in vivo. We therefore characterized the CD19CAR T cells after ex vivo expansion. We found that 40% of AKT-inhibited CD19CAR T cells expressed CD62L and co-expressed CD28. In contrast, only 10% of control untreated CD19CAR T cells expressed CD62L and they were CD28 negative, indicating that AKT-inhibited CD19CAR T cells may have superior anti-tumor activity following adoptive transfer. To test the potency of the AKT inhibitor treated CAR T cells, $0.5x10^6$ CD19+ acute lymphoid leukemic cells (SupB15) that were engineered to express firely lucifierase were inoculated intravenously into NOD/*Scid* IL-2RgammaCnull (NSG) mice. Five days post tumor engraftment, $2x10^6$ CD8+ CD19CAR T cells were intravenously injected into tumor bearing mice. Control mice received either no T cells, non-transduced T cells (Mock), or CD19CAR T cells that were not treated with AKT inhibitor during in vitro expansion. Tumor signals post T cells infusion were monitored by biophotonic imaging. In contrast to the untreated CD19CAR T cells, which exhibited lower and transient anti-tumor activity, AKT-inhibited CD19CAR T cells completely eradicated the $CD19^+$ tumor in all mice (Figure 1) 21 days post CD19CAR T cell infusion. In conclusion, inhibition of AKT signaling during the ex vivo priming and expansion gives rise to a CD19CAR T cell population that possesses superior antitumor activity. These findings suggest that therapeutic modulation of AKT might be a strategy to augment antitumor immunity for adoptive CAR T cell therapy, which could easily be transitioned into the clinic with the availability of pharmaceutical grade AKT inhibitor.

641. Highly Efficient, ZFN-Driven Knockout of Surface Expression of the T-Cell Receptor and HLA Class I Proteins in Human T-Cells for Enhancing Allogeneic Adoptive Cell Therapies

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While adoptive transfer of T-cells modified to express a chimeric antigen receptor or tumor antigen specific T-cell receptor (TCR) has shown great promise for the treatment of malignant cancers, most current clinical applications are limited by the use of autologous T-cell products. Targeting of the TCR and HLA Class I genes in primary T-cells thus represent attractive targets for genome editing in order to produce universal T cells from allogeneic donors. Elimination of the native TCR and HLA class I proteins on T-cells would, respectively, reduce the risk of graft-versus-host disease and host-versus-graft clearance mediated by the adaptive immune system. We have developed clinical grade zinc finger nuclease (ZFN) reagents that can efficiently target the T-cell receptor alpha constant (TRAC) and beta-2-microglobulin (B2M) loci. ZFN encoding mRNAs were introduced into purified T-cells by electroporation. Without selection, T-cells treated with TRAC specific ZFNs showed modification of up to 89% of alleles as gauged by deep sequencing. Flow analysis showed 91% of treated cells were negative for CD3 expression. Results from ZFN mRNA dose titration studies showed that the level of TRAC gene modification by deep sequencing was highly correlated with the percentage of cells that stain negative for CD3 expression by FACS (Spearman rho = 0.96; $p < 0.0001$). B2M is a subunit in all HLA class I molecules, and represents a conserved target for eliminating HLA class I presentation in cells from different donors. Analysis of T-cells transfected with mRNAs encoding B2M specific ZFNs showed up to 94% of alleles were modified, as determined by deep sequencing. FACS analysis showed that 89% of treated cells were negative for expression of HLA-A, B, C. Similar to TRAC modified cells, a marked correlation was observed between the percentage of B2M alleles modified and percentage of cells lacking surface expression of HLA-A,B, and C (Spearman rho = 0.57; $p < 0.0001$). ZFN mediated genome editing was well tolerated. T-cells treated with TRAC or B2M specific ZFNs showed similar viability and growth characteristics as mock transfected cells. Together, these highly efficient ZFN reagents permit the highly efficient double knockout of TCR and HLA class I surface expression in primary human T-cells for potential use in the development of allogeneic cell therapies.

642. Oncolytic Vaccines with Modified Tumor Epitopes for Cancer Immunotherapy

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Oncolytic adenoviruses (OAds) are capable of killing tumor cells while activating the immune system due to their immunogenicity. Hance, they are an excellent platform for oncolytic vaccine. We previously demonstrated that the injection of peptide-coated conditionally replicating adenoviruses (PeptiCRAd) is capable of reducing the growth of established aggressive melanomas (murine B16).

Oncolytic vaccines, like PeptiCRAds, often rely on inducing an immune response against specific tumor antigens. However, many tumor antigens are also self-antigens, hence the peripheral tolerance might impair the activity of tumor-reactive T-cells. Therefore, mutated epitopes represent an optimal tool to break the tolerance, exploiting the cross-reactivity of T-cells. To this end we developed an Epitope Discovery and Improvement System (EDIS) framework to study native epitopes and predict, *in silico,* mutated forms suitable for cancer therapy. The novel aspect of EDIS is the ability to interrogate different prediction servers, integrate the different results and validate these by molecular dynamics simulations.

We started by studying the model epitope *SIINFEKL*. According to our *in silico* predictions, two mutated variants were suggested to be more immunogenic than the native *SIINFEKL* peptide. To test whether this prediction would reflect in enhanced vaccine effect we studied the immune response against these peptides in B16-OVA bearing mice. Mice were challenged with B16-OVA and treated with three different PeptiCRAds coated with *SIINFEKL* and the two predicted derivates. By ELISPOT assay we assessed the anti-peptide response and demonstrated that the two mutated forms were in fact more effective in reducing the growth of established B16OVA tumors.

Finally, we studied the native epitope *SVYDFFVWL* from the tyrosinase-related protein 2 (TRP2), a melanoma antigen in clinical evaluation. By using the EDIS framework we selected two mutated variants that show increased MHC-I binding affinity and we tested them by treating aggressive B16F10 tumors. As expected, treatment with the native TRP2 reduced the growth of the tumors compared to the controls. Suprisingly, one of the two analogues improved significantly the survival of mice and reduced the growth of their tumors compared to the group treated with the native TRP2 epitope. In conclusion, we demonstrated that the integration of different in silico methods increases the accuracy when predicting mutated epitopes for cancer immunotherapy.