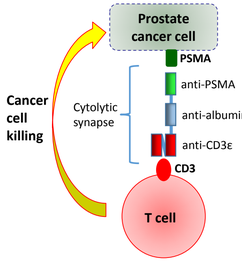
HPN424, a half-life extended, PSMA/CD3-specific TriTAC for the treatment of metastatic prostate cancer

Bryan Lemon, Wade Aaron, Richard Austin, Patrick A. Baeuerle, Adrie Jones, Susan D. Jones, Kathryn Kwant, Che-Leung Law, Anna Muchnik, Kenneth Sexton, Laurie Tatalick, Holger Wesche, Timothy Yu. Harpoon Therapeutics, South San Francisco, CA.

HARPOON Therapeutics

RATIONALE

- Metastatic, castration-resistant prostate cancer (mCRPC) kills 27,000 patients in the US each year
- Once mCRPC has metastasized beyond regional lymph nodes, the 5-year survival rate is 30%
- Abiraterone and enzalutamide have improved the treatment options for mCRPC, but no curative treatment is available and new therapies are urgently needed
- PSMA is expressed in >90% of malignant lesions of mCRPC patients



- In normal tissues, PSMA expression outside the central nervous system is largely restricted to the prostate.
- HPN424 is engineered to direct T cells to kill prostate cancer cells

INTRODUCTION

HPN424 is a PSMA-targeting TriTAC

αPSMA	single domain antibody targets cancer cells expressing PSMA/FOLH1
αALB	single domain antibody binds albumin to extend serum half-life
αCD3	anti-CD3ε scFv engages T cells

➢ HPN424 is a tri-specific single chain molecule of ~50 kDa

BIOPHYSICAL CHARACTERIZATION

Process

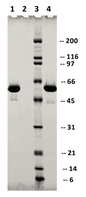
Depth filtration → Protein A affinity → Low pH viral inactivation → Ion exchange step 1 → Ion exchange step 2 → Viral filtration → Ultrafiltration diafiltration

Purity

SDS - PAGE
4 - 20% TRIS Glycine

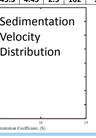
1 Non-Reduced
2 x
3 MW Standards
4 Reduced

Coomassie stain
2.5ug / lane



Analytical Ultra Centrifugation

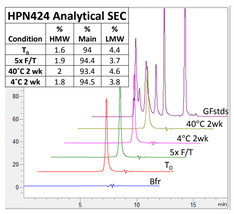
	Main Peak			HMW Peak			Frictional
HPN424	s-value	% Area	MW (kDa)	s-value	% Area	MW (kDa)	Ratio (ffo)
Average	2.63	98.5	45.3	4.43	2.5	102	1.73

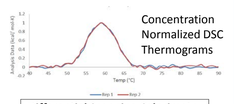


Stability

HPN424 Analytical SEC

Condition	% HMW	% Main	% LMW
T_0	1.6	94	4.4
5x F/T	1.9	94.4	3.7
40°C 2 wk	2	93.4	4.6
4°C 2 wk	1.8	94.5	3.8



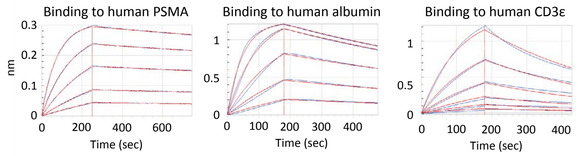


Differential Scanning Calorimetry

HPN424	Tm1 (°C)	Tm2 (°C)
Replicate 1	58.78	61.12
Replicate 2	59.18	61.02
Average	58.98	61.07

IN VITRO BINDING

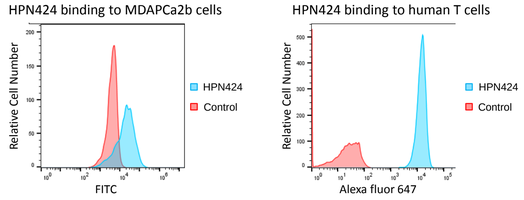
HPN424 binding to human and cyno PSMA, ALB, and CD3ε by biolayer interferometry



	PSMA*	ALB	CD3ε*
Human K_D (nM)	0.5	8	12
Cyno K_D (nM)	indeterminate	7.7	10

*PSMA and CD3ε binding were measured in the presence of 15 mg/ml human serum albumin

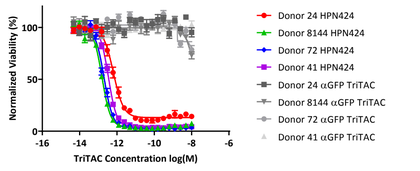
HPN424 binds to cells expressing PSMA and to T cells



IN VITRO PHARMACOLOGY

HPN424 potently directs T cell killing of PSMA expressing cells

Killing of VCaP cells with T cell from four different donors



HPN424 directed killing of four prostate cancer cell lines with four T cell donors

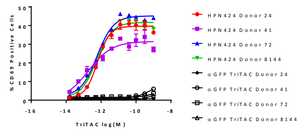
Cell Line	PSMA Expression	EC50 Values (pM) Donor 24	Donor 8144	Donor 72	Donor 41
LNCaP	Positive	1.5	0.22	0.36	0.43
MDAPCa2b	Positive	4.8	0.41	0.49	0.65
VCaP	Positive	0.64	0.16	0.2	0.35
22Rv1	Positive	n/a	0.72	1.4	1.3
HCT116	Negative	>10,000	>10,000	>10,000	>10,000
NCI-1563	Negative	>10,000	>10,000	>10,000	>10,000

- Prostate cancer cell lines were incubated with human T cells and HPN424 or control TriTAC.
- Viability of the cancer cells was assessed by measuring luciferase activity at 48 hours

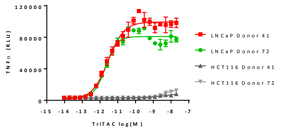
IN VITRO PHARMACOLOGY

PSMA-dependent activation of T cells by HPN424

Induction of CD69 expression on T cells in presence of LNCaP cells with HPN424 but not with an αGFP-TriTAC



HPN424 induction of TNFα expression by T cells in presence of LNCaP cells but not PSMA-negative HCT116 cells



IN VIVO PHARMACOLOGY

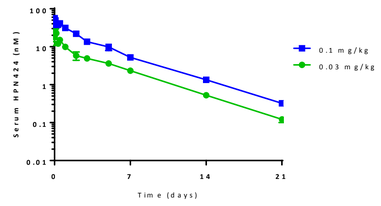
HPN424 potently inhibits growth of 22Rv1 xenografts

Admixture 5 x 10^6 human PBMC + 22Rv1 prostate cancer cells, d0

HPN424 dosing qd x 10, d5 to d14

PHARMACOKINETICS

HPN424 has a half-life of ~ 3.3 days in cynomolgus monkeys



Dose (mg/kg)	Terminal $t_{1/2}$ (h)	C_{max} (ng/mL)	AUC 0-168 h (h*ng/mL)	AUC 0-inf (h*ng/mL)	CL (mL/h/kg)	Vss (L/kg)
0.1	79.9	3,440	154,000	188,000	0.53	61.12
0.03	80.9	1,340	53,000	66,900	0.45	61.02

- Single dose pharmacokinetic dose study with two subjects per dose level
- PK results suggest once or twice weekly dosing in humans

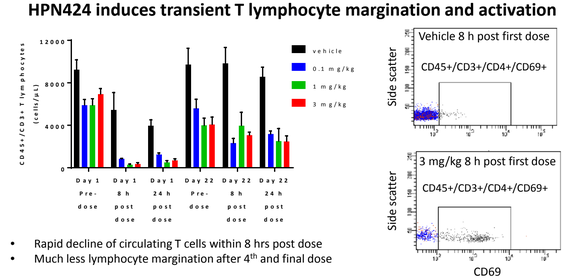
SAFETY

HPN424 is highly tolerated with repeat dosing in cynomolgus monkeys

➢ No observed adverse effect level was 3 mg/kg, qwx4
- Similar results observed with 1 mg/kg and 0.1 mg/kg qwx4 doses
- CD3 and albumin binding domains cross-react with cyno targets
  - Minimal binding of HPN424 to recombinant cyno PSMA
- Pharmacodynamic effects consistent with T cell engagement
  - Transient reduction in circulating T cells, NK cells, and monocytes
  - Upregulation of activation markers (CD25 & CD69) in the remaining circulating T cells
  - Mild and transient increase in cytokines (IFNγ, IL-6, IL-10) with 1st dose, changes after 4th dose much less pronounced

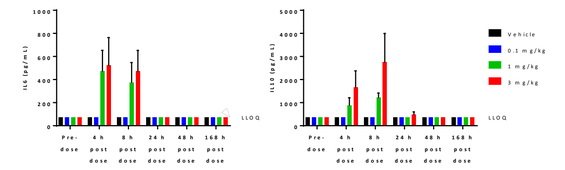
➢ No adverse histopathology findings

HPN424 induces transient T lymphocyte margination and activation



- Rapid decline of circulating T cells within 8 hrs post dose
- Much less lymphocyte margination after 4th and final dose

HPN424 induces limited cytokines, no evidence of cytokine release syndrome



- Transient, dose-dependent increases in IL-6 and IL-10 as similarly reported with other bispecific T cell engagers; no observable trend of increase in IL-2, -4, -5, TNFα, and IFNγ
- First dose effect; no cytokine increase after the 4th and last dose

SUMMARY

- HPN424 TriTAC is a stable, manufacturable, single chain molecule that binds with high affinity and specificity to PSMA, CD3 and albumin
- HPN424 potently activates and redirects T cells to kill PSMA expressing cells in both in vitro and in vivo prostate cancer models
- HPN424 has a long serum half life and was very well tolerated, even at high doses, in cynomolgus monkeys
- With its small size, HPN424 is anticipated to be more able to penetrate solid tumors than antibodies
- HPN424 is expected to be a safe, effective, and convenient treatment for patients with metastatic castration resistant prostate cancer
- HPN424 is anticipated to enter the clinic in 2018

Abstract 1773 AACR Annual Meeting 2018