4-1BB-mediated cancer immunotherapy: 'mission impossible' for non-engineered IgGs?

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Comment on: Buchan SL, Dou L, Remer M, *et al.* Antibodies to Costimulatory Receptor 4-1BB Enhance Anti-tumor Immunity via T Regulatory Cell Depletion and Promotion of CD8 T Cell Effector Function. Immunity 2018;49:958-70.e7.

Increasing tumor-specific immune responses with immunostimulatory monoclonal antibodies (mAbs) is a promising strategy in cancer therapy. Antagonistic mAbs blocking signaling through inhibitory receptors such as cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) and programmed cell death-1 (PD-1) have been shown to induce sustained anti-tumor responses in many cancers across various histologies, and agonistic mAbs targeting activator receptors are undergoing clinical trials (1). Costimulatory receptors of the tumor necrosis factor (TNF) receptor superfamily (TNFRSF), such as 4-1BB (CD137, TNFRSF9) are particularly interesting targets, as these receptors are absent on resting naïve T cells, but transiently expressed after TCR engagement in a precise and narrow time window (2). In addition, 4-1BB is also expressed on B cells, dendritic cells, NK cells, granulocytes and endothelial cells at sites of inflammation. 4-1BB has only one confirmed ligand [4-1BB-Ligand (4-1BBL), TNSF9], which is expressed on antigen presenting cells (2). Crosslinking of 4-1BB by its ligand or an agonistic mAb enhances T cell proliferation, cytokine production, survival and cytolytic activity.

Melero *et al.* (3) were the first to report that agonistic anti-4-1BB mAbs induce regression of large established tumors in mice. The long-lasting and tumor-specific immunity was primarily mediated by CD8+ T cells (3). Two mAbs to human 4-1BB have been developed and are undergoing clinical evaluation. Urelumab (BMS-663513) is an agonistic, non-ligand–blocking, fully human IgG_4 mAb with a hinge region mutation to improve stability. A clinical trial of urelumab as a single agent was associated with serious liver toxicities that resulted in two fatalities, leading to its interruption. The appearance and severity of transaminase elevations were dose-dependent with increased incidence at doses ≥1 mg/kg. Utomilumab (PF-05082566) is an agonistic, ligand-blocking, fully human IgG_2 mAb with a better safety profile than urelumab, but with only modest antitumor activity.

In the November 2018 issue of *Immunity*, Buchan *et al.* (4) set out to delineate the mechanisms through which full-length IgG-based anti-41BB mAbs promote antitumor immunity, and how the specific mechanisms depend on the variable and/or the constant regions of the antibody. The paper begins with the generation and characterization of chimeric mAbs (^{XI}) with variable regions of the rat IgG_{2a} anti-4-1BB LOB12.0 mAb, and either a mouse IgG_1 ($mIgG_1$) or IgG_{2a} ($mIgG_{2a}$) constant region (r4-1BB^{XI}mIgG_1 and r4-1BB^{XI}mIgG_{2a}, respectively). After verifying that both chimeric mAbs had the expected binding activities— both $mIgG_1$- and IgG_{2a}-based chimeras bound 4-1BB similarly, while r4-1BB^{XI}mIgG_1 interacted preferentially with inhibitory FcγRs and r4-1BB^{XI}mIgG_{2a} with activating FcγRs—they studied their effects in three solid tumor models. The r4-1BB^{XI}mIgG_{2a} induces effective antitumor immunity in all the tumor models while the IgG_1-based chimeric mAb had minimal effect. Next, they attempted

to understand the mechanistic differences between the two chimeric anti-4-1BB mAbs that might explain their differential efficacy. They found that intratumoral regulatory T (Treg) cells were effectively reduced in tumor-bearing mice by r4-1BB^{XI}mIgG$_{2a}$, but not r4-1BB^{XI}mIgG$_{1}$. Using bone marrow-derived macrophages from wild-type mice they demonstrated that the IgG$_{2a}$-based chimeric anti-4-1BB mAb induced phagocytosis of 4-1BB-expressing T cells, while IgG$_{1}$-based chimeric mAb did not, and the 4-1BB expression levels directly correlated with the efficiency of target cell depletion. Importantly, in tumor-bearing mice with a conditional deletion of 4-1BB on Foxp3+ Treg cells, the r4-1BB^{XI}mIgG$_{2a}$ lost its Treg cell-depleting activity and long-term protective immunity. Collectively, these studies demonstrated that the r4-1BB^{XI}mIgG$_{2a}$-specific Treg cell depletion depended on the simultaneous binding to both 4-1BB on Treg cells and an FcγR in tumor-infiltrating myeloid cells (*Figure 1*).

Buchan *et al.* (4) designed a series of elegant experiments using genetically engineered mice lacking FcγRs to establish the absolute dependence of the anti-4-1BB mAbs on FcγRs to promote long-lasting immunity. These experiments demonstrated that the therapeutic effect was dependent on the Fc binding profile of the anti-4-1BB mAbs and the availability of specific FcγRs in the tumor microenvironment. Specifically, IgG$_{1}$-based anti-4-1BB mAb exerted its effect primarily by directly activating CD8+ T cells following cross-linking via the inhibitory FcγRIIB, while IgG$_{2a}$-based chimeric anti-4-1BB mAb preferentially bound activating FcγRs and promoted antitumor immunity primarily by depleting Treg cells (*Figure 1*).

The authors then studied the possibility of combining IgG$_{1}$- and IgG$_{2a}$-based anti-4-1BB mAbs to maximize the antitumor effect. The simultaneous administration of r4-1BB^{XI}mIgG$_{1}$ and r4-1BB^{XI}mIgG$_{2a}$ resulted in a reduction in antitumour efficacy compared to the sole administration of r4-1BB^{XI}mIgG$_{2a}$, while sequential administration of r4-1BB^{XI}mIgG$_{2a}$ first to deplete Treg cells, followed by r4-1BB^{XI}mIgG$_{1}$ to activate CD8+ T cells, reduced tumor size and increased the percentage of tumor-free mice. Interestingly, sequential administration of IgG$_{2a}$-based anti-4-1BB mAb followed by anti-PD-1 mAb, similarly increased the long-term response rate to 80%, compared with 20–25% response rates with either of the two monotherapies.

Finally, the authors generated an engineered anti-4-1BB antibody to simultaneously deplete Treg cells and stimulate effector CD8+ T cells by swapping the C$_{H1}$ and

hinge regions from human IgG$_{2}$ in the mIgG$_{2a}$ constant region, a modification known to confer FcγR-independent agonistic properties on costimulatory mAbs (*Figure 1*). The engineered hybrid mIgG$_{2a}$/h$_{2B}$ anti-4-1BB antibody was more effective *in vitro* than the r4-1BB^{XI}mIgG$_{2a}$ mAb, while retaining its phagocytosis-inducing properties. Finally, they demonstrated that the engineered hybrid mIgG$_{2a}$/h$_{2B}$ anti-4-1BB antibody was superior to the IgG$_{2a}$-based anti-4-1BB mAb, in inhibiting the growth of EG7 thymomas in mice.

Although clinical trials will be required to test these ideas in humans, the work of Buchan *et al.* (4) provides several insights that not only inform the development of next-generation anti-4-1BB antibodies, but also show us how to combine depleting and agonistic full-length IgG-based therapies in general. These findings delineate a very intricate scenario, in which, in addition to the tumor immunogenicity and the numbers and locations of CD8+ tumor-infiltrating lymphocytes, the abundance of Treg cells and FcγR-expressing cells in the tumor microenvironment, as well as the ratio of activating to inhibitory FcγR, are important considerations that must be carefully studied in each patient. Furthermore, the competition between therapeutic mAbs, and probably endogenous IgG, can alter the mechanism of action and impact treatment efficacy of full-length IgG-based anti-41BB mAbs. In addition, the dynamic and changing nature of tumors, in the same patient throughout their evolution, or in different tissue locations (primary tumor *vs.* metastatic deposits), introduces additional complexity to the development of immunostimulatory strategies triggering activating receptors, which would probably have to be personalized, on an individual, temporal and even spatial level.

Another important question is whether it will be better to combine these dual properties in one antibody, as shown here in a mouse model, or to use Fc-optimized antibodies sequentially. These results allow speculating that costimulatory strategies that are not based on full-length IgG-based antibodies having wild type Fc regions (5,6), could be more easily systematized and applied in clinical setting. Finally, and considering that toxicity has been the major impediment for first-generation anti-4-1BB mAbs, and that several studies suggest that organ toxicities are mainly dependent on FcγR interactions (6), a similar study would be needed to determine the impact of the IgG isotype on immunological abnormalities associated with full-length anti-4-1BB antibodies.
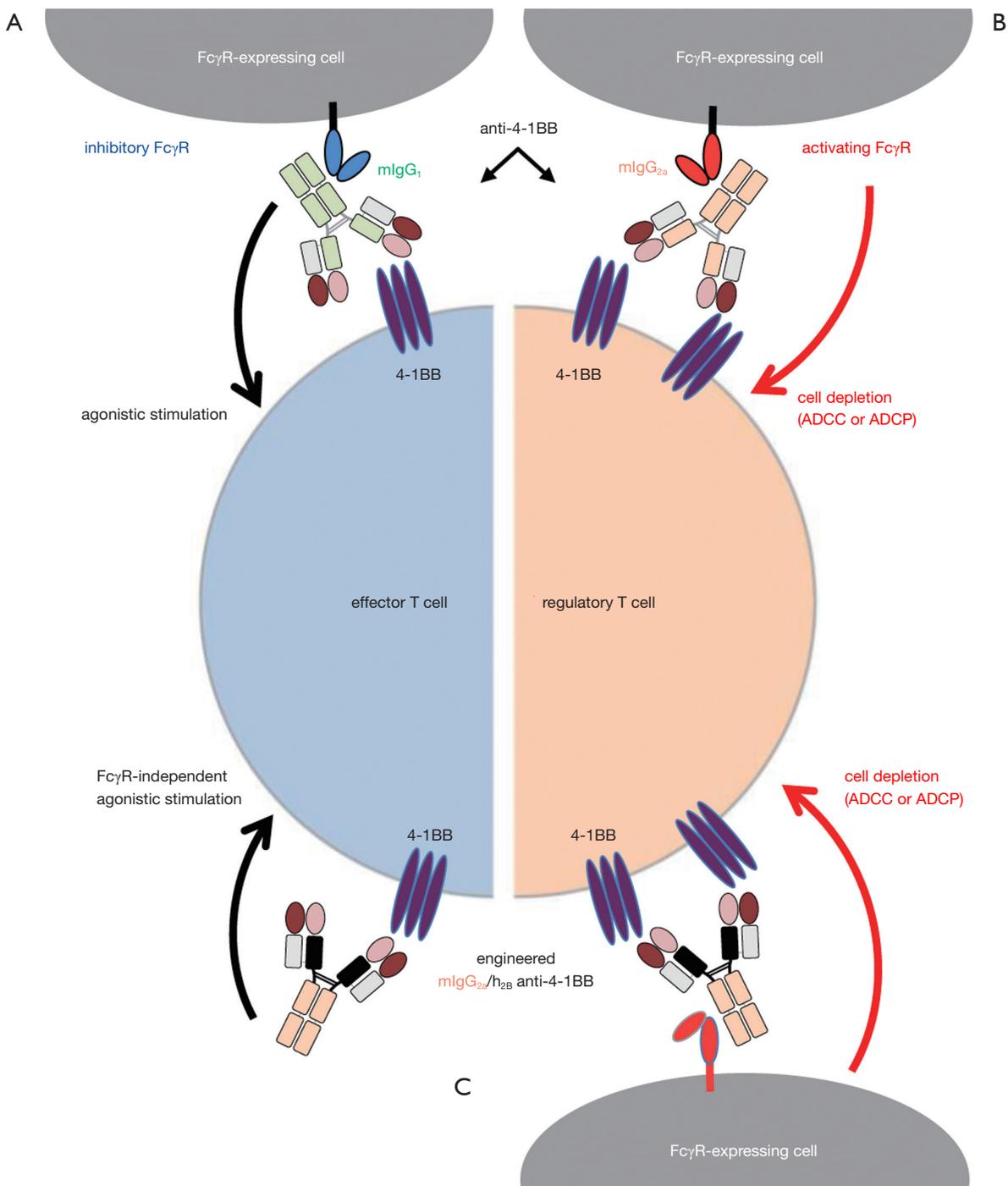
Figure 1 The Fc region of the anti-4-1BB antibodies determines the effector function and its therapeutic efficacy. (A) An anti-4-1BB chimeric mAb with the variable regions of the rat IgG_{2a} LOB12.0 mAb, and the $mIgG_1$ constant region induces the proliferation of the effector T cells, dependent on cross-linking by the inhibitory FcγR receptors; (B) the same anti-4-1BB antibody carrying the $mIgG_{2a}$ constant region depletes Treg cells in the tumor microenvironment via ADCC or ADCP, dependent on activating FcγR receptors; (C) an engineered anti-4-1BB antibody carrying the human IgG_2 C_{H1} and hinge regions and the murine IgG_{2a} constant region allows both FcγR -dependent Treg depletion and FcγR -independent agonistic stimulation of CD8+ effector T cells. $mIgG_1$, mouse IgG_1; $mIgG_{2a}$, mouse IgG_{2a}; Treg, regulatory T; ADCC or ADCP, antibody-dependent cellular cytotoxicity or phagocytosis.

Footnote

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