TOLERIZATION AS A RATIONAL DESIGN APPROACH FOR REDUCING THE IMMUNOGENICITY OF BIOLOGICS

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1. OVERVIEW: Develop safer, more effective mAb drugs to save time and money

Overview: This project aims to develop and validate a rational design approach for engineering protein therapeutic candidates. We proposed to leverage a novel method called "tolerization" for reducing biologic immunogenicity, which introduces Tregitopes into the amino acid sequence with minimal substitutions. Twelve conservative mutations result in the predicted tolerization of the variable regions of an historically immunogenic monoclonal antibody. Future work will focus on in vitro validation of our design approach, which will be broadly applicable to other biologics.

Purpose: Monoclonal antibodies (mAbs) are attractive for the treatment of many diseases; however, patient **anti-drug antibody (ADA) responses inhibit their efficacy** despite "humanization" efforts (**Figure 1A**). Only **17%** of humanized mAbs successfully pass Stage II (efficacy) clinical trials (**Figure 1B**), although an average of **eight years and $700 million** are spent in preclinical mAb development. This project seeks to address the critical unmet need for a **broadly-applicable BioBetter design approach** that improves the efficacy of preclinical mAbs while **unfailingly abolishing immunogenicity**.

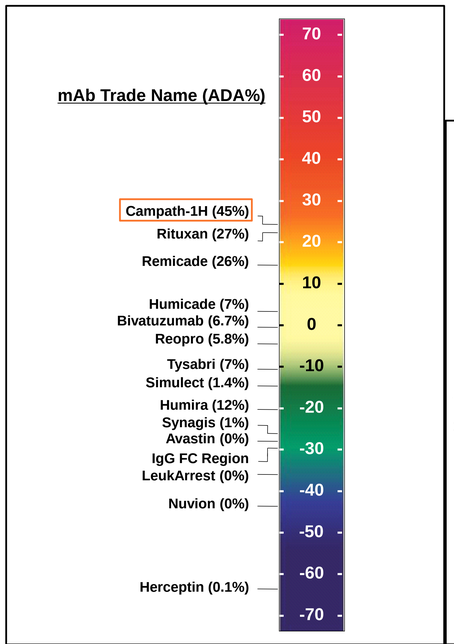


Figure 1A: Predicted and Observed Immunogenicities of FDA-Approved mAb Drugs. EpiMatrix scores for current mAb therapies, based on T cell epitope content, were calculated using our EpiMatrix in silico prediction tools. Proteins are arranged by EpiMatrix score from high immunogenicity (red) to low (blue). Anti-drug antibody (ADA) values for current mAb therapies were obtained from publically-available package inserts. ADA responses generally agree with our immunogenicity predictions.

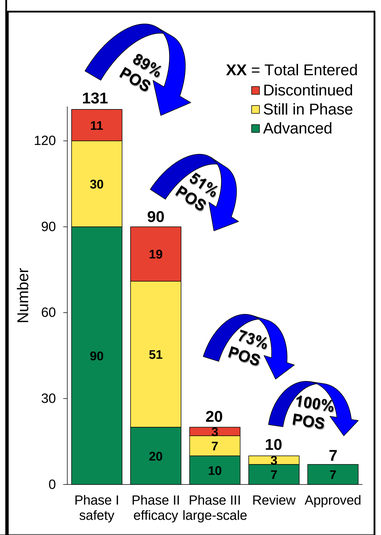


Figure 1B: Therapeutic Human mAb Candidates In FDA Clinical Trials, 1997 – 2008. Probability of Success (POS) values for transitions between clinical stages is defined as the likelihood that candidates entering a clinical stage will advance to the next stage, and is calculated from the candidates with known fates (31% of total cohort). Data from ref.[1] The majority of mAb candidates do not pass Phase II efficacy trials.

4. METHODS: Introduce tolerizing point mutations in six steps

Methods: Using our proprietary **EpiMatrix in silico tools to predict Tregitopes and T cell effector ("Teff") epitopes**, we identified 13 sites within the heavy chain variable region and seven sites within the light chain as **tolerization candidates, requiring only one or two mutations per site**. Candidate mutations were grouped by their relative location in the protein sequence. Groups were then **prioritized** by considering conservation of mutant residue biochemical property (side chain bulk, charge, etc.), predicted impact on the overall immunogenicity score, and number of disrupted Teff epitopes. (**Table 1**). Whenever applicable, mutations were designed to **increase homology with in vitro- and in vivo-validated Tregitopes known to stimulate regulatory T cells**. Prioritized mutations were introduced into the sequence in a stepwise fashion to **tolerize the variable regions**.

Table 1: Alemtuzumab Variable Region Tolerization Steps.

Chain	Step #	Mutation Group ID	# Mutation(s)	# Tregitopes Introduced	# Teff Epitopes Disrupted	EpiMatrix Protein Score Including	Δ EpiMatrix Protein Score from previous step
L	0	ORIGINAL				29.17	n/a
L	1	LA	2	2	2	0.89	-28.28
L	2	LB	3	1	0	-20.61	-21.50
L	FINAL			3	2	-20.61	-49.78
H	0	ORIGINAL				22.44	n/a
H	1	HA	1	3	2	-13.71	-36.15
H	2	HB	2	2	1	-28.88	-15.17
H	3	HC	2	1	1	-43.59	-14.71
H	4	HD	2	1	1	-55.65	-12.06
H	FINAL			7	5	-55.65	-78.09

Key References:

(1) Nelson, A. L.; Dhimolea, E.; Reichert, J. M. *Nat. Rev. Drug Discov.* 2010, *9*, 767–74.
(2) De Groot, A. S. et al. *Blood* 2008, *112*, 3303–11.
(3) Weber, C. a et al. *Adv. Drug Deliv. Rev.* 2009, *61*, 965–76.
(4) De Groot, A. S.; Martin, W. *Clin. Immunol.* 2009, *131*, 189–201.

2. BACKGROUND: Tregitopes elicit antigen-specific tolerance

We have discovered natural, **tolerance-inducing T cell regulatory epitopes** ("Tregitopes") in human IgG sequences that suppress inflammatory T cell responses and **lower ADA levels** (**Figure 2**). Here we consider the potential for "**tolerization**," a dovetail approach to deimmunization **(Figure 3)** by **introducing Tregitopes** into the variable regions of a rat-derived, immunogenic mAb (Campath-1G) **via minimal point mutagenesis**.

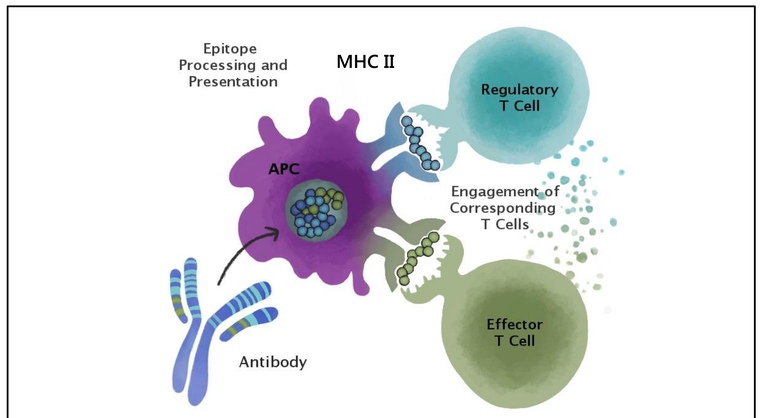
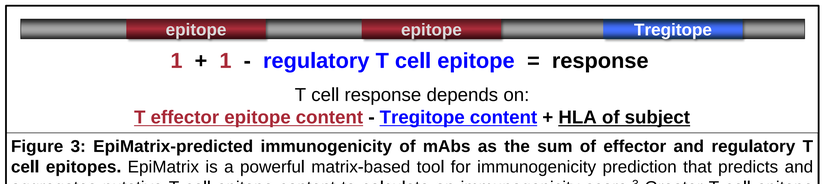


Figure 2: Induction of Immunosuppressive Tolerance through T Cell Regulatory Epitopes ("Tregitopes"). Naturally-occurring Tregitopes within IgG (blue circles) are processed and presented by antigen-presenting cells, where they engage and activate regulatory T cells to suppress the inflammatory response.[2]



T cell response depends on:
T effector epitope content - Tregitope content + HLA of subject

Figure 3: EpiMatrix-predicted immunogenicity of mAbs as the sum of effector and regulatory T cell epitopes. EpiMatrix is a powerful matrix-based tool for immunogenicity prediction that predicts and aggregates putative T cell epitope content to calculate an immunogenicity score.[3] Greater T cell epitope content increases the likelihood that the protein will stimulate an immunogenic response. For mAbs, variable domain scores are adjusted for Tregitope content, resulting in a high correlation between "Tregitope-adjusted" immunogenicity scores and observed anti-therapeutic response in the clinic.[4]

5. RESULTS: Predicted tolerization of Campath-1G variable regions within 12 point mutations

Results: We predict that **only seven point mutations in the heavy chain and five in the light chain are required to engineer maximal tolerance** into the mAb variable regions. The mutations proposed introduce 10 Tregitopes and disrupt seven Teff epitopes, which **reduce the EpiMatrix-calculated heavy and light chain immunogenicity scores by 3.5-fold and 1.7-fold, respectively** (**Figure 5**). Further, the overall score of the tolerized protein is at or lower than known non-immunogenic mAbs as predicted by EpiMatrix.

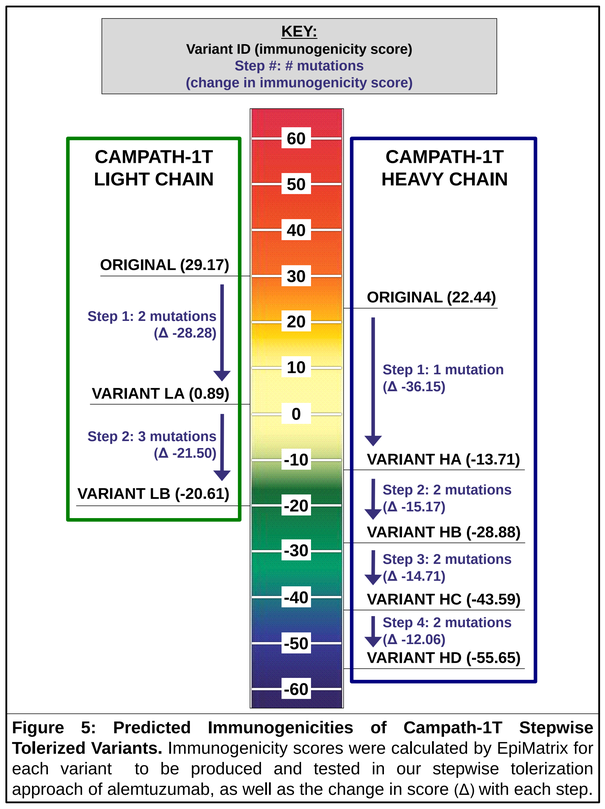


Figure 5: Predicted Immunogenicities of Campath-1T Stepwise Tolerized Variants. Immunogenicity scores were calculated by EpiMatrix for each variant to be produced and tested in our stepwise tolerization approach of alemtuzumab, as well as the change in score (Δ) with each step.

3. APPROACH: Stepwise tolerization of Campath-1G

We propose to tolerize **alemtuzumab (trade name Campath)** as a proof of principle for our design approach (**Figure 4**). Despite humanization, alemtuzumab remains **immunogenic in the clinic** and its therapeutic efficacy is compromised.

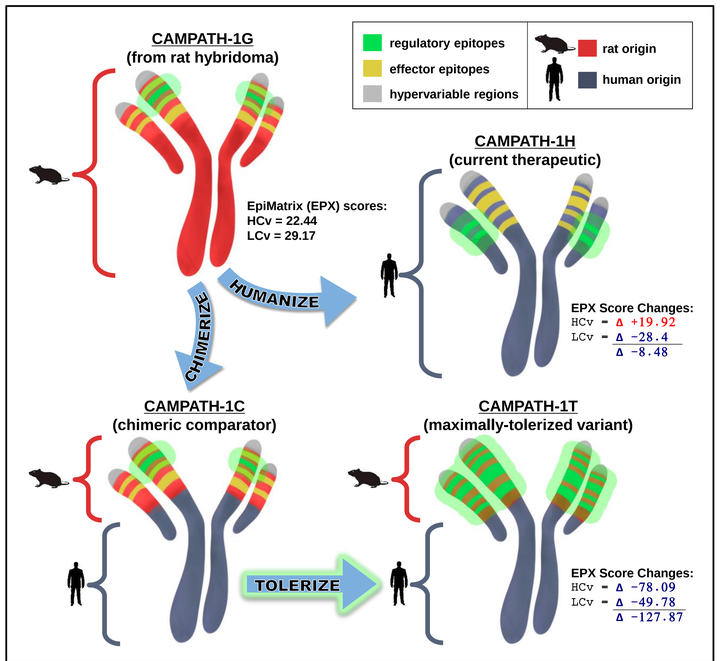


Figure 4: Alemtuzumab Tolerization Approach. Alemtuzumab therapeutics (trade name Campath) and proposed variants are built upon one or more frameworks (red, rat; dark grey, human). Rather than humanizing (Campath-1H), our approach re-designs the original Campath-1G rat variable regions by introducing tolerizing T cell regulatory epitopes ("Tregitopes") into the amino acid sequence with minimal point mutations to create Campath-1T. The proposed Campath-1C variant, chimerized with a human Fc region, allows for direct immunogenic comparisons with and without Tregitope incorporation. Heavy and light chain variable regions (HCv and LCv, respectively) were evaluated for immunogenic potential using our EpiMatrix prediction tools; negative changes in EpiMatrix (EPX) scores (blue font) indicate a reduced likelihood of immunogenicity.

6. CONCLUSIONS: Successful development of a tolerization design approach

Conclusions: We have successfully **developed a biologic design approach to reduce immunogenic potential**. The introduction of **minimal, targeted point mutations** into a protein sequence predicts tolerization that is comparable to entire IgG framework grafts currently practiced.

Future Directions: Synthesis of the original "humanized" therapeutic mAb, as well as full-length tolerized variants (e.g., **Figure 6**), is underway. Future work is planned to **evaluate the structural impacts of the mutations** on the entire protein, and to **test the antibodies for function and immunogenicity in vitro**.

Impact: This project facilitates translational science into the clinic by bypassing the conventional approach of framework switches, and is **applicable to other protein-based therapies**. Our approach can be used singularly or in combination with humanization techniques, and **can save countless hours and dollars** by proactively eliminating the root cause of immunogenicity while preserving efficacy of clinical trial candidates.

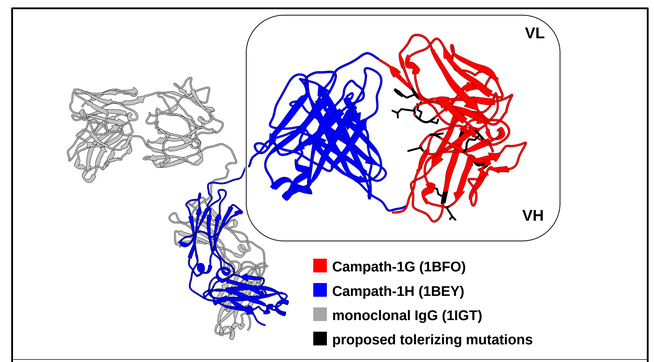


Figure 6: Structural Model of the Campath-1C Proposed Variant. The crystal structure of the rat-derived Campath-1G (1BFO, red) variable regions is presented with the humanized Campath-1H (1BEY, blue) constant regions (enlarged to show detail). A monoclonal IgG structure (1IGT) is presented for context. Residues to be substituted in the variable heavy (VH, 7 total) and light (VL, 5 total) domains, designed to introduce Tregitopes and create Campath-1T, are indicated in black.

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