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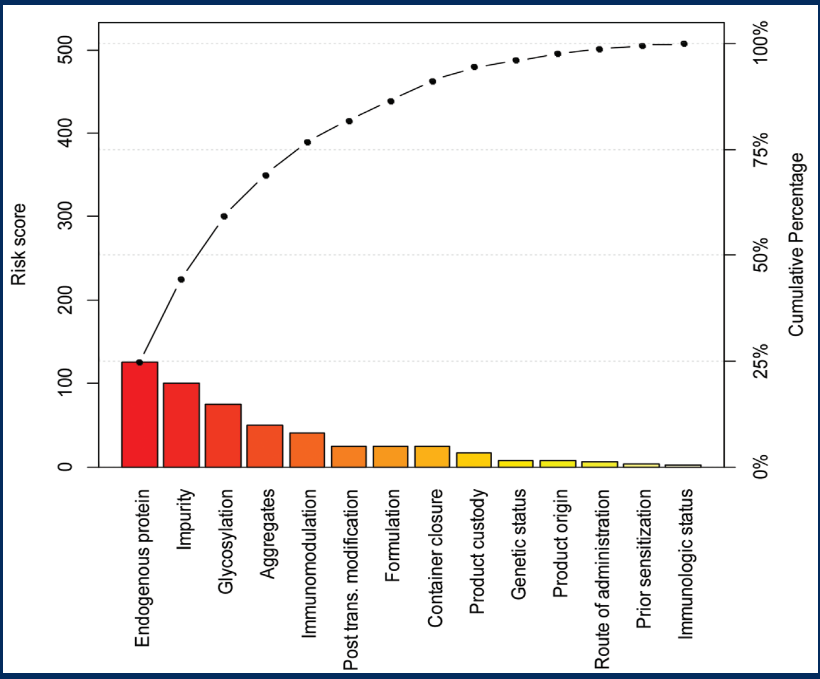
# Statistical Methods for Immunogenicity Assessment




**Harry Yang • Jianchun Zhang**
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# Statistical Methods for Immunogenicity Assessment

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# Statistical Methods for Immunogenicity Assessment

**Harry Yang • Jianchun Zhang**
**Binbing Yu • Wei Zhao**
MedImmune, LLC
Gaithersburg, Maryland, USA

CRC Press
Taylor & Francis Group
Boca Raton London New York

CRC Press is an imprint of the
Taylor & Francis Group, an **informa** business

A CHAPMAN & HALL BOOK

CRC Press
Taylor & Francis Group
6000 Broken Sound Parkway NW, Suite 300
Boca Raton, FL 33487-2742


Version Date: 20150515

International Standard Book Number-13: 978-1-4987-0035-1 (eBook - PDF)

*To our families*

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# *Preface*

Biotechnology-derived therapeutics including monoclonal antibodies, proteins, and peptides hold great promise for treating various diseases such as cancer and inflammatory diseases. They also represent an important class of therapeutic interventions. However, because of their large size, complex structure, and complicated manufacture process, biopharmaceutical products can lead to immunogenic responses, resulting in formation of anti-drug antibodies (ADAs). Immune responses to non-vaccine biologics have the potential to negatively affect both patient safety and product efficacy. For example, a neutralizing antibody is deleterious if it inhibits the efficacy of the product, and can be harmful when it cross-reacts with an endogenous counterpart of the therapeutic in patients. Non-neutralizing antibodies may affect the pharmacokinetic properties of the drug, thus may affect dosing regime. These immunologically-based consequences may cause drug developers to either terminate development or limit the use of otherwise effective therapies. Therefore, immunogenicity assessment is a key component of biopharmaceutical safety and efficacy evaluation, and a prerequisite for the successful development of biopharmaceuticals. Furthermore, immunogenicity is also a complex phenomenon, owing to myriad factors potentially affecting immunogenicity. For the purposes of this book, these factors are grouped into two categories: product-specific factors such as product origin, glycosylation, aggregation, impurities and formulation, and patient-related characteristics such as genetic makeup and immune status and competency. These numerous and varied factors impose challenges to immunogenicity risk assessment and development of risk mitigation strategies. The intrinsic complexity of detection, quantification, characterization, and control or mitigation of ADA argues for advanced statistical methods in both study design and analysis. This book is intended to provide a single source of information on statistical concepts, principles, methods, and strategies for detection, quantification, assessment, and control of immunogenicity.

The book consists of six chapters. Chapter 1 provides an overview of immunogenicity, its impact on biopharmaceutical development, regulatory requirements, statistical methods and strategies used for immunogenicity detection, quantification, risk assessment, and mitigation. Chapter 2 deals with ADA assay development, optimization, validation, and transfer based on sound statistical principles, design, and analysis. It discusses statistical considerations in many aspects of screening, confirmatory, and neutralizing assay development. Chapter 3 is focused on analysis of cut point, a key assay per-

formance parameter in ADA assay development and validation. It covers a wide range of topics from sample size calculation, data normalization, outlier detection and removal, to selection of proper models for cut point analysis. Challenges and limitations of cut point applied to practical clinical sample testing are also explained. In Chapter 4, we illustrate how to apply statistical modeling approaches to establishing associations between ADA and clinical outcomes, and process parameters, predicting immunogenicity risk, and developing risk-mitigation strategies. Various strategies for immunogenicity risk control are presented in Chapter 5. Finally, the majority of computer codes/algorithms of the statistical methods introduced in the book are provided and explained in Chapter 6.

In recent years, assessment of immunogenicity has emerged as an important regulatory initiative as evidenced by a growing number of white papers on the subject, and publication of the FDA and EMA guidelines. It is also a crucial step toward using risk-based strategies in biopharmaceutical product development. To ensure regulatory compliance, gain deep understanding of immunogenicity, and develop effective immunogenicity risk mitigation strategies, it is imperative to apply robust statistical methods and thinking in the detection, quantification, assessment, and mitigation of immunogenicity risk. To that end, a single book covering statistical concepts, principles, methods, and strategies in immunogenicity assessment will provide an invaluable resource for practitioners in biopharmaceutical therapy development. As immunogenicity risk assessment and control are issues faced by professionals who are involved in non-clinical, clinical, and bioprocess development, this book will be helpful to many individuals in various scientific and regulatory disciplines, including statisticians, pharmacokineticists, toxicologists, clinical assay developers, clinicians, biopharmaceutical engineers, and regulatory reviewers.

We are extremely grateful to John Kimmel, executive editor, Chapman & Hall/CRC Press, for giving us the opportunity to work on this book. We would like to express our gratitude to Laura Richman, Dianne Hirsch, and Kicab Castañeda-Méndez for their expert review of the book and helpful comments.

Harry Yang
Jianchun Zhang
Binbing Yu
Wei Zhao
*Gaithersburg, Maryland, USA*

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# 1

# *Introduction*

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## 1.1 Background

The discovery of DNA in 1953, and the many advances made afterwards in cellular and molecular biology in the late 1970s brought into existence the biotechnology industry. Of particular importance was the development of recombinant DNA technology which enabled the creation and production of proteins in a laboratory setting. These technological advances have provided biopharmaceutical companies with the tools needed to develop "targeted therapies" aimed at the biological underpinnings of various diseases. The first recombinant biologic therapy licensed in the United States (U.S.) was recombinant human insulin which was approved by U.S. Food and Drug Administration (FDA) in 1982. Since the approval of recombinant human insulin, more than 200 biological products have been approved over the past several decades, treating diseases ranging from cancers to rare genetic disorders (Guilford-Blake and Strickland (2008)). As of 2013, more than 900 molecules, targeting over 100 diseases including cancer, multiple sclerosis, and rheumatoid arthritis, were at various stages of development (PhRMA (2013)). These biotechnology-derived therapeutics hold a great deal of promise for future medicinal innovation and breakthroughs.

However, despite the promise of therapeutic proteins and monoclonal antibodies to meet unmet medical needs, development of biologics poses a host of unique challenges. Biopharmaceutical products are often large in size, having complex structures which are often modified post-translationally, e.g., glycosylation, and/or during manufacturing to improve product quality, e.g. pegylation. Additionally, most therapeutic proteins are produced in non-human cell lines and therefore are not identical to the homologous human protein. In light of these complexities, it is not surprising that the manufacture of biologics requires complicated and tightly controlled manufacturing processes. An additional consideration is that most biologics are administered intravenously or subcutaneously. As a result, therapeutic proteins and monoclonal antibodies (mAbs) have the potential to induce immune responses when administered to patients.

One common immunogenic response to therapeutic proteins and mAbs is the development of anti-drug antibodies (ADA). While the development of ADAs against therapeutic proteins is common and often has no measurable clinical effects, ADA responses have the potential to negatively affect both patient safety and product efficacy (Shankar et al. (2008)). For instance, for a therapeutic protein that has a non-redundant endogenous counterpart, a neutralizing antibody response can cross-react with the endogenous protein, causing serious consequences (FDA (2014)). One example is recombinant human erythropoietin (rhEPO) which is used to treat anemia. It was shown that neutralizing antibodies (NAbs) directed against rhEPO secondary to administration of the product also blocked the function of endogenous erythropoietin

which was causal in the development of pure red cell aplasia (Casadevall et al. (2002)). ADA binding to the therapeutic can also impact product efficacy. For instance, 50% patients treated with the murine monoclonal antibody OKT3 developed human anti-mouse antibodies (HAMAs) that correlated with decreased efficacy (Kuus-Reichel et al. (1994)). Readers interested in reviews on immunogenicity are referred to van de Weert and Møller (2008) and Baker et al. (2010).

In recent years, various methods and strategies have been developed to reduce and manage immunogenicity of biologic products. Early efforts were centered on methods for measuring ADA. Now, in addition to ADA monitoring, therapeutic protein manufacturers are increasingly focusing on engineering therapeutics with reduced risk of inducing ADA responses. Approaches include development of humanized proteins, removal of T-cell epitopes, and selection of less immunogenic proteins using *in silico*, *in vitro*, and *in vivo* prediction methods. Therapeutic proteins are often produced in non-human cell lines and species, e.g., mice. As such, their protein sequences differ from the human counterpart, thus increasing immunogenic potential of the therapeutic protein when administered to human recipients. Humanization of proteins produced in non-human species is a process to increase the proteins similarity, through modifying non-human sequences to homologous human sequences. In certain cases, humanization has been shown to be effective in reducing the risk of immunogenicity. In one retrospective review of ADA responses to mAbs, murine mAbs were shown to have the highest frequency of ADA responses, and that replacement of the mouse immunoglobin constant regions with human sequences reduced the development of ADAs (Hwang and Foote (2005)). ADA responses to T-cell epitopes is also well recognized. It has been shown that the presence of T-cell epitopes in a therapeutic protein is one driver of ADA responses. When T-cell receptors recognize small fragments derived from protein antigens coupled with major histocompatibility complex (MHC) class II molecules on the surface of antigen-presenting cells (APCs), T-cell responses are activated. Therefore, one way to minimize immunogenic risk is to deactivate T-cell responses to a therapeutic protein. For this purpose, several methods have been utilized to identify and design proteins that have a more acceptable immunogenic profile. The strategies include removal of T-cell epitopes through *in silico*, *in vitro*, and *in vivo* prediction, patient immunosuppression and tolerization (Adair and Ozanne (2002)). Using *in-vitro* experiments, T-cell epitopes can be screened and then proteins with the least T-cell epitopes can be used for subsequent development. Immunosuppression reduces immunogenicity through treating subjects with drugs that suppress T-cell activities; whereas the tolerance approach focuses on desensitizing the immune system to the therapeutic protein so that the therapeutic protein is no longer recognized as foreign.

As pointed out by De Groot and Martin (2009), successful mitigation of immunogenicity potential of a therapy is likely to rely on a combined approach. It uses rational sequence design, and *in vitro* and *in vivo* animal testing to