

Development and characterization of novel CD40 antibody agonists for cancer immunotherapy

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CD40 as a Target for Immunotherapy

CD40 is a key molecule in the regulation of immune responses and its activity can be modulated using antibodies. In particular, agonist CD40 antibodies are highly effective in preclinical tumor models either through direct interaction with CD40-expressing lymphomas, or indirectly through the activation of an anti-tumor immune response. To date, limited clinical data have been reported with strong CD40 agonist antibodies; nonetheless it seems likely that targeting this pathway will require a balance between the benefits of potent immune stimulation to drive anti-tumor responses, and the harm that can result from non-specific immune cell activation. We set out to develop novel human anti-CD40 antibodies with different levels of agonist activity to identify a lead candidate for systemic application. Here we describe two novel fully human anti-CD40 mAbs with good agonist activity resulting in immune cell activation and direct anti-lymphoma activity in a xenograft model.

Development of novel human anti-CD40 mAbs

Anti-CD40 monoclonal antibodies (mAbs) were generated by immunization of human Ig transgenic mice (H2L2 strain of Harbour® transgenic mice) with recombinant and cell surface expressed human CD40.

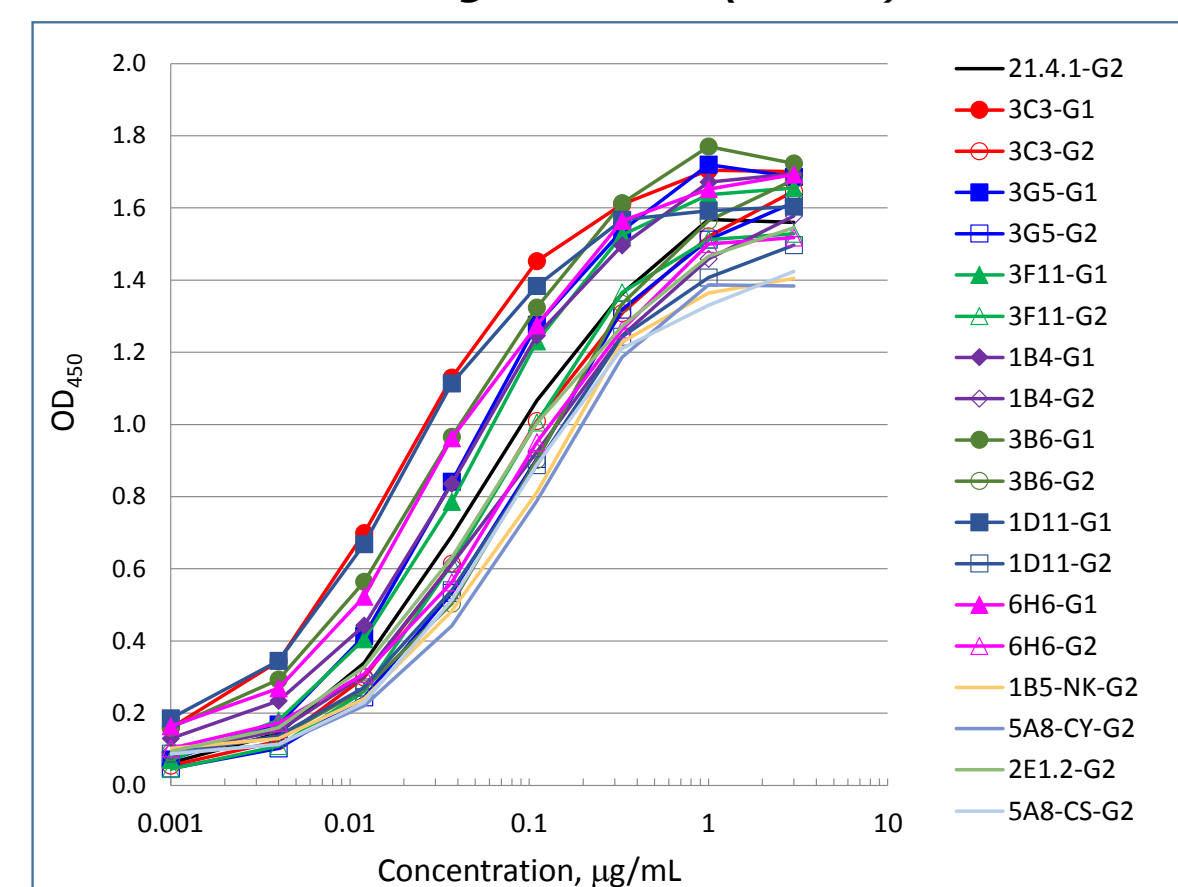
Hybridoma

Binding to human and NHP CD40 (ELISA and FACS)

VL and VH sequenced and inserted into human IgG1 and IgG2 vector

Transient expression

Binding to CD40 (ELISA)



ELISA plates were coated with rhu CD40-Fc and mAb binding was detected with a goat-anti-human IgG F(ab')₂-specific probe.

mAb 21.4.1 is also known as CP-870,893, Vonderheide RH et al. J Clin Oncol 2007;25:876-83

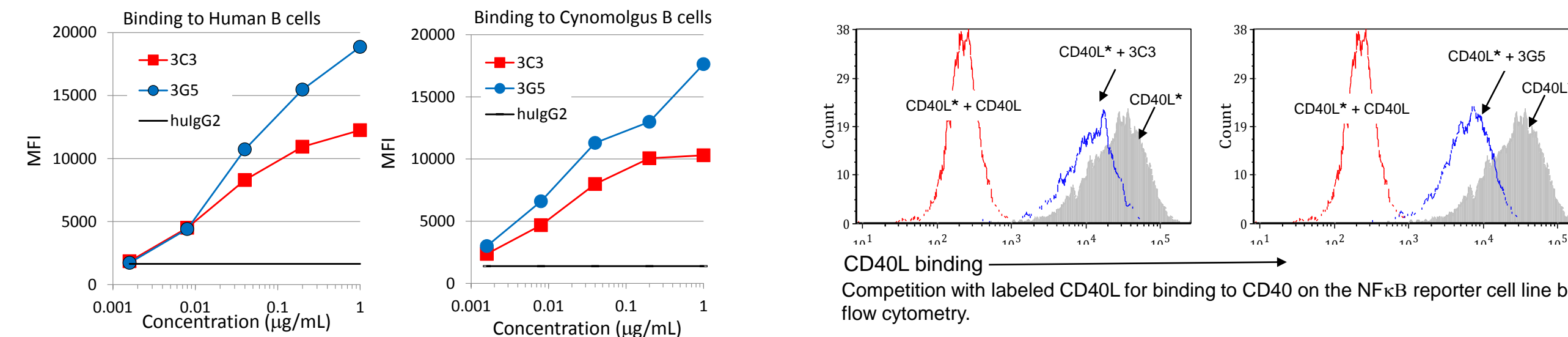
Clones 3G5 and 3C3 were selected for further characterization

mAb Affinities

Clone	Isotype	KD (pM)
3C3	G1	4.0
3C3	G2	10.7
3G5	G1	1.4
3G5	G2	3.3
3F11	G1	19.5
3F11	G2	20.5
1B4	G1	5.6
1B4	G2	10.5
3B6	G1	24.9
3B6	G2	7.9
6H6	G1	3.4
6H6	G2	2.8
1D11	G1	2.1
1D11	G2	7.2

Binding affinity was measured by bio-layer interferometry (BLI) using an Octet™ QKe instrument

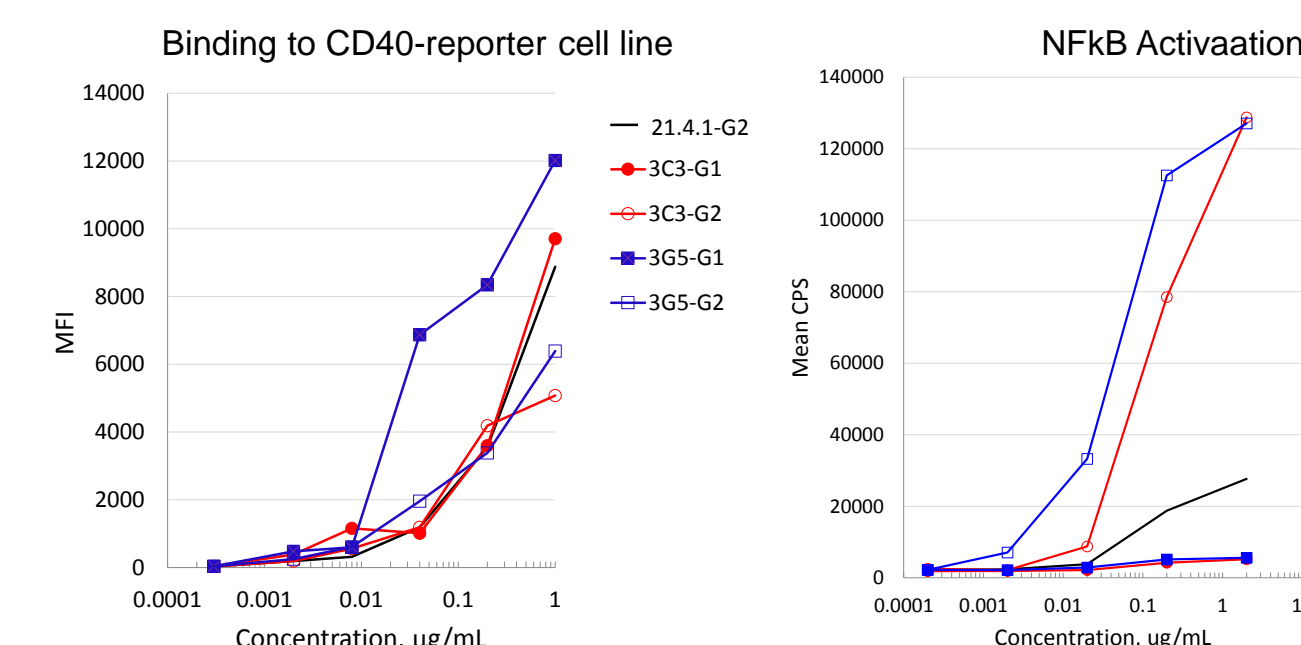
Binding to NHP and CD40L blocking activity



PBMC's from human or cyno were incubated with anti-CD40 mAbs. Binding was probed with a goat anti-human IgG Fc-PE antibody. B cells were identified by subsequent staining with anti-CD20.

3C3 and 3G5 mAbs have similar binding to NHP
Neither mAb competes for the CD40L binding site

CD40 NFκB reporter assay



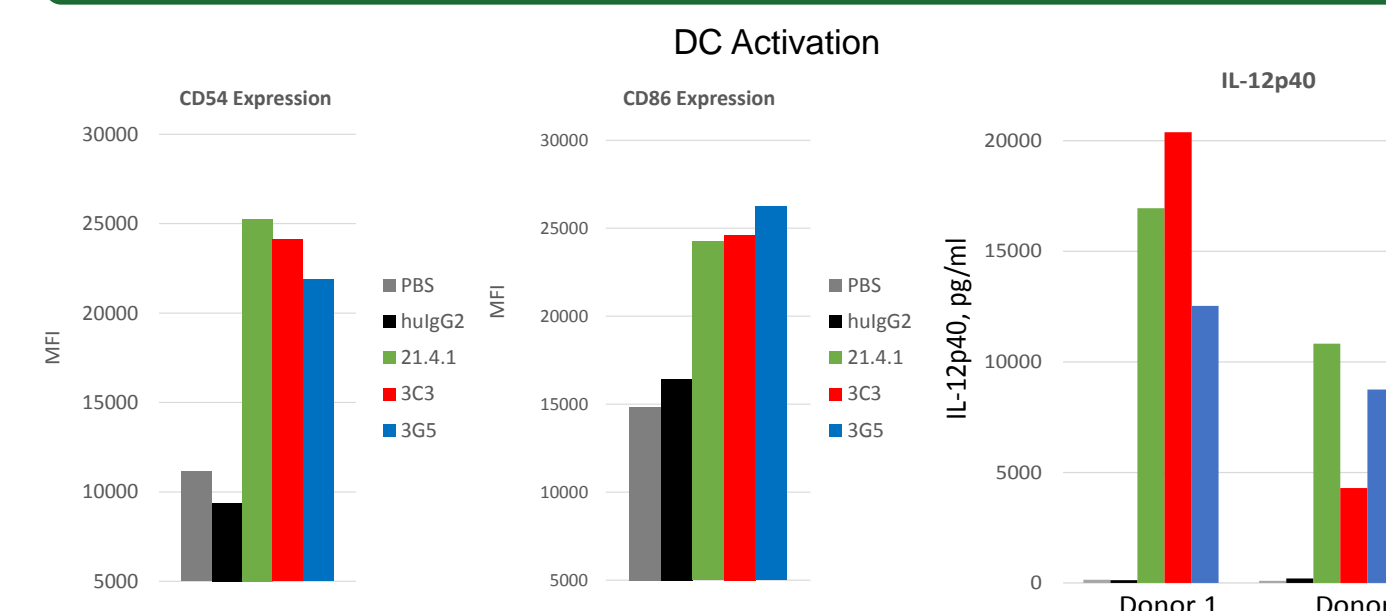
CD40 signaling leads to NFκB activation. This reporter assay provides a specific readout for signaling via CD40

The IgG1 isotype of 3C3 and 3G5 mAbs bind as well or better than IgG2 isotype

Only the IgG2 isotype have agonist activity measured using the reporter assay

CD40 was transfected into a NFκB-luciferase reporter cell line (Signosis). The mAb binding to the cells was determined by flow cytometry. For NFκB activations, the cells were incubated for 6 hours with mAbs. Luciferase expression was detected with the Luciferase Assay System by Promega.

Activation of DCs, B cells, and T cells

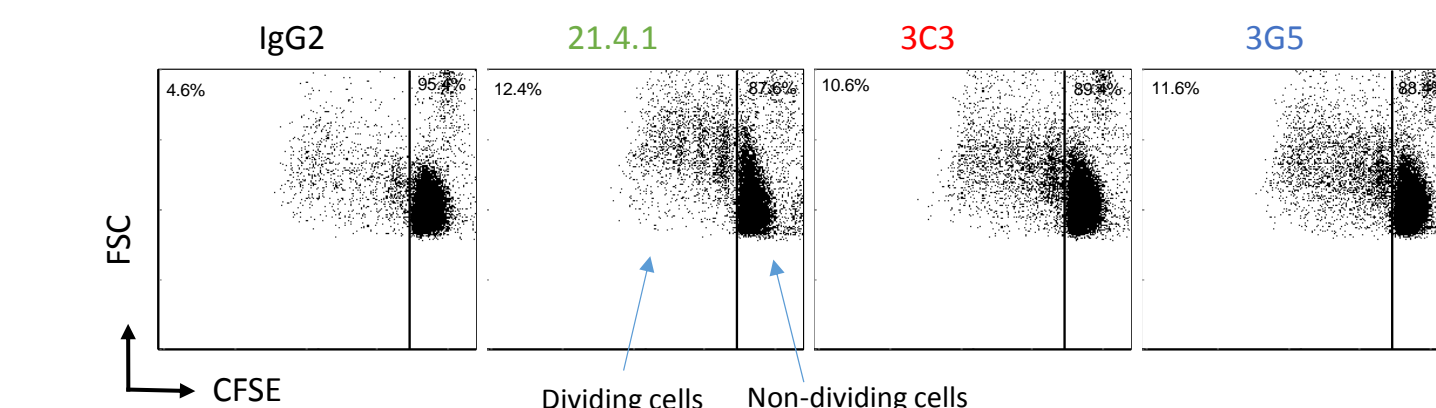


Adherent PBMC were cultured for 7 days in media containing 10ng/mL IL-4 (R&D Systems) and 100ng/mL GM-CSF (R&D Systems). Non adherent cells were harvested and confirmed to be dendritic cells by expression of CD11c. DCs were then incubated in the presence of 10 µg/mL of anti-CD40 mAbs for 72 hours. The cells were collected for flow cytometry and the supernatant was analyzed for cytokines by ELISA.

CD40 signaling on DCs, Macrophages, and B cells leads to their activation to promote immunity

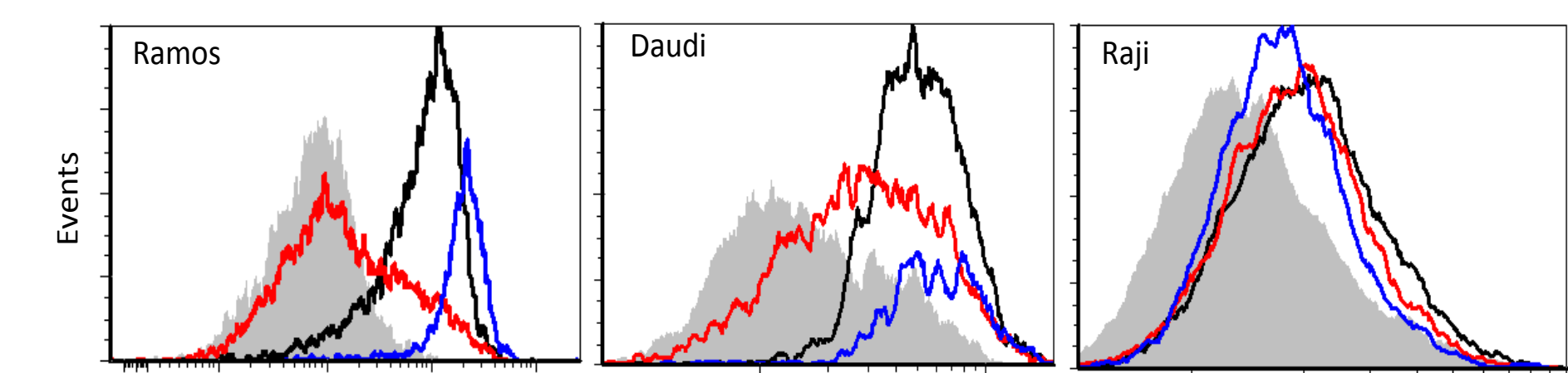
3C3 and 3G5 mAbs activate DCs and B cells through direct interaction with CD40 leading to cytokine production and expression of activation markers

3C3 and 3G5 mAbs induce T cells proliferation through indirect activation of APCs



CFSE labeled PBMCs were cultured in wells coated with CD3 antibody (OKT3) at 0.2 µg/mL. The CD40 mAbs or the isotype control were added at 10 mg/ml and cells were cultured for 6 days. The cells were stained with anti-CD3 and analyzed by flow cytometry.

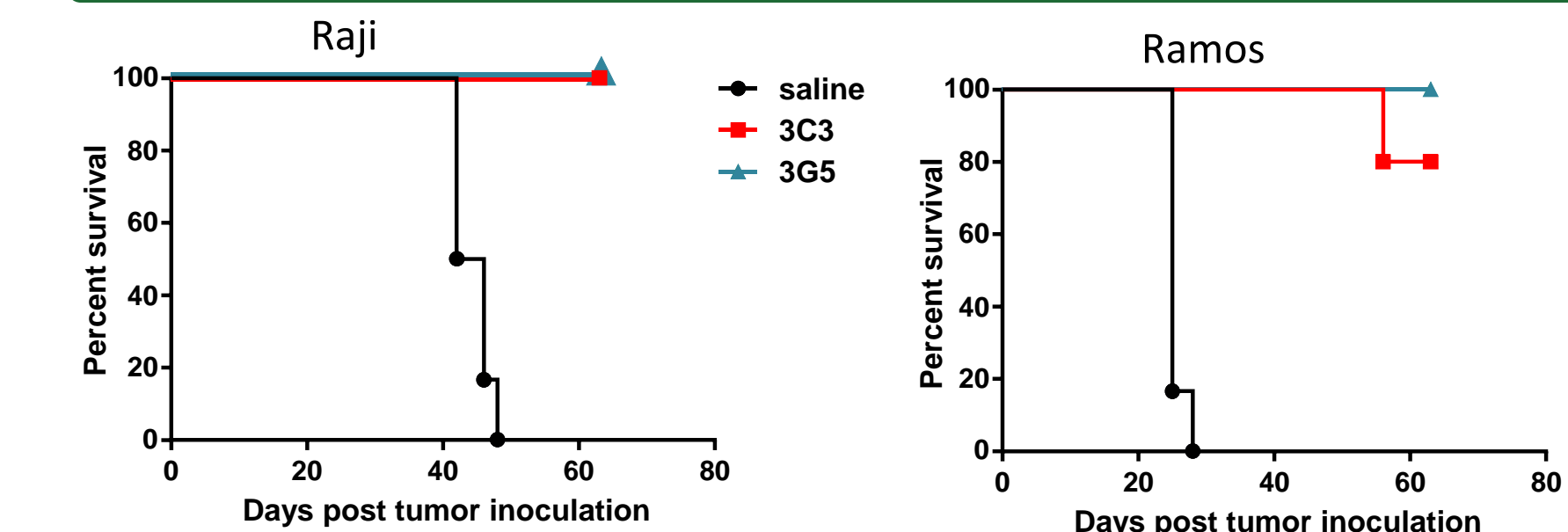
CD95 induction on tumor cells



Ramos, Daudi or Raji cells were incubated overnight with 2µg/mL of anti-CD40 mAbs. The cells were stained with PE-conjugated anti-CD95 antibody and analyzed by flow cytometry. Shaded histogram: untreated, red: 3C3, blue: 3G5, black: 21.4.1

3C3 and 3G5 mAbs induce CD95/Fas expression to a different extent on CD40-expressing B cell lymphoma cell lines

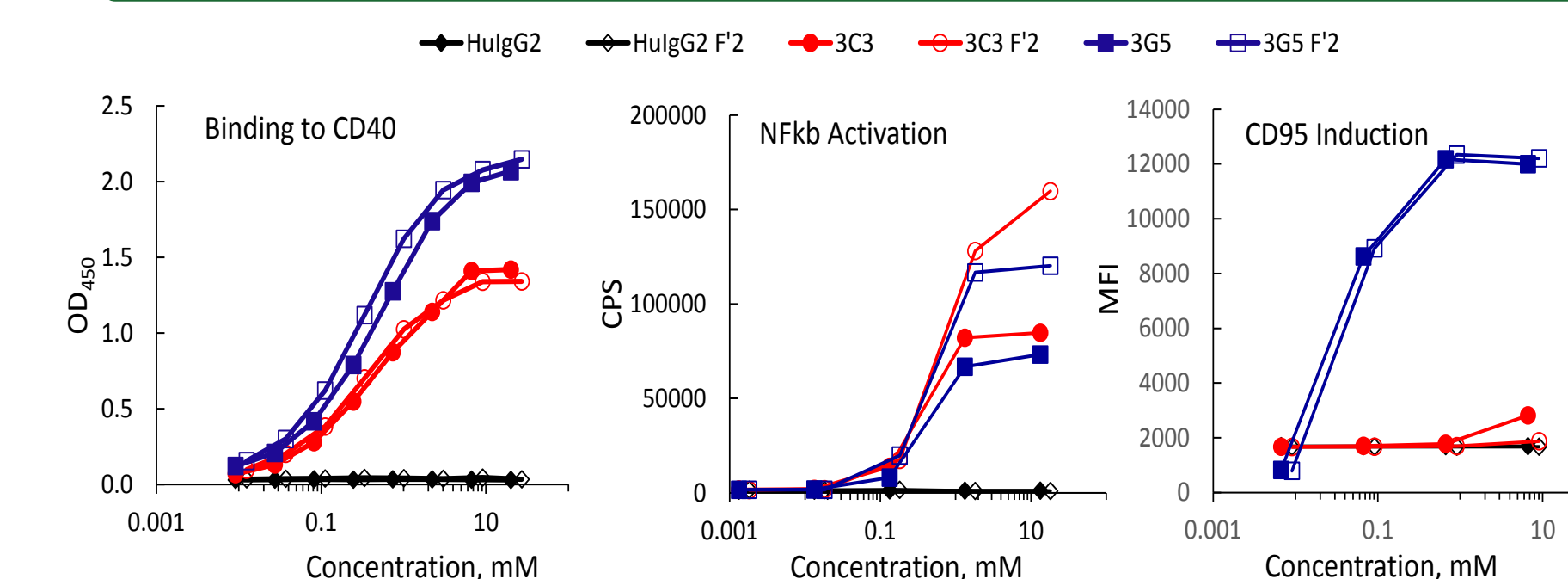
Anti-lymphoma activity



Ramos or Raji cells (1 x 10⁶) were subcutaneously injected into SCID mice, 5 mice per group (Day 0). On day 1, 5 and 11, these mice were treated with CD40 human mAbs or saline via intraperitoneal administration, 0.3 mg per dose.

3C3 and 3G5 mAbs have direct anti-tumor activity against CD40-expressing B cell lymphoblast cell lines in immune-deficient mice

Agonist activity does not require Fc



Fab', fragments of CD40 mAbs were generated by enzymatic digestion and tested for binding and activation with the CD40-expressing NFκB reporter cell line and CD95 induction with Ramos cells.

Agonist mAbs often require Fc receptor mediated crosslinking for efficient activity

3C3 and 3G5 mAbs do not require Fc interaction for agonist activity

Summary and next steps

- CD40 is a promising and powerful target for immunotherapy, but requires an appropriate balance between anti-tumor immune activation and harmful side effects of immune stimulation
- We have identified 2 novel, fully human anti-CD40 mAbs with good agonist activity when compared to the clinically evaluated mAb 21.4.1
- The agonist activity is dependent on expression as an IgG2 isotype and is independent of Fc receptor interactions
- Next steps are to characterize the biological activity and safety profile of 3C3 and 3G5 mAbs in non-human primates and select a lead candidate for clinical development

