**Neuroblastoma**

**Background**

Neuroblastoma is the most prevalent solid tumor in children and the most common tumor in infants less than one year of age. In spite of aggressive multi-modal therapeutic approaches, the five-year survival rate in patients with high-risk neuroblastoma remains poor at 50%.

Although anti-CTLA-4 and anti-PD-1/L1 can suppress neuroblastoma growth in certain mouse models, the role that the immune system has in controlling neuroblastoma growth is not well characterized.

This study provides a comprehensive profile of the neuroblastoma immune response using the murine syngeneic Neuro-2a model and investigates how anti-PD-1 therapy shapes that response in the tumor microenvironment (TME).

**Methods**

- Groups of mice implanted with Neuro-2a tumors were dosed with anti–mouse PD-1 antibody, isotype-matched control antibody, or left untreated.

- Tumor was measured at 7 days post-implantation.

- **MIP-1α** and **MIP-1β** panels were used to profile the following T cell and myeloid subsets in the TME:
  - CD3+ CD4+ and CD8+ T cells, regulatory T cells (Tregs), activated/unactivated CD8+ T cells.

- TMs: Macrophages and tumoral/peritumoral-derived suppressor cells (M1-MSC, M2-MSC), macrophages, M1 and M2 tumor-associated macrophages (TAMs).

- **CD8+** cell pro-inflammatory cytokine responses were measured ex vivo in tumor-derived cells from isotype and anti-PD-1-treated groups.

- **Neuro-2a** tumors consisted relatively few T cells and were dominated by M2-MSC subsets and M2 polarized macrophages.

- Both isotype and anti-PD-1 treatment increased tumor-infiltrating T cells and decreased the prevalence of suppressive myeloid subsets.

- Anti-IL-1 antibody treatment but not isotype control enhanced CD8+ T cell cytokine responses to neuroblastoma implantation models.

**Results**

1. **Neuro-2a** tumors consisted relatively few T cells and were dominated by M2-MSC subsets and M2 polarized macrophages.

2. Both isotype and anti-PD-1 treatment increased tumor-infiltrating T cells and decreased the prevalence of suppressive myeloid subsets.

3. Anti-IL-1 antibody treatment but not isotype control enhanced CD8+ T cell cytokine responses to neuroblastoma implantation models.

**Materials & Methods**

- Female Balb/c mice were purchased from Jackson laboratories and were implanted subcutaneously in the low backs with Neuro-2a cells (p=1–1.7x10^6).

- Mice were treated in extra peritoneal injection with either mice or anti-PD-1 antibodies from the Cell (Medarex), or isotype control (PeproTech) every day for three days as a total dose.

- For immunophenotyping, tumors were processed into single cell suspensions using the GentleMACS Dissociators (Miltenyi Biotec). Samples were analyzed on an Attune Nxt Flow Cytometer (Thermo Fisher Scientific) and data was analyzed using FlowJo software (Treestar).

- For cytokine analysis, tumor-derived cells were cultured at a concentration of 1x10^6/ml treated with IFN-γ (10 ng/ml) and LPS (100 ng/ml) for five hours. The presence of IL-2, IL-12 (interferon-gamma inducing), and tumor necrosis factor alpha (TNF-α) expression was analyzed by flow cytometry.

**Conclusion**

Treatment with anti-PD-1/anti-IL-1 shifts the innate tumor immune profile towards an inflammatory and anti-tumor phenotype characterized by decreased myeloid subsets and increased T cell subsets.