# A novel anti-PD-L1/VEGF bispecific antibody (HLX37) with immune checkpoint inhibition, anti-angiogenic, and antineoplastic activities

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## Background

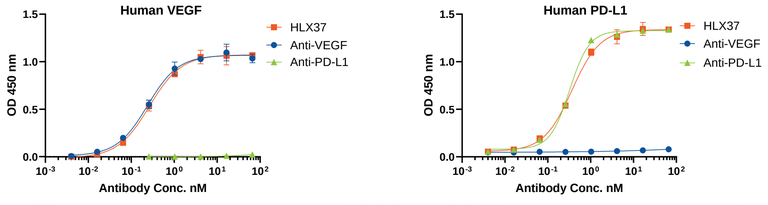
Dual blockade of PD-(L)1 and VEGF has demonstrated significant clinical benefits in several cancer types[1-3]. Bispecific antibodies targeting PD-L1 and VEGF enhance anti-VEGF delivery to tumor sites by binding to tumor PD-L1, promoting synergistic effects of blood vessel normalization and T cell activation within the tumor microenvironment, thus improving anti-tumor activity[4,5]. Here we developed HLX37, a rationally designed PD-L1/VEGF bispecific antibody, featuring an entirely exposed PD-L1 VHH nanobody that facilitates antigen-mediated drug deposition, and confers excellent drug developability. The optimized interdomain linker ensures the bispecific antibody can bind and block both targets with full activity. In the presence of VEGF, HLX37 dramatically promoted cell surface PD-L1 binding and internalization. In addition, VEGF also enhanced PD-1/PD-L1 signal blocking in the reporter gene assay. In vivo anti-tumor studies demonstrated that HLX37 inhibited tumor growth of MDA-MB-231 and NCI-H292 in a dose-dependent manner. Compared to the combination group, HLX37 showed increased tumor distribution, which may be mediated by both PD-L1 and VEGF. In the toxicity study, HLX37 was well tolerated in cynomolgus monkeys after intravenous infusion at 100 mg/kg once weekly for 3 doses. These data suggested that HLX37 has strong preclinical efficacy and favorable safety profile, with enhanced tumor enrichment. HLX37 holds promising potential for further application in various types of cancer.

**Key words**: Anti-PD-L1/VEGF; Immune Checkpoint Inhibition; Anti-Angiogenic

## Method

Flow cytometry and ELISA assays were employed to evaluate the binding of the bispecific antibody. PD-L1 or VEGF blockade assays were conducted using PD-1-Jurkat cells/PD-L1-APC-CHOK1 or KDR cells. Efficacy was assessed in CDX models with immune humanization, including MDA-MB-231 and NCI-H292 tumor cell lines. Pilot pharmacokinetic (PK) and toxicity studies were performed in cynomolgus monkeys.

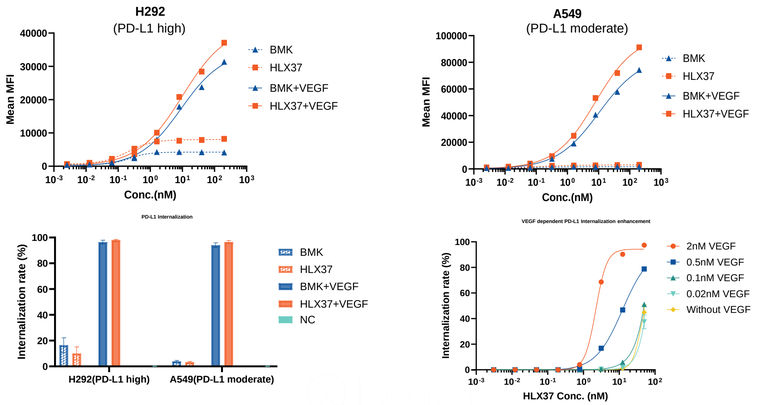
### *Figure 1. Antigen binding activity of HLX37 to human VEGF and PD-L1 protein*




- HLX37 showed similar activity with anti-VEGF and PD-L1 monoclonal antibodies (mAbs) on target binding.

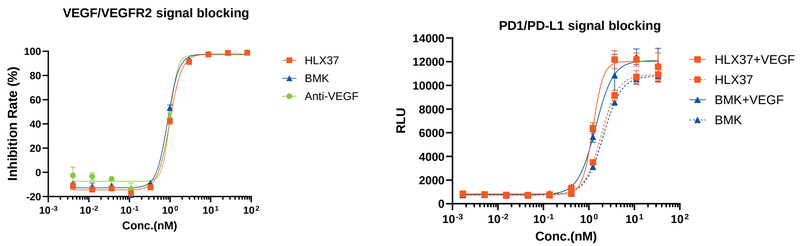
1. Zhou, C. et al. Journal of Thoracic Oncology, Volume 19, Issue 10, S1
2. Wu, J. et al. Annals of Oncology, Volume 35, S361
3. Wu, Y-L. et al. Annals of Oncology, Volume 35, S804
4. Andrew C. Dudley and Arjan W. Griffioen. Angiogenesis (2023) 26:313–347
5. Bourhis, Morgane et al. Frontiers in immunology vol. 12 618837. 19 Mar. 2021

### *Figure 2. Enhanced binding and internalization activity of HLX37 in the presence of VEGF*




- VEGF enhanced binding and internalization of HLX37 to cancer cells with different PD-L1 expression levels.

### *Figure 3. Signal blocking activity of HLX37 determined by reporter gene assay*

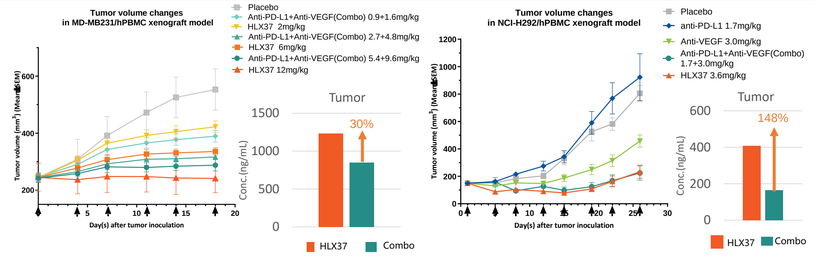



| Protein | HLX37 | BMK | Anti-VEGF |
|---|---|---|---|
| $EC_{50}$ (nM) | 0.99 | 0.87 | 1.0 |
| Max inhibition (%) | 97.76 | 97.69 | 97.06 |

| Protein | HLX37 + VEGF | HLX37 | PM8002 + VEGF | PM8002 |
|---|---|---|---|---|
| $EC_{50}$ (nM) | 1.23 | 1.88 | 1.40 | 2.10 |
| Top signal | 12027 | 10886 | 12101 | 10781 |

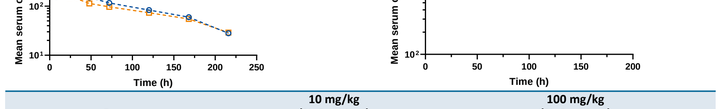
- HLX37 possesses mAb similar VEGF blocking activity and enhanced PD-L1 signal blocking in the presence of VEGF.

### *Figure 4. Anti-tumor activity of HLX37 in PBMC humanized mouse CDX models*




- HLX37 showed significant anti-tumor activity and tumor enrichment in MDA-MB-231 and NCI-H292 CDX models. (N=8/group, Data presented as mean ± SEM)

### *Figure 5. HLX37 showed favorable PK and safety profiles*




| Doses | | 10 mg/kg (N=2, Male) | 100 mg/kg (N=2, Male) | |
|---|---|---|---|---|
| | | Single dose | 1st dose | 3rd dose |
| PK/TK | $t_{1/2}$ (h) | 83.5 | 121.1 | 96.0 |
| | $C_{max}$ (μg/mL) | 243.8 | 2823.6 | 3372.6 |
| | $AUC_{last}$ (h*μg/mL) | 20592.5 | 206711.8 | 278230.3 |
| | Accumulation index | N/A | 1.35 | |

The PK/TK parameters were mean value. N/A, not applicable.

- No abnormal findings were noted on body weight, food consumption, clinical observation, ECG, blood pressure, clinical pathology, immunophenotyping and gross necropsy. Maximal tolerance dose (MTD) was 100 mg/kg.
- The drug exposures increased in a dose-proportional manner.
- No drug accumulation was noted after repeated dose.

## Conclusion

- HLX37, a rationally designed PD-L1/VEGF bispecific antibody, can bind both VEGF and PD-L1 with full activity.
- VEGF significantly enhanced binding and internalization of HLX37 to cancer cells with different PD-L1 expression levels.
- HLX37 possesses mAb similar VEGF blocking activity and enhanced PD-L1 signal blocking in the presence of VEGF.
- HLX37 showed significant anti-tumor activity and tumor enrichment in MDA-MB-231 and NCI-H292 CDX models.
- HLX37 was well tolerated in cynomolgus monkeys with MTD of 100 mg/kg.



Disclosures: SG, XX, WX, YX, GC, ZS, WH, HC, WQ, LJ, YJ and ZJ are employees of Henlius.

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