Coexpression of MHC class I–restricted neoTCRs and ectopic CD8 receptors in precision genome engineered CD4 T cells significantly potentiates antigen-specific effector functions

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

Abstract # 895
bsennino@pactpharma.com

Abstract

Neoepitopes (NeoE) from tumor-exclusive mutations represent compelling targets for personalized neoE-specific autologous TCR-T cell therapies for patients with solid tumors. The imPACT Isolation Technology® is an ultra-sensitive and high-throughput process for capturing neoE-specific CD8 T cells from the blood of patients with solid cancers. Leveraging this technology, neoepitope-specific MHC class I-restricted TCRs (MHC-I neoTCRs) are cloned from individually captured CD8 T cells. Using DNA-mediated (non-viral) gene editing, fresh CD8 and CD4 T cells from the same patient with cancer are engineered to express the MHC-I neoTCR (concomitant with elimination of the endogenous TCR).

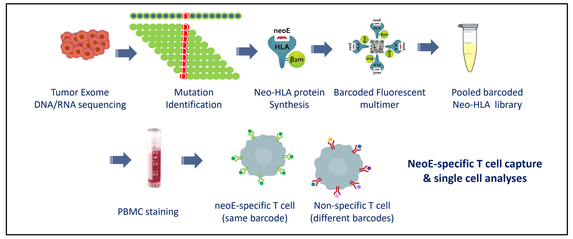
While naturally occurring MHC-I TCRs are presumed to require concurrent CD8 coreceptor help to stabilize peptide-MHC binding, higher affinity TCRs drive CD8-independent target binding and T cell activation. CD4 T cells, when engineered with high affinity neoTCRs, are thus able to recognize peptide-MHC-I targets and trigger effector T cell functions. However, lower affinity TCRs are dependent on CD8 coreceptors to trigger T cell activation. By precision genome engineering CD8 coreceptor genes together with the neoTCR into CD4 T cells, MHC-I neoTCRs are now competent to trigger antigen-specific effector T cell function.

In this study, MHC-I neoTCRs were cloned from neoE-specific T cells captured from the blood of a patient with colorectal cancer. Healthy donor CD8 and CD4 T cells were precision genome engineered to express the cloned MHC-I neoTCRs alone or to include engineering of ectopic CD8 coreceptors in the gene-edited T cells. Flow cytometric analysis was used to evaluate surface expression of neoTCRs and ectopic CD8 coreceptors, respectively. Rescue of neoTCR binding among CD4 T cells for lower affinity, CD8-dependent neoTCRs was observed. Importantly, in response to stimulation with cognate antigen, CD107a and intracellular IFNγ staining revealed 10-100-fold increases in the sensitivity of MHC-I neoTCR-induced effector functions by CD4 T cells, with no effect on specificity. No change in functionality or sensitivity was seen on CD8 T cells by the expression of additional CD8 coreceptor.

These results demonstrate that simultaneous precision genome engineering of the CD8 coreceptor together with CD8-dependent MHC-I neoTCRs into CD4 T cells significantly increases their sensitivity to neoE-HLA target recognition as well as triggering pro-inflammatory and cytotoxic function, yet without compromising antigen-specificity.

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 alone or together with ectopic CD8 coreceptors. Briefly, neoE-specific TCR and CD8 coreceptor 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.
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).

Functional assays: CD8 and CD4 T cells were stimulated with different concentrations of cognate neoE-HLA for 24hour. After stimulation, intracellular IFNγ and CD107a staining in CD4 and CD8 T cells was assessed by flow cytometry. Mismatched neoE-HLA was used as negative control. EC_{50} was calculated as the concentration of neoE-HLA that induced half-maximal response.

Target cell killing assays: CD4 T cells expressing the neoTCR with or without CD8 constructs were isolated and then co-cultured with a tumor cell line expressing the COX6C mutant in one allele (heterozygous) at a final Product to Target (P:T) ratio of 1:1 or 1:2. Target cell killing was evaluated over 4 days using the IncuCyte System.

Results

CD4 T cells expressing high affinity MHC-I restricted neoTCRs show antigen-specific effector function

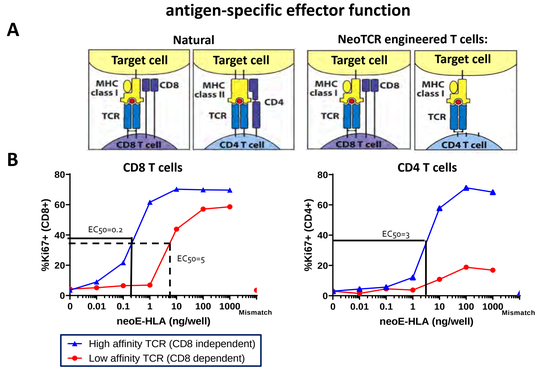


Figure 1. A. Schematic. *TCR binding to peptide-HLA is naturally supported by concurrent coreceptor expression on T cells, CD8 binds MHC-I while CD4 binds to MHC-II. neoTCR P-1 are precision genome engineered to express MHC-I restricted neoTCRs in CD8 and in CD4 T cells (diagram modified from Garland (2005) Science).*
B. Proliferation assay *(Ki67) of CD8 (left) and CD4 T cells (right) express a high affinity (blue) or a low affinity neoTCR (red) cloned from neoE-specific CD8 T cells. These cells were stimulated with increasing concentration of neoE-HLA proteins. Two log-differences in EC_{50} were observed between CD8 T cells expressing low and high affinity TCRs. CD4 T cells expressing high affinity neoTCRs (blue) proliferate upon exposure to neoE-HLA stimulation while no or minimum activity was observed in CD4 T cells expressing low affinity neoTCRs (red).*

Patient-specific neoTCRs of different affinities and cognate tumor cells

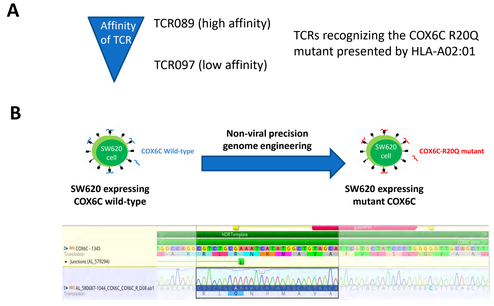


Figure 2. A. The neoTCRs. *Two neoTCRs against the same COX6C R20Q mutation presented by HLA-A02:01, TCR089 (CD8-independent) and TCR097 (CD8-dependent), were isolated from a patient with colorectal cancer by imPACT Isolation Technology®. These neoTCRs were expressed in healthy donor CD4 and CD8 T cells together with CD8 coreceptor constructs. The functional activity of the resulting T cell products was tested in subsequent assays.* ***B. The tumor cells.*** *The SW620 colorectal tumor cell line was precision genome engineered to express the R20Q point mutation in the COX6C gene. Sanger sequencing of COX6C amplicon demonstrates editing for the R20Q mutation.*

CD8 coreceptor expression boosts CD4 T cell sensitivity for low affinity TCRs

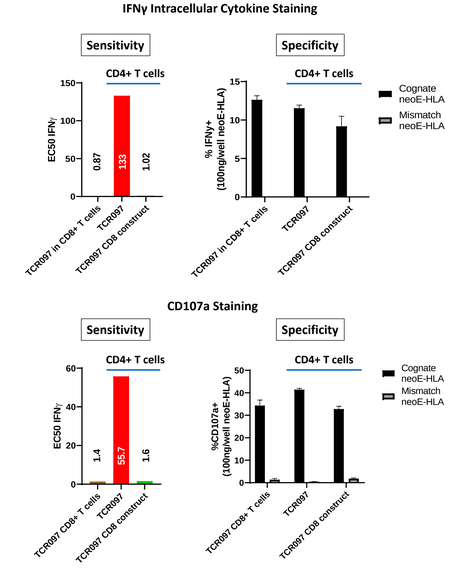


Figure 3. CD4 T cells expressing TCR097 (CD8 dependent, low affinity) *alone or together with a CD8 coreceptor construct were stimulated with neoE-HLA. After 24h IFNγ* ***(A)*** *or CD107a intracellular staining* ***(B)*** *was performed and EC_{50} was determined. Expression of CD8 coreceptors in TCR097 CD4 T cells reduced IFNγ EC_{50} by ~130 fold and CD107a EC_{50} by ~35 fold compared to TCR097 CD4 T cells. Addition of CD8 coreceptor preserved TCR specificity.*

CD8 coreceptor expression boosts CD4 T cell sensitivity for high affinity TCRs

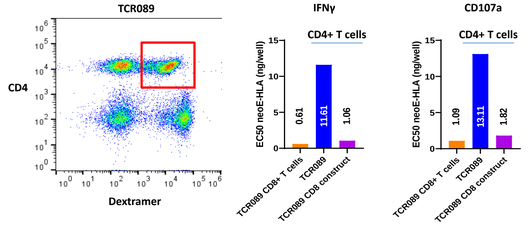


Figure 4. CD4 T cells expressing TCR089 (CD8-independent, high affinity) *alone or together with the CD8 construct were stimulated with cognate neoE-HLA. After 24h, T cell function was assessed by intracellular staining for IFNγ* ***(A)*** *or CD107a* ***(B)*** *and EC_{50} was determined. Expression of CD8 coreceptors in TCR089 CD4 T cells improved IFNγ EC_{50} ~11 fold and CD107a EC_{50} ~7 fold compared to TCR097 CD4 T cells.*

CD8 coreceptor expression rescues killing of tumor target cells expressing low levels of endogenous neoantigen

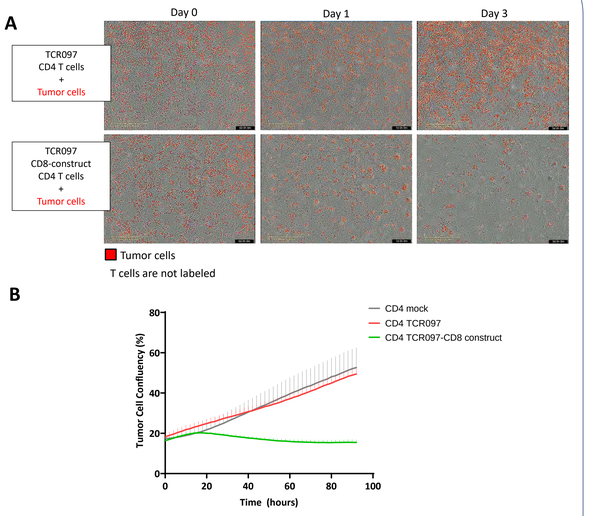


Figure 5. *Time-lapse microscopy of tumor cell death and T cell proliferation. TCR097 CD4 T cells (top) or TCR097 CD8 coreceptor expressing CD4 T cells (bottom) were co-cultured with SW620 tumor cells expressing the R20Q mutated COXC6 together with a red fluorescent protein (nuclear RFP) in a stable manner. Images shown here were collected at time 0 (left panels), 1 day (middle panels) and 3 days (right panels).* ***B.*** *Quantification of tumor cell confluency (percentage of nuclear RFP) over ~4 days. * $p<0.05$ compared to TCR097 CD4 T cells. Negative control = mock CD4 T cells.*

Conclusions

- Engineering CD8 coreceptor together with CD8-dependent MHC-I restricted TCR (poster #7883) increased CD4 T cells sensitivity, as measured by cytokine production and CD107a expression without compromising antigen-specificity.
- Importantly, CD8 coreceptor expression also increased CD4 T cell sensitivity among CD8-independent (high affinity) MHC-I TCRs.
- Simultaneous precision genome engineering of the CD8 coreceptor together with MHC-I neoTCRs into CD4 T cells significantly increases their sensitivity to neoE-HLA target recognition, allowing killing of tumor cells expressing low neoantigen target levels.