Seprehvir, an Oncolytic Herpes Viroimmunotherapeutic, Enhances Therapeutic Efficacy of T Cell Checkpoint Inhibition in Solid Tumors By Increasing T Cell Recruitment and Remodeling the Immunosuppressive Microenvironment

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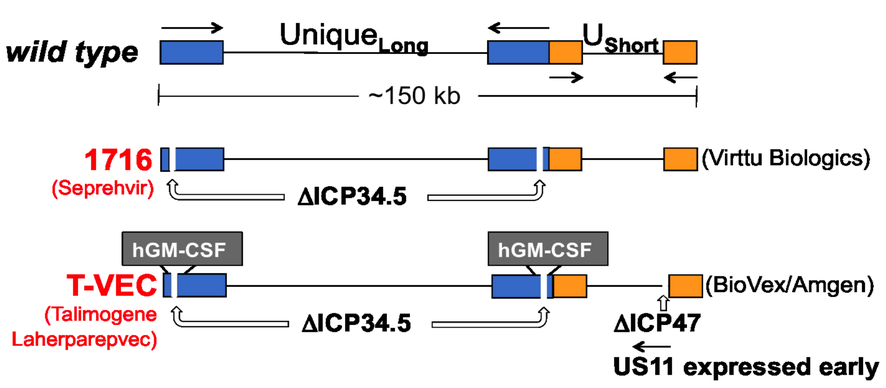
Abstract

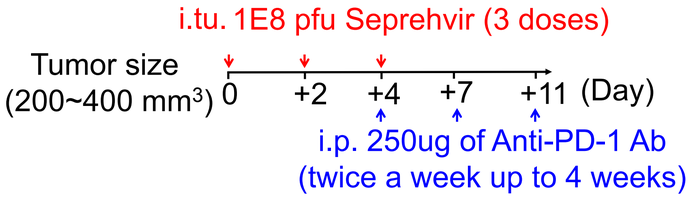
Most solid tumors are characterized by an immunosuppressive microenvironment, limiting the effectiveness of antitumor immunotherapeutics. Programmed cell death protein (PD)-1-mediated T cell suppression via engagement of its ligand, PD-L1, is of particular interest due to recent successes in selected cancers. Oncolytic virotherapy induces tumor shrinkage via a multistep process including direct tumor cell lysis, induction of cytotoxic or apoptosis-sensitizing cytokines, and induction of anti-tumor T cell responses. We recently demonstrated that intratumoral injection of an oncolytic herpes virus induced T cell mediated growth delays and in some cases durable remissions in several immunocompetent mouse models of rhabdomyosarcoma (Leddon et al., Mol Ther-Oncolytics 1, article number: 14010, 2015). We classified models into high and low immunogenicity based on their differential growth rates in syngeneic hosts relative to athymic nude mice. In syngeneic animals, response to single agent Seprehvir (HSV1716), a virus currently in pediatric clinical trials (see www.clinicaltrials.gov: NCT00931931), was directly proportional to tumor immunogenicity, without detectable virus replication. Single agent PD-1 blockade also showed moderate but significant tumor growth delay. Strikingly, combining these two therapies together substantially prolonged overall survival with several complete responses lasting beyond 60 days treatment in the most immunogenic model. To investigate the mechanism of combination, we analyzed the effects of treatment on intratumoral virus spread, recruitment of myeloid and lymphoid cellular subpopulations, and immunocytokines. There were no effects of single or combination therapy on virus recovered from tumors, on myeloid cells populations, or on NK cells. In contrast, the combination of Seprehvir and anti-PD1 antibody resulted in a more proinflammatory microenvironment characterized by increased CD4+ and CD8+ T cells without increased CD4+CD25+Foxp3+ regulatory T cells, increased gene expression for T-bet, interferon-gamma and iNOS and decreased gene expression for IL-10 and MRC-1, with all changes more marked in the more highly immunogenic tumor model. Overall, our data suggest the combination of PD-1 and oncolytic herpes virotherapy may be an effective treatment strategy for some cancers.

Methods



	Purpose in T-VEC	Potential downside relative to Seprehvir
hGM-CSF	Immune stimulation	More rapid virus clearance
ΔICP47	Restored MHC peptide presentation	More rapid virus clearance
US11	Increased replication by blocking autophagy	Increased neurotoxicity

Treatment regimen for virotherapy



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 (RMP1-14) was given twice a week, up to 4 weeks, starting from the 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

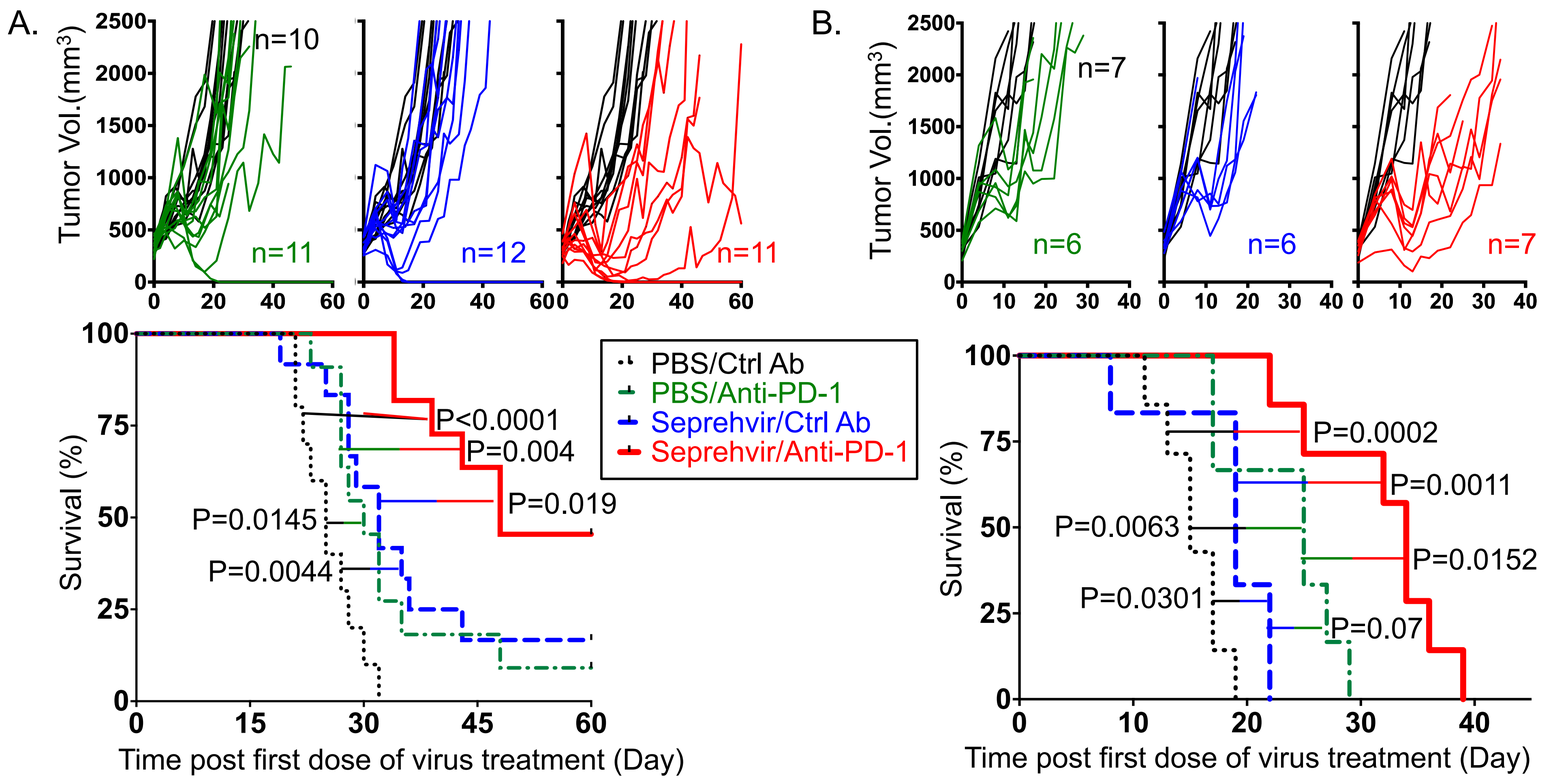


Figure 1. Combination of Seprehvir with anti-PD-1 antibody significantly prolongs survival with several complete responses in male to female M3-9-M tumor model. A) Female or B) Male C57BL/6 mice were injected with M3-9-M cells subcutaneously. The effects of Seprehvir plus anti-PD-1 blockade on antitumor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

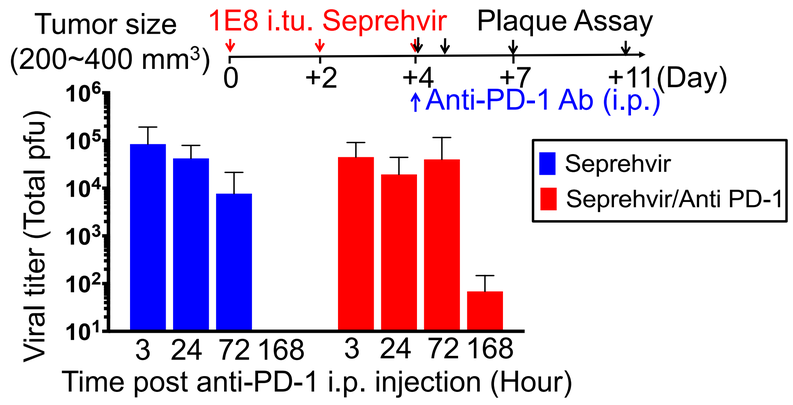


Figure 2. Checkpoint inhibition does not significantly alter intra-tumoral viral kinetics. Female M3-9-M tumor-bearing mice were treated with three doses of 1E8 pfu of Seprehvir intra-tumorally (i.tu.) followed by intra-peritoneal (i.p.) injection of anti-PD-1 or control antibody. Tumors were harvested 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.

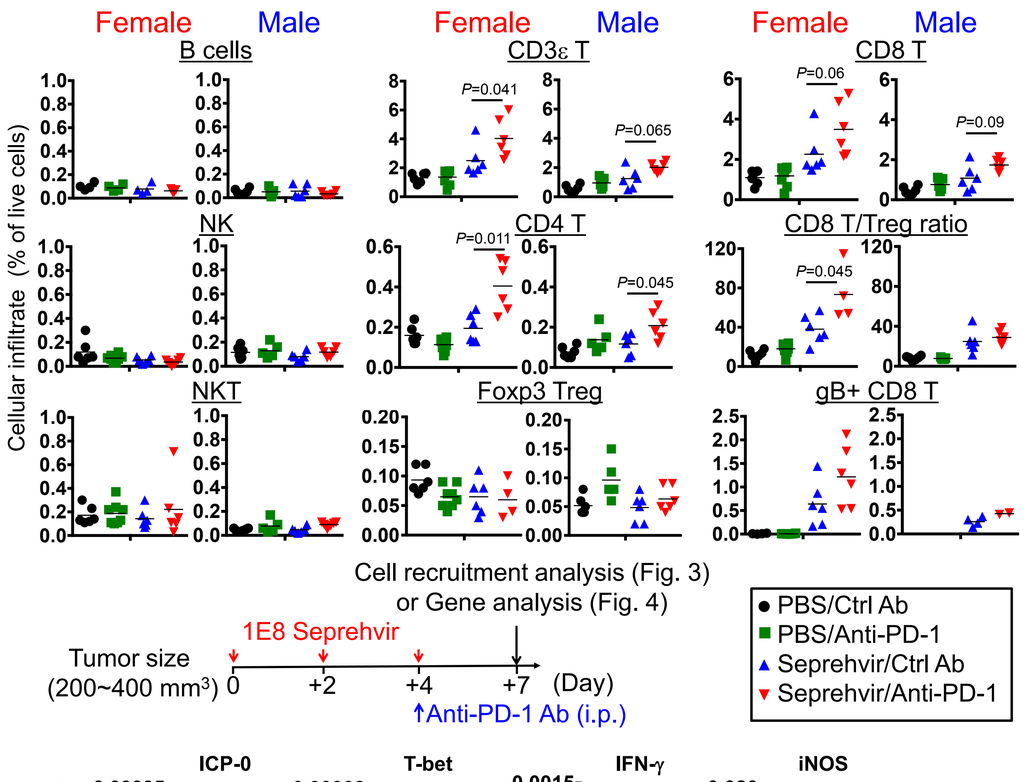


Figure 3. Combination therapy induces more $CD4^+$ and $CD8^+$ T cells but less $CD25^+CD4^+$ Treg cells. Female or Male M3-9-M tumor-bearing mice received three doses of intra-tumoral (i.tu.) Seprehvir injection followed by intra-peritoneal (i.p.) injection of anti- PD-1 or control antibody. Immune cell infiltrates in tumors were evaluated by flow cytometry analyses 72 hours post intra-peritoneal antibody injection. Statistical analysis was performed by unpaired t-test.

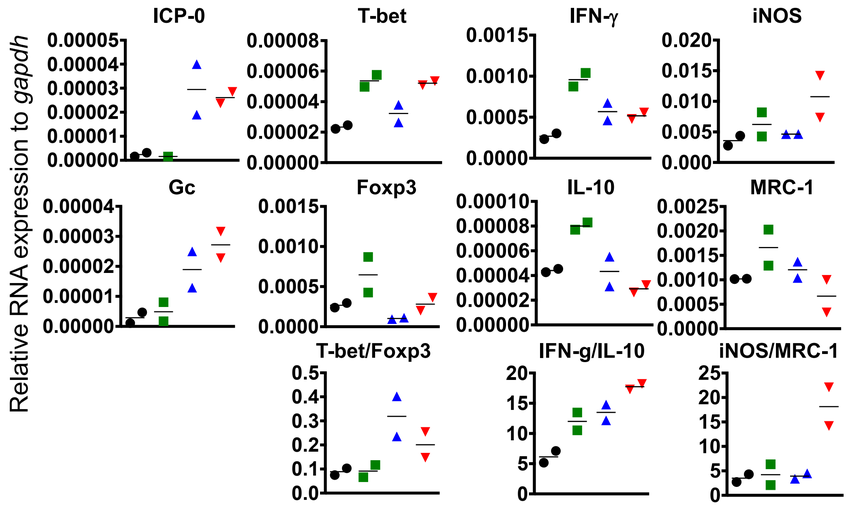


Figure 4. Combination therapy induces higher inflammatory gene expression and lower immune suppressive gene expression. Female M3-9-M tumor-bearing mice received three doses of intra-tumoral (i.tu.) Seprehvir injection followed by intra-peritoneal (i.p.) injection of anti- PD-1 or control antibody. Tumors were harvested 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 PCR. Data are represented as relative RNA expression to *gapdh*.

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 show dramatic impact on virus activity.
- Combination therapy induces more inflammatory responses with less immune regulatory/suppressive responses.

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