CANCER RESEARCH UK

ENHANCING ANTIBODY AGONISM: MODULATING AGONISTIC PROPERTIES OF AN ANTIBODY

THE TECHNOLOGY

Monoclonal antibodies (mAbs) that stimulate anticancer immune responses are proving increasingly effective in cancer treatment, with growing evidence that such responses can be harnessed to provide durable eradication of tumours [1,2].

This versatile and patented technology provides the exciting opportunity to engineer clinical reagents with defined, tuneable therapeutic activity regardless of FcγR expression levels in the local microenvironment [3,4,5].

Through a combination of *in vitro* and *in vivo* approaches, it has been shown that the human IgG2 hinge and CH1 domains (Figure 1) impart FcγR-independent agonistic activity to immunostimulatory mAbs that bind to -CD40 and that this might also apply to other specificities.

Activity is provided by a structurally constrained isoform of hIgG2 due to its unique arrangement of disulfide bonds which confers distinct agonistic (or super agonistic) properties to the mAb.

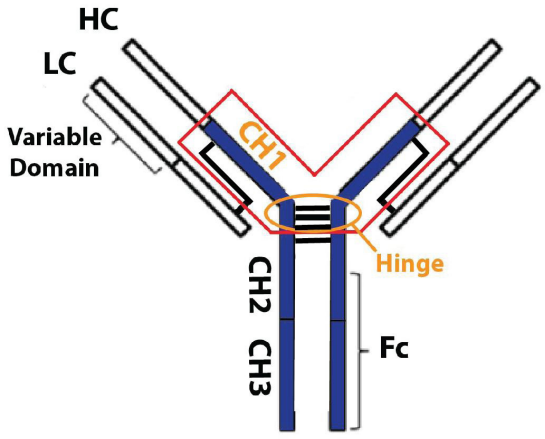

Figure 1: Schematic diagram of hIgG2 *antibody showing the immunoglobulin hinge and CH1 domains.*

SCIENTIFIC EVIDENCE

Agonistic Activity Depends on Both the Human IgG2 Hinge and CH1 Domains

CD40 mAb ChiLob 7/4 was used to examine the effect of human constant regions on agonistic activity (Figure 2 and 3).

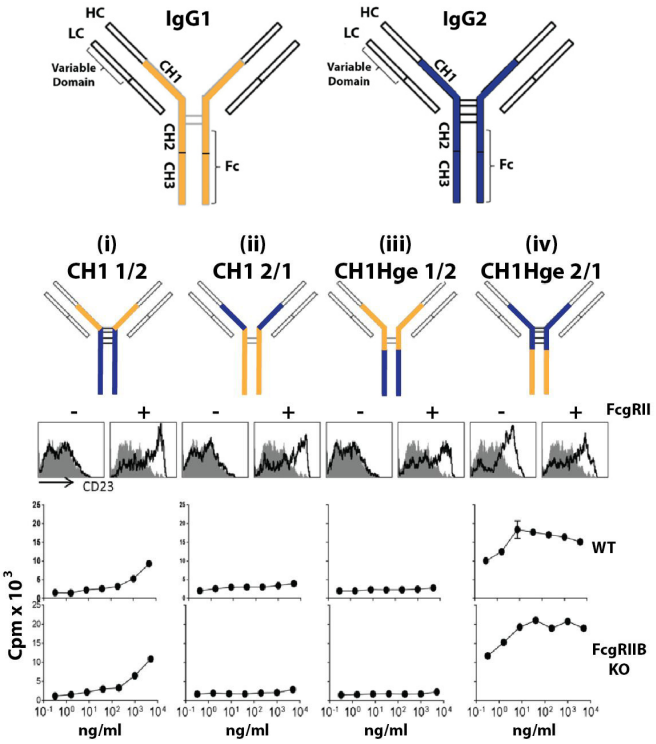

Figure 2: The Hinge and CH1 domains confer activity to ChiLob7/4 h2. Schematics of ChiLob 7/4 h1 (top left, in yellow) and ChiLob 7/4 h2 (top right, in blue) and hybrids (second row) where the CH1 (i, CH1 1/2 and ii, CH1 2/1) or CH1 and hinge regions (iii, CH1Hge 1/2 and iv, CH1Hge2/1) of h1 and h2 were swapped. Third row: CD23 expression on human B cells in the absence or presence of FcγRIIB-expressing crosslinking cells. Bottom: hCD40 Tg FcγRIIB WT or KO B cell proliferation in response to the chimeric mAb (mean and range of duplicates).

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In Figure 2, hybrids were created in which either the CH1 domain alone (CH1, i and ii) or both the CH1 and hinge regions (CH1Hge, iii and iv) of ChiLob 7/4 h1 (in yellow) and ChiLob 7/4 h2 (in blue) were switched (Figure 2 schematic diagrams). Comparative agonistic activity of the different mAbs was assessed by their ability to promote activation of human B cells and proliferation of hCD40 Tg B cells *in vitro* (Figure 2 third and bottom rows) .

Results show that only when both CH1 and the hinge of h1 were replaced with that of h2 (CH1Hge 2/1, iv) robust human B cell activation and proliferation of both FcɣRIIB WT and KO hCD40 Tg B cells was observed, similar to that seen with native h2 (data not shown).

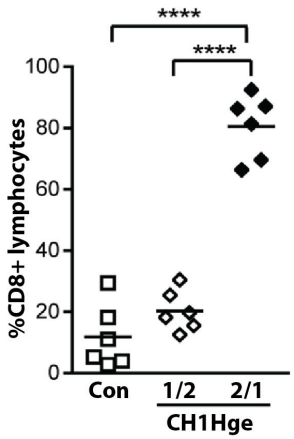

*Figure 3: OT I CD8+ T cell responses in hCD40 Tg mice treated with the indicated mAb. ****p < 0.0001 – OT I CD8+ T cells have a transgenic T cell receptor designed to recognise ovalbumin residues 257-264 (OVA) and they are used to study the response of CD8+ T cells to antigen (OVA-specific response).*

Similarly, *in vivo* (Figure 3), ChiLob 7/4 CH1Hge 2/1 (iv) produced significant increases in OVA-specific CD8+ T cell expansion, whereas ChiLob 7/4 CH1Hge 1/2 (iii) was inactive.

Human IgG2 Activity Is Dependent upon Its Disulfide Bond Configuration and Mutagenesis Produces a Range of IgG2 Agonistic Activities

IgG2 is unique among human IgG in its ability to ''shuffle'' disulfide bonds in its CH1 and hinge regions (Figure 1), resulting in a range of isoforms. The molecule is believed to be synthesized in its ''h2A'' form (Figure 1), which then gradually converts in the blood through a series of intermediates to its ''h2B'' form.

Mutagenesis was used to produce ''locked'' h2A- and h2B-like forms and data suggest that the FcɣR-independent agonistic activity of hIgG2 is contingent upon the precise conformation of disulfide bonds in its hinge and CH1 domains, and specifically on its ability to adopt the more compact h2B form.

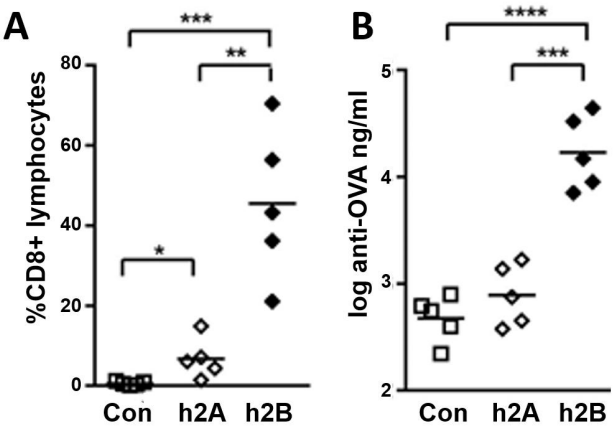

*Figure 4: hCD40 Tg FcɣRIIB KO mice immunised with OVA peptide plus 100 µg of chemically 'locked' h2A or h2B forms of ChiLob 7/4 hIgG2. (A) OVA-specific OT I CD8+ T cell responses, and (B) day 18 serum Ab responses. **p < 0.01, ***p < 0.001.*

Data have demonstrated that ChiLob 7/4 h2 could be manipulated *in vitro* and *in vivo* to achieve a range of agonistic activities. Figure 4 shows differences in ChiLob 7/4 h2A and ChiLob 7/4 h2B activity *in vivo*, where h2B caused significantly greater expansion of OVA-specific CD8+ T cells (Figure 4A) and production of OVA-specific IgG (Figure 4B) when compared to h2A.

Ongoing studies are aiming to determine the precise configurations of the different forms of the hIgG2 to shed light on their precise modes of action.

Moreover, the observation that IgG2 constant regions also conferred FcɣR-independent activity on another anti-hCD40 mAb, SGN40 and also possibly other receptors (data not shown), suggests this may be a general property of this restricted conformation.

INTELLECTUAL PROPERTY

Patent Family WO 2015/145360 protecting antibodies containing modified IgG2 domains which elicit agonist or antagonistic properties.

COMMERCIAL OPPORTUNITY

The patented technology is available to license on a non-exclusive basis.

REFERENCES

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CONTACT

Michela Perani, Business Development Manager

Michela.Perani@cancer.org.uk