ZW427, a Ly6E-targeting antibody-drug conjugate bearing a novel pan-RAS inhibitor payload for the treatment of RAS mutated cancers

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Abstract #7715

Introduction

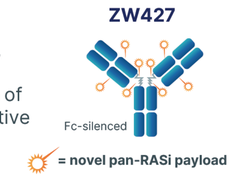
ZW427 is an antibody-drug conjugate (ADC) targeting human lymphocyte antigen 6 family member E (Ly6E) that comprises a proprietary humanized Fc-silenced IgG1 antibody (hu10E02) conjugated to a novel pan-RAS inhibitor via a cleavable linker.

The aim of ZW427 is optimized delivery of a novel pan-RASi to tumor cells for improved response and a differentiated safety profile compared to small molecule pan-RASi.

Mechanism of Action

The novel pan-RAS inhibitor is a tricomplex inhibitor (with CypA and active GTP-bound conformation of RAS [RAS ON]), blocking downstream signaling.

Upon target binding and receptor-mediated internalization of ZW427, intracellular release of bystander-active pan-RASi induces cell death of Ly6E positive cells, and Ly6E negative cells through bystander-mediated killing.



Ly6E is overexpressed in many solid tumors including those commonly featuring oncogenic RAS mutations

Ly6E, a clinically validated target [1], is a GPI-anchored cell surface protein, overexpressed in cancer indications of high unmet medical need bearing RAS mutations, including CRC, PDAC, and LUAD, with minimal presence in normal tissues.

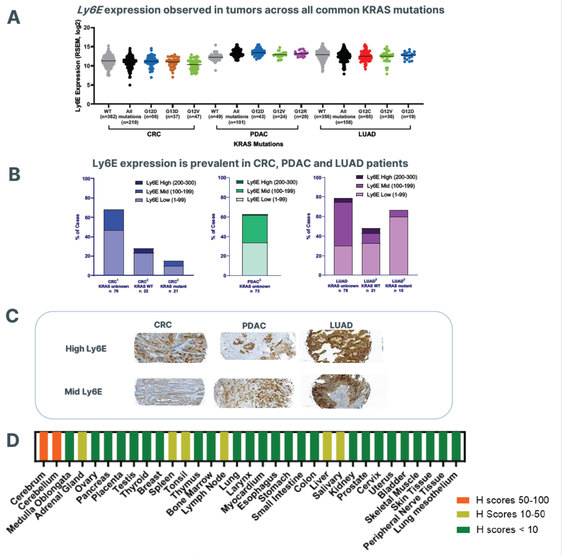


Figure 1. Ly6E expression across CRC, PDAC, LUAD and normal tissues. **(A)** Gene expression with WT or mutant data sourced from TCGA hosted by cBioportal, batch normalized from Illumina HighSeq_RNAseqV2. **(B)** Patient TMA IHC analysis (TMAs sourced from TissueArray.com and Tristar Technologies). TMAs 1, 3, 4 did not include KRAS mutational status of patients. TMAs 2, 5 have low power and should be interpreted alongside gene expression data presented in Fig. 1A **(C)** Representative IHC images of Ly6E expression from select indications at 10x magnification **(D)** Ly6E expression in normal tissues of three donor samples per tissue type. H Scores determined by pathologist based on a non-clinical IHC assay with antibody EPR26038-105. n=number of patient samples. LUAD: Lung Adenocarcinoma, PDAC: Pancreatic Ductal Adenocarcinoma, CRC: Colorectal Cancer.

ZW427 demonstrates strong anti-tumor activity in CDX models of RAS mutated CRC, PDAC and LUAD cancer indications

Single dose activity observed across a range of Ly6E expression

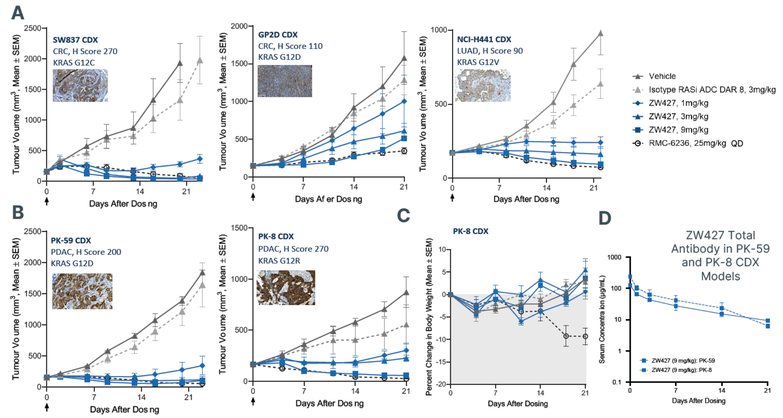


Figure 2. *In vivo* efficacy of ZW427 (hu10E02 Fc-silenced RASi ADC DAR8) was assessed in CDX models of **(A)** CRC and LUAD, **(B)** PDAC (immuno-compromised mice, n=3 or 4 per group, isotype Fc-silenced RASi ADC DAR8 contains highly related payload). All IHC images are at 20x magnification. H Scores determined by pathologist based on a non-clinical IHC assay. Tumor volume data points are excluded if >20% mice euthanized in a group. 9mg/kg dose anticipated to be clinically relevant dose. **(C)** Change in body weight of PK-8 tumor bearing mice treated as described **(D)** Total antibody PK in nude mouse CDX models indicates ZW427 maintains a favorable PK profile, with $T_{1/2}$ of 6.2-6.3 days.

ZW427 promotes targeted and sustained *DUSP6* expression inhibition in tumors *in vivo* and *in vitro*

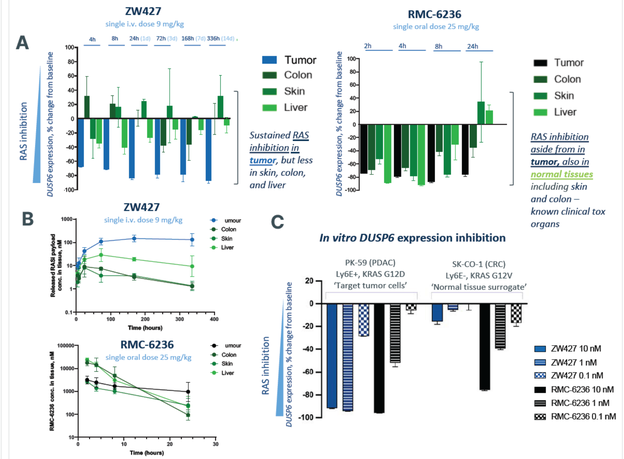


Figure 3. Summary of *in vivo* ZW427 *DUSP6* expression inhibition in tumor and normal tissues. NCI-H441 tumor bearing mice were dosed as indicated and tissues were collected at the designated timepoints **(A)** *DUSP6* expression was quantified by qPCR and **(B)** Released RASi ADC payload or RMC-6236 in different tissues quantified by mass spectrometry. **(C)** Summary of *in vitro DUSP6* expression inhibition in treated RAS mutated cancer cell lines for 24hrs, 37°C.

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[2]
- 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[2]
- 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; MTD: maximum tolerated dose.

ZW427 enables strong *in vitro* cytotoxicity across cancer indications of interest, Ly6E expression levels and RAS mutation type

Target-specific activity across a range of Ly6E expressing RAS mutated CRC, PDAC and LUAD cancer cell lines

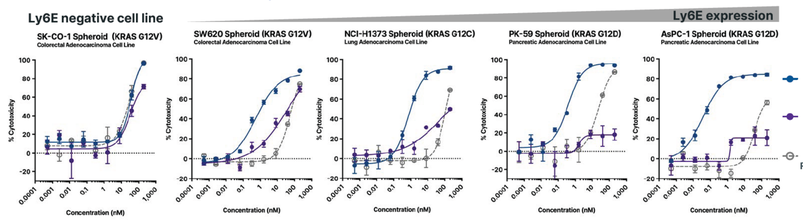


Figure 4. Representative *in vitro* ZW427 cytotoxicity against RAS mutated cancer cell line spheroids (3D) expressing a range of Ly6E after 6 days treatment, 37°C. Target specificity is indicated by differential sensitivity to ZW427 and the isotype RASi ADC and an absence of activity in a Ly6E negative cell line.

Breadth of activity observed for ZW427

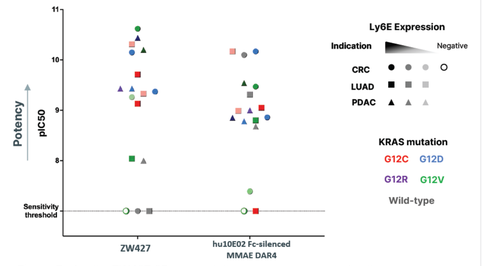
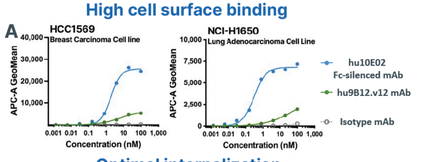


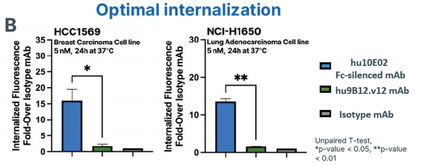
Figure 5. *In vitro* ZW427 3D cytotoxicity summary, across indications (symbols), Ly6E expression (gradient) and RAS mutation type (color).

ZW427 mAb exhibits superior target-specific binding, internalization and spheroid penetration compared to the benchmark mAb and ZW427 displays robust payload delivery and effective bystander activity

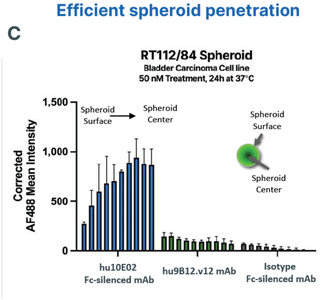
High cell surface binding



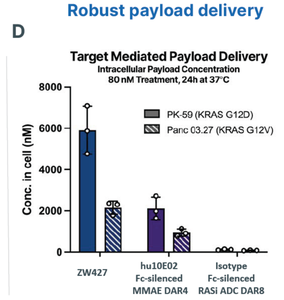
Optimal internalization



Efficient spheroid penetration



Robust payload delivery



Effective bystander activity

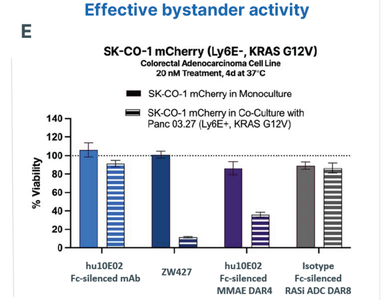


Figure 6. **(A)** mAb cell binding to HCC1569 and H1650 cancer cell lines by flow cytometry. **(B)** Internalization of AF488 labelled mAbs into HCC1569 and H1650 cancer cell line at 5 nM after 24hrs, 37°C. **(C)** Penetration of AF488 labelled mAbs as quantified by high content imaging of RT112/84 cancer cell spheroid layers at 50 nM after 24hrs, 37°C. **(D)** Target-specific payload delivery of RASi or MMAE in PK-59 and Panc 03.27 cancer cell lines at 80 nM after 24hrs, 37°C. **(E)** Bystander activity of ZW427 as shown by the decreased viability of Ly6E negative cancer cell line SK-CO-1 KRAS G12V when co-cultured with Ly6E positive cancer cell line Panc 03.27 KRAS G12V, at 20 nM after 4 days, 37°C. hu9B12.v12 mAb is the antibody of DLYE5953A.

Conclusions

- ZW427 ADC targets Ly6E and is highly differentiated by a rapidly internalizing antibody and novel pan-RAS inhibitor payload.
- ZW427 represents a first-in-class ADC aimed at CRC, PDAC, and LUAD Ly6E-expressing RAS mutated cancer indications.
- Robust ZW427 anti-tumor activity is observed in CRC, PDAC and LUAD xenograft models with a range of Ly6E-expression.
- Favorable RASi-ADC tolerability observed in NHP at exposure levels above those projected to be efficacious for ZW427.
- ZW427 has the potential to enhance the clinical activity and safety profile compared to small molecule pan-RASi.

References

1. S.M. Tolaney et al. *Clin Cancer Res.* **2020**, *26(21)*, 5588-5597
2. G. Garnett et al. *AACR abstract #5140.* **2026**

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