DAR evaluation in mouse plasma using anti-human IgG immunocapture and intact mass spectrometry at the discovery stage

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Summary

We developed a simple and broadly applicable method for *in vivo* DAR evaluation of ADCs by combining anti-human IgG immunocapture with intact MS analysis. This approach enabled reliable purification of ADCs without target-specific reagents and successfully captured time-dependent DAR profiles of trastuzumab emtansine (T-DM1) in mouse plasma. Deconvoluted mass spectra revealed DAR distributions and temporal changes, providing detailed *in vivo* characterization. The method facilitates efficient DAR analysis *in vivo* for efficacy and safety evaluation in the discovery stage and provides valuable insights into the drug release properties of ADC candidates. Our method can also support rapid optimization during the drug discovery process.

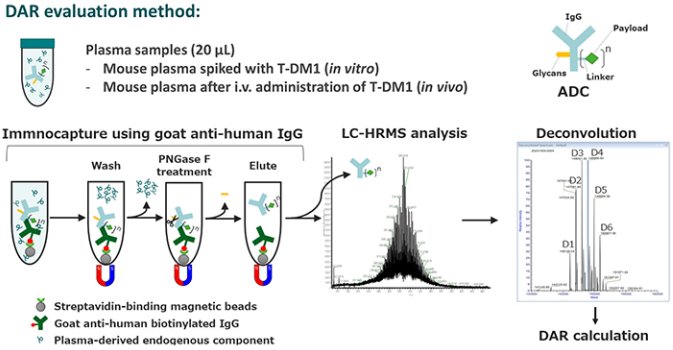
Materials and method

Model compound:
T-DM1, average DAR=3.5

In vitro samples:
Mouse (C57BL) blank plasma spiked with T-DM1; 250 and 25 µg/mL (n=2).

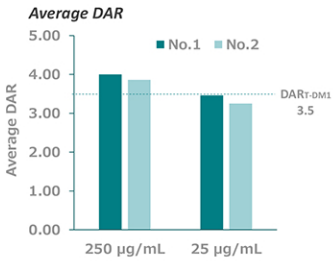
In vivo samples:
i.v.; 15 mg/kg to male C57BL mice (8 weeks old, n=3).
Plasma samples were collected 1, 4, and 7days after administration.
Plasma concentrations of total antibody (TAb) were measured by hybridization ELISA.

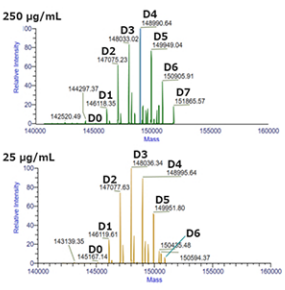
DAR evaluation method:



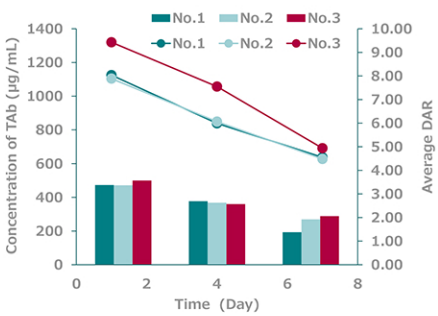
Results

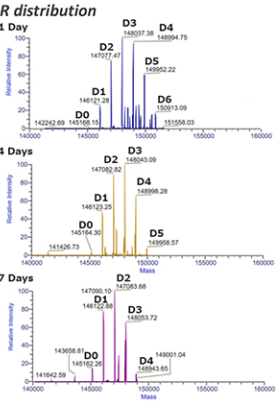
[1] Average DAR and DAR distribution in mouse blank plasma spiked with T-DM1 (*in vitro*)



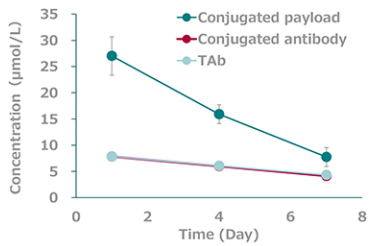


[2] Concentrations of TAb, average DAR and DAR distribution in mouse plasma after i.v. administration of T-DM1 (*in vivo*)





[3] Concentrations of conjugated payload, TAb, and conjugated antibody in mouse plasma after i.v. administration of T-DM1 (n=3, Mean±SD) (calculated)



The concentrations were calculated by following equations;

- Conjugated payload (µmol/L) =
  C_{TAb} (µg/mL) ÷ MW_{ADC} × average DAR × 1000
- Conjugated antibody (µmol/L) =
  C_{TAb} (µg/mL) ÷ MW_{ADC} × (1-$Ratio_{D0}$) × 1000
- TAb (µmol/L) = C_{TAb} (µg/mL) ÷ MW_{ADC} × 1000

C_{TAb} : Concentration of TAb
MW_{ADC} : Molecular weight of ADC
$Ratio_{D0}$: Intensity of DAR0 ÷ sum of intensity of DAR0 to DARn

- ✓ Using the DAR evaluation method combining anti-human IgG immunocapture with intact MS analysis, the average DAR and DAR distribution of T-DM1 in *in vitro* mouse plasma could be assessed.
- ✓ The method was also applicable to the time-course evaluation of average DAR and DAR distribution in mouse plasma *in vivo*.
- ✓ By evaluating average DAR together with TAb concentrations, the concentrations of conjugated antibody and conjugated payload can be calculated, allowing a better understanding of PK/PD and TK/TD of ADCs.

Conclusion

- ◆ We developed a simple and broadly applicable method for *in vivo* DAR evaluation of ADCs at the discovery stage.
- ◆ Our method can support rapid optimization of ADCs during the drug discovery process.

Acknowledgement

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COI disclosure information

We have no financial relationship to disclose for our presentation contents.

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