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ZW220, a NaPi2b-directed TOPO1i ADC, demonstrates compelling preclinical activity in NSCLC, ovarian and uterine cancer models, with a favorable toxicology profile in NHPs

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ADC Therapeutic Development
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Conflict of Interest

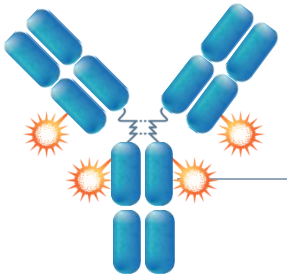
Andrea Hernández Rojas

I have the following relevant financial relationships to disclose:
Employee of: Zymeworks Inc.

Background

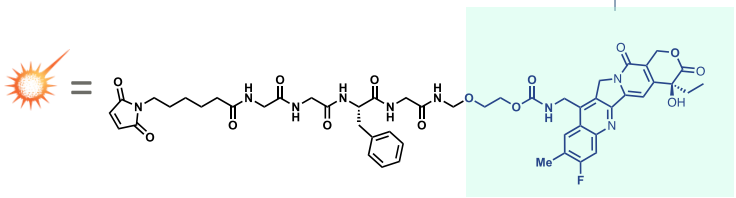
ZW220 is a novel TOPO1i ADC which may help address unmet medical need in patients with NaPi2b-expressing tumors, a target overexpressed in ovarian cancer and NSCLC, among other cancers

ZW220 ADC

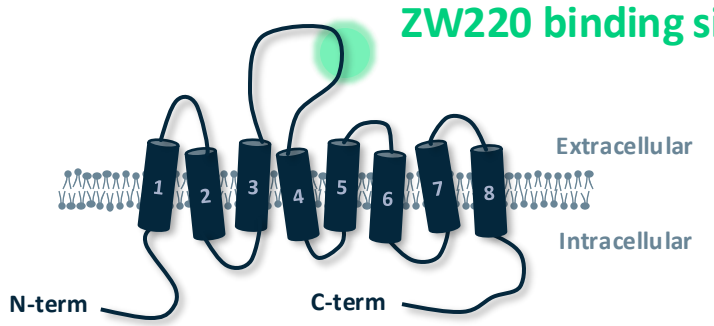


> Humanized IgG1 mAb
> **Cross-reactive to human, monkey, mouse, and rat NaPi2b**
> **FcγR silenced** ('LALADS')

> **Novel TOPO1i payload, ZD06519**
> **Moderate potency**
> **DAR 4**



NaPi2b is involved in phosphate homeostasis and is overexpressed in various cancers



- NaPi2b is expressed in various cancers, including **ovarian, lung, uterine/endometrial and thyroid cancers**, with limited expression in normal tissues[1]
- Normal tissue expression observed in lung, liver, and small intestine, where it is involved in inorganic phosphate homeostasis

[1] Lin *et al.* 2015. *Clin Cancer Res*

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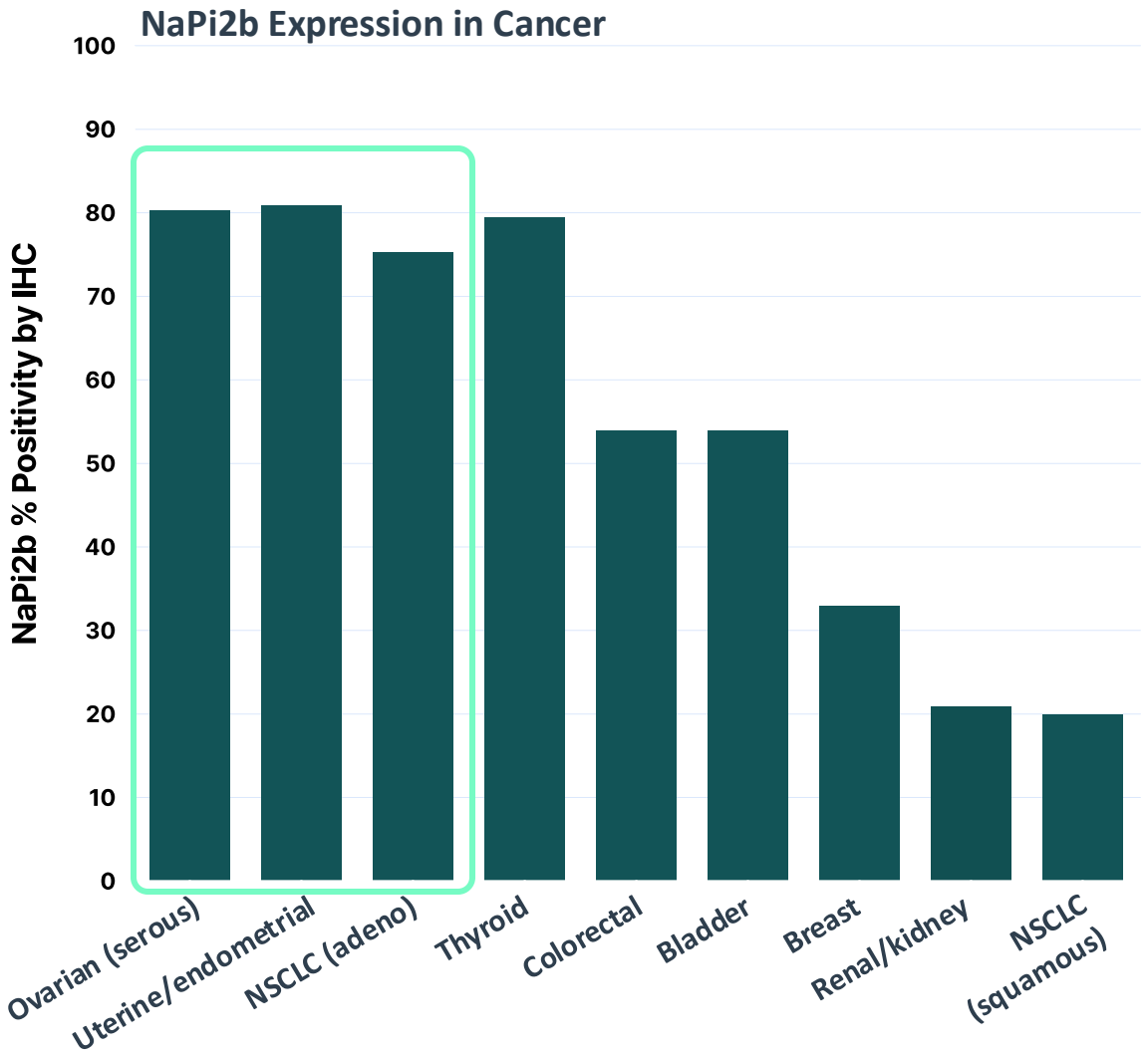
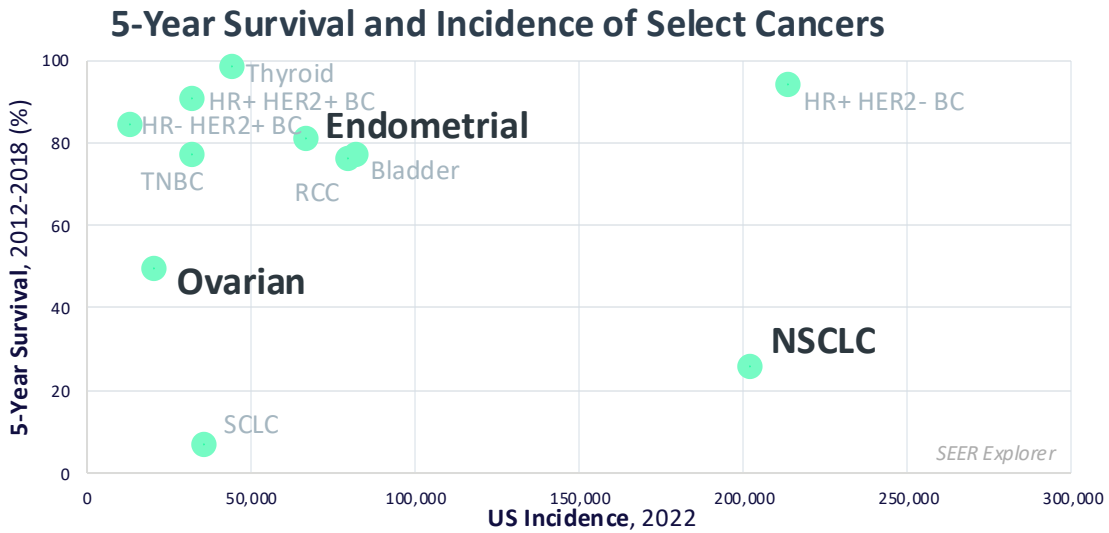
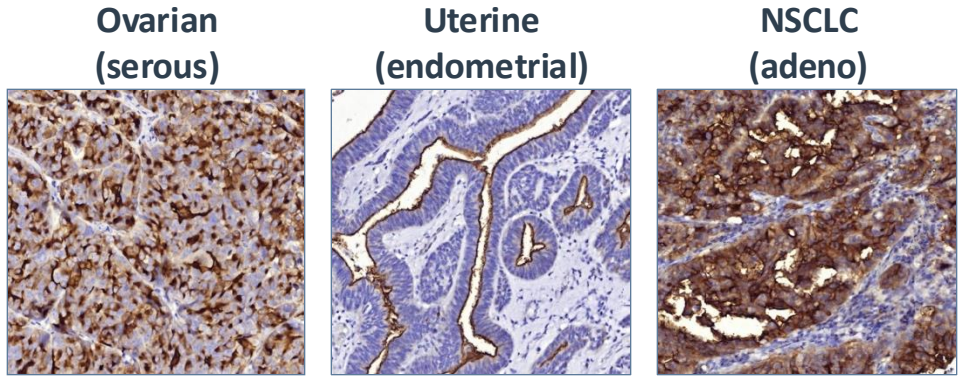
NaPi2b is associated with various human cancers with unmet medical need, such as ovarian and lung cancer







NaPi2b expression in patient-derived xenograft (PDX) samples
IHC: Zymeworks internal studies

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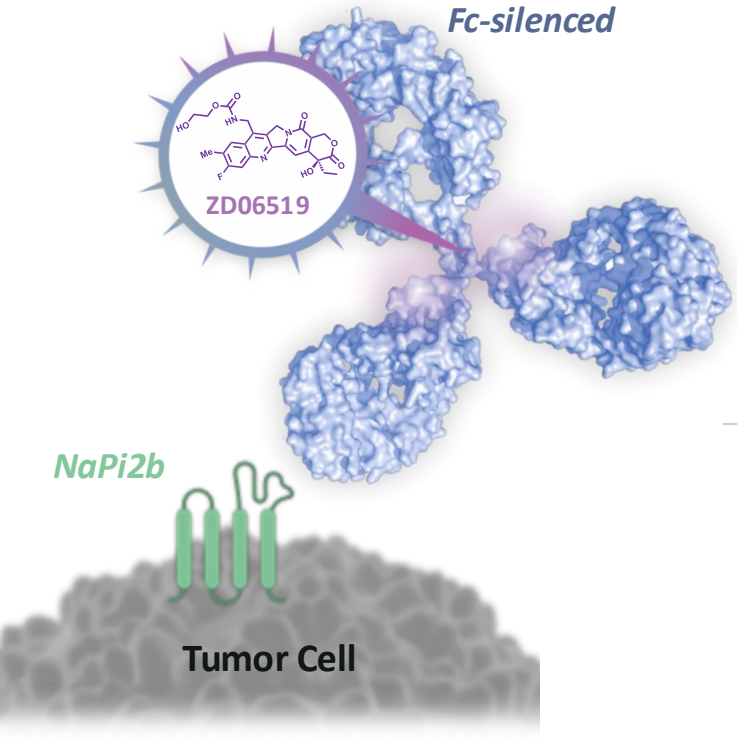
NaPi2b expression in select cancer indications: Banerjee *et al.* 2023. ESMO Abstract 145, Hakim *et al.* 2021. *Anal Cell Pathol*, Horsley *et al.* 2024. Cancer Res #5085, Lin *et al.* 2015. *Clin Cancer Res*, Liu *et al.* 2018. *Biomed Pharmacother*, Lopes dos Santos *et al.* 2013. *PLoS One*, Ye *et al.* 2017. *Cell Death Dis*, Yu *et al.* 2018. IASLC Poster 12636

ZW220, a NaPi2b-targeting TOPO1i antibody drug conjugate

ZW220



	Design Feature	Benefit
Antibody	**Strong internalizing antibody**	Strong binding, internalization, and payload delivery can result in improved anti-tumor activity and the ability to **target lower levels of NaPi2b**
	FcγR silenced	Potential to **minimize toxicities** driven by cellular uptake via FcγRs
Payload	**Topoisomerase-1 inhibitor mechanism**	Proven ADC mechanism in solid tumors across multiple tumor associated antigens
	Moderate TOPO1i payload potency	Potential for high doses in humans; **Well-tolerated ADC,** NHP MTD ≥90 mg/kg & rat MTD ≥200 mg/kg
	Strong bystander activity	Beneficial when treating tumors with low and heterogenous expression of NaPi2b
Linker and Conjugation	**Moderate stability of the antibody-linker**	Minimize antibody-driven toxicities including potential on-target or off-tumor toxicities
	DAR 4	Intermediate DAR confers balance between anti-tumor activity and **reduced potential for on-target toxicities**

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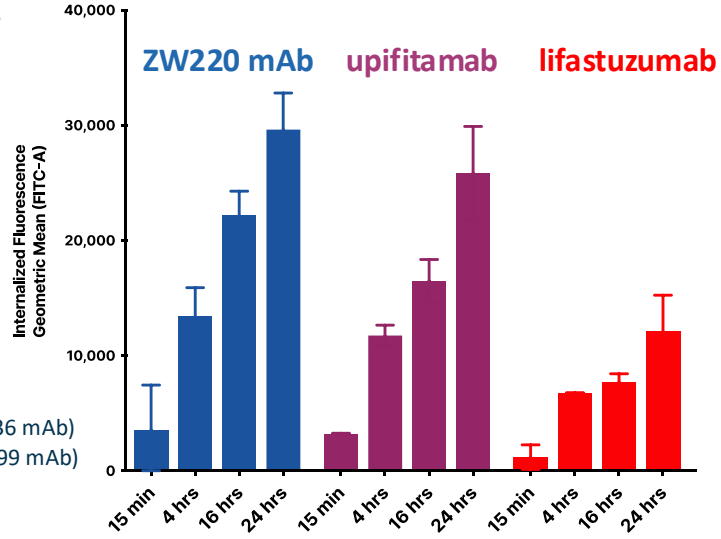
ZW220 is rapidly internalized and effectively diffused into NaPi2b-expressing 3D spheroids

ZW220 internalization and colocalization with lysosomes

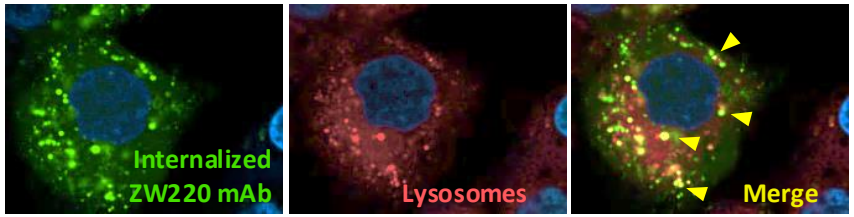
Flow cytometry
AF488-labelled mAbs



Upifitamab (XMT-1536 mAb)
Lifastuzumab (RG-7599 mAb)

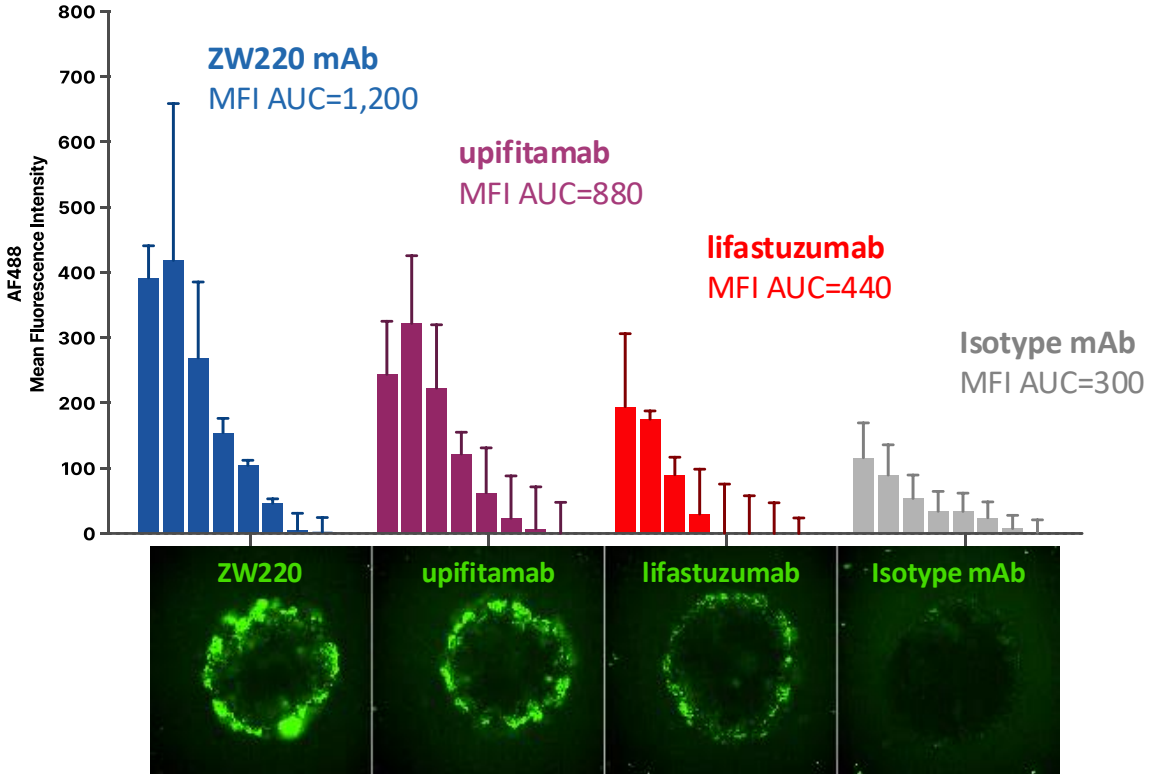
OVCAR-3 ovarian cancer cell line

Lysosomal trafficking



IGROV-1 ovarian cancer cell line

Effective penetration in heterogeneous tumor spheroids



HCC78 lung cancer cell line heterogeneous spheroid (>1,000 cells/spheroid)
AF488-labelled antibodies, 24-hour antibody incubation at 37°C

Data: Zymeworks internal studies

Internalization and lysosomal colocalization by high content imaging 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.
Internalization of AF488-labelled mAbs in OVCAR-3 cells by flow cytometry (external fluorescence quenched prior to analysis). ZW220 ADC shows comparable internalization profile to unconjugated ZW220 mAb (ADC data not shown)
Spheroid penetration by high content imaging of ZW220 mAb, upifitamab and lifastuzumab NaPi2b-targeting control antibodies, and palivizumab isotype mAb control, after 24 hours in HCC78 cell line-containing spheroid model

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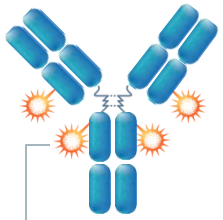
ZW220 is an Fc-silenced ADC with lower affinity for normal FcγR-expressing cells

Fc-silencing has the **potential to minimize toxicities** driven by target-independent cellular uptake via FcγRs

ZW220 Fv:
NaPi2b-binding

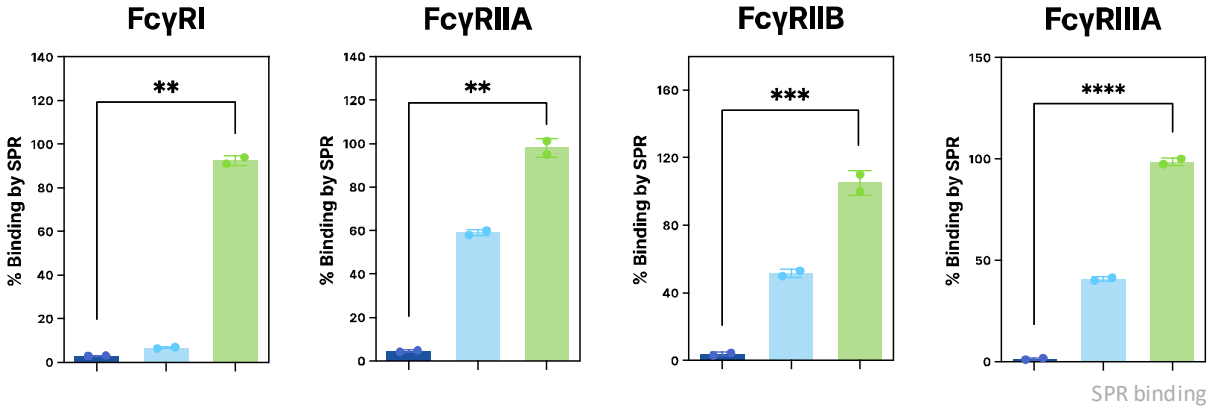


ZW220 Fc:
Effector-silenced
LALADS mutations

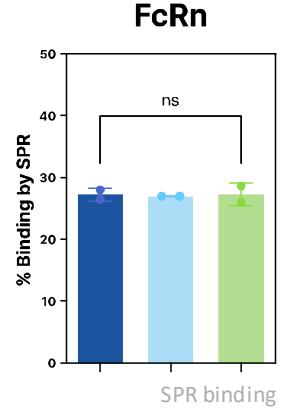
- ZW220 mAb
- LALA mAb[a]
- WT Fc mAb[a]

LALA = L234A/L235A
Widely used Fc effector function attenuating mutations (developed circa 90s)[1,2]

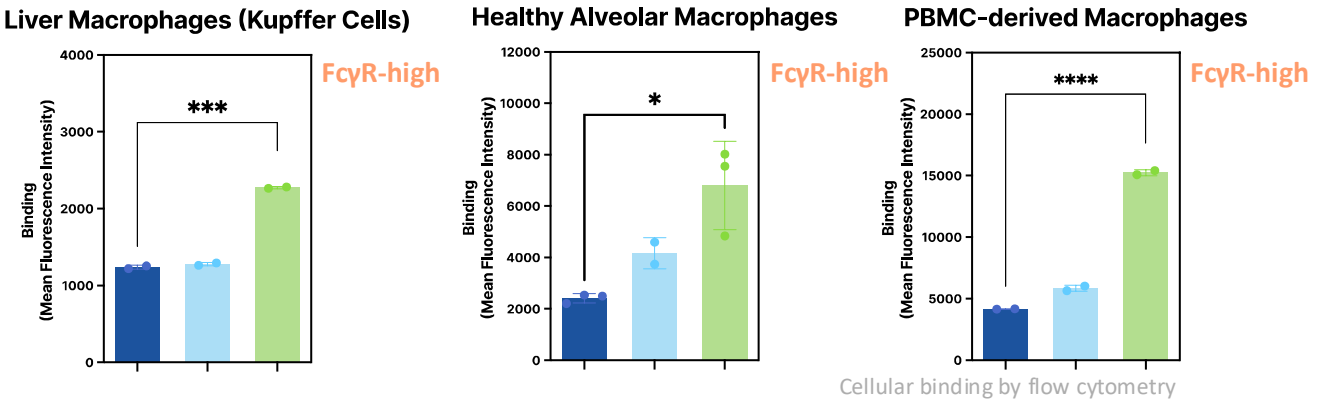
Abrogated binding to FcγRs



Sustained FcRn binding



Reduced binding affinity for FcγR-expressing normal cells



Data: Zymeworks internal studies

[a] Wild-type and LALA versions of ZW220 mAb evaluated

The binding affinities of ZW220 (LALADS) to human FcγRs (Fc gamma receptors - FcγRI, FcγRIIA, FcγRIIB, and FcγRIIIA) and human FcRn (neonatal fragment crystallizable, Fc, receptor), were determined using surface plasmon resonance (SPR) - 12A10 (humanized antibody used in ZW220 ADC) with wild-type Fc region, LALA mutation, and LALA-DS Fc-silencing mutation ("ZW220") were evaluated; select FcγRs shown. Binding to FcγR-expressing normal cells evaluated using flow cytometry (24 hours at 4°C to prevent internalization)

[1] Wilkinson et al. 2021. PLoS One
[2] Hale, G. 2024. Immunological Reviews

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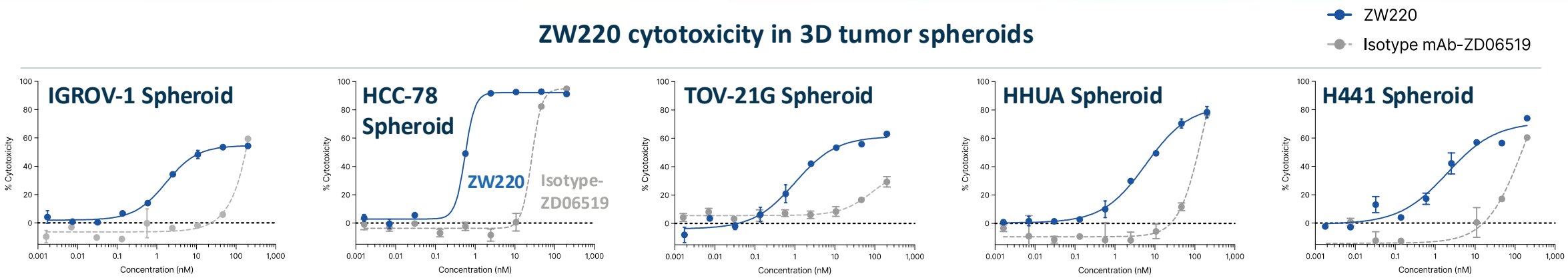
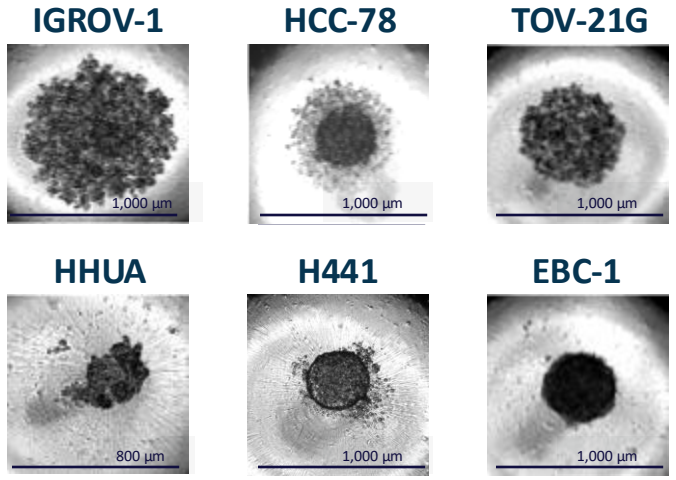
ZW220 shows robust activity in indications of interest *in vitro,* across a wide range of NaPi2b expression

ZW220 cytotoxicity in 3D tumor spheroids



Ovarian | Lung (NSCLC) | Ovarian | Endometrial | Lung (NSCLC)

Untreated spheroids



Cell line spheroids	NaPi2b/cell	EC_{50} (nM) in 3D tumor cell spheroids	
		ZW220	Isotype mAb-ZD06519 non-targeting control DAR 8
IGROV-1 (ovarian)	**1,770,000**	1.3 ± 0.4	44.7 ± 12.8
HCC-78 (NSCLC)	**820,000**	0.7 ± 0.2	32.4 ± 8.0
TOV-21G (ovarian)	**350,000**	0.9 ± 0.3	116.7 ± 28.9
HHUA (endometrial)	**80,000**	2.1 ± 1.0	150.1 ± 68.7
H441 (NSCLC)	**41,000**	7.0 ± 2.7	128.6 ± 91.5
EBC-1 (NSCLC)	**0**	54.7 ± 7.9	113.7 ± 51.4

4-6 day incubation with ADCs at 37°C; viability assessed by CTG

Data: Zymeworks internal studies

Representative dose-response cytotoxicity curves for ZW220, relative to untreated, in a panel of NaPi2b+/- tumor cell line spheroids, viability assessed using Cell Titer Glo reagent. Select phase contrast images of untreated NaPi2b+/- tumor cell line spheroids, acquired moments prior to ADC treatment. Mean EC50 values (2-7 biological replicates) for ZW220 and non-targeting ('isotype') control ADC palivizumab-ZD06519 DAR 8 in NaPi2b+/- tumor cell line spheroids. NaPi2b/cell quantification performed by flow cytometry using AF647-labelled NaPi2b control mAb

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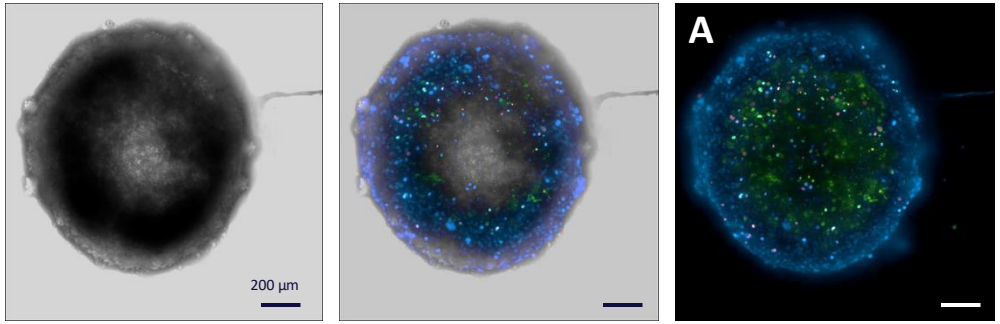
ZW220 inhibits the growth of heterogeneous NaPi2b-expressing spheroid models

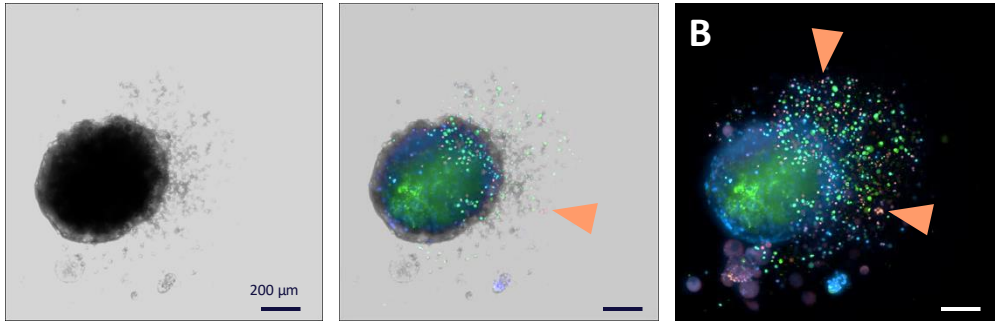
ZW220 shows significant killing of heterogeneous NaPi2b-expressing spheroids
HCC-78, **NaPi2b-high** cell line, co-cultured with **NaPi2b-negative** carcinoma cell line 1:1

Untreated



- Minimal cell death
- Compact morphology

ZW220
50 nM



- Reduction in spheroid size compared to untreated
- ↑ Apoptotic core and dead cells

6-day incubation with test article

ZW220 is **highly active and demonstrates bystander killing** *in vitro*, with significant **size reduction of heterogeneous NaPi2b spheroids**

Nuclear Stain (Live Cells)
Hoechst 33342 stain

Apoptotic Cells
YO-PRO-1 stain

Dead Cells + Apoptotic Cells
PI stain

Data: Zymeworks internal studies

Representative images of heterogeneous NaPi2b-expressing spheroids treated with ZW220, free TOPO1i payload, or untreated (blank medium). Spheroids were formed by 1:1 co-culture of NaPi2b-negative cell line (ZW220-insensitive under monoculture conditions) and HCC78 NSCLC cell line (NaPi2b-high, ZW220-sensitive in monoculture) and treated with test article for 6 days. Following incubation, spheroids were stained with fluorescent viability and cytotoxicity markers and imaged and analyzed by high content imaging, using Operetta instrument.

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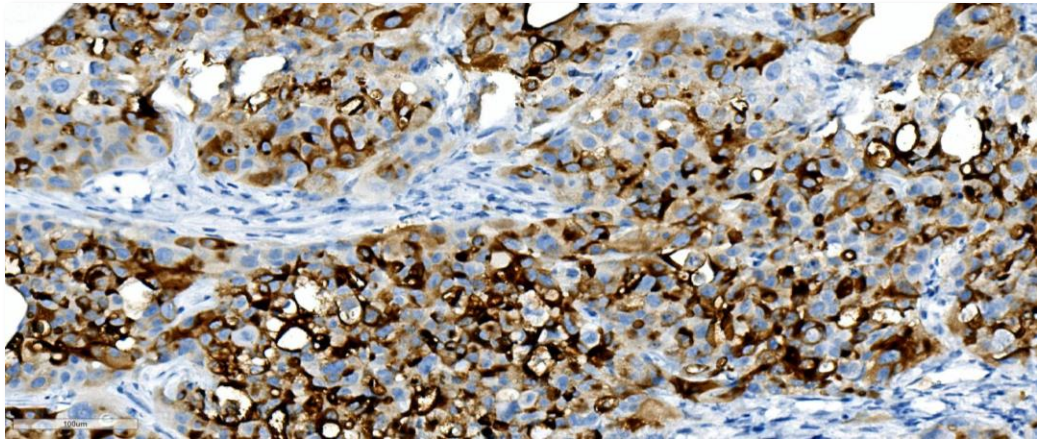
ZW220 exhibits strong bystander-mediated killing *in vitro*

NaPi2b heterogeneity

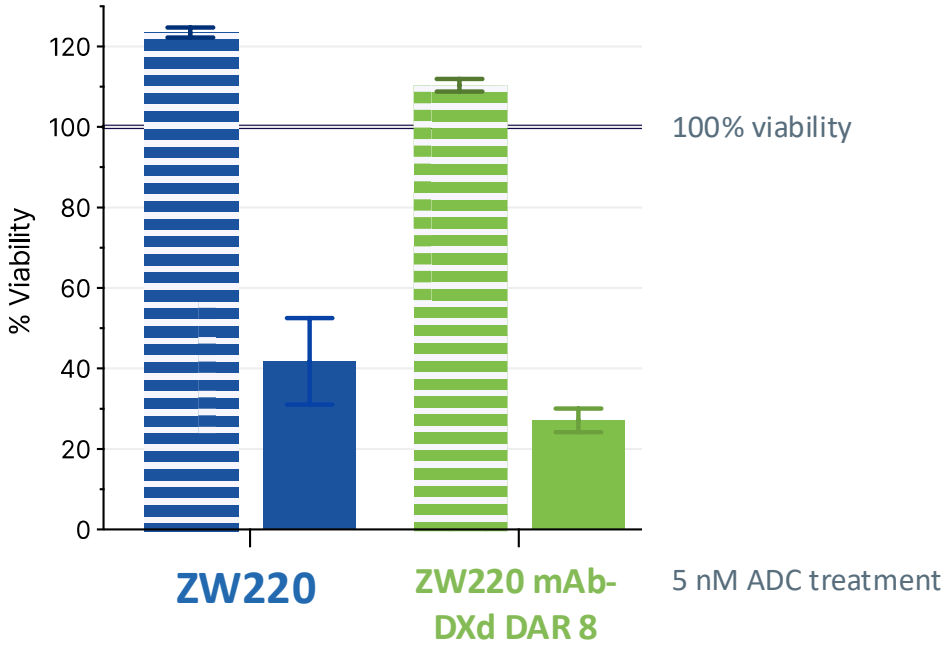
Non-small cell lung cancer
H441 CDX IHC



low/no NaPi2b staining

strong NaPi2b staining

Viability of EBC-1 cells (NaPi2b⁻)
Bystander activity in co-culture with IGROV-1 (NaPi2b⁺)

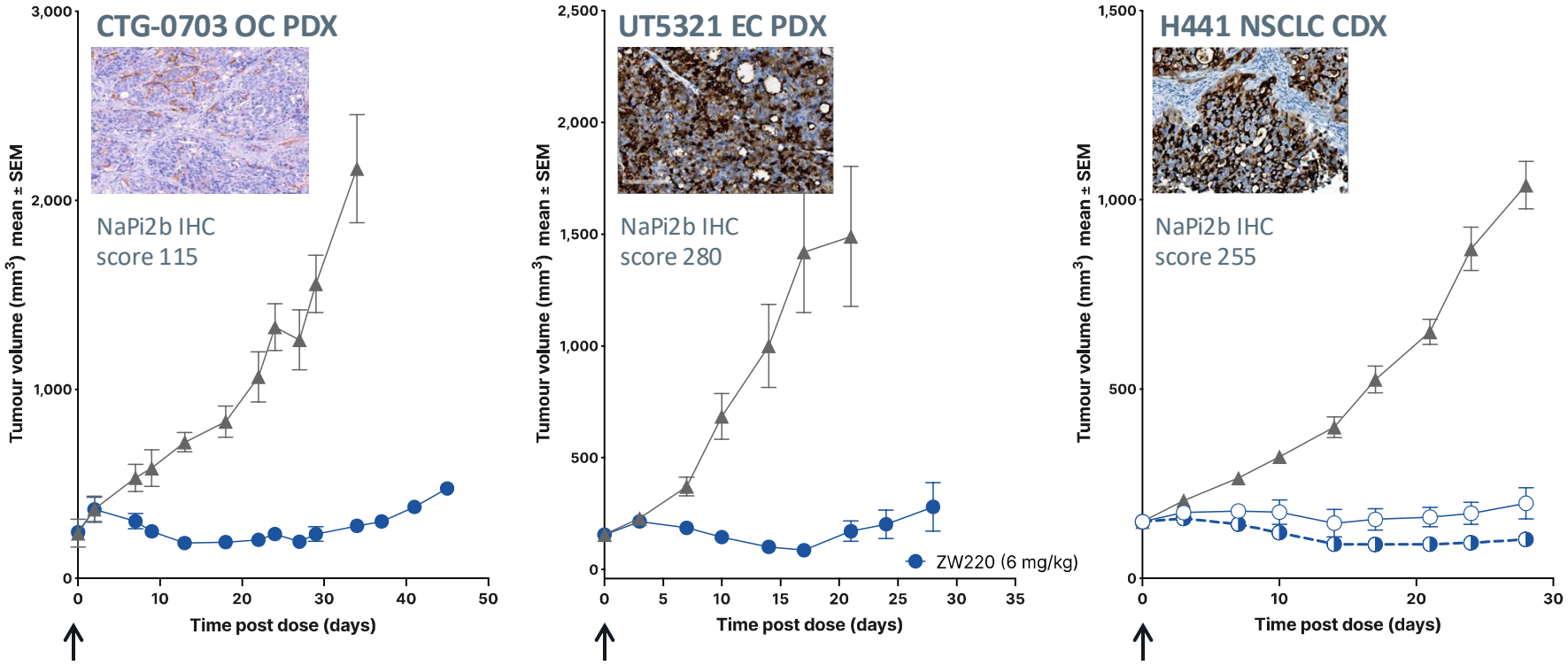


Data: Zymeworks internal studies

ZW220 exhibits comparable bystander activity to lifa-vedotin DAR 4 and ZW220 mAb-DXd DAR 8 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 IGROV-1 and EBC-1 cells, stably and homogenously expressing GFP by lentiviral transduction, 4-night incubation with ADCs, dead cell exclusion with YO-PRO-3, viability analysis by flow cytometry. Commercial anti-NaPi2b antibody used for immunohistochemistry (IHC) staining of lung cell line-derived xenograft (CDX) model, archival sample.

ZW220 demonstrates anti-tumor efficacy in NaPi2b-expressing ovarian, endometrial and NSCLC *in vivo* models

Select ovarian, endometrial and lung xenograft models



ZW220 was active at 6 mg/kg in a majority of ovarian, endometrial and NSCLC models tested

- Activity is largely target-dependent
- ZW220 mAb appears inactive
- 4 to 8 models evaluated per indication
- 6 mg/kg is considered a conservative dose for ZW220 based on tolerability data

Data: Zymeworks internal studies

Antitumor activity in patient derived xenograft (PDX) models of ovarian cancer, uterine (endometrial) cancer, and non-small cell lung cancer (NSCLC), 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.

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ZW220 is well-tolerated in non-human primates and rats

Rat toxicology (non-GLP)

2-dose non-GLP NHP toxicology study ZW220, Q3Wx2

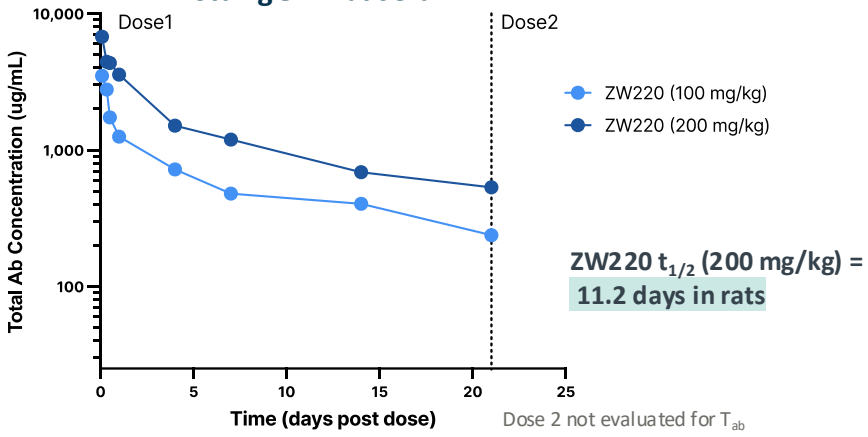
Dose levels:
100 mg/kg
200 mg/kg

Dose 1 Day 1 → Dose 2 Day 22

4F + 4M per group — Terminal necropsy day 28

Rat MTD = ≥ 200 mg/kg

Total IgG in rat serum



ZW220 $t_{1/2}$ (200 mg/kg) = 11.2 days in rats

Dose 2 not evaluated for T_{ab}

- No mortalities or abnormal clinical signs
- **Body Weight & Food Consumption:** ↓ relative to controls at ≥ 100 mg/kg, dose-related
- **Clinical Chemistry:** ↑ phosphorous at ≥ 100 mg/kg (F)
- **Hematology:** ↑ platelets at 200 mg/kg (F); ↑ reticulocytes at 200 mg/kg
- **Organ Weights:** ↓ thymus (absolute and relative to brain/body weight) at ≥ 100 mg/kg
- **Histopathology:** ↓ lymph cellularity in thymus, mandibular and mesenteric lymph node at ≥ 100 mg/kg; ↓ lymph cellularity in spleen at 200 mg/kg; and ↓ cellularity in ileal GALT at 200 mg/kg (F)

Non-human primate (NHP) toxicology (non-GLP)

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

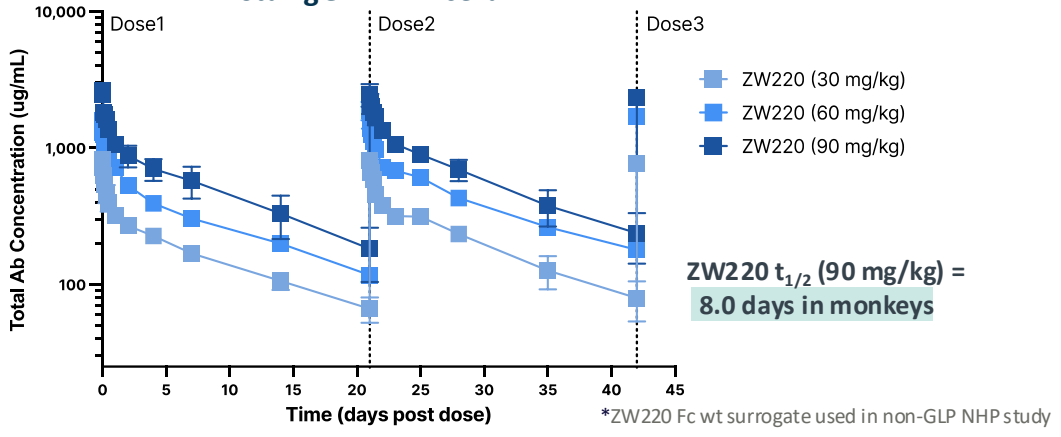
Dose levels:
30 mg/kg
60 mg/kg
90 mg/kg

Dose 1 Day 1 → Dose 2 Day 22 → Dose 3 Day 43

3M per group — Terminal necropsy day 50

NHP MTD = ≥ 90 mg/kg

Total IgG in NHP serum



ZW220 $t_{1/2}$ (90 mg/kg) = 8.0 days in monkeys

*ZW220 Fc wt surrogate used in non-GLP NHP study

- No mortalities or effects on food consumption, clinical pathology parameters, organ weights or histopathology
- **Clinical Signs:** transient fecal abnormalities (soft/loose/watery stool) observed at 90 mg/kg
- **Body Weight:** ↓ after 1st (≥ 30 mg/kg) and 3rd (≥ 60 mg/kg) doses relative to controls

Data: Zymeworks internal studies

Relevant study design and result parameters from repeat dose non-GLP toxicology studies in male cynomolgus monkeys and male + female rats, performed to assess the tolerability and pharmacokinetic profile of ZW220 (n=3 monkeys/group and n=6 female + 6 male rats/group). Circulating antibody levels in NHP determined by ligand binding assay (MSD) measuring human IgG in serum following single intravenous dosing of ADC, following 1st, 2nd and 3rd dose (1st time point only) for monkey study and following 1st dose only for rat study. Half life (T1/2) and clearance rate calculated from total IgG (Tab) data.

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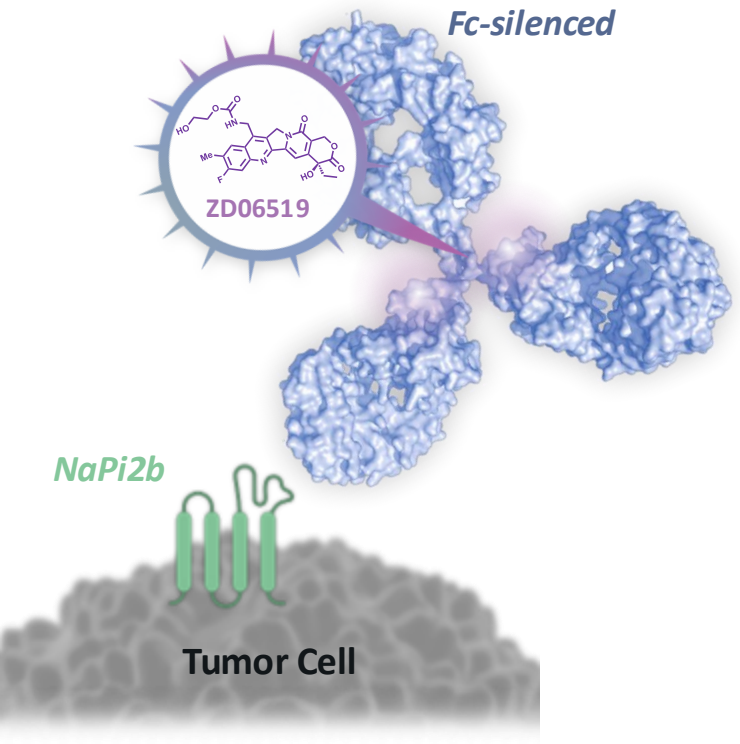
ZW220 – a differentiated, low DAR, Fc-silenced NaPi2b TOPO1i ADC

ZW220 has the potential for improvement over previous NaPi2b MTI ADCs and other novel NaPi2b TOPO1i ADCs on the basis of efficacy, tolerability and payload mechanism



Purposeful design

- **Strong internalizing antibody** with effective tissue penetration – can result in improved activity and the ability to target lower NaPi2b levels
- **Fc-silenced antibody** – potential to minimize off-target toxicities driven by FcγRs-mediated cellular uptake
- **Moderate potency TOPO1 inhibitor payload with strong bystander** activity
- **Intermediate DAR of 4** – can minimize antibody-driven toxicities
- Moderate stability of the antibody-linker

Compelling preclinical profile

- **Strong anti-tumor activity** in models with a breadth of NaPi2b expression
- Differentiated safety profile compared to MTI and exatecan (high potency TOPO1i) ADCs
- **NHP MTD ≥90 mg/kg and rat MTD ≥200 mg/kg**; potential for high doses in humans

Expected IND filing in 1H 2025

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Acknowledgments

Thank you to the ZW220 preclinical project team and the ADC therapeutic development group at Zymeworks!

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- Jamie Rich
- Paul Moore

Intellectual Property
- Jan-Philip Meyer

Vancouver is situated on the traditional, ancestral, and unceded territories of the xʷməθkʷəy̓əm (Musqueam), Sḵwx̱wú7mesh (Squamish), Stó:lō, and Səl̓ílwətaʔ / Selilwitulh (Tsleil-Waututh) Nations.

Zymeworks' ADC Therapeutic Development group

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