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Discovery and characterization of novel TNFR2 antibodies to modulate T cell activities in immunosuppressive environment

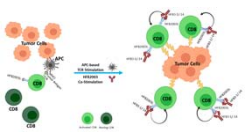
Shuo Wei, Ross Fulton, Guang Yang, Juying Li, Qian Zhang, Matthieu Delince, Greg Rose, Charina Ortega, Pascaline Mary, He Zhou, , Andreas Raue, Dean Lee, Roshan Kumar, Stephanie Beq, Jennifer Watkins-Yoon, Nicola Beltraminelli, Francisco Adrian, Liang Schweizer

HiFiBiO THERAPEUTICS

Summary

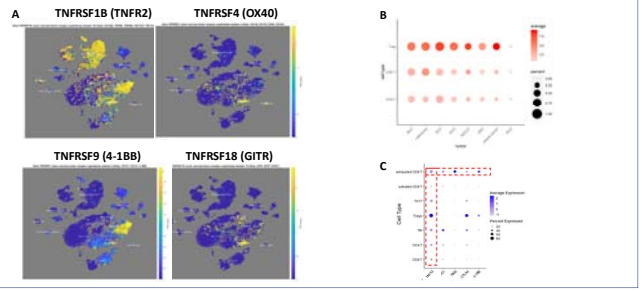
umor necrosis factor receptor-2 (TNFR2) has recently emerged as a promising herapeutic target for immuno-oncology. TNFR1 and TNFR2 are receptors for umor necrosis factor (TNFa). However, unlike TNFR1, TNFR2 is expressed xclusively on immune cells. TNFR2 is a potent co-stimulatory molecule expressed n the surface of CD8 and CD4 T cells in the tumor micro-environment. An gonistic antibody against TNFR2 has the potential to further enhance effector T ell functions and their anti-tumor response. We applied our single-cell platform elliGO™ for antibody discovery and discovered a panel of antibodies binding NFR2 on distinct epitopes (HFB3-18:CRD1, HFB3-1:CRD2, HFB3-14:CRD3).

he selected antibodies bind to TNFR2 without competing with its ligand TNFα, stimulate activated CD4 and CD8 T-cells and nhance their proliferation in-vitro. Interestingly, HFB3-18 requires cross-linking of Fc receptors for its activity while HFB3-1 and IFB3-14 do not. The most potent antibody, HFB3-1, was humanized and the resulting humanized, HFB200301, was tested in uman TNFR2 knock-in mice bearing syngeneic subcutaneous MC38 tumors. HFB200301 displays potent anti-tumor activity and ombination with PD1 blockade resulted in enhanced survival in this model, indicating that TNFR2 co-stimulation and PD1 lockade could lead to a synergistic anti-tumor immune response. HFB200301 was further evaluated in non-human primate oxicity model. When administrated to adult cynomolgus monkeys up to 150mg/kg, HFB3-200301 displays favorable harmacokinetic and safety profiles. All together these data support the development of HFB200301 as a novel therapeutic gent in Oncology.



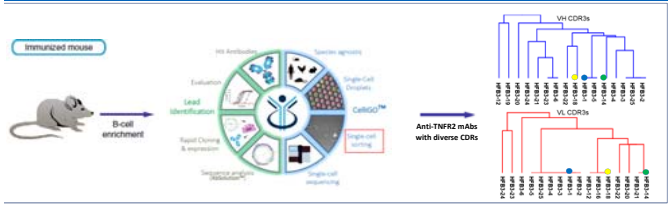
Lead Antibody	Target cells	MOA	Indications
Selective humanized IgG1 with cyno cross-reactivity	T Cells	Co-stimulation of CD4 and CD8 T cells	Advanced solid tumors

TNFR2 expression in TILs



igure 1. Single cell analysis of TNFR2 expression . (A) Expression of TNFR2 and other immuno-stimulating genes, OX40, 4-1BB and GITR. TNFR2 is expressed most CD8 T cells and a large proportion of CD4 T cells, including Tregs, and monocytes. **(B)** scRNA-sequence analysis on multiple cancer types indicates NFR2 expression mainly on Treg, CD4 and CD8 cells. BCC, basal cell carcinoma; SCC, sarcoma cell carcinoma; HCC, hepatocellular carcinoma; NSCLC, non mall cell lung cancer; CRC, colorectal cancer; RCC, renal cell carcinoma. **(C)** Expression of TNFR2 on exhausted CD8 cells aligns with those from other nmune T cell targets, such as PD-1, TIM-3, CTLA-4 and 4-1BB, in the sample of SCC. *References: Breast cancer (Azizi et al., 2018 Cell); BCC/SCC (Yost et al., 2019 Nat. ed.); NSCLC (Zilionis et al., 2019 Immunity); Melanoma (Schelker et al., 2017 Nat. Comm.); HCC (Qiming et al., 2019 Cell); CRC/RCC (Wu et al., 2020 Nature)*

Discovery of anti-TNFR2 antibodies



igure 2. Discovery of anti-TNFR2 antibodies using HiFiBiO CelliGO™ platform. Splenocytes from C57BL/6 mice immunized with human recombinant TNFR2 CD–Fc fusion protein were isolated, B-cell enriched and subjected to CelliGO™ droplet-based single cell screening for TNFR2 binding. The screening esulted in 17 potent TNFR2 binders with diverse CDRs. Color-labeled HFB3-1, -14 and -18 were selected based on their different epitopes on TNFR2.

Results

Epitope mapping, binding and crossreactivity of anti-TNFR2 antibodies

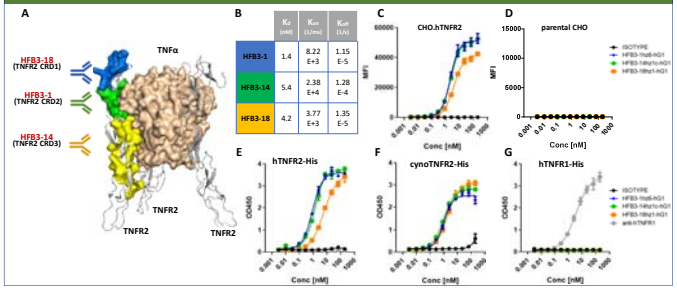


Figure 3. In vitro binding characterization of HFB3 antibodies. (A) Diverse epitopes identified by HDX-MS. **(B)** Kd values of HFB3 antibodies to rhTNFR2-His. **(C-D)** Cellular binding of humanized HFB3 antibodies to human TNFR2. **(E-G)** ELISA assay demonstrates the binding EC_{50} of humanized HFB3 antibodies to recombinant human and cynomolgus TNFR2 range from a sub- to single digit-nM, without recognizing TNFR1. All data represented as mean and SD (N=3).

Binding and functional effect of anti-TNFR2 antibodies on CD4 and CD8 T cells

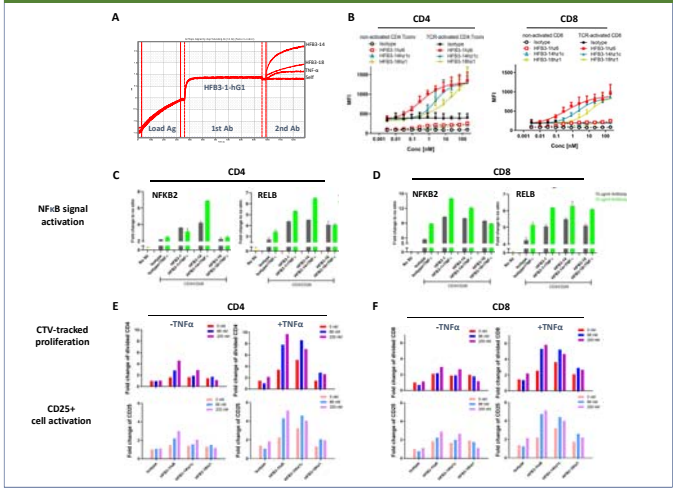


Figure 4. In vitro functional characterization of HFB3 antibodies. (A) Epitope binning shows HFB3 antibodies do not compete with ligand TNF-α. **(B)** Humanized HFB3 antibodies preferentially binds to TCR-primed CD4 and CD8 T cells after CD3/28 co-stimulation. **(C,D)** Co-stimulation of isolated conventional CD4 and CD8 T cells with HFB3 antibodies triggers TNFR2 downstream NFκB signaling, measured as gene expression upregulation of NFKB2 and RELB. Treatment of T cells with HFB3 antibodies in the presence of TNF-α enhances NFκB signaling responses (green bars). **(E,F)** Co-stimulation of isolated CD3 T cells with humanized HFB3 antibodies triggers cell proliferation and cell activation of CD4 and CD8 T cells. Cell proliferation is monitored by with cell trace violet (CTV), while cell activation is detected by changes of CD25 positive population in CD4 or CD8 T cells. Treatment of HFB3 antibodies in the presence of TNF-α enhances cell proliferation and cell activation of CD4 and CD8 T cells. All data denoted as mean and SD (N=3) or representative data from 3 independent experiments. In addition, HFB3 antibodies have no/minimal ADCC effect comparable to isotype (data not shown).

Results (cont.)

Pharmacokinetic and antitumor activity of HFB200301 (HFB3-1hz6-hG1) in mice

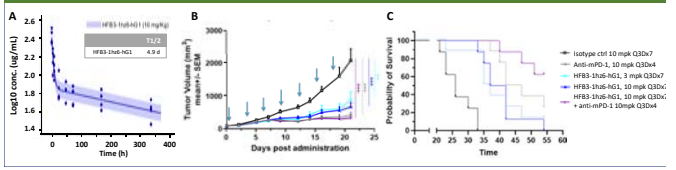


Figure 5. In vivo evaluation of HFB3 antibodies. (A) Pharmacokinetic profile of development candidate HFB3-1hz6-hG1. **(B,C)** Efficacy and survival studies i MC38 tumor model in hTNFR2 knock-in (TNFR2 KI) mice (n=8/group). Tumor growth inhibitions (TGI) is statistically significant for both 3 and 10 mg/kg HFB3 1hz6-hG1, anti-PD-1 (RMP-14), and HFB3-1hz6-hG1/PD-1 combination groups, that result in extended lifespan of the HFB3-treated animals. Particularly HFB3-1hz6/PD-1 combination results in increased survival than PD-1 alone. HFB3-1hz6-hG1 and anti-PD-1 antibodies were injected 7 x 10 mg/kg or 4 x 1 mg/kg (vertical arrows) respectively, every 3 days intraperitoneally from day 0. Tumor inoculation was at day -7. Data are analyzed using ANOVA comparin treatment groups to isotype control. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001

Exploratory toxicological evaluation of HFB200301 in NHPs

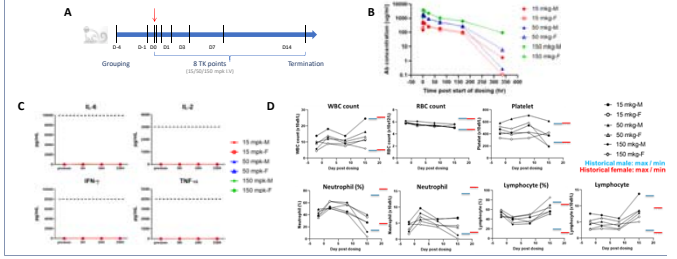
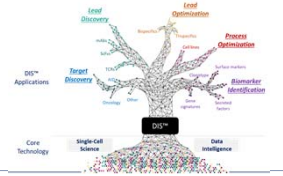


Figure 6. Exploratory toxicological evaluation of HFB3-1hz6-hG1 in NHPs. (A) Scheme of experimental design. Two cynomolgus monkeys per group were injected with a single dose of 15 mg/kg (low), 50 mg/kg (medium) and 150 mg/kg (high) of HFB3-1hz6-hG1, after which plasma was collected at different time points until 336h (day 14). **(B)** Toxicokinetic analysis of HFB3-1hz6-hG1. **(C)** No elevation of cytokines was observed at the 15, 50 or 150 mg/kg of HFB3 1hz6-hG1 in comparison to reported data (dotted lines) from CD3xCD20 bispecific IgG at ≤ 3 mg/kg **(D)** Cell count analysis of HFB3-1hz6-hG1. No abnormality was found in cell count examination, as compared to historical data range from normal monkeys indicated by the blue and red lines.

Drug Intelligent Science (DIS™)



DIS approach captures single-cell biology from target to patients

- Target discovery benefiting from patient single-cell analysis
- High-quality and differentiated antibody leads empowered by single B cell analysis
- Innovative lead optimization in picoliter droplets and rapid process optimization
- Identification of predictive biomarkers at single-cell level to select responsive patient populations

CONCLUSION

We have discovered a number of anti-TNRFR2 antibodies capable of co-stimulating CD4 and CD8 T cells and selected a candidate, HFB200301, for further development. Highlight of HFB200301:

- *First-in-class Immuno-stimulatory humanized anti-TNFR2 antibody*
- *Exhibit potent anti-tumor activity in MC38 syngeneic model, alone and in combination with anti-PD-1*
- *Co-stimulate cell proliferation and activation on CD4 and CD8 cells*
- *Specifically binds to human TNFR2 with sub-nanomolar affinity*
- *Cross-reactive with monkey TNFR2 ortholog*
- *Favorable acute toxicity profile to cynomolgus monkeys*
- *Favorable developability and pharmacokinetic profiles*

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For additional information, please email contact@hifibio.com or visit Hifibio.com