ZW220, a novel NaPi2b-targeting antibody-drug conjugate bearing a topoisomerase I inhibitor payload

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Introduction

ADC structure

ZW220 is an antibody-drug conjugate (ADC) targeting human NaPi2b. The ADC is comprised of a novel fully humanized IgG1 antibody covalently conjugated to a novel topoisomerase I inhibitor payload, a camptothecin derivative, via endogenous interchain cysteines. The drug-linker consists of a maleimidocaproyl (MC) anchor and a GGFG-aminomethyl (AM) protease cleavable sequence. Drug to antibody ratios (DAR) of 8 and 4 have been evaluated with ZW220 ADC.

Mechanism of action

Following NaPi2b binding and receptor-mediated internalization of ZW220, intracellular payload release induces targeted cell death in NaPi2b-positive cells, and subsequent death of NaPi2b-negative cells through bystander-mediated killing.

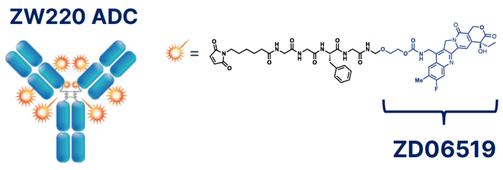


Figure 1. Structure of ZW220, a NaPi2b-targeting ADC comprised of novel monospecific IgG1 mAb, covalently conjugated to 8 or 4 drugs per antibody (MC-GGFG-AM-ZD06519 drug linker); DAR 8 pictured.

NaPi2b is overexpressed in ovarian and lung cancers

Expression

NaPi2b is highly expressed in ovarian and lung carcinomas; some NaPi2b expression is also found in endometrioid[1], thyroid[1], colorectal[2] and breast carcinomas[3]. Normal tissue expression of NaPi2b is observed in lung, liver, and small intestine.

Function

NaPi2b is a multi-pass transmembrane sodium-dependent phosphate transport protein, encoded by SLC34A2 gene, involved in phosphate homeostasis.

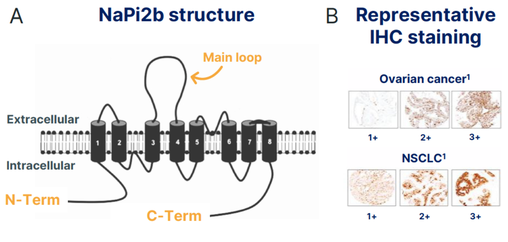
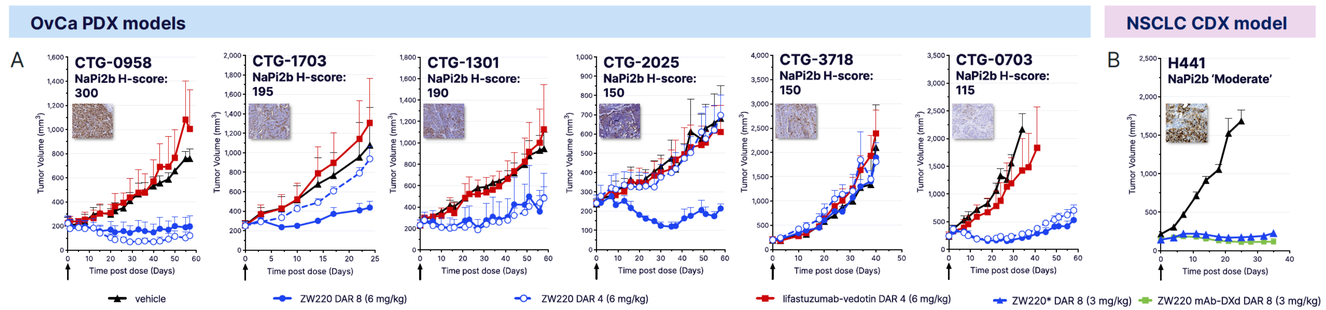


Figure 2. (A) Structural model of NaPi2b multi-pass transmembrane protein, adapted from Bobeck et al. 2015[4]. **(B)** Immunohistochemistry of representative tumor samples (stained using mouse NaPi2b antibodies) shows NaPi2b expression in human nonsquamous non-small cell lung cancer (NSCLC) and nonmucinous ovarian cancer[5].

ZW220 demonstrates robust anti-tumor activity in ovarian carcinoma and NSCLC xenograft models with a range of NaPi2b-expression



C OvCa anti-tumor activity (6 mg/kg single dose)

NaPi2b H-score	ZW220 DAR 8	ZW220 DAR 4	lifa-vedotin
>150	100% (3/3)	67% (2/3)	0% (0/3)
≤150	67% (2/3)	33% (1/3)	0% (0/3)
All	**83% (5/6)**	**50% (3/6)**	**0% (0/6)**

D NaPi2b-targeting ADCs evaluated in vivo

ADC	Antibody	Linker (payload)	DAR	Payload class	Mechanism of action
ZW220	12A10	(ZD06519)	8/4	camptothecin	topoisomerase-1 inhibition
ZW220	12A10	deruxtecan (DXd)	8	camptothecin	topoisomerase-1 inhibition
lifa-vedotin	lifastuzumab	vedotin (MMAE)	4	auristatin	microtubule disruption

Figure 3. (A) Antitumor activity in patient derived xenograft (PDX) models of ovarian cancer, n=3 mice/cohort. IV administration on Day 0. Immunohistochemistry (IHC) images from same study tissues stained using a commercial anti-NaPi2b antibody. H-scores determined by pathologist. **(B)** Antitumor activity in cell line derived xenograft (CDX) model of non small cell lung cancer, H441, n=6 mice/cohort. IV administration on Day 0. IHC images from archival CDX tissues stained using a commercial anti-NaPi2b antibody. **(C)** Scope of ZW220 anti-tumor activity in OvCa PDX models, as defined by %TGI > 50%, in models with higher NaPi2b expression (H-score >150) and lower NaPi2b expression (H-score ≤150). Anti-tumor activity was determined by %tumor growth inhibition calculated as $[(1-TV_{treatment}/TV_{vehicle}) \times 100]$ at Day 28, or at the closest evaluable time point. **(D)** NaPi2b-targeting ADCs evaluated in vivo, prepared internally.
*ZW220-analog DAR 8 is comprised of ZW220 mAb conjugated to a closely related drug-linker.

ZW220 exhibits rapid internalization, potent target-mediated cytotoxicity and bystander killing in tumor cell lines

ZW220 is efficiently internalized and colocalizes with lysosomes

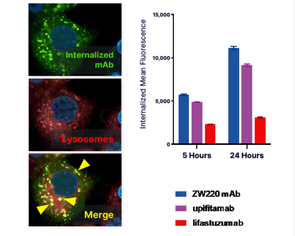


Figure 4. (A) Internalization of AF488-labelled ZW220 mAb (green), staining of lysosomes with LysoTracker Deep Red AF647 (red), and colocalization (yellow) after 24 hours in IGROV-1 cells by high content imaging. **(B)** Internalization of AF488-labelled mAbs in H441 cells after 5 and 24 hours by flow cytometry (external fluorescence quenched prior to analysis). ZW220 DAR 8 and 4 show comparable internalization profile to unconjugated ZW220 mAb (ADC data not shown).

ZW220 induces cell growth inhibition in lung and ovarian NaPi2b⁺ tumor cell line spheroids

A

Cell line spheroids	NaPi2b/cell	EC$_{50}$ (nM) in 3D tumor cell spheroids: ZW220 DAR 8	ZW220 DAR 4	Pali-ZD06519 non-targeting control
IGROV-1 (OvCa)	1,770,000	0.5 ± 0.2	1.3 ± 0.4	44.7 ± 12.8
HCC-78 (NSCLC)	820,000	0.6 ± 0.2	0.7 ± 0.2	32.4 ± 8.0
TOV-21G (OvCa)	350,000	0.6 ± 0.1	0.9 ± 0.3	116.7 ± 28.9
H441 (NSCLC)	41,000	1.6 ± 0.2	7.0 ± 2.7	128.6 ± 91.5
EBC-1 (NSCLC)	0	53.7 ± 14.3	54.7 ± 7.9	113.7 ± 51.4

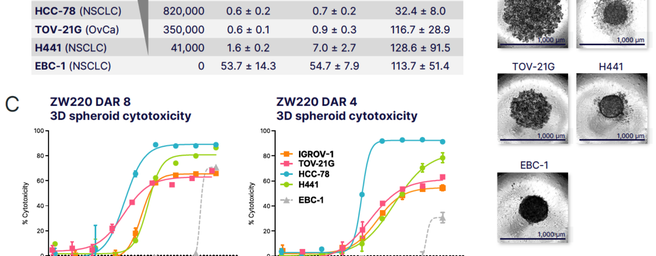


Figure 5. Mean EC$_{50}$ values (2-7 biological replicates) for ZW220 DAR 8 and 4 ADCs and non-targeting control ADC Palivizumab-ZD06519 DAR 8, in NaPi2b$^{+/-}$ tumor cell line spheroids. NaPi2b/cell quantification performed by flow cytometry using AF647-labelled NaPi2b monoclonal antibody. **(B)** Select phase contrast images of untreated NaPi2b$^{+/-}$ tumor cell line spheroids, acquired moments prior to ADC treatment. **(C)** Representative dose-response curves for ZW220 DAR 8 and 4 in a panel of NaPi2b^{+} tumor cell line spheroids and NaPi2b^{-} tumor cell line spheroid control.

ZW220 exhibits strong bystander activity in co-culture assay

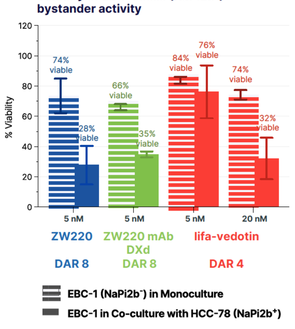


Figure 6. At equivalent treatment concentrations, ZW220 exhibits stronger bystander activity than lifa-vedotin, and comparable bystander activity to ZW220 mAb-DXd ADC control. Bystander activity, as shown by the decreased viability of NaPi2b^{-} cells when co-cultured with NaPi2b^{+} cells, was assessed in a co-culture assay with HCC-78 cells (NaPi2b^{+}) and EBC-1 cells (NaPi2b^{-}), 4-night incubation with ADCs, viability analysis by flow cytometry.

ZW220 mAb binds to NaPi2b with high affinity and specificity

Strong binding to endogenous human NaPi2b-expressing cell lines

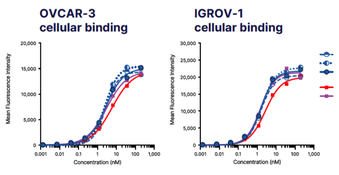


Figure 7. ZW220 mAb and ADC DAR 8 and 4 demonstrate comparable on-cell apparent binding affinities. ZW220 binding is comparable to lifastuzumab and upifitamab unconjugated antibody controls. Binding of NaPi2b mAbs and ADCs to endogenous expressing NaPi2b tumor cell lines was assessed by flow cytometry.

ZW220 cross-reacts to cynomolgus monkey and mouse NaPi2b

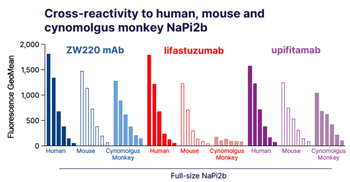
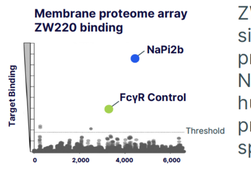


Figure 8. ZW220 mAb and ADC (ADC data not shown) show comparable binding to human, mouse and cynomolgus monkey NaPi2b. Binding of mAbs to transfected HEK293 cells expressing human, mouse and cynomolgus monkey NaPi2b assessed by flow cytometry. Titration of 200-0.001 nM antibody shown.

Specific binding to NaPi2b in membrane proteome array screen



ZW220 mAb shows no significant binding to proteins besides human NaPi2b in a native human membrane proteome array (MPA) specificity screen.

Figure 9. ZW220 specificity was profiled by measuring binding by flow cytometry to HEK293 cells expressing a library of ~6,000 human membrane proteins including 94% of all single-pass, multi-pass, and GPI-anchored human proteins. Binding hits above threshold in initial MPA screen were subsequently individually validated and ZW220 mAb binding was not found to be significant.

ZW220 is well tolerated in non-human primates

- Repeat-dose non-GLP toxicology study of ZW220 DAR 8 and DAR 4 in male NHPs resulted in no mortalities.
- No adverse clinical observations, effect on body weights, macroscopic observations or organ weights were noted.
- No clinical or anatomic pathology findings related to administration of ZW220.
- MTD of DAR 8 is 45 mg/kg; MTD of DAR 4 is 90 mg/kg.

ZW220 3-dose non-GLP non-human primate (NHP) toxicology study, Q3Wx3

Test article	Dose	Mortality	Clinical observations	Histo-pathology	Clinical chemistry	Hema-tology	MTD	$T_{1/2}$ (day)*
ZW220 DAR 8	15 mg/kg	None	None	None	None	None	45 mg/kg	8.7
	30 mg/kg	None	None	None	None	None		7.7
	45 mg/kg	None	Fecal abnormalities (soft/loose)	None	None	None		8.0
ZW220 DAR 4	30 mg/kg	None	None	None	None	None	90 mg/kg	10.3
	60 mg/kg	None	None	None	None	None		9.8
	90 mg/kg	None	Fecal abnormalities (soft/loose/watery)	None	None	None		8.0

Figure 10. Relevant study parameters from a repeat dose non-GLP toxicology study in male cynomolgus monkeys performed to assess the tolerability and pharmacokinetic profile of ZW220 DAR 8 and 4 (n=3 animals/group).
*Half life ($T_{1/2}$) calculated from Total IgG data in Figure 11B.

ZW220 has a favorable pharmacokinetic profile

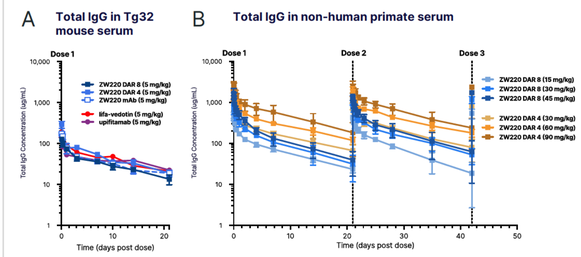


Figure 11. (A) Circulating antibody levels in Tg32 transgenic mouse model (expressing human neonatal Fc receptor, hFcRn) determined by ligand binding assay (MSD) measuring human IgG in serum following single intravenous dosing of antibody or ADC. **(B)** Circulating antibody levels in NHP determined by MSD assay following 1st, 2nd and 3rd dose (1st time point only).

Conclusions

- ZW220 demonstrates robust preclinical anti-tumor activity in ovarian and lung cancer xenograft models with low NaPi2b expression levels (H-score ≥ 115).
- ZW220 is tolerated at high doses in non-human primates, with an MTD of 45 mg/kg for DAR 8 ADC, and 90 mg/kg for DAR 4 ADC.
- Potential for improvement over NaPi2b-targeting microtubule inhibitor-based ADCs on basis of efficacy, tolerability, and payload mechanism.
- Robust preclinical data package supports the continued development of ZW220 as a best-in-class NaPi2b ADC.

References

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