Permanent Abstract Number: 895
Title: Coexpression of MHC class I-restricted neoTCRs and ectopic CD8 receptors in precision genome engineered CD4 T cells significantly potentiates antigen-specific effector functions
Presenter: Barbara Sennino
Authors: Barbara Sennino, Andrew Conroy, Bhamini Purandare, Kyle Jacoby, Olivier Dalmas, Songming Peng, Alex Franzusoff, Stefanie Mandl
Session Category: Immunology
Session Title: Adoptive Cell Therapy 1
Session Date and Time: Sunday Apr 26, 2020 1:30 PM - 5:00 PM
Location: San Diego Convention Center, Exhibit Halls A-F, Poster Section 46
Poster Board Number: 24

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 co-receptor 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 co-receptors to trigger T cell activation. By precision genome engineering CD8 co-receptor 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 co-receptors in the gene-edited T cells. Flow cytometric analysis was used to evaluate surface expression of neoTCRs and ectopic CD8 co-receptors, 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 IFNy 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 co-receptor.

These results demonstrate that simultaneous precision genome engineering of the CD8 co-receptor 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.

Permanent Abstract Number: 2192
Title: Non-viral genome engineering method allows highly efficient, single-step removal and precise insertion of multiple large genes
Presenter: Kyle Jacoby
Authors: Kyle Jacoby, William Lu, Diana Nguyen, Barbara Sennino, Andrew Conroy, Bhamini Purandare, Alex Franzusoff, Stefanie Mandl
Session Category: Immunology
Session Title: Adoptive Cell Therapy 2
Session Date and Time: Monday Apr 27, 2020 9:00 AM - 12:30 PM
Location: San Diego Convention Center, Exhibit Halls A-F, Poster Section 47
Poster Board Number: 24

Recent advances in gene editing have enabled the targeted engineering of primary cells to insert entire transgenes without the use of viral vectors. Using these methods, novel genes can be inserted in a seamless manner into the specified genomic locus, realizing the goal of precise targeted genomic modifications. Non-viral modification of primary cells holds potential to significantly reduce costs and time needed for the generation of therapeutic cell products. It has also allowed the generation of personalized cell therapies involving patient-specific manufacturing of DNA constructs, such as for the developed of patient-derived, neoepitope-specific TCR-T cells.

Proof-of-concept experiments have shown that non-viral genome engineering methods can integrate small genetic elements such as *GFP*, while the delivery of larger therapeutically relevant payloads at high efficiency has previously been challenging. Here we describe a proprietary single-step, DNA-mediated method developed for seamlessly engineering fully natural neoepitope-specific TCRs (neoTCR) into the endogenous TCR locus of primary human T cells at high efficiencies (i.e. >50% gene editing efficiency). This method allows the delivery of the two genes comprising the neoTCR without the need for selection necessitated by less efficient approaches.

To further evaluate this approach, fresh human donor T cells were engineered to express the patient-specific neoTCR plus two additional gene products, CD8α and CD8β (i.e. precise genome engineering of four ectopic genes). The data shows that these modifications were made at high efficiency, resulting in fully functional CD8 and CD4 T cells. Surface expression of CD8 coreceptor together with the neoE-targeted TCR increased T cell signaling sensitivity of the engineered neoTCR-T cells by 10-100 fold.

The potential for off-target cleavage or unexpected genomic outcomes was assessed using multiple methods, including a newly developed primary T cell GUIDE-seq assay. Despite multi-locus editing, no evidence of off-target insertion or unexpected genomic rearrangements were observed.

In summary, these results demonstrate the applicability of a single step, highly efficient method for manufacturing fresh human T cells into neoTCR-T cell therapies, engineered with multiple functionalities. This proprietary precision genome engineering technology supports the on-going Phase 1 clinical trial of personalized autologous, NeoTCR-P1 engineered T cell therapies for patients with solid tumors (NCT03970382).

Permanent Abstract Number: 3253
Title: A high-throughput platform to produce neoE-HLA libraries for capturing neoE-specific T cells from the peripheral blood of patients with solid tumors
Presenter: Olivier Dalmas
Authors: Olivier Dalmas, Zheng Pan, Christine Shieh, Allison Xu, Jason Kwa, Katharine Heeringa, Yan Ma, John Collins, Duo An, Boi Quach, Songming Peng, Alex Franzusoff
Session Category: Immunology
Session Title: Adoptive Cell Therapy 3
Session Date and Time: Monday Apr 27, 2020 1:30 PM - 5:00 PM
Location: San Diego Convention Center, Exhibit Halls A-F, Poster Section 45
Poster Board Number: 25

A critical step in the development of personalized neoTCR-T cell therapies is operational selection of tumor-exclusive neoantigen peptide targets (neoepitopes or neoE) presented by each person's own HLA proteins (neoE-HLA targets). We have developed a high-throughput, automated process for the cloning, expression and purification of soluble proteins comprising neoE peptide with beta2m and the HLA heavy chain fused into a single polypeptide (neoE-HLA proteins). Of the 13 thousand HLA alleles in the human population, with 6 HLA alleles expressed in each person, our HLA catalog enables rapid production of neoE-HLA protein candidate libraries, representing >4 of 6 HLAs for ≥95% of all patients, regardless of ethnicity. We apply our proprietary bioinformatics pipeline to predict and prioritize tumor-exclusive neoE-HLA candidates for each patient. We then generate neoE-HLA libraries in single production runs for up to several hundred neoE-HLA candidates per patient. These soluble neoE-HLA proteins are then assembled into barcoded snare multimers. These barcoded snare libraries are used to interrogate matched PBMCs from that patient to capture rare (>1 per 106) to abundant antigen-experienced CD8 T cells which specifically bind the cognate neoE-HLA tumor targets, using the imPACT Isolation Technology®.

This platform is capable of producing barcoded snares predicted to represent high affinity (≤500 nM) and low affinity (>500nM) neoE binding to their cognate HLAs. These capabilities permit deep and broad interrogation of the tumor-targeted neoE-specific T cell repertoire in the blood of patients with solid cancers. Data shown here demonstrate that >85% of patients with different cancer types, indeed, harbor a surprising repertoire of tumor-targeted neoE-specific T cells. These antigen-experienced T cells reveal the pre-existing immune response to each person's cancer, and how that repertoire evolves in longitudinal analyses of patients that respond/do not respond to their cancer therapies.

In summary, PACT's proprietary neoE-HLA prediction pipeline and the proprietary high-throughput neoE-HLA protein production platform enables the discovery of tumor-targeted, neoE-specific T cells from patients. We leverage the neoTCRs cloned from these captured T cells to engineer fresh T cells from the patient into a full dose of tumor-targeted personalized neoTCR-T cells (NeoTCR-P1 T cell therapy). This fully personalized NeoTCR-P1 cell therapy is manufactured in fully enclosed, automatable systems for each individual patient with solid tumors. These breakthrough technologies support the on-going Phase 1 clinical trial of personalized engineered autologous NeoTCR-P1 T cell therapies for patients with six different solid tumor types (NCT03970382).

Oral Presentation

Title: Landscape analysis of neoepitope-specific T cell responses to immunotherapy
Presenter: Cristina Puig-Saus
Authors: Cristina Puig-Saus, Barbara Sennino, Bhamini Purandare, Duo An, Boi Quach, Songming Peng, Huiming Xia, Sidi Zhao, Zheng Pan, Yan Ma, Justin Saco, Sameeha Jilani, Christine Shieh, Katharine Heeringa, Olivier Dalmas, Robert Moot, Diana Nguyen, William Lu, Kyle Jacoby, Andrew Conroy, Jasreet Hundal, Malachi Griffith, Stefanie Mandl, Alex Franzusoff, Antoni Ribas.
Session Category: session SY26
Session Title: TCR Targeting of Mutational Neoantigens
Session Date and Time: Wednesday April 29, 2020 10:15 AM-12:00 PM
Location: San Diego Convention Center, **Ballroom 20 A-C - Upper Lvl**

In infectious disease, polyclonal T cell responses against immunodominant epitopes drive successful immune responses. In cancer, neoepitopes (neoE) derived from non-synonymous mutations, similarly to the immunodominant epitopes in viral infections, are potentially highly immunogenic because the T cells recognizing these antigens are not subjected to the mechanisms of tolerance. Indeed, early studies support that neoE derived from non-synonymous mutations are the primary target of T cell responses induced by immune checkpoint blockade therapy and have been successfully targeted by adoptively transferred T cell therapies (ACT) in multiple cancer histologies. However, there is limited knowledge on the immunodominance and evolution of neoE's, or the clonality of the T cell responses against these neoE. Furthermore, little is known regarding the correlation between the presence and expansion of neoE-specific T cells and the clinical response to immunotherapy in patients.

To characterize the neoE-specific T cell responses induced after immunotherapy, we collected peripheral blood mononuclear cells (PBMCs) over time (longitudinally) and established expanded tumor infiltrating lymphocyte cultures (TILs) and autologous tumor cell lines from the patient's tumor biopsies. We performed whole exome and RNA sequencing of the tumor and normal tissue controls for the computational prediction and ranking of patient-specific neoEs. We then generated a library of capture reagents consisting of the patient HLA class I molecules loaded with predicted neoE (Peng et al. AACR 2019) and isolated neoE-specific T cells from the patients' PBMC or TIL samples. Once isolated, the paired neoE-specific TCR alpha and beta chains (neoTCR) from isolated T cells were obtained by single cell sequencing. For functional characterization of the neoTCRs, healthy donor primary human T cells were modified to express the neoTCR using CRISPR-based, non-viral precision genome engineering by replacing the endogenous TCR with the respective neoTCR (Jacoby et al., AACR 2019, Sennino et al., AACR 2019). These gene-edited T cells were then used in co-culture experiments with the patient autologous cell lines.

We analyzed T cell responses in three patients (PT1, PT2, and PT3) with metastatic melanoma receiving immunotherapy. PT1 had a fast and durable anti-tumor response to anti-PD-1 therapy. Sequencing identified 2556 somatic coding mutations. A library of 243 neoE-specific pMHC capture reagents across 3 HLA types, HLA-A*03:01, A*24:01, and C*12:03 was generated and used for screening of PBMCs or TILs derived from multiple longitudinal time points. Several hundred neoE-specific T cells were isolated. Importantly, this neoE-specific T cell response was comprised of 17 different neoE-specific T cells clones targeting only 5 different HLA-neoE complexes supporting the immunodominance hypothesis.

On the other hand, PT2 and PT3 showed marginal responses to immunotherapy. Patient two progressed after being treated with anti-PD1. This patient had 24 somatic coding mutations. Seventeen neoE-HLA reagents across 3 HLAs, B*35:03, C*12:03, and C*08:01 were generated and used to capture neoE-specific T cells from TILs and PBMCs. While 14 different TCRs targeting 7 HLA-neoE complexes were identified from expanded TILs, no neoE-reactive T cells were captured from the peripheral blood. PT3 presented with progressive disease after being treated with local TVEC. This patient had 61 somatic coding mutations; 78 neoE-specific pHLA capture

reagents covering HLA-A*02:01, A*03:01, B*07:02, C*05:01, and C*07:02 were generated and used to screen for neoE-specific T cells in the patient's TIL and PBMCs. In contrast to PT2, 2 different neoTCRs targeting the same HLA-neoE complexes were isolated from PBMCs, but none from TILs.

To further characterize the T cell responses from patients that responded or did not respond to immunotherapy, we generated 17 separate T cell products, each expressing a different neoTCR isolated from PT1, PT2 and PT3. For PT1, we characterized 14 different neoTCRs specific for neoE's in the mutated *IL8, PUM1 and TPP2* genes. All 14 T cell products displayed specific cytotoxicity against the matched autologous melanoma cell line established from a biopsy of patient one (50-75% tumor growth inhibition compared to melanoma cell line growth in co-culture with a mismatched control TCR, 96 hour assay using a product to target ratio (P:T) of 1:1, $p < 0.000001$ for each comparison). No cytotoxic effect against an unmatched human melanoma cell line was observed. Furthermore, neoE TCR T cells upregulated 4-1BB and OX-40, secreted IFNγ, IL-2, TNFα, and IL6, and induced T cell proliferation and degranulation. Again, no unspecific T cell activation was observed when T cells were co-cultured with unmatched targets. Interestingly, precision genome engineered T cell products expressing neoTCRs identified from patients that did not respond to therapy (PT2 and PT3), also potently killed autologous tumor cells. Three neoTCRs were studied (1 TCR for PT1 and 2 TCRs for PT3), and all three showed specific cytotoxicity against the matched autologous melanoma cell line (35-100% tumor growth inhibition compared to melanoma cell line growth in co-culture with a mismatched control TCR, 96 hour assay using P:T 5:1, $p < 0.05$ for each comparison). Additionally, upon co-culture with the matched melanoma cell line, but not against an unmatched melanoma cell line control, neoE TCR T cells upregulated 4-1BB and OX-40, secreted IFNγ, IL-2, TNFα, and IL6, and induced T cell proliferation and degranulation. These data demonstrate that even patients that did not respond to immunotherapy harbor neoTCRs that, when expressed in 'fresh' T cells, are able to kill the autologous tumor cell lines.

Using newly developed techniques to isolate and capture neoE-specific single T cells, as well as non-viral gene editing, we isolated and characterized neoE-specific T cells that can recognize the cancer cells and induce an anti-tumor response. We also studied the neoE immunodominance and TCR clonality over time of the natural T cell repertoire that induce anti-tumor responses to ICB therapy. Our results show that in a patient with a good response to anti-PD-1, there is a polyclonal response that targets a limited number of neoE-HLA complexes (2% of the neoE tested in the case of patient one) highlighting the immunodominance of these epitopes. Interestingly, different T cell clonotypes targeting the same mutations evolve over time, suggesting functional differences amongst the TCRs. In addition, our results demonstrate that even patients that did not respond to these therapies harbor neoE-specific T cells, as we were able to isolate neoE-specific T cells that recognized and killed patient-derived cancer cells. This suggests that even in patients that do not respond to immunotherapy, neoE-specific TCRs can be isolated and could be potentially used for personalized ACT. Finally, our results also show how non-viral precision genome engineering can successfully redirect T cells to neoE-expressing tumors, enabling the personalized ACT.

(under review for late-breaking abstract)

A Phase 1a/1b, Open-Label First-In-Human Study Of The Safety, Tolerability And Feasibility Of Gene-Edited Autologous NeoTCR-T Cells (NeoTCR-P1) Administered To Patients With Locally Advanced Or Metastatic Solid Tumors

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Introduction: Neoepitopes (neoE) derived from private tumor-exclusive mutations represent compelling targets for personalized TCR-T cell therapy. An ultra-sensitive and high-throughput process was developed to capture tumor mutation-targeted CD8 T cells from patient blood. NeoTCRs cloned from the captured CD8 T cells, when engineered into fresh CD8 and CD4 T cells, effected killing of patients' autologous tumor cells in vitro. These observations have been leveraged for the development of a fully personalized adoptive T cell therapy (NeoTCR-P1). A Phase 1 clinical trial testing NeoTCR-P1 in subjects with solid tumors is ongoing (NCT03970382).

Study Design: During the initial trial phase, escalating doses of NeoTCR-P1 T cells administered without and with IL-2 in the regimen, and following conditioning chemotherapy, will be evaluated in subjects with advanced or metastatic solid tumors (melanoma, urothelial cancer, colorectal cancer, ovarian cancer, HR^+ breast cancer, and prostate cancer). The objective of the Phase 1a study is to establish a recommended Phase 2 dose. Primary endpoints include the incidence and nature of DLTs and overall process feasibility. The proliferation, persistence, and trafficking of NeoTCR-T cells will be characterized. In the expansion trial phase, preliminary anti-tumor activity of NeoTCR-P1 will be assessed in selected tumors. The combination of NeoTCR-P1 dosing plus nivolumab will be tested in a Phase 1b study.

Conclusion: This is the first clinical study of an autologous, fully personalized adoptive T cell therapy directed against private tumor-exclusive mutations, generated without using recombinant viral vectors.