TIDAL-01: A selected TIL process that enriches for neoantigen reactive TIL in solid tumors

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Background: Tumor infiltrating lymphocyte (TIL) therapy is capable of mediating durable complete responses in melanoma. While solid tumors such as colorectal cancer (CRC), non-small cell lung cancer (NSCLC), ovarian and breast have been shown to contain neoantigen reactive TIL, the success of bulk TIL therapy in these tumors has been limited. Enhancing tumor reactivity through the selective expansion of neoantigen-reactive subpopulations, has demonstrated success in cancers outside of melanoma underscoring the potential of a neoantigen selected TIL approach in indications with lower tumor mutational burdens[1,2]. Here we demonstrate that the TIDAL-01 process, which utilizes tumor-specific mutation containing peptides to select neoantigen-reactive TIL produces TIL products significantly enriched in neoantigen reactivity.

Methods: Fresh tumors were cut into fragments or dissociated and cultured in a primary expansion (preREP). Antigen presenting cells (APCs) were isolated and expanded from patient matched blood. Whole exome and RNA sequencing was performed on tumor tissue and autologous PBMCs and used to prioritize neoantigen mutations. Peptides encoding the mutations were synthesized, loaded onto APCs and co-cultured with autologous TIL. Thereafter, neoantigen-reactive TIL were selected by fluorescence activated cell sorting (FACS), based on the upregulation of the activation markers CD134 and CD137 and expanded with a rapid expansion protocol (REP). Bulk and unselected TIL were expanded alongside for comparison. Neoantigen reactivity was quantified and deconvoluted by cytokine secretion, degranulation, upregulation of CD134/CD137 by flow and when practical, killing of autologous tumor cell lines or organoids.

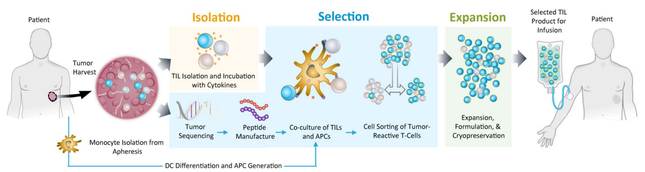
Results: Successful TIL expansion was achieved in 31/34 (91%) tumors (14/17 CRC, 10/10 NSCLC, 3/3 ovarian and 3/3 melanoma) using both tumor fragments and dissociated tumors. CRC tumors accounted for half of the samples (17/34), and the tumor mutational burden within these samples varied substantially, ranging from 229 to 5436 mutations. Upregulation of CD134 and CD137 and increased IFN-γ production was observed in all samples upon co-culture with peptide loaded APCs. Peptide restimulation and deconvolution revealed that the TIDAL-01 process is capable of enriching for both CD4 and CD8 reactivities. Selected TIL products produced up to 50x more IFN-γ, TNF-α and Granzyme B than bulk TIL and at least 2x higher levels of degranulation, indicative of greater killing potential.

Conclusions: TIL from metastatic CRC, melanoma, NSCLC and ovarian tumors were successfully expanded from the majority of patients. Co-culture of TIL and peptide loaded APCs followed by FACS significantly enriched for neoantigen reactivity compared to bulk TIL, demonstrating the potential of the TIDAL-01 process to produce selected TIL products for the treatment of melanoma and non-melanoma tumors.

Methods

TIDAL-01 process

- Mutations were defined by whole exome sequencing and prioritized peptides containing the potential neoantigens were manufactured.
- TIL from cryopreserved dissociated tumors were enriched for neoantigen reactivity by FACS following coculture with autologous antigen presenting cells (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) in a small-scale research representation of the cGMP-compliant clinical manufacturing process.



Key Selection Attributes:

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

Neoantigen reactive TIL can be isolated and expanded from both high and low TMB tumors

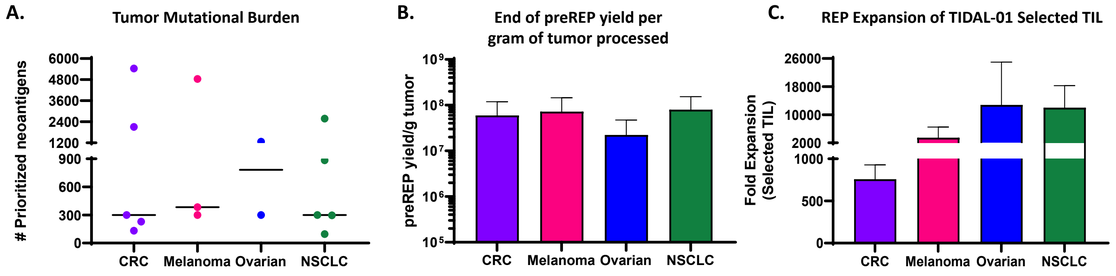


Figure 1. Tumor mutational burden and TIL expansion from CRC, Melanoma, NSCLC and Ovarian tumors. (**A**) Non-synonymous mutations were identified by WES of tumor and normal (PBMC) using and tumor specific neoantigens prioritized by the BFX pipeline using whole exome and transcriptome (RNAseq) sequencing (n=15). (**B**) Average preREP yield by tumor type per gram of tissue from fresh tumor fragments or single cell suspensions (n=31; 15 CRC, 3 melanoma, 3 ovarian and 10 NSCLC). (**C**) Average fold expansion of TIDAL-01 selected TIL at the end of REP for CRC (n=5) melanoma (n=2), ovarian (n=2) and NSCLC (n=2). Error bars depict Standard Error of the Mean (SEM).

TIDAL-01 Selected TIL display an effector memory phenotype and are functional upon TCR stimulation

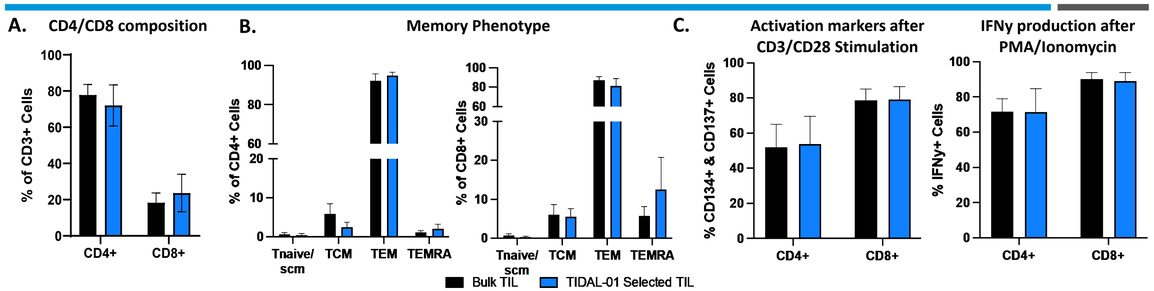


Figure 2. Phenotype and response to polyclonal TCR stimulation of bulk and TIDAL-01 selected TIL analyzed to date. (**A**) Frequency of CD4 and CD8 within bulk (black) and TIDAL-01 selected (blue) TIL products from CRC (n=3), melanoma (n=2), ovarian (n=1) & NSCLC (n=2). (**B**) Effector/memory subsets were defined within the CD4 (left) and CD8 (right) populations based on the expression of CD45RA and CCR7. TEM = effector memory T cells (CD45RA-CCR7-), TCM = central memory T cells (CD45RA-CCR7+), TSCM = Naïve/stem cell memory T cells (CD45RA+CCR7+) and TEMRA = effector T cells (CD45RA+CCR7-). **(C)** Bulk and TIDAL-01 selected TIL from CRC (n=3), melanoma (n=1) and ovarian cancer (n=1) were stimulated overnight with soluble CD3/CD28 activator (left) or for 5 hours with PMA/Ionomycin (right) and expression of CD134 and CD137 and IFNγ assessed by flow cytometry. Bars depict mean values and error bars the SEM.

TIDAL-01 selected TIL are oligoclonal and display focused TCR diversity relative to bulk and bystander TIL

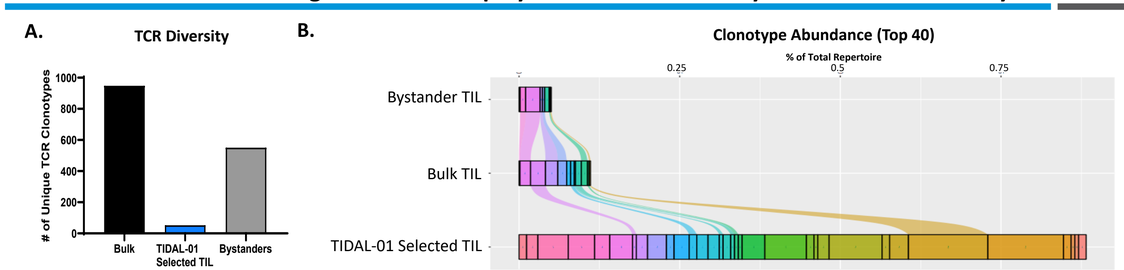


Figure 3. TIDAL-01 selected TIL from an ovarian tumor are enriched for tumor reactive TCRs and have reduced TCR diversity relative to bulk and bystander TIL. Single cell RNA sequencing was performed on bystander (grey), bulk (black) and TIDAL-01 selected TIL (blue) at the end of REP. A representative plot of (**A**) diversity and (**B**) abundance of TCR clonotypes are shown. Each color block represents a unique TCR and colored lines connecting samples indicate shared TCR clonotypes. The frequency of the Top 40 most abundant clonotypes across all samples are displayed.

TIDAL-01 selected TIL display enhanced cytokine secretion and cytotoxic potential against neoantigens

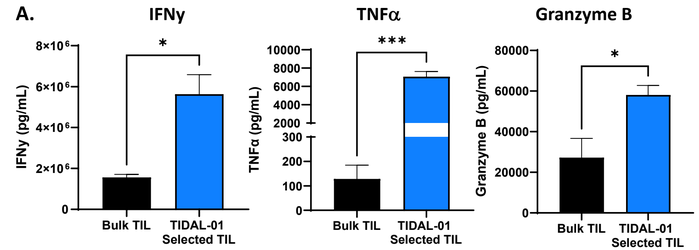


Figure 4. TIDAL-01 selected TIL produce more cytokine and have greater potential for cell killing than bulk TIL in response to neoantigen specific peptides. (**A**) Secretion of IFNγ (left), TNFα (middle) and Granzyme B (right) was quantified in supernatants collected 24 hrs after co-culture of bulk or TIDAL-01 selected TIL + peptide pulsed autologous APC (E:T of 5:1) from a CRC tumor. Error bars depict standard error of the mean. * P < 0.05, *** P < 0.001, Student's T test. (**B**) Flow cytometric analysis of CD107a expression in CD4+ (left) and CD8+ (right) TIL from bulk (black) and TIDAL-01 selected TIL (blue) from a CRC tumor, 5 hrs post co-culture with unpulsed or peptide pulsed APCs at an effector to target ratio of 5:1. (**C**) Average fold change in IFNγ reactivity of bulk and TIDAL-01 selected TIL (pulsed co-culture relative to unpulsed co-culture) within the CD4 and CD8 compartments for CRC (n=3), melanoma (n=1) and ovarian (n=1) samples. Error bars depict standard error of the mean. ** P < 0.01, *** P < 0.001, Two-way ANOVA.

Conclusions

1. TIDAL-01 selected TIL successfully enriches for neoantigen reactive TIL in multiple indications
2. TIDAL-01 selected TIL possess novel TCR repertoires compared to Bulk TIL
3. TCRs associated with TIDAL-01 selected TIL drive superior T cell cytokine production in selected T cells in comparison to Bulk TIL
4. CD107a and Granzyme B appear to be sensitive markers of the superior cytotoxic potential of TIDAL-01 selected TIL compared to Bulk TIL

References

1. Enrichment of Neoantigen Reactive TIL in a CRC Patient Sample by FACS: The TIDAL-01 Process. [Abstract 404] SITC 2022
2. Expansion and Identification of neoantigen reactive tumor infiltrating lymphocytes (TIL from metastatic colorectal cancer (CRC). [Abstract 387] SITC 2022