Nonclinical Safety Evaluation of the Immunotoxic Potential of Drugs and Biologics

Guidance for Industry

DRAFT GUIDANCE

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For questions regarding this draft document, contact (CDER) David McMillan, 240-402-1009, or (CBER) Office of Communication, Outreach and Development, 800-835-4709 or 240-402-8010.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
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Pharmacology/Toxicology

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Nonclinical Safety Evaluation of the Immunotoxic Potential of Drugs and Biologics Guidance for Industry[1]

This draft guidance, when finalized, will represent the current thinking of the Food and Drug Administration (FDA or Agency) on this topic. It does not establish any rights for any person and is not binding on FDA or the public. You can use an alternative approach if it satisfies the requirements of the applicable statutes and regulations. To discuss an alternative approach, contact the FDA staff responsible for this guidance as listed on the title page.

I. INTRODUCTION

The purpose of this guidance is to assist sponsors in their nonclinical evaluation of the immunotoxic potential of drugs and biologics by supplementing the recommendations on nonclinical immune system assessments provided across the following guidance documents:

- International Council for Harmonisation (ICH) guidances for industry:
  - *S8 Immunotoxicity Studies for Human Pharmaceuticals* (April 2006)[2]
  - *M3(R2) Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals* (January 2010)
  - *S6(R1) Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals* (May 2012)
  - *S5(R3) Detection of Toxicity to Reproduction for Human Pharmaceuticals* (November 2017)[3]

[1] This guidance has been prepared by the Center for Drug Evaluation and Research in cooperation with the Center for Biologics Evaluation and Research at the Food and Drug Administration.

[2] We update guidances periodically. To make sure you have the most recent version of a guidance, check the FDA guidance web page at https://www.fda.gov/regulatory-information/search-fda-guidance-documents.

[3] When final, this guidance will represent the FDA's current thinking on this topic. For the most recent version of a guidance, check the FDA guidance web page at https://www.fda.gov/regulatory-information/search-fda-guidance-documents.

  - *S11 Nonclinical Safety Testing in Support of Development of Paediatric Medicines* (January 2019)[4]

  - *S9 Nonclinical Evaluation for Anticancer Pharmaceuticals* (March 2010)

  - *S9 Nonclinical Evaluation for Anticancer Pharmaceuticals – Questions and Answers* (June 2018)

  - *S1A The Need for Long-term Rodent Carcinogenicity Studies of Pharmaceuticals* (March 1996)

- Guidance for industry *Considerations for Developmental Toxicity Studies for Preventive and Therapeutic Vaccines for Infectious Disease Indications* (February 2006)[5]

This guidance covers the evaluation of functional, histomorphologic, and cellular aspects of the immune system in nonclinical studies for new drugs, therapeutic proteins, and recombinant/plasma-derived blood proteins regulated by the Center for Drug Evaluation and Research (CDER) and the Center for Biologics Evaluation and Research (CBER). Cell and gene therapies, adjuvanted vaccines, and other biologics are not within the scope of this guidance, although some of the principles in this guidance may also be applicable to these product types; for these products, direct consultation with the review division is strongly recommended. Consult ICH S9 and ICH S9 Questions and Answers for guidance on the need for specific types of studies to support the development of anticancer pharmaceuticals. Evaluation of all assessments discussed in this guidance may be indication-specific and is not necessarily expected for every product with potential immune effects. This guidance replaces the withdrawn guidance for industry *Immunotoxicology Evaluation of Investigational New Drugs* (October 2002).

II. BACKGROUND

The immune system is a complex and highly regulated system that involves many biological structures (e.g., proteins, cells, tissues, and organs distributed throughout the body) and complex physiological responses (e.g., innate, adaptive, cell-mediated, and humoral immunity) with the primary purpose of protecting the body from infections, diseases, tumors, and foreign substances. The ability of drugs and biologic products[6] to modify the activity of the immune system is an important part of evaluating the safety and efficacy of these products. Safety evaluation of these drugs and biological products should include evaluating both the intended (pharmacological) and the unintended (toxicological) actions on the immune system. The extent of the effects on the

[4] When final, this guidance will represent the FDA's current thinking on this topic.

[5] Additional information can be found in *WHO Guidelines on Nonclinical Evaluation of Vaccine Adjuvants and Adjuvanted Vaccines*, World Health Organization, 2013.

[6] For purposes of this guidance, references to *drugs* and *drug and biological products* includes drugs approved under section 505 of the Federal Food, Drug, and Cosmetic Act (the FD&C Act) (21 U.S.C. 355) and biological products licensed under 351 of the Public Health Service Act (PHS Act) (42 U.S.C. 262) that are drugs.

immune system should be adequately characterized to properly inform the overall risk-benefit of the product. Effects can include both a reduction or an increase in activity, as well as changes in the immune balance (e.g., a shift from Th1 to Th2). For drugs that are designed to affect the immune system, the sponsor should provide data from immunological assays to demonstrate the pharmacological effects of the drug. The choice of assays should be guided by the expected pharmacology of the drug. In addition, it may be important to evaluate the possibility of off-target or unintended effects on the immune system when results from standard studies suggest unexpected effects.

FDA supports the principles of the 3Rs (replace/reduce/refine) for animal use in testing when feasible. FDA encourages sponsors to consult with review divisions when considering a nonanimal testing method believed to be suitable, adequate, and feasible. FDA will consider whether the alternative method is adequate to meet the nonclinical regulatory need.

Finally, sponsors are reminded that they should, to the extent practicable, follow existing guidance on placing immunotoxicology studies in the electronic common technical document (eCTD) format. Data that refer to specific immunotoxicology studies should be included in the eCTD in section 4.2.3.7.2. Data on antigenicity should be included in section 4.2.3.7.1. Data evaluating the immune system that are part of a general repeated-dose toxicity study should be included with those data in section 4.2.3.2. See the FDA eCTD technical specification *The Comprehensive Table of Contents Headings and Hierarchy*[7] for further details.

The term *immunomodulator* is used frequently in drug development; however, its definition is not universally agreed upon. For the purposes of this document, the term immunomodulator could be any therapeutic that modifies the immune response, including those that act in a manner that is not overtly immunosuppressive or immunostimulatory and may have subtle or even mixed effects. For example, products that can affect immune cell signaling via common downstream signaling pathways (e.g., MEK, RAS, NF-κB), or products in the "IMiD" class, such as thalidomide, can have immunomodulatory effects. Alterations in immune system parameters that are detected in general toxicology studies can warrant further investigation, on a case-by-case basis, depending on the characteristics of the specific development program (e.g., indication, patient population, intended pharmacology). Many of the principles outlined below may be considered for such a situation.

For product-specific recommendations related to this guidance, FDA recommends that sponsors contact the appropriate review division.

[7] Accessible at https://www.fda.gov/downloads/drugs/ucm163175.pdf.

III. ASSESSING THE POTENTIAL FOR PRODUCTS TO REDUCE THE ACTIVITY OF THE IMMUNE SYSTEM

A. General Immunotoxicity Assessment

Historically, many of the endpoints for immunological safety evaluation were optimized to detect immunosuppression/reduction of immune system activity. Some of the classical assays to evaluate immunosuppression are mentioned in ICH S8. The majority of these classical assays are still commonly used with enhancements or modifications. The decision on which assay to use should be based on a weight-of-evidence (WOE) approach, as discussed in ICH S8.

For small molecules for which the intended pharmacological action is not targeted to the immune system—and therefore extensive pharmacological studies of the immune system were not conducted during development—the initial evaluation for immunotoxicity should also follow ICH S8. For situations not directly covered by ICH S8 (e.g., autoimmunity, sensitization), FDA recommends a WOE approach.

If the WOE approach suggests potential immunotoxicity, but a specific affected part of the immune system is not identified, then FDA recommends a common secondary assay that requires functionality of several key immune cell subtypes (e.g., antigen-presenting cells, T-helper cells, B cells), such as the T-cell-dependent antibody response (TDAR) assay. The TDAR assay has been successfully used in mouse, rat, dog, minipig and cynomolgus monkey, using Keyhole Limpet Hemocyanin (KLH) as the test antigen. This assay has been significantly extended and improved since the finalization of ICH S8. KLH is a common choice of antigen based on the extensive historical database, growing standardization, and experience across multiple labs. Other antigens (e.g., sheep erythrocytes and tetanus toxoid) have been used in drug development. In any TDAR assay, FDA recommends a positive control compound. Sponsors should justify the choice of species tested, antigen chosen, and conditions of the assay.

Sponsors should follow ICH S6(R1) for development of therapeutic proteins that may reduce the activity of the immune system as a consequence of the intended pharmacology. For drugs such as cytotoxic or myelosuppressive anticancer pharmaceuticals, follow-up assays discussed in ICH S8 are generally not warranted.

B. Carcinogenicity and Immunosuppression

Immunosuppression is associated with an increased risk of certain tumor types in humans. These tumors are primarily associated with loss of control of chronic/latent pathogen infections, although direct interference with tumor surveillance could also result in an increased risk for tumors.

Sponsors should follow the recommendations in ICH S1A and ICH S9 on the need for a carcinogenicity assessment. Standard 2-year carcinogenicity studies are not specifically designed to detect carcinogenicity caused by drug-induced decreases in tumor surveillance, particularly when the increased tumor risk is caused by recrudescence of latent viral oncogenes, infectious agents, or chronic inflammatory states. Therefore, if an assessment is warranted for a product

with immunosuppressive potential, sponsors should complete a WOE-based risk assessment in addition to the standard carcinogenicity studies. A WOE-based risk assessment is particularly relevant for drugs and biologic products that lack the intended pharmacological activity in rodents and for biologics for which significant formation of anti-product antibodies diminishes interpretability of rodent studies. This WOE-based risk assessment should address relevant attributes of the drug and the drug target. This includes the potential for a therapeutic to increase tumor promotion, growth, and metastases, and specific evaluation of the impact of the product on immune cell subpopulations.

When the product adversely impacts key components of the immune system, such as critical cells involved in tumor surveillance (e.g., natural killer (NK) cells, T cells, B cells), sponsors should consider a functional assessment of these key components. If this assessment suggests an increased risk based on specific concerns, sponsors should consider follow-up in vivo studies to specifically address the concerns raised. For small molecule products in particular, the WOE-based risk assessment should also address the carcinogenic relevance of any compound-specific toxicology findings not related to the product's intended effect on the immune system (e.g., off-target activity). FDA recommends that sponsors discuss these concerns with the review division before embarking on extended studies.

IV. ASSESSING THE POTENTIAL FOR PRODUCTS TO INCREASE ACTIVITY OF THE IMMUNE SYSTEM

A. Immunostimulation

Evaluating increases in immune system effects normally requires a safety evaluation paradigm that differs from the evaluation of immune suppression and may involve specific assays or alternative methodology for translation to first-in-human trials. See below for examples.

Immunostimulatory products are defined in this section as products that are intended to either directly stimulate signaling in an immune cell subtype or indirectly enhance the immune system response by blocking or activating an endogenous regulator of the immune system response. Toxicities of these products are often the result of exaggerated pharmacological activity.

Excessive release of cytokines can cause severe adverse reactions as shown by the near-fatal clinical responses to the monoclonal antibody TGN 1412.[8] There are now commonly used in vitro models available to evaluate the potential for this risk.[9] As alternative models are developed and refined, additional assays may become available.

Because of immunological differences in expression and sensitivity between humans and nonclinical test species, additional safety considerations may be needed for therapeutics intended

[8] See the UK report *Expert Group on Phase One Clinical Trials*, Duff, GW, 2006, (accessible at https://webarchive.nationalarchives.gov.uk/20070807213430/http://www.dh.gov.uk/en/Publicationsandstatistics/Publications/PublicationsPolicyAndGuidance/DH_063117).

[9] See the guidance for industry *Immunogenicity Assessment for Therapeutic Protein Products* (August 2014).

to modulate the immune response, which can lead to adverse reactions such as excessive cytokine release. For therapeutic biologic products intended to stimulate an immune response either directly or indirectly, a starting dose based on a minimal anticipated biologic effect level (MABEL) or a pharmacological effect level (PEL) may be more appropriate than a starting dose based on toxicology endpoints such as the no observed adverse effect level (NOAEL). The same approach may apply to small molecule products intended to stimulate specific immune system outcomes, depending on the relevance of available animal models. As a MABEL or PEL approach relies heavily on pharmacological data, FDA expects sponsors to provide the following types of specific immune system pharmacological data using human cells before initiating clinical trials:

- In vitro assays assessing immune activation, cytokine release, and ligand-receptor interactions in human cells that include assessment of concentration response curves for determining the effective concentration (EC) values, such as EC_{20}, EC_{50}, and EC_{80}, or for receptor occupancy values from direct or competitive binding assays. The specific assays used to evaluate functional parameters will depend on the biology of the intended pharmacological effect.

- An assessment of the potential for cytokine release syndrome caused by therapeutic proteins using unstimulated human cells in both plate-bound (or other assays that can assess the contribution of crosslinking of receptors) and soluble formats with appropriate positive and negative controls.[10] These assays are considered critical for hazard identification. If the assays used to characterize the primary pharmacology of the product have already demonstrated that the product has a clear potential to directly cause cytokine release (e.g., a CD3 bispecific T cell redirector), these assays are usually not necessary, as the hazard has already been identified. Similarly, if one assay is positive, then an assay in the other format may not be needed.

- When products do not directly bind to surface receptors with recognized involvement in immune system activation, then assays such as cytokine release assays are generally not warranted.

Although a positive response in a cytokine release assay may not preclude further development of a drug, it could impact the selection of the appropriate start dose and inform clinical monitoring, the need for potential interventions, and dose escalation and stopping criteria. The selection of an appropriate start dose could be based on a variety of considerations, such as predicted C_{max} values that result in the lowest in vitro pharmacological activity (e.g., EC_{20} or EC_{50} values) from various activation assays or receptor occupancy estimates. In addition, appropriate and relevant in vivo pharmacology/disease models may be useful endpoints to include in the justification of a starting dose for an immune system-modulating product. Products predicted to have the potential for cytokine release are likely to require increased clinical monitoring during the early clinical development of the product and a starting dose closer to a MABEL than to a PEL.

[10] See the guidance for industry *Immunogenicity Assessment for Therapeutic Protein Products*.

B. Non-Target-Related Antibody-Mediated Immune Stimulation

Typically, adverse antibody-mediated stimulation reactions are considered hypersensitivity reactions. However, based on the pharmacology of the product, other concerns may be warranted. Should there be a concern regarding antibody-mediated immune enhancement, there are various assays that may be helpful to evaluate the potential impact of the therapeutic agent on these parameters (e.g., enzyme-linked immunosorbent assay (ELISA), immunoassay, modified TDAR).

For IgE, multiple mechanisms may result in adverse events. Anaphylactic reactions occur when a drug binds to IgE on mast cells and induces a degranulation reaction. Symptoms may range from mild to fatal. Anaphylactoid reactions are caused by multiple mechanisms, including direct interaction between a drug and a receptor on the mast cell surface. The symptoms in humans are indistinguishable from those of a true anaphylactic reaction. The difference is that no sensitization is required for an anaphylactoid reaction, and these reactions often show a reproducible dose-response relationship. Overall, no nonclinical models are available to reliably predict either anaphylactic or anaphylactoid reactions.

Although not traditionally considered as a means to understand the potential risks associated with increased IgM/G production, an antigen-based model (e.g., the TDAR assay) can be modified to detect increased antibody production to address specific concerns. This may be especially concerning for products with the potential for long-term effects, including significant enhancement of secondary or memory responses.

For a discussion of the assessment of anti-product antibodies, see the guidance for industry *Immunogenicity Assessment for Therapeutic Protein Products*.

C. Autoimmune-Type Reactions

There are a variety of cellular responses to drugs that are observed in humans that suggest the potential for reaction against the body's own healthy cells and tissues. These include a variety of skin reactions, T-cell-mediated hypersensitivity (autoimmune diseases such as lupus and myasthenia gravis), and drug reaction with eosinophilia and systemic symptoms (DRESS). There are no nonclinical models available to reliably predict these adverse reactions.

D. Dermal Sensitization

FDA no longer recommends that sponsors conduct the murine local lymph node assay to assess the sensitization potential of topical drug products due to the limitations of the assay. FDA currently accepts a dermal sensitization study conducted in guinea pigs using the clinical formulation to assess the sensitization potential of a topical drug product. Several in chemico and in vitro assays for skin sensitization are described in the Organisation for Economic Co-opertion

and Development (OECD) test guidelines (OECD 2019;[11] OECD 2018;[12] OECD 2018).[13] As an alternative screen for skin sensitization for individual chemicals, FDA will consider a battery of studies (e.g., in silico, in chemico, in vitro) that have been shown to adequately predict human skin sensitization with an accuracy similar to existing in vivo methods.

E. Innate Immunity

Use the WOE approach discussed in ICH S8 for follow-up evaluation of innate immune system effects. Based on the pharmacology of the drug, it may be necessary for sponsors to address known or expected effects on innate immunity (e.g., oligonucleotide modifications can affect innate immunity).

V. DEVELOPMENTAL AND JUVENILE STUDIES

A. Overview

Sponsors should consult appropriate guidances for recommendations on the need for juvenile studies and developmental studies, as well as the appropriate battery of developmental studies. Developmental toxicity evaluation for infectious disease vaccines should follow the guidance for industry *Considerations for Developmental Toxicity Studies for Preventive and Therapeutic Vaccines for Infectious Disease Indications*.

Developmental and reproductive toxicology studies for therapeutic proteins are referenced by ICH S5(R3), ICH S6(R1) and ICH S11. Juvenile and pre- and postnatal development studies are not typically warranted for products intended to treat patients with cancer. Products being developed for these indications should follow ICH S9 and ICH S9 Questions and Answers.

If there is concern that the formation of a functional immune system could be compromised, and if the existing data do not already characterize the potential for risk to the target patient population, then sponsors should include in these studies additional testing of a therapeutic product on the developing immune system. In general, sponsors should consider the following factors when conducting juvenile or developmental toxicology animal studies that warrant including an assessment of immune endpoints:

[11] OECD, 2019, Test No. 442C: In Chemico Skin Sensitisation: Assays Addressing the Adverse Outcome Pathway Key Event on Covalent Binding to Proteins, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, https://doi.org/10.1787/9789264229709-en.

[12] OECD, 2018, Test No. 442D: In Vitro Skin Sensitisation: ARE-Nrf2 Luciferase Test Method, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, https://doi.org/10.1787/9789264229822-en.

[13] OECD, 2018, Test No. 442E: In Vitro Skin Sensitisation: In Vitro Skin Sensitisation Assays Addressing the Key Event on Activation of Dendritic Cells on the Adverse Outcome Pathway for Skin Sensitisation, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, https://doi.org/10.1787/9789264264359-en.

- If appropriate endpoints can be built into the pre- and postnatal development study and they identify a risk, then a dedicated developmental immunotoxicity juvenile animal study may not be necessary.

- Selection of the most appropriate species to be used to assess immune system functional development.

- As there are differences in the timing of immune system developmental landmarks across species, sponsors should adjust dosing to cover the intended developmental period.

- Risk-benefit considerations for the intended clinical indication and duration of dosing.

B. Developmental Animal Studies

For small molecules, proteins, and other biologics, sponsors should consider developmental immunotoxicity in certain cases (e.g., if the product is known to target a component of the immune system). Follow-up assessments may be necessary in the following circumstances:

- The drug product has been shown to elicit immunotoxicity in nonclinical studies with adult animals, as outlined in ICH S8.

- There is reasonable evidence that the mechanism of action or the pharmacology of the drug product could affect the developing immune system.

- The drug or drug class is known to directly affect the immune system.

C. Nonhuman Primate Enhanced Pre- and Postnatal Development

The nonhuman primate (NHP) enhanced pre- and postnatal development (ePPND) study combines endpoints from both embryo-fetal development (EFD) studies and pre- and postnatal development (PPND) studies, for which dosing is extended from the gestation period to parturition.

- Developmental immunotoxicity study endpoints may include standard parameters as listed in ICH S8. Sponsors may perform additional specialized immunohistochemistry as needed.

- Sponsors can include specialized endpoints for immunotoxicity if there is a concern to the developing immune system. The choice of functional endpoints should be scientifically justified to address potential effects on relevant immune cells at the earliest appropriate developmental time points (e.g., B and T cells, macrophages, natural killer cells). In NHPs, for example, immunophenotyping can be obtained as early as postnatal day 28, and immune system function can usually be assessed between 3 to 6 months. See ICH S5(R3) and ICH S6(R1) for information on timing, group size, and parameters to be evaluated.

- Immune function testing of offspring should address the concern of immunotoxicity to the innate or adaptive immune system (or both) as needed. Examples of immunotoxicity testing could include the TDAR assay, immunophenotyping, NK cell activity, macrophage/neutrophil function, CD3 cell proliferation, etc. Assays should be appropriate to the age and species selected.

D. Juvenile Animal Studies

If an evaluation of existing nonclinical toxicity studies indicates the potential for enhanced toxicity in pediatric patients, sponsors should consider juvenile animal studies for products being developed in some indications. This is especially true in organs and/or tissues that undergo substantial development and/or maturation after birth, such as the immune system.

- Sponsors should consider timing and duration of exposure to cover important developmental windows for the immune system as well as the intended ages of enrolled pediatric patients.

- Sponsors should base assay selection on the pharmacology of the drug or the immunotoxicity seen in adult animals. And sponsors should conduct functional assays, such as the TDAR, after appropriate times of development.