

significant, strictly inducer-dependent antitumor activity in a well-established mouse model of B cell lymphoma.

Conclusions The zinc-finger-based transcriptional control system investigated in this study provides small molecule-inducible control over a therapeutically relevant anti-CD20 CAR in primary T cells in a time- and dose-dependent manner. The tight regulation of CAR expression will pave the way for safer cellular therapies.

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P01.23 CREATING A CELL-CULTURE BASED REPORTER SYSTEM FOR THE EVALUATION OF MOLECULAR SIGNALING MECHANISMS OF INHIBITORY CHIMERIC ANTIGEN RECEPTORS

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Background Adoptive transfer of T cells expressing chimeric antigen receptors (CARs) is a novel treatment option for patients with B-cell lineage derived cancers. Other cancer entities are less successfully targeted due to the lack of antigens that are expressed on cancer cells but not healthy tissue cells. One way to address this issue is the concept of inhibitory chimeric antigen receptors (iCARs) that deliver an inhibitory signal upon antigen encounter on off-target cells. The activating effect of CARs depends on a multitude of factors including expression levels, affinity, different signaling domains and steric effects. It has to be expected that co-expression of an iCAR would result in even more complexity. Consequently, there is a demand for a robust high-throughput cellular system to evaluate iCAR-formats.

Materials and Methods Our approach is based on a previously published Jurkat based triple parameter reporter cell (TPR) system. This setup allows for molecular monitoring of the T cell activation state by measuring fluorescent reporter gene expression via flow cytometry. Inhibitory effects of receptors are determined as the ratio of cellular geometric mean fluorescent intensity (gMFI) in the presence of the inhibitory ligand versus without inhibitory ligand.

Results To test if inhibitory receptors would measurably reduce reporter activation, PD-1 as a well-characterized inhibitory receptor was expressed on Jurkat TPRs. When PD-L1 was present during stimulation reporter activation was reduced by 26–34% proving feasibility of our approach. Intracellular domains of other inhibitory receptors including BTLA, ILT-2 and KIR2DL1 were evaluated. All three domains outperformed PD-1 in a series of experiments with a mean reduction of gMFI by 48–57%, 50–53% and 38–41% respectively. To assess if the reporter platform could be used to study downstream signaling pathways of inhibitory constructs we created a SHP-2 knock-out reporter cell line using the

CRISPR/Cas9 gene editing technique. To simulate different degrees of activating signal strength, peptide-MHC complex recognizing activating receptors were created. The reporter activation correlated with the concentrations of peptide in the stimulation cultures. Co-expression of iCAR and CAR could be achieved using selection for two separate antibiotic resistance genes introduced into the respective vector. Preliminary experiments showed greatly reduced inhibitory efficacy of iCAR molecules due to an adhesion effect resulting from the high affinity extracellular domain of the iCARs that lead to tighter cell-cell contact and stronger stimulation through the CAR.

Conclusions We present a highly flexible and controllable Jurkat-based reporter cell platform for the thorough study of inhibitory signaling mechanisms. This project was supported by the Austrian Science Fund, FWF; Project P32411

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P01.24 THE SELECTIVE HDAC6 INHIBITOR ITF3756 INCREASES THE DIFFERENTIATION TO CENTRAL MEMORY T CELLS WITH REDUCED EXHAUSTION PHENOTYPE

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Background Central memory T cells show superior persistence and antitumor immunity compared to effector memory and effector T cells. T effector cells respond quickly to tumors, but they are terminally differentiated and undergo apoptosis upon killing activity. T memory differentiate rapidly into T effector cells and maintain a pool of cells that can continuously differentiate thus sustaining a more lasting response. In adoptive cell therapy (ACT), T cells infused into patients may have a limited time of activity if they are terminally differentiated, and may rapidly undergo exhaustion and apoptosis. The development of new strategies based on novel agents able to generate memory T cells ex-vivo is important for a successful clinical application of ACT. We have studied the effect of a potent and selective HDAC6 inhibitor, ITF3756, on CD8 T cells differentiation during an *in vitro* induced exhaustion process.

Materials and Methods To induce exhaustion purified human CD8+ cells were stimulated twice with anti-CD3/CD28 beads (1:2) during 5 days, with or without ITF3756 1µM or 2µM added at all times of stimulation. At day 3 and 5 the expression of exhaustion, memory and effector T cells markers were analyzed by flow cytometry. Cells were also collected at day 5 for genes expression analysis. Expression of exhaustion, T phenotype, metabolic pathway and inflammatory cytokines were investigated by qPCR. Paired two-tailed t-tests was used to determine statistical significance between control versus treatment group at day 3 and 5 in 10 different donors. P-values ≤ 0.05 were considered significant.

Results ITF3756 1µM increased significantly the T central memory phenotype (CD45RO+CD62L+CCR7+) and decreased significantly the T effector phenotype (CD45RO+CD62L-CCR7-). The expression of CD62L in T central memory cells was significantly increased in agreement with the high expression of this marker in naïve and memory T cells. ITF3756 treatment decreased significantly the expression of