WHITE PAPER

Strengthening investigational new drug applications with a mixed lymphocyte reaction assay.

The desirable goal for every drug developer is to progress feasible drug candidates to the clinical phase, constituting a critical milestone for the significant investment of time and resources. To increase the likelihood of rapidly moving therapeutic candidates into clinical trials, regulatory bodies have provided recommendations for *in vitro* assays and to leverage high throughput screening technologies. This whitepaper discusses the requirements of regulatory bodies and how a rapid immunogenicity assessment screening can provide invaluable support for your application.

What is an Investigational New Drug filing?

The Investigational New Drug (IND) application is submitted to the United States Food and Drug Administration (FDA) by a drug manufacturer seeking permission to conduct clinical trials of a new drug or biological product in humans. It is the first step in the regulatory process that ultimately leads to the approval of a new drug for marketing in the United States. The purpose is to provide information about the drug, including its chemistry, manufacturing, controls, pharmacology and toxicology, and clinical protocols. The FDA reviews the application to ensure that the proposed clinical trials are safe and that the drug's benefits outweigh its risks. The IND process helps ensure that new drugs are rigorously tested before making them available and helps to identify potential safety issues before the drug candidate progresses to clinical trials. Filing an IND requires completing a series of forms detailing the study, providing information about the investigator, and certifying that the investigation is registered in the national database of clinical trials.[1] The stated purpose of an IND is "to ensure that subjects will not face undue risk of harm" in a clinical investigation involving drug use.[2]

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Why assessing the immunogenicity of therapeutics candidates is crucial for IND filing?

Assessing immunotoxicity in the drug development process and demonstrating safety in the IND application is crucial because the immune system is critical in protecting the body from foreign substances. Many drugs and biologics can interact with the immune system, potentially causing adverse effects or harmful immune reactions. Therefore, it is crucial to evaluate the potential immunotoxicity of a drug early during the development process to ensure its safety and efficacy.

The FDA guidance for the industry entitled "Immunotoxicology Evaluation of Investigational New Drugs" is consistent with the FDA's good practices regulation and represents the agency's current immunotoxicology evaluation.[3] In the guidance, the FDA defines Toxicology Studies as the preclinical data to assess whether the product is reasonably safe for human initial testing. Evidence of immunotoxicity usually can be observed in standard nonclinical toxicology studies, but in some cases, additional further studies are essential, including follow-ups at the clinical stage.

The human immune system is a complex set of cells and organs that drugs can adversely affect. The potential impairment of the immune system can result in increased susceptibility to infections and tumors, allergic responses to drugs, autoimmune reactions, or other forms of immune system disease. Immunotoxicology studies can be conducted in animals to determine the potential of an investigational drug to affect the immune system adversely. However, the recent Modernization Act 2.0 ends a federal mandate that experimental drugs must be tested on animals before being used in human clinical trials, boosting the importance of *in-vitro* models that can simulate the immune microenvironment.[4] The FDA guidance offers recommendations regarding the following aspects:

1. The appropriate timing for performing immunotoxicology studies.
2. The observable impacts in standard nonclinical toxicology studies that suggest a drug may possess immunotoxic properties.
3. The various study types that aid in discerning the characteristics of immunotoxicity.

In the IND application, the drug manufacturer must provide data on its potential effects on the immune system, including its ability to avoid immunosuppression or hyperactivation. This information is vital for the FDA to evaluate the drug's safety profile and decide whether to allow clinical trials to proceed. Suppose a drug is known to affect the immune system. In that case, the clinical trial may need to monitor participants' immune function more closely to ensure that any adverse effects are detected and managed appropriately.

Overall, immunotoxicity is a significant concern in drug development because it can cause adverse reactions in patients and impede the drug's development process. Drug manufacturers use various assays and evaluations to mitigate these risks. It is at this stage where compound screening *in vitro* models, such as the Mixed Lymphocyte Reaction (MLR) functional assay, can provide information on the potential of a drug to induce an immune response, measuring T-cell proliferation and cytokine production (Figure 1). The screening compounds early in the drug development allow drug manufacturers to identify and optimize medications with lower immunogenicity before proceeding to clinical trials. While in vitro assays cannot replace clinical trials, they can help reduce the risk of adverse patient effects and streamline drug development.

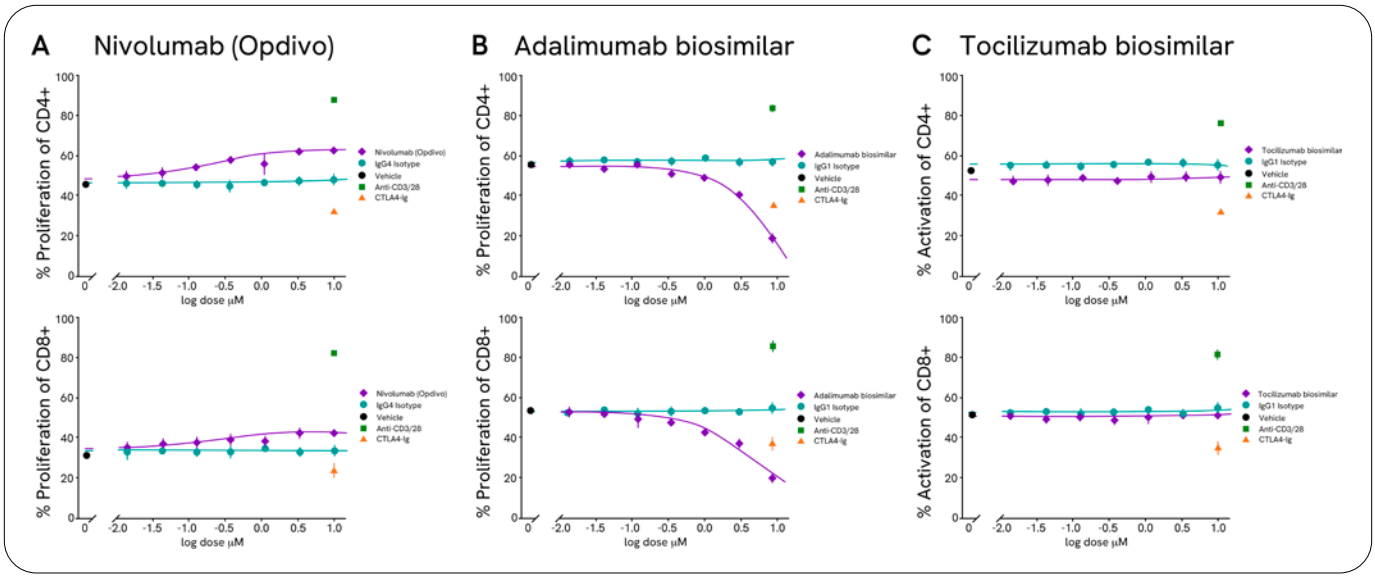

Figure 1. Representative plots of an ImmuSignatureTM MLR assay: Allogenic MoDC-challenged CD3+ T cells were co-cultured in the presence of antibody-based drugs. The proliferation of the CD4+ and CD8+ population is quantified by CellTrace Violet staining through flow cytometry. **A)** Increased proliferation of CD4+ and CD8+ T cells treated with nivolumab compared with IgG4 isotype control. Single donor. **B)** Inhibition of CD4+ and CD8+ T cell proliferation treated with adalimumab compared with IgG1 isotype control. Single donor. **C)** Percentage of CD25+ as activator marker in T cells in the presence of tocilizumab biosimilar. No increase or decrease in T cell activation was detected compared to the isotype control. Single donor. Single-dose assay controls such as anti-CD3/28 mAb and CTLA4-Ig and vehicle is also included.

Why is the MLR functional assay recommended for evaluating immunotoxicity in IND applications?

Immunogenicity is a significant concern in drug development because it can lead to adverse effects such as hypersensitivity reactions, autoimmunity, or reduced efficacy. The MLR assay can help identify, at an early stage, drugs that may have a higher risk of inducing an immune response and therefore require closer monitoring during clinical trials. The allogeneic MLR assay measures the ability of T-cells from a sample of donors to respond to and proliferate in the presence of antigens from another individual presented by antigen-presenting cells. By investigating or testing multiple aspects of the T cell function, the MLR assay comprehensively evaluates potential immunotoxicity.

For the evaluation of T cells' immune response, the MLR functional assay is a relevant model for the assessment of immune response highlighted in the Immunotoxicity Testing Guidance provided by the FDA.[5]

Advantages and limitations of the MLR functional assay for an IND filing.

The MLR functional assay is highly sensitive and can detect low levels of T cell activation, making it helpful in identifying potential immunogenicity even in cases where the response is weak. This assay can detect T cell responses to both major histocompatibility complex (MHC)-restricted and non-MHC-restricted epitopes. It measures a broad T cell response to a drug candidate, which is essential for assessing its immunogenic potential, including adverse immunostimulation, immunosuppression, or hypersensitivity, as described in the 2001 Draft Guidelines of the FDA for Immunotoxicology Evaluation of Investigational New Drugs.[6]

However, despite its advantages, the MLR functional assay has some limitations when interpreting the data. The results of the MLR functional assay can be variable due to differences in donor cells and antigen presentation, making it difficult to interpret results and establish cut-off values. However, because of the method's allogeneic basis, these variations can explain how the therapeutic candidate will behave in a broader general population. The MLR functional assay has limited specificity, and a positive response does not necessarily indicate clinical immunogenicity or adverse effects in patients.

High throughput Multiplexing Assessment for the MLR Functional Assay

The potential for high throughput multiplexing assessment to speed up the MLR functional assay and, ultimately the IND application process. Traditional methods of cytokine analysis require individual ELISA assays for each cytokine, which can be time-consuming and costly. High throughput multiplexing assessment allows for the simultaneous analysis of multiple readouts in a single sample, reducing the time and cost associated with traditional ELISA assays.

Some benefits of high throughput multiplexing assessment include increased efficiency and reduced costs minimizing the need for numerous assays. Additionally, high throughput multiplexing assessment can provide a more comprehensive evaluation of the immune response, allowing for a more accurate immunogenicity assessment.

Conclusion

In conclusion, it is imperative to thoroughly assess the immunotoxicity of therapeutic candidates for IND filings, ensuring the drug's safety and potential effects on the immune system. As per FDA recommendations, employing the MLR assay for evaluating T cell immune responses can support the initial stages of clinical applications. Incorporating high-throughput multiplexing techniques of the MLR functional assay enhances the speed, reproducibility, and data collection, expediting the entire IND application process. Furthermore, outsourcing the MLR functional assay at any stage of the pre-clinical pipeline can provide rapid and essential insights that facilitate informed decision-making in therapeutic candidate development.

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