Seprehvir, an Oncolytic Herpes Immunotherapeutic, Enhances GD2-Directed Chimeric Antigen Receptor (CAR) T-Cell Therapy in GD2-Expressing Solid Tumor Xenografts

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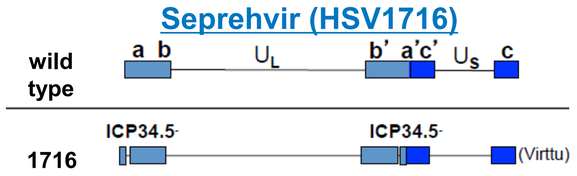
Abstract

While chimeric antigen receptor (CAR) T-cell therapies have shown remarkable anticancer efficacy in patients with relapsed and refractory lymphoid leukemias, their effectiveness in patients with solid tumors has been more challenging. Among the barriers thought to interfere with CAR T cell efficacy are impaired homing to tumors and poor CAR T cell persistence, likely attributable to the immunosuppressive microenvironment. Due to their pro-inflammatory effects, oncolytic viruses are strong candidates to potentiate the competence of CAR T cells within solid tumors. Seprehvir (HSV1716) is an HSV-1 attenuated by deletion of the RL1 gene encoding the neurovirulence protein ICP34.5. The virus has a long track record of safety in clinical trials and is currently being tested in adolescents and young adults with refractory solid tumors (NCT00931931). We hypothesized that intra-tumoral administration of Seprehvir enhances GD2-directed CAR T cell efficacy. We characterized the chemokine and cytokine profiles of human GD2-positive Ewing sarcoma and neuroblastoma cell lines before and after Seprehvir inoculation. We performed transwell migration assays of third-generation (containing CD28, OX40, and CD3z signaling domains) GD2-directed human CAR T-cells before and after the addition of Seprehvir in these models in vitro. We then performed in vivo survival studies using athymic nude mice and cyclophosphamide (CPM) lymphodepletion prior to CAR therapy. Our results suggest that infection of these pediatric solid tumor models with Seprehvir induces an immune response, which includes the T-cell attractant chemokines CXCL-10 (IP-10) and CCL-5 (RANTES) and T-cell activating cytokines such as IFN-g and TNF-a, while down-regulating such inhibitory cytokines as TGF-b. Flow cytometry analysis revealed variable tumoral GD2 surface expression on each of these models, while the CAR T-cells displayed high CXCR-3 and CCR-5 surface expression, allowing for chemotactic signaling through CXCL-10 and CCL-5, respectively. The CAR T-cells displayed increased migration toward oHSV-infected tumor cells over non-infected cells. Mice treated with combination therapy had significantly delayed tumor growth and prolonged survival when compared to CAR treatment alone. Despite being athymic nude mice, the majority of mice cured by combination therapy were resistant to tumor re-challenge, suggesting the long-term persistence of CAR T cells. These results indicate that the addition of Seprehvir may be a valuable adjunct to CAR T-cell therapy and should be further explored in clinical trials.

Methods

Seprehvir (HSV1716)



CAR T-Cells

3rd generation GD2-directed human CAR T-cells
(CD28, OX40, and CD3ζ signaling domains)

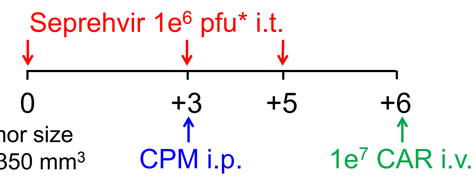
Tumor Models

Human EWS: **A673**
Human NBL: **SK-N-AS**

In Vitro:

- ✓ Characterized oHSV-induced chemokine/ cytokine gene expression by RT-PCR
  - ➢ Tumor cells cultured with Seprehvir at multiplicity of infection (MOI) = 10 x 12 hours
- ✓ Determined tumoral GD2 expression by flow cytometry
- ✓ Determined CAR T-cell CXCR-3 and CCR-5 expression by flow cytometry
- ✓ Performed transwell migration assays:
  - ➢ Tumor cells cultured with Seprehvir at MOI = 1 x24 hours
  - ➢ Red fluorescent PKH23-stained CAR T-cells added to 5 μm pore inserts x 2 hours
  - ➢ Negative control: media alone
  - ➢ Positive controls: media with 75 ng/ml CXCL-10 (IP-10) or 10 ng/ml CCL-5 (RANTES)
  - ➢ Cells quantified through microscopic visualization
  - ➢ Results represent averages of n replicates for each sample

In Vivo:



- ➢ Athymic nude mice with subcutaneous flank tumors
- ➢ PBS or Seprehvir intra-tumorally (i.t.) x 3
- ➢ Intra-peritoneal (i.p.) PBS or cyclophosphamide (CPM) x 1 prior to CAR treatment
- ➢ Intravenous (i.v.) PBS or CAR T-cells x 1

*pfu=Plaque Forming Unit

In Vitro Results

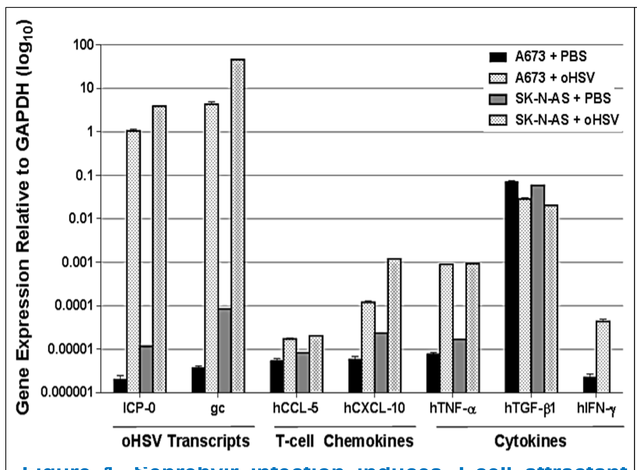


Figure 1. Seprehvir infection induces T-cell attractant chemokines and T-cell activating cytokines *in vitro.*

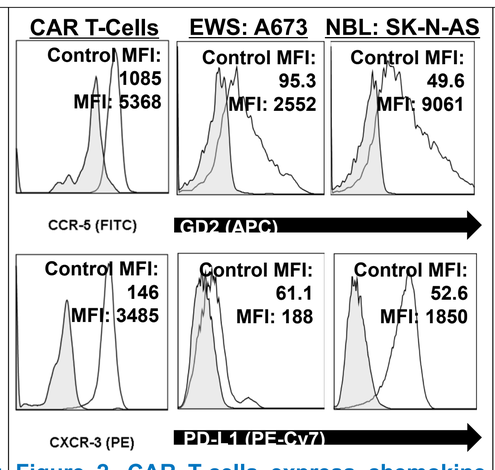


Figure 2. CAR T-cells express chemokine receptors (left). Tumor cells variably express GD2 and PD-L1 (right).

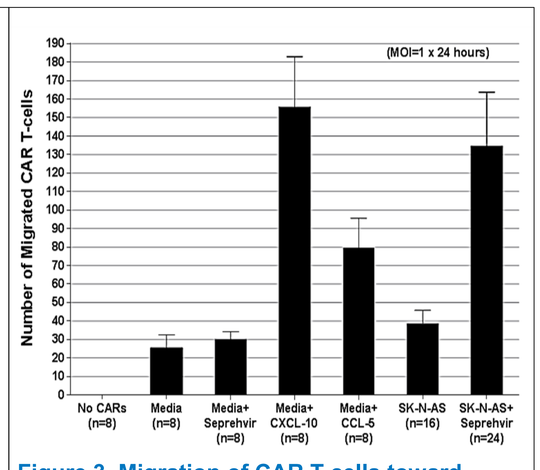


Figure 3. Migration of CAR T-cells toward SK-N-AS with and without Seprehvir infection.

Background

High Risk Neuroblastoma (NBL)

- ➢ Most common non-CNS pediatric solid tumor
- ➢ Multimodal and targeted therapy
- ➢ ~15% total childhood cancer deaths
- ➢ <10% survival for ~50% of children who relapse

Ewing Sarcoma (EWS)

- ➢ Among most prevalent solid tumor afflicting older children and adolescents
- ➢ ~30% refractory to conventional therapy
  - ➢ ~30% survival for patients with metastases

GD2

- ➢ Disialoganglioside expressed on NBL and EWS
- ➢ Strategic immunotherapeutic target

Chimeric Antigen Receptor (CAR) T-Cells

- ➢ Engineered T-cells targeted against tumor antigen
- ➢ Remarkable efficacy in relapsed/ refractory lymphoid leukemias
- ➢ Little clinical success against solid tumors
  - ➢ Modest migration to tumor
  - ➢ Lack of activation, proliferation, and persistence
  - ➢ Attributable to solid tumor immunosuppressive microenvironment

Oncolytic Herpes Simplex I Virotherapy (oHSV)

- ➢ Tumor-selectivity
- ➢ Recent FDA approval
- ➢ Several open clinical trials
- ➢ 2 antitumor efficacy mechanisms
  - ➢ Direct lytic effect
  - ➢ Induction of immune response

In Vivo Results

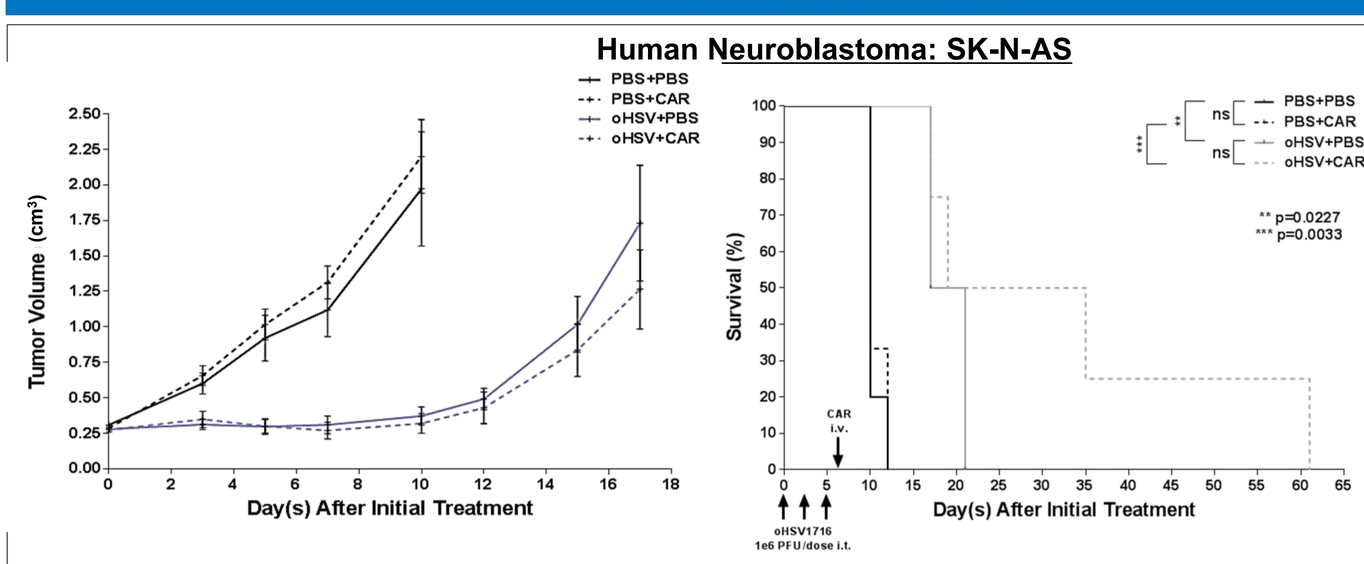


Figure 4. Seprehvir significantly delays tumor growth and enhances anti-tumor efficacy of GD2-directed human 3rd generation CAR T-cells against the neuroblastoma xenograft model SK-N-AS.

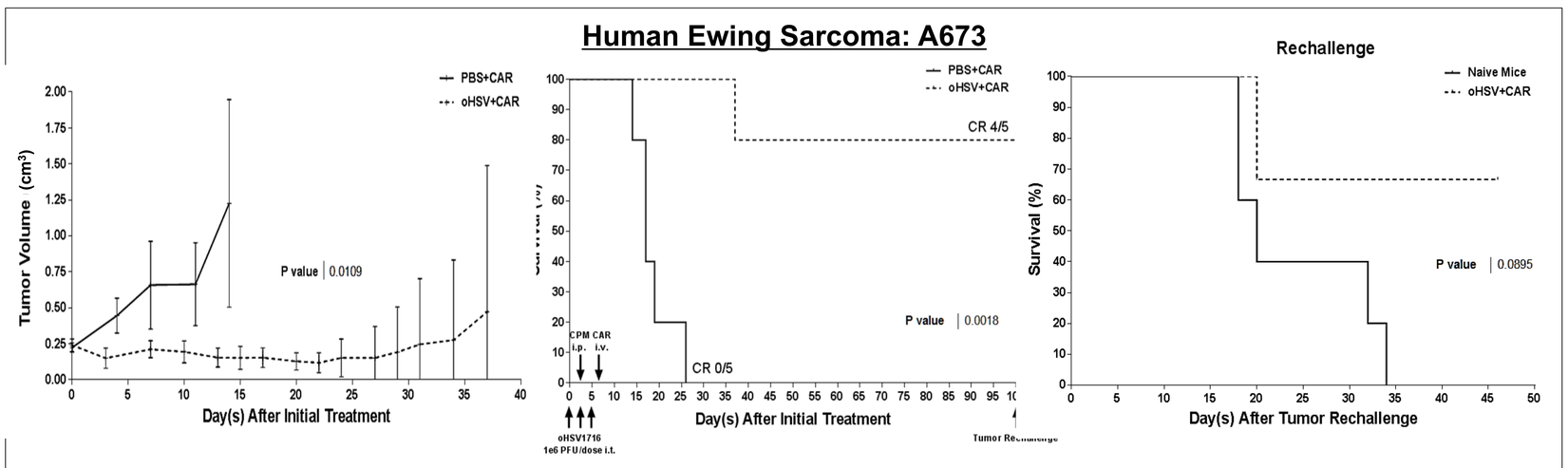


Figure 5. Seprehvir significantly delays tumor growth and enhances anti-tumor efficacy of human GD2-directed CAR T-cells against human Ewing sarcoma xenograft model A673. Cured mice were re-challenged with tumor cells after 100 days survival.

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Conclusions

- ➢ oHSV infection induces release of chemokines and cytokines that promote CAR T-cell migration and activation.
- ➢ oHSV enhances GD2-directed human CAR T-cell antitumor efficacy against GD2-expressing pediatric solid tumors.

Implications

- ➢ **oHSV is a promising adjunct to CAR T-cell therapy for pediatric solid tumors.**
- ➢ **The combination of oHSV and CAR T-cell therapy should be further explored in clinical trials.**

Acknowledgements

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