Novel Monoclonal Antibodies (mAbs) Targeting Human CCR8 Provide Potential Candidates for Next Generation Cancer Immunotherapy

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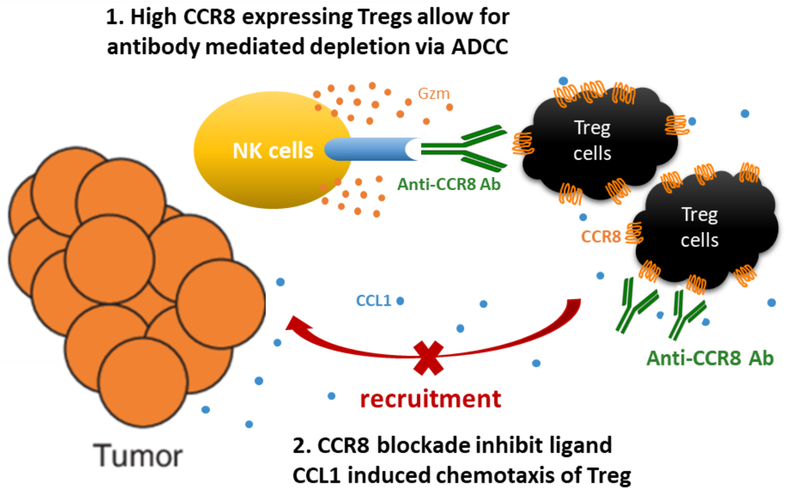
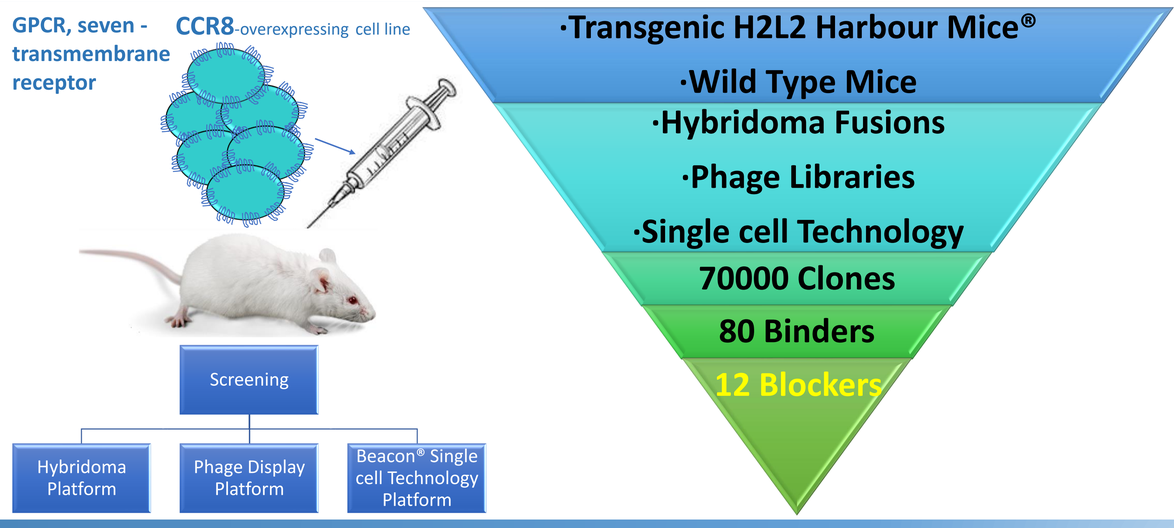
Abstract

- The tumor resident CCR8-expressing CD4 and Foxp3 positive Treg (CCR8+ Treg) has been demonstrated to be a major driver for immunosuppression[1]. Clinical studies have shown that CCR8 is selectively up-regulated by tumor resident Tregs in several tumor types including clear cell renal cell carcinoma (ccRCC)[2] and breast cancer[3]. In these tumor types, resident Tregs exhibited strong CCR8 expression both at the mRNA level as well as on the cell surface. CCR8 was rarely observed on Tregs in peripheral blood mononuclear cells (PBMCs). High expression of the CCR8 in Tregs was associated with poor prognosis in breast cancer patients. These results suggest CCR8 as a promising therapeutic target; and an anti-CCR8 mAbs could selectively inhibit a subpopulation of tumor resident Tregs in the tumor microenvironment (TME), to augment antitumor immunity.
- CCR8 has been an extremely challenging target by virtue of it being a G-protein coupled receptor (GPCR), and previous attempts of generating a cross-reactive (cynomolgus and human) have yielded limited success. Here, we report the generation of anti-CCR8 mAbs using cell immunized H2L2 Harbour Mice® as well as wild type mice and screened by hybridoma, phage display and Beacon® single cell technology platforms. These mAbs, in normal human IgG1 or enhanced antibody-dependent cell-mediated cytotoxicity (eADCC) versions, specifically bind to cell lines that over-express both human and cynomolgus CCR8, as well as ccRCC tumor-infiltrating lymphocytes-Treg cells (TIL-Tregs). The anti-CCR8 mAbs potently inhibit CCL1-induced calcium flux and cell migration, and induced *in vitro* ADCC against CCR8 over-expressing cell line. Moreover, these mAbs also showed significant inhibition of tumor growth in a mouse syngeneic model of colon cancer. Combination of anti-CCR8 mAbs with Keytruda® showed even better antitumor activity than single agent in NCG mice xenograft model with human PBMC and human triple negative breast cancer (TNBC) cell line. These lead candidates present an exciting opportunity for the development of an innovative immunotherapy targeting intra-tumoral suppressive Treg cells.

Therapeutic Rationale For Targeting CCR8 In Cancer



Immunization and Screening Strategy



With Cross Cynomolgus CCR8 Reactivity, Anti-CCR8 mAbs Potently Antagonize CCL1-CCR8 Binding and Deplete CCR8-Expressing Cells

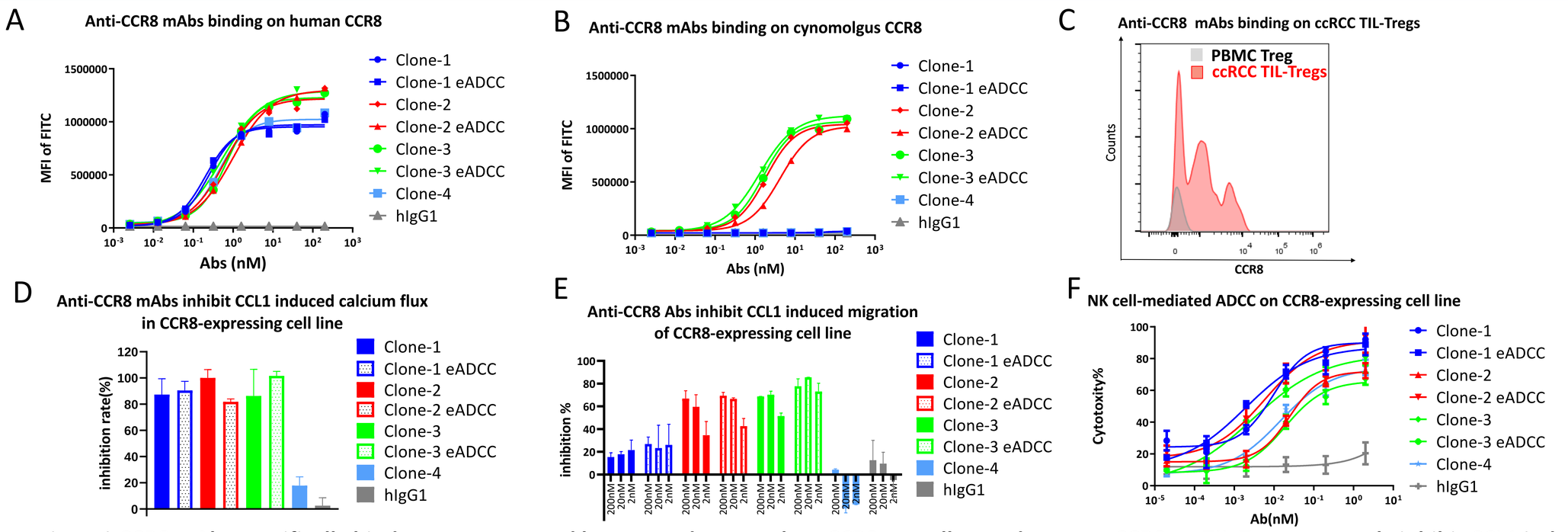
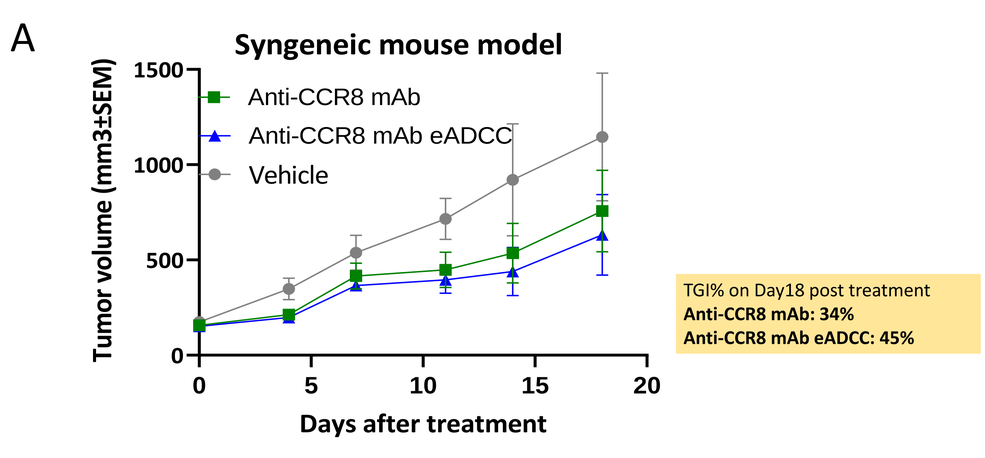


Figure 1. Therapeutic anti-CCR8 mAbs specifically bind to overexpressed human and cynomolgus CCR8 as well as endogenous CCR8 on TIL-Tregs , potently inhibit CCL1 induced calcium flux and cell migration and provides potent NK cell-mediated ADCC killing activity. (A) Anti-CCR8 mAbs specifically bind to human CCR8 over-expressing CHO-K1 stable cell line and (B) some clones show high cross-reactivity to cynomolgus CCR8. (C) Moreover, clones with cynomolgus cross-reactivity could recognize endogenous CCR8 on ccRCC TIL-Tregs. (D) After adding ligand CCL1 to CCR8 over-expressing CHO-K1 cell line, anti-CCR8 mAbs potently inhibit intracellular calcium flux mediated by CCR8. (E) Chemotaxis is shown by migration of CCR8 over-expressing Baf3 cell line. Anti-CCR8 mAbs potently block the migration of Baf3-CCR8 cells toward CCL1. (F) Anit-CCR8 mAbs are able to potently deplete *in vitro* CCR8-expressing cells by NK cell-mediated ADCC killing activity.

Anti-CCR8 mAbs Single Agent Impairs Tumor Growth and Combination Therapy Enhanced Antitumor Efficacy



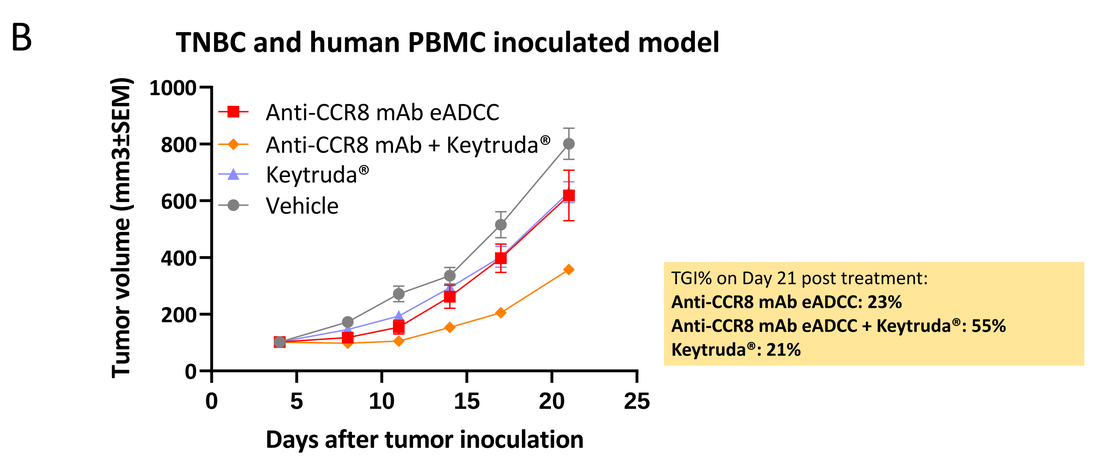


Figure 2. Anti-CCR8 mAb inhibited tumor growth in preclinical models. (A) High-dosed anti-CCR8 mAbs inhibited tumor growth in mouse syngeneic colon cancer model. (B) Low-dosed anti-CCR8 mAb enhanced the antitumor activity of pembrolizumab in NCG xenograft model with human PBMC and human triple negative breast cancer cell line.

Summary

Harbour BioMed has generated novel anti-human CCR8 mAbs:

- ✓ **Target human CCR8 on intra-tumoral Treg cells, and inhibit tumor growth as a single agent as well as a combination therapy with Keytruda®**
- ✓ **Potently deplete CCR8 expressing cells**
- ✓ **Block binding of CCL1 to CCR8 thereby inhibit CCL1-induced suppressive function of TIL-Tregs**
- ✓ **First clearly demonstrate the cyno-cross reactivity**

References

1. Barsheshet Y, et al. P NATL ACAD SCI USA, 2017;114:6086–6091
2. Yoshida T, et al. US10550191B2, 2020
3. George P, et al. Immunity, 2016; 45: 1122–1134

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