

## REGULATION OF NEOANTIGEN SPECIFIC T CELL INFILTRATION AND SPATIAL TUMOR IMMUNE ARCHITECTURE OF MYELOMA AND ITS PREMALIGNANT PRECURSORS

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**Background** Entry of neoantigen-specific T cells into tumors is critical for immunotherapy but underlying mechanisms are poorly understood. Spatial aspects of immune infiltration impact outcome in solid tumors, but the impact of spatial immune patterns in hematologic malignancies such as multiple myeloma is unknown.

**Methods** We combined several high dimensional spatial analysis platforms with in-vitro/in-vivo modeling in humanized mice of human MM and its precursor monoclonal gammopathy of undetermined significance to gain mechanistic insights into entry of neoantigen-specific T cells into MM tumors.

**Results** Multiplex immune fluorescence immunohistochemistry (mIF-IHC) analysis of bone marrow biopsies from 95 patients with plasma cell malignancy or its precursor states (MGUS n=13, SMM n=12 and MM n=70) revealed a decline in TCF1+ stem memory like cells and increase in granzymeB+ effector T cells in transition from MGUS to MM. Spatial analyses of tumor/immune infiltration identified formation of microclusters with areas of T cell exclusion in MM but not MGUS. Multifocal growth of MM but not MGUS was reproduced in humanized MISTRG6 mice, indicating this as an intrinsic property associated with malignancy. Consistent with IHC data, MM tumors were resistant to entry of T cells in vitro. In these systems, T cells entry required T cell activation and was enhanced upon CD28-mediated costimulation. T cell entry also required CD2/CD58 interaction and was abrogated upon disruption of these interactions. Importantly, entry of neoantigen-specific T cells into antigen-expressing tumors required in situ stimulation with antigen-presenting dendritic cells (DCs). Upon adoptive transfer into humanized mice, while neoantigen-specific T cells selectively enrich at tumor site, entry of these T cells into tumor masses in vivo again depends on tumor-associated DCs. Spatial analysis of IHCs from MM biopsies revealed that T cell infiltration follows a gradient from CLEC9A+ DCs. Nanostring analyses revealed that CLCC9<sup>high</sup> regions are enriched for immune activation genes. Patterns of T and myeloid infiltration also correlated with both overall and progression free survival in multivariable analyses.

**Conclusions** These data identify several distinct aspects of spatial immune alterations that correlate with evolution of malignancy and outcome in MM. They also demonstrate that entry of neoantigen-specific T cells into MM tumors depends on target recognition, costimulation, CD2/58 axis and in situ antigen presentation by tumor-associated DCs. These data therefore identify a novel role for specific antigen presentation by tumor-associated DCs in the effector phase of cancer immunity cycle, with broad implications for immunotherapy and T cell redirection.

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**Ethics Approval** Animal studies including injections of patient samples into mice were approved by the institutional animal care committee at Emory University.

For patients samples, the participants gave informed consent before taking part.

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