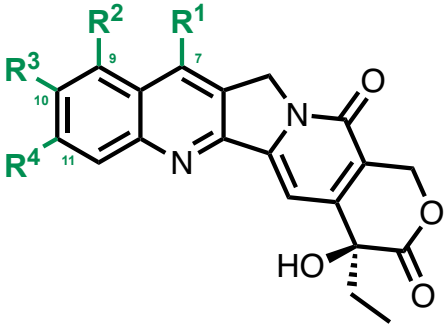
TOPO1i ADC Platform: From Concept to Pipeline

World ADC London 2022

Thursday, 31st March 2022

60 Years of Camptothecins

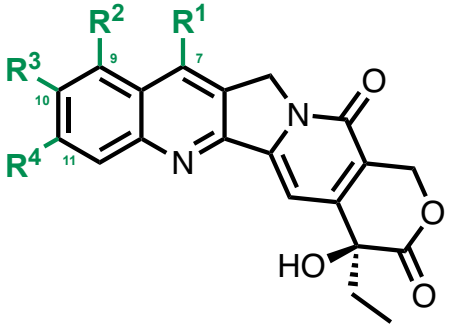


Potent inhibitors of topoisomerase I:

- Discovered in the early 1960 by M. E. Wall and M. C. Wani of Research Triangle Institute (RTI)
- Isolated from *Camptotheca acuminata* (The Happy Tree)
- Prevent DNA religation which results in double strand breaks and apoptosis

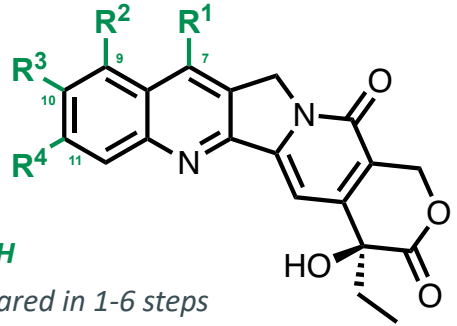
- **3 approved small molecules** (Topotecan, Irinotecan, Belotecan)
- **2 approved ADCs** (Enhertu, Trodelvy)
- **Several ADCs**, **SMDCs**, and **NPs** at different stages of development

TOPO1i ADC Platform: **From Concept to Pipeline**



1. Synthesis of new payloads
2. In vitro characterization of payloads
3. Synthesis of drug linkers
4. Conjugation
5. In vitro activity of ADCs
6. Additional assays and analysis
7. In vivo studies

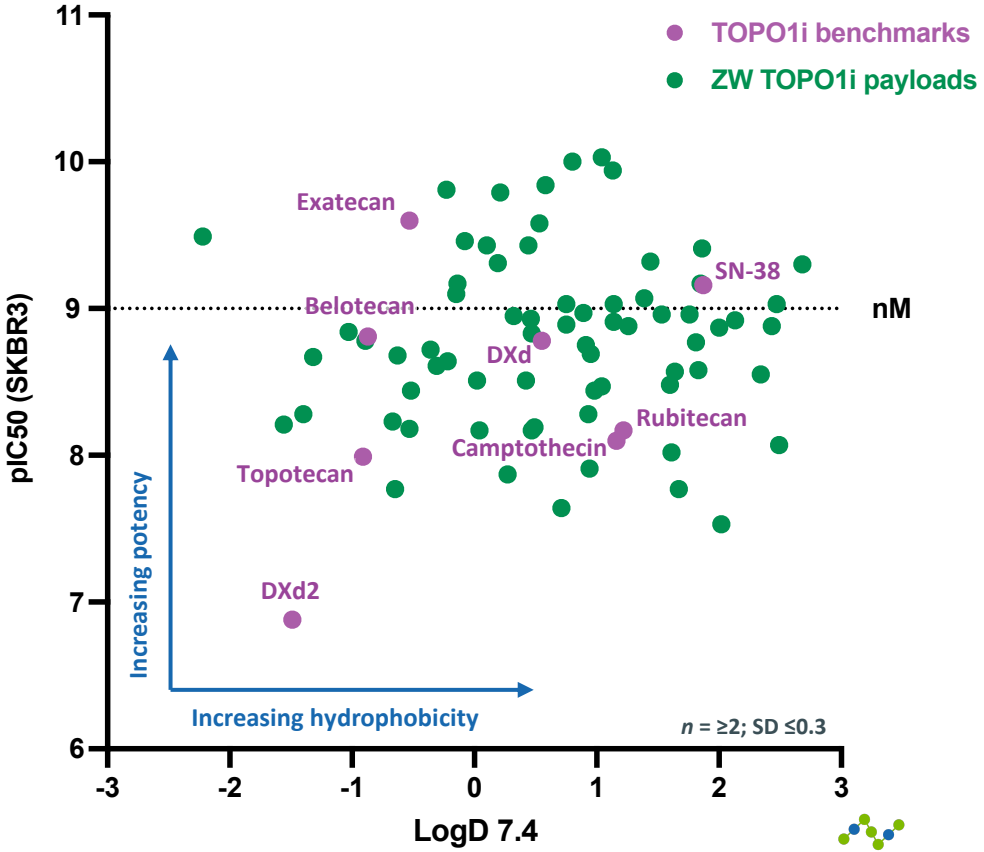
Zymeworks TOPO1i Payloads Span Range of Potency and Hydrophilicity



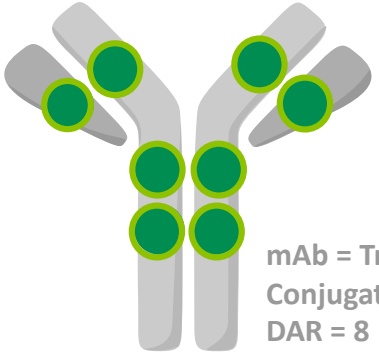
R^2 = H

Prepared in 1-6 steps

- ✓ ~100 new TOPO1i payloads prepared
- ✓ Range of potency and hydrophobicity
- ✓ Two linking strategies (from *R^1* and *R^3* groups)



ZW TOPO1i Drug-Linkers Yield ADCs with Desired Physicochemical Properties and Exceptionally Low Aggregation

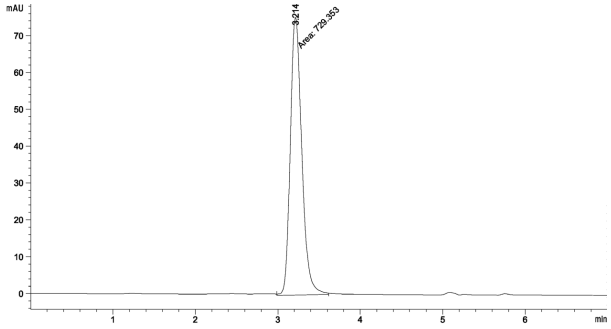


mAb = Trastuzumab
Conjugation = cysteine
DAR = 8

ADCs with ZW TOPO1i DLs:

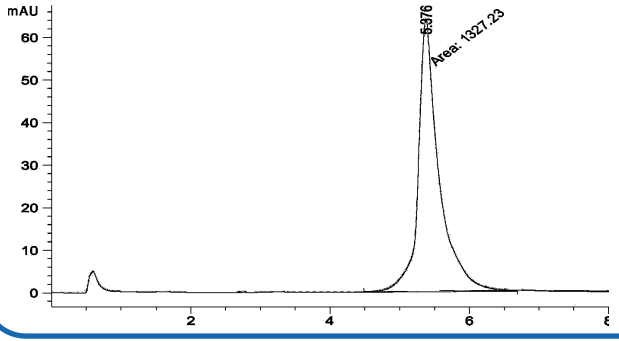
- ✓ No aggregation for DAR8 (*challenge for this class*)
- ✓ Hydrophilic
- ✓ Robust freeze thaw stability

Representative HPLC-SEC profile:



100% monomer

Representative HPLC-HIC profile:



Complete DAR8

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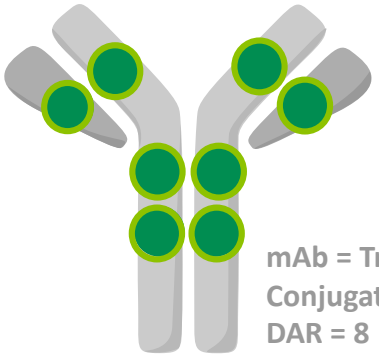
ZW TOPO1i Drug-Linkers Yield ADCs with Desired Physicochemical Properties and Exceptionally Low Aggregation



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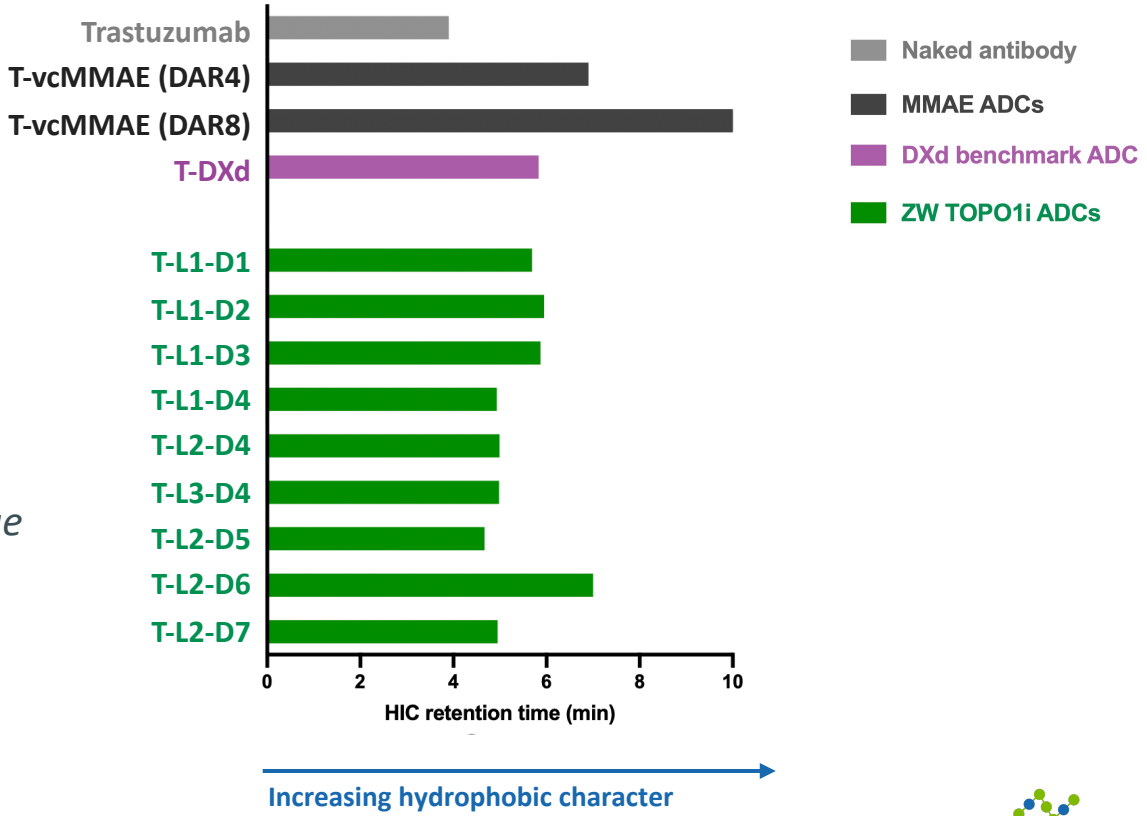

ADCs with ZW TOPO1i DLs:

- ✓ No aggregation for DAR8 (*challenge for this class*)
- ✓ Hydrophilic
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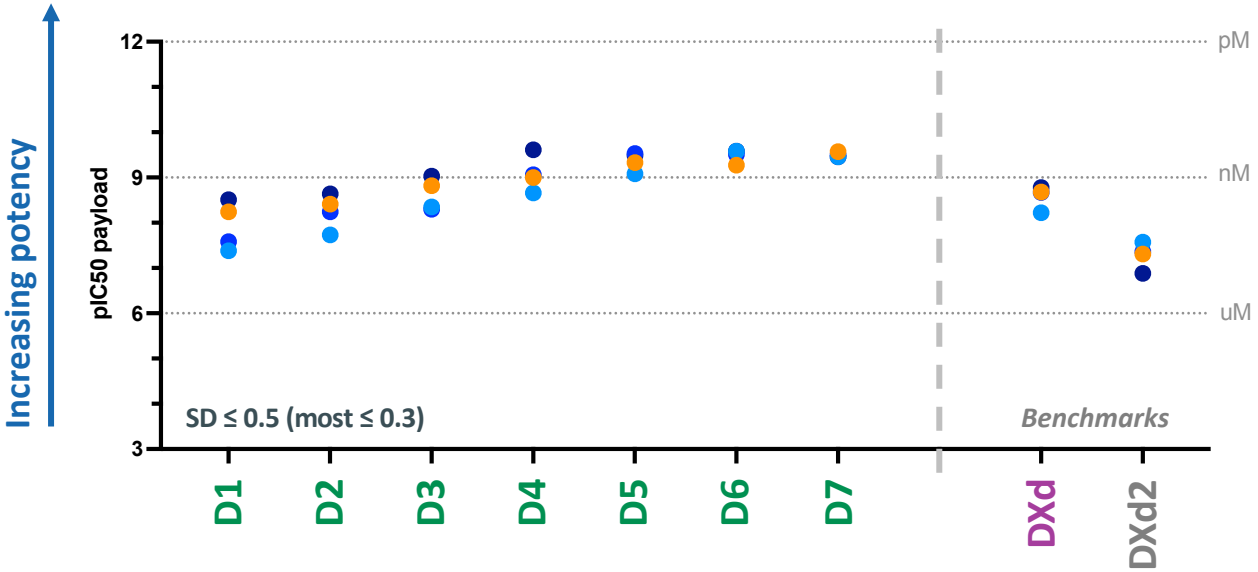
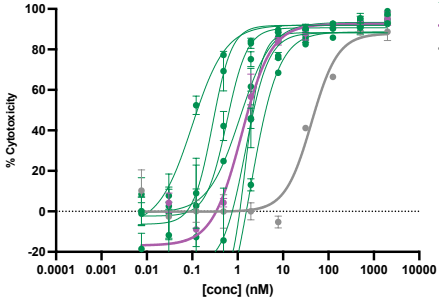
Payloads Showed Similar Potency to Benchmarks on Multiple Cell Lines



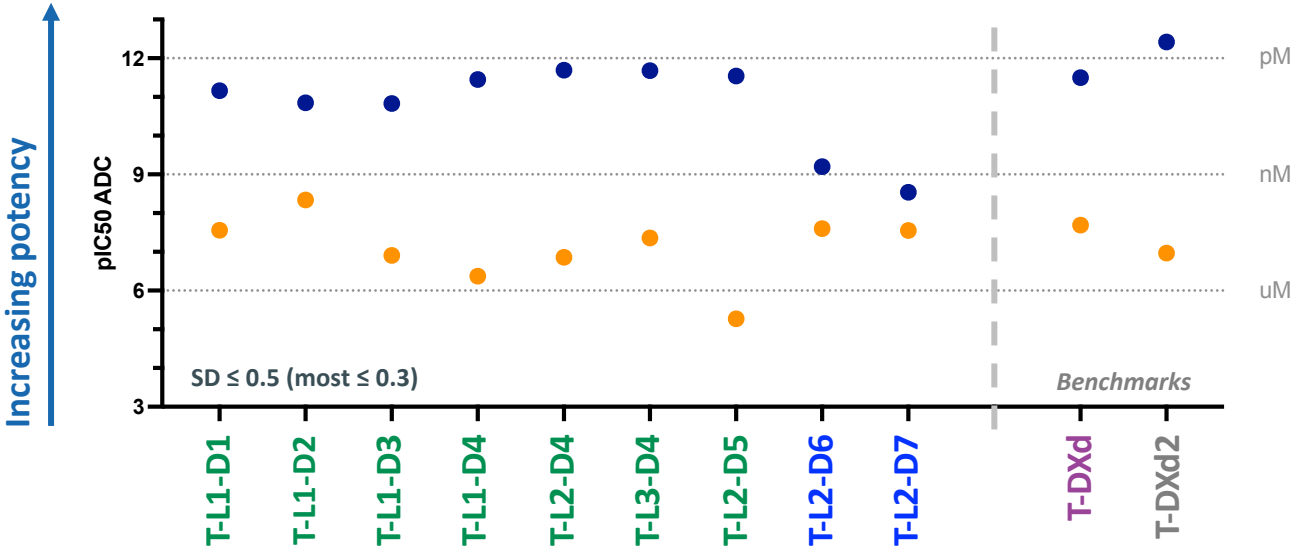
- pIC50 SK-BR-3
- pIC50 Calu-3
- pIC50 SK-OV-3
- pIC50 MDA-MB-468

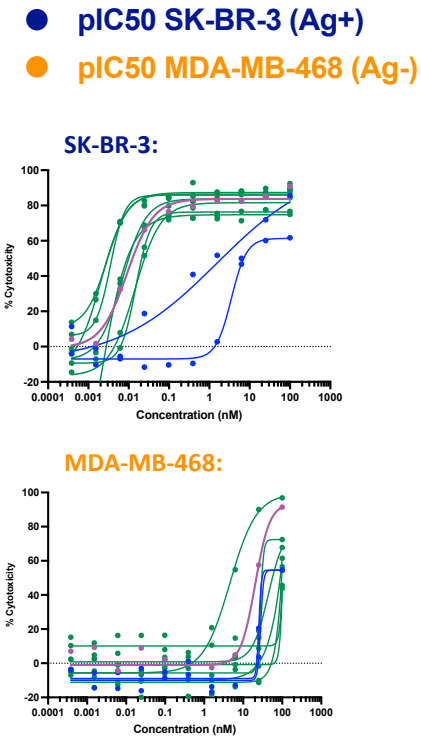


- ZW TOPO1i payloads
- DXd
- DXd2

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

Most ADCs Showed Good Potency and Selectivity

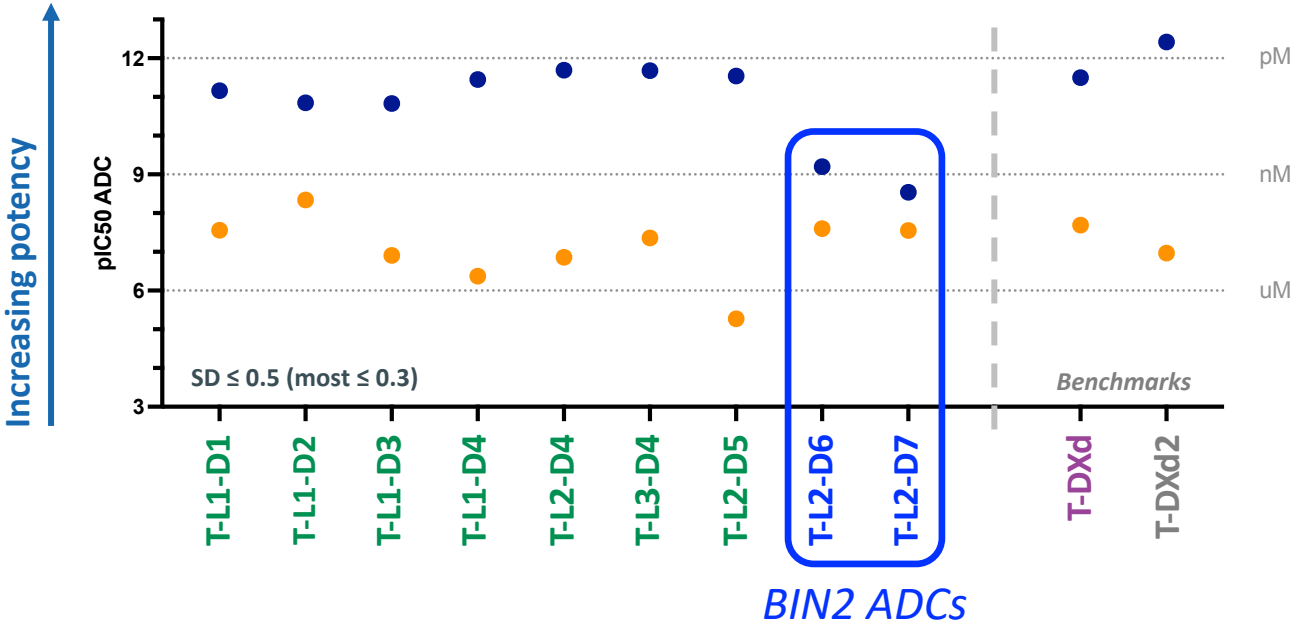


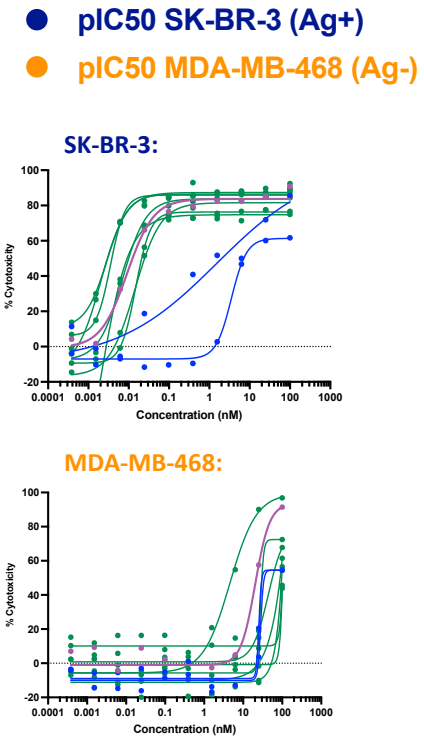


Representative pIC50 in an Ag+ cell line sensitive to TOPO1i ADCs and an Ag- cell line

>70 cell lines tested in 2D assays with 8 different TAA TOPO1i ADCs (~25% sensitive)

Most ADCs Showed Good Potency and Selectivity





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

>70 cell lines tested in 2D assays with 8 different TAA TOPO1i ADCs (~25% sensitive)

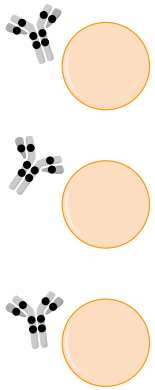
Strong Bystander Activity for Most Zymeworks TOPO1i ADCs

In vitro bystander assay:

Mono-culture

MDA-MB-468

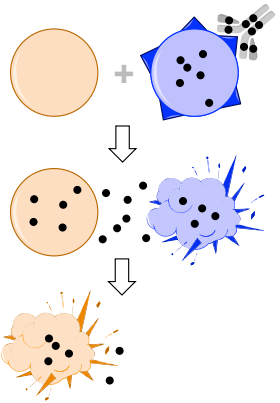
(HER2-)



Co-culture

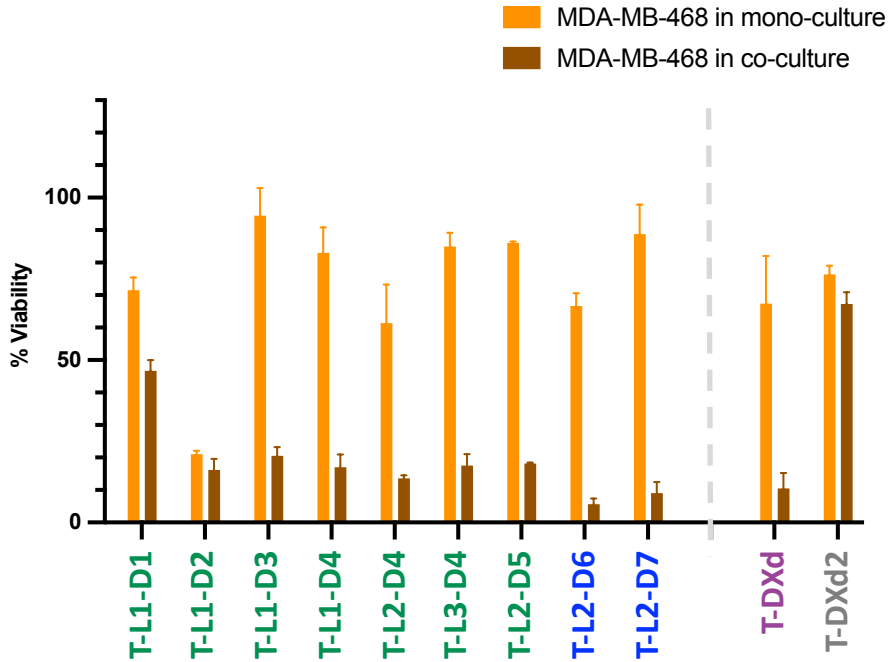
MDA-MB-468 + SK-BR-3

(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



Lower bystander!

Unstable / non-specific?

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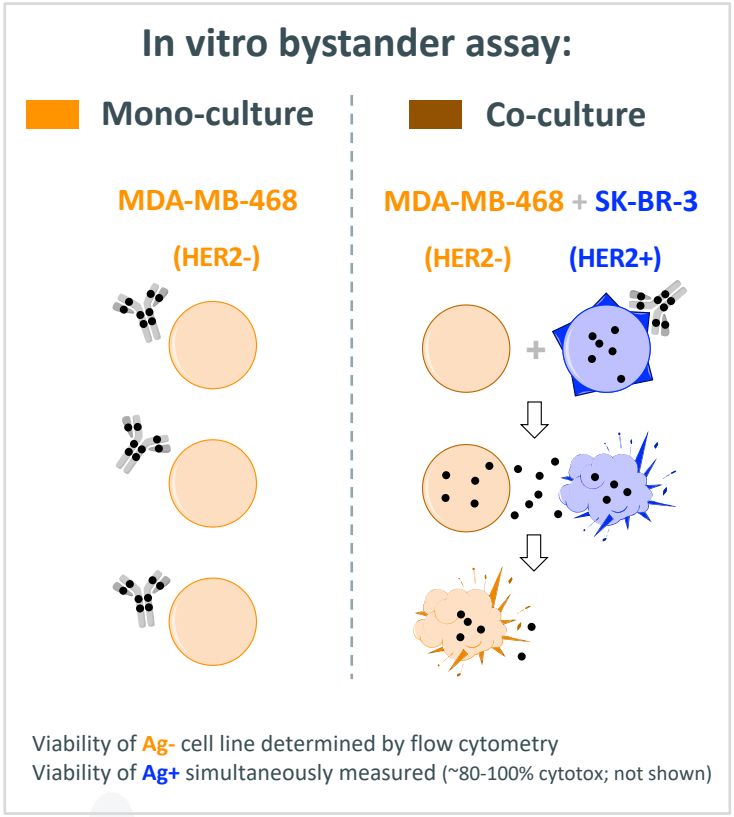
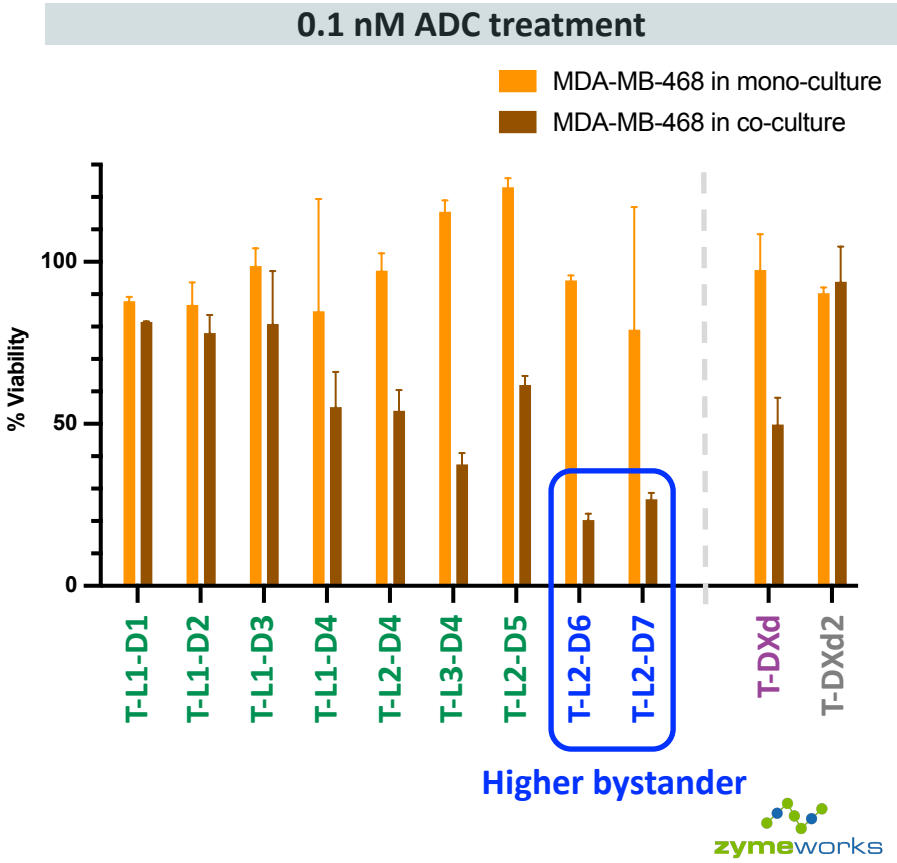
Higher Bystander Activity for Bin 2 ADCs



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



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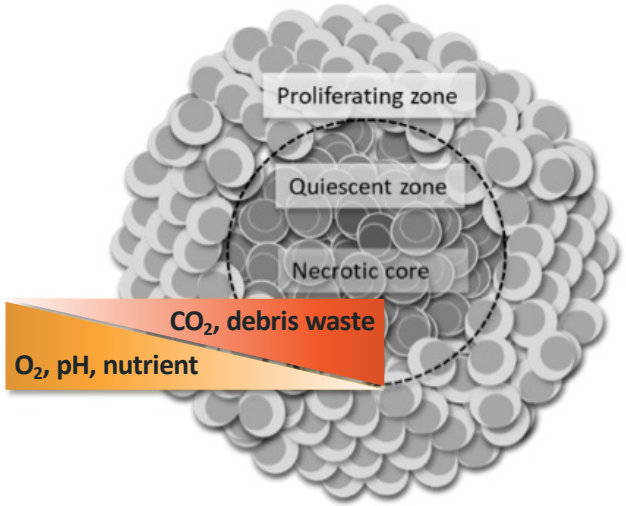
Fit for Purpose Spheroid Cytotoxicity Assay was Developed to Screen TOPO1i ADCs

Key spheroid features:

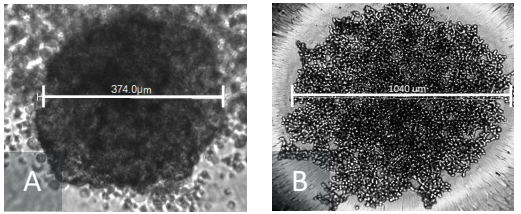
- Spatial organization
- Layers of distinct cell populations
- Formation of different gradients from outer to inner regions
- More complex cell signaling
- Potential to recapitulate drug resistance and metabolic adaptation



Adapted from: Pinto B, Henriques AC, Silva PMA, Bousbaa H. *Pharmaceutics.* **2020**, *12*, 1186

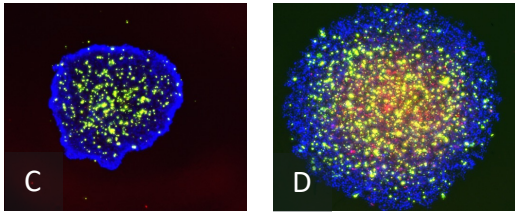
Size (200-1200 µm)

Cell line 1 374 µm diameter	Cell line 2 1040 µm diameter



Spheroid imaging and viability

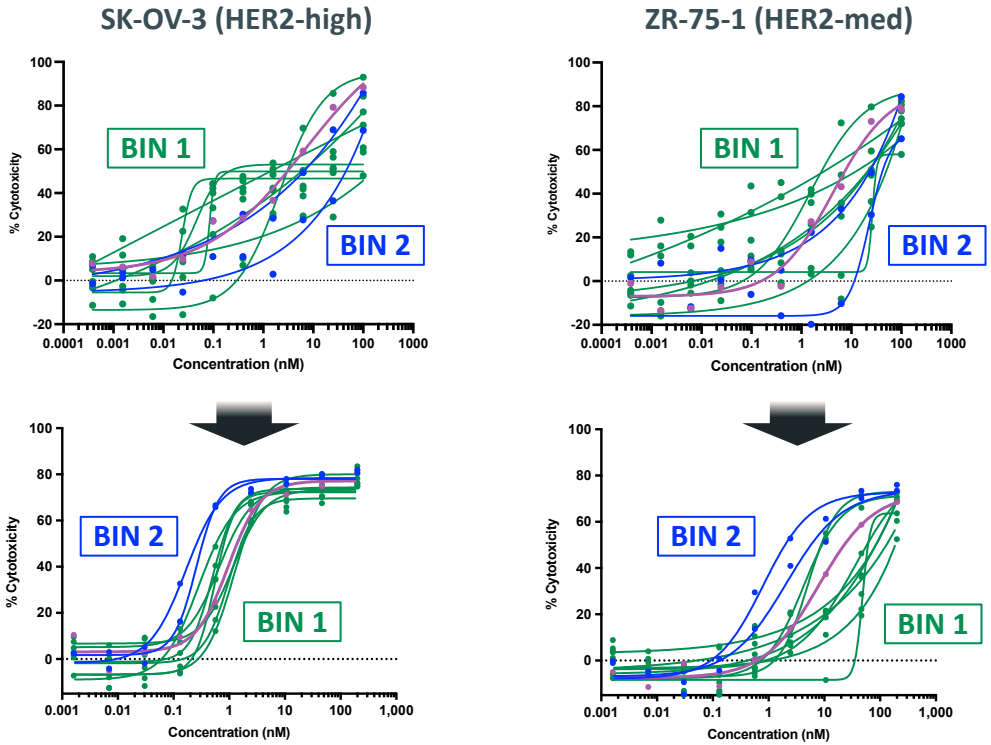
Cell line 3	Cell line 4



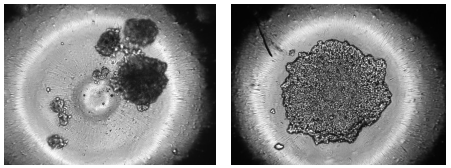
Pictured:
- A-B – Spheroid phase contrast images; pre-ADC treatment
- C-D – Live cells (Hoechst nuclear stain, blue), Apoptotic cells (YO-PRO-1, green), dead cells (YO-PRO-3, red); blanks (no treatment)

Cell Titer Glo is used to quantify spheroid viability post ADC treatment

Spheroid Cytotoxicity Assay Altered Dose-Response Relationship and Relative Potency Ranking of ADCs



- T-TOPO1i ADCs (BIN 1)
- T-TOPO1i ADCs (BIN 2)
- T-DXd



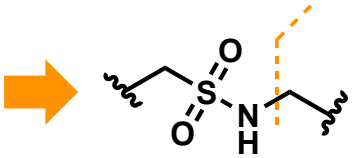
Phase contrast images acquired pre-ADC treatment

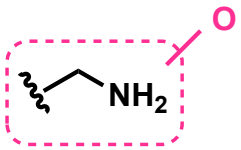
- Representative examples
- >30 cell lines paneled in 3D assays with 8 different TAA TOPO1i ADCs

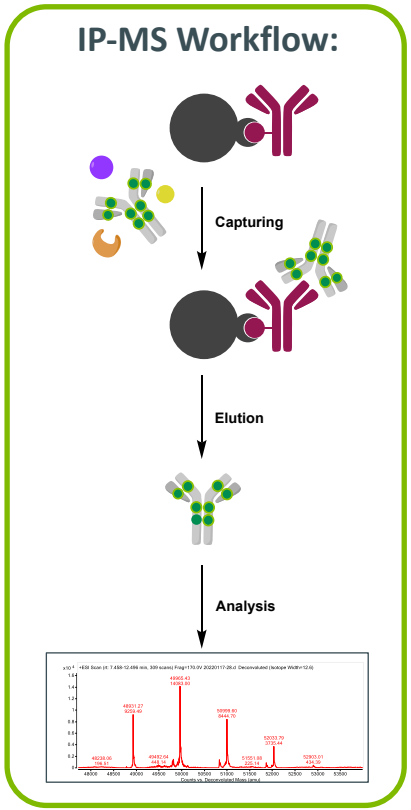
- Potency differences may be due to better bystander killing

ADC Plasma Stability Assays Revealed Liabilities for 2 Drug-Linkers

ADC	Observed payload instability (7 d, mouse plasma) [1]
T-DXd	none
T-L1-D1	none
T-L1-D2	drug-linker fragmentation
T-L1-D3	none
T-L1-D4	none
T-L2-D4	none
T-L3-D4	none
T-L2-D5	drug-linker oxidation
T-L2-D6	none
T-L2-D7	none



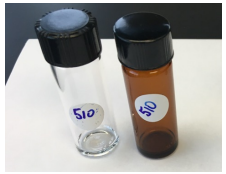




ZW TOPO1i Payloads and ADCs Showed Good Photostability

Package leaflet / package insert of Enhertu:

- ⚠ Cover the infusion bag to ***protect from light***
- ⚠ If not used immediately, ***protect from light***



Payload	Payload photostability (16 days) [1]
D3	90%
D4	78%

[1] As % of intact payload left after 16 days, room temp, no agitation, lab light

ADC	ADC photostability (14 days) [2]
T-DXd	86%
T-L1-D3	100%
T-L1-D4	100%
T-L2-D4	100%
T-L3-D4	94%

[2] As % of intact LC+D left after 14 days, room temp, no agitation, lab light

- No decomposition observed in amber vials
- Drug-linker stocks and ADCs protected from light as a precaution

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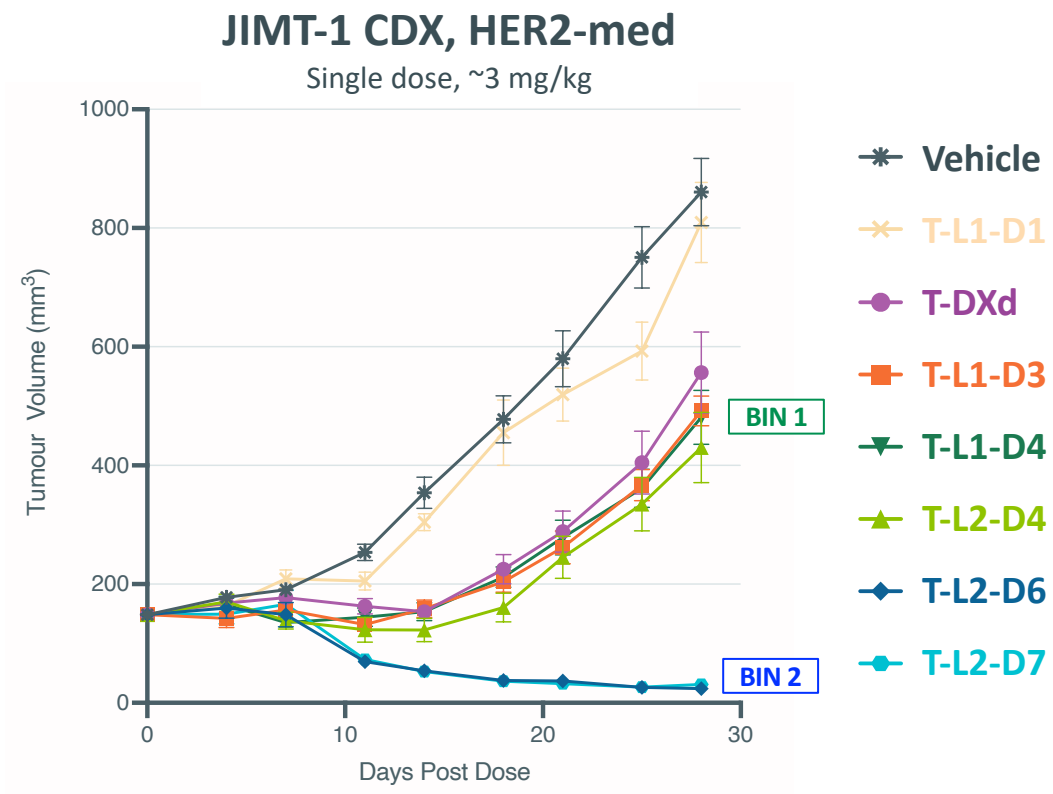
Most ZW TOPO1i ADCs Resulted in Comparable or Increased Efficacy vs. Benchmark in a JIMT-1 Study, Further Highlighting Two Separate Bins



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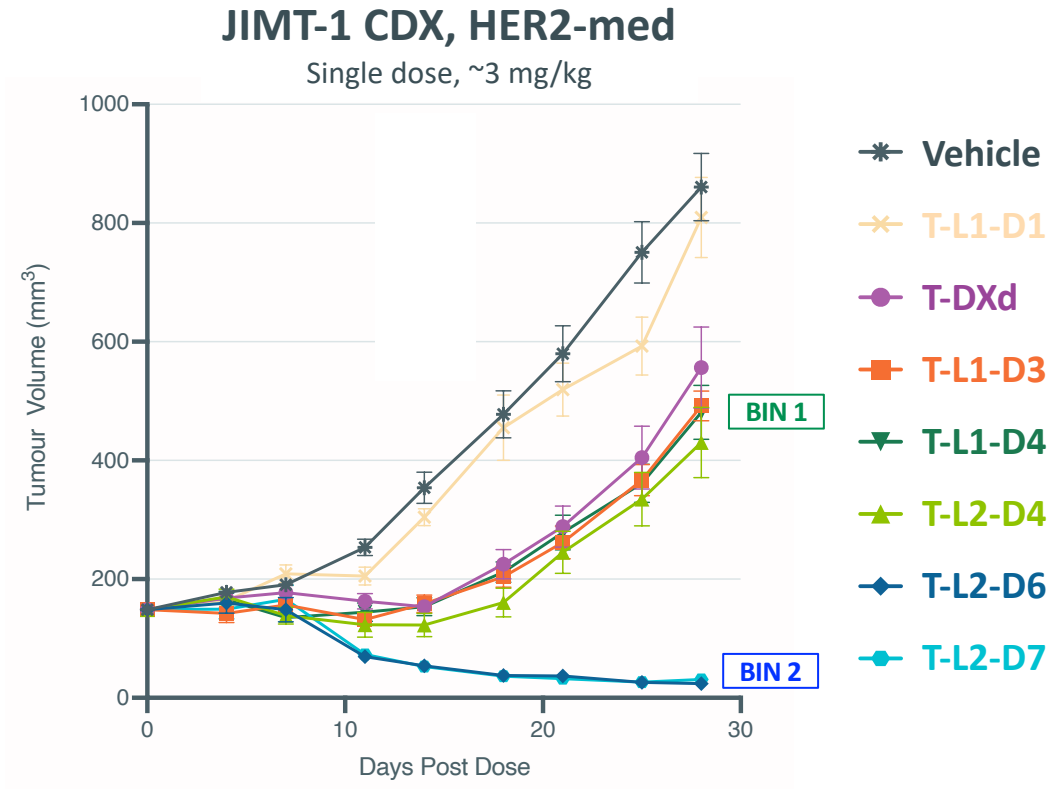
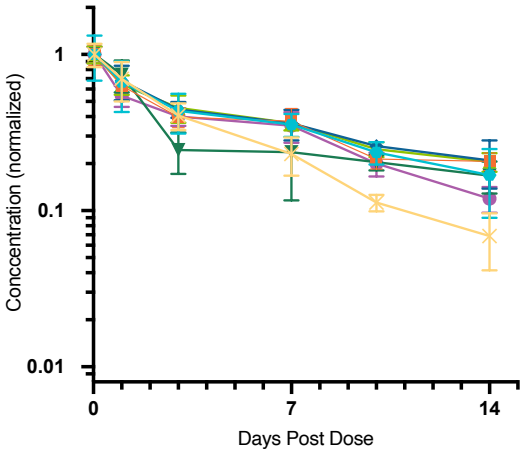
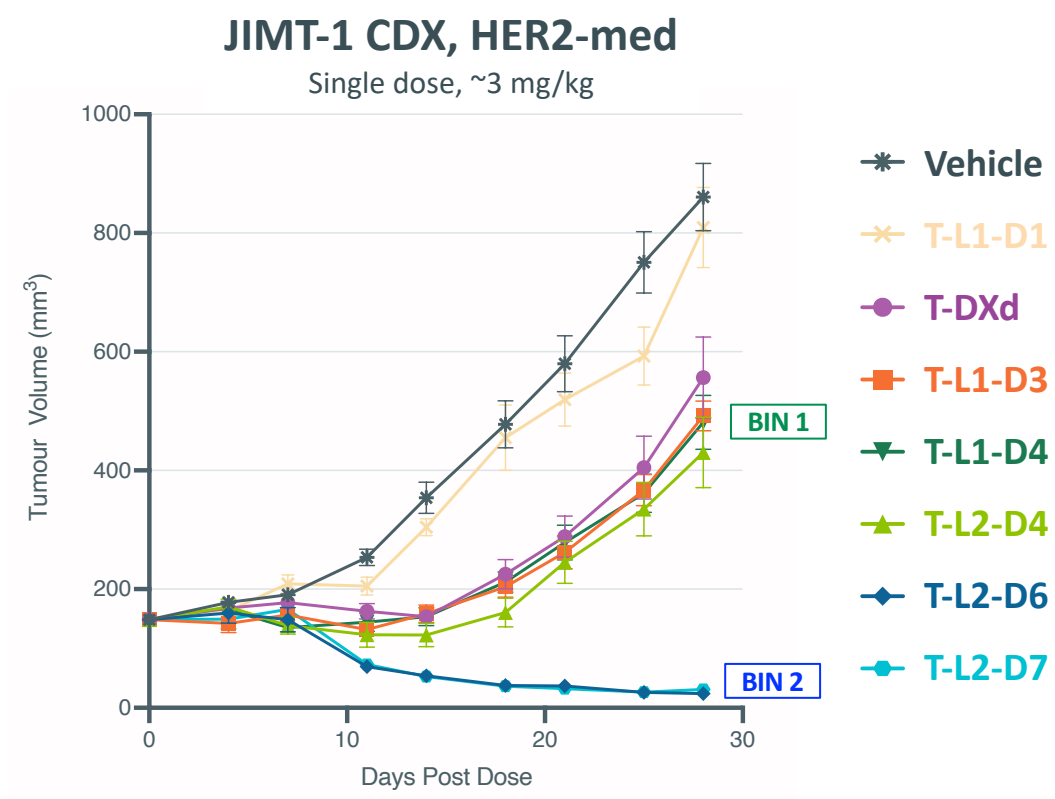


Total IgG by ELISA:

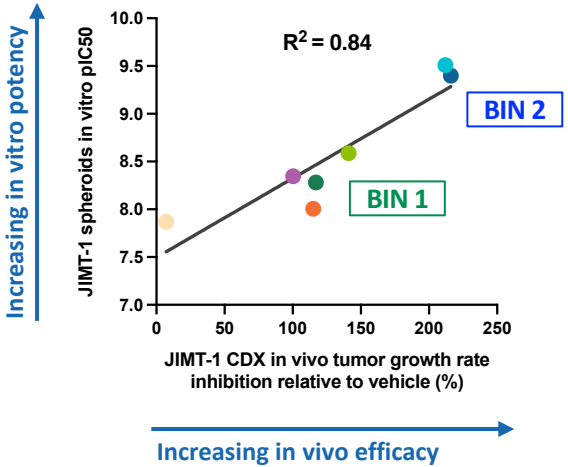


All test articles (except T-L1-D1) showed comparable PK profile, within assay variability

Most ZW TOPO1i ADCs Resulted in Comparable or Increased Efficacy vs. Benchmark in a JIMT-1 Study, Further Highlighting Two Separate Bins



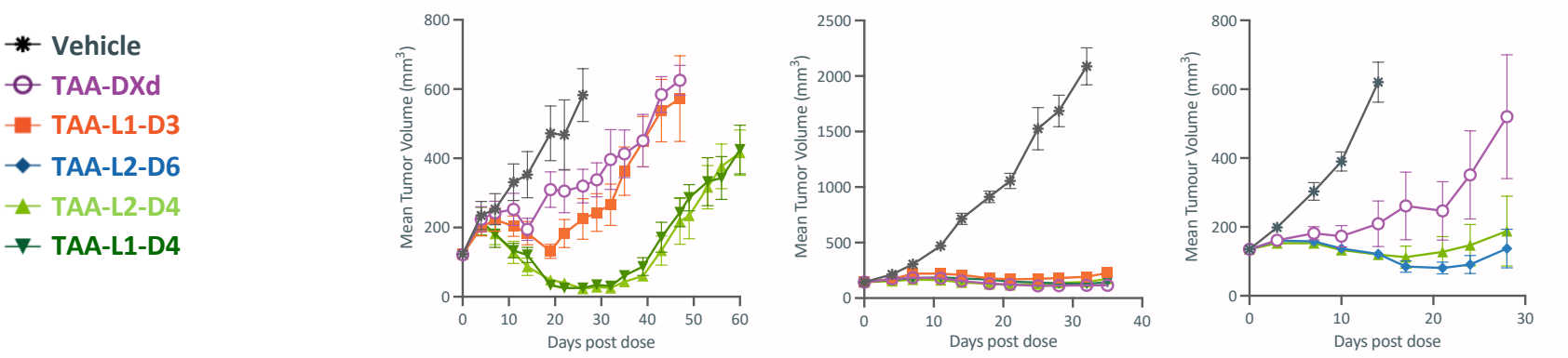
In vitro to in vivo correlation:



3D in vitro cytotoxicity better correlates with in vivo efficacy

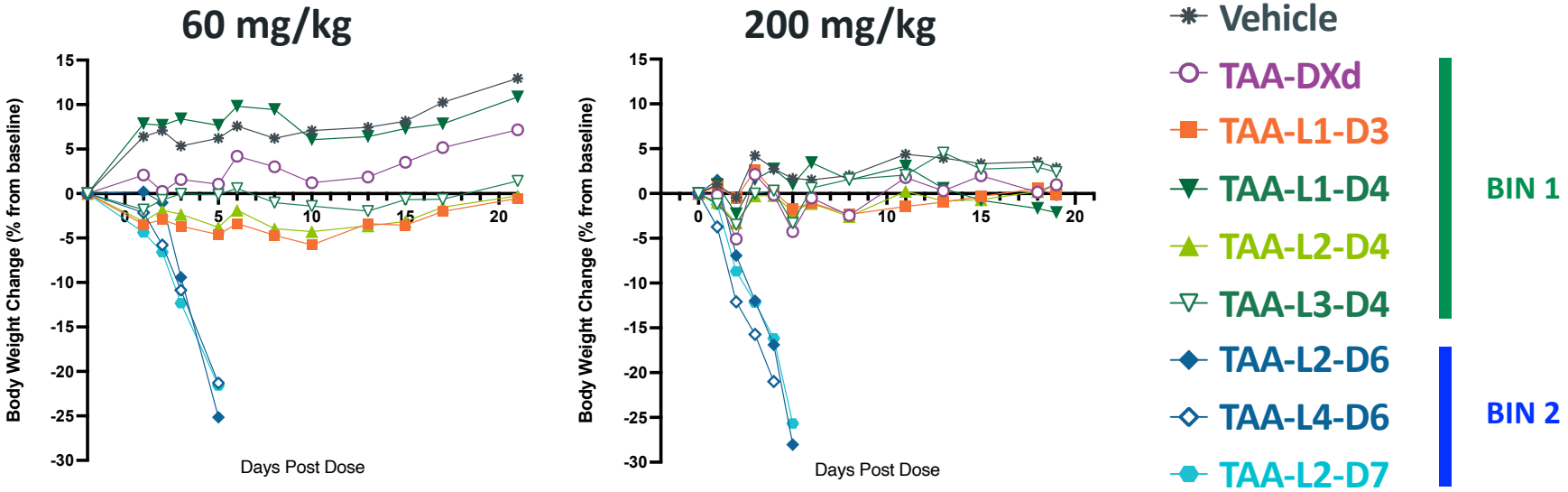
ZW TOPO1i ADCs Demonstrate Anti-Tumor Activity Comparable to DXd in Multiple *in vivo* Models

- Strong anti-tumor activity for DAR8 ADCs in cell line derived xenografts models across three targets with a single dose at 3 mg/kg



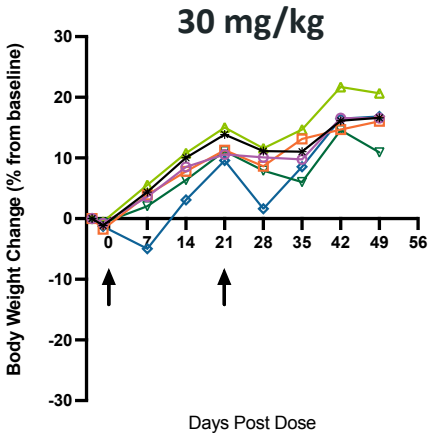
TAA	TAA1	TAA2	TAA3
Model	Ovarian CDX	Lung CDX	Solid tumor CDX
Target Expression Level	Med/Low, Heterogeneous	High	High/Med, Heterogeneous
Mice per group	6	6	6

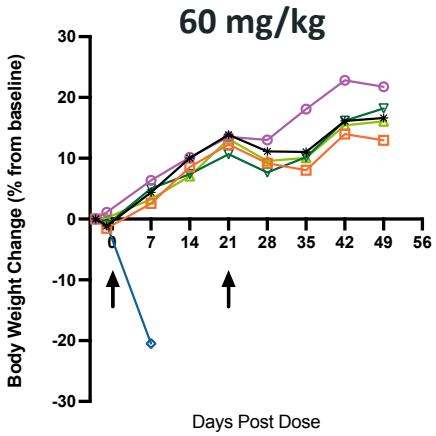
Four ZW TOPO1i ADCs are Tolerated in a High-Dose Murine Screening

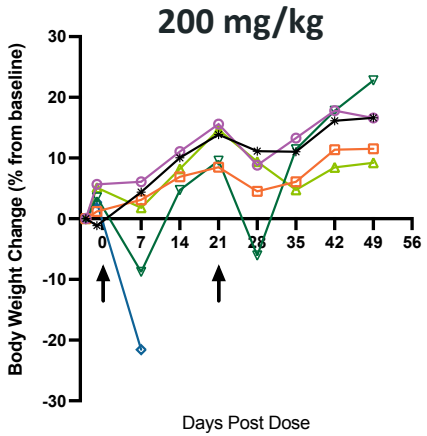


- Balb/c female mice, 8 weeks old
- 60 and 200 mg/kg
- Intraperitoneal injection, single dose
- 3 animals per group

Two ZW TOPO1i ADC Leads Identified in a Rat Tox Study *







- Vehicle
- TAA-DXd
- TAA-L3-D3
- TAA-L3-D4
- TAA-L4-D4
- TAA-L4-D6

- Female SD rats, 8 weeks old
- 30, 60 and 200 mg/kg
- IV injection, Q3Wx2
- 6 animals per group

**** Selection based on:***
body weight (shown), clinical signs, mortality, food consumptions, hematology, coagulation, clinical chemistry, urine analysis, histopathology, gross pathology, ophthalmoscopy, organ weights (not shown)

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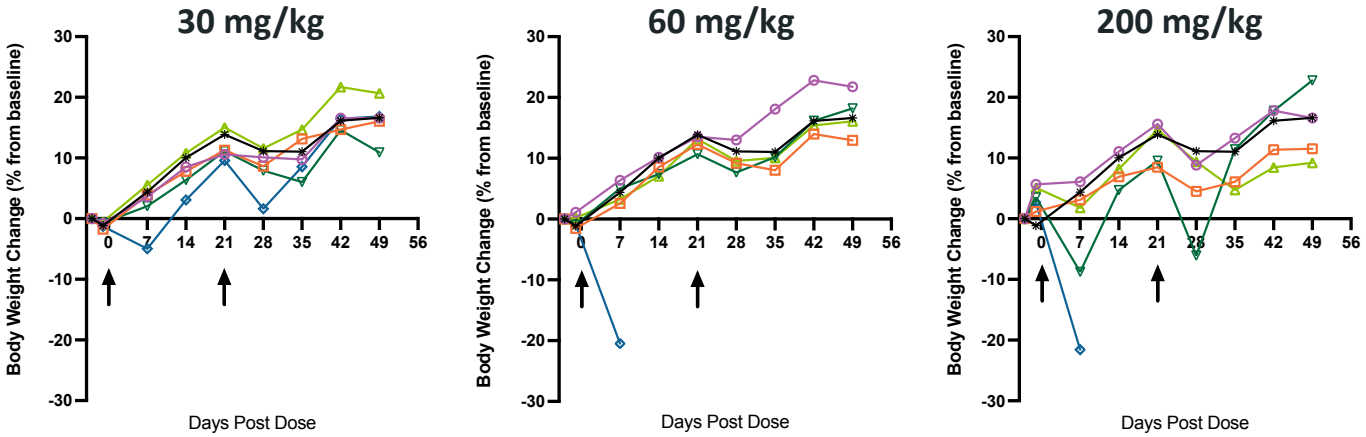
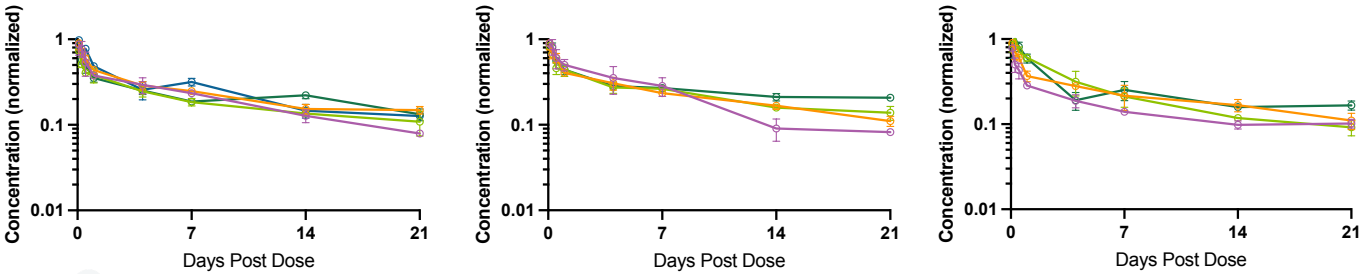
Two ZW TOPO1i ADC Leads Identified in a Rat Tox Study *



Toxicokinetic analysis showed comparable profile across the different test articles:



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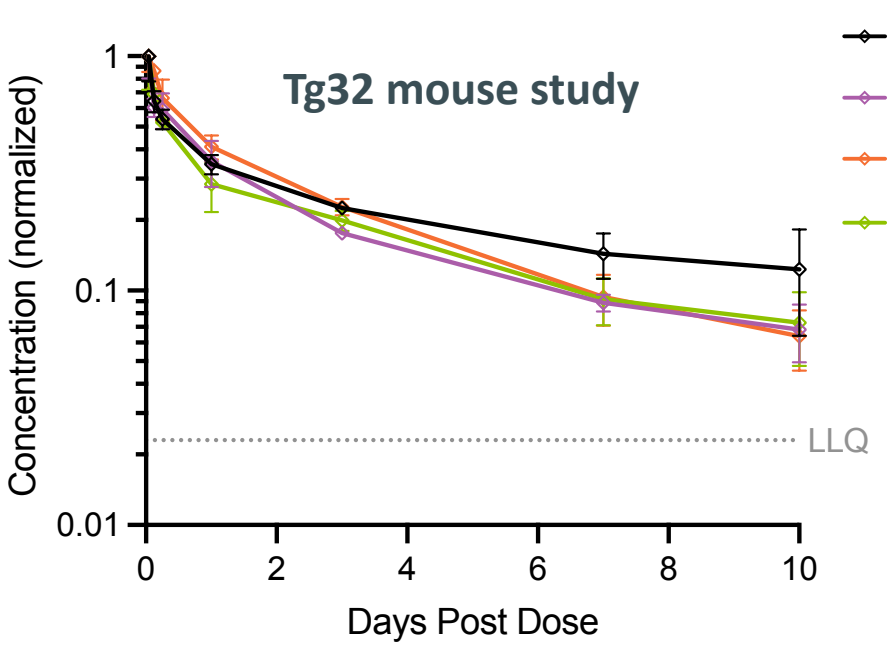
Microscopic Findings Confirm Good Tolerability and Dose/Response for TAA-L3-D3 and TAA-L4-D4

Test article	TAA-DXd			TAA-L3-D3			TAA-L3-D4			TAA-L4-D4		
Dose (mg/kg)	**30**	**60**	**200**	**30**	**60**	**200**	**30**	**60**	**200**	**30**	**60**	**200**
Bone marrow			X					X	X			X
Large intestine									X			
Small intestine		X	X				X	X	X		X	X
Lymph node		X	X		X	X	X	X	X		X	X
Spleen			X									
Thymus			X					X	X		X	X
Pancreas											X	X
Salivary gland						X					X	X

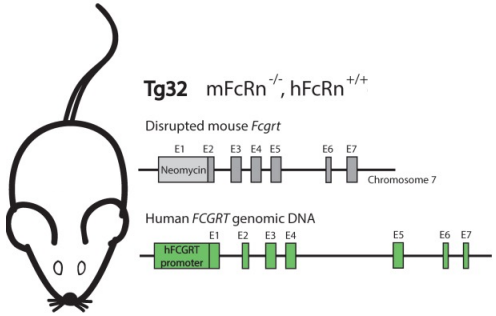
Microscopic findings* were observed in the GI tract, bone marrow, thymus, spleen, pancreas, and salivary glands. Microscopic findings had resolved by 28 days following the second dose.

* Severity not shown

ZW and DXd ADCs Showed Comparable PK Profiles in Tg32 Mice



- Naked TAA mAb
- TAA-DXd
- TAA-L3-D3
- TAA-L4-D4



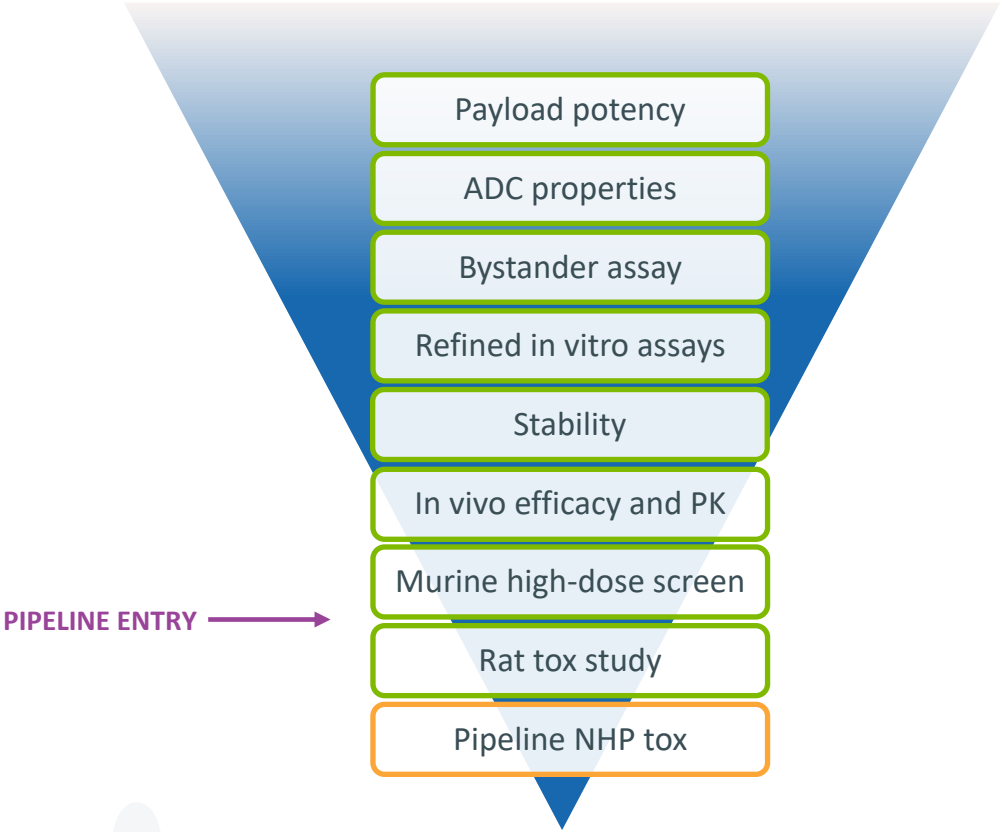
	Tg32 mice
FcRn	Human (random insertion)
Serum albumin	Murine

- 5 mg/kg, single dose
- Intravenous injection
- 4 animals per group

- Homozygous *Tg32* mice have the most human-like protection of humanized IgG
- Useful in evaluating the pharmacokinetics of human IgG

Adapted from: Nilsen, J.; Sandlie, I.; Roopenian, D.C.; Andersen, J.T. *Current Opinion in Chemical Engineering,* **2018**, *19*, 68-76

TOPO1i ADC Platform: **From Concept to Pipeline**



- Rapid effort to identify leads from ~100 TOPO1i payloads
- Comparable efficacy to industry leading DXd platform across different targets
- Two lead drug-linkers identified after rat tox study
- Pipeline NHP tox studies initiated
- Multiple pipeline programs in development

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