Development of ZW418, a biparatopic PTK7-targeting antibody-drug conjugate incorporating a novel pan-RAS inhibitor payload for the treatment of non-small cell lung cancer

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Introduction

Mutations in the RAS oncogene family occur in approximately 25–30% of lung adenocarcinomas and represent one of the most common oncogenic drivers in non-small cell lung cancer (NSCLC).[1] These alterations lead to constitutive activation of downstream MAPK signaling, driving tumor progression and therapeutic resistance. Protein tyrosine kinase 7 (PTK7) is prevalently overexpressed in NSCLC regardless of RAS mutational status and is minimally present in healthy tissues.[2]

Leveraging the large, multi-domain extracellular region of PTK7, we designed a biparatopic antibody (Bip Ab) to engage two non-overlapping epitopes. This antibody facilitates superior cell-surface decoration and enhanced receptor-mediated internalization compared to conventional monospecific antibodies[3] and is thus well suited to targeted delivery of a RAS-inhibitor (RASi) via an ADC approach.

By selectively delivering a potent novel RASi payload to PTK7-expressing cells, ZW418 may not only bypass systemic dose-limiting toxicities typically associated with small molecule RASi, but can maximize anti-tumor effect, offering a greater therapeutic window for RAS-driven malignancies.

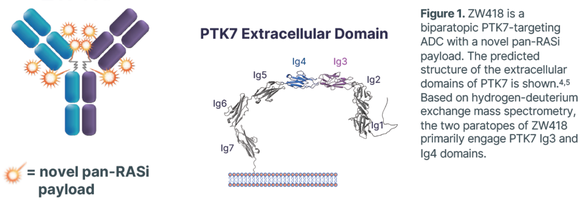


Figure 1. ZW418 is a biparatopic PTK7-targeting ADC with a novel pan-RASi payload. The predicted structure of the extracellular domains of PTK7 is shown.[4,5] Based on hydrogen-deuterium exchange mass spectrometry, the two paratopes of ZW418 primarily engage PTK7 Ig3 and Ig4 domains.

PTK7 is prevalently overexpressed in NSCLC regardless of KRAS mutation

PTK7 Expression in NSCLC Subtypes

PTK7 Expression in KRAS WT vs KRAS Mutant NSCLC

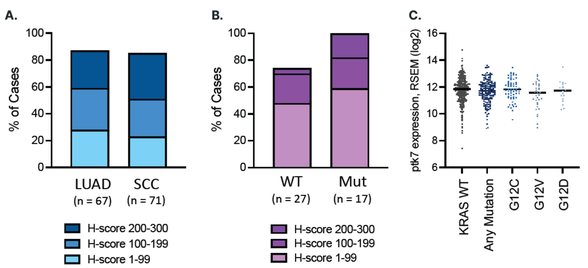
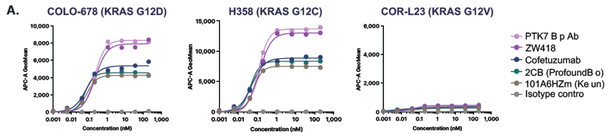


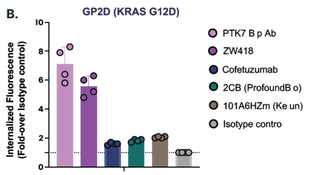
Figure 2. PTK7 protein expression in NSCLC tissue microarrays stratified by **(A)** subtype (LUAD, adenocarcinoma; SCC, squamous cell carcinoma) or **(B)** KRAS mutation. Expression was determined by IHC using an antibody developed at Zymeworks. H-score was calculated according to: (1 x i) + (2 x ii) + (3 x iii) where i, ii, and iii represent the % of cancer cells with weak, moderate, and strong membrane staining. **(C)** PTK7 gene expression in lung adenocarcinoma stratified by KRAS mutation (TCGA, LUAD PanCancer Atlas dataset sourced from cBioPortal, Batch normalized from Illumina HiSeq_RNAseqV2. WT (n = 356), Any Mutations, KRAS mutation other than G12C, G12V and G12D (n = 154), G12C (n = 62), G12V (n = 33), G12D (n = 18).

Biparatopic targeting of PTK7 results in superior ADC properties relative to clinical benchmarks

Increased tumor cell binding



Enhanced internalization



E.

Cell line	KRAS mut	Average PTK7/cell
COLO678	G12D	157,507
GP2D	G12D	129,149
PK-8	G12R	94,818
H358	G12C	75,450
LU99	G12C	65,199
H1373	G12C	42,128
COR-L23	G12V	negligible

Cell line	KRAS WT	Average PTK7/cell
H446	WT	120,556
H446 PTK7 KO	WT	negligible
HaCaT	WT	low

Improved spheroid penetration

Greater ADCC effect

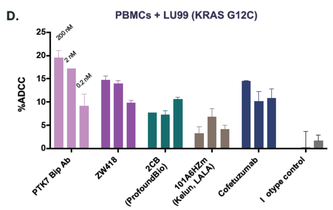


Figure 3. (A) Binding assay: Binding of PTK7 targeting antibodies or ADCs to cancer cell lines was assessed by flow cytometry. **(B) Internalization assay:** Antibodies were fluorescently labeled with Fab-AF488 at a 1:1 molar ratio for 24 hrs at 4°C. Fluorescence signal was measured after quenching and normalized to untreated control. **(C) Spheroid penetration assay:** Antibodies were fluorescently labeled with Fab-AF488 at a 1:1 molar ratio. Coupled antibodies were added to formed spheroids at 50 nM and incubated for 24 hrs at 37°C. Penetration of AF488 labelled mAbs were quantified by high content imaging of spheroid layers after 24 hrs. **(D) ADCC assay:** LU99 tumor cells were stained and mixed with human PBMC effector cells at a ratio of 25:1. The cell mixtures were treated with test articles at 200 nM, 2 nM and 0.2 nM. Viability staining was performed using a fixable LIVE/DEAD™ violet dye and cytotoxicity was assessed. Percent ADCC was determined by subtracting the percent cytotoxicity of the untreated co-cultures from the percent cytotoxicity of the test article treated co-cultures. **(E) PTK7 receptor quantification** in different cancer cell lines by flow cytometry.

ZW418 has selective cytotoxicity in KRAS mutant cell lines of NSCLC

Cytotoxicity

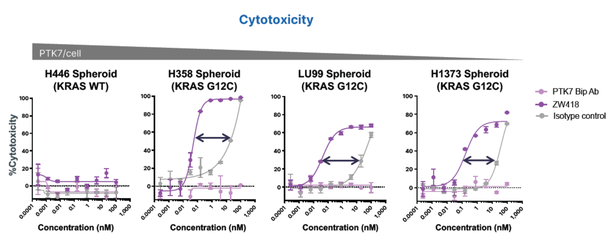


Figure 4. Cytotoxicity was assessed after treatment of lung adenocarcinoma tumor cell lines with PTK7 antibody or RASi ADC for 6 days, with cell viability determined using CellTiterGlo®.

ZW418 exhibits strong anti-tumor activity in CDX models of NSCLC

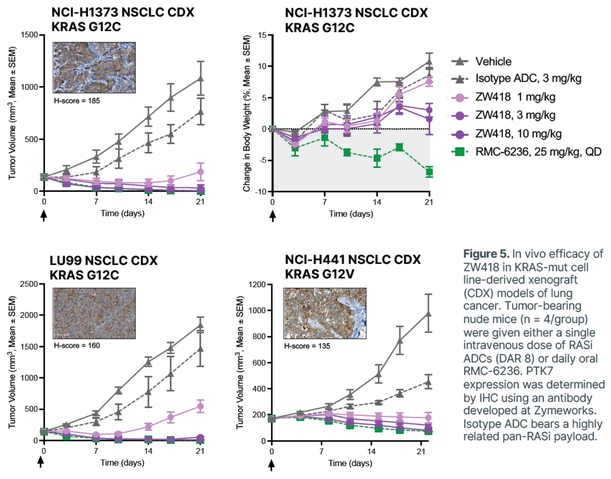


Figure 5. In vivo efficacy of ZW418 in KRAS-mut cell line-derived xenograft (CDX) models of lung cancer. Tumor-bearing nude mice (n = 4/group) were given either a single intravenous dose of RASi ADCs (DAR 8) or daily oral RMC-6236. PTK7 expression was determined by IHC using an antibody developed at Zymeworks. Isotype ADC bears a highly related pan-RASi payload.

Zymeworks' biparatopic PTK7 antibody and ZW418 have desirable pharmacokinetic profiles

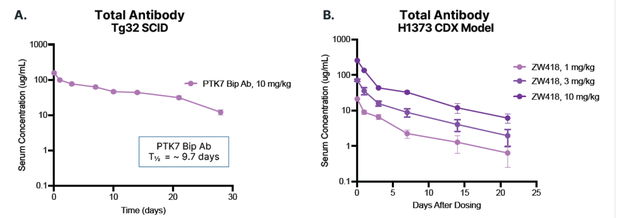


Figure 6. (A) A single-dose non-tumor bearing study in Tg32 SCID mice with hFcRn at 10 mg/kg dose shows that ZW418 antibody exhibits favourable total antibody PK and **(B)** ZW418 displays dose-proportional total antibody PK at 3 dose levels in a single-dose xenograft study in BALB/c nude mice.

ZW418 exhibits target-dependent cytotoxicity through RAS pathway inhibition, while sparing normal tissues

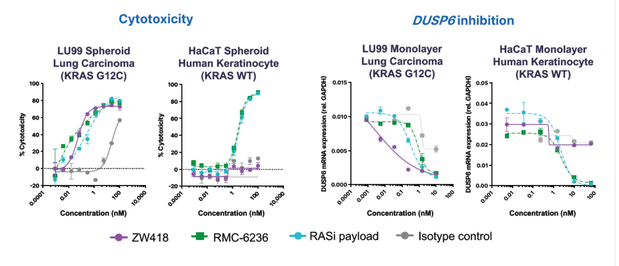


Figure 7. Cytotoxicity was assessed by treating cell lines with ZW418, RMC-6236, RASi, or isotype control for 6 days. Cell viability was determined using CellTiterGlo®. *DUSP6* mRNA levels were measured by seeding 1 million cells/well overnight followed by treatment with different concentrations of test articles for 24 hours. *DUSP6* mRNA was determined by qPCR of RNA extracts.

ZW418 treatment leads to intracellular payload accumulation of RASi payload

Substantially higher intracellular accumulation of RASi payload compared to MMAE

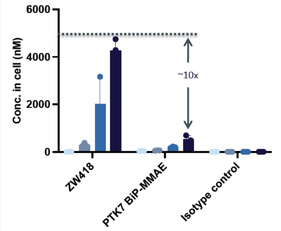


Figure 8. Intracellular payload concentrations (nM) in a panel of cell lines with varying PTK7 expression levels following treatment with 10 nM of ZW418 (DAR 8), a PTK7 BiP-MMAE ADC (DAR 4), or a non-targeting isotype-RASi control (DAR 8). Treatment with ZW418 results in substantially higher intracellular payload accumulation than treatment with PTK7-MMAE. Intracellular payload concentrations were determined by mass spectrometry.

RASi ADC platform demonstrates favorable tolerability in rodents and NHP

RASi ADC platform tolerated in rodents with MTD ≥ 200 mg/kg

- Pan-RASi ADCs of varying potency assessed; highest dose tested was 200 mg/kg [6]
- No mortality, body weight loss or clinical pathology effects
- No observations of GI or skin toxicity

RASi ADC platform tolerated in NHP with MTD ≥ 120 mg/kg

- Pan-RASi ADCs of different potencies assessed [6]
- TAA-RASi ADCs cross-reactive to NHP target
- Highest dose tested 120 mg/kg
- No mortality or body weight loss
- No in-life observations of GI toxicity or skin toxicity

NHP study design and observations

ADC (DAR8)	Dose (mg/kg); schedule	Clinical Signs & Body Weights	Clinical Pathology	MTD
TAA-RASi ADC 1	90; single dose	No treatment related signs or effects	Effects consistent with a transient inflammatory response	≥ 120 mg/kg
	120; single dose			
TAA-RASi ADC 2	90; q3wx2			
	120; q3wx2			

NHP study design: ADCs dosed at single administration or every 3 weeks for 2 doses (2M and 1F cynomolgus monkey per group), with termination 7 days post-final dose; histopathology pending.
Rodent study design: Single dose of ADCs to female BALB/c mice. In-life observations included serum chemistry and hematology, with gross necropsy and histopathology assessment at 1 and 3 weeks post dose.
NHP: non-human primate; TAA:tumor associated antigen.

Conclusions

- ZW418 is a first-in class biparatopic ADC designed to target PTK7 expressing RAS-mutated NSCLC.
- PTK7 is prevalently overexpressed in RAS-mutated NSCLC.
- ZW418 demonstrates target-dependent delivery of novel pan-RASi payload to tumor cells, resulting in potent inhibition of RAS activity and cytotoxicity.
- ZW418 shows strong antitumor activity in all PTK7 expressing RAS mutant xenograft models tested, at doses as low as 1 mg/kg, with favorable PK.
- RASi-ADC platform shows an encouraging nonclinical safety profile, supporting doses and drug exposures that are expected to be highly efficacious.

References

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6. Garnett et al. *Cancer Res* . **2026**, 86(7_Supplement), 1642.

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