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Immunogenicity-Mediated Abnormal Toxicokinetic Exposure and Toxicity Risk of Antibody-drug Conjugate

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Background

Antibody drug conjugate (ADC) consists of two distinct components: an antibody and a payload linked through a specialized linker. Unlike traditional antibody-based therapies, the complex structure of ADCs presents unique challenges in characterizing Toxicokinetics (TK). Moreover, therapeutic proteins are often accompanied by significant immunogenicity risks. Anti-drug antibodies (ADA) may not only affect exposure, but also induce undesired or unexpected immune responses, raising serious safety concerns. Therefore, immunogenicity complicates the determination of TK profiles, safety assessment, and efficacy evaluation for ADCs. TriApex has extensive expertise in ADC toxicology and bioanalysis. This study investigates abnormal TK data observed in nonclinical ADC studies conducted internally, with a focus on animals demonstrating atypical responses. These findings aim to provide valuable insights into toxicity risk assessment and guide further nonclinical studies.

Methods

According to the structure of ADC, both antibody and payload are required to be detected. ELISA is commonly employed for detecting ADC and total antibody (Fig 1). Simultaneously, LC-MS/MS is utilized to analyze the payload. In addition, ADA are detected through Affinity Capture Elution (ACE) assay (Fig 2). We have systematically collected and analyzed TK, immunogenicity, and related toxicological data from 3 GLP-compliant ADC studies conducted by TriApex. This comprehensive analysis enabled us to identify the underlying causes of abnormal exposure patterns observed in repeat-dose toxicity studies and to evaluate the associated safety risks. Our findings provide crucial insights for optimizing ADC evaluation strategies and enhancing preclinical safety assessments.

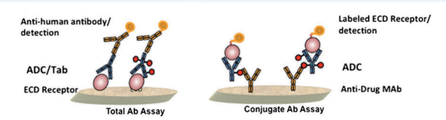

Fig 1. Schematic diagram for TK

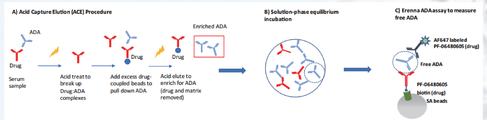

Fig 2. Schematic diagram for ADA

Results

1. TK Data Summay

In low-dose groups, abnormal TK exposures (e.g., elevated payload concentrations) were observed in ADC-treated animals (Tab 1).

Tab 1. Summary of the TK data in abnormal animals

Test Article	TA-A	TA-B	TA-C
Animal No.	1/10 (1♂)	4/10 (2♂2♀)	5/10 (2♂3♀)
Day of TK collection	D22 (QW, 4th dosing)	D71 (Q2W, 6th dosing)	D22 (QW, 4th dosing)

* Note: 5 animals/sex in the low-dose group

Toxicokinetic data revealed that all abnormal animals exhibited significantly elevated payload exposure levels (C_{max} and AUC_{0-t}) compared to normal animals within the same group. However, both total antibody and ADC exposure levels in abnormal animals were substantially lower than those observed in normal animals, even falling BLQ (Fig 3).

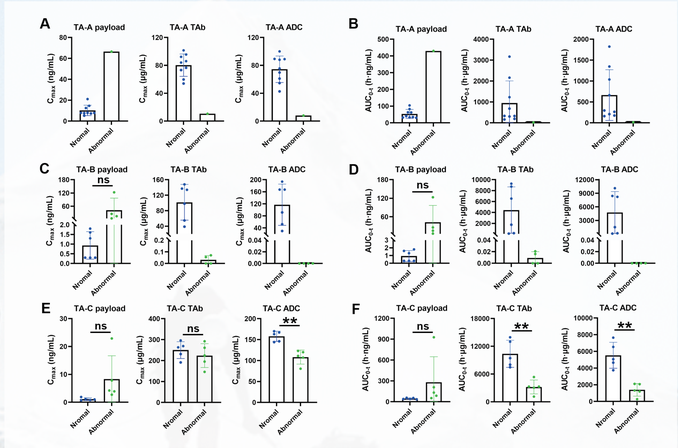

Fig 3. TK parameters of payload, total antibody, and ADC for TA-A, TA-B, and TA-C (ns, no significance ,**P < 0.01). For TAb and ADC concentration of TA-B, two and four abnormal animals were BLQ, respectively.

2. Immunogenicity Findings

All ADC compounds induced high ADA positivity rates: 80% for TA-A and TA-B, and 100% for TA-C. ADA titers increased progressively with repeated dosing (TA-A: D14; TA-B: D42; TA-C: D28) (Tab 2).

Tab 2. Summary of the toxicological data in abnormal animals

Test Article	TA-A	TA-B	TA-C
Positive rate of ADA	8/10	8/10	10/10
ADA titer	512	3-2187	8-17496
Day of ADA collection	D14 (after 2nd dosing)	D42 (after 3rd dosing)	D28 (after 4th dosing)

3. Toxicokinetic Exposure and Toxicity Risk

Toxicological data indicated a lower risk of toxicity in the low-dose group compared to the medium- and high-dose groups within the same study. However, the abovementioned abnormal animals may have other risks of toxicity compared with normal animals in the same group (Tab3).

Tab 3. Summary of the toxicity risks in abnormal animals

Kind of Risks	Contents
Related with TA	➢ Amplification of target-related pharmacological effects (e.g., TA-C: Decreased cellularity of lymphocytes in immune organs). ➢ Myelosuppression linked to abnormally high payload exposure (e.g., TA-A: Elevated white blood cell indicators and liver enzyme levels).
Unrelated with TA	➢ Unscheduled death caused by hypersensitivity reactions (HSR), which occur following repeated administration and are not directly attributed to the test article (e.g., TA-A). • Symptom of HSR: trembling with anxiety, reduced activity, lying still, pale skin, and decreased body temperature. • Prediction indicators of HSR: positive ADA, positive CIC, complement activation, significant reductions in complement receptor 1 (CR1) and decreased platelets counts in animals experiencing HSR. • Countermeasures upon the occurrence of HSR: stop administration immediately after symptoms appear, and inject dexamethasone, nicotinamide, promethazine hydrochloride, and adrenaline. ➢ In cases where death is attributed to HSR, a comprehensive analysis of TK data, ADA levels, CIC status, clinical observations, and histopathological findings is necessary to further assess potential toxicity risks.

Although the immunogenicity of antibody therapeutics in animals offers limited predictive value for humans, similarities can be observed in the clinical manifestations of HSR caused by immunogenicity. Therefore, animals with TK abnormalities and ADA positivity require close monitoring to assess their risk of HSR.

Conclusion

Due to the unique structure of ADCs, data of different components (ADC, total antibody, and payload) reflect their respective contents and meanings, collectively forming a complete picture of the TK of ADCs in vivo. Considering the influence of antibodies on the TK characteristics of ADCs and the fact that all abnormal animals in the study were ADA-positive, the abnormalities in TK data are likely associated with ADA-mediated structural anomalies or increased cellular clearance of ADCs. However, the specific underlying mechanisms warrant further investigation. In general, Immunogenicity-mediated abnormal toxicokinetic exposure is a significant factor affecting ADC safety in preclinical studies. Understanding the correlation between immunogenicity, TK exposure, and toxicity endpoints can enhance risk assessment for clinical development of ADCs.

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