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Generation of an innovative BsAb targeting CCR8/CTLA4

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Introduction

CTLA-4-expressing regulatory T cells (Tregs) are abundantly present in tumor tissues and suppress anti-tumor responses. Currently approved CTLA-4 inhibitors, such as ipilimumab, induce severe irAEs, which limited their use. Therefore, next-generation of CTLA-4 inhibitors with reduced toxicity and increased efficacy is highly demanded, given the fact that CTLA-4 inhibition has showed unique advantages in inducing immune memory response and prolonging anti-tumor activity.

CCR8 is recently found to be predominantly expressed on intra-tumor Tregs but little on peripheral Tregs or activated Tconv. Anti-CCR8 antibodies have been shown significant anti-tumor activity in pre-clinical studies. We have hypothesized that a strong anti-CCR8 arm may provide preferential inhibition of CTLA-4 on CCR8+CTLA-4+ intra-tumor Tregs and have synergistic effects on Tregs in TME. Therefore, we have developed a novel BsAb, GBD201, targeting CCR8/CTLA-4. The innovative bsAb exhibited potent depletion of intra-tumor Tregs but spared peripheral Tregs and Tconv. In hCCR8/hCTLA-4 mice, GBD201 showed better efficacy and safety profile compared to ipilimumab.

Molecular structure and mechanisms of GBD201

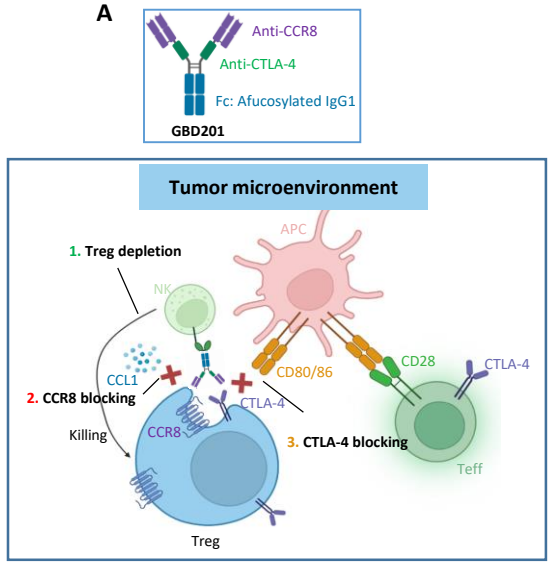


Figure 1. Molecular structure and multiple mechanisms of GBD201. A. The schematic structure of GBD201. B. The MOAs of GBD201 include the followings: 1) Preferential Treg depletion in TME by specifically binding to CCR8+CTLA-4+ Tregs and enhancing ADCC function. 2) Inhibition of CCR8-CCL1 interaction to block Treg migration and differentiation in TME. 3) Partially blockade of CTLA-4 binding to its ligands CD80/CD86, which is dependent on CCR8 expression.

CCR8 & CTLA-4 are co-expressed on tumor Tregs

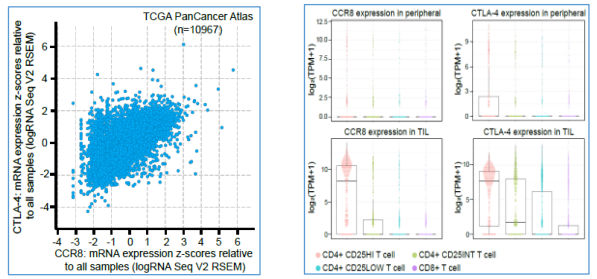


Figure 2. CCR8 and CTLA-4 are highly co-expressed on tumor Tregs. A) Correlation analysis of CCR8 and CTLA-4 mRNA expression levels in tumors from TCGA dataset using cBioportal. **B)** Expression of CCR8 and CTLA-4 in T cells of NSCLC patients by single-cell sequencing according to literature (Xinyi Guo *et al., Nature Medicine*, 2018).

Table 1. Ag-binding affinity measured by Octet

Antigen	Antibody	KD (M)	Kon (1/Ms)	Kdis (1/s)
CCR8 (Met1-Lys35)-mFc	BMK1*	3.436E-09	1.371E05	4.711E-04
	Parental Anti-CCR8-AF**	2.612E-09	1.547E05	4.040E-04
	GBD201	1.592E-09	1.556E05	2.476E-04
CTLA-4-mFc	Ipilimumab	1.339E-09	2.674E05	3.582E-04
	Parental Anti-CTLA-4	8.075E-10	2.277E05	1.839E-04
	GBD201	9.868E-10	3.072E05	3.032E-04

** BMK1 is an afucosylated anti-CCR8 mAb currently in clinical stage.*
***AF: afucosylated*

Differential binding property of GBD201 to CCR8/CTLA-4 double-positive cells vs. CTLA-4 single-positive cells

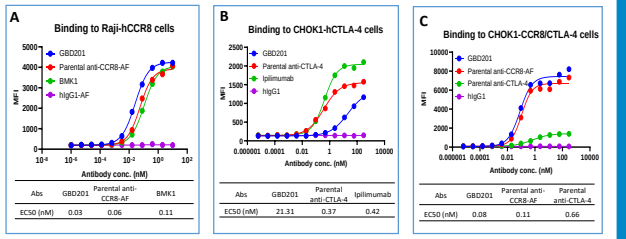
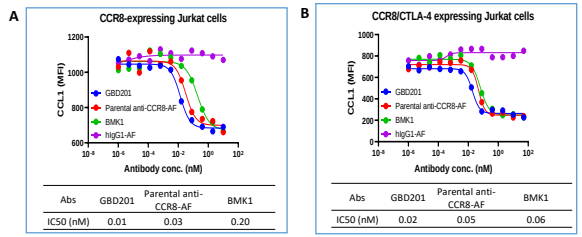


Figure 3. GBD201 preferentially bound to CCR8-expressing cells. A. GBD201 bound strongly to hCCR8-expressing cells (EC50 = 0.03 nM). **B.** GBD201 relatively weakly bound to hCTLA-4-expressing cells (EC50 = 21.31 nM). **C.** GBD201 potently bound to hCCR8/hCTLA-4 double positive cells (EC50 = 0.08 nM).
BMK1 is a clinical asset of afucosylated anti-CCR8 mAb.

GBD201 efficiently blocked the CCL1/CCR8 interaction

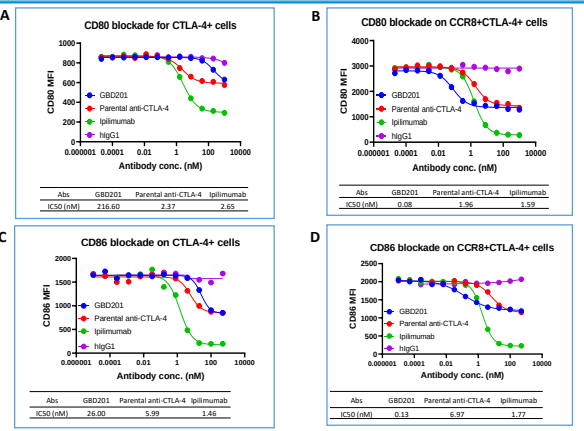


Abs	GBD201	Parental anti-CCR8-AF	BMK1
IC50 (nM)	0.01	0.03	0.20

Abs	GBD201	Parental anti-CCR8-AF	BMK1
IC50 (nM)	0.02	0.05	0.06

Figure 4. GBD201 significantly inhibited the binding of CCL1 to CCR8. GBD201 blocked CCL1/CCR8 interaction on CCR8 single-positive cells (**A**) and CCR8/CTLA-4 double-positive cells (**B**).

GBD201 is a unique partial blocker for CTLA-4



Abs	GBD201	Parental anti-CTLA-4	Ipilimumab
IC50 (nM)	216.60	2.37	2.65

Abs	GBD201	Parental anti-CTLA-4	Ipilimumab
IC50 (nM)	0.08	1.96	1.59

Abs	GBD201	Parental anti-CTLA-4	Ipilimumab
IC50 (nM)	26.00	5.99	1.46

Abs	GBD201	Parental anti-CTLA-4	Ipilimumab
IC50 (nM)	0.13	6.97	1.77

Figure 5. GBD201 partially blocked the interactions of CTLA-4 with its ligands (CD80 or CD86). GBD201 showed very weak blocking activity on CTLA-4 single-positive cells (**A, C**) but potent blocking activity was seen on CCR8/CTLA-4 double-positive cells (**B, D**). The partial blockade and weak binding to CTLA-4 single positive cells were intentionally designed to reduce the toxicity of CTLA-4 inhibition in the periphery.

GBD201 induced strong ADCC responses

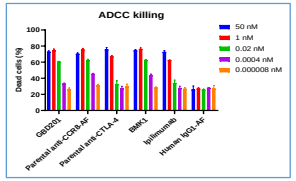


Figure 6. GBD201 induced potent ADCC killing of CCR8/CTLA-4 double-positive cells. Human PBMC were mixed with target cells (CCR8/CTLA-4 double-positive cells) at the E:T ratio of 20:1. After 2h incubation, dead cells was stained by propidium Iodide and analyzed by FACS.

In vivo characterization of GBD201 for efficacy vs. toxicity

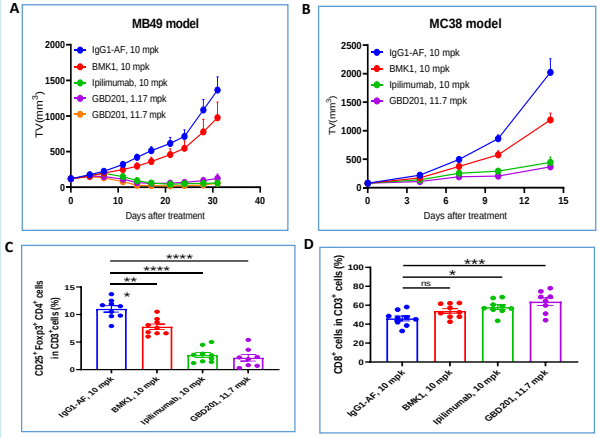


Figure 7. GBD201 showed potent in vivo anti-tumor efficacy in syngeneic models. hCCR8/hCTLA-4 C57BL/6 mice were implanted s.c. with MB49 (**A**) or MC38 (**B**) cells and treated with Abs from 11 days (for MB49) or 7 days (for MC38) after tumor inoculation. GBD201 showed potent tumor inhibition in MB49 model (**A**) and MC38 model (**B**). The tumor infiltration lymphocytes (TILs) from MC38 model were analyzed for Treg (**C**) and CD8+T cells (**D**). Significant Treg depletion and increased CD8+ cells were observed after GBD201 treatment.
BMK1, anti-CCR8 mAb.

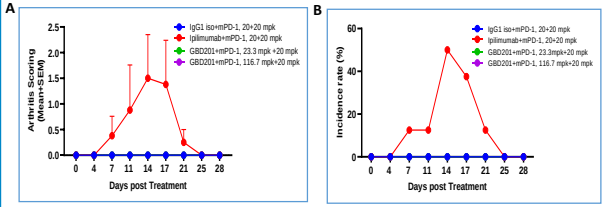


Figure 8. GBD201 showed better tolerance compared with ipilimumab in the induction of arthritis. hCCR8/hCTLA4 BALB/c mice were injected with Abs i.p. on days 0, 4, 7, 11, 14 and 17. The arthritis scores for paws (**A**) and arthritis incidence rate (**B**) were evaluated. No arthritis was observed in the GBD201-treated groups (both low or high doses).

Summary

- GBD201 preferentially targets CCR8$^+$CTLA-4$^+$ Tregs in TME but not CTLA-4$^+$ Tregs in the periphery, reducing the peripheral toxicity caused by CTLA-4 inhibition.
- GBD201 is a full blocker for CCR8, and potently inhibits CCR8/CCL1 interaction, resulting in the inhibition of Treg migration and functions.
- GBD201 has been fine tuned for the activity of CTLA-4 arm, by using a partial blocker and a unique molecular design, to balance the efficacy and safety. GBD201 showed much better safety profile compared to Ipilimumab in the arthritis induction model.
- GBD201 showed potent anti-tumor activity in mouse models. GBD201 may potentially be a next generation of CTLA-4 inhibitor by preferentially directed to TME.

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