LCMS BASED STRATEGIES FOR IN VIVO DAR CHARACTERIZATION IN NHP SERUM

Poster Number: Day3-P25
Want a copy? Questions?

Jing Huang[1], Yifan Wang[1], Pei Zhang[1], Wenqin Xu[1], Tongtong Chen[1], Ming Liu[1], Ke Li[1], Xiaoying Jin[1], Lan Li[2]
[1] WuXi AppTec, 1318 WuZhong Avenue, Suzhou, Jiangsu, China.
[2] WuXi AppTec, 288 Fute Zhong Road, Waigaoqiao Free Trade Zone, Shanghai, China.

NOVELTY

In vivo characterization of drug-antibody ratio (DAR) provides direct insights into the dynamic process of payload release from antibody drug conjugates (ADCs) following drug administration. This information is crucial for evaluating the effectiveness and safety of ADCs. However, measuring in vivo DAR is exceptionally challenging due to the complexity of ADC structure and the stringent sensitivity requirements. This poster showcases two effective and precise LCMS-based approaches for evaluating DAR in NHP (non-human primates) plasma samples, as applied in a case study of an ADC with an average DAR of 4.

The first approach is a bottom-up method, involving quantitation of a proteolytic peptide of total antibody as well as the conjugated payload for determining the average DAR. The second approach is a middle-down method, where the ADC is reduced to individual light and heavy chains. The deglycosylated light and heavy chains are then quantified to determine the average DAR and obtain DAR distribution information for each chain. The poster also includes a comparison between these two approaches.

INTRODUCTION

Antibody drug conjugates (ADCs) are a class of conjugates formed by coupling monoclonal antibodies with highly toxic small molecular toxins through chemical linkers. There has been significant progress in ADC drug development in recent years. Due to the heterogeneity of ADCs and their unique behavior in metabolism and drug release in vivo, measuring the drug-antibody ratio (DAR) plays a crucial role in the discovery, development, and manufacture of ADCs [1]. While there are mature analytical methods for in vitro DAR evaluation, such as hydrophobic interaction chromatography (HIC), native mass spectrometer (MS), Size exclusion chromatography (SEC)-native MS, there are still specific challenges in studying DAR in vivo, including sensitivity issues, complexity of biological samples, and structure diversity of ADC [2,3,4]. Therefore, the availability of precise analytical methods for in vivo ADC DAR evaluation is of great importance in supporting early-phase screening and demonstrating in vivo behavior of ADCs.

Here, we report two effective and precise LCMS-based approaches which have been applied for evaluating DAR in NHP (non-human primates) plasma samples (Figure 1), in a case study of an ADC with an average DAR of 4. One method is a bottom-up approach, involving highly efficient immuno-capture and digestion of ADCs to quantify surrogate peptides of total antibody and conjugated payloads by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) for determining the average DAR. The second method is a middle-down approach, where the ADC is reduced to individual light and heavy chains. The deglycosylated light and heavy chains are then quantified by liquid chromatography coupled with Time-of-Flight mass spectrometry (LC-TOF-MS) to calculate the average DAR of ADC and obtain DAR distribution information for light chain and heavy chain, respectively.

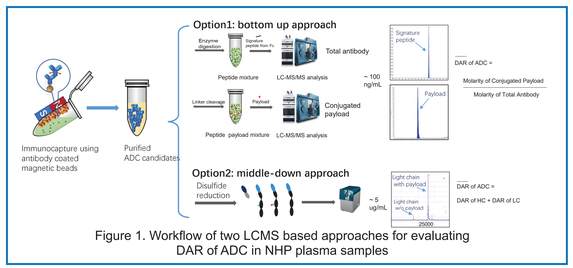

Figure 1. Workflow of two LCMS based approaches for evaluating DAR of ADC in NHP plasma samples

METHOD

Bottom up approach:
Total antibody, including both the naked antibody and conjugated antibody, present in NHP serum, was captured using Goat anti-Human IgG(H+L) Secondary Antibody immobilized on magnetic beads. Following this, the beads were washed to remove any contaminants, and then eluted to obtain the captured antibodies. For half of the sample elution, denaturation, reduction, alkylation, and trypsin digestion were performed to obtain signature peptides of the antibody. LC-MS/MS analysis was then utilized to monitor one signature peptide that accurately represents the total antibody. The remaining sample elution were subjected to digestion with Cathepsin B to release the payload, and LC-MS/MS was employed to monitor the payload that represents the conjugated payload. To determine the average DAR of the real samples, the concentration of the conjugated payload was divided by the total antibody concentration.

Middle down approach:
Total antibody, including both the naked antibody and conjugated antibody, present in NHP serum, was captured using Goat anti-Human IgG(H+L) Secondary Antibody immobilized on magnetic beads. Subsequently, rapid PNGaseF was employed to achieve deglycosylation. The target antibody was then eluted using a solution comprising 10%FA in 20%ACN. To facilitate further analysis, the elution was mixed with a TCEP solution and subjected to additional incubation. LC-TOF-MS analysis was subsequently utilized to accurately quantify the deglycosylated light chain and heavy chain, facilitating the determination of the average DAR of the NHP serum samples.

RESULTS

Bottom up approach:
Method performance:
A robust LC-MS/MS method was developed and comprehensively validated for the accurate and precise quantitative determination of total antibody and conjugated payload in NHP serum (Figure 2). The method exhibited a lower limit of quantification (LLOQ) of 0.5 µg/mL, demonstrating its high sensitivity in detecting and quantifying the analytes of interest. The validation results provided strong evidence for the accuracy, precision, specificity, and desired stability properties of the method. Furthermore, the method proved to be suitable for obtaining accurate concentration measurements of total antibody and conjugated payload in the intended matrix and species. These measurements were utilized for calculating the average DAR of the ADC at each time point.

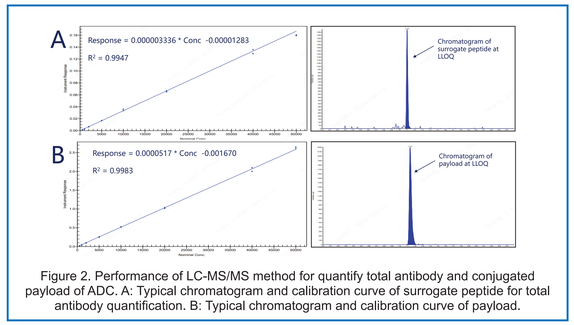

Figure 2. Performance of LC-MS/MS method for quantify total antibody and conjugated payload of ADC. A: Typical chromatogram and calibration curve of surrogate peptide for total antibody quantification. B: Typical chromatogram and calibration curve of payload.

Sample analysis
The average DAR value in real samples can be determined by dividing the concentration of the conjugated payload by the concentration of the total antibody. Upon administration of the ADC to NHP, the average DAR value in the real samples was observed to be approximately 4 at 0.5 hours. As the ADC undergoes metabolism in vivo, the average DAR value gradually decreases over time. After 168 hours of administration, the average DAR value in the samples was found to be around 1 (Figure 4).

Middle down approach:
Method performance:
A robust LC-TOF-MS method was developed and qualified to measure the average DAR of ADCs in NHP serum (Figure 3). To ensure accuracy, a set of quality control (QC) samples prepared with a reference standard of the ADC were included in the analysis. The results obtained from the QC samples demonstrated that the method exhibited good accuracy, further illustrating its reliability and suitability for DAR determination. Moreover, the method demonstrated a LLOQ of 0.5 µg/mL, indicating its high sensitivity in detecting and quantifying the ADC analytes of interest. This sensitivity enables the method to accurately measure DAR values even at low concentrations in NHP serum samples.

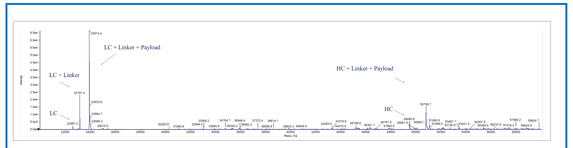

Figure 3. Typical mass spectrum of ADC subunit with LC-TOF-MS method

Sample Analysis:
The average DAR of NHP plasma samples was calculated using the following approach. **DAR of ADC = DAR of HC + DAR of LC**
DAR of HC = 1 * (peak area of HC+1 payload) /total peak area of HC + 2 * (peak area of HC+2 payloads) /total peak area of HC + … + x * (peak area of HC + x payloads) /total peak area of HC
Total peak area of HC = (peak area of HC +0 payload) + (peak area of HC +1 payload) + (peak area of HC +2 payloads) + … + (peak area of HC + x payloads)
DAR LC = 1 * (peak area of LC+1 payload) /total peak area of LC+ … + x * (peak area of LC + x payloads) /total peak area of LC

Total peak area of LC = (peak area of LC +0 payload) + … + (peak area of LC + x payloads)

Upon ADC injection, the average DAR of the sample at early time point is found to be close to 4, which matches the DAR of the ADC reference standard. As the ADC undergoes metabolism in vivo, the average DAR value gradually decreases, indicating the detachment of payloads from the antibody (Figure 4).

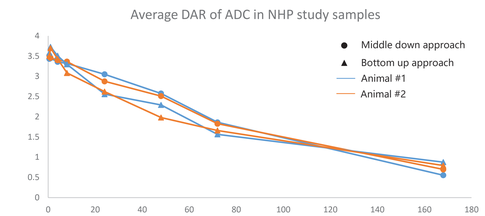

Figure 4. Comparison of average DAR in NHP study sample with two LCMS-based methods

CONCLUSION

Two LCMS-based methods were developed to accurately measure the in vivo Drug-to-Antibody Ratio (DAR) of ADCs with an average DAR of 4. These methods exhibited excellent precision and were effectively employed to track changes in DAR over time in a toxicokinetic study. Notably, the results obtained from both methods were consistent with each other, further demonstrating their reliability.

Furthermore, these methods provided valuable information regarding the distribution of light and heavy chains across different species. The DAR data obtained through these two LCMS methods provide crucial insights into the mechanisms and metabolism of ADCs in vivo. These findings contribute to a better understanding of the in vivo behavior and metabolic fate of ADCs, facilitating the optimization and development of future ADC-based therapeutics.

REFERENCES

[1] B. Gorovts, S.C.Alley, S.Bilic, et al., Bioanalysis (2013) 5(9), 997–1006.
[2] A. Wakankar, Y. Chen, Y.Gokarn, et al., mAbs (2011) 3 161-172.
[3] K. Xu, L. Liu, O.M. Saad, et al., Anal. Biochem. (2011) 412 56-66.
[4] S.Fekete, A.Beck, J.-L. Veuthey, et al., J. Pharmaceut. Biomed. Anal. (2015) 113 43-55.

药明康德
WuXi AppTec