Background Medulloblastoma is the most common malignant brain tumor in children. Despite recent advances in our understanding of its tumor biology, one-third of affected children do not survive this disease. Even amongst survivors of medulloblastoma, there are significant issues with treatment-related health sequelae, and thus more effective and safer therapies are urgently needed. Oncolytic herpes simplex viruses (oHSV), which exploit the dysregulated cellular programs in malignant cells as a replicative advantage, also evoke innate immune responses. As such, they provide an increasingly appealing option for treating many malignancies, including brain cancers.

Methods Here, we studied the effects of the oHSV C134 in two syngeneic medulloblastoma mouse models, one that aligns with the sonic hedgehog (SHH) subtype (MYCN) and another that aligns with group 3 subtype tumors (CMYC). We treated intracranial tumors with C134 or vehicle to evaluate changes in overall survival, then applied single cell RNA-sequencing (scRNA-seq) and flow cytometry to study tumor samples across multiple post-treatment timepoints and characterize the immune responses evoked by C134 treatment.

Results Treatment with C134 increased survival in C57BL/6 mice bearing tumors for the CMYC model (C134 median = 38.5 days, range = 24-75 days, n = 10 vs. vehicle median = 19 days, range = 17-24 days, n = 10, p<0.0001) as well as the MYCN model (C134 median = 17.5 days, range = 14-23 days, n = 10 vs. vehicle median = 13 days, range = 10-14 days, n = 10, p<0.0001). Flow cytometry demonstrated similarities in immunophenotypic response to C134 between the two models including increased M1-like macrophages, CD4+ T cells, CD8+ T cells, NKT cells, and myeloid-derived suppressor cells. Collectively these data provided evidence for a complex immune response. scRNA-seq data analysis allowed higher resolution immune response characterization, indicating over twenty cell types (figure 1). Here, we identified statistically significant (FDR < 0.05) increases in proportions of macrophages, monocytes, lymphocytes, and dendritic cells after C134 treatment. Differential gene expression revealed that cytokines, MHC class I, Ib, and II genes, and interferon-response genes exhibited marked expression changes in lymphocytes, macrophages, microglia, and dendritic cells in response to C134.

Conclusions Our findings suggest a multifaceted immune response contributes to the efficacy of C134 oHSV treatment to prolong survival in two mouse models of medulloblastoma. A wide array of gene expression changes occurs in response to C134 treatment across immune cell types, time points, and medulloblastoma models, illuminating potential mechanisms involved in C134’s antitumor effects.

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Ethics Approval All animal experiments were approved by the Nationwide Children’s Hospital Institutional Animal Care and Use Committee (AR17-00039).