T-Cell Dependent Immunogenicity of Protein Therapeutics: Preclinical Assessment and Mitigation

L.P. Cousens[1], V. Jawa[2], M. Awwad[3], E. Wakshull[4], H. Kropshofer[5], W. Martin[1], A.S. De Groot[1,6]

[1]EpiVax, Inc. USA, [2]Amgen Inc. USA, [3] Merrimack Pharmaceuticals USA, [4]Genentech USA, [5]Roche Switzerland, [6]University of Rhode Island USA

ABSTRACT

Purpose: Adverse effects associated with immune responses to some biologic therapies have become a topic of some concern. While assessment of accurate product safety profile currently relies on the clinical immunogenicity data, drug developers are working to develop strategies to evaluate immune responses to protein therapeutics during both preclinical phases of development. Of the many factors that contribute to protein immunogenicity, T cell-dependent (Td) responses appear to play a critical role in the development of antibody responses to biologic therapeutics.

Methods and Results: Focusing on the T cell contribution to immunogenicity, a range of methodologies to predict and measure Td immune responses to protein drugs are available. The advantages and limitations of these technologies will be discussed. Case studies will be presented to illustrate the importance of Td immunogenicity and the practical application of these methods. This analysis has led us to propose a framework for the prediction and measurement of Td immune responses as a critical component of a risk assessment strategy. An evidence-based roadmap is proposed here (Figure 1) for identifying Td responses in protein therapeutics and step-wise assessment of immunogenicity by (i) sequence-driven assessment using in silico algorithms, (ii) *in vitro* assays, and (iii) *in vivo* models. Lastly, we introduce the emergence of methods for mitigating Td immunogenicity, such as de-immunization and tolerance induction.

Conclusions: A wide range of Td immunogenicity screening methods examining different aspects of the process by which a protein therapeutic may trigger an immune response are available. However, no single method has emerged as a definitive tool for determining whether or not a protein therapeutic will elicit a detrimental immune response in patients. Given the complexity of the immune system, a singular solution may not be realistic. Rather, the field is evolving to apply strategic combinations of multiple methods to most closely predict and mitigate immunogenicity risk. Td immunogenicity screening is a rapidly advancing science with implications in drug development, reducing risks to patients and costs to industry. As more preclinical immunogenicity testing is performed and clinical correlations become available, accuracy of preclinical immunogenicity screening methods and utility to industry are bound to improve.

METHODS

In Silico

In silico methods model different aspects of the T cell activation process to predict immunogenicity. One common method is to predict the probability that a particular linear peptide sequence (epitope) will bind to a specific HLA class II allele.

In Silico Method	Advantages	Limitations
• Epitope mapping identifies within a protein linear amino acid sequences that are predicted to bind to HLA II • Prediction tools for DR, DP and DQ are available; DR is correlated with immunogenicity	• Identify target regions from primary sequence • Reported prediction accuracy up to 85% • Can predict across multiple HLA DR alleles for broad population coverage • High throughput and low cost	• Does not model DM epitope editing, post-translational factors, etc. • Cannot distinguish T cell phenotype (e.g. effector or regulatory) • In silico predictions strengthened by in vitro validation

In silico immunogenicity prediction is a good first step to screen therapeutic candidates and to define the scope of downstream in vitro and in vivo testing.

In Vivo

Recent development of new humanized mouse models may be applied in the future to more closely predict immune response in humans.

In Vivo Method	Advantages	Limitations
• Transgenic mice express human HLA II, APCs present "human" epitopes • Mice engrafted with human tissues (*i.e.* BLT model) express a repertoire of human immune cells	• Provide functional and testable elements of a complex human immune response • Excellent model for comparing two homologous proteins, such as a therapeutic and modified variant	• Chimerism in the monocyte lineage results in a mixture of mouse and human APCs • Currently cannot recapitulate a full human B cell response • HLA expression within each cohort of mice is restricted to that of the human donor

In Vitro

In vitro methods validate in silico predictions or model certain aspects of the Td immune response that cannot be modeled computationally.

In Vitro Method	Advantages	Limitations
• **HLA binding assays** Measure binding affinity of target peptide relative to a reference peptide (competition) or to unbound peptide (direct) • Certain florescence-based methods allow for real-time measurements of the peptide binding rate	• More straightforward and easier to run than live cell experiments • Low cost, high throughput • Can be used alone or as validation for in silico predictions	• Sensitive to peptide length, purity and interaction with solvents • Reference peptide varies for each HLA in competition based assays • Direct binding and real-time measurements can be complex and costly
• **T cell Assays** (i.e. ELISA and ELISpot) measure cytokine production by T cells to target peptide • **CFSE staining** allows measurement of cell proliferation by specific T cell populations • **Tetramer assays** enumerate antigen-specific T cells	• Can provide information on magnitude and type of T cell response • Flow cytometry allows staining of multiple cell markers and cytokines – allowing for in depth characterization of responses • Can incorporate whole proteins or specific peptides	• ELISA assays are relatively low sensitivity, do not identify the cytokine-producing cell • Assays are sensitive to culture environment • MHC class II tetramers can be technically challenging
• **Naïve PBMC Assays** measure T cell responses that have not previously been exposed to antigen • **Exposed Blood Assays** measure memory T cell responses for antigens to which the donor has been previously exposed	• Incorporate whole immune process • Naïve blood assays allow for testing against a novel therapeutic	• Repeated antigen exposure or addition of exogenous cytokines to support T cell viability may skew results • Require a sufficient donor pool to represent HLA spectrum • Sensitive to individual variation
Artificial lymph node models incorporate PBMC into 3D structure that replicates the natural immune environment	• Better recapitulate the natural immune environment	• Complex and costly equipment • Still in development

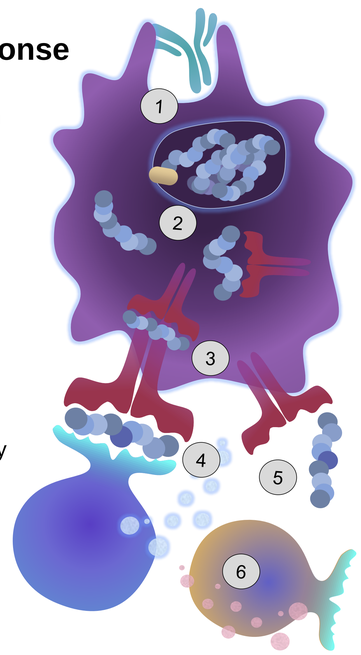
In vitro assays provide a biologic context for the prediction, measurement, and characterization of an immune response without the complexities of animal testing or risks inherent in human trials.

BACKGROUND

- Anti-drug immune responses to protein therapeutics may reduce drug efficacy or trigger adverse events
- Determinants of a drug's immunogenicity may be internal (*i.e.* sequence-based) or external (*i.e.* patient health status)
- T cell responses provide critical "help" to downstream antibody responses, therefore their prediction and mitigation can be a valuable component of risk assessment

The Td Immune Response

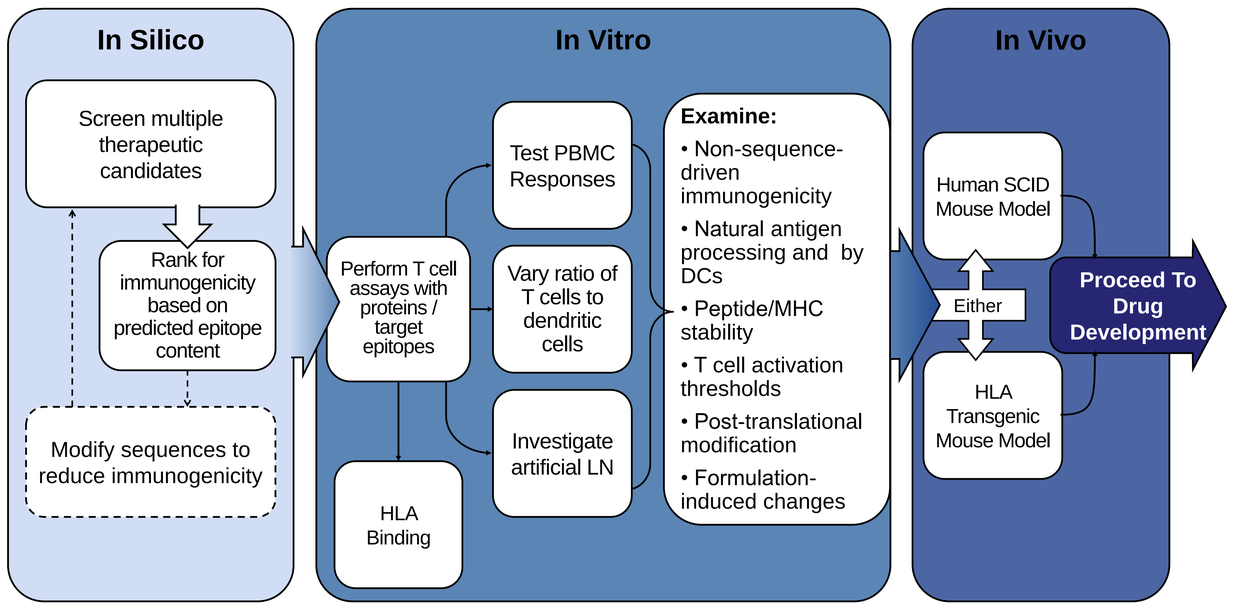
① Therapeutic proteins are taken up by antigen presenting cells (APC).
② Whole proteins processed into short, linear peptides are loaded on to MHC.
③ MHC-binding peptides are presented by APCs to T cells.
④ T cell receptors specifically recognizing the peptide-MHC complex will be activated.
⑤ Stability of peptide-MHC binding, presence of secondary signals, and peptide-specific T cell abundance may further influence T cell activity.
⑥ T cell responses influence B cell activation and other downstream processes.



APPLICATIONS

A tiered approach to predict the immunogenic potential of a protein therapeutic in development, as shown below, can leverage the advantages of multiple methods to predict immunogenic risk in a rational, cost-effective manner.

- Starting with in silico epitope mapping, a protein can be quickly characterized for potential immunogenicity. Early in development, this information can be applied to alter the protein sequence to reduce immunogenicity.
- In vitro methods can validate in silico predictions and test the magnitude or quality of a T cell response to the protein therapeutic.
- Finally, humanized or transgenic mouse models can be used to test for some aspects of therapeutic safety before proceeding to clinical trials.



- This approach has been demonstrated by Koren et al. (Clin Immunol. 2007 Jul;124(1):26-32.) where the in vitro and in silico immunogenicity assessment of four fusion proteins was well correlated with clinical immunogenicity data.
- Using in silico and in vitro methods one can identify amino acid sequences with HLA binding potential. Specific substitutions to lower HLA binding affinity can be engineered to **deimmunize** while retaining therapeutic function.
- Alternatively, immunogenicity to a therapeutic protein may be mitigated by **tolerization.** This may be achieved by inserting regulatory T cell epitopes (Tregitopes) into the protein sequence to counteract the effector T cell response.

CONCLUSIONS

- Anti-drug immune responses and their impact on safety and efficacy of protein therapeutics remain a serious concern of drug developers and regulatory bodies alike. Immunogenicity screening is an important component of any risk assessment.
- A multitude of in silico, in vitro and in vivo methods, each with their own strengths and limitations, have been developed to model and predict different drivers of T cell dependent immunogenicity.
- Although no one method emerges as a definitive predictor of an adverse immune response in human patients, or a substitute for well-controlled human trials, a strategic combination of techniques can be leveraged to provide a detailed and instructive understanding of a product's immunogenic potential.

REFERENCE

Jawa, Cousens, Awwad, Wakshull, Kropshofer, and De Groot. **T cell dependent immunogenicity of protein therapeutics: Preclinical assessment and mitigation. Clin Immunol.** 2013 Dec;149(3):534-55.
http://bit.ly/The_TCWP