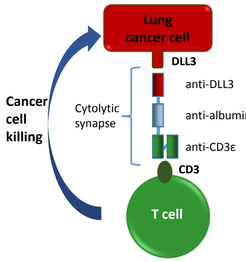
HPN328: An Anti-DLL3 T Cell Engager for Treatment of Small Cell Lung Cancer

Wade Aaron, Richard Austin, Manasi Barath, Evan Callihan, Michael Cremin, Thomas Evans, Maria Gamez Guerrero, Vaishnavi Ganti, Golzar Hemmati, Avneel Hundal, Kathryn Kwant, Che-Leung Law, Bryan Lemon, Llewelyn Lao, Mary Ellen Molloy, Jessica O'Rear, Purbasa Patnaik, Laura Sun, Holger Wesche, Stephen Yu, and Timothy Yu

Harpoon Therapeutics, South San Francisco, CA

RATIONALE

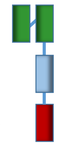
- Each year more than 29,000 patients in the US are diagnosed with small cell lung cancer (SCLC)
- Median progression-free survival is 2–3 months and median overall survival 8–13 months with a 5-year OS rate of <5%
- Standard of care (SOC) for patients with extensive stage SCLC is radiation, etoposide, cisplatin or carboplatin, and atezolizumab. Although SCLC is often responsive to the SOC treatment, relapse is common.
- DLL3 is a Notch inhibitory ligand and is expressed in more than 70% of SCLCs



- DLL3 has little to no surface expression in normal adult tissues outside of the CNS
- HPN328 is engineered to direct T cells to kill small cell lung cancer cells expressing DLL3

INTRODUCTION

HPN328 is a DLL3-Targeting TriTAC



αCD3 — anti-CD3ε scFv engages T cells

αALB — single domain antibody binds albumin to extend serum half-life

αDLL3 — single domain antibody targets cancer cells expressing DLL3

➢ HPN328 is a tri-specific single chain molecule of ~50 kDa

BIOPHYSICAL CHARACTERIZATION

Process

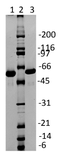
Depth filtration → Protein A affinity → Low pH viral inactivation → Ion exchange step 1 → Ion exchange step 2 → Viral filtration → Ultrafiltration diafiltration

Purity & Stability

SDS-PAGE

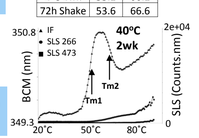
1. Reduced
2. MW Std
3. Non-reduced

10-20% TRIS-Glycine
3µg / lane
Coomassie Stain



Intrinsic Fluorescence Static Light Scattering

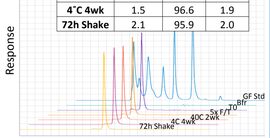
Condition	Tm1 (°C)	Tm2 (°C)
T0	53	66.2
5 X FT	53.2	66.4
40°C 2wk	53.3	66.2
4°C 4wk	53.1	66.1
72h Shake	53.6	66.6



* Low SLS, Tagg > 80˚C all conditions

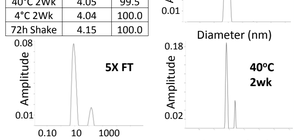
Analytical SEC

Condition	% HMW	% Main	% LMW
T_a	2.7	95.4	1.9
5 X F/T	2.8	95.7	1.5
40˚C 2wk	2.2	95.6	2.2
4˚C 4wk	1.5	96.6	1.9
72h Shake	2.1	95.9	2.0



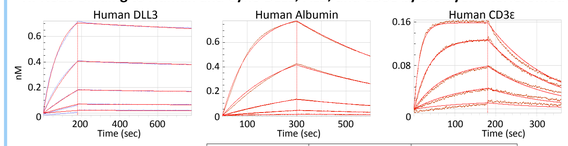
Dynamic Light Scattering (Intensity Distribution)

Condition	Peak1 R_h (nm)	Peak1 Mass%
T0	4.49	100.0
5 X FT	4.10	99.8
40°C 2Wk	4.05	99.5
4°C 2Wk	4.04	100.0
72h Shake	4.15	100.0



BINDING

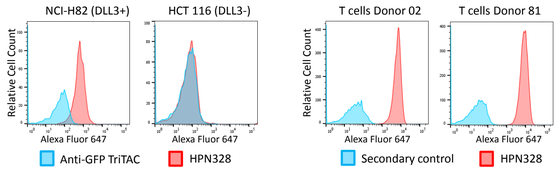
HPN328 Binding to Human and Cyno DLL3, ALB, and CD3ε by Biolayer Interferometry



	DLL3*	ALB	CD3ε*
Human K_D (nM)	0.78 ± 0.05	28 ± 1	7.9 ± 1
Cyno K_D (nM)	0.98 ± 0.1	26 ± 2	6.4 ± 0.5

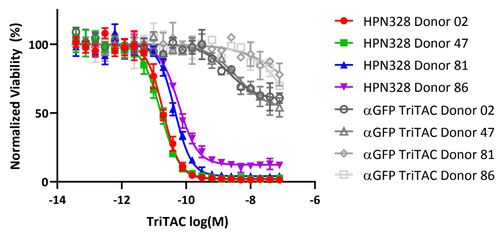
* DLL3 and CD3ε binding were measured in the presence of 15 mg/ml human serum albumin

HPN328 Binds to T Cells and DLL3-Positive but Not Negative Target Cells



IN VITRO PHARMACOLOGY

HPN328-Mediated TDCC Against DLL3 Positive Cell Line NCI-H82



Luciferase-labelled target cells (DLL3 positive or negative) were co-incubated with purified human T cells, human serum albumin (15 mg/mL), and a titration of HPN328. The viability of the target cells was measured at 48 hours.

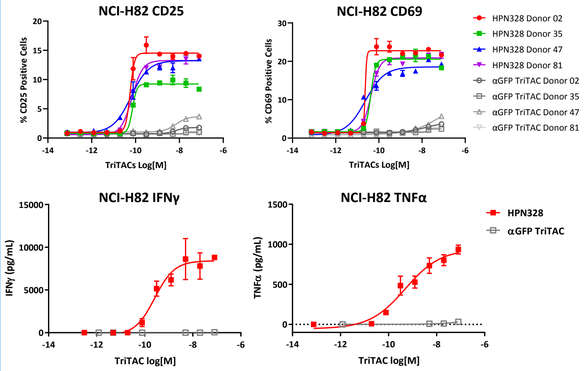
HPN328 Exhibits Potent Cytotoxicity against SCLC Cells

	DLL3 Expression	EC50 (pM)*			
		Donor 02	Donor 47	Donor 81	Donor 86
SHP77	Positive	63	36	85	188
NCI-H82	Positive	20	16	45	59
DMS53	Positive	70	72	80	220
NCI-2171	Positive	162	76	286	<80,000
HCT 116	Negative	>80,000	>80,000	>80,000	>80,000
NCI-H292	Negative	>80,000	>80,000	>80,000	>80,000

*EC50 values generated in the presence of 15 mg/mL human serum albumin

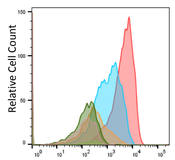
IN VITRO PHARMACOLOGY

HPN328- and DLL3-Dependent Activation of T Cells: Upregulation of CD69 & CD25; Secretion of TNFα & IFNγ



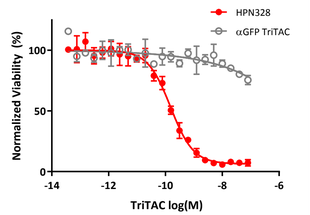
HPN328 Binds to Cynomolgus T Cells and Mediates TDCC Against Cells Expressing Cynomolgus DLL3

Flow Cytometry Analysis



HPN328 binds to the T cells in whole cyno blood

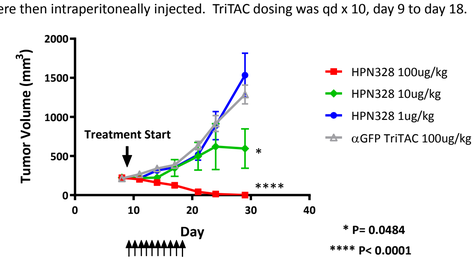
HEK 293_CynoDLL3 in TDCC with Cyno PBMCs



IN VIVO PHARMACOLOGY

Anti-DLL3 TriTACs Potently Inhibit Growth of NCI-H82 Xenografts

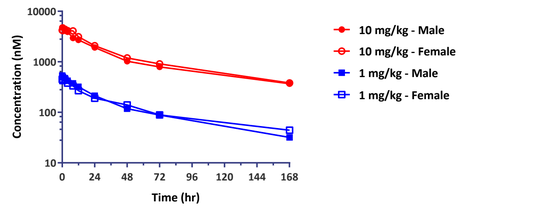
5 x 10^6 NCI-H82 were implanted on day 0. On day 8, 2x10^7 human T-cells per mouse were then intraperitoneally injected. TriTAC dosing was qd x 10, day 9 to day 18.



* P= 0.0484
**** P< 0.0001

PHARMACOKINETICS & *IN VIVO* STABILITY

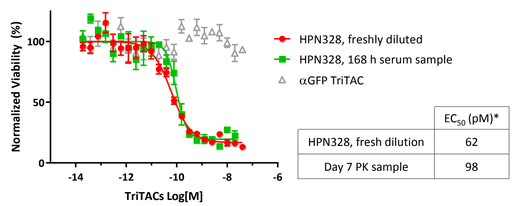
HPN328 Has a Half-Life of 2.8 to 3.3 days in Cynomolgus Monkeys



Dose (mg/kg)	Terminal $T_{1/2}$	C_{max} (nM)	AUC_{0-inf} (h*nM)	CL (mL/h/kg)	V_{ss} (mL/kg)
1	68	493	23800	0.8	64
10	79	4492	236500	0.8	72

- Test subjects were given a single i.v. dose of HPN328
- HPN328 was well tolerated in this study: there were no clinically significant or adverse test article-related changes in hematology or clinical chemistry, and no apparent adverse findings at terminal and recovery necropsy
- The serum concentrations of HPN328 were measured using anti-idiotype antibodies in an electrochemiluminescent assay and were plotted vs. time (see above) and tested in T cell killing assay (below)

HPN328 in Cynomolgus Monkey Serum Collected 1 Week After Dosing Retains TDCC Activity



	EC_{50} (pM)*
HPN328, fresh dilution	62
Day 7 PK sample	98

*assay performed with human T cells in the presence of 8.4 % cynomolgus monkey serum

HPN328 SUMMARY

- A TriTAC that binds to human and cynomolgus DLL3, CD3ε, and albumin
- Directs T cells to kill DLL3 expressing cells *in vitro*
- Inhibits tumor growth in models of small cell lung cancer
- Has a terminal serum half-life in cyno monkeys of 68-79 hrs; stability of ≥ 1 week in circulation in cyno monkeys
- Anticipated to be an efficacious, safe, and convenient therapeutic for patients with DLL3 expressing malignancies
- Projected to enter a first in human clinical trial in the second half of 2020

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