Human anti-CD40 agonistic antibodies with enhanced FcγRIIb engagement activate immune cell and promote anti-tumor efficacy

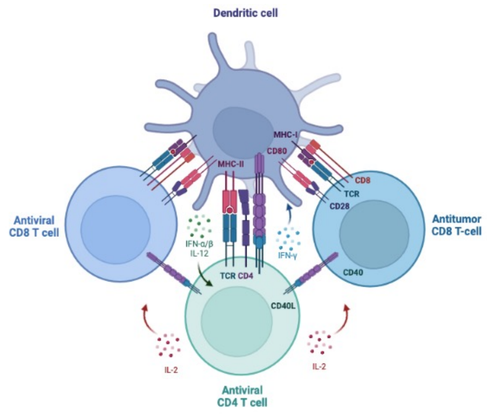
Hansika Wadhwa, Linya Wang, Tom Z. Yuan, Mouna Villalta, Zhen Han,, Kara Y Chan, Marisa Yang, Tammy Htoy, Paul VanDyke, Carson Holliday, Hector Franco, Hoa Giang, Fumiko Axelrod, and Aaron Sato

Twist Bioscience, South San Francisco, CA, USA

ABSTRACT

Checkpoint inhibitors represent a major advance in cancer immunotherapy, and combinatorial immunotherapies with secondary drivers of anti-tumor immunity provide beneficial effects for patients that do not show a strong endogenous immune response. CD40 is a member of the TNF family of receptors that has been shown to play a crucial role in enhancing B cell and dendritic cell activity and fostering anti-tumor immune responses. CD40-CD40L pathway is important in mediating a broad variety of immune and inflammatory responses including T cell-dependent immunoglobulin class switching, memory B cell development, and germinal center formation. Engagement of the inhibitory Fcg-receptor (FcgR) IIb shows promise for in vivo antitumor activity of agonistic anti-CD40 monoclonal antibodies, and Fc region mutant designs of anti-CD40 antibodies had been discovered to enhance FcgRIIb engagement. With Twist's precision DNA writing technologies, we have created phage display VHH and scFv libraries with diversity greater than 1×10^{10} for optimal discovery. In this study, we performed high affinity binding of the antibodies by SPR and cell surface binding. The leads are reformatted on human IgG2, IgG4, and IgG1 mutant. The in vitro properties of the CD40 agonistic antibodies demonstrate enhanced FcgRIIb engagement by NFkB activation. B cell activation is also detected by upregulation of CD86 and IL6 secretion. In humanized hCD40 mice model, we observed the engineered anti-CD40 agonists enhance anti-tumor immune function in vivo. These studies suggest that our antibodies can be potential drug for cancer immunotherapy.

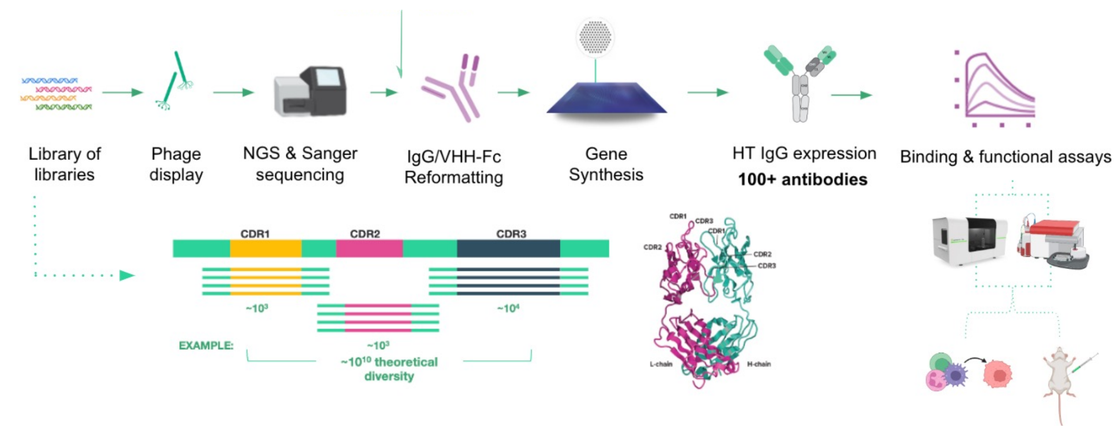
INTRODUCTION



CD40 is a TNF receptor superfamily member expressed on both immune and non-immune cells. Its ligand CD40L is a protein that is primarily expressed on activated T cells and is also a member of the TNF superfamily of molecules. CD40-CD40L pathway is important in mediating a broad variety of immune and inflammatory responses including T cell-dependent immunoglobulin class switching, memory B cell development, and germinal center formation. **Targeting the CD40L and CD40 pathway is a powerful means of attenuating autoreactive and alloreactive immune responses.**

CD40L and CD40 pathway contributes to an enhancement of cellular immune responses by virtue of an interaction between CD40L expressed on activated antigen-specific CD4+ T cells and CD40 expressed on dendritic cells (DC). CD40 signaling into dendritic cells thereby transmits a signal to activate the APC, which results in upregulation of CD80, CD86, and other co-stimulatory molecules for the optimal stimulation of CD8+ antigen-specific T cell responses. In addition, CD40 also expresses in the macrophage and B cells. In macrophage, the primary signal for activation is IFN-γ from Th1 type CD4+T cells. The secondary signal is CD40L on the T cell which binds CD40 on the macrophage cell surface. As a result, the macrophage expresses more CD40 and TNF receptors on its surface which helps increase the level of activation. B cells can also present antigens to helper T cells. If activated T cells recognize the peptide presented by the B cell, the CD40L on the T cell binds to the B cell's CD40 receptor, causing B cell activation. B cell can undergo division, antibody isotype switching, differentiation to plasma cells, and being able to mass-produce specific antibodies against an antigenic target.

DISCOVERY WORKFLOW



Accelerated workflow for the discovery of anti-CD40 antibodies. Twist's oligo synthesis technology can synthesize discrete oligo pools that are optimized during the design phase to maximize antibody library quality. Oligo pool's sequences are written directly from natural human diversity. Despite the natural sequences from human diversity, some may have isomerization, cleavage sites, deamination, and glycosylation sites but these liabilities can be removed upfront. Top binders from phage selections are converted to full length IgGs where their codons are optimized for mammalian expression and cloned into custom high copy mammalian expression vectors. IgG DNA are transiently transfected into HEK293 cells to produce antibodies. Antibodies are triaged in a series of binding and functional assays.

LIBRARIES AND ASSAYS

Project	Panning	Library	Top cell binders	NFkB activator	Primary immune cell activators
SC175	Protein panning Biotinylated hCD40	VHH hShuffle + VHH HI	13	7	4
SC177	Protein panning Biotinylated hCD40	Hyperimmune scFv	5	3	3
TB644	Protein panning Biotinylated hCD40-Fc	VHH hShuffle + VHH HI	10	3	2

Table 1. Summary of libraries and triage results for antibody characterization.

SCIENTIFIC RESULTS

1. Anti-CD40 antibodies show agonistic activity in cell-based functional assay

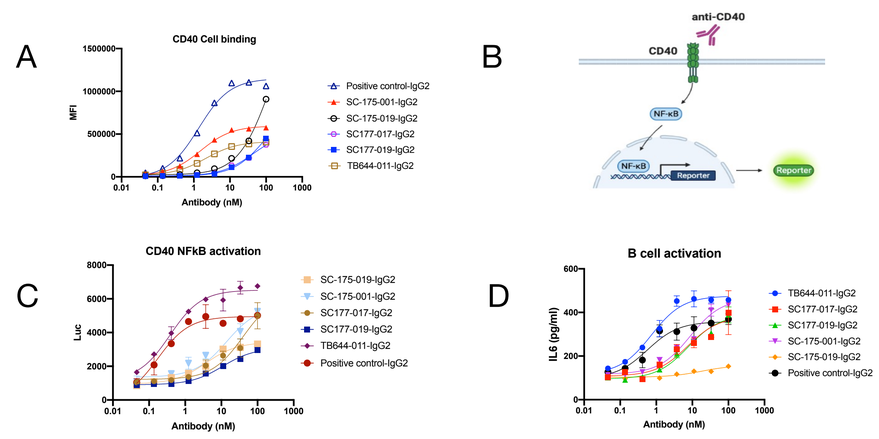


Figure 1. *In vitro* characterization of anti-CD40 antibodies. (A) Anti-CD40 antibodies bind to CD40-overexpressing HEK cell with high specificity. (B) NFkB reporter cells are utilized to evaluate NFkB activation under antibody treatment. (C) Anti-CD40 antibodies induce NFkB activation, showing agonistic activity. (D) Primary B cell activation is stimulated by anti-CD40 antibodies. IL6 release is measured by ELISA.

2. IgG1 Fc 2141-V11 mutant upregulates FcγRIIb engagement

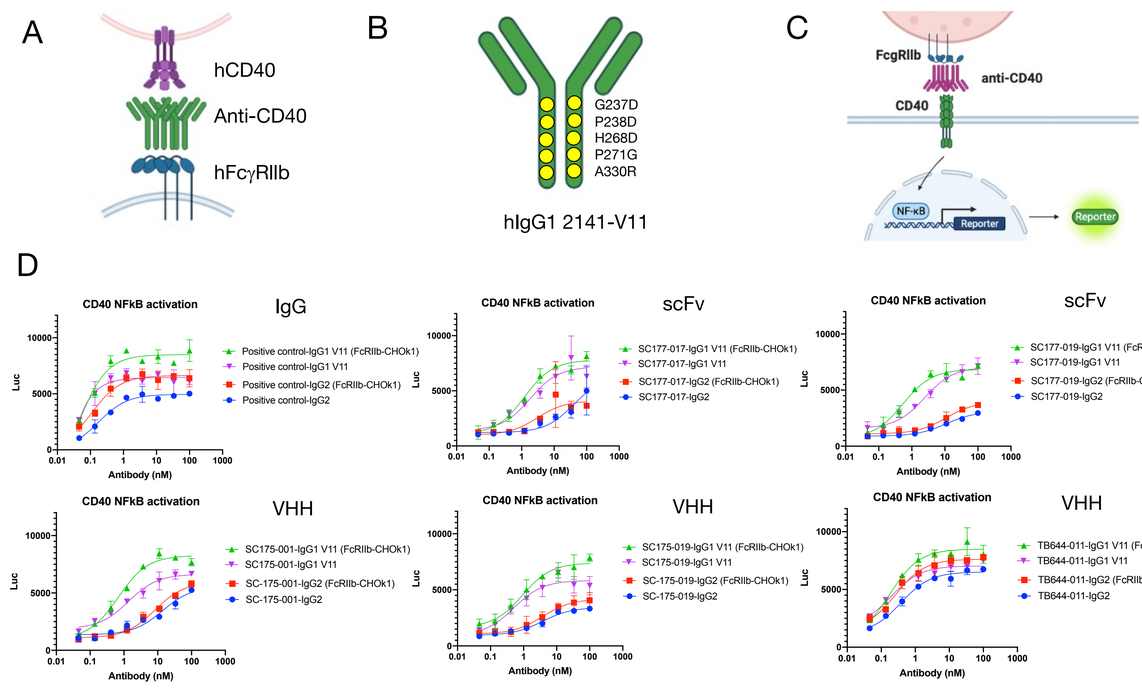


Figure 2. Human CD40 mAbs require FcγRIIb engagement for enhanced activity. (A) anti-CD40 antibodies cluster via Fc binding to FcγRIIb. The engagement upregulates agonistic activity of anti-CD40 antibodies. (B) Mutant hIgG1 2141-V11 enhances FcγRIIb engagement. Twist anti-CD40 clones are reformatted to hIgG1 2141-V11. (C) NFkB reporter cells are co-cultured with or without FcγRIIb CHO-k1 cells to evaluate FcγRIIb engagement. (D) CD40 leads with mutant hIgG1 2141-V11 show higher agonistic activity to activate NFkB.

3. Twist CD40 clones suppress tumor growth

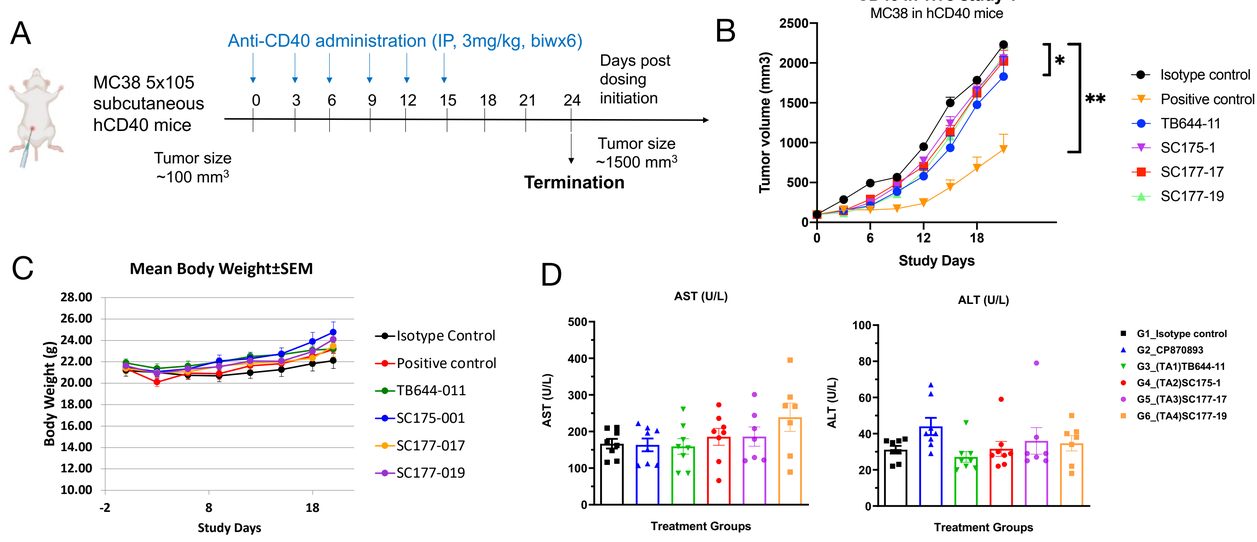


Figure 3. Anti-CD40 lead suppresses the growth of MC38 tumors in hCD40 mice . (A) The humanized CD40 mice are inoculated with MC38 cells. Dosing initiate at tumor volume average of ~100 mm^3 with 3 mg/kg via intraperitoneal injection once every 3 days for 6 cycles (Q3Dx6). Tumor sizes are measured 3 times a week. (B) Anti-CD40 treatment downregulates tumor growth, showing its efficacy in tumor suppression. *P ≤ 0.05 vs. isotype; **P ≤ 0.01 vs. isotype. (n=8). (C) No significant body weight changes were observed. (D) No significant liver toxicity was observed in detection of AST and ALT in the serum.

Linya Wang, PhD @ lwang@twistbioscience.com