Determination of drug-to-antibody ratio of antibody-drug conjugate in biological samples using microflow-liquid chromatography/high-resolution mass spectrometry

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Summary

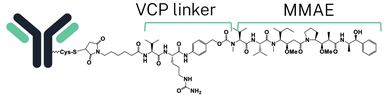
For pharmacokinetic studies, it is necessary to quantify both the antibody and the drug on the ADC circulating in the body, because the drug bound to the antibody plays an important role in the pharmacological activity of the ADC.

ADCs in biological samples are quantified with whole antibodies using ligand binding assays such as ELISA.

However, the humanized antibodies used to bind the payload in ADCs are often derived from the human IgG subfamily, making it difficult to isolate the target ADC in biological samples such as plasma or serum.

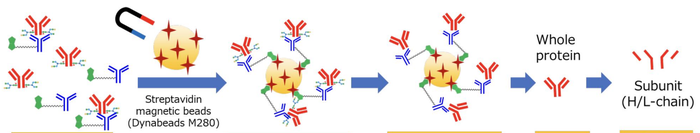
Here, we examined species-specific differences in DAR retention when commercially available Vorsetuzumab Mc-VC-PAB-MMAE ADC (Vorsetuzumab MMAE ADC) was incubated in plasma (Human, Cynomolgus monkey , and mouse).

Instruction and Method



Name	AntiCD70 (Vorsetuzumab)-MC-VA-PAB-MMEA ADC
ADC type	Cys-linked random ADC
Supplier	Creative Biolabs
DAR	4.12 (HIC)
Target molecule	CD27 ligand
Payload	MMAE
Linker	maleimide-valine-citrullin-PAB

To develop a suitable pretreatment and analytical workflow for ADCs, we employed Vorsetuzumab maleimidocaproyl valine-citrulline p-aminobenzyloxycarbonyl monomethyl auristatin E.



Vorsetuzumab MMAE ADC was incubated in mouse, monkey, human plasma, and PBS in triplicates for 0, 24, 48, and 72 hours at 37°C. To obtain biotinylated human CD27 (hCD) and Vorsetuzumab MMAE ADC conjugates, biotinylated hCD27 was reacted with aliquots of the Vorsetuzumab MMAE ADC samples (0, 24, 48, 72 hours). Dynabeads M-280 streptavidin was then suspended in the resulting mixtures and incubated for 15min at room temperature. On-bead deglycosylation was performed using rapid PNGaseF. The deglycosylated Vorsetuzumab MMAE ADCs on the magnetic beads were eluted with acetonitrile/H2O/formic acid.

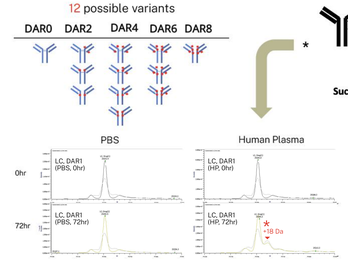
Biological samples
In vitro assay
In vivo animal PK
Human clinical samples

Immunocapture automation device
Duo Prime
(Kingfisher, Thermo)
12 sample/run, 1.5 hr

Microflow-LC/HRMS
(M5/TT6600+, SCIEX)
10uL/min, ~30 min/run

The obtained eluates were reduced, and the reduced ADCs were subjected to microflow LC-or conventional UFLC-HRMS system. For the DAR calculation, the HRMS data were processed with Byos software.

DAR calculation of various variants and metabolites



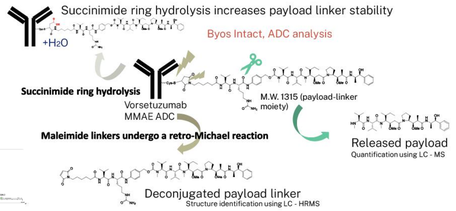
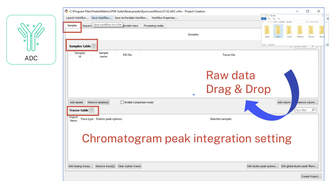
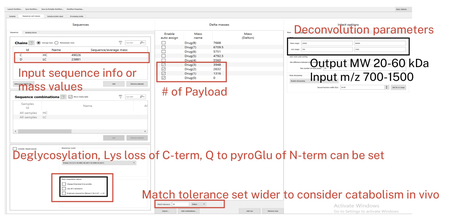
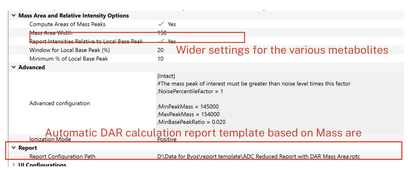


Fig.1 Vorsetuzumab MMAE ADC metabolites are very complex due to mixture of linker hydrolysates, released payloads, and deconjugated payload lnkers.

Fig.2 Byos ADC analysis parameter settings







DAR calculation in a report

Protein name	Drug Count	Peak #	1	2	3
HC	0		0.00	0.00	0.00
	1		0.70	0.69	0.71
	2		0.84	0.87	0.84
	3		1.18	1.18	1.16
LC	0		0.00	0.00	0.00
	1		1.08	1.08	1.08
		Level 1	3.80	3.82	3.79
		Totals			

Batch analysis, Auto DAR calculation, Customizable report template

Conflict of interest statement

The authors declare no competing financial interest.

Results and conclusion

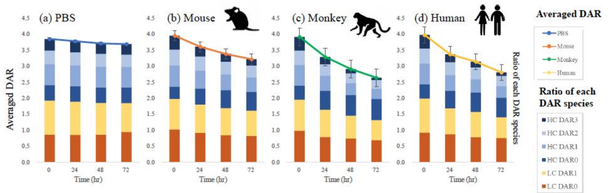


Fig.3 DAR retentions of Vorsetuzumab MMAE ADC in mouse, monkey, and human plasma and in PBS

DARs of Vorsetuzumab MMAE ADC in mouse, monkey, and human plasma decreased in a time-dependent manner. However, it remained stable during incubation in PBS, indicating that the stability of the ADC would depend on biological matrices. We found that the stability of DAR was species dependent, most stable in mouse, followed by human and monkey. DAR3 of the heavy chain was the most unstable, and the lower DAR species of each subunit increased in a time-dependent manner. The time-dependent release of MMAE was observed in mouse plasma, and the released MMAE quantity was consistent with the expected MMAE release back-calculated from the DAR change observed.

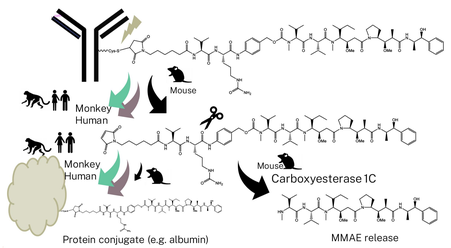


Fig. 4 Proposed metabolites generated from Vorsetuzumab MMAE ADC incubated in mouse, monkey, and human plasma and in PBS

Many deconjugated payload-linker moieties may bind to plasma proteins in monkey and human plasma instead of undergoing hydrolysis. Dong et al. reported that albumin adduct with deconjugated payload-linker of cysteine-linked ADC showed time-dependent increase in human and mouse plasma, indicating that albumin could be a significant acceptor of deconjugated maleimide containing payload-linker.

- Byos easily analyzed DAR in biological samples and improved the accuracy of ADC drug development
- In vivo payload release and DAR conversion of the ADC Vorsetuzumab MMAE ADC were species-dependent manner.

References

1. Inoue, K. et al. Bioanalysis 2022, 14(24), 1533-1545. Dong, L. et al. Anal. Chem. 2018, 90, 5989-5994.