HPN536, a T cell-engaging, Mesothelin/CD3-specific TriTAC for the treatment of solid tumors

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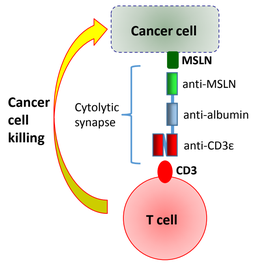
RATIONALE

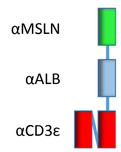
- Mesothelin (MSLN) is a GPI-linked membrane bound tumor antigen
- Normal tissue expression of MSLN is restricted to single-cell, mesothelial layers lining the pleural, pericardial, and peritoneal cavities
- MSLN is overexpressed in ovarian, pancreatic, lung and triple-negative breast cancers and mesothelioma
- Overexpression of MSLN is associated with poor prognosis in lung adenocarcinoma and triple-negative breast cancer



- MSLN has been used as cancer target antigen for numerous modalities, including immunotoxins, vaccines, antibody drug conjugates and CAR-T cells: early signs of clinical efficacy have validated MSLN as target, but therapies with improved efficacy are needed to treat MSLN-expressing cancers
- HPN536 is engineered to direct T cells to kill MSLN-expressing cancer cells

BACKGROUND

HPN536 is a MSLN-targeting TriTAC



anti-MSLN single domain antibody targets cells expressing MLSN

anti-albumin single domain antibody extends half-life

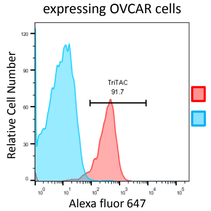
anti-CD3ε scFv engages T cells

molecular weight of ~50 kDa

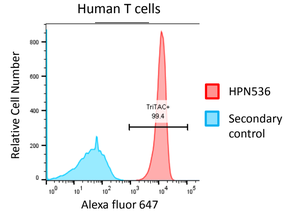
HPN536 binds human and cyno MSLN, albumin, and CD3ε

In vitro affinity measurements		MSLN	CD3	ALB
	human K_D (nM)	1.4	15	8
	cyno K_D (nM)	5	9	8

HPN536 Binding to MSLN-expressing OVCAR cells



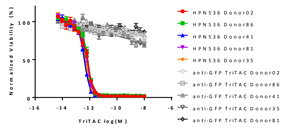
HPN536 Binding to Human T cells



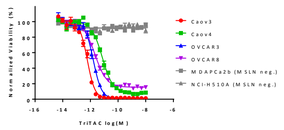
IN VITRO PHARMACOLOGY

HPN536 directs T cells to kill MSLN expressing cells

Killing of OVCAR3 cells using T cells from 5 different donors



Killing of MSLN-expressing ovarian cancer cell lines

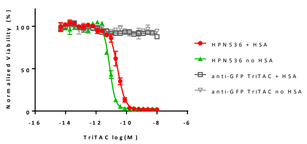


Tumor origin	Cell Line	EC50 (pM)	MSLN sites per cell
Ovarian	Caov3	0.6	51262
	Caov4	7.3	101266
	OVCAR3	1.6	40589
	OVCAR8	2.2	40216
	SKOV3	3.6	10617
Pancreatic	Hs766T	7.8	5892
	CaPan2	3.2	27413
	HPaFII	15	17844
NSCLC	NCI-H596	1.5	103769
	NCI-H292	3.8	5977
	NCI-H1563	2.6	17221
Mesothelioma	NCI-H2052	8.0	nd
	NCI-H2452	2.3	nd
Engineered (non-tumor)	293 human MSLN	0.9	128091
	293 cyno MSLN	0.7	140683

nd not determined

HPN536 directs cell killing in presence of human serum albumin

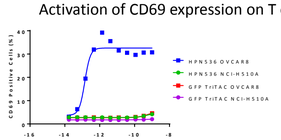
Killing of NCI-H2052 cells in presence or absence of 15 mg/ml HSA



Cell line	EC50 no HSA (pM)	EC50 with HSA (pM)	EC50 shift (fold)
OVCAR8	2.7	8.7	3.2
SKOV3	3.9	11	2.8
NCI-H2052	8.0	26	3.2
NCI-H24522	2.3	6.3	2.7
Caov3	0.8	3.6	4.3
OVCAR3	1.6	3.8	2.4

HPN536 activates T cells in presence of MSLN expressing cells

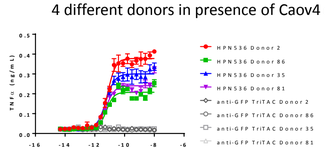
Activation of CD69 expression on T cells



T cell donor	EC50 for CD69 expression (pM)
Donor 35	0.1
Donor 2	0.4
Donor 81	0.3
Donor 86	0.4

- OVCAR8 cells express MSLN; NCI-H510A cells are MSLN negative

Secretion of TNFα into medium by T cells from 4 different donors in presence of Caov4 cells



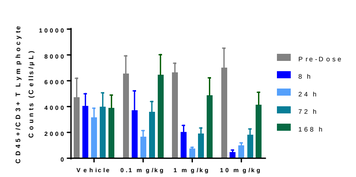
Cell line	TNFα EC50 values (pM) Donor 2	Donor 86	Donor 35	Donor 81
Caov3	5.2	5.4	5.9	4.9
Caov4	7.2	6.0	5.5	5.5
OVCAR3	9.2	4.0	1.7	8.9
OVCAR8	1.3	9.1	5.1	5.0

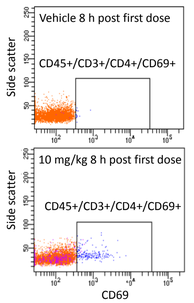
SAFETY

A single 10 mg/kg dose of HPN536 is well tolerated in cynomolgus monkeys

- Findings were similar at 0.1, 1 and 10 mg/kg
- Findings were trending towards reversible 3 weeks after dosing
  - Reduced severity / incidence (small sample size)
- Most findings were associated with the known mechanism of action

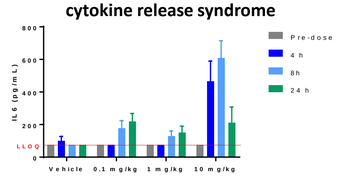
Transient T lymphocyte margination & activation mediated by HPN536





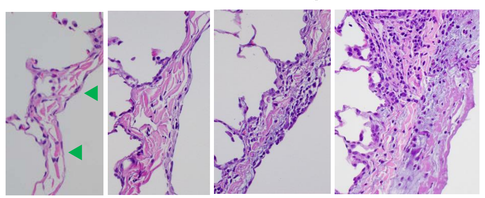
- Transient, dose-dependent decline in circulating T cells within 8 h post dosing
- Reversible after 7 days
- T cell activation 8 h after first dose at 10 mg/kg

Dose-dependent increase in IL-6 with no evidence of cytokine release syndrome



- Transient, dose-dependent increase in IL-6 similar to other bispecific T cell engaging molecules
- No observable trend in IL-2, -4, -5, -10, TNFα, and IFNγ

MSLN expressing tissues have hyperplasia and inflammation 1 week after dosing with HPN536

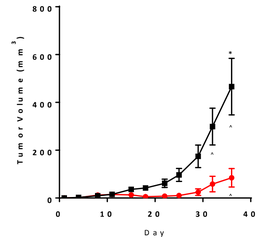


- Representative histopathological images of lung mesothelia
- MSLN is expressed in the mesothelia[1] (mesothelium in the control is marked by arrow heads ◀)
- Note increase in thickness of mesothelial cell layer, increase in inflammatory cells and addition of acellular matrix as severity grade increases
- There is a trend to resolution of these findings 3 weeks after dosing (data not shown)

[1] Hassan R, Ho M. 2008. "Mesothelin targeted cancer immunotherapy". Eur. J. Cancer. 44: 46–53.

IN VIVO PHARMACOLOGY

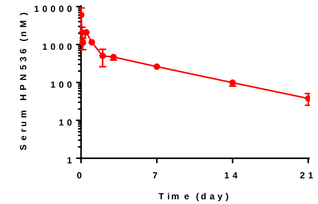
NCI-H292 tumor growth inhibition in mice implanted with human PBMCs and treated with HPN536



- 10^7 NCI-H292 cells and 10^7 human PBMCs co-implanted subcutaneously in NCG mice, 8 mice / group

PHARMACOKINETICS

HPN536 has a half-life of ~5 days in cynomolgus monkeys



Dose Level	Terminal $t_{1/2}$	C_{max} (nM)	AUC, 0-inf (hr*nM)	Clearance (mL/hr/kg)	Vss (mL/kg)
10 mg/kg	112	6,130	355,000	0.58	70.0

- C_{max} exceeds the in vitro killing EC50 by 400,000 fold
- Suggests once weekly dosing in humans

HPN536 SUMMARY

- Binds human and cynomolgus MSLN, CD3ε, and human serum albumin
- Redirects T cells to kill MSLN expressing cells in vitro
- Inhibits tumor growth in mouse models
- Is well tolerated in cynomolgus monkeys after a single dose with data suggestive of target engagement in vivo
- Anticipated to be an efficacious, safe, and convenient therapeutic for patients with MSLN expressing malignancies
- Projected to enter the clinic within the next 12 months

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