486 COMBINED PD-L1/TGFβ BLOCKADE ALLOWS EXPANSION AND DIFFERENTIATION OF STEM CELL-LIKE CD8 T CELLS IN IMMUNE EXCLUDED TUMORS

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Background TGFβ signaling is associated with non-response to immune checkpoint blockade in patients with advanced metastatic cancers, particularly in patients with the immune excluded phenotype. While previous work demonstrates that converting tumors from excluded to inflamed phenotypes requires attenuation of PD-L1 and TGFβ signaling, the underlying cellular mechanisms remain largely unclear.

Methods We performed single-cell RNA-seq (n=5 animals per treatment) on myeloid, stromal, tumor, and T cells, as well as TCR-seq (n=7 animals per treatment) on T cells from anti-PD-L1 and anti-TGFβ antibody-treated mice bearing subcutaneous EMT6 tumors on day 7 after initiation of treatment. We performed flow cytometry to characterize and quantify T cell subtypes from treated tumors (n>=10 animals per treatment). Furthermore, we measured T cell motility (n=8 animals per treatment) in a dorsal skinfold chamber from IFNγ-YFP reporter mice bearing EMT6-mApple tumor cells with anti-gp120 as control or anti-PD-L1 and anti-TFGβ combination treatment. To determine the impact of IFNγ response on the effect of combination treatment, we tracked tumor growth in mice with anti-IFNγ treated EMT6 tumors (n=10 per treatment) or with IFNGR1 KO EMT6 tumors (n=10 per group).

Results We show that TGFβ and PD-L1 restrain intratumoral stem cell-like CD8 T cell (TSCL) expansion and maintain progenitor-exhausted and dysfunctional CD8 T cells. Upon combined TGFβ/PD-L1 blockade, TSCL expand and can differentiate into IFNγhi CD8 T effector cells that show enhanced motility and accumulate in the tumor microenvironment. Ensuing IFNγ signaling transforms myeloid, stromal, and tumor niches to yield a broadly immune-supportive ecosystem. Blocking IFNγ either through knockout of IFNGR1 in tumor cells or blocking IFNγ abolishes the effect of anti-PD-L1/TGFβ combination therapy.

Conclusions Our data suggest that TGFβ works with PD-L1 to prevent TSCL expansion and replacement of exhausted CD8 T cells, thereby maintaining the T cell compartment in a dysfunctional state.

Ethics Approval All animal activities in the research studies here presented were conducted under protocols approved by the Genentech Institutional Animal Care and Use Committee (IACUC).

http://dx.doi.org/10.1136/jitc-2023-SITC2023.0486