Preclinical Studies Reveal that Protease-Mediated Payload Release and Immuno-Oncology/Immunomodulatory Effects Contribute to the Antitumor Activity of HLX43

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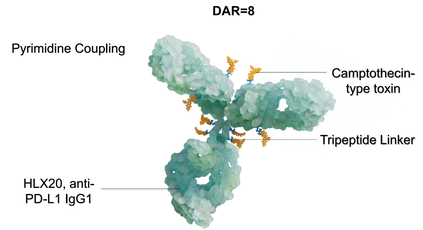
Introduction

Despite the clinical success of programmed cell death protein 1/programmed death-ligand 1 (PD-1/PD-L1) inhibitors in cancer treatment, resistance driven by spatial tumor heterogeneity and an immunosuppressive tumor microenvironment (TME) remains a critical challenge[1]. To overcome this, HLX43 was engineered to uniquely integrate targeted chemotherapy with immuno-oncology principles. It directly addresses heterogeneity through a dual mechanism: the antibody backbone blocks PD-1/PD-L1 to reactivate immunity, while its TME-activatable linker is cleaved by extracellular proteases such as matrix metalloproteinases (MMPs) and cathepsins (CTSL/B). This liberates a potent payload, exerting a robust bystander effect to eradicate adjacent PD-L1-negative cells[2]. Furthermore, payload-induced immunogenic cell death (ICD) synergizes with checkpoint blockade to comprehensively remodel the TME[3].

Molecular Structure of HLX43



HLX43 is a novel PD-L1 targeting ADC. It comprises HLX20, an engineered anti-PD-L1 humanized IgG1 Fc mute antibody. This antibody is conjugated via a TME-activatable tripeptide linker (TMALIN®) to a potent camptothecin-derived topoisomerase I inhibitor payload (C24) at a drug-to-antibody ratio (DAR) of 8.

This specific design confers a dual mechanism of action (MOA), integrating targeted chemotherapy with immuno-oncology principles:

- **Immune Restoration:** The antibody component blocks the PD-1/PD-L1 interaction to potentially restore antitumor immunity.
- **Targeted & Bystander Killing:** The linker is cleavable by TME-enriched proteases, such as cathepsins B and L. This facilitates direct tumor cell killing upon ADC internalization, while also allowing efficient extracellular payload release to exert a potent bystander effect on neighboring, heterogeneous cancer cells.

Study Objective: Elucidating the MOAs of HLX43

Previously reported studies demonstrated that HLX43 retains the favorable stability and binding affinity of the parental HLX20 antibody[4]. Here, we comprehensively describe the multifaceted MOAs of HLX43, integrating immune checkpoint blockade with cytotoxic payload–mediated antitumor effects.

Results

HLX43 demonstrates potent cytotoxicity and bystander killing against tumor cells, inducing DNA damage, apoptosis, and immunogenic cell death. Importantly, its payload release is specifically activated by TME-enriched proteases (MMPs, CTSL/B), safely sparing PD-L1+ immune cells. Furthermore, HLX43 blocks PD-1/PD-L1 signaling, significantly increasing cytotoxic CD8+ T cell infiltration while reducing immunosuppressive Tregs. Together, these multifaceted mechanisms result in robust *in vivo* tumor growth inhibition across multiple models, remarkably independent of baseline PD-L1 expression. .

HLX43 exhibits potent cytotoxicity and bystander killing on tumor cells

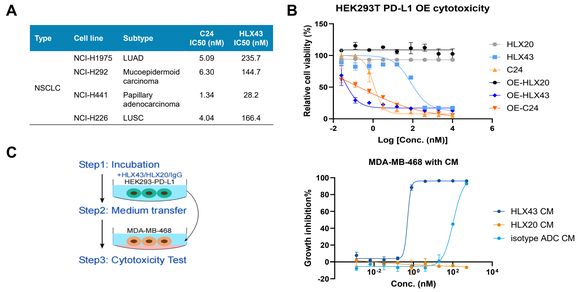


Figure 1. The *in vitro* cytotoxicity of HLX43. A. HLX43 exhibits cytotoxicity in various NSCLC cells with different PD-L1 expression. All cell lines were treated as indicated for 96 hours and cell viability was assessed by CTG assay. **B.** Enhanced tumor cell killing activity of HLX43 in PD-L1 overexpressing/positive tumor cells. OE-PD-L1 overexpression. C24: payload. **C.** The bystander cytotoxicity was evaluated in PD-L1 negative MDA-MB-468 cells with HLX43 treated condition medium.

HLX43 exhibits potent in vivo tumor growth effects independent of PD-L1 expression

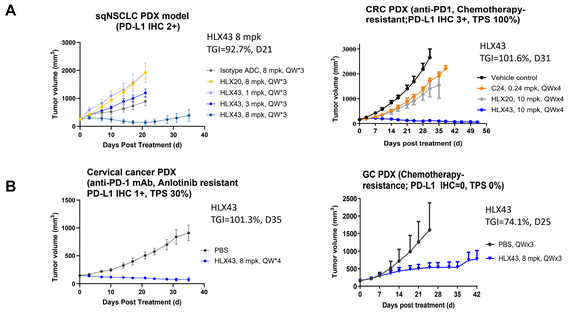


Figure 2. HLX43 exhibits anti-tumor activity independent of PD-L1 expression in PD-1 mAb or chemotherapy resistant PDX models. **A**. HLX43 exhibits dose dependent and potent anti-tumor efficacy in PD-L1 positive sqNSCLC and CRC tumors. **B**. HLX43 inhibited tumor growth *in vivo* in cervical cancer and gastric cancer PDX without PD-L1 expression.

HLX43 exhibits topoisomerase inhibitor ADC hallmarks: DNA damage–driven apoptosis and immunogenic cell death

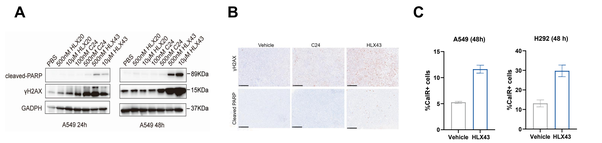


Figure 3. HLX43 induces DNA damage, cell apoptosis and immunogenic cell death.
A. Western blot of DNA damage markers including cleaved PARP and phospho-gamma-H2AX in A549 cells with HLX43 or C24 incubation 24 and 48 hours after treatment. **B.** IHC of cleaved PARP and phospho-gamma-H2AX in NSCLC PDX with HLX43 treatment. **C.** HLX43 causes increased surface calreticulin in NSCLC cells.

Cytotoxicity of HLX43 is enhanced by protease-mediated payload release

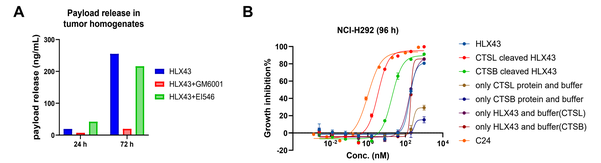


Figure 4. HLX43 payload release is regulated by MMPs and cathepsin (CTSL and CTSB enzymes. A. The release of payload was measured in breast tumor homogenates with HLX43 or HLX43 plus enzyme inhibitors. HLX43 payload release could be inhibited by GM6001 (pan-MMP inhibitor) but not by EI546 (elastase inhibitor). **B.** HLX43 *in vitro* cytotoxicity in NCI-H292 cells is enhanced by incubation of CTSL or CTSB with HLX43.

Activated and PD-L1-positive T cells, dendritic cells, and macrophages are resistant to HLX43 in vitro

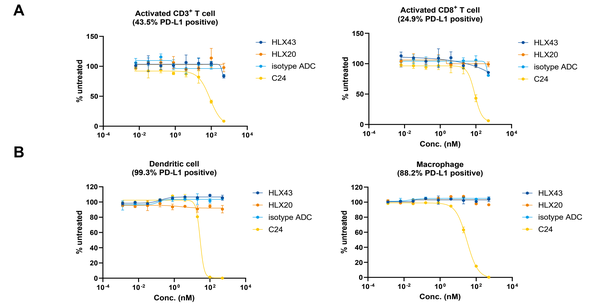


Figure 5. Immuno-toxicity of HLX43 on immune cells. A. *In vitro* isolated CD3⁺T and CD8⁺T were treated with PHA for PD-L1 induction. **B.** Dendritic cell and macrophage were isolated from human PBMC and differentiated, activated by IFNγ for PD-L1 induction. The % of PD-L1 positive cells were analyzed by FACS. Comparing to the payload C24, HLX43 did not present target specific cytotoxicity on PD-L1 positive immune cells.

HLX43 retains checkpoint inhibition activity, suppressing Tregs and promoting tumoral T-cell infiltration and cytotoxicity

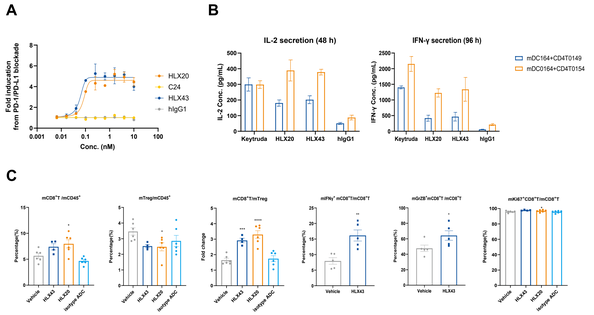


Figure 6. HLX43 blocks PD-1/PD-L1 signaling and promotes immune cell activation.
A. HLX43 blocks PD-1/PD-L1 signaling as potent as its parental HLX20, anti-PD-L1 mAb, *in vitro* in Jurkat-PD-1/NFAT-Luc reporter assay. **B**. HLX43 acts similar to HLX20 and promotes T cell cytotoxic cytokines including IL-2 and IFNγ secretion in MLR assay with two human PBMC donors. **C**. Tumoral immune cell profiling in hPD-L1 OE MC38 CDX in hPD-L1 knock-in mice with HLX43, isotype ADC or HLX20 treatment. HLX43 increased tumor CD8⁺T cell infiltration and Treg reduction as indicated by increased CD8⁺T/Treg ratio. The cytotoxic IFNγ-positive and Granzyme B-positive CD8⁺T cell% also increased after HLX43 treatment. The proliferating CD8⁺T was not changed significantly by HLX43 at 2~2.5 mg/kg.

Conclusions

- **Convergent Antitumor Mechanisms:** HLX43 integrates immune checkpoint blockade with cytotoxic payload–mediated activity. It induces direct tumor cell killing through DNA damage–driven apoptosis, while protease-mediated extracellular linker cleavage in the tumor microenvironment enables payload diffusion and a potent bystander effect on neighboring PD-L1–negative cancer cells.
- **Profound TME Remodeling:** Beyond direct cytotoxicity, HLX43 induces immunogenic cell death (ICD), evidenced by calreticulin upregulation, and reactivates T-cell responses through PD-1/PD-L1 blockade. In vivo studies confirm this TME remodeling, showing increased infiltration of cytotoxic CD8⁺ T cells with enhanced effector function (elevated IFNγ and Granzyme B), along with a marked reduction in immunosuppressive Tregs.
- **Therapeutic Promise & Synergy:** This unique combination of targeted chemotherapy and immuno-oncology mechanisms drives robust efficacy even in PDX models with heterogeneous PD-L1 expression. HLX43's convergent mechanisms position it as a promising therapeutic candidate with strong potential to synergize with other immuno-oncology agents and enable broader, more durable clinical responses across multiple tumor types.

Reference

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Disclosures

All authors are employees of Henlius.

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