Enrichment of Neoantigen Reactive TIL in a CRC Patient Sample by FACS: The TIDAL-01 Process

Jake Nikota, Larissa A. Pikor, Anna Fritzsche, Zachary Jilesen, Sara Fallahi, Barbara Sennino, and David F. Stojdl

Turnstone Biologics, San Diego, CA, USA

Abstract #404

Background

Tumor infiltrating lymphocyte (TIL) therapy has proved to be most effective in melanoma. Additionally, enhancing tumor reactivity by selective expansion of individual TIL subpopulations, screened for neoantigen reactivity, has demonstrated some success in breast cancer[1]. However, while other solid tumors such as colorectal cancer (CRC) have been shown to contain neoantigen reactive TIL[2], the ability to selectively enrich these cells has been challenging. Here we demonstrate that the TIDAL-01 process, which utilizes tumor-specific mutation containing peptides to select neoantigen reactive TIL by fluorescence-activated cell sorting (FACS), can lead to a neoantigen targeted selected TIL product in CRC.

Methods

TIL were expanded from a cryopreserved dissociated CRC tumor sample and antigen presenting cells (APCs) were generated from patient-matched blood. Mutations were identified by sequencing and 13-40 amino acid peptides containing the mutation were generated. TIL were selected by FACS from a co-culture of TIL and peptide pulsed APCs based on the activation markers CD134 and CD137. Selected TIL were cultured with a rapid expansion protocol (REP) and the expanded TIL were phenotyped and co-cultured with neoantigen pulsed APCs to confirm reactivity. The unselected TIL were expanded by REP as a bulk control for comparison.

Results

A clear positive population of activated TIL (3.93%) were selected from the co-culture and expanded >1000-fold. The final TIL product was 93.6% CD4 cells and 4.60% CD8 cells and >90% of CD4 and CD8 cells expressed markers of an effector memory phenotype. Single cell sequencing of the T cell receptor repertoire revealed 12 clonotypes that were enriched in sorted TIL vs bulk TIL. In response to co-culture, 31.2% of CD8 cells and 25.6% of CD4 cells in the selected TIL product were positive for IFN-γ by intracellular cytokine staining. IFN-γ and TNF-α were 53 and 360-fold higher in the co-culture supernatants of selected TIL compared to bulk TIL, respectively. As a measure of killing potential, 20.9% of CD8 cells degranulated in co-culture based on CD107 expression and granzyme B secretion was increased 16.5-fold over bulk TIL. Deconvolution of the peptide pool identified one CD4 and one CD8 antigen driving the neoantigen reactivity.

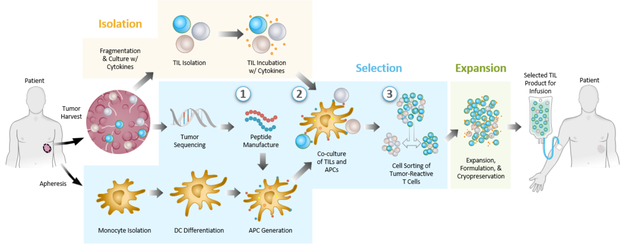
Conclusions

These data provide non-clinical proof of concept that neoantigen enriched TIL can be selected by FACS and expanded into a TIL product that contains a marked increase in neoantigen reactive TIL compared to bulk expanded TIL from a CRC tumor.

Methods

TIDAL-01 process

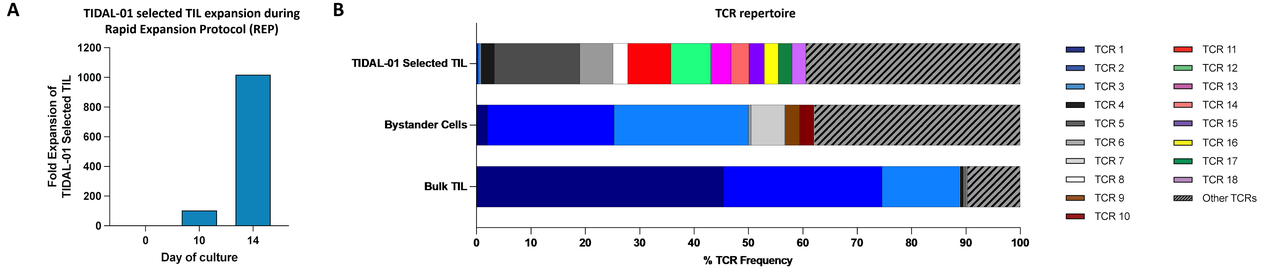
- Patient-specific mutations were predicted and peptides containing potential neoantigens were manufactured
- TIL from cryopreserved dissociated tumor cells of a CRC patient were enriched for neoantigen reactivity by FACS following co-culture with autologous APCs pulsed with the predicted peptides
- Sorted neoantigen positive (+/+) and negative (-/-, or bystander cells) TIL and bulk TIL (as control) were expanded using a Rapid Expansion Protocol (REP)



Key attributes:

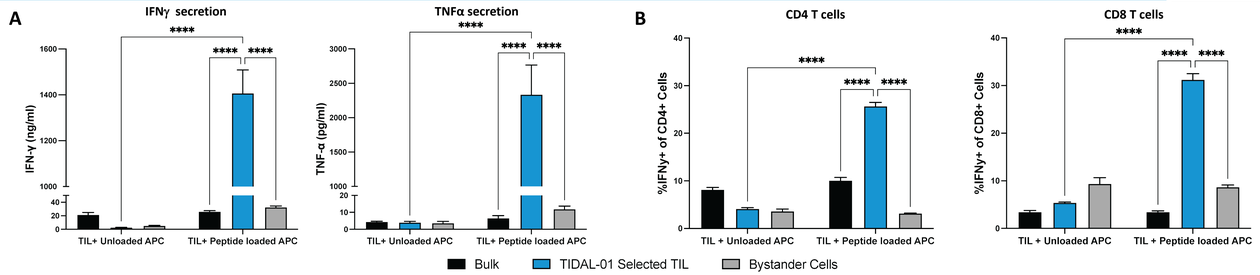
1) Unbiased mutation calling with peptides generated against a large breadth (~200) relevant tumor neoantigens
2) Co-culture with autologous DCs to optimize peptide-based antigen presentation and capture greatest TCR diversity within TIL population
3) Sort cells with validated markers to enrich for both CD4 and CD8 TILs with the highest tumor reactivity; removal of bystander cells

TIDAL-01 process enriches tumor-reactive TIL from a CRC sample with low TIL content



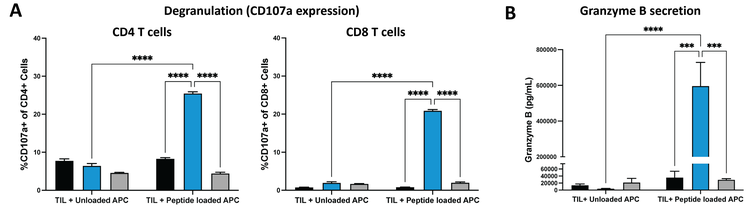
A TIL were sorted on CD134 and CD137 expression after coculture with APCs pulsed with a pool of 190 peptides containing predicted mutations and seeded into a REP. At the end of the REP (day 14) TIDAL-01 Selected TIL expanded 1000-fold relative to the number of TIL at Day 0 of culture. (**B**) scRNAseq of the expanded Bulk TIL, TIDAL-01 Selected TIL, and Bystander Cells at the end of REP revealed enrichment of several clonotypes in TIDAL-01 Selected TIL, including some that were very low frequency in the bulk population. By comparison, the profile of the Bystander Cells was similar to the Bulk TIL. Each color on the bar graph represents a unique TCR. The top 18 most frequent clonotypes are shown in solid colors while all other clonotypes identified by scRNA-seq for each sample are shown in the single hashed bar.

TIDAL-01 selected TIL displays superior cytokine expression compared to bulk TILs



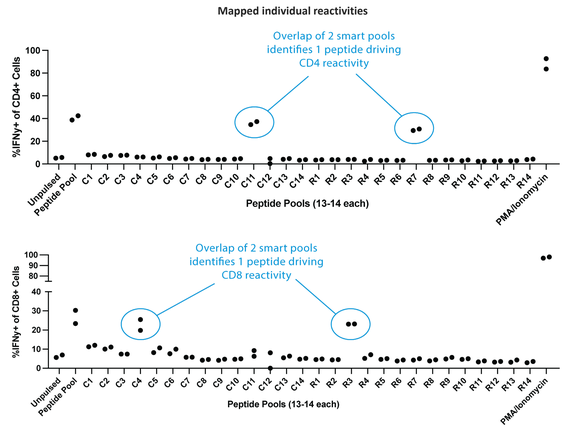
(**A**) IFNγ (left) and TNFα secretion (right) by Bulk TIL (black), TIDAL-01 Selected TIL (blue), and Bystander Cells (grey) TIL after co-culture with unloaded APC or APC pulsed with peptide pool. TIL were incubated with unloaded or peptide loaded APC for 24 hours at an effector to target (E:T) of 5:1 and supernatants collected and analyzed using LEGENDPlex™ assays. Selection of patient tumor-reactive TCRs results in superior cytokine expression in TIDAL-01 selected TIL in comparison to Bulk TIL in response to patient-tumor-specific neoantigens. (**B**) TIDAL-01 selected TIL show enhanced IFN-γ expression in both CD4+ & CD8+ cells. Bulk TIL (black), TIDAL-01 Selected TIL (blue), and Bystander Cells (grey) were cultured either alone or co-cultured at an effector to target ratio of 5:1 with unloaded or peptide loaded APCs. T cell activation was quantified by IFN-γ production after 5 hrs. of co-culture in the presence of GolgiPlug™ and GolgiStop™ in CD4+ (left) and CD8+ T cells (right). Error bars represent standard deviation of triplicate wells. ****P <0.0001, Two-way ANOVA.

TIDAL-01 selected TIL display greater cytotoxic potential compared to bulk TIL



(**A**) CD107a expression by Bulk TIL (black), TIDAL-01 Selected TIL (blue), and Bystander Cells (grey) co-cultured with unloaded or peptide loaded APCs at an effector to target ratio of 5:1. CD107a expression was assessed after 5 hrs of co-culture by flow cytometry. Expression of CD107a is a surrogate marker for the ability of T-cells to liberate multiple types of tumor-killing cytolytic enzymes. (**B**) Granzyme B production was quantified from supernatants collected after 24 hrs of co-culture and analyzed using a LEGENDPlex™ assay (left panel). Expression of the cytolytic enzyme Granzyme B represents key mechanism by which T cells kill tumor cells. TIDAL-01 Selected TIL produces significantly more CD107a and granzyme B than Bulk TIL or Bystander Cells when co-cultured with peptide loaded APCs, demonstrating enhanced reactivity that is specific to the peptide pool. Error bars depict standard deviation of triplicate wells. *** P < 0.0005, **** P < 0.0001, Two-way ANOVA.

TIL activation is driven by minority of tumor-derived neoantigen peptides



Individual peptide reactivities were deconvoluted and identified for CD4+ (top) and CD8+ (bottom) restricted reactivity within TIDAL-01 Selected TIL. TIDAL-01 Selected TIL was co-cultured for 5 hrs at an effector to target ratio of 5:1 with APC and IFN-γ production was quantified by flow cytometry within the CD4+ and CD8+ compartments. APC were either unloaded (unpulsed), loaded with the entire pool of 190 peptides (peptide pool) or loaded with smart peptide pools comprised of 13-14 peptides. Unloaded APC were used as the baseline and PMA/Ionomycin treated T cells as the positive control (maximal response). Within CD4 and CD8 T cells, a single peptide produced IFN-γ to levels equivalent to that produced in the pool co-culture. The overlap of the reactive pools was used to identify the specific reactive peptide.

Conclusions

- TIDAL-01 process successfully enriches for neoantigen reactive TIL in a CRC patient
  - TIL with less than 4% reactivity to predicted neoantigens were successfully selected and expanded
- TIDAL-01 process leads to a TIL product possessing a novel TCR repertoire compared to Bulk TIL
- TCRs associated with TIDAL-01 selected TIL drive superior T cell cytokine secretion in both CD4 and CD8 T cells in comparison to Bulk TIL
- TCRs associated with TIDAL-01 selected TIL show superior T cell degranulation compared to Bulk TIL

References

1) Zacharakis, N., Huq, L. M., Seitter, S. J., Kim, S. P., Gartner, J. J., Sindiri, S., Hill, V. K., Li, Y. F., Paria, B. C., Ray, S., et al. (2022). Breast Cancers Are Immunogenic: Immunologic Analyses and a Phase II Pilot Clinical Trial Using Mutation-Reactive Autologous Lymphocytes. J Clin Oncol, JCO2102170.

2) Parkhurst, M. R., Robbins, P. F., Tran, E., Prickett, T. D., Gartner, J. J., Jia, L., Ivey, G., Li, Y. F., El-Gamil, M., Lalani, A., et al. (2019). Unique Neoantigens Arise from Somatic Mutations in Patients with Gastrointestinal Cancers. Cancer Discov 9, 1022-1035.