Rationale for the development of a differentiated Trop2 ADC

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Abstract 6297

Introduction

Thailanstatins are naturally occurring anti-proliferative compounds that target spliceosomes and modulate pre-mRNA splicing. Alterations in splicing machinery and mRNA splicing are common in cancer and represents a potential susceptibility that can be exploited by targeted delivery of a splicing modulator to tumors with antibody drug conjugates (ADCs).

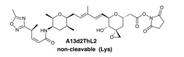
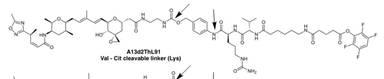
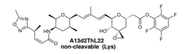
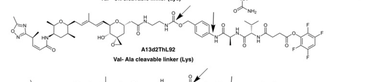
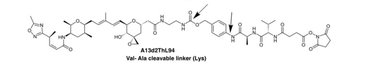
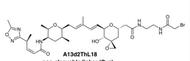
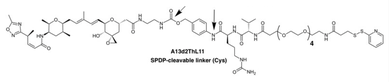
- Lee SC, Abdel-Wahab O. *Nat Med*. 2016;22(9):976-986.
- Effenberger KA, Urabe VK, Jurica MS. *Wiley Interdiscip Rev RNA*. 2017;8(2):10.1002/wrna.1381.

Chemistry

The lead small molecule of Peak Bio's R&D toxin and ADC platform is a novel analog of Thailanstatin called analog 13 (A13), that led to the development of a family of seven related linker-toxins (L-Ts).

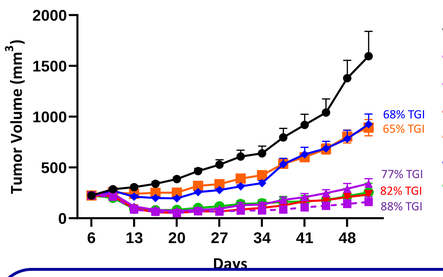
The ADCs derived from these L-Ts have been extensively characterized *in vitro* and *in vivo* as Her2 and Trop2 ADCs.

- AACR; Cancer Res 2021;81(13_Suppl): Abstract nr 1832

	Structure of linker-toxins	
Lysine	Non-cleavable	Cleavable
	L2 	L91 
	L22 (PH1) 	L92 
		L94 
Cysteine	L18 	L11 

Linker Screen

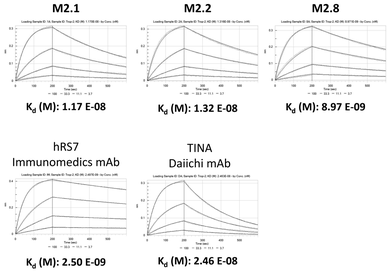
The PH-1 family of L-Ts were conjugated to the Trop2-specific monoclonal antibody (hRS7) and the resulting ADCs were tested for therapeutic efficacy against pre-implanted Trop2- expressing NCI-N87 gastric carcinoma tumors



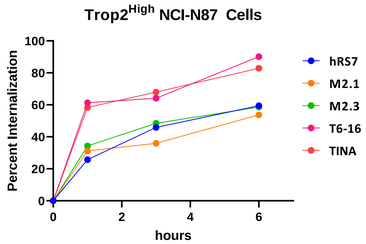
ADC Label	Experimental DAR
L2 DAR 8	7.8
L2 DAR 4	3.7
L22	4.5
L18	3.7
L92	3.0
Kadcyla	3.5

Non-cleavable Lysine linked PH-1 Linker-Toxins were associated with maximal Tumor Growth Inhibition (TGI) in vivo

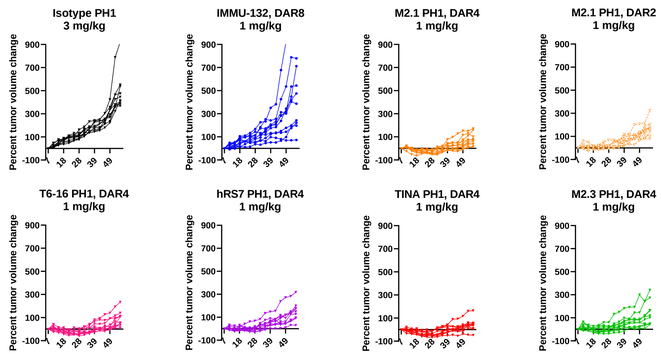
Antibody Binding Affinity



Antibody Internalization



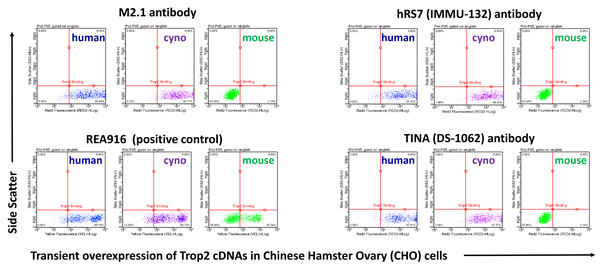
Comparison of TGI efficacies of IMMU-132 vs various anti-Trop2 PH1 ADCs



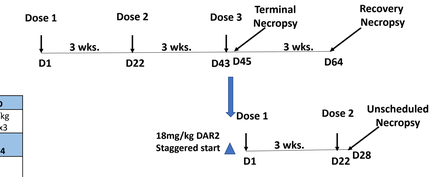
At equivalent dose level of 1mg/kg QWx3 dose-
TGI of hRS7 PH1 DAR 4 > hRS7 SN38 (IMMU-132) DAR 8 (p<0.05)
TGI of M2.1 PH1 DARs 2 and 4 > IMMU-132 DAR 8 (p<0.05)

Significant TGI at 1 mg/kg and tumor shrinkage at 3 mg/kg observed with multiple TROP2 antibodies- hRS7, TINA, T6-16, M2.1 and M2.3

M2.1 and M2.8 mAbs recognize human and cynomolgus monkey Trop2



Differentiated NHP safety profile for Trop2 PH1 ADCs

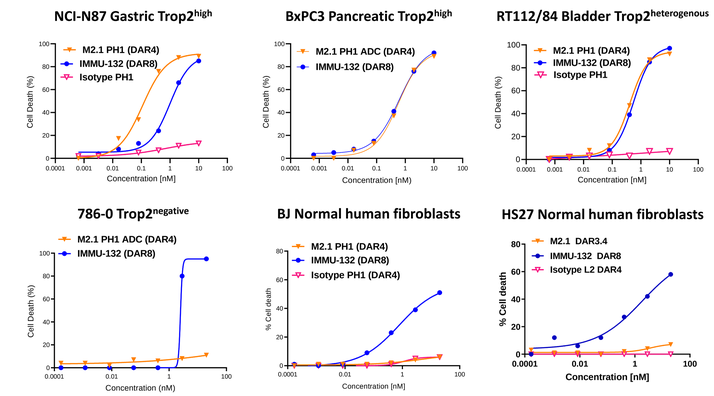


	Below MTD	MTD	Not tolerated	Below MTD	MTD
Dose level/ Regimen	2 mg/kg Q3Wx3	6 mg/kg Q3Wx3	18mg/kg Q3Wx2	2 mg/kg Q3Wx3	6 mg/kg Q3Wx3
Drug-to-antibody ratio	DAR 2	DAR 2	DAR 2	DAR 4	DAR 4
number of animals	3	3	3	3	3
General observations					
Morbidity/ mortality	-	-	+	-	-
Body weight loss	-	-	Transient	-	-
Inappetance/ loss of activity	-	-	+	-	-
Skin redness/ rash	-	-	n=2	-	n=1
Skin scaling	-	n=1	n=3	n=2	n=2
Serum chemistry					
ALT	-	-	marked increase	-	-
AST	-	mild increase, transient	moderate increase, transient	mild increase, transient	moderate increase, transient
TBIL	-	-	mild increase	-	-
GLOB	-	-	mild increase	-	-
ALB	-	-	moderate decrease	-	-
A/G	-	-	mild decrease	-	-
Na	-	-	minimal decrease	-	-
Cl	-	-	minimal decrease	-	-
Hematology and Coagulation times					
PLT	minimal decrease, transient	mild decrease, transient	mild decrease, transient	mild decrease, transient	mild decrease, transient
FIB	-	-	minimal decrease	-	-
APTT	minimal prolonged	minimal prolonged	minimal prolonged	minimal prolonged	minimal prolonged

Histopathology	Below MTD		MTD		Not tolerated	Below MTD		Below MTD	
Dose level/ Regimen	2 mg/kg Q3W x 3		6 mg/kg Q3W x 3		18 mg/kg Q3W x 2	2 mg/kg Q3W x 3		6 mg/kg Q3W x 3	
Drug-to-antibody ratio	DAR 2		DAR 2		DAR 2	DAR 4		DAR 4	
Necropsy Group	Terminal	Recovery	Terminal	Recovery	Unscheduled	Terminal	Recovery	Terminal	Recovery
number of animals	2	1	2	1	3	2	1	2	1
Kidneys-									
Necrosis, proximal tubule, medullary array	-	-	-	-	-	-	-	Minimal (n=1)	-
Vaculoation, proximal tubules, bilateral	-	-	-	-	Mild (n=1)	-	-	-	-
Liver-									
Necrosis, hepatocyte, centrilobular	-	-	-	-	Moderate to marked (n=2)	-	-	Minimal (n=1)	-
Vacuolation, hepatocyte, centrilobular	-	-	-	-	-	-	-	Mild (n=1)	-
Infiltrate, neutrophilic, centrilobular	-	-	-	-	-	-	-	Minimal (n=1)	-
Bone Marrow (sternum)-									
Cellularity decreased	-	-	Moderate (n=1)	-	-	-	-	Mild (n=1)	-
Cellularity increased, granulocytic	Minimal (n=1)	-	Minimal (n=1)	-	-	Minimal (n=1)	-	-	-
Adrenal glands									
Vacuolation, decreased, cortex, diffuse	-	-	-	-	Mild (n=1)	-	-	Mild (n=1)	-
Pancreas									
Vacuolation, acinar cells	-	-	-	-	Minimal (n=1)	-	-	-	-
Thymus									
lymphocytes, decreased	-	-	-	-	Moderate (n=1)	-	-	-	-

- *Histopathology performed unilaterally for all tissues*
- *No evidence of typical Trop2 ADC toxicities such as neutropenia, peripheral neuropathy, stomatitis or ILD above MTD*
- *Tolerated at 6 mg/kg Q3W times 3 repeat doses with mild-minimal or transient observations. Recovery necropsy after 3 weeks confirms reversibility*

Peak Bio Trop2 PH1 ADCs exhibits low off-target activity

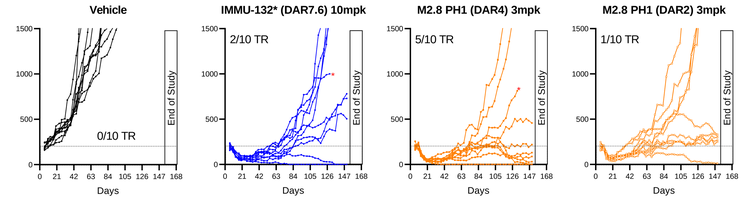


Optimized Trop2 PH1 ADC has potent in vitro in multiple indications

Cell No.	Cell line Type *	Absolute IC50		% inhibition at top conc.	
		M2.8 PH1 (nM)	Cisplatin (µM)	M2.8 PH1 (nM)	Cisplatin (µM)
1	Lung 1	0.56	2.74	90.78%	99.49%
2	Lung 2	1.63	3.08	90.31%	99.96%
3	Lung 3	1.88	9.00	74.29%	95.28%
4	Lung 4	3.35	2.40	58.86%	99.78%
5	Lung 5	3.69	4.15	63.30%	91.17%
6	Lung 6	3.78	5.04	68.28%	99.88%
7	Lung 7	4.65	2.36	81.52%	99.71%
8	Lung 8	54.10	0.13	62.22%	99.26%
9	Pancreatic 1	1.21	15.35	88.52%	93.41%
10	Pancreatic 2	1.50	0.39	88.62%	99.98%
11	Pancreatic 3	7.52	0.70	82.82%	99.94%
12	Esophageal 1	1.21	1.72	82.86%	99.97%
13	Gastric 1	1.32	2.36	85.07%	97.52%
14	Gastric 2	4.03	10.03	88.93%	92.97%
15	Gastric 3	10.55	1.02	85.99%	99.96%
16	Bladder 1	1.36	3.89	86.91%	99.21%
17	Bladder 2	1.77	4.12	95.19%	99.97%
18	Bladder 3	1.97	1.29	93.54%	99.99%
19	Ovarian 1	3.25	2.60	83.63%	99.10%
20	Breast 1	7.77	7.03	83.38%	99.67%
21	Breast 2	12.10	5.26	70.82%	94.25%
22	Breast 3	12.30	1.52	77.55%	99.53%
23	Uterine 1	9.68	0.93	74.10%	99.96%
24	Uterine 2	23.82	1.27	63.34%	99.86%
25	Uterine 3	27.40	0.51	61.40%	99.95%

* Patent pending

M2.8 PH1 ADC has potent in vivo activity



Model: Nude mice bearing human NCI-N87 gastric tumors

Horizontal dotted line indicates mean tumor volume of 200 mm^3 size at which treatment was initiated. Tumor shrinkage below this line was considered regression

Dosing regimen: QW for 2 weeks

TR= tumor regression
TGI= tumor growth inhibition

IMMU-132* Represents clinical grade IMMU-132 or Sacituzumab govitecan

At DAR4- tumor regression in 50% of treated mice over a period of ~5 months

At DAR2- Stable disease in 50% of treated mice over a period of ~5 months

Group	TGI ± Std Err (day 20)	p Value Vs M2.8 PH1 (DAR4) (day 20)	TGI ± Std Err (Day 41)	p Value vs M2.8 PH1 (DAR4) (day 41)
M2.8 PH1 (DAR 4)	87.7 ± 1.0		90.3 ± 1.8	
M2.8 PH1 (DAR 2)	80.5 ± 1.8	1.40e-04	78.5 ± 3.2	1.42e-03
IMMU-132* (DAR 7.6)	79.0 ± 2.1	2.79e-05	83.3 ± 2.4	3.54e-02

Conclusions:

- *Trop2 ADCs conjugated with PH1 toxin exhibit significant TGI at drug-to-antibody ratio of 2 and 4. These ADCs are internalized rapidly, exhibit low off-target activity in normal cells and Trop2-negative cells while exhibiting equal or better cytotoxic potency and TGI relative to first-in-class therapeutic ADC in tested cell lines and xenograft model, respectively*
- *The target validation studies suggest that Trop2 PH1 ADCs were sufficiently differentiated in areas of preclinical efficacy and well tolerated in a toxicologically relevant host.*