#5537. Anti-mPD-1 Reduces the Immuno-suppressive Phenotype of Myeloid Subsets in the Murine Hepa 1-6 Tumor

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Introduction

- Hepatocellular carcinoma (HCC) is the third leading cause of cancer related deaths and is refractory to standard chemo and radiation therapies.
- Hepa 1-6 is a murine model for HCC characterized by suppressive Arg1 expressing M2 tumor-associated macrophages (TAM) and reduced costimulatory molecule expression in dendritic cells (DC), which impairs T cell activity.
- To expose new options for therapeutic intervention, we performed immunophenotypic analysis in subcutaneous Hepa 1-6 tumor-bearing mice treated with anti-mPD-1 mediated checkpoint inhibition.
- Flow cytometry was used to measure tumor infiltrates and provide a deep dive into the biology of the immune response.

Materials and Methods

- Hepa 1-6 cells were implanted subcutaneously into the axilla region. Anti-mPD-1 antibody (clone RMP1-14) was acquired from Bio X Cell and dosed IP twice weekly for a total of 4 doses. Tumor progression was monitored over time by caliper measurements. Animal care and use was performed in conformance with the Guide for the Care and Use of Laboratory Animals in an AAALAC-accredited facility.
- For flow cytometric analysis, tumors were dissociated into single cell suspensions (gentleMACS™, Miltenyi Biotec), and labeled with fluorescent antibodies. Cells/gram of tumor were quantified using Precision Count Beads™ (BioLegend). Data was acquired on Attune™ NxT (ThermoFisher Scientific) and Cytoflex LX (Beckman Coulter) flow cytometers and then analyzed using Flowjo software (BD).
- For cytokine and granzyme B analysis, tumor-derived cells were stimulated ex vivo with PMA and ionomycin for 5 hours in the presence of brefeldin A. Following incubation, cells were collected and immuno-stained with fluorescent antibodies targeting cell surface receptors. The cells were then fixed, permeabilized, and stained for intracellular targets prior to sample acquisition.

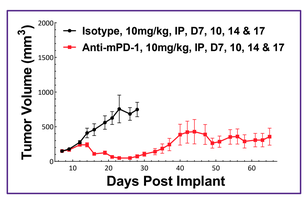


Figure 1. Tumor growth curves for anti-mPD-1 and isotype control treated mice. Hepa 1-6 is responsive to checkpoint blockade by anti-mPD-1.

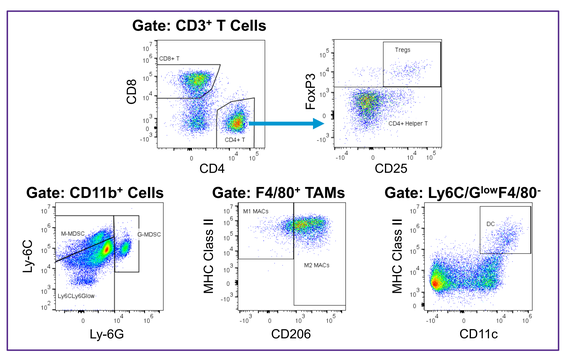


Figure 2. Representative immune subset gates for Hepa 1-6 tumor-derived cell analysis. T cell subset gates (top) and myeloid subset gates (bottom).

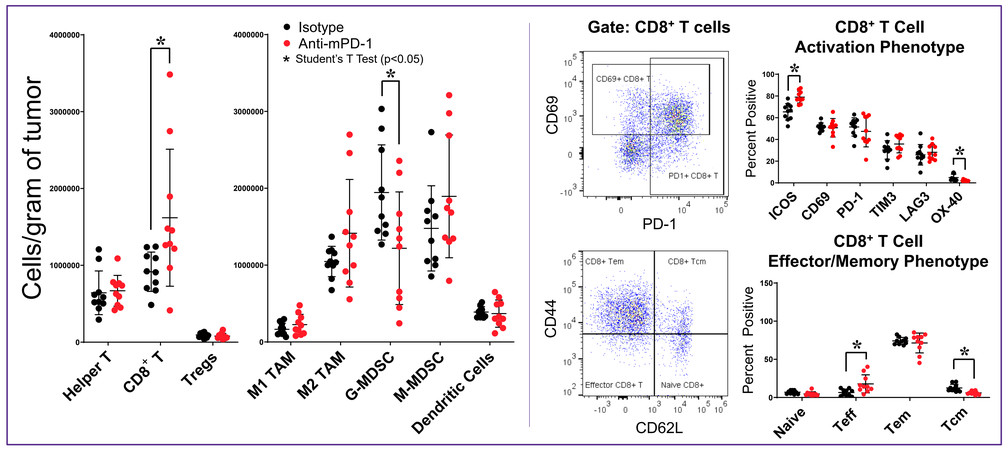


Figure 3. Immune infiltration and T cell phenotype in the Hepa 1-6 tumor is modulated by anti-mPD-1 treatment. Tumors were collected 4 days after the last antibody dose and analyzed by flow cytometry. Tumor immune infiltration of T cell and myeloid subsets (left) and T cell activation marker and effector/memory gating and analysis (right) .

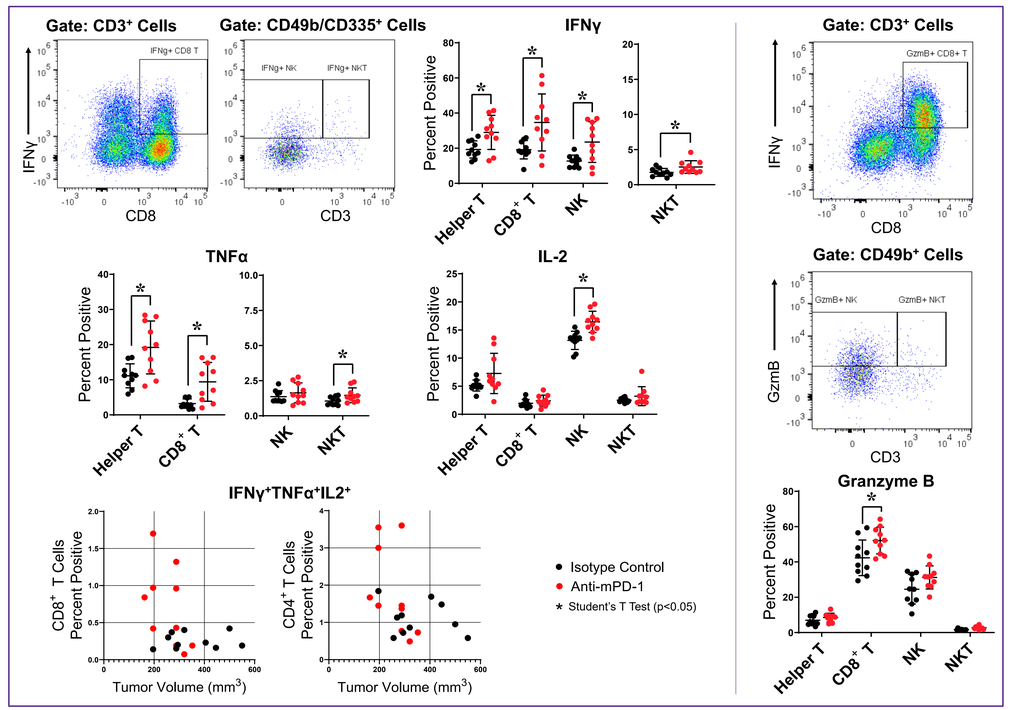
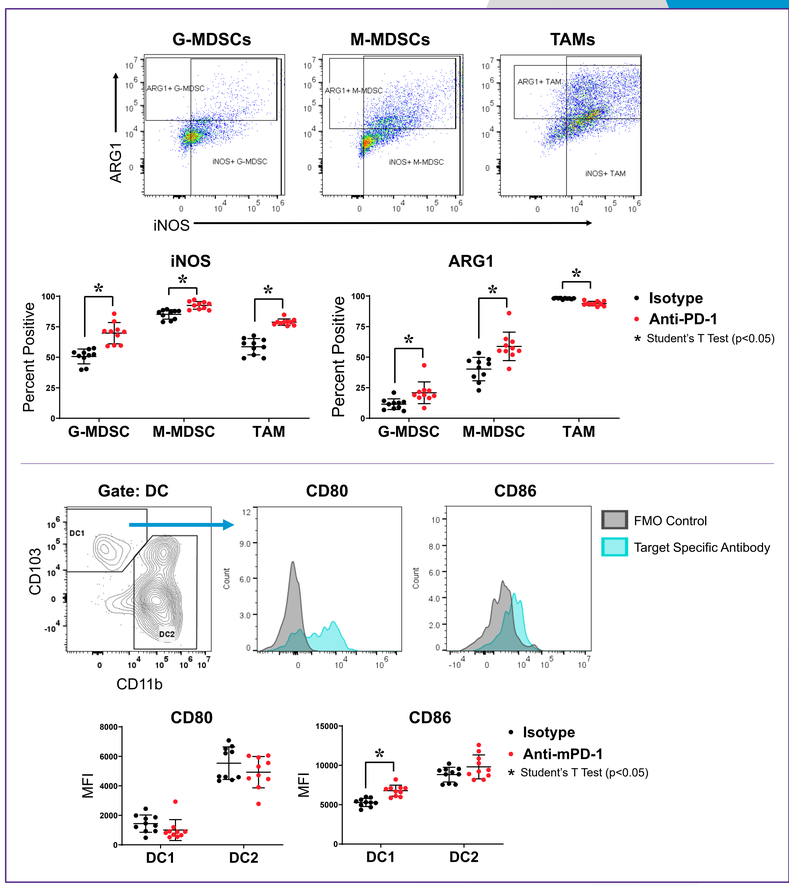


Figure 4. Cytokine and granzyme B modulation in T cell and NK/NKT cell subsets. Gating and analysis of intracellular cytokines (left) and granzyme B (right) levels in helper T cells, CD8+ T cells, NK cells and NKT cells. Polyfunctional T cell analysis and association with tumor volume (bottom left).



Figures 5. Myeloid subset functional marker analysis. Gating and analysis of intracellular iNOS and ARG1 expression in G-MDSC, M-MDSC and TAM subsets (top). Gating and analysis of DC1 (CD103+) and DC2 (CD11b+) dendritic cell subsets for CD80 and CD86 costimulatory receptors (bottom).

Results and Conclusions

- Hepa 1-6 tumors were responsive to anti-mPD-1 treatment with an increased time to progression of 60% compared to the control group.
- Anti-mPD-1 increased tumor infiltrating CD8+ T cells, upregulated ICOS expression, and increased the frequency of polyfunctional T cells producing IFNγ, TNFα, and IL-2. Granzyme B and IFNγ responses were also increased in tumor-derived NK and NKT cells.
- In the myeloid compartment, anti-mPD-1 reduced the number of tumor-infiltrating G-MDSCs, increased levels of the M1 TAM marker iNOS, and increased CD86 surface expression on DC subsets.
- In summary, anti-mPD-1 enhanced lymphocyte activity, possibly through the reprogramming of M2 tumor-associated macrophages towards an M1 phenotype as well as through enhanced antigen presentation in dendritic cells. Future efforts to reveal additional insight into mechanisms that enhance anti-tumor activity will inform new therapeutic development.

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