AN OVERVIEW OF CDE TECHNICAL GUIDELINE FOR NONCLINCIAL STUDY OF ADCs: IMPORTANCE AND STRATEGY OF STUDYING RELEASE OF PAYLOAD-CONTAINING COMPONENTS FROM ADC AND THEIR FURTHER METABOLISM

25th North American ISSX meeting

Chongzhuang Tang[1], Zhiqiang Li[1], Yifei He[1,2], Linwei Wang[1, 2], Xingxing Diao[1,2], Mingshe Zhu[1,3]

1 XenoFinder Co., Ltd., China, 2 Shanghai Institute of Materia Medica, Chinese Academy of Sciences, China, 3 MassDefect Technologies, United States.

XenoFinder SIMM

Introduction

- Antibody-drug conjugates (ADCs) are a rapidly growing class of anti-cancer therapeutics. In the past two years, more than 90 new ADC drug candidates entered clinical trials, about 50% of which were from Asian countries. The research and development of ADC drugs generally follow relevant guidelines such as ICH S9 and ICH S6.
- To better guide and promote the research and development of ADC drugs, the Center for Drug Evaluation (CDE) in China issued "Technical Guideline for Non-clinical Studies of ADCs (ADC Guideline)" in July 2022. ADC Guideline elaborates on the pharmacology, pharmacokinetics, toxicology studies of ADCs, dose projection in the first in human study, and nonclinical study strategy in support of clinical development and marketing applications.

Objectives of this presentation

- Provided an overview of ADC Guideline from drug metabolism considerations.
- Presented an integrated analytical strategy of studying release of payload-containing components from an ADC and their metabolism by following ADC Guideline.

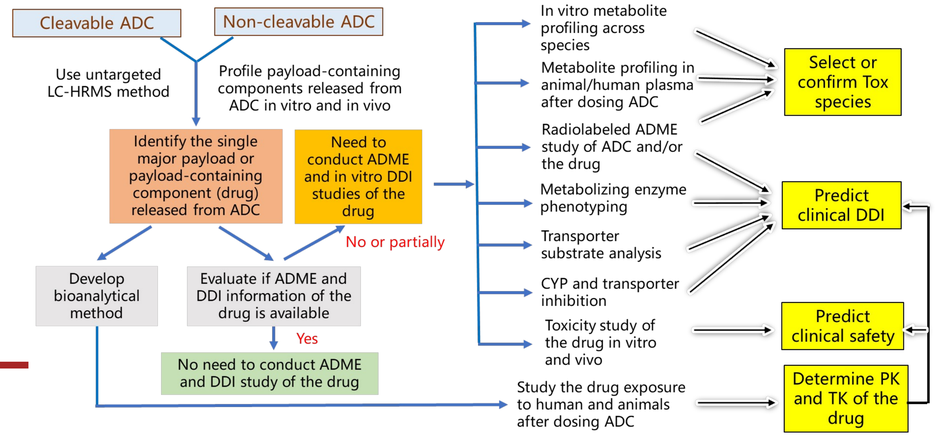
The purpose and scope of of ADC Guideline

- Purpose: Help and guide the conduct of ADC non-clinical studies to obtain scientifically sound and standardized experimental data to support conducting subsequent clinical trials and approval for marketing.
- Scope: Non-clinical study areas not covered by other relevant guidelines.
- Content: Non-clinical studies include pharmacology, safety pharmacology, pharmacokinetics (including ADME and DDI), and toxicology (including general toxicology, genotoxicity, reproductive toxicity, carcinogenicity, immunogenicity/immunotoxicity, photo toxicity, tissue cross-reactivity, toxicokinetics).

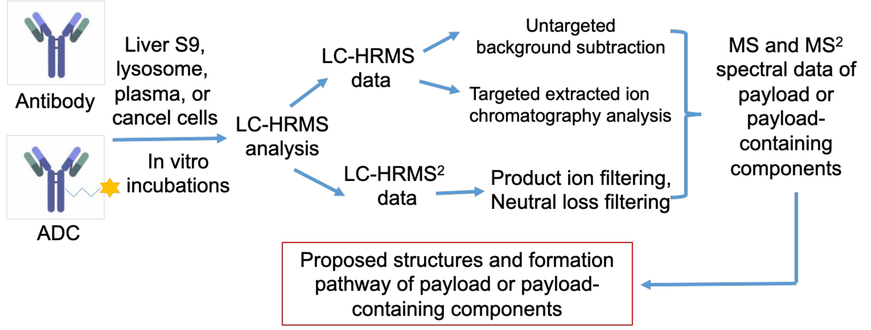
Drug metabolism studies recommended by ADC Guideline

In early discovery	•Obtain information on payload and payload-containing components that have pharmacological activity and are released from internalization and cleavage of ADC. • It is necessary to identify the released payload or payload-containing component for a marketed ADC. If results show that it is a new molecular, preclinical studies of the compound should be performed.
Preclinical	• If the released small molecule compound from ADC is a new molecule, studies of its plasma protein binding, tissue distribution, metabolism, excretion/mass balance, related metabolizing enzymes and transporters should be carried out. • In addition, in vivo metabolism of the new molecule should be considered, especially radiolabeled ADME study. • It is not necessary to study tissue distribution; however, for an ADC targeted a special organ such as brain, it is needed to study the tissue distribution using a radiolabeled ADC.
Clinical	• If the payload or payload-containing component is a new molecule and has a disproportionate metabolite in human, it is necessary to evaluate its non-clinical safety following ICH M3 or ICH S9 and based on its indications.

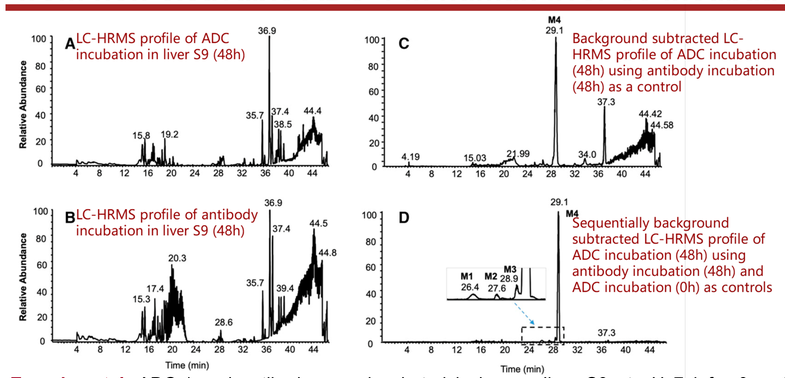
Analytical strategy of studying release of payload or payload-containing component (drug) from ADC



LC-HRMS workflow for detection and identifcation of paylaod or payload containing components from ADC in vitro



Untargeted detection of payload-containing catabolites from non-cleavable ADC-1 in human liver S9 incubation



Experimental: ADC-1 and antibody were incubated in human liver S9 at pH 7.4 for 0 and 48h followed by LC-HRMS analysis and data processing using background subtraction. The antibody (48h) and ADC (0h) incubation samples were used in background subtraction processing as control samples.

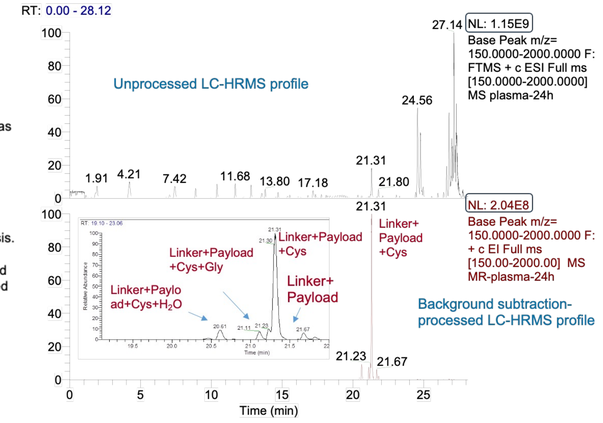
Results: Sequential background subtraction enabled removal of endogenous components and non-payload-containing peptides from ADC, resulting comprehensive and selective detection of payload-containing components.

DMD: DOI: https://doi.org/10.1124/dmd.122.001135

Characterization of payload-containing components generated in ADC incubation with monkey plasma

Case study:

- A test ADC had very short T1/2 in monkey. The study was designed to find out what happened.
- The ADC and related antibody were incubated in monkey plasma for 24h, followed by LC-HRMS analysis.
- Detection of payload-related componens were accomplihed by procesing LC-HRMS data usingbackground subtration



DMPK studies to support discovery, development and registration of ADC

	Discovery Lead optimization	Preclinical Development Support clinical candidate characterization and IND filing	Clinical Development Support development and NDA filing
ADME	- Identify drug released from ADC in S9 and lysosomes across species - Analyze drug released from ADC in target-expressed cells - Metabolite profiling of ADC in plasma	- Metabolism of drug in hepatocytes across species - Plasma protein binding of drug - Radiolabeled tissue distribution of ADC in rat and tumor-bearing mice - Radiolabeled ADME study of drug in animals	- Metabolite profiling in human plasma for MIST - Profiling and identification of drug metabolites in human, urine and/or feces after dosing ADC
DDI		- In vitro CYP inhibition and induction of drug - In vitro transporter inhibition of drug - Metabolizing enzyme phenotyping of drug - In vitro P-gp/BCRP substrate analysis of drug	- Transporter substrate analysis of drug - DDI prediction in human using PBPK - Clinical DDI study of ADC
PK	- PK of ADC in pharmacological model - PK screening of ADC and drug in rodent	- Full PK of ADC and drug in rodent - Full PK of ADC and drug in non-rodent - TK of ADC and drug	- Full PK of ADC and drug in human - Population PK of ADC - Hepatic and renal impairment study of ADC - Immunogenicity

Summary

- China CDE issued the draft “Technical Guideline for Non-clinical Studies of ADCs” in 2022, which is the first and only regulatory guideline focused on DMPK, toxicology and pharmacology of ADC in the world.
- It is critical to identify the major released payload or payload-containing component from an ADC in early discovery, which will determine what and how to conduct ADME, DDI, PK and bioanalytical studies of the ADC.
- An integrated strategy of studying release of the payload or payload-containing components released from an ADC and follow up studies are developed based on recommendations by ADC Guideline.
- Examples shown demonstrate that the LC-HRMS-based background subtraction processing is very effective in studying in vitro release of payload or payload-containing components from ADCs in vitro.

Contact inforamtion: Mingseh Zhu, Ph.D.
minshe.zhu@zoryaconsulting.com