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REVIEW

Influence of antibody–drug conjugate cleavability, drug-to-antibody ratio, and free payload concentration on systemic toxicities: A systematic review and meta-analysis

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Abstract
While in theory antibody drug conjugates (ADCs) deliver high-dose chemotherapy directly to target cells, numerous side effects are observed in clinical practice. We sought to determine the effect of linker design (cleavable versus non-cleavable), drug-to-antibody ratio (DAR), and free payload concentration on systemic toxicity. Two systematic reviews were performed via PubMed search of clinical trials published between January 1998—July 2022. Eligible studies: (1) clinical trial for cancer therapy in adults, (2) ≥ 1 study arm included a single-agent ADC, (3) ADC used was commercially available/FDA-approved. Data was extracted and pooled using generalized linear mixed effects logistic models. 40 clinical trials involving 7,879 patients from 11 ADCs, including 9 ADCs with cleavable linkers ($N = 2{,}985$) and 2 with non-cleavable linkers ($N = 4{,}894$), were included. Significantly more composite adverse events (AEs) ≥ grade 3 occurred in patients in the cleavable linkers arm (47%) compared with the non-cleavable arm (34%). When adjusted for DAR, for grade ≥ 3 toxicities, non-cleavable linkers remained independently associated with lower toxicity for any AE ($p = 0.002$). Higher DAR was significantly associated with higher probability of grade ≥ 3 toxicity for any AE. There was also a significant interaction between cleavability status and DAR for any AE ($p = 0.002$). Finally, higher measured systemic free payload concentrations were significantly associated with higher DARs ($p = 0.043$). Our results support the hypothesis that ADCs with cleavable linkers result in premature payload release, leading to increased systemic free payload concentrations and associated toxicities. This may help to inform future ADC design and rational clinical application.

Keywords Antibody–drug conjugates · Cleavable and non-cleavable linkers · Payloads · Drug to antibody ratio · Systemic toxicities · Meta-analysis

1 Introduction

Antibody–drug conjugates (ADCs) are monoclonal antibodies connected to a cytotoxic agent known as the payload via a chemical linker. It was hoped that ADCs would be "magic bullets," delivering high-dose cytotoxic chemotherapy directly to cancer cells without affecting surrounding normal tissues. However, this has not borne out in clinical practice. Though many factors affect toxicity, the toxicities of currently approved ADCs appear to be driven primarily by premature release of the payload into the bloodstream by the linker, by an excessively prominent bystander effect [1], or even payload released by the lysed tumor cells [2].

ADC linkers can be divided broadly into two groups: cleavable and non-cleavable. Cleavable linkers such as hydrazone, disulfide, or peptide linkers rely on physiologic factors

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(i.e., cathepsin, glutathione (GSH), and low pH) within the cell to cleave the linker. Because these conditions can occur independently of antigen internalization, cleavable linkers are often less stable in the blood, resulting in various off-target effects [3]. In contrast, non-cleavable linkers, such as the thioether or maleimidocaproyl linkers, require internalization by the target cell, so that the antibody, rather than the linker, can be degraded by the lysosome before the drug is released. This latter mechanism does not produce efficient bystander killing and thus results in lower toxicity profiles [4]. For these reasons, other novel linkers, including conditionally released linkers, are currently in rapid development [5, 6].

Preclinical studies have shown that compared to ADCs with non-cleavable linkers, those with cleavable linkers likely release free payload prematurely, leading to increased systemic toxicity. In this study, we sought to delineate the potential effect of linker design on systemic toxicity by analyzing the results of clinical trials using ADCs constructed with both types of linkers. We hypothesized that ADCs with cleavable linkers would be associated with greater systemic toxicities than those with non-cleavable linkers. To test this hypothesis, we conducted a systematic review of adverse events (AEs) occurring in cancer patients treated with commercially available ADCs. We then carried out a meta-analysis on all eligible phase II-III clinical trials. We also evaluated the potential effect of drug-to-antibody ratio (DAR) and systemic free payload concentration on toxicity in the context of the cleavability of the linkers used.

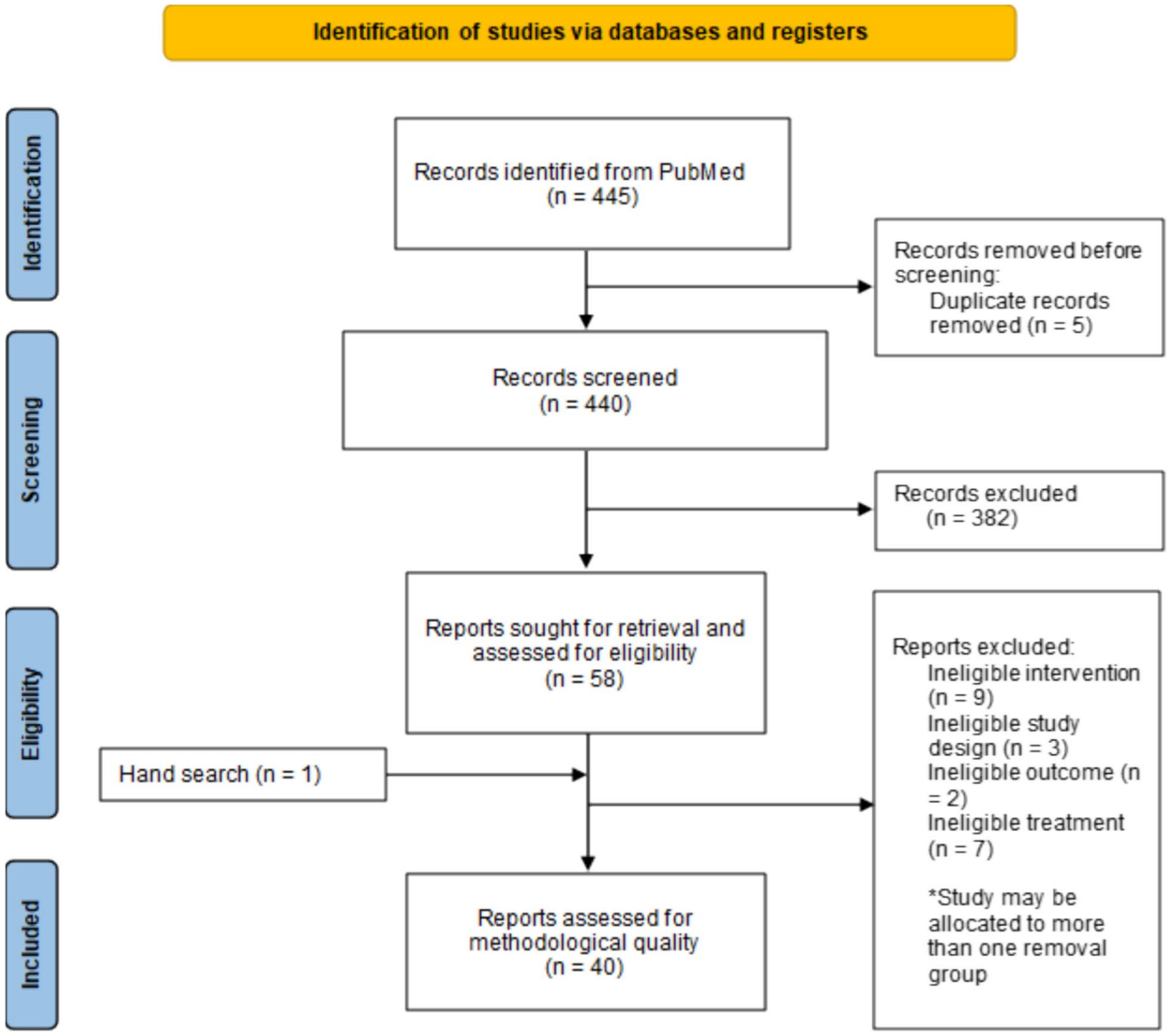

Fig. 1 PRISMA flowchart describing the result of the search and selection process

2 Materials and methods

2.1 Search methods and study selection

Following the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) 2020 updated guidance, two systematic reviews were performed through a PubMed search on July 5, 2022. The first identified studies reporting clinical toxicity rates. Studies eligible for inclusion met the following criteria: (1) clinical trial for cancer therapy in adults ages 18 or older, (2) participants in at least one arm of the study were treated with single-agent ADC, (3) the ADC used was commercially available and FDA-approved for cancer treatment as of July 5, 2022, (4) the study reported treatment-related adverse events, and (5) the study was published in English. Studies where the ADC was administered in combination with other chemotherapy agents were excluded because the ADC's individual contribution to the overall toxicity profile of the regimen could not be fully delineated. A second systematic review identified studies reporting the DARs and estimated systemic free payload concentrations for all FDA-approved ADCs.

2.2 Statistical analysis: meta-analysis of clinical toxicity rates

To compare the incidence of toxicities in patients treated with ADCs constructed with cleavable vs non-cleavable linkers, generalized linear mixed effects logistic models were conducted for each toxicity, including:

1. *By linker type*. Univariable mixed effects logistic regression models were constructed evaluating the association between frequency of each specific toxicity for the binary outcome variables (any grade vs none; grade ≥ 3

Table 1 Included ADCs with linker type, drug-to-antibody ratio, and systemic maximum free payload concentration

Antibody drug conjugate	Indication(s)	Antigen	Payload agent	Number of studies	Number of patients	Linker	Drug-to-antibody ratio (DAR)	Systemic maximum free payload concentration (kg/L)
Belantamab mafodotin	Multiple myeloma	BCMA	MMAF	1	97	Non-cleavable	4	0.0004
Brentuximab vedotin	Hodgkin lymphoma, anaplastic large cell lymphoma, CD30 + peripheral T cell lymphoma, CD30 + mycosis fungoides	CD30	MMAE	11	660	Cleavable	4	0.0026
Enfortumab vedotin	Urothelial cancer	Nectin-4	MMAE	3	515	Cleavable	3.8	0.0040
Gemtuzumab ozogamicin	Acute myeloid leukemia	CD33	Calichea-micin	2	175	Cleavable	2.5	0.0229
Inotuzumab ozogamicin	Acute lymphoblastic leukemia	CD22	Calichea-micin	2	205	Cleavable	6	0.0556
Loncastuximab tesirine	Large B-cell lymphoma	CD19	PBD dimer	1	145	Cleavable	2.3	0.0003
Moxetumomab pasudotox	Hairy cell leukemia	CD22	PE38	1	80	Cleavable	1	*No systemic accumulation observed*
Sacituzumab govitecan	Breast cancer Urothelial cancer	Trop-2	SN38	3	479	Cleavable	7.6	0.0120
Tisotumab vedotin	Cervical cancer	Tissue factor	MMAE	2	156	Cleavable	4	0.0026
Trastuzumab deruxtecan	Breast cancer Gastric cancer	HER2	Deruxtecan	3	570	Cleavable	8	0.4347
Trastuzumab emtansine	Breast cancer	HER2	Emtansine	12	4,797	Non-cleavable	3.5	0.0012

BCMA B-cell maturation antigen, *MMAE* monomethyl auristatin, *MMAF* monomethyl auristatin F, *PBD* pyrrolobenzodiazepine, *PE38* the 38 kDa fragment of Pseudomonas exotoxin A, *SN38* active metabolite of irinotecan

Table 2 Studies included in meta-analysis

ADC name	Study first author, year	Cancer type	Study design/phase/site	ADC group sample size	Modified NOS rating
Belantamab mafadotin	Lonial, 2021	Multiple myeloma	RCT/2/multicenter	97	Good
Brentuximab vedotin	Pro, 2012	Systemic ALCL	Single-arm study/2/Multicenter	58	Fair
	Younes, 2012	Hodgkin lymphoma	Single-arm study/2/Multicenter	102	Good
	Horwitz, 2014	PTCL	Single-arm study/2/Multicenter	35	Fair
	Monjanel, 2014	Hodgkin lymphoma, ALCL	Retrospective cohort study/2/Single center	45	Good
	Duvic, 2015	pcALCL, MF	Single-arm study/2/Single center	54	Good
	Kim, 2015	MF, Sezary syndromeS	Single-arm study/2/Multicenter	32	Fair
	Prince, 2017	MF, pcALCL	RCT/3/Multicenter	64	Good
	Walewski, 2018	Hodgkin lymphoma	Single-arm study/4/Multicenter	60	Good
	Stefoni, 2020	Hodgkin lymphoma	Single-arm study/2/Multicenter	18	Good
	Kuruvilla, 2021	Hodgkin lymphoma	RCT/3/Multicenter	153	Good
	Song, 2021	Hodgkin lymphoma, ALCL	Single-arm study/2/Multicenter	39	Fair
Enfortumab vedotin	Rosenberg, 2019	Urothelial cancer	Single-arm study/2/Multicenter	125	Good
	Powles, 2021	Urothelial cancer	RCT/3/Multicenter	301	Good
	Yu, 2021	Urothelial cancer	Single-arm study/2/Multicenter	89	Good
Gemtuzumab ozogamicin	Taksin, 2007	AML	Single-arm study/2/Multicenter	57	Fair
	Amadori, 2016	AML	RCT/3/Multicenter	118	Good
Inotuzumab ozogamicin	Kantarjian, 2013	ALL	Single-arm study/1.5/Single center	41	Good
	Kantarjian, 2019	ALL	RCT/3/Multicenter	164	Good
Loncastuximab tesirine	Caimi, 2021	DLBCL	Single-arm study/2/Multicenter	145	Good
Moxetumomab pasudoxtox	Kreitman, 2021	Hairy cell leukemia	Single-arm study/2/Multicenter	80	Good
Sacituzumab govitecan	Bardia, 2019	Breast cancer	Single-arm study/1.5/Multicenter	108	Good
	Bardia, 2021	Breast cancer	RCT/3/Multicenter	258	Good
	Tagawa, 2021	Urothelial cancer	Non-randomized multicohort study/2/Multicenter	113	Good
Tisotumab vedotin	Hong, 2020	Cervical cancer	Single-arm study/1.5/Multicenter	55	Fair
	Coleman, 2021	Cervical cancer	Single-arm study/2/Multicenter	101	Good
Trastuzumab deruxtecan	Modi, 2020	Breast cancer	Single-arm study/2/Multicenter	184	Good
	Shitara, 2020	Gastric cancer	RCT/2/Multicenter	125	Good
	Cortés, 2022	Breast cancer	RCT/3/Multicenter	261	Good
Trastuzumab emtansine	Burris, 2011	Breast cancer	Single-arm study/2/Multicenter	112	Good
	Krop, 2012	Breast cancer	Single-arm study/2/Multicenter	110	Good
	Verma, 2012	Breast cancer	RCT/3/Multicenter	495	Good
	Yardley, 2015	Breast cancer	Single-arm study/4/Multicenter	215	Good
	Kashiwaba, 2016	Breast cancer	Single-arm study/2/Multicenter	73	Good
	Krop, 2017	Breast cancer	RCT/3/Multicenter	403	Good
	Watanabe, 2017	Breast cancer	Single-arm study/2/Multicenter	232	Fair
	Montemurro, 2019	Breast cancer	Single-arm study/3b/Multicenter	2002	Good
	Von Minckwitz, 2019	Breast cancer	RCT/3/Multicenter	743	Good
	Cortés, 2020	Breast cancer	RCT/1.5/Multicenter	80	Fair
	Emens, 2020	Breast cancer	RCT/2/Multicenter	69	Good
	Cortés, 2022	Breast cancer	RCT/3/Multicenter	263	Good

ALCL anaplastic large cell lymphoma, *ALL* acute lymphoblastic leukemia, *AML* acute myeloid leukemia, *DLBCL* diffuse large B-cell lymphoma, *MF* mycosis fungoides, *NOS* Newcastle–Ottawa scale, *pcALCL* primary cutaneous ALCL, *PTCL* peripheral T cell lymphoma, *RCT* randomized controlled trial

versus grade ≤ 2) and ADC linker type (cleavable vs non-cleavable).

2. *Linker type adjusted for drug-to-antibody ratio*. Multivariable mixed effects logistic regression models were constructed evaluating the association between frequency of each specific toxicity for the binary outcome variables (any grade vs none; grade ≥ 3 vs grade ≤ 2) and ADC linker type (cleavable vs non-cleavable) plus drug-to-antibody ratio (numeric predictor) with the potential interaction between ADC linker type and drug-to-antibody ratio when estimable. The model includes the interaction when estimable since cleavability status and drug-to-antibody ratio are not necessarily independent factors but arise from the design of each medication.

3. *Linker type adjusted for estimated systemic free payload concentration*. Multivariable mixed effects logistic regression models were constructed evaluating the association between frequency of each specific toxicity for the binary outcome variables (any grade vs none; grade ≥ 3 vs grade ≤ 2) and ADC linker type (cleavable vs non-cleavable) plus systemic free payload concentration (numeric predictor) with the potential interaction between ADC linker type and payload systemic free concentration when estimable. Again, the model includes the interaction when estimable since cleavability status and systemic free payload concentration are not necessarily independent factors but arise from the design of each medication.

Heterogeneity in the estimated probability of each specific toxicity between studies was assessed with the I [2] statistic, describing the percentage of variation in probability of each specific toxicity across the studies arising from differences in the included trials (heterogeneity) rather than sampling error (chance).

3 Results

Study inclusion A literature search and review of references identified 440 relevant publications after duplicates were removed. After eligibility assessment, a total of 40 clinical trials involving 7,879 patients were used to perform our meta-analysis, as shown in Fig. 1 [7–46]. Eleven (11) commercially-available FDA-approved ADCs were included (Table 1). Nine of these studies reported the results of treatment with ADCs with cleavable linkers ($N = 2{,}985$), whereas two used non-cleavable linkers ($N = 4{,}894$). Table 2 lists the ADC agent, target disease, study design, number of patients

Table 3 Toxicity ≥ Grade 3 by Cleavability of Linker, Drug-to-Antibody Ratio, and Interaction of Cleavability*Drug-to-Antibody Ratio

	# Studies Reporting	# Patients At Risk	# Patients With Toxicity	Cleavability Association (p-value)	Noncleavable = Lower Toxicity	DAR (p-value)	Lower DAR = Less Toxicity	Interaction Cleavability* DAR (p-value)	Interaction Moderating Association	I2 (%)
Any Toxicity ≥ Grade 3	30	7,193	3,169	0.002	Yes	0.000	Yes	0.002	Yes	91.895
HEMATOLOGIC										
Neutropenia ≥ Grade 3	30	4,772	614	0.021	Yes	0.000	Yes	0.042	Yes	83.002
Leukopenia ≥ Grade 3	16	2,551	173	0.008	Yes	0.115	NS	0.017	Yes	71.442
Anemia ≥ Grade 3	31	7,185	349	0.001	Yes	0.027	Yes	0.001	Yes	77.075
Hematologic ≥ Grade 3	6	3,975	624	0.959	NS	0.249	NS	ID	ID	93.092
Thrombocytopenia ≥ Grade 3	26	6,522	517	0.282	NS	0.782	NS	0.257	NS	92.705
Lymphopenia ≥ Grade 3	8	947	90	0.612	NS	0.274	NS	ID	ID	44.802
GASTROINTESTINAL										
Nausea ≥ Grade 3	32	7,060	101	0.557	NS	0.000	Yes	0.556	NS	1.329
Vomiting ≥ Grade 3	29	6,653	91	0.560	NS	0.508	NS	0.576	NS	53.511
Diarrhea ≥ Grade 3	31	6,990	120	0.802	NS	0.058	NS	0.874	NS	62.225
Elevated AST ≥ Grade 3	25	6,500	163	0.525	NS	0.239	NS	0.564	NS	71.383
Elevated ALT ≥ Grade 3	21	5,834	106	0.911	NS	0.266	NS	ID	ID	57.674
Constipation ≥ Grade 3	23	5,730	20	0.636	NS	0.718	NS	0.637	NS	0.277
Liver Toxicity ≥ Grade 3	20	11,508	243	0.508	NS	0.256	NS	ID	ID	82.614
CONSTITUTIONAL										
Pyrexia ≥ Grade 3	23	5,219	37	0.004	Yes	0.024	No	0.006	Yes	46.414
Fatigue ≥ Grade 3	33	7,352	225	0.903	NS	0.239	NS	0.847	NS	60.924
Headache ≥ Grade 3	15	4,305	30	0.554	NS	0.710	NS	0.552	NS	35.244
OTHER										
Peripheral Neuropathy ≥ Grade 3	22	2,985	107	0.005	Yes	0.042	Yes	ID	ID	84.451
ILD ≥ Grade 3	12	3,906	21	0.257	NS	0.833	NS	ID	ID	0.000
Ocular Toxicity ≥ Grade 3	6	807	50	0.926	NS	0.556	NS	0.925	NS	0.000
Hypokalemia ≥ Grade 3	15	2,593	58	0.871	NS	0.753	NS	0.863	NS	41.073

DAR = drug-to-antibody ratio, ID = insufficient data to evaluate interaction, NS = not significant, ILD = interstitial lung disease, ALT = alanine transaminase, AST = aspartate aminotransferase

p<0.05 Expected direction
p<0.01
P<0.05 Unexpected direction
p<0.01

treated with the ADC, and modified Newcastle–Ottawa scale study quality rating. Table 3 lists the 21 specific toxicities examined. It also indicates the number of included studies reporting the specific toxicity, patients at risk, and the number of patients experiencing toxicities for grade ≥ 3 (Table 3) and any grade (Table 4).

As quantified in Tables 3 and 5, at least half the studies reported thrombocytopenia, neutropenia, anemia, increased AST and ALT, nausea, vomiting, diarrhea, and fatigue, as well as any toxicity at any grade and grade ≥ 3 AEs. Other toxicities were reported less frequently.

Systemic toxicity rates with ADCs, stratified by cleavable vs non-cleavable linker The meta-analytic point estimate of the proportion of patients experiencing each of the 21 toxicities with a 95% confidence interval is displayed in Fig. 2A for toxicities ≥ grade 3, and in Fig. 2B for any grade toxicity, both stratified by linker type. Composite AEs ≥ grade 3 occurred in 43% of patients overall, 47% in the cleavable linker-treated patients and 34% in the non-cleavable-treated patients, and these differences were significant (weighted risk difference −12.9%; 95% CI: −17.1% to −8.8%). Specific toxicities ≥ grade 3 with significantly lower proportions favoring non-cleavable linkers were neutropenia (−9.1%; 95% CI −12% to −6.2%) and anemia (−1.7%; 95% CI −3.3% to −0.1%). There was no significant difference in rates of grade ≥ 3 thrombocytopenia, increased AST/ALT, or fatigue. For all grade toxicities, there were no significant differences in rates of nausea, vomiting, diarrhea, hypokalemia, or headache.

Linker type and drug-to-antibody ratio We further examined the potential association between ADC linker type and drug-to-antibody ratios and the estimated probabilities of systemic toxicity. Since the linker type and drug-to-antibody ratio are design features of each ADC and thus are not independent factors, the interaction between linker type and drug-to-antibody ratio was modeled and estimated whenever feasible. A summary of the results for the 21 toxicities are represented as a heatmap (Tables 3 and 4). The p-values are color-coded for level of significance and direction of association.

For grade ≥ 3 toxicities (Table 3), non-cleavable linkers remain significantly and independently associated with lower toxicity for any AE ($p = 0.002$), neutropenia ($p = 0.021$), leukopenia ($p = 0.008$), anemia ($p = 0.001$), pyrexia ($p = 0.004$), and peripheral neuropathy ($p = 0.005$) when adjusted for DAR and their interaction where estimable. In addition, higher DAR was significantly and independently associated with higher probability of grade ≥ 3 toxicity for any AE, neutropenia, anemia, nausea ($p < 0.001$), and peripheral neuropathy. Higher DAR was significantly and independently associated

Table 4 Toxicity Any Grade by Cleavability of Linker, Drug-to-Antibody Ratio, and Interaction of Cleavability * Drug-to-Antibody Ratio

	# Studies Reporting	# Patients At Risk	# Patients With Toxicity	Cleavability Association (p-value)	Noncleavable = Lower Toxicity	DAR (p-value)	Lower DAR = Less Toxicity	Interaction Cleavability* DAR (p-value)	Interaction Moderating Association	I2(%)
Any AE	27	6528	6173	0.987	NS	0.059	NC	0.992	NS	91.023
HEMATOLOGIC										
Neutropenia	27	3949	916	0.475	NS	0.000	Yes	0.562	NS	88.8
Leukopenia	16	2551	375	0.167	NS	0.034	Yes	0.229	NS	88.888
Anemia	28	6812	1075	0.111	NS	0.002	Yes	0.133	NS	89.24
Hematologic	6	3975	1026	0.691	NS	0.008	Yes	ID	ID	92.09
Thrombocytopenia	22	6228	1380	0.823	NS	0.390	NS	0.709	NS	96.593
Lymphopenia	7	908	125	0.997	NS	0.697	NS	ID	ID	53.794
GASTROINTESTINAL										
Nausea	37	7609	2816	0.228	NS	0.000	Yes	0.271	NS	92.775
Vomiting	29	6808	1264	0.097	NS	0.000	Yes	0.106	NS	88.301
Diarrhea	34	7301	1374	0.811	NS	0.013	Yes	0.766	NS	92.89
Elevated AST	26	6542	1172	0.923	NS	0.227	NS	0.935	NS	94.804
Elevated ALT	23	6108	846	0.781	NS	0.392	NS	ID	ID	94.345
Constipation	24	5945	1125	0.366	NS	0.182	NS	0.402	NS	91.765
Liver (all)	26	13084	2344	0.925	NS	0.240	NS	0.938	NS	97.491
CONSTITUTIONAL										
Pyrexia	24	5609	989	0.854	NS	0.341	NS	0.808	NS	82.785
Fatigue	34	7584	2635	0.091	NS	0.385	NS	0.092	NS	94.253
Headache	15	4546	1042	0.016	No	0.483	NS	0.023	Yes	86.633
OTHER										
Peripheral neuropathy	23	3050	658	0.054	NS	0.358	NS	ID	ID	93.793
ILD	12	1937	78	0.562	NS	0.020	Yes	ID	ID	84.672
Ocular (all)	9	1290	285	0.008	Yes	0.006	No	0.009	Yes	93.742
Hypokalemia	15	2065	248	0.280	NS	0.553	NS	0.298	NS	81.218

AE = adverse event, DAR = drug-to-antibody ratio, ID = insufficient data to evaluate interaction, NS = not significant, ILD = interstitial lung disease, ALT = alanine transaminase, AST = aspartate aminotransferase

p<0.05
p<0.01
P<0.05
p<0.01

Expected direction

Unexpected direction

with lower probability of grade ≥ 3 pyrexia. There were significant interaction terms between cleavability status and DAR for any AE ($p = 0.002$), neutropenia ($p = 0.042$), leukopenia ($p = 0.017$), anemia ($p = 0.001$), and pyrexia ($p = 0.006$). These were all moderating interactions, indicating a lower toxicity then would be predicted from the additive effects of a linear increase in DAR and linker cleavability type.

For any grade toxicity (Table 4), non-cleavable linkers were not significantly and independently associated with toxicity adjusted for DAR and their interaction except lower ocular and higher headache toxicity of any grade. However, higher DARs were significantly and independently associated with a higher probability of any grade toxicity for neutropenia, anemia, leukopenia, all hematological toxicities, nausea, vomiting, diarrhea, and interstitial lung disease. Although the sample size is limited, higher DAR was significantly and independently associated with lower probability of any grade ocular toxicity.

As observed in the heatmap of these data, the direction of the associations when significant were typically in the pre-specified clinically expected direction: higher DAR was associated with higher probability of grade ≥ 3 toxicity. As reported in Tables 3 and 4, most of the I [2] statistics are > 50% indicating at least moderate to high levels of heterogeneity in reported probabilities of each specific toxicity between studies.

Linker type, systemic free payload agent concentration, and toxicity Next we considered the potential association between ADC linker type and the systemic free payload concentration, including the potential interaction between linker type and systemic free payload concentration when estimable. These results are summarized as a heatmap of the p-values for the significance of the estimated coefficients for each factor in Tables 5 (toxicities ≥ grade 3) and 6 (any grade toxicity). The p-values are color-coded for level of significance and direction of regression coefficient associations between systemic free payload concentrations and linker type. For grade ≥ 3 toxicities (Table 5), non-cleavable linkers remain significantly and independently associated with lower toxicity for only peripheral neuropathy ($p = 0.001$). Neutropenia, leukopenia, and pyrexia no longer had a significant independent association with linker type after adjustment for the systemic free payload concentration. In fact, the probability of any AE ≥ grade 3 ($p = 0.009$) and anemia ($p = 0.011$) was higher in patients treated with

Table 5 Toxicity ≥ Grade 3 by Cleavability of Linker, Systemic Free Payload Concentration, and Interaction of Cleavability* Systemic Free Payload Concentration

	# Studies Reporting	# Patients At Risk	# Patients With Toxicity	Cleavability Association (p-value)	Noncleavable = Lower Toxicity	Free Payload Concentration	Lower Systemic Free Payload Concentration = Less Toxicity	Cleavability* Systemic Free Payload Concentration (p-value)	Interaction Moderating Association	I2 (%)
Any Toxicity ≥ Grade 3	29	7113	3145	0.009	No	0.096	NS	0.004	Yes	94.018
HEMATOLOGIC										
Neutropenia ≥ Grade 3	29	4692	610	0.792	NS	0.08	NS	0.065	NS	89.391
Leukopenia ≥ Grade 3	15	2471	166	0.078	NS	0.549	NS	0.020	Yes	76.438
Anemia ≥ Grade 3	30	7105	341	0.011	No	0.022	Yes	0.001	Yes	77.183
Hematologic ≥ Grade 3	5	3575	584	0.688	NS	0.986	NS	ID	ID	94.512
Thrombocytopenia ≥ Grade 3	25	6442	512	0.167	NS	0.72	NS	0.275	NS	93.271
Lymphopenia ≥ Grade 3	7	867	74	0.153	NS	0.604	NS	ID	ID	10.131
GASTROINTESTINAL										
Nausea ≥ Grade 3	31	6980	99	ID	ID	ID	ID	ID	ID	
Vomiting ≥ Grade 3	29	6653	91	0.738	NS	0.897	NS	0.553	NS	53.984
Diarrhea ≥ Grade 3	30	6910	120	0.601	NS	0.284	NS	0.816	NS	64.674
Elevated AST ≥ Grade 3	25	6500	163	0.781	NS	0.232	NS	0.513	NS	71.765
Elevated ALT ≥ Grade 3	20	5754	105	0.690	NS	0.86	NS	ID	ID	62.729
Constipation ≥ Grade 3	22	5650	20	0.999	NS	0.518	NS	0.999	NS	0.520
Liver Toxicity ≥ Grade 3	20	11508	243	0.276	NS	0.582	NS	ID	ID	83.777
CONSTITUTIONAL										
Pyrexia ≥ Grade 3	22	5139	36	0.174	NS	0.035	Yes	0.027	Yes	60.354
Fatigue ≥ Grade 3	32	7272	225	0.551	NS	0.239	NS	0.883	NS	56.331
Headache ≥ Grade 3	14	4225	30	0.908	NS	0.954	NS	0.911	NS	34.930
OTHER										
Peripheral Neuropathy ≥ Grade 3	22	2985	107	0.000	Yes	0.015	No	ID	ID	81.545
ILD ≥ Grade 3	12	3906	21	0.355	NS	0.428	NS	ID	ID	0.000
Ocular Toxicity ≥ Grade 3	6	807	50	1.000	NS	0.519	NS	1.000	NS	0.000
Hypokalemia ≥ Grade 3	14	2513	56	0.903	NS	0.624	NS	0.849	NS	43.456

ID = insufficient data to evaluate interaction, NS = not significant, ILD = interstitial lung disease, ALT = alanine transaminase, AST = aspartate aminotransferase

p<0.05 Expected direction
p<0.01
P<0.05 Unexpected direction
p<0.01

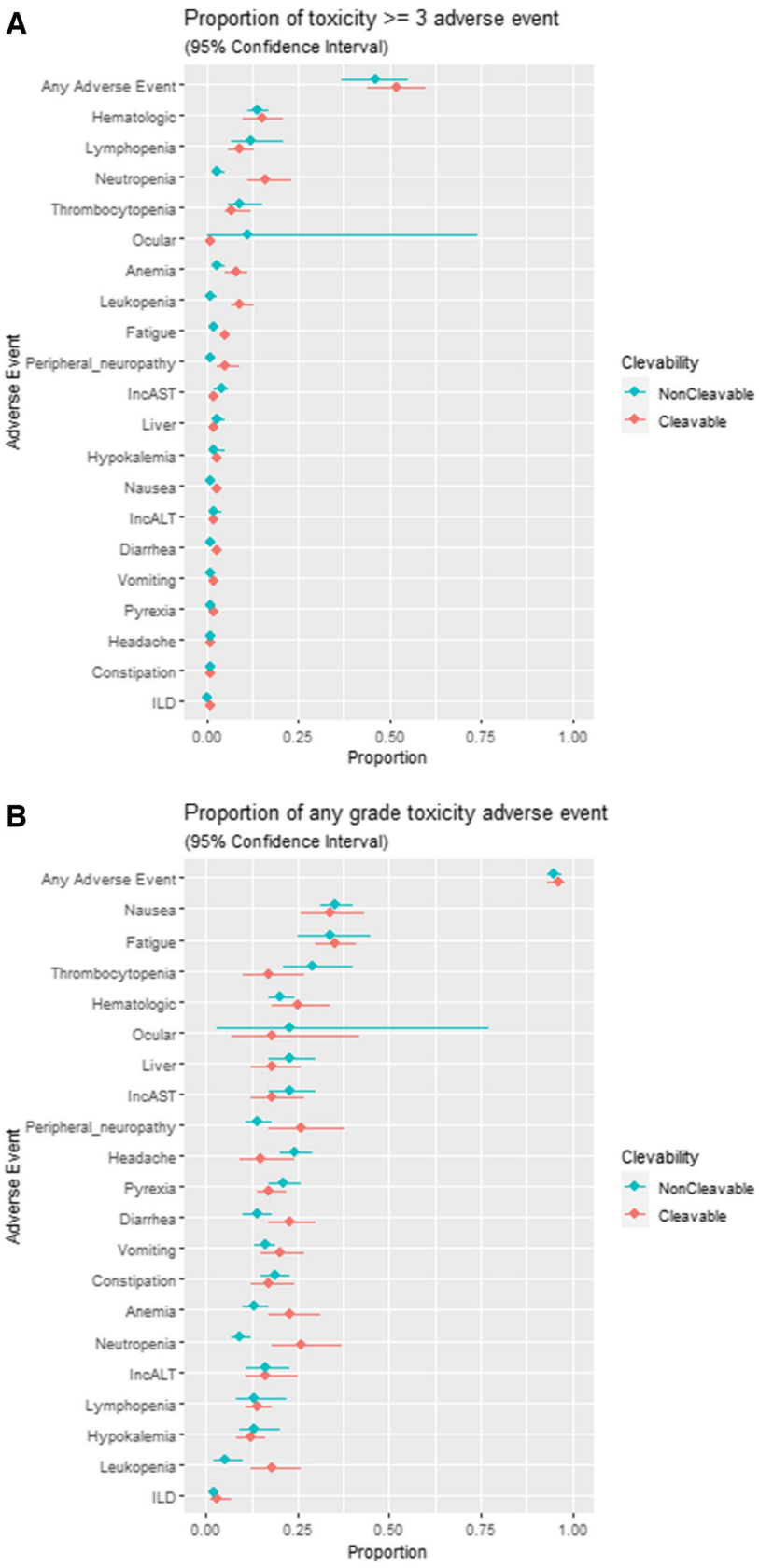

Fig. 2 A. Proportion of patients experiencing each of the 21 toxicities ≥ grade 3 with 95% confidence interval. ILD = interstitial lung disease, IncAST = increased aspartate aminotransferase, IncALT = increased alanine aminotransferase. **B**: Proportion of patients experiencing each of the 21 toxicities any grade with 95% confidence interval. ILD = interstitial lung disease, IncAST = increased aspartate aminotransferase, IncALT = increased alanine aminotransferase

non-cleavable linkers when adjusted for systemic free payload concentrations. Similarly, higher systemic free payload concentrations were significantly and independently associated with higher probability of anemia ($p = 0.022$) and pyrexia ($p = 0.035$). For any grade toxicities (Table 6), non-cleavable linkers remain independently associated with lower peripheral neuropathy ($p = 0.025$) but no other toxicity when adjusted for systemic free payload concentration. The main effect of higher systemic free payload concentration was significantly associated with increased probability for any toxicity ($p = 0.043$), anemia ($p = 0.028$), lymphopenia ($p = 0.005$), nausea ($p < 0.001$), vomiting ($p = 0.006$), constipation ($p = 0.041$), and interstitial lung disease ($p < 0.001$) after adjustment for linker type.

Again the I[2] statistics tend to be > 50% indicating at least moderate to high levels of heterogeneity in probabilities of the toxicities between studies.

ADC linker type, DAR, and free payload concentration As noted, linker-type, DAR, and systemic free payload concentration are not necessarily independent varying characteristics for each ADC. To further investigate this, we described the relationships between cleavability type, DAR, and measured systemic free payload concentration for the 11 FDA-approved ADCs under evaluation in this study. These characteristics are summarized in Table 1. Non-cleavable linkers have a numerically lower mean estimated DAR (3.75 ± 0.35 versus 4.78 ± 2.18, $p = 0.544$) and lower estimated systemic free payload concentration (1.89×10^{-5} $m^2/L \pm 1.41 \times 10^{-5}$ m^2/L versus 1.62×10^{-3} $m^2/L \pm 3.64 \times 10^{-3}$ m^2/L, $p = 0.567$). These differences are not statistically different, likely due to the small number of agents being compared (2 non-cleavable and 9-cleavable). However, the higher measured systemic free payload concentrations were significantly associated with higher DARs ($p = 0.043$) as depicted in Fig. 3.

4 Discussion

In this review and meta-analysis, we sought to delineate how features of ADC design, including linker cleavability, DAR, and systemic free payload concentration, may contribute to their associated systemic toxicities. The results support the hypothesis that ADCs with cleavable linkers are associated with more systemic toxicities than those with non-cleavable

Table 6 Toxicity Any Grade by Cleavability of Linker, Systemic Free Payload Concentration, and interaction of Cleavability * Systemic Free Payload Concentration

	# Studies Reporting	# Patients At Risk	# Patients With Toxicity	Cleavability Association (p-value)	Noncleavable = Lower Toxicity	Free payload Agent Concentration	Lower Sysmetic Free Payload Concentration = Less Toxicity	Cleavability* Sysmetic Free Payload Concentration (p-value)	Interaction Moderating Association	I2 (%)
Any AE	26	6448	6094	0.905	NS	0.043	Yes	0.908	NS	91.235
HEMATOLOGIC										
Neutropenia	26	3869	912	0.762	NS	0.051	NS	0.495	NS	92.730
Leukopenia	15	2471	367	0.993	NS	0.078	NS	0.201	NS	89.662
Anemia	27	6732	1058	0.385	NS	0.028	Yes	0.129	NS	91.408
Hematologic	5	3575	972	0.559	NS	0.127	NS	ID	ID	91.962
Thrombocytopenia	21	6148	1371	0.191	NS	0.078	NS	0.656	NS	96.494
Lymphopenia	6	828	109	0.294	NS	0.005	Yes	ID	ID	0.000
GASTROINTESTINAL										
Nausea	36	7529	2788	0.656	NS	0.000	Yes	0.417	NS	93.544
Vomiting	29	6808	1264	0.180	NS	0.006	Yes	0.197	NS	90.726
Diarrhea	33	7221	1357	0.546	NS	0.605	NS	0.877	NS	94.425
Elevated AST	26	6542	1172	0.924	NS	0.938	NS	0.879	NS	95.092
Elevated ALT	22	6028	829	0.880	NS	0.757	NS	ID	ID	94.772
Constipation	23	5865	1107	0.755	NS	0.041	Yes	0.435	NS	91.433
Liver (all)	26	13084	2344	0.918	NS	0.932	NS	0.884	NS	97.613
CONSTITUTIONAL										
Pyrexia	23	5529	964	0.425	NS	0.242	NS	0.842	NS	80.858
Fatigue	33	7504	2608	0.118	NS	0.806	NS	0.109	NS	94.630
Headache	14	4466	1016	0.244	NS	0.099	NS	0.004	Yes	72.445
OTHER										
Peripheral neuropathy	23	3050	658	0.025	Yes	0.136	NS	ID	ID	93.436
ILD	12	1937	78	0.211	NS	0.000	Yes	ID	ID	0.000
Ocular (all)	9	1290	285	0.110	NS	0.658	NS	0.066	NS	97.013
Hypokalemia	14	1985	235	0.544	NS	0.928	NS	0.297	NS	82.609

AE = adverse event, ID = insufficient data to evaluate interaction, NS = not significant, ILD = interstitial lung disease, ALT = alanine transaminase, AST = aspartate aminotransferase

p<0.05 Expected direction
p<0.01
P<0.05 Unexpected direction
p<0.01

linkers. Interestingly, even though higher DAR was associated with higher grade ≥ 3 toxicity, the apparent protective effect of the non-cleavable linker persisted even after adjusting for DAR. However, we found that the association between non-cleavable linkers and lower toxicity was *not* observed after adjusting for systemic free payload concentration. This suggests that systemic free payload concentration is the main factor driving toxicity in ADC-treated patients.

Notably, trastuzumab deruxtecan (T-DXd) has a considerably higher systemic free payload concentration than the other agents studied here. T-DXd has a tetrapeptide cleavable linker that may make it more vulnerable to premature release of its payload. Such a prematurely released payload might explain why T-DXd may be effective in tumor control regardless of HER2 expression. Emerging clinical evidence supports this hypothesis. In a small trial of T-DXd in non-small cell lung cancer patients, the activity of T-DXd was shown to be independent of HER2 over-expression in HER3 + , 2 + , or 1 + tumors [47]. More recently, clinical trial data presented during the 2021 San Antonio Breast Cancer Symposium showed that T-DXd was active in breast cancer patients regardless of HER2 expression, including HER2 0 tumors [48].

By contrast, trastuzumab emtansine (T-DM1), another anti-HER2 ADC with a non-cleavable linker, has not been shown to have activity in patients with low HER2 tumor expression. This may partly explain why in the DESTINY-03 trial[35], T-DM1 (HER2 dependent) was shown to have lower efficacy compared to T-DXd (HER2 dependent and independent). Consonant with these observations, T-DM1 was also shown to have much lower systemic toxicity compared to T-DXd, likely because of the relative stability of the T-DM1 linker. We've previously proposed that the off-target effects observed with T-DM1 may be the result of payload released from lysed tumor cells [2]. It is tempting to speculate that for these reasons, ADCs with cleavable linkers may in general have higher anti-tumor efficacy albeit higher systemic toxicities.

Our results arise from a meta-analysis using multiple agents used to treat highly disparate patient populations with different malignancies. We suspect this explains most of the heterogeneity observed in probabilities of specific toxicities between studies reflected in the I [2] statistics. Nonetheless, differences in ADC chemical design are shown to be significantly associated with specific clinical toxicities despite this tremendous heterogeneity.

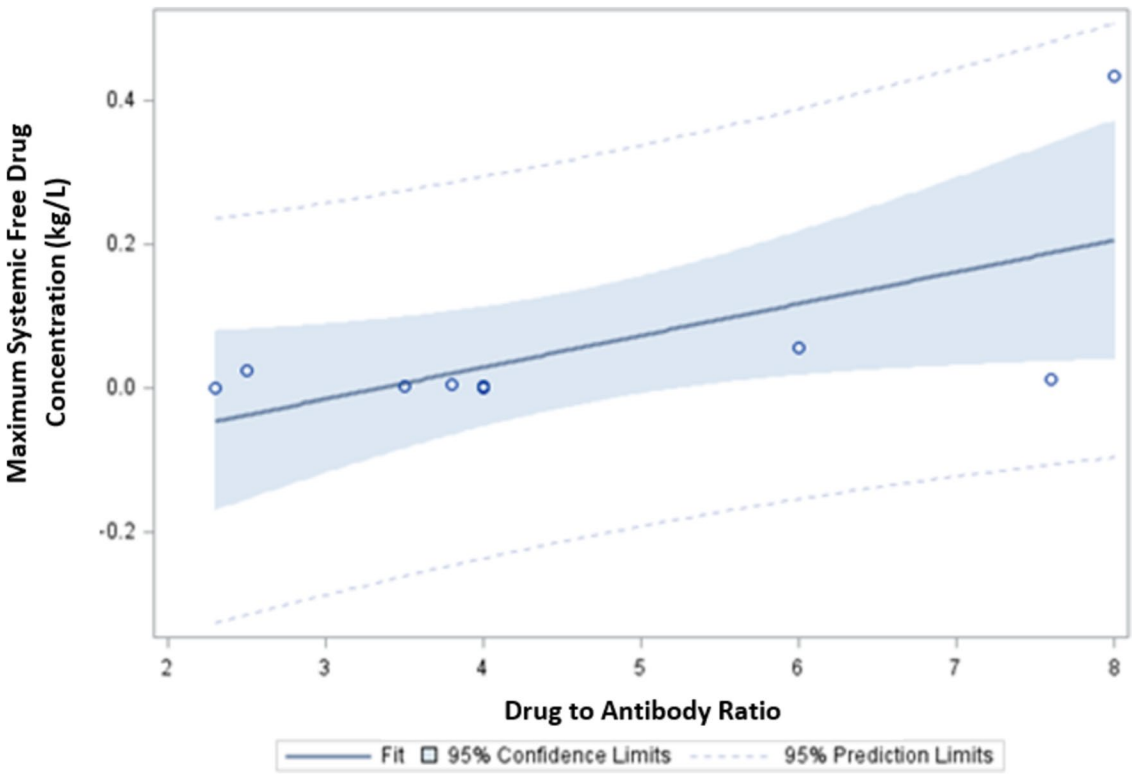

Fig. 3 Association between DAR and maximum systemic free payload concentration

5 Conclusion

In summary, ADCs are rapidly becoming the standard of care for patients across disease sites. The results here show that linker choice and the potential for premature payload release among ADCs can affect their systemic toxicity and efficacy. It will, therefore, be critical in the design of future ADCs to find the appropriate balance between the highest potential efficacy and associated systemic toxicities [49]. In this regard, contemporary studies are focused on the development of novel ADC linkers that can be released conditionally within the tumor microenvironment to increase both the specificity of drug delivery and anti-tumor efficacy. The ideal ADC design should aim for high therapeutic index that balances off-target toxicities, taking into consideration factors such as linker cleavability, DAR, and payload membrane permeability [50]. The results presented here suggest one critical consideration in achieving this balance during future ADC development for cancer patients will be linker design and the potential for premature payload release.

Author Contribution S.C.T. conceived of the idea and supervised the study design and manuscript writing. C.W., M.M., and R.P. assisted with data collection and manuscript writing. T.L., Y.Z., and W.H. designed the computational framework and analyzed the data. W.H. also assisted with manuscript writing. N.M. helped supervise the manuscript.

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Declarations

Conflict of Interest The authors declare no competing interests.

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