87 cutaneous T-cell lymphoma cell lines with similar uncharacteristic features.


**P01.03 RNAI MEDIATED PD-1 KNOCKDOWN INDUCES A TCF-1 POSITIVE POPULATION IN ACTIVATED HUMAN CD8 T CELLS WITH STEM-LIKE ASSOCIATED MARKER PROFILE**

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**Background** Adoptive Transfer of antigen specific T cells (ATT) is a powerful tool in the treatment of cancer. However, there are still hurdles to satisfactory efficacy. One of them is the upregulation of immune-inhibitory receptors like programmed cell death protein 1 (PD-1). Silencing PD-1 at the mRNA level would not only prevent expression and therefore programmed cell death protein 1 (PD-1), but also inhibit tonic signalling. This should increase proliferation, cytotoxicity, cytokine production and metabolic activity via the AKT pathway. Another well-known hurdle to ATT efficacy is the poor persistence of effector T cells in patients. Stem-like memory subsets of CD8 T cells such as those marked by TCF-1 expression may therefore represent an advantageous effector population for ATT, as they show longer persistence, higher proliferative activity, responsiveness to checkpoint inhibitors and the ability to differentiate into new effector T cells.

Increasing the proportion of this population is thought to be beneficial in anti-tumor therapy. Here, we present data showing that specific downregulation of PD-1 using a novel RNA interference (RNAi) technology increases the frequency of a CD8 T cell population with a stem-like associated marker profile.

**Materials and Methods** INTASYL™ compounds incorporate drug-like properties into RNAi, resulting not only in enhanced cellular uptake but also eliminates the need for transfection reagents. TCR53-transduced T cells, suitable for ATT, were incubated with PD-1 targeting INTASYL compound PH-762 for 24h. As controls, cells were either treated with a non-targeting compound (NTC) or left untreated (UTC). Following PH-762 loading, T cells were co-cultured with the autologous tumor cell line RCC-53 for 96h. PD-1 knockdown efficacy was assessed along with other markers of interest via flow cytometry before and after co-culture.

**Results** PH-762 treatment reduced PD-1 surface expression in TCR53 T cells after 24h by ~50% compared to UTC or NTC. PH-762 mediated PD-1 silencing increased the subset of TCF-1 positive T cells at 24h post compound treatment and continued through 96h of co-culture with tumor cells. The TCF-1 positive cells expressed stem-like markers including high expression levels of CD127 and CCR7 together with CD95 and lower levels of perforin.

**Conclusions** Increasing the proportion of stem-like CD8 T cells holds promise for optimizing ATT. PD-1 knockdown in TCR53 CD8 T cells for ATT by PH-762 induced the emergence of a T cell population expressing stem-like phenotypic markers including TCF-1. Further experiments are underway to assess the effects of the induced stem-like properties on a functional level, including proliferative activity and effector cell differentiation.

**REFERENCES**


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**P01.04 APEX-MEDIATED BIOTINYLLATION AS A POTENT TOOL FOR EVALUATION OF CHEMOKINE-RECEPTOR INTERACTIONS AND RESPECTIVE BINDING INHIBITORS**

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**Background** The immunosuppressive tumor microenvironment (TME), which is strongly shaped by regulatory T cells (Treg), represents a major drawback for anti-cancer immunity and cancer immunotherapy. The migration of Treg into the TME is mediated by the CC chemokine receptor 4 (CCR4) whose main ligand, CCL22, is overexpressed in many tumor entities and is associated with unfavorable prognosis. Therefore, therapeutic blockade of the CCR4-CCL22 axis to suppress Treg migration is a promising strategy to overcome tumor-derived immune suppression. To study such chemokine-receptor interactions and to assess the binding capacity of potential inhibitors, we aimed to establish a screening tool using APEX-mediated biotinylation.

**Materials and Methods** In the presence of hydrogen peroxide, the enzyme ascorbate peroxidase (APEX) oxidizes biotin-phenol to short-lived, highly reactive radicals that biotinylate structures in close proximity. Using a streptavidin-linked fluorophore that strongly binds biotin, the biotinylated structures can subsequently be analyzed and quantified via flow cytometry. To analyze the binding capacity of inhibitors of the CCR4-CCL22 axis, a CCL22-APEX fusion protein was created that binds to CCR4-expressing cells. Thus, the level of