Report from PACT: Moving successful virus-specific T-cell therapy for hematopoietic stem cell recipients to late phase clinical trials

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Introduction
Viral infections are the cause of about 30% of transplant-related deaths in recipients of T-cell depleted, allogeneic hematopoietic stem cell transplants (HSCT).\(^1\) Epstein-Barr virus (EBV), cytomegalovirus (CMV) and adenoviruses (Ads) are the most common culprits, but other common viruses like parainfluenza virus, respiratory syncytial virus, influenza viruses, polyomaviruses and human herpesvirus 6 together contribute significant morbidity and mortality.\(^2,3,4\) Small molecule therapies are often ineffectual, always costly and frequently produce significant adverse effects.

Virus-specific T cells derived from stem cell donors can prevent and treat post transplant viral infections, in the recipients for whom they were intended, and also in partially HLA-matched, third party recipients.\(^5,7\) The low toxicity and long term protection provided by virus-specific T cells compares favorably with the significant toxicities and short-term effects of most antivirals.\(^5,6,8,10\) Is it time therefore to begin the transfer of T-cell manufacturing from academic phase I/II clinical trials into hospital or industry supported facilities so that virus-specific T cells can be made available to all high-risk HSCT recipients?

Several barriers prevent the broader use of virus-specific T-cell therapies after stem cell transplantation: While T-cell therapies for EBV, CMV and Ads have clearly demonstrated their safety and efficacy both as prophylaxis and as therapy, for many other viruses the antigens that induce protective T cells have yet to be identified. Moreover these infections may occur in less than 5% of patients making it difficult to perform the rigorous comparative effectiveness studies that will be required to show lower overall cost, fewer adverse effects and equivalent or superior efficacy. Before any of these barriers can be breached effectively by the academic institutions who are the major developers of these T-cell therapeutics, we must select and optimize manufacturing strategies that are robust and scalable and have the lowest possible cost. This article will largely deal with selection and optimization of manufacturing strategies.

Choice of protective viral antigens. The mere existence of circulating virus-specific T cells does not mean they are protective, since viral antigens may be cross-presented by professional antigen-presenting cells (APCs) even when absent or ineffectively presented by the infected cells themselves. Protective antigens are often virion proteins, like hexon and penton of adenovirus and pp65 of CMV, or immediate early proteins that are presented by newly infected cells before they produce infectious virus or express their ubiquitous immune evasion genes.\(^5,11-13\) T cells specific for immediate early antigens, like CMV-IE, should also eliminate cells in which viruses reactivate from latency. For EBV, T cells must also recognize and kill proliferating B cells expressing latent cycle proteins. EBV transformed B-cell line (EBV-LCL)-activated T cells are protective but recognize a broad range of latent and early lytic cycle proteins, which tend to
vary depending on the donor’s haplotype, and single antigens that provide protective immunity are yet to be identified. Our group is testing T cells specific for the latency proteins, EBNA1 and LMP2, and the immediate early ZEBRA protein, for their ability to protect against EBV. For other viruses, protective antigens may be predicted, and tested in animal models if available, but they can be validated only in human clinical trials.

**Problems with current manufacturing strategies.** Several strategies for the manufacture of virus-specific T cells have been described. The first studies used T cells that had been activated and expanded in vitro to remove alloreactive T cells that could cause graft-versus-host disease, while increasing virus-specific T-cell number and effector functions to increase their speed of action. This T-cell activation and expansion used cells infected with live viruses, namely CMV-infected fibroblasts and EBV-LCLs as APCs. Subsequently, EBV-LCLs were used as APCs to expand T cells specific for other viruses, since LCLs can be made from almost any donor, are available in unlimited numbers and can be transduced to express and present heterologous viral antigens such as CMV-pp65. This approach, while effective, introduced undesirable pathogens (EBV, CMV) into the manufacturing process, which itself was extremely prolonged. The production time for EBV-LCLs is about 6 weeks, while the CTLs required an additional 4 weeks of culture followed by 2 weeks for quality control (QC) testing. This timing is feasible for prophylaxis of viral infections, but inappropriate for reactive therapy. Finally, the approach was hard to scale; CTLs needed to be grown in 24 well plates, which introduces a risk of contamination and does not satisfy GMP requirements. Fortunately, strategies to remove live virus and viral vectors, shorten manufacturing time and scale the culture conditions are now available.

**Reducing the time for T-cell manufacture and eliminating viral vectors.** Investigators have extracted antigen-specific T cells directly from blood using HLA-peptide streptamers, or by capturing cells that secrete γ-IFN in response to overnight antigen stimulation (γ-catch). Streptamers select T cells that recognize peptide epitopes in association with specific HLA alleles, an approach that is limited by their availability for a small number of HLA class I alleles. The gamma-capture technique is more universal and can capture both CD4+ and CD8+ T cells specific for multiple epitopes in any antigen, regardless of HLA phenotype. Both techniques are rapid and clinically effective, since small numbers of T cells undergo massive expansion after infusion and can reconstitute immunity to CMV, adenoviruses and EBV. A phase 2 trial of streptamer selected CMV-specific T cells (Cytovir-CMV) is currently sponsored by Cell Medica UK. However, their broader use may be limited by the large blood volumes required to select sufficient numbers of virus-specific T cells from unrelated donors, and by the low numbers of circulating T cells specific for non-persistent viruses.

**Rapid expansion of virus-specific T cells.** Virus-specific memory T cells undergo 2 to 3 logs of expansion after a single in vitro stimulation. Thus as little as 20 mLs of blood containing 10^5 virus-specific T cells could yield 10^7 or 10^8 virus-specific T cells after 8 to 10 days, given appropriate stimulation and the right culture conditions. This expansion has been achieved by stimulation of peripheral blood mononuclear cells with autologous dendritic cells nucleofected with plasmids expressing antigens for four different viruses (CMV, EBV, BK, and Adv), and expansion in highly scalable (from 10 to 100 cm^2) gas-permeable, Wilson-Wolf G-Rex rapid expansion devices. Sufficient virus-specific non-alloreactive T cells could be infused within 10 days of culture initiation. This strategy is currently under clinical evaluation. These small volumes of blood could readily be stored from stem cell donors at the time of transplant and T cells could be initiated prophylactically or at the first indication of virus reactivation or infection.
Off the shelf virus-specific T cells. Haque and colleagues showed that partly HLA-matched third party EBV-specific T cells produced a 65% response rate in PTLD after solid organ transplantation, with a 42% complete response rate.\textsuperscript{23-25} Similarly, O’Reilly and colleagues have used partially HLA-matched EBV-targeted T cells as treatment for EBV lymphoproliferative disease in allogeneic HSCT recipients and achieved complete or partial remissions in 68%.\textsuperscript{26,27} In an NHLBI-Production Assistance for Cellular Therapies (PACT)-sponsored study, we used banks of third party T cells specific for EBV, CMV and Ads in HSCT recipients and observed high (>70%) response rates for all three viruses, which were achieved even when only a single HLA antigen was matched between virus-specific line and third party recipient. Hence, these cells provide a stand alone off the shelf product that may also serve to gain time for the rapid manufacture of a patient-specific product.

Summary. There are now multiple effective strategies for the activation and expansion, or direct selection of virus-specific T cells. The stage is set for their evaluation in large-scale clinical trials.

Conflict of interest statement: Cliona M. Rooney is on the scientific advisory board of Cell Medica.

Reference List


clinical applications using gas-permeable rapid expansion cultureware (G-Rex). 

J.Vis.Exp. 2011;(51).


