A Sensitive MSD-ECL Platform for In Vivo Pharmacokinetic Profiling of ADCs

Carla N. Castro, Jonas Hummel, Tamara Sahner, Melanie Heisler, Gojko Bijelic, Philipp Metzger, Cynthia Obodozie, Holger Weber
Reaction Biology Europe GmbH, Engesserstrasse 4, 79108 Freiburg, Germany

Introduction

Antibody-drug conjugates (ADCs) are a rapidly evolving class of targeted cancer therapeutics that combine the specificity of monoclonal antibodies with the potency of small-molecule cytotoxic drugs. It is critical for ADCs to be stable in the systemic circulation in order to prevent premature release of the payload, which can lead to off-target toxicity and reduced therapeutic efficacy.

The pharmacokinetic (PK) behavior of ADCs is influenced by several factors, such as antibody structural heterogeneity, linker type, and payload physicochemical properties. Stability is a key quality attribute that directly affects dosing strategies, the therapeutic window, and clinical outcomes. Therefore, robust and sensitive analytical platforms are essential for monitoring ADC integrity and payload release over time.

While traditional ELISA-based methods are widely used, they often lack the sensitivity and dynamic range required to detect subtle changes in ADC composition during circulation.

In this study, we use the Meso Scale Discovery® (MSD) platform, which uses plate-based electrochemiluminescence (ECL) technology, to evaluate the in vivo PK and stability of ADCs. By applying this technology to preclinical in vivo models, we aim to generate high-resolution PK profiles and stability data that support the rational design and optimization of ADCs for clinical development

Method

Standard 1-Spot SECTOR plates were used to detect the antibody backbone by coating them with a specific capture antibody. Small Spot Streptavidin SECTOR plates were used to detect the intact ADC, capturing the molecule via anti-payload antibodies. In both assays, detection was performed using a sulfo-tagged anti-human IgG antibody.

After optimizing the setup, it demonstrated a broad dynamic range of 4–5 orders of magnitude and a lower limit of detection of less than 100 pg/mL for both the antibody and the intact ADC. Subsequent in vivo PK studies involved administering ADCs via a single intravenous injection to SCID beige mice. Serum samples were diluted 1:1000 in PBS containing 1% BSA, and 25 µL per well was applied to the MSD plates.

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Results

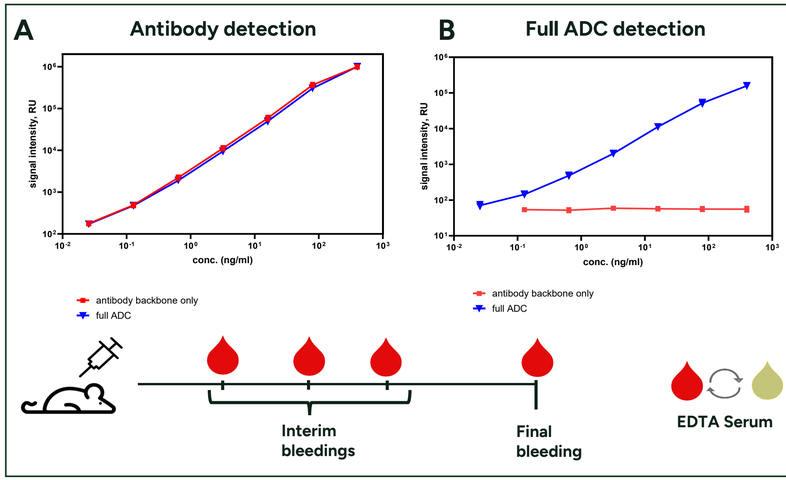
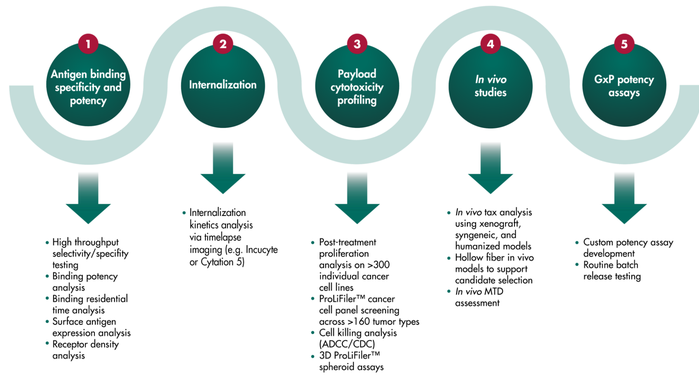


Fig. 1: Standard curves to detect antibody (A) or full ADC (B).

MSD technology was used to detect the antibody by coating an anti-idiotypic antibody to a standard MSD plate(A) or to detect payload by coating with a biotinylated anti-payload antibody to a Streptavidin plate (B). Anti-human IgG detection antibody was used for both determinations. We show here the sensitivity of several (4-5) logs.

We support every step of your ADC discovery program



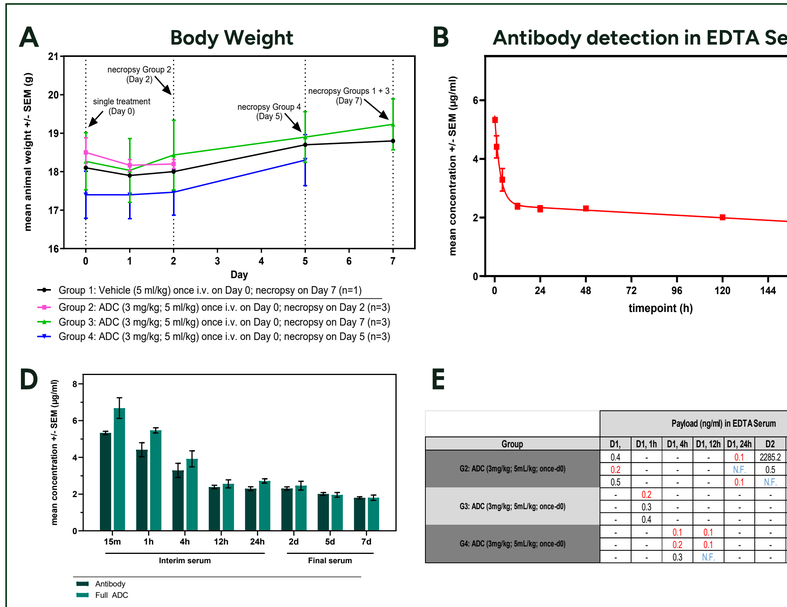


Fig. 2: Case study: in vivo PK study showing the dynamic of an ADC.
We show here a scheme of an in vivo PK study, with the different bleedings upon single treatment, and the requirements in terms of sample volume for the analysis. Animal weight showed not significant differences among groups (A). Antibody (B) and full ADC detection (C) in interim and final serum samples show similar curves. Comparison between both determinations (D) and the negligible amounts of free-payload as analyzed by LC-MS (E) show stability of ADC. Table depicting PK values that can be derived from ADC detection vs time (F)

E

	Payload (ng/ml) in EDTA Serum							
Group	D1,	D1, 1h	D1, 4h	D1, 12h	D1, 24h	D2	D5	D7
G2: ADC (3mg/kg; 5mL/kg; once-d0)	0.4	-	-	-	0.1	2285.2	-	-
	0.2	-	-	-	N.F.	0.5	-	-
	0.5	-	-	-	0.1	N.F.	-	-
G3: ADC (3mg/kg; 5mL/kg; once-d0)	-	0.2	-	-	-	-	-	N.F.
	-	0.3	-	-	-	-	-	N.F.
	-	0.4	-	-	-	-	-	N.F.
G4: ADC (3mg/kg; 5mL/kg; once-d0)	-	-	0.1	0.1	-	-	N.F.	-
	-	-	0.2	0.1	-	-	N.F.	-
	-	-	0.3	N.F.	-	-	N.F.	-

F

		Full ADC 3 mg/kg PO	Ab 3 mg/kg PO
Parameter	Unit	Estimate	
Adj_R^2		0.973	0.994
AUCextr	%	61.8	70.2
AUCinf	h*ug/mL	*1010*	*1240*
AUCinf/Dose	h*kg*ug/mL/mg	*338*	*412*
AUClast	h*ug/mL	387	368
AUClast/Dose	h*kg*ug/mL/mg	129	123
CL/F	ml/min/kg	0.0493	0.0405
Cmax	ug/mL	6.68	5.33
Cmax/Dose	kg*ug/mL/mg	2.23	1.78
MRT	h	348	480
No._points_λz		4	3
SE_Cmax	ug/mL	0.561	0.0896
Span		0.597	0.359
t1/2	h	241	335
tlast	h	168	168
tmax	h	0.25	0.25
Vz/F	l/kg	1.03	1.17
λz	1/h	0.00287	0.00207
λz_lower	h	24	48
λz_upper	h	168	168

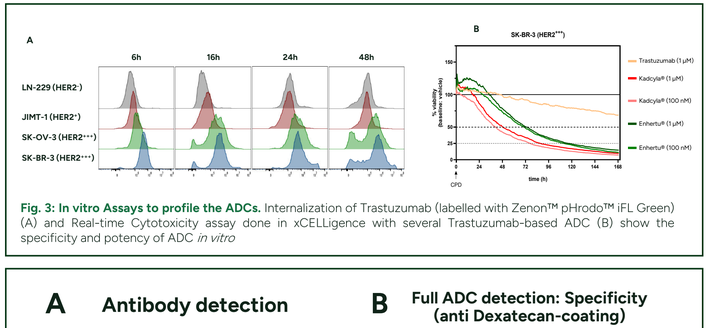


Fig. 3: In vitro Assays to profile the ADCs. Internalization of Trastuzumab (labelled with Zenon™ pHrodo™ iFL Green) (A) and Real-time Cytotoxicity assay done in xCELLigence with several Trastuzumab-based ADC (B) show the specificity and potency of ADC *in vitro*

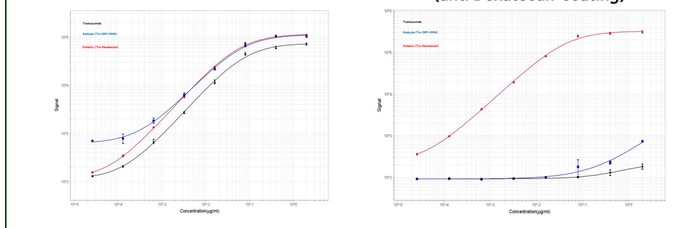


Fig. 4: Exemplary standard curves for the detection of Antibody and full ADC. These data sets show sensitivity of the detection of Trastuzumab (A) and the full ADC (Enhertu®) (B). The latter, also shows the specificity of the determination by using a second trastuzumab-related ADC (Kadcyla®) which has a different payload

Contact Information

Holger Weber, PhD
Head of In Vivo Pharmacology
Reaction Biology Europe
Engesserstr. 4
79108 Freiburg, Germany
+49-151-24000023
Holger.Weber@reactionbiology.com
www.reactionbiology.com

Conclusion

MSD technology allows a broad dynamic range with 4-5 order of magnitude and a lower limit of detection in the order of 100 pg/ml. Also the high dilution factor needed (around 1:1000 permits minimal blood volume collection per time point, thereby reducing the number of animals required. The assay demonstrated excellent reproducibility with minimal variability between replicates. To further increase the relevance of the results, future studies may use mFcRn$^{-/-}$ hFcRn transgenic mice or HSA/hFcRn/hFcγR triple transgenic models. These models more accurately reflect the serum half-life of human IgG and correlate well with data from cynomolgus monkeys and humans.

In summary, this MSD-based assay platform offers a highly sensitive and reproducible approach for in vivo PK analysis of ADCs that spares animals. There is potential to further refine this approach using humanized mouse models to improve clinical predictability.