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An Integrated DMPK and Bioanalytical Platform for Comprehensive Characterization of Antibody-Drug Conjugates

CROWN BIOSCIENCE

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Introduction

Antibody-drug conjugates (ADCs) represent a transformative class of targeted oncology therapeutics, with their efficacy and safety profiles critically dependent on their pharmacokinetic (PK) behavior and biotransformation *in vivo*. However, their complex structure introduces significant challenges in characterization, necessitating a holistic DMPK strategy to understand their *in vitro* stability, *in vivo* PK and biodistribution, and biotransformation[1]. The success of this strategy hinges on the precise quantification of key analytes, including total antibody (Tab), conjugated antibody (ADC), free payload, and the drug-to-antibody ratio (DAR), in diverse biological matrices[2]. Here, we present a robust ADC DMPK evaluation platform, which combines integrated *in vitro* drug stability, payload release assessment, and *in vivo* PK/PD studies with a versatile bioanalytical platform, demonstrating its utility through a direct comparison of three clinically relevant ADCs with distinct designs: Trastuzumab Deruxtecan (T-DXd), Trastuzumab Emtansine (T-DM1) and Enfortumab Vedotin (EV). This comprehensive approach is designed to delineate *in vivo* PK behavior and biotransformation pathways, providing critical pharmacology and safety data to de-risk the translational path of ADC candidates from discovery through to preclinical stage[3].

Methods

For three approved ADCs with distinct linkers and payloads, including T-DXd, EV, and T-DM1, as two types of drug release triggers (T-DXd and EV are cleavable linker, T-DM1 is non-cleavable linker), the *in vitro* stability was evaluated in plasma from multiple species (human, cyno monkey, SD rat, CD-1 mouse) at 37°C over 7-21 days. Samples were collected at 0, 1, 2, 4, 7, 14, 21 days to evaluate payload release evaluated in the corresponding plasma samples. For *in vivo* characterization, PK studies were conducted in three mouse models, including WT mice, hFcRn, hAlb/hFcRn model, following a single intravenous dose (10 mg/kg, N=3) of the three ADCs. To support these studies, a comprehensive bioanalytical toolbox was employed: Ligand Binding Assays (LBA) for Tab and ADC quantification, LC-MS/MS for sensitive free payload measurement (LLOQ: 10-50 pg/mL), and hybrid immunocapture LC-HRMS/LC-MS/MS for determining average DAR value and monitoring biotransformation.

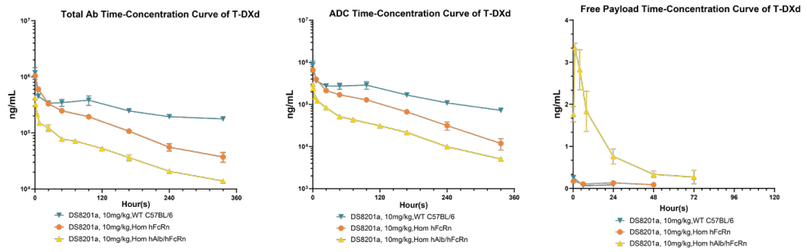
Results

T-DXd, with a cleavable peptide linker and high baseline DAR (~7.6), demonstrated high stability *in vitro*, showing less than a 40% DAR decrease (to ~4.4) over 7 days (**Figure 3A**) and minimal free DXd release (<2% across species) (**Table 1**). Its *in vivo* DAR gradually declined to ~5.8 by Day 5 (**Figure 3B**), with highly overlapped PK curves for Tab and conjugated ADC (**Figure 1A**). EV, incorporating a protease-cleavable linker and a baseline DAR of ~3.5, exhibited faster degradation *in vitro*, with DAR dropping to ~1.6 (**Figure 3C**) and significant MMAE release (37.4% at Day 21 in mouse plasma) (**Table 1**). The high release in mice is likely due to plasma esterase cleavage of the Val-Cit linker, whereas human/monkey/rat lack this enzyme activity, thus showing minimal release. *In vivo*, ADC levels displayed a more rapid decline than Tab, accompanied by a marked DAR decrease to ~0.5 by Day 5 (**Figure 3D**). T-DM1, featuring a non-cleavable thioether linker and DAR ~3.5, displayed very high plasma stability and negligible free DM1 release (<1% at Day 21 across species plasma) (**Table 1**). Its *in vivo* PK profiles for free payload also confirmed minimal payload release in circulation (**Figure 1B**). The PK profile comparison across different mouse models demonstrated significantly shorter half-lives in hFcRn and hAlb/hFcRn models compared to WT mouse (Refer to poster 3387). For ADCs lacking anti-payload reagents, we can develop a hybrid approach combining DAR-insensitive Tab assay with LC-HRMS based DAR profiling. This flexible bioanalytical strategy enabled comprehensive characterization across diverse ADC formats.

Results Continued

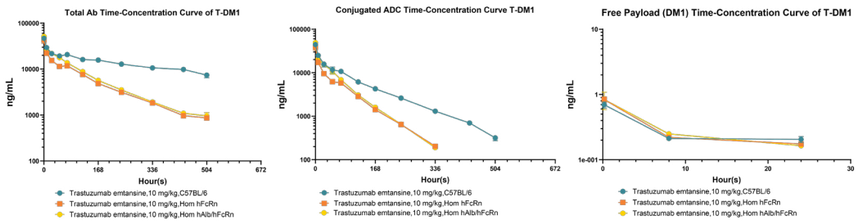
Figure 1. *In Vivo* PK Curve and Parameters Across 3 ADCs (A) T-DXd (B) T-DM1 (C) EV

A



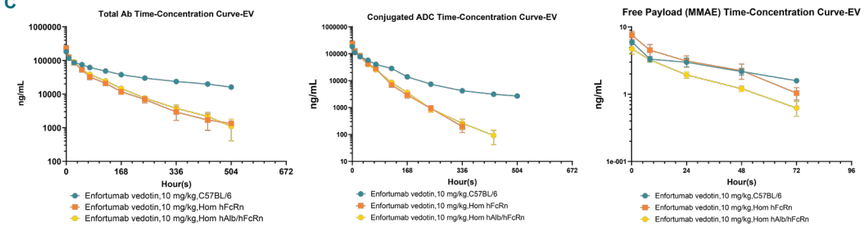
PK Parameter	Total antibody			ADC			Free payload		
	C57BL/6	Hom hFcRn	Hom hAlb/hFcRn	C57BL/6	Hom hFcRn	Hom hAlb/hFcRn	C57BL/6	Hom hFcRn	Hom hAlb/hFcRn
$T_{1/2}$ (hr)	310.4	96.71	192.6	134	66.65	110.1	22.16	68.22	18.97
AUC_{last} (hr*ng/mL)	93374676	51119607	19541990	63792094	32274687	11267588	2.281	5.522	62.02
AUC_{INF} (hr*ng/mL)	172723863	56521264	20688403	77821927	33521651	11366098	5.073	14.09	69.44
CL(mL/hr/kg)	0.059	0.178	0.073	0.129	0.3	0.132	NA	NA	NA

B



PK Parameter	Total antibody			ADC			Free payload(DM-1)		
	WT C57BL/6	Hom hFcRn	hAlb/hFcRn	WT C57BL/6	Hom hFcRn	hAlb/hFcRn	WT C57BL/6	Hom hFcRn	hAlb/hFcRn
$T_{1/2}$ (hr)	331	112	110	92.0	59.5	55.5	15.8	11.9	11.2
$AUCI_{last}$ (hr*ng/mL)	7078798	2617619	3194165	2381166	1158826	1506450	6.65	6.91	7.20
AUC_{INF} (hr*ng/mL)	10682396	2757919	3347015	2424038	1176551	1534224	11.3	9.91	9.85
CL(mL/hr/kg)	0.94	3.64	3.01	4.16	8.56	6.57	NA	NA	NA

C



PK Parameter	Total antibody			ADC			Free payload		
	WT C57BL/6	Hom hFcRn	hAlb/hFcRn	WT C57BL/6	Hom hFcRn	hAlb/hFcRn	WT C57BL/6	Hom hFcRn	hAlb/hFcRn
$T_{1/2}$ (hr)	346	84.7	93.7	214	45.5	50.8	50.3	30.5	30.0
AUC_{last} (hr*ng/mL)	19369867	8865000	9814710	9869875	6522509	6262833	193	209	130
AUC_{INF} (hr*ng/mL)	27378323	9209317	10013026	10761009	6536559	6267808	308	255	159
CL(mL/hr/kg)	154	96.6	101	158	64.8	72.1	NA	NA	NA

Results Continued

Figure 2. *In Vitro* Plasma Stability Across 3 ADCs (A) T-DXd (B) T-DM1 (C) EV

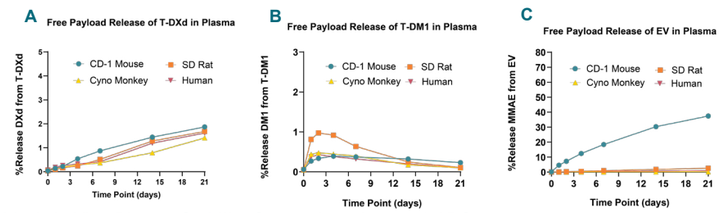
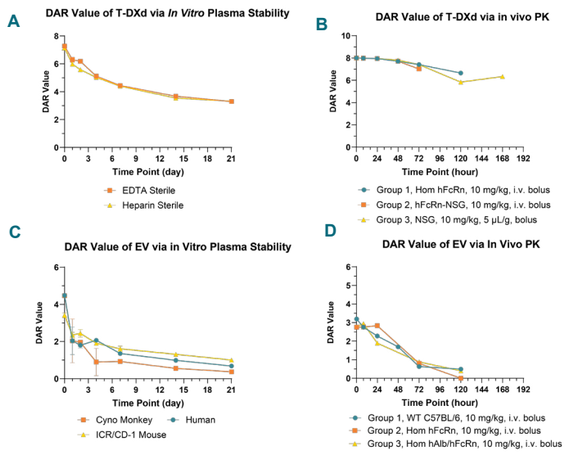


Table 1. *In Vitro* Plasma Stability Comparison Across 3 ADCs

ADC/free payload	Incubation Time (Days)	Free Payload Release Concentration (ng/mL)				Free Payload Release Rate (%)			
		Mouse	Rat	Monkey	Human	Mouse	Rat	Monkey	Human
T-DXd/DXd	21	47.2	42.2	35.6	40.6	1.87	1.68	1.41	1.61
EV/MMAE	21	605	41.1	3.50	15.6	37.4	2.54	0.22	0.97
T-DM1/DM1	21	3.93	1.81	1.69	1.64	0.23	0.11	0.99	0.10

Figure 3. *In Vitro* and *In Vivo* DAR Value Comparison

(A) *In Vitro* Plasma DAR Value Evaluation of T-DXd Across Different Anticoagulants **(B)** *In Vivo* DAR Value Evaluation of T-DXd Across Different Species **(C)** *In Vitro* Plasma DAR Value Evaluation of EV Across Different Species **(D)** *In Vivo* DAR Value Evaluation of EV Across Different Species



Conclusion

Our integrated DMPK platform provides comprehensive and robust ADC characterization capability, providing critical insights into ADC stability, biotransformation, and exposure profiles. This supports direct correlation between ADC design, *in vitro* properties, and *in vivo* PK behavior, thereby de-risking candidate selection and accelerating the development of novel ADC therapeutics.

References

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