Poster #2899
Presented at the
AACR 2022 Annual Meeting
New Orleans, LA
April 8-13, 2022

Targeting regulatory T cells with HFB101110, a novel anti-human CCR8 antibody for the treatment of solid tumors

Roshan Kumar[1*], Julie Prigent[1], Hombline Poullain[1], Ayrin Kök[1], Carine George[1], Sami Ellouze[1], Yun-Yueh Lu[1], Rebecca Silver[1], Clarisse Monchecourt[1], Ross Fulton[1], Qian Zhang[1], Nicola Beltraminelli[1], Bernhard Moser[2], Francisco Adrian[1], Liang Schweizer[1]

*Contact information : r.kumar@hifibio.com 1. HiFiBiO Therapeutics 2. Division of Infection & Immunity, Cardiff University School of Medicine, Cardiff UK

SUMMARY

Regulatory T cells (Tregs) contribute to immunosuppression within the tumor microenvironment and have been associated with poor outcomes in a range of cancers. Targeted depletion of tumor-infiltrating Tregs (TITRs) is an attractive therapeutic strategy with the potential to enhance antitumor immunity in patients who do not respond to current treatments. However, such approaches have been limited to date by a lack of markers that are highly specific for TITRs.

CCR8 is a chemokine G protein-coupled receptor (GPCR) that has recently been shown to be specifically expressed on TITRs as compared to Tregs in the periphery or other T cells within tumors. Here, we report the development and characterization of HFB101110, a humanized monoclonal antibody against CCR8 with potent and specific antibody-dependent cellular cytotoxicity (ADCC) activity. HFB101110 specifically recognizes an epitope on the N-terminus extracellular domain of CCR8 and does not recognize the closely related chemokine receptor CCR4. HFB101110 acts through a dual mechanism of action, by both depleting CCR8+ cells via ADCC and blocking binding of the CCL1 chemokine to its receptor CCR8. Blockade by HFB101110 inhibited calcium flux and chemotaxis induced by the interaction between CCL1 and CCR8. Furthermore, HFB101110 showed potent single-agent anti-tumor activity associated with depletion of intratumoral Tregs in a human CCR8 knock-in mouse model. HFB101110 mediated specific *ex vivo* killing of Tregs from primary patient samples both in the presence, and in some cases the absence, of allogeneic NK cells. HFB101110 showed favorable pharmacokinetic properties and a favorable developability profile. HFB101110 was well-tolerated in both wild-type mice and cynomolgus monkeys.

HFB101110 is currently being developed as a novel immunotherapy for the treatment of solid tumors 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	Tumor-infiltrating Regulatory T Cells	1) Depletion via ADCC 2) Blockade of CCL1 binding	Advanced solid tumors

TARGET RATIONALE

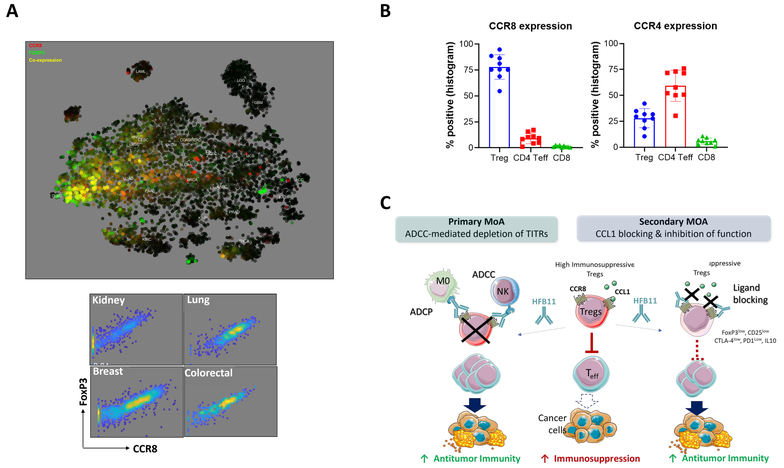

Figure 1. CCR8 expression and therapeutic hypothesis. (A) Expression of CCR8 and the regulatory T cell specific transcription factor FOXP3 in patient samples from the Cancer Genome Atlas (TCGA). CCR8 expression is highly correlated with FOXP3 expression in a variety of cancers, indicating specific expression in tumor-infiltrating regulatory T cells (TITRs). **(B)** Flow cytometry quantification of CCR8 and CCR4 expression in regulatory T cells, CD4 T effector cells, and CD8 T cells isolated from renal cancer and lung cancer patient samples. CCR8 shows a more Treg-specific expression pattern as compared to CCR4. (**C**) Therapeutic hypothesis for HFB101110. The antibody recognizes CCR8 on the surface of highly immunosuppressive TITRs and depletes them through antibody-dependent cellular cytotoxicity (ADCC), thereby reversing Treg-mediated immunosuppression and promoting antitumor immunity. As a secondary mechanism, the antibody blocks binding of the CCL1 ligand to CCR8 and inhibits its immunosuppressive functions.

ANTIBODY DISCOVERY

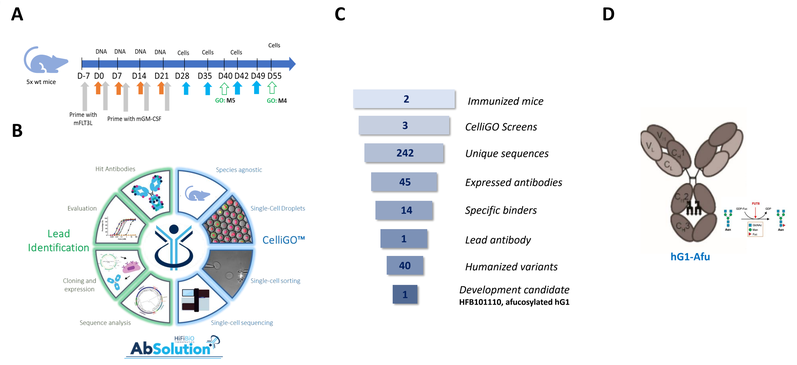

Figure 2. Discovery and development of anti-CCR8 mAb HFB101110. (A) Immunization of wild-type mice with hCCR8. Mice were primed with mFLT3L and mGM-CSF, and immunized with DNA encoding hCCR8 followed by boosting with mouse 300.19 cells expressing hCCR8. **(B)** HiFiBiO's droplet-based microfluidic CelliGo™ single B cell screening platform was used to screen immune repertoires from immunized mice and identify anti-hCCR8 antibodies. See Gerard *et al.*, *Nat Biotech* **38** 715-721 (2020) for further details on the CelliGo™ platform and antibody discovery process. **(C)** Summary of hit characterization and lead selection leading to HFB101110. **(D)** A human IgG1 afucosylated antibody format was chosen for HFB101110 to enhance ADCC activity.

IN VITRO CHARACTERIZATION

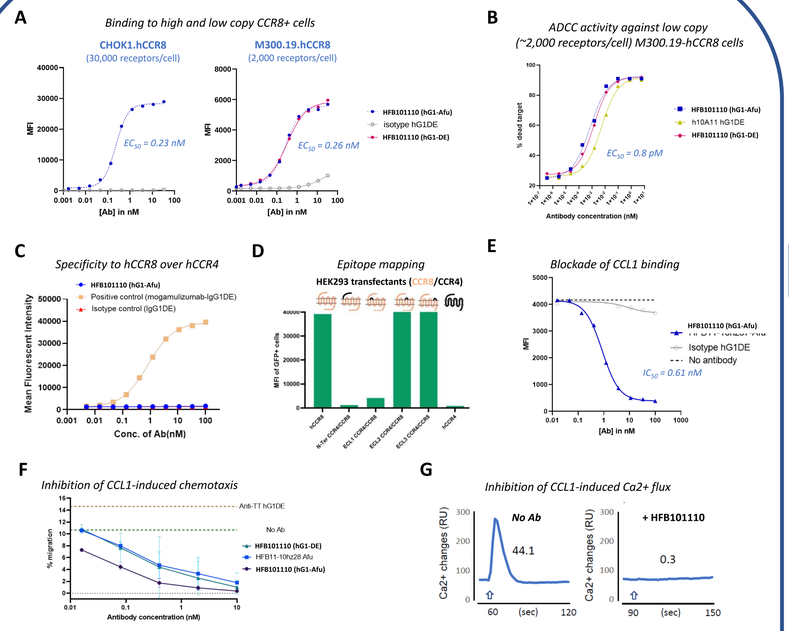

Figure 3. *In vitro* characterization of anti-CCR8 mAb HFB101110. (A) Binding of HFB101110 to high-copy (CHOK1-hCCR8, ~30,000 receptors/cell, left) or low-copy (M300.19-hCCR8, ~2,000 receptors/cell, right) cells as measured by flow cytometry. The parental antibody to HFB101110 formatted as an hIgG1 antibody with normal glycosylation and DE mutations in the Fc region to enhance ADCC activity (HFB101110-hIgG1-DE) was used as a positive control for afucosylated HFB101110. HFB101110 was able to bind both high- and low-copy hCCR8-expressing cells with similar sub-nM EC_{50} values. **(B)** ADCC activity of HFB101110 against M300.19-hCCR8 cells. Exogenous primary NK cells isolated from PBMCs were added at an Effector:Target ratio of 3:1, and dead cells were quantified by flow cytometry using NucGreen staining after 16h of co-culture. Mean values of experiments using NK cells from 3 different donors are shown. HFB101110-hIgG1-DE and another anti-hCCR8 DE-formatted hIgG1 antibody were used as comparators. **(C)** The binding of HFB101110 to the related chemokine receptor CCR4 was evaluated by flow cytometry, with the anti-CCR4 antibody mogamulizumab used as a positive control. HFB101110 showed no binding to hCCR4, indicating specific recognition of hCCR8 by this antibody. **(D)** Domain swapping experiments to identify the region of CCR8 recognized by HFB101110. Portions of hCCR8 were replaced with homologous domains of the related chemokine receptor CCR4, and the resulting chimeric proteins were expressed in 293 cells and binding of HFB101110 was assessed by flow cytometry. Based on these experiments, it was determined that HFB101110 recognizes the N-terminal extracellular domain of hCCR8. **(E)** Blockade of hCCL1 binding to hCCR8 by HFB101110, measured by flow cytometry. **(F)** HFB101110-mediated blockade of chemotaxis of CCR8+ cells induced by recombinant hCCL1, as measured by a transwell migration assay. CCL1 was present at a concentration of 30 nM while varying the amount of antibody present. HFB101110-hIgG1-DE as well another humanized variant were used as comparators. **(G)** HFB101110-mediated blockade of calcium flux induced by the addition of hCCL1 to CCR8+ cells. 10 nM hCCL1 was added to CCR8+ cells at the time indicated by the arrow, in the presence or absence of 10 ug/mL HFB101110.

EX VIVO KILLING OF PRIMARY TILs

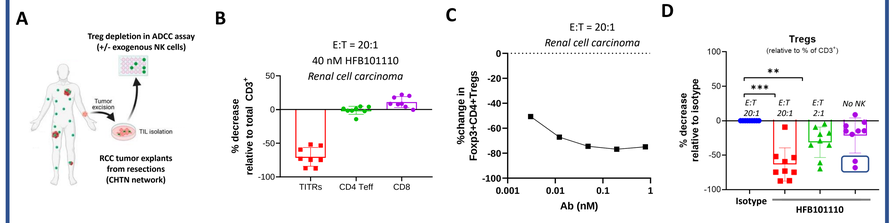

Figure 4. *Ex vivo* killing of primary tumor-infiltrating lymphocytes mediated by HFB101110. (A) Schematic of sample collection and ADCC assay. **(B)** Depletion of different T cell subsets after overnight co-culture of primary tumor-infiltrating lymphocytes (TILs) with primary NK cells at an Effector : Target ratio of 20:1. Remaining cells were quantified by flow cytometry. **(C)** Dose-response of Treg-depleting activity of HFB101110. **(D)** Depletion of Tregs in ADCC assay as a function of amount of exogenous NK cells added. Two out of nine samples profiled (highlighted in the blue box) showed substantial Treg depletion even in the absence of exogenous NK cells, suggesting that endogenous NK cells present in the tumor sample are able to mediate ADCC.

IN VIVO EFFICACY

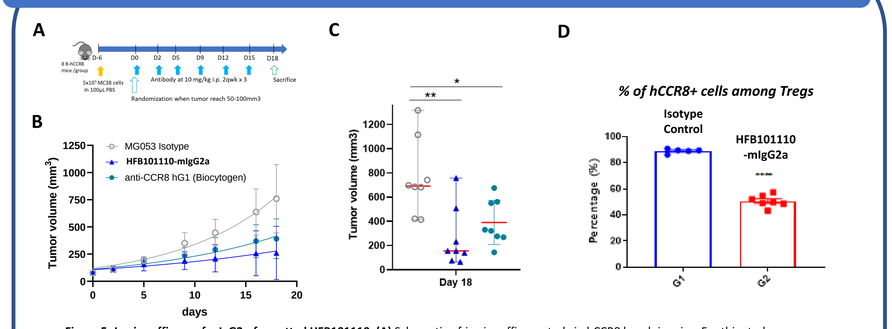

Figure 5. *In vivo* efficacy of mIgG2a-formatted HFB101110. (A) Schematic of *in vivo* efficacy study in hCCR8 knock-in mice. For this study, a non-afucosylated version of HFB101110 formatted as an mIgG2a antibody was used, to facilitate engagement with mouse Fc receptors. **(B)** HFB101110-mIgG2a delays tumor growth in an MC38 tumor model in hCCR8 knock-in mice. A comparator anti-hCCR8 antibody formatted as an hIgG1 was used as a control. **(C)** Tumor volume of individual mice at the time of sacrifice (d18). **(D)** Levels of hCCR8+ Tregs in tumors isolated from mice at the time of sacrifice. HFB101110, mIgG2a treatment results in reduced numbers of intratumoral Tregs as compared to the isotype control.

SAFETY & PHARMACOKINETICS

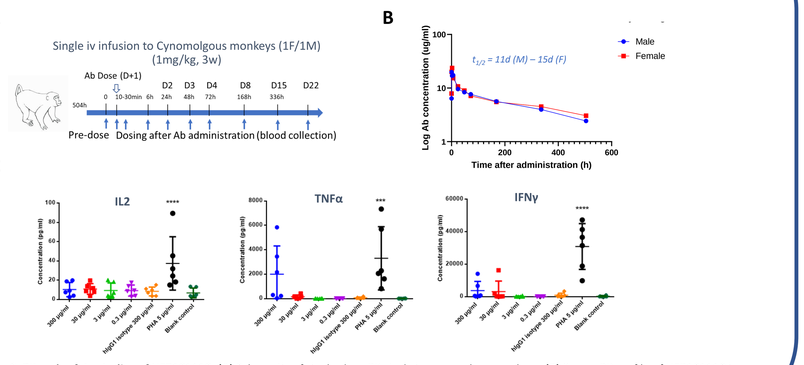

Figure 6. PK and safety studies of HFB101110. (A) Schematic of single-dose PK study in cynomolgus monkeys. **(B)** Serum PK profile of HFB101110 in cynomolgus monkeys. No anti-drug antibodies were detected after 15 days. **(C)** *In vitro* cytokine release study from human PBMCs using a soluble antibody format. No significant cytokine release was observed.

CONCLUSION

HFB101110 is a potent and specific binder of CCR8 and blocker of CCL1 binding, and is able to direct robust ADCC-mediated killing of CCR8+ regulatory T cells both *ex vivo* and *in vivo*. It holds promise as a novel immunotherapy to reverse Treg-mediated immunosuppression, and is currently being developed for applications in solid tumors.