Development of a Novel TOPO1i ADC Platform: From Concept to Pipeline Application

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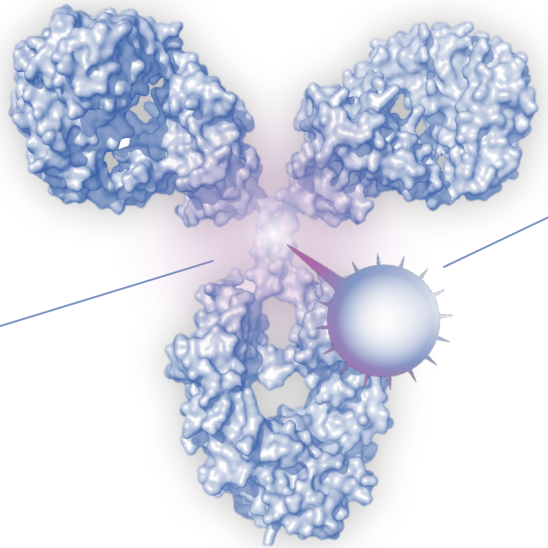
Anatomy of an Antibody-Drug Conjugate (ADC)

Antibody

- Pharmacokinetics
- Tumor targeting
- Internalization
- Fc binding profile
- Glycan profile

Linker

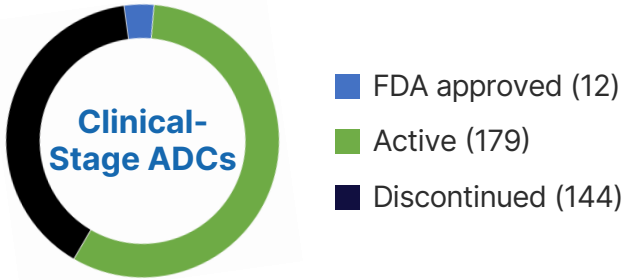
- Cleavable vs. non-cleavable
- Conjugation chemistry
- Stability
- Hydrophobicity



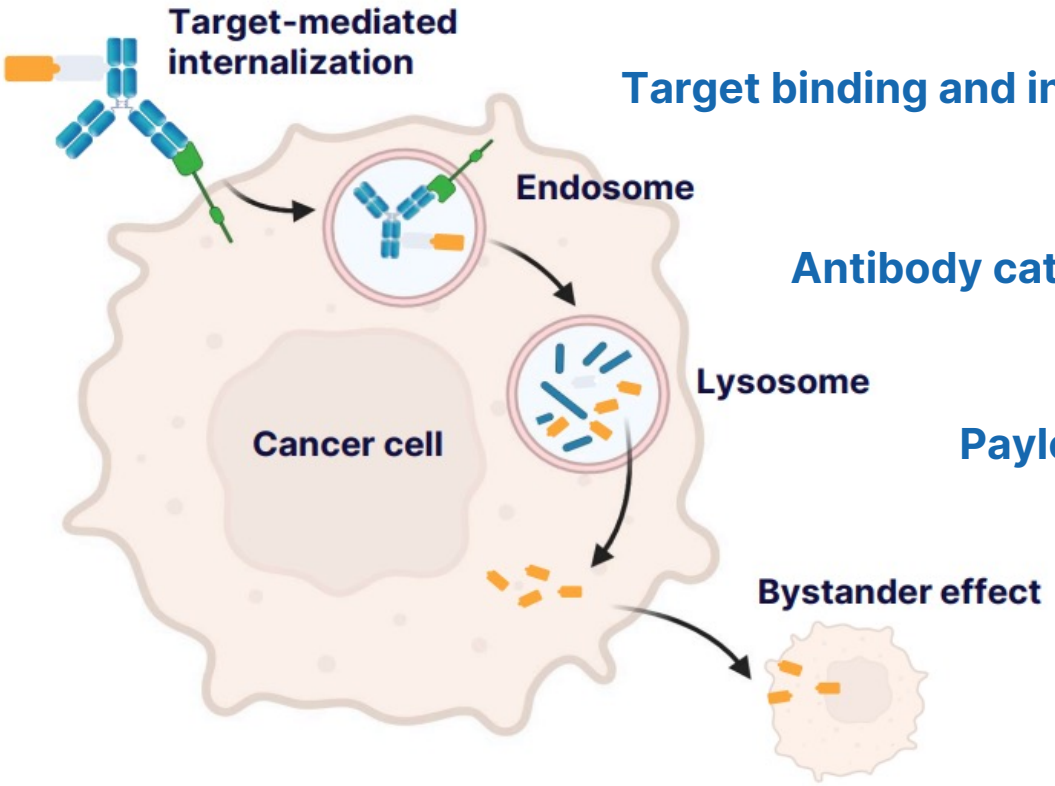
Payload

- Typically small molecules
- Mechanisms of action
- Potency
- Bystander activity
- Drug to Antibody Ratio (DAR)

>335 ADCs have reached the clinic



Conventional representation of the ADC mechanism



Target binding and internalization

Antibody catabolism and payload release

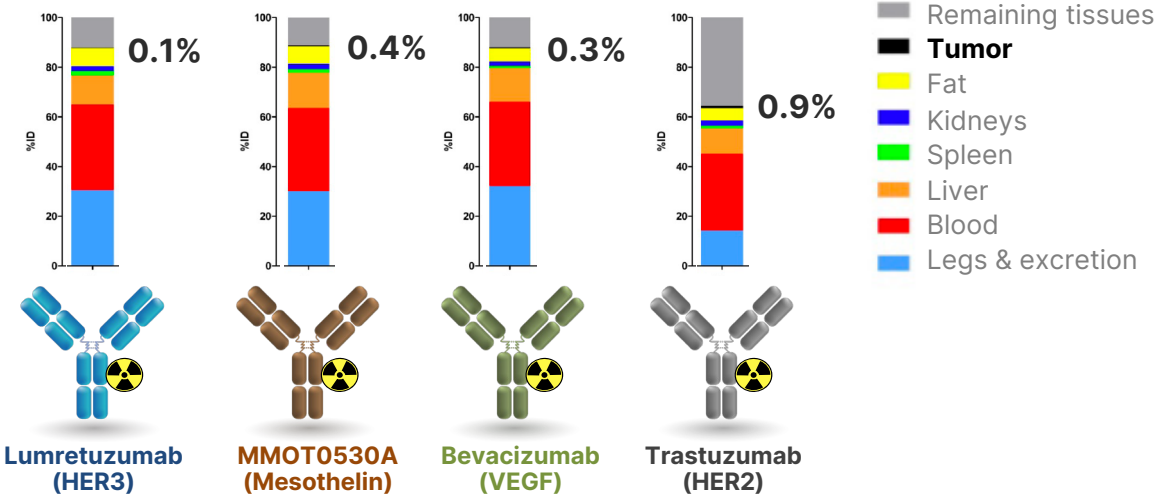
Payload exerts cytotoxic effect

Membrane permeable payloads can kill neighboring cancer cell

ADCs are commonly described as **“Magic Bullets”**

ADCs are not simple “magic bullets”

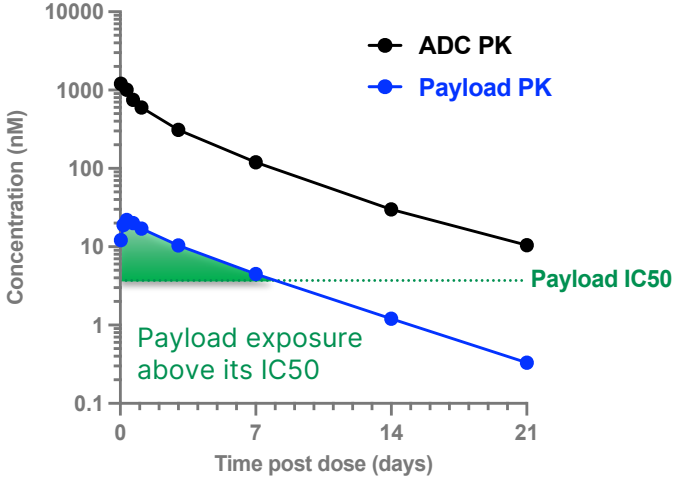
Irrespective of the target, radiolabeled antibodies show high normal tissue distribution and generally <1% tumor uptake in humans



Absolute **uptake in healthy tissues and tumor 4 days after dosing**

F. Bensch et al. *Theranostics* **2018**, *8*, 4295-4304

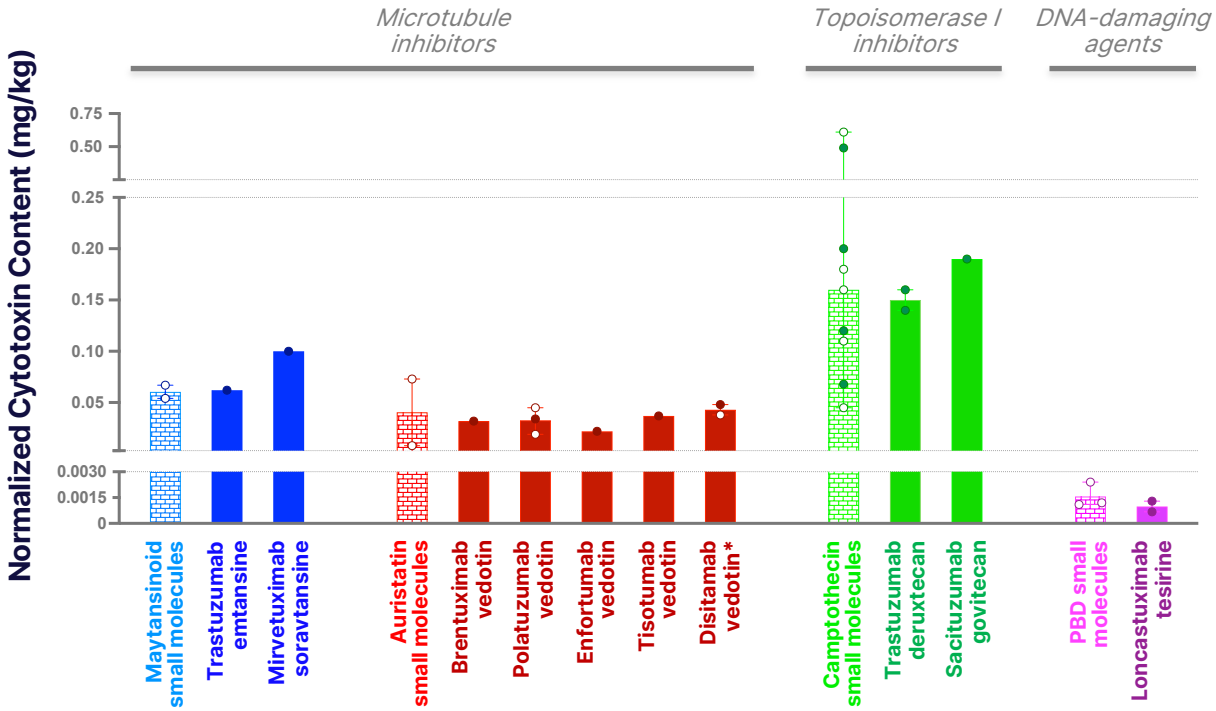
ADCs significantly alter payload PK



- Payload half-life extended from hours (typical small molecule PK) to days
- Payload exposure contributes to clinical efficacy and tolerability

Data from DESTINY-Gastric01.
Original concept: E. Tarcsa et al. *Drug Discov. Today Technol.* **2020**, *37*, 13-22

Human MTD of approved ADCs is comparable to human MTD of related small molecules



Small molecules	Approved ADCs
Maytansinoid	Maytansinoid
Auristatin	Auristatin
Camptothecin	Camptothecin
PBD	PBD

- ● *MTD for approved drug*
- ○ *MTD for experimental drugs*

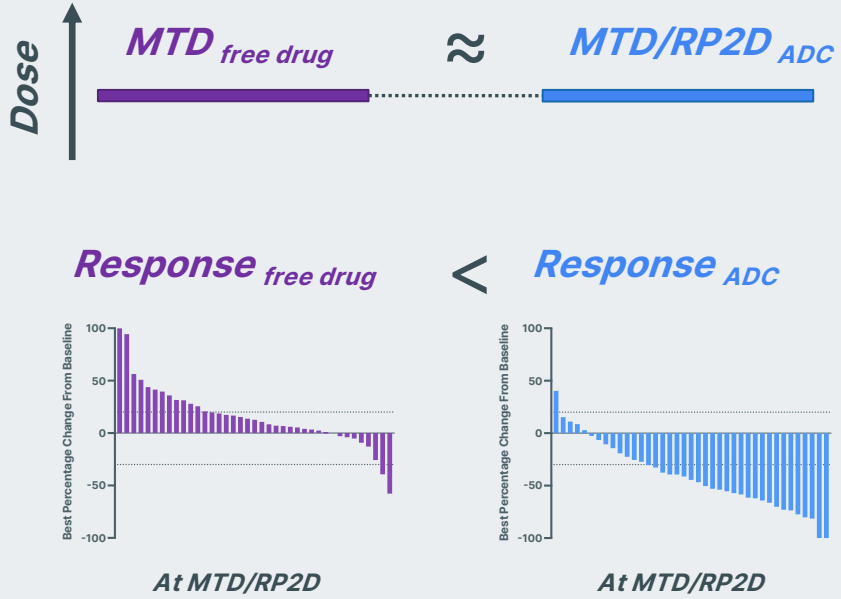
Normalized cytotoxin content

$$= \frac{Dose_{ADC} \cdot DAR \cdot MW_{payload}}{MW_{ADC}}$$

R. Colombo, J. R. Rich. *Cancer Cell,* **2022,** *40*, 1255-1263

Revised representation of ADC therapeutic window (in humans)

Revised representation based on emerging clinical data

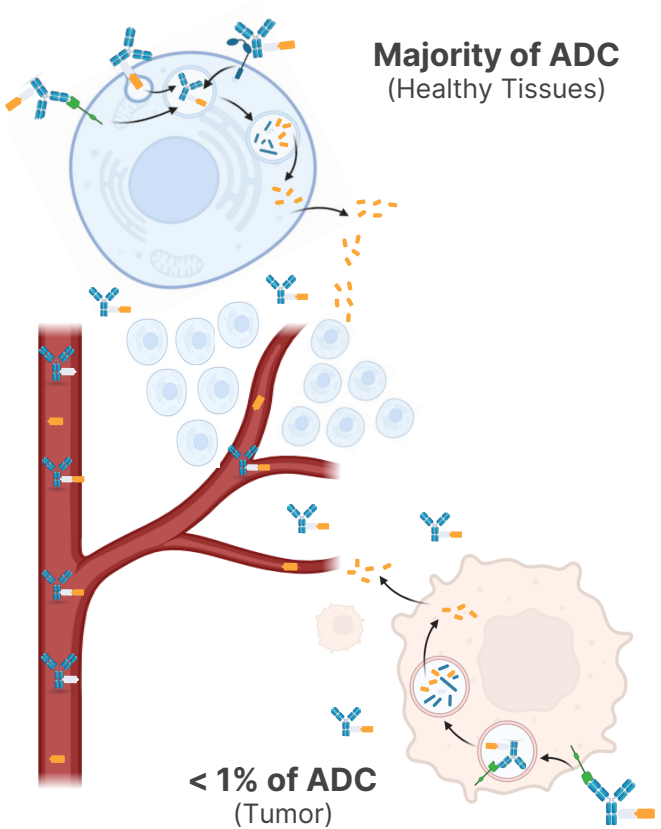


- **ADCs do not significantly increase the maximum tolerated dose (MTD)** of their conjugated payloads

- **Minimum efficacious dose (MED) not established** in clinical studies
- When dosed at their MTD/RP2D, **ADCs can offer improved efficacy over related unconjugated small molecules** (and, in certain cases, standard of care)

R. Colombo, J. R. Rich. *Cancer Cell,* **2022,** *40*, 1255-1263

We tend to optimize ADCs for the 1% and not the for the 99%



In pursuit of "magic bullets":

- ***Payload*** should be **as potent as possible**
  Highly potent payloads lead to toxic ADCs with a poor therapeutic index
- ***Linker*** should be **as stable as possible** in circulation
  All approved ADCs feature linker instability
- ***Antibody*** should **have high affinity as possible** to a target **only expressed in the tumor**
  Leads to binding site barriers and poor tumor penetration

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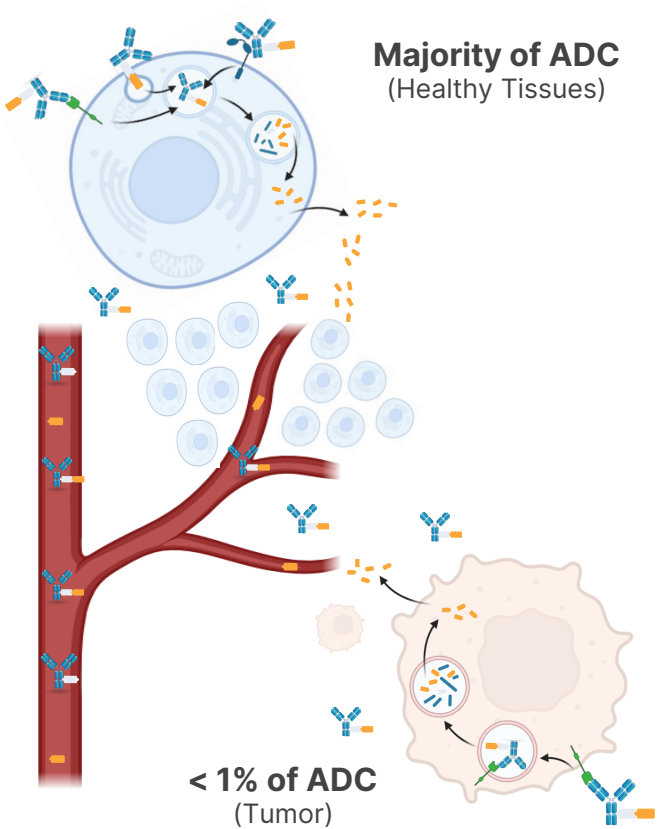

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- ***Antibody*** should **have high affinity as possible** to a target **only expressed in the tumor**
  Leads to binding site barriers and poor tumor penetration

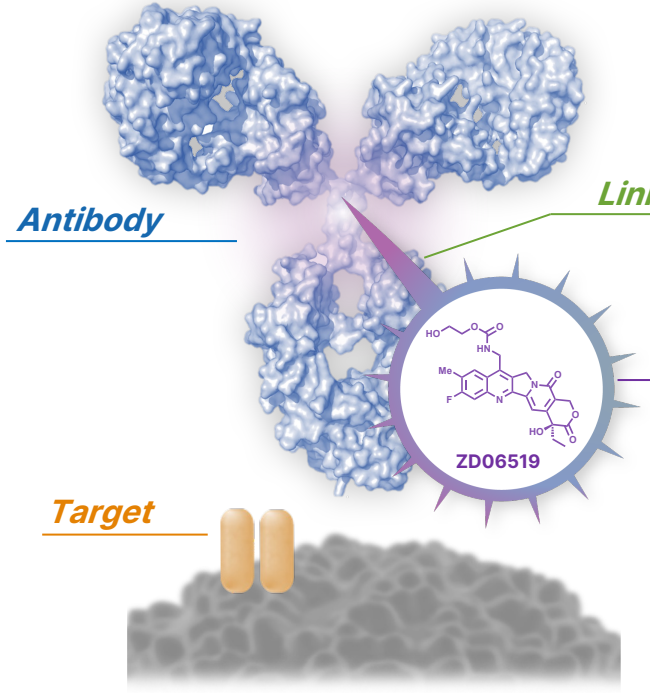
Considering disposition is mainly off-tumor:

- ***Payload*** should be **drug-like**
- ***Linker*** should be **traceless** for **bystander activity**
- ***Linker*** should **not** be **overly stabilized**
- ***Antibody*** should be **optimized** for tumor penetration and payload delivery

Emerging clinical data applied to the design of Zymeworks' TOPO1i ADCs

- **Antibody** selected for optimal **internalization, tumor penetration,** and **payload delivery**

…it's not just affinity



- Leveraging validated **peptide-cleavable linkers** and **stochastic conjugation**

…it's not just stability

- Development of **proprietary TOPO1i payload (ZD06519)**

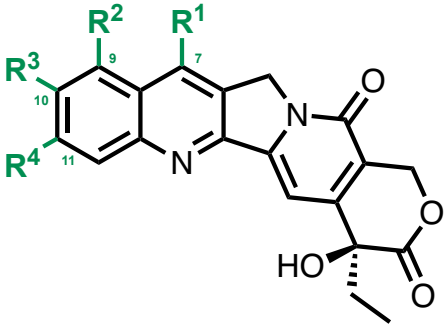
…it's not just potency

- **Right ADC design for target and indication**

…one size does not fit all

TOPO1i: topoisomerase-1 inhibitor

Camptothecins have been known for 60 years



Potent inhibitors of topoisomerase I:

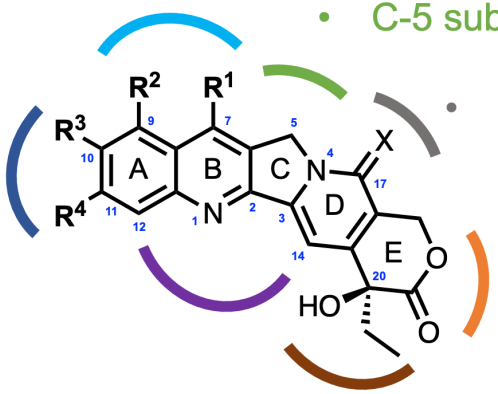
- Discovered in the 1960's by M. E. Wall and M. C. Wani
- Isolated from *Camptotheca acuminata* (The Happy Tree)
- Prevent DNA religation which results in double strand breaks and apoptosis

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

Leveraging 60 years of camptothecin SAR knowledge

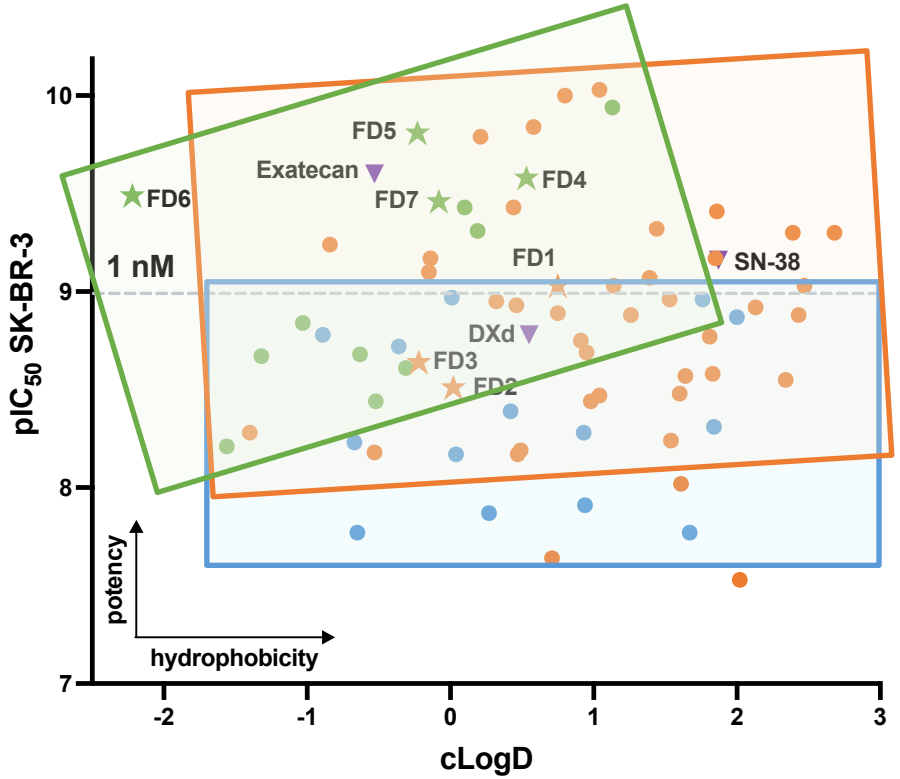
- C-7 and C-9 positions tolerate a variety of functional groups, including fused rings
- C-7 substitution improves solubility

- Large substituents at C-10 and C-11 reduce potency
- C-10 substitution by electron rich groups preferred
- 10-NH2 increases potency
- 11-F increases potency
- 10,11-methylenedioxy improves potency

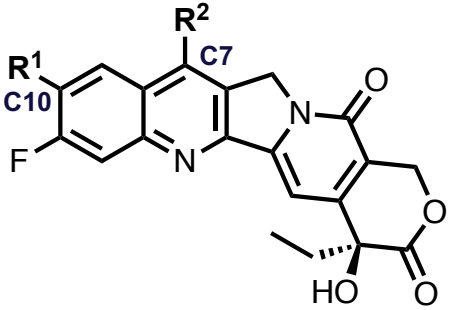


- C-5 substitutions reduce potency
- C-17 O or S required for potency
- Lactone form significantly more potent than open form
- C-12, N-1, and C-14 substitutions reduce potency
- 20-OH group and 20-S configuration are critical for potency

Selection of lead payloads from library of camptothecin analogs



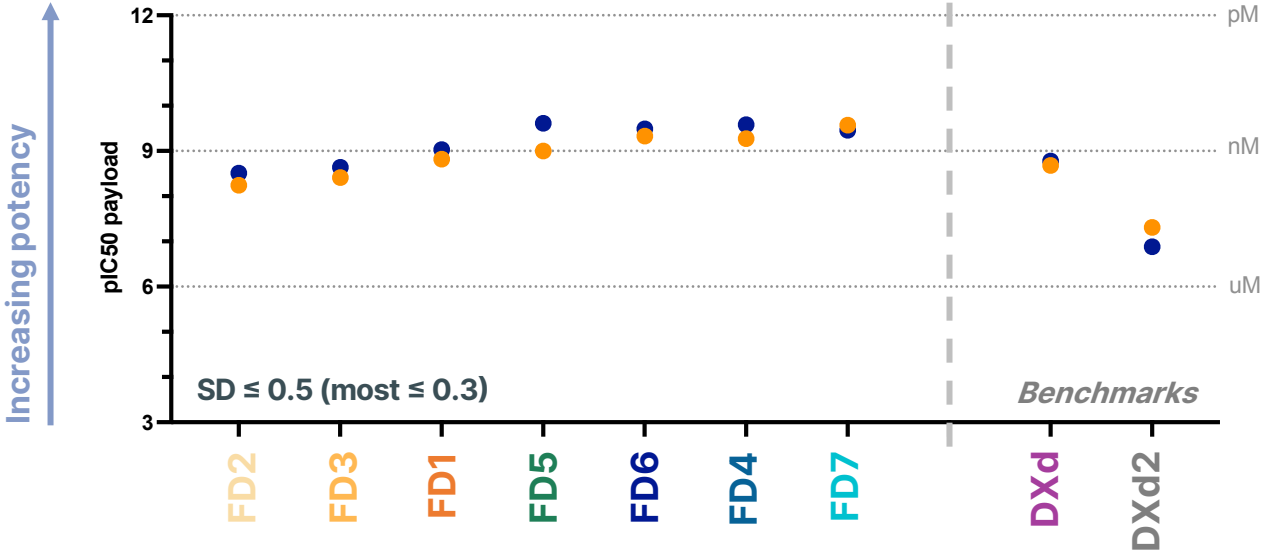
R^1 = Me
R^1 = OMe
R^1 = NH_2

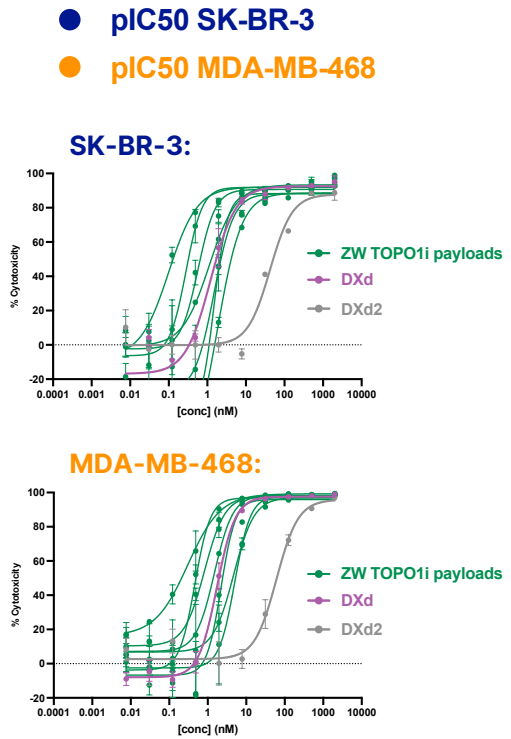


R^2 = Amines, Ureas, Carbamates, Sulfonamides

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

Payloads showed potency between 10 and 0.1 nM in multiple cell lines



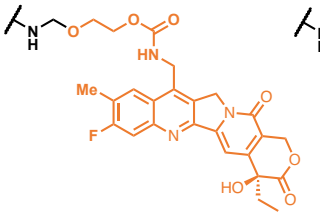


Representative pIC50s; >70 cell lines tested

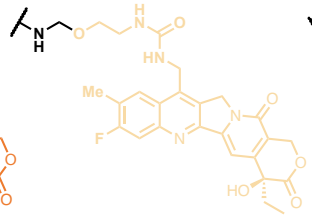
$pIC50 = -\log_{10}(IC50)$

Drug-linkers were generated using C7 or C10 attachment points

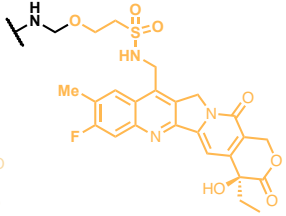
C-7 hemiaminal ether linked payloads



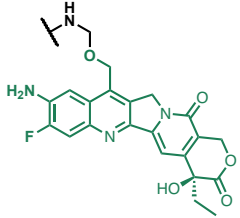
DL1 = MT-GGFG-AM-FD1
DL2 = MC-GGFG-AM-FD1



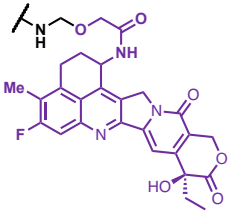
DL3 = MT-GGFG-AM-FD2



DL4 = MT-GGFG-AM-FD3

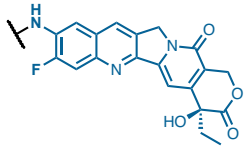


DL5 = MT-GGFG-AM-FD5
DL6 = MC-GGFG-AM-FD5

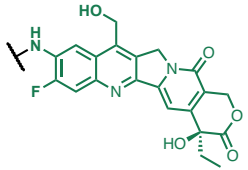


DL7 = MT-GGFG-AM-DXd
DL8 = MC-GGFG-AM-DXd
(Deruxtecan)

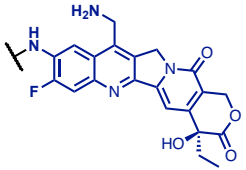
C-10 amide linked payloads



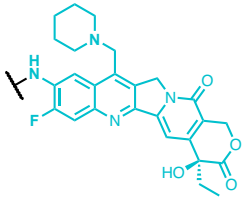
DL9 = MT-GGFG-FD4
DL10 = MC-GGFG-FD4



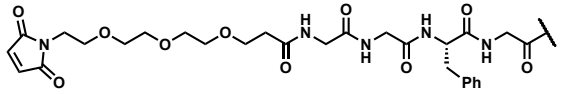
DL11 = MT-GGFG-FD5
DL12 = MC-GGFG-FD5



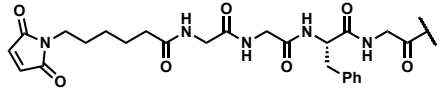
DL13 = MT-GGFG-FD6



DL14 = MT-GGFG-FD7

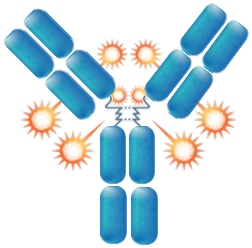


MT-GGFG-

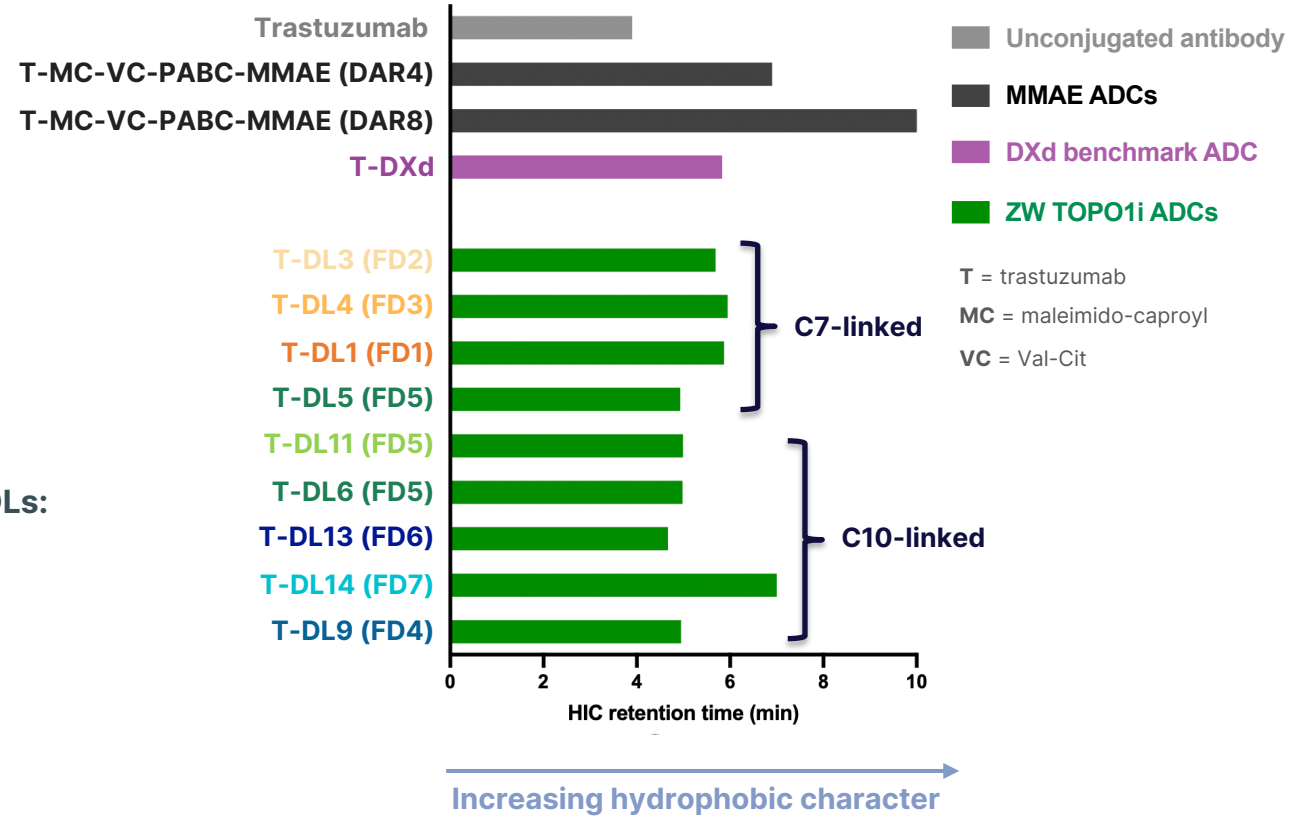


MC-GGFG-

Drug-linkers yield trastuzumab ADCs with desired physicochemical properties and exceptionally low aggregation



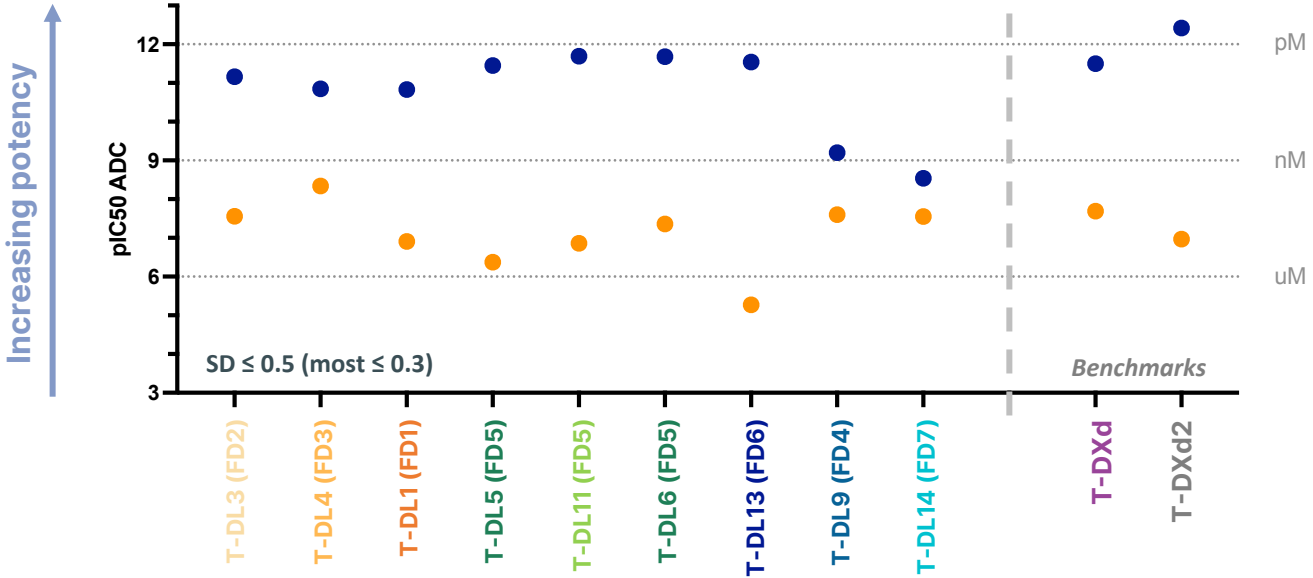
mAb = trastuzumab
conjugation = cysteine
DAR = 8

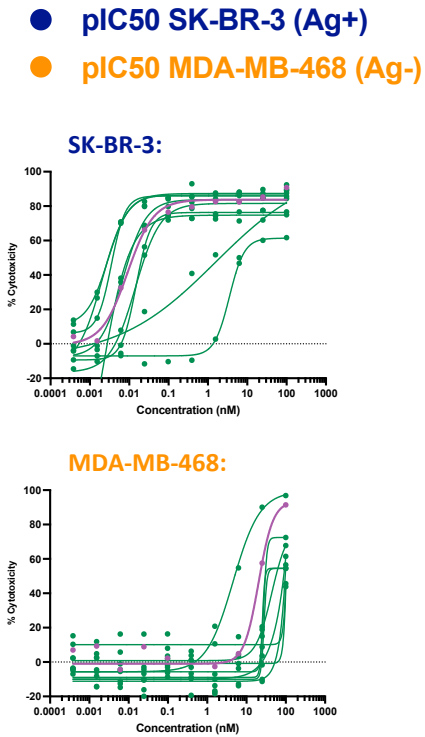


ADCs with Zymeworks TOPO1i DLs:

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

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

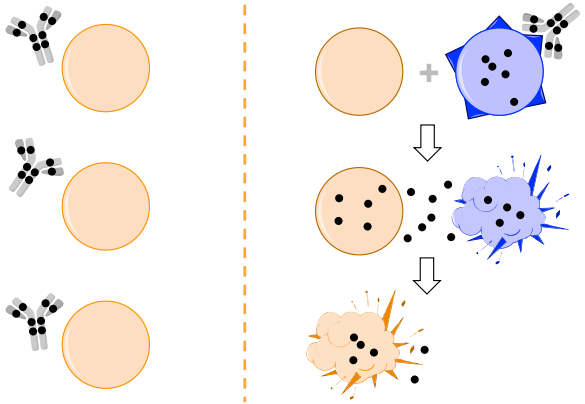
MDA-MB-468

(HER2-)

Co-culture

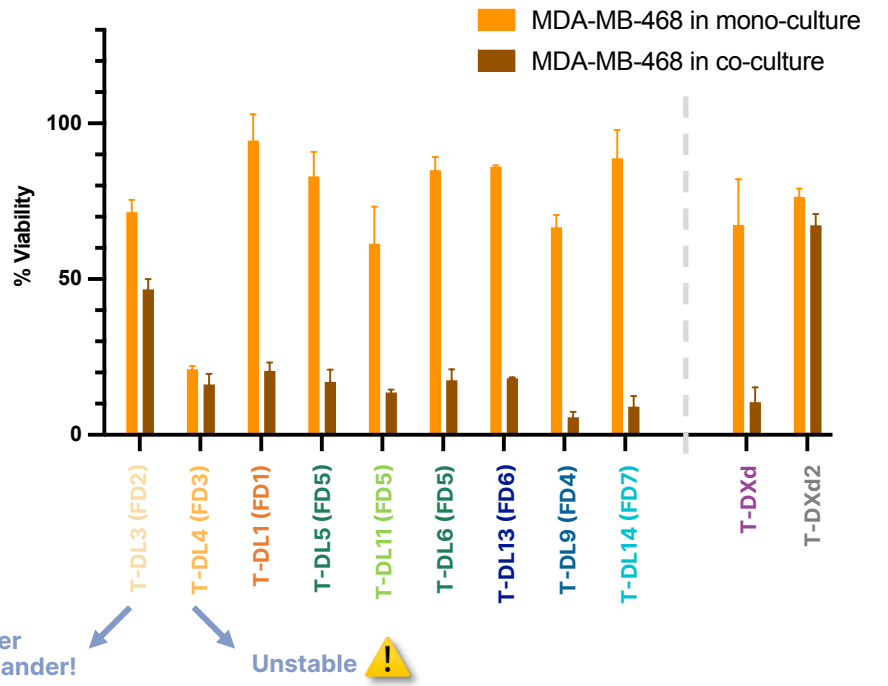
MDA-MB-468 + SK-BR-3

(HER2-) (HER2+)



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

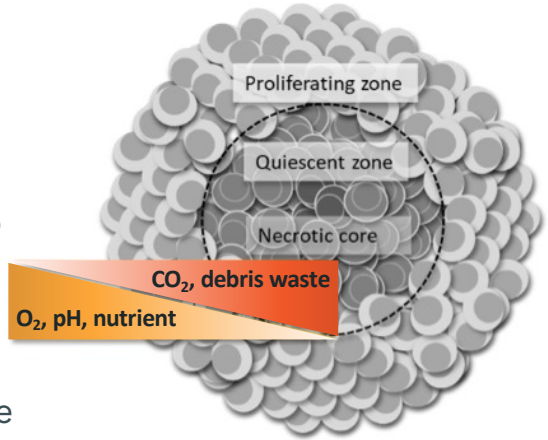
1 nM ADC treatment



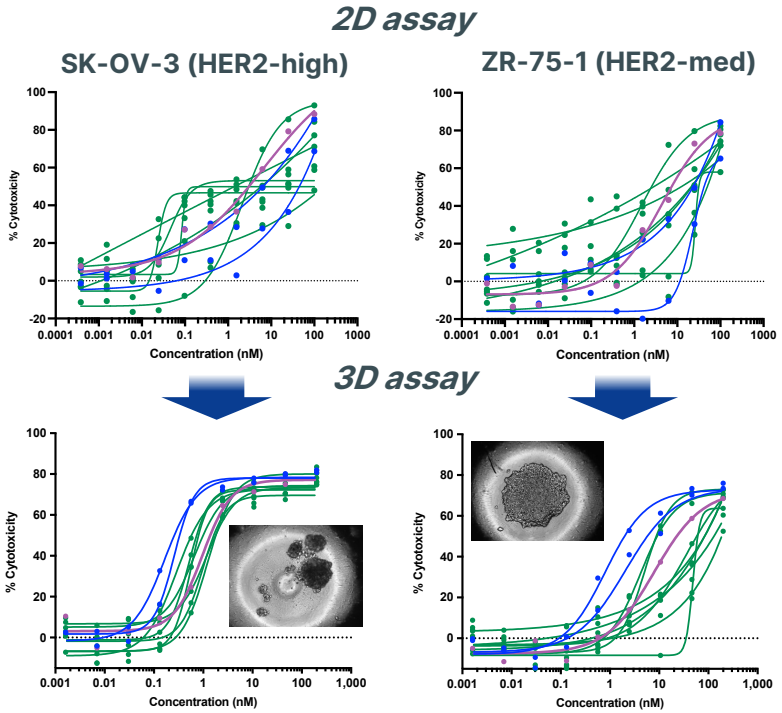
Spheroid cytotoxicity assay was developed to screen TOPO1i ADCs

Key spheroid features:

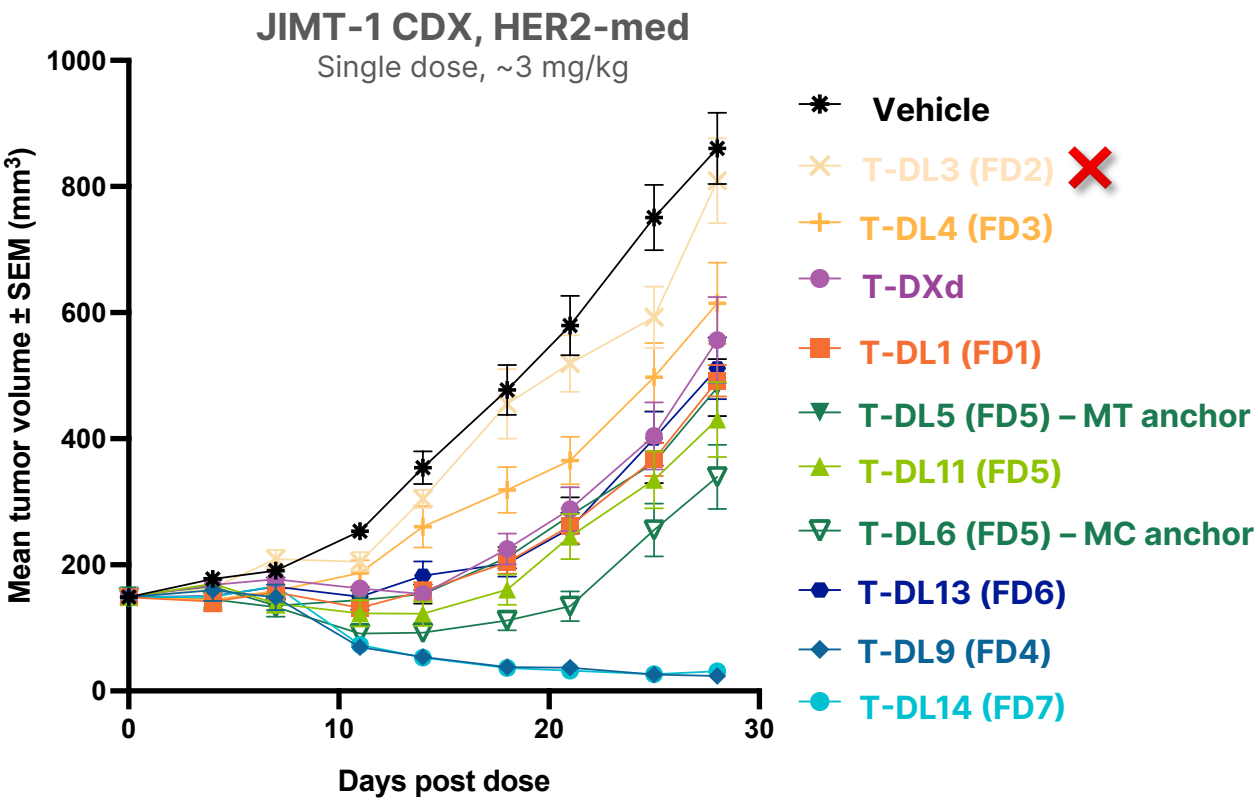
- Spatial organization
- Layers of distinct cell populations
- Formation of different gradients from outer to inner regions
- More complex cell signaling
- Potential to recapitulate drug resistance and metabolic adaptation



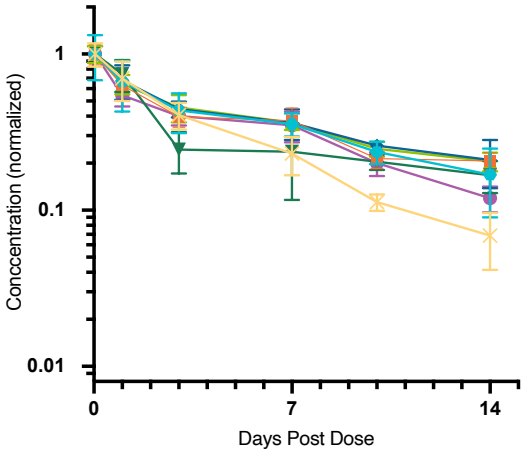
Adapted from: Pinto B, Henriques AC, Silva PMA, Bousbaa H. *Pharmaceutics.* **2020**, *12*, 1186



Most ADCs demonstrated comparable or increased efficacy vs. T-DXd benchmark in a JIMT-1 xenograft study

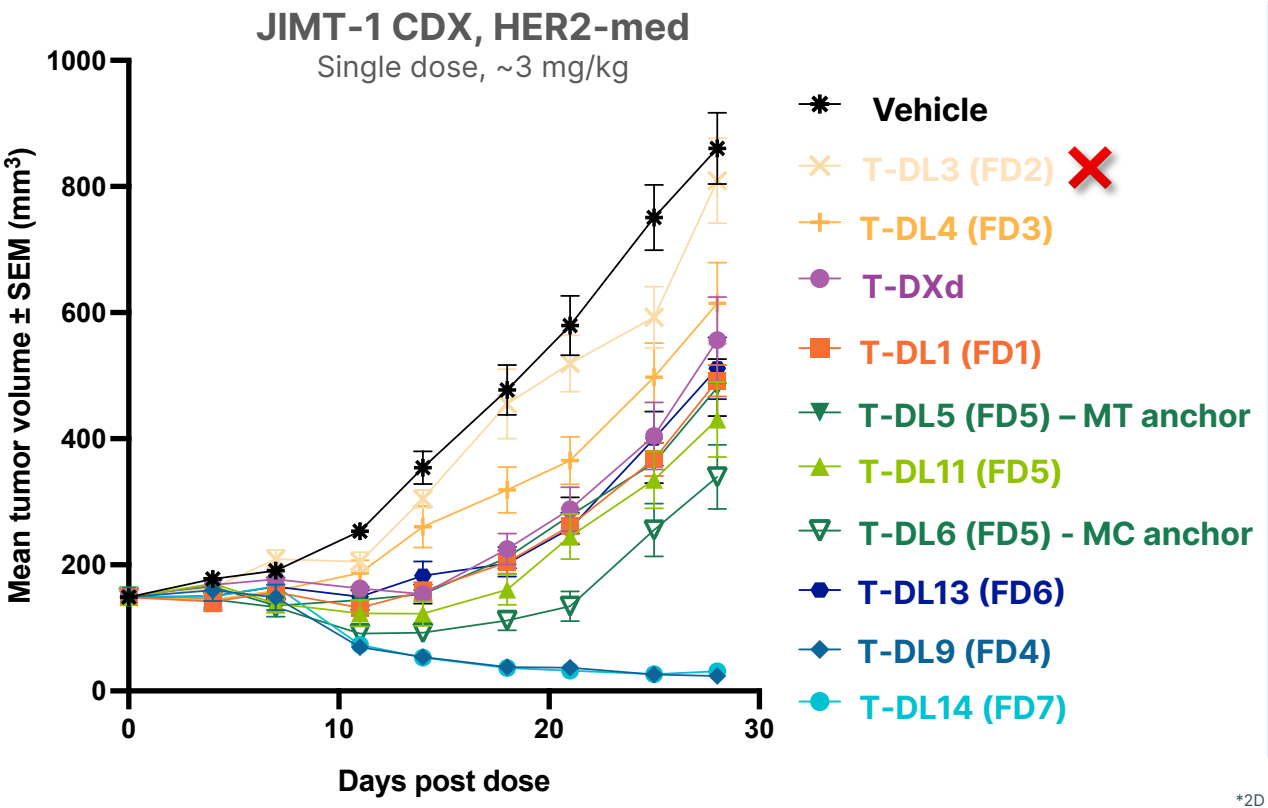


Total IgG by ELISA:

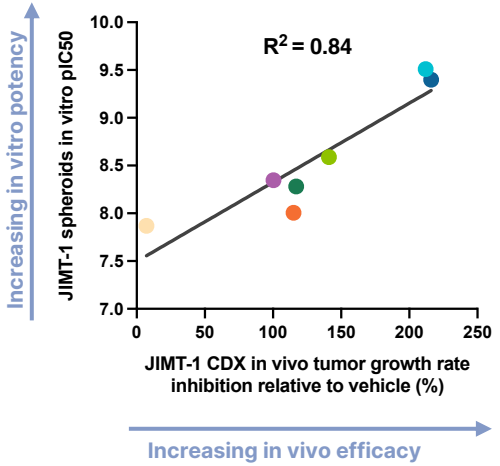


All test articles (except DL3) showed comparable PK profile

Most ADCs demonstrated comparable or increased efficacy vs. T-DXd benchmark in a JIMT-1 xenograft study



In vitro to in vivo correlation:



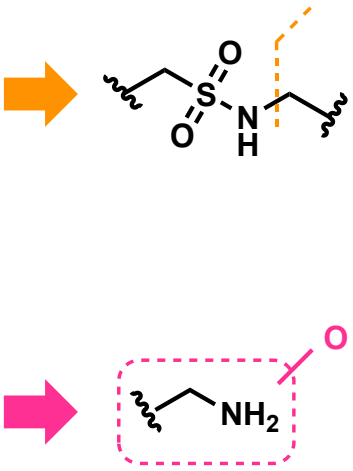
3D in vitro cytotoxicity correlates with in vivo efficacy*

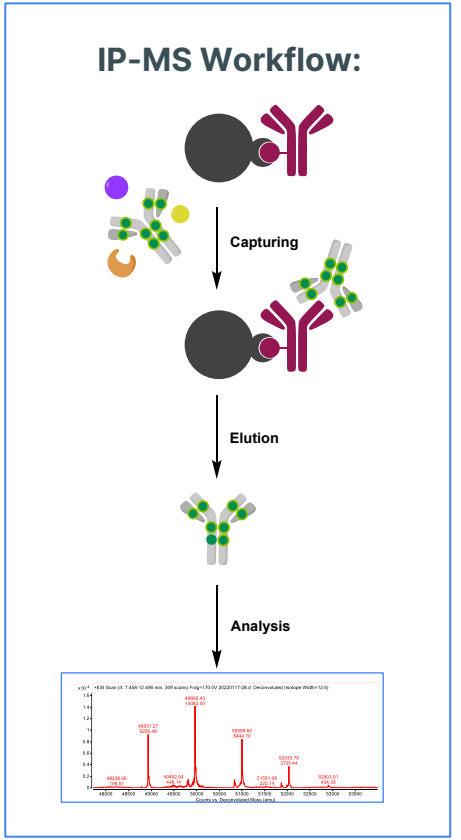
*2D in vitro cytotoxicity on JIMT1 resulted in pIC50s < 7 with incomplete curves

Plasma stability assays revealed liabilities for two drug-linkers

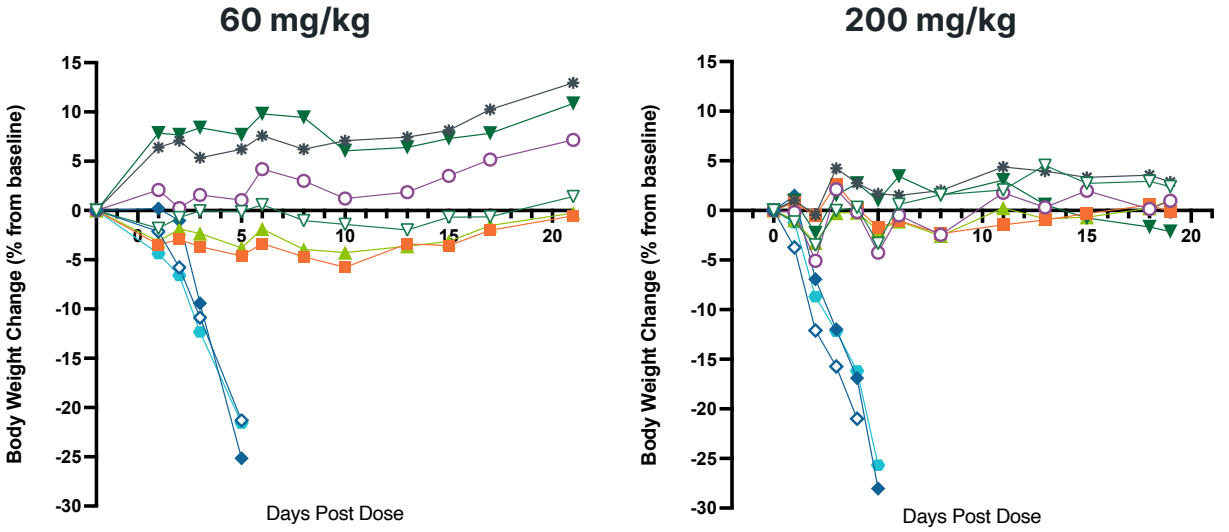
ADC	Observed payload instability (7 d, mouse plasma)
T-DXd	none
T-DL3 (FD2)	none
T-DL4 (FD3) ✗	drug-linker fragmentation
T-DL1 (FD1)	none
T-DL5 (FD5)	none
T-DL11 (FD5)	none
T-DL6 (FD5)	none
T-DL13 (FD6) ✗	drug-linker oxidation
T-DL9 (FD4)	none
T-DL14 (FD7	none

✗ design criteria not met





Four ADCs were tolerated at high-doses in mice



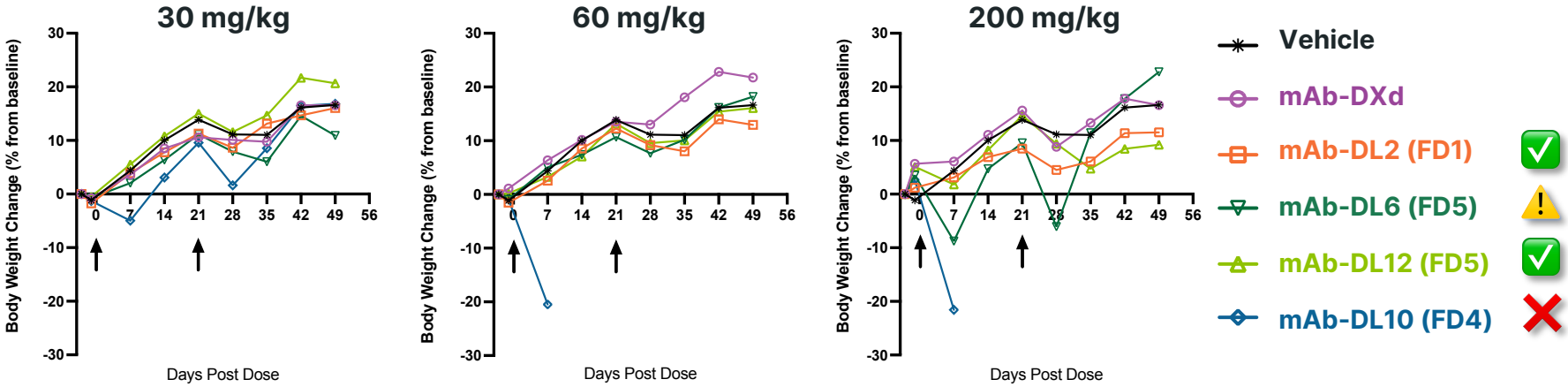
- Vehicle
- mAb-DXd
- mAb-DL1 (FD1)
- mAb-DL5 (FD5) – MT anchor
- mAb-DL11 (FD5)
- mAb-DL6 (FD5) – MC anchor
- mAb-DL9 (FD4) – MT anchor
- mAb-DL10 (FD4) – MC anchor
- mAb-DL14 (FD7)

✅ 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

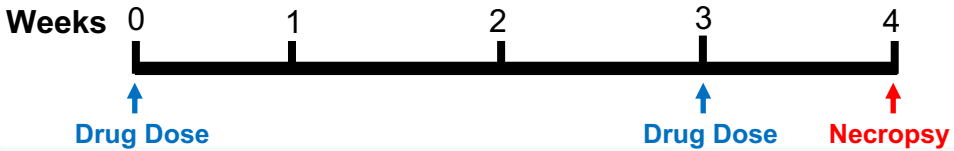
✅ **design criteria met**

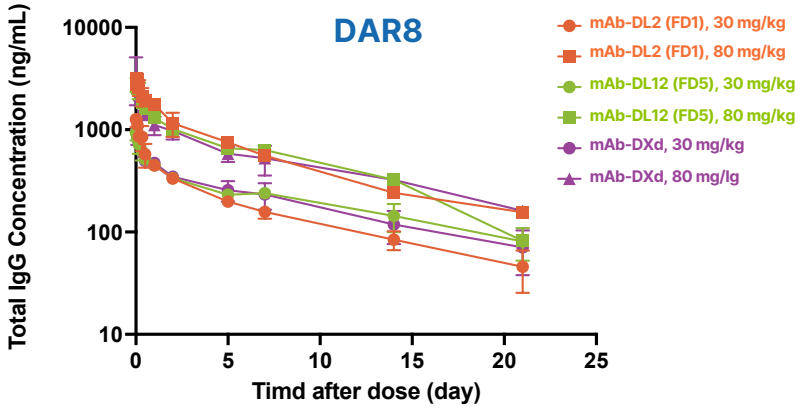
⚠️ **not better than mAb-MC-GGFG-FD5**

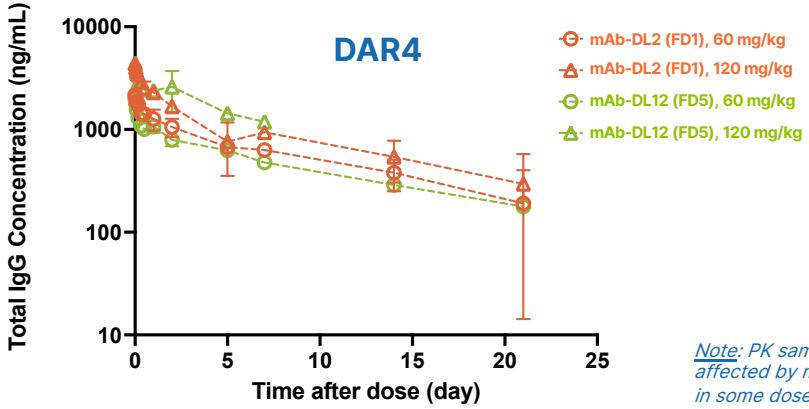
❌ **design criteria not met**

Two dose NHP ADC toxicity study support the selection of MC-GGFG-AM-FD1 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-DL2 (FD1)	4	60	Y
5			**120**	**Y**
6		8	30	Y
7			80	N
9	mAb-DL12 (FD5)	4	60	Y
10			**120**	**N**
11		8	30	Y
12			80	N

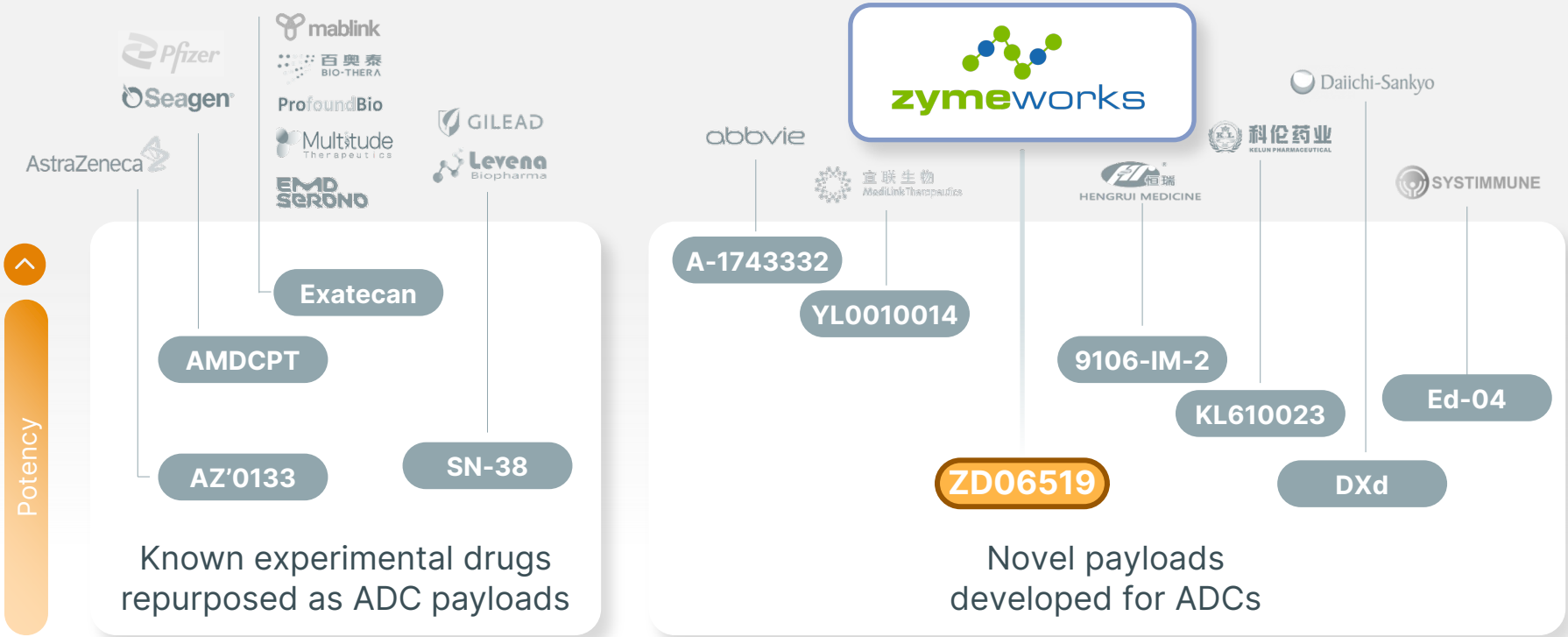






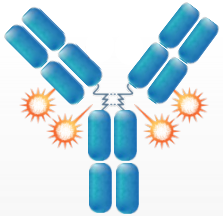
Note: PK sampling affected by mortality in some dose groups

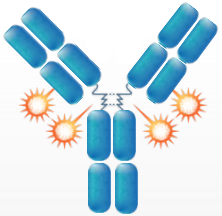
ZD06519 (FD1) payload was selected with ADCs in mind



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

ZD06519 payload is being utilized in multiple pipeline programs



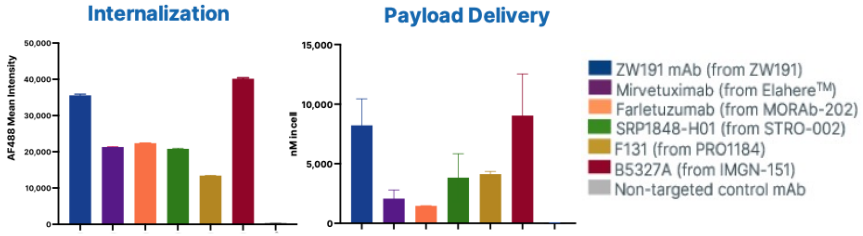


	ZW191	ZW220	ZW251
Target	FRα	NaPi2b	GPC3
Format/Technology	Monospecific/TOPO1i ADC	Monospecific/TOPO1i ADC	Monospecific/TOPO1i ADC
Potential Indications	Ovarian cancer, other gynecological cancers, and other solid tumors	Ovarian cancer, NSCLC	Liver cancer
Stage	IND-enabling	IND-enabling	IND-enabling
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

Novel anti-FRα mAb selected for enhanced internalization and payload delivery

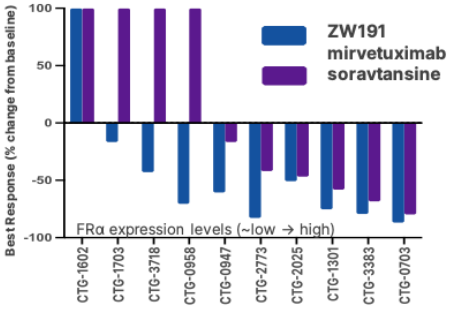


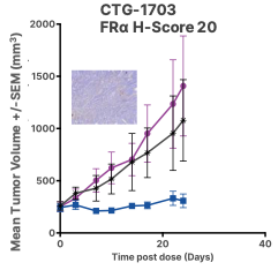
ZW191 shows a compelling tolerability profile of 60 mg/kg in NHP

Dose mg/kg	Clinical observations	Histopathology	Clinical Chemistry	Hematology & coagulation	Adverse effects	HNSTD
10	None	None	↑ AST, ALT (n=1)	No effects	None	60 mg/kg
30	Emesis/vomitus	↓ Thymic lymphocytes, ↓ PACS	↑ AST, ALT			
60	Liquid/discolored feces Emesis/vomitus ↓ activity level (n=1)	↓ Thymic lymphocytes, ↓ PACS	↑ AST, ALT ↑ CK			

ZW191 Demonstrates activity across multiple tumor types and range of FRα expression (PDX models)

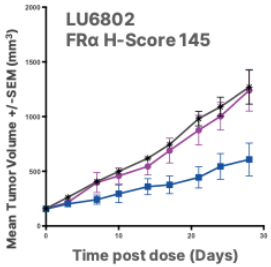
Ovarian Cancer



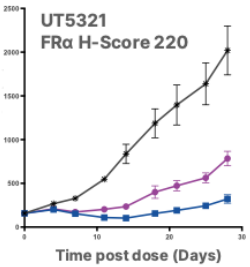


In vivo efficacy of ZW191 and mirvetuximab soravtansine was assessed in Nude or NOD/SCID mice, n=3 per group, single dose 6mg/kg.

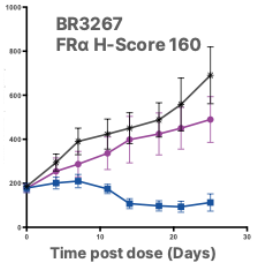
NSCLC



Endometrial

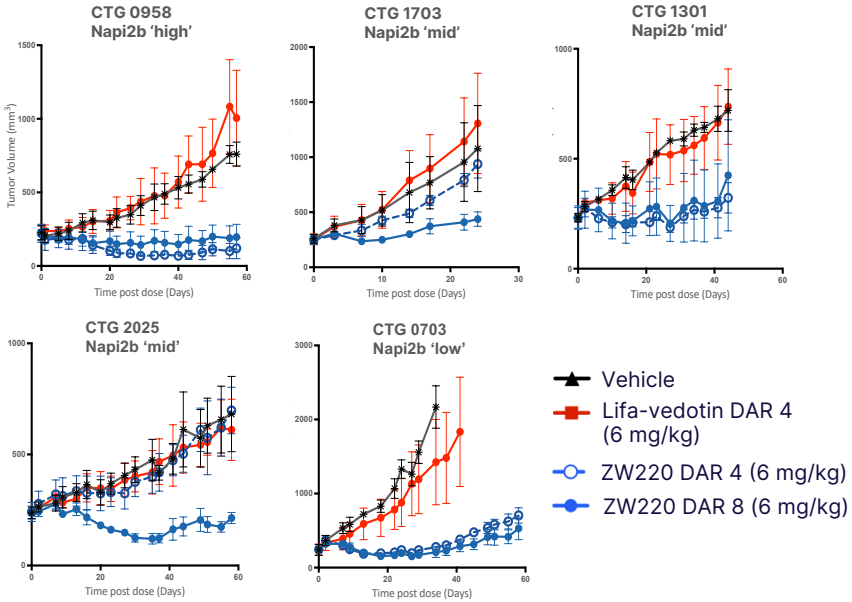


TNBC



ZW220, a DAR4 NaPi2b-targeting ADC

ZW220 demonstrates robust activity in NaPi2b-expressing ovarian cancer PDX models



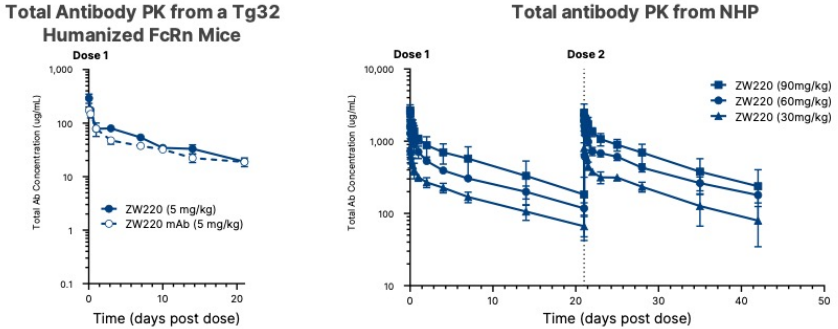
- ZW220 is more efficacious than Lifatuzumab-vedotin
- DAR 4 ADC is equivalent to DAR 8 ADC in 3/5 models

ZW220 is well tolerated in non-Human Primates with an MTD of 90 mg/kg

ZW220 3-dose non-GLP NHP toxicology study, Q3Wx3

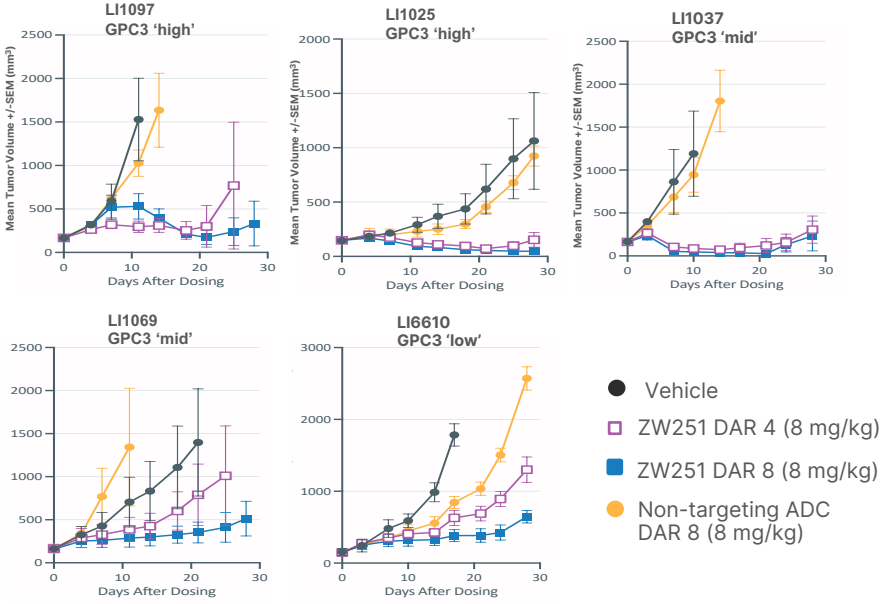
Test article	Dose	Tolerated?	Histopathology; Clinical Chemistry; Hematology	MTD
ZW220	30 mg/kg	Yes	None	90 mg/kg
	60 mg/kg	Yes	None	
	90 mg/kg	Yes	None	

ZW220 has a favorable pharmacokinetic profile



ZW251, a DAR4 glypican-3-targeting ADC

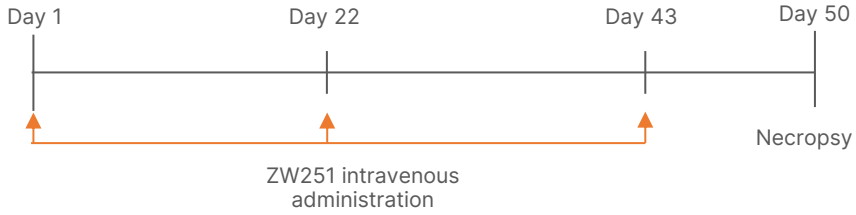
ZW251 Demonstrates Compelling Anti-Tumor Activity in GPC3-Expressing Liver Cancer PDX Models



- 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

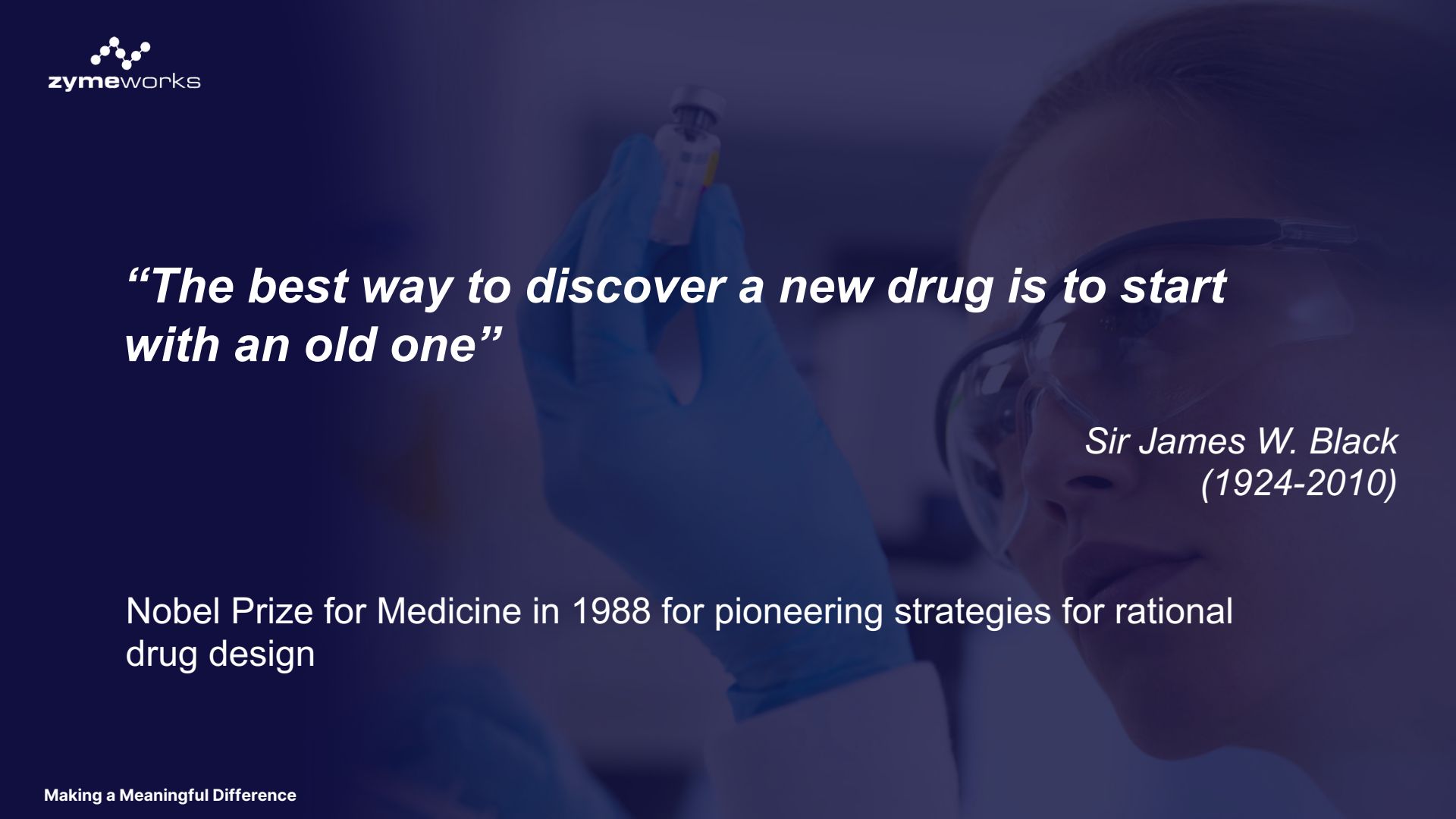
Repeat dose non-GLP NHP toxicology study



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.

zymeworks



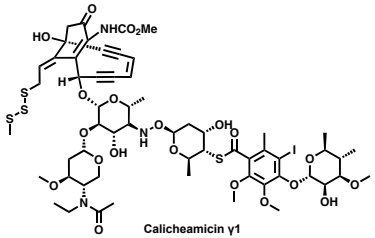
"The best way to discover a new drug is to start with an old one"

Sir James W. Black
(1924-2010)

Nobel Prize for Medicine in 1988 for pioneering strategies for rational drug design

The history of ADC payloads began in the 1960-1980s

Calicheamicin (1987)



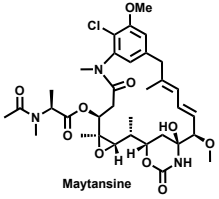
Isolated from Micromonospora echinospora

13 years

2000, reapproved in 2017: Gemtuzumab ozogamicin

2017: Inotuzumab ozogamicin

Maytansinoid (1972)



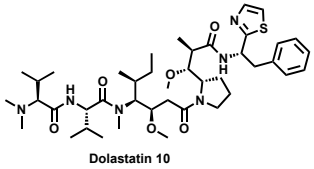
Isolated from Maytenus serrata

41 years

2013: Trastuzumab emtansine

2022: Mirvetuximab soravtansine

Auristatin (1987)



Isolated from Dolabella auricularia

24 years

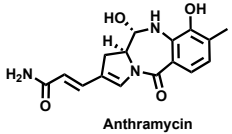
2011: Brentuximab vedotin

2019: Polatuzumab vedotin

2019: Enfortumab vedotin

2021: Tisotumab vedotin

PBD (1965)

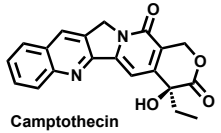


Isolated from Streptomyces refuineus

56 years

2021: Loncastuximab tesirine

Camptothecin (1966)



Isolated from Camptotheca acuminata

53 years

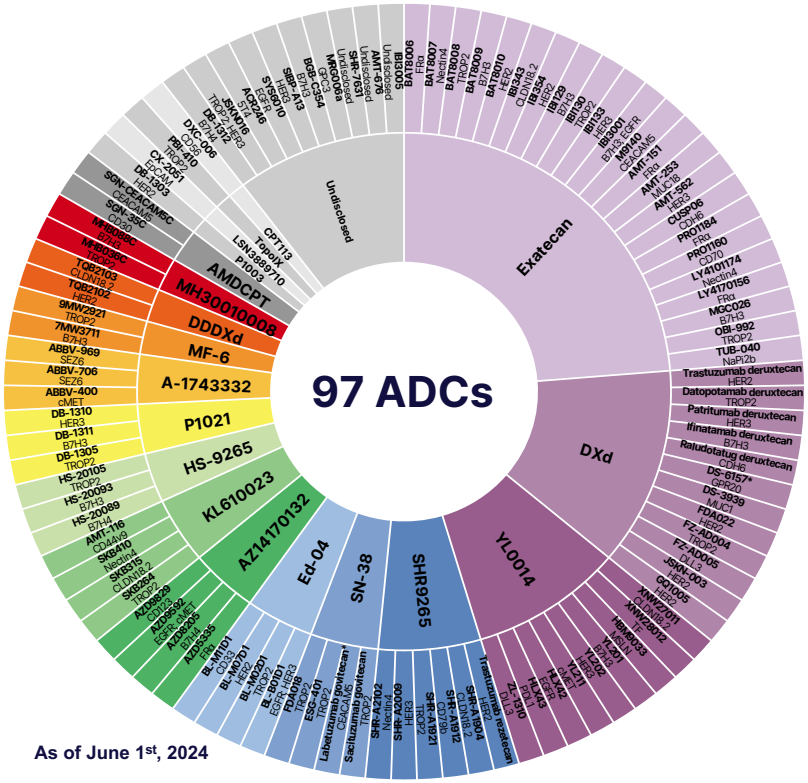
2019: Trastuzumab deruxtecan

2020: Sacituzumab govitecan

Year of first approval of ADCs by FDA: www.fda.gov. First isolation of the natural products: **Calicheamicin**, *J .Am. Chem. Soc.* **1987**, *109*, 3464; **Maytansine**, *J .Am. Chem. Soc.* **1972**, *94*, 1354, later proven to be an endophytic bacterial metabolite; **Dolastatin 10**, *J. Am. Chem. Soc.* **1987**, *109*, 6883, later proven to be produced by the cyanobacterium Symploca species VP642; **Anthramycin**, *J .Am. Chem. Soc.* **1965**, *87*, 5791; **Camptothecin**, *J .Am. Chem. Soc.* **1966**, *88*, 3888.

Camptothecin (TOPO1i) ADCs currently dominate the field

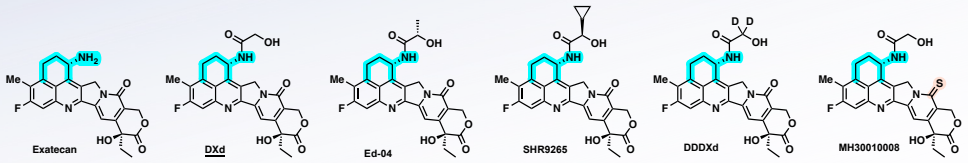
Clinical TOPO1i ADCs:



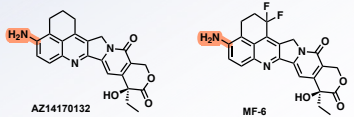
As of June 1st, 2024

* discontinued

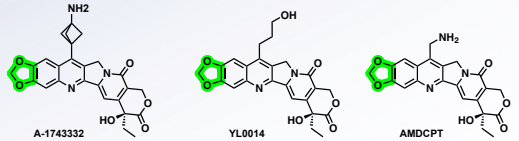
Exatecan and its derivatives



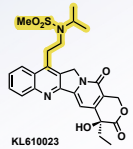
10-anilino



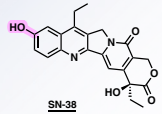
10,11-methylendioxy



Belotecan derivative



SN-38

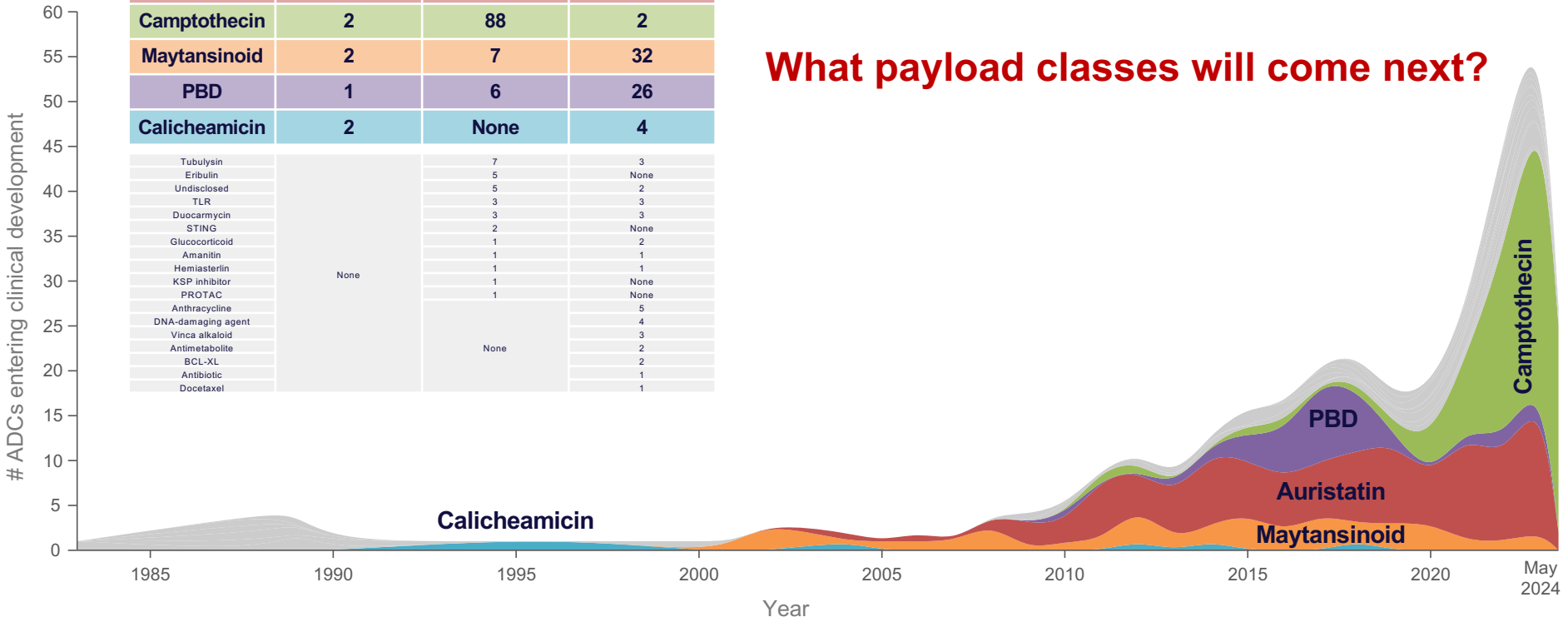


Adapted and updated from: M. E. Petersen, M. G. Brant, et al. *Mol. Cancer Ther.* **2024**; https://doi.org/.MCT-23-082210.1158/1535-7163

Payload choice for clinical ADCs has evolved over time

	FDA approved	In development	Discontinued
Auristatin	4	59	50
Camptothecin	2	88	2
Maytansinoid	2	7	32
PBD	1	6	26
Calicheamicin	2	None	4
Tubulysin	None	7	3
Eribulin		5	None
Undisclosed		5	2
TLR		3	3
Duocarmycin		3	3
STING		2	None
Glucocorticoid		1	2
Amanitin		1	1
Hemiasterlin		1	1
KSP inhibitor		1	None
PROTAC		1	None
Anthracycline		None	5
DNA-damaging agent			4
Vinca alkaloid			3
Antimetabolite			2
BCL-XL			2
Antibiotic			1
Docetaxel			1

What payload classes will come next?



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