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

Taylor Aiken1,2, Julie Voeller3, Amy Erbe1,2, Alexander Rakhmilevich1,2, Paul Sondel1,2
1 UW School of Medicine and Public Health, Department of Pediatrics
2 UW School of Medicine and Public Health, Department of Human Oncology
3 Baylor College of Medicine

METHODS
Immunotherapy Regimen

Cell Lines

4946D-GD2 neuroblastomas 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 tumor digests 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 9446D-GD2 tumor digests. Tumor cells (CD2+CD24+), CD4 T cells (CD4+CD45+), Tregs (CD25+FoxP3+CD4+CD45+), CD8 T cells (CD8+CD45+), Macrophages (F4/80+Ly6G+), and MDSCs (Ly6G+Ly6C+) are measured in relation to total live, singlet cells.

RESULTS

Evaluation of MHC class I expression on tumor digests.

A) MHC class I expression (CD45+GD2+CD8+CD4+CD45+). B) MHC class I expression (CD45+GD2+CD8+CD4+CD45+).

B) MHC class I expression does not improve response to combination immunotherapy

A) MHC class I expression does not improve response to combination immunotherapy.

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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