PD1-positive tumor-infiltrating lymphocytes (TIL) for the next generation of adoptive T cell therapy

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INTRODUCTION

Adoptive T cell therapy (ACT) with autologous tumor-infiltrating T cells (TIL) has demonstrated high response rates in patients with metastatic melanoma.[1] TIL products recognize tissue-specific antigens, neoantigens, and non-cancer related antigens.[2, 3] Neoantigen-specific T cells are considered the main contributors to the anti-tumor activity of TIL.[4] Strategies enriching TIL for the neoantigen-specific T cells are expected to yield more potent therapeutic products, especially in epithelial cancers which contain a high proportion of non-cancer specific T cells.[5] Several studies have demonstrated that expression of PD1 on TIL identifies the neoantigen-specific T cells.[6, 7, 8] Presented here is the development of a new process to produce neoantigen-specific-enriched TIL products for clinical application.

MATERIALS & METHODS

- PD1-positive ($PD1^+$) cells were sorted via flow cytometry directly from fresh tumor digests and expanded in vitro
- Samples from six melanomas, three sarcomas, six breast cancers, and eight lung cancers were evaluated
- 3 populations were studied:
  - $PD1^+$ sorted TIL
  - $PD1^-$ sorted TIL
  - Bulk TIL (whole tumor unsorted digest)
- TIL were evaluated for yield (cell count), phenotype (flow cytometry), TCR Vβ repertoire (RNA-sequencing), non-specific functionality (anti-CD3 and PMA), and tumor reactivity and killing (co-culture assays)

Figure 1. A streamlined protocol for expanding PD1+ TIL to clinically relevant levels

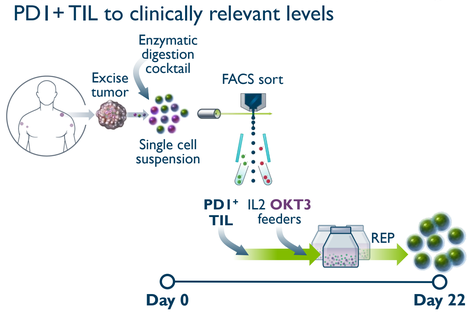

Figure 1. The tumor is excised from the patient and transported to Iovance research laboratories. Upon arrival, the tumor is digested, and the single-cell suspension stained for CD3 and PD1. PD1+ TIL are sorted by FACS using an FX500 instrument (Sony). The PD1+ cell fraction is placed into a flask with an anti-human CD3 antibody (OKT3; 30ng/ml) and irradiated allogeneic PBMCs (feeders) at 1:100 (TIL: feeder) ratio and rapidly expanded for 22 days (REP).

References

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RESULTS

Frequency of PD1+ TIL varies across tumor samples but *in vitro* expansion process reliably yields more than 1 billion TIL

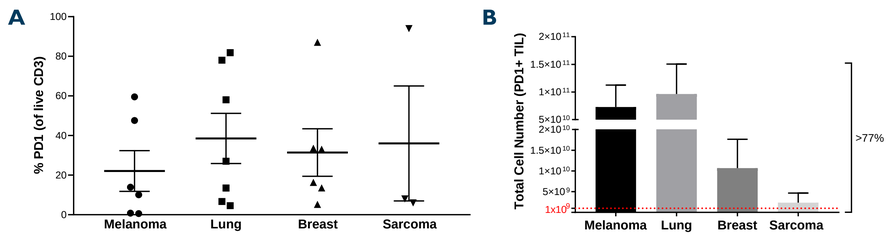


Figure 2: Selected and bulk TIL were expanded from melanoma (n=6), lung cancer (n=7), breast cancer (n=6), and sarcoma (n=3). **(A)** Frequencies of $PD1^+$ cells in fresh tumor digests are shown for each individual sample. Horizontal and vertical lines represent the mean values and standard errors, respectively. **(B)** $PD1^+$ and $PD1^-$ sorted cells, and bulk digests were expanded as described in Figure 1. Cells were counted at the completion of the REP and fold expansions (final cell count/seeding cell count) calculated that were used to extrapolate total cell counts. For Bulk TIL, seeding cell count was estimated using the percentage of T cells in the tumor digests. Mean values are plotted as bars and standard errors shown as vertical lines.

$PD1^+$ TIL demonstrate a different phenotypic profile, compared to $PD1^-$ TIL

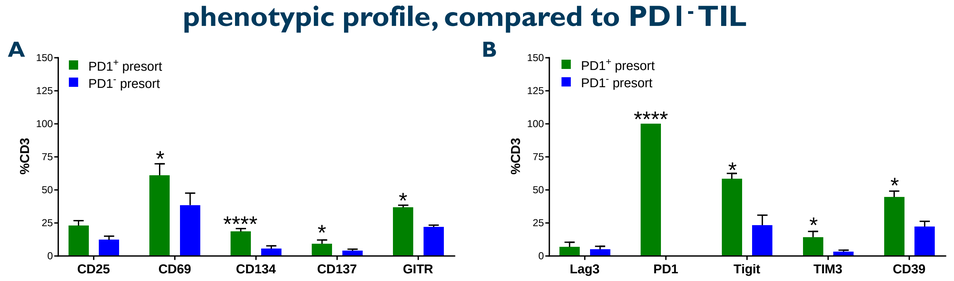
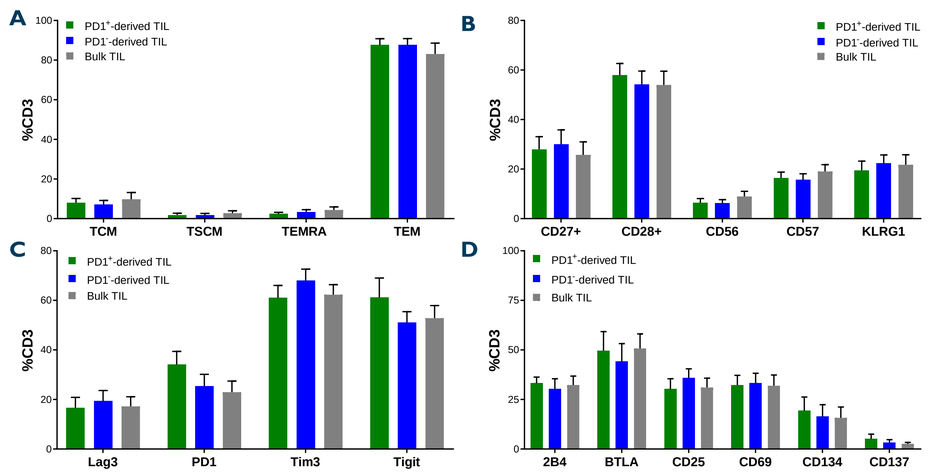
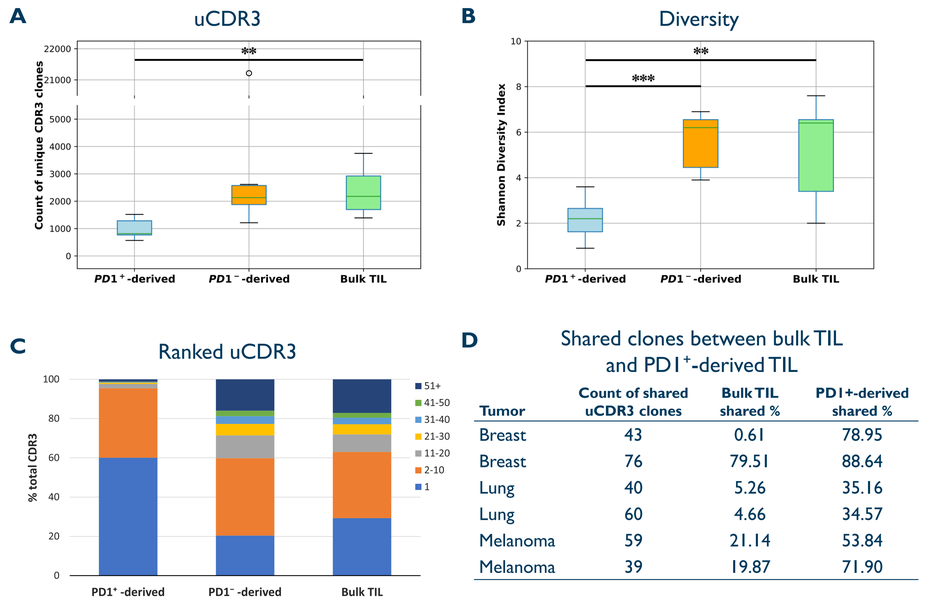


Figure 3. Digested tumors from melanoma (n=2), lung (n=2), and breast (n=2) were assessed phenotypically by flow cytometry, prior to sorting. **(B-C)** Live lymphocytes were gated on $CD3^+$ cells and assessed for $PD1^+$ and $PD1^-$. The PD1+ and PD1- populations were assessed for cell surface expression of **(B)** activation and **(C)** exhaustion markers. Mean values are plotted as bars and standard errors shown as vertical lines. Statistical significance was assessed by a paired student t-test ****P<0.0001, *p<0.05.

In vitro expanded $PD1^+$ TIL are phenotypically similar to bulk TIL



Expanded $PD1^+$ TIL are oligoclonal and comprise a fraction of the clones present in bulk TIL



D

Shared clones between bulk TIL and $PD1^+$-derived TIL

Tumor	Count of shared uCDR3 clones	Bulk TIL shared %	PD1+-derived shared %
Breast	43	0.61	78.95
Breast	76	79.51	88.64
Lung	40	5.26	35.16
Lung	60	4.66	34.57
Melanoma	59	21.14	53.84
Melanoma	39	19.87	71.90

Expanded PD1+ TIL are functional as determined by IFNγ secretion and CD107a mobilization in response to non-specific stimulation

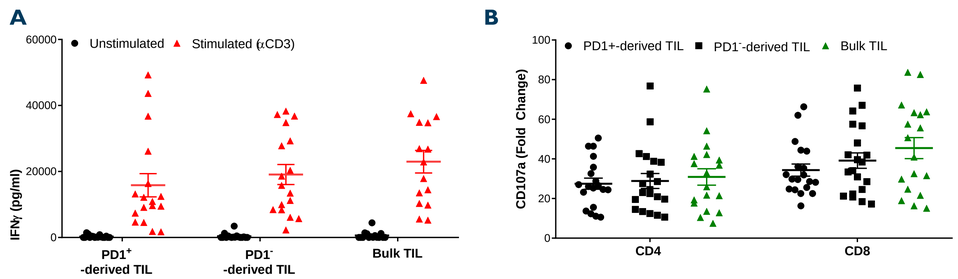


Figure 6: (A) $PD1^+$-derived TIL, $PD1^-$-derived TIL, and bulk TIL from melanoma (n=5), lung (n=6), and breast (n=6) were stimulated for 18 hours with plate-bound anti-CD3. Supernatants were assessed for IFNγ secretion by ELISA. Results are plotted for individual samples. **(B)** $PD1^+$-derived TIL, $PD1^-$-derived TIL, and bulk TIL from melanoma (n=5), lung (n=7), breast (n=6), and sarcoma (n=1) were assessed for CD107a cell surface expression in response to PMA stimulation for 4 hours on the CD4+ and CD8+ cells, by flow cytometry. Results are plotted for individual samples. Horizontal lines represent the mean percentages of each subset and vertical lines represent the standard errors.

Expanded $PD1^+$ TIL demonstrate an enhancement in autologous melanoma cell killing and tumor reactivity relative to $PD1^-$ TIL

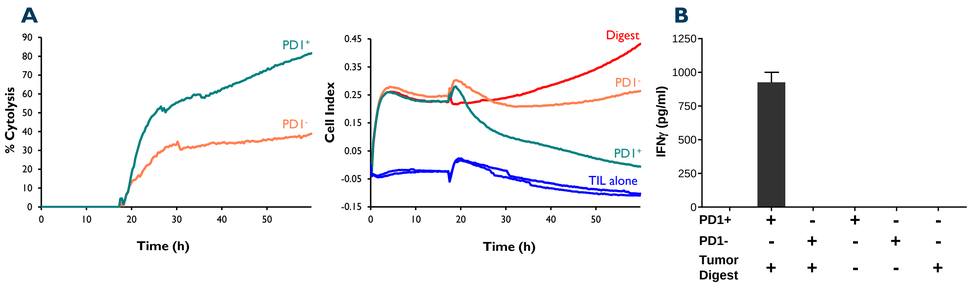


Figure 7: Tumor reactivity was assessed on PD1 selected TIL product from one melanoma sample. **(A)** Whole tumor digest was cleaned up using a dead cell removal kit (Miltenyi). 1e5 live cells were plated per well of a 96 well plate and permitted to adhere for 18 hours at 37°C in the xCELLigence instrument (ACEA Biosciences, Inc.). 1e5 $PD1^+$- and $PD1^-$-derived autologous TIL were added to their respective wells, resulting in a 1:1 (TIL:target) cell ratio, and incubated for 48 hours. Killing of the autologous target cells was recorded as increased impedance resulting from cell detachment. Cell killing (% cytolysis) (left most graph) was calculated using the formula *% Cytolysis= [1-(NCIst)/(AvgNCIRt)]x100*, where NCI_{st} is the Normalized cell index for the sample and NCI_{Rt} is the average of the Normalized Cell Index for the matching reference wells (digest alone). Right graph shows the normalized cell indices of the samples. **(B)** 1e5 cells from the whole tumor digest were cocultured with 1e5 TIL (or digest and TIL alone) for 18 hours. Supernatants were assessed for IFNγ release by ELISA (R&D systems). Bars represent the mean values of duplicate wells and vertical lines represent the standard errors.

Figure 4. $PD1^+$-derived TIL, $PD1^-$-derived TIL, and bulk TIL from melanoma (n=5), lung (n=7,) breast (n=6) and sarcoma (n=3) were assessed phenotypically by flow cytometry for the cell surface expression of T cell markers. **(A)** Four effector/memory subsets were identified based on the levels of (CD45RA and CCR7) on the $CD3^+$ cells. T_{EM}=effector memory (CD45RA-, CCR7-), T_{CM}=central memory (CD45RA-, CCR7+), TSCM= stem cell memory (CD45RA+, CCR7+), T_{EMRA}=effector T cells (CD45RA+,CCR7-). **(B)** Markers for differentiation, **(C)** exhaustion and **(D)** activation were also assessed. Bars represent the mean percentages of each subset in all 3 TIL preparations and vertical lines represent the standard errors.

Figure 5: PD1 selected and bulk TIL from melanoma (n=2), breast (n=2) and lung (n=2) were analyzed by RNA-sequencing. **(A)** Unique CDR3 (uCDR3) peptide sequences were numbered and boxplots were generated using the pandas and matplotlib libraries of Python 3.6.3, Anaconda, Inc. **(B)** Shannon Diversity indices were calculated for each sample by iRepertoire and boxplots were generated using the pandas and matplotlib libraries of Python 3.6.3, Anaconda, Inc). Bars represent the mean percentages of each subset and vertical lines represent the standard errors. Statistical significance was assessed by a paired student t-test ***P<0.001, **p<0.01. **(C)** The uCDR3 frequencies were ranked in descending order and reported or summed in intervals indicated (top ranking uCDR3, CDR3s ranked 2-10, 11-20, etc.) for each of the samples sequenced. The frequencies were then averaged by group and plotted using Excel v. 1803. **(D)** Shared uCDR3 clones were identified in the complementary Bulk TIL and $PD1^+$-derived samples. The sum of the frequencies of each of the shared unique CDR3 clones is reported in the "shared %" columns.

SUMMARY

- Expanded $PD1^+$ TIL demonstrate oligoclonality, compared to $PD1^-$- derived TIL and bulk TIL, a sign of antigen-driven clonal expansion at the tumor site
- Preliminary data demonstrate autologous tumor cell killing by $PD1^+$ but not $PD1^-$-derived TIL
- Functionality of the expanded $PD1^+$ TIL was confirmed by robust IFNγ and CD107a expression in response to non-specific stimulation
- Importantly, *in vitro* expansion of $PD1^+$ TIL resulted in products phenotypically comparable with bulk TIL, indicating a strong therapeutic potential
- T cell markers regulated at the surface of expanded $PD1^+$ TIL relative to pre-sort TIL included PD1 and CD25 and suggest a high activation level
- We intend to investigate this TIL product in clinic