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COMMENTARY

Immunogenicity in Clinical Practice and Drug Development: When is it Significant?

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Managing immunogenicity in clinical practice and during drug development was a recent topic at the ASCPT 2019 annual meeting. This commentary expands on the discussion to facilitate a broader engagement across the community. The intent is to provide a rationale for ongoing research into the current gaps in assessing and interpreting immunogenicity in drug development and managing clinical immunogenicity for an approved drug. The following are highlighted: (i) Immunogenicity Considerations in Clinical Practice, (ii) Immunogenicity Testing and Current Limitations, (iii) Immunogenicity Risk Assessment and Mitigation, and (iv) Quantitative Systems Pharmacology (QSP) models of Immunogenicity.

IMMUNOGENICITY CONSIDERATIONS IN CLINICAL PRACTICE

Biologics revolutionized the treatment of many serious conditions, such as cancer, rheumatoid arthritis, and inflammatory bowel disease (IBD); however, the issue of immunogenicity (i.e., the development of antidrug antibodies (ADAs) against these protein-based therapies) continues to plague patients and providers. Although limiting the benefit of a clinical response and invoking safety/tolerability issues due to immunogenicity to a therapeutic protein is of great concern for all patients in which treatment options are limited, it is perhaps of greatest concern in pediatrics, as a limited number of therapeutic proteins are approved for pediatric indications. Long-term outcomes of diseases treated with such therapeutics may be severely impacted by immune responses to them, necessitating hypervigilance against ADA formation and the consequent loss of treatment response to the few agents approved in this vulnerable patient population.

At the Children's Mercy Hospital (Kansas City, MO), of the 620 children with IBD, over 60% depend on biologics for treatment. Statistically, up to 65% of these patients will develop ADAs during the course of IBD treatment.[1] Thus, many providers prefer proactive ADA monitoring, at least annually; however, limited insurance coverage of testing for ADAs frequently precludes this judicious practice and/or necessitates the use of different ADA assays, creating added challenges for assay interpretation. For example, consequent to prominent third-party payers labeling therapeutic drug monitoring for biologics "experimental" or "investigational," the institution was forced to change preferred ADA assays three times in the last 24 months. With each change, providers were expected to familiarize themselves with a new assay type, the upper and lower limits of assay quantification, report output, and interpretability of values between different assays, in order to make sense of the information reported.

Even when prescribers succeed in correctly interpreting drug level and ADA information, there are challenges associated with third-party payer re-imbursement. This is especially problematic when drug trough levels are low and dose escalation or interval shortening is warranted to prevent ADA formation and loss of treatment response.[2] Payers frequently use US Food and Drug Administration (FDA) labeling, which focuses on a specific dose and interval, rather than on a therapeutic level, to challenge the need for different dose/interval escalation requests. In such scenarios, the only course of action available to prescribers is to add an immunomodulator in attempt to increase drug concentrations and prevent ADA formation; however, this decision comes with increased risks for added potential adverse events and malignancy (e.g., hepatosplenic T-cell lymphoma, attributed to treatment with biologics and/or immunomodulator and universally fatal in IBD (see Beaugerie *et al*., **Supplementary Material**).

IMMUNOGENICITY TESTING AND CURRENT LIMITATIONS

One of the major challenges in the utilization of information on immunogenicity for biologics in drug development and clinical practice is related to the analytical methodologies used to assess the incidence of ADA formation and the impact of immune reactions. The FDA recently released updated guidance on "Immunogenicity Testing of Therapeutic Protein Products—Developing and Validating Assays for Anti-Drug Antibody Detection" (reference in **Supplementary Material**).

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During drug development, immunogenicity of a biologic is usually assessed with a three-tiered approach, consisting of a screening assay designed to minimize false-negatives (tier 1), followed by a more stringent confirmatory assay designed to minimize false-positives (tier 2), and finally various ADA characterization assays (tier 3). Tier 1 and tier 2 assays are usually ligand-binding immunoassays, for which the biggest limitation is the reliance on positive controls for ADAs created in non-human species by exposure to the biologic agent and isolation of the resulting ADAs. This response is polyclonal, differs by animal, and inherently results in differences in the formed ADAs from those expected in humans due to species foreignness. As a consequence, immunogenicity assays are semiquantitative assessments, including unique positive controls for each established assay. Therefore, with regard to incidence rates or intensity of response, the results of these assays cannot be compared between different therapeutic proteins or for the same therapeutic protein when different assays are utilized.

Data from the development of adalimumab biosimilars represent a good example, in which each of the three biosimilar products were individually compared with the adalimumab reference product (i.e., Humira) in patients with moderate-to-severe rheumatoid arthritis, under stable methotrexate background therapy.[3–5] All three studies reported similar ADA incidence rates and neutralizing capacity between the respective biosimilar and the reference product within each study, but the ADA incidence rates for the identical adalimumab reference product (Humira) were vastly different across studies with 53% (54% neutralizing), 32% (50% neutralizing), and 38.2% (29% neutralizing) in the three independent studies.

Immunogenicity testing in clinical practice is typically performed as reflex testing based on drug-level monitoring or clinical suspicion. Although immunoassays represent the most common analytical platform, other methodologies demonstrate similar performance. This is exemplified by clinical immunogenicity testing for the anti-TNF biologics for which a variety of analytical platforms are available, including: homogenous mobility-shift assays, gene-reporter assays, and assays that utilize surface plasmon resonance or mass spectrometry.[6]

IMMUNOGENICITY RISK ASSESSMENT AND MITIGATION

In 2014, the FDA published a "Guidance for Industry-Immunogenicity Assessment for Therapeutic Protein Products," (reference in **Supplementary Material**) given the dramatic expansion of developing and approved protein therapeutics, as well as the advent of biosimilars, and the severe adverse clinical consequences pertaining to immune responses to several protein therapeutics. The most severe ADA consequences, including anaphylaxis, cross-reactive antibody-mediated neutralization of nonredundant endogenous proteins, and neutralization of life-saving therapeutics, demand the development of preventive or therapeutic mitigation strategies. Additionally, ADA may cause severe, although not immediately life-threatening responses, including delayed hypersensitivity responses due to immune-complex formation and complement activation. Clinical signs may include fever, rash, arthralgia, myalgia, hematuria, proteinuria, serositis, central nervous system involvement, and hemolytic anemia in the face of ongoing robust ADA to therapeutic proteins. This has been observed in cases in which there are attempts to "dose over" the ADA thereby fully saturating ADA and allowing the residual, free protein therapeutic to access target tissues, with examples in immune tolerance protocols for Factor IX in hemophilia B and α-glucosidase in Pompe disease (**Supplementary Material**). The overarching principle espoused is that the clinical consequences of immune responses to protein therapeutics, generally mediated by ADA (**Figure 1**), determine the appropriate mitigation strategy.

There are two principal options for ADA mitigation: induction of immune tolerance to the therapeutic protein once in clinical development (principle 1) or de-immunization of

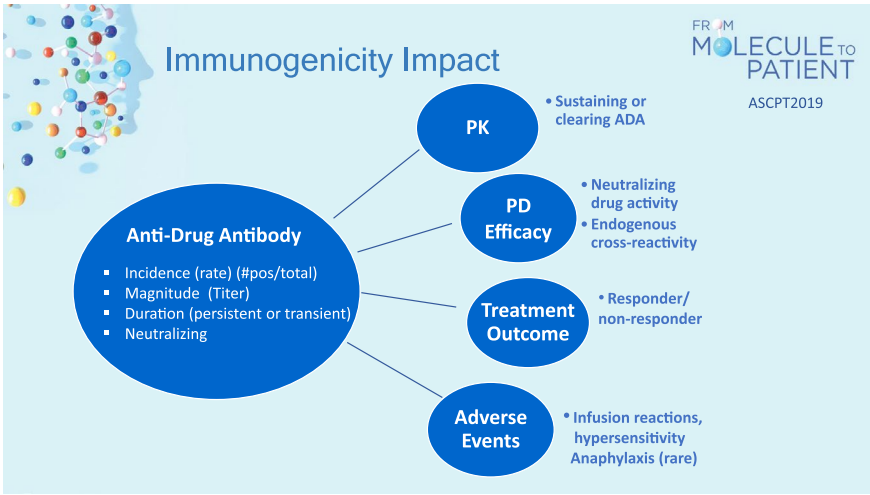

Figure 1 Immunogenicity impact. 2019 ASCPT Annual Meeting artwork designed by GRAPHEK Design Studio. ADA, antidrug antibody; PD, pharmacodynamic; PK, pharmacokinetic.

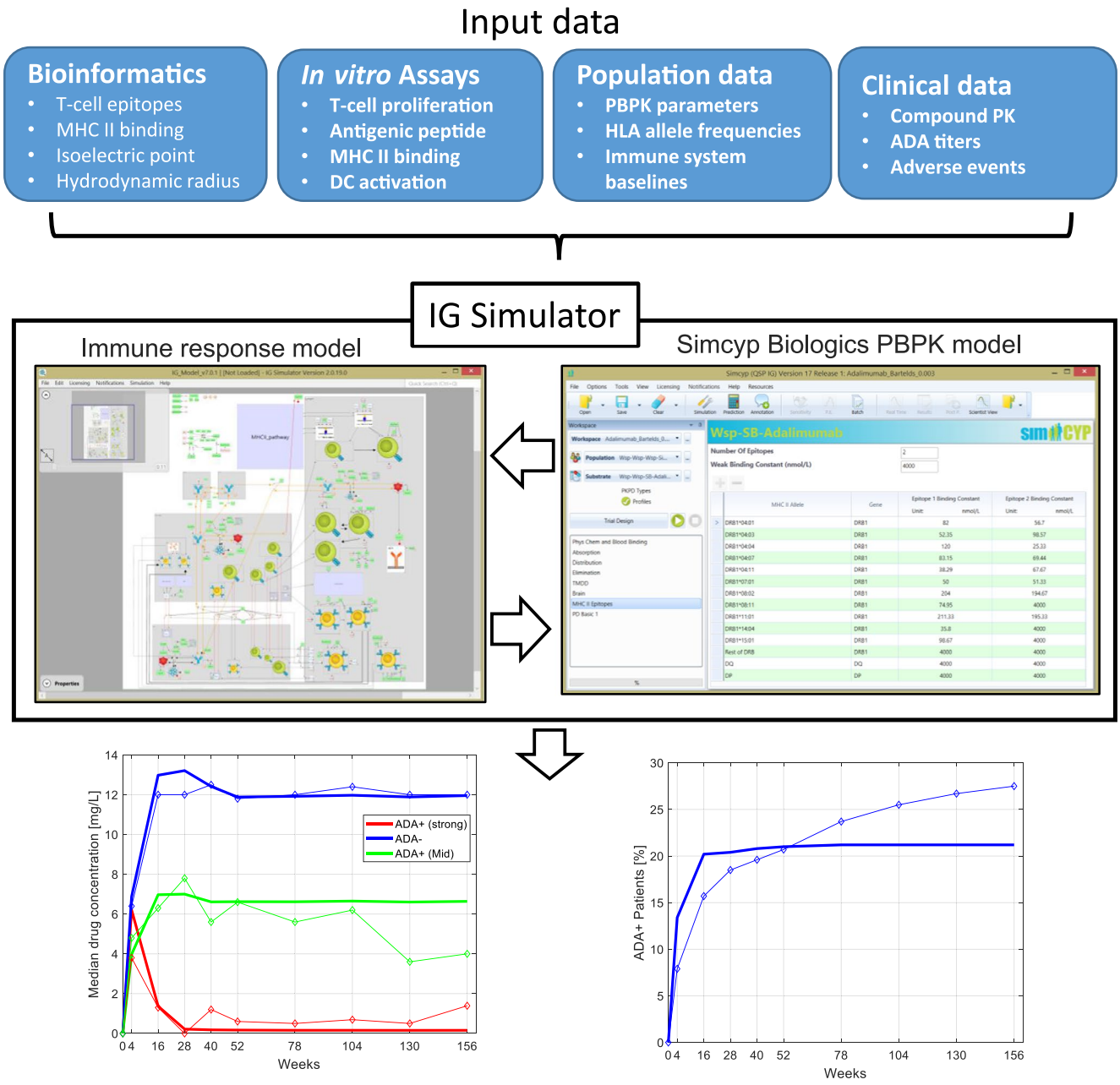

Figure 2 Overview of the IG Simulator (Adapted from Kierzek *et al.*, 2019[8] CPT:PSP). The Quantitative Systems Pharmacology model includes mechanistic model of immune response and Simcyp Biologics physiologically-based pharmacokinetic. The model has sufficient mechanistic granularity to use major histocompatibility (MHC) II binding constants predicted by bioinformatics or determined *in vitro*, as well as results of other *in vitro* assays, such as T-cell proliferation. Population data for virtual clinical trial simulation include frequencies of *HLA* genes, immune system baselines, and physiological parameters in target population. When the model is applied to extrapolation from first-in-human data or extrapolation between different clinical populations, clinical data on pharmacokinetic and antidrug antibody (ADA) titers are used. Mechanistic model integrates diverse inputs and simulates virtual trial—a population of individuals subject to specific dosing regimen. The figure shows simulation of adalimumab clinical trial data (solid dark line) and comparison with clinical data (line with symbols). Because individual virtual patient time profiles for both adalimumab and ADA are available, simulation results can be analyzed and reported using the same criteria as used in the clinic.

the protein therapeutic via use of predictive algorithms and *in vitro* studies to identify and remove immunogenic epitopes while maintaining product activity prior to or during product development (principle 2). These principles are discussed below with specific examples in the **Supplementary Material**.

Principle 1: When the immune response to a protein therapeutic is life-threatening, immune tolerance induction may be life-saving. Immune tolerance is broadly defined as "selective elimination of pathogenic immune responses to relevant antigens by any of a variety of approaches, while preserving protective immunity and does not require

ongoing treatment with the intervention." Immune tolerance induction strategies include: 1) antigen-specific tolerance approaches; 2) antigen-targeted tolerance approaches; and 3) immune regenerative tolerance approaches.

Principle 2: Protein engineering is a longer-term strategy that may be used to remove immunogenic epitopes of a protein therapeutic or in designing a therapeutic with the essential activity of an endogenous protein, but lacking in sequence homology.

Because risk is a function not only of consequences, but also of probability of generating an immune response, it is important to consider the patient and protocol-specific risk factors, as well as the critical product quality attributes that may facilitate or diminish the likelihood of ADA generation. These risk factors are described in the **Supplementary Material (Figure S1)**.

QUANTITATIVE SYSTEMS PHARMACOLOGY MODELS OF IMMUNOGENICITY

Computational approaches are making an increasing impact on decision making in drug development. Application of *in silico* methods to predict immunogenicity is currently limited to bioinformatics prediction of peptides that bind strongly to major histocompatibility (MHC) II receptors by bioinformatics and *in vitro* studies to inform protein engineering approaches. This approach, however, does not consider a number of other important factors related to the drug, the patients, or the route of administration. Moreover, bioinformatics does not predict the impact of ADAs on pharmacokinetics (PKs) and is, therefore, not applicable to informing changes to dosing regimens and co-therapy in the management of immunogenicity in patients.

A Quantitative Systems Pharmacology (QSP) approach can open an avenue toward prediction of ADA impact on PK in patient populations; thus, enabling model-informed management of immunogenicity. Following seminal work,[7] a number of companies recognized that development of a QSP model of immunogenicity is a noncompetitive effort and formed a consortium.[8] The immunogenicity simulator, referred to as the IG Simulator, developed by the IG QSP Consortium is one example of a QSP model, among others,[7,9] that integrates literature-based, mechanistic models of immune response and ADA synthesis with a physiologically-based pharmacokinetic model of biologics[10] (**Figure 2**). The simulator uses data on T-cell epitopes, MHC II affinities and patient HLA genotype as input, thereby enabling extrapolation from *in vitro* assays and bioinformatics to predictions of ADA incidence along with PK effects in patient populations. The IG Simulator outputs virtual trials, where the effect of, among others, different dosing regimens, patient characteristics, and co-therapy can be examined. A recent publication describing a QSP model to predict ADA for a biotherapeutic in phase I provides an example of this approach.[9] Additionally, as a drug development program progresses, a QSP model can be further informed by clinical data and used for extrapolation to later stages and special populations, including pediatric. Thus, QSP models enable integration of a wide range of *in vitro* assays, clinical data, and bioinformatics predictions. This could be used to simulate immunogenicity in both drug development and clinical practice.

CONCLUSIONS

Clinicians face challenges in maintaining efficacy for approved biologics when patients develop ADAs and there are impacts on product efficacy and/or patient safety. The ability to adequately prevent the loss of efficacy requires ADA testing in the postmarketing setting, where access to ADA assays and technical assay limitations can be problematic. Routine ADA monitoring and dose/interval escalation to mitigate immunogenicity effects in the clinic could be encouraged through publishing ADA assay methodologies, increased access to the drug sponsors'/vendors' testing methods and/or using inclusive labeling practices. Improving ADA detection technologies is also warranted, specifically to identify the means to normalize ADAs to a reference product/standard, in much the same way a reference standard is used for other clinical assays. The best proactive approach to immunogenicity mitigation is to develop more predictive tools and, where possible, design out immunogenic sequences early in drug development. Additionally, once ADAs present in early clinical development, deriving methods to induce product-specific tolerance to maintain efficacy and making these methods available to clinicians would benefit patients. Ultimately, increasing availability of QSP models to integrate knowledge on basic immune system biology to simulate virtual trials has potential to inform drug development decisions, drug labels, and clinical practice.

Supporting Information. Supplementary information accompanies this paper on the *Clinical and Translational Science* website (www.cts-journal.com).

Figure S1. Immunogenicity risk factors. 2019 ASCPT Annual Meeting artwork designed by GRAPHEK Design Studio.
Supplementary Materials

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