ABSTRACT
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# Discovery of the novel, homogeneous payload platform Dolasynthen for Antibody-Drug Conjugates

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## Abstract

Dolasynthen is a novel, proprietary, homogeneous payload platform enabling the creation of antibody-drug conjugates (ADC) with DARs ranging from 2-24. We previously reported a high drug-to-antibody ratio (DAR) payload platform, Dolaflexin, which is a polymer-based, polydisperse scaffold incorporated into the ADCs XMT-1522 and XMT-1536 currently in clinical trials. We disclose here efficacy, safety and PK data for Dolasynthen, a second-generation Auristatin F-hydroxypropylamide (AF-HPA) payload platform with a defined, fully homogeneous structure that allows for the creation of ADCs with diverse, defined DAR values.

SAR data showed a strong correlation between the structure of the payload platform and observed activity *in vivo* and allowed for further optimization of hydrophilic modifiers and other physicochemical enhancing components. PK evaluation in non-human primates showed a strong correlation of the ADC exposure to the net charge of the payload platform. The ADCs showed exceptional plasma stability and good exposures in tumor bearing mouse models and NHP tolerability studies. Lead ADCs showed excellent efficacy in mouse xenograft models and desired tolerability in NHPs. The Dolasynthen payload platform was used to generate homogeneous ADCs that showed the expected pM activity *in vitro* and excellent efficacy *in vivo*. Data suggest that fully homogeneous Dolasynthen ADCs present attractive features that make them suitable for future development.

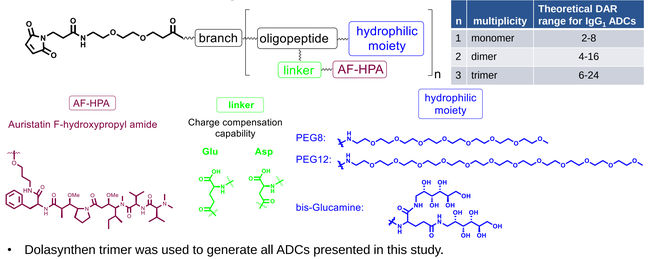
## Materials and Methods

- A set of payload platforms was synthesized that use the Auristatin F-hydroxypropylamide (AF-HPA) cytotoxic warhead. These payload platforms contain highly biodegradable and biocompatible polypeptide frameworks and can be varied with respect to overall charge and the identity and characteristics of hydrophilic moieties to achieve favorable physicochemical properties for the resulting ADCs.
- Two $IgG_1$ monoclonal antibodies were used for this study: anti-NaPi2b mAb and Trastuzumab.
- ADCs 1-9 and 12 were prepared by partial reduction of native disulfide interchain bridges of $IgG_1$ antibodies with TCEP and conjugation of the payload platforms through stochastic bioconjugation. The resulting ADCs were analyzed by SEC and showed <1% hMW content.
- ADC10 was obtained by conjugation to mutant Cys following full reduction and re-oxidation of the native disulfide bridges to generate a fully homogeneous product.
- ADC11 was made by HIC fractionation to achieve the desired DAR 6.
- ADCs 1-9 were evaluated in OVCAR-3 mouse xenograft efficacy models at a 3 mg/kg single dose administration, and non-human primates (NHP) PK studies.
- Trastuzumab ADCs dosing in the mouse efficacy studies was performed as follows: all ADC were dosed at 0.067 mg/kg payload dose that translated to 1 mg/kg mAb dose for ADC12 and 2 mg/kg for ADC10 and ADC11.

| ADC# | mAb | Hydrophilic moiety | Charge Compensation Unit | DAR[1] |
|---|---|---|---|---|
| ADC1 | anti-NaPi2b mAb | PEG8 | Glu | 15[a,c] |
| ADC2 | anti-NaPi2b mAb | PEG8 | Asp | 17[a] |
| ADC3 | anti-NaPi2b mAb | PEG8 | none | 15[b] |
| ADC4 | anti-NaPi2b mAb | PEG12 | none | 16[c] |
| ADC5 | anti-NaPi2b mAb | PEG12 | Glu | 16[c] |
| ADC6 | anti-NaPi2b mAb | PEG12 | Asp | 15[b] |
| ADC7 | anti-NaPi2b mAb | bis-Glucamine | none | 9[a] |
| ADC8 | anti-NaPi2b mAb | bis-Glucamine | none | 14[a] |
| ADC9 | anti-NaPi2b mAb | bis-Glucamine | Glu | 16[c] |
| ADC10 | Trastuzumab with engineered cysteines | PEG8 | Glu | 6[c] |
| ADC11 | Trastuzumab | PEG8 | Glu | 6[c] |
| ADC12 | Trastuzumab | PEG8 | Glu | 12[c] |

[1]DAR was determined by the following analytical methods (a) CE SDS (b) LC/MS or (c) HPLC.

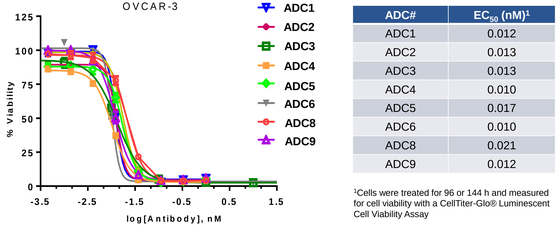
## Dolasynthen Generic Structure




| n | multiplicity | Theoretical DAR range for $IgG_1$ ADCs |
|---|---|---|
| 1 | monomer | 2-8 |
| 2 | dimer | 4-16 |
| 3 | trimer | 6-24 |

- Dolasynthen trimer was used to generate all ADCs presented in this study.

## *In Vitro* Activity of Dolasynthen ADCs

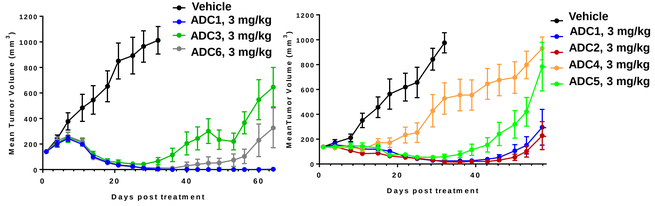



| ADC# | $EC_{50}$ (nM)[1] |
|---|---|
| ADC1 | 0.012 |
| ADC2 | 0.013 |
| ADC3 | 0.013 |
| ADC4 | 0.010 |
| ADC5 | 0.017 |
| ADC6 | 0.010 |
| ADC8 | 0.021 |
| ADC9 | 0.012 |

[1]Cells were treated for 96 or 144 h and measured for cell viability with a CellTiter-Glo® Luminescent Cell Viability Assay

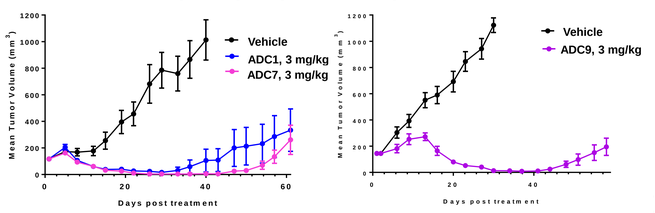
- All ADCs showed *in vitro* potency in antigen expressing cells in the range of 10-21 pM.
- The structure and the charge compensation feature of the payload did not impact activity at equivalent DARs.

## *In Vivo* Efficacy of Dolasynthen PEG Variants in OVCAR-3



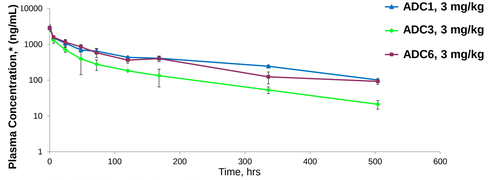

- High DAR ADCs showed higher efficacy when charge compensated capability was included.
- Charge compensated ADCs containing PEG8 (#1 and 2) outperformed ADCs containing PEG12 (#4, 5 and 6).
- The identity of the charge compensation residue had little effect upon the efficacy in OVCAR-3 xenograft model.

## *In Vivo* Efficacy of Hydrophilic Moiety Variants in OVCAR-3




- The presence of bis-glucamine in ADCs 7 and 9 imparted higher potency and a prolonged tumor growth inhibition when compared to PEG-containing ADC1.

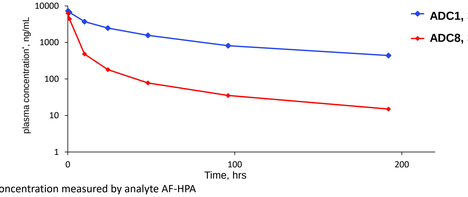
## *In Vivo* Mouse PK of key ADCs




* Plasma concentration measured by analyte AF-HPA

- ADCs that contain charge compensation moiety (ADC1 and ADC6) showed higher *in vivo* stability in the mouse relative to ADCs without charge compensation (ADC3).

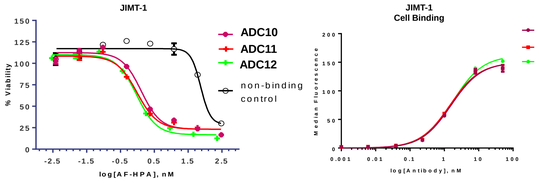
## *In Vivo* NHP PK and Tolerability




* Plasma concentration measured by analyte AF-HPA

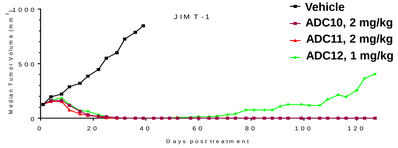
- High DAR Dolasynthen ADCs that were charged compensated showed a significantly higher exposure in NHP following a 3 mg/kg dose.
- A 3 mg/kg dose of Dolasynthen ADC administered to NHP was well tolerated. There was no evidence of neutropenia and no evidence of significant pulmonary toxicity.

## *In Vitro* Activity and Binding of Trastuzumab Dolasynthen ADCs



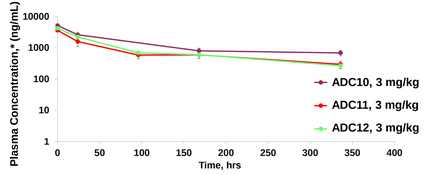

- All ADCs showed antigen-specific activity in HER2 expressing cell line JIMT-1.
- Conjugation of Dolasynthen payload did not impair cell binding of ADCs.

## *In Vivo* Efficacy of Stochastic and Homogeneous ADCs




- Fully homogeneous DAR 6 and the fractionated DAR 6 ADCs showed full tumor inhibition at 2 mg/kg dose while stochastic DAR12 ADC regrew after day 60.
- *In vivo* efficacy data suggests that increased homogeneity is advantageous for Dolasynthen ADCs

## *In Vivo* Mouse PK for Trastuzumab ADCs




| ADC# | AUC*0-T (days*ng/mL) |
|---|---|
| 10 | 14,000 |
| 11 | 10,200 |
| 12 | 10,200 |

* Plasma concentration measured by AF-HPA analyte.

* Plasma concentration measured by AF-HPA analyte.

- Fully homogeneous DAR6 ADC with engineered cysteines showed higher exposure relative to stochastic DAR6 or DAR12 ADC following dosing at 3 mg/kg in a 14-day *in vivo* study.

## Conclusions

- Dolasynthen, a novel, fully homogeneous AF-HPA based payload platform showed potent *in vivo* antitumor activity and excellent tolerability in NHP.
- Dolasynthen platform is amenable to generation of fully homogeneous ADCs.
- The structurally defined framework described herein constitutes a platform that shows potential for future clinical use.