ZW327, a novel Ly6E-targeting antibody-drug conjugate bearing a topoisomerase 1 inhibitor payload

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Abstract #6198

Introduction

ZW327 is an antibody-drug conjugate (ADC) targeting human lymphocyte antigen 6 family member E (Ly6E). ZW327 is comprised of a novel humanized Fc-silenced IgG1 antibody (hu10E02) conjugated to a proprietary topoisomerase 1 inhibitor ZD06519[1], a camptothecin (CPT) derivative, via endogenous interchain cysteines with a drug to antibody ratio (DAR) of 8. The linker in ZW327 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 ZW327, intracellular release of bystander-active ZD06519 induces cell death of Ly6E positive cells, and Ly6E negative cells through bystander-mediated killing.

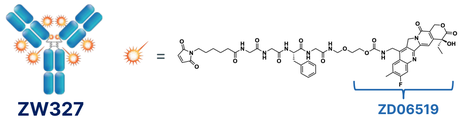


Figure 1. ZW327 is comprised of a novel Ly6E targeting Fc-silenced IgG1 mAb conjugated to a proprietary camptothecin derivative at a DAR of 8 using a protease cleavable linker.

Ly6E is overexpressed in many solid tumors

Ly6E is overexpressed in indications of high unmet medical need, including NSCLC[2] (LUAD+LUSC), TNBC[2], HNSCC[2], and GI cancers: ESCC, PDAC[2], GEJ, CHOL, COAD[2], and STAD[2] tissues with minimal presence in normal tissues.

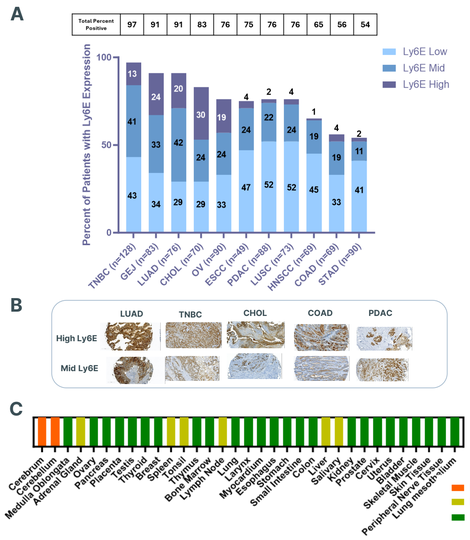


Figure 2. **(A)** Ly6E expression across a range of cancer indications. Expression noted in 54-97% patients across indications. Designation of low, mid and high Ly6E expression is based on frequency and intensity of staining, n=number of patient samples. NSCLC: Non-Small Cell Lung Cancer, LUAD: Lung Adenocarcinoma, LUSC: Lung Squamous Cell Carcinoma, TNBC: Triple-Negative Breast Cancer, HNSCC: Head and Neck Squamous Cell Carcinoma, GI: Gastrointestinal Cancers, ESCC: Esophageal Squamous Cell Carcinoma, PDAC: Pancreatic Ductal Adenocarcinoma, GEJ: Gastroesophageal Junction, CHOL: Cholangiocarcinoma, COAD: Colon Adenocarcinoma, STAD: Stomach Adenocarcinoma **(B)** Representative IHC images of mid and high Ly6E expression from select indication clinical tissue microarrays. **(C)** Ly6E expression in normal tissues of three donor samples per tissue type. H-scoring by pathologist. Low Ly6E expression in some organs of the immune system and moderate expression in cerebrum and cerebellum.

ZW327 developability

Property	Assay	Result
mAb Titer	pA HPLC	~3g/L
Fab Tm (°C) (mAb)	DSC	~84
mAb #pI	cIEF	8.8
ADC &HIC RT (min)	HIC	6.7
mAb pH 3.5 stability (3h)	UPLC-SEC	%Monomer Comparable to T0
ADC *HuFcRn/HuFcgR binding	SPR	Comparable to WT/Abrogated
ADC stability in Succ5Su, His5Su buffers (4°C and 40°C), 14 days	UPLC-SEC, MS, internalization	%Monomer and DAR comparable to T0, no functional impact
ADC Mouse(Cyno) plasma stability, 37°C, 14days	MS including peptide mapping, internalization	No notable PTMs, minor cleavage product without functional impact

Table 1. Summary of ZW327 developability assessment. mAb self-aggregation and non-specific binding assessed by AC-SINS and NS-ELISA respectively, were within the acceptable range. &Trastuzumab HIC RT is 5 min; #main isoform; *FcgRI, FcgRIIIa, FcgRIIa, FcgRIIb were tested.

ZW327 demonstrates robust anti-tumor activity in PDX and CDX models across a range of Ly6E expression levels, superior to benchmark DLYE5953A

Complete regressions at single 6mg/kg dose in 13/14 models tested across low, mid and high Ly6E expression in TNBC and NSCLC models (8 shown)

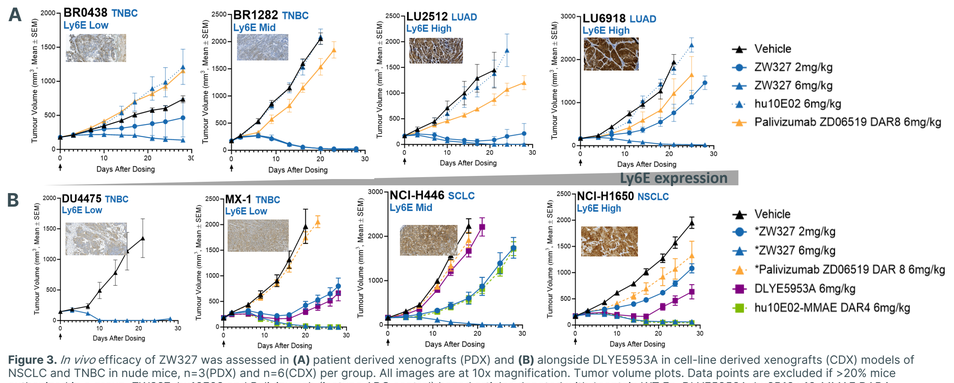
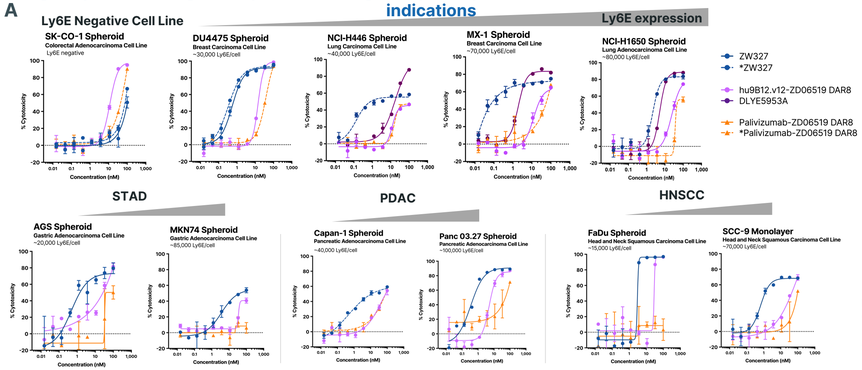


Figure 3. *In vivo* efficacy of ZW327 was assessed in **(A)** patient derived xenografts (PDX) and **(B)** alongside DLYE5953A in cell-line derived xenografts (CDX) models of NSCLC and TNBC in nude mice, n=3(PDX) and n=6(CDX) per group. All images are at 10x magnification. Tumor volume plots. Data points are excluded if >20% mice euthanized in a group. ZW327, hu10E02 and Palivizumab (isotype ADC control) based articles denoted with *contain WT Fc, DLYE5953A: hu9B12.v12-MMAE DAR4. Additional 6 models were tested, ZW327 has predominantly led to regression at 6mg/kg (CDX: NCI-H2228, SW900, HCC156 and PDX: BR1282, LU0876, LU2071). 6 mg/kg dose anticipated to be clinically relevant based on ZW327's preclinical tolerability profile.

ZW327's ZD06519 payload and novel antibody enable strong cytotoxicity across a range of solid tumor indications

Target-specific strong activity across a range of Ly6E expression in TNBC, NSCLC, as well as additional indications



Activity is observed in a broad range of indications, consistently superior to benchmark ADC

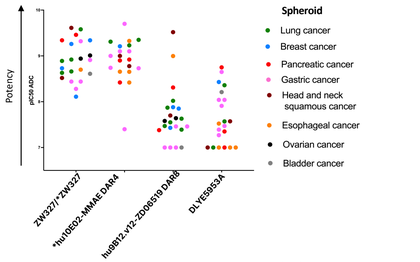
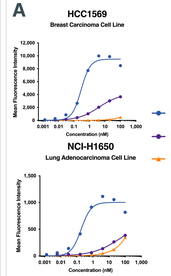


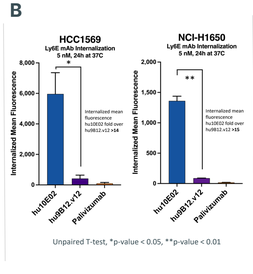
Figure 4. **(A)** *In vitro* ZW327 cytotoxic activity against spheroid (3D) cancer cell lines expressing a range of Ly6E. Target specificity is indicated by the differential sensitivity to Palivizumab and absence of activity in Ly6E negative cell line. **(B)** pIC50 values (3D) across 8 indications for ZD06519 and MMAE based ADCs. ZW327, hu10E02 and Palivizumab based articles denoted with * contain WT Fc.

ZW327's novel antibody drives superior binding, internalization and spheroid penetration compared to benchmark mAb hu9B12.v12. ZW327 exhibits bystander activity

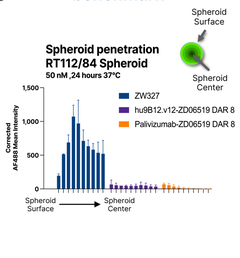
High cell surface binding



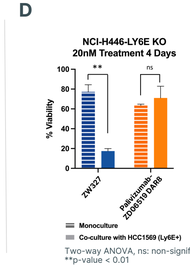
Optimal internalization



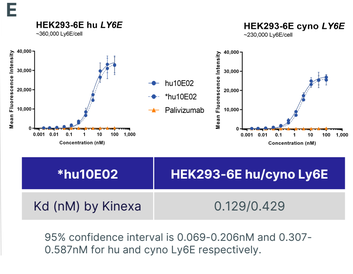
Superior penetration to benchmark



Effective bystander activity



Hu/cyno Ly6E cross-reactivity



*hu10E02	HEK293-6E hu/cyno Ly6E
Kd (nM) by Kinexa	0.129/0.429

95% confidence interval is 0.069-0.206nM and 0.307-0.587nM for hu and cyno Ly6E respectively.

Figure 5. **(A)** mAb cell binding to HCC1569 and NCI-H1650 cells by flow cytometry. **(B)** Internalization of AF488 labelled antibodies into HCC1569 and NCI-H1650 after 24hrs at 5nM and 37°C. **(C)** Penetration of AF488 labelled ADCs as quantified by high content imaging of spheroid layers at 50 nm after 24hrs, 37°. **(D)** Bystander activity of ZW327 as shown by the decreased viability of Ly6E negative cells NCI-H446-LY6E KO when co-cultured with Ly6E positive cells HCC1569. **(E)** mAb cell binding to stable HEK293-6E hu and cyno *LY6E* cell lines by flow cytometry and Kd determination by Kinexa (cells were incubated with mAb at 5nM and 0.5nM for 7 days at 4°C at different cell densities), *hu10E02 contains WT Fc.

ZW327 is well-tolerated in NHP at 60 mg/kg

- MTD ≥ 60 mg/kg in a 2-dose non-GLP non-human primate (NHP) toxicology study.
- Clinical observations were limited to post-dose minimal decrease in body weight and food consumption at both 30 and 60 mg/kg.
- Hematology and histopathology findings at 30 and 60 mg/kg were considered mild, primarily reflective of decreased lymphocyte cellularity in lymphoid tissues.

Dose (mg/kg) q3w x 2	Clinical Pathology	Histopathology
30	↓ lymphocyte count	↑ thyroid weight; ↓ thymus weight; ↓ lymph. cellularity in thymus, spleen & lymph nodes
60	↓ lymphocyte count; ↓ phosphorus	

Table 2. Summary of clinical pathology and histopathology for non-human primates treated with 30 or 60 mg/kg of ZW327 for two doses every three weeks

ZW327 has a favorable PK profile

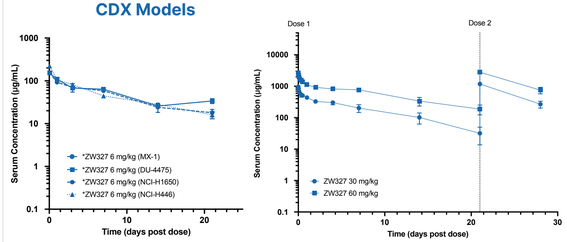


Figure 6. **(A)** Total antibody PK in nude mice CDX models indicates ZW327 maintains a favorable PK profile, **(B)** Total antibody PK from NHP shows ZW327 to have a favorable PK profile at both doses with T1/2 of 5.1-7.6 days; PK-Pharmacokinetics

Conclusions

- ZW327 is a Ly6E-targeting ADC differentiated by its novel antibody and proprietary topoisomerase 1 inhibitor payload.
- ZW327 represents a first-in-class ADC against Ly6E and has potential in diverse indications including NSCLC, TNBC, HNSCC[2] and GI cancers (COAD[2], CHOL, STAD[2], GEJ, ESCC) including PDAC[2].
- Robust anti-tumor activity observed in low, mid, and high Ly6E-expressing CDX and PDX models treated with ZW327.
- Favorable pharmacokinetics and good tolerability in non-human primates at exposure levels above those projected to be efficacious for ZW327.
- ZW327 has the potential to improve on the clinical activity reported for DLYE5953A in Ly6E-expressing cancers[3].

References

1. Petersen et al. Design and evaluation of ZD06519, a novel camptothecin payload for antibody drug conjugates. Mol Cancer Ther. **2024** May 2; *23(5)*:606-618
2. US9290578B2
3. Tolaney et al. A Phase I Study of DLYE5953A, an anti-Ly6E antibody covalently linked to monomethyl auristatin E, in patients with refractory solid tumors. Clin Cancer Res. **2020** Nov 1; *26(21)*: 5588-5597

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