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**REVERSE ABCOPAL EFFECT: INTERTUMORAL HETEROGENEITY SUPPRESSES SYSTEMIC CD8 T CELL-MEDIATED ANTITUMOR IMMUNITY AND CONFERS PD-1 INHIBITOR RESISTANCE IN SYNCHRONOUS MELANOMA**

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**Background** Despite recent advancements in systemic therapy, only a minority of metastatic patients develop meaningful clinical responses to immune checkpoint inhibitors. Inherent genetic instability of melanoma generates genomically and microenvironmentally distinct metastases. These different tumor microenvironments (TMEs) contain numerous T cell suppression mechanisms, such as upregulation of the PD-1/PD-L1 exhaustion pathway. However, as synchronous metastases share one host immune system, intertumoral heterogeneity may result in increasing cross-talk between metastases that impairs systemic antitumor immunity and promotes PD-1 immunotherapy resistance.

**Methods** YUMM 1.7 (less immunogenic) and YUMMER 1.7 (more immunogenic cell line derived from YUMM following UVB irradiation) melanoma cell lines were simultaneously injected into opposite flanks of the same mice as a model of synchronous melanoma. We assessed tumor growth in wild-type, interferon-gamma (IFN- $\gamma$ ) knockout, and CD8-depleted mice as well as in response to PD-1 inhibitor. We characterized the TME with flow cytometry and performed TCR sequencing on tumor-infiltrating CD8 T cells.

**Results** Distinct TMEs were observed for YUMM and YUMMER tumors simultaneously grown in the same mouse. The presence of the less immunogenic YUMM tumor allows the more immunogenic YUMMER tumors to escape IFN- $\gamma$  and CD8 T cell-mediated rejection, despite abundant tumor-infiltrating, clonally expanded CD8 T cells. Identical immunodominant CD8 T cell clones were found in both YUMM and YUMMER tumors within the same mouse. Synchronous YUMMER-infiltrating CD8 T cells exhibit suppressed phenotypes, including increased persistence of surface PD-1 and decreased surface CD107a expressions. Simultaneously, these synchronous YUMMER tumors additionally upregulate macrophage surface PD-L1 expression, which potentially contributes to tumor immune escape. Lastly, synchronous YUMMER tumors become resistant to PD-1 inhibition, in direct contrast to control YUMMER tumors.

**Conclusions** In a host with multiple melanoma lesions, immunogenicity of all tumors contribute to the systemic antitumor immune response. We show that two synchronous tumors with synonymous mutations (<40%), as is the case with metastatic patients, lead to skewed CD8 T cell expansion of the same clones in both tumors. The presence of a less immunogenic tumor prevents CD8 and IFN- $\gamma$  mediated rejection of the more immunogenic tumor. Furthermore, CD8 T cells in the more immunogenic tumor exhibit decreased effector function and increased resistance to PD-1 blockade, as tumor-infiltrating macrophages concurrently become more immunosuppressive. These results are highly suggestive of a “reverse abscopal effect,” by which immunologically “cold” tumors generate systemic immunosuppression that facilitate PD-1 immunotherapy resistance and immune escape of all other tumors in synchronous metastatic melanoma patients.

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**Ethics Approval** Animal experiments were approved by the University Committee on Animal Resources and performed in accordance with University of Rochester approved guidelines.

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