947 THE ACTIVATION STATES OF TUMOR-RESIDENT TYPE 2 DENDRITIC CELLS IMPACT THE STRENGTH OF OVARIAN CANCER IMMUNE RESPONSES

[1]Fiona Chatterjee*, [1]Vincent Butty, [1]Emi A Lutz, [1]K Dane Wittrup, [2]Stefani Spranger. *[1]Massachusetts Institute of Technology, Cambridge, MA, USA; [2]Koch Institute for Integrative Cancer Research at MIT, Cambridge, MA, USA*

Background Ovarian cancer is the fifth leading cause of cancer-related death in women in the United States.[1] To date, checkpoint blockade therapy (CBT) has failed to be effective in ovarian cancer.[2] CBT efficacy typically requires a strong pre-existing tumor-specific T cell response.[3 4] However, analysis of patient data indicates that tumor-infiltrating T cells in ovarian cancer are often poorly activated.[5] Understanding the mechanisms governing this poor T cell activation could lead to novel ovarian cancer therapeutics.

Methods We utilized a transplantable, syngeneic, murine ovarian cancer cell line driven by $Ccne1^{OE}$ $p53^{-/-R172H}$ $Ak2^{OE}$ and $Kras^{G12V}$ (CPAK).[6] CPAK tumor cells were implanted intraperitoneally to model metastatic ovarian cancer or subcutaneously to model productive systemic immunity. CPAK cells were engineered to express the model $CD8^+$ T cell antigen SIY to enable studies of tumor-specific T cells. CBT consisted of αCTLA-4 and αPD-L1 therapy. Immune cells were profiled using flow cytometry and single-cell RNA sequencing (scRNA-seq).

Results Intraperitoneal (IP) CPAK-SIY tumors were unresponsive to CBT. However, mice bearing subcutaneous (SQ) CPAK-SIY tumors treated with CBT displayed delayed tumor growth compared to control animals. Flow cytometric analysis of tumor-infiltrating $CD8^+$ T cells illustrated that while tumor-reactive T cells were activated in IP and SQ tumors, tumor-reactive T cells in IP tumors failed to upregulate high levels of effector molecules such as granzyme B. Unbiased analysis of dendritic cells (DCs) within IP tumors using scRNA-seq revealed a population of DCs that expressed CD103 and CD11b and a type 2 conventional DC (cDC2) gene signature. Gene signature analysis indicated these $CD103^+$ $CD11b^+$ double-positive DCs were a suppressive state of cDC2s induced by TGFb that reside in gut tissue and induce Tregs.[7] Analysis of tumor-infiltrating DCs in IP tumors revealed that the proportion of double-positive DCs increased during tumor growth while the proportion of cDC2s decreased. We hypothesized that the polarization of cDC2s away from these suppressive double-positive DCs may improve ovarian cancer immunity. Previous work from our group demonstrated that cDC2s acquire an activation state characterized by an interferon-stimulated gene signature (ISG^+ DCs) upon exposure to interferon-beta (IFNβ) and that ISG^+ DCs are potent activators of $CD8^+$ T cells.[8] In our ovarian cancer model, addition of IFNβ to IP tumors enhanced tumor-specific T cell responses.

Conclusions Metastatic ovarian tumors are refractory to CBT and are infiltrated by poorly activated $CD8^+$ T cells. Our results suggest that the poor T cell activation is a consequence of the cDC2 activation state within the tumor.

Acknowledgements We would like to thank MIT's Department of Comparative Medicine and the Koch Institute's Swanson Biotechnology Core Facility. This work was supported by Break Through Cancer and the Margaret A. Cunningham Immune Mechanisms in Cancer Research Fellowship.

REFERENCES

1. Siegel RL, Miller KD, Wagle NS, Jemal A. Cancer statistics, 2023. *CA Cancer J Clin* 2023;**73:**17–48, doi:10.3322/caac.21763.
2. Kandalaft LE, Odunsi K, Coukos G. Immunotherapy in Ovarian Cancer: Are We There Yet? *J Clin Oncol* 2019;**37:**2460–2471, doi:10.1200/JCO.19.00508.
3. Tumeh PC, *et al.* PD-1 blockade induces responses by inhibiting adaptive immune resistance. *Nature* 2014;**515:**568–571, doi:10.1038/nature13954.
4. Cristescu R, *et al.* Pan-tumor genomic biomarkers for PD-1 checkpoint blockade-based immunotherapy. *Science* 2018;**362**, doi:10.1126/science.aar3593 (2018).
5. Vazquez-Garcia I, *et al.* Ovarian cancer mutational processes drive site-specific immune evasion. *Nature* 2022;**612:**778–786, doi:10.1038/s41586-022-05496-1.
6. Iyer S, *et al.* Genetically Defined Syngeneic Mouse Models of Ovarian Cancer as Tools for the Discovery of Combination Immunotherapy. *Cancer Discov* 2021;**11:**384–407, doi:10.1158/2159-8290.CD-20-0818.
7. Bain CC, *et al.* TGFbetaR signalling controls CD103(+)CD11b(+) dendritic cell development in the intestine. *Nat Commun* 2017;**8:**620, doi:10.1038/s41467-017-00658-6.
8. Duong E, *et al.* Type I interferon activates MHC class I-dressed CD11b(+) conventional dendritic cells to promote protective anti-tumor CD8(+) T cell immunity. *Immunity* 2022;**55:**308–323 e309, doi:10.1016/j.immuni.2021.10.020.

Ethics Approval All mouse experiments were approved by MIT's Committee on Animal Care (CAC) – PHS Animal Welfare Assurance # D16–00078 (A3125–01).

http://dx.doi.org/10.1136/jitc-2023-SITC2023.0947