Why early mAb Sequence Optimization can Improve Developability and Reduce Costs

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With global sales of monoclonal antibodies (mAbs) reaching $168.70 billion in 2021 and continuing to rise at an estimated annual growth rate of 11.5%[1], one major issue is that they are still very expensive, reflecting the high costs associated with development and manufacture. For example, the price of treatment with Humira®, which was approved by the Federal Drug Administration (FDA) in 2002, is around $38,000 per year. In the case of the more recently approved KEYTRUDA®, where this rises to more than $100,000 per year, they are not accessible for many patients even in developed nations. mAb-based therapies are expensive to develop and manufacture than small molecule-based drugs, with costs typically ranging between $95 and $200 per gram.

Biopharmaceutical companies looking to produce more affordable mAb therapies are therefore searching for ways to reduce their cost of goods (CoGs). One strategy is to select high affinity, high titer lead candidates which do not have potential development and manufacturability issues. Taking into account developability and manufacturability during the selection process enables the identification of lead candidates having a better fit to existing manufacturing platforms, reducing the likelihood of having to spend time solving technical issues and developing additional steps during process development, ultimately leading to shorter timelines and lower development and manufacturing costs. Further, liabilities which could degrade the therapeutic efficacy can be reduced, resulting in longer shelf life and improved serum stability.

To effectively perform optimal lead selection and sequence optimization requires an array of computational and high-throughput empirical workflows to enable early evaluation of antibody sequences for potential liabilities and to devise methods for their repair. Biopharmaceutical companies often lack these capabilities and therefore need to partner with client-focused, technology-driven specialists who are aware that the primary objective for their client is to generate results from toxicology and first-in-human (FIH) studies as rapidly and inexpensively as possible.

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Selecting Optimum Lead Candidates

Natural B-cell response results in somatic hypermutation of antibodies which are generally of high affinity and specificity. The B-cell response, however, has no selective pressure for developability characteristics which are either necessary, or at least highly desirable, during production, storage, and clinical administration of the therapeutic.

Most failures during the discovery and early clinical development of mAb-based therapies are due to lack of efficacy and/or safety issues. However, intrinsic molecular properties can result in lead candidates which are difficult to manufacture or keep stable in formulation. This creates process development and manufacturing challenges which significantly impact on costs and timelines. Considering potential manufacturing issues of lead candidates early in development using in silico computational predictive tools can improve lead selection and/or drive the design of lead optimization. Sequences and structures can be analyzed for germline background diversity, isoelectric point (pI), potential post-translational modifications (PTMs), surface properties, and, perhaps most importantly, stability which might have a negative impact on a lead candidate's process development and formulation prospects. Lead candidates can be ranked from best to worst from a developability perspective and, if necessary, candidate sequences can be optimized usually through framework modifications to increase their developability potential without impacting efficacy. To improve lead selection further, the best lead candidates can be run though high-throughput *in vitro* assays to confirm their manufacturing potential and make the pathway to FIH trials more efficient.

At Just – Evotec Biologics, our J.MD™ Services include the use of our proprietary Abacus™ software suite for improving lead selection (see Figure 1 for an overview of the workflow). The software provides tools for humanization, germline background, sequence diversity, machine-learned (ML) and statistical stability prediction, ML immunogenicity prediction, positional frequency analysis, PTM motif prediction, patent report generation, lead optimization engineering, physical properties, and more. Abacus can incorporate its analyses along with surface structure properties into a score table, allowing for candidate ranking. This ensures that lead candidates with optimal developability characteristics can be identified early and pursued. If optimization is required, Abacus suggests modification locations, potential residue replacements, tracks the changes, builds the combinatorial variants, and can generate the cloning aids to eliminate design error. Variants are then produced and evaluated using assays that indicate how well each molecule can be expressed, purified, and formulated. If the number of candidates is reasonable and manufacturing is being undertaken by Just – Evotec Biologics, the initial candidates are produced in stable pools which generate representative material in a very short timeline.

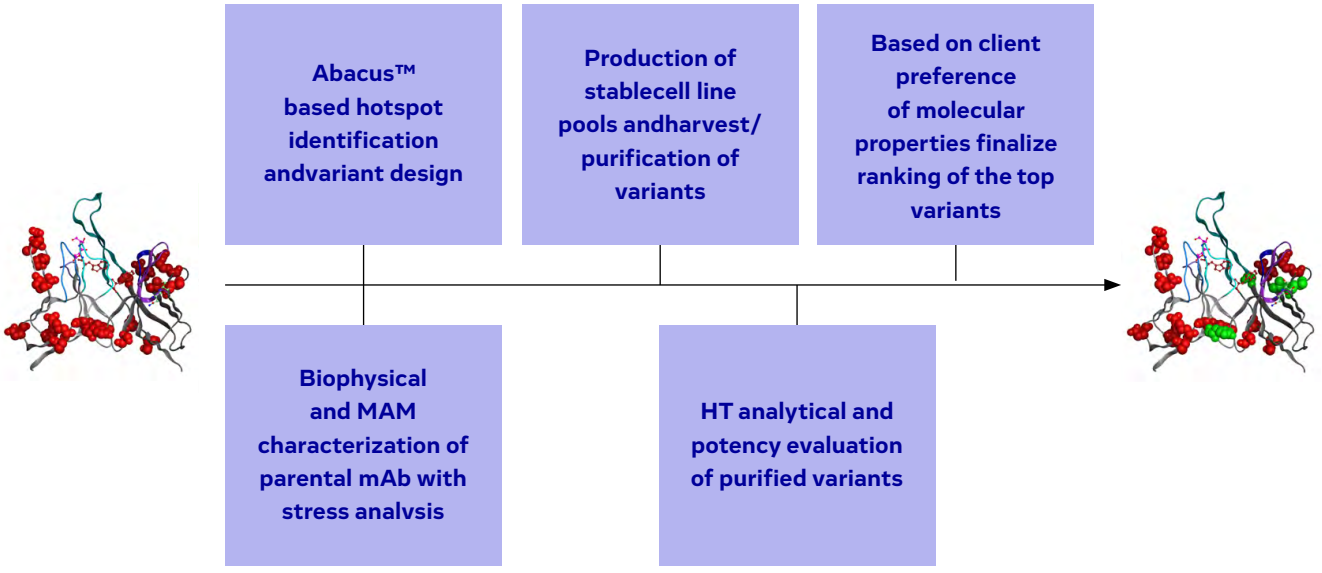

Figure 1: *Sequence optimization strategy utilized to identify improved variants*

Case Study

Applying Just-Evotec Biologics' J.MD Services approach and the Abacus software suite to a set of broadly neutralizing, anti-HIV mAb lead candidates, several potentially destabilizing residues were identified which were predicted to cause yield and stability issues. Framework residue substitutions were identified, and variants were generated for two mAb candidates (mAb1 and mAb2) and their behavior when subjected to low pH conditions typically used for viral inactivation (VI) during downstream processing (Figure 2). The non-optimized mAb1 parental sequence showed 40% aggregation after the exposure to low pH, whereas one of the optimized variants showed only 3.6% aggregation. Similarly, the non-optimized mAb2 parental sequence showed 88% aggregation after exposure to low pH, while one of the corresponding optimized variants showed only 3.4% aggregation. Both variants maintained broad anti-HIV efficacy. Additionally, incubation of the mAb1 variant at 100 mg/mL for 6 weeks at 40°C showed a 9-fold decrease in sub-visible particles compared to the parental mAb1 molecule. Such a significant reduction in aggregation during the VI step clearly increases the downstream process yield leading to the potential for a reduction in CoGs for the optimized mAbs. The improvement in stability of these variants also translated to serum half-life studies, potentially enabling lower patient doses to be considered.

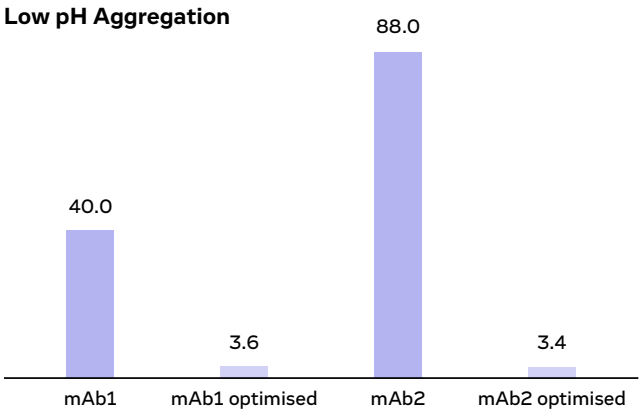

Figure 2: *Comparison of optimized versus non-optimized candidates for two mAbs during exposure to low pH conditions used during a Viral Inactivation processing step (bars in figure refer to % aggregation).*

Future Perspective

Biopharmaceutical companies can now perform molecular optimization using *in-silico* methods to select the best lead candidates as early as possible during development to ensure that their mAb therapies can be developed efficiently for FIH trials and manufactured affordably. This will predict many issues that a molecule may have during process development and formulation. It is usually preferable to select a candidate that fits standard platform approaches as this will then streamline the process and mitigate the need for extensive process development at additional time and cost.

Collaborating with a client focused, technology-driven partner such as Just – Evotec Biologics that can provide early development services which include combining leading edge AI/ML-based *in silico* tools with powerful, high-throughput, empirical characterization can reduce the process development burden. This will help deliver manufacturable mAbs at a competitive CoGs and ensure that life changing mAb therapies can become more accessible to a wider patient population.

References

1. Monoclonal Antibodies (MAbs) Global Market Report 2022 https://www.thebusinessresearchcompany.com/report/monoclonal-antibodies-global-market-report. Accessed June 2022.