While the immune system protects organisms from infections, it can also react adversely to certain therapeutic interventions such as biologics that target various components of the immune system. Monitoring the responsiveness of the immune system to therapeutic interventions is crucial. This process, called immune monitoring, helps determine the safety and efficacy of therapeutic agents like vaccines or biologics.

While immune monitoring involves number of techniques, employing the right technique—at the right time—is essential to accurately characterize the effectiveness of a therapeutic approach in clinical trials.

A Complex System

Our complex immune system is composed of a variety of cells characterized by unique surface markers that are either constitutively expressed or induced upon activation. In addition to surface markers, immune cells also secrete a variety of soluble mediators, such as cytokines and chemokines, that shape the outcome of the immune response. These markers and soluble mediators, which are characteristic of various immune cells, can serve as targets for drug development. When immune cells or their soluble mediators are targeted, there is a potential for an adverse reaction that needs to be monitored.

Citing the Cytokine Storm

The importance of immune monitoring can be highlighted in an example where a company called TeGenero developed an antibody named TGN1412 to treat a variety of immune conditions. This biologic worked against CD28, a protein that is expressed on T-Cells and some of its subtypes. Following the issue-free completion of in vitro and preclinical studies in monkeys, TeGenero decided to take TGN1412 into human Phase I clinical studies in a set of healthy volunteers in the UK. Using a concentration of drug determined during in vitro and preclinical studies, they found that within two hours these healthy volunteers developed a serious condition known as “cytokine storm” that led to the quick discontinuation of the trial.

Without any obvious issues in preceding in vitro and preclinical studies, why did this antibody turn out to be problematic in humans? Researchers revisited this study and asked exactly how the antibody was presented to the immune system. Through in vitro testing using aqueous and air-dried forms of the antibody, researchers found that air-dried TGN1412 is capable of inducing substantial levels of a “cytokine storm.” In addition, it was also found that the tissue distribution of CD28 is slightly different between humans and monkeys, which were used as the preclinical species. This underscores the necessity for a detailed in vitro and preclinical characterization of therapeutic molecules.
Technologies for Examining Soluble Mediators

As biologics such as antibody therapeutics and vaccines are emerging as effective means to treat cancer and inflammatory diseases, assays to determine their safety and efficacy become crucial in immune monitoring. Several platforms can support these efforts depending upon their suitability to measure soluble mediators as well as cell-based biomarkers.

▶ Multiplex Assays

Multiplex assays that can simultaneously measure several soluble biomarkers such as chemokines and cytokines have gained particular relevance in immune monitoring for many reasons. The compatibility of antibody reagents used in multiplex assays is an important factor that determines the success of these assays.

▶ Enzyme-Linked Immunospot (ELISPOT) Assay Analysis

ELISPOT is a technique that looks at single cells that specifically secrete cytokines in response to a given stimulus, such as an antigen. In addition to providing information on the number of activated cells, the intensity of the signal can correlate to the amount of cytokine(s) secreted in response to a stimulus. ELISPOT is widely used in immune monitoring to support clinical trials evaluating the safety of biologics and efficacy of vaccine candidates.

An Important Cell-Based Platform: Flow Cytometry

Flow cytometry is a widely used technique in immune monitoring with its ability to look at specific subsets of immune cells using phenotypic and functional markers. Furthermore, fluorescence-activated cell sorting (FACS) allows select subsets of PBMCs to be isolated and analyzed for their functionality.

Despite its wide applications, flow cytometry is a complex technique. Substantial amounts of whole blood or other tissue material are required to complete flow cytometry-based complex functional assays. While some flow cytometry-based assays can be performed using cryopreserved PBMCs that allow batch testing of clinical samples, some cell types have to be analyzed using whole blood samples, which poses logistical challenges to global clinical trials. Additionally, management of whole blood and cryopreserved PBMC samples requires standardized procedures implemented across global clinical trial sites to minimize variability from site to site. Even with stable, viable samples, flow cytometry also demands well-trained staff to accurately analyze and interpret data. Some of the commonly used flow cytometry-based methods in immune monitoring include:

▶ Intracellular Cytokine Staining (ICS)

ICS measures intracellular cytokine levels following in vitro or ex vivo stimulation with a mitogen or an antigen. PBMCs or whole blood are treated with a stimulant followed by an inhibitor of the Golgi complex to prevent the secretion of any cytokines. Subsequently, cells are fixed, permeabilized and stained with antibodies specific for cytokines. When ICS is combined with phenotypic markers, specific subsets of immune cells that respond to a stimulus can be analyzed using flow cytometry.

▶ Tetramer Analysis

This technique employs MHC tetramers or multimers to detect T-cells specific for a particular MHC-peptide complex. Antigenic peptides are presented to T-cells on MHC Class I or Class II molecules. The ability to synthesize soluble MHC Class I or Class II molecules, load them with antigenic peptides and use them as a tool to examine T cells that recognize a particular MHC-peptide complex has been widely used to monitor vaccine efficacy. A number of methods have been employed to increase the sensitivity of tetramer analysis.
Mass Cytometry (CyTOF)

By combining principles of flow cytometry with mass spectrometry, CyTOF (cytometry by time-of-flight) is able to avoid the spectral overlaps that are common to flow cytometry because mass spectrometry can distinguish between two elements, even those extremely close in terms of molecular weight. The technique does not require color compensation like traditional flow cytometry and the easier design of panels makes it an attractive alternative to traditional flow cytometry. However, data acquisition is slower than traditional flow cytometry with only 1,000 events per second and CyTOF requires much cleaner samples because of the sensitivity of this platform. Restrictions include a limited set of available commercial antibodies, more complex data analysis and the inability to recover cells following measurement, since they are destroyed in the process.

Assessing T-Cell Mediated Anti-Tumor Immunity and Identification of T-Cell Epitopes

Designing vaccines to target various forms of cancer is a growing area where the immune system is harnessed to fight off cancer. When a normal cell becomes tumorigenic, several cellular pathways are dysregulated. Proteins that are dysregulated in cancer cells can be targets for the immune system. Epitopes that are generated from such dysregulated proteins can be unique to cancer cells, therefore, identifying these unique epitopes is critical to designing novel peptide-based vaccines that can stimulate cancer-specific T cells.

One approach to identifying cancer-specific epitopes involves isolating MHC-peptide complexes from cancer cells and characterizing them by mass spectrometry. Once such peptides are identified, they are validated using in vitro approaches. Binding of peptides to MHC molecules can be assessed using a variety of techniques including T2 stabilization assay where stabilization of "empty" MHC molecules on the surface of T cells by is determined by flow cytometry. To determine their functionality, these T cells can be co-cultured with cancer cells or other target cells pulsed with the synthetic peptides.

If the epitope is generated within the cancer cells and presented in conjunction with MHC molecules on the surface, it will stimulate peptide-specific T cells, which can be assessed using in vitro assays such as ELISPOT or ICS. Tumor-specific reactivity can be confirmed by co-culturing T cells with normal healthy cells from various tissue types and looking at T cell activation. Once the authenticity of these peptides is confirmed, they can be evaluated for their safety and efficacy in clinical trials using assays discussed here.

Assays to Measure the Functionality of Antibody Therapeutics

Antibody-based therapeutics bring about their effector activities through a number of mechanisms. Two activities that are routinely assessed are: (i) Complement Dependent Cytotoxicity (CDC), where antibodies recruit complement proteins after binding to their target resulting in lysis of target cells, and (ii) Antibody Dependent Cellular Cytotoxicity (ADCC), where Natural Killer (NK) cells bind to the Fc region of an antibody, which is bound to its cellular target, and mediate target cell lysis. Cell-based in vitro assays with a variety of radioactive and nonradioactive readouts can measure CDC and ADCC activities mediated by complement proteins and NK cells from patients after treatment with antibody therapeutics.
The Crucial Role of Immune Monitoring in Clinical Trials

When administering a therapeutic agent like a biologic in a patient, we need a set of reliable immune monitoring tools to ensure that the therapeutic agent is safe and efficacious. While several assays can be used for immune monitoring, the ultimate choice depends on the suitability of the assay to answer specific questions asked in the unique therapeutic approach.

References

Learn more about our vaccine testing solutions at www.covance.com/vaccines

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The Americas + 1.888.COVA NCE + 1.609.452.4440
Europe / Africa + 00.800.2682.2682
Asia Pacific + 800.6568.3000
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