ZW251, a novel glypican-3-targeting antibody-drug conjugate bearing a topoisomerase I inhibitor payload, demonstrates compelling preclinical activity in hepatocellular carcinoma models

Abstract #177

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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 tolerability profile in a non-GLP non-human primate toxicology study.

ZW251 Antibody Drug Conjugate

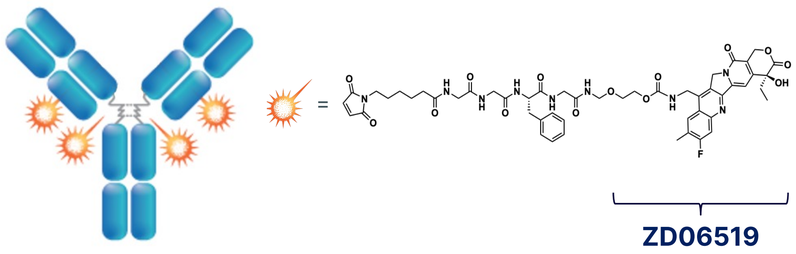
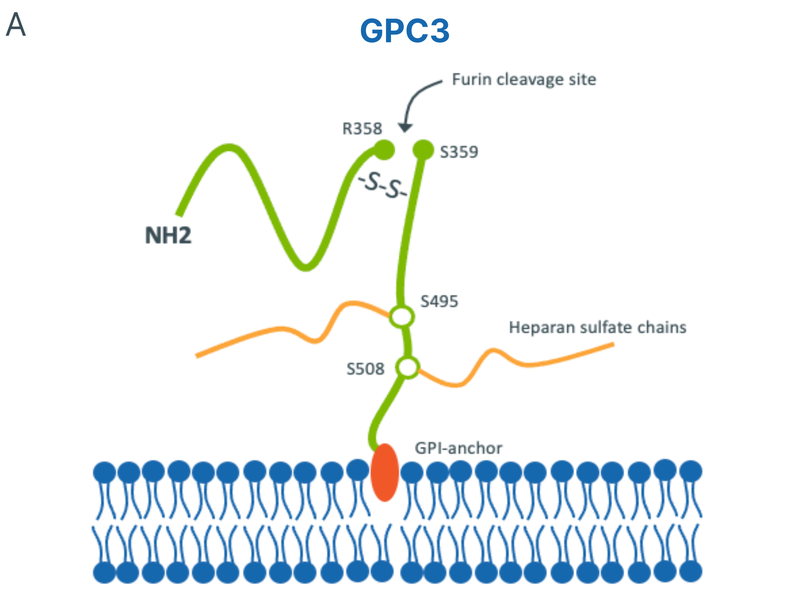


Figure 1. ZW251 ADC composition and linker-payload structure.

- Humanized anti-GPC3 monoclonal antibody
- ZD06519 topoisomerase 1 inhibitor payload
- Interchain disulfide conjugation
- Optimized drug-antibody ratio (DAR) of 4

GPC3 is a Compelling ADC Target for Hepatocellular Carcinoma



	GPC3 Prevalence in HCC	GPC3 Expression Pattern in HCC
Ye *et al.* Transl Cancer Res 2020	86% (n = 229)	N/A
Abou-Alfa *et al.* J. Hepatol 2016	87% (n = 185)	57% IHC 2+/3+ 30% IHC 1+ 13% IHC 0
Wang *et al.* Oncotarget 2016	96% (n = 69)	3% +++ 75% ++ 17% + 4% Negative
Liu *et al.* World J Gastroenterol 2010	95% (n = 55)	N/A
Wang *et al.* Arch Pathol Lab Med 2008	76% (n = 111)	55% Diffuse 21% Focal 24% Negative
Yamauchi *et al.* Mod Pathol 2005	84% (n = 56)	84% ++ 0% + 16% Negative
Capurro *et al.* Gastroenterol 2003	83% (n = 23)	N/A

Figure 2. (A) GPC3 structure. **(B)** Representative IHC staining in human normal liver and HCC samples. **(C)** Prevalence and intensity of GPC3 expression in patients with HCC

- 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]
- Demonstrated anti-GPC3 antibody accumulation in HCC patient tumors[3]
- **GPC3 is highly expressed in most HCC tumors[2] and exhibits limited expression in healthy tissues**
- GPC3 is also expressed in subsets of patients with other solid tumor diseases[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.
4. Moek, K.L., Fehrmann, R.S.N., van der Vegt, B., de Vries, E.G.E., de Groot, D.J.A. Glypican 3 Overexpression across a Broad Spectrum of Tumor Types Discovered with Functional Genomic mRNA Profiling of a Large Cancer Database. *Am J Pathol.* **2018**, *188*, 1973-1981.

ZW251 Selectively Binds, Internalizes, and Kills GPC3 Expressing Tumor Cell Lines

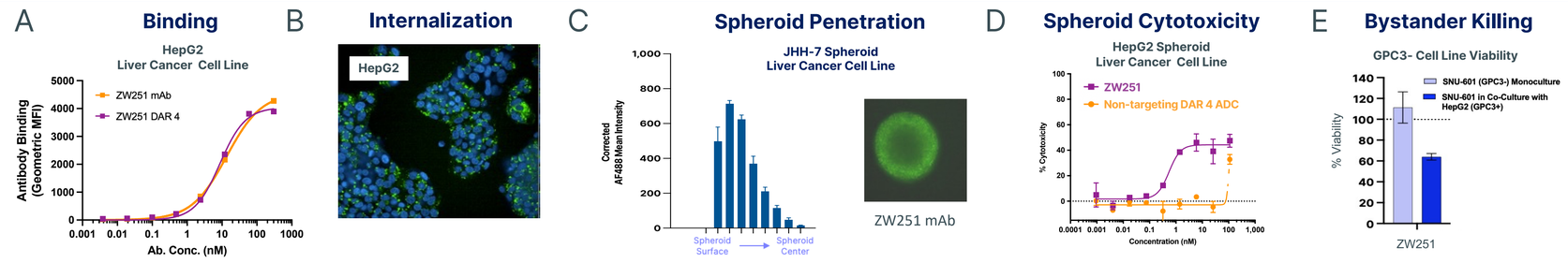
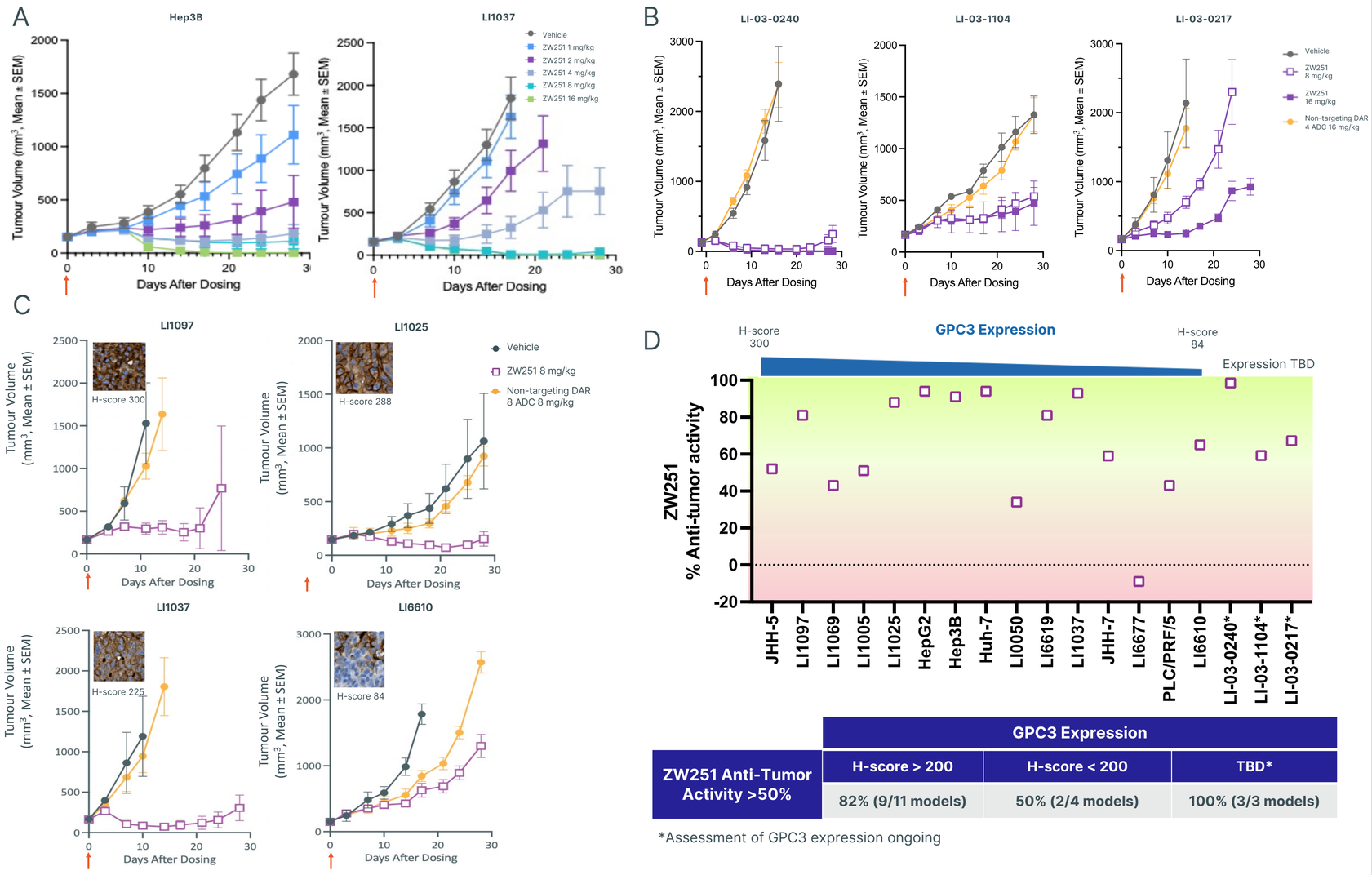


Figure 3. (A) Binding of to a GPC3-expressing cancer cell line was assessed by flow cytometry. **(B)** Internalization into a GPC3-expressing cell line was visualized after 24h treatment with ADC coupled to an anti-human IgG Fab-488 and subsequent surface quenching. **(C)** Spheroid penetration was visualized after 24h treatment with ZW251 mAb coupled to an anti-human IgG Fab-488. **(D)** Cytotoxicity was assessed by treating spheroids with ADC for 4 days and assessed for viability using CellTiterGlo®. **(E)** 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 1 nM ZW251 for 4 days.

ZW251 Demonstrates Robust In Vivo Anti-Tumor Activity in a Broad Panel of HCC Xenograft Models



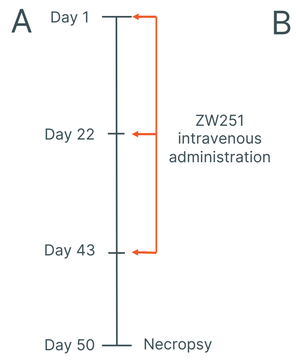
ZW251 Anti-Tumor Activity >50%	GPC3 Expression		
	H-score > 200	H-score < 200	TBD*
	82% (9/11 models)	50% (2/4 models)	100% (3/3 models)

*Assessment of GPC3 expression ongoing

Figure 4. (A) Dose response activity of ZW251 in mice engrafted with Hep3B CDX or LI1037 PDX tumors, 8 mice per group. **(B)** Activity of ZW251 at 8 and 16 mg/kg in mice engrafted with HCC PDX models, 3 mice per group. **(C)** Representative studies showing activity of ZW251 at 8 mg/kg in mice engrafted with a range of HCC PDX models, 3 mice per group. **(D)** Breadth of ZW251 anti-tumor activity across all tested CDX/PDX models of HCC. Anti-tumor activity at 8 mg/kg was determined by %tumor growth inhibition calculated as $[(1-TV_{treatment}/TV_{vehicle}) \times 100]$ at Day 21, or at the closest evaluable time point. GPC3 expression was determined by IHC using codrituzumab followed by pathologist scoring.

- Dose response anti-tumor activity observed in CDX and PDX models of HCC
- Single dose at 8 mg/kg results in anti-tumor activity in 5/6 CDX models and 9/12 PDX HCC models, including those with lower or heterogenous GPC3 expression
- **Compelling breadth of anti-tumor activity activity observed with ZW251 DAR 4 ADC**
- **Broad target-mediated *in vivo* activity across a range of HCC models highlights the therapeutic potential of ZW251 in HCC**

ZW251 Well Tolerated in Repeat Dose Non-Human Primate Toxicology Study and Exhibits Dose-Proportional PK



B

Test article	Dose	Mortality	Clinical observations	Histopathology	Clinical Chemistry	Hematology	MTD	$T_{1/2}$ (day)
ZW251 DAR 4	20 mg/kg	None	None	None	None	Decreased reticulocytes	120 mg/kg	4.6
	60 mg/kg	None	None	None	None	Decreased reticulocytes		4.8
	120 mg/kg	None	Fecal abnormalities (loose feces)	Thymus and Lymph node	None	Decreased reticulocytes		5.4

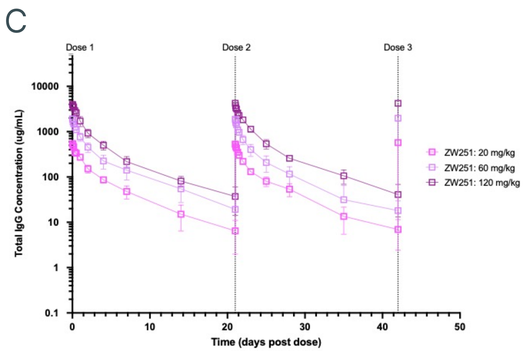
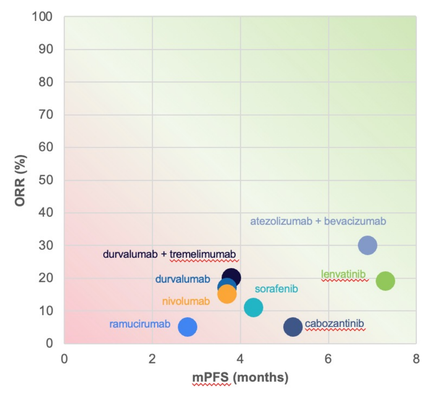


Figure 5. (A) Study design of a repeat dose non-GLP toxicology study in cynomolgus monkeys performed to assess the tolerability and pharmacokinetic profile of ZW251. **(B)** Toxicology results of non-human primates administered with ZW251. **(C)** Total human antibody levels in non-human primate serum as measured by ELISA

- Dose proportional pharmacokinetics observed with total antibody levels in non-human primate serum in a multi-dose study
- Treatment-related lower mean reticulocyte counts observed and deemed non-adverse in all dose groups; non-adverse decreased thymus cellularity and mesenteric lymph node cellularity seen with microscopic observation in one animal administered 120 mg/kg
- No mortality or adverse clinical observations, body weight effects, food consumption observed; no on-target toxicity observed
- **Impressive tolerability in non-human primates of ZW251 up to 120 mg/kg suggests potential for high first-in-human dosing of ZW251**

Clinical Efficacy of Approved Agents Emphasizes Significant Unmet Need in HCC



	Line	ORR	mPFS (months)	mOS (months)	Reference
Atezolizumab + bevacizumab	1L	30%	6.9	19.2	Finn RS *et al.* ASCO GI 2021
Durvalumab + tremelimumab	1L	20%	3.8	16.4	Abou-Alfa GK *et al.* ASCO GI 2022
Durvalumab	1L	17%	3.7	16.6	Abou-Alfa GK *et al* ASCO GI 2022
Nivolumab	1L	15%	3.7	16.4	Yau T *et al.* Lancet Oncol 2022
Sorafenib	1L	5-11%	3.8-4.3	13-15	Abou-Alfa GK *et al.* ASCO GI 2022
Lenvatinib	1L	19%	7.3	13.6	Kudo M *et al.* The Lancet 2018
Regorafenib	2L	11%	3.1	10.6	Bruix J *et al.* The Lancet 2018
Cabozantinib	2L	4.6%	5.2	10.2	Abou-Alfa GK *et al.* NEJM 2018
Ramucirumab	2L	4.6%	2.8	8.5	Zhu AX *et al.* Lancet Oncol 2019

Figure 6. Clinical efficacy of approved therapeutic agents against HCC

- Therapeutic outcomes associated with approved drugs highlight an unmet need in HCC
- **ZW251 offers the potential of a new mechanism-of-action for patients, and an opportunity to improve upon the existing standard of care (SOC)**
- **An ADC approach allows potential therapeutic strategy of ZW251 as a monotherapy, or in combination with existing SOC**

Conclusions

- ZW251 exhibits robust anti-tumor activity in a large panel of HCC CDX and PDX models, including those with lower and heterogenous GPC3 expression
- ZW251 demonstrates impressive tolerability in a repeat dose NHP toxicology study
- ZW251 DAR 4 offers an optimal balance between tolerability and breadth of anti-tumor activity
- **ZW251 DAR 4 was selected for further development, with a planned IND submission in H2 2025**
- **ZW251 may provide an opportunity to address unmet need in HCC and other GPC3-expressing tumors**

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