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Commentary

The therapeutic window of antibody drug conjugates: A dogma in need of revision

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Despite a prevailing dogma wherein antibody drug conjugates (ADCs) increase the maximum tolerated dose of potent cytotoxin payloads while lowering the minimum effective dose, mounting clinical evidence argues that the tolerated doses of ADCs are not significantly different from those of related small molecules. Nonetheless, when dosed at or near the maximum tolerated dose, certain ADCs demonstrate improved efficacy. Understanding the challenges and opportunities for this class of biotherapeutics will help improve the design of next-generation ADCs.

Antibody drug conjugate (ADC) therapies have developed rapidly in recent decades, with 12 products approved worldwide and more than 140 ADCs in clinical trials. Analysts estimate that the ADC market will reach >15 billion USD by 2030, driving a significant increase in interest in this field. The theory behind ADCs is simple yet elegant: by combining the specificity of a monoclonal antibody with the cytotoxicity of a potent small molecule drug, ADCs can precisely deliver toxins to tumors while sparing normal tissues, increasing the therapeutic window of a drug. In support of this concept, preclinical data demonstrate that conjugating a drug to an antibody decreases the minimum effective dose (MED) and increases the maximum tolerated dose (MTD) of the drug. Clinical data, in contrast, do not align with this thinking. We argue instead that the MTDs of ADCs and the corresponding small molecules in humans are roughly the same after normalization for cytotoxin content. Therefore, the therapeutic window expansion concept (Figure 1A) widely propagated in almost all reviews and articles on ADCs is inaccurate. Notably, when dosed at or near their MTDs, certain ADCs showed significantly better therapeutic responses in patients than the unconjugated small molecules.

Can ADCs really achieve higher MTDs than small molecules?

The MTD of a drug is the highest tolerable dose without serious side effects (dose-limiting toxicities). Historically, defining MTD was the primary objective of phase 1 oncology trials. More recently, especially for new targeted drugs (including ADCs), emphasis has been placed on determining the recommended phase 2 dose (RP2D), which better captures chronic toxicities emergent after multiple treatment cycles (e.g., edema, effusion, pneumonitis, ocular toxicities) and certain grade 2 side effects (e.g., diarrhea, mucositis, cytopenia, neuropathy, severe fatigue) that may become intolerable over time (Tolcher, 2022).

ADCs currently approved or in clinical development employ payloads that are close structural relatives of highly cytotoxic small molecules discovered in preceding decades, many of which were previously evaluated as standalone chemotherapies. Comparing the clinical doses of small molecules and ADCs requires conversion to a common unit that accounts for variation in molecular weight and drug-to-antibody ratio (DAR). Therefore, all MTD/RP2D values were converted to mg/kg of cytotoxin content, considering the familiarity of this unit across a wide audience.

A summary of the MTDs/RP2Ds for 10 approved ADCs (normalized by the conjugated payload) and clinically evaluated cytotoxins closely related to the conjugated payload is reported in Figure 1B. A comprehensive analysis of all ADCs in active clinical development with established MTD/RP2D values is provided in Figure 1C and Table 1. After converting ADC and small molecule doses to a common unit, it is evident that ADCs fail to meaningfully expand the therapeutic window by increasing the MTD of their conjugated drugs. An appreciation that ADCs do not significantly enhance the MTDs of their payloads may provide insight into several existing observations in this field:

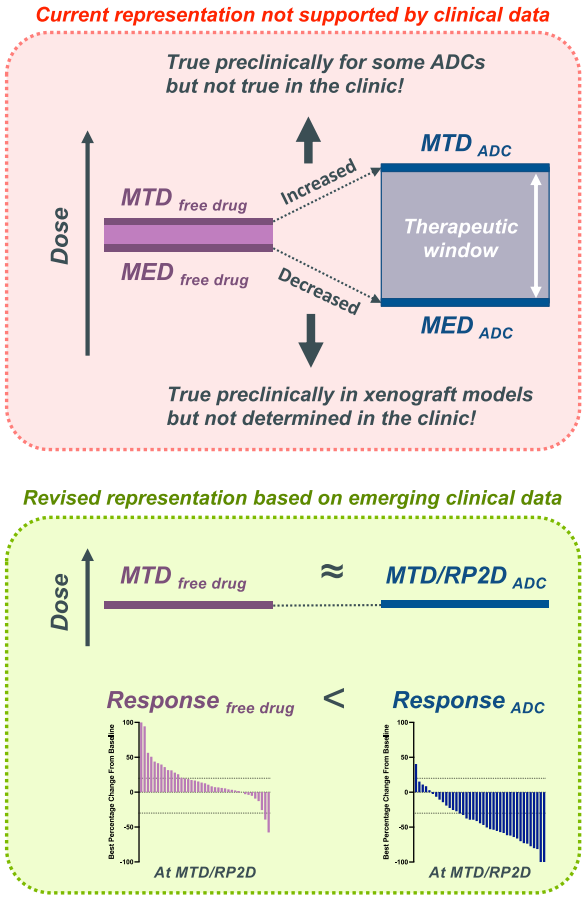
(1) ADCs that feature a common drug linker often encounter similar MTDs because of payload-associated platform toxicities, independent of the target antigen. This highlights that most off-target adverse events are antibody independent (Lucas et al., 2019).

(2) On-target off-tumor toxicities resulting from antibody-target engagement in normal tissues are common. In this case, the MTD may be lower than for other ADCs employing the same drug linker. For example, Dato-DXd (a DAR4 DXd-ADC targeting TROP2) does not achieve an equivalent cytotoxin dose to other DXd-containing ADCs (e.g., T-DXd, HER3-DXd, B7H3-DXd, CDH6-DXd), likely due to drug-related adverse events (e.g., rash, stomatitis, and mucositis) consistent with TROP2 expression in normal tissues. Similar toxicities on skin and oral mucosa are also observed in patients treated with other TROP2 ADCs.

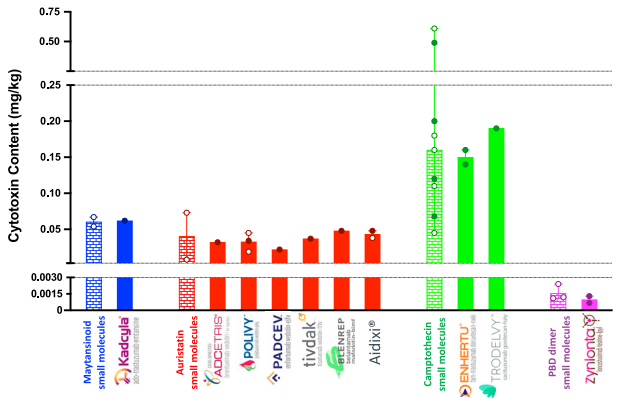
(3) Engineered ADCs designed to limit binding to healthy tissues (e.g., CX-2009, CX-2029, BA3011, BA3021) haven't improved normalized MTDs over canonical ADCs with the same drug linkers.

(4) Reducing the DAR of an ADC in several cases leads to a roughly proportional increase in the tolerated ADC dose but yields little improvement after normalization for the amount of cytotoxin. For example, ALT-P7 (DAR2 MMAE ADCs) has a similar normalized MTD compared to the other DAR4 MMAE ADCs. Similarly,

A **Current and revised views of ADC therapeutic window**



B **Normalized human MTDs/RP2Ds of approved ADCs *vs* small molecules**



C **Normalized human MTDs/RP2Ds of investigational ADCs *vs* small molecules**

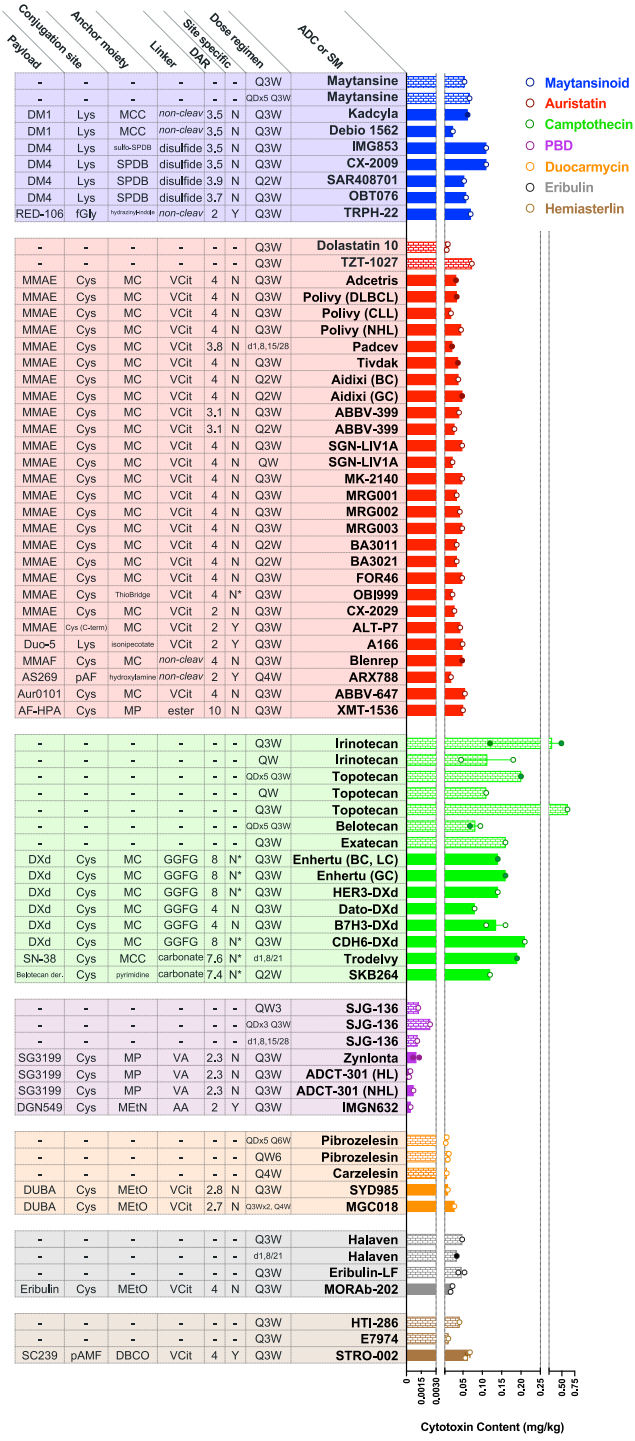


Figure 1. Analysis of clinical ADC and small molecule MTDs prompts revision of the therapeutic window dogma

(A) Commonly reproduced schematic representation of an ADC therapeutic window (red box). The concept of a wider therapeutic window for ADCs seems to be based on preclinical evidence rather than clinical outcomes. Revised representation of ADC therapeutic advantage (green box). ADCs don't significantly improve the tolerated doses, but they have shown better efficacy than related small molecule free drugs when dosed at MTDs/RP2Ds. MTD, maximum tolerated dose; MED, minimum effective dose; RP2D, recommended phase 2 dose.

(B) Graphical representation of human MTD/RP2D values of 10 approved ADCs normalized by cytotoxic content compared to related small molecules.

(legend continued on next page)

B7H3-DXd (DAR4) doesn't differentiate from other DAR8 DXd ADCs.

While somewhat surprising, given that ADCs are broadly perceived to broaden the therapeutic window of their payloads, it is clear from clinical data that there is no increase in MTD. A disconnect between clinical and preclinical tolerability of T-DM1 and its related small molecule maytansine was previously noted (Poon et al., 2013). This seminal observation from a decade ago is now corroborated by the clinical data of almost 40 active ADCs spanning over seven classes of payloads (Figures 1B and 1C; Table 1).

Why do payload and ADC MTDs not vary substantially?

The reasons why ADCs fail to increase the MTD of their payloads remain elusive. A possible explanation resides in the critical role played by the antibody in protecting the conjugated drug from elimination and metabolism. In a simplified view, the "full dose" of the payload is attached to the antibody until ADC target and non-target-mediated cellular uptake and catabolism release the free payload or payload metabolites, which are in turn eliminated via canonical small molecule routes.

ADC drug linkers can also be cleaved extracellularly in plasma or in the tumor microenvironment, providing a direct source of the payload in circulation without prior ADC endocytosis. While newer ADCs employ more stable linkers than earlier generations, some effective ADCs (including approved ADCs) are built using linkers with relatively short half-lives in plasma. For example, sacituzumab govitecan contains a labile carbonate linker that releases its payload (SN-38) in plasma with a half-life of ~20 h.

ADCs prepared via thiol-maleimide chemistry, which comprise the majority of ADCs currently approved or in development, can undergo deconjugation of the entire drug linker from the antibody. This process, known as retro-Michael reaction, is especially relevant for ADCs with linkers containing the maleimidocaproyl (MC) moiety. Examples include T-DXd and the other deruxtecan ADCs, vedotin ADCs, and many other ADCs (see "anchor moiety" in Figure 1C) in development. For these ADCs, up to 50%–75% of the drug linker is deconjugated in plasma over ~7 days, and the deconjugated drug linker rapidly reacts with thiol-containing plasma molecules, mainly albumin, forming new conjugates. Albumin has a long half-life in humans because, like IgG, it is actively recycled via FcRn binding. Consequently, the payload is not immediately released into circulation but is maintained in the bloodstream until the albumin conjugate is catabolized. Drug-linker transfer from ADC to albumin could contribute to certain toxicities via albumin-conjugate non-specific disposition and by increasing payload half-life. Conversely, it may contribute to antitumor efficacy via albumin-conjugate direct tumor uptake and gradual catabolism to release the payload in circulation. Proper assessment of the role of albumin in toxicity and/or efficacy tied to drug-linker deconjugation remains elusive because the biology of albumin in preclinical models is very distinct from the human setting (Nilsen et al., 2018).

Are ADCs an improvement over their payloads?

The 12 ADCs approved to date demonstrate the importance of this class of therapeutics, and the recent data from DESTINY-Breast03 and DESTINY-Breast04 highlight the potential for T-DXd to transform the breast cancer treatment paradigm (Cortés et al., 2022; Modi et al., 2022). On the other hand, the more than 100 discontinued ADC programs illustrate the challenge of identifying the right combination of antibody, target, drug-linker, DAR, and indication to create a successful therapeutic.

In oncology trials, the MTD is traditionally considered the most relevant surrogate for the pharmacologically active dose, and the MED is not determined at all. Efficacy (and not MED) is the endpoint of phase 2 and 3 trials, where patients are treated with the dose regimen determined in phase 1. In the 1970s, FDA cancer drug approvals were based on objective response rate (ORR), but from the early 1980s approvals have been granted on more direct evidence of clinical benefit, including improvement in progression-free survival (PFS) and overall survival (OS). These parameters are only determined for a single regimen (or a very limited number of different regimens), usually at the MTD or RP2D. Albeit survival benefits are not always predicted by ORR, assessment of tumor response is widely accepted by oncologists in guiding cancer treatment, as ORR is directly attributable to the drug effect. ORR has also been the most common surrogate endpoint to support FDA accelerated approvals. Considering ORR is often used as primary endpoint in early ADC trials and ORR data are available for small molecule drugs developed in the past three to four decades, we compared ORRs of small molecules and ADCs when used to treat similar patient populations (Figure 2).

To date, there are no direct head-to-head randomized trials comparing ADCs to their payloads. The closest example is T-DXd (bearing the topoisomerase I inhibitor payload DXd) in the DESTINY-Gastric01 trial, where most patients (~90%) in the physician's choice group were treated with irinotecan, a topoisomerase I inhibitor in the same drug class as DXd; an ORR of 42% was reported in T-DXd-treated patients versus 12.5% in the physician's choice group. For several other tumor types, cross-trial data are available to qualitatively compare ADCs to related small molecules. Inferences should be taken with caution when comparing different trials; however, sufficient clinical data are available to conclude that multiple ADCs have shown better ORRs than related small molecule therapies (Figure 2).

What mechanisms do contribute to the success of ADCs?

The pharmacokinetics (PK) of small molecules are fundamentally altered by

(C) Graphical representation of human MTD/RP2D values of ADCs in active clinical development normalized by cytotoxic content, for which values are available, compared to related small molecules.
Symbols and abbreviations: ADC, solid bars; small molecule, shaded bars; approved drug MTD/RP2D, solid dots; experimental drug MTD/RP2D, empty dots. fGly, formylglycine; pAF, *p*-acetylphenylalanine; *Non-cleav*, non-cleavable; MC, maleimidocaproyl; MCC, maleimidomethyl cyclohexane-1-carboxylate; MP, maleimidoproyl; MEtO, maleimidoethyloxy; MEtN, maleimidoethylamino; SPDB, 4-mercaptobutanoyl; DBCO, dibenzocyclooctyne; N*, full saturation of the interchain cysteines, not engineered for site-specific conjugation.

Table 1. Normalized cytotoxin dose analysis of ADCs and related small molecules

Drug	Type	Payload	Antibody target	MW (Da)	DAR	ADC clinical MTD/ RP2D (mg/kg)[a]	Normalized cytotoxin dose (mg/kg)[a,b]	Dose schedule[c]
Maytansinoid								
Maytansine	SM	–	–	692	–	–	0.054	Q3W
							0.067[d]	QDx5 every 3 weeks
Kadcyla (trastuzumab emtansine, T-DM1)	ADC	DM1	HER2	~149,000	3.5	3.6	0.062	Q3W
Debio 1562 (naratuximab emtansine, IMGN529)	ADC	DM1	CD37	~150,000[g]	3.5	1.4	0.024[i]	Q3W
IMGN853 (mirvetuximab soravtansine)	ADC	DM4	FRα	~150,000[g]	3.5	6.0	0.11	Q3W, AIBW
CX-2009 (praluzatamab ravtansine)	ADC	DM4	CD166	~150,000[g]	3.5	6.0	0.11	Q3W
SAR408701 (tusamitamab ravtansine)	ADC	DM4	CEACAM5	~150,000[g]	3.8	2.7	0.053	Q2W
OBT076	ADC	DM4	CD205	~150,000[g]	3.7	3	0.058	Q3W
TRPH-222	ADC	RED-106	CD22	~150,000[g]	1.8	7.5	0.070	Q3W
Auristatin								
Dolastatin 10	SM	–	–	785	–	–	0.008–0.011	Q3W
TZT-1027	SM	–	–	702	–	–	0.073	Q3W
Adcetris (brentuximab vedotin)	ADC	MMAE	CD30	~153,000	~4	1.8	0.032	Q3W
Polivy (polatuzumab vedotin)	ADC	MMAE	CD79b	~153,000	~4	1.8 (DLBCL)	0.034	Q3W
						1.0 (CLL)	0.019	Q3W
						2.4 (NHL)	0.045	Q3W
Padcev (enfortumab vedotin)	ADC	MMAE	Nectin-4	~152,000	3.8	1.2	0.022	days 1, 8, and 15 of a 28-day cycle
Tivdak (tisotumab vedotin)	ADC	MMAE	Tissue factor	~153,000	~4	2.0	0.037	Q3W
Aidixi (disitamab vedotin, RC48)	ADC	MMAE	HER2	~150,000[g]	~4	2.0 (BC)	0.038	Q2W
						2.5 (GC)	0.048	Q2W
ABBV-399 (telisotuzumab vedotin)	ADC	MMAE	cMet	~150,000[g]	3.1	1.9	0.028	Q2W
						2.7	0.040	Q3W
SGN-LIV1A (ladiratuzumab vedotin)	ADC	MMAE	LIV1	~150,000[g]	~4	1.25	0.023	Q1W
						2.5	0.048	Q3W
MK-2140 (zilovertamab vedotin, VLS-101)	ADC	MMAE	ROR1	~150,000[g]	~4	2.5	0.048	Q3W
MRG001	ADC	MMAE	CD20	~150,000[g]	~4	1.8	0.034	Q3W
MRG002	ADC	MMAE	HER2	~150,000[g]	~4	2.6	0.050	Q3W
MRG003	ADC	MMAE	EGFR	~150,000[g]	~4	2.5	0.048	Q3W
BA3011 (mecbotamab vedotin)	ADC	MMAE	AXL	~150,000[g]	~4	1.8	0.034	Q2W
BA3021 (ozuriftamab vedotin, CAB-ROR2-ADC)	ADC	MMAE	ROR2	~150,000[g]	~4	1.8	0.034	Q2W
FOR46	ADC	MMAE	CD46	~150,000[g]	3.7	2.7	0.048	Q3W, AIBW
OBI999	ADC	MMAE	Globo H	~150,000[g]	~4	1.2	0.023	Q3W

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Table 1. ***Continued***

Drug	Type	Payload	Antibody target	MW (Da)	DAR	ADC clinical MTD/ RP2D (mg/kg)[a]	Normalized cytotoxin dose (mg/kg)[a,b]	Dose schedule[c]
CX-2029	ADC	MMAE	CD71	~150,000[g]	2	3.0	0.028	Q3W
ALT-P7	ADC	MMAE	HER2	~150,000[g]	2	4.5	0.043	Q3W
A166	ADC	Duo-5	HER2	~150,000[g]	2	4.8	0.049	Q3W
Blenrep (belantamab mafodotin)	ADC	MMAF	BCMA	~152,000	~4	2.5	0.048	Q3W
ARX788	ADC	AS269	HER2	~150,000	2	1.5	0.019	Q3W
ABBV-647 (cofetuzumab pelidotin)	ADC	Aur0101	PTK7	~150,000[g]	4	2.8	0.055	Q3W
XMT-1536 (upifitamab rilsodotin)	ADC	AF-HPA	NaPi2b	~175,000[f]	10–12	0.97	~0.050	Q3W
Camptothecin								
Irinotecan	Prodrug	SN-38	–	587	–	–	0.12–0.49[h]	Q3W
						–	0.045–0.18[h]	QW
Topotecan	SM	–	–	421	–	–	0.20[d]	QDx5 every 3 weeks
							0.11	QW
							0.61	Q3W
Belotecan	SM	–	–	434	–	–	0.068–0.095[d]	QDx5 every 3 weeks
Exatecan mesylate (DX-8951f)	SM	–	–	532	–	–	0.16	Q3W
Enhertu (trastuzumab deruxtecan, T-DXd)	ADC	DXd	HER2	~156,000	~8	5.4 (BC, LC)	0.14	Q3W
						6.4 (GC)	0.16	Q3W
HER3-DXd (patritumab deruxtecan, U3-1402)	ADC	DXd	HER3	~158,000	~8	5.6	0.14	Q3W
Dato-DXd (datopotamab deruxtecan, DS-1062a)	ADC	DXd	TROP2	~150,000[g]	~4	6	0.08	Q3W
DS-7300 (ifinatamab deruxtecan, B7H3-DXd)	ADC	DXd	B7H3	~150,000[g]	~4	8–12	0.11–0.16	Q3W
DS-6000 (raludotatug deruxtecan, CDH6-DXd)	ADC	DXd	CDH6	~150,000[g]	~8	8	0.21	Q3W
Trodelvy (sacituzumab govitecan)	ADC	SN-38	TROP2	~160,000	7.6	10	0.19	days 1, 8 of a 21-day cycle
SKB-264	ADC	Belotecan derivative	TROP2	~160,000	7.4	5	0.12	Q2W
Pyrrolobenzodiazepine								
SJG-136 (SG2000)	SM	–		557	–	–	0.0012	Q3W
							0.0024[d]	QDx3 every 3 weeks
							0.0011	days 1, 8, and 15 of a 28-day cycle
Zynlonta (loncastuximab tesirine, ADCT-402)	ADC	SG3199	CD19	~150,000[g]	2.3	0.15 for 2 cycles then 0.075	0.0013 then 0.00067	Q3W

(*Continued on next page*)

Table 1. *Continued*

Drug	Type	Payload	Antibody target	MW (Da)	DAR	ADC clinical MTD/ RP2D (mg/kg)[a]	Normalized cytotoxin dose (mg/kg)[a,b]	Dose schedule[c]
ADCT-301 (camidanlumab tesirine)	ADC	SG3199	CD25	~150,000[g]	2.3	0.045 for 2 cycles then 0.030 (HL)	0.00040 then 0.00027	Q3W
						0.080 (T-cell NHL)	0.00071	Q3W
IMGN632 (pivekimab sunirine)	ADC	DGN549	CD123	~150,000[g]	2	0.045	0.00042	Q3W
Duocarmycin								
Pibrozelesin (KW-2189)	SM	–	–	698	–	–	0.0054–0.0081[e]	QDx5 every 6 weeks
							0.011–0.014	Q6W
Carzelesin (U-80244)	SM	–	–	729	–	–	0.0081	Q4W
SYD985 (trastuzumab duocarmazine)	ADC	Duba	HER2	~150,000[g]	2.8	1.2	0.011	Q3W
MGC018 (vobramitamab duocarmazine)	ADC	Duba	B7H3	~150,000[g]	2.7	3	0.028	Q3W
Eribulin[j]								
Halaven (Eribulin mesylate, E7389)	SM	–	–	826	–	–	0.048	Q3W
							0.034	days 1, 8 of a 21-day cycle
Eribulin liposomal formulation (Eribulin-LF, E7389-LF)	SM	–	–	730	–	–	0.038–0.054	Q3W
MORAb-202 (farletuzumab ecteribulin)	ADC	Eribulin	FRα	~150,000[g]	4	0.9–1.2	0.018–0.023	Q3W
Hemiasterlin								
Taltobulin (HTI-286)	SM	–	–	474	–	–	0.041	Q3W
E7974	SM	–	–	437	–	–	0.012	Q3W
STRO-002 (luveltamab tazevibulin)	ADC	SC239	FRα	~150,000[g]	4	4.3–5.2	0.056–0.068	Q3W

ADC, antibody drug conjugate; SM, small molecule; –, not applicable; AIBW, using adjusted ideal body weight, DLBCL, diffuse large B-cell lymphoma; CLL, chronic lymphocytic leukemia; NHL, non-Hodgkin lymphoma; BC, breast cancer; GC, gastric cancer; LC, lung cancer.

[a]FDA-recommended scaling factors were used to convert mg/m^2 to mg/kg.

[b]The normalized cytotoxin content of a payload conjugated to an ADC was calculated as following: $Dose_{payload} = Dose_{ADC}{*}DAR{*}MW_{payload}/MW_{ADC}$.

[c]Because ADCs are usually dosed once every 3 weeks (Q3W), small molecule MTDs, when established, were selected to match the same regimen. When a small molecule was dosed daily for 3–5 days (QDx3 or QDx5) every 3 weeks, the MTD was reported as cumulative dose over the 3 weeks, because this schedule can approximate a Q3W regimen.

[d]Reported as cumulative dose over 3 weeks.

[e]Reported as cumulative dose over 6 weeks.

[f]Estimated based on the published structure of the drug-linker.

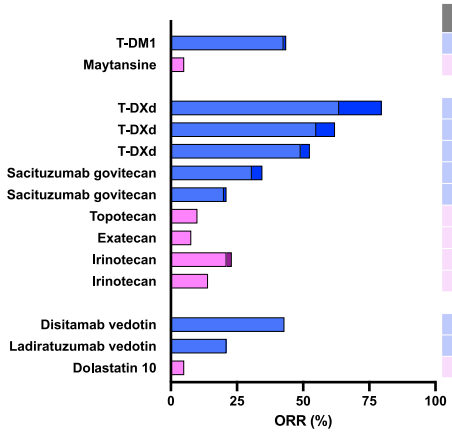
[g]Molecular weight of the ADC not available; ~150 kDa used as estimated value.

[h]The amount of SN-38 delivered via Irinotecan was calculated using the equation $Dose_{SN\text{-}38} = Dose_{irinotecan}{*}MW_{SN\text{-}38}/MW_{irinotecan}$ and then adjusted for amount of irinotecan that is metabolized to release SN-38 (2%–8% of the dose, see Palakurthi, 2015).

[i]With growth factor support.

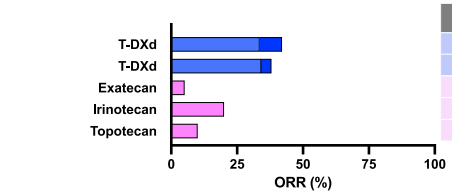
[j]Notably, eribulin is the only approved small molecule chemotherapy used unmodified as an ADC payload.

A Breast cancer



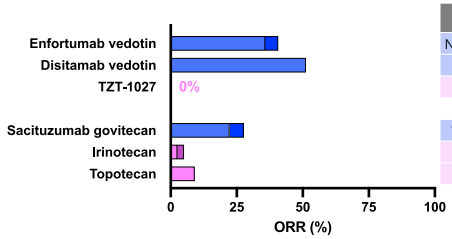
Target	Phase	Prior lines of therapy	Patients	Publication	Note
HER2	3	1	495	2012	*EMILIA (HER2-high)*
-	2	1+	31	1979	
HER2	3	1	261	2021	*DESTINY-Breast03 (HER2-high)*
HER2	2	2+	184	2021	*DESTINY-Breast01 (HER2-high)*
HER2	2	1-9 (median 3)	373	2022	*DESTINY-Breast04 (HER2-low)*
TROP2	3	2+	235	2020	*ASCENT (TNBC)*
TROP2	3	0-8 (median 3)	272	2022	*TROPiCS-02 (HR+/HER2–)*
-	2	1	40	1999	
-	2	1+	39	2003	
-	2	1+	52	2004	*QW regimen*
-	2	1+	51	2004	*Q3W regimen*
HER2	1b	2+	21	2021	*C003 CANCER (HER2-high)*
LIV1	1b	2+	44	2018	*SGNLVA-001 trial (TNBC)*
-	2	0	39	2003	

B Gastric cancer



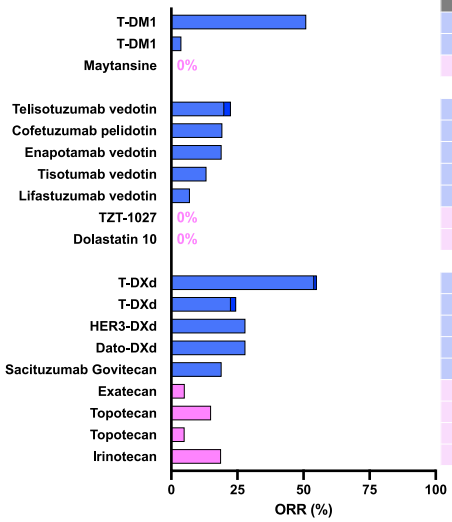
Target	Phase	Prior lines of therapy	Patients	Publication	Note
HER2	2	2+	129	2021	*DESTINY-Gastric01 (HER2-high)*
HER2	2	1+	79	2021	*DESTINY-Gastric02 (HER2-high)*
-	2	0+	39	2005	
-	2	1+	35	2004	QWx4 then 2-week rest
-	2	0+	20	1997	

C Urothelial cancer



Target	Phase	Prior lines of therapy	Patients	Publication
NECTIN4	3	1+	301	2021
HER2	2	1+	43	2021
-	1	1+	2	2006
TROP2	2	0+	112	2021
-	2	1+	40	2008
-	2	0+	44	1998

D Non-small cell lung cancer



Target	Phase	Prior lines of therapy	Patients	Publication	Note
HER2	2	0-4 (median 2)	15	2018	*HER2 amplified and/or mutant*
HER2	2	1+	29	2019	*HER2 IHC 3+ and 2+*
-	2	1+	19	1980	
c-MET	1	0-7 (median 3)	40	2021	*Q2W + Q3W*
PTK7	1	2+	31	2021	*Q2W + Q3W*
AXL	2	0+	26	2019	
TF	1-2	1-4 (median 2)	15	2019	
NaPi2b	1	1+	45	2020	
-	2	1+	32	2007	
-	2	0	19	2000	
HER2	2	0-7 (median 2)	91	2021	*HER2-mutant*
HER2	2	1-8 (median 3)	49	2021	*HER2 IHC 3+ and 2+*
HER3	1	1-10 (median 4)	57	2021	*EGFR-mutant*
TROP2	1	1+	50	2021	
TROP2	1	2-7 (median 3)	47	2017	
-	2	0	39	2003	*18% minor response*
-	2	0	40	1996	*Intravenous*
-	3	1	414	2006	*Oral*
-	2	2-3	32	2013	

Figure 2. ADCs demonstrate improved efficacy compared to related small molecules

Objective response rates (ORRs) for selected ADCs (blue) and related small molecules (pink) in (A) breast, (B) gastric, (C) urothelial, and (D) non-small cell lung cancers. ADC partial response, light blue; ADC complete response, dark blue; small molecule partial response, light pink; small molecule complete response, dark pink.

conjugation to an antibody. ADCs prolong the cytotoxin half-life, including by protecting it from renal clearance. On the other hand, ADCs suffer from issues similar to other biologics, which limit their tumor penetration and accumulation. These include significant on-target off-tumor uptake and non-specific clearance, limited extravasation across capillary walls, low diffusion inside the tumor masses due to increased tumor interstitial fluid pressure, and the "binding site barrier" phenomenon (antibodies bind to their targets faster than their diffusion rates preventing deeper penetration) (Nessler et al., 2021). For unconjugated antibodies, some of these obstacles may be overcome by increasing dosing, but this strategy is not available for ADCs, since the conjugated cytotoxin dictates the MTD. Indeed, it has been reported that less than 1% of an ADC reaches the tumor in humans, with the remainder potentially causing unwanted toxicities. Therefore, ADC tumor targeting alone may not account for improved efficacy over related small molecules.

Tarcsa and coauthors analyzed population PK models of approved ADCs, focusing on the role of the free payload in circulation (Tarcsa et al., 2020). They concluded that ADCs may rely on additional mechanisms for efficacy, including extended payload release in circulation at efficacious levels. ADC payloads often achieve a plasma steady-state concentration above the experimentally determined half-maximal effective concentration of the free drug. The antigen expression and ADC properties influence ADC PK and catabolism, which regulate rate and location of payload release, in turn impacting the plasma level and tumor concentration of free payload. The degree of payload bystander activity, conjugation chemistry (susceptibility to deconjugation), and linker type (cleavable or not cleavable) are therefore crucial design parameters. Ongoing trials of ADCs targeting known antigens but using different conjugation modalities or payloads could help to inform which ADC feature(s) may improve efficacy while maintaining acceptable tolerability.

Correlation between efficacy and expression suggests additional mechanisms

Recent clinical results from patients with different levels of tumor antigen expression support the idea that circulating payload can lead to a baseline antitumor effect. For instance, a number of ADCs have shown efficacy in patients with either low or negative tumor antigen expression. While different tumor sensitivities to payload, imprecise antigen quantification, or small sample size of some datasets cannot be ruled out as alternative origins of this observation, for certain ADCs it is plausible that the payload concentration in circulation is sufficient to elicit a therapeutic response. The antibody-targeting component (i.e., ADC direct delivery) may enhance efficacy over the baseline activity provided by free cytotoxins, particularly in tumors with high antigen expression. The hypothesis put forward is supported through comparison of trial data for trastuzumab, T-DM1, and T-DXd in HER2+ breast cancer patients with brain metastases: T-DXd and T-DM1 demonstrated intracranial ORRs of 64% and 33%, respectively, in a subgroup analysis of DESTINY-Breast03, while in separate trials trastuzumab did not generate an objective response (Garcia-Alvarez et al., 2021; Hurvitz et al., 2022). The intact blood-brain barrier (BBB) prevents penetration of antibodies but may not limit small molecules, depending on their physicochemical properties. Although other factors may contribute to BBB penetration, the propensity of (circulating) cytotoxic catabolites of T-DXd (i.e., DXd; compatible with penetration) and T-DM1 (i.e., DM1 and Lys-MCC-DM1; less compatible with penetration), compared to trastuzumab (no cytotoxic catabolite), may provide the best explanation for the observed differences in activity against brain metastases. Deconvoluting the origins of the relative clinical benefits for similar, albeit different, ADCs is challenging even after careful consideration of the different ADC structures and properties.

Conclusions and perspectives

The growing list of marketed ADCs demonstrates the feasibility of combining the right target, antibody, conjugation modality, DAR, linker, payload, and disease indication to generate clinically useful drugs. However, current ADCs don't significantly increase MTDs of their conjugated drugs across multiple oncology disease states despite broad diversity. Thus, ample opportunity exists to explore mechanisms that may provide further improvements in efficacy and tolerability. Below are selected key considerations and implications for the field:

(1) If the payload, linker, DAR, and conjugation modality are unchanged, the tolerated dose of an ADC will be at best similar to other ADCs prepared with the same technology (platform toxicities).

(2) The roughly equivalent MTDs observed for ADCs and related small molecules across multiple drug classes is intuitively easy to accept but more difficult to rationalize. Is there an explanation for the similar tolerability despite extensive PK differences of ADCs and small molecules?

(3) Alternative dosing regimens have been successfully leveraged to mitigate safety risks while maintaining efficacy of ADCs (Liao et al., 2021). This suggests that an in-depth analysis of the PK/PD profiles is essential to optimize ADC clinical dosing strategies.

(4) It is important to better understand the mechanism of action for clinically active ADCs (antibody-targeted delivery, sustained free drug concentration in circulation, albumin transfer, or a combination of multiple mechanisms) and the interrelationship between ADC structural components and their PK/PD to influence the design of the next generation of ADCs.

(5) More predictive *in vitro* and *in vivo* models are needed to allow better clinical translatability. Current workflows may filter out compounds that could become successful drugs or select candidates with suboptimal properties.

(6) Novel ADC conjugation sites, conjugation chemistry, and linker technology have been hailed to improve MTDs, often on the basis of a better stability yielding increased preclinical tolerability, but this is not yet supported by clinical evidence. Pending trial results for these newer ADCs will establish if these solutions can successfully improve over current technologies.

(7) A significant amount of work has been performed to reduce well-understood platform toxicities of ADCs bearing microtubule inhibitors, by modifying either the conjugation or the payload. Although successful in limiting hematological toxicities, these strategies fail to meaningfully improve MTDs owing to the emergence of new issues, including ocular and pulmonary toxicities. Understanding and overcoming the origins of such tissue-specific

damage may enable further incremental MTD improvements.

(8) Altering payload potency via structural modifications may shift the therapeutic window of the drug but not necessarily widen it. Exploring payloads with better "drug-like" properties (such as solubility, permeability, metabolic stability, and transporter substrate profile) could improve ADC clinical attrition rates.

(9) Antibodies should be matched with payloads based on tumor type, localization, and antigen expression relative to normal tissue to allow dosing of ADCs at sufficient levels to overcome non-tumor tissue antigenic sinks.

(10) Antibody conjugates with new classes of payloads have been developed, including steroids, PROTACs, TLR agonists, STING agonists, viral peptides, and RNAs. Clinical data for these new conjugates could broaden the utility of antibody delivery. While promising, it is likely that off-target effects could also interfere with these new modalities. For example, immunostimulatory antibody conjugates (ISACs) bearing TLR agonists haven't yet lived up to expectations, with three out of four therapeutic candidates discontinued in the clinic.

(11) Numerous ADC payloads have been shown to induce immunogenic cell death (ICD) (Nessler et al., 2021). This immunostimulatory phenomenon is a relatively new area of research with the potential to impact the future direction of the ADC field. However, there are still significant gaps in our understanding of payload-induced ICD.

(12) The combination of ADCs with immune-checkpoint inhibitors (ICIs) or other cytotoxic agents has become popular because it provides patients with multiple opportunities to respond to a single agent. Although clinical benefits have been observed (Nicolò et al., 2022), it is important to establish if the effect is caused by synergistic, additive, or independent drug action. So far there is no clinical evidence of synergistic or even additive interaction of ICIs with each other or other drugs (Palmer et al., 2022), but better biomarkers and patient selection could help in improving drug combinations, including ADCs.

Our analysis is intended to stimulate discussions and further consideration by researchers and clinicians working with this class of therapeutics. Refined engineering strategies guided by clinical data, along with new modalities and applications, could elevate antibody conjugates as a robust and predictable therapeutic modality.

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DECLARATION OF INTERESTS

R.C. and J.R.R. are employees and shareholders of Zymeworks Inc. and work to develop antibody drug conjugates for therapeutic applications.

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