

## REVIEW

# Adoptive cellular immunotherapy for childhood malignancies

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**Clinical trials have established that T cells have the ability to prevent and treat pathogens and tumors. This is perhaps best exemplified by engraftment of allogeneic T cells in the context of hematopoietic stem-cell transplantation (HSCT), which for over the last 50 years remains one of the best and most robust examples of cell-based therapies for the treatment of hematologic malignancies. Yet, the approach to infuse T cells for treatment of cancer, in general, and pediatric tumors, in particular, generally remains on the sidelines of cancer therapy. This review outlines the current state-of-the-art and provides a rationale for undertaking adoptive immunotherapy trials with emphasis on childhood malignancies.**

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## Introduction

The infusion of antigen-specific T cells for the child with cancer is particularly appealing as cellular immunotherapy offers an approach to treating malignancies that avoids the distressing long-term toxicities associated with cytotoxic conventional chemotherapy and radiation therapy.<sup>1–3</sup> Reconstituting or augmenting cellular immunity through the infusion of T cells remains the pursuit of academic centers, and unlike the development of monoclonal antibodies for immunotherapy, there is scant interest from major biopharmaceutical companies toward the development and commercialization of cellular therapies. Thus, due to the economics and regulations associated with conceiving and executing adoptive immunotherapy, clinical advances chiefly depend on small-scale, single-center, investigator-initiated adoptive immunotherapy studies. As a consequence, immunotherapists developing targeted approaches for childhood malignancies must rely upon extrapolating data from studies enrolling adult patients and upon the clinical experience of adoptive

immunotherapy for opportunistic diseases. This monograph will help the reader review these immunotherapy data to frame the issues regarding T-cell therapy for childhood malignancies, but to complete the picture, the reader is encouraged to read additional recent reviews.<sup>4–9</sup>

Adoptive immunotherapy, derived from the term ‘adoptive immunity’,<sup>10</sup> is typically taken to involve the infusion of lymphocytes or antibodies. T cells are the most common lymphocytes infused, but some protocols use natural killer cells.<sup>11</sup> Furthermore, as defined T-cell subsets can be transferred investigators have used adoptive immunotherapy to not just enhance an effect, but to suppress or prevent an immune response. The first clinical trials infusing T cells, building on animal experimentation in the 1950s<sup>12,13</sup> coincided with the declaration of the ‘war on cancer’ in the 1970s. One of the earliest successful adoptive immunotherapy experiences involved children, as the first patient to receive unprimed donor lymphocyte infusion for a hematologic malignancy in relapse after HSCT was a boy with refractory ALL who obtained and sustained a complete remission after receiving multiple transfusions of lymphocytes from his sister. Indeed, the field of adoptive immunotherapy has long been intertwined with the practice of HSCT. For, HSCT is not just a platform for delivery of radiation and drugs, but is the most successful example of immunotherapy for malignancies, including many pediatric cancers. Practitioners of HSCT well understand the practicalities and challenges of suppressing recipient T-cell function to obtain successful engraftment and infusing donor-derived hematopoietic stem cells (HSC) to restore desired T-cell effects. Since the morbidity and mortality of allogeneic HSCT due to opportunistic infection and relapse appear to be solvable with infusions of donor-derived T cells, many centers that practice HSCT now provide cell-based therapies beyond the infusion of HSC. In the sections to follow, I highlight some of the general advances in the field of adoptive immunotherapy and apply the principles learned to the use of T cells to treat childhood malignancies.

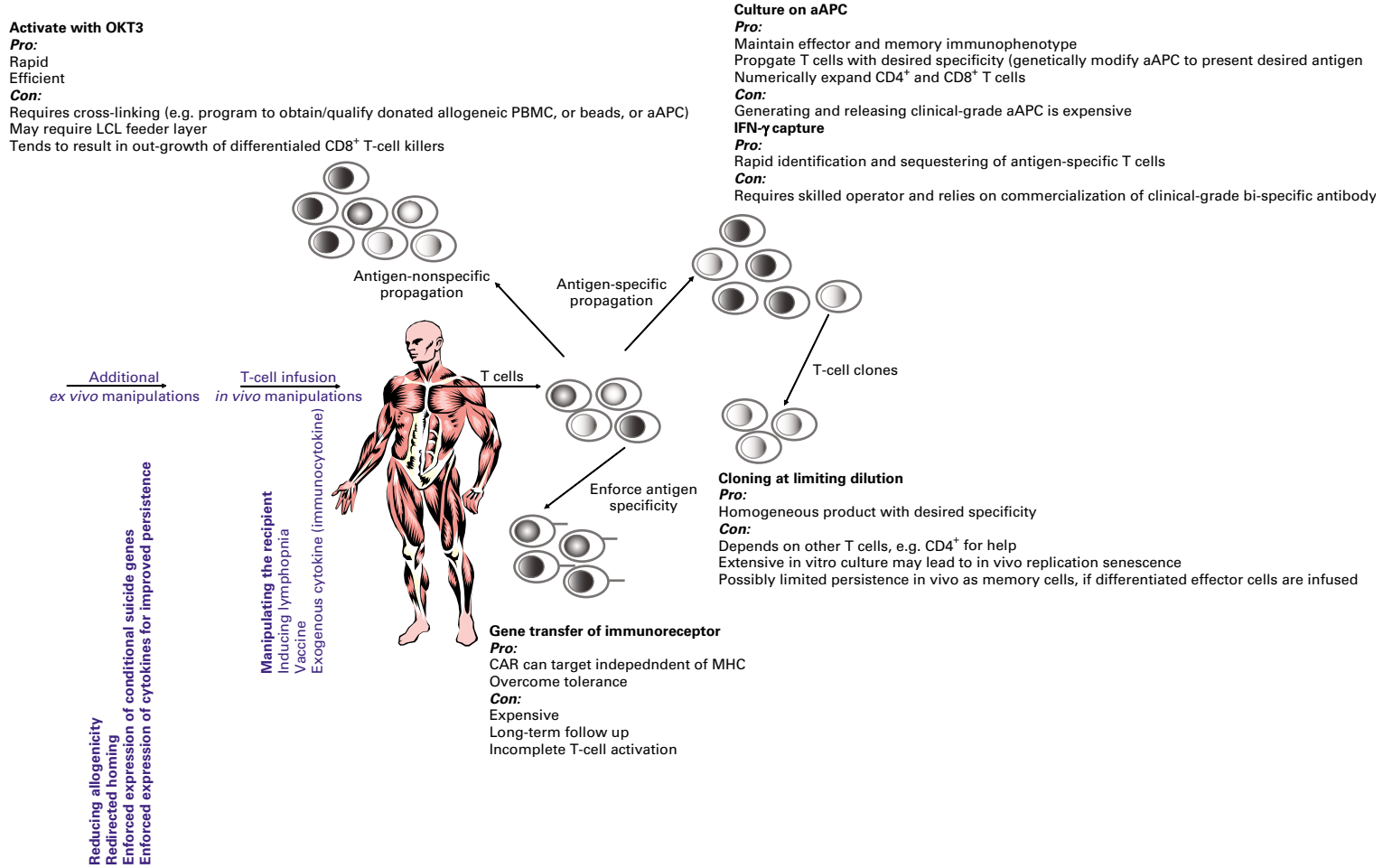
## Adoptive cellular immunotherapy in general

There are typically three approaches that can be undertaken to generate clinical-grade T cells to specifically augment a child’s cellular immune response for a

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**Figure 1** Schematic of *ex vivo* manipulations that can be undertaken on harvested clinical-grade T cells to improve the therapeutic effect (and reduce toxicity) before reinfusion. These include (i) sustained propagation (antigen-dependent and antigen-independent) to achieve clinically meaningful numbers, (ii) defining antigen-specificity (for example, by gene transfer of immunoreceptor transgene), (iii) defining the T-cell product (for example, selection of T-cell subsets prior to propagation and cloning) and (iv) manipulation of tissue culture conditions (for example, reducing culture time to achieve minimal manipulation, addition of cytokines).

malignancy: (i) identifying and isolating autologous or allogeneic antigen-specific T cells to homogeneity or near-homogeneity, (ii) infusion of polyclonal populations which include desired subpopulations of antigen-specific T cells, and (iii) genetic modification to render T cells specific for a specific antigen. These approaches are conceptualized in Figure 1, and examples are provided for each of these methods with their associated pros and cons which reflect the tumor type and the practicality of generating desired T cells. For example, the generation of cloned T cells from healthy subjects, with uniform expression of an immunoreceptor of defined specificity, typically requires prolonged *ex vivo* time in culture which can skew T-cell differentiation, typically toward CD8<sup>+</sup> effector cells, with limited *in vivo* survival in the absence of help, and may render these cells sensitive to replicative senescence. Indeed, the recognition that therapeutic efficacy is dependent not just on presence of T cells with increased cytotoxic profiles, but on the persistence of tumor-specific cells *in vivo*, has prompted investigators to design platforms to infuse populations of cells with long-lived potential by including memory T cells with their increased proliferative potential. This in turn puts the emphasis on developing manufacturing schemes to infuse minimally manipulated T cells to avoid terminal differentiation of most, if not all, of the cells in the inoculum into perforin/granzyme<sup>+</sup> effectors.

#### Adoptive cellular immunotherapy for CMV

Immunotherapy for opportunistic infections can be used as a template for designing immunotherapy for childhood malignancies. The CMV-specific T-cell clones infused in the seminal work by Riddell and Greenberg were generated by numerically expanding viral specific T cells, identified through an initial co-culture period on CMV-infected autologous fibroblasts.<sup>14,15</sup> This technology resulted in the successful prevention of opportunistic CMV infection after allogeneic HSCT and provides the following two points for treatment of childhood malignancies. (i) Donor-derived T-cell clones recognizing immunogenic structural virion proteins were sufficient to safely prevent CMV-disease without the apparent emergence of escape variants. (However, as we shall see later in this review, the application of a homogeneous population of T cells for treatment of tumors may not prevent the emergence of antigen-negative tumor-escape variants.) (ii) Long-lived survival of the adoptively transferred CD8<sup>+</sup> CMV-specific T cells, when extensively expanded *ex vivo*, are dependent on the development of a concomitant helper response from endogenous CD4<sup>+</sup> CMV-specific T cells. Indeed, subsequent clinical data have demonstrated that infusion of CD4<sup>+</sup> as well as CD8<sup>+</sup> CMV-specific polyclonal T-cell lines (generated from short-term culture) can provide improved immune reconstitution.<sup>16</sup> Since the oncology patient may not be capable of generating an endogenous CD4<sup>+</sup> helper response to tumor, these data imply that infused tumor-specific T cells should contain CD4<sup>+</sup> tumor-specific T cells, or that a helper response be applied in the form of exogenous IL-2.

#### Adoptive cellular immunotherapy for EBV

The infusion of T cells for prevention and treatment of EBV-related diseases is one of the most successful examples of adoptive immunotherapy for malignancies. And, since many of the leaders of EBV-specific T-cell therapy practice in hospitals caring for children with cancer, much of the clinical work has been performed in the pediatric population. The ability to generate EBV-specific T cells in response to need was made possible by the recognition that EBV-transformed autologous B cells (lymphoblastoid cell line (LCL)) could be lethally  $\gamma$ -irradiated and used *in vitro* as stimulator cells to generate virus-specific cytotoxic T cells to recognize the immunogenic latency III antigens (EBNA-3A, 3B and 3C proteins) presented *in vivo* on the lymphoproliferative disease (LPD) of EBV-infected B cells. The concept of recursive stimulation of T cells with autologous LCL resulted in many centers undertaking infusions of EBV-specific T cells in a variety of iatrogenic clinical settings in which recipients are at risk for opportunistic EBV-disease, such as T-cell depleted allogeneic HSCT or chronic immunosuppression for engraftment of solid organs. The trial data demonstrate that prophylactic infusions of these viral-specific T cells can prevent clinically-significant EBV infection as well as treat bulky post transplant LPD/lymphoma, EBV-associated NK-cell malignancies (angiocentric lymphomas), and HIV-associated lymphomas.<sup>17–25</sup> The implications of clinical trials infusing EBV-specific T cells for adoptive immunotherapy of pediatric neoplasms are potentially far-reaching and five sequelae are described. (i) Bulk (nonclonal) populations of allogeneic T cells, enriched for specificity for EBV, can be safely infused without causing GVHD. (ii) Infused gene-marked EBV-specific T cells are capable of long-lived functional immune responses at least a year after infusion.<sup>26,27</sup> (iii) LCL, when considered as an artificial aAPC, provided the ground work for other immortalized cells, not expressing immuno-dominant EBV antigens, to be used to present tumor antigens in tissue culture. For example, bead-derived or cell-based aAPC with enforced expression of desired HLA molecules have been used to expand tumor-specific T cells.<sup>28–33</sup> As an alternative source of APC, investigators have also used T cells, which can be nonspecifically propagated *ex vivo* to vast numbers by cross-linking CD3 with OKT3, as a source of antigen present cells (T-APC).<sup>34–37</sup> (iv) The safe and efficacious infusions of EBV-specific T cells for LPD has facilitated the targeting of other EBV-derived antigens associated with Hodgkin's disease (HD), and nasopharyngeal carcinoma (type II EBV malignancies) with investigators developing technologies to generate T cells with specificity for latency membrane protein (LMP) 1, LMP2 and EBNA-1.<sup>38–46</sup> (v) Since the generation of EBV-specific T cells on LCL requires weeks of cell-culture time, investigators have recently developed a paradigm for the *a priori* manufacture of HLA-restricted EBV-specific T cells. Early clinical experience indicates that despite HLA-disparity, these pre-prepared allogeneic T lymphocytes can be immediately, safely and effectively infused.<sup>47–49</sup> This approach is among the first examples of using T cells as an 'off-the-shelf' reagent.

### Adoptive cellular immunotherapy for adenovirus

Adenoviral infections are significant causes of morbidity and mortality after HSCT, and especially after T-depleted HSCT. The recent data regarding T-cell therapy for prevention/treatment of this viral infection are notable for two observations with implications for T-cell therapy of pediatric malignancies. (i) Two types of APC, monocytes and genetically modified LCL, have been sequentially combined to sculpt the immune response *in vitro* to generate populations of T cells with multiple specificities (adenovirus, CMV and EBV).<sup>50,51</sup> This has implications for developing populations of tumor-specific T cells with multiple specificities to reduce the risk of inducing antigen-escape variants after adoptive transfer. (ii) The second approach uses paramagnetic beads to isolate adenoviral-specific T cells based upon Miltenyi Biotec's  $\gamma$ -interferon secretion and capture assay.<sup>52,53</sup> The adenoviral-specific T cells are stimulated with adenoviral antigen and 'captured' using commercially available bivalent reagents that cross-link CD45 on T cells and secreted  $\gamma$ -interferon (IFN). The advantage of this technology for tumor therapy is the ability to rapidly identify potentially heterogeneous populations of antigen-specific T cells.<sup>54</sup> By analogy, 'capture' protocols might be developed using stimulator cells expressing pediatric tumor antigens to identify responding tumor-specific T cells that secrete  $\gamma$ -IFN upon co-culture.

### Adoptive cellular immunotherapy for *Aspergillus*

A recent report by Velardi and co-workers describes the adoptive transfer of donor-derived *Aspergillus*-specific T cells after haplo-identical HSCT.<sup>55</sup> This approach has implications for pediatric immunotherapy as the investigators selected T-cell clones with desired specificity and rejected clones with unwanted alloreactivity. The clones were then pooled and safely infused. These data have implications for reducing alloreactivity in the context of infusing donor-derived tumor-specific T cells for pediatric malignancies.

### Adoptive cellular immunotherapy using allodepleted T cells

Investigators have also developed technologies for reducing undesired alloreactive T cells in bulk-populations of cells and these approaches impact the design of pediatric immunotherapy in the allogeneic setting. For example, Brenner and co-workers used a CD25-specific immunotoxin to deplete alloreactive lymphocytes prior to infusion.<sup>56</sup> An alternative approach is to induce energy by stimulating donor-derived T cells with stimulator cells expressing recipient HLA antigens in the presence of costimulatory blockade.<sup>57</sup> Another method to reduce the potential for GVHD from infused polyclonal populations of T cells is to use veto cells to induce tolerance *ex vivo* prior to infusion.<sup>58</sup>

### Adoptive cellular immunotherapy using NK cells

NK cells were described in 1975 as cells that 'spontaneously' lyse tumor cells.<sup>59,60</sup> However, it was the inability

to lyse certain tumors that resulted in the 'missing self' hypothesis that explained, in part, the NK cells innate function to kill in response to the absence of MHC class I.<sup>61</sup> Understanding that the ability of NK cells to kill is the aggregate of cell-surface activation and inhibitory receptors binding to tumor MHC and non-MHC molecules, allowed investigators to use the genetics of haploidentical HSCT to preferentially activate donor-derived NK cells in recipients.<sup>62</sup> This results in desired NK-cell inhibitory receptor-blockade or receptor activation leading to improved survival in patients with AML undergoing T-cell depleted haplo-identical HSCT. The donor killer Ig-like receptors (KIR) recipient-HLA mismatch allows engrafted donor-derived NK cells to kill tumor cells and host dendritic cells resulting in reduced relapsed and GVHD, respectively.<sup>63,64</sup> Miller *et al.*<sup>65</sup> have extended this observation by infusing haploidentical NK cells after lymphodepleting chemotherapy in patients with refractory AML. This study demonstrated a complete response in 5 of 19 patients with minimal toxicity, with 4 of the 5 responding patients KIR ligand mismatched. These encouraging data need to be followed-up for the treatment of pediatric malignancies.<sup>66</sup> NKT cells are immunoregulatory cells which recognize glycolipids presented in the context of the MHC class I-like CD1d molecule resulting in secretion of anti- and pro-inflammatory cytokines altering innate and acquired immune responses. NKT cells are appreciated for their ability to regulate autoimmunity and tumor surveillance and a role for selectively activating NKT *in vivo* is beginning to be explored.<sup>67,68</sup>

### Adoptive cellular immunotherapy for melanoma

While melanoma is generally not a pediatric malignancy, the infusions of tumor-specific T cells has resulted in seven important principles that can be generalized for the field of adoptive immunotherapy for pediatric tumors. (i) The first observation is that autologous tumor-specific T cells can be identified in patients with cancer.<sup>69</sup> However, other less immunogenic tumors may not result in circulation of T cells with specificity of pediatric tumor-associated antigens (TAA). (ii) Infusion of TAA-specific T cells can result in desired antitumor effects as well as unwanted autoimmunity and damage to normal tissues.<sup>70</sup> (iii) Persistence of *ex vivo* propagated CD8<sup>+</sup> T cells required help in the form of exogenous IL-2.<sup>71</sup> (iv) Infusion of homogeneous populations of T cells with one defined specificity (for example, to MART-1) may lead to emergence of tumor-escape MART-1<sup>neg</sup> variants. (v) Adoptive transfer of T cells after lymphodepleting chemotherapy can lead to the desired homeostatic expansion of infused T cells.<sup>72-74</sup> (vi) Infusion of polyclonal populations of tumor-specific T cells, in contrast to clones,<sup>75</sup> resulted in the *in vivo* outgrowth of subsets of T cells with memory phenotype and propensity to be long-lived.<sup>76</sup> (vii) T cells can be genetically modified to express melanoma-specific TCR to obtain a therapeutic response.<sup>77</sup> This is one of the signature examples of a therapeutic success of gene therapy and supports the rationale for using gene transfer to render T cells specific for a desired antigen.

## Adoptive cellular immunotherapy using genetically modified T cells

Two major hurdles for the clinical application of T-cell immunotherapy for a few pathogens and tumors have been solved, namely, the ability to safely and reliably numerically expand primary T cells and produce T cells with desired specificity. However, for most tumors, and especially pediatric malignancies, immunologic tolerance, and modulation of tumor MHC and costimulatory molecule expression, prevents identifying T cells with desired antigen specificity. Therefore, we and others have introduced a chimeric antigen receptor (CAR) with specificity for desired antigens. The CAR, described 20 years ago,<sup>78</sup> is typically a fusion molecule combining the scFv, or antigen-recognition domain, of an antibody with a T-cell activation endodomain, most often derived from CD3- $\zeta$ . Clinical studies using genetically modified T cells expressing CAR to redirect specificity have been reported, or are underway.<sup>79–83</sup> Indeed, in a trial I helped initiate at the City of Hope, patients with relapsed follicular lymphoma are receiving autologous T cells with specificity for human CD19, an antigen broadly expressed on B-lineage malignancies.<sup>84</sup> These trials using genetically modified T cells are now facilitated in their design and execution by the development of lentiviral vectors<sup>85</sup> and advanced nonviral gene transfer strategies with improved integration frequencies, such as using *Sleeping Beauty* transposition,<sup>86,87</sup> and the availability of immortalized aAPC expressing tumor antigens.<sup>88,89</sup> Recently, there have been a number of important developments using the CAR technology with implications for childhood malignancies, such as the development of CARs with specificity for HER2<sup>+</sup> medulloblastoma,<sup>90</sup> CD30<sup>+</sup> HD<sup>91</sup> and CD171<sup>+</sup> neuroblastoma.<sup>92,93</sup> The ability to redirect specificity has also extended to the transfer of pairs of  $\alpha\beta$  TCR to confer specificity in the context of HLA<sup>94–100</sup> and new approaches are available to limit the inappropriate mis-pairing of introduced TCR chains with endogenous TCR chains so minimizing the expression of aberrant TCR that have not been selected in the thymus and thus have potential autoreactivity.<sup>101–104</sup>

The genetic manipulation of T cells with implications for tumor therapy is not limited to redirecting specificity, for transgenes can be expressed to improve persistence of the T cells after infusion. For example, to increase the proliferative potential (i) the CAR endodomain can be systematically altered to provide a fully competent activation signal,<sup>105–111</sup> or (ii) T cells can be modified to enforce expression of stimulatory cytokine transgenes<sup>112–116</sup> or (iii) the CAR can be introduced into T cells which can respond/proliferate via endogenous TCR to defined (often viral) antigens.<sup>117–119</sup> Recognizing that tumor-specific T cells may not traffic to sites of disease, cells can be genetically modified to express desired homing receptors.<sup>120</sup> Gene transfer can also be undertaken to render T cells resistant to the anti-inflammatory tumor micro-environment, such as by expressing a dominant-negative TGF- $\beta$  receptor<sup>121,122</sup> or decreasing the sensitivity of the adoptively-transferred T cells to Fas-induced apoptosis.<sup>123</sup> Finally, given the potential for gene transfer to cause unwanted genotoxicity, investigators have included suicide genes<sup>124–132</sup> which

conditionally render the cells sensitive to ablation in the event of an adverse event such as GVHD.<sup>133–136</sup>

## Adoptive cellular immunotherapy in the future

There are a number of challenges to broadening the application of adoptive cellular immunotherapy for childhood cancers. The therapeutic effect of infused tumor-specific T cells for pediatric malignancies will depend on the persistence of the adoptively transferred cells, their ability to home, and their ability to operate and repetitively kill within the tumor microenvironment. The marriage of T-cell therapy with gene therapy offers a potential solution for many of these problems. As viral-based and nonviral gene transfer strategies gain widespread acceptance and improve their safety profile, so we will see the advent of clinical trials of T cells expressing one or more transgenes. As other single-agent, immunotherapies are applied (such as vaccines, mAbs, cytokines other than IL-2, and immunocytokines) pediatric immunotherapists will be able to undertake trials infusing combinations of one or more of these immunotherapeutics. There are already several proofs-of-principal for combination immunotherapies beyond merging T cells with chemotherapy and T cells with IL-2. For example, we have recently demonstrated that CD19-specific T cells can be combined with CD20-specific immunocytokine to improve *in vivo* T-cell persistence and antitumor effect.<sup>137</sup> Nadler and co-workers have combined vaccine and T cells.<sup>138</sup> June and co-workers have combined vaccine, *ex vivo* T-cell propagation and infusion, with autologous HSCT to enrich the recipient with desired antigen-specific autologous T cells.<sup>139</sup> However, even with the advent of combinatorial therapies and development of genetic tools, two significant hurdles remain to be overcome, (i) broadening the knowledge-base and (ii) reducing the cost of manufacturing clinical-grade T cells, in other words operating in compliance with current good manufacturing practices.<sup>140</sup> Thus, training programs, and especially Pediatric Fellowships, will not only need to educate the next generation of oncologists who can develop new immunotherapy ideas, but also these programs will need to teach trainees how to put these ideas into practice.

## Adoptive cellular immunotherapy is the future

New therapies are urgently needed for pediatric cancers which are refractory to conventional chemotherapy and radiation therapy and for upfront treatment of malignancies so that cytotoxic agents can be reduced in intensity or eliminated. Indeed, long-term follow-up studies for pediatric cancer survivors clearly demonstrate that we have reached a point of diminishing return for the application of cytotoxic agents that eliminate pediatric neoplasms by generically damaging DNA replication. However, biopharmaceutical companies are unlikely to develop new-targeted biological agents for 'orphan' diseases, such as pediatric cancer, due to the unfavorable economic return on their investments. Yet, this is not all bad news, as the lack of company involvement widens the academic investigator's

freedom to operate, as T-cell trials can be undertaken in nonprofit medical centers outside the purview of biopharmaceuticals and their concern about protecting market share and investments; a practice which sadly often leads to the exclusion of children from industry-sponsored trials.

In the era of personalized medicine, T cells offer unsurpassed potency, specificity and long biologic half-lives. To realize the full potential of T-cell therapies, we need to breakdown the barriers to undertake multi-institution efficacy trials and to fully invest in applying this technology in children. However, while there are scientific hurdles to be overcome, perhaps the most immediate rate-limiting step for the widespread practice of adoptive cellular immunotherapy for childhood malignancies is adequately financing clinical trials and reducing regulatory burdens.

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