P167
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Site Specific Dolasynthen ADCs demonstrate consistent exposure across a wide range of drug-to-antibody ratios

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Abstract

Key defining attributes of an antibody-drug conjugate (ADC) include the choice of targeting antibody, linker, and the drug-to-antibody ratio (DAR). The choice of DAR, within the constraints of acceptable physicochemical properties for the given platform, is a function of balancing delivery of sufficient payload to targeted cells with the ability to achieve sustained in vivo exposures. Previous reports have described lower DAR mc-VC-MMAE conjugates, DAR = 1-2, that demonstrated higher in vivo exposure and lower clearance when compared to higher DAR (e.g. 4-8) counterparts. In theory, high DAR conjugates may be especially desirable when targeting low antigen expressing tumors or when lower potency payloads are used, as each binding and internalization event results in greater payload delivery. Here we report a systematic exploration of DAR across a much wider range than has been previously reported, by combining THIOMAB® protein engineering technology with the Dolasynthen platform.

Homogeneous, site-specific ADCs spanning a discrete range of DARs – 2, 4, 6, 12, and 18 – were made by conjugation of Trastuzumab IgG1 THIOMAB constructs with 1, 2, or 3 engineered cysteines to monomeric or trimeric Dolasynthen. The cytotoxicity of the resulting well-defined ADCs was assessed in vitro in cell lines with high or low expression of HER2 antigen. Pharmacokinetic data for all test articles in mice were generated in tumor bearing mice.

In high HER2 expressing cell lines, in vitro cytotoxicity by payload was comparable across DARs. In a lower HER2 expressing system, the higher DAR ADCs performed better. In vivo, our data demonstrated comparable pharmacokinetics for the Dolasynthen conjugates across all DARs. These results illustrate the utility of a DAR ranging platform, such as Dolasynthen when evaluating ADCs as it enables the interrogation of a range of antibody and payload dosing regimens.

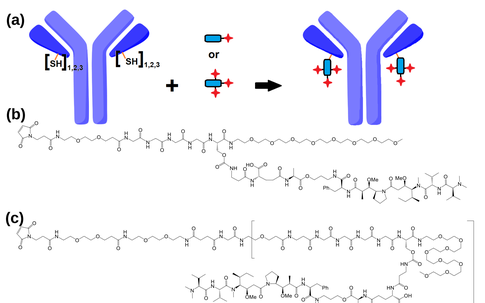
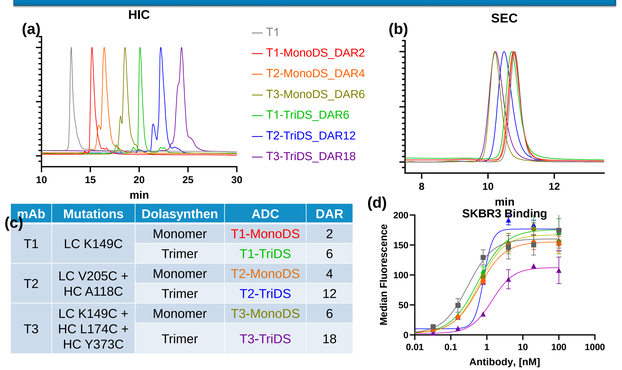


Figure 1. (a) THIOMABs with 1, 2, or 3 engineered cysteines were conjugated with monomeric or trimeric Dolasynthen yielding DAR 2, 4, 6, 12, and 18 conjugates. **(b)** Monomeric Dolasynthen **(c)** Trimeric Dolasynthen

Characterization of ADCs



(c)

mAb	Mutations	Dolasynthen	ADC	DAR
T1	LC K149C	Monomer	T1-MonoDS	2
		Trimer	T1-TriDS	6
T2	LC V205C + HC A118C	Monomer	T2-MonoDS	4
		Trimer	T2-TriDS	12
T3	LC K149C + HC L174C + HC Y373C	Monomer	T3-MonoDS	6
		Trimer	T3-TriDS	18

Figure 2. (a) HIC traces for each ADC; Tosoh Butyl-NPR column. **(b)** SEC traces for each ADC; Tosoh TSK-gel column. **(c)** ADCs prepared with DARs ranging from 2 to 18. **(d)** ADCs tested for binding to SKBR3 cells

- HIC analysis shows increasing DAR correlates with increasing retention time
- No significant aggregation was observed for any ADC
- ADCs bound cells similarly with DAR18 ADC showing a minor shift in EC50

In Vitro Results

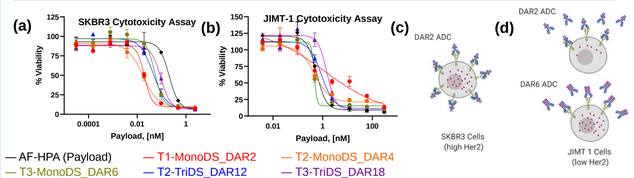
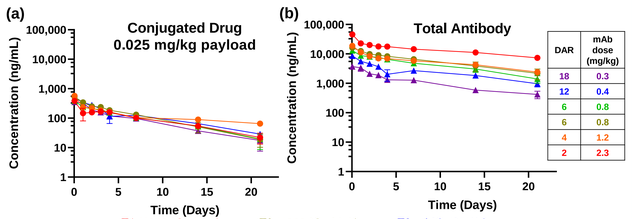


Figure 3. (a) Cytotoxicity assay for SKBR3 cells treated with ADCs. **(b)** Cytotoxicity assay for JIMT-1 cells treated with ADCs. **(c)** Proposed internalization pathway for high receptor density and **(d)** low receptor cell lines

- High HER2 expressing model, SKBR3 (5,714 k/cell)[1], shows comparable cytotoxicity by payload for DAR 2, 6, and 18 ADCs→ Equivalent payload is internalized regardless of DAR
- Lower HER2 expressing model, JIMT-1 (73.7 k/cell)[1], has aberrant kill curves for DAR 2 ADCs→ Limits the amount of drug internalized when using low DAR ADCs

PK



DAR	mAb dose (mg/kg)
18	0.3
12	0.4
6	0.8
6	0.8
4	1.2
2	2.3

Figure 4. (a) Conjugated Drug PK **(b)** Total antibody PK for THIOMAB-Dolasynthen ADCs

- All ADCs were dosed in JIMT-1 tumor bearing CB.17 SCID mice at a matched payload dose of 0.025 mg/kg
- Conjugated Drug PK is indistinguishable between DARs using the Dolasynthen platform
- Total antibody PK shows similar clearance of ADCs regardless of DAR
- This is in contrast to previous reports of the relationship between higher drug loading, later HIC retention time, and faster PK for ADCs[2]

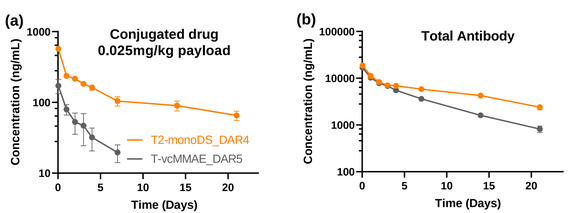


Figure 5. (a) Conjugated Drug PK **(b)** Total antibody PK for Trastuzumab-vcMMAE ADC vs. THIOMAB-Dolasynthen ADC

- All ADCs were dosed in JIMT-1 tumor bearing CB.17 SCID mice at a matched payload dose of 0.025 mg/kg
- Stochastically cysteine-conjugated vcMMAE ADC (Trastuzumab-vc-PAB-MMAE DAR 5) has much faster clearance and reduced exposure when compared to the THIOMAB-Dolasynthen ADC at DAR 4

Conclusions

- The Dolasynthen platform, in combination with THIOMAB technologies afforded a straightforward system for creating ADCs with a broad range of DARs.
- All ADCs had excellent physicochemical properties and similar binding profiles.
- In vivo PK demonstrated excellent exposure across all ADCs tested, regardless of DAR.
  - The Dolasynthen platform does not suffer from the inverse relationship of DAR and exposure reported in other ADC platforms.
- The combination of these technologies represents a powerful tool for interrogating what DAR is most suitable for a specific project.

Materials and Methods

Trastuzumab THIOMAB variants were prepared with 1 (LC K149C), 2 (LC V205C, HC A118C), or 3 (LC K149C, HC L174C, HC Y373C) engineered point mutations. The antibodies were prepared in a pH 5 sodium acetate buffer and frozen at -80C. To conjugate the THIOMABs, the appropriate antibody and payload were combined in pH6 50 mM triethyl ammonium acetate, 1 mM EDTA using 3-5 molar equivalents of payload per engineered cysteine, the incubated at 37°C for 2 hours. After conjugation, the ADCs were purified using CHT Type II[3]. ADCs were screened by HIC, SEC, and cell binding assays. Other conjugates were prepared by reducing trastuzumab with 3-5 molar equivalents of TCEP in 50 mM HEPES, 1 mM EDTA, pH 7 for 90 minutes at 37°C followed by conjugation to 5-7 molar equivalents of maleimide-vc-PAB-MMAE for 60 minutes at 37°C. Excess linker-payload was removed by iterative rounds of ultrafiltration and dilution using a 30 kDa MWCO centrifugal filter.

Resulting ADCs were tested for binding and in vitro cytotoxicity in JIMT1 and SKBR3. Binding of the ADCs to Her2 compared to the corresponding naked antibodies were assessed by flow cytometry analysis. SKBR3 or JIMT1 cells in triplicates were incubated with the ADCs or the corresponding naked antibodies on ice for 1 hour, followed by incubation with the fluorophore conjugated secondary antibodies against human IgG. The cells were washed one time with ice cold PBS and fixed with ice cold PBS containing 1% paraformaldehyde. Cells were run on a MACSQuant instrument to determine fluorescence intensity. Median Fluorescence for each treatment was graphed using GraphPad Prism software. For cytotoxicity, SKBR3 or JIMT1 cells were seeded on 96 well plates and treated with the indicated test articles. After 3 days incubation (37°C, 5% CO_2) , cell viability was determined by the Cell Titer-Glo assay

ADCs were dosed in JIMT-1 tumor bearing CB.17 SCID mice at a matched payload dose of 0.025 mg/kg. PK samples were collected by 10 µL plasma using serial bleeds with n=3 per arm at 1 hour and 1, 2, 3, 4, 7, 14, and 21 days post-dose. Total antibody concentrations were determined by ligand binding assay and conjugated drug was determined by LC MS.

References

1.Internal Flow Cytometry Assay; 2. Lyon RP. etal, *Nat. Biotech.* **33**, 733-736 (2015);
3. Bio-Rad Bulletin 6086 Ver E