

PepMix™ Peptide Pools for Clinical Applications: T Cell Therapy for Viral Infections after Hematopoietic Stem Cell Transplant

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Overlapping peptide pools that encompass the entire sequence of an antigen (PepMix™) can effectively reactivate polyclonal, CD4+ and CD8+ T cells directed against one or a combination of target antigens. We plan to use peptide pool-activated, CD4+ and CD8+ T cells specific for a spectrum of viral antigens to prevent viral infections in stem cell recipients in an FDA-approved clinical trial.

Introduction

Antigen-specific T cells are effective for the treatment of viral infections in the hematopoietic stem cell transplant (HSCT) setting. The ideal antigen-specific T cell population should consist of CD4+ and CD8+ T cells, to provide both helper and cytotoxic effector functions, and should target a broad spectrum of epitopes from a variety of antigens expressed by the target virus to minimize viral immune escape. The activation and expansion of such broad spectrum antigen-specific T cells requires that a whole antigen source, presented by autologous antigen presenting cells (APCs), be used as a stimulus in order to efficiently activate T cell populations of the desired profile from all individuals, irrespective of their HLA background.

Typically antigens are introduced into APCs using viral vectors, but this poses regulatory hurdles and introduces unwanted vector-derived antigens that may compete for presentation with weaker viral antigens. Overlapping peptide libraries (PepMixes™, JPT Peptide Technologies) provide an alternative antigen source that is simple to use, less expensive than viral vectors and does not introduce unwanted stimuli. PepMixes™ (15mer peptides overlapping by 11 amino acids) that span the entire sequence of a target antigen can present all possible HLA class I-restricted epitopes and multiple class II-restricted epitopes¹. These can be presented in association with HLA class I molecules by virtually any cell, and within PBMCs, monocytes and B cells express HLA class II molecules and thus also present to CD4+ T cells.

T cells specific for the viruses most commonly responsible for clinical disease in stem cell recipients, could be activated with combinations of PepMixes™ suitable for phase 1 clinical trials. When cultured in a specialized cell culture device (G-Rex) in the presence of pro-survival cytokines, multivirus-specific T cells expanded *in vitro* and despite the presence of multiple competing peptide pools, we found no evidence of antigenic competition when we compared the specificity of T cell lines stimulated using single PepMixes™ with T cells stimulated with up to 15 PepMixes™. The expanded cultures contained cells that were Th1-polarized, produced multiple effector cytokines upon stimulation, and killed virus-infected targets without evidence of alloreactivity².

Based on this preliminary data the FDA recently approved our clinical protocol to prevent or treat EBV, CMV, Adenovirus, HHV6, and/or BK virus infections after-HSCT using T cell lines generated with our new manufacturing technology. In this Phase I dose escalation study we will determine the feasibility and safety of administering rapidly-generated pooled PepMix™-activated CTLs to HSCT recipients at 3 dose levels (5×10^6 , 1×10^7 , and 2×10^7 cells/m²) and we will assess whether CTL infusions produce clinical benefit and support the reconstitution of antiviral immunity post-infusion.

The goal of the current study was to establish the parameters that would support optimal *in vitro* CTL expansion to ensure our ability to consistently produce sufficient T cells to treat HSCT recipients at all 3 proposed dose levels and to determine the starting donor blood volumes that would be required for T-cell manufacture.

Results

To activate multivirus-specific T cells, PBMCs were pulsed with pooled PepMixes™ spanning immunogenic antigens derived from each target virus - CMV (IE1 and pp65), AdV (Hexon and Penton), EBV (LMP2, EBNA1, BZLF1), BKV (VP1 and large T), and HHV6 (U90, U11 and U14). T cells were then cultured in a G-Rex10 culture device in the presence of IL4 and IL7 for expansion. To identify the optimal seeding cell density for T cell expansion we seeded GRex-10s with 15, 20 or 25×10^6 pooled PepMix™-activated PBMCs. As shown in **Figure 1**, G-Rex devices seeded with 15×10^6 PepMix™-activated PBMCs expanded by a mean of 5.5 ± 0.8 fold over a 10-day period, resulting in an average of $82.9 \pm 11.9 \times 10^6$ T cells (n=6). In contrast, in devices seeded with 20 and 25×10^6 PBMCs expansion was inferior (4.8 ± 0.9 and 4.2 ± 0.6 fold, respectively), with a mean of $95.4 \pm 19.7 \times 10^6$ and $101.5 \pm 14 \times 10^6$ T cells generated. Importantly, there was detectable activity against all the stimulating viral antigens, as detected by IFN γ ELISpot assay, which was similar in magnitude irrespective of whether the cultures were initiated with 15, 20 and 25×10^6 PepMix™-activated PBMCs (**Figure 2**). Over the course of this 10-day culture period we estimate an approx. 10-fold enrichment in virus-specific cells and a corresponding reduction in alloreactive T cells to levels observed in repetitively stimulated conventional CTLs, which have a proven safety record *in vivo*^{3,4}.

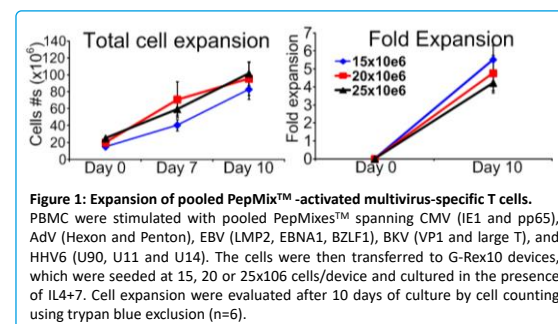
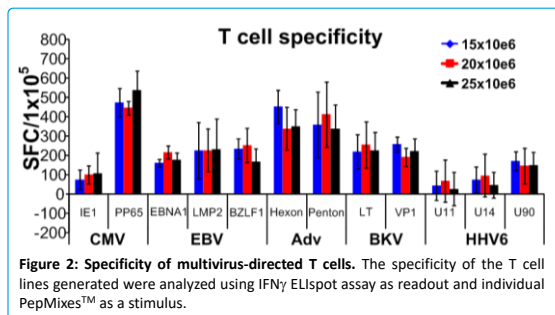


Figure 1: Expansion of pooled PepMix™-activated multivirus-specific T cells. PBMC were stimulated with pooled PepMixes™ spanning CMV (IE1 and pp65), AdV (Hexon and Penton), EBV (LMP2, EBNA1, BZLF1), BKV (VP1 and large T), and HHV6 (U90, U11 and U14). The cells were then transferred to G-Rex10 devices, which were seeded at 15, 20 or 25×10^6 cells/device and cultured in the presence of IL4+7. Cell expansion were evaluated after 10 days of culture by cell counting using trypan blue exclusion (n=6).

PepMix™ Peptide Pools

Materials & Methods

Activation of viral antigen-specific T cells. 15, 20 or 25 x 10⁶ PBMCs were pelleted in a 15ml falcon tube and pulsed with pooled PepMixes™ (100ng of each peptide) spanning 12 viral antigens from 5 viruses for 30 minutes at 37°C. PBMC were then resuspended in 30ml of CTL medium (RPMI 1640, 45% Click's (Irvine Scientific, Santa Ana, CA), 2mM GlutaMAX TM-I, and 10% fetal calf serum (Hyclone) containing 400U/ml IL4 and 10ng/ml IL7 and cultured in a GRex-10. On day 6 or 7 the cultures were counted, and if there were >50x10⁶ total cells, the culture was split into a second G-Rex and both flasks were supplemented with CTL media and IL4+7. On day 10 of culture cells were harvested, counted using trypan blue exclusion, and then tested for specificity by IFN γ ELISpot, as previously described⁵⁻⁷.



Discussion & Conclusions

In the current study we have shown that a single blood draw of only 20ml from a stem cell donor should be adequate to produce sufficient multivirus-specific T cells for the treatment of the stem cell recipient at all three dose levels proposed in our study, given that 1ml of blood should yield between 1-2x10⁶ PBMCs.

Our new CTL manufacturing approach is more rapid and simpler than previous *ex vivo* expansion strategies as we have removed the need for the production of a distinct population of professional APCs and changed the antigenic stimulus from viral transgenes to chemically synthesized PepMixes™. In addition, the absence of antigenic competition between T cells of different specificities within our lines has allowed us to expand the viral target range to include BK virus and HHV6, which are both frequently detected and clinically problematic after HSCT. Our clinical trial, scheduled to begin accrual later this year, will compare the safety and clinical activity of these pooled PepMix™-activated multivirus-directed T cell lines with results from our previous trials using conventionally generated T cell products.

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Her research interests are focused on developing novel immune-based therapies to treat both viral infections and cancer. She has performed clinical trials using T cell lines with specificity for EBV, CMV, and Adenovirus to prevent viral infections in allogeneic HSCT recipients and more recently has turned her attention to targeting non-viral tumor associated antigens.

The Company

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