Discovery of a Novel Antibody-drug Conjugate Linker-payload with a Distinct Killing Mechanism via Prolonged Unfolded Protein Response Activation

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Background:

A specific class of compounds possessing the 3-(4-hydroxyphenyl)indoline-2-one pharmacophore demonstrated potent anti-tumor activity against cancer cells with elevated TRPM4 expression or estrogen receptor positivity through the sustained activation of unfolded protein response (UPR). This killing mechanism is distinct from existing toxins. This study aimed to optimize this scaffold for use as antibody-drug conjugate (ADC) payloads. By selecting antigens with low gastrointestinal expression, this strategy could potentially overcome ADC resistance or synergize with existing ADCs, leading to more durable patient responses and improved survival outcomes.

Methods:

The cytotoxicity of lead toxin (HLX91-048) and its related ADCs was evaluated in both immortalized cell lines and treatment-resistant patient-derived organoids (PDOs). Flow cytometry was employed to analyze the binding and internalization of the ADCs. *In vivo* efficacy was tested in the BT474 breast cancer model and the DS-8201 resistant NCI-N87R gastric cancer model. The tolerability of HLX91-048 based HER2 ADC was compared in parallel to a DS-8201 analog in rat pilot pre-toxicology studies.

Results:

HLX91-048 demonstrated sub-nanomolar to nanomolar IC_{50} values across tumor cell types. Its ADC exhibited enhanced cytotoxicity and superior plasma stability versus DS-8201 analogs. Dose-dependent tumor regression was observed in BT474 and NCI-N87R models. Notably, HLX91-048 based ADC retained sensitivity in some treatment-resistant PDOs that were unresponsive to DS-8201 analog. In rat pre-toxicology studies, both HLX91-048 based ADC and a DS-8201 analog were well-tolerated at 60 mpk weekly for three weeks.

Conclusions:

The HLX91-048 based linker-payload represents a first-in-class ADC platform featuring a highly differentiated killing mechanism that demonstrated exceptional efficacy and safety in preclinical evaluations. Preliminary toxicology studies in non-human primates have been scheduled.

Toxin HLX91-048 Based HER2 ADC And Its Unique Killing Mechanism

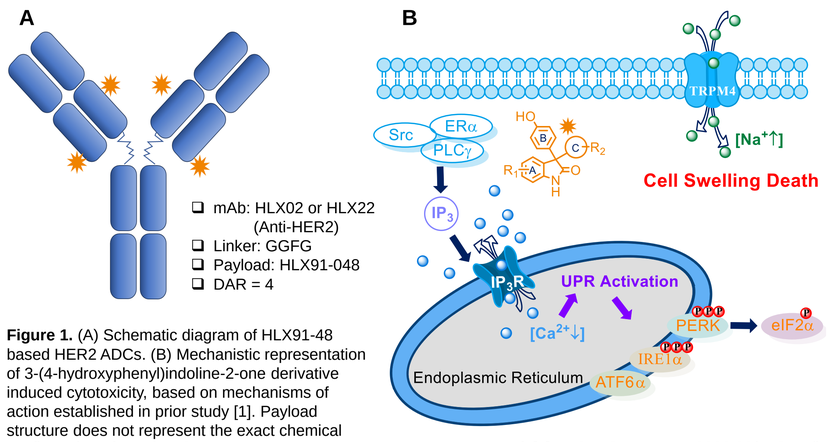


Figure 1. (A) Schematic diagram of HLX91-48 based HER2 ADCs. (B) Mechanistic representation of 3-(4-hydroxyphenyl)indoline-2-one derivative induced cytotoxicity, based on mechanisms of action established in prior study [1]. Payload structure does not represent the exact chemical configuration of HLX91-48.

Ref: [1] AACR; Cancer Res 2023;83(7_Suppl).

Toxin HLX91-048 and Its Conjugates Induced UPR Activation

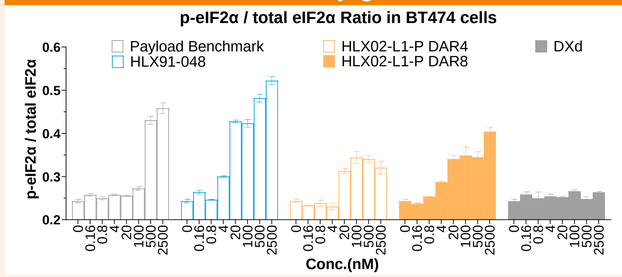


Figure 2. HER2⁺ BT474 cells were treated with escalating concentrations of indicated toxins and ADCs for 16 hours, then UPR activation was quantified via HTRF assay by measuring the ratio of phosphorylated eIF2α (p-eIF2α) to total eIF2α.

Cytotoxicity of Toxin HLX91-048 in ERα⁺ and ERα⁻ Cell Lines

ERα⁺ Cell line	MCF7^{Y537S}	PC-3	MDA-MB-361	BT-20	Caov-3	HCC1428
IC_{50} (nM)	1.1	0.4	1.2	0.8	4.2	1.9
I_{max} (%)	96.6	93.9	100	99.5	92.1	53.9
ERα⁻ Cell line	BT-549	IGROV-1	HCC116	HL-60	MDA-MB-231	HT29
IC_{50} (nM)	3.2	1.9	1.6	>500	>500	>500
I_{max} (%)	84.8	71.8	46.4	10.5	0.7	19.8

Table 1. Toxin HLX91-048 exhibited potent activities against most of ERα⁺ cell lines and some ERα⁻ cell lines.

In Vitro Characterization of HLX02-L1-P (HLX91-48 Based HER2 ADC)

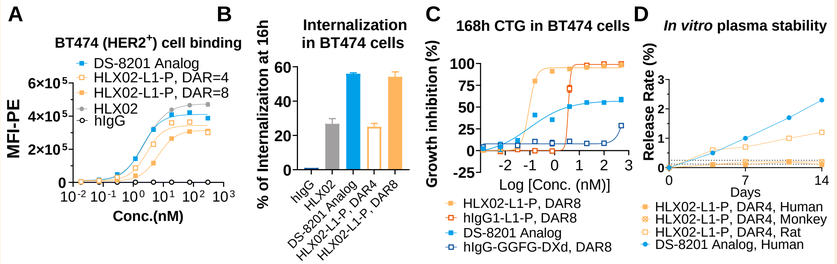
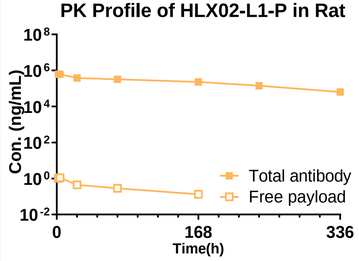


Figure 3. *In vitro* profiling of HLX02-L1-P binding affinity (A), internalization rates (B), and cytotoxic activity (C) of ADCs against HER2⁺ BT474 cells. (D) Plasma stability of ADCs across preclinical species.

Stability of Linker L1-P Confirmed by Low Levels of Circulating Payload



Analyte	Total antibody	Free payload
$T_{1/2}$ (h)	280	83.7
C_0 or C_{max} (ng/mL)	614123.8	1.099
AUC_{last} (h*ng/mL)	121008580	57.4
$AUC_{0\text{-}inf}$ (h*ng/mL)	146457794	73.3
CL (mL/h/kg)	0.205	-
$MRT_{0\text{-}inf}$ (h)	366	103
V_{ss}(mL/kg)	74.9	-

Figure 4. Pharmacokinetic profiles of total antibody and free payload following intravenous administration of HLX02-L1-P (30 mg/kg, DAR=4) in Sprague-Dawley rats.

HLX91-048 Based HER2 ADCs Demonstrated Potent Antitumor Efficacy in BT474 Xenograft and DS-8201 Resistant NCI-N87R Models

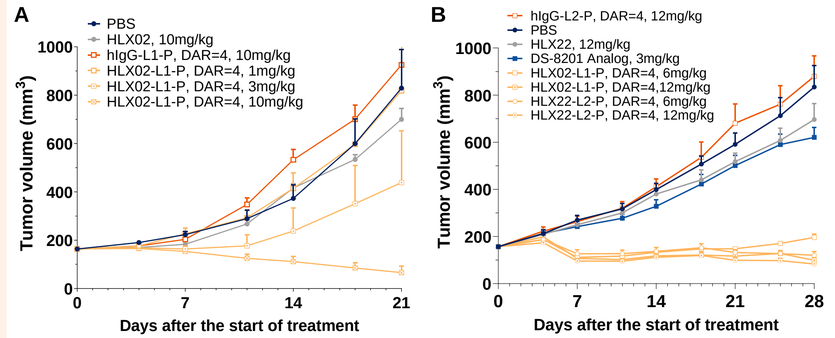


Figure 5. HER2 ADCs conjugated with HLX91-048-derived linker-payloads (L1-P & L2-P) demonstrate dose-dependent antitumor efficacy in HER2⁺ BT474 model (A) and DS-8201-resistant NCI-N87R model (B). All ADCs were given intravenously once weekly.

HLX02-L1-P and HLX22-L2-P Were More Potent than DS-8201 Analog Against Treatment Resistant Patient-derived Organoids *in Vitro*

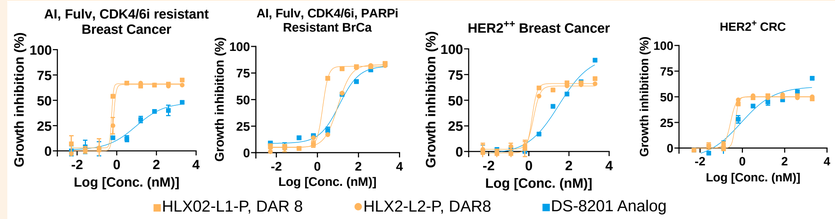


Figure 6. Cytotoxic efficacy of indicated ADCs against different PDOs assessed via CTG assay after 144h treatment

HLX02-L1-P Exhibited Favorable Tolerability at 60 mpk QW×3 in SD Rats

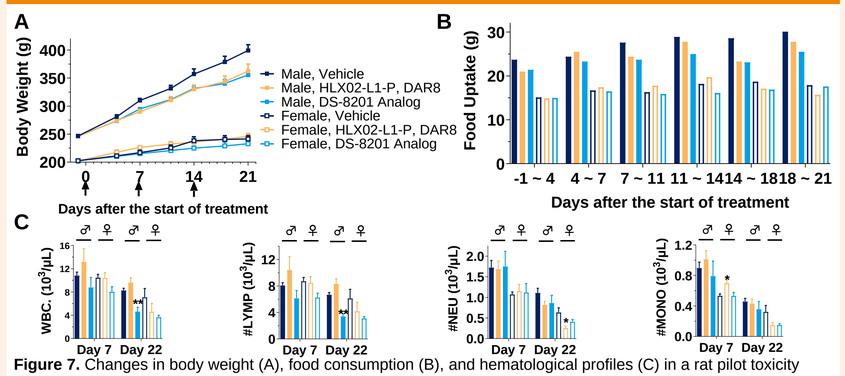


Figure 7. Changes in body weight (A), food consumption (B), and hematological profiles (C) in a rat pilot toxicity study comparing HLX02-L1-P and DS-8201 Analog. ADCs were given intravenously once weekly for 3 weeks (n=5/sex/group). Data presented as mean ± SEM; *p<0.05, **p<0.01 vs. control.

Disclosures: LR, CY, JC, ZY, ZX, ZW, TX, ZY, YF, HD, HS, SJ, YJ, YW, YX, MZ and HC are employees of Henlius.

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