ASSESSING SLEEPING BEAUTY TRANSPRESSIONS FOR T-CELL IMMUNOTHERAPY BY SUPERCOMPUTER-BASED HIGH-THROUGHPUT PROFILING OF INTEGRATION EVENTS

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MINANT VIRUS-SPECIFIC T-CELLS IN RECIPIENTS OF HAPLOIDENTICAL FICACY OF ADOPTIVELY TRANSFERRED DONOR DERIVED IMMUNODO-

FACTORS CONTRIBUTING TO VIRAL INFECTIONS AND THE LIMITED EF-

Epitopes eliciting IFNγ [4] TC were mapped using a matrix of peptide pools, and their HLA-restriction determined.

Six of 11 donors tested responded to CMV epitopes presented by HLA alleles shared with the recipient, while 5 responded exclusively to epitopes presented by donor-unique HLA alleles. Patients receiving transplants from the latter 5 donors experienced viremia correlated with CMV viremia, CMV chorioretinitis and/or pneumonitis. CMV-CTL isolated from each of the six patients over 1 year post HCT were specific for epitopes presented by HLA alleles shared by their donors. In patients transplanted from donors whose CMV-CTL were restricted by donor-unique HLA alleles, the engrafted TC responded to an epitope that was different from donor CMV-CTL, but restricted by an HLA allele shared with the donor, suggesting that these TC may have developed from donor precursors differentiating in the host thymus. In general, responses against epitopes presented by HLA C0401, B3501 and A0101 were less robust.

Overall, in a significant proportion of cases (5/11), CMV-CTL from HLA haploptope disparate donors are restricted by donor-unique HLA alleles, and the development of effective CMV-specific responses in the patient is delayed until the evolution of new TC from host thymic precursors. These data can be used to predict CMV infections and develop adoptive immunotherapy strategies to overcome infectious mortality in haploidentical transplants.

ADOPTIVE TRANSFER OF RAPIDLY-GENERATED MULTIVIRUS-SPECIFIC T CELLS TO TREAT ADENOVIRUS, EBV AND CMV INFECTIONS OF HEMATOPOIETIC STEM CELL TRANSPLANT RECIPIENTS


We have demonstrated that small numbers of ex vivo-expanded, triviruss T cells, targeting EBV, CMV, and Adv are safe, proliferate in vivo and protect against all 3 viruses post-HSCT. However, broader implementation is limited by the need for infectious virus (EBV), clinical adenovector, and prolonged (6 wk) manufacture. In addition, competition between viral antigens limits extension to other viruses. We are now evaluating whether infusion of rapidly-generated donor trivirus T cells (rCTL), stimulated only once with DCs nucleofected with DNA plasmids encoding immunogenic EBV (LMP2, EBNA1, BZLF1), Adv (Hexon, Penton), and CMV (pp65, IE1) antigens, and expanded in the presence of IL4+7 in a gas permeable device (G-Rex), is safe and similarly effective in HSCT recipients with active infections.

With NHLBI-PACT support, 18 clinical rCTL lines have been generated. From 15x10^6 PBMCs, we prepared a median of 241.6x10^6 rCTLs (range 100-420x10^6) in 9-12 days. The rCTLs were polyclonal, comprising both CD4 (31±15%) and CD8 (62±17%) cells, with specificity for CMV (IE1: 360±36; pp65: 697±29 SFC/2x10^5), EBV (LMP2: 222±11, EBNA1: 90±11 and BZLF1: 121±910) and Adv (Hexon, Penton), and CMV (pp65, IE1) antigens, and expanded in the presence of IL4+7 in a gas permeable device (G-Rex), is safe and similarly effective in HSCT recipients with active infections.

This patient also had detectable Adv in blood that resolved. Clearance of both viruses corresponded with an increase in detectable CMV and Adv-specific T cells in peripheral blood (PB). Four weeks later Pt1 again reactivated CMV (increase from 0 to 10,000 copies/ml) while receiving antivirals. Concurrently the frequency of CMV-reactive T cells increased from 12 to 528 SFC/4x10^5 and 3 Tr virus-specific T cells coexpressing with viral clearance. Pt 2 was similarly able to clear CMV (1500 copies/ml) with a corresponding increase (from 0 to 96 and 0 to 51 SFC/4x10^5) in both IE1 and pp65-specific T cells, respectively. The 3rd patient received rCTL for Adv and is too early to evaluate. Infusion of rCTLs has thus been safe to date and is associated with the appearance of virus-specific cells in PB and subsequent viral clearance. We are now extending this platform to additional viruses, thereby broadening the spectrum of pathogens that can be targeted with a single T cell line.