Development of a first-in-class camptothecin-based antibody-drug conjugate targeting ALPP/ALPPL2 with potent antitumor activity and excellent tolerability

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Introduction:

ALPP (alkaline phosphatase, placental) and ALPPL2 (alkaline phosphatase, placental-like 2) emerge as promising targets due to their expression across multiple solid tumors, including ovarian (60%), endometrial (50%), pancreatic (30%), and gastric cancer (15%) along with minimal expression in normal adult tissues. We developed a novel ALPP/ALPPL2-directed antibody-drug conjugate (ADC) incorporated a proprietary camptothecin-derived payload linked through a protease-cleavable linker to a high-affinity monoclonal antibody. Preclinical studies demonstrate robust antitumor activity with favorable safety, supporting its potential to benefit patients with refractory advanced solid tumors.

Methods:

Parental antibody binding to ALPP extracellular domain was evaluated by biolayer interferometry and ELISA. Selectivity against related phosphatases (hALPL and hALPI) was confirmed via enzyme-linked immunosorbent assay. ALPP/ALPPL2-ADC cytotoxicity was assessed using CellTiter-Glo (CTG) in ALPPL2-positive cells (HEp2, NCI-H1651). Bystander killing was quantified in co-cultures of HEp2 cells and Jurkat controls by measuring Jurkat viability with CTG assay. *In vivo* efficacy was examined in Capan-1 pancreatic adenocarcinoma cell line-derived xenograft (CDX) model and LD1-0017-200702 gastric patient-derived xenograft (PDX) models. Pharmacokinetics and toxicology were characterized in cynomolgus monkeys up to 40 mg/kg, administered every three weeks for three cycles.

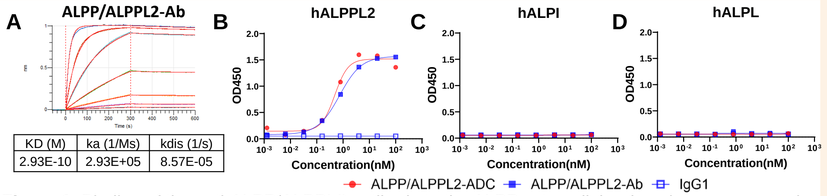
Results:

The parental ALPP/ALPPL2 antibody demonstrated high specificity for ALPP/ALPPL2, with no cross-reactivity to related phosphatases (hALPL and hALPI). The ALPP/ALPPL2-ADC (drug antibody ratio of 8), incorporating a proprietary camptothecin-based linker-payload, exhibited target mediated cytotoxicity in HEp2 (ALPP/ALPPL2-high) and NCI-H1651 (ALPP/ALPPL2-medium) cells *in vitro*. The ADC also showed a >40-fold greater bystander killing effect compared with a deruxtecan-based ADC. *In vivo*, a single 1 mg/kg dose induced profound tumor regression in the Capan-1 CDX model, while in the gastric adenocarcinoma PDX model refractory to an ADC with an MMAE payload, a single 8 mg/kg dose achieved deep tumor remission. In non-human primates, the ALPP/ALPPL2 showed linear pharmacokinetics and excellent tolerability (highest non severely toxic dose = 40 mg/kg).

Conclusions:

ALPP/ALPPL2-ADC is a promising therapeutic candidate for solid tumors, particularly gynecologic cancers. As a first-in-class camptothecin-based ADC targeting ALPP/ALPPL2, it combines high antibody specificity with robust antitumor efficacy and favorable tolerability, supporting advancement into clinical development.

ALPP/ALPPL2 Ab/ADC Binds to Human ALPP/ALPPL2 with High Affinity and Specificity



KD (M)	ka (1/Ms)	kdis (1/s)
2.93E-10	2.93E+05	8.57E-05

Figure 1. Binding of the anti-ALPP/ALPPL2 antibody to the ALPP extracellular domain was assessed by BLI (A) and ELISA (B). Both the antibody and the corresponding ADC showed no binding to human ALPL or ALPI (C–D).

ALPP/ALPPL2 ADC Based on the Hanjugator™ Platform Showed Enhanced Internalization Compared to Parental Antibody

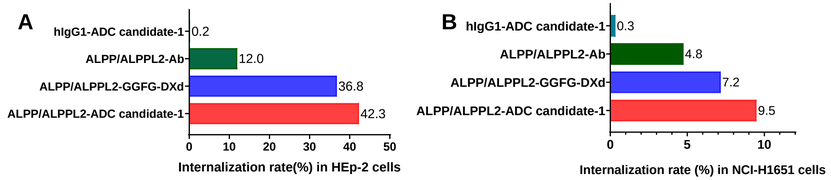
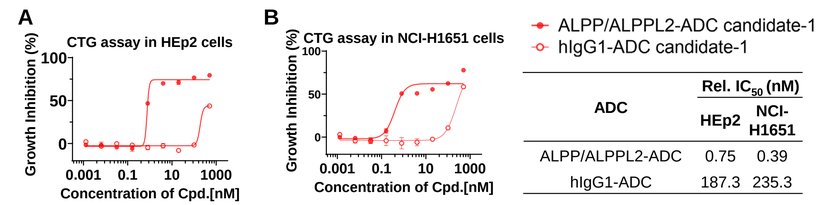


Figure 2. pHrodo-labeled indicated antibodies or ADCs (40 nM) were incubated with ALPP/ALPPL2-high HeLa derivative HEp2 cells (A) and ALPP/ALPPL2-medium nsq-NSCLC NCI-H1651 cells (B) for 16 hours. Antibody/ADC internalization was quantified by flow cytometry. All ADCs used in this experiment were DAR 8.

ALPP/ALPPL2 ADC Exhibits Target-Dependent Cytotoxic Activity *In Vitro*



ADC	Rel. IC_{50} (nM)	
	HEp2	NCI-H1651
ALPP/ALPPL2-ADC	0.75	0.39
hIgG1-ADC	187.3	235.3

Figure 3. HEp2 (A) and NCI-H1651 (B) cells were treated with the ALPP/ALPPL2 ADC or the corresponding IgG control ADC (DAR 8) for 96 hours. Cell viability was measured using the CTG assay.

Hanjugator™ Platform Enables ALPP/ALPPL2 ADCs With >10-Fold Improved Bystander Effect Compared With Deruxtecan Analogues

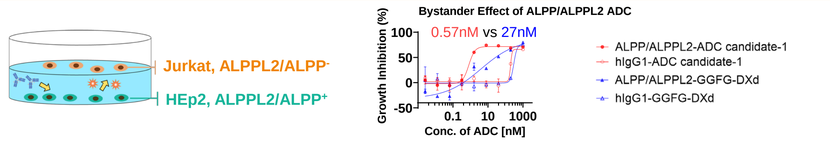
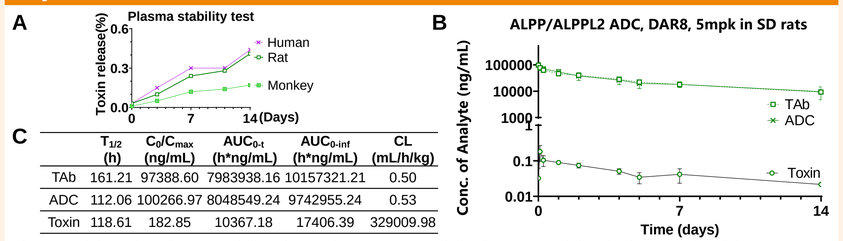


Figure 4. ALPP/ALPPL2+ adherent HEp2 cells were co-cultured with ALPP/ALPPL2- suspension Jurkat cells and treated with the indicated ADCs for 96 hours. Jurkat cell viability was quantified using the CTG assay to evaluate the bystander killing activity of indicated ADCs.

Hydrophilic Design of Hanjugator™ Confers Superior Stability and Low Payload Loss of ALPP/ALPPL2 ADC *in vitro* and *in vivo*



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	$T_{1/2}$ (h)	C_0/C_{max} (ng/mL)	AUC_{0-t} (h*ng/mL)	AUC_{0-inf} (h*ng/mL)	CL (mL/h/kg)
TAb	161.21	97388.60	7983938.16	10157321.21	0.50
ADC	112.06	100266.97	8048549.24	9742955.24	0.53
Toxin	118.61	182.85	10367.18	17406.39	329009.98

Figure 5. (A) *In vitro* plasma stability of ALPP/ALPPL2 ADCs following a 2-week incubation at 37 °C. (B) Pharmacokinetic profiles and (C) summary parameters of total antibody, intact ADC, and free payload following intravenous administration of ALPP/ALPPL2 ADC (5 mg/kg, DAR = 8) in Sprague–Dawley rats.

ALPP/ALPPL2 ADC Demonstrates Superior Antitumor Efficacy Compared with a Deruxtecan-Based ADC in CDX/PDX Models

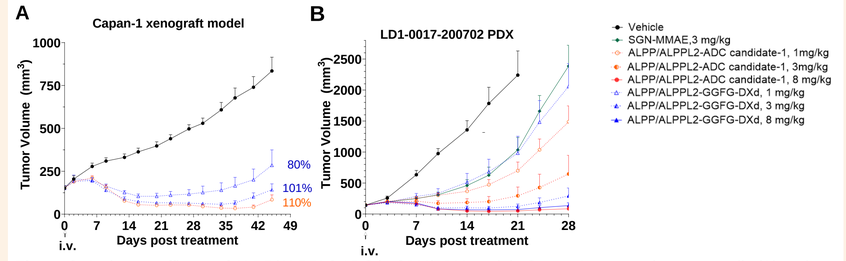
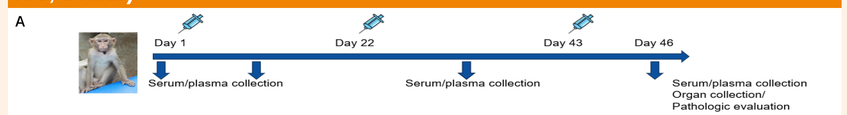


Figure 6. Antitumor efficacy of ALPP/ALPPL2 ADC in CDX/PDX models.Capan-1 pancreatic cancer cells (A) and LD1-0017-200702 gastric cancer PDX tumors (female, 72 years old) (B) were implanted subcutaneously into 6–8-week-old BALB/c nude mice. Treatment was initiated when tumor volume reached ~150 mm³. All ADCs were administered as a single intravenous dose on Day 0 (DAR 8, n = 6 per group).

High Tolerability of ALPP/ALPPL2 ADC in NHPs (HNSTD = 40 mpk, Q3W x 3, DAR8)



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Analyte	Day	C_{max} (ng/mL)	AUC_{0-t} (h*ng/mL)	$T_{1/2}$ (h)
TAb	1	927500 ± 145351	53350000 ± 9544457	119.25 ± 21.12
	22	1083750 ± 147061	62750000 ± 16162199	149 ± 37.03
Toxin	1	4.00 ± 0.88	415 ± 89.9	-
	22	4.95 ± 0.96	495 ± 161	-

Figure 7. Pilot toxicology study with companion toxicokinetic (TK) assessment of the ALPP/ALPPL2-ADC in cynomolgus monkeys.(A) Schematic overview of the pilot toxicology study design.(B) Toxicokinetic profiles of total antibody (TAb) and free payload following administration of ALPP/ALPPL2-ADC at 40 mg/kg.(C) Summary toxicokinetic parameters for the 40 mg/kg dosing group following single and repeated administrations.

Design and Differentiating Advantages of the ALPP/ALPPL2 ADC

Hydrophilic Unit — Linker — Topoi

- Sub-nanomolar binding affinity to ALPP/ALPPL2 with clean profile in MPA assay
- > 10× bystander effect vs. deruxtecan to address tumor heterogeneity
- Robust *in vivo* antitumor activity: superior to deruxtecan in a CDX model
- Efficacious across different ALPP/ALPPL2 expression levels
- NHP pilot toxicity studies, HNSTD = 40mpk Q3W x 3

Figure 8. Schematic overview of the rationally designed ALPP/ALPPL2-targeting ADC.

Disclosures: LR, ZL, YF, ZJ, YW, YX, SJ, CY, SG, QW, LZ, ZQ, YH, HC and YJ are employees of Henlius.

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