ZW191

A Potential Best-in-Class TOPO1i ADC for Treatment of FRα-Expressing Solid Tumors

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World ADC London 2023

ZW191: Folate Receptor Alpha Topoisomerase-1 Inhibitor ADC

Target

- Folate receptor alpha (FRα, FOLR1) is a clinically validated ADC target
- FRα is over-expressed on the cell surface of ovarian cancer, other gynecological cancers, and additional solid tumors with unmet medical need

Antibody

- Internally discovered, novel IgG1 monospecific antibody
- Optimal internalization, payload delivery and tumor penetration

Drug Linker

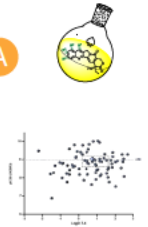
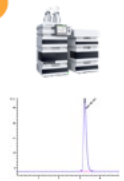
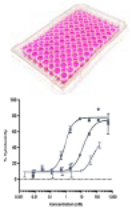
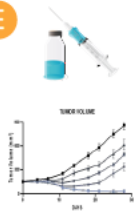
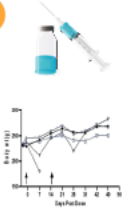
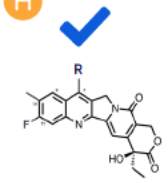
- Novel bystander-active topoisomerase-1 inhibitor
- Cysteine conjugated, DAR8, protease cleavable, traceless drug-linker

Status

- Compelling activity and tolerability profile
- GMP process development underway

Robust Interrogation Yields Pipeline Ready Topoisomerase 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 

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ADC biophysical characterization

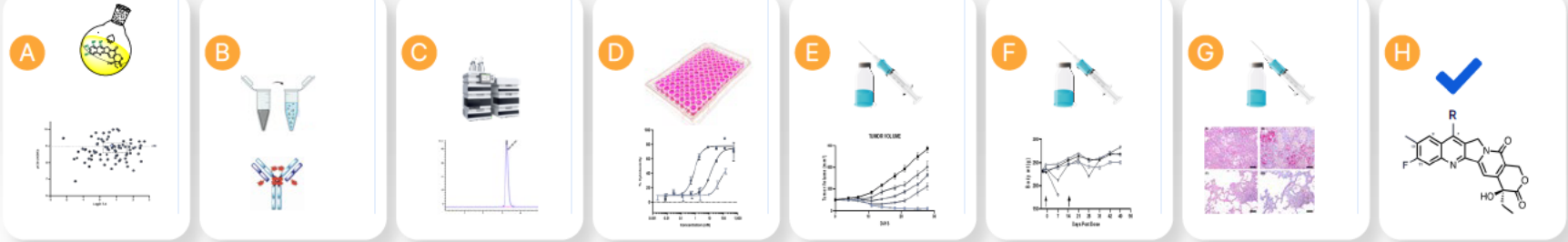
In vitro potency & stability

In vivo anti-tumor activity & PK

Rodent tolerability

Non-human primate toxicology & TK

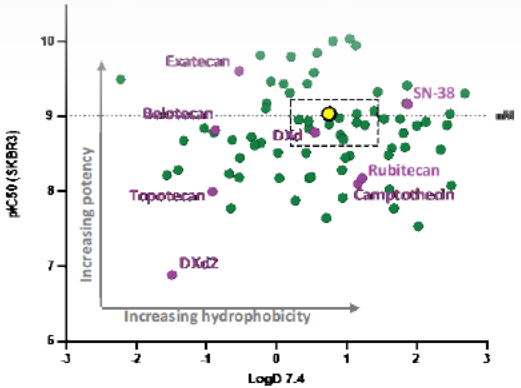
Lead selection



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



Robust Interrogation Yields Pipeline Ready Topoisomerase ADC Platform

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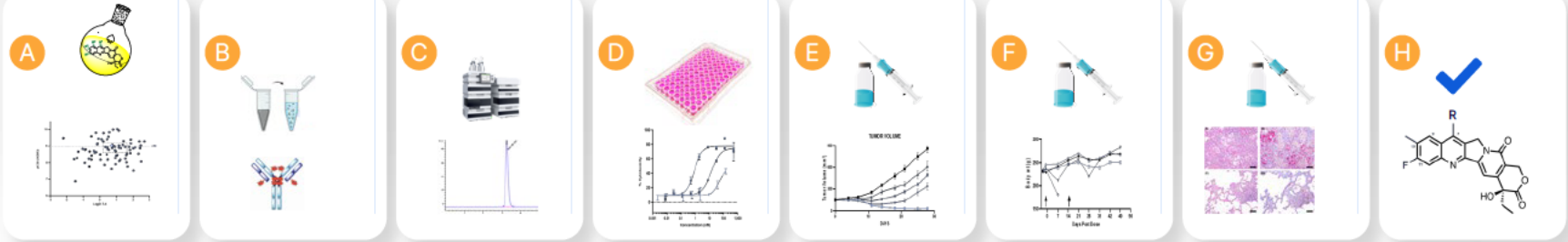


LINKER

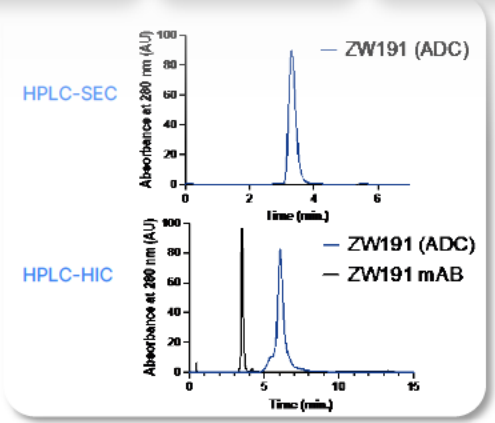
Traceless, plasma-stable, cleavable peptide

- 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



From Platform to Pipeline

zymeworks

100 Payloads

8 Tumor targets

~80 Cell lines

>20 CDX models

>20 PDX models

3 PK studies

5 Tox & TK studies

3 Pipeline programs
ZW191, ZW220, ZW251

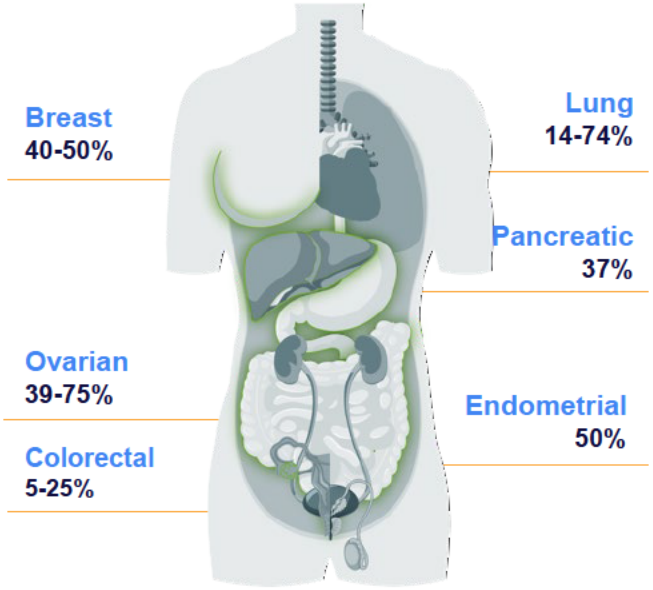
Additional early-stage assets

	ZW191	ZW251	ZW220
Target	**FRα**	**GPC3**	**NaPi2b**
Format/Technology	Monospecific/TOPO1i ADC	Monospecific/TOPO1i ADC	Monospecific/TOPO1i ADC
Potential Indications	Ovarian cancer, other gynecological cancers, and other solid tumors	Liver cancer	Ovarian cancer, NSCLC
Stage	IND-enabling	Late discovery	Late discovery
Next Milestone	IND 2024	Pilot NHP toxicology study initiated	Pilot NHP toxicology study initiated

Folate Receptor Alpha is a Relevant and Exploitable Target in Cancer

Structure	Glycosylphosphatidylinositol (GPI)-anchored membrane protein
Normal Tissue Expression	Apical surfaces of tissues including, intestine, lung, Fallopian tube, placenta, choroid plexus. Luminal surface of kidney.
Cancer Tissue Expression	Elevated expression in numerous gynecological cancers including ovarian, and in NSCLC, TNBC.
Ligands	Folate
Function	Internalization of folate via endocytosis

FOLATE RECEPTOR ALPHA EXPRESSING CANCERS



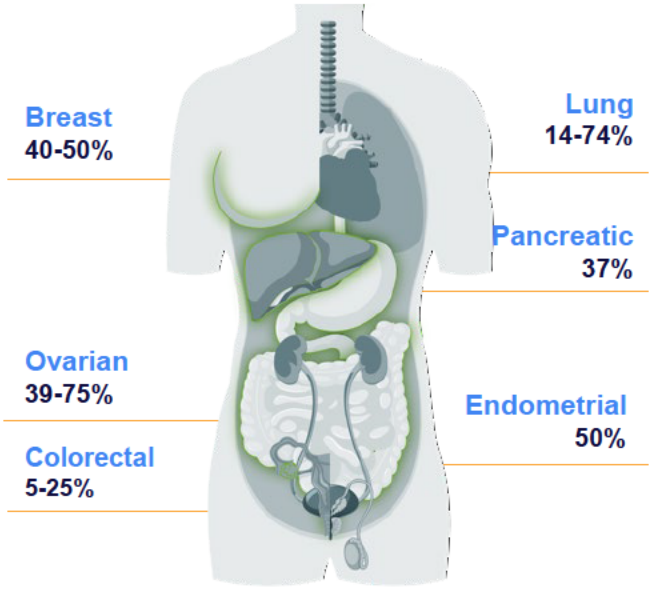
Expression levels cited from multiple sources including: Senol S et al 2015; Ayada et al. Med Mol Morphol 2018; Oza AM SGO 2021; O'Shannessy DJ et al Oncotarget 2012; Nunez MI et al 2012; D'Angelica et al. Mod Path 2011; Nature Review: Clinical Oncology; Vol. 17 June 2020.

Folate Receptor Alpha is a Relevant and Exploitable Target in Cancer

Elahere approval validates FRα as an ADC target, bringing benefit to patients, but with multiple points for improvement and expansion

	Mirvetuximab Soravtansine	**Potential for ZW191**
Indication:	Ovarian	Ovarian, NSCLC, Breast, Endometrial…
FRα expression:	High (36%)	High, Mid, Low (~80%)
Efficacy:	32% ORR	↑ ORR, ↑ DOR
Tolerability:	Ocular tox	Improved

FOLATE RECEPTOR ALPHA EXPRESSING CANCERS



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Topoisomerase 1 Inhibitor ADCs have Potential for Significant Impact in FRα-Expressing Cancers

Ovarian Cancer is Chemosensitive

Various drug classes are active in OvCa

- Alkylating agents
- DNA cross-linking agents
- Microtubule inhibitors
- Topoisomerase inhibitors
- Antimetabolites
- PARP inhibitors

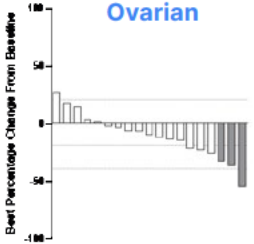
ADCs have validated efficacy in OvCa

FRa: ELAHERE, MORAb-202, STRO-002

Other targets: Upifitamab Rilsodotin, Tisotumab vedotin, DS-6000

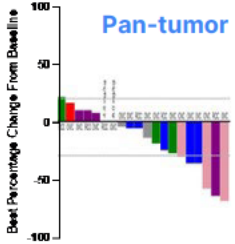
CRLX101 (NDC)

Ovarian

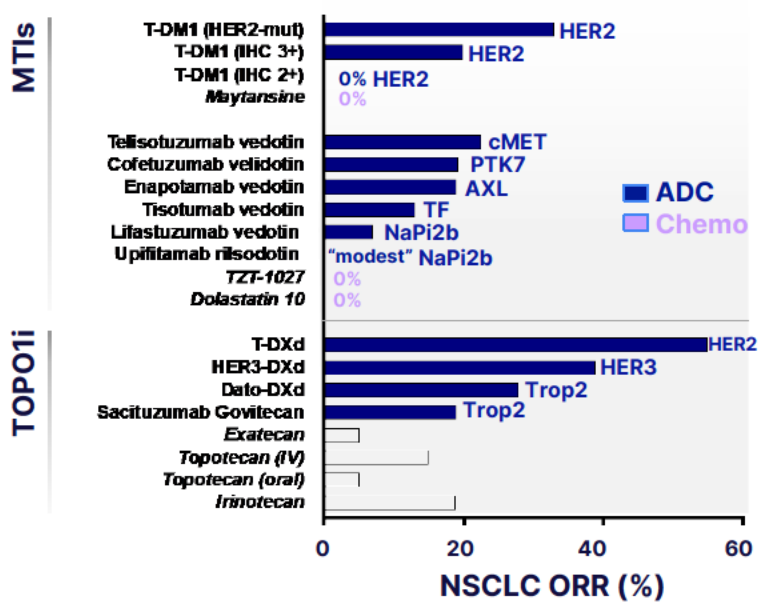


DS-6000 (CDH6-DXd)

Pan-tumor



NSCLC: TOPO1i MoA Demonstrates Superior Activity



Ovarian cancer and NSCLC respond to ADCs and Topoisomerase 1 inhibition

ZW191 Novel mAb Discovery and Engineering

Rabbit immunization recombinant FRa

~50K Immune cells mined

15 Primary hits found

10 Molecular rescued hits

10 Antibodies tested as ADCs

Various Humanized variants generated

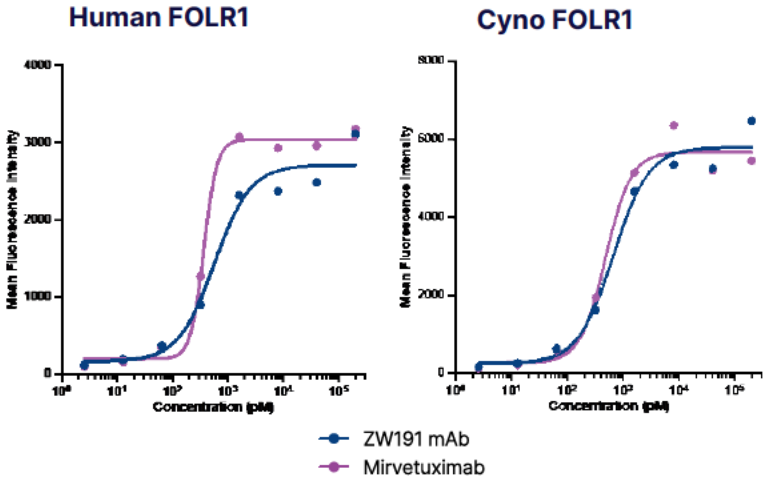
1 Lead humanized mAb selected

ZW191 Antibody Properties

Species	Fully humanized (originally rabbit chimera)
Subclass	IgG1
MW (Da)	~145,000
Structure	Full-sized mAb

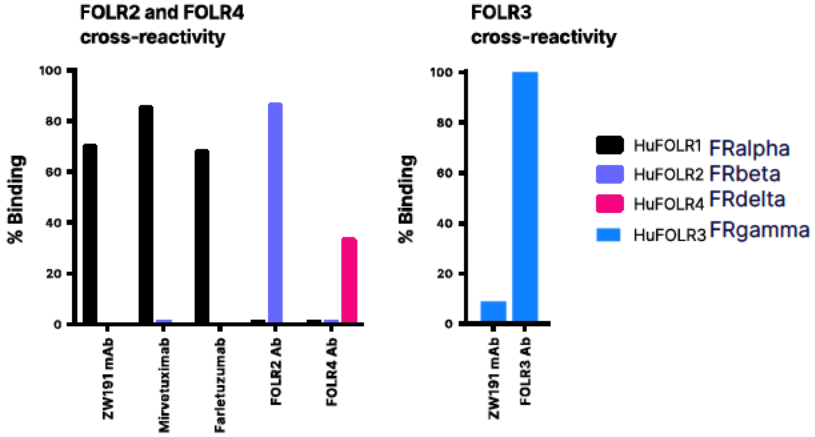
ZW191 Binds with High Specificity to Human FRα and Cross-Reacts with Cyno FRα

Human and Cyno FRα Cross Reactivity



- ZW191 retains strong binding across human and cyno monkey FRα

ZW191 mAb does not show cross-reactivity to other FOLR family members FOLR2, FOLR3 and FOLR4



- Left: Binding to HEK293 Hu FOLR1, FOLR2 and FOLR4 transients
- Right: Binding to soluble Hu FOLR3 by ELISA

ZW191 mAb Binds with High Specificity to FRα

Pre-Screen

Library screen

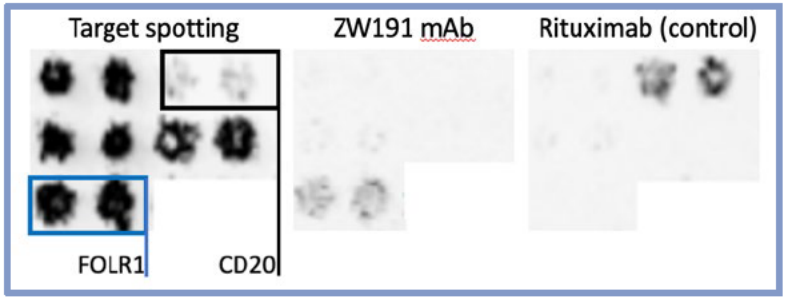
- Test molecule screened (as part of a pooled multiplex)
- Full library of 6,200+ plasma membrane, secreted and cell–tethered secreted proteins, ~400 heterodimers
- Fixed HEK293 cell microarray format
- 'Library hits' identified

Fixed confirmation screen

- Repeat specificity on hits
- Fixed cell microarray format

Live confirmation screen

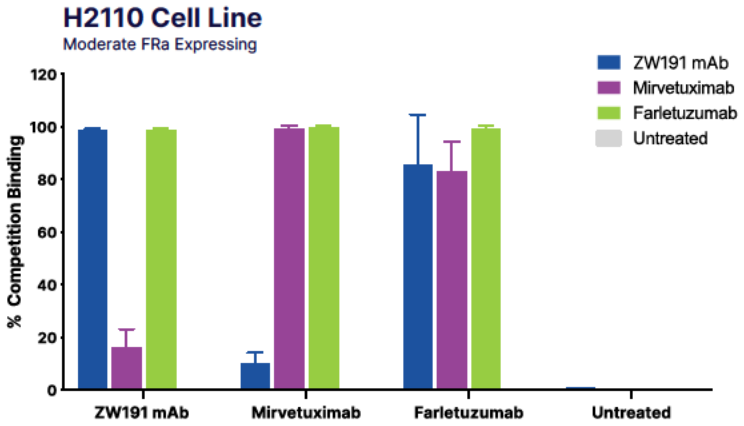
- Repeat specificity on hits
- Live cell microarray format



FRα identified as the only significant target for ZW191 mAb

ZW191 Exhibits Distinct FRα Binding Properties

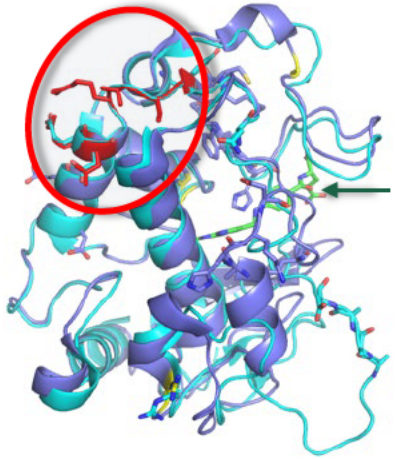
ZW191 mAb demonstrates a binding profile distinct from clinical benchmark ADC mAbs



- ZW191 mAb is non-competitive with Mirvetuximab for FRα binding
- ZW191 and Mirvetuximab compete with Farletuzumab for FRα binding

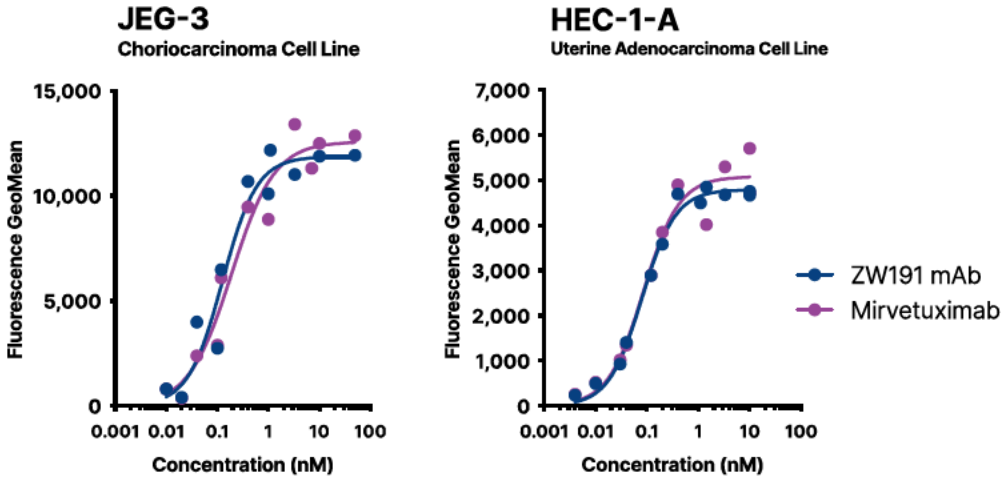
ZW191 epitope unaffected by folate binding

Cyan: Folate unbound form
Blue: Folate bound form



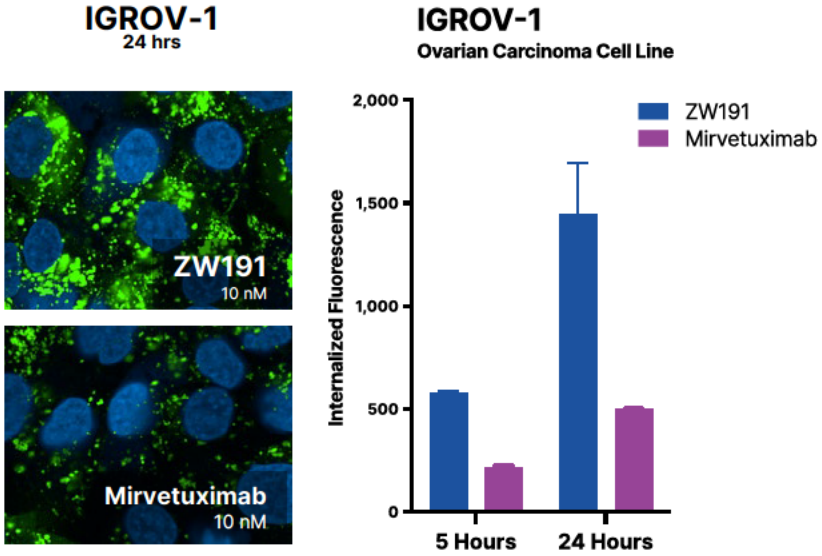
ZW191 mAb Exhibits Strong Binding to FRα-Expressing Cells

ZW191 mAb Binding is Comparable to Mirvetuximab Benchmark

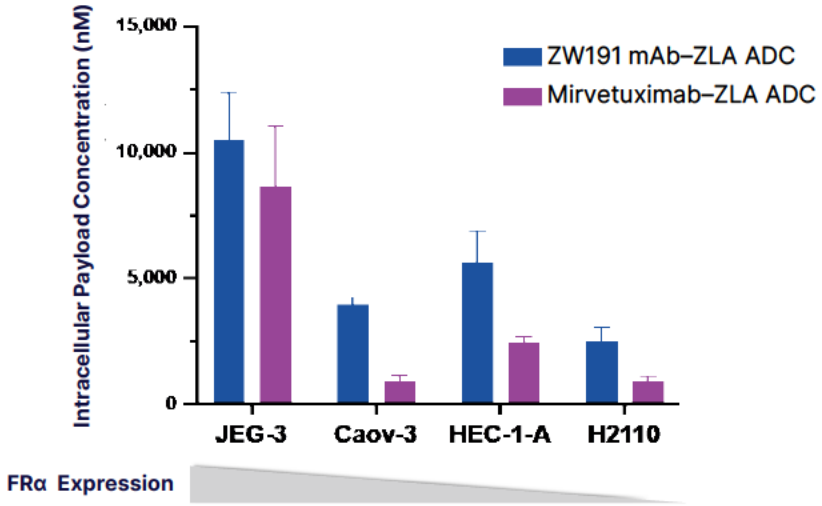


ZW191 Demonstrates Effective Internalization and Payload Delivery

Superior Internalization to Mirvetuximab



Superior Payload Delivery to Mirvetuximab

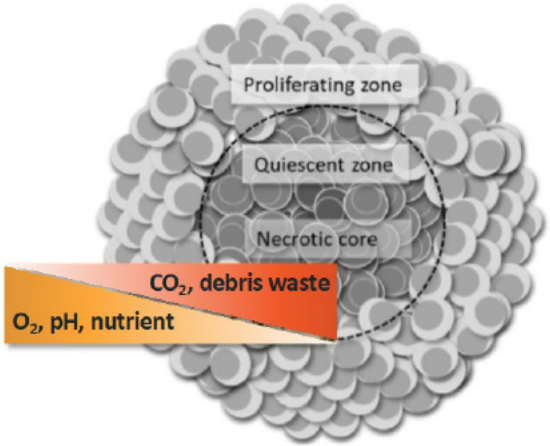


Payload delivery study utilizes ZymeLink Auristatin (ZLA) payload

Tumor Spheroids are an Informative Model to Assess Antibody Distribution and ADC Cytotoxicity

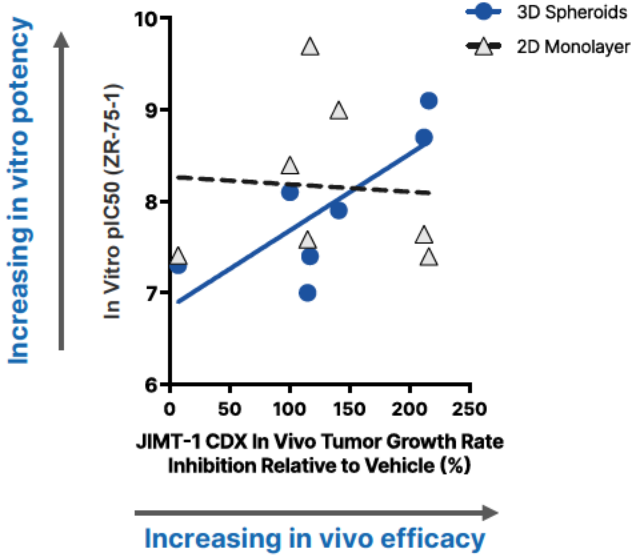
Key spheroid features:

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



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

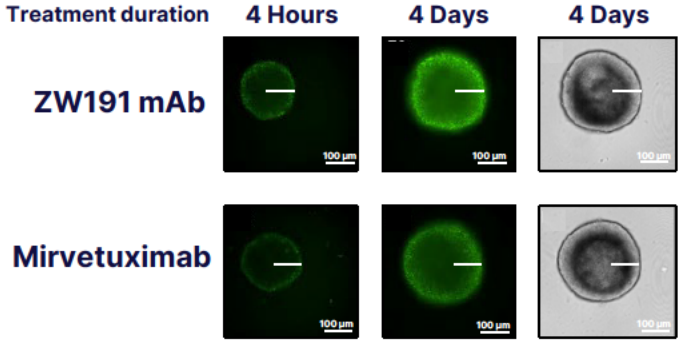
3D Spheroid Cytotoxicity Better Predicts In Vivo ADC Activity Than 2D Cytotoxicity:



ZW191 Demonstrates Effective Tumor Spheroid Penetration

JEG-3 Tumor Spheroids

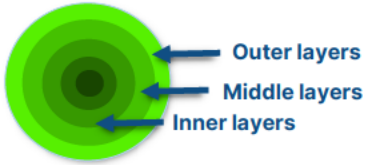
~1,100,000 FRα/cell



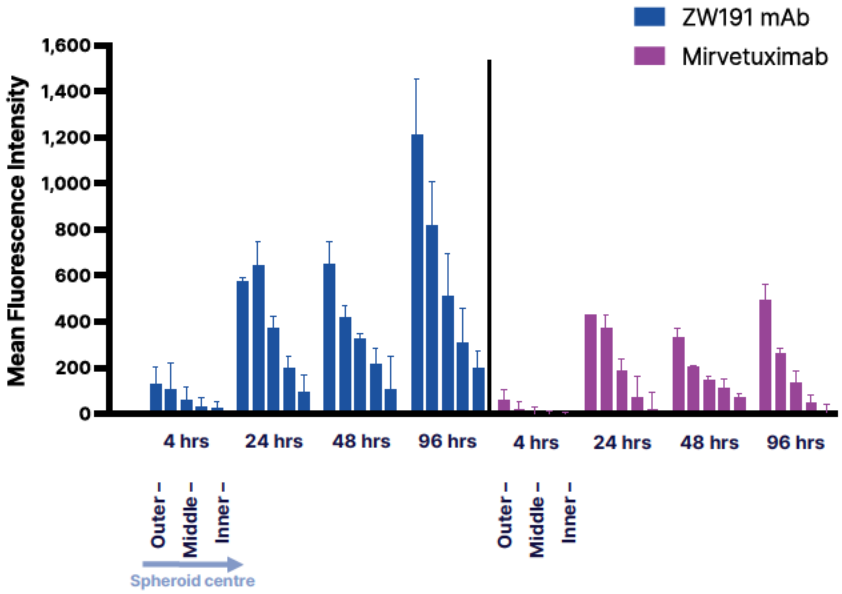
- Spheroids were grown for 3 days at 37°C prior to test article treatment

Spheroid penetration analysis:

- Central spheroid section selected using Z-stack microscopy
- Fluorescence measured in multiple outer, middle and inner layers of spheroid section using high content imaging



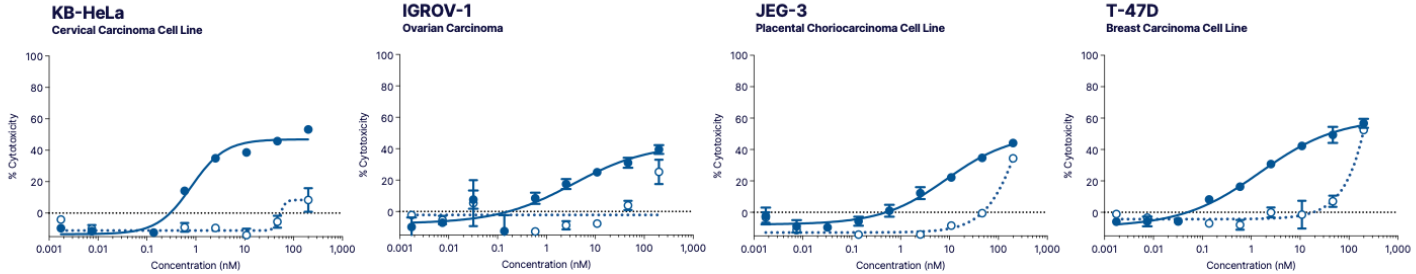
Fluorescence Intensity in JEG-3 Tumor Spheroids



ZW191 Demonstrates Strong Target-dependent Potency in a Range of FRα-expressing Tumor Cell Lines from Different Cancer Indications

2D Monolayer

ZW191
Isotype ZW TOPO1i ADC



3D Spheroids

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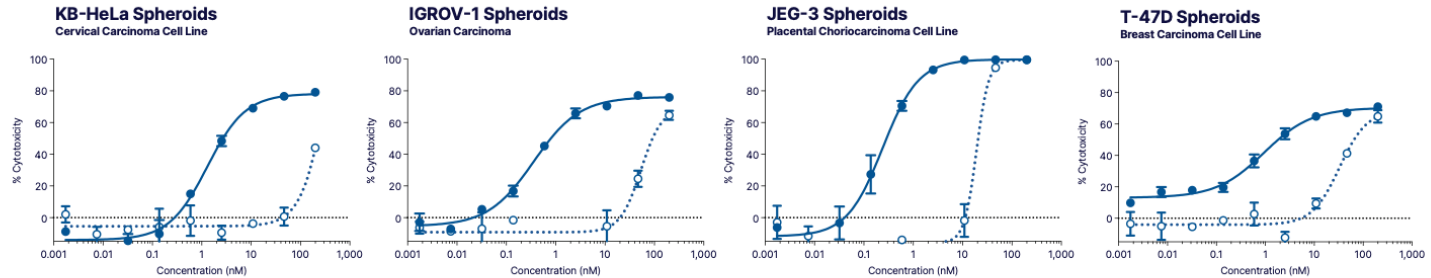
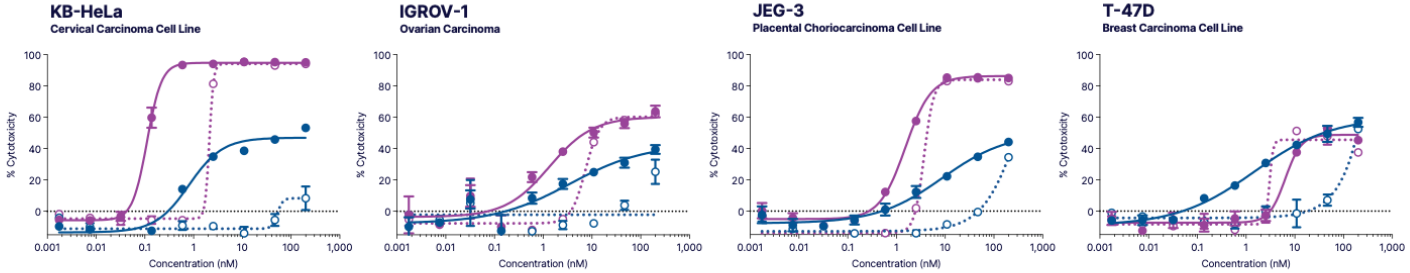


ZW191 Demonstrates Strong Target-dependent Potency in a Range of FRα-expressing Tumor Cell Lines from Different Cancer Indications

2D Monolayer

- ZW191
- Isotype ZW TOPO1i ADC
- Mirvetuximab Soravtansine
- Isotype-sSPDB-DM4 ADC



3D Spheroids

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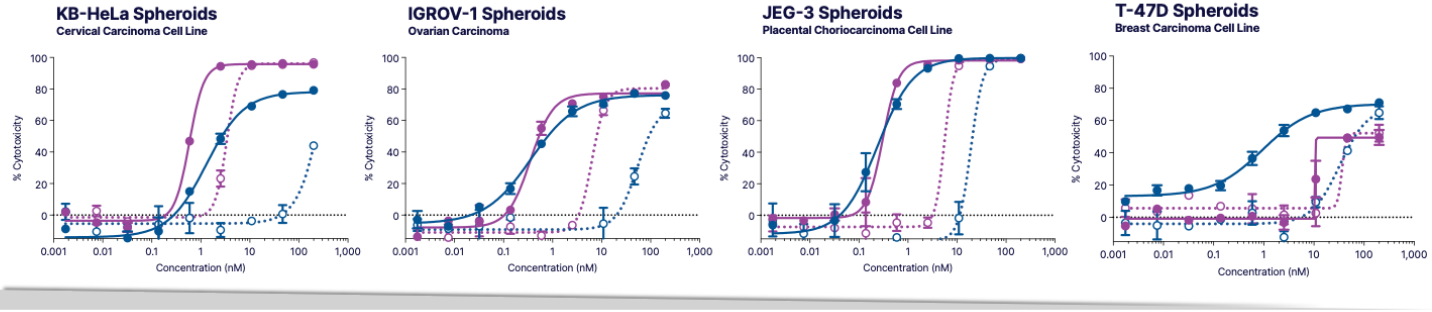
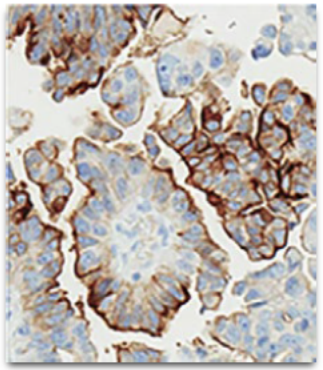
- Isotype ZW TOPO1i ADC
- Mirvetuximab Soravtansine
- Isotype-sSPDB-DM4 ADC



ZW191 Exhibits Strong Bystander Activity In Vitro

FRα Heterogeneity

Ovarian Cancer

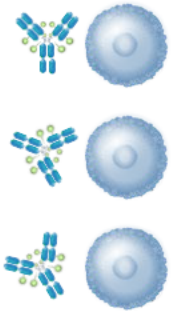


IHC images sourced from Martin et al. 2017. Gynecologic Oncology

ZW191 Bystander Activity in In Vitro Tumor Cell Co-culture Assay

Monoculture

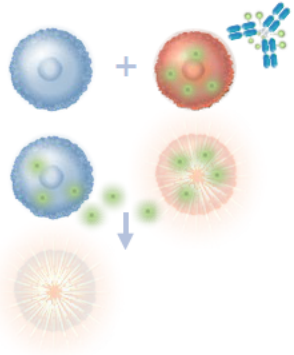
FRα- cells
EBC-1



No cytotoxicity in FRα- cells

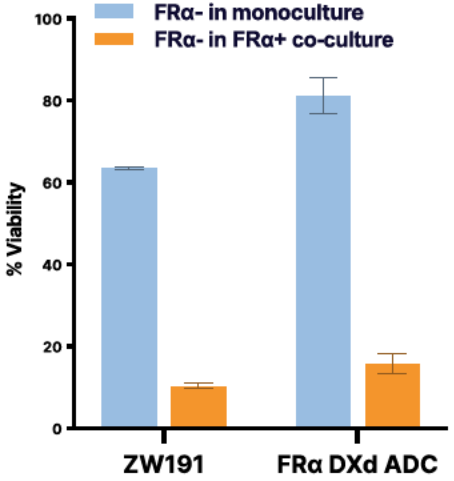
Co-culture

FRα- cells EBC-1 + FRα + cells IGROV-1



Cytotoxicity in FRα⁻ cells when co-cultured with FRα⁺ cells

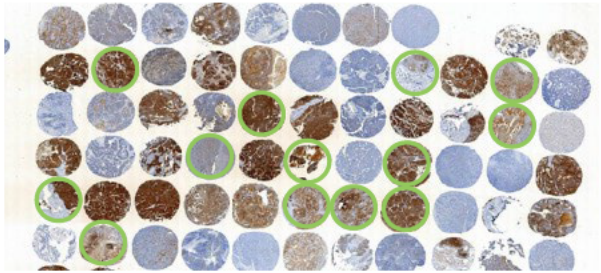
Viability of FRα Negative Cells



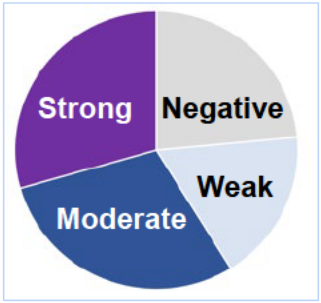
DXd control ADC contains same mAb as ZW191, conjugated to DXd

Ovarian PDX Models were Selected across a Range of FRα Expression

PDX TMA FRα Expression (IHC)



Breakdown of FRα Expression in PDX TMA



- ✓ Strong and moderate expression models prioritized
- ✓ Weak model also evaluated

IHC uses a research level assay, independent from validated FOLR1-2.1 Ventana assay

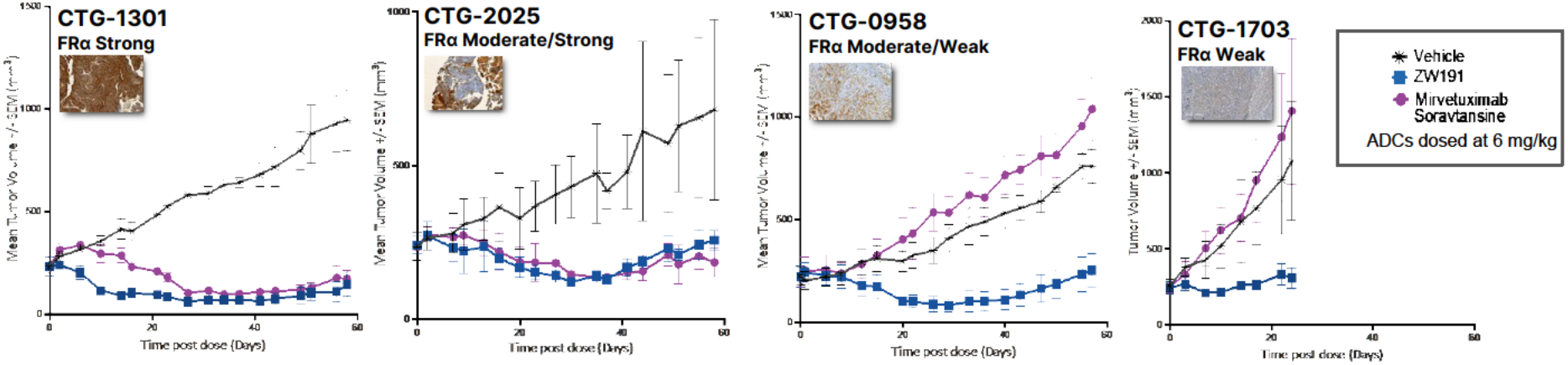
Ovarian Cancer PDX Models Selected

Model	FRa Expression
0703	Strong
1301	Strong
2733	Strong
2025	Moderate/strong
3416	Moderate
3331	Moderate
2299	Moderate
3383	Moderate
0947	Moderate
0958	Moderate/weak
3718	Moderate/weak
1602	Weak
1703	Weak

Study Design

Test Article	Single Dose (mg/kg)	n
Vehicle	N/A	3
ZW191	6	3
Mirvetuximab Soravtansine	6	3

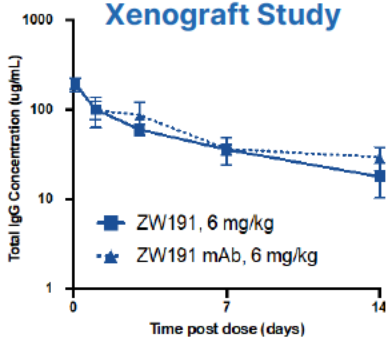
ZW191 Demonstrates Efficacy across a Range of FRα-Expressing Ovarian Cancer PDX



- ZW191 is highly efficacious in models with **strong** FRα expression, similar to Mirvetuximab Soravtansine
- ZW191 is highly efficacious in models with **weaker** FRα expression, superior to Mirvetuximab Soravtansine

IHC is from archive PDX samples using a research level assay, independent from validated FOLR1-2.1 Ventana assay

ZW191 PK from Xenograft Study



- 6 mg/kg dose and exposure projected to be clinically relevant
- ZW191 maintains the favorable PK profile of its mAb

ZW191 is Well-tolerated in Rodent & Non-human Primates

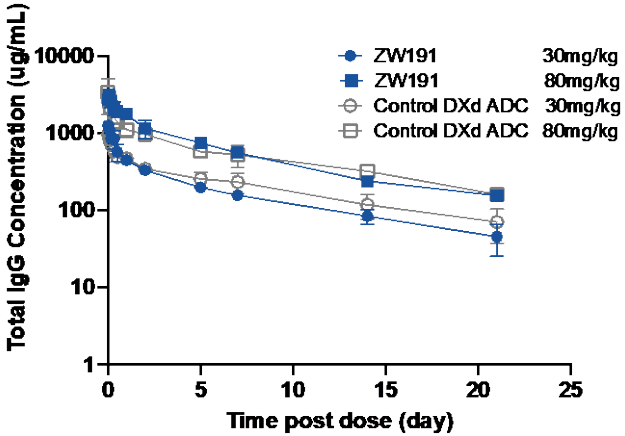
- Non-antigen binding species:
  - Rats + mice: Tolerated at 200 mg/kg
- Antigen-binding species:
  - NHP: Tolerated at 30 mg/kg

ZW191 demonstrates a favorable tolerability profile

Two-dose (Q3W) Non-Human Primate non-GLP Toxicology Study			
Test Article	**Dose mg/kg**	**Tolerated?**	**Histopath; Clin. Chemistry; Hematology**
ZW191	30	Yes	Thymus, stomach; AST ↑; BUN ↑; ABRETIC↓
	80	No	Thymus, kidney, testis, and brain; AST ↑; BUN ↑; ABRETIC↓; ABLYMP↓
ZW DAR4 ADC	120	Yes	Thymus, adrenal glands, prostate, brain, lymph nodes; ABRETIC↓; ABNEUT ↓

No increased severity or distinct adverse effects compared to control DXd ADC

ZW191 PK is comparable to control DXd ADC



ZW191: A Differentiated FRα Targeting ADC

Development underway and on track for 2024 IND

Therapeutic Rationale

FRα is a clinically validated ADC target in ovarian cancer with good potential in other gynecological and solid tumors.

Topoisomerase-1 inhibition is a clinically validated MOA in ovarian cancer and other solid tumors

Product Differentiation

Compelling internalization, payload delivery, tumor penetration and anti-tumor activity

Novel topoisomerase-1 inhibitor likely to provide a **differentiated safety profile** compared to MIRV and STRO-002

Opportunity

Potential best-in-class opportunity to improve over MIRV in FRα-high ovarian cancer

Potential first and best-in-class opportunity in FRα-high endometrial, NSCLC, TNBC, and FRα-mid/low solid tumors

Next Milestones

GMP process development underway

GLP toxicology study scheduled

IND 2024

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