Advantages of Polyacetal Polymer-Based Antibody Drug Conjugates Employing Cysteine Bioconjugation

#4331

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Summary

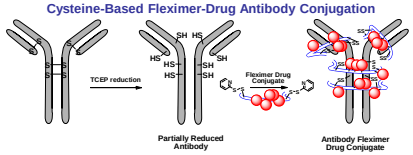
The application of polymers to antibody drug conjugates (ADC) can provide numerous advantages, including 1) significantly higher capacity for drug payload without sacrificing PK or tolerability; 2) utilization of alternative payloads not suitable for direct conjugation approaches; 3) improvement of physicochemical properties of resulting ADCs; and 4) utilization of protein recognition scaffolds beyond the commonly used IgGs.

Herein we present results of a novel, biodegradable polyacetal polymer-based conjugation system to create next-generation ADCs. The basis of this new conjugation system is a hydrophilic, fully biodegradable polyacetal carrier (PHF or poly(1-hydroxymethylethylene hydroxymethylformal, or Fleximer®) modified with chemically orthogonal linkers. One linker is used to covalently attach a targeting moiety (mAb or alternative) via cysteine conjugation, while a second, chemically distinct linker is used to attach a drug payload and to control the mechanism and rate of drug release.

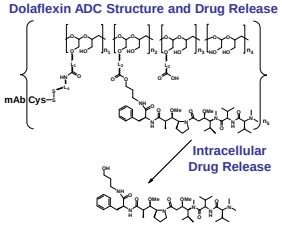
Previously we have reported highly efficacious polyacetal ADCs prepared by bioconjugation of the polymer to random lysine residues; in this report we present an alternative cysteine-based bioconjugation strategy. It is known that direct drug-cysteine linked ADCs result in destabilization of the protein, as the conjugation process necessarily disrupts inter-chain disulfide bridges. In contrast, the Fleximer conjugation approach via cysteines in the antibody hinge region allows for the formation of inter-chain bridge structures involving the polymer backbone, which provide restabilization of the overall construct, as evidenced by analytical methods such as SDS-PAGE and HPLC.

To demonstrate the benefits of this approach, we prepared Her-2 targeted ADCs with protein recognition scaffolds ranging in size from 15 kDa to 150 kDa, all targeting the Her-2 antigen, and bearing a proprietary Dolastatin derivative, XMT-1267, coupled to a Fleximer scaffold (Dolaflexin™). These Dolaflexin ADCs were highly active and selective *in vitro* in Her-2 expressing cell lines. Furthermore, trastuzumab-Dolaflexin ADCs tested *in vivo* exhibited prolonged plasma exposure and tumor specific accumulation in the Her-2 expressing BT474 mouse xenograft model. The ADC was well-tolerated, and resulted in 100% tumor-free survivors at doses as low as 2 mg/kg.

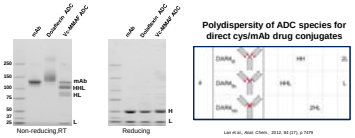
Cysteine-Based Fleximer-Drug Antibody Conjugation



Dolaflexin ADC Structure and Drug Release



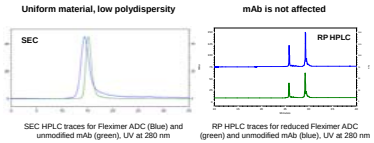
Dolaflexin Conjugation Stabilizes ADC by Bridging Adjacent Sulfhydryl Groups

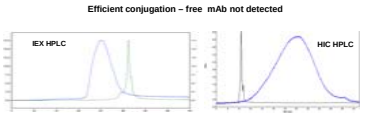


Dolaflexin ADC Characterization (Cysteine-Linked Fleximer/XMT-1267 ADCs)

Parameter	Analysis	Methods
ADC size	150-200 kDa	SDS-PAGE, SEC
ADC polydispersity	PDI<1.5	SEC
Drug/mAb ratio, (DAR)	~ 20	HPLC
Fleximer/mAb ratio	3-4	HPLC
Free drug content, % total	<0.1%	HPLC
Free mAb content, % total	<0.5%	HPLC
Aggregated fraction, %	<2%	SEC
Free thiol groups	Not detected	UV-vis

HPLC Characterization of Dolaflexin ADCs

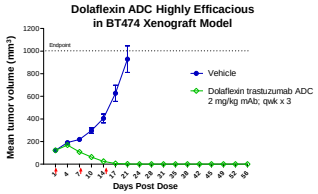


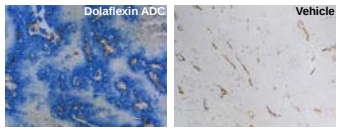


Dolaflexin ADC Cytotoxicity *In Vitro* (IC_{50}/72h, nM, Toxin Based/ADC based)

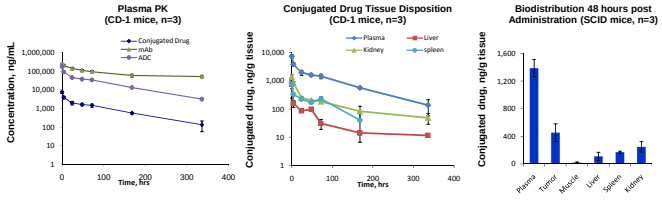
Test Agent	Targeting Ligand	Ligand Size	DAR	SKBR3	BT474	NCI-N87	MCF7
Dolaflexin ADC, toxin based/ADC based	Trastuzumab	~150 kDa	18.5	0.3/0.02	1.3/0.07	1.5/0.08	67.4/3.64
Dolaflexin ADC, toxin based/ADC based	Trastuzumab Fab	~50 kDa	18.6	0.3/0.02	1.7/0.09	4.0/0.22	>100/>5.4
Dolaflexin ADC, toxin based/ADC based	Anti-Her2 Affibody	~14 kDa	12	0.4/0.03	-	3.9/0.33	>100/>8.3
Dolaflexin, toxin based	n/a	n/a	n/a	23.2	36.9	28.7	>100
Toxin XMT-1267, toxin based	n/a	n/a	n/a	0.3	0.8	1.0	8.5

Dolaflexin ADC *In Vivo* Pharmacology





Dolaflexin ADC Plasma PK and Tissue Distribution



Dolaflexin ADC Plasma PK (SCID Mice, n=3)

ADC Dose (mg/kg)	ADC* AUC 0-t (µg*day/mL)	Half-Life, (days)	
		Toxin*** (ADC Release)	ADC** (mAb Based)
10	204	5.1	12

*) Conjugated Toxin BioA by LC/MS/MS; **) Total mAb analysis by ELISA;
***) Both toxin (LC/MS/MS) and mAb (ELISA) BioA data have been used for the estimate

Discussion and Conclusions

- We have demonstrated that conjugation of the Fleximer-XMT-1267 (Dolaflexin) based cytotoxic drug conjugated to cysteine residues in the hinge region of the antibody results in highly reproducible, well-characterized ADCs which do not show the destabilization of the protein characteristic of conventional, direct cysteine-drug ADCs.
- Trastuzumab-Dolaflexin ADCs with a DAR of 20 are highly potent and selective *in vitro* in a variety of cell lines, consistent with antigen-dependent binding and internalization. Similarly, alternative Her-2 targeting moieties including a trastuzumab Fab fragment or a Her-2 targeted Affibody can be conjugated via cysteine residues to Dolaflexin, resulting in highly active and selective Her-2 targeted drug conjugates.
- In *in vivo* studies, the trastuzumab-Dolaflexin ADC with DAR of 20 was highly efficacious and displayed excellent pharmacokinetics, tolerability, tumor penetration, and target-specific tissue accumulation.
- Additional examples of Dolaflexin-based ADCs employing antibodies other than trastuzumab will be reported in due course.

Acknowledgements

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3. Yurkovetskiy A, Yin M, Bodyak N, Stevenson C, Thomas J, Hammond C, Qin L, Zhu B, Gumerov D, Ter-Ovanesyan E, Uttard A, Lowinger TB. Polyacetal-based immunoconjugates: new-generation ADCs with high drug loading, alternative payloads, and alternative protein recognition scaffolds. 2012 Annual AACR Meeting (Chicago, IL), #4633