**Introduction**

PACT Pharma is developing NeoTCR-P1, a personalized adoptive T cell therapy, which is composed of antigen-derived, patient-autologous, CD8 and CD4 T cells that have been precision genome engineered to express an adaptive T cell receptor recognizing a neoepitope presented exclusively on the surface of the patient’s tumor cells. (Jacoby et al. Abstract: 4858)

Upon reinfusion of a defined dose into the patient, NeoTCR-P1 T cells are anticipated to traffic to tissues harboring tumor cells expressing the neo peptide in the context of the autologous HLA molecules. NeoTCR-P1 T cells will target T cell proliferation and secretion of effector molecules from the engineered T cells.

To demonstrate these activities, ex vivo mechanism-of-action studies were performed with NeoTCR-P1 T cells derived from the blood of healthy donors or patients with cancer. T cells were engineered to express the novel neo12 TCR or the F5 TCR isolated from a melanoma patient’s PBMCs using the IMAC technology, and FSC TCR, a clinically validated TCR against the tumor antigen. Phenotypic analysis was performed to determine the T cell subset distribution of the NeoTCR-P1 final cell product. Antigen-specific activity was characterized by measuring target-specific killing, proliferation and cytokine production.

**Methods**

Edited T cells: CD8 and CD4 T cells from healthy donors or patients with cancer were precision genome engineered (Jacoby et al. Abstract: 4858) to express the neo12 TCR or the F5 TCR.

Co-Culture assay: NeoTCR-P1 T cells were co-cultured with K562 cells expressing HLA-A02 pulsed with different concentrations of peptides (0-1000 nM) or with K562 cells constitutively expressing peptide-HLA complex at a final P:T ratio of 4:1.

Cytokine secretion was measured in the cell supernatant at 24h with a cytokine bead assay. Mismatched: surrogate tumor target cells expressing MART1-HLA-A2 complex; Positive control: NeoTCR-P1 T cells expressing the neo12 TCR were co-cultured with tumor cells expressing 246-254 and the specific neoantigen (neo12) and HLA-A02 complex. At baseline, added (red) and non-added (green) TCR IgG were round and smaller in size than tumor cells (grey). After reprogramming expressing cancerous cells, neoTCR T cells became elongated, formed immunological synapses and killed the target tumor cell. The neo12 TCR specifically killed tumor cells to show that cytokine activity. Images were taken at 24h intervals.

Specific killing of antigen-expressing surrogate tumor target cells and antigen-specific proliferation of NeoTCR-P1 T cells

**Results**

NeoTCR-P1 T cells are mainly of the “younger” Tscm & Tcm phenotype

NeoTCR-P1 T cells rapidly convert to effector cells on antigen exposure

NeoTCR-P1 polyfunctional responses are strongly driven by proteins associated with effector function

**Conclusions**

- Final NeoTCR-P1 product consist mainly of T cells of the “younger” Tscm and Tcm phenotype
- NeoTCR-P1 cells rapidly convert to effector cells upon contact with neoantigen
- NeoTCR-P1 cells can be manufactured from T cells of both healthy donors or patients with cancer
- NeoTCR-P1 cells are highly polyfunctional, even when exposed to low concentrations of cognate peptide stimulation
- Taken together, these ex vivo mechanism-of-action studies demonstrate that PACT NeoTCR-P1 cells rapidly turn into highly active tumor-killing lymphocytes upon encounter of cognate tumor cells expressing the tumor-exclusive mutated antigen, with the potential to eradicate tumor cells throughout the body.