Detecting T-cell Reinvigoration and Persistence Using Patient-derived *Ex Vivo* Three-dimensional Spheroid Models

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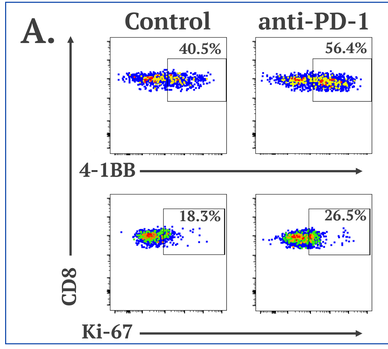
Background

Immune checkpoint blockade is shifting the paradigm for cancer treatment. However, this class of therapeutics is limited by insufficient or dysfunctional antitumor T-cells with impaired memory formation. Adoptive cell therapy is a treatment option for patients with exhausted resident T-cells, yet the effective use of this immunotherapy for the treatment of solid tumors is still in early stages. A durable patient response is possible when T-cell products successfully persist following recursive tumor cell exposure and resist differentiation and exhaustion [1]. Due to the variability of personalized cellular immunotherapies, verification of T-cell function would facilitate selection of the most desirable product for clinical use. Herein, we report a tissue agnostic ex vivo three-dimensional model which recapitulates the tumor microenvironment for the assessment of T-cell performance.

Reinvigoration of Tumor Infiltrated Lymphocytes

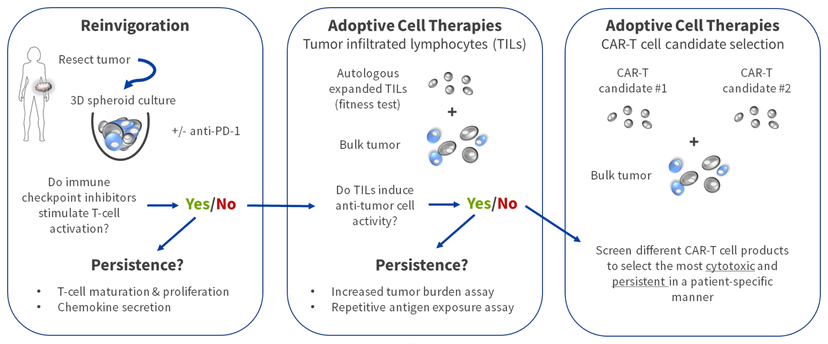


data not available due to limited cell material

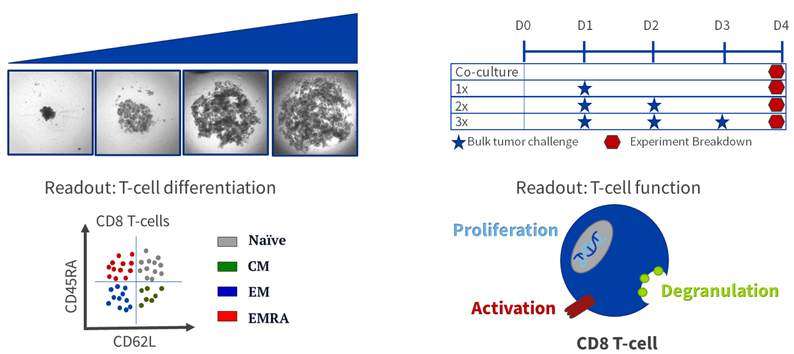
Figure 1. Anti-PD-1 induced reinvigoration of ovarian patient TILs. (A) Representative flow cytometry plots of Patient 4 TILs from spheroids comparing negative control and anti-PD-1 after 48-72 hours. **(B)** TILs were evaluated for indicated markers and fold change was determined by normalizing percent of parent of anti-PD-1 treatment over the negative control.

Ex Vivo Response to Immunotherapy

Which patients are good candidates for immuno-therapy?



Persistence assays



Persistence of Adoptive Cell Therapies

Memory and fitness of expanded TILs

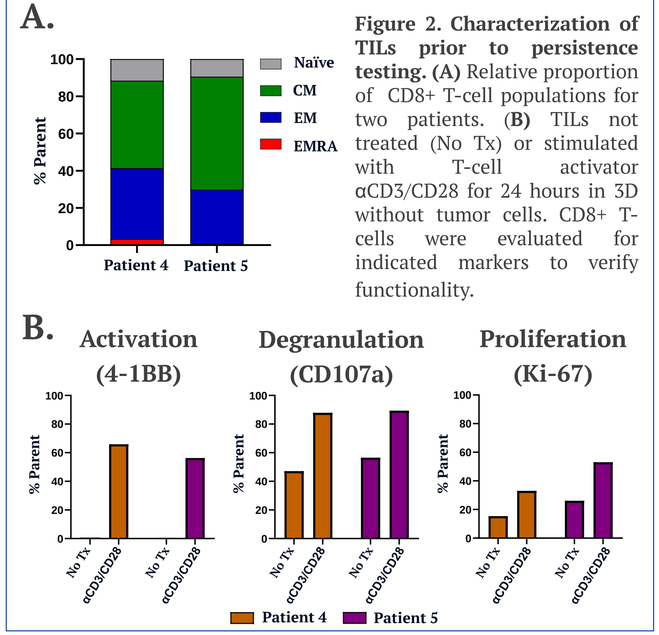


Figure 2. Characterization of TILs prior to persistence testing. (A) Relative proportion of CD8+ T-cell populations for two patients. **(B)** TILs not treated (No Tx) or stimulated with T-cell activator αCD3/CD28 for 24 hours in 3D without tumor cells. CD8+ T-cells were evaluated for indicated markers to verify functionality.

Increased tumor burden assay

Repeated antigen exposure assay

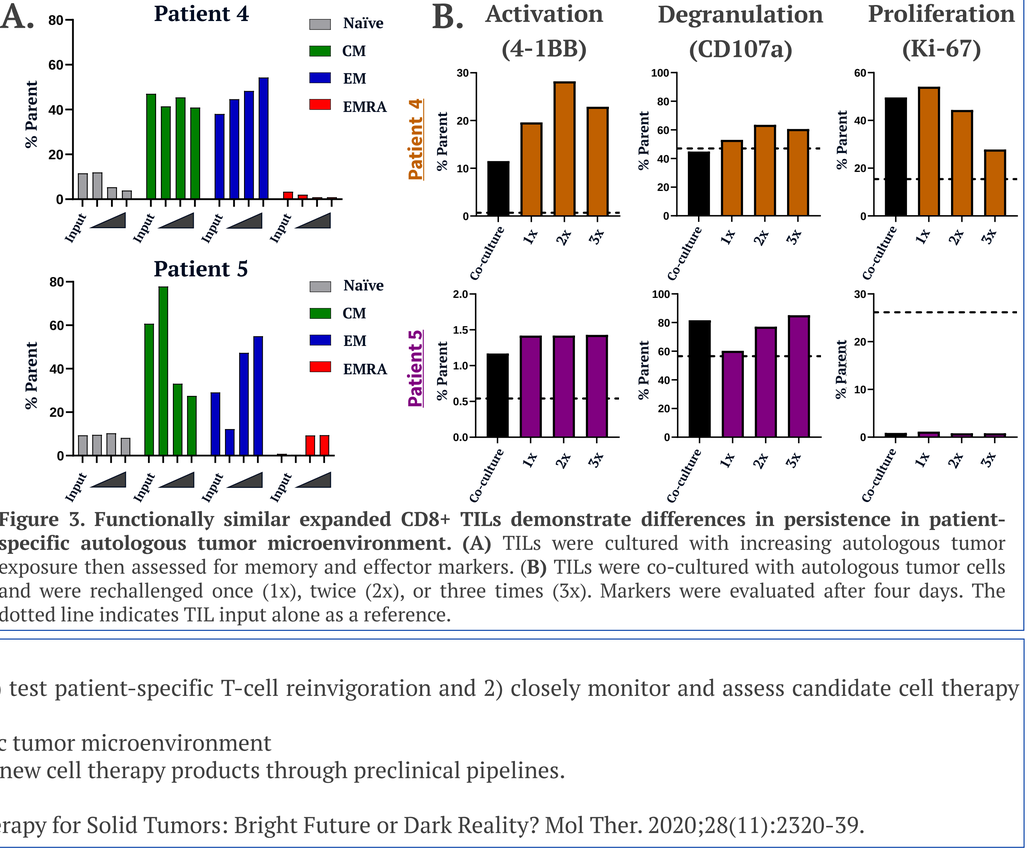


Figure 3. Functionally similar expanded CD8+ TILs demonstrate differences in persistence in patient-specific autologous tumor microenvironment. (A) TILs were cultured with increasing autologous tumor exposure then assessed for memory and effector markers. **(B)** TILs were co-cultured with autologous tumor cells and were rechallenged once (1x), twice (2x), or three times (3x). Markers were evaluated after four days. The dotted line indicates TIL input alone as a reference.

Conclusions:

- This complex three-dimensional platform has the ability to 1) test patient-specific T-cell reinvigoration and 2) closely monitor and assess candidate cell therapy products during development.
- This platform monitors T-cell performance in a patient-specific tumor microenvironment
- These methods can provide a cost-effective means to expedite new cell therapy products through preclinical pipelines.

Reference:

1. Wagner J, Wickman E, DeRenzo C, Gottschalk S. CAR T Cell Therapy for Solid Tumors: Bright Future or Dark Reality? Mol Ther. 2020;28(11):2320-39.