Indole-Biaryl Pyrrolobenzodiazepines (I-BiPs): A Potent and Well-tolerated Class of DNA Mono-alkylating Payload for Antibody-Drug Conjugates (ADCs)

ABSTRACT #173

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Mersana THERAPEUTICS

Abstract

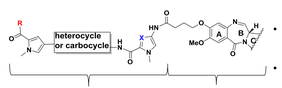
Although it has been known for years that pyrrolobenzodiazepine (PBD) monomers and polyheterocycle DNA binders can be synergistically combined to form potent DNA mono-alkylators,[1] such PBD hybrids have not yet been used as ADC payloads. We describe here a new class of PBD mono-alkylator hybrids (referred to as I-BiPs) and demonstrate their use as ADC payloads.

A series of biaryl heterocyclic DNA binders were linked to a PBD monomer and the cytotoxic activities of the resulting molecules were evaluated in a panel of solid tumor cell lines. The selected molecules were incorporated into linked payloads. The payloads were conjugated to monoclonal antibodies and the resulting ADCs that displayed desirable physicochemical properties and *in vitro* activities were evaluated in rodent tolerability studies and *in vivo* xenograft models. Payload and ADC processing was assessed in tumor cells in culture as well as in the presence of lysosomal extract and in plasma. Bystander capability of the cytotoxic payload released from the ADC was also assessed in co-culture experiments using antigen-positive and antigen-negative cell lines. The lead ADCs were evaluated in *in vivo* xenograft models, PK, and rat tolerability head-to-head against a known DNA mono-alkylator class (IGN mono-alkylators[2]).

SAR studies led to the identification of a key indole unit in the DNA binder portion that significantly improved potency while providing a site for antibody conjugation. The lead I-BiP series exhibited low picomolar activity in a broad panel of solid tumor cell lines, including cell lines resistant to anti-tubulin agents. These *in vitro* cytotoxicities correlated with *in vivo* activity and the corresponding I-BiP ADCs were also highly active *in vivo* in auristatin-resistant xenograft models. Co-culture experiments with I-BiP ADCs showed that the extent of bystander killing could be modulated *via* simple structural variations on the indole unit. Unlike typical PBD dimers and IGN mono-alkylators, I-BiP ADCs are more hydrophilic and therefore are not limited to DAR 2; DAR 4-5 I-BiP ADCs with high monomeric content were readily achieved without resorting to site-specific conjugation. Pronounced anti-tumor activity was observed for I-BiPs ADCs at single IV doses of 1 or 3 mg/kg in a variety of solid tumor xenograft models. In toxicology studies in rats, I-BiP ADCs were well tolerated after multiple doses.

Given their potent antitumor efficacy in a variety of solid tumor models, favorable therapeutic index and hydrophilicity relative to PBD dimers and IGNs, I-BiPs are a promising new class of DNA damaging payload for ADCs.

Discovery of I-BiPs

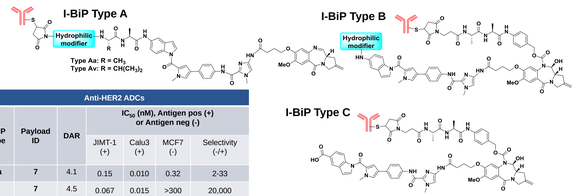


- Modification at the PBD C-ring or "X" resulted in modest improvements in activity (2-3 fold)
- Modification at "R" favored rigid/planar groups. 5-amino indole most active *in vitro*.
- Most active DNA binder units carried biaryl motif
- Representatives from I-BiP series are highlighted below in blue.

Payload ID	R	Heterocycle / Carbocycle	X	PBD C-ring	IC_{50} (nM) in HER2 + or - Cell Lines			
					JIMT-1 (+)	SKBR3 (+)	NCI-N87 (+)	MCF7 (-)
1	HO		CH		3.0	2.0	4.0	8.0
2	HO		N		1.3	0.83	1.7	5.5
3	HO		N		0.80	0.49	1.0	1.3
4	H_2N		N		26.8	9.4	24.1	--
5	H_2N		N		37.1	1.4	6.4	14.9
6	H_2N		N		0.84	0.15	0.43	4.0
7	H_2N		N		0.037	0.0069	0.030	0.025
8	HO_2C		N		1.5	0.13	2.5	7.2

- $5x10^3$ cells/well in 96-well plate treated 5 days with test articles in 37°C, 5% CO_2
- Viability measured by CellTiter-Glo Luminescent (Promega)
- IC_{50} calculated based on Least squares fitting method using Nonlinear regression by GraphPad Prism

ADC Characteristics and *In Vitro* Activity

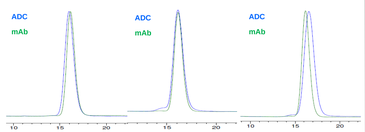


Anti-HER2 ADCs						
I-BiP Type	Payload ID	DAR	IC_{50} (nM), Antigen pos (+) or Antigen neg (-)			
			JIMT-1 (+)	Calu3 (+)	MCF7 (-)	Selectivity (-/+)
Aa	7	4.1	0.15	0.010	0.32	2-33
B	7	4.5	0.067	0.015	>300	20,000
C	8	4.6	>300	0.01	71	7,000

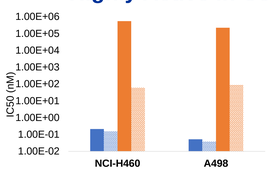
Anti-NaPi2b ADCs					
I-BiP Type	Payload ID	DAR	IC_{50} (nM)		
			OVCAR3 (+)	JIMT-1 (-)	Selectivity (-/+)
Aa	7	4.1	0.03	3.2	100
B	7	4.0	0.03	>300	>10,000
C	8	3.6	0.24	>300	>1,000

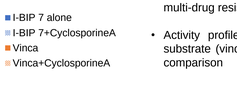
- $5x10^3$ cells/well in 96-well plate treated 5 days with Test articles in 37°C, 5% CO_2
- Viability measured by CellTiter-Glo Luminescent (Promega)
- IC_{50} calculated based on Least squares fitting method using Non
- linear regression by GraphPad Prism

SEC of I-BiP ADC (Type Aa) SEC of I-BiP ADC (Type B) SEC of I-BiP ADC (Type C)



Highly Active in Cell Lines Expressing P-gp





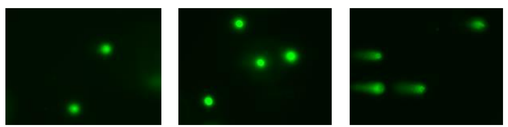
- I-BiP payload **7** is highly active in multi-drug resistant cell lines
- Activity profile of a known P-gp substrate (vinca) is also shown for comparison

- $5x10^3$ cells/well in 96-well plate treated 5 days with Test articles with or without 1mM Cyclosporine A (CAS Number 59865-13-3) in 37°C, 5%CO_2
- Viability measured by The CellTiter-Glo® Luminescent Cell Viability Assay (Promega)
- IC_{50} calculated based on Least squares fitting method using Nonlinear regression by GraphPad Prism

Mechanism of Action (MOA) Profiling

- I-BiPs exert their effect via DNA damage as shown below in a standard COMET assay. In this assay, damaged DNA manifests as a "comet tail" under electrophoresis and can be visualized via fluorescence microscopy.
- DNA damage was assessed using OVCAR3 cells expressing the NaPi2b antigen following treatment with I-BiP Type Aa ADCs

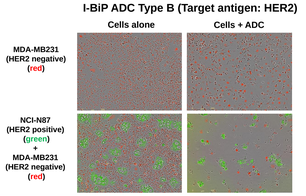
Untreated Non-Binding Control ADC Anti-NaPi2b ADC

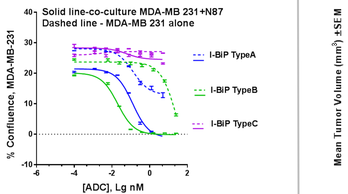


- Ovcar3 cells treated for 24h, 37°C, 5%CO_2
- Comet Assay was performed under alkaline electrophoresis conditions at 33V / 300 mA for 15 minutes, following the manufacture's protocol (Cat# STA-350, Cell Biolabs, Inc)
- Detection by Fluorescence Microscopy

I-BiP ADCs Exhibit Bystander Killing

I-BiP ADC Type B (Target antigen: HER2)





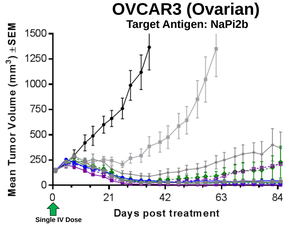
I-BiP ADC	IC50 (nM)			Bystander Effect Observed
	N87	MDA-MB231	MDA-MB231 + N87 Coculture	
Type Aa	0.05	Inactive	0.12	Yes
Type B	0.05	30	0.02	Yes
Type C	0.05	Inactive	Inactive	No

- MDA-MB-231 labelled by nuclear red [Cat.# 4476 CellPlayer NucLight Red (Lenti, EF-1 alpha, puro) Essen BioScience]
- N87 labelled by nuclear green [Cat# 4567 CellPlayer™ NucLight Green (Lenti, EF-1 alpha, puro) Essen BioScience]
- Co-culture MDA-MB-231: N87(3:1) (4500:1500 cells per well) & each cell line alone have been treated with test articles for 5 days monitoring by IncuCyte
- Viability measured by % Confluence of each cell line using IncuCyte Software

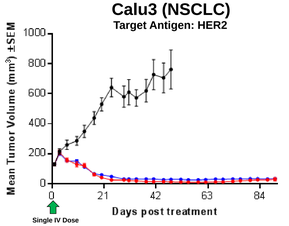
- The "bystander effect" is the process by which an ADC payload is able to diffuse out of the target antigen-expressing cell and also kill neighboring cells that do not express the target antigen
- Modifications to the DNA binder unit of I-BiP payloads permit selection of ADCs with bystander killing (as in Type A and B) or without it (as in Type C)

Efficacy in Solid Tumor Models

OVCAR3 (Ovarian) Target Antigen: NaPi2b



Calu3 (NSCLC) Target Antigen: HER2



Group	mAb Target	ADC	DAR	Payload Dose (mg/kg)	mAb Dose (mg/kg)
Vehicle	--	--	--	--	--
NaPi2b	NaPi2b	I-BiP Type Aa	4.1	0.03	1.5
NaPi2b	NaPi2b	I-BiP Type Aa	4.1	0.06	3.0
Control*	NaPi2b	I-BiP Type Aa	4.6	0.06	2.5
NaPi2b	NaPi2b	I-BiP Type B	4.0	0.03	1.5
NaPi2b	NaPi2b	I-BiP Type B	4.0	0.06	3
Control*	NaPi2b	I-BiP Type B	5.2	0.06	2.2
NaPi2b	NaPi2b	I-BiP Type C	2.6	0.03	2.2
NaPi2b	NaPi2b	I-BiP Type C	2.6	0.06	4.4
Control*	NaPi2b	I-BiP Type C	3.6	0.06	3

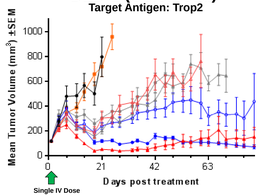
* Non-binding control ADC

Group	mAb Target	ADC	DAR	Payload Dose (mg/kg)	mAb Dose (mg/kg)
Vehicle	--	--	--	--	--
HER2	I-BiP Type Av		4.8	0.026	1.0
HER2	IGN^{2b}		2.3	0.011	1.0

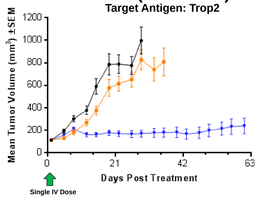
- I-BiP ADCs were highly efficacious at a single IV dose of 1-3 mg/kg (mAb dose)
- IGN mono-alkylator ADCs were prepared as described in ref 2c.
- The *in vivo* efficacy of I-BiP ADCs were comparable to the IGN^{2b} mono-alkylator ADC

Efficacy in Auristatin-Resistant Tumor Models

DLD-1 (Colorectal) Target Antigen: Trop2



HT29 (Colorectal) Target Antigen: Trop2



Group	mAb Target	ADC	DAR	Payload Dose (mg/kg)	mAb Dose (mg/kg)
Vehicle	--	--	--	--	--
Dolaflexin[a]	Trop2	AF-HPA	16.5	0.3	3.0
Trop2	Trop2	IGN^{b}	3.4	0.02	1.0
Trop2	Trop2	IGN^{b}	3.4	0.06	3.0
Control[c]	Trop2	IGN^{b}	2.0	0.03	3.0
Trop2	Trop2	I-BiP Type Aa	5.4	0.03	1.0
Trop2	Trop2	I-BiP Type Aa	5.4	0.09	3.0
Control[c]	Trop2	I-BiP Type Aa	5.5	0.09	3.0

(a) Dolaflexin is a high DAR ADC platform developed by Mersana that delivers an auristatin-based payload known as AF-HPA
(b) IGN ADCs prepared as described in ref 2c
(c) Non-binding control ADC

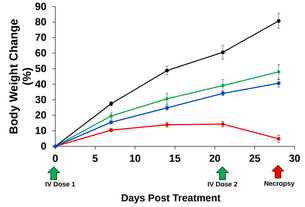
Group	mAb Target	ADC	DAR	Payload Dose (mg/kg)	mAb Dose (mg/kg)
Vehicle	--	--	--	--	--
Dolaflexin[a]	Trop2	AF-HPA	16.5	0.3	3.0
	Trop2	I-BiP Type Av	5.4	0.09	3.0

- The I-BiP ADC was highly active in both auristatin resistant models whereas the DF ADC was completely ineffective—despite the fact that the DF ADC carried 3-fold higher payload dose/antibody
- The *in vivo* efficacy of the I-BiP ADC is comparable to the IGN mono-alkylator ADC^{2b}

Tolerability in Rat

Repeat Dose vs IGN Mono-alkylator

Body Weight Change



ADC	DAR	Group	Dose 1 (mg/kg)		Dose 2 (mg/kg)	
			mAb	Payload	mAb	Payload
Vehicle	--		--	--	--	--
IGN^{2b}	2.5		1.6	0.02	3.2	0.04
I-BiP Type Aa	4.1		2	0.04	2	0.04
I-BiP Type B	4.0		2	0.04	2	0.04

- mAb = Anti-NaPi2b (Note: rat cross-reactive)
- ADCs dosed by payload
- I-BiP ADCs tolerated at least 2-fold higher than IGN

Conclusions

- Although they are highly potent, I-BiPs differ from other well-known DNA damaging agents in terms of MOA (mono-alkylator), physicochemical properties (DAR 4-5 readily achievable), and tolerability (I-BiP > IGN >> PBD dimer).
- Such attributes make I-BiPs an attractive new ADC payload class.

References

1. (a) *Bioorg. Med. Chem. Lett.*, **1998**, *8*, 3019-3024; (b) *J. Med. Chem.*, **2013**, *56*, 2911-2935
2. (a) *Mol. Cancer Ther.*, **2016**, *15*, 1870-1878; (b) *Mol. Cancer. Ther.* **2018,** *17*, 650-660; (c) U.S. Patent 0082114A1, March 24, 2016