acquired SAA in children or young adults if a fully matched unrelated donor can be identified.

90

GENETIC REPROGRAMMING AND EDITING OF T CELLS USING THE SLEEPING BEAUTY SYSTEM AND DESIGNER ZINC FINGER NUCLEASES

Torikai, H.1, Reik, A.2, Yuen, C.1, Zhou, Y.1, Kellar, D.1, Hils, H.3, Warren, E.H.1, Tyborski, S.S.1, Gregory, P.D.2, Holmes, M.C.2, Rekha, E.J.2, Lee, D.A.4, Champlin, R.E.1, Bonni, C.1, Cooper, L.F.1, 1 Dana Farber/Children's Hospital Cancer Center, Houston, TX; 2 Sangamo BioSciences, Inc., Richmond, CA; 3 Fred Hutchinson Cancer Research Center, Seattle, WA; 4 The University of Texas MD Anderson Cancer Center, Houston, TX; 5 San Raffaele Scientific Institute, Milan, Italy

We have shown that Sleeping Beauty transposon/transposase can be harnessed to stably express a CD19-specific chimeric antigen receptor (CD19RCAR) to reprogram T cell specificity to B-cell tumors independent of human leukocyte antigens (HLA), a technology that is currently being evaluated clinically (IND#14193). However, the time required to manufacture patient-specific T cells to sufficient numbers ex vivo can be incompatible with the window of opportunity to treat patients with rapidly-progressing malignancies. An alternative approach is to prepare allogeneic T cells as third party effector cells for infusion on demand. However, immune-mediated rejection by recipient T cells recognizing disparate HLA and graft-versus-host-disease mediated by the endogenous αβ T-cell receptor (TCR) on the infused T cells may compromise this alternative approach to adoptive immunotherapy. To this end, we investigated whether HLA and TCR expression can be eliminated in T cells by designed zinc-finger nucleases (ZFNs). ZFNs are chimeric endonucleases comprised of a zinc finger DNA binding domain designed to bind a specific DNA sequence fused to the cleavage domain of Fok I endonuclease. The introduction of a double strand break (DSB) at an intended target site requires the design of two ZFNs that bind in a specific spatial orientation to allow dimerization on DNA. Cellular repair of DSB by error-prone non-homologous end joining results in the permanent disruption of target gene expression. For an initial proof-of-concept we designed ZFN pairs targeting the HLA-A locus. Since only transient expression of ZFNs is needed to permanently disrupt a target gene, we tested the electro-transfer of in vitro-transcribed ZFN mRNA in primary T cells. We show that administration of the mRNA encoding the ZFNs targeting HLA-A resulted in over 40% HLA-A negative primary T cells, which could be readily enriched by paramagnetic bead separation to obtain a pool of > 90% HLA-A<sup>-</sup>T cells. By using the same approach, we tested ZFN pairs targeting the TCRα or TCRβ locus, resulting in a highly enriched αβ TCR<sup>-</sup>cell population (> 90% ab TCR<sup>-</sup>). Importantly, both sets of ZFNs were shown to successfully modify CD3<sup>+</sup> T cells and result in the efficient elimination of αβ or αβ TCR expression from CAR<sup>+</sup> T cells which maintain redirected specificity for CD19. These data support the potential for allogeneic CD19-specific T cells as “off-the-shelf” biologics that can be infused on demand as “drugs”.

91

INVERSE CORRELATION BETWEEN BMI AND TIME-TO-ENGRAFTMENT AFTER PEDIATRIC HSCT: IMPLICATIONS FOR THE ROLE OF ADIPOCYTES IN HEMATOPOIETIC STEM AND PROGENITOR CELL (HSPC) FUNCTION

Wang, L.D.1,2, Duncan, C.N.1,1 Dana Farber/Children's Hospital Cancer Center, Boston, MA; 2 Harvard Stem Cell Institute, Boston, MA

Recent studies have shown that adipocytes directly negatively regulate hematopoietic stem cell (HSC) function. It has also been shown that visceral adiposity correlates with bone marrow cell content and therefore increased adipocyte-HSC interaction, which may link high body mass index (BMI) with increased negative regulation of HSC function. Clinically, this has significant implications for stem cell transplant patients, particularly as Americans in general and the pediatric population in particular becomes more obese. By investigating the relationship between BMI and transplant mortality have been done; the results of these studies are somewhat contradictory. Additionally, interpretation of these studies is complicated by the fact that transplant outcome is an endpoint significantly downstream of HSC function, and is influenced by many other factors affected by obesity. We sought to address directly the issue of HSC function as influenced by adiposity, using the clinical endpoint of engraftment as a proxy measure for hematopoietic stem and progenitor cell (HSPC) function.

We performed an analysis of BMI and time to engraftment (TTE) in all patients transplanted at our institution over a two-year interval. We analyzed data separately from allogeneic bone marrow recipients and autologous peripheral blood stem cell recipients. Only data from the first transplant was analyzed when a subject had multiple HSCTs performed. Linear regression was used to evaluate the relationship between BMI and TTE. Sixty-one patients had an allogeneic bone marrow transplant (29 MRD and 32 MURD) and 33 patients had an autologous HSCT during the study period. There was an inverse correlation between BMI and TTE in the allogeneic group (r = -0.2971, p = 0.02). BMI did not correlate with TTE in the autologous HSCT group. BMI also did not correlate with overall survival in either the autologous or the allogeneic group. Importantly, graft cell dose did not correlate with TTE in either population.

The negative correlation between BMI and time to engraftment was unexpected and may be the result of proliferative effects of adipocytes on more mature hematopoietic progenitors. To assess this possibility in a more controlled setting, we have undertaken an analysis of HSC and MPP frequency and function by performing myeloablative transplants in a murine diet-induced obesity (DIO) model.

STEM CELL BIOLOGY

92

TARGETED CLEARANCE OF HUMAN HEMATOPOIETIC STEM CELL NICHES VIA INHIBITION OF SFC SIGNALING USING MONOCLONAL ANTIBODY SR-1

Czechowicz, A.1, Bhardwaj, R.1, Pang, W.1, Park, C.Y.2, Weiszman, I.L.3 1 Stanford University, Stanford, CA; 2 Memorial Sloan-Kettering Cancer Center, New York, NY

Upon transplantation, hematopoietic stem cells (HSCs) home to specialized bone marrow niches, self-renew and differentiate and create new, complete hematolymphoid systems. Unfortunately, HSCT/BMT has been limited in part due to the toxic conditioning regimens, such as irradiation and chemotherapy, deemed necessary for HSC engraftment. We have previously shown that one important barrier to HSC engraftment is availability of HSC niche space. In the absence of pre-transplant conditioning, 99% of HSC niches are occupied with host HSCs preventing donor HSC engraftment under these conditions is minimal. We have shown in mouse models, that elimination of host HSCs using anti-mouse-ckit monoclonal antibody ACK2 allows for 90% donor HSC engraftment with minimal toxicity in immunodeficient animals, which is sufficient to cure most hematolymphoid disorders.

We examined the effects of SFC-signaling in human HSCs using various monoclonal antibodies and verified that anti-human-ckit monoclonal antibody SR-1 uniquely inhibits SFC binding. We cultured purified human BM HSC and CB HSC (CD34<sup>+</sup>CD38<sup>-</sup>CD90<sup>+</sup>CD45RA-Lin-) with SR-1 or 4G7 (a non-active anti-human-ckit clone) and show that SR-1 uniquely inhibits human HSC proliferation in vitro. Interestingly, SR-1 did not induce human HSC cell death via apoptosis, but rather shifted the differentiation profile and increased Glycophorin A and CD41+ cells (RBC and platelets respectively), and decreased output of CD14, CD13, and CD33 cells (macrophages/myeloid cells).

To examine the depletion capability of SR-1 in vivo, robust human-mouse hematopoietic chimeras were generated using newborn NOD/SCID/IL-2R<sup>−/−</sup> null mice IV transplanted with 1000 human CB HSC post 100Gy. By BM aspirate, average pre-treatment baseline total human bone marrow engraftment (%CD45<sup>+</sup>) was 58.6%, and myeloid chimerism (%CD13/33) was 25.4%. The mice were then treated IV with 500ug of SR-1 every other day for 1 week. Human total and myeloid engraftment 8 weeks post treatment decreased by 92.4% and 96.9% respectively, most likely due to depletion of human HSCs that maintain these populations. The remaining persistent human cells in these animals were primarily