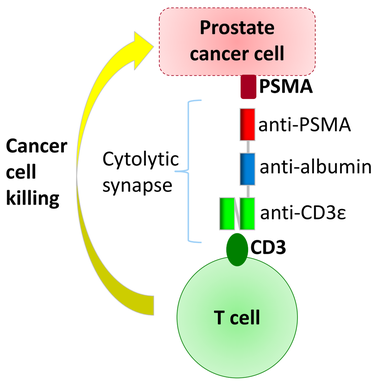
HPN424, a half-life extended, PSMA/CD3-specific TriTAC for the treatment of metastatic prostate cancer

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

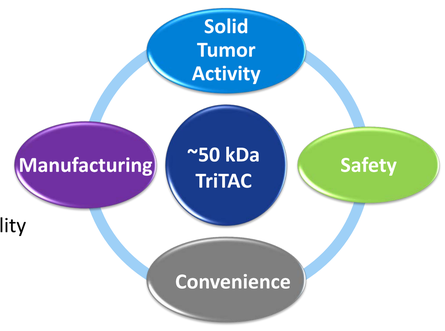
RATIONALE

- Metastatic, castration-resistant prostate cancer (mCRPC) kills 71,000 patients in the European Union 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 mCRPC lesions
- 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



TriTAC platform is optimized for treatment of solid tumors

- Small size for diffusion-controlled solid tumor penetration
- Optimized CD3 binding to address T cell-mediated clearance



- Single polypeptide
- Protein A purification
- Robust stability
- No potential for Fcγ receptor binding
- Monovalent CD3 binding minimizes non-specific T cell activation
- Albumin binding for extended serum half-life

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

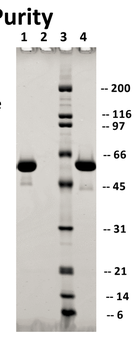
➢ HPN424 is a tri-specific single chain molecule of ~53 kDa

BIOPHYSICAL CHARACTERIZATION

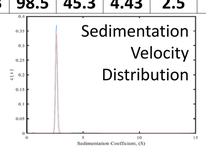
Process

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

Purity

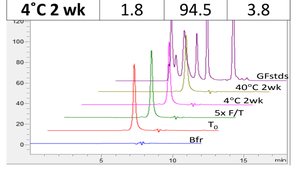


Analytical Ultra Centrifugation							
	Main Peak			HMW Peak			
HPN424	s-value	% Area	MW (kDa)	s-value	% Area	MW (kDa)	Frictional Ratio (ffo)
Average	2.63	98.5	45.3	4.43	2.5	102	1.73

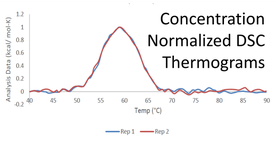


HPN424 Exhibits High Stability

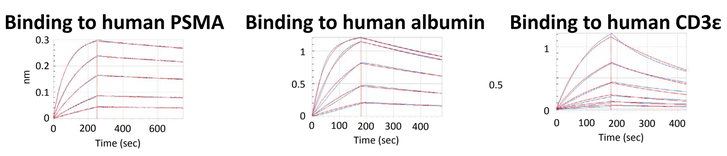
HPN424 Analytical SEC			
Condition	% HMW	% Main	% LMW
T_0	1.6	94	4.4
5x F/T	1.9	94.4	3.7
40˚C 2 wk	2	93.4	4.6
4˚C 2 wk	1.8	94.5	3.8



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**



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

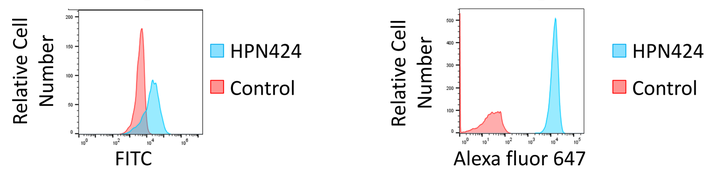


	PSMA*	ALB	CD3ε*
Human K_D (nM)	0.5	8	12
Cyno K_D (nM)	indeterminate	7.7	10

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

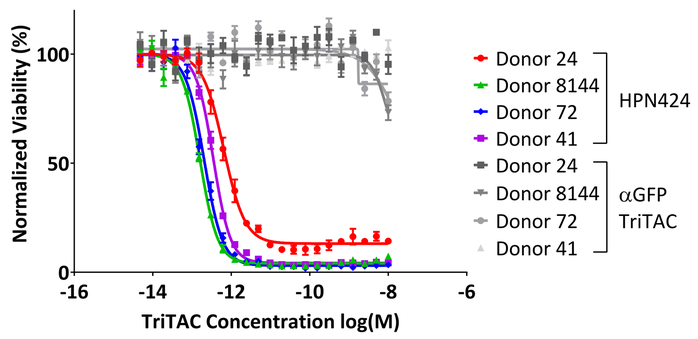
IN VITRO PHARMACOLOGY

HPN424 binds to cells expressing PSMA and to T cells



HPN424 potently directs T cell killing of PSMA expressing cells

Killing of VCaP cells with T cell from four different donors



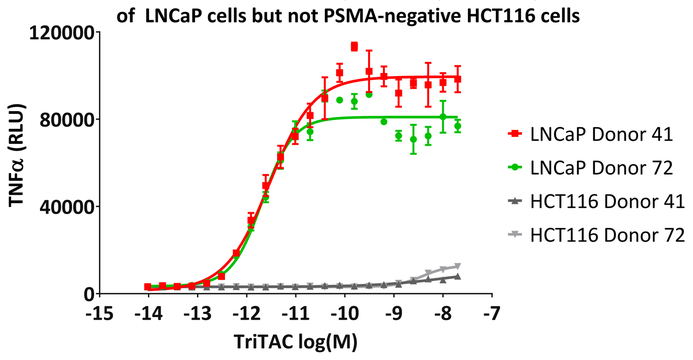
HPN424 directed killing of four prostate cancer cell lines with four T cell donors

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

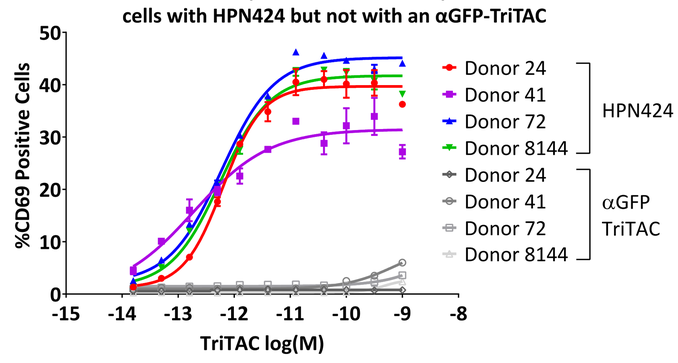
- Prostate cancer cell lines were incubated with human T cells and HPN424 or control TriTAC.
- Viability of the cancer cells was assessed by measuring luciferase activity at 48 hours

PSMA-dependent activation of T cells by HPN424

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



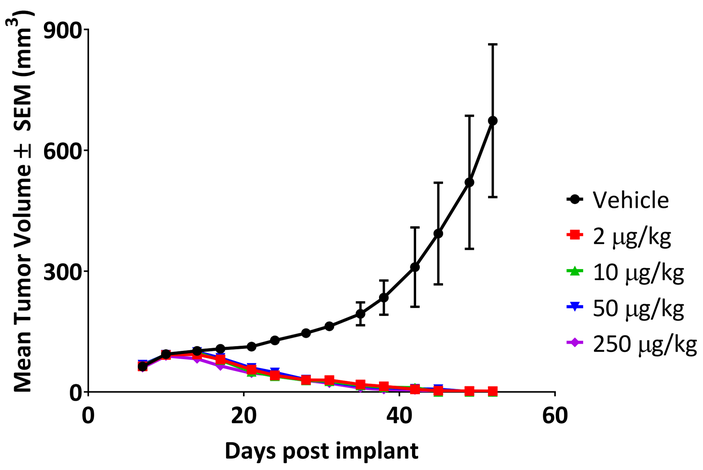
Induction of CD69 expression on T cells in presence of LNCaP cells with HPN424 but not with an αGFP-TriTAC



IN VIVO PHARMACOLOGY

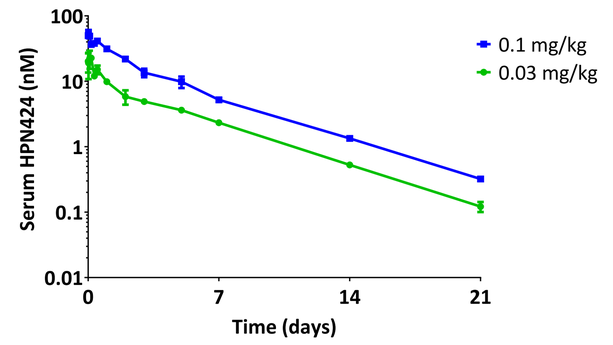
HPN424 potently inhibits growth of 22Rv1 xenografts

- NCG mice were implanted subcutaneously with a mixture of 5 x 10^6 human PBMC and 5 x 10^6 22Rv1 prostate cancer cells on day 0
- Mice were dosed i.v. with HPN424 or vehicle once per day from days 5 to 14
- Significant tumor growth inhibition (P<0.0001) was observed in all groups versus vehicle starting at day 17



PHARMACOKINETICS

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



Dose (mg/kg)	Terminal $t_{1/2}$ (h)	C_{max} (nM)	AUC 0-168 h (h*nM)	AUC 0-inf (h*nM)	CL (mL/h/kg)	Vss (L/kg)
0.1	79.9	65.5	3,530	3,570	0.53	0.051
0.03	80.9	25.4	1,260	1,270	0.45	0.047

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

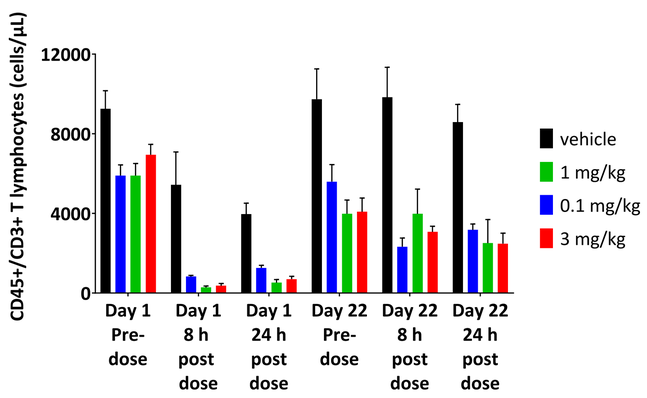
SAFETY

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

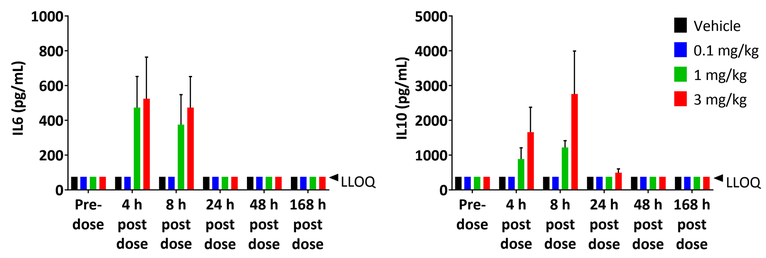
➢ No adverse histopathology findings

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 199 CIMT Annual Meeting 2018

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