A Best-in-Class HER2xHER2 Novel Biparatopic Antibody-Drug Conjugate with an Efficacious, Low-Toxicity Design that Maximizes Antibody Functionality

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Background

HLX22 is a novel anti-HER2 antibody that binds to a unique site on HER2 subdomain IV, which is different from trastuzumab. When combined with HLX02 (a trastuzumab biosimilar), HLX22 increases internalization of HER2 homodimers and HER2/EGFR heterodimers, thereby reducing cell proliferation signals. This combination showed stronger antitumor effects than HLX02 plus HLX11 (a pertuzumab biosimilar) both *in vitro* and *in vivo*. and it has demostrated benefits in clinical trails. Based on the binding sites of HLX22 and HLX02, we developed a biparatopic antibody-drug conjugate (ADC) with an effective, low-toxicity payload, allowing higher dosing and better receptor coverage to improve tumor cell killing.

Method

Cytotoxicity of HER2xHER2 biparatopic ADC was assessed using the CellTiter-Glo (CTG) assay in tumor cell lines. The binding and internalization of the ADC were evaluated by flow cytometry in cells with varying HER2 expression levels. *In vivo* efficacy of HER2xHER2 biparatopic ADC was evaluated in JIMT-1 and patient-derive xenografts models. Preliminary toxicology in cynomolgus monkeys was assessed at a dose of 120 mg/kg, administered every three weeks for three cycles.

Maximizing Antibody Function of ADC by Fine Tune Payload potency

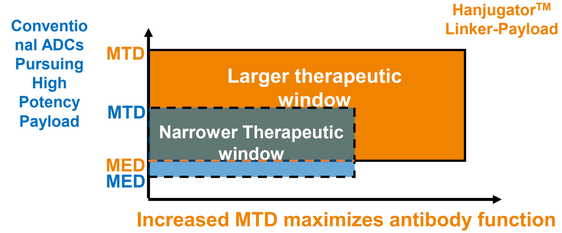


Figure 1. Schematic representation illustrating how precisely calibrated payload potency improves ADC therapeutic window by maximizing antibody-mediated pathway blockade.

Figure 2. A novel HER2 epitope antibody is used to generate the biparatopic antibody

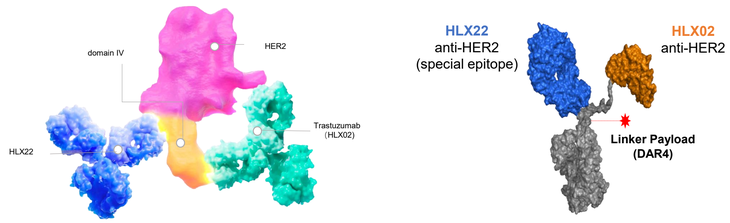
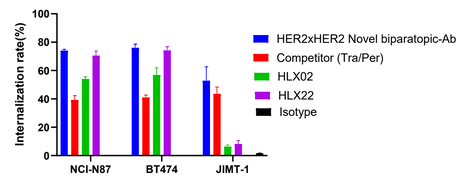
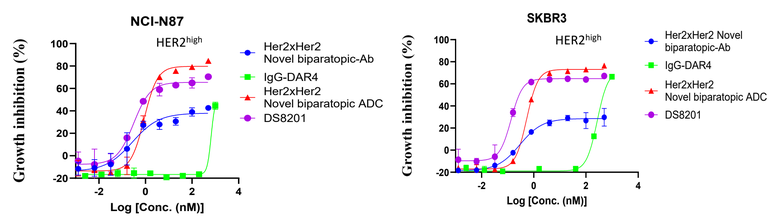


Figure 3. The novel HER2xHER2 biparatopic antibody induces greater internalization compared with competitor and HLX02 in both HER2-high/moderate cells



Sample	NCI-N87	BT474	JIMT-1
Her2xHer2 Novel biparatopic ADC-Ab	74.05	75.97	52.82
Competitor (Tra/Per)	39.25	40.99	43.5
HLX02	54.04	56.87	6.25
HLX22	70.54	74.09	8.18
Isotype	0.09	0.03	1.67

Figure 4. The HER2xHER2 novel biparatopic ADC demonstrated strong antibody inhibition ability and potent cytotoxicity



Compound	IC50 (nM)
Her2xHer2 Novel biparatopic-Ab	0.27
IgG-DAR4	662.4
Her2xHer2 Novel biparatopic-ADC	0.88
DS8201	0.31

Compound	IC50 (nM)
Her2xHer2 Novel biparatopic-Ab	0.29
IgG-DAR4	270
Her2xHer2 Novel biparatopic-ADC	0.53
DS8201	0.13

Figure 5. Novel HER2xHER2 biparatopic ADC showed a significantly better efficacy than DS-8201 in JIMT-1 model at a lower HNSTD dosage.

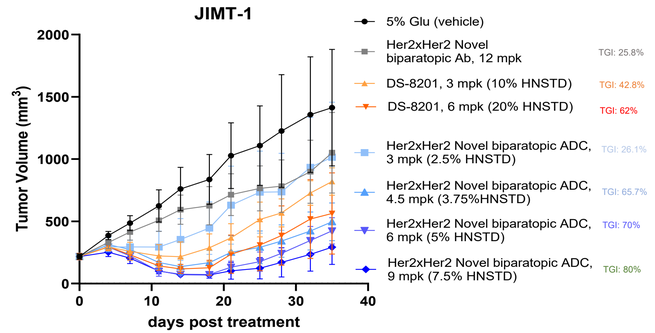
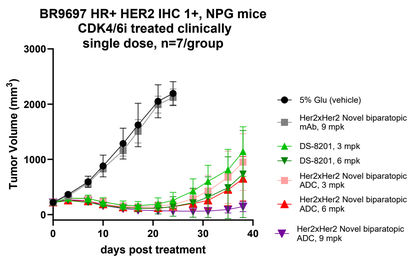


Figure 6. More sustained tumor inhibition is achieved by Her2xHer2 novel biparatopic ADC treatment compared with DS - 8201 in the HER2 low - expression tumor model at a lower HNSTD dosage.



Until day 38, high dose of Her2xHer2 novel biparatopic ADC (DAR4) at 9 mpk (7.5% HNSTD)showed a trend of superior efficacy than DS-8201 (DAR8) at 6 mpk (20% HNSTD). More tumor regression in Her2xHer2 novel biparatopic ADC (DAR4) than DS-8201.

Conclusion

Results: The HER2xHER2 biparatopic ADC demonstrated superior internalization efficiency than competitor (a HER2-targeted bispecific antibody) and trastuzumab deruxtecan in BT474 and NCI-N87 cell lines. The biparatopic ADC showed better anti-tumor efficacy than trastuzumab deruxtecan across multiple cell lines. The ADC induced significant tumor regression at a single 6 mg/kg dose in various xenograft models outperforming trastuzumab deruxtecan head-to-head, including HER2-positive and HER2-low models. Preliminary toxicology shows good tolerability in cynomolgus monkeys following 3 doses of the ADC at 120 mg/kg.

Conclusion: We have developed a potential best-in-class HER2xHER2 novel biparatopic ADC that exhibits a superior therapeutic index, antibody-mediated signaling blockade. The preclinical findings support clinical development in breast and gastric cancers, with the hope that the improved therapeutic index of this agent confers survival benefit.

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