A Novel TNFRSF25 Agonist, mPTX-35, Synergizes with mHS-110, mHS-130 and anti-PD-1 to Delay Tumor Growth

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Introduction

PTX-35 is a potential first-in-class agonist antibody targeting death receptor 3 (DR3) or tumor necrosis factor receptor superfamily member 25 (TNFRSF25). T-cell mediated immune responses are initiated by presentation of cognate antigens in the context of appropriate co-stimulatory molecules. In contrast to other T cell co-stimulators, TNFRSF25 is preferentially expressed in activated CD4+ and CD8+ T cells, after prior engagement of the T cell receptor (TCR). Given that T cell costimulation by TNFSFRSF25 agonism is antigen-dependent, we are combining it with cancer-associated antigens (HS-110). In addition, anti-tumor activity may be enhanced in combination with other co-stimulators and checkpoint inhibitors. In this study, we evaluated whether potent anti-tumor activity can be achieved through a combination of a TNFRSF25 agonist (mPTX-35), cancer antigens (mHS-110), an OX40 agonist (mHS-130) and a checkpoint inhibitor (mPD-1 inhibitor) in B16F10 mouse melanoma model.

PTX-35 is a humanized agonist antibody targeting TNFRSF25. mPTX-35 is a mouse monoclonal antibody targeting TNFRSF25 in mice. Stimulation of TNFRSF25 results in expansion of antigen-specific CD8+ T-cells, which drives killing of tumor cells.

HS-110 is an allogeneic cell-based therapy that is designed to secrete tumor antigen chaperone gp96-Ig with a variety of cancer-associated antigens. HS-110 is being tested in combination with PD-1 inhibitors in a phase 1/2 clinical trial in non-small cell lung cancer (NSCLC) patients (NCT02439450). mHS-110 is a genetically engineered murine B16.F10 melanoma cell line that expresses gp96-Ig with ovalbumin (OVA) and cancer associated antigens.

HS-130 is an allogeneic cell-based therapy designed to express a costimulatory molecule, Fc-OX40L fusion protein. HS-130 is being tested in combination with HS-110 in a phase 1 clinical trial in patients with solid tumors (NCT04116710). mHS-130 is a genetically engineered murine B16F10 melanoma cell line that expresses Fc-OX40L fusion protein.

Mechanism of Action of PTX-35

Potential synergy with HS-110, HS-130 and anti-PD-1

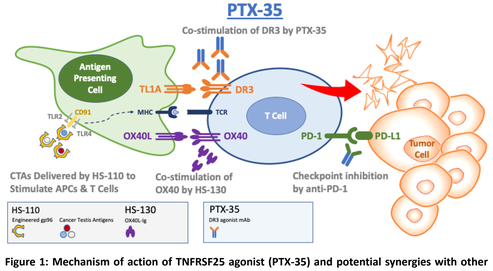


Figure 1: Mechanism of action of TNFRSF25 agonist (PTX-35) and potential synergies with other co-stimulators and checkpoint inhibition

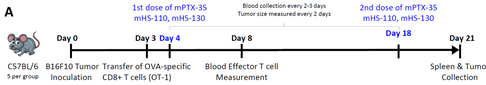
TNFRSF25, as well as many co-stimulators and checkpoint molecules are expressed in activated T lymphocytes. Their specific ligands are expressed in professional antigen presenting cells (APCs) or tumor cells. The costimulation by a TNFRSF25 agonist (PTX-35) may act in synergy with tumor antigens chaperoned by gp96-Ig (HS-110)*, an OX40 agonist (HS-130) or a checkpoint inhibitor (PD-1 inhibitor) in enhancing T cell activation and tumor growth inhibition.

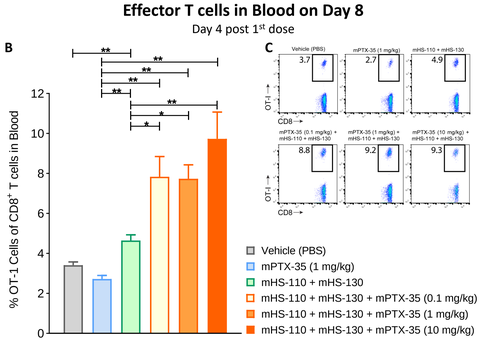
* The gp96-Ig destines these tumor associated antigens for uptake by the scavenger receptor CD91 on antigen presenting cells (APCs) for cross-presentation to T-cell receptor (TCR) on T-cells via major histocompatibility complex (MHC), which leads to the clonal expansion of antigen-specific T cells. gp96 is also a damage associated molecular pattern (DAMP) that activates APCs via TLR2/4 stimulation.

Method

C57BL/6 mice were inoculated with murine B16F10 melanoma that expresses ovalbumin (B16F10-OVA) via subcutaneous injection (S.C.), and adoptively transferred with syngeneic ovalbumin-specific CD8$^+$ T cells (OT-1) labeled with green fluorescent protein. mHS-110, mHS-130, mPTX-35 and anti-PD-1 were administered in different combinations. A second dose was administered 14 days later. Effector and effector memory T cells in peripheral blood and spleen, as well as effector T cells in the tumor-microenvironment were characterized. Mann-Whitney two-tailed test was used for pairwise comparisons (p values not adjusted for multiple comparisons). Two-way ANOVA was used for comparing multiple groups across time points. Statistical analysis was performed using GraphPad Prism 8.

Combination of mPTX-35, mHS-110 and mHS-130





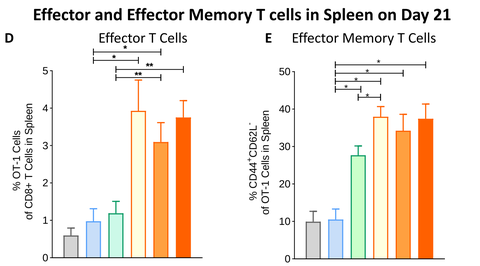


Figure 2: Effector and effector memory T cells in blood and spleen

(A) Experimental design **(B, C)** Percentage of OT-1 T cells in CD8$^+$ T cells in blood on day 8. **(D)** Percentage of OT-I T cells in CD8$^+$ T cells in spleen on day 21. **(E)** CD44$^+$ CD62L$^-$ OT-1 cells in OT-1 cells in spleen on day 21. Error bars represent SEM (5 mice/ group). Mann-Whitney two-tailed test used for pairwise comparisons *p<0.05, **p<0.01.

Combination of mPTX-35, mHS-110 and mHS-130

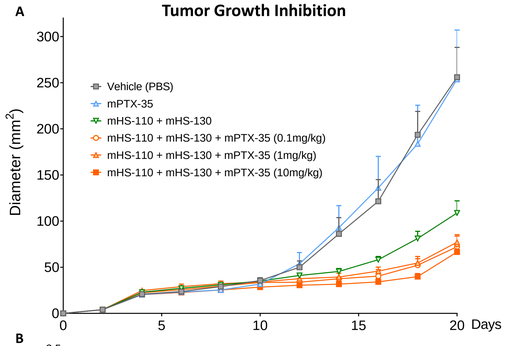


Figure 3: Tumor growth inhibition

(A) Tumor size (calculated by the largest multiplied by the smallest diameter). 2-way ANOVA was performed to determine statistical significance from vehicle control. **(B)** Tumors weight on day 21 post-tumor inoculation. Mann-Whitney two-tailed test was used for pairwise comparisons. *p<0.05. p values did not adjust for multiple comparisons.

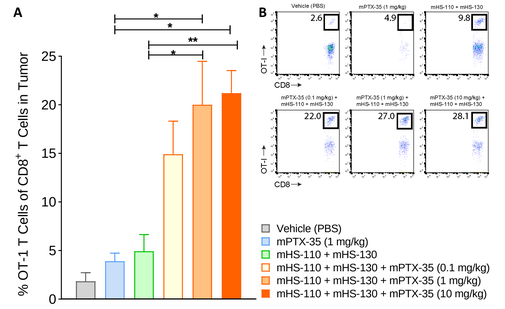
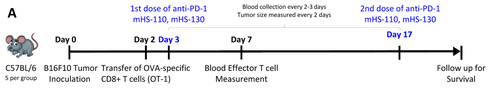


Figure 4: Tumor infiltrating CD8+ T lymphocytes (TILs)

(A) Percentage of OT-1 T cells among CD8+ T cells in the tumor on day 21 post-tumor inoculation. Error bars represent SEM. Each group had 5 mice. Mann-Whitney was used for pairwise comparisons. *p<0.05, **p<0.01. **(B)** Representative FACS plots.

Combination of mPTX-35, mHS-110 and anti-PD-1



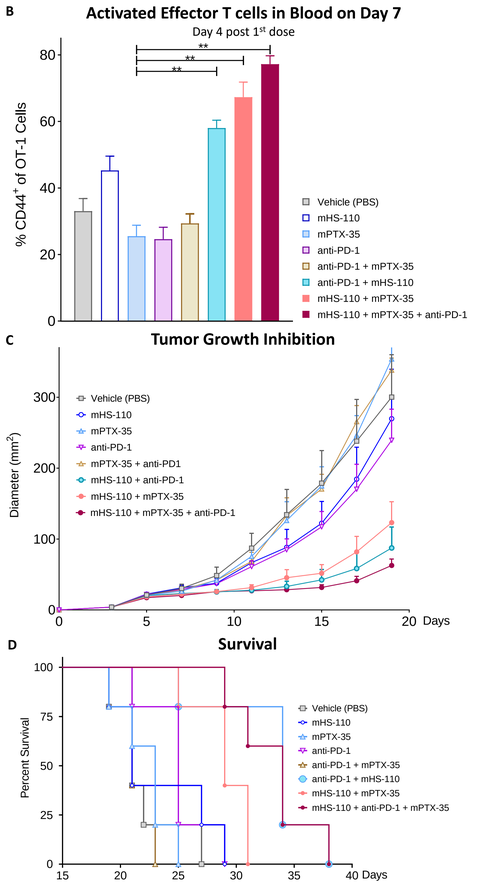


Figure 5: (A) Experimental design **(B)** Percentage of CD44$^+$ OT-1 cells among OT-1 cells in blood on day 7. Error bars represent SEM (5 mice/group). Mann-Whitney two-tailed test used for pairwise comparisons. p values did not adjust for multiple comparisons. **p<0.01. **(C)** Tumor size calculated by the largest diameter multiplied by the smallest diameter. 2-way ANOVA was performed. **(D)** Overall survival was monitored over 38 days. mPTX-35 dosed at 1mg/kg.

PTX-35 IND-Enabling Studies

Type		Measured Parameter	Value
Pharmacology		EC_{50} on human Jurkat-DR-3 cells	760 ng/mL
		MABEL in mice	0.01 mg/kg
		NOEL in mice	0.001 mg/kg
		PAD in mice (range)	0.1 – 10 mg/kg
Safety Pharmacology		Human PBMCs stimulated with anti-CD3 and PTX-35	No increase in any deleterious cytokines, thirty-five different analytes tested
Tissue cross-reactivity		Membrane of lymphoid cells	Human, monkey, mouse
Toxicology & Toxico-kinetics	28-day DRF in mouse i.v. bolus	NOAEL	200 mg/kg
		C_{max}	2,440 µg/mL
		T_{max}	24 hrs
		$AUC_{(0\text{-}216\ hrs)}$	390,000 hr x µg/mL
	2-week DRF in NHP i.v. bolus	NOAEL	96 mg/kg/dose
		C_{max}	2,590 µg/mL
		$AUC_{(0\text{-}360\ hrs)}$	261,500 hr x µg/mL
	8-week in NHP* i.v. bolus	NOAEL	100 mg/kg
		C_{max}	2,660 µg/mL
		$AUC_{(0\text{-}336\ hrs)}$	298,000 µg x hr/mL
		TDAR (KLH and Tetanus)	No suppression at doses evaluated

*KLH immunization challenge demonstrated that PTX-35 had no deleterious effect on immunity or the ability to mount an immune response. MABEL = the minimum anticipated biological effect level; NOEL = no observed effect level; NOAEL = no observed adverse effect level; DRF = dose range finding; NHP = Non-human primate; KLH = Keyhole limpet hemocyanin. TDAR = T cell-dependent antibody response.

Conclusions

- In a B16F10 melanoma mouse model, mPTX-35, in combination with mHS-110 and mHS-130, resulted in significant tumor growth reduction, activation of effector and effector memory CD8$^+$ T cells and expansion of tumor-infiltrating CD8$^+$ T lymphocytes (TILs).
- Addition of anti-PD-1 antibody to mPTX-35 and mHS-110 resulted in a significant increase of overall survival, further reduction in tumor burden and enhanced antigen-specific CD8$^+$ T cell responses.
- PTX-35 has a favorable safety profile in non-human primates and first-in-human study in cancer patients is planned.

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