Editorial

An engineering feat of small proportions

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Comment on: Zhang Q, Ouyang Q, Li W, *et al.* Efficacy and safety of margetuximab plus chemotherapy *vs.* trastuzumab plus chemotherapy in Chinese patients with pretreated HER2-positive advanced metastatic breast cancer: results from a randomized, open-label, multicenter, phase II bridging study. Transl Breast Cancer Res 2022;3:31.

Received: 02 September 2022; Accepted: 21 September 2022; Published: 31 October 2022.

doi: 10.21037/tbcr-22-44

View this article at: https://dx.doi.org/10.21037/tbcr-22-44

Spanning over 2,000 years to materialize, the Great Wall of China ranks among the greatest architectural feats ever accomplished by humans. While people are aware of this engineering wonder, lesser known is its historical bifunctionality. Much as defense was the bedrock of this monumental effort, the structure is also symbolic of human subsistence and portage. Now, even more than 400 years ago when the wall was completed, the reality of molecular engineering, too, is a technological marvel. Central to this theme is the creative packaging of cloned antibodies as therapeutic agents. In reviewing the accompanying paper by Professor Jiang *et al.* (1), we delve (briefly) into hybridoma technology, learn (more) about the effector functions of the antibody, and indulge in subtle details to an (even greater) degree.

Essential to understanding the transformative possibilities of monoclonal antibodies (mAbs) as life-prolonging anti-cancer agents was the emergence of a method to generate large quantities of identical immunoglobulins (Ig). These epitope-specific proteins are configured as two fragments, one with a pair of identical antigen-binding regions, (F)ab, appended by a hinge to a crystallizable domain (F)c. Even though the bulk of the antibody's therapeutic activity is modulated by (F)c, innate cellular and protein components mediate the immune response. Among the Ig isotypes, IgG and specifically IgG1, stimulates the most robust effector response via binding interactions between (F)c and members of the (F)c gamma receptor (F)cγR family. While beneficial, the immune response must also be stringently balanced to protect against toxic autoimmune reactions; and hence the presence of activating and inhibitory receptors. The relevance of this binary system may be superseded by the importance attached to the portion of (F)c which regulates (or facilitates) engagement of the immune components. So salient is this issue, it raises the question whether microscale modifications of trastuzumab's amino acid configuration give rise to a new "designer" molecule with greater anti-tumor activity. The results reported in this proffered paper (hereafter referred to as the Bridging study), which corroborate findings of the SOPHIA trial (2), suggest that this is so.

At the focus of both clinical trials are two nearly indistinguishable human epidermal growth factor receptor 2 (HER2)-targeted mAbs. Although of identical isotype and epitope specificity, the individual differences relate to their hybridoma and cosmetic platforms. Regarding the former, technological advances of the murine hybridoma resulted in the development of chimeric mAbs (such as margetuximab), which linked murine fragments of both light and heavy chains of (F)ab to human (F)c domains (3). Further improvement occurred with "humanization", a process which entails transfecting the exact nucleotides that encode the complementarity-determining region of a mouse gene into a human Ig gene (4). The resulting complementarity-determining region (CDR)-grafted antibody (such as trastuzumab) features only the murine antigen-binding surface in the human Ig molecule. In essence, both types of hybridomas should decrease (though not necessarily to the same extent) formation of anti-idiotypic antibodies compared to the fully murine Ig. With respect to the second platform, appreciating the critical role of (F)cγRs in regulating IgG effector functions gave impetus not only

to determine which parts of (F)c facilitate recruitment of various immune components but also an opportunity to investigate whether tailored alteration of the amino acid makeup imparts enhanced activity. The latter was again explored in the Bridging study.

Aside from hybridoma technique, the major difference between margetuximab and trastuzumab involves substitution of only five amino acids (L235V/F243L/R292P/Y300L/P396L) in the chimeric protein's (F)c domain. That the microscopic chemical changes integrated into margetuximab appear to support the improvement in clinical outcomes is reinforced by the comparable median progression-free survivals (mPFS) observed in the Bridging and SOPHIA trials, 5.52 [95% confidence interval (CI): 4.14–8.34] months *vs.* 4.14 (95% CI: 2.76–5.52) and 5.8 (95% CI: 5.5–7.0) months *vs.* 4.9 (95% CI: 4.2–5.6) months, respectively (1,2). Concordance notwithstanding is the question whether between-study differences, most notably, participants of Asian ethnicity (100% *vs.* 6.3%), total number of subjects enrolled (123 *vs.* 536), and prior anti-HER2 antibody treatment (excluding trastuzumab, ≤25% *vs.* ≥90%), could impart any meaningful effect.

While some population demographics (e.g., ethnicity) may not confound outcomes, others (e.g., agents given in prior lines of therapy) can have muddling effects. For example, both studies included "heavily" pretreated patients though only a quarter of the enrollees in Bridging received at least two mAb-targeted therapies compared to SOPHIA where nearly all subjects received three antibodies with HER2 specificity. Furthermore, the considerable disparity regarding ado-trastuzumab emtansine (T-DM1) (and possibly pertuzumab) is of interest because *prior* treatment with this (these) agent(s) appeared to surreptitiously benefit margetuximab more than trastuzumab (2). Although not necessarily the corollary, it is intriguing to speculate that the absence of previous tandem mAb therapy underestimated the beneficial effects of margetuximab observed in the Bridging study rather than the observed benefit being linked to previous tyrosine kinase inhibitor (TKI) therapy. Second, it is well accepted that binding and activating immune cells depend on (F)c/(F)cγR interactions (5). In addition, a single nucleotide polymorphism of the (F)cγRIIIa gene generates two allotypes which have been found to alter binding affinity and therefore, antibody function (6). One in particular, the (F)cγRIIIa-158 valine (V)/phenylalanine (F) gene polymorphism corresponds to appearance of the V/V, V/F, or F/F phenotypes. Of significance, studies have observed trastuzumab binding to high-affinity (F)cγRIIIA-158 V/V (compared to lower affinity F genotypes) correlated with improved clinical benefit (7,8). Margetuximab's (F)c domain was engineered to increase binding to F variants, especially F/F and V/F. Worth noting, approximately 90% of the population of the Bridging study were F/F or V/F genotypes; about 7% had the V/V polymorphism. Comparative percentages of these genotypes in the SOPHIA trial were 82% and 14%, respectively. Protocol-specified (in SOPHIA) exploratory analysis of progression-free survival (PFS) by (F)cγRIIIA-158 genotype appears to add credence to the engineering rationale of margetuximab. Though with a median-follow-up of only 2.8 months, mPFS among margetuximab-treated carriers of F/F or V/F genotypes compared to their trastuzumab-treated counterparts were 6.9 (95% CI: 5.55–8.1) *vs.* 5.1 (85% CI: 4.14–5.59) months, P=0.005. What remains uncertain, however, is whether F-zygosity matters. Unclear also, mPFS was not improved (in SOPHIA) among those with the V/V phenotype, 4.8 (2.46–5.65) *vs.* 5.6 (2.86–11.04) months, again respectively; hazard ratio (HR), 1.78 (95% CI: 0.87–3.62). A similar negative trend in risk reduction, HR, 1.15 (95% CI: 0.07–18.59) associated with margetuximab was also found in the Bridging study. Even though a few reasons have been proposed to cover this unexpected outcome, it is also rational to consider whether alterations in (F)c subverted (at least partially) the antibody's affinity for the V/V activating receptors. Third, while uncertain, it appears that at least half of the population in the two studies did not have metastatic disease at diagnosis. The relevance of this information relates to the supposition that a relatively short duration from the end of (neo)adjuvant therapy to relapse could have amplified the poorer outcomes of those randomized to trastuzumab again. This is alluded in the Bridging study where the authors state they "*counted adjuvant therapy as one line of therapy if progressed within 6 months*". Fourth, nearly all analyzed components have CIs which cross the null effect suggesting differences between the mAbs are not significantly different. Still, there is a hint that margetuximab is less effective in subjects with no prior T-DM1 or the V/V genotype. These data are confounding but may be instructive for another reason, the issue related to sequencing of anti-HER2 mAbs. For instance, given the relative frequency of the F/F and V/F polymorphisms in the general population, margetuximab may be better positioned to be given in subjects who do not harbor the V/V genotype. Furthermore, margetuximab may be of greater benefit after disease progression on trastuzumab and T-DM1. But perhaps most instructive is the issue related to the development of neutralizing

antibodies. Immunologic dogma forebodes generation of anti-idiotypic antibodies against any innovated antibody that is not identical to the native molecule. Partial validation of this principle comes from a report of neutralizing antibodies against another chimeric mAb (9). In a study from the United Kingdom, 23% of subjects developed anti-CD20 antibodies after one dose of rituximab. Although disease-related remission rates at six months were not different, relapse rates were significantly higher for patients who developed autoantibodies. Small as the study was, the findings should not be overlooked as cross-reactivity (and diminution of activity) with other humanized mAbs occurred in 80% of the patients. The inference of these findings is the use of humanized antibodies before chimeric molecules may be the more rational approach.

Presupposing that the improvements in clinical outcome were due, inherently, to enhanced cellular effector functions do not exclude the possibility of other operative mechanisms. Nonetheless, the perception that (F)c-engineering can retrofit mAbs with improved anti-cancer properties appear to be the realization of a chimerical dream. And though modest in scope, the early accomplishments are not necessarily inconsequential. Moreover, the emergence of novel immunotherapies from the laboratory to the clinic is still in its formative years.

While (F)cγRs have the potential to modify the efficacy of antibodies, it is important to emphasize that activating and inhibitory receptors are more than one dimensional. For example, several therapeutic mAbs have been purposely engineered with decreased affinity for (F)cγRIIIa. Unlike margetuximab, all of the anti-PD-L1 antibodies have been designed in this manner in order block engagement of PD-1 and PD-L1 without depleting PD-L1-expressing antigen-presenting cells and T-cells (10). Another explicit example of a component with divergent consequences is (F)cγRIIb (CD32B). Not mentioned previously, an additional feature of the altered five amino acids in margetuximab relates to decreasing the affinity for this receptor. Correspondingly, several noteworthy studies have shown that this inhibitory receptor was associated with impaired rituximab and trastuzumab activities (11,12). Expressed on macrophages, antibody-dependent cellular phagocytosis was reduced, putatively by internalization of the antibodies. On the other hand, binding of IgG1 to (F)cγRIIb has been shown to enhance the immunomodulatory effects of stimulatory mAbs through formation of receptor clusters and triggering tumor cell death (13).

Although the current publication adds to the importance and demonstrates that (F)c-engineering can lead to improved cancer outcomes, the paper also reveals that margetuximab may be a technological, but not a clinical, breakthrough. The latter assertion is supported by mPFS prolongation favoring the margetuximab arm of 41 and 27 days in the Bridging and SOPHIA trials, respectively; although preliminary, differences in duration of mOS were not specified in Bridging and 54 days in SOPHIA.

The reality is that mAbs are elegant constructs and optimizing effector function will require precision engineering. Glycoengineering by altering the specific configuration of the (F)c glycan can increase the affinity for (F)cγRs is one rendition; another variant that can enhance binding affinity involves removing the core fucose (afucosylation). And because enhancement of cellular effector function co-exists with potential undesirable effects, another empiric consideration encompasses the development and testing of (F)c-void or -muted antibodies. These are only a few examples of ways anti-cancer antibodies could also be improved; many more will inevitably become manifest. In hindsight, analogous to the initial phases of building the Great Wall is the fact that it is much too soon to envision the full extent of antibody engineering feats yet to come.

Acknowledgments

Funding: None.

Footnote

Provenance and Peer Review: This article was commissioned by the editorial office, *Translational Breast Cancer Research*. The article did not undergo external peer review.

Conflicts of Interest: The author has completed the ICMJE uniform disclosure form (available at https://tbcr.amegroups.com/article/view/10.21037/tbcr-22-44/coif). The author has no conflicts of interest to declare.

Ethical Statement: The author is accountable for all aspects of the work, including ensuring that any questions related to the accuracy or integrity of any part of the work have been appropriately investigated and resolved.

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doi: 10.21037/tbcr-22-44
Cite this article as: Higa GM. An engineering feat of small proportions. Transl Breast Cancer Res 2022;3:35.

