Inhibiting Protein Translation: A Novel ADC Payload With Strong Antitumor Activity in Mono- and Dual-Payload Formats

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Abstract

Background:
Antibody-drug conjugates (ADCs) have shown clinical success, but resistance, often driven by the payload, can limit long-term efficacy. To address this, Hexagon Bio is mining its natural product library to discover new payloads suitable for mono- or dual-payload ADCs. We report on a protein translation inhibitor (PTI) with strong antitumor activity when conjugated to the anti-TROP2 antibody sacituzumab, both as a mono payload and in combination with the topoisomerase inhibitor Dxd (dual payload). The PTI also shows promise when conjugated to other antibodies, including those targeting low-abundance antigens.

Materials and methods

By mining small molecules produced by Hexagon Bio's 120,000-strain microbial library, several potent cytotoxic compounds were identified. The lead PTI was evaluated as a free agent in cell-free translation assays and as an ADC. It was conjugated to sacituzumab at drug-to-antibody ratios (DARs) of 4 and 8 and tested in:

- In vitro cytotoxicity (CellTiter-Glo, 120 hrs) and immunogenic cell death (ICD) assays (detection of hallmarks of ICD: extracellular ATP, secreted high mobility group box 1 and cell surface calreticulin, 120-168 hrs)
- In vivo efficacy models (NCI-N87, HT1376)
- Pharmacokinetics/pharmacodynamic (PK/PD) studies
- Rodent tolerability studies

To assess broader applicability, the PTI was also conjugated to other antibodies (anti-HER3, -Nectin4, -ITGB6, -B7H3, -HER2), and cytotoxicity was evaluated. A dual-payload ADC combining the PTI on sacituzumab was also generated and tested.

Results

As a free agent, the PTI potently inhibited protein translation in cell-free assays (EC_{50}~ 15 nM). When conjugated to sacituzumab, the ADC exhibited strong in vitro cytotoxicity (sub- to single-digit nM) across TROP2-expressing cell lines, including those that have low antigen levels and are resistant to Dxd. The ADC also induced hallmarks of ICD, suggesting it may combine well with checkpoint inhibitors. In vivo, PTI-based ADCs delivered complete responses at ≥ 5 mg/kg (DAR 4) in both Dxd-sensitive (NCI-N87) and Dxd-resistant (HT1376) models, outperforming sacituzumab-Dxd (DAR 8). PK profiles were favorable, with circulating t1/2 in line with that of naked sacituzumab. In preliminary tolerability studies, the DAR 4 ADC was well tolerated in mice and rats, suggesting a reasonable therapeutic index.

PTI-conjugated ADCs remained cytotoxic when paired with other antibodies, including those targeting antigens expressed as low as 20 K receptors per cell, supporting its translatability. The dual-payload ADC (PTI + Dxd) outperformed the Dxd-only ADC in vitro, including in Dxd-resistant cell lines, demonstrating its potential to broaden efficacy and overcome resistance.

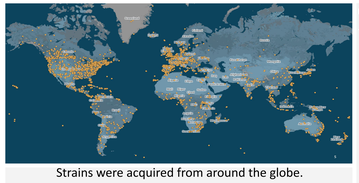
Conclusions

Our findings highlight the promise of a novel PTI as a versatile ADC payload, demonstrating potent antitumor activity alone or in combination with Dxd. Its compatibility with diverse antibodies, and favorable PK/tolerability profiles support its further evaluation as an approach to overcome resistance and improve patient outcomes.

Clinical ADC Sequencing Studies Suggest ADC Resistance Is Primarily Due to Resistance to the Payload

"The problem is, regardless of the antibody, there are sequencing issues when a tumor has already been exposed to a payload of similar class. **New payloads are desperately needed to advance ADCs forward."**
Patricia LoRusso, Yale School of Medicine, ASCO 2025

Hexagon's >100,000 Microbial Strains Are an Unparalleled Source for Novel Cytotoxic ADC Payloads



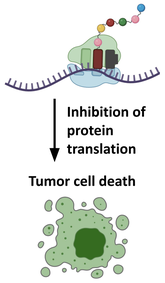
Strains were acquired from around the globe.
We have sequenced **over 80,000** genomes.
Approximately 400 putative mechanisms of action predicted.

Platform identifies mechanisms orthogonal to existing ADC payloads

~400 cytotoxic MoAs essential to cancer cell growth / survival:

- **DNA/RNA-associated pathways**
  - Nucleotide biosynthesis
  - mRNA splicing
  - tRNA, rRNA modification
- **Protein-associated pathways**
  - Protein biosynthesis: Initiation, elongation, termination
  - Sumoylation
- **Energy production**
  - Electron transport
  - Citric acid cycle
  - Glutamine metabolism
- **Fat/lipid synthesis**
  - Cholesterol, sphingolipid, triglyceride biosynthesis
- **Plus many other mechanisms**
  - Cell cycle, vacuolar protein sorting, and dozens more

Protein Translation Inhibition Is Predicted to Be a Vulnerability for Tumor Cells, Particularly Those That Are Dependent on Oncogene Expression

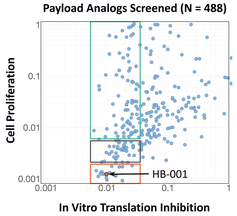


Tumor development and progression depends on continuous proteome remodeling and sustained production of oncogenic proteins. Inhibiting this supply disrupts critical survival pathways, leading to tumor cell death

Tumors likely to be most sensitive to protein translation inhibition are those that:

- Rely on continuous supply of oncogenic drivers
- Overexpress oncogenic proteins with short half-lives
- Express elevated levels of translational machinery needed to sustain their oncoproteome

Our Optimization Efforts Have Resulted in an PTI Analog With Desirable Properties

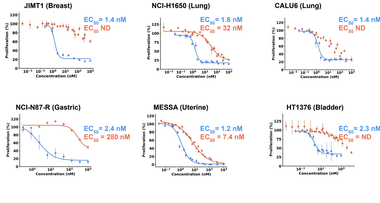


		HB-001
Payload properties and in vitro activity	ALogP | ALogD(7.4) | Topological polar surface area Å^2	2.3 | 2.2 | 146
	Calculated pKa (%ionized at pH 7.4)	6.9 (24%)
	In vitro translation EC_{50} (span)	14 (95%)
	72 hr in vitro cell killing EC_{50} (geo mean)	1.4 nM
	Liver microsome stability Mouse | Rat | Cyno | Hu t1/2 (min)	103 | 19 | 17 | 58
Payload in vivo PK	Mouse 0.3 mg/kg iv PK Cl | Vss | t1/2	2.6 L/h/kg | 10 L/kg | 5.4 h
ADC bystander activity	4:1 KPL4/CALU6 bystander EC_{50}	3.7 nM

Payload Has Broad Cytotoxic Activity, Including Cell Lines That Are Resistant to Tubulin or Topoisomerase Inhibitors

Cancer cell lines, incl. MMAE/Dxd resistant (n=103)	Protein Translation Inhibitor	Tubulin inhibitor MMAE	Topo-I inhibitor Dxd
Bladder			
Bowel			
Breast			
Cervix			
Esophagus			
Kidney			
Lung			
Lymphoid			
Myeloid			
Ovarian			
Pancreas			
PNS			
Prostate			
Uterus			
Median IC50	1-15 nM	15-100 nM	>100 nM

HB-001, Dxd

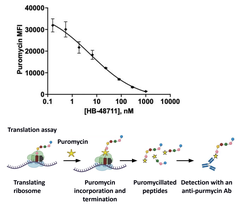


ND = Not Determined

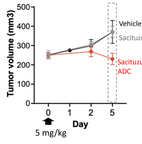
CellTiter-Glo luminescent assay measured cell viabilities at 72h treatment (N=2-3 biological replicate, N=2-3 technical replicates). Dose response curves were generated for 9 concentrations of each test compound and the half-maximal inhibitory concentration (IC50) was calculated.

On-target Translation Inhibition Is Demonstrated In Vitro and In Vivo Using Fluorescent Puromycin Pulse Chase

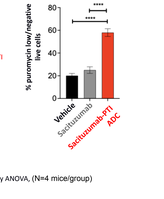
Inhibition of protein translation is detectable within 4 hrs of HB-001 treatment (earliest time point tested)



Tumor cell death



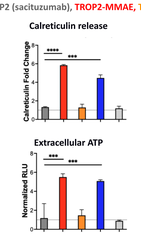
Translation Inhibition in tumors (Day 5) Puromycin-pulse assay



Data are +/- SEM, ***p<0.0001 by One Way ANOVA, (N=4 mice/group)

TROP2 PTI ADC Induces Markers of Immunogenic Cell Death and Treatment Increases Intratumoral Cytotoxic T cells in Vivo

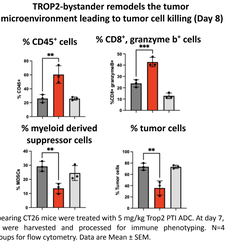
TROP2 (sacituzumab), **TROP2-MMAE**, **TROP2-Dxd**, **TROP2-PTI**, PBS control



Data are mean +/- SD (N=1-2 biological replicates, N=2-3 technical replicates)
*p values based on One way ANOVA (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001)*

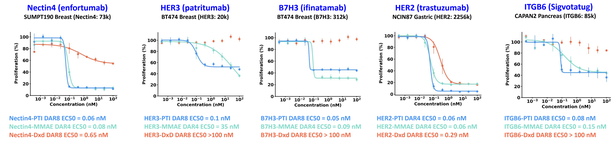
Vehicle, **TROP2-PTI ADC**, TROP2 Ab

TROP2-bystander remodels the tumor microenvironment leading to tumor cell killing (Day 8)

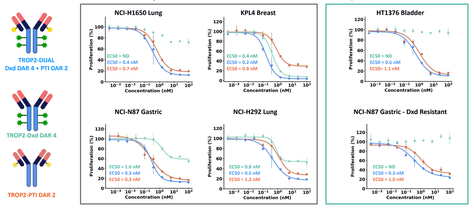


Tumor-bearing CT26 mice were treated with 5 mg/kg Trop2 PTI ADC. At day 7, tumors were harvested and processed for immune phenotyping. N=4 mice/groups for flow cytometry. Data are Mean ± SEM.

PTI Payload Is Active In Vitro When Conjugated to Other Validated ADC Antibodies

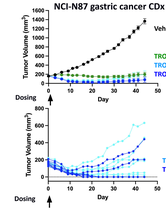


Dual-Payload ADC Comprising PTI and Dxd Outperforms an ADC with Dxd Alone (In Vitro)



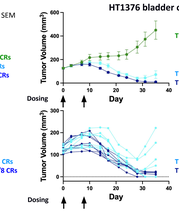
TROP2 PTI ADC Delivers Complete Responses Following a Single Dose and Is Active in CDx Models That Are Non-responsive to a Dxd-based ADC

Single Dose of TROP2 PTI ADC Is Sufficient to Deliver Complete Responses

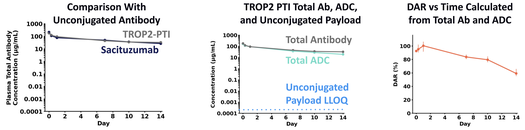


Data are Mean ± SEM (N=7-8 mice/group)

TROP2 PTI ADC Is Active in a Model That Is non-Responsive to TROP2 Dxd ADC

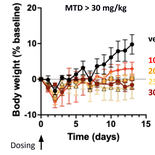


TROP2 PTI ADC Is Stable and Has Good PK in Sprague-Dawley Rat



Test Article	Dose (mg/kg)	Analyte	Cl (mL/h/kg)	Vss (mL/kg)	t1/2 (h)	Cmax (µg/mL)	AUClast (µg*h/mL)	AUCinf (µg*h/mL)
Sacituzumab	5	Total Ab	0.22	53	189	214	17900	25200
TROP2-PTI ADC	5	Total Ab	0.18	52	216	181	18900	28900
		Total ADC	0.24	45	139	167	16800	20600
		Unconj payload	ND	ND	ND	<0.0002 ug/mL	ND	ND

TROP2 PTI ADC Is Well Tolerated in Rats at >10-fold Higher Exposure* Than What Is Required for Efficacy



Mortality	No mortality up to 30 mg/kg
Clinical observations	Skin discoloration and scabs at 25 and 30 mg/kg
Clinical chemistry	Transient decrease of total protein (day 7, 1.1x). Minor decrease in albumin (day 7 and 14, 1.3x)
Hematology	Minor decrease in hemoglobin (day 14, 1.2x). Increase in WBC (day 7 and 14, 3x), monocytes (day 14, 3x) and neutrophils (day 14, 10x)
Tissue histopath (25 mg/kg)	No remarkable findings in colon, heart, kidneys, lymph nodes, rectum, spleen, bone marrow and thymus.

Exposure (AUClast µg*h/ml) in mice at 5 mg/kg: 10100; in rats at 30 mg/kg: 100,800

Novel PTI Payload Offers Approach to Overcome Resistance to Existing ADCs

Payload Property	Tubulin Inhibitor	Topo-1 Inhibitor	Protein Translation Inhibitor
Mechanism is not used in SoC chemotherapy (i.e., no prior exposure)	✘	✘	✔
Low vulnerability to resistance related to target mutation or expression	✔	✘	✔
Low vulnerability to resistance related to drug efflux by BCRP	✔	✘	✔
Highly potent across a broad range of cancer cell lines	✘	✔	✔
Induction of immunogenic cell death markers	✔	✘	✔
Absence of classical ADC toxicities: hematologic, ocular, pulmonary, etc.	✘	✘	✔
First-in-class potential across validated tumor targets	✘	✘	✔

Ongoing and Upcoming Activities:
PTI ADCs:
- **Tolerability in NHPs**
- **Further characterization of PTI ADC efficacy**

Dual payload ADCs
- **In vivo efficacy and tolerability studies**

Other new payloads
- **In vitro and in vivo efficacy testing**