In vitro study of payload release from ADC using LC-HRMS and untargeted data processing: Identification of a new payload formation pathway of DS-8201a

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XenoFinder

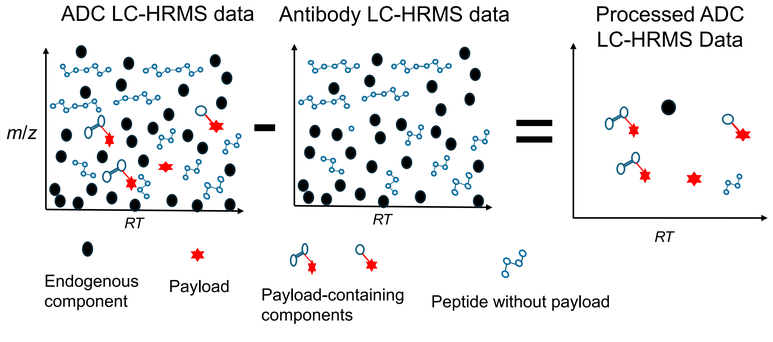
Introduction

- In vitro study of payload release from an ADC plays very important roles in support of lead optimization, bioanalytical development, ADME and DDI studies of ADC.
- Targeted LC-HRMS methods commonly used for MetID of small molecules drugs based on their predicted molecular weights, fragmentation patterns or mass defects are not suited for identification of payload-containing components.
- Low levels of payload-containing components in the presence of large amounts of peptides from ADC hydrolysis and formulation chemicals made their detection very challenging.
- The main objective of this study was to apply untargeted LC-HRMS data processing workflow (Xeno-Discovery) to investigate the payload (DXd) release from an ADC (DS-8201a) in human liver S9 fractions.

Experimental

- DS8201a (ENHERTU®) and its antibody (trastuzumab) at 1.0 mg/mL were incubated separately with human liver S9 at pH 5.0 and 37℃ for 0, 24 and 48 h.
- LC-MS and LC-MS/MS datasets of these incubation samples were acquired using DDA on a Thermo QE HF.
- Detection of payload-containing components was accomplished by using untargeted background subtraction processing software (developed in-house) to process acquired LC-MS datasets.
- Structure elucidation of payload-containing components was performed via interpreting their MS/MS data.

Fig 1. Principal of comprehensive detection of payload-containing components using LC-HRMS and background subtraction



Results

Fig 2. Unprocessed and processed LC-MS profiles (base peak ion chromatograms) of ADC and antibody incubations in human liver S9

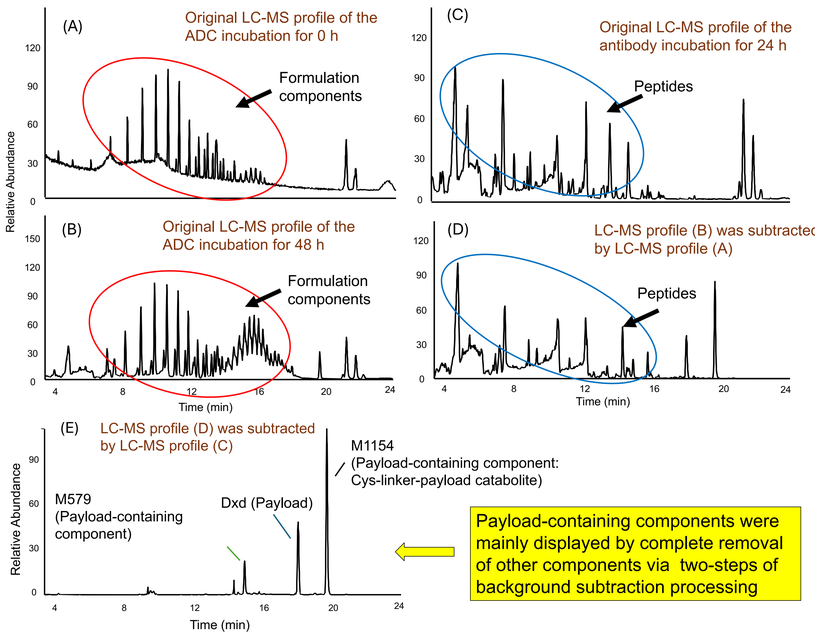


Fig 3. MS/MS spectra and proposed structures of payload-containing components from DS-8201a with human liver S9

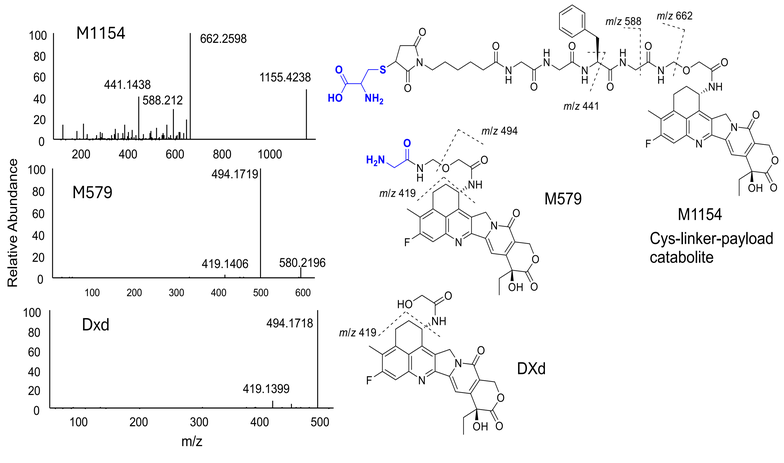


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Fig 4. Proposed formation pathways of DXd from DS-8201a in human based on the results of this in vitro study

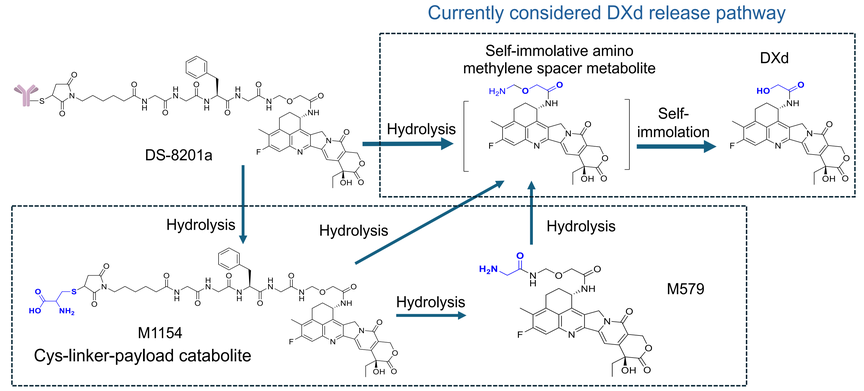
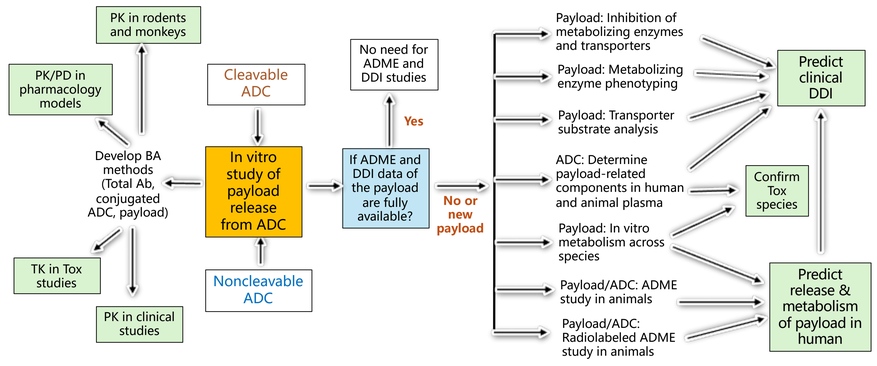


Fig 5. Roles of in vitro study of payload release in support of ADC DMPK investigation in drug discovery and development



Conclusions

- Results demonstrate that untargeted background subtraction processing is capable of sensitively and selectively profiling payload-containing components in vitro regardless of their molecular weights, mass defects, charge states and fragmentation patterns.
- DS-8201a underwent the direct cleavage on the tetrapeptide linker to generate an amino methylene spacer-containing metabolite that immediately underwent self-immolation to release DXd, confirming the previously proposed DXd formation pathway of DS-8201a.
- A new DXd release pathway from DS-8021a was identified in human S9 incubation, which was mediated by ADC protein hydrolysis to generate a Cys-linker-payload catabolite that led to DXd through continuous hydrolyses to form the self-immolative spacer-containing metabolite.