Antitumor mechanisms of local radiation and combination immunotherapy in an immunologically cold model of neuroblastoma

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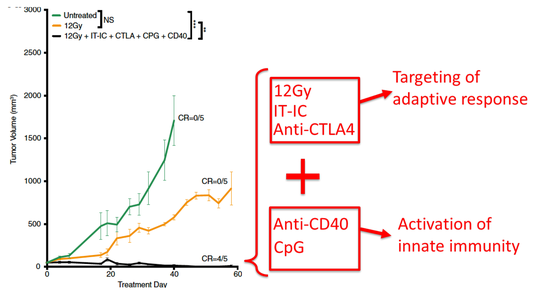
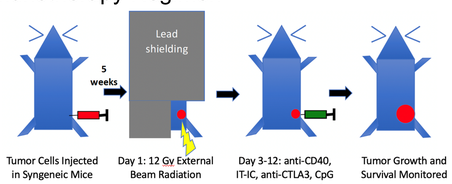
BACKGROUND

We recently described an effective *in situ* vaccination strategy in immunologically cold neuroblastoma that combines local radiation (RT), intratumoral immunocytokine (IT-IC), and immunotherapeutic agents targeting the innate and adaptive immune response.[1] This study sought to better characterize the mechanism of this regimen in directing an effective immune response.



METHODS

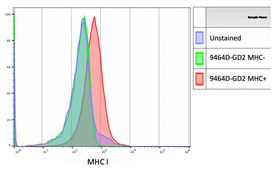
Immunotherapy Regimen



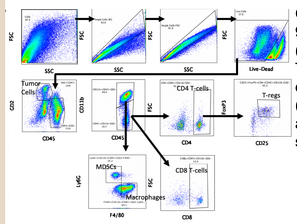
External beam RT (12 Gy) was delivered to 100mm^3 in vivo tumors on day 1. Treated mice received 500µg anti-CD40 IP on day 3; 25µg IT-IC on days 6-10; 200µg anti-CTLA4 IP on days 6, 9, 12; and 50µg CpG IT on days 6, 8, 10.[1-4]

Cell Lines

9464D-GD2 neuroblastoma cells have a low tumor mutation burden and low MHC class I expression and are considered immunologically cold. We developed an MHC class I expressing clone of 9464D-GD2 (9464D-GD2 MHC class I+) using flow cytometry sorting and confirmed stable MHC class I expression via flow cytometry of tumor digests.



Flow Cytometry Gating Strategy



Gating strategy for immunophenotyping of 9464D-GD2 tumor digests. Tumor cells (GD2$^+$CD45$^-$), CD4 T-cells (CD4$^+$ CD45$^+$), Tregs (CD25$^+$FoxP3$^+$CD4$^+$CD45$^+$), CD8 T-cells (CD8$^+$ CD45$^+$), Macrophages (F4/80$^+$Ly6G$^-$), and MDSCs (Ly6G$^+$F4/80$^+$) are measured in relation to total live, singlet cells.

MHC class I expression does not improve response to combination immunotherapy

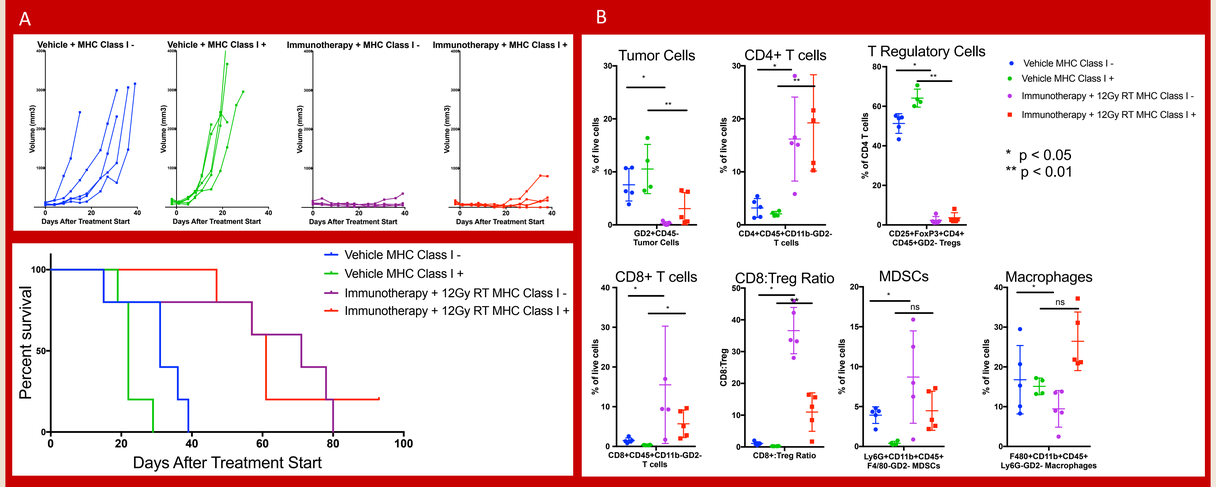


Figure 1. Effect of MHC class I expression on response to RT and combination immunotherapy (IT-IC, anti-CTLA4, anti-CD40, CpG). A) MHC class I expression in 9464D-GD2 derived tumors did not alter tumor growth or survival following treatment. B) MHC class I expression did not alter immune subsets following treatment of 9464D-GD tumors with radiation and combination immunotherapy. Increased numbers of CD8$^+$ and CD4$^+$ T-cells was observed in both MHC class I+ and MHC class I- cell lines. T regulatory cells were also effectively depleted in both treated groups. MDSCs were upregulated in treated MHC class I+ tumors but not MHC class I- tumors. Similarly, macrophages were decreased in MHC class I+ tumors but not MHC class I- tumors.

RESULTS

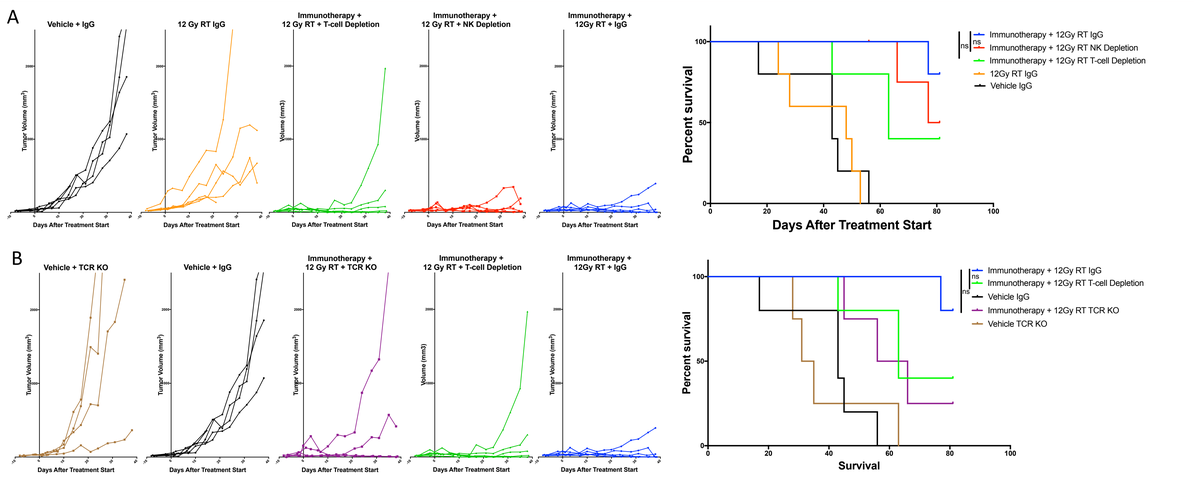


Figure 2. Depletion of immune subsets during treatment of 9464D-GD2 tumors with radiation and combination immunotherapy (IT-IC, anti-CTLA4, anti-CD40, CpG). A) Depletion of NK cells via depleting antibodies (anti-NK1.1, clone PK136) and T cells via T cell depleting antibodies (anti-CD4, clone GK1.5 and anti-CD8, clone 2.43) does not decrease early efficacy (< day 20) of combination treatment, but may decrease survival compared to non-depleted tumors. B) TCR knockout mice that are bearing 9464D-GD2 tumors and are deficient in αβ T cells exhibit similar tumor growth pattern to T cell depleted mice, with neither having significant impact on early efficacy, with a trend toward survival benefit in non-depleted mice.

CONCLUSIONS

- MHC class I expression did not significantly alter immunophenotype or response to treatment in 9464D-GD tumors treated with radiation and combination immunotherapy
- NK and T cell depletion did not alter early response to radiation and combination immunotherapy, though the later adaptive response may be affected by T cell depletion

ADDITIONAL KEY INFORMATION

Future Directions

- Exploration of role of innate immunity and myeloid lineages in treatment response
- Use of combination immunotherapy regimen in metastatic models to assess systemic response.

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