Commentary

Selective FcγR engagement by human agonistic anti-CD40 antibodies

Peter Ellmark[1], Sara M. Mangsbo[2], Malin Lindstedt[3]

[1]Alligator Bioscience AB, Lund, Sweden; [2]Department of Immunology, Genetics, and Pathology, Uppsala University, Uppsala, Sweden; [3]Department of Immunotechnology, Lund University, Lund, Sweden

Correspondence to: Malin Lindstedt. Professor in Immunotechnology, Department of Immunotechnology, Lund University, Medicon Village (building 406), S-22381 Lund, Sweden. Email: malin.lindstedt@immun.lth.se.

Comment on: Dahan R, Barnhart BC, Li F, *et al*. Therapeutic Activity of Agonistic, Human Anti-CD40 Monoclonal Antibodies Requires Selective FcγR Engagement. Cancer Cell 2016;29:820-31.

Submitted Sep 23, 2016. Accepted for publication Oct 09, 2016.
doi: 10.21037/tcr.2016.10.87
View this article at: http://dx.doi.org/10.21037/tcr.2016.10.87

Dahan *et al*. recently published a study where they investigated the influence of FcγRs on the activity of human monoclonal anti-CD40 antibodies using a mouse model with humanized FcγRs and CD40 (1). Using a humanized model system they elegantly demonstrate the importance of FcγRIIB for optimal efficacy of human anti-CD40 antibodies. The results partly contrast previously published material (2-4) where the importance of FcγRIIB is not unambiguous and is suggested to depend also on the antibody itself as well as the overall tissue expression load of the FcγR family. If the data presented by Dahan *et al*. can be transferred and confirmed in un-modified *in vitro* human assays it could impact the design of TNFR family directed antibodies in the future.

The success of antibodies targeting CTLA-4 and PD-1/PD-L1 has established immunotherapy as one of the pillars of cancer treatment that will have an impact on treatment of most types of advanced cancer. The main driver for development of new immunotherapeutic drugs is to identify therapies that acts complementary or synergistically with checkpoint inhibitors. One category of targets that holds promise in this regard is the co-stimulatory receptors of the TNFR super family (SF). These co-stimulatory receptors are expressed on T cells, NK cells and antigen presenting cells as well as other cell types and include targets to which there are numerous clinical development programs e.g., agonistic antibodies targeting CD40, CD137, OX40 and GITR (5). As for all immunomodulatory targets, the optimal way to target these receptors is based on the understanding of the biological processes and signaling mechanisms. The strength of the stimulatory signal induced via members of the TNFR-SF relies on receptor clustering and thus for antibody activation, on the level and quality of cross-linking induced by the antibodies (6). Generally lacking intrinsic signaling domains, TNFR-SF co-stimulators rely on stabilization of clusters of adaptor proteins (TRAF) to activate down-stream signaling pathways. There may be subtle differences between different members of the TNFR-SF in terms of pre-ligand assembly and association with additional cell surface receptors, but in general it seems that an increase in properly clustered TNFR-SF receptors results in a stronger signal (7,8).

FcγR expressing cells are important for the activity of these TNFR-SF targeting antibodies (8,9). An antibody can by itself only cross link two TNFR-SF receptors, and to induce a strong signal further cross linking via FcγR expressed on other cells (in trans) can be relevant. The FcγR/IgG biology differs between mice and humans, making the role of certain FcγR, the micro-milieu and the density of receptors mediating cross-linking difficult to translate between the species. The subject is further complicated by the fact that engagement of FcγR receptors may also induce ADCC, antibody-dependent cellular phagocytosis (ADCP) and complement-dependent cytotoxicity (CDC) (10). Typically, human IgG1 is a strong inducer of NK/Macrophage dependent ADCC, depending on the nature of the target, the cell type and the receptor density. IgG4 antibodies may also induce ADCC but to a lower extent than IgG1 (11). IgG2 antibodies induce

limited ADCC, but may confer other properties to TNFR-SF agonists, rendering them cross-linking independent and fully agonistic without the requirement for FcγR binding (2,3). The net effect will likely depend on the distribution of cells expressing the receptor, the possibility of the target cells to engage FcγR-expressing immune cells, the intrinsic antibody property to mediate clustering, as well as the receptor density and the sensitivity of different populations of immune cells to ADCC/ADCP/CDC. Consequently, the optimal choice of Fc may differ for agonistic antibodies targeting different TNFR-SF members, e.g., OX40, GITR, CD137 and CD40 (11).

It has been suggested that CD40 agonists require cross linking mediated by FcγRIIB for optimal functional activity *in vivo* in mouse models (4,12). However, other FcγR can induce the same function *in vitro* (4) and it is thus not clear from these studies if FcγRIIB in itself is important, or if it is the bio-distribution/tissue prevalence of the FcγRIIB that support the agonistic function of CD40 antibodies. Further, the pre-clinical efforts aiming at guiding the selection of the right Fc for a certain TNFR-SF are hampered by the differences in the IgG types and FcγR in mice and humans, which differ both in expression levels, distribution and affinity. These issues need to be resolved in order to understand the translational relevance of the pre-clinical data generated in this field.

In the recent publication in *Cancer Cell* (1), Dahan *et al*. address some of these issues using a CD40/FcγR humanized mice to study the effects of CD40 agonists with different variants of Fc. This model allows for functional studies where both CD40 and the FcγR are fully human, resolving the issue of different affinities between human and murine Fc-FcγR. Their data support that the IgG1 formats of tested CD40 agonists are more potent than the IgG2 isoforms, which is slightly contradicting to the conclusions from the study by White *et al*. (3) with regards to the ChiLob7/4 antibody. The difference is attributed to the fact that White *et al*. evaluated the CD40 agonists in mice with murine FcγR. Dahan *et al*. further provide data showing that the enhancement of FcγRIIB-binding is the most efficient strategy to enhance the activity of agonistic CD40 antibody. They demonstrated that the effects were stronger in FcγRIIB$^+$ than in FcγRIIA$^+$/FcγRIIB$^+$ hCD40 transgenic mice, however, the mechanisms behind the preference for FcγRIIB have not been elucidated. Bruhns *et al*. suggest that it can depend on the ability of FcγRIIB to bind actin and induce receptor capping (13). However, *in vitro*, any FcγR will provide sufficient cross-linking. Until a mechanistic explanation is presented it cannot be excluded that the dependency on FcγRIIB in mouse models is a consequence of the biodistribution/expression pattern of the FcγRII receptors or the physical proximity between the APCs that provides the FcγR-mediated cross-linking. Expression patterns and cell population densities may affect both innate and adaptive immune responses. Further, these mice may be more prone to develop IgM responses than IgG (14) and, importantly, the ADCC biology between the hFcγR mouse and wildtype mice may be affected. The translational relevance of the benefits of selectively enhancing the FcγRIIB binding may thus depend on how similar the biodistribution of CD40 and FcγR are in mice and humans. Dahan *et al*. further demonstrate that the increased activity of CD40 agonist, designed to bind selectively to FcγRIIB, increases thrombocytopenia and that this increase is linked to the agonistic effect. The authors demonstrate that the therapeutic window was improved compared to the IgG2 variant of CP8070.893 (1). However, in terms of toxicity, cytokine release is the most common adverse event related to treatment with CP870.893 (15), and further pre-clinical studies evaluating the cytokine release pattern, as well as assessment of the liver toxicity, in these models would be helpful to understand the potential clinical consequences of using Fc-engineered CD40 agonists.

Further, the finding that CP870.893 depends on FcγR binding for its agonistic function contradicts the study by Richman *et al*. (2) where they utilize a Fab2-variant of CP870.893 instead of an aglycosylated IgG1 version of CP870.893 and use a human *in vitro* model to assess the FcR dependence. To establish the relevance of FcγRIIB cross-linking presented by Dahan *et al*., it would be informative to assess the same reagents using relevant human *in vitro* systems to understand if the biology of these mice translates to the human situation.

In conclusion, the study by Dahan *et al*. certainly provides new insights into biology of CD40 agonists; however, since conflicting data have been generated the question of how to design the optimal CD40 agonistic antibody in terms of risk versus benefit remains open.

Acknowledgments

Funding: Sara M. Mangsbo is founded by FP7 MCA-ITN 317445, Göran Gustafssons Stiftelse and the Swedish Society for Medical Research SSMF.

Footnote

Provenance and Peer Review: This article was commissioned and reviewed by the Section Editor Xia Fang (Department of hematology, Shanghai Tongji Hospital, Tongji University School of Medicine, Shanghai, China).

Conflicts of Interest: P Ellmark is employed by Alligator Bioscience AB; SM Mangsbo is the founder and CSO of Immuneed AB; the other author has no conflicts to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Cite this article as: Ellmark P, Mangsbo SM, Lindstedt M. Selective FcγR engagement by human agonistic anti-CD40 antibodies. Transl Cancer Res 2016;5(Suppl 4):S839-S841. doi: 10.21037/tcr.2016.10.87