TOPO1i ADC Platform from Concept to Pipeline Application

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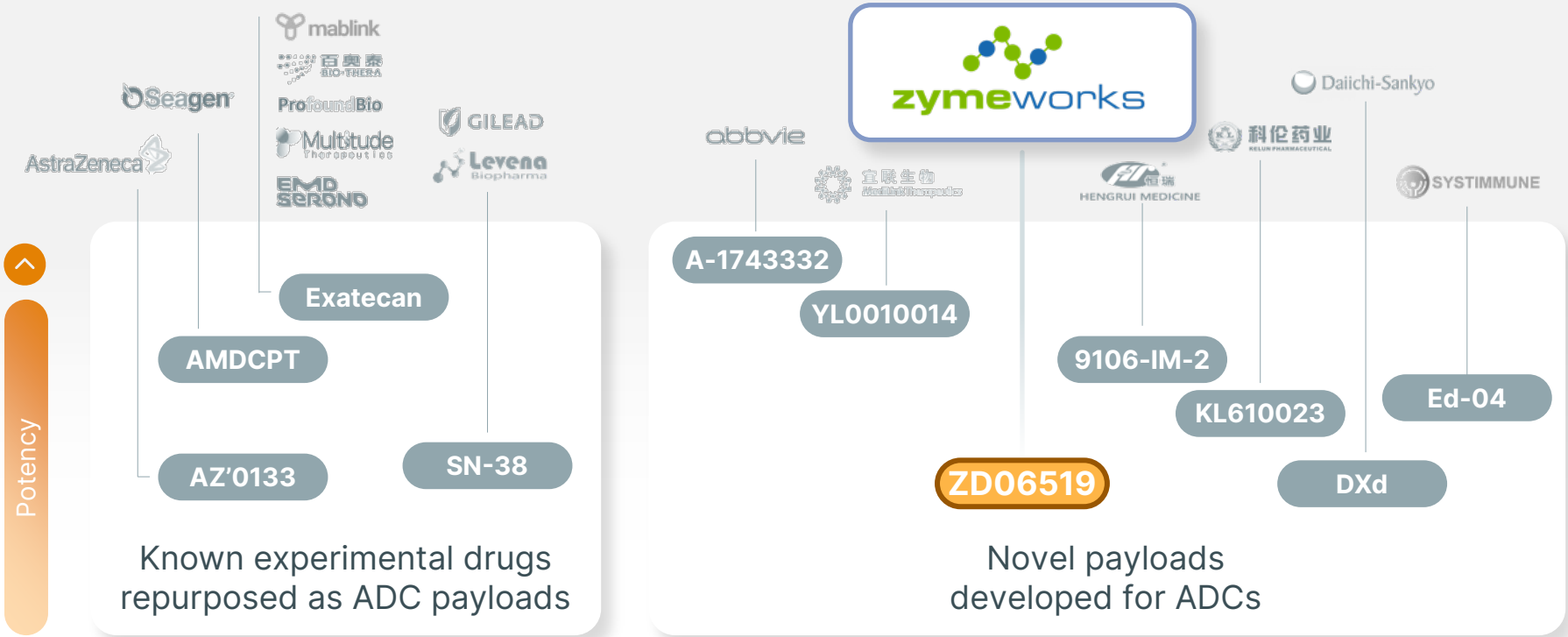
Festival of Biologics Basel 2023

Zymeworks Novel Camptothecin Payload was Selected with ADCs in Mind



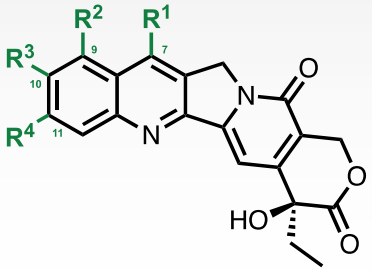
Design of novel payloads enables incorporation of properties tailored for ADC mechanism

Platform Design Criteria Draw on Validated ADC Technologies

PAYLOAD

Novel camptothecin with moderate potency and strong bystander activity

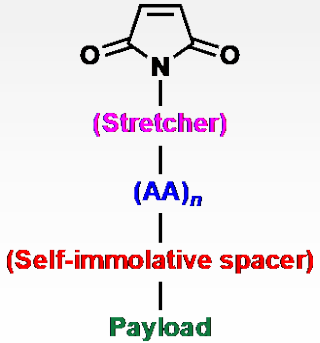
- Acknowledges complex mechanisms driving TOPO1i ADC action
- Sufficient tolerability to achieve ADC dose > 5 mg/kg



LINKER

Traceless, plasma-stable, cleavable peptide

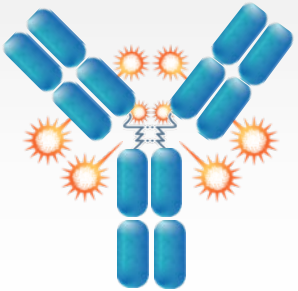
- Common to majority of approved ADCs
- Compatible with desired bystander activity



CONJUGATION

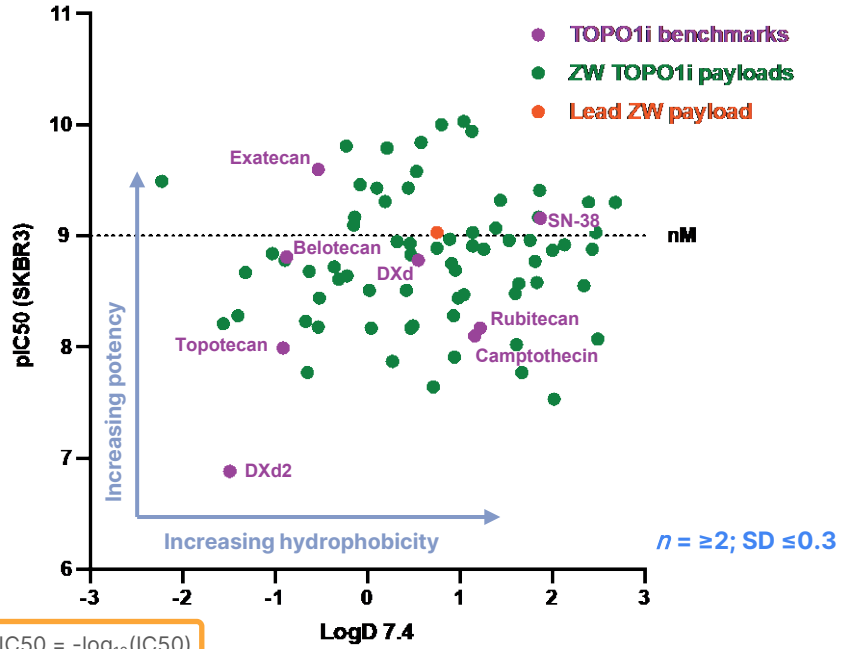
Thiol-maleimide chemistry

- Stochastic conjugation utilized in *all* approved ADCs
- Facilitates DAR optimization
- Good balance of stability, safety, and anti-tumor activity



Evaluation of Payloads Enable Selection of Drug-linker Panel for Conjugation

Payload selection driven by potency, hydrophobicity, and ADME characteristics

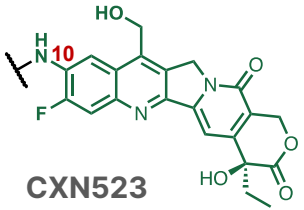


n = ≥2; SD ≤0.3

pIC50 = $-\log_{10}(IC50)$

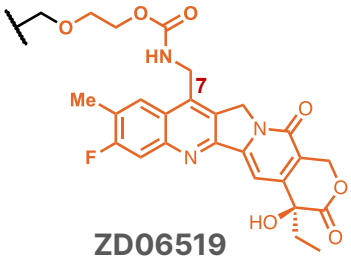
Payloads were functionalized using two different linker attachment points

C10 amide



CXN523

C7 aminal

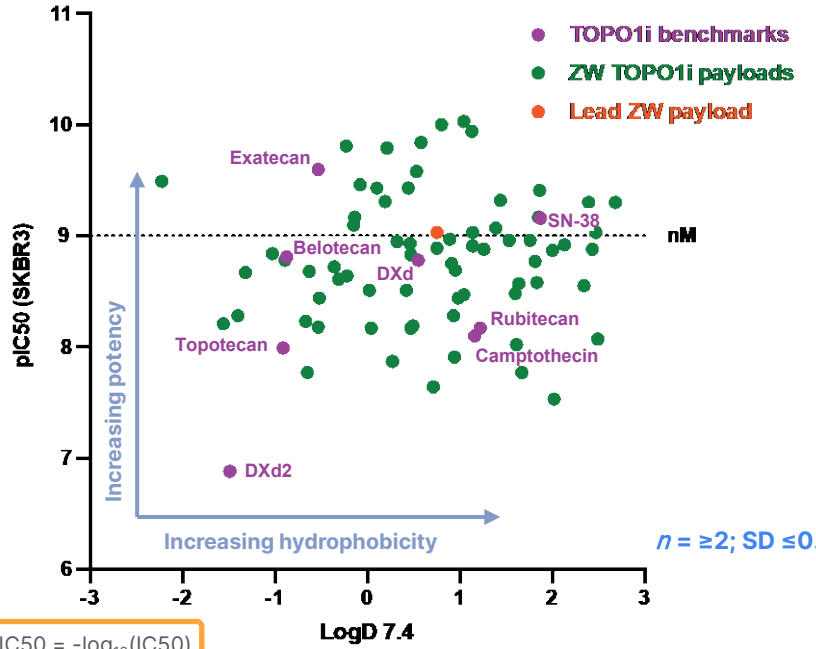


ZD06519

Zymeworks' Topoisomerase I inhibitor

Evaluation of Payloads and ADCs Enable Selection of Drug-linker Panel for Extended Characterization

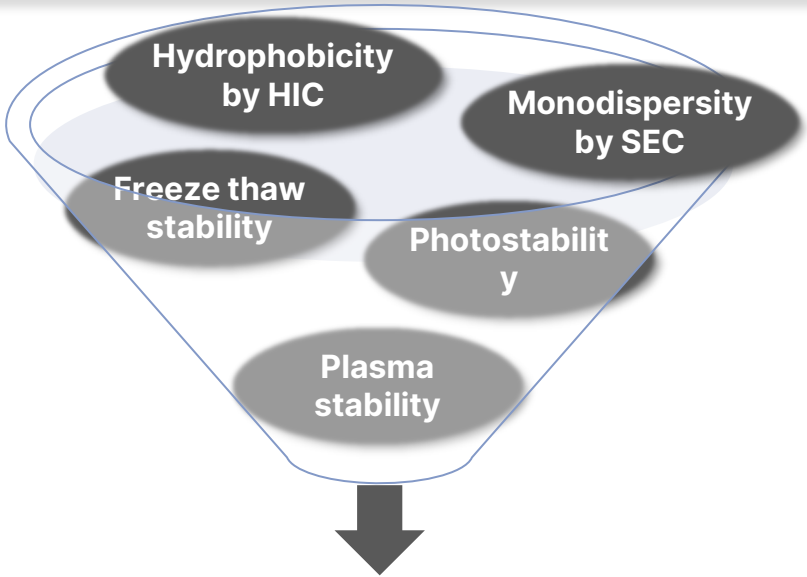
Payload selection driven by potency, hydrophobicity, and ADME characteristics



n = ≥2; SD ≤0.3

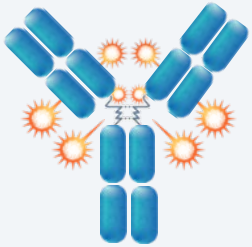
pIC50 = $-\log_{10}(IC50)$

- Selected payloads were conjugated to trastuzumab at DAR8
- Biophysical characterization enabled selection of a panel of leads



7 payloads selected for further evaluation

Zymeworks TOPO1i Drug-linkers Yield ADCs with Desired Physicochemical Properties and Exceptionally Low Aggregation



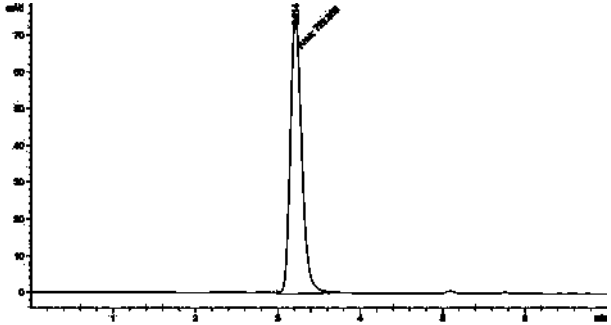
mAb = trastuzumab
conjugation = cysteine
DAR = 8

ADCs with Zymeworks TOPO1i DLs:

- ✓ No aggregation for DAR8 (*challenge for this class*)
- ✓ Hydrophilic
- ✓ Robust freeze thaw stability

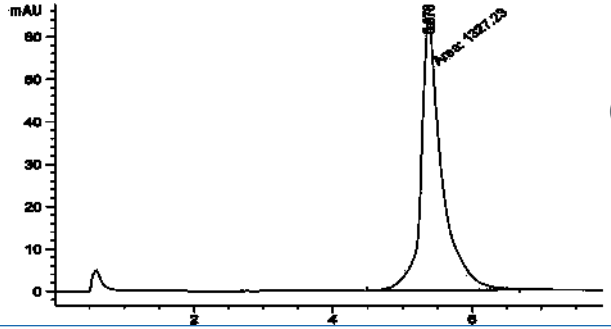
*DL = Drug-linker

Representative HPLC-SEC profile:



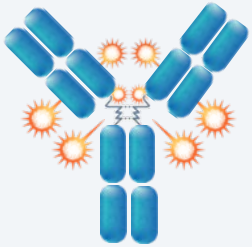
100% monomer

Representative HPLC-HIC profile:



Complete DAR8

Zymeworks TOPO1i Drug-linkers Yield ADCs with Desired Physicochemical Properties and Exceptionally Low Aggregation



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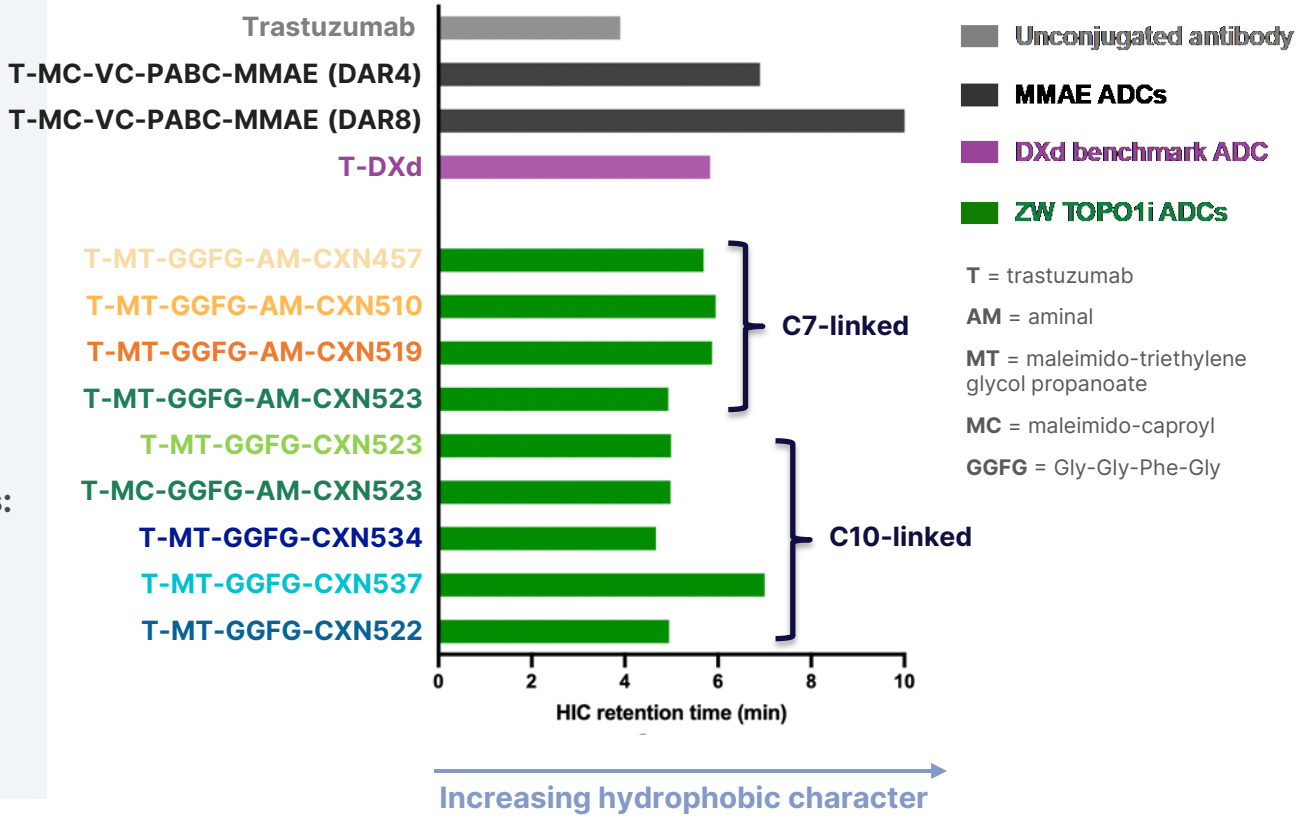
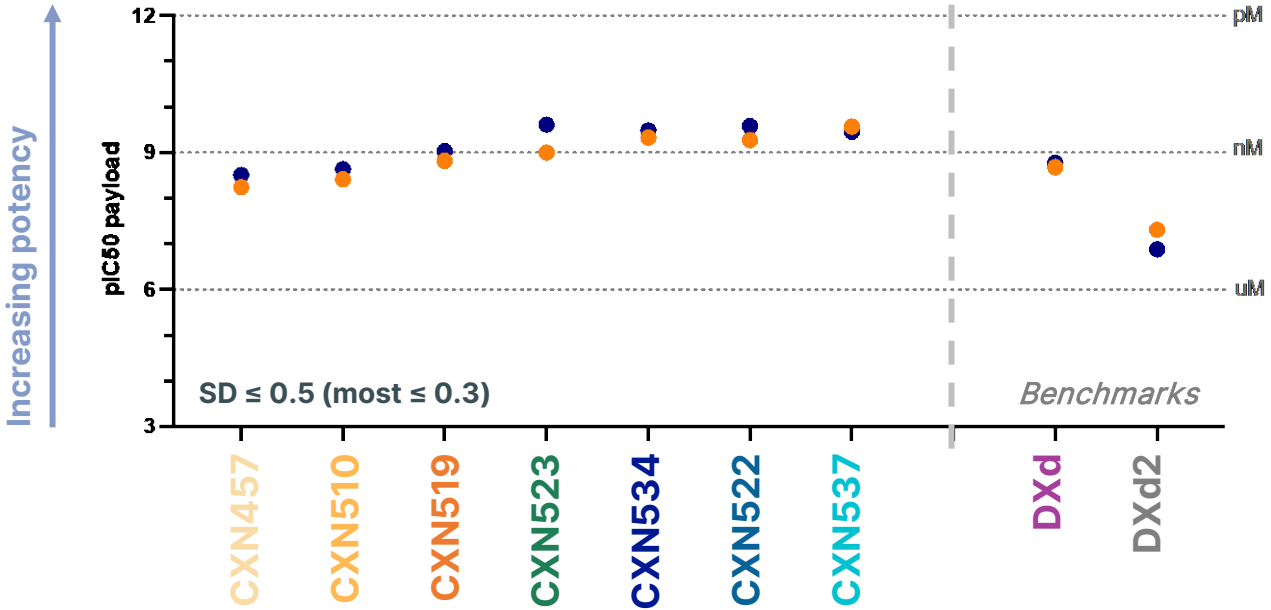
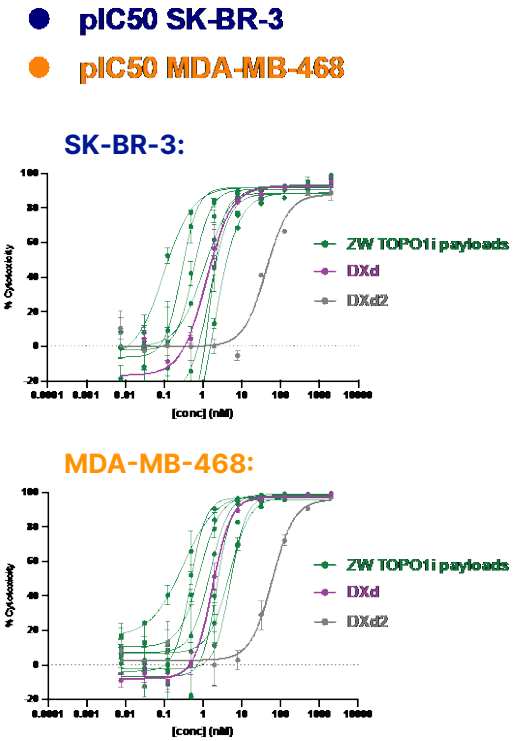


Payloads Showed Similar Potency to Benchmarks on Multiple Cell Lines

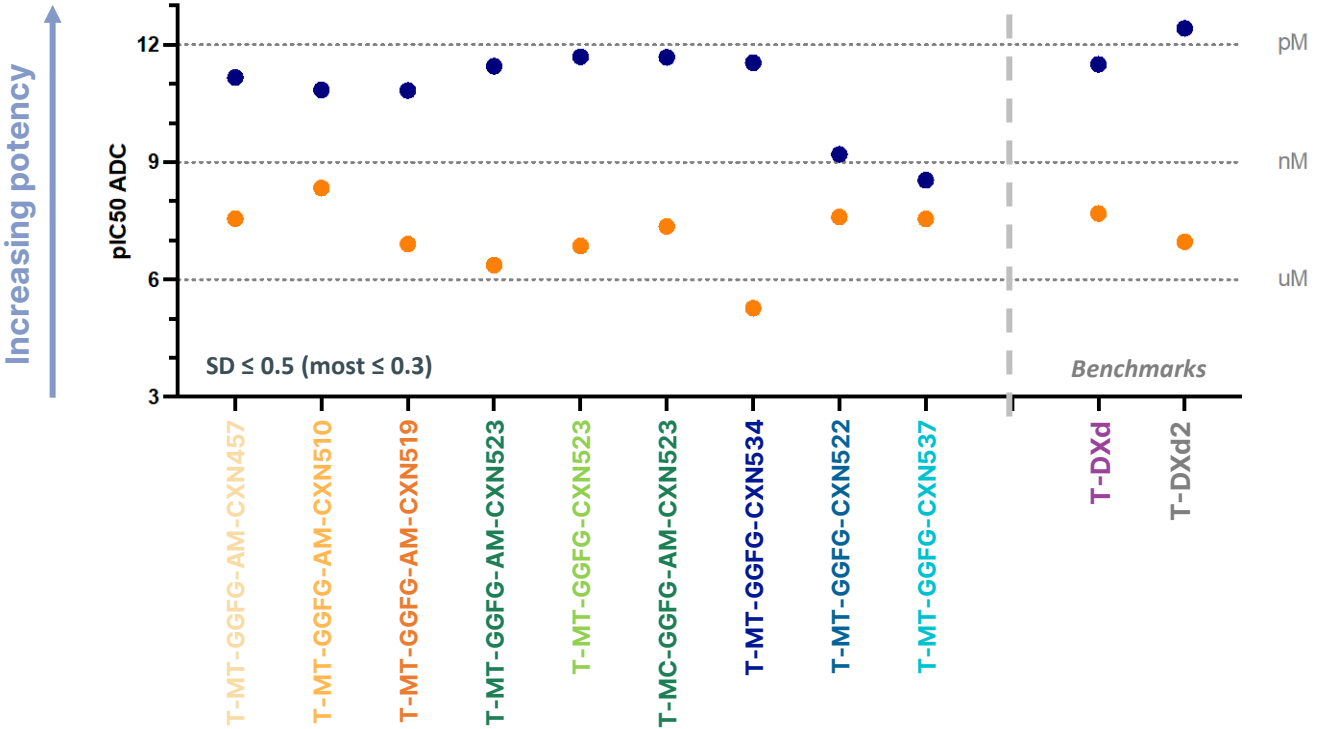


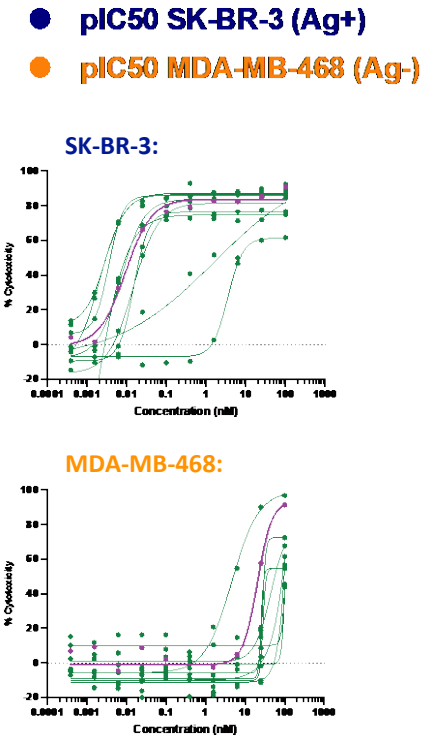


$pIC50 = -\log_{10}(IC50)$

Representative pIC50s; >70 cell lines tested

Most ADCs Showed Good Potency and Selectivity





Representative pIC50 in an **Ag+ cell line sensitive to TOPO1i ADCs** and an **Ag- cell line**

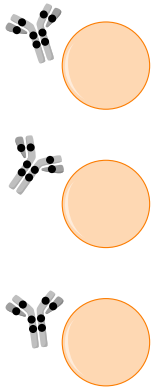
Strong Bystander Activity for Most Zymeworks TOPO1i ADCs

In vitro bystander assay:

Mono-culture

MDA-MB-468

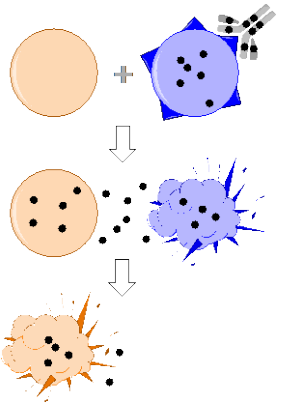
(HER2-)



Co-culture

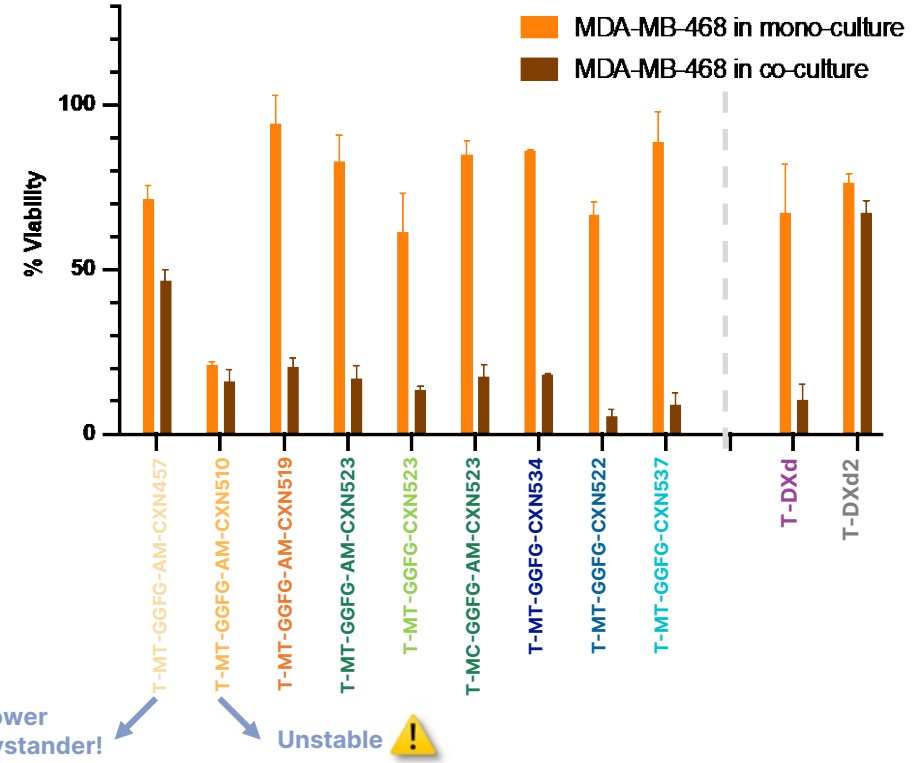
MDA-MB-468 + SK-BR-3

(HER2-) (HER2+)



Viability of **Ag-** cell line determined by flow cytometry
Viability of **Ag+** simultaneously measured (~80-100% cytotox; not shown)

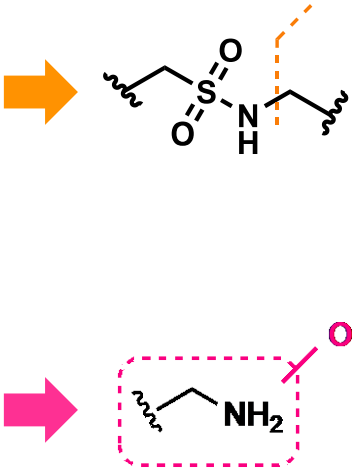
1 nM ADC treatment



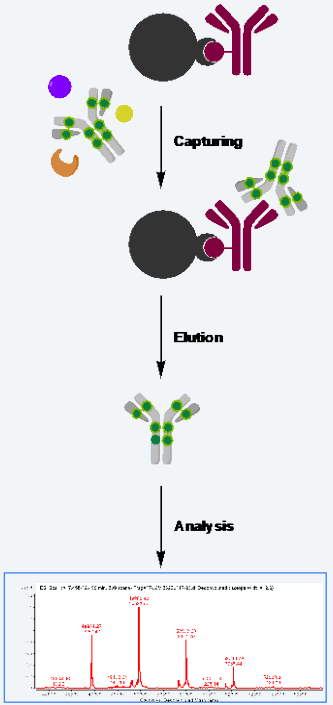
ADC Plasma Stability Assays Revealed Liabilities for Two Drug-linkers

ADC	Observed payload instability (7 d, mouse plasma) [1]
T-DXd	none
T-MT-GGFG-AM-CXN457	none
T-MT-GGFG-AM-CXN510 ✗	drug-linker fragmentation
T-MT-GGFG-AM-CXN519	none
T-MT-GGFG-AM-CXN523	none
T-MT-GGFG-CXN523	none
T-MC-GGFG-AM-CXN523	none
T-MT-GGFG-CXN534 ✗	drug-linker oxidation
T-MT-GGFG-CXN522	none
T-MT-GGFG-CXN537	none

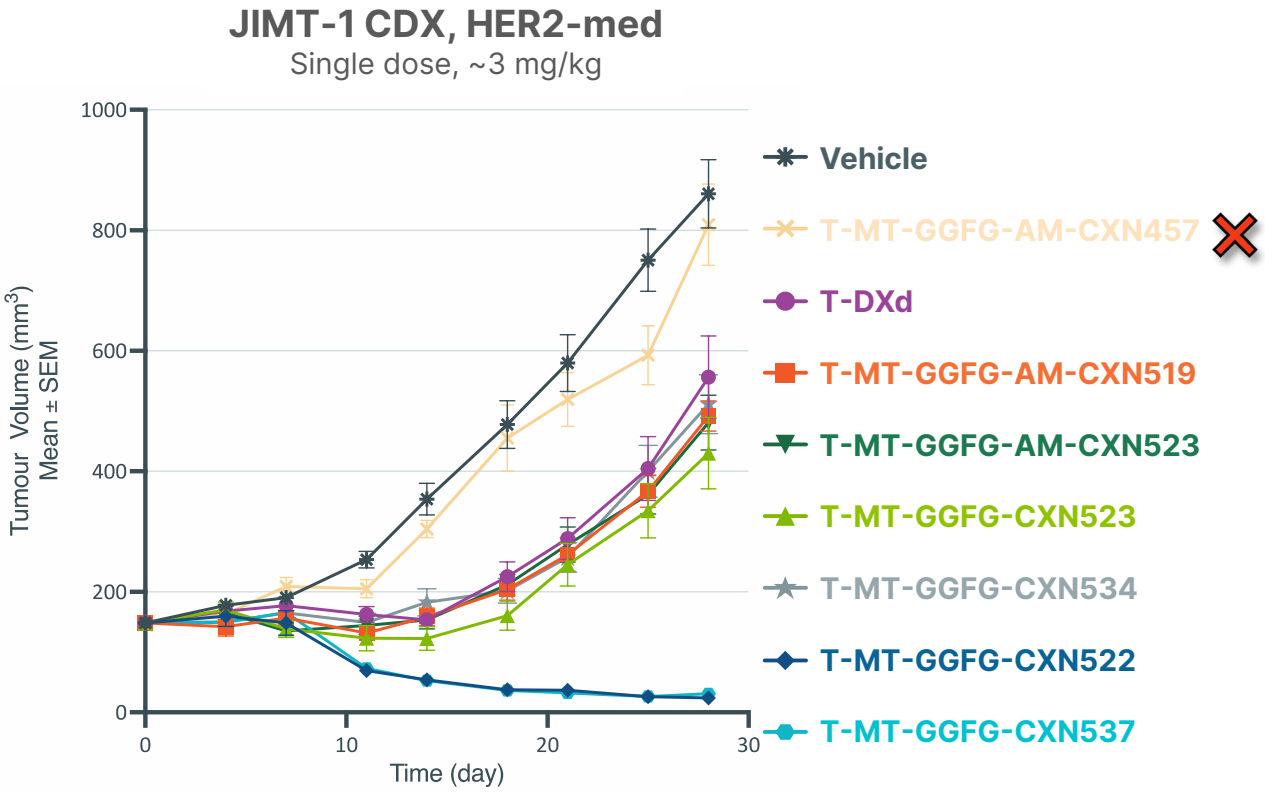
✗ **doesn't meet design criteria**



IP-MS Workflow:



Most Zymeworks TOPO1i ADCs Resulted in Comparable or Increased Efficacy vs. Benchmark in a JIMT-1 Xenograft Study



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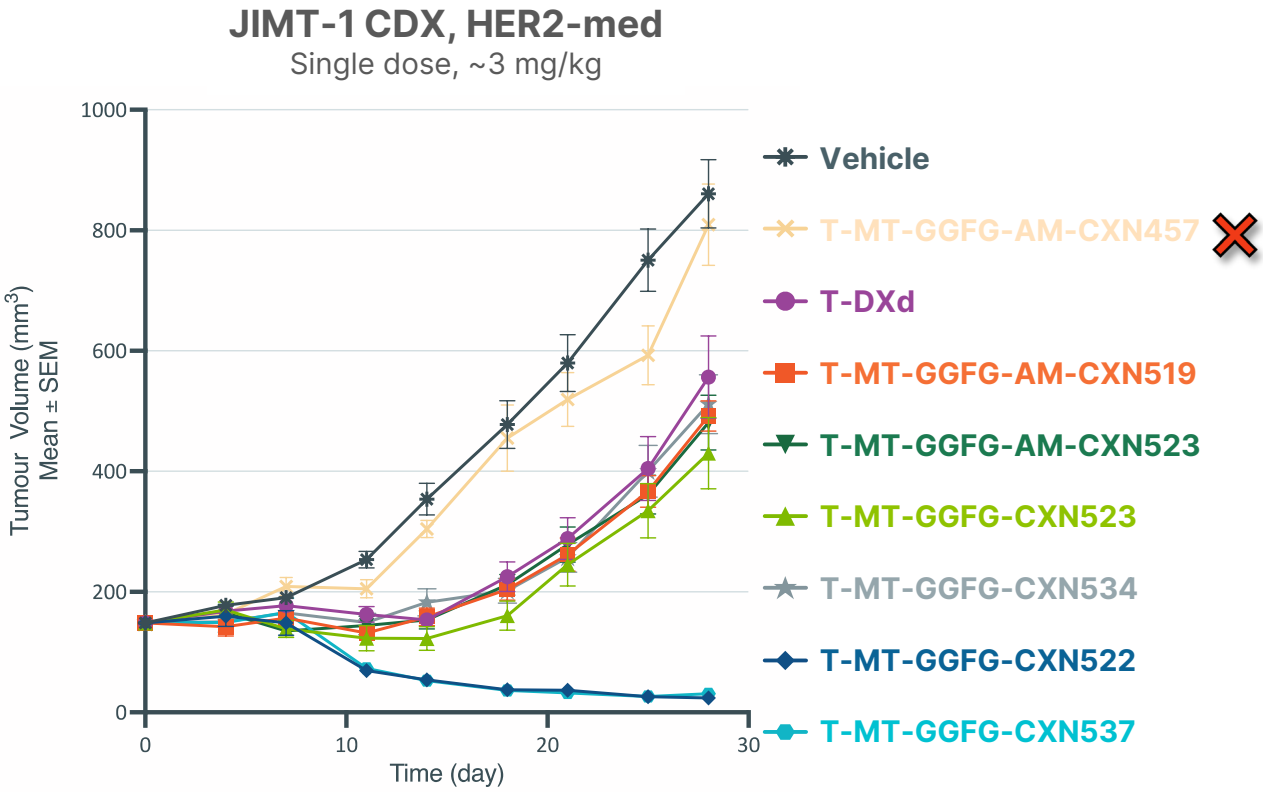
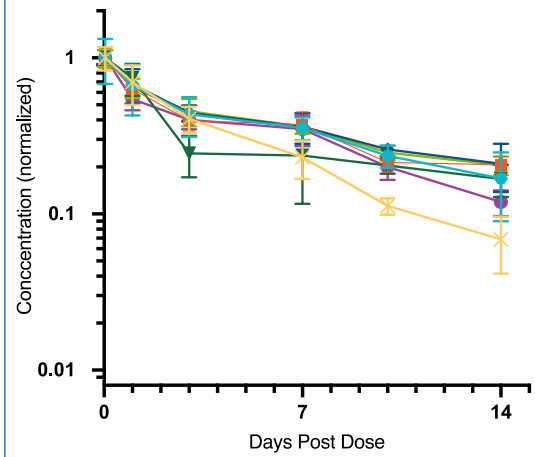
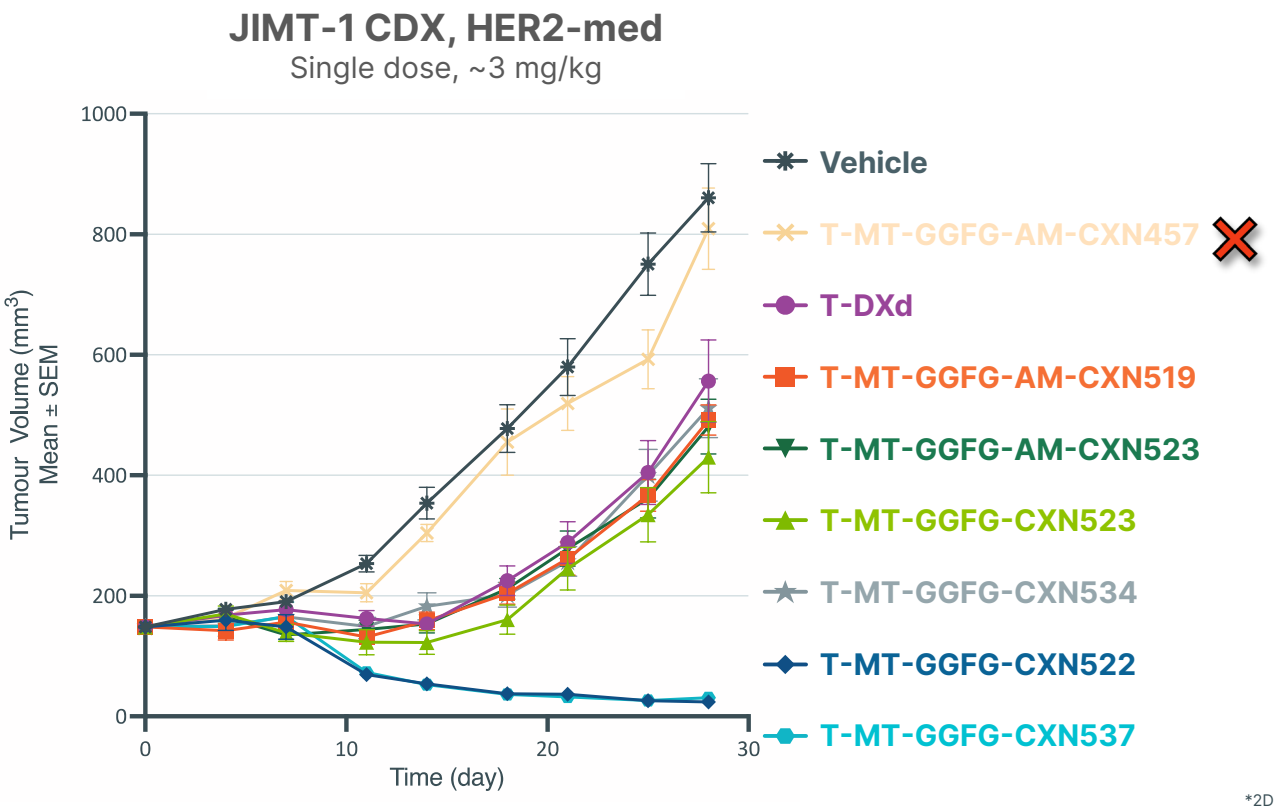


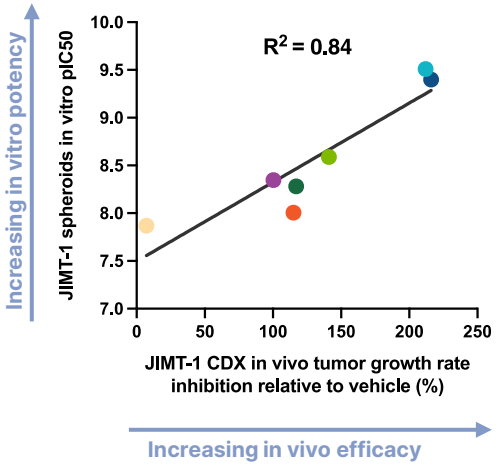


All test articles (except CXN457) showed comparable PK profile

Most Zymeworks TOPO1i ADCs Resulted in Comparable or Increased Efficacy vs. Benchmark in a JIMT-1 Xenograft Study



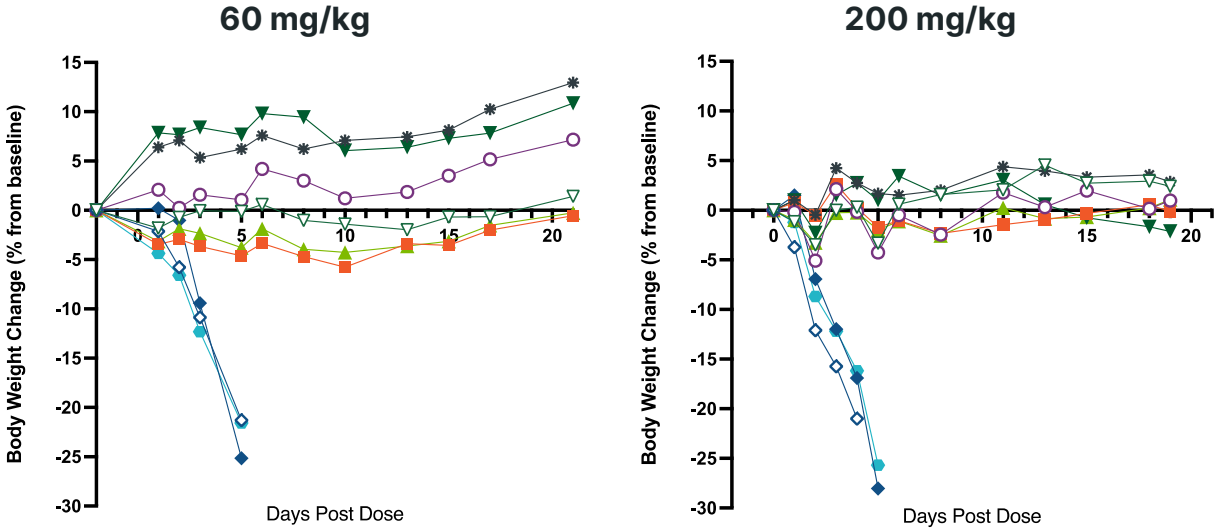
In vitro to in vivo correlation:



3D in vitro cytotoxicity correlates with in vivo efficacy*

*2D in vitro cytotoxicity on JIMT1 resulted in pIC50s < 7 with incomplete curves

Four Zymeworks TOPO1i ADCs were Tolerated at High Doses in Mice



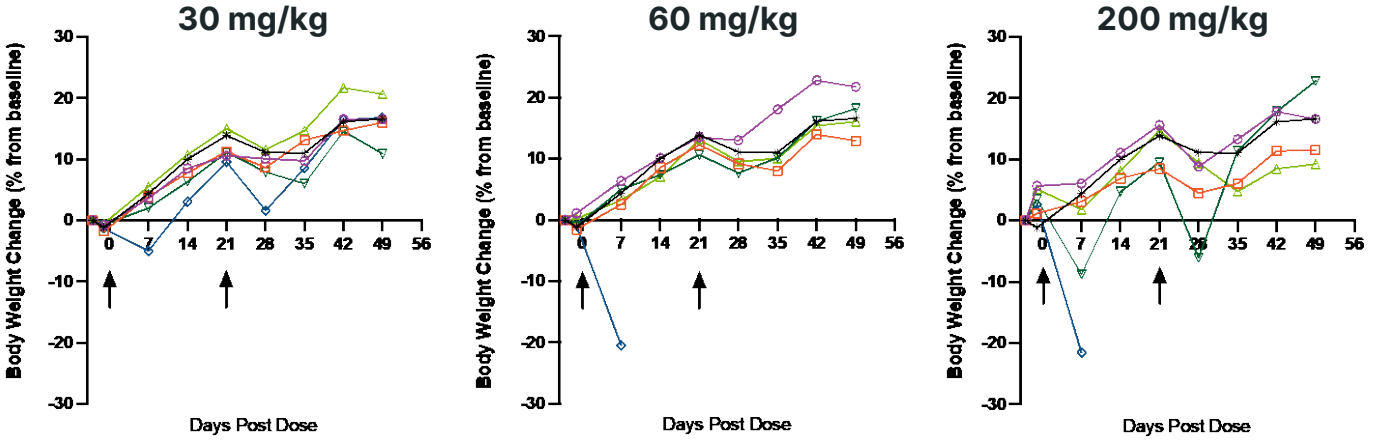
- Vehicle
- mAb-DXd
- mAb-MT-GGFG-AM-CXN519
- mAb-MT-GGFG-AM-CXN523
- mAb-MT-GGFG-CXN523
- mAb-MC-GGFG-AM-CXN523
- mAb-MT-GGFG-CXN522
- mAb-MC-GGFG-CXN522
- mAb-MT-GGFG-CXN537

✅ **design criteria met (tolerated at 200 mg/kg)**

❌ **design criteria not met (not tolerated at 200 and 60 mg/kg)**

- **TAA = Folate receptor α**
- Balb/c female mice, 8 weeks old
- 60 and 200 mg/kg
- Intraperitoneal injection, single dose
- 3 animals per group

Top Two TOPO1i ADCs Identified in a Rat Tox Study



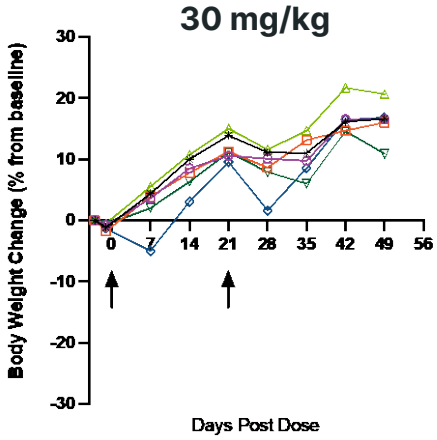
- **TAA = Folate receptor α**
- Female SD rats, 8 weeks old
- 30, 60 and 200 mg/kg
- IV injection, Q3Wx2
- 6 animals per group

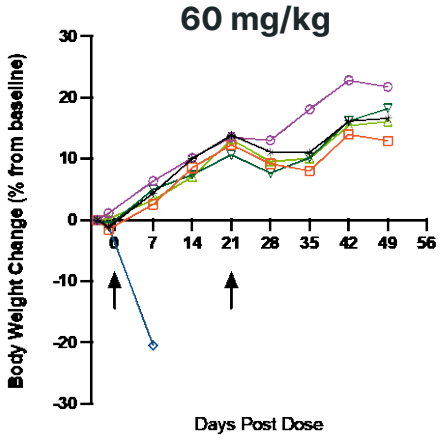
design criteria met

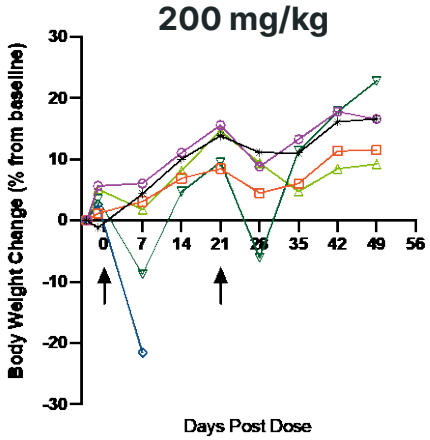
not better than ZW191 mAb-MC-GGFG-CXN523

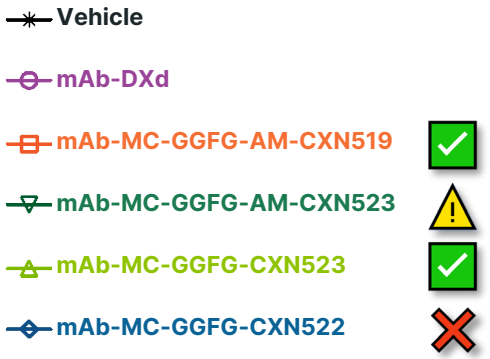
design criteria not met

Top Two TOPO1i ADCs Identified in a Rat Tox Study

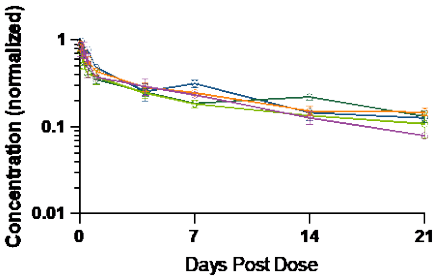


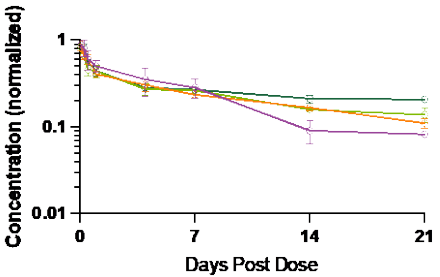


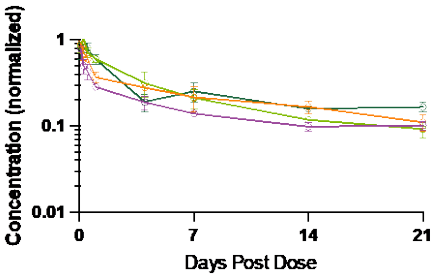




Toxicokinetic analysis showed comparable profile across the different test articles:



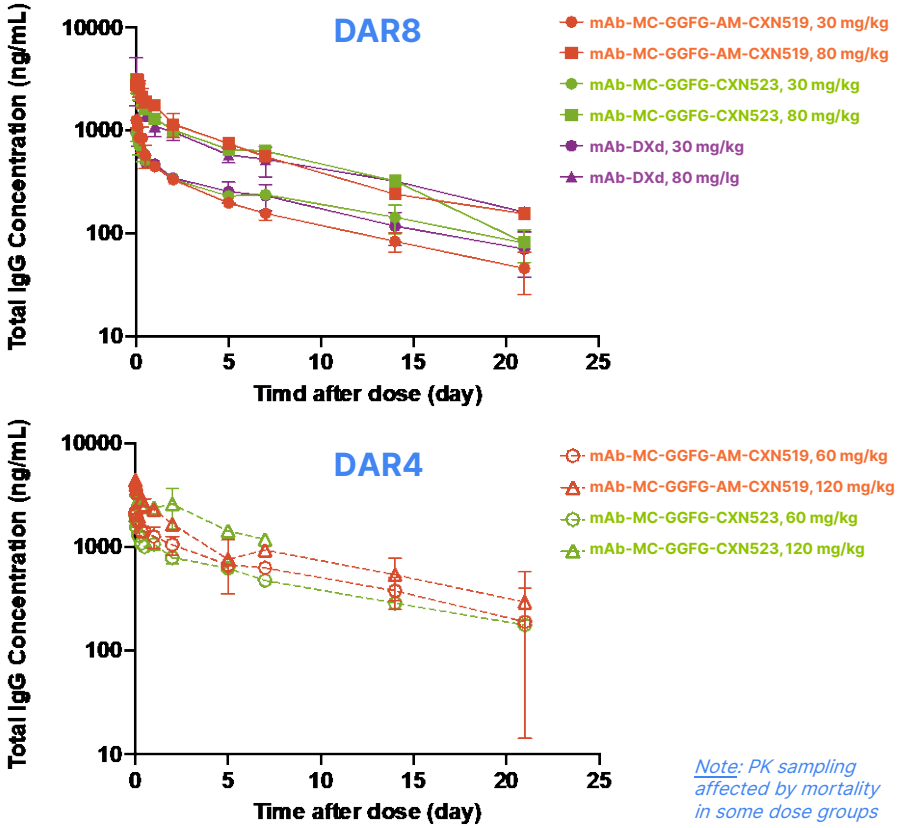




- **TAA = Folate receptor α**
- Female SD rats, 8 weeks old
- 30, 60 and 200 mg/kg
- IV injection, Q3Wx2
- 6 animals per group

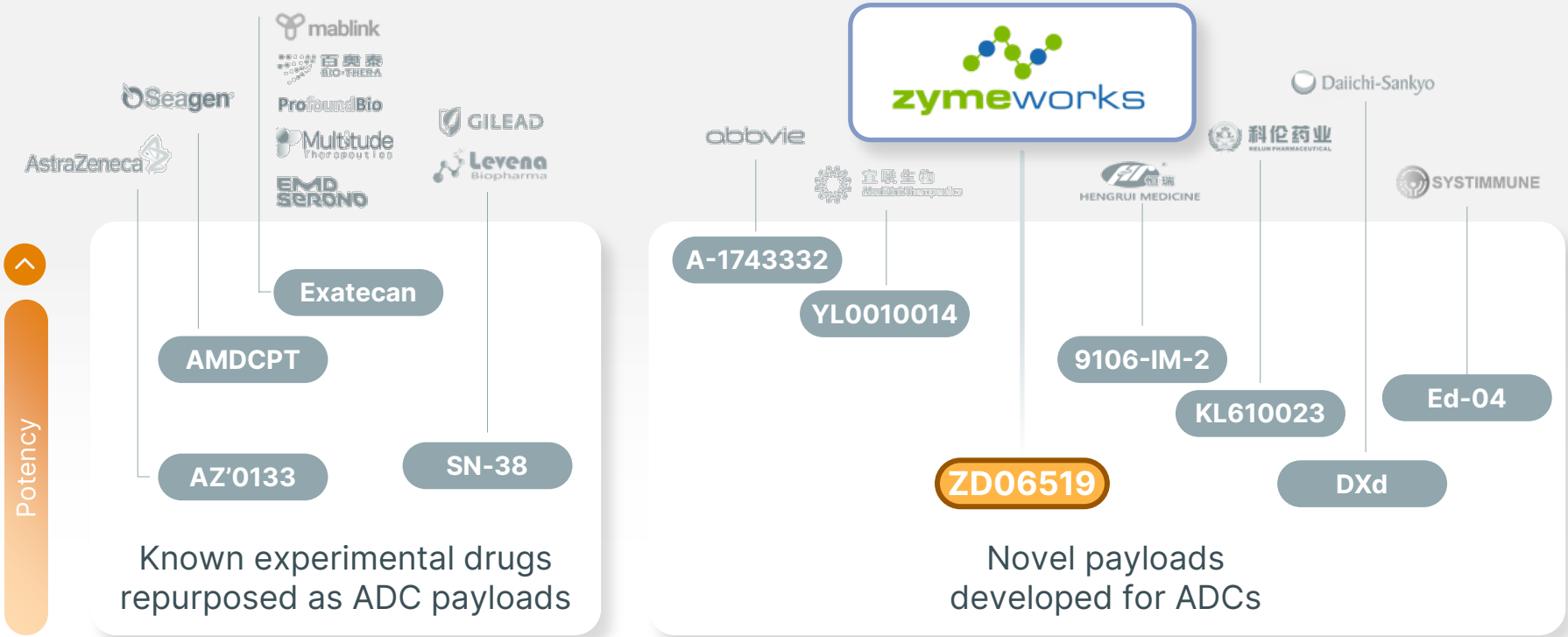
Two Dose NHP ADC Toxicity Study Support the Selection of MC-GGFG-AM-CXN519 as Platform Lead Drug-linker

Group	Test Article	DAR	Dose (mg/kg)	Tolerated?
1	Vehicle	-	-	-
2	mAb-DXd	8	30	Y
3			80	N
4	mAb-MC-GGFG-AM-CXN519	4	60	Y
5			**120**	**Y**
6		8	30	Y
7			80	N
9	mAb-MC-GGFG-CXN523	4	60	Y
10			**120**	**N**
11		8	30	Y
12			80	N



Note: PK sampling affected by mortality in some dose groups

Zymeworks Novel Camptothecin Payload was Selected with ADCs in Mind



Design of novel payloads enables incorporation of properties tailored for ADC mechanism

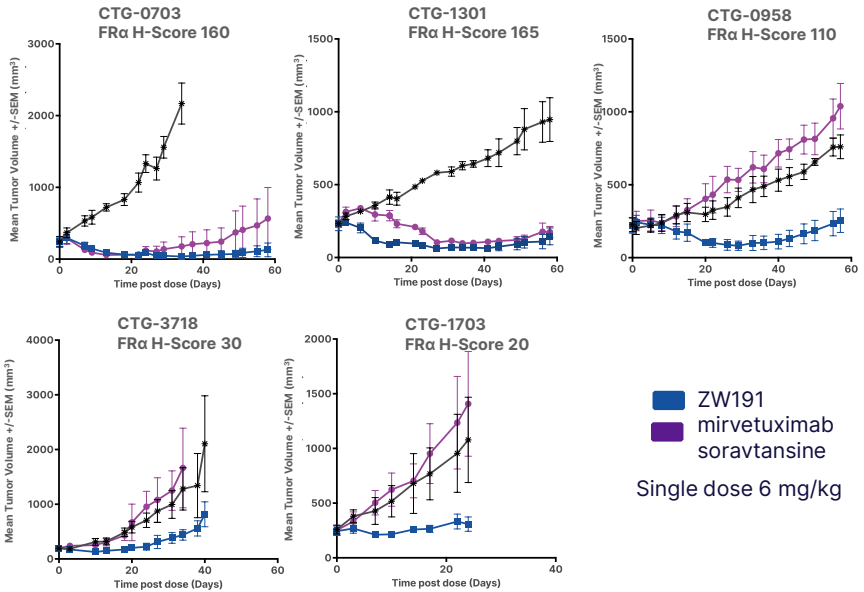
ZD06519 Payload is Being Utilized in Multiple Pipeline Programs

	ZW191	ZW220	ZW251
Target	FRα	NaPI2b	GPC3
Format/Technology	Monospecific/TOPO1i ADC	Monospecific/TOPO1i ADC	Monospecific/TOPO1i ADC
Potential Indications	Ovarian cancer, other gynecological cancers, and other solid tumors	Ovarian cancer, NSCLC	Liver cancer
Stage	IND-enabling	IND-enabling	Lead format evaluation
Next Milestone	IND 2024	On track for 2025 IND	On track for 2025 IND

Additional early-stage assets in development

ZW191, a DAR 8 FRα-targeting ADC

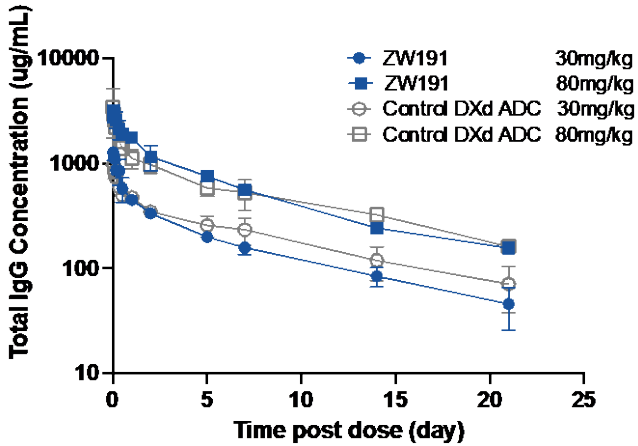
ZW191 Demonstrates Strong Efficacy in Ovarian Cancer PDX Models with a Range of Frα Expression



Higher FRα-expressing models: ZW191 at least as efficacious as MirvDM4

Lower FRα-expressing models: ZW191 substantially more efficacious than MirvDM4

ZW191 is Well Tolerated at 30 mg/kg in Non-Human Primates

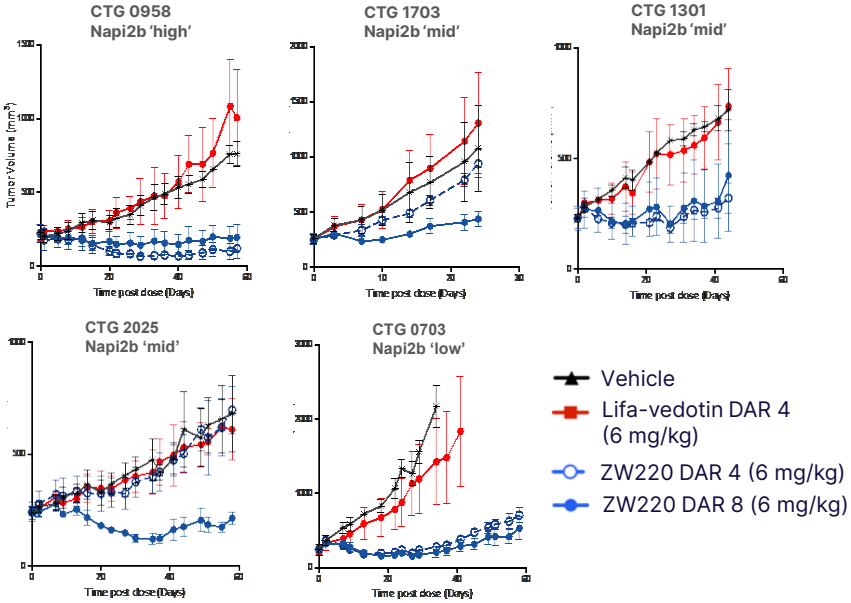


Test article	Doses	
ZW191 (DAR 4)	60 mg/kg	120 mg/kg
ZW191 (DAR 8)	30 mg/kg	80 mg/kg

- No increased severity of adverse events compared to DXd ADC
- **DAR 8 ADC selected for preclinical development**

ZW220, a DAR 4 NaPi2b-targeting ADC

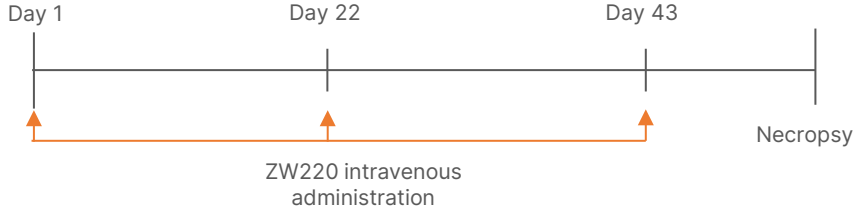
ZW220 Demonstrates Robust Anti-Tumor Activity in NaPi2b-Expressing Ovarian Cancer PDX Models



- ZW220 is more efficacious than Lifatuzumab-vedotin
- DAR 4 ADC is equivalent to DAR 8 ADC in 3/5 models

ZW220 is Well Tolerated at 90 mg/kg in Non-Human Primates

Three dose non-GLP NHP toxicology study

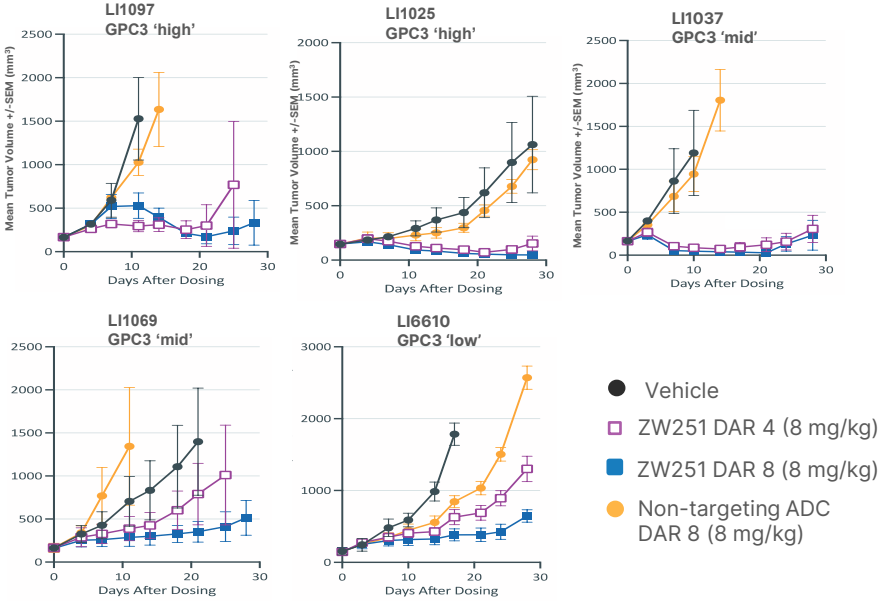


Test article	Doses		
ZW220 DAR 8	15 mg/kg	30 mg/kg	45 mg/kg
ZW220 DAR 4	30 mg/kg	60 mg/kg	90 mg/kg

- Minimal changes in body weight, hematology parameters, and clinical chemistry parameters in all treatment groups.
- No mortality observed in any treatment group prior to necropsy.
- **DAR 4 ADC selected for pre-clinical development**

ZW251, a Glypican-3-targeting ADC

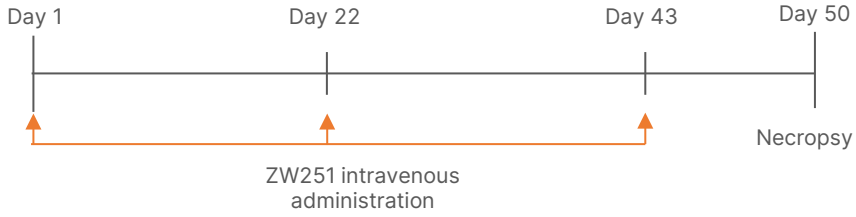
ZW251 Demonstrates Compelling Anti-Tumor Activity in GPC3-Expressing Liver Cancer PDX Models



- A Single 8 mg/kg dose of either ZW251 DAR 4 or DAR 8 results in robust efficacy.
- DAR 4 ADC is equivalent to DAR 8 ADC in 3/5 models.

ZW251 is Well Tolerated in Non-Human Primates

Repeat dose non-GLP NHP toxicology study



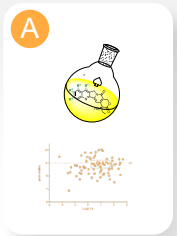
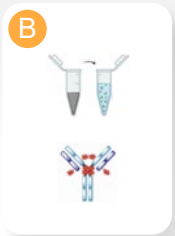
Test article	Doses		
ZW251 DAR 8	10 mg/kg	30 mg/kg	60 mg/kg
ZW251 DAR 4	20 mg/kg	60 mg/kg	120 mg/kg

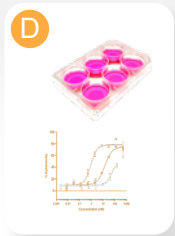
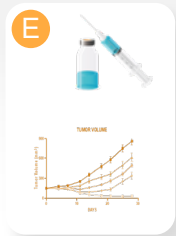
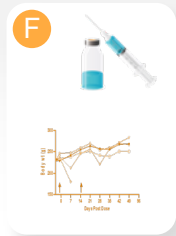
- Minimal changes in body weight, hematology parameters, and clinical chemistry parameters in all treatment groups.
- No mortality observed in any treatment group prior to necropsy.

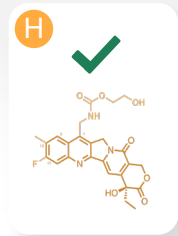
Robust Interrogation Yields Pipeline Ready TOPO1i ADC Platform

From concept to platform:

Payload synthesis & screening	Conjugation of select payloads	ADC biophysical characterization	In vitro potency & stability	In vivo anti-tumor activity & PK	Rodent tolerability	Non-human primate toxicology & TK	Lead selection
A	B	C	D	E	F	G	H



From platform to pipeline:

8	80	>25	>25	3	5
Tumor targets	Cell lines	CDX models	PDX models	PK studies	Tox & TK studies

3 Pipeline programs
ZW191, ZW220, ZW251

Additional early-stage assets

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