Antibody Humanization using Composite Human Antibody™ (CHAb) Technology

Developing an antibody therapeutic that can be administered without triggering an unwanted immune response is a key hurdle when working with non-human antibodies. While advances in protein engineering—such as antibody humanization—have helped address this issue by making the antibody variable domains appear more human, it has become clear that not all humanization approaches are created equal. Although all strategies aim to reduce immunogenicity, different methodologies can significantly impact the final outcome.

Abzena's Solution to Humanization

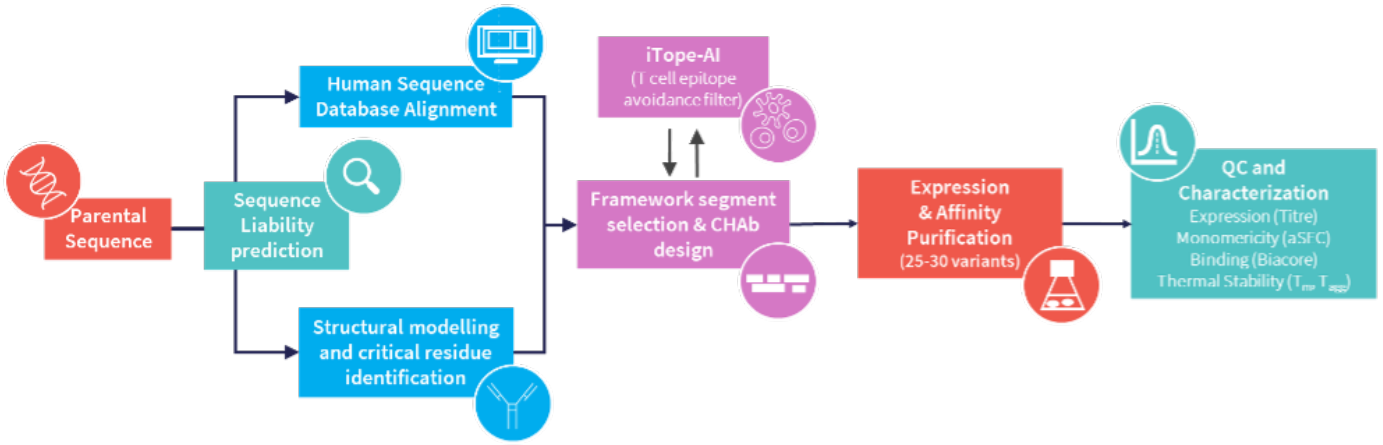
At Abzena, we have created a distinctive solution called Composite Human Antibody™ (CHAb) technology. This technology integrates humanization and deimmunization to produce therapeutic antibodies with reduced immunogenicity while preserving the affinity and efficacy of the parental antibody.

What is CHAb Technology? Some humanization approaches, such as CDR grafting, simply increase the percentage of human sequence in an antibody, assuming that this inherently lowers immunogenicity. Composite Human Antibody™ (CHAb) technology takes a more proactive route. In addition to carefully combining segments of human germline sequences (to achieve high homology to human antibodies), CHAb technology systematically identifies and reduces potential immunogenic "hotspots." This dual focus directly addresses key factors that lead to anti-drug antibody formation.

iTope-AI and Composite Human Antibody™ Technology: ITope-AI, Abzena's proprietary in silico immunogenicity risk assessment tool, is at the core of our CHAb platform. iTope-AI evaluates antibody sequences by examining overlapping linear peptides for their potential to bind a broad panel of human MHC Class II alleles (HLA-DR, DP, and DQ), identifying potential T-cell epitopes that drive immunogenicity. We can strategically modify or remove these epitopes to minimize their impact and reduce immunogenicity risk by integrating this algorithm into the humanization design process.

Humanization and Function: Abzena applies structural modeling to identify critical residues—sometimes outside the CDRs—that must be preserved for the antibody to retain function. Since each antibody is unique, the number and location of these pivotal residues can vary. As a result, we typically create 20–30 variants exploring how different residue combinations affect binding properties.

Once expressed, these variants undergo target-binding assessments (e.g., Biacore, flow cytometry) to ensure they maintain or match the parental molecule's binding capabilities.



Humanization, Sequence Liabilities and Manufacturability: A successful therapeutic candidate must retain function and be manufacturable to the necessary quality standards. Abzena begins by examining the parental antibody sequence for potential liabilities (e.g., N-linked glycosylation sites, free cysteines) and, when possible, removing them during humanization. If these liabilities lie in the CDRs, we apply a combination of structural insights and iTope-AI data to analyze multiple potential substitutions that maintain affinity while mitigating liabilities.

- **Standalone Liability Removal:** We can also remove sequence liabilities independently of the humanization process for any antibody.
- **Biophysical Stability:** Proper framework selection using stable human variable domain scaffolds helps drive favorable manufacturability. After expression and purification, we analyze thermal stability (Tm and Tagg) to confirm that potential lead variants display robust biophysical profiles.

The choice of Fc: An antibody's Fc domain plays a vital role in overall antibody function—governing immune effector mechanisms and pharmacokinetics. Defining your molecule's desired mode of action is paramount to selecting the right isotype or determining whether specific mutations could enhance or reduce particular Fc functionalities (e.g., effector function, FcRn binding).

Beyond Humanization: Humanization is just one step toward creating a successful therapeutic. Depending on your program needs, additional engineering—such as affinity maturation or combining multiple specificities to form a bispecific—may be required.

Analytical and Bioassay Support

Candidate selection requires a holistic view of all of the parameters described above - immunogenicity, function, and manufacturability - and more. By applying a range of stage appropriate analyses, we are able to rapidly select candidates that have the optimal properties for development.

At Abzena, we are able to support you at all the stages of this process, whether it be from assessing a molecule's functionality or confirming the reduction in immunogenicity using our EpiScreen® 2.0 immunogenicity risk assessment assays through to extended stability analysis such as freeze/thaw studies and other manufacturability studies.

Composite Human Antibodies™ (CHAb) Key Points

- >20 years experience of humanizing antibodies from a wide range of species.
- Rational sequence design increases the humanness of a sequence and potentially minimizes CD4+ T-cell epitopes, reducing immunogenicity compared to other humanization approaches. Confirmation of T-cell epitope avoidance can be tested using Abzena's EpiScreen® 2.0 ex vivo PBMC-based platform.
- Careful framework selection and variant design together with sequence liability removal ensures excellent retention of affinity as well as good manufacturability profiles.
- Clinically validated technology with more than 12 antibodies reaching clinical development, and at least one having received regulatory approval.
- Abzena's CHAb technology is complemented by state-of-the-art screening & analytical methods to ensure your molecule shows the functionality and manufacturability profile that is essential for clinical success.
- Humanization should always be considered within the broader concept of developability.
- Significant experience in Fc engineering to modulate the function of an antibody.
- CHAb approaches humanization from two angles:
  - Increases the proportion of human sequence content, and;
  - Reduces the potential for immunogenicity through the identification and elimination of T-cell epitopes.

Summary

To obtain a viable humanized therapeutic candidate requires careful consideration and a detailed understanding of your molecule. Abzena's CHAb humanization platform, can provide a pathway to a successful therapeutic candidate, providing a molecule with reduced immunogenicity, while retaining binding affinity.

ABZENA

Take your project to the next level

At Abzena, our extensive experience in antibody development enables us to anticipate potential challenges and design innovative solutions. We offer personalized strategies to align with your goals, ensuring the development of safe, effective, and high-quality therapeutic antibodies. Contact us today to learn how our Composite Human Antibody (CHAb) technology can transform your humanization project by minimizing immunogenicity and ensuring optimal developability for clinical success.

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