A Symmetrical Hexavalent Trispecific Antibody, HH160, Targeting PD-1, CTLA-4 and VEGF-A for Enhanced Anti-Cancer Effects

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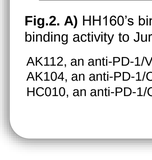
Introduction

Antiangiogenic agents, such as the anti-vascular endothelial growth factor A (VEGF-A) antibody Bevacizumab, are among the first targeted therapies in cancer treatment[1,2]. Immune checkpoint inhibitors (ICIs), including anti-PD-1/L1 and anti-CTLA-4 antibodies, have revolutionized cancer therapy[3,4]. Combinations of anti-VEGF agents and ICIs have demonstrated enhanced efficacy in treating various types of cancers, including hepatocellular carcinoma, non-small cell lung cancer, and cervical cancer, indicating synergistic anti-tumor mechanisms[5,6,7]. Here, we report a novel symmetrical hexavalent trispecific antibody (TriAb), HH160, which simultaneously targets PD-1, CTLA-4, and VEGF-A. This design aims to enhance therapeutic effects, increase dosing convenience, achieve tumor-specific drug distribution, and reduce treatment-related side effects.

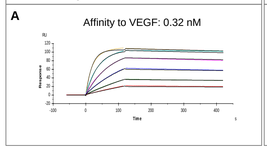
HH160 (anti-PD-1/CTLA-4/VEGF-A TriAb)



- A symmetrical structure with a hexavalent IgG1 isotype. Anti-PD-1 and anti-CTLA-4 nanobodies fused to the N- and C-termini of a conventional anti-VEGF-A antibody, respectively
- The Fc region is engineered to minimize effector functions such as ADCC, ADCP, and CDC, inducing much less secretion of irAEs related cytokines, such as IL-6, IL-8, potentially leading to reduced side effects

HH160 exhibits binding and blocking activities comparable to those of Bevacizumab

HH160 showed similar VEGF-A binding affinity to that of Bevacizumab



HH160 showed comparable VEGF/VEGFR2 inhibition activity to reference Abs

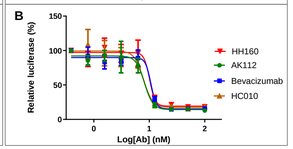
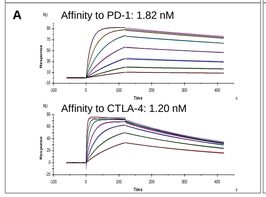


Fig.1. A) HH160's binding activity to VEGF-A analyzed using SPR. **B)** HH160's blocking activity analyzed using a luciferase reporter assay. HH160 blocked VEGF-A binding to luciferase-expressing HEK293T-VEGFR2 cells, resulting in reduced relative luciferase activity.

HH160 effectively binds to both PD-1 and CTLA-4

Monovalent binding of HH160 to PD-1 and CTLA-4



HH160's effective binding to Jurkat-PD-1-CTLA-4 cells

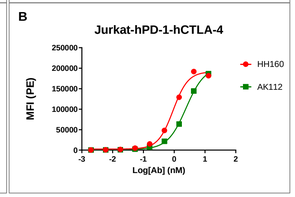


Fig.2. A) HH160's binding activities to PD-1 and CTLA-4 analyzed using SPR; **B)** HH160's binding activity to Jurkat-PD-1-CTLA-4 cells analyzed using FACS.

AK112, an anti-PD-1/VEGF BiAb (bispecific antibody), sequence was sourced from AK112 sequence;
AK104, an anti-PD-1/CTLA-4 BiAb, sequence was sourced from AK104 sequence;
HC010, an anti-PD-1/CTLA-4/VEGF-A TriAb, sequence was sourced from patent (WO2017193032A2).

HH160 synergistically and potently blocks the activities of both PD-1 and CTLA-4

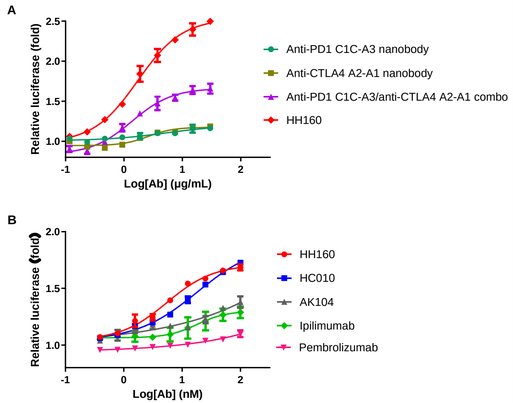


Fig. 3. Blocking activity of HH160 in a luciferase reporter assay. A) HH160 synergistically blocked the binding of Jurkat-PD-1-CTLA-4 cells (target cells) to luciferase-expressing Raji-PD-L1 reporter cells (effector cells), resulting in stronger luciferase activity compared to individual antibodies (anti-PD-1 C1C-A3 or anti-CTLA-4 A2-A1 nanobody) or their combination. **B)** HH160 showed superior blocking efficacy compared to Pembrolizumab, Ipilimumab, and reference Abs (TriAb HC010 or BiAb AK104), demonstrating its enhanced functional potency. Effector : target cell ratio = 6:1 for both panels.

HH160 triggers stronger T cell activation

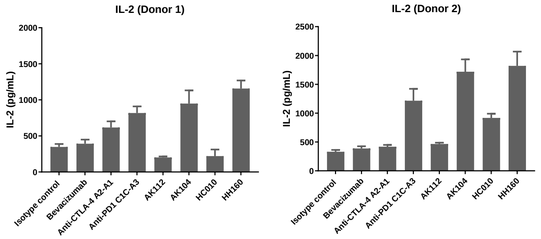


Fig.4. Mixed lymphocyte reaction (MLR) assay. HH160 induced higher IL-2 production in the MLR assay compared to reference antibodies.

HH160 induces minimal IL-6 and IL-8

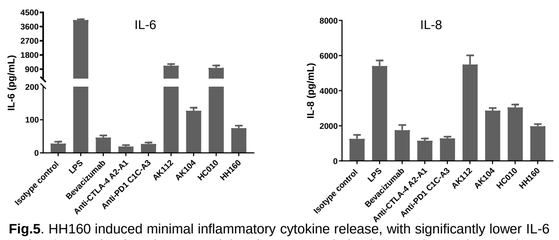


Fig.5. HH160 induced minimal inflammatory cytokine release, with significantly lower IL-6 and IL-8 secretion from human peripheral monocyte-derived macrophages (HPMMs) compared to AK112, AK104 and HC010.

HH160 exhibits high internalization efficiency in Jurkat-PD-1-CTLA-4 cells potentially leading to increased immune function

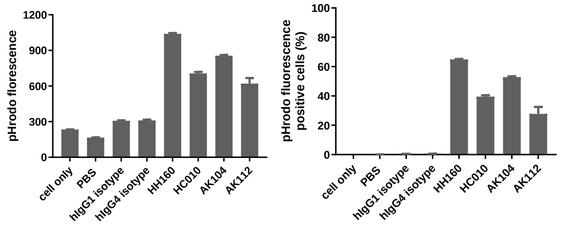


Fig.6. Antibody internalization assay. Antibodies labeled with pH-sensitive pHrodo dyes were tested for endocytosis in Jurkat cells expressing both PD-1 and CTLA-4 (Jurkat-PD-1-CTLA-4 cells).

HH160 induces strong CTLA-4 mediated VEGF-A internalization potentially leading to tumor specific VEGF-A depletion

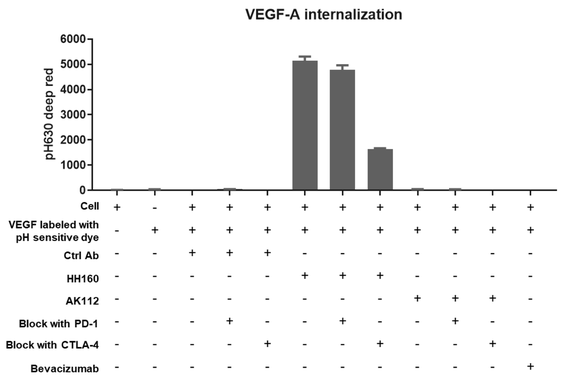


Fig.7. VEGF-A internalization assay. VEGF-A was labeled with the pH-sensitive pHrodo dye pH630 Deep Red, and its internalization in Jurkat-PD-1-CTLA-4 cells was evaluated following antibody treatment. CTLA-4 blocking reduced the HH160-induced internalization, indicating this is CTLA-4 mediated.

HH160 exhibits favorable PK profile in BALB/c mice

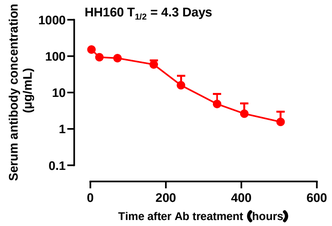


Fig.8. Serum PK of HH160 Ab in BALB/c mice. HH160 was administrated at a single dose of 10 mg/kg (i.v.), n=4.

HH160 exhibits potent anti-tumor effect in a syngeneic mouse tumor model

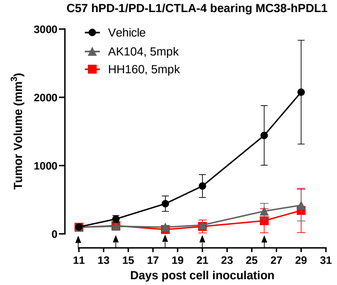


Fig.9. Anti-tumor activity in a syngeneic mouse model. C57 BL hPD-1/PD-L1/CTLA-4 triple transgenic mice bearing MC38-PD-L1 tumor cells were treated with HH160 or AK104 twice weekly (BIW, i.p.). N=4/group, except on day 29, where n=3 for the AK104 group due to one mouse succumbing to tumor progression.

HH160 demonstrates robust anti-tumor effect in a CDX mouse tumor model

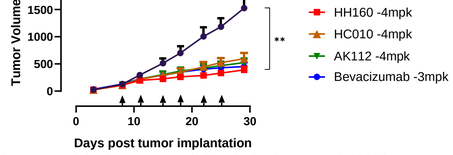


Fig.10. Anti-tumor activity in a tumor cell line-derived xenograft (CDX) mouse model. BALB/c nude mice bearing Colo205 cells were treated with HH160, HC010, AK112, or Bevacizumab with BIW (i.p.), n=5/group.

Summary

- **HH160 demonstrated compelling preclinical efficacy and safety**
  - HH160 triggered stronger T cell activation, as evidenced by elevated IL-2 secretion than reference antibodies
  - HH160 induced significant VEGF internalization, suggesting lysosomal degradation and a potential tumor-specific VEGF sweeping effect
  - HH160 exhibited markedly lower IL-6 and IL-8 secretion, two key cytokines implicated in irAEs
  - HH160 showed potent and synergistic anti-tumor activity
- **As a TriAb with a novel molecular design, HH160 combines robust efficacy with a potentially improved safety profile, positioning it as a promising best-in-class candidate for cancer therapy**

References

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