A Deep Learning Tool and Service for Next Generation Antibody Humanization

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Abstract

Here, we introduce a cutting-edge, species agnostic service for next-generation humanization of antibodies, based on the novel AIGX generative deep learning model. Through a commercial project focused on the discovery and development of anti-RAGE antibodies, we show that this solution generates humanized mouse and rat sequences with significantly greater success than using traditional methods such as CDR grafting and publicly available algorithms such as BioPhi. Genovac's capacity to achieve superior results across species and to enable humanization of large numbers of candidates represents a significant advancement in antibody humanization, leading to lowered costs and a shortened timeline to IND filing.

Anti-RAGE Antibody Discovery

- Commercial partner: Dr. Stefan Vetter and Dr. Estelle Leclerc, North Dakota State University School of Pharmacy
- Target: Receptor for Advanced Glycation End Products (RAGE)
  - Type I transmembrane protein
  - Implicated in Alzheimer's disease, cancer, diabetes, vascular and lung disease
  - Known ligands include S100 proteins, DNA, amyloids, phosphatidylserine clusters, and AGE productions

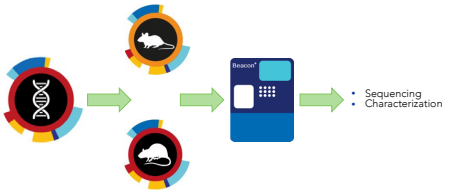

Figure 1. Anti-RAGE antibody discovery. Wild-type mice and rats were genetically immunized with the RAGE extracellular domain. Target-specific single B-cells were isolated using the Bruker Beacon® Optofluidic System and variable region sequences recovered. Following testing and characterization, 8 mouse and 8 rat parental antibodies were selected for humanization and further development.

Anti-RAGE Humanization

Figure 2. Eight mouse and eight rat parental antibodies were selected for humanization using sequence information and results of the characterization experiments. Five humanized variants were created for each parental antibody using Genovac's humanization. Parental and humanized variants were expressed in high-throughput, small-scale culture and the resulting supernatants used to measure binding kinetics.

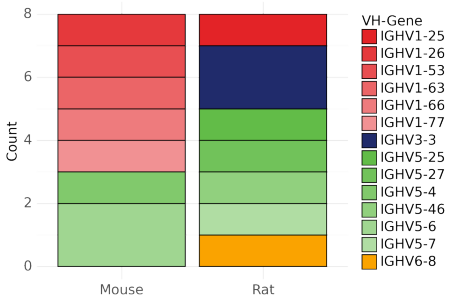

Figure 3. VH gene usage of the mouse and rat parental antibodies selected for humanization.

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GENOVAC ENPICOM DECODING THE IMMUNE SYSTEM

Anti-RAGE Humanization

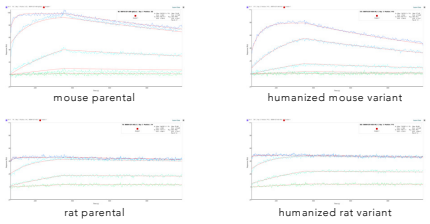

Figure 4. Representative Carterra® LSA sensorgrams for anti-RAGE parental and humanized variant antibodies.

Success Criteria for Humanization

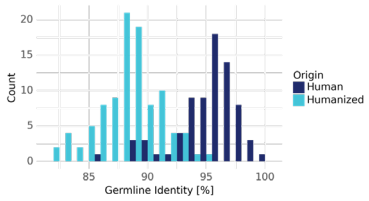

Figure 5. Percent germline identity for approved human and humanized therapeutic antibodies.

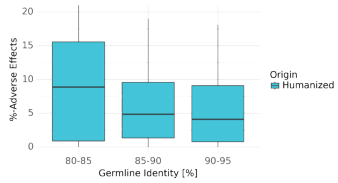

Figure 6. Percentage of adverse effects vs. percent germline identity for approved humanized therapeutic antibodies. Figure 5 and 6 data from Clavero-Alvarez *et al.* (2018) Sci Rep, 8, 14820 and Marks *et al.* (2021) Bioinformatics, 37, issue 22.

Binding Affinity (KD)	**within 2-fold of parental antibody – or – within 5-fold of parental antibody**
Germline Identity	**80-85% 85-90% 90-95%**

Table 1. Success criteria for successful humanization were defined based on retention of the parental antibody's binding affinity (KD) and the percent identity to the germline sequence.

Superior Humanization Efficiency

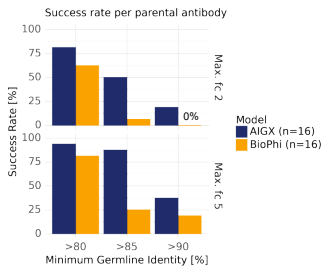

Figure 7. Success rate per parental antibody. The deep learning AIGX humanization model included in Genovac's humanization offers significantly higher success rates in humanizing antibodies compared to BioPhi at >85% and >90% germline identity. Max. fc 2 = maximum 2-fold change in binding affinity; Max fc 5 = maximum 5-fold change in binding affinity.

Superior Humanization Efficiency

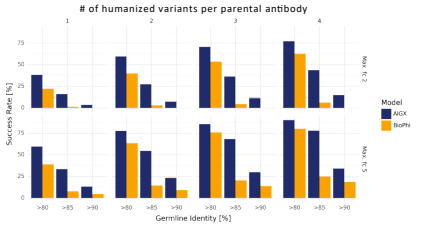

Figure 8. Average humanization success rate vs. number of humanized variants. The AIGX humanization algorithm consistently yields a greater number of successfully humanized variants as defined by a 2- or 5-fold maximum change in binding affinity (KD) and >80%, >85%, or >90% germline identity compared to BioPhi.

Enhancing Affinity with Deep Learning

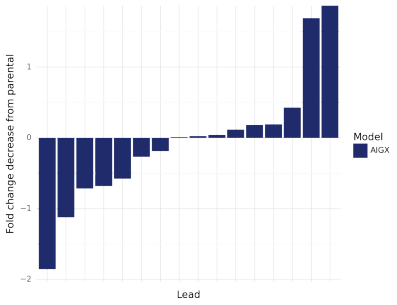

Figure 9. Best humanized variant (lowest KD) for each parental antibody. For each candidate, at least one AIGX humanized antibodies binding affinity within 2-fold of the parental antibody. In 40% of the cases, the affinity of a humanized variant improved vs. the parental, despite the model having no affinity task-specific training.

> […] a model without any task-specific training data or knowledge of the antigen can guide antibody evolution toward higher binding affinity, with competitive performance compared to protein-specific or task-specific methods.
>
> Hie *et al.* (2024) Nat Biotechnol 42, 275

Humanization-Forward Antibody Development

Figure 10. The superior efficiency of the AIGX algorithm enables humanization of large numbers of parental antibodies immediately after a discovery campaign, saving considerable time and cost in a therapeutic antibody development program.

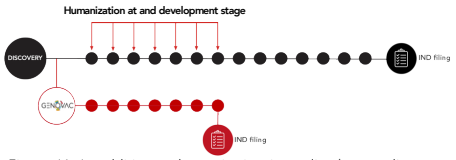

Figure 11. In addition to humanization immediately post-discovery, the efficiency and flexibility of Genovac's humanization enables deployment of the tool at any step in the discovery process.

Genovac's Humanization Powered By ENPICOM

Genovac's humanization is the first in a suite of antibody engineering tools based on ENPICOM's AIGX family of protein language models. Additional services will be launched in the coming months.

- Humanization
- Affinity maturation
- Manufacturability
- Functional Enhancement
- Epitope prediction
- Paratope prediction