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 HLX

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

A B

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).

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

A B C D

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.

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

A B

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*

HLX02-L1-P, DAR 8 HLX2-L2-P, DAR8 DS-8201 Analog

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