162 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.

Methods Spheroids were generated using primary patient-derived tumor cells or patient-derived xenografts and cultured with immune cells, expanded tumor-infiltrating lymphocytes or CAR-Ts. Reinvigoration was determined via increased T-cell activation and proliferation as detected by flow cytometry. Low effector to target ratios were utilized to mimic a high tumor burden. T-cell persistence was tested following multiple rounds of tumor cell challenge in a repetitive antigen exposure assay. Granzyme B levels, degranulation and annexin V were evaluated to measure antitumor efficacy. T-cell susceptibility to differentiate was determined by detecting memory markers CD62L and CCR7. Finally, T-cell products that were prone to exhaustion were identified by monitoring expression of checkpoint proteins.

Results Patient samples capable of reinvigoration responded to PD-1 blockade as determined by increases in T-cell proliferation, 4–1BB, and degranulation. Decreased expression of the memory markers CD62L and CCR7 was observed on CD45RO+/CD8+ T-cells when challenged with high tumor burden, but not with low tumor burden exposure. Responding T-cells persisted with higher absolute numbers compared to low tumor burden models. Using our repetitive antigen challenge platform, we detected no significant change in T-cell function with a single rechallenge of tumor cell exposure. Upon multiple challenges, varying degrees of T-cell degranulation, granzyme B levels, and T-cell viability were observed compared to lesser challenged T-cells demonstrating inherent differences in donor T-cell persistence.

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 can provide a cost-effective method to expedite new cell therapy products through preclinical pipelines.

REFERENCES

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.

Ethics Approval Written informed consent was obtained from patients in accordance with the Institutional Review Board (IRB) approved biology protocols by Prisma Health, formally known as Greenville Health System, Cancer Institute (IRB-Committee C). Where applicable, additional tissue for this study was procured from commercial vendors who maintain strict ethical compliance, including fully de-identified materials and stringent IRB and Ethics Committee compliance.

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