T cells precision engineered to express neoepitope-specific TCRs cloned from a patient with colorectal cancer, specifically target and kill relevant neoantigen-expressing tumor cells

Andrew Conroy, Barbara Sennino, Adam Litterman, John Gagnon, Bhamini Purandare, Zheng Pan, Olivier Dalmas, Kyle Jacoby, Songming Peng, Alex Franzusoff, Stefanie Mandl
PACT Pharma, 2 Corporate Drive, South San Francisco, CA 94080, USA.

Abstract

Background: Neoepitopes (neoE) derived from private tumor-exclusive mutations represent compelling targets for personalized TCR-T cell therapy to eradicate tumor cells throughout the body. The imPACT Isolation Technology® is an ultra-sensitive and high-throughput process for the capture of mutation-targeted CD8 T cells from patient blood. NeoE-specific TCRs of native sequence (neoTCRs), cloned from the captured T cells, were evaluated for tumor-targeted functionality by non-viral precision genome engineering of fresh human CD8 and CD4 T cells for neoTCR expression [Sennino et al. AACR 2019; Jacoby et al. AACR 2019].

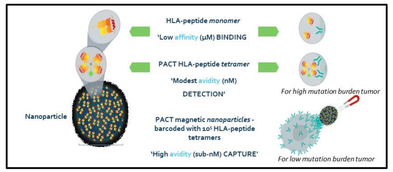
Methods: NeoTCRs were isolated from the blood of a patient with colorectal cancer using the imPACT Isolation Technology® [Peng et al. AACR 2019]. Subsequently, healthy donor CD8 and CD4 T cells were precision genome engineered to replace endogenous TCRs with the native neoTCR sequence for expression at native TCR levels. Precision genome engineering was used to generate a colon cancer tumor cell line (SW620) with the patient-specific non-synonymous mutation resulting in expression of the neoantigen (COX6C-R20Q) at native levels. NeoTCR-T cells were co-cultured with SW620 expressing the COX6C-R20Q mutation or the COX6C wild-type (WT) peptide. Functional readouts were T cell proliferation, cytokine secretion and tumor cell killing.

Results: Seven neoTCR clonotypes against the mutated COX6C peptide (COX6C-R20Q) presented in the context of HLA-A2 were cloned from imPACT-captured neoE-specific CD8 T cells. Primary human T cells were engineered with the 7 different TCR specificities against the COX6C-R20Q. Each of the seven candidate neoTCR-engineered T cell products displayed specific cytotoxicity against tumor cells expressing endogenous levels of the COX6C-R20Q neoantigen. At 96 hours, using a Product to Tumor cell ratio (P:T) of 1:1, 85-90% tumor elimination was observed ($p < 0.000001$ for each comparison). Significant tumor cell killing was detected with an P:T ratio as low as 1:5. neoTCR-T cells also proliferated and secreted interferon-gamma (IFNγ) in response to co-culture with the relevant tumor target. Importantly, neoTCR-T cell activity was absent when co-cultured with tumor cells expressing wild-type COXC6 protein.

Conclusions: These results demonstrate, that the imPACT Isolation Technology® used to capture antigen-experienced, neoE-specific T cells from the blood of patients with cancer *authenticates* that these neoE-HLA targets are relevant for engineering neoTCR-T cells therapies. Leveraging this approach, PACT is developing autologous personalized adoptive T cell therapy (NeoTCR-P1 product). A Phase 1 clinical trial to test NeoTCR-P1 T cells in subjects with solid tumors is currently ongoing (NCT03970382).

Methods

imPACT Isolation Technology®: Diagram illustrating the capturing of NeoE-specific T cells from the patient blood



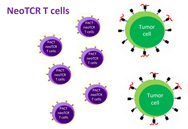
Gene editing: CD8 and CD4 T cells from healthy donors were precision genome engineered to express the neoTCRs. Briefly, neoE-specific TCR sequences were cloned into homologous recombination (HR) DNA templates. These HR templates were used with site-specific nucleases to engineer primary human T cells. The single-step (non-viral) precision genome engineering results in the seamless replacement of the endogenous TCR with the patient's neoE-specific TCR (of native sequence), whose expression is under endogenous regulation.

PACT precision genome engineering was furthermore used to generate stable tumor cell lines expressing the COX6C R20Q mutated neoantigen under control of endogenous regulatory elements. The mutated neoantigen differed from the wild-type only by a single amino acidic (R20Q).

Co-Culture assay: NeoTCR-P1 T cells were co-cultured with a tumor cell line expressing the COX6C mutant or wild-type at a final Product to Target (P:T) ratio of 1:1 or 5:1. Target cell killing was evaluated over 6 days using the IncuCyte system. Cytokine secretion was measured in the cell supernatant at 48h using the BD Cytokine Bead Array (CBA) Human Th1/Th2 Cytokine Kit II.

Schematic of cell-based assay:

Target tumor cells:
- SW620 expressing mutant COX6C
- SW620 expressing wild-type COX6C (control)



Read-outs:
- **Target cell killing**
- **Cytokine secretion**

Results

imPACT Isolation Technology® identification of NeoE-specific T cells from blood of a treatment-naïve patient with colorectal cancer

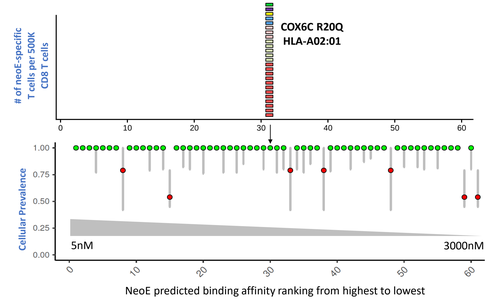


Figure 1. A. *NeoE-specific T cells isolated from PBMC sample of a treatment-naïve patient with colorectal cancer using imPACT Isolation Technology®. NeoE derived from non-synonymous mutations were predicted using the Whole Exome Sequencing (WES) and RNAseq from the tumor biopsy and WES from PBMC and were ranked according to the predicted HLA-binding affinity, the clonality of the mutation and the level of expression. HLA-NeoE capture reagents for the top-ranked NeoE were used to isolate the NeoE- specific T cells.*
B. *Seven different neoTCRs were identified against the COX6C R20Q mutation presented by HLA-A02:01. These neoTCRs were expressed in healthy donor T cells. The functional activity of the resulting T cell products was tested in subsequent assays.*

Generation of tumor cell line expressing COX6C-R20Q mutant

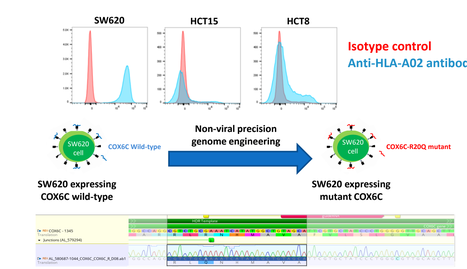


Figure 2. A. *Three commercially available colorectal cancer cell lines were tested for HLA-A02 expression. The SW620 cell line was selected for the highest HLA-A02 expression.* ***B.*** *SW620 tumor cells were precision genome engineered to express the R20Q point mutation in the COX6C gene. Sanger sequencing of COX6C amplicon demonstrates homozygous editing for R20Q mutation.*

Engineered (CD8 & CD4) NeoTCR-T cells specifically kill tumor cells expressing endogenous levels of neoantigen

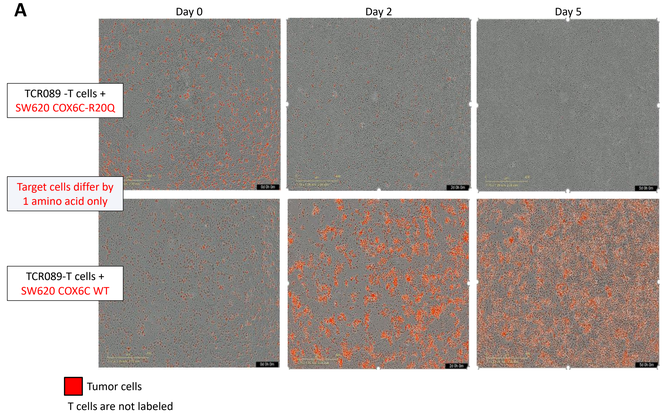


Figure 3. A. *Time-lapse microscopy of tumor cell death and T cell proliferation. NeoTCR-T cells were co-cultured with SW620 tumor cells expressing the R20Q mutated or wild-type COXC6 together with a red fluorescent protein (nuclear RFP) in a stable manner. Images shown here were collected at time 0 (left panels), 2 days (middle panels) and 5 days (right panels). T cells were not labeled in this experiment, but antigen-specific proliferation can be appreciated visually by increased numbers of T cells over the course of 5 days (top images).*
B. *Quantification of tumor cell confluency (percentage of nuclear RFP) over 6 days. * p<0.05 compared to controls (t test with Holm-Sidak method for multiple comparison correction). Negative controls were either tumor cell line cocultured with mock control T cells, only media (RPMI) or T cells expressing an unrelated neoTCR (Neo12 T cells), respectively.*

Engineered (CD8 & CD4) NeoTCR-T cells specifically kill tumor cells expressing endogenous levels of neoantigen at low P:T ratio

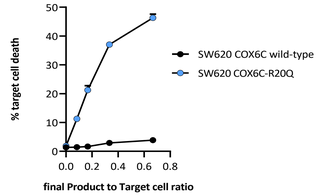


Figure 4. *TCR089 expressing T cells were co-cultured with SW620 tumor cells expressing the wild-type or the mutant COX6C at different product neoTCR T cell: target cell ratios; target cell killing was measured after 24 hours.*

NeoTCR-T cells specifically secrete cytokines upon coculture with tumor cells expressing endogenous levels of neoantigen

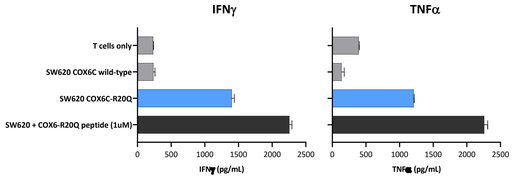


Figure 5. *Graphs showing the amount of secreted IFNγ and TNFα secreted after 24 hours. TCR089 expressing T cells were co-cultured with SW620 tumor cells expressing the wild-type or the mutated COX6C. TCR089 T cell alone were used as negative control. TCR089 T cell cocultured with SW620 tumor cells pulsed with 1μM of peptide (COXC6-R20Q) were used as positive control. No secretion was observed with the SW620 expressing the wild-type.*

Conclusions

- Using the ultra-sensitive and high-throughput imPACT Isolation Technology® neoepitope-specific TCRs (neoTCRs) were successfully isolated from PBMCs of a treatment-naïve patient with colorectal cancer.
- To study functional activities mediated by the isolated neoE-specific TCRs, non-viral precision genome engineering was used to replace the endogenous TCR of primary human T cells with the isolated neoTCRs to generate neoE-specific T cells (NeoTCR-T cells).
- Non-viral precision genome engineering was also used to generate a tumor cell line that expressed the mutated neoantigen at native levels by introducing a point mutation in the wild-type gene.
- Newly generated NeoTCR-T cells expressing TCRs isolated using the imPACT technology® specifically killed tumor cells expressing the mutated neoantigen but not the wild-type parental cell line.

Leveraging the imPACT Isolation Technology, PACT is developing autologous personalized adoptive T cell therapy (NeoTCR-P1 product). A Phase 1 clinical trial to test NeoTCR-P1 T cells in subjects with solid tumors is currently ongoing (NCT03970382).