Prometheus 2.0™: Engineering manufacturable humanized antibodies from diverse sources including rabbit and camelid antibodies

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INTRODUCTION

Humanization is a critical step in the therapeutic development of all antibodies from non-human sources. It involves the grafting of critical non-human amino acids onto a human antibody framework. Two important considerations are immunogenicity and manufacturability. In previous work we analyzed manufacturability data (titer, self-interaction, melting temperature and aggregation) for more than 100 therapeutic antibodies. As reported by others, there is a clear trend for antibodies that reach approval to have a lower number of "red-flag" observations, while the degree of "human-ness" proved to be a poor correlator with clinical immunogenicity (Figure 2). We therefore identified a cohort of well-tolerated, highly manufacturable human antibody frameworks to use as acceptor scaffolds during humanization using our Prometheus™ Service.

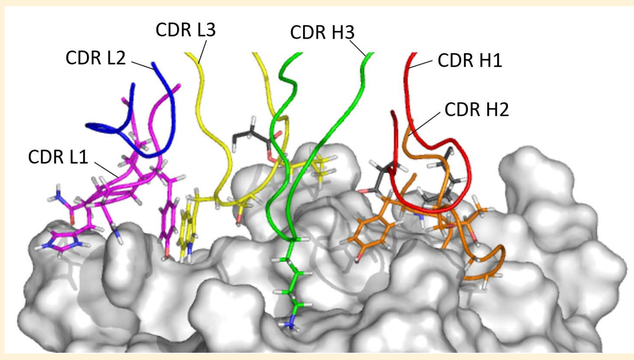

Fig 1. Model of antibody-antigen interface.

Like many humanization platforms our initial approach was focused on humanizing mouse or rat antibodies, which benefited from the high structural similarity of murine and human antibodies. Increasingly, however, therapeutic developers are starting discovery campaigns using non-murine models, such as rabbit and camelids (e.g. alpaca, vicuna). These species have a range of advantages over more traditional sources of monoclonal antibodies, such as longer CDRs, non-mammalian tolerization and single-domain architecture; however, structural differences have made humanization challenging.

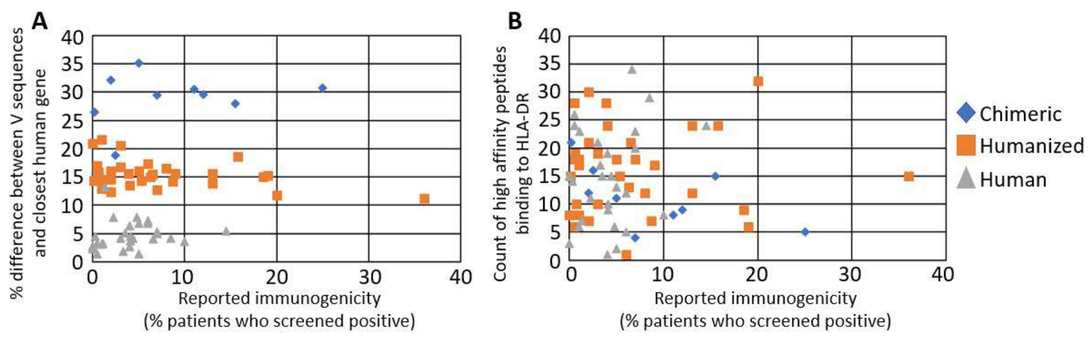

Fig 2. Clinical immunogenicity of 72 approved antibodies plotted against a measure of human-ness (A) or *in silico* predicted immunogenicity (B). Human content determined by alignment to germline. *In silico* immunogenicity determined by IEDB consensus method. There is no correlation between *in silico* predictions and clinically measured immunogenicity.

HUMANIZATION USING PROMETHEUS™ METHOD

A murine antibody with poor expression and high aggregation propensity was humanized using the Prometheus™ method. 25 humanized variants were created with overall more favorable manufacturability than the chimeric antibody based on the parental clone (Figure 3).

Activity testing showed that 19 of the 25 variants retained activity within two-fold of the chimeric, and nine even showed an increase in affinity of up to two-fold (Figure 4).

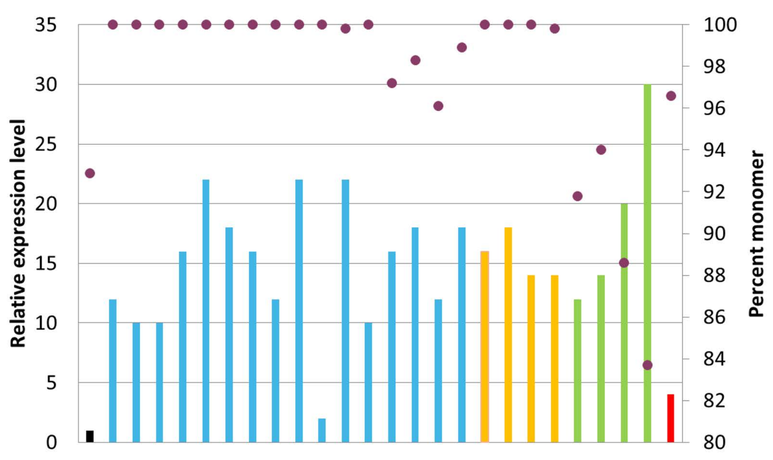

Fig 3. Expression yields relative to the chimeric antibody shown as bars and monomer content shown as purple dots. Black bar is chimeric antibody, blue bars have favorable VH and VL, yellow bars have unfavorable VL only, green bars have unfavorable VH only and red bars have both unfavorable VH and VL.

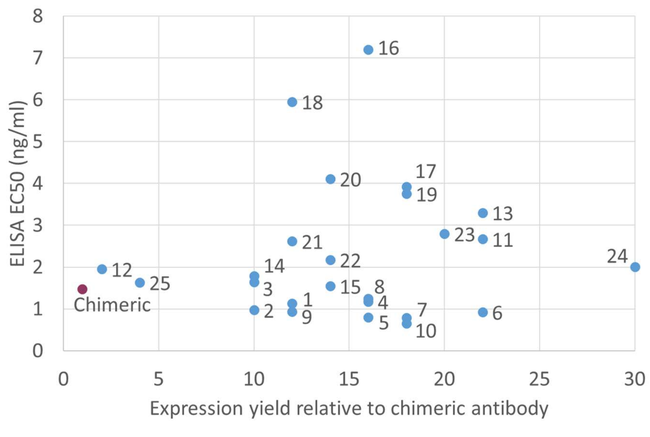

Fig 4. Expression levels and binding activity of the 25 humanized versions were compared to the chimeric.

EXPANSION TO NON-MURINE ORIGIN SPECIES

Through bioinformatic analysis of antibody structure and germline conservation, we expanded this humanization platform to non-murine species. Rabbit antibodies feature longer than average CDRs and can present with kappa 1 light chains containing an additional framework cysteine forming a disulphide between VL and CL. Camelid VHH are single-domain binders, and therefore human frameworks need to be engineered to present without the requirement for pairing a VL.

Addressing these challenges, we were able to humanize rabbit and camelid antibodies against a range of targets. The resulting antibodies showed maintained activity comparable to the chimeric antibody based on the parental clone (Figure 5). In addition, the humanized rabbit antibodies showed improved expression over the chimeric, while expression levels were comparable for camelid, which generally express at very high levels (Figure 6).

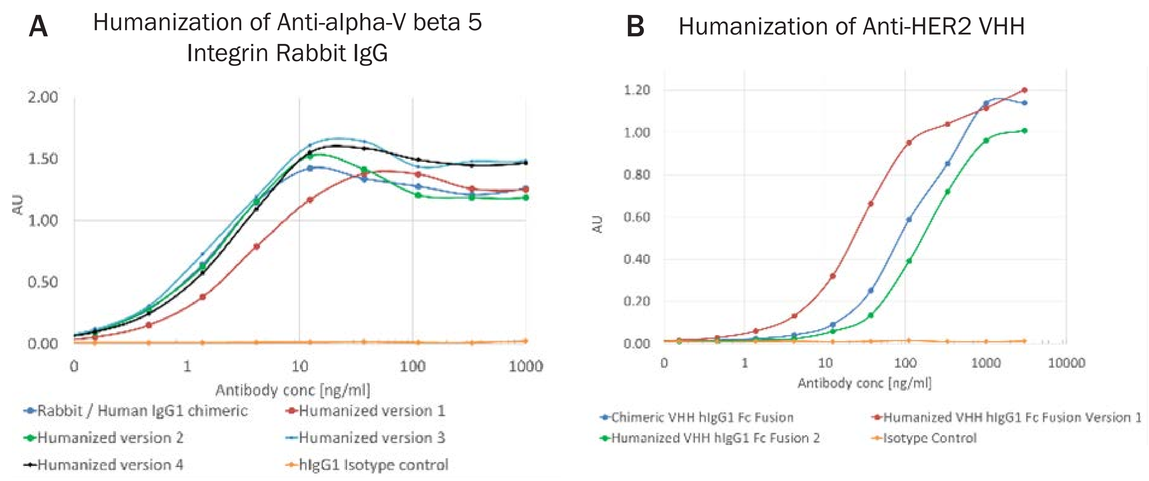

Fig 5. ELISA activity data for three representative antibodies of humanized antibodies compared to chimeric antibodies based on the parental clone of a) rabbit and b) camelid origin.

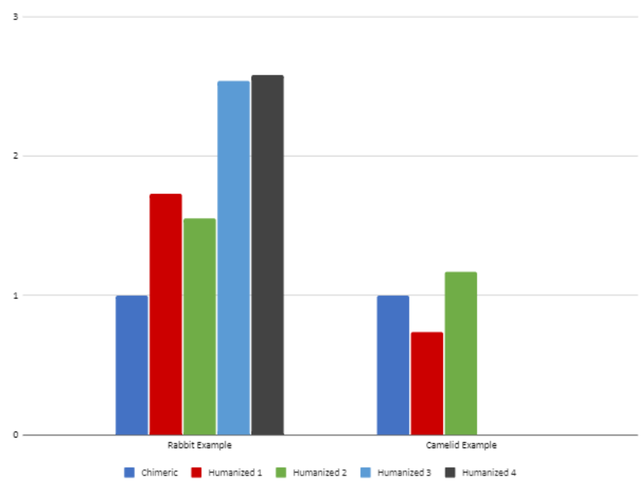

Fig 6. Relative expression levels of humanized antibodies for the representative rabbit and camelid examples shown in this study.

SUMMARY

We have successfully expanded our humanization service to popular non-murine antibody species. Prometheus 2.0™ incorporates strategies to overcome structural differences of rabbit and camelid antibodies to generate humanized antibodies with functional characteristics comparable to the original antibody, while maintaining, and often even improving, manufacturability characteristics. The launch of Prometheus 2.0™ enables researchers to benefit from the functional and structural advantages antibodies from a wide range of source organisms bring, and will help improve the characteristics of next-generation biotherapeutics.

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