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HFB200603, a novel anti-BTLA monoclonal antibody that provides therapeutic potential for immune escape and synergizes with anti-PD-1 treatment

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HiFiBiO THERAPEUTICS

Summary

BTLA is a co-inhibitory immune checkpoint molecule sharing sequence and structural homology with PD-1 and CTLA4. Ligation of HVEM with BTLA induces recruitment of SHP1 and SHP2, and triggers inhibition of T cell proliferation and cytokine production. In melanoma, HVEM is described to have a broader expression than PD-L1 and constituted a negative prognostic marker; in PD-L1 negative NSCLC, the expression of HVEM has been shown to contribute to immune escape. BTLA-HVEM axis could play an important role in the immune escape, thus BTLA blockade in combination with PD-1/PD-L1 blockade could represent an effective therapeutic option.

Using our proprietary microfluidic-based single cell platform CelliGO™, we identified a series of anti-human BTLA antibodies that were characterized for their binding affinity, cross-reactivity, selectivity and functional activity. Amongst them, HFB200603 was identified as a single-digit nanomolar binder to human and cynomolgus BTLA, capable of reversing HVEM-mediated immune suppression in a BTLA-HVEM reporter system and in a primary CD4⁺ T cell proliferation assay. HFB200603 showed synergistic effect with anti-PD-1 to enhance IFN-γ production in an MLR assay and demonstrated favorable developability and pharmacokinetic profiles. Profiling of tumor infiltrating lymphocytes demonstrated that BTLA⁺PD-1⁺ T cells are present in melanoma, NSCLC, and HCC. Blockade of the BTLA-HVEM interaction with HFB200603 alone or in combination with anti-PD-1 led to increases in IFN-γ, CXCL9, IP-10 and other proinflammatory cytokines in primary dissociated tumor cultures.

Based on its favorable pharmacological activity and excellent developability, HFB200603 is currently being developed as a potential novel immunotherapy coupled with a patient biomarker strategy derived from HiFiBiO's Drug Intelligent Science (DIS™) single-cell immune profiling platform.

Lead Antibody	Target cells	MOA	Indications
Selective humanized mAb with cyno cross-reactivity	T and B Cells	Blocking BTLA-HVEM interaction	Advanced solid tumors

BTLA expression in TILs

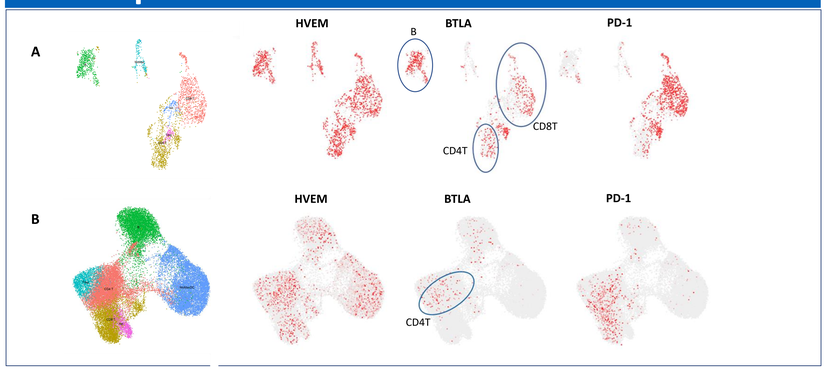


Figure 1. Single cell analysis of BTLA expression . ScRNA-seq analysis of BTLA, HVEM and PD-1 in melanoma **(A)** and NSCLC **(B)**. *References: Melanoma (Schelker et al., 2017 Nat. Comm.); NSCLC (Zilionis et al., 2019 Immunity)*

Discovery of anti-BTLA blocking antibodies

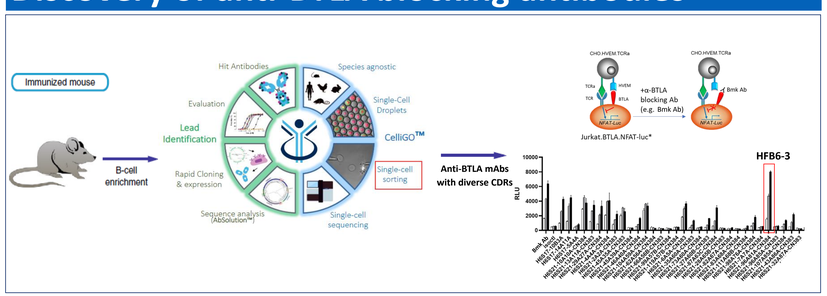
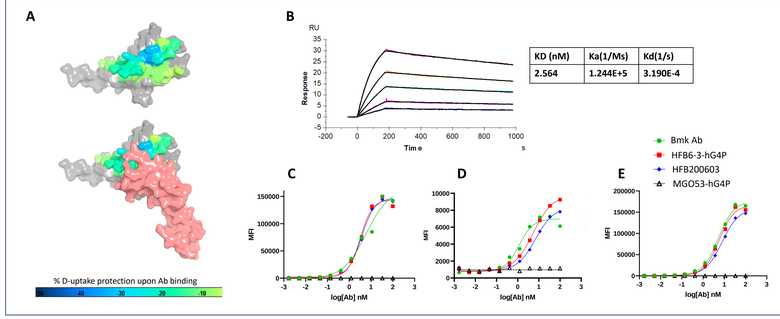


Figure 2. Discovery of anti-BTLA antibodies using HiFiBiO CelliGO™ platform. Splenocytes from SJL mice immunized with human recombinant BTLA ECD–Fc fusion protein were isolated, B-cell enriched and subjected to CelliGO™ droplet-based single cell screening for BTLA binding. The screening resulted in 71 potent BTLA binders with diverse CDRs. Functional blockers were selected based on their ability to reverse HVEM-mediated TCR suppression in a Jurkat.BTLA.NFAT-luc reporter cells. Bmk Ab is a benchmark anti-BTLA blocking antibody. HFB6-3 is the parental antibody of HFB200603.

Results

Epitope mapping, binding and cross-reactivity of HFB200603



KD (nM)	Ka(1/Ms)	Kd(1/s)
2.564	1.244E+5	3.190E-4

Figure 3. In vitro binding characterization of HFB200603 antibody. (A) HFB200603 epitope on BTLA identified by HDX-MS. BTLA in gray; HVEM in pink; HFB200603 epitope on BTLA in green-blue. By comparing the Deuterium labeling with or without HFB200603, the epitope region (blue or green based on the relative labeling difference) can be mapped on BTLA structure. **(B)** Kd values of HFB200603 antibody binding to rhBTLA-His. **(C-E)** Cellular binding of HFB200603 to human BTLA in HEK293T cells overexpressing hBTLA **(C)** and in human primary T cells **(D)** and to cynomolgus BTLA in Expi293 cells overexpressing cynoBTLA **(E).** MGO53-hG4P is an isotype ctl Ab.

Functional effect of HFB200603 *in vitro*

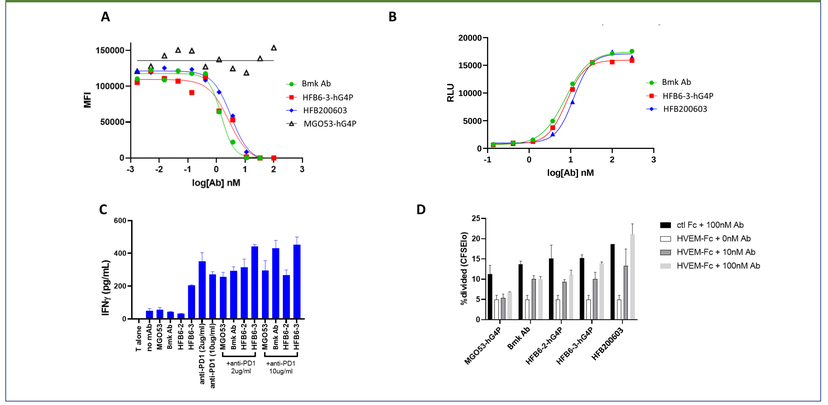


Figure 4. In vitro functional characterization of HFB200603. (A) HFB200603 blocking soluble HVEM binding to cellular BTLA. **(B)** HFB200603 reversal of HVEM-mediated TCR suppression in a HVEM-BTLA blockade reporter assay as in Fig 2. **(C)** HFB6-3 synergized with anti-PD-1 in inducing IFNγ production in a MLR assay. HFB6-3 is the parental antibody of HFB200603. **(D)** HFB200603 reversal of HVEM-mediated suppression of CD4⁺ T cell proliferation.

Exploratory toxicological evaluation of HFB200603 in NHPs

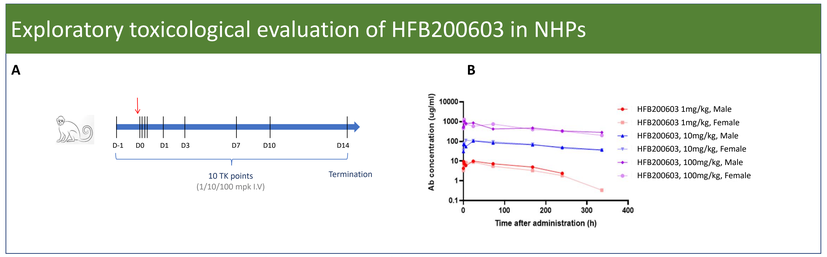


Figure 5. Exploratory toxicological evaluation of HFB200603 in NHPs. (A) Scheme of experimental design. Two cynomolgus monkeys per group were administered with a single dose of 1 mg/kg (low), 10 mg/kg (medium) and 100 mg/kg (high) of HFB200603 via i.v. infusion, after which plasma was collected at different time points until 336h (day 14). **(B)** Toxicokinetic analysis of HF200603. No obvious abnormalities in food consumption, body weight, body temperature, clinical observation; no macroscopic changes at necropsy; increase in exposure with dose in a dose proportional or near dose proportional manner with no ADA detectable.

Results (cont.)

Combinatorial effect of HFB200603 and anti-PD-1 in dissociated tumor culture

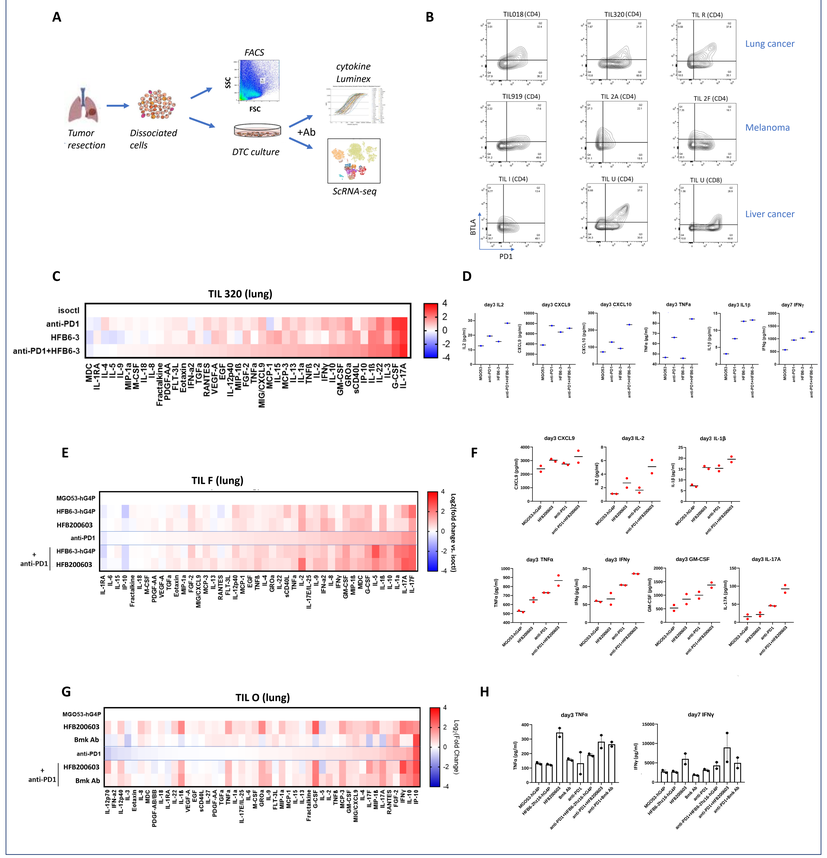


Figure 6. Combinatorial effect of HFB200603 and anti-PD-1 in ex vivo primary dissociated tumor cell (DTC) culture. (A) Scheme of ex vivo primary tumor culture analysis. **(B)** representative flow cytometry analysis of BTLA and PD-1 expression on tumor infiltrating T cells of melanoma, lung cancer and liver cancer. **(C, E&G)** Cytokine heatmaps (Luminex). Dissociated tumor culture was treated with HFB6-3 (or HFB200603) and anti-PD-1 for 72hrs. Supernatant was analyzed with 48-plex human cytokine/chemokine assay. Heat map was plotted as $\log_2$(fold change of treatment Ab vs isotype ctl). MGO53-hG4P is an isotype ctl Ab. TIL320 **(C)**, TIL F **(E)** and TIL O **(G)** are three lung cancer tumor samples. **(D, F&H)** Combinatorial effect of HFB200603 and anti-PD-1 on IFNγ related cytokine and pro-inflammatory cytokine production in selected lung cancer tumor cultures.

Conclusion

We have discovered anti-BTLA antibodies capable of blocking BTLA-HVEM interaction and selected a clinical candidate, HFB200603, for further development. Highlight of HFB200603:

- *A humanized anti-BTLA mAb binding specifically to human/cyno BTLA with single-digit nanomolar affinity and potent ligand-binding blocking activity*
- *Exhibit single agent effect and combinatorial effect with anti-PD-1 in inducing IFNγ and proinflammatory cytokine production in primary tumor culture*
- *Favorable acute toxicity profile in cynomolgus monkeys*
- *Favorable developability and pharmacokinetic profiles*
- *Innovative translational strategy applying HiFiBiO's Drug Intelligent Science (DIS™) single-cell immune profiling platform*
- *HFB200603 IND filing is anticipated in the second half of 2022*

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