A novel anti-CD3xCD28xSTEAP1 trispecific T-cell engager (HLX3902) with enhanced and durable antitumor responses in prostate cancer

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Background

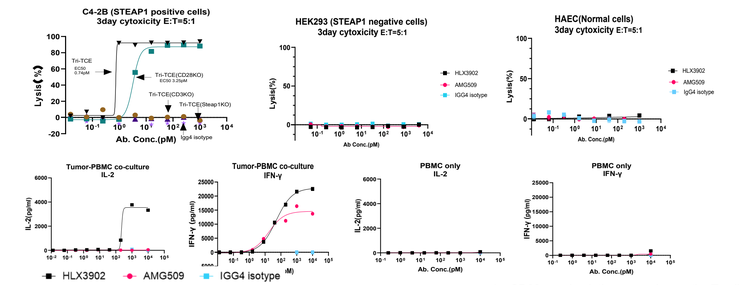
Immunotherapy has demonstrated limited efficacy in prostate cancer, largely due to the immunosuppressive tumor microenvironment, poor T-cell infiltration, and a lack of robust tumor-specific targets [1,2]. To overcome these barriers, we developed HLX3902, a CD3×CD28×STEAP1 trispecific T-cell engager, which redirects and fully activates T cells by delivering both primary (CD3) and co-stimulatory (CD28) signals directly to prostate cancer cells that highly express STEAP1, thereby enhancing T-cell activation, proliferation, persistence, and antitumor efficacy. Preclinical evaluation showed that HLX3902, with optimized CD3 and CD28 signaling domains, exhibited potent target-dependent T-cell activation and cytotoxicity *in vitro*. It demonstrated superiority over a comparator T-cell bispecific engager, as an inducer of enhanced T-cell activation, proliferation and in sustained tumor cell killing. Consistent with its intended purpose, the incorporation of CD28 co-stimulation led to improved T-cell persistence and functionality in repeated antigen challenge assays. *In vivo*, HLX3902 exhibited superior and sustained antitumor activity in both C4-2/hPBMC xenograft and abiraterone-resistant PDX/hPBMC models, accompanied by increased T-cell infiltration, activation, and persistence in the tumor microenvironment. Importantly, toxicology studies in cynomolgus monkeys showed that HLX3902 was well tolerated, with a manageable safety profile supporting further clinical development.

Key words: Trispecific T cell engager; CD28 co-stimulation; STEAP1; Prostate cancer

Method

T cell–dependent cellular cytotoxicity (TDCC) was assessed across a range of effector-to-target (E:T) ratios. Target cell viability was quantified via luciferase assay or flow cytometry after 3 to 5 days of co-culture. Concurrently, cytokine secretion in supernatants was analyzed using a multiplex bead-based flow cytometric assay. To evaluate T-cell proliferation and memory formation, PBMCs were co-cultured with C4-2B cells for 3.5 and 5 days and subsequently analyzed by flow cytometry. The sustained cytotoxic potency of T cells was examined in a repeated antigen challenge model, wherein T cells were serially re-stimulated with fresh LNCaP-Luc cells and 5 nM antibody. After each cycle, cytotoxicity, T-cell activation, proliferation, and memory subset expansion were evaluated using luciferase assay and flow cytometry. In vivo, antitumor efficacy was investigated in C4-2/hPBMC xenograft and abiraterone-resistant PDX models. Additionally, a pilot pharmacokinetic and toxicity study was performed in cynomolgus monkeys.

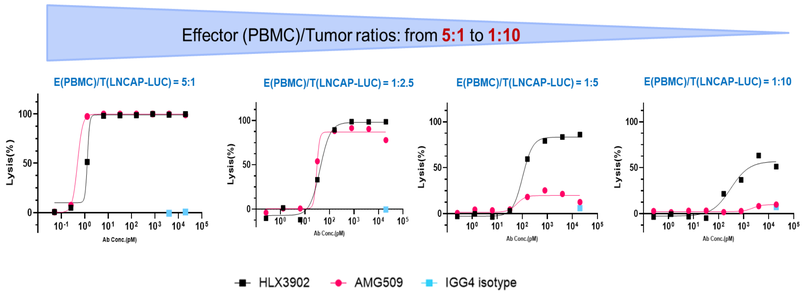
Figure 1. HLX3902 Exhibited Target-dependent T-cell activation and Cytotoxicity



✓ CD3 Activation is STEAP1-dependent ✓ CD28 Activation is CD3-dependent ✓ CD28 co-stimulation can enhance the T cell activation and cytotoxicity

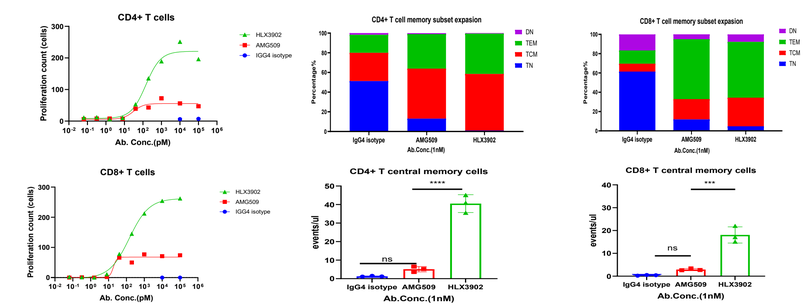
1.Kwon, W.-A.; Joung, J.Y. Cancers 2025, 17, 1064.
2. Kelly WK. et al. Cancer Discov. 2024 Jan 12;14(1):90-103.

Figure 2. HLX3902 Exhibited Better Cytotoxicity Potency at Low E/T Ratios Compared with Bispecific T Cell Engager

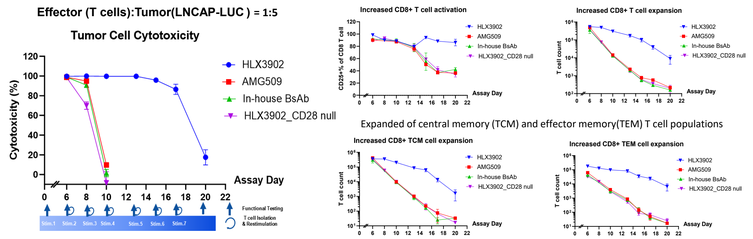


- HLX3902 displays superior cytotoxic potency in low E:T co-cultures.

Figure 3. Enhanced T Cell Function and Sustained In Vitro Cytotoxicity Compared with Bispecific T Cell Engagers

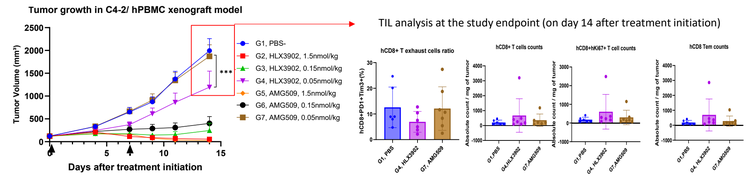


- HLX3902 demonstrates superior T cell proliferation and enhanced expansion of memory T cells.



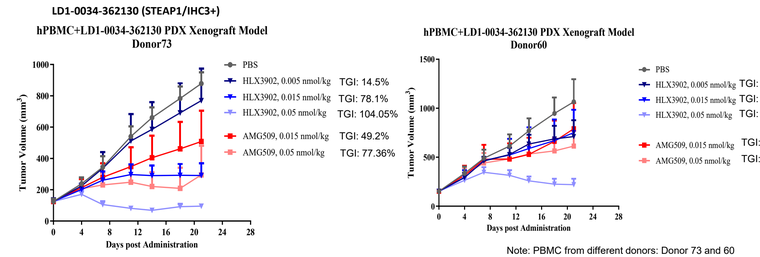
- HLX3902 Trispecific with CD28 showed more sustained cytotoxicity compared to CD3 bispecific in repeated antigen challenge assays. This was accompanied by more persistent T-cell activation, proliferation, and expansion of TCM and TEM.

Figure 4. Anti-tumor activity of HLX3902 in C4-2/hPBMC Xenograft Model



- HLX3902 exhibited superior antitumor activity relative to comparator T Cell Bispecific, accompanied by increased T-cell infiltration and persistence in the tumor microenvironment.

Figure 5. Anti-tumor activity of HLX3902 in an Abiraterone Resistant PDX/hPBMC model



Note: PBMC from different donors: Donor 73 and 60

- HLX3902 showed better efficacy within a dose range. At a dose of 0.05 nmol/kg (0.01 mg/kg), which is the target clinical dose of AMG509, HLX3902 exhibited significantly stronger efficacy than AMG509.

Conclusion

- HLX3902, a novel trispecific T-cell engager with optimized CD3 and CD28 signaling, effectively co-engages T cells and STEAP1-expressing tumor cells.
- HLX3902 elicits potent, target-dependent T-cell activation and cytotoxicity, demonstrating enhanced tumor cell killing at low effector-to-target (E:T) ratios *in vitro*.
- HLX3902 promotes T-cell proliferation and survival. The incorporation of CD28 signaling significantly improves T-cell persistence and sustained functional activity against repeated antigen challenges.
- HLX3902 demonstrated superior antitumor activity in both C4-2/hPBMC xenograft and abiraterone-resistant PDX/hPBMC models. In the C4-2/hPBMC model, this enhanced efficacy was associated with increased T-cell infiltration, proliferation, and persistence in the tumor microenvironment.
- HLX3902 was well-tolerated in cynomolgus monkeys, supporting potential for clinical development, It is positioned as a best - in - class molecule with an Investigational New Drug submission in Q1 2026.

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