Direct selection of PD-1+ CD39+ tumor infiltrating lymphocytes (TIL) from tumor dissociates enrich for functional tumor-reactive cells

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Abstract #4052

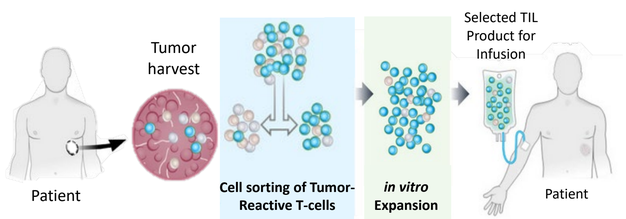
Background: The autologous cell therapy field has investigated numerous novel strategies and processes to improve response rates and expand the use of adoptive cell therapies (ACT) to patient suffering from a broad range of cancers. Bulk tumor infiltrating lymphocytes (TIL) can be harvested from solid tumors, expanded *in vitro,* and infused to patients; resulting in substantial clinical benefits in some patient populations. Tumor reactivity of TIL can vary greatly, representing a key limiting factor of bulk TIL therapies. The ability to generate an autologous cell product enriched in tumor reactive cells while limiting the presence of potentially detrimental and/or competitive non-reactive cells is highly desirable for next generation TIL products. Here we demonstrate that direct selection of antigen experienced TIL from tumor dissociates is feasible and generates functional cells enriched for tumor reactivity from various tumor indications.

Methods: Patient-derived tumor material from various indications, including ovarian, kidney, colorectal cancer and lung tumors were first processed into single cell suspensions to maximize TIL recovery. Antigen experienced TIL were immediately selected based on the co-expression of PD-1 and CD39[(1-2)], using fluorescence-activated single cell sorting (FACS). The sorted cells or the unselected counterparts were expanded in a rapid expansion protocol (REP), then analyzed for phenotypic and functional characteristics and reactivity against autologous tumor cells.

Results: The PD-1+ CD39+ selection strategy consistently allowed sorting of a population of viable TIL that could be cultured *in vitro* from tumors of various cancer indications. The selected TIL successfully expanded in a rapid expansion protocol. TCR sequencing analysis revealed that the PD-1+ CD39+ sorted populations were enriched in distinct subsets of TCR clonotypes compared to the unselected populations. Expanded PD-1+ CD39+ selected cells demonstrated the ability to produce key effector cytokines upon restimulation in polyclonal assays. Importantly, the PD-1+ CD39+ expanded TIL were enriched for tumor reactive T cells and showed improved cytotoxic activity against autologous tumors.

Conclusions: PD-1+ CD39+ selected TIL can be successfully isolated and expanded *in vitro*, generating a TIL product of superior reactivity in multiple cancer indications. PD-1+ CD39+ selected TIL showed increased cytokine secretion and cytotoxic activity against autologous material in comparison to unselected TIL, indicating that this selection strategy rapidly enriches for functional tumor-reactive lymphocytes, which is likely to be a key feature of successful ACT for solid tumors.

Methods



- Tumors are processed in single cell suspensions
- TIL co-expressing PD-1 and CD39 (PD-1+ CD39+) are selected by fluorescent activated cell sorting (FACS)
- Selected TIL were expanded *in vitro* in a Rapid Expansion Protocol (REP)
- Selected TIL were characterized for:
  - Cell composition and phenotype
  - TCR repertoire by single cell RNA sequencing
  - Function in polyclonal stimulation and killing assay using autologous tumor material

PD-1+ CD39+ TIL can be isolated and expanded from various tumor indications

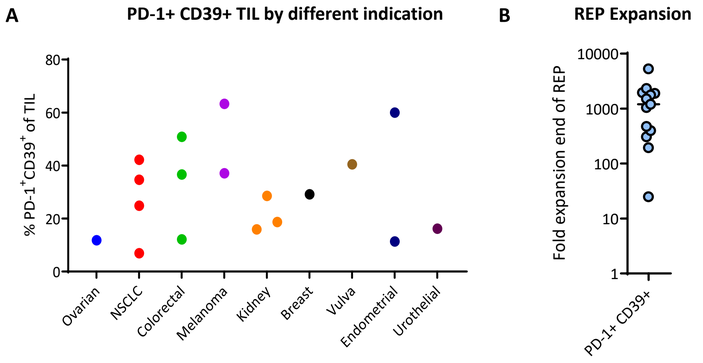


Figure 1. (**A**) Percentage of TIL co-expressing PD-1 and CD39 in fresh or frozen single cell suspensions from different cancer indications. (**B**) Expansion of PD-1+ CD39+ selected TIL after a 14-day REP protocol (in presence of irradiated PBMCs, OKT3 and IL-2) calculated as fold increase between the number of cells after sorting (day 0) and the number of cells harvested at the end of the 14-day REP. Each dot represents data from a patient tumor.

PD-1+ CD39+ selected TIL are composed primarily of CD4 and CD8 effector memory T cells

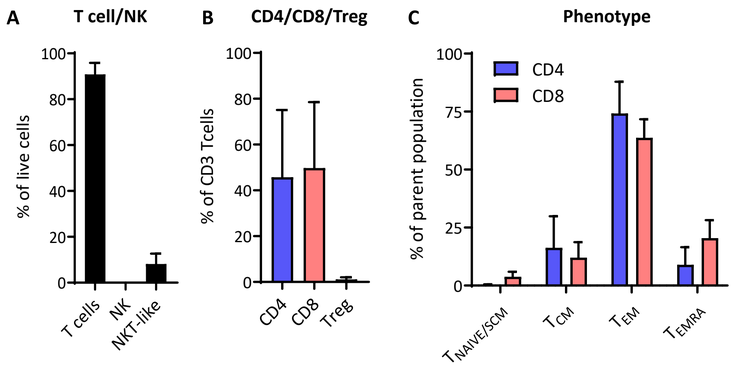


Figure 2. (**A**) Percentage of cells expressing CD3+ CD56- (T cells), CD56+ CD3- (NK) or CD3+CD56+ (NKT-like) in Selected TIL after REP. (**B**) Percentage of CD4 T cells, CD8 T cells and regulatory T cells (Tregs, defined as CD25+ Foxp3+ CD127-) within the T cell population. (**C**) Percentage of naïve/stem-cell memory T cells ($T_{NAIVE/SCM}$ = CD45RA+ CCR7+), central memory T cells (T_{CM} = CD45RA- CCR7+), effector memory T cells (T_{EM} = CD45RA- CCR7-) and effector memory T cells expressing CD45RA (T_{EMRA} = CD45RA+ CCR7-), within the CD4 and CD8 T cell populations in Selected TIL after REP. Mean +/- SD

PD-1+ CD39+ selected TIL repertoire is oligoclonal and distinct from the unselected population

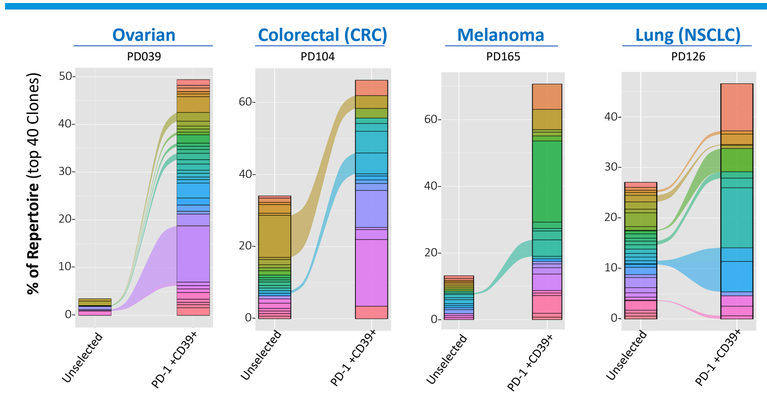


Figure 3. Single cell RNA sequencing was performed on unselected and PD-1+ CD39+ selected TIL at the end of the REP expansion. Graphs show the diversity and abundance of TCR clonotypes in unselected and PD-1+ CD39+ selected TILs for each patient. Within each sample set, TCRs are annotated by different colors and lines connecting the same colors indicate shared TCR clonotypes between the TIL products generated from PD-1+ CD39+ or unselected TIL. The frequency of the Top40 most abundant clonotypes within each sample set are displayed.

PD-1+ CD39+ selected TIL displays superior reactivity against autologous tumor cells

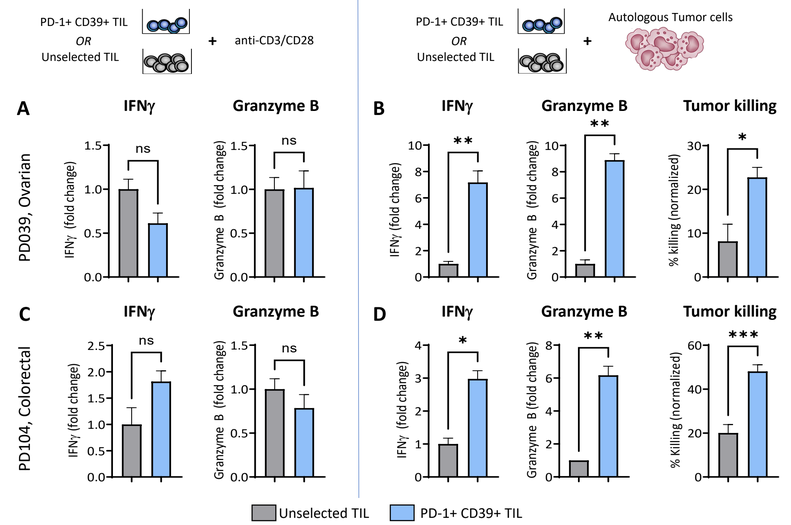


Figure 4. (**A**, **C**) IFNγ and granzyme B production by PD-1+ CD39+ selected TIL product and unselected TIL after overnight stimulation with anti-CD3/CD28 antibodies (ns = No significant difference). (**B**, **D**) IFNγ and granzyme B production and tumor cell killing by PD-1+ CD39+ selected TIL and unselected TIL after overnight stimulation with autologous tumor cells (CD45- DTCs). IFNγ and granzyme B production data is expressed as fold change as compared to the unselected TIL. Cell killing data was normalized based on the viability of tumor cell cultured alone (0% killing). Mean +/- SD, error bars display standard deviation of triplicates. * = p <0.05 ** = p <0.01 *** = p <0.001, paired t-test. A-B : Ovarian tumor sample; C-D: Colorectal tumor sample.

Conclusions

- TIL co-expressing PD-1 and CD39 (PD-1+ CD39+) can be successfully isolated and expanded from various solid tumor indications
- The PD-1+ CD39+ TIL are composed of CD4 and CD8 T cells displaying a predominantly effector memory phenotype
- The PD-1+ CD39+ direct selection enriches for a specific subset of TCR clonotypes that is distinct from the unselected population
- The PD-1+ CD39+ direct selected TIL product display increased cytokine secretion and killing activity against autologous tumor cells as compared to the unselected population
- The PD-1+ CD39+ direct selection method can generate a functional TIL product enriched for tumor reactivity, an important feature of successful ACT for solid tumors.

References

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2- Palomero J, et al. *Biomarkers of tumor-reactive CD4+ and CD8+ TILs associate with improved prognosis in endometrial cancer*. J Immunother Cancer 2022;10:e005443. PMID: 36581331