the efficacy of Tregs for GVHD prevention could not be determined. Importantly, however, no severe Treg-related acute toxicities were observed. Furthermore, this study showed that in vitro expanded Tregs survive in vivo, at least transiently [76].

The main obstacles to broadening the use of Tregs beyond phase I studies include the difficulties in the isolation and expansion of these cells. Some groups have explored the in vitro use of rapamycin to expand Tregs. Rapamycin inhibits the proliferation and function of conventional T cells, thereby allowing the Treg expansion even from a mixed population [77,78]. Unfortunately, rapamycin also induces Foxp3 in conventional T cells [79], and this induction is temporary. In addition, expanding these cells to sufficient numbers can be difficult.

The target cell dose and optimal timing for Treg infusions in the HSCT setting remain to be defined. Because Tregs must be isolated from the stem cell donor and require 2-3 weeks of in vitro expansion, cell production often may be too slow in patients with severe and rapidly progressive disease. Thus, to date the clinical implementation of Tregs as therapy for tolerance induction beyond the adult phase I setting has been limited by the lack of a rapidly available clinical-grade well-defined cell product and uncertainties regarding the activity and potency of the product in vivo.

Genetically Modified Hematopoietic Cells

T lymphocytes transduced with chimeric antigen receptors

The development of non–HLA-restricted chimeric antigen receptors (CARs) is a strategy for overcoming post-HSCT tumor immune evasion [80]. CAR technology involves the genetic reprogramming of T cells through artificial immune receptors that reproducibly and efficiently redirect the antigen specificity of polyclonal T lymphocytes toward target antigens expressed by tumor cells. When expressed by T cells, CARs mediate antigen recognition and tumor cytolysis in an MHC-unrestricted fashion and can target any molecule (protein, carbohydrates, or glycolipids) expressed on the surface of tumor cells, thus bypassing one of the major tumor escape mechanisms based on the down regulation of MHC molecules [81-83].

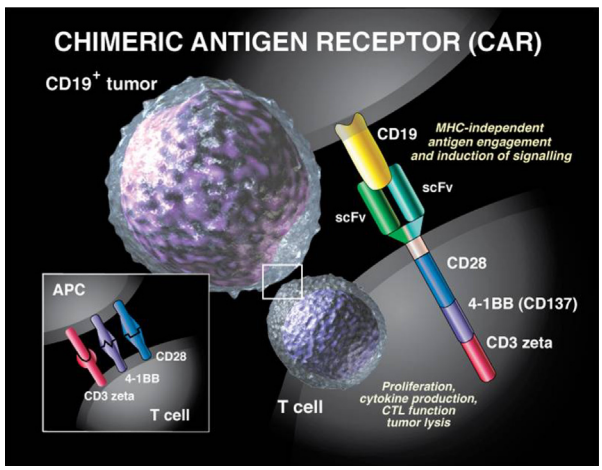

Figure 1. A CAR consists of a single chain variable fragment of an antibody (scFv) that recognizes a protein (such as CD19 on B cells and ALL cells) coupled to the CD3 ζ activation domain and costimulatory domains from CD28 and/or 4-1BB. This combines the MHC-independent recognition of a tumor antigen with the activating potential of T cell receptor signaling, allowing for redirection of T cells to cancer cells and extensive in vivo proliferation. © Sue Seif. Reproduced with permission.

CARs combine an extracellular specific antigen-binding moiety, usually a single-chain antibody (scFv), with intracellular signaling components (Figure 1). First-generation CARs provided only signal 1 (T cell activation) using a portion of the ζ-chain of the TCR/CD3 complex. An important early pediatric study targeted neuroblastoma with such a first-generation CAR [84].

The main problem with the approach using first-generation CARs was a lack of persistence and expansion of the T cells in vivo, possibly because full activation of T cells requires additional costimulatory signals. Accordingly, second- and third-generation CARs have been generated that add costimulatory domains, such as 41-BB and CD28. In both murine models and human studies, the addition of costimulation signals and cytokines promoting T cell expansion and/or survival has been shown to improve the antitumor efficiency of the engineered T cells and their survival in the tumor milieu [81,82,85,86]. Another strategy for optimizing the survival of genetically modified cells uses virus-specific CTLs transduced with the tumor-specific CAR [87,88]. Finally, the process by which manufactured T cells are ex vivo expanded also may play a key role in these cells' ability to expand and persist after infusion. Beads expressing anti-CD3 and anti-CD28 antibodies have been used to create cells using good manufacturing practices in recent trials at the University of Pennsylvania, Children's Hospital of Philadelphia, Baylor College of Medicine, Fred Hutchinson Cancer Research Center, Seattle, M.D. Anderson Cancer Center, the National Institutes of Health, and Memorial Sloan-Kettering Hospital, and these cells have demonstrated significant improvements over previous results in terms of expansion and efficacy. Moreover, bead-manufactured T cells have significantly greater in vivo proliferative capacity and longer telomeres than T cells expanded using OKT3 and IL-2, as shown in xenograft models [89].

Gamma retrovirus and lentiviruses have been used to transduce CARs into T lymphocytes for use in the clinical setting. These vectors efficiently infect T lymphocytes, integrate into the host genome, and produce robust expression of the gene in human T cells and their progeny [81-83,87,90,91]; however, limitations of these vectors include their genetic capacity and high manufacturing costs. The Sleeping Beauty (SB) transposon system has been proposed as an alternative gene transfer method, with the aim of reducing the time and costs of production and increasing the cargo capacity of the vector, thereby favoring the inclusion of multiple genes [92].

In terms of clinical efficacy, CAR-modified T cells with redirected specificity toward $CD19^+$ cells hold promise for immunotherapy in relapsed ALL. The initial results from the first trial in pediatric patients treated with CD19-CAR^+ T cells were published recently. Two children with relapsed/refractory ALL received T cells transduced with anti-CD19 CARs; in 1 case, the donor-origin T cells were obtained from the patient after she relapsed after allogeneic HSCT. Approximately 1 month after adoptive T cell transfer, both children achieved remission of leukemia, with minimal residual disease (MRD) levels $<0.01\%$. Both children had a dramatic expansion and persistence of CAR^+ T cells, with 33%-70% of T cells expressing CAR at peak expansion, although only the second patient received lymphodepleting chemotherapy before T cell infusion. The first patient has remained in complete remission (CR) more than 18 months after the CAR T cell infusion, and did not undergo allogeneic HSCT. In this patient clinical remission was associated with persistent molecular remission. The second patient relapsed approximately 2 months after treatment, with blast cells that no longer expressed CD19, emphasizing the emergence of a novel mechanism of tumor escape [91].

The foregoing findings demonstrate that vigorous in vivo expansion of CD19-CAR^+ T cells with persistent B cell depletion can result in sustained antileukemia activity in children with advanced ALL. More recently, Cruz et al. [93] reported on 8 patients given allogeneic (donor-derived) CD19.CAR-transduced virus-specific T cells between 3 months and 13 years after HSCT. No infusion-related toxicities were observed. The CD19.CAR-transduced virus-specific T cells persisted for a median of 8-9 months. Objective antitumor activity was evident in 2 of 6 patients with relapsed disease, whereas 2 patients who received cells while in remission remained disease-free at the time of the report [93].

In the era of highly active CAR-modified T cell therapy with the advent of second- and third-generation CARs, dramatic clinical responses have been accompanied by significant toxicities [94]. In particular, life-threatening, sometimes fatal, toxicities owing to the concomitant cytotoxic activity of CAR-T cells on normal tissues have been reported in clinical trials [95-97]. Another specific example of "off-tumor/on-target" toxicity occurs with the prolonged depletion of normal B cells occurring after the infusion of CD19 CARs, which causes agammaglobulinemia. A systemic inflammatory response syndrome or cytokine release syndrome (CRS), or cytokine storm, has been reported in the first two patients infused with CAR-transduced T cells [91]. Both pediatric patients experienced dramatic elevations in IL-6 and IFN-γ levels. One patient had severe CRS, accompanied by biochemical evidence of a macrophage activation syndrome (MAS), including a rise in serum ferritin level to 45,000 ng/dL and an elevated d-dimer level. Of note, both CRS and MAS improved dramatically after administration of the IL-6 receptor blocking agent tocilizumab, an approach that is becoming more widely adopted. More recently, in a study of pediatric patients with relapsed or refractory ALL at Children's Hospital of Philadelphia, 13 of 16 patients (81%) achieved CR at 1 month post-treatment [98]. Eleven of the 13 patients who achieved CR were also MRD-negative, with 2 showing $\leq 0.1\%$ MRD on flow cytometry. Although T cells collected from the 11 patients who had relapsed after allogeneic HSCT were of donor origin, no GVHD occurred, suggesting tolerization of the donor-derived T cells in the HSCT recipients. Were this to become a problem, the use of suicide genes in the construct used to transduce cells could reverse it. Levels of CRS vary among treated patients, ranging from mild to severe (ie, hypotension requiring intensive care). Four patients were treated with tocilizumab and demonstrated prompt resolution of MAS and CRS. The same pattern of IL-6 elevation and rapid response to tocilizumab has been reported with use of the bispecific T cell engaging antibody blinatumomab [99]. This points to CRS/MAS and the key role of IL-6 in mediating the toxicities of therapies that drive nonphysiological T cell activation.

In the allogeneic HSCT setting, a specific concern refers to the potential alloreactivity of CAR-transduced T cells. The experience at Children's Hospital of Philadelphia, with two-thirds of patients receiving allogeneic but tolerized T cells collected from the recipient without GVHD, demonstrates the potential efficacy of this approach to treat MRD or relapse in the post-HSCT setting. This has also been reported by the National Institutes of Health and M.D. Anderson Cancer Center groups [100].

Similar to CAR-transduced T cells, a transgenic approach introducing human and murine tumor antigen-specific T cell receptors in patient or donor T cells is currently being explored in preclinical studies [101,102]. To date, however, results from phase I/II studies in humans have been reported solely in adults.

CAR NK cells and CAR CIK cells

NK cells may be CAR-modified as well. Potential advantages of this approach are the lack of GVHD in the allogeneic HSCT setting and possibly a reduced risk of eliciting a CRS, as has been observed with the use of CAR T cells [91]. Disadvantages may include the limited numbers of ex vivo–generated NK cells, as well as MK cells' shorter half-life than T cells and no memory function. Imai et al. [103] reported a transduction efficacy of 43%-93% when using a retrovirus CD19.CAR construct to transduce ex vivo expanded NK cells. Although several preclinical studies have explored NK cell engineering with CD19.CAR and CD20.CAR constructs [104], other preclinical studies have used other targets, including GD2 [105,106], Her-2 neu [107], EpCam [108], EBNA [109], and NKG2D [110]. Two clinical studies exploring the use of NK CAR cells, one at St Jude Children's Research Hospital and the other at the National University Hospital in Singapore, are currently enrolling patients. Both of these studies involve administering genetically modified haploidentical NK cells transduced with CD19.CAR to patients with ALL [111,112].

Finally, recent preclinical studies exploring therapy with CAR-modified CIK cells (eg, targeting CD123 and CD33 expressed by AML) have provided evidence of both safety and antitumor efficacy in murine models [113]. The clinical safety and efficacy of CAR-modified CIK cells remains to be evaluated in phase I/II studies, however.

Dendritic Cells

Dendritic cells (DCs) are highly specialized antigen-presenting cells (APCs) that are essential to regulating the balance between beneficial and detrimental alloreactivity (graft-versus-leukemia and immune reconstitution versus GVHD). The flexibility and plasticity of DCs allow in vitro engineering to prepare DC therapy that favors immune regulatory or stimulatory responses, which may enable the prevention of infection and tumor relapse after HSCT without causing GVHD.

The majority of DC vaccination trials to date have been performed with patient-derived hematopoietic progenitor cells or monocytes that when cultured with the appropriate cytokine cocktails develop into DCs or monocyte-derived DCs in vitro. These trials have demonstrated that DC-based vaccines are safe and can elicit the expansion of circulating tumor-associated antigen-specific $CD4^+$ and $CD8^+$ cells [114]. The overall clinical efficacy of DC vaccination as a single treatment has been disappointing so far, however. Most of the clinical trials studying DC vaccination after HSCT have involved adult patients (eg, patients with myeloma, non-Hodgkin lymphoma, ALL, AML). Some clinical responses have been observed, with no evidence of GVHD [115-118]. Preventive CMV peptide-loaded DCs have been associated with the induction of specific T cell responses against CMV and the prevention of CMV reactivation [119]. At this time, phase I/II studies in pediatric patients (without HSCT) have shown that vaccination with immature or mature DCs is generally safe, although responses have been limited [120,121].

Mesenchymal Stromal Cells

Mesenchymal stromal cells (MSCs), first reported in 1968 by Friedenstein et al. [122], have the capacity for multilineage differentiation and are pivotal in regulating hematopoiesis in the hematopoietic stem cell niche. Along with their role in supporting hematopoiesis, MSCs have immunomodulatory properties, as has been shown in numerous in vitro and in vivo studies [123]. MSCs can be generated from various human tissues, including bone marrow, amniotic fluid, adipose tissue, and UCB, and these cells can be manufactured and expanded ex vivo and infused safely into patients. In the pediatric population, clinical experience with MSCs has been in patients with acute GVHD. In 2004, Le Blanc et al. [124] reported a seminal case of a 9-year-old child with steroid- and second-line therapy–refractory grade IV acute GVHD of the gut and liver who was treated successfully with 2×10^6/kg third-party bone marrow-derived MSCs. Building on this experience, the Developmental Committee of the European Group for Blood and Marrow Transplantation reported a multicenter experience in 55 adult and pediatric patients treated with MSCs for grade II-IV acute GVHD [125]. Bone marrow–derived MSCs were obtained mainly from third-party HLA-mismatched donors. Most patients had already received 1 or more second-line treatments, and thus there was a variable interval between the onset of GVHD to the initiation of MSC infusion. No infusion-related toxicities were reported. CR and partial remission were documented in 30 and 8 patients, respectively, resulting in an overall response rate of 69%. Overall survival (OS) at 2 years post-HSCT was higher in complete responders compared with partial responders and nonresponders (52% versus 16%; $P = .018$) [125]. Lucchini et al. [126] reported results of MSC treatment in 11 children with steroid-refractory GVHD, showing complete responses in 4 children. In a recent study, Ball et al. [127] reported the outcomes of 37 children with steroid-refractory grade III-IV acute GVHD treated with MSCs. The rate of CR was 65%, with a cumulative incidence of transplantation-related mortality of 17% in patients who achieved CR and 69% in those who did not achieve CR ($P = .001$). After a median follow-up of 2.9 years, the OS was 37%; however, OS was 65% in the patients who achieved CR, compared with 0% in those who did not achieve CR ($P = .001$). Furthermore, in a combined adult/pediatric study including 15 children with grade II-IV acute GVHD treated with a median of 3 MSC infusions, Introna et al. [128] reported an overall response rate of 67% and a CR rate of 27%.

Several groups have reported outcome in studies using the Prochymal MSC product (Osiris Therapeutics, Columbia MD). Prasad et al. [129] reported on 12 children with grade III-IV steroid-refractory and second-line agent–refractory gastrointestinal GVHD who received sequential MSC infusions ($2\text{-}8 \times 10^6$/kg) on a compassionate need basis. The overall response rate was 75%. Two-year survival was 40% for the whole cohort and 68% for the 7 patients with CR. In a large placebo-controlled randomized study in both adults and children, the Prochymal product was studied in combination with different agents for patients with steroid-refractory acute GVHD grade II-IV. Although the final analysis has not been published, the addition of Prochymal did not appear to improve CR rates in this cohort [130].

Despite these encouraging results and the apparent safety of MSC therapy, the heterogeneity of the patient population and limited size of studies reported to date precludes any firm conclusions regarding the efficacy of MSCs in

steroid-refractory GVHD. Thus, evaluation in well-designed, randomized controlled trials is critical. In particular, these studies should address the timing, dosing and frequency of MSC administration. Optimally monitored, randomized controlled studies in children will be critcially important for providing convincing evidence of the therapeutic potential of MSC products for treating of acute GVHD.

HARMONIZING CLINICAL TRIAL DESIGN FOR CELL THERAPY STUDIES IN PEDIATRICS TO MOVE BEYOND PHASE I

For complex biological therapies to become the standard of care for pediatric patients after HSCT, these studies must first move beyond phase I. The previous phase I/II trials using these novel cellular therapies generally have different endpoints and definitions of response, making comparisons difficult and sometimes impossible. In the past, many early-phase trials were designed to identify the maximal tolerated dose and to address safety issue; however, now it is critical to use such studies to simultaneously evaluate the proof of concept, as well as to demonstrate that the strategy will have broad applications. In this context, harmonizing clinical trial designs using novel cell therapies for specific diseases may be better compared with one another, realizing of course that a direct comparison in phase III is superior. Organizing well-prepared consensus meetings is critical to the harmonization of clinical trial design. At such meetings, achieving a consensus is critical for at least the following topics:

- Inclusion/eligibility criteria in phase I/II studies
- Disease/complication-specific readouts in phase I/II, for example, MRD measurements in leukemia patients at standard time points and biomarker response after MSC treatment for GVHD
- Standardization of the primary and secondary endpoints, including time points
- Standardized monitoring of clinical and immunologic markers (humoral, cellular, and functional), preferably in accredited quality-controlled laboratories
- Biobanking of samples (eg, plasma, cells, tissue, bone marrow, DNA) at defined time points.

Given the importance of cell expansion and in vivo persistence, these key outcome measures need to be implemented whenever feasible.

To reach a consensus on harmonizing the clinical trial design, setting up a technical platform within the Westhafen Intercontinental Group may be of additional value with regards to state-of-the-art biomonitoring to ensure quality control among laboratories. Furthermore, the fact that novel cell therapy trials are often first performed in adults should be taken into account. Thus, it is important for international pediatric groups to work with regulatory agencies to advocate the early use of these therapies in children. Consensus on a harmonized clinical trial design may make it easier for research groups to obtain ethical approval for specific trial designs, which ultimately can be used in similar trials worldwide. The role of the Westhafen Intercontinental Group will be to plan and prepare these meetings for the international pediatric community, and to discuss and publish their consensus recommendations. The goal is to conduct multicenter, multinational cell therapy trials in the pediatric population so that ultimately such therapies will become standard of care for our patients after HSCT.

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