1024 ILLUMINATING STEM MEMORY T CELL (TSCM) HETEROGENEITY IN HUMAN CANCER: CHARACTERIZATION OF A UNIQUE CD45RO+ TSCM POPULATION IN HUMAN OVARIAN CANCER

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Background Stem memory T cells (Tscm) are a unique T cell subset positioned between naïve and central memory (CM) T cells, that express the surface markers CCR7, CD45RA and CD95, and are CD45RO negative.[1] Tscm cells can self-renew, are multipotent, and appear to be critical for effective antitumor immunity, but little is known about Tscm immunobiology in human tumors.[1-4] Most studies examining canonical Tscm cells are performed using mouse models, expanded tumor-infiltrating lymphocytes (TILs), or peripheral blood T cells, and few studies examine Tscm-like cells directly from human tumors, despite evidence for their crucial role in solid tumor immunotherapeutic treatment.[2-6]

Methods Therefore, we hypothesized that Tscm cells in human ovarian cancer exist and explored Tscm heterogeneity in ovarian cancer tumor digests using flow and mass cytometry. This investigation resulted in the identification of a novel CD45RO + expressing Tscm subset, which was further interrogated using FACS sorted TIL populations to assess self-renewal capabilities and effector function. Additionally, bulk RNA-seq and TCR sequencing were completed on sorted TIL populations to examine the RO+ Tscm subset.

Results Like canonical CD45RO- (RO-) Tscm cells, the RO+ subset can self-renew and is maintained by the homeostatic cytokines IL-7 and IL-15; however, RNA-seq, TCR sequencing, and functional assessment of the RO+ Tscm population position it as a unique subset between RO- Tscm and CM T cell subsets. RNA-seq revealed that RO+ Tscm cells are transcriptionally distinct from other subsets, exhibiting intermediate expression of naïve and effector associated genes. TCR sequencing indicates that the RO+ subset shares a high degree of clones with downstream memory populations and has an intermediate clonality relative to canonical RO- Tscm and CM populations. Notably, our results demonstrate that RO+ Tscm cells exhibit greater effector potential than the RO- Tscm subset, with RO+ Tscm cells producing more IFNγ upon stimulation. These results, paired with the observation that RO+ Tscm cells express CD137, a biomarker of tumor-specificity, more frequently than other differentiation subsets, indicate that this subset may have enhanced anti-tumor capabilities.

Conclusions In conclusion, we (1) demonstrate that a heterogeneous Tscm population exists in human ovarian cancer and (2) identify a distinct RO+ Tscm subset that appears to be transcriptionally, phenotypically, and functionally intermediate between canonical RO- Tscm and CM populations. Importantly, we show that the RO+ Tscm subset is multipotent and exhibits effector-like attributes, making it a desirable target for future research in the setting of cancer immunotherapy.

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REFERENCES

1. Gattinoni L, Lugli E, Ji Y, Pos Z, Paulos CM, Quigley MF, Restifo NP. A human memory T cell subset with stem cell–like properties. *Nature medicine* 2011;**17**(10),1290–1297.
2. Gattinoni L, Speiser DE, Lichterfeld M, Bonini C. T memory stem cells in health and disease. *Nature Medicine* 2017;**23**(1):18–27.
3. Krishna S, Lowery FJ, Copeland AR, Bahadiroglu E, Mukherjee R, Jia L, Rosenberg, SA. Stem-like CD8 T cells mediate response of adoptive cell immunotherapy against human cancer. *Science* 2020;**370**(6522):1328–1334.
4. Biasco L, Izotova N, Rivat C, Ghorashian S, Richardson R, Guvenel A, Amrolia PJ. Clonal expansion of T memory stem cells determines early anti-leukemic responses and long-term CAR T cell persistence in patients. *Nature Cancer* 2021;**2**(6):629–642.
5. Jansen CS, Prokhnevska N, Master VA, Sanda MG, Carlisle JW, Bilen MA, Kissick H. An intra-tumoral niche maintains and differentiates stem-like CD8 T cells. *Nature* 2019;**576**(7787):465–470.
6. Siddiqui I, Schaeuble K, Chennupati V, Marraco SAF, Calderon-Copete S, Ferreira DP, Held W. Intratumoral Tcf1+ PD-1+ CD8+ T cells with stem-like properties promote tumor control in response to vaccination and checkpoint blockade immunotherapy. *Immunity* 2019;**50**(1):195–211.

Ethics Approval Viably frozen, human high-grade serous ovarian tumor or ascites samples were purchased from the University of Pennsylvania (UPenn) Ovarian Cancer Research Center (OCRC) Tumor BioTrust Collection. All donor samples used in this study were de-identified and approved for use by the UPenn Institutional Review Board (IRB 702679, UPCC 17909).

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