Checkpoint Blockade Enhances Oncolytic Herpes Virotherapy in Immunosuppressive Sarcoma Models

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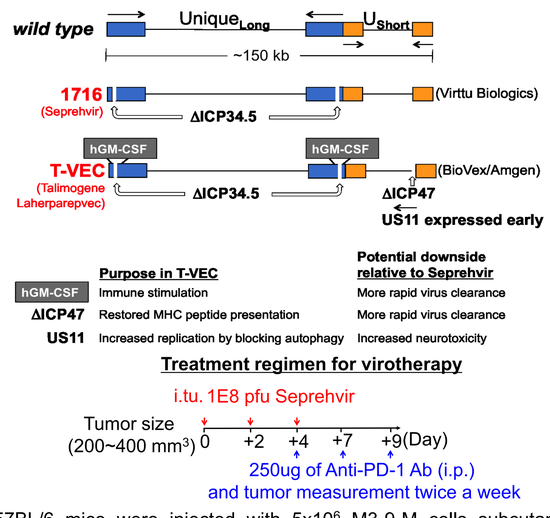
abstract

Most solid tumors are characterized by an immunosuppressive microenvironment, limiting the effectiveness of antitumor immunotherapeutics. Programmed cell death protein (PD)-1-mediated T cell suppression via engagement of its ligand, PD-L1, is of particular interest due to recent successes in selected adult cancers. The utility of PD1-directed therapy for pediatric cancers is unknown, especially given the paucity of mutations and thus infrequent neoantigens in many types of childhood tumors. Oncolytic virotherapy induces tumor shrinkage via a multistep process including direct tumor cell lysis, induction of cytotoxic or apoptosis-sensitizing cytokines, and induction of antitumor T cell responses. We recently demonstrated that intratumoral injection of an oncolytic herpes virus induced growth delays and in some cases durable remissions in several mouse models of rhabdomyosarcoma. The effects were T cell-mediated, as surviving mice were resistant to tumor rechallenge and efficacy was lost in athymic nude hosts (Leddon et al., Mol Ther-Oncolytics **1**, Article number: 14010, 2015). We found these tumor models express PD-L1, suggesting that T cell checkpoints may in part limit virus-induced antitumor immunity. Here we show the implantable C57BL/6 rhabdomyosarcoma model, M3-9-M, showed a moderate response to single-agent Seprehvir (HSV1716), a virus currently in pediatric clinical trials (NCT00931931, NCT02031965). Single-agent PD-1 blockade also showed moderate but significant tumor growth delay with no complete responses. Combining these two therapies together substantially prolonged overall survival with several complete responses post 60 days treatment. Interestingly, mice that received combination therapy did not show more T cell recruitment to the tumor, but instead displayed higher immune inflammatory responses and a less immunosuppressive microenvironment, as measured by decreased Tregs and suppressive cytokines. Overall, our data suggest the combination of PD-1 and oncolytic herpes virotherapy may be an effective treatment strategy for some cancers.

methods



C57BL/6 mice were injected with 5x10^6 M3-9-M cells subcutaneously. Tumors were treated intra-tumorally (i.tu.) with Seprehvir when sizes reached 200~400 mm^3. Intra-peritoneal (i.p.) injection of anti-PD-1 antibody were given twice a week after last dose of virus treatment. Tumor growth was monitored twice a week. Mice were sacrificed when tumors reached 2,500 mm^3 in volume or grew over 2cm in length. pfu=Plaque Forming Unit

results

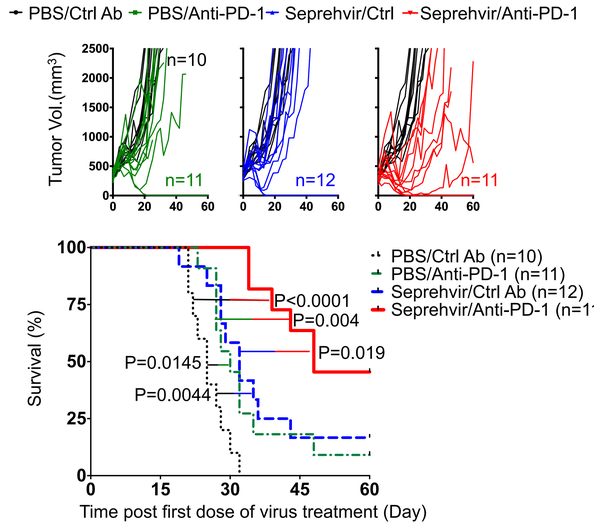


Figure 1. Combination of Seprehvir with anti-PD-1 antibody significantly prolongs survival with several complete responses in male to female M3-9-M tumor model. Female C57BL/6 mice were injected with 5x10^6 M3-9-M cells subcutaneously. The effects of Seprehvir plus anti-PD-1 blockade on antitumor efficacy were evaluated by measuring tumor volumes over time. Survival data were evaluated for statistical significance with Log-rank (Mantel-Cox) test.

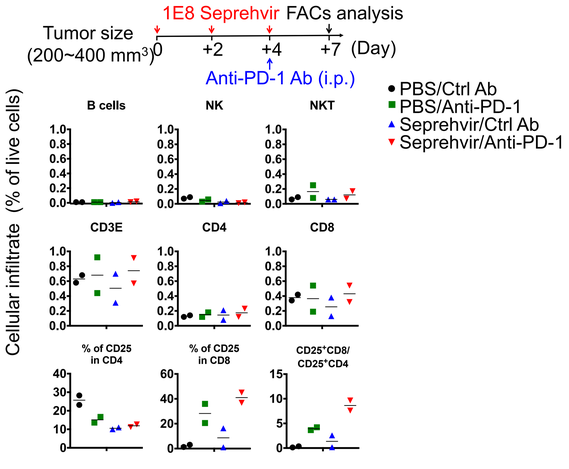


Figure 4. Combination therapy induces more CD25$^+$CD8$^+$ memory T cells but less CD25$^+$CD4$^+$ Treg cells. Female M3-9-M tumor-bearing mice were received three doses of intra-tumoral (i.tu.) Seprehvir injection followed by intra-peritoneal (i.p.) injection of anti-PD-1 or control antibody. Immune cell infiltrates in tumors were evaluated by flow cytometry analyses 72 hours post intra-peritoneal antibody injection.

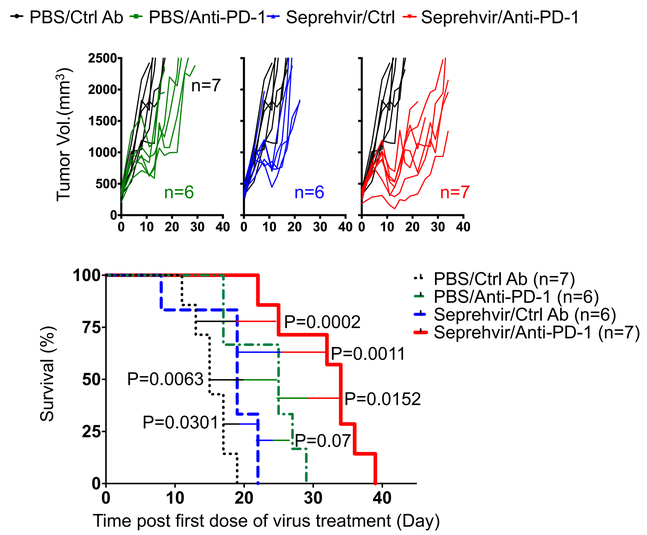


Figure 2. Combination of Seprehvir with anti-PD-1 antibody significantly prolongs survival in less immunogenic male to male M3-9-M tumor model. Male C57BL/6 mice were injected with 5x10^6 M3-9-M cells subcutaneously. The effects of Seprehvir plus anti-PD-1 blockade on antitumor efficacy were evaluated by measuring tumor volumes over time. Survival data were evaluated for statistical significance with Log-rank (Mantel-Cox) test.

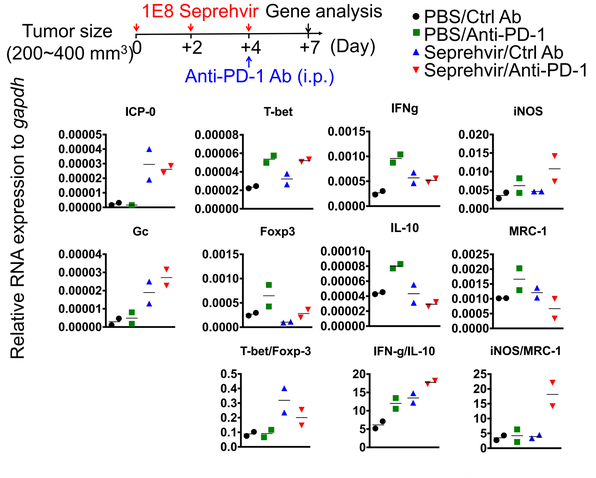


Figure 5. Combination therapy induces higher inflammatory gene expression and lower immune suppressive gene expression. Female M3-9-M tumor-bearing mice were received three doses of intra-tumoral (i.tu.) Seprehvir injection followed by intra-peritoneal (i.p.) injection of anti-PD-1 or control antibody. Tumors were harvest 72 hours post intra-peritoneal antibody injection. T-bet (Th-1-related gene), Foxp3 (Treg-related gene), IFNg, IL-10, iNOS (M1 macrophage-related gene) and MRC-1 (M2 macrophage-related gene) were quantified by real-time. Date are represented as relative RNA expression to *gapdh*.

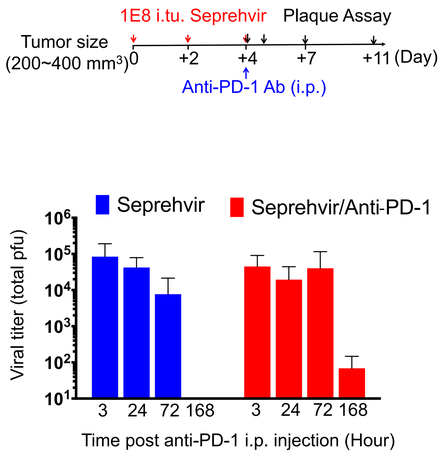


Figure 3. Checkpoint inhibition does not significantly alter intra-tumoral viral kinetics. Female M3-9-M tumor-bearing mice were treated with three doses of 1E8 pfu of Seprehvir intra-tumorally (i.tu.) followed by intra-peritoneal (i.p.) injection of anti-PD-1 or control antibody. Tumors were harvested 3, 24, 72 and 168 hours after intra-peritoneal antibody injection for plaque assay. Data are expressed as total plaque-forming units (pfu) per tumor.

conclusions

- Combination of oHSV treatment with immune checkpoint inhibitor anti-PD-1 significantly prolongs survival in both male to male and male to female rhabdomyosarcoma models.
- Greater antitumor efficacy was observed in male to female murine rhabdomyosarcoma, suggesting that combination therapy favors more immunogenic microenvironments.
- Combination therapy did not result in more T cell recruitment or affect *in vivo* virus activity.
- Combination therapy induces more inflammatory responses with less immune regulatory/suppressive responses.

acknowledgements

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