zymeworks

ZW220

A Potential First-in-Class TOPO1i ADC for the Treatment of NaPi2b-Expressing Solid Tumors

Andrea Hernandez Rojas, Scientist & Group Lead, In Vitro Biology

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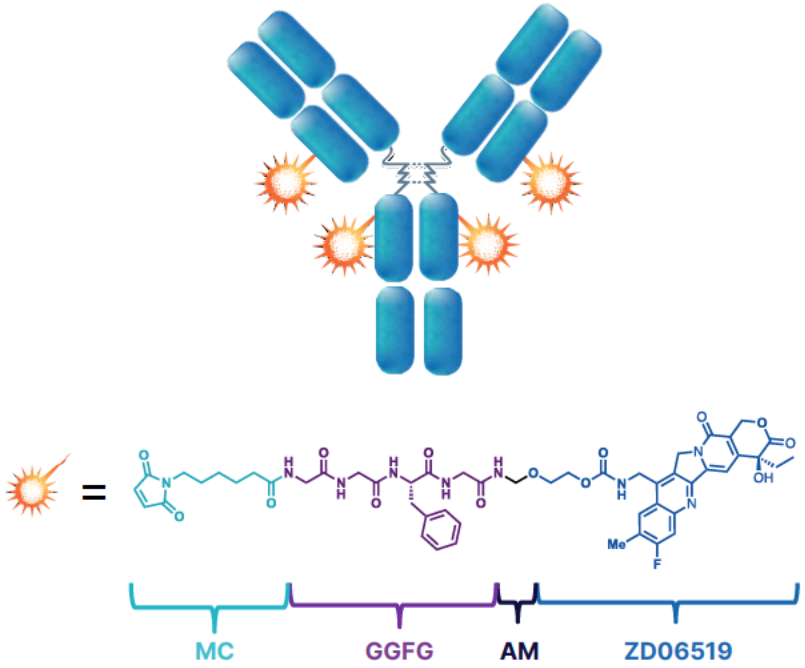
ZW220: A Novel NaPi2b Topoisomerase I Inhibitor ADC



ZW220 Design

- Internally discovered, humanized IgG1 mAb against NaPi2b (SLC34A2)
- Novel, bystander-active, topoisomerase-1 inhibitor payload
- Stochastic, cysteine conjugation, average DAR 4
- Protease cleavable drug linker
- ZW220 is an ADC for the treatment of NaPi2b-expressing solid tumors, such as ovarian cancer

ZW220: A Novel Napi2b Topoisomerase I Inhibitor ADC

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

LINKER

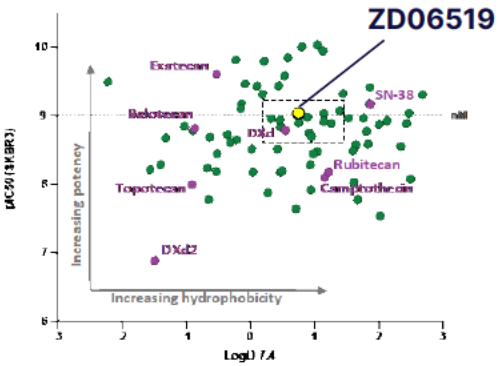
Traceless, cleavable peptide linker

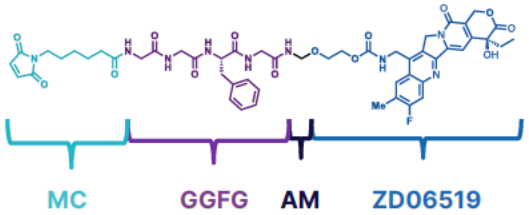
- Common to majority of approved ADCs
- Compatible with desired bystander activity

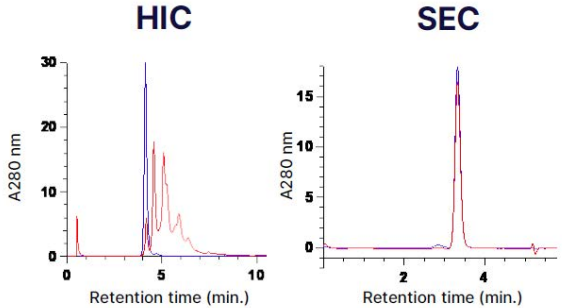
CONJUGATION

Thiol-maleimide chemistry

- Stochastic conjugation utilized in all approved ADCs
- Facilitates DAR optimization
- Good balance of stability, safety, and anti-tumor activity







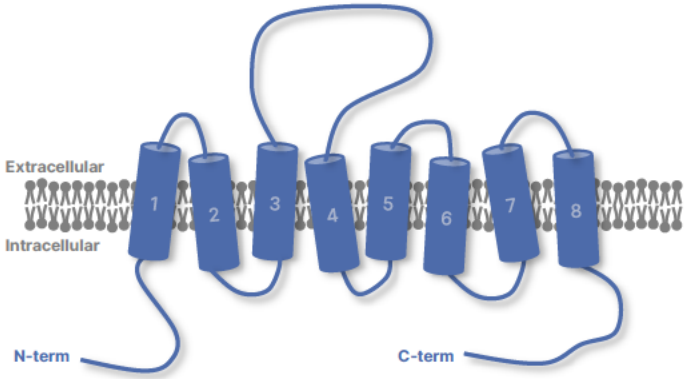
ZW220
ZW220 mAb

Hydrophobic interaction chromatography (HIC)
Size exclusion chromatography (SEC)

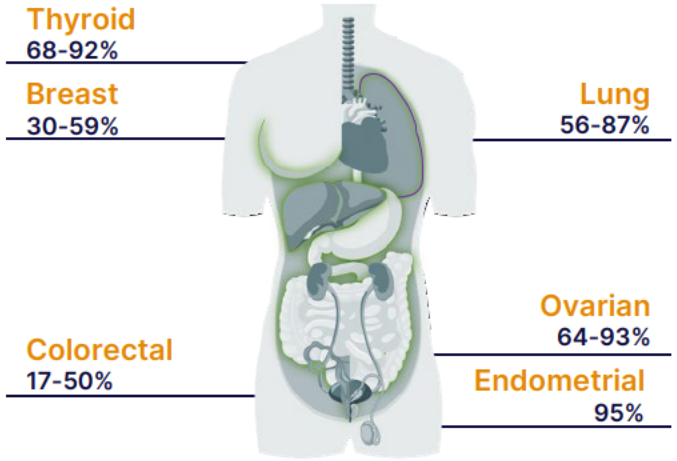
NaPi2b is a Relevant and Exploitable Target for Solid Malignancies

NaPi2b is involved in inorganic phosphate homeostasis

- NaPi2b is a phosphate transport multi-pass transmembrane protein, encoded by SLC34A2 gene[1]
- Normal tissue expression of NaPi2b is observed in lung (type II alveolar cells), liver, kidney and small intestine, amongst other tissues



NaPi2b is associated with various human cancers

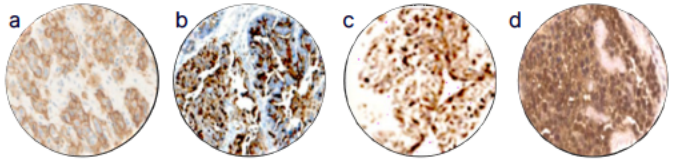


- NaPi2b is highly expressed in ovarian and lung carcinomas
- Some expression is also found in endometrioid[1], thyroid[1,2], colorectal[3,4] and breast carcinomas[5]
- Proto-oncogene in several human malignancies[2]

[1]Lin *et al.* 2015. *Clin Cancer Res*
[2]He *et al.* 2020. *Oncogene*
[3]Yang *et al.* 2022. *Pathol Res*
[4]Liu *et al.* 2018. *Biomed Pharmacother*
[5]Chen *et al.* 2010. *Anticancer Res*

Napi2b is Highly Expressed and Prevalent in Serous Ovarian Adenocarcinoma and Lung Adenocarcinoma

Ovarian cancer expression

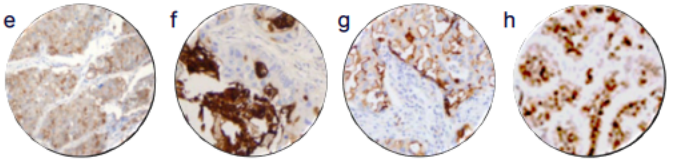


NaPi2b is primarily expressed in epithelial serous ovarian cancer, clear cell ovarian cancer, and endometroid cancer, with low expression observed in mucinous ovarian cancer

OC % positivity*	Intensity (serous OC)	Reference
64%	52% high NaPi2b, TPS ≥75 48% low NaPi2b, TPS <75	a) Banerjee *et al.* 2023. ESMO Abstract 145
80%	N.D.	b) Lopes dos Santos *et al.* 2013. *PLoS One*
88%	23% 3+, 69% 2+, 4% 1+ IHC	c) Lin *et al.* 2015. *Clin Cancer Res*
93%	31% 3+, 44% 2+, 24% 1+ IHC	d) Levan *et al.* 2017. *BMC Cancer*

*OC % positivity calculated from
a) 56 HGSOC (high-grade serous ovarian cancer) samples
b) 39 serous, 5 mucinous, 4 endometroid, 2 clear cell OC samples
c) 26 serous, 10 mucinous, 20 endometroid, 11 clear cell OC samples
d) 83 serous, 25 mucinous, 15 endometroid, 7 clear cell, 6 undiff. malignant and borderline OC samples

Lung cancer expression



In non-small cell lung cancer (NSCLC), NaPi2b is predominantly expressed in lung adenocarcinoma (ACA), with low expression in lung squamous cell carcinomas (SCC)

NSCLC % positivity*	Intensity (NSCLC ACA)	Reference
56%	N.D.	e) Lopes dos Santos *et al.* 2013. *PLoS One*
66%	66% high NaPi2b, H-score ≥50	f) Heynemann *et al.* 2022. *Clin Lung Cancer*
83%	17% H-score = 0, 4% 50-100, 35% 100-200, 43% 200-300	g) Yu *et al.* 2018. IASLC Poster 12636
87%	42% 3+, 35% 2+, 10% 1+ IHC	h) Lin *et al.* 2015. *Clin Cancer Res*

*NSCLC % positivity calculated from
e) 117 NSCLC samples
f) 208 NSCLC adenocarcinoma samples
g) 23 NSCLC adenocarcinoma samples
h) 31 NSCLC adenocarcinoma samples

N.D. = not determined

Ovarian and Lung Cancer Respond to ADCs and TOPO1i Inhibition

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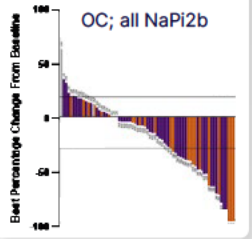
Microtubule inhibitor-bearing

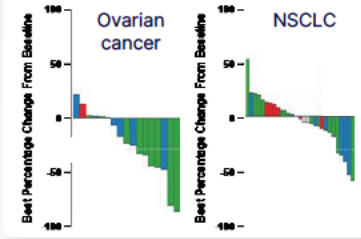
Upifitamab rilsodotin (UpRi) NaPi2b

NCT03319628[1]



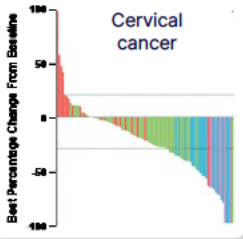
Lifastuzumab vedotin (Lifa-V) NaPi2b

NCT01363947[2]



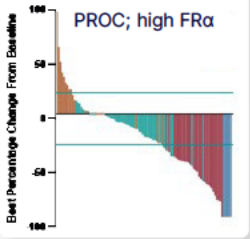
Tisotumab vedotin (TV) tissue factor

InnovaTV 204[3]



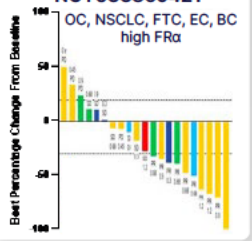
Mirvetuximab soravtansine (ELAHERE) FRα

SORAYA[4]



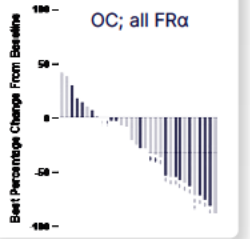
MORab-202 (Farletuzumab-ecteribulin) FRα

Pan-tumor NCT03386942[5]



Luveltamab tazevibulin (STRO-002) FRα

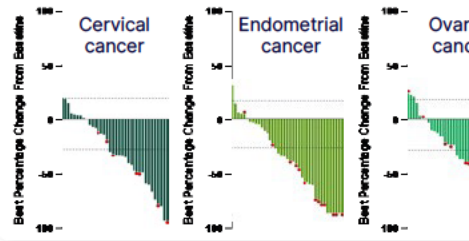
NCT03748186[6]



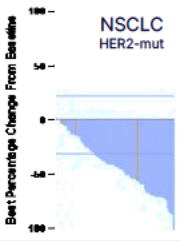
Topoisomerase I inhibitor-bearing

Trastuzumab deruxtecan (ENHERTU; T-DXd) HER2

DESTINY-PanTumor02[7]

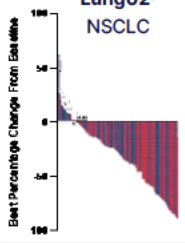


DESTINY-Lung01[8]

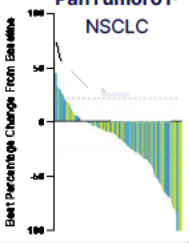


Datopotomab deruxtecan (DXd) TROP2

TROPION-Lung02[7]

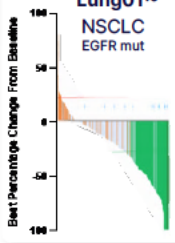


TROPION-PanTumor01[9]

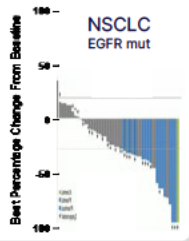


Patritumab deruxtecan (U3-1402) HER3

HERTHENA-Lung01[10]

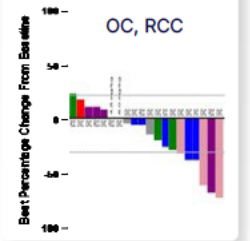


NCT03260491[11]



DS-6000 (DXd) CDH6

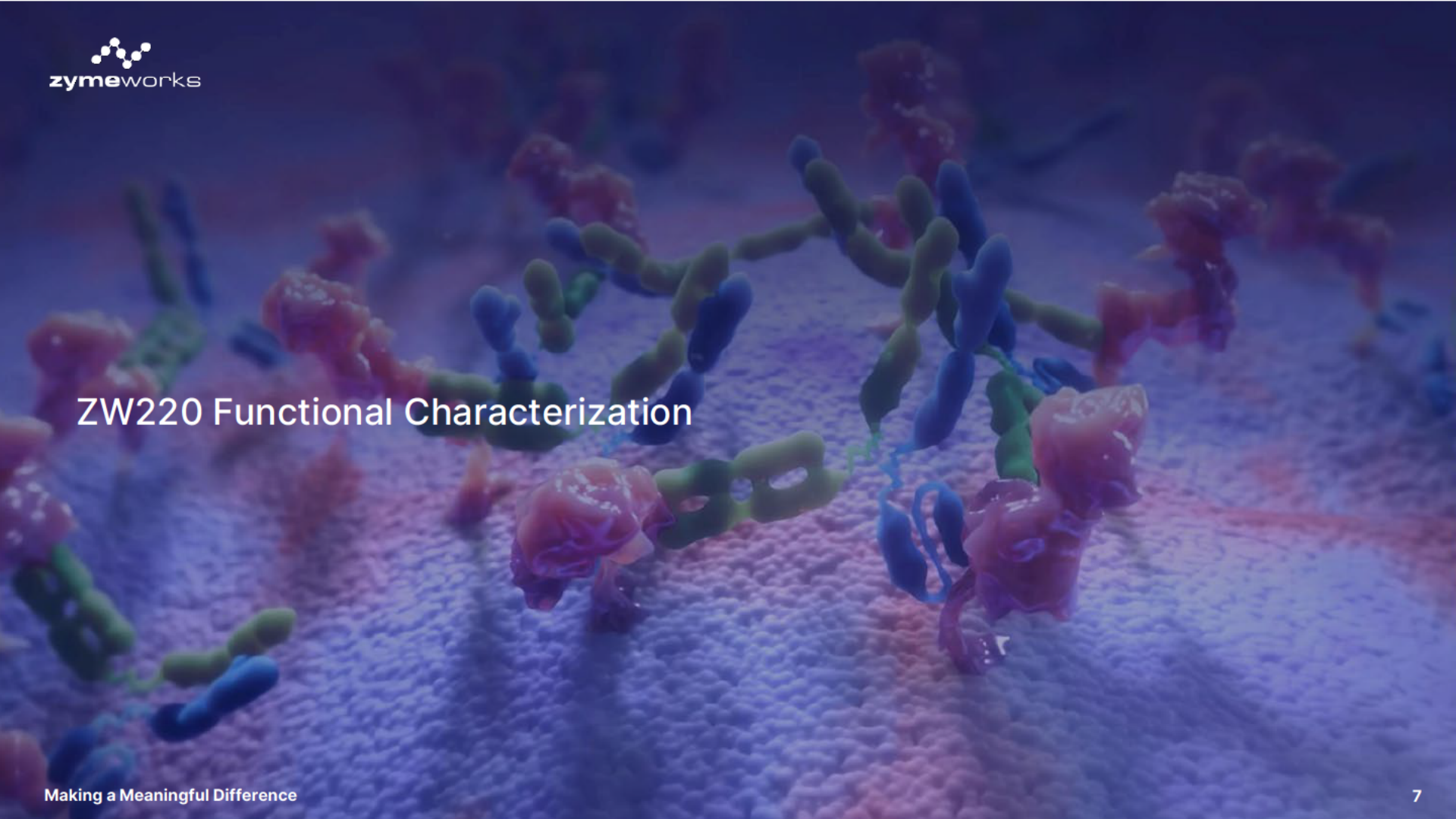
NCT04707248[12]



- 2 approved MTI ADCs in gynecologic oncology: tisotumab vedotin (cervical cancer) and mirvetuximab soravtansine (FRα+ PROC)
- Topoisomerase 1 inhibitor ADCs have potential for significant impact in NaPi2b-expressing cancers

FTC=follicular thyroid carcinoma EC=endometrial cancer BC=breast cancer RCC=renal cell cancer PROC=platinum-resistant ovarian cancer

[1]Richardson *et al.* SGO 2022 Abstract 76
[2]Gerber *et al.* 2020. *Clin Cancer Res*
[3]Coleman *et al.* 2021. *Lancet Oncol*
[4]Matulonis *et al.* 2023. *J Clin Oncol*
[5]Shimizu et al. 2021. *Clin Cancer Res*
[6]Oaknin et al. 2023. *J Clin Oncol*
[7]AstraZeneca. ASCO 2023 Investor Presentation
[8]Li et al. 2022. *N Engl J Med*
[9]Meric-Bernstam *et al.* ASCO 2021
[10]Yu *et al.* 2023. *J Clin Oncol*
[11]Yu *et al.* ESMO 2020
[12]Hamilton *et al.* ASCO 2022



ZW220 Functional Characterization

NaPi2b-targeting ZW220 mAb was Discovered and Humanized Internally

ZW220 mAb generation

- Hybridoma technology
- Mice immunized with transient hNaPi2b-expressing CHO cells

Immunization to humanized antibody lead progression

ZW220 mAb properties

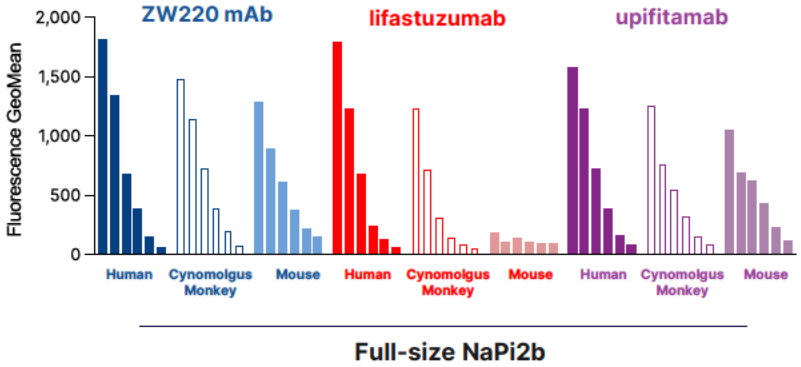
Species	Fully humanized (originally mouse chimera)
Subclass	IgG1
MW (Da)	145,000
Affinity (Kd)	0.1 nM (monospecific FSA) to NaPi2b in IGROV-1 cell line by MSD

CHO = chinese hamster ovary cells FSA = full-sized antibody MSD = Meso Scale Discovery platform

ZW220 mAb Binds to Human NaPi2b with High Specificity

ZW220 cross-reacts to cynomolgus monkey and mouse NaPi2b

Cross-reactivity to human, cynomolgus monkey and mouse NaPi2b

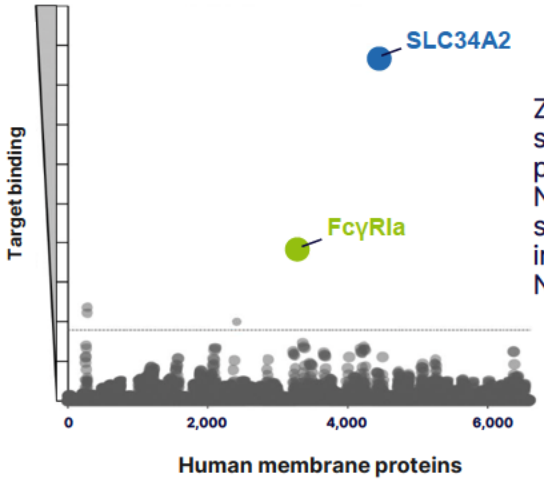


ZW220 mAb shows comparable binding to human, cynomolgus monkey and mouse NaPi2b by flow cytometry

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

Specific binding to NaPi2b in membrane proteome array screen

ZW220 mAb binding in membrane protein array specificity screen

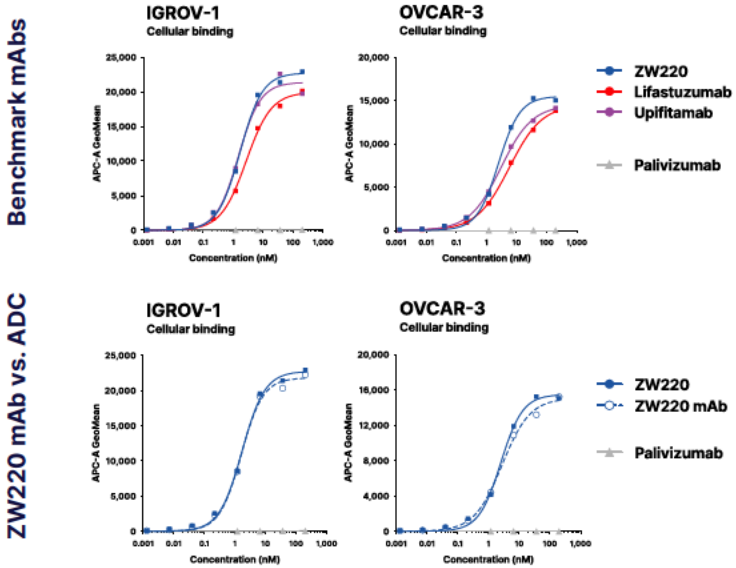


ZW220 mAb shows no significant binding to proteins besides human NaPi2b in a native MPA specificity screen, including NaPi2a and NaPi2c

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. Screen performed by Integral Molecular.

ZW220 Demonstrates Strong Binding to NaPi2b and Rapid Internalization

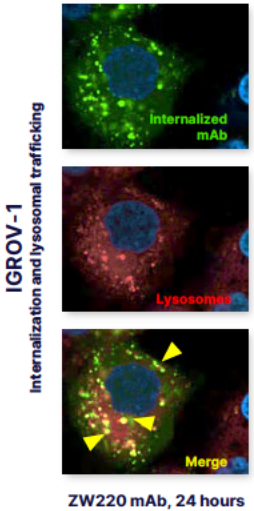
ZW220 exhibits comparable binding to upifitamab and lifastuzumab

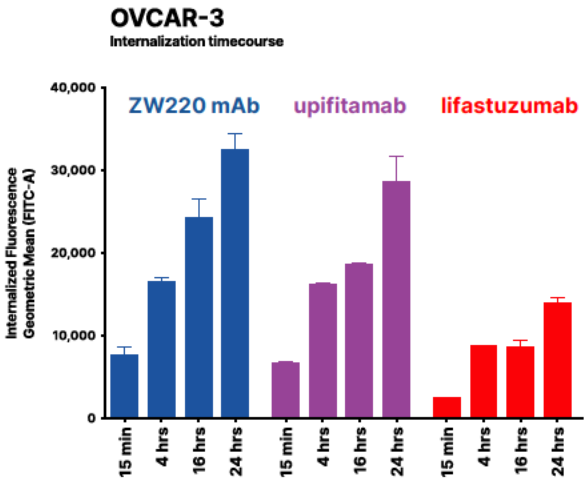


ZW220 mAb and ADC show comparable binding affinities

(Top) ZW220 DAR 4 ADC binding is comparable to lifastuzumab and upifitamab unconjugated antibody controls. (Bottom) ZW220 mAb and ADC DAR 4 demonstrate comparable on-cell apparent binding affinities. Palivizumab non-targeting control did not demonstrate binding to human cancer cell lines. Binding of NaPi2b mAbs and ADCs to endogenous expressing NaPi2b tumor cell lines was assessed by flow cytometry.

ZW220 is efficiently internalized and colocalizes with lysosomes

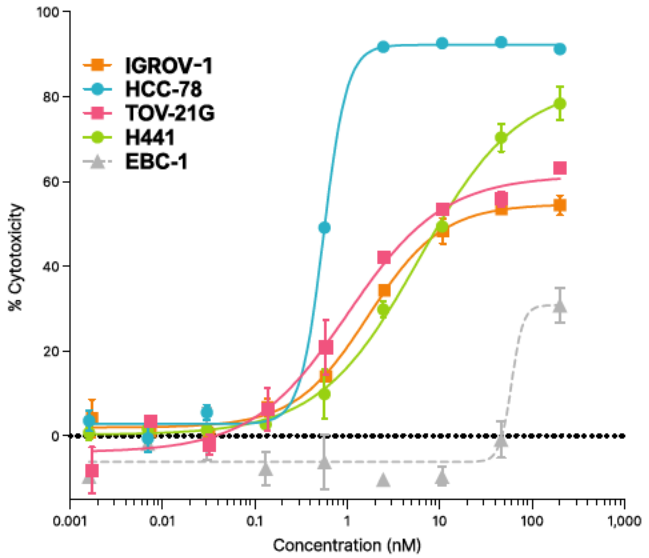




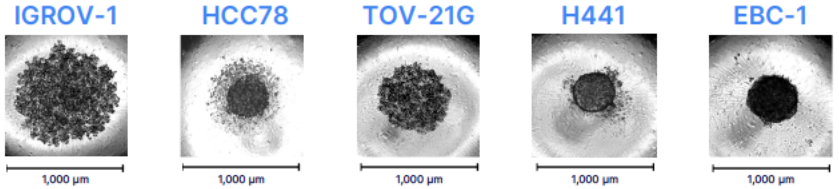
(Left) Internalization of AF488-labelled ZW220 mAb (green), staining of lysosomes with LysoTracker Deep Red AF647 (red), and colocalization (yellow) after 24 hours in IGROV-1 cells by high content imaging. (Right) Internalization of AF488-labelled mAbs in OVCAR-3 cells by flow cytometry (external fluorescence quenched prior to analysis). ZW220 DAR 4 shows comparable internalization profile to unconjugated ZW220 mAb (ADC data not shown).

ZW220 Induces Cell Growth Inhibition in Ovarian and Lung Cancer Napi2b⁺ Spheroids

ZW220 DAR 4 cytotoxicity in 3D spheroids



Untreated spheroids



Cell line spheroids	NaPi2b/cell	EC_{50} (nM) in 3D tumor cell spheroids	
		ZW220 DAR 4	Pali-ZD06519 non-targeting control
IGROV-1 (OvCa)	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 (OvCa)	350,000	0.9 ± 0.3	116.7 ± 28.9
H441 (NSCLC)	41,000	7.0 ± 2.7	128.6 ± 91.5
EBC-1 (NSCLC)	0	54.7 ± 7.9	113.7 ± 51.4

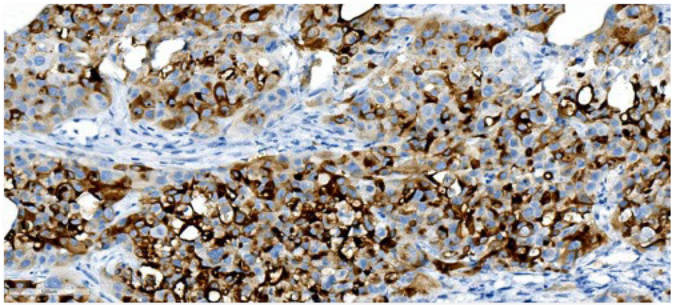
ZW220 DAR 4 ADC exhibits strong target-dependent cytotoxicity in high, moderate and low expression cancer cell lines

(Left) Representative dose-response cytotoxicity curves for ZW220 DAR 4, relative to untreated, in a panel of NaPi2b$^{+/-}$ tumor cell line spheroids. (Right) 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 DAR 4 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.

ZW220 Exhibits Strong Bystander-mediated Killing *In Vitro*

NaPi2b heterogeneity

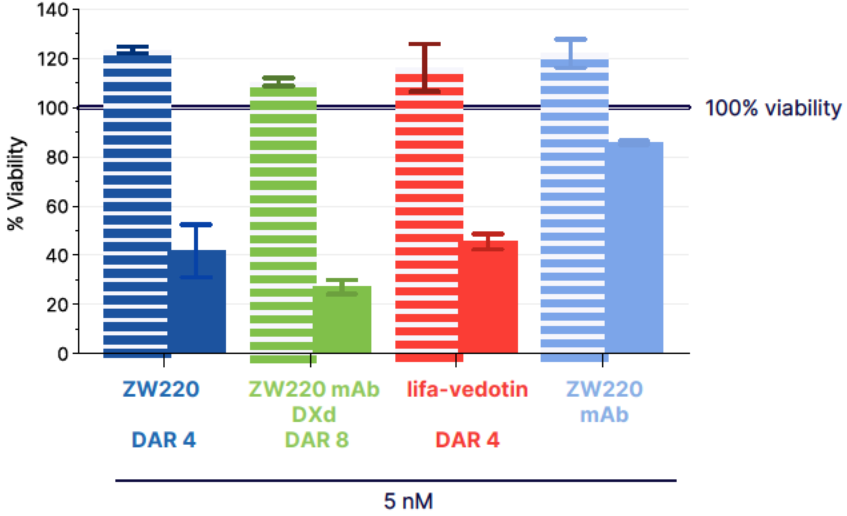
Non-small cell lung cancer
H441 CDX IHC



low/no
NaPi2b staining

strong
NaPi2b staining

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



EBC-1 (NaPi2b⁻) in monoculture

EBC-1 in co-culture with IGROV-1 (NaPi2b⁺)

(Left) Commercial anti-NaPi2b antibody used for immunohistochemistry (IHC) staining of lung cell line-derived xenograft (CDX) model, archival sample. (Right) ZW220 DAR 4 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.

ZW220 Demonstrates Anti-tumor Efficacy in Napi2b-expressing Ovarian Patient-derived Xenograft Models

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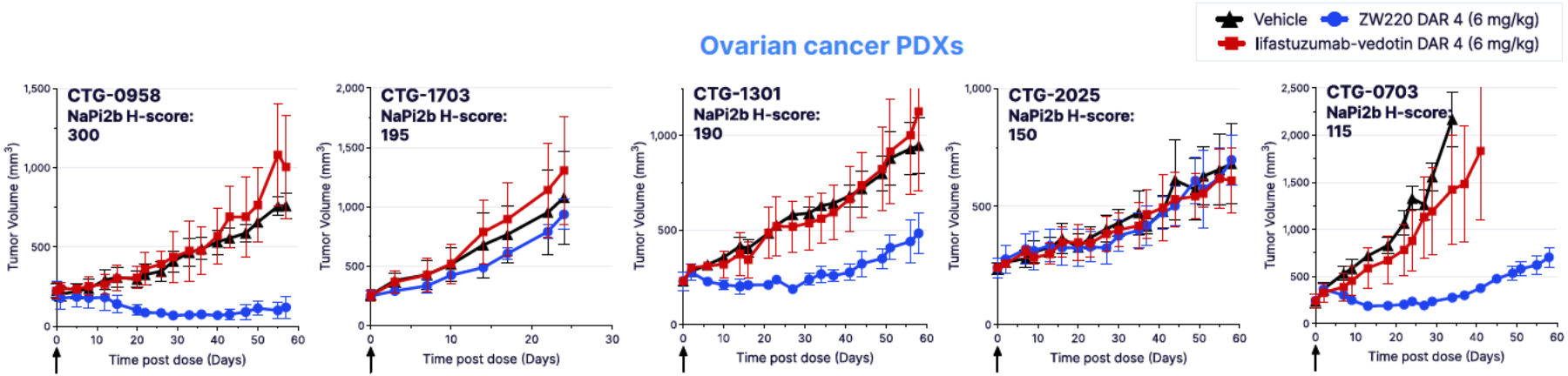
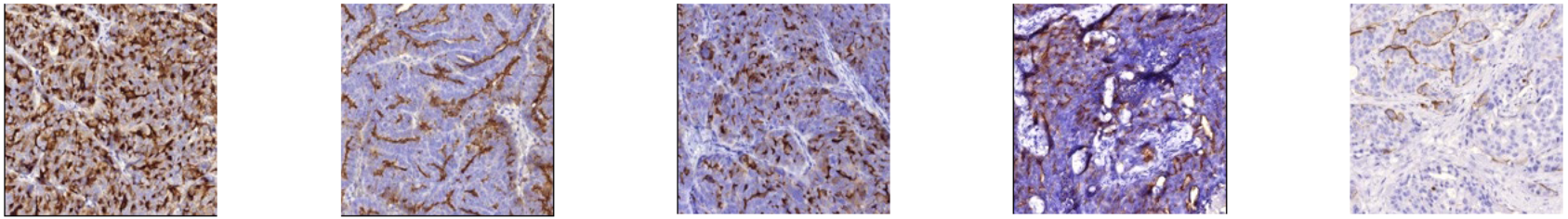
Ovarian cancer PDXs



NaPi2b IHC



H-score: 300 | 195 | 190 | 150 | 115

Anti-tumor 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.

ZW220 is Well-tolerated in a Repeat Dose Non-human Primate (NHP) Toxicology Study

NHP tolerability

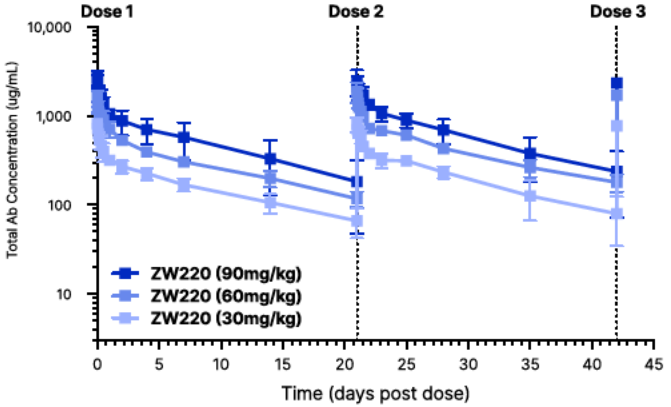
3-dose non-GLP NHP toxicology study, Q3Wx3

Test article	Dose	Mortality	Clinical Observations	Histo-pathology	Clinical Chemistry	Hema-tology	MTD
ZW220 DAR 4	30 mg/kg	None	None	None	None	None	90 mg/kg
	60 mg/kg	None	None	None	None	None	
	90 mg/kg	None	Fecal abnormalities (soft/loose/watery)	None	None	None	

- Repeat-dose non-GLP toxicology study 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

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 4 (n=3 animals/group). Dosing regime: Q3Wx3

Total IgG in NHP serum



Test article	$T_{1/2}$ (days)	Cl (mL/day/kg)
ZW220 DAR 4	9.4	7.6

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). Half life ($T_{1/2}$) and clearance rate calculated from Total IgG NHP data.

ZW220: A Differentiated NaPi2b-targeting ADC

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

- **Novel, bystander-active, TOPO1 inhibitor payload**
  - Unique approach addresses NaPi2b heterogeneous expression
  - Differentiated safety profile compared to MTI ADCs
- **Novel antibody against NaPi2b (SLC34A2)**
  - Strong and specific target binding
  - Efficient internalization and cellular trafficking
- **Average DAR 4, cysteine-based conjugation**
  - Stochastic conjugation utilized in all approved ADCs
  - Good balance of stability, tolerability, and anti-tumor activity
- **On track for IND 1H 2025**

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ZW220 preclinical project team and ADC therapeutic development group at Zymeworks[1]

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- Raffaele Colombo
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- Manuel Lasalle
- Vincent Fung
- Samir Das
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Antibody Discovery & Engineering
- Dunja Urosev
- Saki Konomura

In vivo Biology & PK
- Sam Lawn
- Kaylee Wu
- Devika Sim
- Winnie Cheung

In vitro Biology
- Jodi Wong
- Lemlem Degefie
- Araba Sagoe-Wagner
- Laurence Madera
- Allysha Bissessur
- Catrina Kim
- Ambroise Wu
- Andrea Hernández Rojas

Analytics
- Luying Yang
- Diego Alonzo
- Linglan Fu
- Janice Tsui
- Rehan Higgins

Toxicology
- Sara Hershberger[2]
- Marcie Wood[2]
- Gerry Rowse
- Daya Siddappa

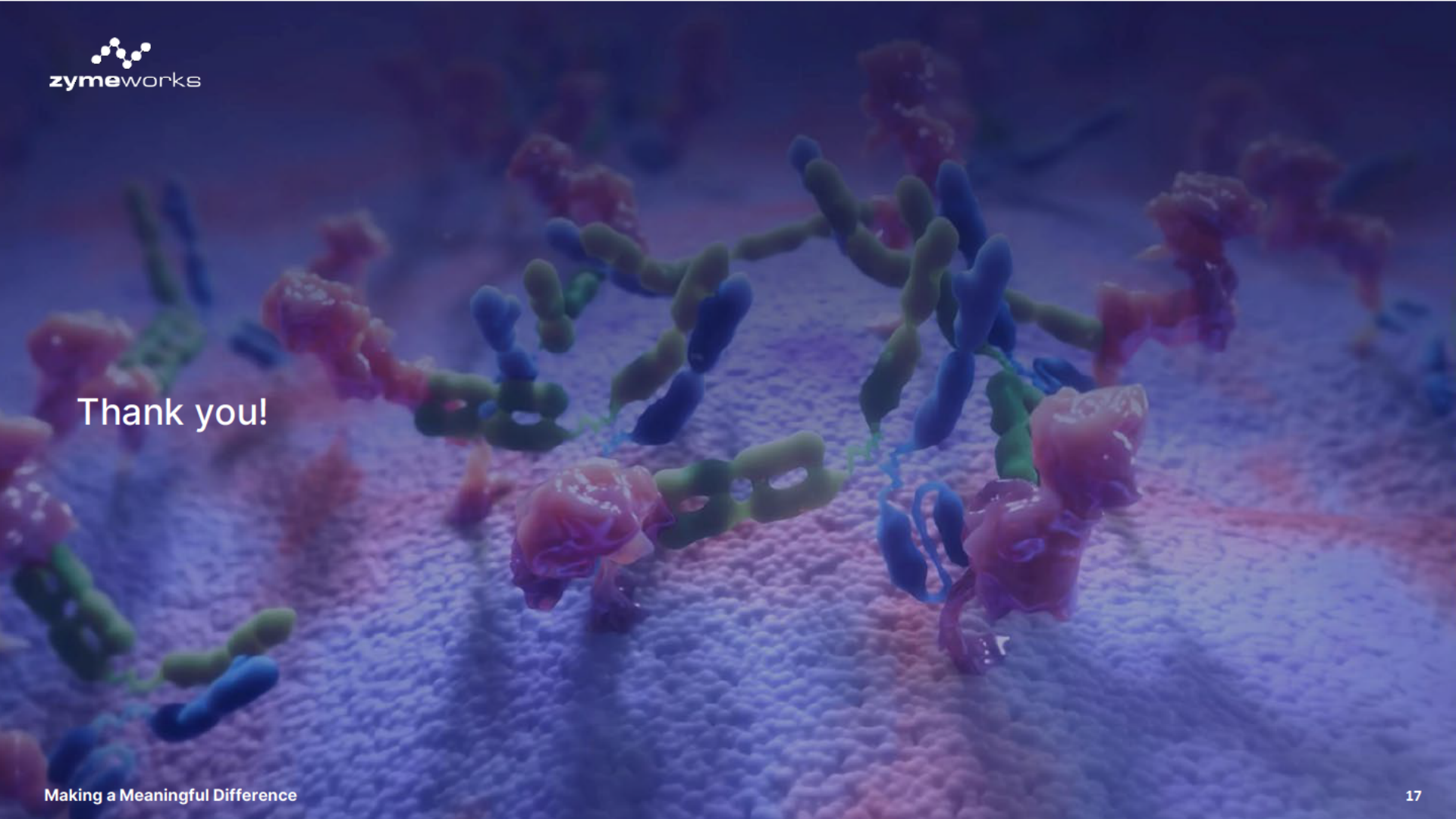
Research Leadership
- Stuart Barnscher
- Jamie Rich
- Paul Moore

Project Management
- Chi Wing Cheng
- Kari Frantzen

Intellectual Property
- Neena Kuriakose

Business Development
- Steve Seredick
- Lisa Mullee

[1]Zymeworks Inc., Vancouver, BC, Canada [2]ToxStrategies, LLC, Katy, TX, United States


zymeworks
Thank you!