Abstract Number: 5810

The Impact of Scaffold, Linker, Homogeneity and Payload Selection on the Efficacy and Tolerability of Anti-Tubulin ADCs

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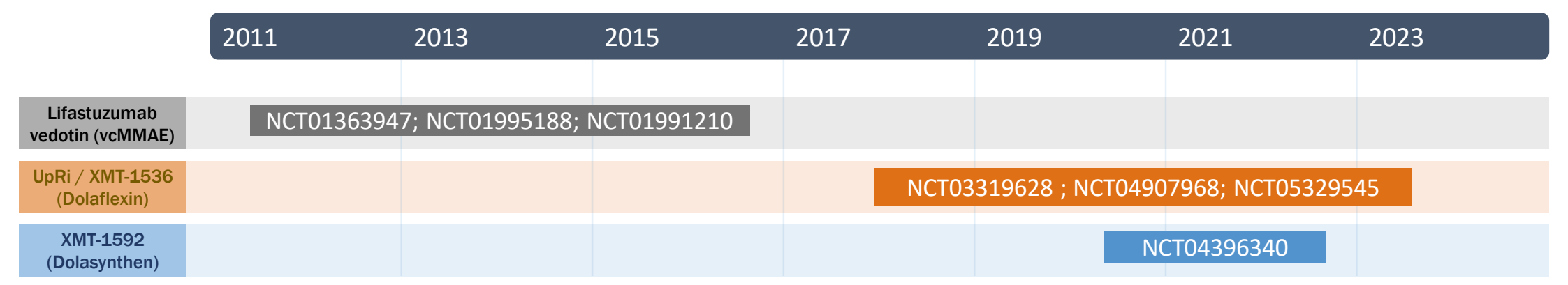
Abstract

Antibody-drug conjugates (ADCs) achieve targeted drug delivery to a tumor and have demonstrated clinical success in many tumor types. The properties of an ADC, including its activity and safety profile, can be highly influenced by its characteristics and individual components, commonly viewed as the antibody, payload, linker, and drug-to-antibody ratio (DAR). However, multiple other characteristics can also significantly influence an ADC's overall drug-like properties, including DAR homogeneity, method of bioconjugation, hydrophilicity, and charge balance. Due to the multiparametric nature of ADC platform optimization, there are few examples in preclinical research, and even fewer in the clinical realm, where comparisons across platforms have been investigated for the same target, both preclinically and clinically, to elucidate how deliberate modifications in molecular design can improve drug-like properties and clinical outcomes. To that end, here we describe the improvements observed in preclinical characteristics, as well as their translational relevance to corresponding clinical characteristics. Two NaPi2b ADCs were produced using two different platforms, Dolaflexin (XMT-1536) and Dolasynthen (XMT-1592), conjugated to the same antibody and employing the same payload. The Dolasynthen platform was deliberately designed, taking into account preclinical and clinical learnings from earlier ADC platforms, to improve both safety and efficacy.



Timeline of clinical trials with NaPi2b targeting ADCs. Lifastuzumab vedotin (vcMMAE) was the first ADC targeting NaPi2b to enter the clinic but was discontinued[1]. Upifitamab rilsodotin (XMT-1536) was the first ADC from Mersana to target NaPi2b delivering a differentiated payload demonstrated to avoid toxicities commonly associated with anti tubulin ADCs (neutropenia, peripheral neuropathy, ocular toxicity)[2]. XMT-1592 was Mersana's second NaPi2b targeting ADC, designed to further improve upon XMT-1536. Development of XMT-1592 was discontinued before completing the Phase 1 dose escalation due to portfolio reprioritization considerations[3]. Based on the efficacy not meeting prespecified primary endpoint criterion, development of XMT-1536 was discontinued[4].

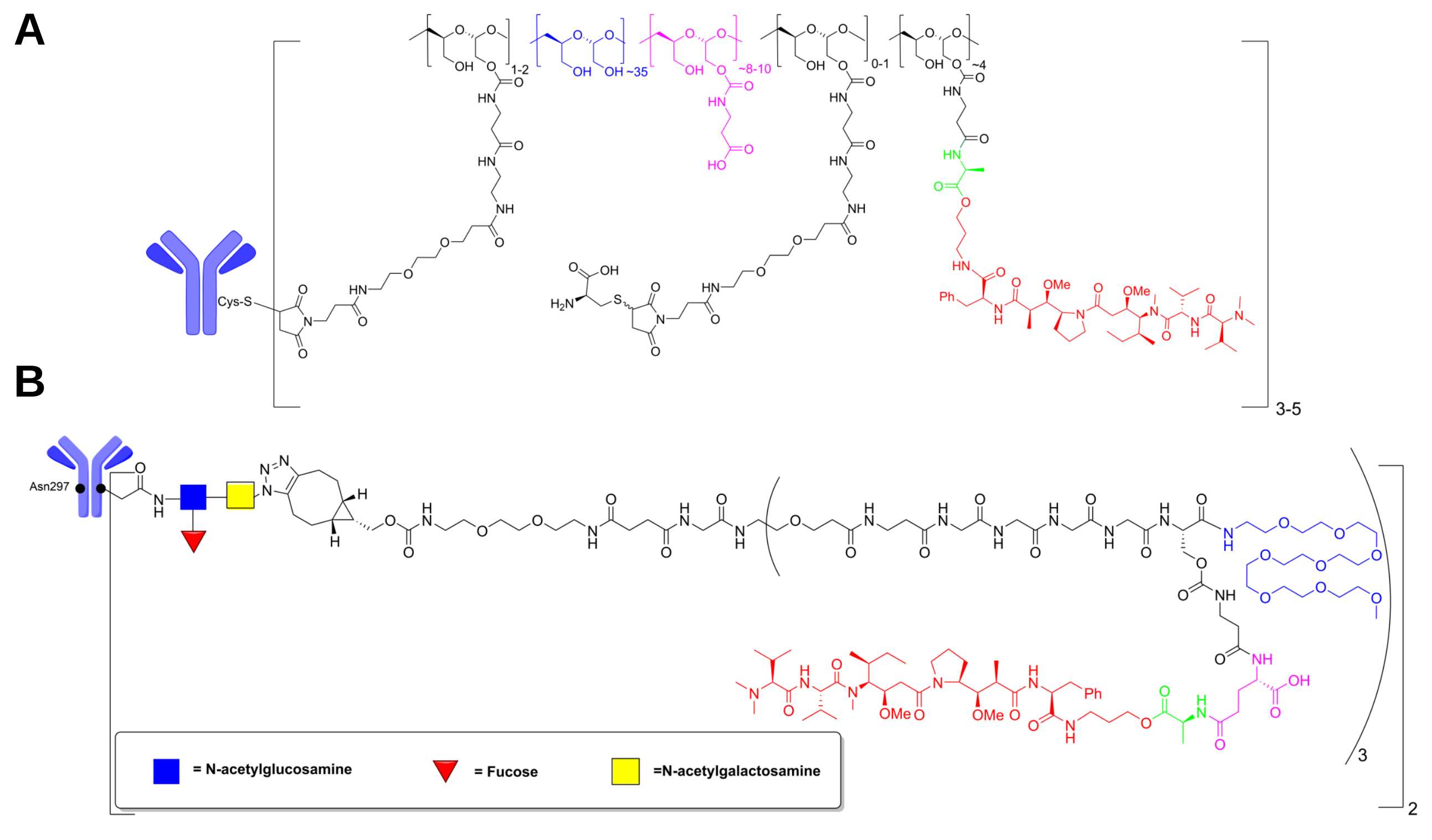


Figure 1. (A) XMT-1536: a heterogeneous Dolaflexin ADC that contains a hydrophilic polymer backbone (blue), β-alanine moieties (purple) and the alanine linker ester (green) with the number of AF-HPA payloads per scaffold defined as an average that, following conjugation to the antibody, generates an ADC with a range of DAR species.[5] **(B)** XMT-1592: a homogeneous Dolasynthen ADC that includes a fully synthetic, well-defined scaffold with a specific and defined DAR.[6]

	XMT-1536	XMT-1592
Platform	Dolaflexin	Dolasynthen
Generation	1st generation cytotoxic ADC	2nd generation cytotoxic ADC
mAb	NaPi2b targeting mAb (XMT-1535)	
Payload	AF-HPA payload with controlled bystander effect; highly potent anti-tubulin inhibitor selectively toxic to rapidly dividing cells and designed to avoid dose-limiting neuropathy or neutropenia	
Bioconjugation Method	Stochastic (random)	Site-specific
Bioconjugation Chemistry	Maleimide conjugation to native cysteine	Click chemistry / irreversible
DAR	Heterogeneous DAR ~10	Homogeneous DAR 6
Fcγ receptor binding	Fully intact	Significantly reduced

Table 1. Similarities and differences of XMT-1536 & XMT-1592. The goals for Dolasynthen as the next-generation platform included**:** DAR customization for target, antibody-like PK, enhanced tumor payload delivery, increased efficacy, reduced platform toxicity and expanded therapeutic index.

ADC, antibody-drug conjugate; AF-HPA, Auristatin F hydroxypropyl amide; DAR, drug-to-antibody ratio; Fcγ, fragment crystallizable gamma receptor

Preclinical Efficacy: XMT-1536 vs. XMT-1592

OVCAR-3 CDX

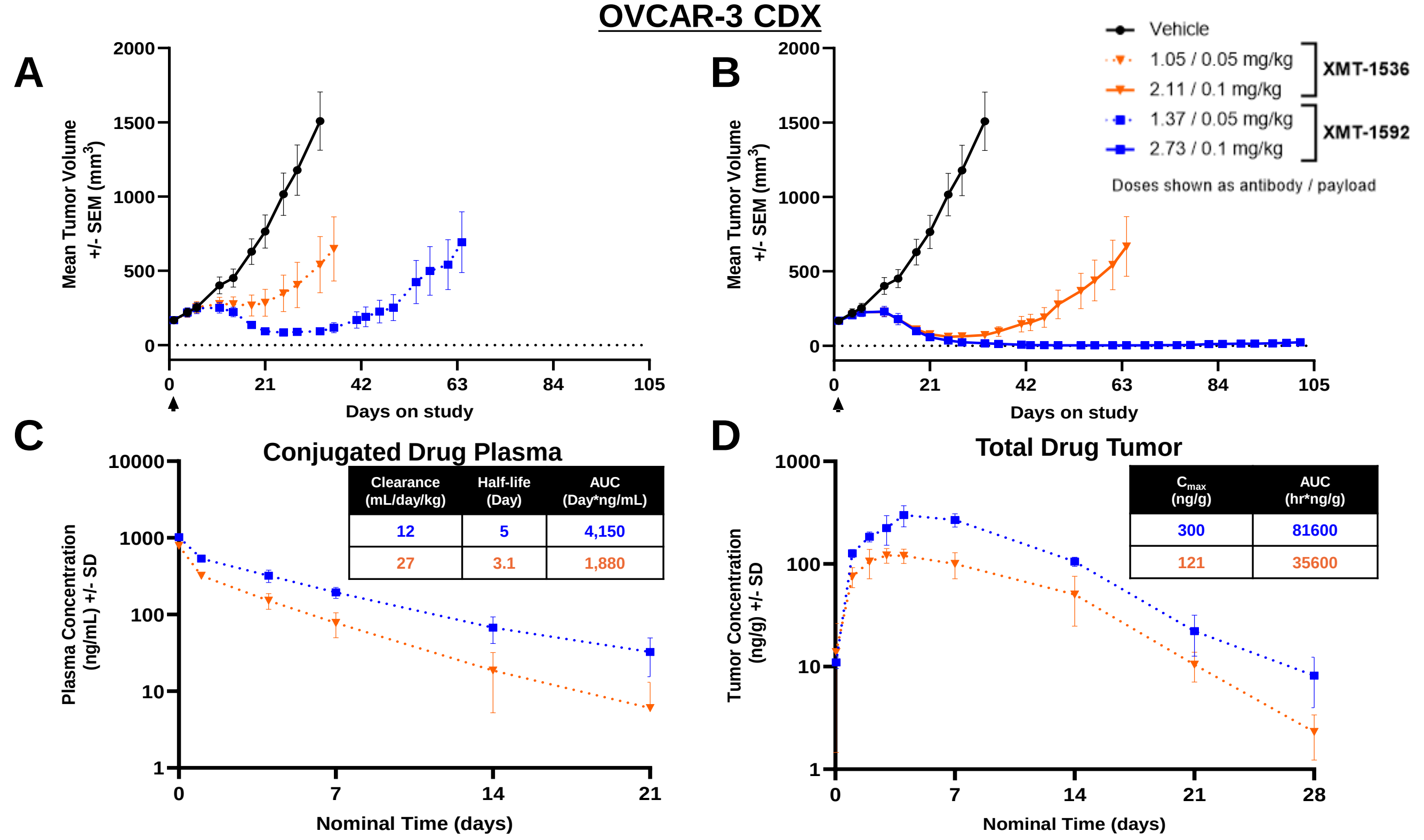


Figure 2. XMT-1592 demonstrates ~2-fold greater potency in vivo and an improved PK profile in the OVCAR-3 cell-line derived xenograft (CDX) model at matched payload doses. Mice (n=8/group) were given a single IV injection of XMT-1592 or XMT-1536 at **(A)** 0.05 mg/kg or **(B)** 0.1 mg/kg matched payload doses and tumor growth was monitored over time. **C.** From the same study as in **(A)**, plasma was collected over 21 days (n=4/group/timepoint) and conjugated drug was measured. **D.** A separate cohort of OVCAR-3 tumor-bearing mice were treated with a single IV dose of 0.05 mg/kg XMT-1592 or XMT-1536, tumors were harvested over 28 days (n=4-5 /group/timepoint), and total drug was measured.

Total drug = conjugated payload + free payload + active metabolite*(MW payload/MW active metabolite)

CTG-0852 NSCLC PDX

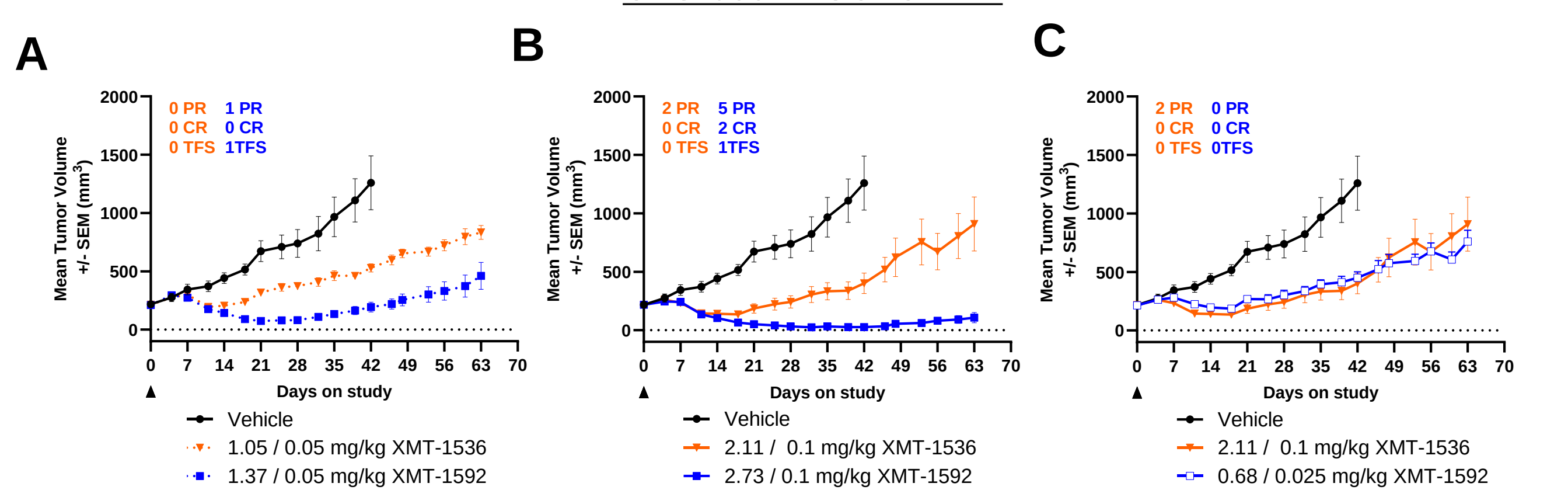


Figure 3. XMT-1592 exhibits ~4-fold greater potency in CTG-0852, a NSCLC patient-derived xenograft (PDX) model at matched payload doses. Mice (n=8/group) were given a single IV injection of XMT-1592 or XMT-1536 at **(A)** 0.05 mg/kg or **(B)** 0.1 mg/kg matched payload doses and tumor growth was monitored over time. **C.** A single IV injection of XMT-1592 at 0.025 mg/kg results in comparable efficacy as 0.1 mg/kg XMT-1536, a 4-fold higher dose.

PR: Partial responders, CR: complete responders, TFS: tumor-free survivors. Doses shown as antibody / payload.

Preclinical Safety: XMT-1536 vs. XMT-1592

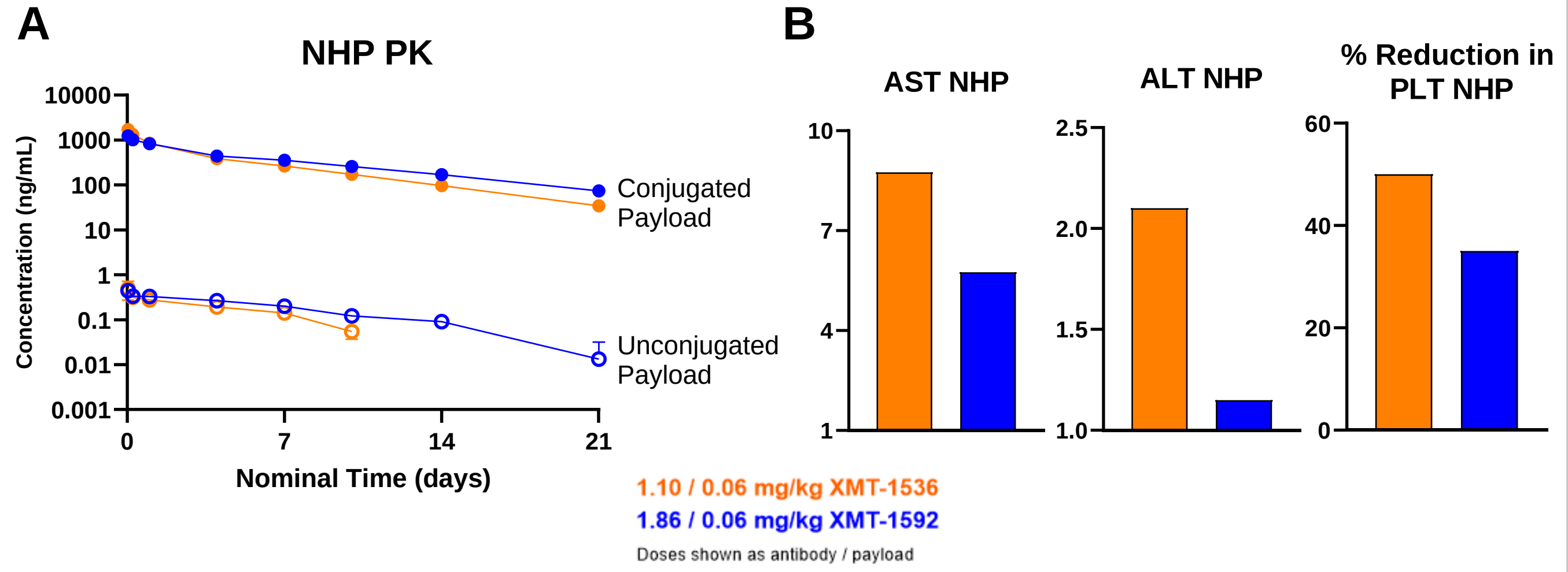


Figure 4. XMT-1592 demonstrated improvements in preclinical safety studies. Non-human primates were dosed at equal payload dose of 0.06 mg/kg of XMT-1592 or XMT-1536. **(A)** Conjugated payload and unconjugated payload, plasma PK following a single dose. **(B)** Comparison of key clinical pathology parameters associated with platform toxicity between XMT-1536 and XMT-1592.

AST: Aspartate amino transferase. ALT: Alanine amino-transferase. PLT: Platelets. Y-axis values are fold change vs baseline.

Dolaflexin (XMT-1536) Clinical Experience

UPLIFT (ENGOT-ov67/GOG-3048): Study Design[4]

Patient Population

- HGSOC[a] progressing after standard treatments; measurable disease per RECIST v1.1; ECOG PS 0 or 1; enrolling regardless of NaPi2b expression

Key Inclusion Criteria

- Platinum-resistant[b] ovarian cancer
- 1–4 prior lines of therapy
- Grade ≤2 peripheral neuropathy
- Available archived or fresh tissue for retrospective NaPi2b evaluation

Key Exclusion Criteria

- 1–2 prior lines AND bevacizumab-naive
- Primary platinum-refractory disease

UpRi
36 mg/m²
up to
max 80 mg;
IV q4w

Primary Endpoint

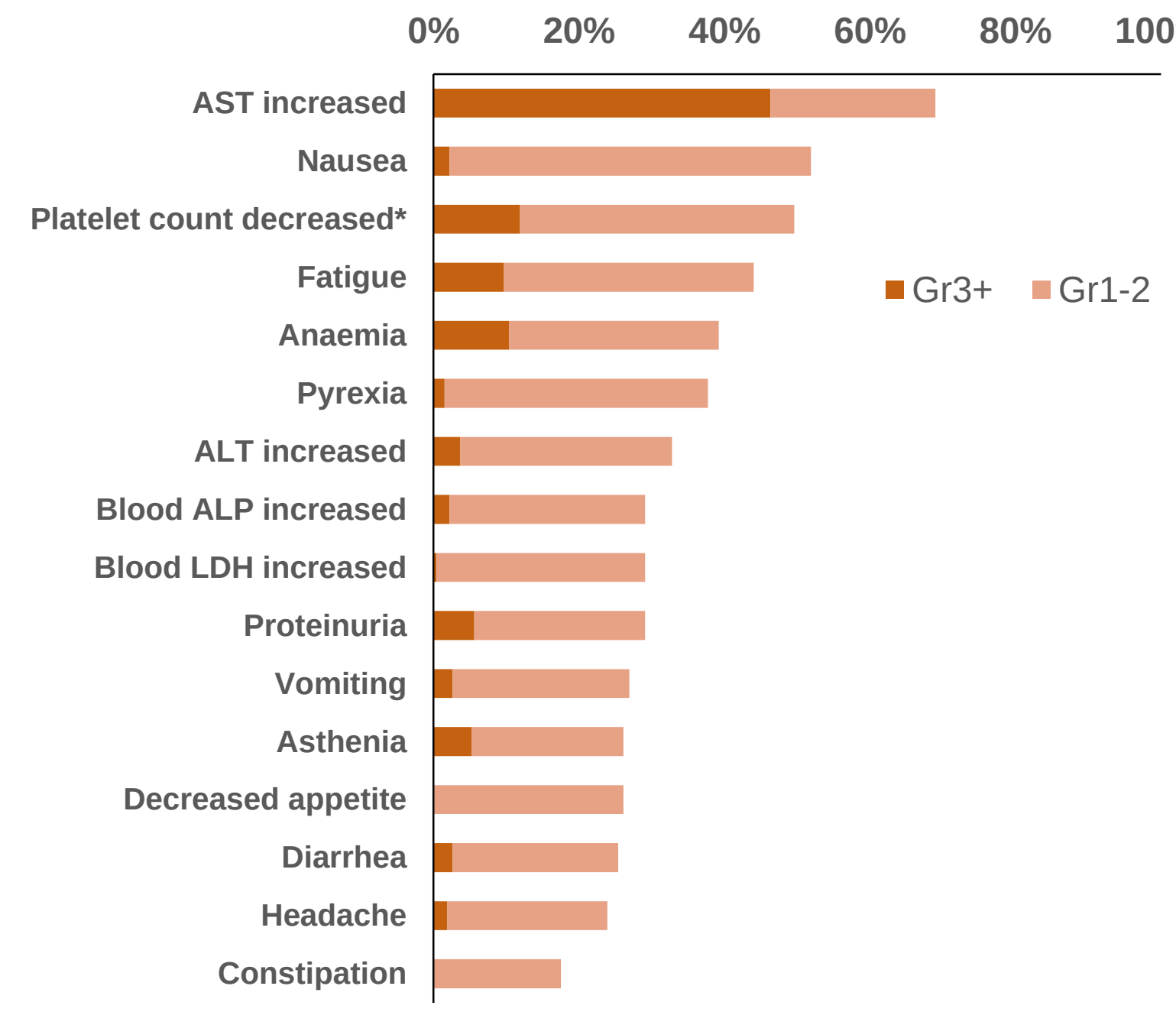
- Confirmed INV-assessed ORR in NaPi2b-positive (TPS ≥75)

Secondary Endpoints

- Confirmed INV-assessed ORR in overall population
- Confirmed ORR by BICR in the NaPi2b positive and in the ITT population
- INV-assessed DOR in the NaPi2b positive population
- Safety

	NaPi2b-Positive Population (TPS ≥75)	ITT Population
N	141	268
ORR[a], n (%);	22 (15.6%)	35 (13.1%)
Two-sided 95% CI	10.0%, 22.7%	9.3%, 17.7%
CR, n (%)	2 (1.4%)	3 (1.1%)
PR, n (%)	20 (14.2%)	32 (11.9%)
DCR[b]	93 (66.0%)	157 (58.6%)
Median DOR , Months	7.4	7.4
Two-sided 95% CI	4.2,NR	3.6, 10.4

Table 2. Investigator-Assessed Objective Response Rate in NaPi2b Positive, and ITT Population



- Gr3 peripheral neuropathy and neutropenia occurred in <1%; no G3+ ocular toxicity.
- Pneumonitis occurred in 9.7% of patients, 0.7% Gr3 (no G4 or G5); believed to be on-target toxicity due to NaPi2b expression on Type II pneumocytes in the lungs
- Based on aggregate analysis of bleeding cases, treatment emergent G3+ hemorrhage occurred in 5.6% of patients, including 5 G5 (fatal) cases
- Hypothesize that the high-DAR sub-population within UpRi disproportionately delivered payload to endothelial cells, resulting in off-target toxicities, including thrombocytopenia and bleeding

Figure 5. Treatment Related Adverse Events (TRAEs) observed in ≥15% of patients (N=268)

[a] HGSOC including fallopian tube and primary peritoneal cancer. [b] Platinum-resistant is defined as disease that has progressed within 6 months of last dose of platinum. DoR, duration of response; ECOG, Eastern Cooperative Oncology Group; HGSOC, high-grade serous ovarian cancer; ORR, overall response rate; q4w, every 4 weeks; RECIST, Response Evaluation Criteria in Solid Tumors; UpRi, upifitamab rilsodotin; TPS, Tumor Proportion Score. [a]ORR is determined by investigator radiologic review and defined as the proportion of patients who achieve a confirmed CR or PR per RECIST v1.1.The exact two-sided 95% CI is calculated based on binomial distribution using the Clopper-Pearson method; [b]DCR is defined as the proportion of patients who achieve a confirmed CR, PR, or SD.NR, Not Reported.

Dolasynthen has a More Stable DAR Profile

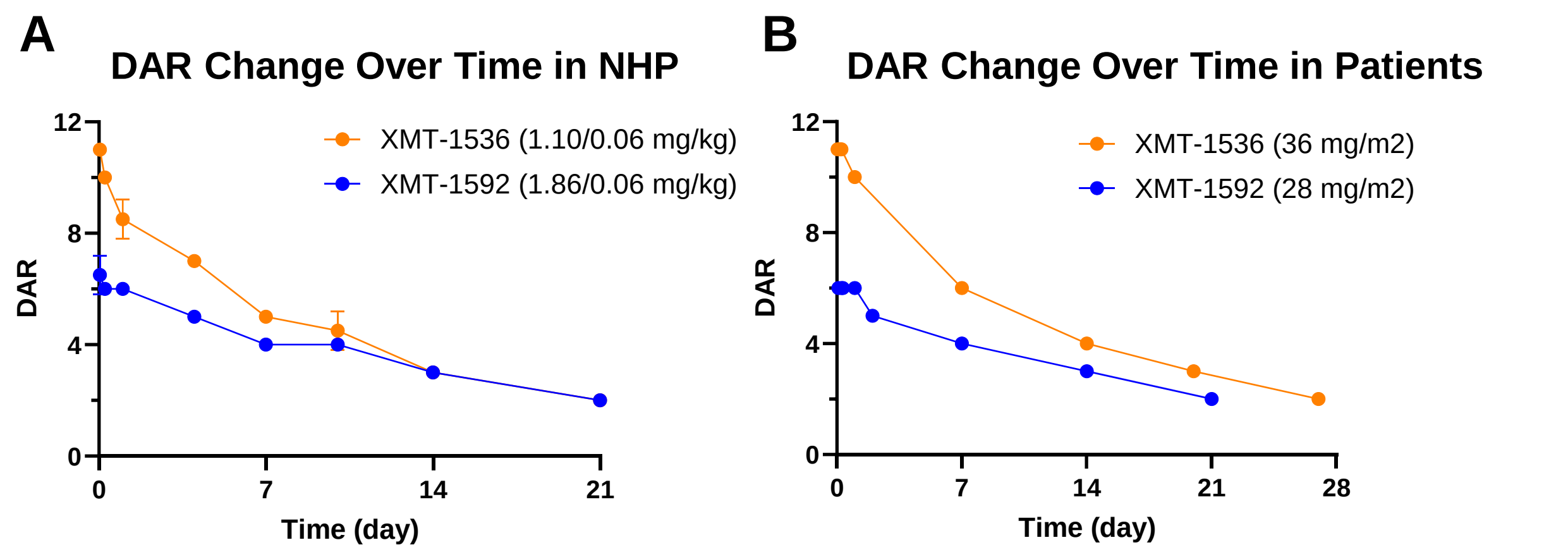


Figure 6. Plots show DAR change over time in NHP following the first dose in a repeat dose NHP study **(A)** and in patients following the first dose of either XMT-1536 at 36 mg/m² or XMT-1592 at 28 mg/m² **(B)**. Rapid decrease of XMT-1536 average DAR is likely not due to payload deconjugation (free payload levels remain low; data not shown). It is hypothesized that the high DAR species within XMT-1536 are cleared more rapidly, leading to a reduction in DAR over time.

DAR = [Concentration (Conjugated payload)/MW(payload)]/[Concentration (Total Ab)/MW(mAb)]

Dolasynthen (XMT-1592) Clinical Experience

XMT-1592 Phase 1 Dose Escalation Trial Design[3]

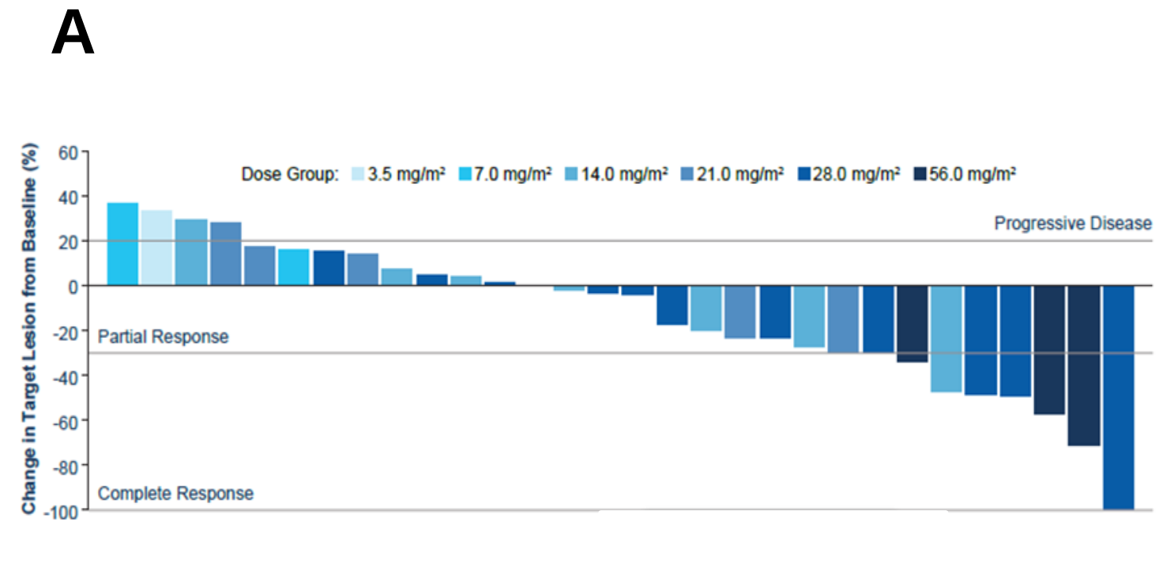
DL1 q3w	DL2 q3w	DL3 q3w	DL3.5 q3w	DL4 q3w	DL5 q3w
3.5 mg/m²	7 mg/m²	14 mg/ m²	21 mg/ m²	28 mg/m²	56 mg/m²
N = 1	N = 2	N=4	N = 5	N = 11	N = 4

DL3 q4w
14 mg/ m²
N=4

- Enrolled patients with OC with histological diagnosis of high-grade serous epithelial ovarian, fallopian tube, or primary peritoneal cancers; non-squamous NSCLC
  - Measurable disease by RECIST 1.1
  - ECOG performance status 0 or 1
  - Available archival tumor tissue blocks, or freshly cut tissue slides for retrospective NaPi2b testing
- Dosing: XMT-1592 administered IV every 3 or 4 weeks
- Primary objectives: MTD or RP2D; safety and tolerability
- Escalation design: Modified version of the Simon accelerated titration design

	All Patients All Dose Levels	Ovarian Cancer ≥28 mg/m²
Patients, N	30[e]	13[e]
ORR, n (%)	5 (17%)	4 (31%)
SD, n (%)	19 (63%)	7 (54%)
DCR, n (%)	24 (80%)	11 (85%)
Median DOR, months	7.9	7.9

Table 3. Confirmed best overall response regardless of NaPi2b Expression



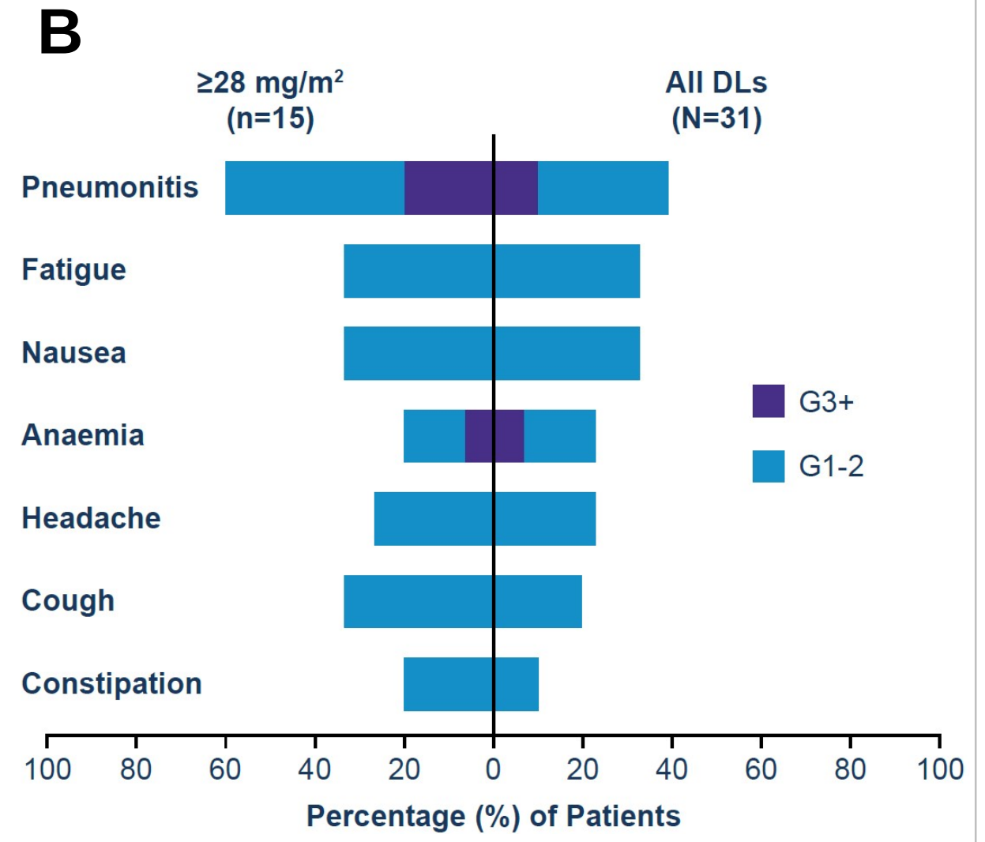


Figure 7.
(A) Efficacy – Best percent change from baseline in target lesions in patients dosed with XMT-1592 (N=30, all dose-levels). Waterfall plot of evaluable patients regardless of NaPi2b expression.
(B) Safety TRAEs in ≥15% of patients dosed with XMT-1592. TRAEs were mostly low grade.

No severe peripheral neuropathy, neutropenia, or ocular toxicity, which are associated with other anti-tubulin ADCs[2].
No thrombocytopenia or treatment-related bleeding events, in contrast to XMT-1536.
Pneumonitis observed (including 1 G5) likely an on-target toxicity based on NaPi2b expression in Type II pneumocytes.

DL, dose level; ECOG PS, Eastern Cooperative Oncology Group performance status; NSCLC, non-small cell lung cancer; OC, ovarian cancer; qw, every week; RECIST, Response Evaluation Criteria in Solid Tumors; RP2D, recommended phase 2 dose.
e Evaluable patients; excludes one patient at 56 mg/m² who was non-evaluable by RECIST due to withdrawal of consent before first scan.
CR, complete response; DCR, disease control rate; DOR, duration of response; NaPi2b, sodium-dependent phosphate transporter 2b; ORR, objective response rate; SD, stable disease

Clinical Comparisons. Dolaflexin vs Dolasynthen

Treatment-Related AEs		First Generation XMT-1536 (n=268)[d] 36mg/m² N (%)		Next Generation XMT-1592 (n=31) All Dose Levels N (%)	
		All grades	Grade 3 ≥	All grades	Grade 3 ≥
Presumed off-target platform toxicities	**AST Elevation N (%)**	185 (69.0%)	124 (46.3%)	3 (9.7%)	0
	Platelet Count Decrease / Thrombocytopenia N (%)	133 (49.6%)	32 (11.9%)	0	0
	Nausea N (%)	139 (51.9%)	6 (2.2%)	10 (32.3%)	0
	Fatigue N (%)	118 (44.0%)	26 (9.7%)	10 (32.3%)	0
Presumed on-target toxicity	**Pneumonitis N (%)**	26 (9.7%)	2 (<1%)	12 (38.7%)	3 (9.7%)

Table 4. Comparison table between XMT-1536 and XMT-1592 TRAEs. Based on two independent studies; select AEs. XMT-1592 had reduced incidence of off-target/platform AE's toxicities compared to XMT-1536; however, the incidence of pneumonitis, considered on-target due to NaPi2b expression on Type II pneumocytes, was increased with XMT-1592.

[d] Data cut: May 31, 2023 (UPLIFT: Phase 2 trial).AST, aspartate aminotransferase; G, grade; TRAE, treatment-related adverse event

Subsequent Investigative Studies

A biodistribution study was conducted in male Sprague Dawley rats to investigate potential differences in exposure to payload. Rats were administered a single IV 9 mg/kg mAb dose of XMT-1536 or XMT-1592 on Day1 and sampled to Day 29. Lung tissue from each rat was divided, half for bioanalysis, half for histology.

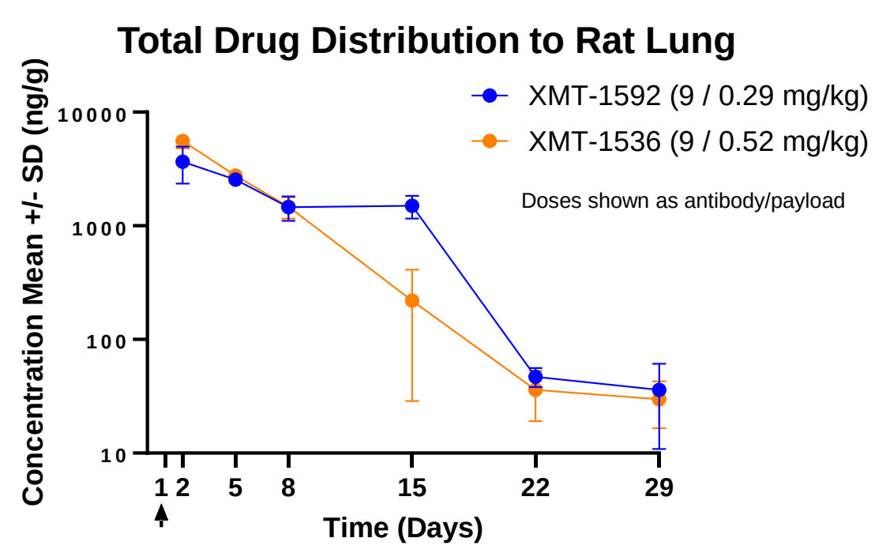


Figure 8. XMT-1536 & XMT-1592 Total drug distribution to rat lung. Distribution of total drug to the lungs based on bioanalysis of tissue homogenate were comparable between XMT-1536 and XMT-1592 dosed groups, despite XMT-1536 delivering a greater payload dose.

Total drug = conjugated payload + free payload + active metabolite*(MW payload/MW active metabolite)

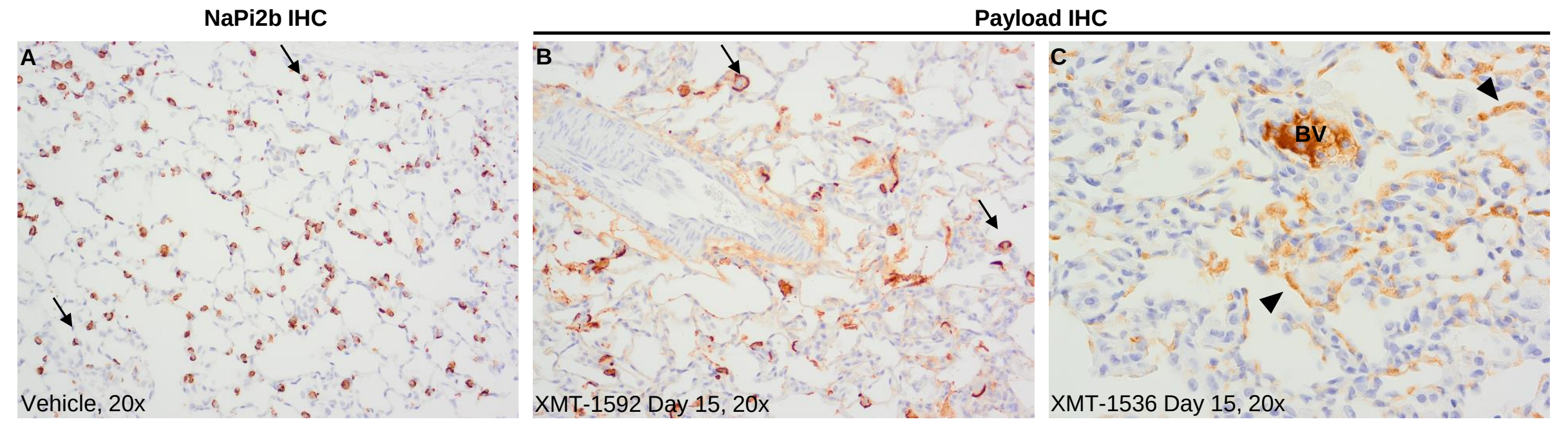


Figure 9. The Dolasynthen platform increased delivery of the payload to target expressing cells despite the lower payload dose with XMT-1592.
(A) NaPi2b is highly expressed in Type II pneumocytes (arrows), as illustrated by NaPi2b immunohistochemistry (IHC) in vehicle-treated rat lung.
(B) Using an anti-payload antibody, staining of drug in the lungs of animals administered XMT-1592 revealed a pattern of expression consistent with co-localization of drug in cells expressing the NaPi2b target (Type II pneumocytes, arrows).
(C) Payload IHC in the lungs of rats administered XMT-1536 revealed less staining in Type II pneumocytes, consistent with less lung-specific pathology. For XMT-1536, payload was primarily within blood vessels (BV) including capillaries (arrowheads).

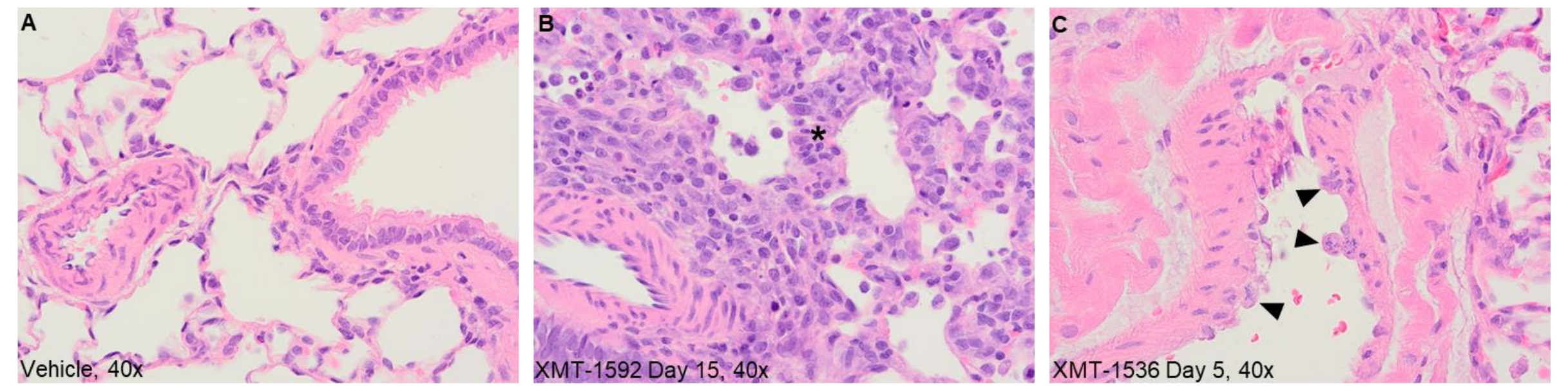
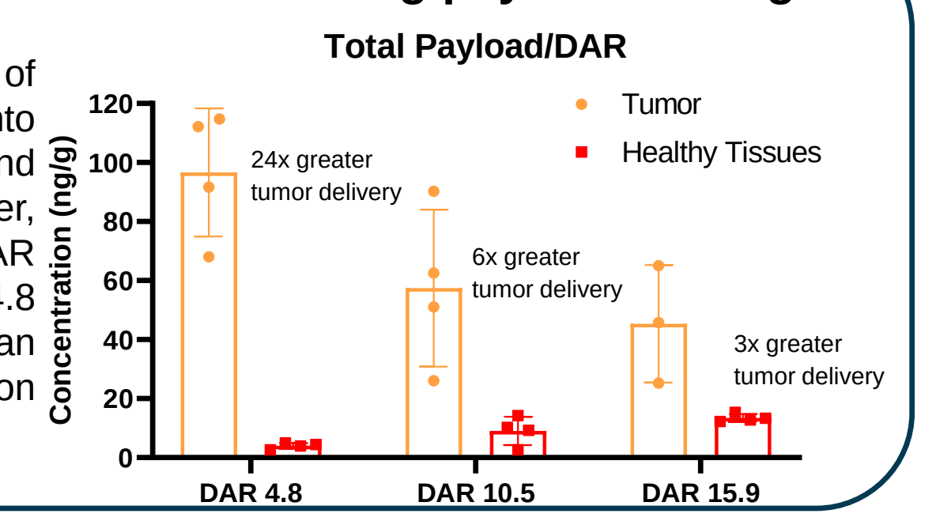


Figure 10. Presence of payload in Type II pneumocytes preceded alveolar histopathology findings in H&E-stained lung sections and correlated with the presence of lung injury, which was considered NaPi2b-target related.
(A) The alveolar walls of vehicle-control rats were thin, with minimal cells or debris within alveolar spaces.
(B) Rats administered XMT-1592 had significant thickening of alveolar walls (*) and cells and/or debris within alveolar lumina.
(C) Rats administered XMT-1536 had histologic findings that were focused on endothelial cells (arrowheads) and manifested earlier than alveolar findings, which were observed with XMT-1592.

High-DAR Dolaflexin sub-populations are less effective at delivering payload to Target

Figure 11. JIMT-1 Tumor bearing mice were dosed with 3 mg/kg of HER2-targeting Dolaflexin ADC that had been fractionated into subpopulations of different DAR. At 168hrs, mice were euthanized and perfused with saline prior to sampling of tumor and healthy tissues (liver, spleen, kidney) for bioanalysis of total payload. The plot of DAR normalized concentration in tumor vs healthy tissues depicts the DAR 4.8 sub-population delivering 24x greater payload to tumor tissues than healthy tissues combined; contrastingly the DAR 15.9 sub population delivers only 3-fold more payload to tumor than healthy tissues.



Summary

Dolasynthen is Mersana's 2nd generation ADC platform with demonstrable benefits over the 1st generation Dolaflexin platform as well as other ADC platforms.

- XMT-1592 (Dolasynthen) had an improved preclinical profile vs. XMT-1536 (Dolaflexin).
- Clinically, XMT-1592 (Dolasynthen) differed from XMT-1536 (Dolaflexin) with reduced platform-related TRAEs, but with increased incidence of pneumonitis, likely an on-target toxicity based on NaPi2b expression in Type II pneumocytes.

Subsequent analysis and investigative studies show that:

- XMT-1592 (Dolasynthen) shows a more stable DAR profile whereas XMT-1536 (Dolaflexin) has a rapid decrease in DAR over time, hypothesized to be due to faster clearance of high DAR species from the plasma.
- High DAR sub-populations of Dolaflexin are less effective at delivering payload to target.
- XMT-1592 (Dolasynthen) has increased efficiency delivering payload to NaPi2b-expressing Type II pneumocytes than XMT-1536 (Dolaflexin).
- These findings are consistent with the different clinical profiles of XMT-1536 (Dolaflexin) and XMT-1592 (Dolasynthen).

Histologic findings in Type II pneumocytes in animal models have not been seen with multiple other Dolasynthen ADCs.

Further efforts to target NaPi2b with cytotoxic ADCs should carefully consider target expression on Type II pneumocytes and the potential for dose-limiting lung toxicity.

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