ZW251

A novel glypican-3 targeting ADC bearing a topoisomerase I inhibitor payload

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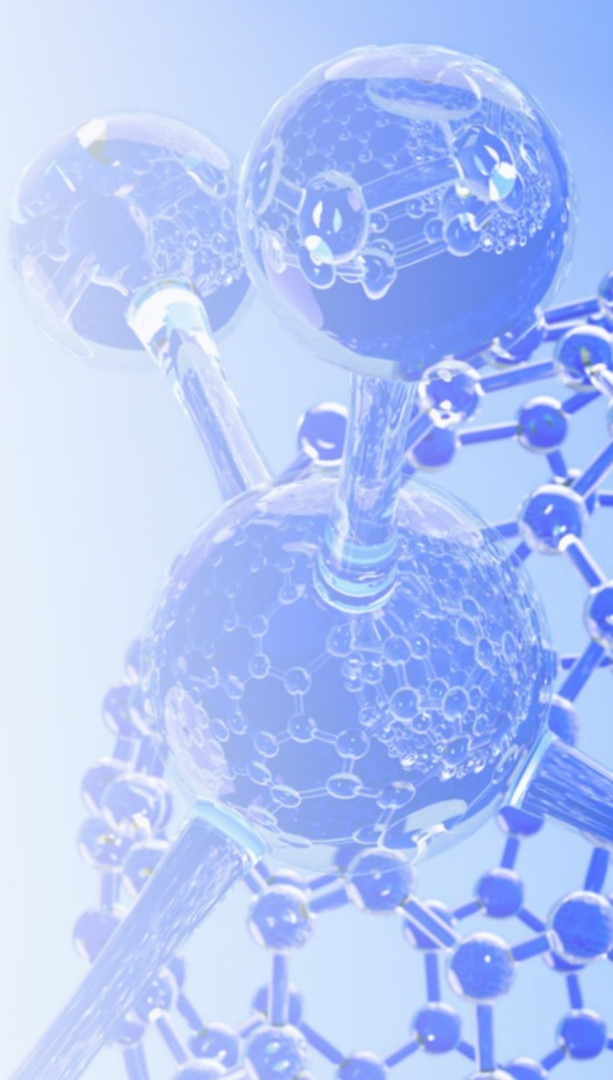
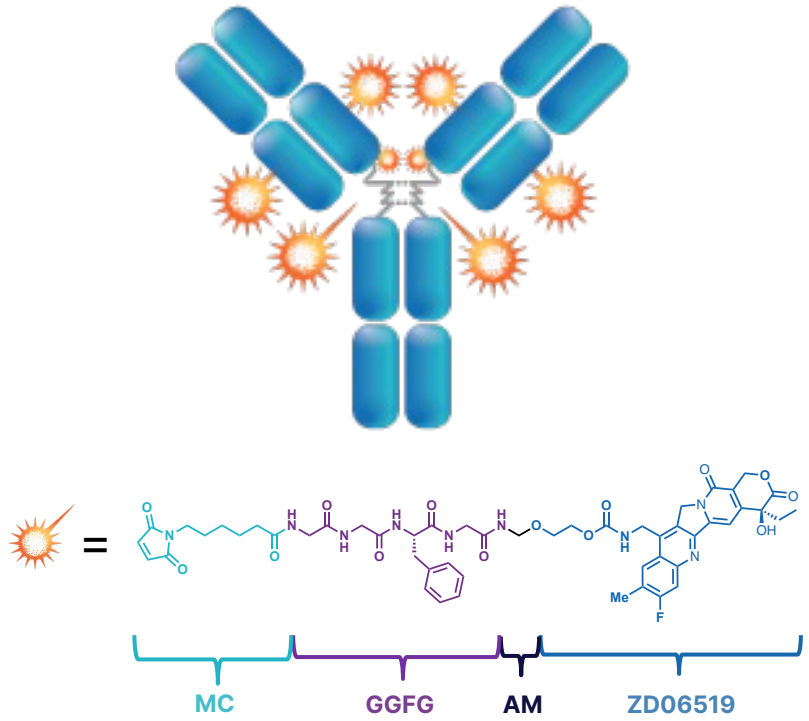


ZW251 – A novel glypican-3 targeting ADC bearing a topoisomerase I inhibitor payload



DAR 8 pictured for illustrative purposes

ZW251 composition

- Humanized IgG1 monoclonal antibody against glypican-3 (GPC3)
  - Specifically selected for strong binding and internalization
- ZD06519 topoisomerase I inhibitor payload
  - Desired balance of stability, activity, and tolerability, with bystander-active properties
- Drug-to-antibody-ratio (DAR) 4 and 8 molecules evaluated
- **ZW251 is an ADC designed for the treatment of hepatocellular carcinoma (HCC)**

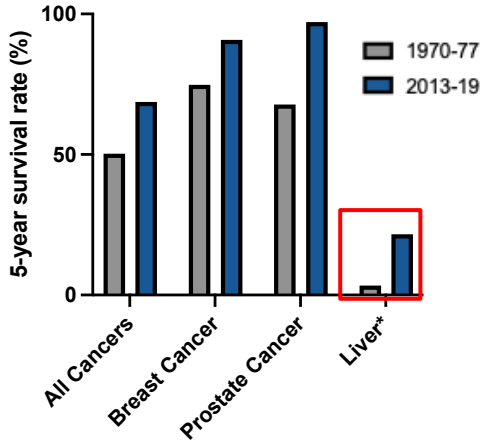
Hepatocellular carcinoma has a high burden of disease

Liver cancer has a high burden

~40,000 cases diagnosed each year

~30,000 deaths each year

Changes in 5-year Cancer Survival Rates
1970-77 vs 2013-19



*Liver and intrahepatic bile duct cancers
US SEER Data

Limited systemic treatment options exist for HCC

- **2007:** sorafenib
- **2017:** regorafenib, nivolumab
- **2018:** lenvatinib, pembrolizumab
- **2019:** cabozantinib, ramucirumab
- **2020:** nivolumab + ipilimumab, atezolizumab + bevacizumab
- **2022:** durvalumab + tremelimumab

Dates of approval for treatment of HCC obtained from FDA.gov

HCC is difficult to treat

Different drug classes can be active in HCC

- Alkylating agents[1]
- Anthracyclines[1]
- Topoisomerase inhibitors[2]
- Multi-kinase inhibitors[1]
- Anti-angiogenics[1]
- Checkpoint inhibitors[3]

Limited targeted therapy classes approved in HCC[3]

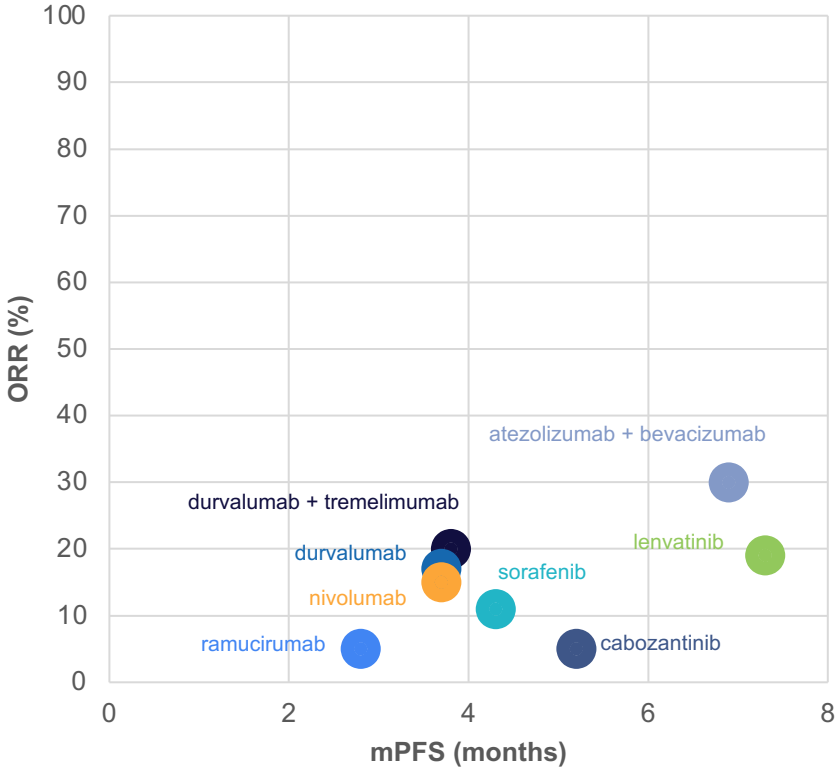
Anti-angiogenics:
- *Cyramza*
- *Avastin*

Checkpoint inhibitors:
- *Opdivo, Keytruda*
- *Tecentriq, Imfinzi*
- *Imjudo, Yervoy*

ADC format may enhance efficacy of small molecule chemotherapeutics with a first-in-class opportunity

[1]Grazie *et al.* 2017. World J Hepatol; [2]Martin *et al.* 2009. World J Surg Oncol; [3]Llovet *et al.* 2021. Nat Rev Dis Primers

Currently approved therapies in HCC have low efficacy

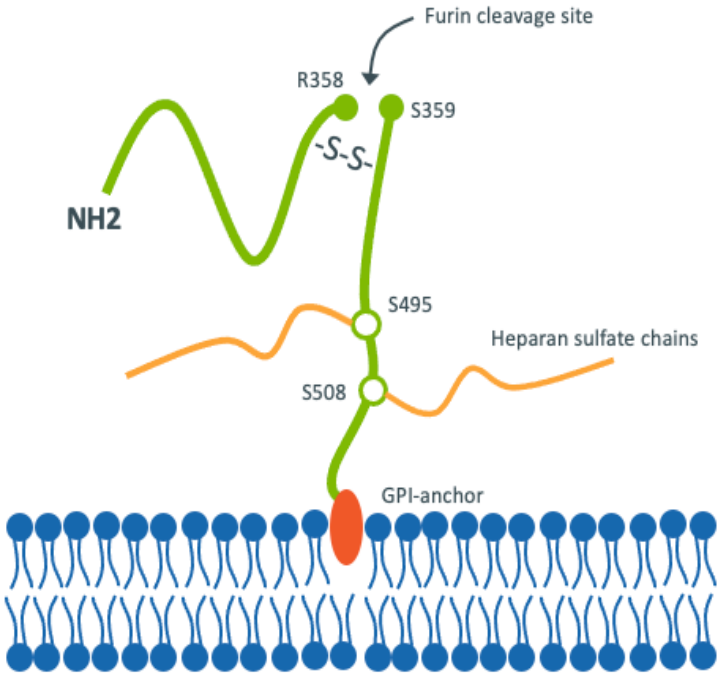


	Line of treatment	ORR	mPFS (months)
Atezolizumab + bevacizumab[1]	1L	30%	6.9
Durvalumab + tremelimumab[2]	1L	20%	3.8
Durvalumab[2]	1L	17%	3.7
Nivolumab[3]	1L	15%	3.7
Sorafenib[2]	1L	5-11%	3.8-4.3
Lenvatinib[4]	1L	19%	7.3
Regorafenib[5]	2L	11%	3.1
Cabozantinib[6]	2L	4.6%	5.2
Ramucirumab[7]	2L	4.6%	2.8

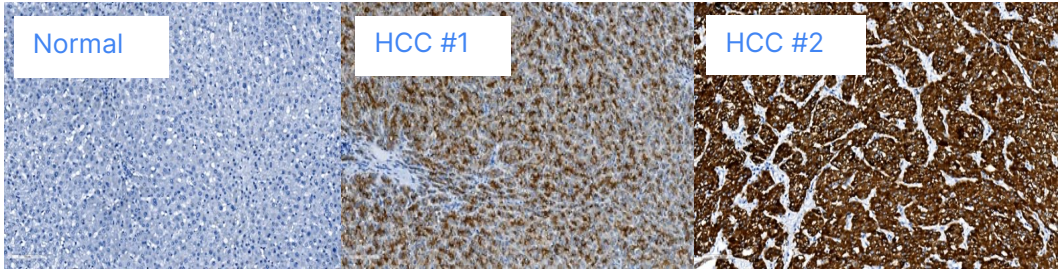
Limited therapeutic options for later lines of treatment

[1]IMbrave150; Finn RS *et al.* ASCO GI 2021
[2]HIMALAYA; Abou-Alfa *et al.* ASCO GI 2022
[3]CheckMate459; Yau *et al.* Lancet Oncol 2022
[4]REFLECT; Kudo *et al.* Lancet 2018
[5]RESOURCE; Bruix *et al.* Lancet 2017
[6]CELESTIAL; Abou-Alfa *et al.* NEJM 2018
[7]REACH-2; Zhu *et al.* Lancet Oncol 2019

GPC3 is prevalent and highly expressed in hepatocellular carcinoma



Cell-surface GPI-anchored oncofetal glycoprotein

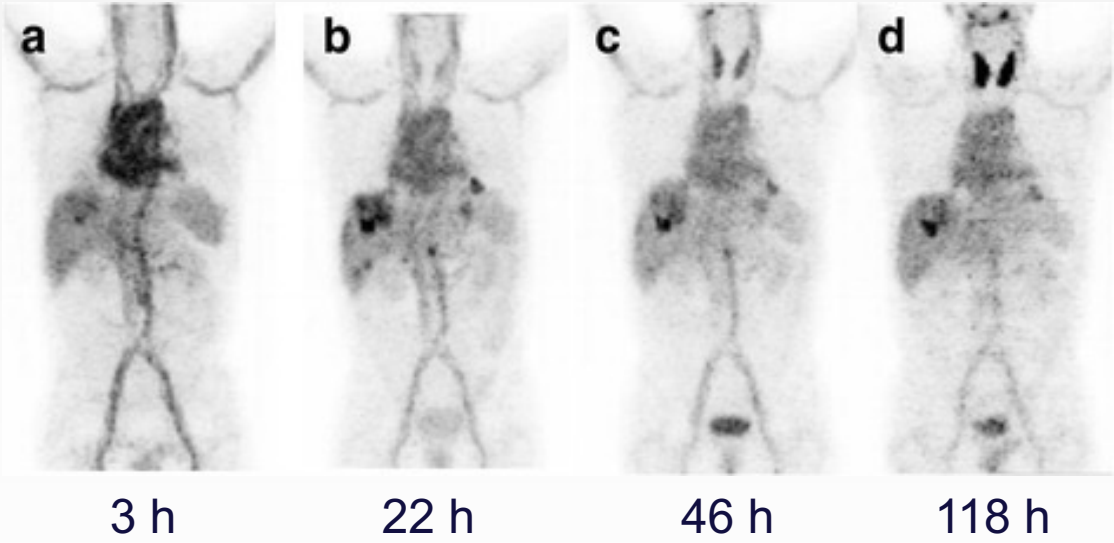


HCC % Positivity	Intensity	Reference
87%	57% IHC2+/3+	Abou-Alfa *et al*. 2016. *J Hepatol*
96%	75% '++', 3% '+++'	Wang *et al*. 2016. *Oncotarget*
84%	84% '++'	Yamauchi *et al*. 2005. *Mod Pathol*
76%	N.D.	Wang et al. 2008. *Arch Pathol Lab Med*

N.D. – not determined

GPC3-targeting antibody can rapidly localize into HCC lesions

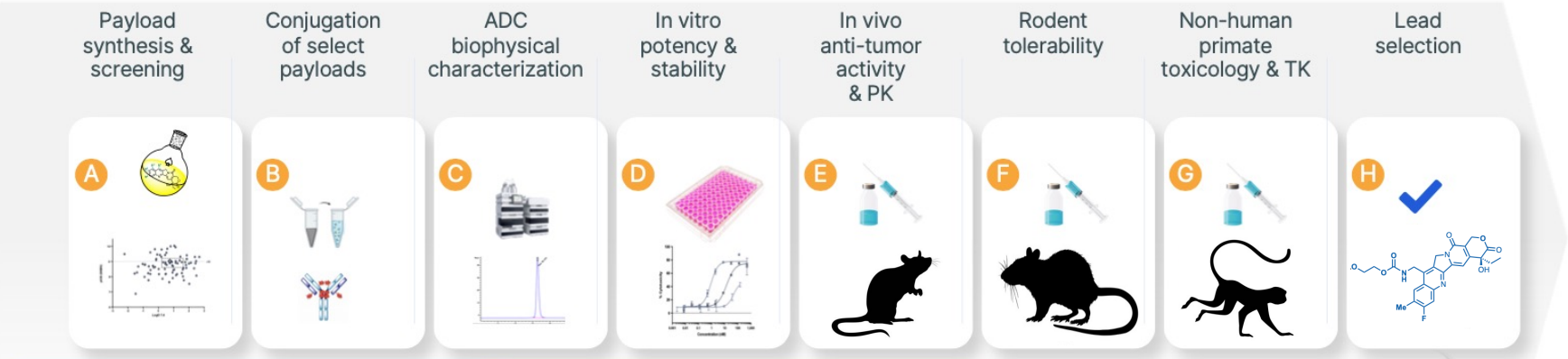
Uptake of iodine radiolabeled codrituzumab (anti-GPC3) in an HCC patient



- Uptake observed in 13 of 14 HCC patients with a range of GPC3 expression
- Rapid localization to liver tumor lesions within hours, peaking at 24 h
- No preferential normal organ accumulation except for the thyroid
- **Antibody-based targeting of GPC3 enables a selective approach to HCC**

Carrasquillo *et al.* 2018. EJNMMI Res

ZW251 utilizes a pipeline-ready topoisomerase I inhibitor ADC platform



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

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

CONJUGATION

Thiol-maleimide chemistry

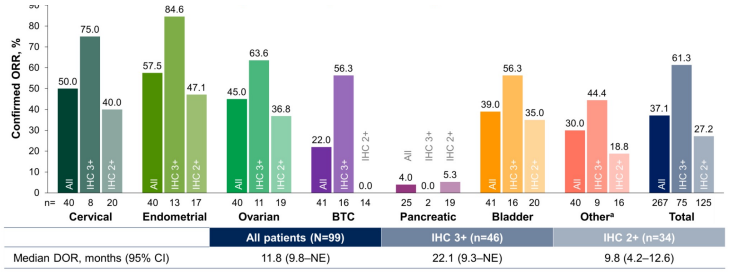
- Stochastic conjugation utilized in *all* approved ADCs
- Facilitates DAR optimization
- Good balance of stability, safety, and anti-tumor activity

Adapted from Lawn *et al.* World ADC London 2023

Topoisomerase I inhibitor payloads are providing meaningful benefit to solid-tumor patients

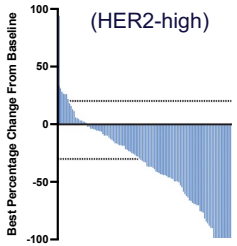
Enhertu (HER2-DXd)

DESTINY-PanTumor02[1]

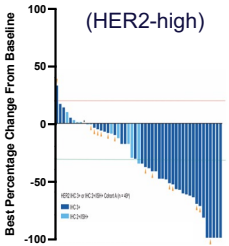


	All patients (N=99)	IHC 3+ (n=46)	IHC 2+ (n=34)
Median DOR, months (95% CI)	11.8 (9.8–NE)	22.1 (9.3–NE)	9.8 (4.2–12.6)

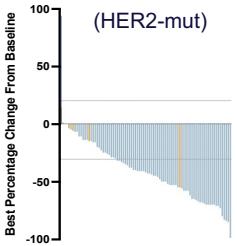
DESTINY-Gastric01[2] (HER2-high)



DESTINY-CRC01[3] (HER2-high)

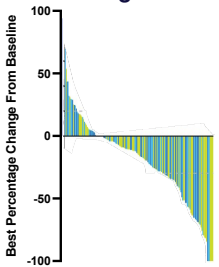


DESTINY-Lung01[4] (HER2-mut)

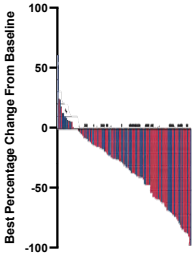


Dato-DXd (Trop2-DXd)

TROPION-PanTumor01[5] Lung

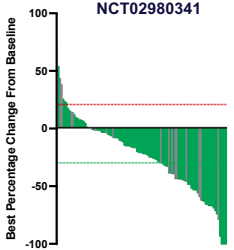


TROPION-Lung02[6]

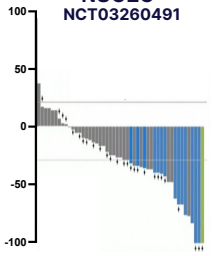


U3-1402 (HER3-DXd)

Breast, HR+/HER2-[7] NCT02980341

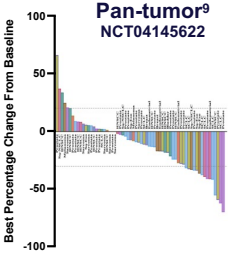


NSCLC[8] NCT03260491

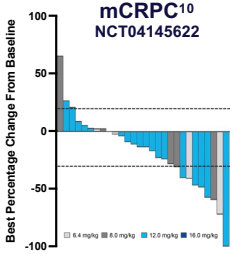


DS-7300 (B7H3-DXd)

Pan-tumor[9] NCT04145622

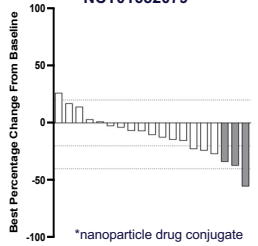


mCRPC[10] NCT04145622



CRLX101 (NDC*)

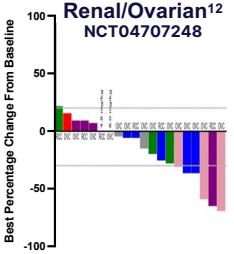
Ovarian[11] NCT01652079



*nanoparticle drug conjugate

DS-6000 (CDH6-DXd)

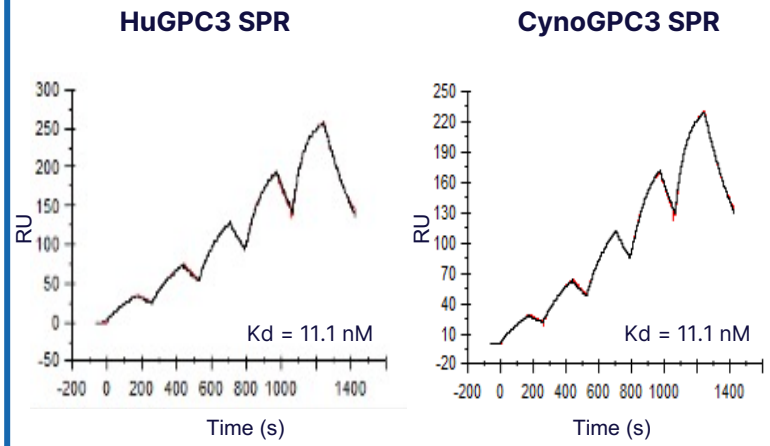
Renal/Ovarian[12] NCT04707248



[1]Meric-Bernstam *et al.* ASCO 2023
[2]Shitara *et al.* ASCO 2021
[3]Yoshino *et al.* ASCO 2021
[4]Li *et al.* NEJM 2021
[5]Meric-Bernstam *et al.* ASCO 2021
[6]Goto *et al.* ASCO 2023
[7]Krop *et al.* ASCO 2022
[8]Yu *et al.* ESMO 2020
[9]Johnson *et al.* ESMO 2021
[10]Patel *et al.* ASCO GU 2022
[11]Pham *et al.* Clin Cancer Res 2015
[12]Hamilton *et al.* ASCO 2022

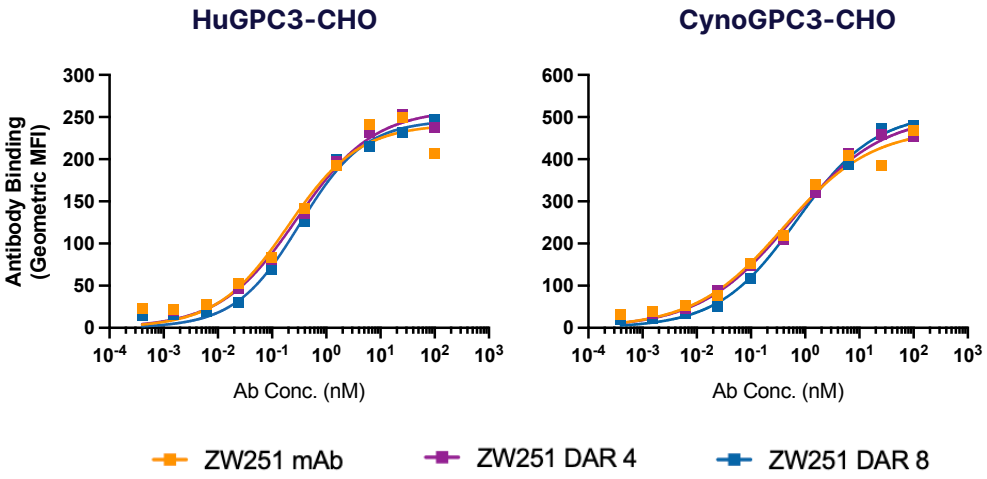
ZW251 demonstrates desired GPC3 binding and cross-reactivity

SPR binding to GPC3



Binding of soluble GPC3 extracellular domain to immobilized ZW251 mAb measured by surface plasmon resonance (SPR)

Binding to GPC3-transfected CHO



Binding of ZW251 to transfected CHO cells expressing human or cynomolgus monkey GPC3 assessed by flow cytometry.

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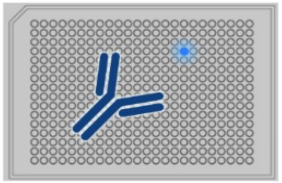
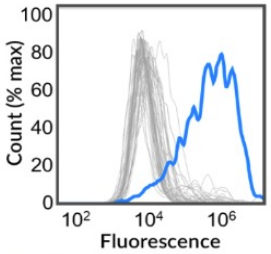
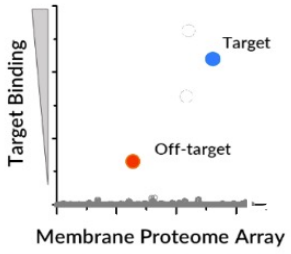
ZW251 mAb binds GPC3 with a high degree of specificity

ZW251 membrane proteome specificity screen

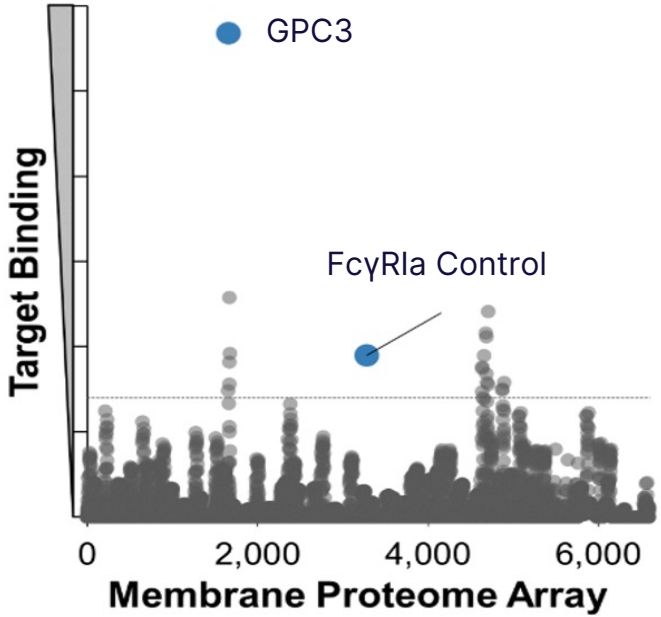
Array of 6000 human membrane proteins expressed on HEK293 cells → **Measure binding** to cells by flow cytometry → **Profile specificity** across human membrane proteome







- Includes 94% of all human single-pass, multi-pass, and GPI-anchored proteins, including GPCRs, ion channels, and transporters
- Specificity profiled by measuring binding to HEK293 cells expressing an array of membrane proteins
- Hits in initial screen were subsequently validated to confirm binding



Screening performed at Integral Molecular

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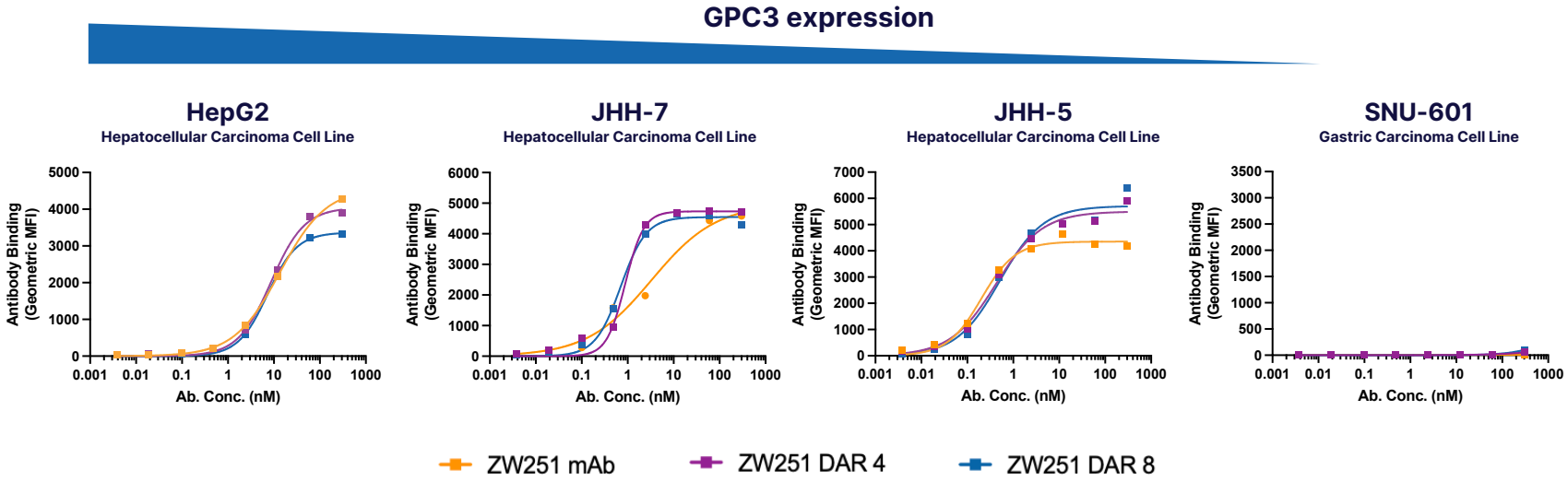
ZW251 demonstrates binding to GPC3-expressing tumor cells

Binding to tumor cell lines



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.

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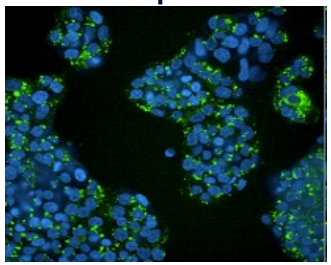
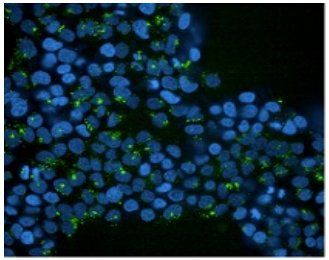
ZW251 internalizes into tumor cells resulting in cytotoxicity

Internalization

HepG2

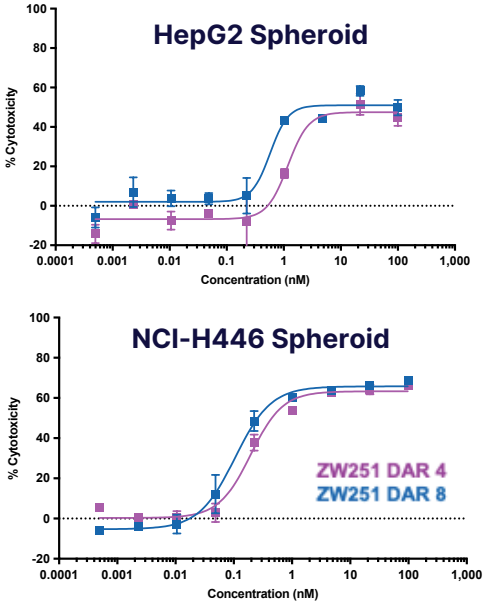


JHH-7



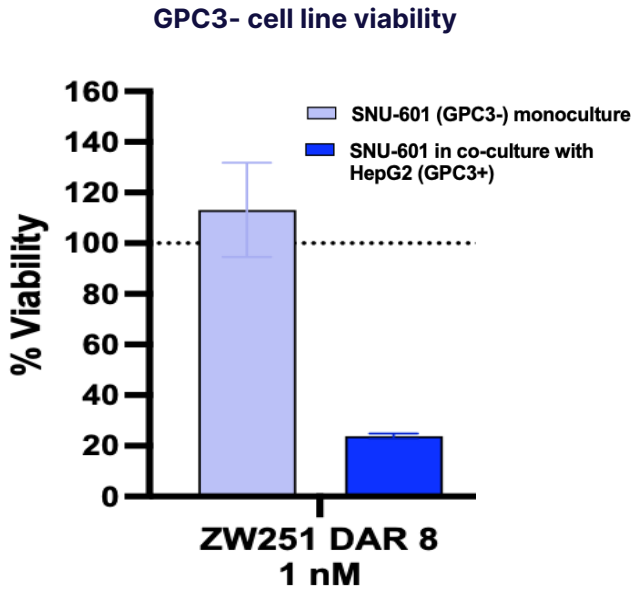
ZW251 internalization visualized after 24h treatment with ADC coupled to an anti-human IgG Fab-488 and subsequent surface quenching.

Tumor spheroid cytotoxicity



Cytotoxicity assessed by treating cell line spheroids with ZW251 for 4 days and assessed for viability using CellTiterGlo®.

Bystander killing



Bystander effect assessed by measuring viability of SNU-601 GPC3- cells in monoculture, or co-culture with GPC3+ HepG2 cells, following treatment with ZW251 for 4 days.

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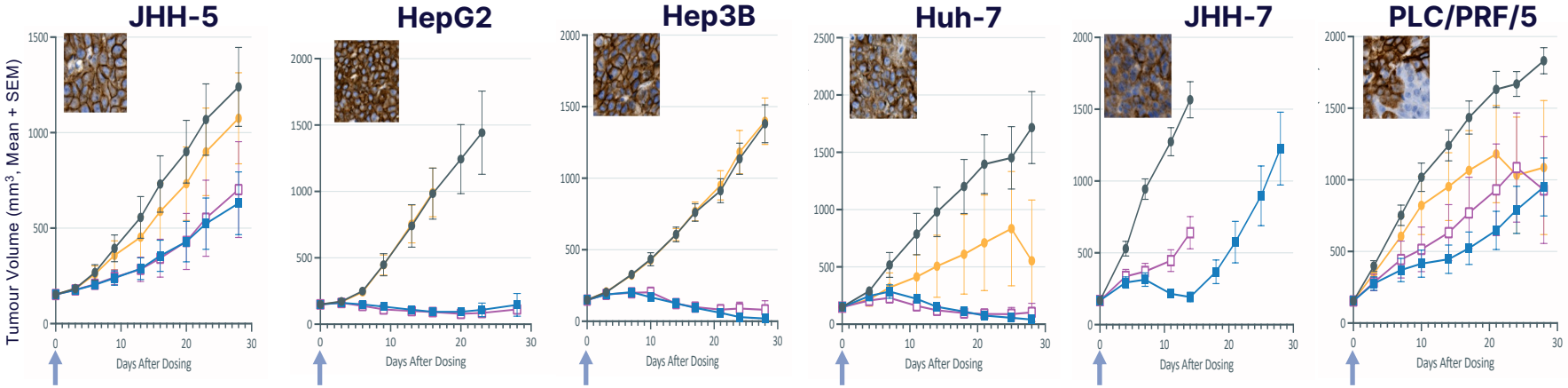
ZW251 demonstrates anti-tumor activity in a range of HCC CDX models

ZW251 activity in HCC CDX models

GPC3 expression (High to Low)



- BALB/c nude mice bearing subcutaneously engrafted HCC CDX tumors
- 5-6 mice per treatment group
- Single 8 mg/kg intravenous administration on Day 0

Vehicle
ZW251 DAR 8
ZW251 DAR 4
Non-targeting ADC DAR 8

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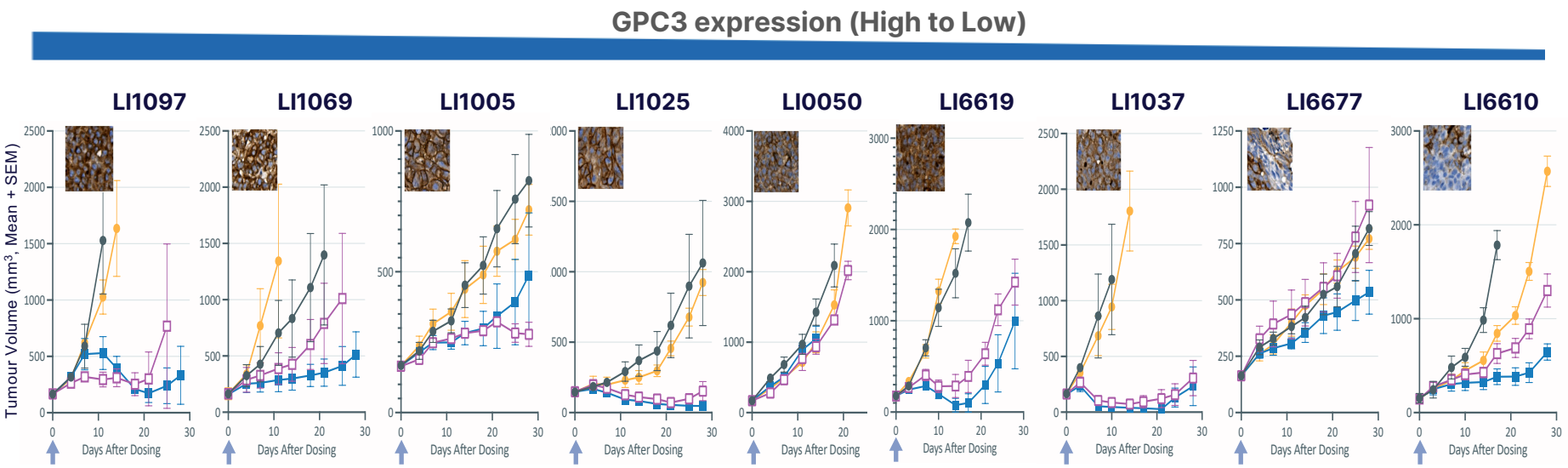
ZW251 demonstrates anti-tumor activity in a broad range of HCC PDX models

ZW251 activity in HCC PDX models



- BALB/c nude mice bearing subcutaneously engrafted HCC PDX tumors
- 3 mice per treatment group
- Single 8 mg/kg intravenous administration on Day 0

Vehicle
ZW251 DAR 8
ZW251 DAR 4
Non-targeting ADC DAR 8

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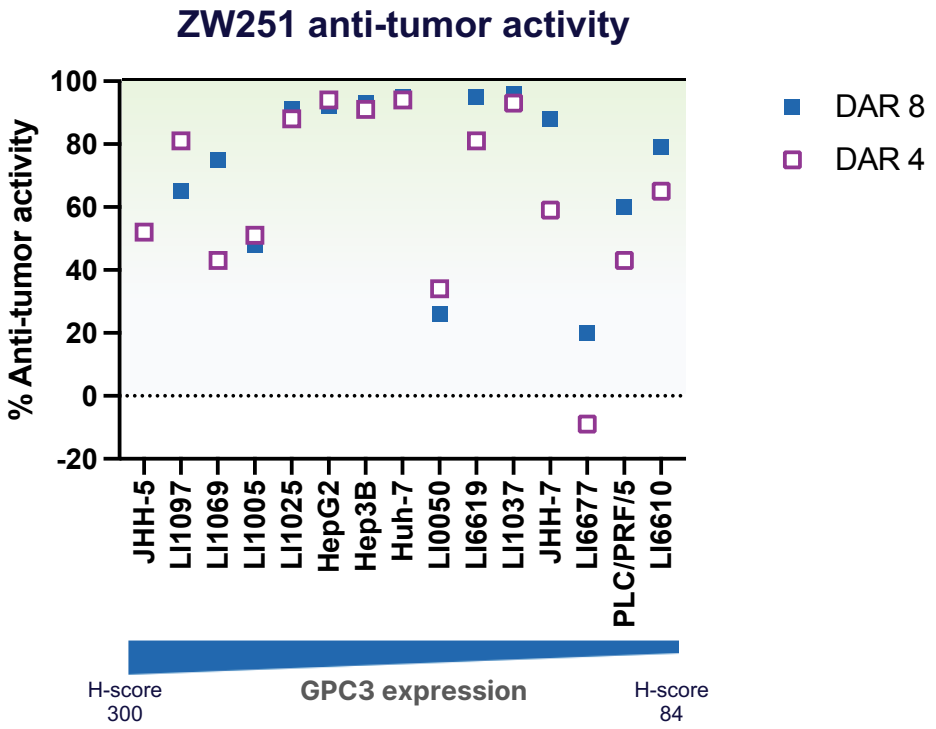
ZW251 exhibits broad range anti-tumor activity in a wide range of HCC xenograft models



% anti-tumor activity was determined by % tumor growth inhibition (%TGI) calculated as [(1-$TV_{treatment}/TV_{vehicle}$) x 100] at Day 21, or at the closest evaluable time point.

- ZW251 anti-tumor activity observed in 6/6 CDX and 7/9 PDX models of HCC
- Increased activity observed with higher drug loading
- ZW251 may perform better in higher expressing models
- Anti-tumor activity observed in models with H-scores as low as 84
- **ZW251 demonstrates compelling pre-clinical efficacy against models of HCC**

	ZW251 anti-tumor activity	
H-score	**DAR 8**	**DAR 4**
> 200	**82% (9/11)**	**82% (9/11)**
< 200	**75% (3/4)**	**50% (2/4)**

Scope of ZW251 anti-tumor activity, as defined by %TGI > 50%

ZW251 tolerability was assessed in a non-human primate toxicology study

Repeat dose non-GLP NHP study design

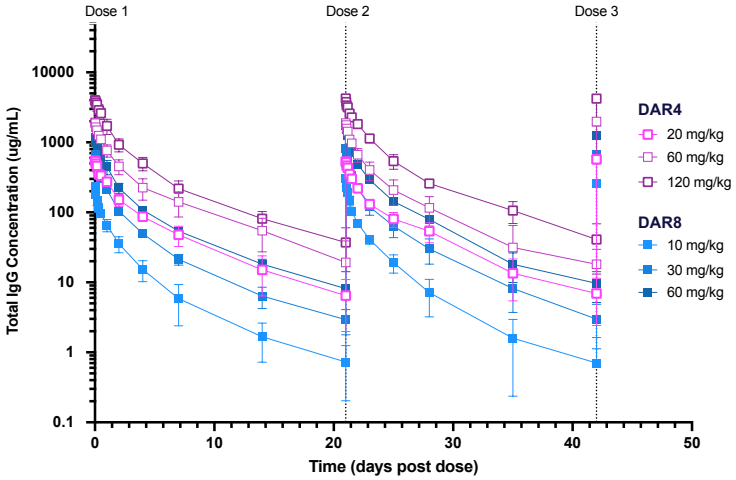
Test article	Doses		
ZW251 DAR 8	10 mg/kg	30 mg/kg	60 mg/kg
ZW251 DAR 4	20 mg/kg	60 mg/kg	120 mg/kg

Assessment of ZW251:

- Toxicology
  - Mortality
  - Body weight
  - Food consumption
  - Cage side/clinical observation
  - Coagulation
  - Hematology
  - Clinical chemistry
  - Macroscopic observations
  - Tissue histopathology
- Pharmacokinetics
  - C_{max}
  - AUC
  - $t_{1/2}$

ZW251 is well-tolerated in a repeat dose non-human primate toxicology study

Test article	Dose	Mortality	Clinical observations	Histopathology	Clinical Chemistry	Hematology	MTD	$T_{1/2}$ (day)
ZW251 DAR 8	10 mg/kg	None	None	None	None	Decreased reticulocytes	60 mg/kg	4.4
	30 mg/kg	None	None	None	None	Decreased reticulocytes		4.7
	60 mg/kg	None	Fecal abnormalities (loose/soft feces)	None	None	Decreased reticulocytes		5.0
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



- 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 (DAR4)
- **No mortality or adverse clinical observations, body weight effects, food consumption observed; lack of on-target toxicity observed**
- Impressive tolerability in non-human primates suggests potential for high first-in-human dosing of ZW251

ZW251 is a potential first-in-class GPC3-targeting topoisomerase I inhibitor ADC

- **Humanized IgG1 monoclonal antibody against glypican-3 (GPC3)**
  - Selective binding to GPC3 with desired human/cyno cross-reactivity
  - Internalization into GPC3-expressing tumor cells
- **ZD06519 topoisomerase I inhibitor payload**
  - ADC cytotoxicity of target-expressing tumor cells
  - Bystander killing of adjacent target-negative tumor cells
- **Drug-to-antibody-ratio (DAR) 4 and 8 molecules evaluated**
  - Broad anti-tumor activity against HCC CDX/PDX models with a range of GPC3 expression
  - Impressive tolerability in a repeat-dose non-human primate toxicology study

ZW251 is a promising therapeutic candidate for patients with hepatocellular carcinoma

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