ZW251, a Novel Glypican 3-Targeting Antibody-Drug Conjugate Bearing a Topoisomerase 1 Inhibitor Payload

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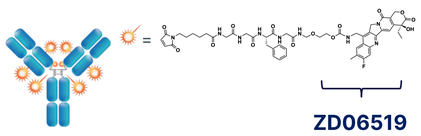
ZW251: Anti-Glypican-3 Antibody-Drug Conjugate

ZW251 is an antibody drug conjugate (ADC) consisting of a topoisomerase 1 inhibitor payload conjugated to an antibody targeting Glypican-3 (GPC3). Topoisomerase 1 inhibiting ADCs have demonstrated wide clinical benefit in solid tumors and ZW251 aims to apply this against a target expressed in hepatocellular carcinoma (HCC), a disease with high unmet need and limited treatment options. We demonstrate that ZW251 exhibits desired target-mediated activity *in vitro,* robust anti-tumor activity against a panel of CDX/PDX HCC models, and favorable pharmacokinetics (PK) in Tg32 human FcRn expressing mice. ZW251 is being assessed in an ongoing non-human primate toxicology study.

ZW251 Antibody Drug Conjugate



- **Humanized monoclonal anti-GPC3 antibody**
- **ZD06519 topoisomerase 1 inhibitor payload**
- **Drug-antibody-ratio (DAR) 4 and 8 evaluated**

Figure 1. ZW251 ADC composition and linker-payload structure. DAR 8 pictured.

GPC3 is a Compelling ADC Target for Hepatocellular Carcinoma

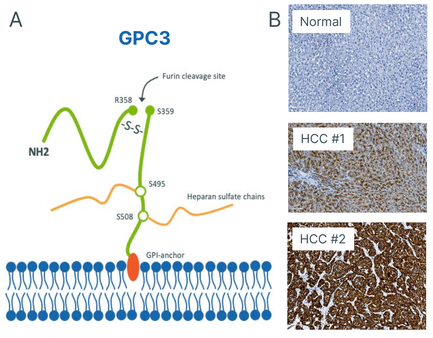


Figure 2. (A) GPC3 structure and **(B)** IHC staining in human normal liver and HCC samples.

- Cell-surface GPI-anchored oncofetal glycoprotein.[1]
- Involved in the Wnt/β-catenin signaling pathway.[1]
- Expressed in fetal tissues and down regulated in adult tissues.[2]
- Clinical validation of tumor accumulation of an anti-GPC3 antibody in HCC patients.[3]
- **GPC3 is highly expressed in most HCC tumors[2] and exhibits limited expression in healthy tissues.**

ZW251 Binds to GPC3 With High Affinity and Specificity

ZW251 Binds Human and Cynomolgus Monkey GPC3 with High Affinity

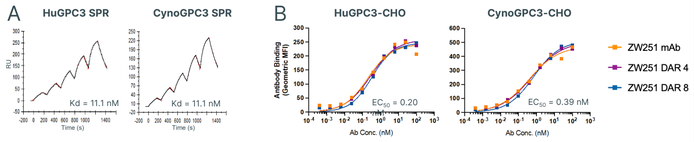


Figure 3. (A) Binding of soluble GPC3 extracellular domain to immobilized ZW251 mAb measured by surface plasmon resonance. (B) Binding of ZW251 to transfected CHO cells expressing human or cynomolgus monkey GPC3 assessed by flow cytometry.

ZW251 Selectively Binds to GPC3 Expressing Tumor Cell Lines

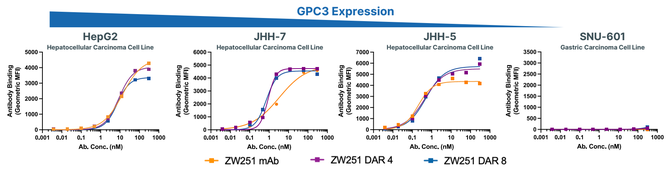
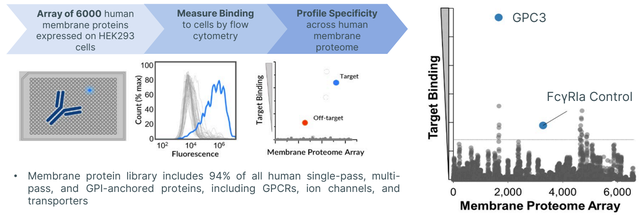


Figure 4. Binding of ZW251 mAb and ADC to cancer cell lines with a range of GPC3 expression was assessed by flow cytometry. SNU-601 was utilized as a GPC3- cell line.

ZW251 mAb Shows Selective Binding to GPC3 in a Membrane Proteome Array



- Membrane protein library includes 94% of all human single-pass, multi-pass, and GPI-anchored proteins, including GPCRs, ion channels, and transporters

Figure 5. ZW251 specificity was profiled by measuring binding to HEK293 cells expressing an array of human membrane proteins Binding hits above threshold in initial MPA screen were subsequently individually validated to confirm ZW251 mAb binding.

ZW251 is Internalized and Exhibits Killing of Cancer Cells

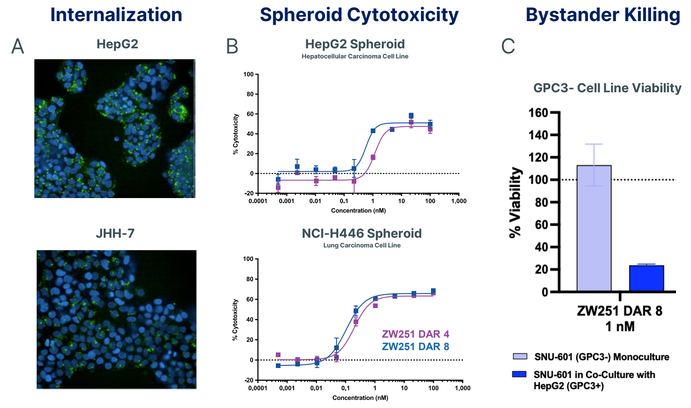


Figure 6. (A) ZW251 internalization into GPC3-expressing cell lines was visualized after 24h treatment with ADC coupled to an anti-human IgG Fab-488 and subsequent surface quenching. **(B)** Cytotoxicity assessed by treating cell line spheroids with ZW251 for 4 days and assessed for viability using CellTiterGlo®. **(C)** Bystander effect assessed by measuring viability by flow cytometry of SNU-601 GPC3- cells in monoculture, or co-culture with GPC3+ HepG2 cells, following treatment with ZW251 for 4 days.

ZW251 Demonstrates Robust In Vivo Anti-Tumor Activity in a Broad Panel of Cell Line-Derived (CDX) and Patient-Derived Xenograft (PDX) Models of HCC

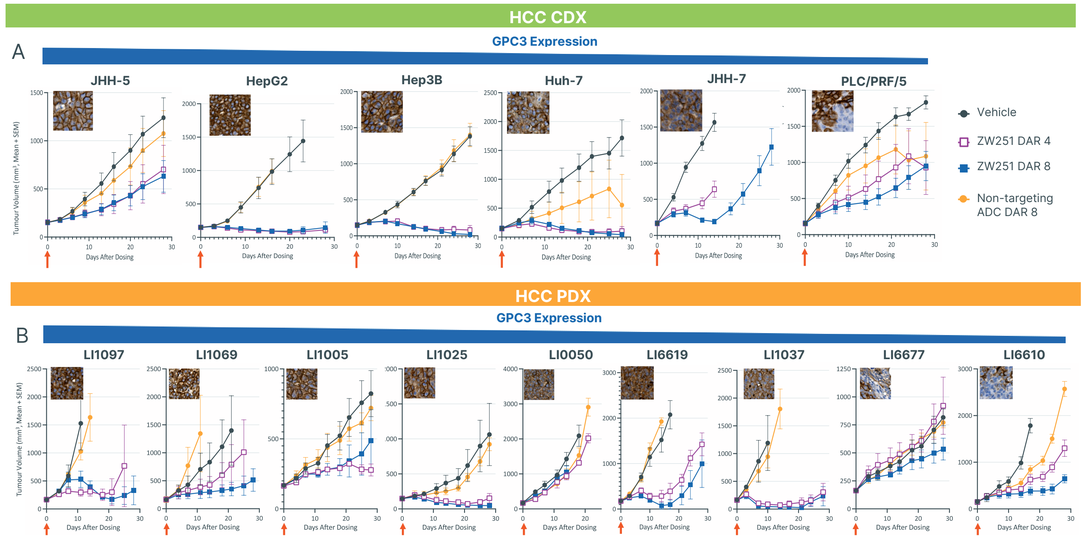


Figure 7. *In vivo* anti-tumor activity of ZW251 in BALB/c nude mice subcutaneously engrafted with **(A)** CDX models, 5-6 mice per group, or **(B)** PDX models, 3 mice per group, of HCC. Mice were given a single intravenous 8 mg/kg dose of ZW251 when tumor volumes reached ~150-200 mm³. Growth plots were terminated >20% of mice (CDX) or 1 mouse (PDX) was lost within a treatment group. **(C)** GPC3 expression per model was determined by IHC using codrituzumab anti-GPC3 monoclonal antibody followed by pathologist scoring. Anti-tumor activity was determined by %tumor growth inhibition (%TGI) calculated as $[(1-TV_{treatment}/TV_{vehicle}) \times 100]$ at Day 21, or at the closest evaluable time point. **(D)** Scope of ZW251 anti-tumor activity, as defined by %TGI > 50%, in CDX/PDX models with higher GPC3 expression (H-score >200) and lower GPC3 expression (H-score < 200).

C

	Expression (H-Score)	ZW251 Tumor Growth Inhibition	
		DAR 8	DAR 4
JHH-5	300	52%	52%
HepG2	278	92%	94%
Hep3B	267	93%	91%
Huh-7	265	95%	94%
JHH-7	198	88%	59%
PLC/PRF/5	138	60%	43%
LI1097	300	65%	81%
LI1069	300	75%	43%
LI1005	295	48%	51%
LI1025	288	91%	88%
LI0050	250	26%	34%
LI6619	248	95%	81%
LI1037	225	96%	93%
LI6677	142	20%	-9%
LI6610	84	79%	65%

D

	ZW251 Anti-Tumor Activity	
H-score	DAR 8	DAR 4
> 200	82% (9/11)	82% (9/11)
< 200	75% (3/4)	50% (2/4)

- Single dose of ZW251 at 8 mg/kg results in anti-tumor activity in 6/6 CDX models and 7/9 PDX models of HCC.
- ZW251 anti-tumor activity is observed in models with lower or heterogenous GPC3 expression.
- **Broad target-mediated *in vivo* activity across a range of HCC models highlights the therapeutic potential of ZW251 in HCC.**

ZW251 Exhibits Favorable Pharmacokinetics in Tg32 Mice

Total IgG in Tg32 Mouse Serum

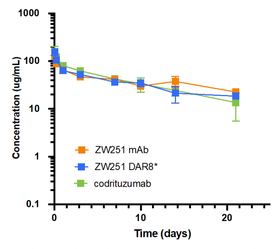


Figure 8. Circulating antibody levels in Tg32 mice determined by ELISA measuring human IgG in mouse serum following single intravenous 5 mg/kg dose of of antibody or ADC. *Analog utilizes ZW251 mAb conjugated to a closely related linker-payload.

- Tg32 mice express human FcRn and are considered a relevant PK model.
- ZW251 mAb exhibits comparable PK to a clinical-stage antibody.
- PK of ZW251 mAb unaffected by conjugation.

Non-Human Primate Toxicology Study

Repeat Dose Non-GLP NHP Toxicology Study

Test Article	Doses		
ZW251 DAR 8	10 mg/kg	30 mg/kg	60 mg/kg
ZW251 DAR 4	20 mg/kg	60 mg/kg	120 mg/kg

Figure 9. Study design of a repeat dose non-GLP toxicology study in cynomolgus monkeys performed to assess the tolerability and pharmacokinetic profile of ZW251. Parameters evaluated include cage side and clinical observations, body weight, food consumption, coagulation parameters, hematology parameters, clinical chemistry parameters, macroscopic examination, organ weights, tissue histopathology, and toxicokinetics in all treatment groups.

- Minimal changes in body weight, hematology parameters, and clinical chemistry parameters in all treatment groups.
- **No mortality observed in any treatment group prior to necropsy.**

Conclusions

- ZW251 is a potentially first-in class glypican-3 targeting antibody drug conjugate.
- ZW251 exhibits robust anti-tumor activity in a large panel of HCC CDX and PDX models at both DAR 4 and DAR 8.
- Anti-tumor activity (tumor growth inhibition > 50%) evident for ZW251 in 82% of models with GPC3 H-score > 200 and 50-75% of models with GPC3 H-score < 200.
- No mortality was observed in a repeat dose NHP toxicology study with doses up to 60 mg/kg (DAR 8) or 120 mg/kg (DAR 4).

References

1. Shih TC, Wang L, Wang HC, Wan YY. Glypican-3: A molecular marker for the detection and treatment of hepatocellular carcinoma. *Liver Res.* **2020**, *4*, 168-172.
2. Hanlin L. Wang, Florencia Anatelli, Qihui"Jim" Zhai, Brian Adley, Shang-Tian Chuang, Ximing J. Yang; Glypican-3 as a useful diagnostic marker that distinguishes hepatocellular carcinoma from benign hepatocellular mass lesions. *Arch Pathol Lab Med.* **2008**, *132*, 1723–1728.
3. Carrasquillo, J.A., O'Donoghue, J.A., Beylergil, V. et al. I-124 codrituzumab imaging and biodistribution in patients with hepatocellular carcinoma. *EJNMMI Res.* **2018**, *8*, 20.