Advantages of Polyacetal Polymer-based Antibody Drug Conjugates: Application to Low Expression Targets

Alex Yurkovetskiy, Natalya Bodyak, Mao Yin, Joshua D. Thomas, Patrick Conlon, Cheri A. Stevenson, Alex Uttard, LiuLiang Qin, Dmitry R.Gumerov, Elena Ter-Ovanesyan, Venu R. Gurijala, Dennis McGillicuddy, Roberta E Glynn, Michael DeVit, Laura L. Poling, Peter U. Park, Timothy B. Lowinger

Mersana Therapeutics Inc., 840 Memorial Drive, Cambridge, MA 02139

#2645

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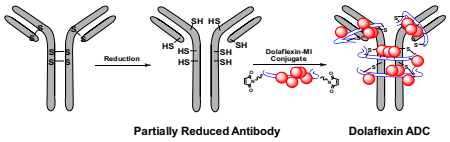
Summary

We have developed a potent and effective drug conjugation platform for ADC application - Dolaflexin™. Dolaflexin consists of a proprietary dolastatin derivative chemically conjugated to poly(1-hydroxymethylethylene hydroxymethylformal) or PHF.

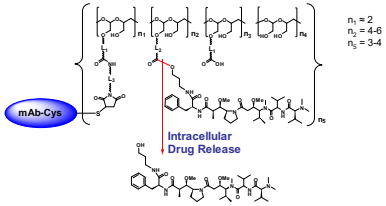
Here, we report the characterization of a novel trastuzumab Dolaflexin ADCs, employing a maleimide-based bioconjugation approach. The resulting ADCs with a fixed drug-antibody ratio of 15 or 20 in the current examples, exhibit enhanced stability and excellent pharmacokinetics, with a prolonged plasma half-life and tumor-specific accumulation. Active drug release and accumulation in tumor tissue was also confirmed by LC/MS/MS methods.

The activity of this novel trastuzumab Dolaflexin ADCs was evaluated in multiple tumor xenograft models with significant variations in target antigen expression levels. Models including BT474 breast cancer, NCI-N87 gastric cancer, and JIMT1 breast cancer models were utilized, and comparisons to a variety of controls and ADC reference standards were made. Significant advantages of the polyacetal polymer-based ADCs in comparison to conventional ADCs, particularly in models with low target antigen expression, were observed.

Cysteine-Based Fleximer-Drug Antibody Conjugation



Dolaflexin ADC Structure and Drug Release

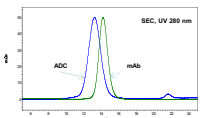


Cysteine-Linked Trastuzumab Dolaflexin ADCs Characterization

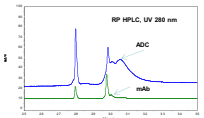
Parameter	Analysis	Methods
Drug/mAb ratio, (DAR)	15, 20	LC-MS/UV
Fleximer/mAb ratio	3-4	LC-MS/UV
Free drug content, % total	<0.1%	LC-MS
Free Dolaflexin	< 1%	LC-MS
Free mAb content, % total	<1%	HIC HPLC
ADC size	180-200 kDa	SDS-PAGE, SEC
ADC polydispersity	PDI<1.2	SEC
Aggregated fraction, %	<2%	SEC
Free thiol groups	Not detected	UV

HPLC Characterization of Dolaflexin ADCs

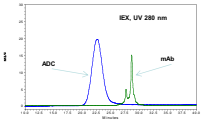
Uniform material, low polydispersity

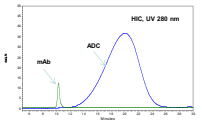


Bioconjugation mostly HC specific



Efficient bioconjugation – no free mAb





Dolaflexin Conjugation Does Not Adversely Affect ADCs Target Binding

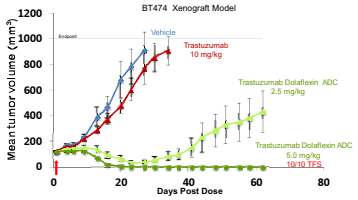
Trastuzumab ADCs/hErbB2 binding parameters measured by SPR at 25°C

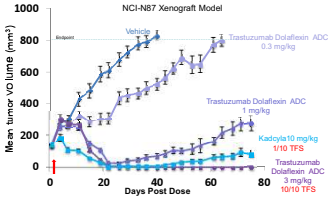
Analyte	DAR	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (pM)
Trastuzumab	n/a	9.90(1)e5	2.60(1)e-5	26.2(1)
Kadcyla	3.5	4.61(2)e5	3.17(9)e-5	68.7(3)
Trastuzumab Cys/Dolaflexin	15	4.61(3)e5	2.60(9)e-5	56.3(3)
Trastuzumab Cys/Dolaflexin	20	5.30(3)e5	2.43(1)e-5	45.9(3)

Notes: The number in parentheses represents the error in the last digit.
For example, 9.9(1)e5 equals (9.9 ± 0.1) x 10^5.

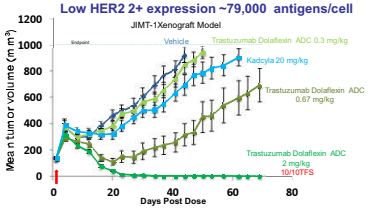
Trastuzumab Dolaflexin ADCs *In Vivo* Pharmacology

High HER2 3+ expression ~500,000 antigens/cell

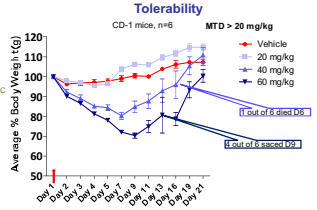




Low HER2 2+ expression ~79,000 antigens/cell



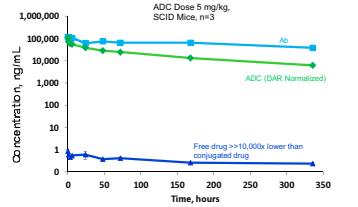
Tolerability



Plasma PK and Tissue Distribution

Plasma PK

ADC Dose 5 mg/kg, SCID Mice, n=3

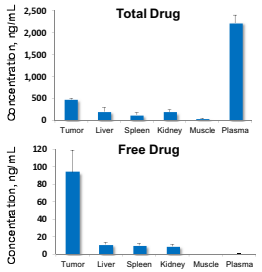


ADC Dose (mpk)	ADC* AUC 0-inf (ug*day/mL)	Half-Life, (days)	
		ADC* (ADC toxin release)	mAb** (mAb ELISA)
5	301	5.2	12

*) Conjugated Toxin BioA by LC/MS/MS; **) Total mAb analysis by ELISA

Total and Free Drug Tissue Accumulation

BT474 Xenograft Bearing SCID Mice, ADC Dose 5 mg/kg, 48 Hrs, n=4



Discussion and Conclusions

- Dolaflexin conjugation to trastuzumab via interchain cysteines and thioether linker does not adversely affect ADCs target binding
- Trastuzumab Dolaflexin ADCs have excellent PK and is well tolerated (MTD > 20 mg/kg)
- Trastuzumab Dolaflexin ADCs demonstrate tumor-specific accumulation and drug release
- Trastuzumab Dolaflexin ADCs showed potent anti-tumor activities in both high HER2-expressing BT-474 (HER2 3+) and low HER2-expressing JIMT-1 (HER2 2+) breast tumor models
- In JIMT-1 model trastuzumab Dolaflexin ADCs showed tumor regression after a single dose of 0.67 mg/kg compared to Kadcyla® which was inactive even at 20 mg/kg

Acknowledgements

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References

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2. Yurkovetskiy A, Bodyak N, Mao Yin, Thomas JD, Conlon P, Stevenson C, Uttard A, Liu Qin, Gumerov D, Ter-Ovanesyan E, DeVit M, Lowinger TB. Advantages of Polyacetal Polymer-Based Antibody Drug Conjugates Employing Cysteine Bioconjugation. 2013 Annual AACR Meeting (Washington, DC), Abstract #4331