OCT 20-23 2024

aaps PharmSci 360

Development, Comparison of Quantitative Analytical Methods to Evaluate Plasma Antibody-Drug-Conjugates

October 22, 2024

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Session Description and Objectives

Three LBA methods, hybrid LC-MS/MS method and LC-MS/MS method were developed to determine the total antibodies and conjugated antibody (Methods 1-3), conjugated payload (method 4) and free payload (method 5). The detection limit and assay range of each method were evaluated. The plasma samples treated with Telisotuzumab Vedotin or Disitamab Vedotin were used as examples to compare the pros, cons and application scopes of each method. We suggest to use the combination of methods 2 and 4 for better assessment of the stability and PK of the ADCs and their molecular species.

Upon completion, we hope you will be able to learn:

- Five ADC analytical strategies (LBA, hybrid LC-MS/MS and LC-MS/MS) for quantitation
- The advantages, disadvantages and application scopes of above methods
- The difference between conjugated antibody and conjugated payload

Biography and Contact Information

Ning is the technical director to lead discovery LM BA group in WuXi AppTec DMPK at New Jersey, US. Her team provides bioanalytical services for biomarkers and various modalities, including ADCs, oligonucleotides, antibodies, fusion proteins, to support drug discovery and development programs. Her team also has capabilities to provide clinical chemistry and hematology services. Prior to joining WuXi in 2015, Ning had worked in Merck over 19 years. She received her MS degrees from University of Rochester and Beijing Normal University and is the author or co-author of more than 26 peer-reviewed publications.

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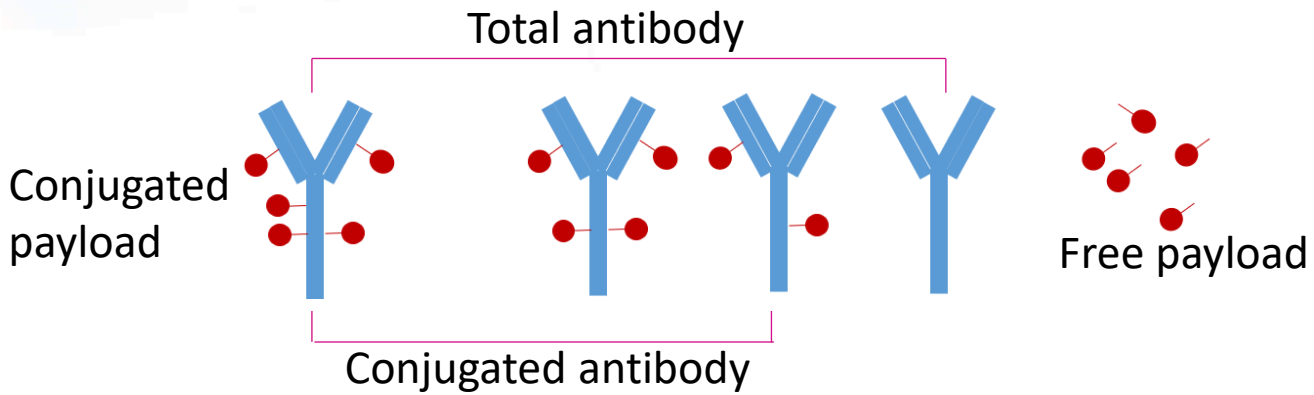
Introduction

- Antibody-drug-conjugates (ADCs) are complex molecules co-existing with multiple molecular species including total antibody, conjugated antibody/conjugate payload, free payload and metabolites.
  - DAR (Drug-Antibody-Ratio) has uniformity issue



- Multiple bioanalytical strategies are used to assess drug stability and pharmacokinetic properties, Each method has its pros and cons.
- Telisotuzumab Vedotin and Disitamab Vedotin are two FDA granted breakthrough ADC therapies for treating solid tumors. They both linked by cleavable linker to MMAE.
  - Monomethyl auristatin E (MMAE), a potent cytotoxic agent through inhibiting microtubule formation, is the most commonly used payload of ADC.
  - Telisotuzumab Vedotin (Teliso-V): anti-c-Met antibody-MMAE conjugate (ADC) that targets solid tumors overexpressing MET (DAR=4.16)
  - Disitamab Vedotin (DV): anti-Her2 antibody-MMAE conjugate (ADC) that targets solid tumors overexpressing Her2 (DAR=4)

Ligand Binding Assays for Total or Conjugated Antibody

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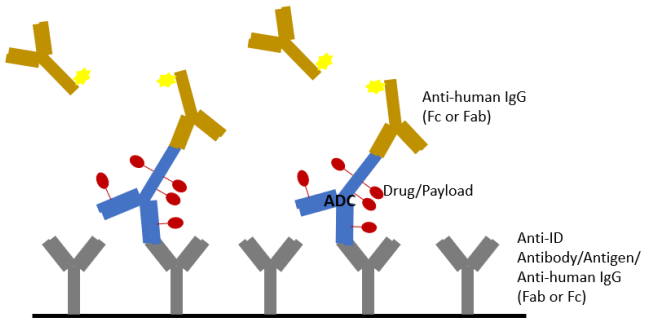
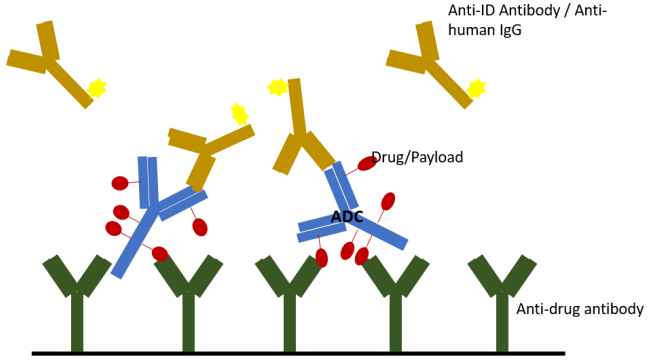
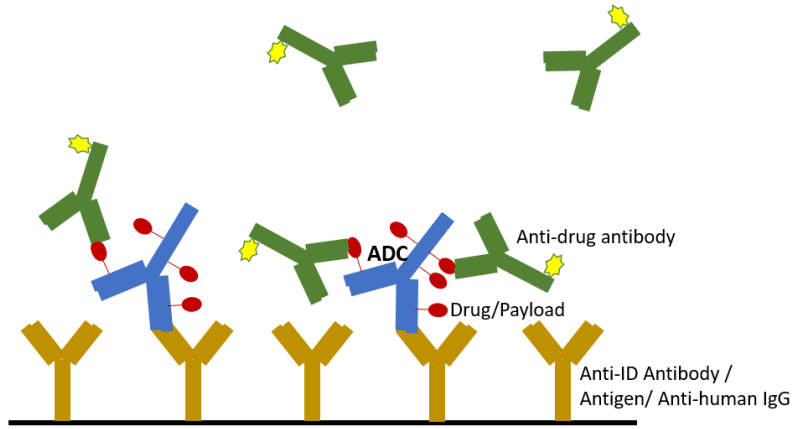
Method 1: Total Antibody



Method 2: Conjugated Antibody



Method 3: Conjugated Antibody/Payload



<u>Method Design and Development</u>

- Capture and detection antibody selection
- Combination study to determine optimal antibody concentrations
- Matrix effect

<u>Method Qualification</u>

- Prozone Range, Linearity & Sensitivity
- Accuracy & Precision (ULOQ, LLOQ, MRD)
- Parallelism (Linearity of Dilution)
- Stability (F/T, Benchtop, LT)
- Selectivity

Method Development and Qualification for LBA

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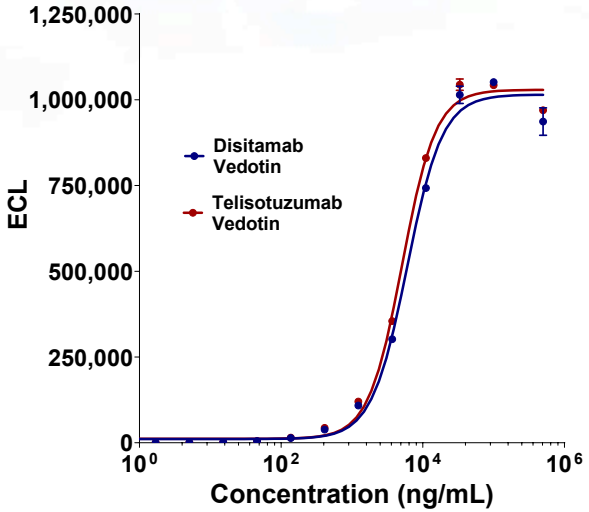
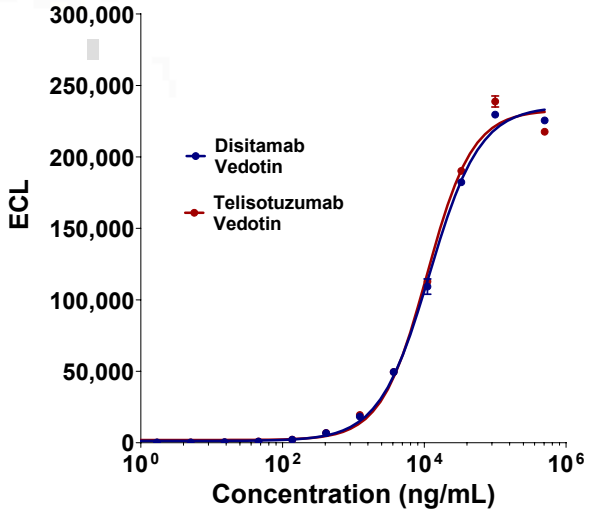
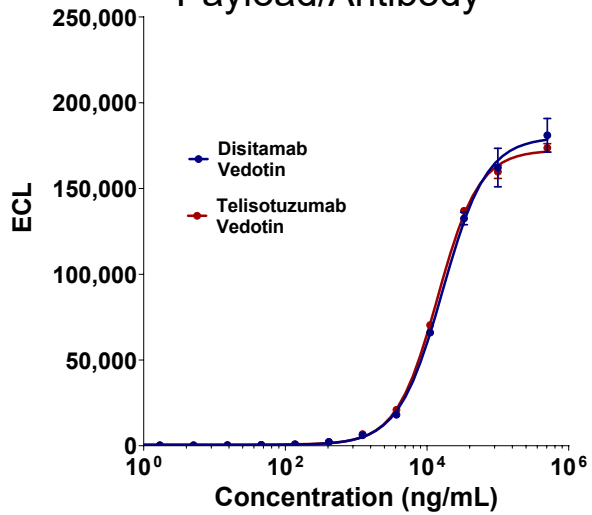
Method 1: Total Antibody



Method 2: Conjugated Antibody



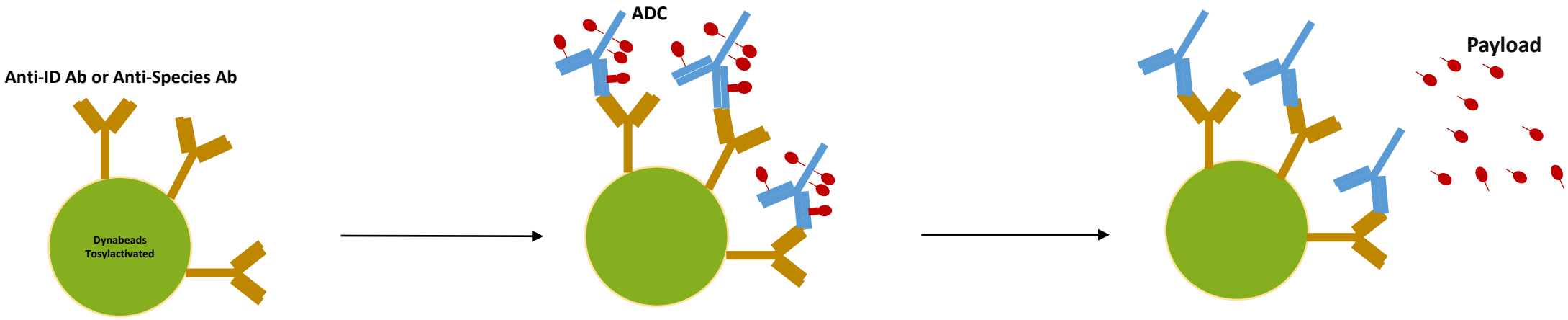
Method 3: Conjugated Payload/Antibody



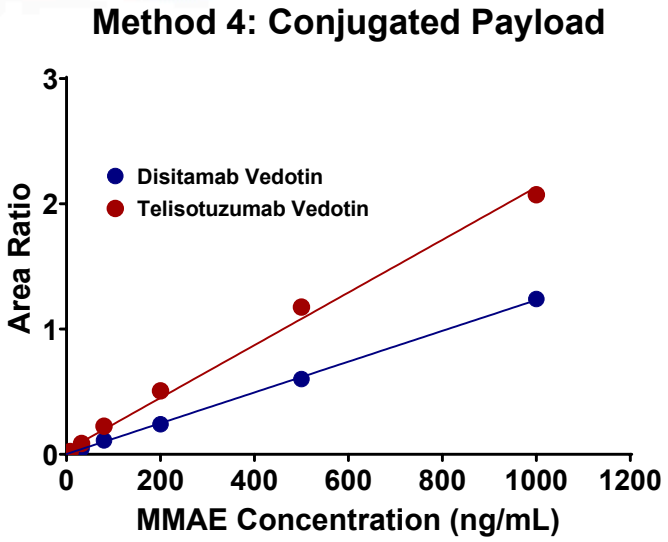
- All three assays passed bioanalytical acceptance criteria.
- Three LBA assays have similar assay ranges and sensitivities with LLOQ at 5.1-15 ng/mL and ULOQ at 33,333 to 100,000 ng/mL (MRD100).
  - MRD25 was also feasible for all three assays

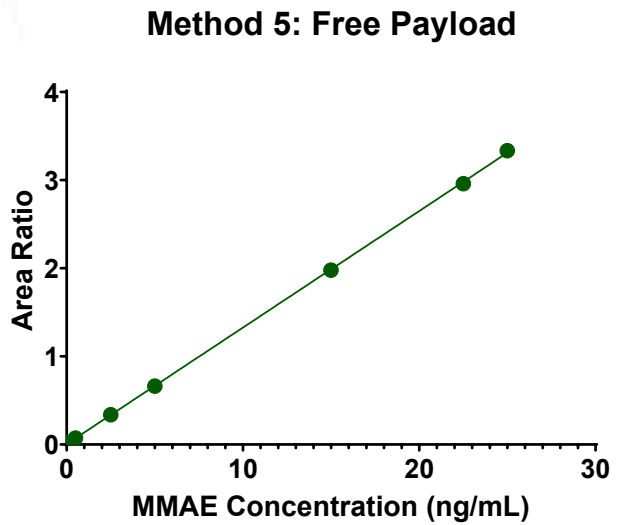
Hybrid LC-MS/MS for Conjugated Payload

- Coat Anti-ID Ab or Anti-Species Ab to the Magnetic Beads
- ADC Binds to Captured Antibody
- Wash to Remove Non-binding components
- Cleave and Elute the Payloads
- LC-MS/MS to Measure Conjugated Payload Concentration



Method Development and Qualification for LC-MS/MS





HPLC condition:

- Shimadzu LC-30
- CORTECS, C18+ 50 X 2.1 mm, 2.7 µm
- Mobile Phase A: 1% FA in water
- Mobile Phase B: 1% FA in methanol

MS Condition:

- 6500+ LC-MS/MS System
- ESI Positive Ion Detection
- Multiple Reaction Monitoring (MRM)

IS: D8-MMAE

DF: Conjugated: 72.5; Free: 4.5

Compound	Precursor ion (*m/z*)	Product ion (*m/z*)	CE	Retention Time (min)
MMAE	718.4	152.2	40	1.5
D8-MMAE	726.5	152	40	1.5

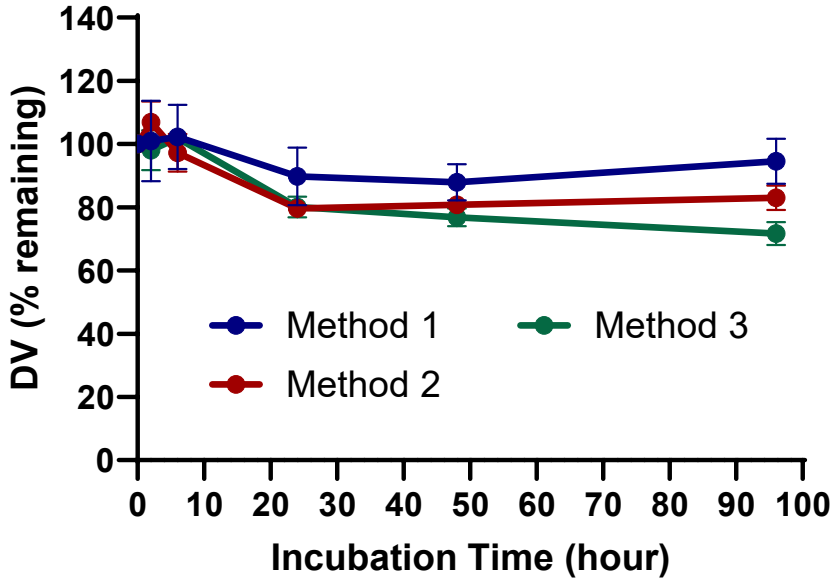
- Both assays passed bioanalytical acceptance criteria.

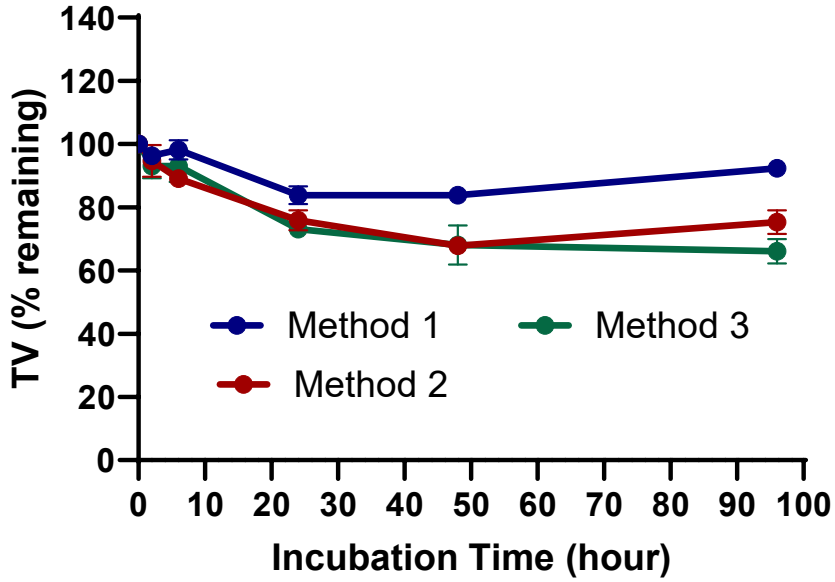
Summary of Analytical Methods for ADCs

	Method 1	Method 2	Method 3		Method 4	Method 5
	Ligand Binding Assay				Hybrid LC-MS/MS	LC-MS/MS
Measurement	**Total antibody**	**Conjugated antibody**	**Conjugated antibody/payload**	**Measurement**	**Conjugated payload**	**Free payload**
Capture	Antigen, anti-human IgG or anti-Id Ab	Bio-anti-MMAE Ab	Anti-human IgG Ab	**Immunocapture method**	Anti-human IgG or anti-Id Ab	NA
Detection	Anti-Id or anti-human IgG Ab	Sulfo-tagged anti-human Ab	Bio-anti-MMAE Ab Sulfo-tagged ST	**Detection Method**	LC-MS/MS	LC-MS/MS

Case Study: Sample Analysis for Plasma Stability Study - LBA

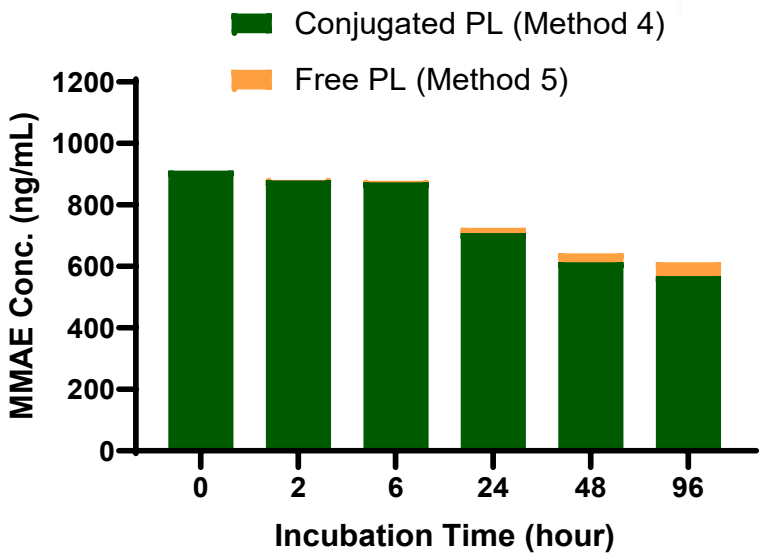
- Mouse plasma treated with 40 ug/mL Telisotuzumab Vedotin or Disitamab Vedotin was incubated @ 37°C for 96 hours
- Samples were collected at pre-dose, 2-, 6-, 24- and 96-hours post-dose and analyzed via 5 methods



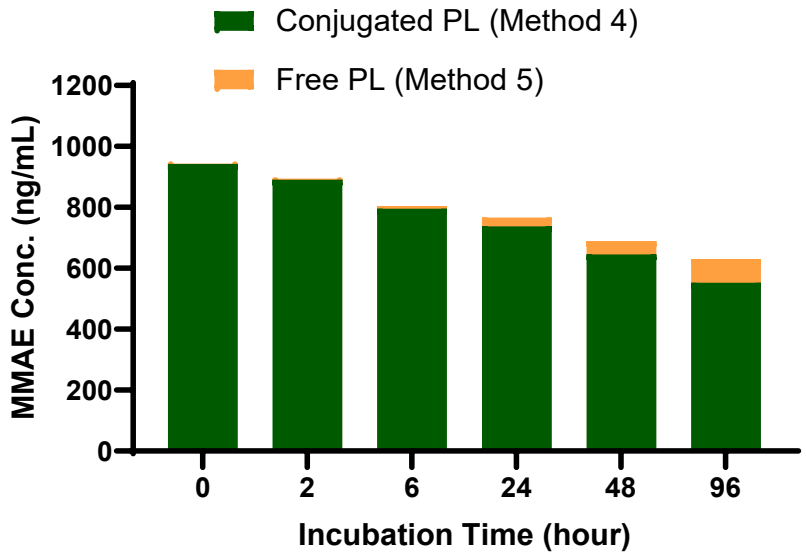


Case Study: Sample Analysis for Plasma Stability Study – LC-MS/MS

Disitamab Vedotin



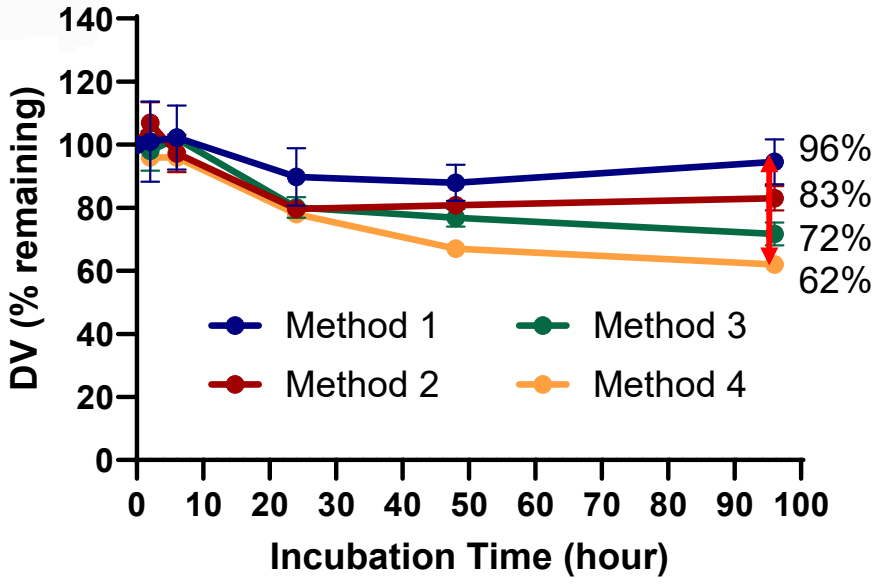
Telisotuzumab Vedotin

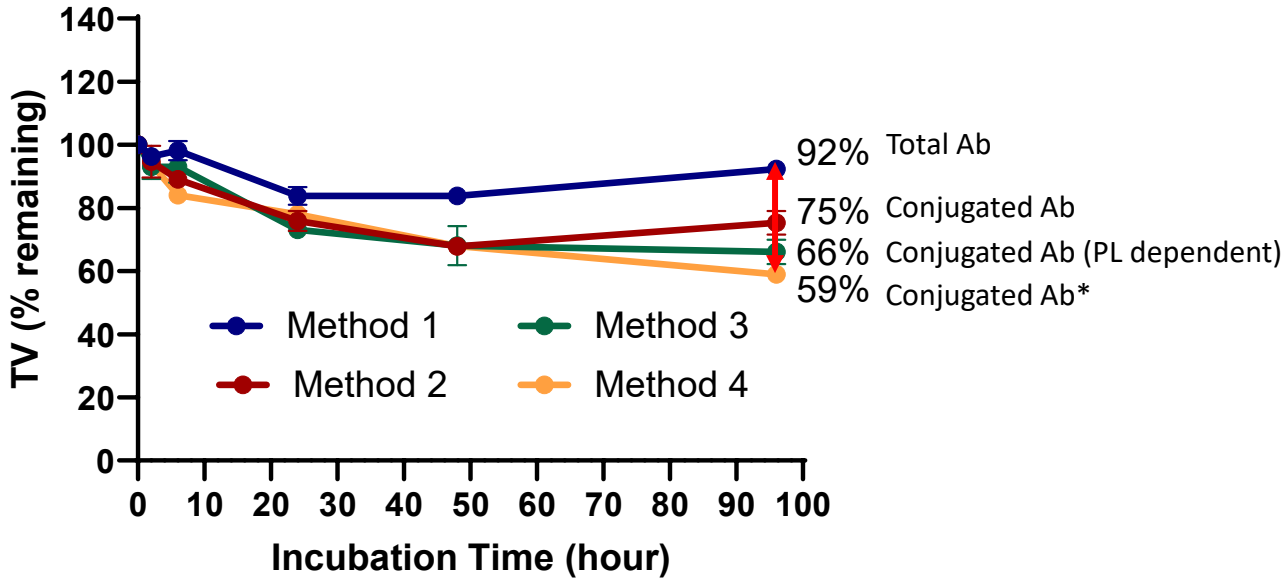


Decreased level of Conjugated MMAE and increased level of free MMAE observed

- *MMAE has 6 metabolites*
- *Discrepancy of MMAE levels across species*

What We May Learn from the Study





** This concentration is calculated by converting conjugated payload concentration to conjugated antibody concentration via initial DAR (provided by vender)*

Results and Pathforward

- Three LBA assays have similar assay ranges and sensitivities with LLOQ at 5.1-15 ng/mL and ULOQ at 33,333 to 100,000 ng/mL (MRD100).
- Five assays measure different species of the ADC.
- Although methods 2-4 are all considered as the methods for conjugated drug, they exhibit different aspects of the test article.
  - Method 2 measures conjugated antibody regardless the number of the payload on each.
  - Method 4 measures total number of the conjugated payload without the content of the antibody concentration.
  - Method 3 has potential of inaccurate measurement of conjugated antibody and payload since the potential aggregation and DAR uniformity issue could generate signal amplification unproportional to the molecule concentration.
  - Combination of methods 2 and 4 provides greater clarity and better assessment of the stability and PK of the ADCs and the migration of their molecular species.
- Pathforward
  - Collecting information of additional ADCs with MMAE as payload
  - Methods for conjugated payload of ADCs with uncleavable linkers

Contributors

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Questions