View from the Fc: Five Rules for mAb Development Risk Reduction

Understanding your product and avoiding nasty surprises in mAb development

The human immune system is an immensely complex network, and our understanding of the interactions between its various components remains partial today. Developers of immunomodulatory and immunotherapy drugs seek to target and harness specific aspects of this network without disrupting or skewing its overall functioning. However, current approaches to measuring immune function fall short in capturing these impacts and worse do not even make full use of the available tools.

The most widely used class of immunotherapy drugs are monoclonal antibodies (mAbs). Only one in five mAbs that enter clinical development achieve commercialization, and the cost of developing an antibody drug, including the cost of failures, is estimated at $2.6bn[1]. With over 1,400 antibody-based drugs reported to be in clinical development, any reduction in the failure rate would have huge economic benefits as well as reduce the risk of harm to clinical trial participants.

SeromYx is an immunology service provider with a unique focus on understanding, profiling and measuring Fc-driven effector function. Our business currently covers two segments of the biopharma industry, vaccines and monoclonal antibodies, that is to say, pharmaceutical interventions reliant on the humoral immune response. The purpose of this paper is to summarize some of the lessons learned from our work for mAb developers.

1. Fc Functions: Three is not a Crowd

Standard industry practice is to evaluate three Fc effector functions: NK cell-driven cytotoxicity (“ADCC”), complement-dependent cytotoxicity (CDC), and monocyte-driven phagocytosis (“ADCP”). This approach vastly understates the range of Fc functions that can be induced by antibodies along two dimensions, the range of functions engaged and the number of cell types capable of performing these functions. As an example, “ADCC” assays usually measure only NK-cell driven cytotoxicity, but the activating FcγRIIIa found on NK cells is also expressed constitutively by macrophages, NK, monocytes and γδ T cells, and inducibly by dendritic cells (DCs). Even if we limit ourselves only to FcγRs (most mAbs in development use IgG backbones) and only Type-I FcRs, we count at least 23 cell type/FcγR combinations, so three assays to measure three combinations is really just scratching the surface.

Why does this matter? Safety and efficacy. For example, a common adverse event with mAbs and cell therapies are infusion reactions and in more severe cases, cytokine release syndrome. Many of these events are likely Fc-mediated and may require vigilant in-patient monitoring and pre-treatment which is onerous and carries risks of its own. Broader Fc functional profiling might allow the selection of candidates with this undesirable effect reduced or eliminated. Such broader profiling might also enhance understanding of the mechanism of action (MOA), as well as allow the selection of more potent candidates where Fc function is key to efficacy; for example, cancer immunotherapies can activate both neutrophils and NK cells. Similarly, other Fc functions, such as phagocytosis and DC activation play a role in antigen presentation and T cell activation, with potential safety and efficacy implications.

This is not just theoretical. In an internal R&D study, SeromYx tested a panel of CD20 mAbs including three approved antibodies and Fc variants of them. Our assays for ADCP, CDC and ADCC recapitulated what has been published for these antibodies, but also showed previously unreported activity across some molecules, specifically

the activation of neutrophils, DCs and eosinophils (Figure 1). What contribution to clinical efficacy, duration and depth of response or adverse events do these functions make? We do not know, but looking forward, antibody engineers would not want to include function inadvertently. SeromYx offers the broadest range of Fc function outcome assays in the industry to help antibody developers understand their product.

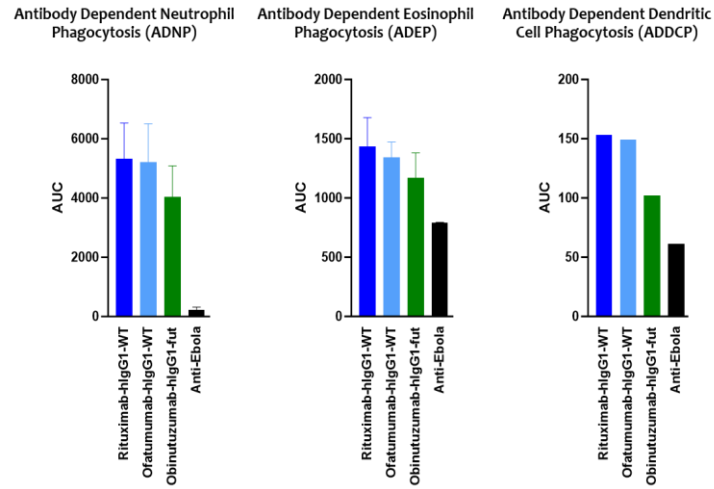

Figure 1: SeromYx Platform uncovers novel Fc effector functions for approved CD-20 mAbs.

2. Screening: a mAb is more than the Sum of its Parts

Often a panel of antibodies is constructed by first focusing on the Fab region, doing high throughput screening for antigen binding and affinity, and then optimizing affinity further. The lead Fabs selected are then joined with an Fc selected from a limited library and based on the desired role of Fc function, if any, in the molecule's MOA. Why this process has emerged is unclear to us: it may reflect the relative importance antibody designers attach to the two regions ("Get the Fab right and an approvable drug will follow") or it may reflect the paucity of high throughput tools available to measure Fc/FcR interactions. Regardless of cause, this process relies on a key but false assumption that the Fab and Fc regions are functionally independent of each other. A Fab on different IgG subclass backbones will have different affinities for its target epitope. And changes to Fab antigen affinity impact the function elicited by the Fc, with a higher affinity Fab eliciting lower Fc function in some cases. These observations strongly argue for early screening of intact molecules rather than fragments to select the best candidates for further development. Currently SeromYx is able to screen thousands of molecules for antigen-specific Fc function, offering a screening platform for mAb developers that was previously not feasible.

3. More Screening: Nature vs. Nurture

There is a second argument in favor of screening entire molecules early in the candidate selection process. Not only are the characteristics of Fab and Fc mutually dependent, but the Fc function is also epitope dependent. It is well established that propensity to fix complement is to some extent dependent on epitope distance from the cell membrane. A further, startling, example: in our hands, a panel of 600 antibodies to the same antigen exhibited an over three log difference in phagocytic activity (Figure 2). This finding is startling because the mAbs share an identical IgG backbone. Assuming phagocytosis is desirable, selecting the right mAb candidate translates either to much higher efficacy for a given dose or much lower dosing. A focus solely on Fab affinity for antigen, in the

screening phase will both miss Fc functionally potent candidates and select for candidates that may have undesirable function. This may in turn require Fc engineering at a later stage to achieve desired characteristics which involves both risk and potential expense, including royalties in due course. This burden might be reduced if one could select from the best nature has to offer by early high throughput screening, which SeromYx can provide.

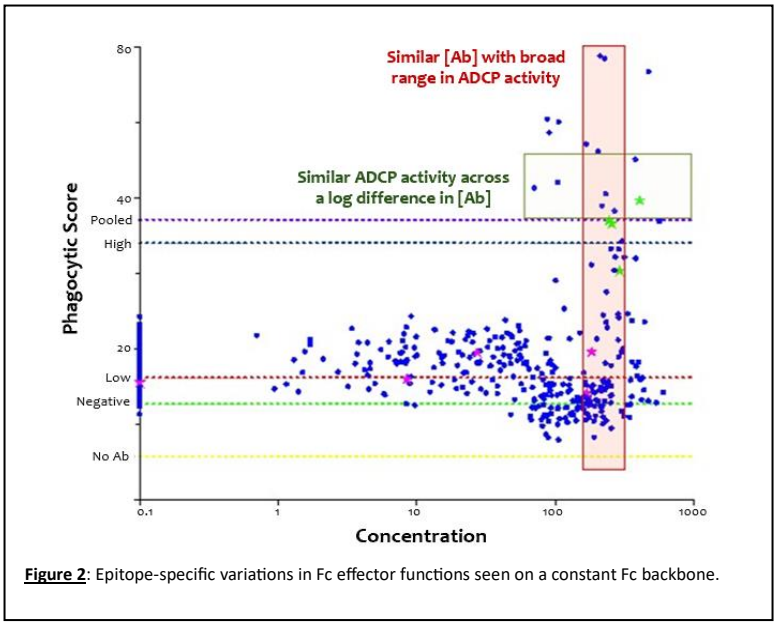

Figure 2: Epitope-specific variations in Fc effector functions seen on a constant Fc backbone.

4. Engineering: "Design In" vs. "Measure Out"

Antibody developers have engineered three Fc strategies to enhance their mAbs: to eliminate ("silence") undesired function, to enhance desired function, and to extend half-life to improve dosing and cost of goods. It is fair to say that these efforts have met partial success, but results are often short of the goal and can be unpredictable. Today, about half of the mAb pipeline employs Fcs that are engineered in some way, and over 100 Fc point mutations have been developed[2].

Taking each goal in turn, in applications where Fc function is undesirable, developers have often selected the IgG2 and 4 subclasses that show lower affinity for FcRs and thus are less prone to induce function. The problem is that this is no guarantee. With the exception of FcγRI, all Fc/FcR interactions are low affinity in the absence of antigen: it is the formation of immune complexes that drives function in vivo, and this can impart function to mAbs on an IgG2 or 4 backbone. Worth remembering that the infamous TGN1412 used an IgG4 backbone[3]. A second method to "silence" function is to use an aglycosylated Fc, but again mAbs with aglycosylated Fcs have been shown to "regain" function in immune complexes[4]. Finally, there are a number of point mutations intended to ablate function, such as LALA. In our hands, at least, such mutations reduce but do not eliminate Fc function.

Turning to enhancing function, there are a number of established techniques, including afucosylation (to enhance ADCC)[2]; and hexamerization (to enhance complement-dependent killing)[2]. However, given the complexity of the interactions, it has often proven difficult to get precisely the desired effect and no other, with off target toxicity often rising with enhanced function. Of note, enhancing binding to FcγRIIa has proven very challenging due to its near-identical binding site on the inhibitory receptor FcγRIIb, and mutations targeting FcγRIIIa often also enhance binding to FcγRIIb.

Finally, half-life extension is promoted by enhancing antibody binding to FcRn, for which there are several technologies available such as the YTE triple mutation[2]. In our hands, some of these technologies reduce function compared to the unmodified parental antibody, for example reductions in ADCC and other activities (Figure 3). In turn, this implies the need to carefully evaluate whether the loss of function is critical to the intended MOA before selecting clinical candidates.

In summary, the complexity and context-dependence of Fc function and the impact of engineered changes to the Fc means there is no substitute for broad functional screening for outcomes.

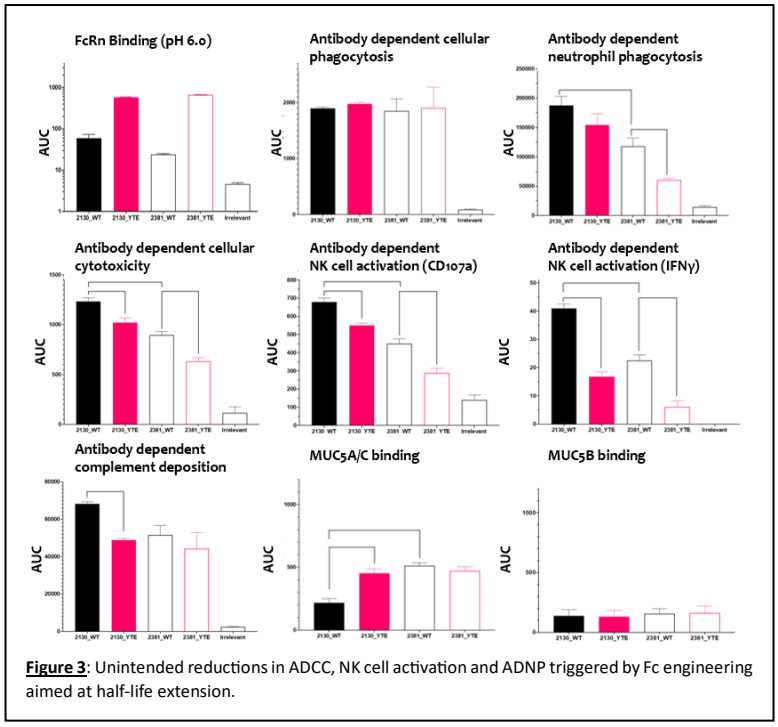

Figure 3: Unintended reductions in ADCC, NK cell activation and ADNP triggered by Fc engineering aimed at half-life extension.

5. Mimicking Life: as Physiological as Possible

All *in vitro* assays are artifacts, but some are more "artifactual" than others. For Fc functional assays to provide developers with the best guidance possible short of *in vivo* studies, they need to replicate the physiology to the furthest extent possible. To us this means, first, that all assays purporting to measure Fc function are conducted in the presence of the relevant antigen, which includes carefully screening for antigen quality because immune complexes drive Fc-mediated function. Remarkably, Fc/FcR binding assays are typically conducted in the absence of antigen even though they do not reflect natural Fc/FcR interactions, and the degree of particular functions does not always correlate with Fc/FcR affinity. There are plenty of examples of published molecules that show no function in the absence of antigen but do when in immune complexes. Second, to the extent possible, assays should measure the relevant function directly e.g. cell killing, rather than be reporter assays. And third, for cell-based assays using primary donors, if possible, donors with known function and allotypes (where relevant) should be used.

Summary

Designing monoclonal antibodies and related modalities such as ADCs and bispecifics with appropriate Fc function is critical to product safety and efficacy, but fiendishly hard. Outcomes are contextual, meaning that what works in one disease state/epitope setting may not hold in another, so the "rules" keep changing. And the factors driving Fc function are strongly interrelated, meaning that changes with one intent often have unexpected and undesirable consequences elsewhere. SeromYx offers the broadest and robust platform for Fc functional characterization (Figure 4).

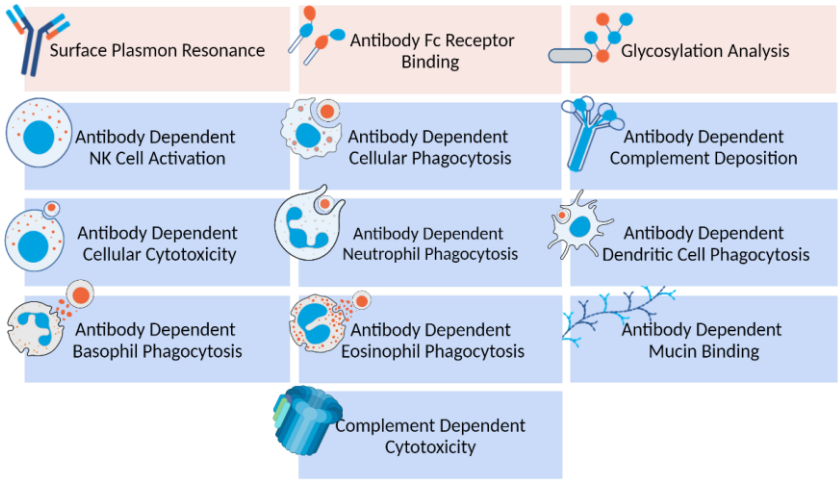

Figure 4: SeromYx Platform offers the broadest array of robust Fc function assays.

Where does our platform fit in? We provide a unique resource for antibody discovery and development with an industrial quality system. While we add value throughout the mAb discovery and development process, from pre-IND to BLA enabling (Figure 5), we encourage developers to utilize our capabilities earlier so that the best candidates can be identified and nominated for the clinic. By measuring outcomes in a robust way, we offer a distinct advantage to developers so that they can maximize the rewards of approvals and minimize the risk of clinical failures due to underexploited and/or incompletely characterized biology.

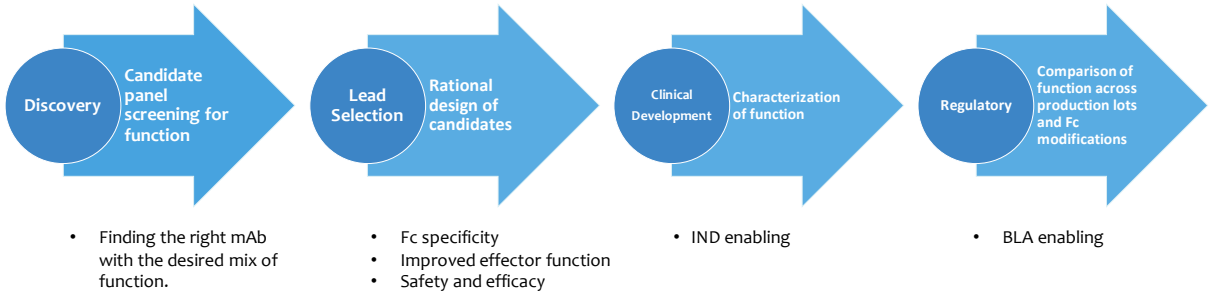

Figure 5: SeromYx Platform adds value throughout the mAb discovery and development process.

References

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