ZW191 – a FRα-targeting antibody-drug conjugate with strong preclinical activity across multiple FRα-expressing indications

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Introduction

ZW191

ZW191 is an antibody-drug conjugate (ADC) targeting human Folate Receptor Alpha (FRα). ZW191 is comprised of a novel fully humanized IgG1 antibody covalently conjugated to a novel topoisomerase I inhibitor (TOPO1i) ZD06519, a rationally designed moderate potency, bystander active camptothecin (CPT) derivative, via endogenous interchain cysteines with a drug to antibody ratio (DAR) of 8. The linker in ZW191 consists of a maleimidocaproyl (MC) anchor and a GGFG-aminomethyl (AM) protease cleavable sequence.

Mechanism of Action

Upon target binding and receptor-mediated internalization of ZW191, intracellular release of bystander-active ZD06519 induces cell death of FRα-positive cells, and FRα-negative cells through bystander-mediated killing.

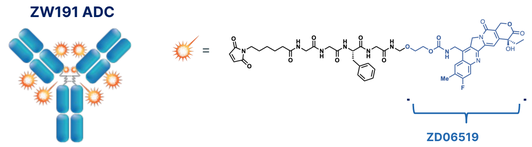


Figure 1. ZW191 comprises a novel FRα targeting IgG1 mAb conjugated to a novel camptothecin derivative at a DAR of 8 using a protease cleavable linker

FRα is expressed across multiple indications with high unmet clinical need

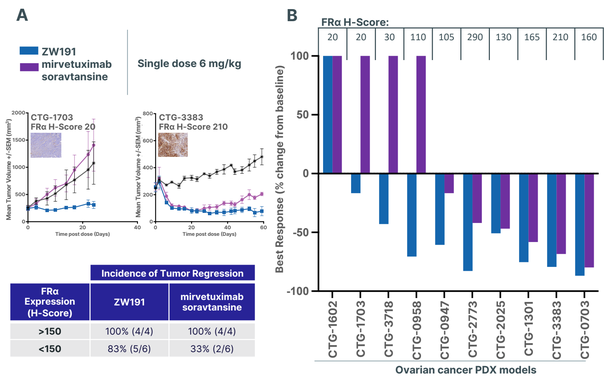
- FRα is a clinically validated ADC target, as demonstrated by the recent approval of mirvetuximab soravtansine (Elahere™) for FRα-positive* ovarian cancer[1]
- Approximately two thirds of ovarian cancer patients are ineligible for Elahere™ based on their tumors expressing relatively low levels of Frα
- FRα is prevalently expressed across additional indications including non-small cell lung cancer (NSCLC)[2,3,4,5] (approximately 70%), endometrial cancer (EC)[6,7,8] (approximately 50%), and multiple other tumors
- There is significant potential for a novel FRα-targeted ADC to improve responses in FRα-positive* ovarian cancer over Elahere™, and to widen the patient population to those with lower FRα-expressing ovarian cancer and additional FRα-expressing indications

* As determined by Ventana FOLR1 assay ≥75 % IHC 2/3+

ZW191 demonstrates strong activity across ovarian cancer, NSCLC, endometrial cancer, and triple-negative breast cancer (TNBC) PDX models expressing relatively high and low levels of FRα

Superior activity in ovarian cancer PDX

- ZW191 demonstrates superior activity to mirvetuximab soravtansine in models with relatively low FRα
- ZW191 demonstrates superior or comparable activity to mirvetuximab soravtansine in models with relatively high FRα

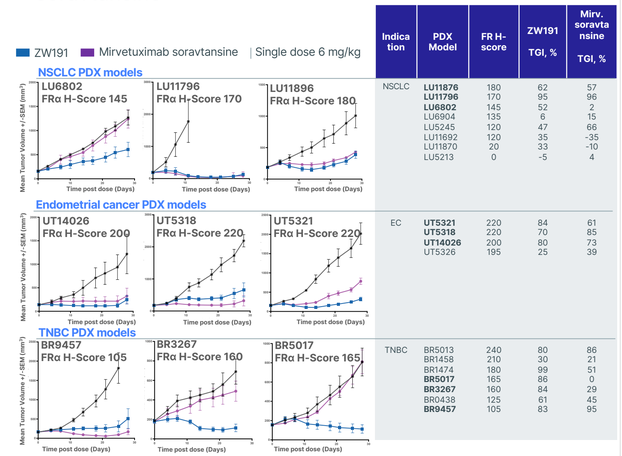


Incidence of Tumor Regression		
FRα Expression (H-Score)	ZW191	mirvetuximab soravtansine
>150	100% (4/4)	100% (4/4)
<150	83% (5/6)	33% (2/6)

Figure 2. *In vivo* efficacy of ZW191 and mirvetuximab soravtansine was assessed in patient derived xenograft (PDX) models of ovarian cancer in Nude mice, n=3 per group. H-scores determined by pathologist from research level IHC assay **(A)** Tumor volume plots **(B)** Waterfall plots of mean best response from each of 3 mice per treatment group, ordered by response to mirvetuximab soravtansine. Tumor regression is defined as <0% change from baseline.

Strong activity in NSCLC, EC, and TNBC PDX

- ZW191 activity observed across a range of relative FRα expression levels
- ZW191 activity is superior or comparable to mirvetuximab soravtansine



Indication	PDX Model	FR H-score	ZW191 TGI, %	Mirv. soravtansine TGI, %
NSCLC	**LU11876**	180	62	57
	LU11796	170	95	96
	LU6802	145	52	2
	LU6904	135	6	15
	LU5245	120	47	66
	LU11692	120	35	-35
	LU11870	20	33	-10
	LU5213	0	-5	4
EC	**UT5321**	220	84	61
	UT5318	220	70	85
	UT14026	200	80	73
	UT5326	195	25	39
TNBC	BR5013	240	80	86
	BR1458	210	30	21
	BR1474	180	99	51
	BR5017	165	86	0
	BR3267	160	84	29
	BR0438	125	61	45
	BR9457	105	83	95

Figure 3. *In vivo* efficacy of ZW191 and mirvetuximab soravtansine was assessed in patient derived xenograft (PDX) models of NSCLC, EC, and TNBC in Nude or NOD/SCID mice, n=3 per group. H-scores determined by pathologist from research level IHC assay. Tumor volume plots from a selection of models are shown. Table summary of tumor growth inhibition (TGI) compared to vehicle control at day 28 or nearest evaluable timepoint from each model. Models in bold indicate those selected for tumor volume plots.

ZW191 is well-tolerated in non-human primates (NHP) at 60 mg/kg

GLP repeat-dose NHP toxicology study, Q3Wx3

- No mortality or body weight effects
- No ophthalmic effects
- All effects were non-adverse and reversible

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 ↓ pancreatic acinar cell secretion	↑ AST, ALT	no effects		
60	liquid/discolored feces emesis/vomitus ↓ activity level (n=1)	↓thymic lymphocytes ↓pancreatic acinar cell secretion	↑ AST, ALT ↑ CK	no effects		

GLP NHP study design: dosed every 3 weeks for 3 doses, with a 7-week recovery period; n= 8 for 10 mg/kg group, n=12 for 30 mg/kg and 60 mg/kg groups

- Favorable preclinical pharmacokinetics support an expectation of strong anti-tumor activity at a well tolerated exposure

ZW191's novel mAb drives superior internalization, payload delivery, and tissue penetration

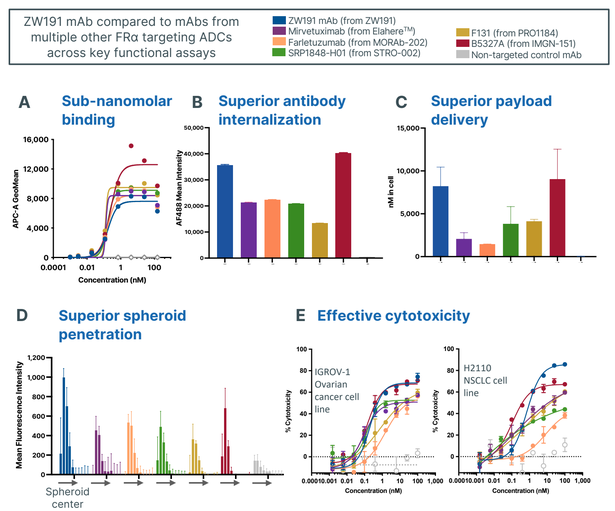


Figure 4. *In vitro* functional assessment of the antibody properties of ZW191 and other FRα-targeted ADCs and a non-targeted control mAb (all WT Fc to facilitate comparison). **(A)** Cell binding to JEG-3 cells by flow cytometry **(B)** Internalization of AF488 labelled antibodies to KB-Hela cells after 24 hrs at 100 nM **(C)** Mass-spec. quantification of internalized payload following 24 hour treatment of IGROV-1 cells with 10 nM of ADCs comprising ZW191 mAb or other FRα-targeted mAbs conjugated to ZymeLink™ Auristatin (ZLA) **(D)** Penetration of AF488 labelled antibodies as quantified by high content imaging of spheroid layers at 24 hours post-treatment at 50 nM **(E)** Cytotoxicity of ZW191 mAb and other FRα-targeted antibodies conjugated to the common linker-payload ZymeLink™ Auristatin (ZLA), as assessed by Cell-Titer-Glo 4 days post-treatment.

ZW191's novel ZD06519 payload enables bystander activity and cytotoxicity across multiple indications

Strong bystander activity

- Drives activity in cases of heterogeneity of target expression

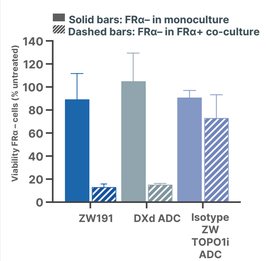


Figure 5. Bystander activity of ZW191 as shown by the decreased viability of FRα negative cells (EBC1) when co-cultured with FRα positive cells (IGROV-1). DXd ADC comprises the same mAb as ZW191 conjugated to MC-GGFG-DXd DAR8. Isotype ZW TOPO1i ADC is a non-targeting ADC bearing the ZD06519 payload.

Strong cytotoxicity against FRα expressing ovarian, lung and endometrial cancer tumor cells

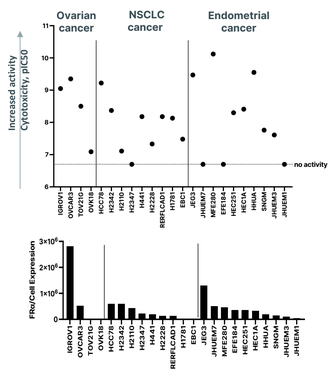


Figure 6. *In vitro* cytotoxicity against cancer cell lines across ovarian cancer, NSCLC and endometrial cancer expressing a range of FRα levels. **(A)** Potency of ZW191 as expressed by pIC50 from 6 day treatment cytotoxicity assays of 3D spheroid cell lines **(B)** Expression of FRα as determined by flow cytometry quantification by interpolation of stained cancer cells against a standard curve of stained IgG beads.

Conclusions

ZW191 is a FRα-targeting ADC differentiated by its novel antibody and novel topoisomerase I inhibitor payload

- Superior internalization, payload delivery and spheroid penetration to other FRα-targeted mAbs
- Bystander active payload drives activity in settings with heterogeneous FRα expression

A compelling preclinical activity profile supports ZW191 development across multiple tumor types

- Activity in FRα-high/mid/low ovarian cancers
- Activity in other FRα-expressing indications, including NSCLC, endometrial cancer, and TNBC
- Well-tolerated, with an HNSTD of 60 mg/kg in NHP

Planned IND submission in 2024

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