20

THE CLINICAL USE OF CORD BLOOD-DERIVED VIRUS-SPECIFIC CYTOTOXIC T LYMPHOCYTES REACTIVE AGAINST CYTOMEGALOVIRUS (CMV), ADENOVIRUS, AND EPSTEIN-BARR VIRUS (EBV)

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CMV, Adenovirus (Ad) and EBV are viral pathogens causing morbidity and mortality in patients after hematopoietic stem cell transplantation (HSCT) and cord blood transplantation (CBT). We have shown that adoptive immunotherapy with peripheral blood donor-derived multivirus-specific Cytotoxic T Lymphocytes (mCTL) directed against EBV, CMV and Ad can effectively prevent and treat the clinical manifestations of these viruses after HSCT. We have now extended these studies by expanding mCTL from umbilical cord blood (CB) to restore cellular immunity to CMV, EBV and Ad simultaneously after CBT. However, the development of mCTL for patients undergoing CBT requires the priming and extensive expansion of naïve T cells rather than the more limited and simple direct expansion of pre-existing memory T cell populations from virus-experienced donors. We have developed a novel protocol utilizing an initial round of stimulation with autologous CB-derived dendritic cells transduced with a recombinant Ad5f35 vector carrying a transgene for the immunodominant CMV antigen, pp65 (Ad5f35pp65) in the presence of IL12, IL15 and IL7. This is followed by 2 rounds of weekly stimulation with autologous EBV-LCL transduced with the same vector in the presence of IL15 and IL2. After 3 rounds of stimulation, 2 CTL cultures generated for clinical use contained a mean of 48% (range 24-72%) CD8+, and 42% (range 11-72%) CD4+ cells with mean 30.5% (range 23-46%) CD45RA-/CD62L+ T cells. In ^{51}Cr release and/or IFN-γ ELISPOT assays, both mCTL lines showed specific activity against CMV, EBV and Ad targets. So far we have treated 2 patients in this phase I study, both on dose level 1 ($5x10^6/m^2$). Patients received 1 infusion of CTL on day 63 post-CBT. Only patient 1 is currently evaluable as patient 2 was infused too recently to test. We observed a 10-fold increase in CMV-specific T cells 5 weeks-post CTL. This patient was transiently positive for CMV by PCR at 4 weeks post CTL but was negative within 16 days of receiving a second dose of mCTL with a corresponding rise in CMV-specific CTL detected in the peripheral blood. The patient became Ag+ for Ad in his stool associated with diarrhea which resolved spontaneously without additional therapy. In summary, we have developed a protocol for the generation of mCTL from CB: infusion of small numbers of these cells increased virus-specific T cells in the peripheral blood post CTL infusion dramatically earlier than observed immune reconstitution post CBT.

21

DRAMATIC REGRESSION OF CHRONIC LYMPHOCYTIC LEUKEMIA IN THE FIRST PATIENT TREATED WITH DONOR-DERIVED GENETICALLY-ENGINEERED ANTI-CD19-CHIMERIC-ANTIGEN-RECEPTOR-EXPRESSING T CELLS AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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We have initiated a clinical trial in which patients receive infusions of allogeneic T cells that are genetically modified with a gammaretroviral vector to express a chimeric antigen receptor (CAR) that recognizes the B-cell antigen CD19. The first patient treated on this trial was a 65 year-old man with chronic lymphocytic leukemia (CLL) who relapsed after HLA-matched unrelated donor hematopoietic stem cell transplantation. Following the relapse, the patient received 4 donor lymphocyte infusions (DLIs) with a maximum $CD3^+$ cell dose of $2.9x10^7$/kg and then a second stem cell transplant from the original donor. An objective remission of the leukemia did not occur after any of the DLIs or the second transplant. Five months after the second transplant, when his CLL was progressing, the patient received an infusion of $6.2x10^7$ ($1x10^6$ cells/kg) allogeneic anti-CD19-CAR-transduced T cells derived from his unrelated transplant donor. Thirty-nine percent of the infused cells expressed the anti-CD19 CAR, and the cells produced interferonγ and IL-2 in a CD19-specific manner. The patient did not receive any other therapy in conjunction with the CAR-transduced T cells. From 6 to 12 days after the CAR-transduced T cell infusion, the patient experienced fevers, fatigue, mild hypoxemia, and intermittent mild hypotension. Increases in serum magnesium, phosphorous, and uric acid consistent with tumor lysis syndrome occurred. A decrease in cardiac left ventricular function developed, which was improving at last follow-up. The patient's blood B cell count decreased from 286 cells/μL before the CAR-transduced T cell infusion to 0 cells/μL 26 days after the cell infusion. Before the CAR-transduced T cell infusion, CLL cells made up 80-90% of the patient's hypercellular bone marrow. A bone marrow biopsy performed 26 days after the cell infusion showed a normocellular marrow, nearly absent B-lineage cells, and no evidence of CLL. CT scans revealed a greater than 50% decrease in the size of multiple lymph nodes after the CAR-transduced T cell infusion, but residual adenopathy was present. CAR-transduced cells were not detected in the patient's blood by quantitative PCR during the first week after the T cell infusion, but made up 0.98% of blood mononuclear cells 11 days after the infusion. These results are encouraging for further development of anti-CD19-CAR-expressing T cells as a treatment for relapse after allogeneic stem cell transplantation.

22

ALLOGENEIC VIRUS-SPECIFIC T CELLS WITH HLA ALLOREACTIVITY DO NOT PRODUCE GRAFT-VERSUS-HOST DISEASE IN HUMAN SUBJECTS

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We and others have recently established that T cell reactivity with non-self HLA (HLA alloreactivity) arises not only from naive T cells but also from the antigen-experienced T cell pool, including Epstein-Barr virus (EBV) and cytomegalovirus (CMV)-specific T cells. Virus-specific T cells could therefore mediate graft-versus-host disease (GvHD) if infused into partially HLA mismatched recipients. We reviewed our clinical experience with adoptive transfer of allogeneic hematopoietic stem cell transplant donor-derived virus-specific T cell lines in 153 recipients, including 73 partially HLA-mismatched recipients. The degree of HLA mismatching varied from one allele to a full haplotype. *De novo* GvHD did not develop after infusion of cytotoxic T lymphocytes (CTL), and the incidence of GvHD reactivation was 6.5% and not significantly different between recipients of HLA matched or mismatched CTL. Thus, virus-specific CTL did not mediate GvHD, even in recipients of partially matched CTL. Next we analyzed the HLA alloreactivity of four donor-infused bivirus-specific T cell lines, using activated T cells, that are known to lack CMV and EBV antigen expression, as antigen presenting cells (TAPC). We used a panel of 44 TAPC covering the most frequent HLA class I and II alleles. The CTL lines were labeled with CFSE and stimulated with TAPC for 6 hours, after which production of TNFα and IFNγ/IL-2 by CD4+ and CD8+ T cells in the CFSE-positive fraction was analyzed by flow cytometry. All CTLs responded to a number of TAPC, with some APC being recognized strongly. The majority elicited only weak or no response from the CTLs. We then assessed whether the CTLs recognized TAPC expressing the recipient's HLA alleles. We found moderate reactivity of the CTL with 1-5 TAPC expressing recipient HLA alleles. Taken together, our data indicate that reactivity of virus-specific CTLs with hematopoietic APC does not correlate with the risk of developing GvHD, and that virus-specific CTL can safely be infused into HLA class I and/or II mismatched recipients.

23

PHASE I CLINICAL TRIAL TARGETING CD20+ NON-HODGKIN'S LYMPHOMA (NHL) AFTER AUTOLOGOUS STEM CELL TRANSPLANT WITH ANTI-CD3 × ANTI-CD20 BISPECIFIC ANTIBODY ARMED T CELLS

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