Neuroblastoma (NB) is the most common extracranial solid tumor of early childhood. Arising from poorly differentiated neuroblast-like cells, these tumors can develop anywhere within the sympathetic nervous system and are most commonly found in children younger than two years of age. Although overall survival for neuroblastoma has shown significant improvement with advanced therapies including surgery, radiation, chemotherapy and immunotherapy, the prognosis for high-risk patients remains poor. Even though testing of oncolytic herpes viruses in neuroblastoma therapy has shown antitumor activity in many preclinical models, most of the studies were conducted in the immunosuppressed xenograft setting, in which the therapeutic efficacy was mainly attributed to direct oncolysis from virus replication. As virotherapies are known to consist of both oncolytic and immunological phases, we sought models of pediatric cancer that are resistant to the lytic effects so that we could study the immunologic effects in isolation. Murine neuroblastoma cell lines, designated 9464D and 975A2, derived from TH-MYCN transgenic mice were found to be relatively resistant to infection by Seprehvir (HSV1716) treatment in vitro. Likewise, virus replication was also substantially impaired in vitro and in vivo. Nevertheless, we found a significant increase of CD11b+ myeloid cells, including myeloid derived suppressor cells (MDSCs) and M2-like tumor-associated macrophages (TAM) after one dose of intra-tumor injection of Seprehvir. We also observed an induction of PD-L1 expression in MDSCs, which may limit the function of adaptive immunity. Although repetitive (3 doses) intra-tumor injection of Seprehvir prompted a significant influx of CD8+ T cells into both 9464D (increased from 0.7% to 6.7%) and 975A2 tumors (increased from 0.4% to 15%), we observed only moderate anti-tumor efficacy, indicating that these newly recruited CD8 T cells may not function properly in killing tumor cells. Our results suggest that future studies should examine the roles of virus-induced myeloid cell recruitment in the tumor microenvironment (pro-tumorigenic or anti-tumorigenic) and how these affect newly recruited CD8+ T cell function in order to further exploit effective adjuvants to achieve optimal therapeutic efficacy.

**Conclusions**

- **oHSV treatment** of 9464D and 975A2 produces minimal virus replication and tumor cell killing in vitro and in vivo.
- Intra-tumor injection of Seprehvir increases CD8+ T cell recruitment but has only a modest impact on anti-tumor efficacy, suggesting that the activities of these newly recruited CD8+ T cells are impaired.
- Intra-tumor injection also induces arginase I and PD-L1 expression in TAMs and MDSCs, which may be contributing factors for suppression of CD8+ T cell effector function.
- Depletion of TAMs by clodrosome reduces tumor burden and shows a trend towards better outcome when combined with oHSV therapy.

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