IMPROVE YOUR PRECLINICAL PROSPECTS

Using Flow Cytometry in Preclinical Development

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How do you find the next great treatment or medication? Many new pharmaceutical drugs and biologics in development or undergoing FDA approval harness the power of the immune system. These therapies treat cancer, autoimmune diseases, and infections, and identification of high quality drug candidates and an understanding of their mechanism of action (MOA) require using techniques that reveal the inner workings of the immune system.

Flow cytometry is an unparalleled technique for preclinical development. It provides researchers with a broad, yet detailed insight into the immune system. Flow cytometry can be completed on a large scale, which gives scientists the power to screen dozens of potential candidates for their effectiveness.

What can you measure with flow cytometry? Many preclinical studies are completed in vitro with peripheral blood cells, and analysis of these samples using flow cytometry gives researchers a snapshot of how different immune cells react to stimulation. More importantly, flow cytometry analysis may be the first way to check if cells of interest are behaving appropriately. Flow cytometry is also a powerful tool for unraveling the mechanism of action of an experimental biologic or drug in development. Cell sorting is similar to flow cytometry and is very useful in preclinical research because different cell types can be sorted into pure populations. These aseptically purified samples can be analyzed through downstream functional assays or used in other applications entirely.

Flow cytometry offers scientists the ability to customize assays to their preclinical needs, and many of these assays can be developed and validated for analysis of clinical trial samples. This white paper illustrates

the power of flow cytometry in preclinical development and contemplates the use of these techniques to yield promising therapeutic candidates

Stimulating Observations

How does a cell react to a drug or biologic candidate? Flow cytometry stimulation assays allow researchers to observe changes in the way cells react to an experimental stimulus. In vitro stimulation of cells followed by flow cytometry analysis enables scientists to concurrently measure a wide array of biomarkers in multiple cell populations that change in response to a stimulus like a biologic or drug. These changes can be monitored over the course of different time points, and the effects of different concentrations of stimulus can also be observed.

Cells of the immune system produce many unique molecules that can cause inflammation, destroy pathogens, or steer immune cells toward damaged tissue. These molecules, including cytokines, chemokines, and antibodies, can serve as biomarkers in flow cytometry assays. Analysis of the biomarkers produced by different immune cells during stimulation can indicate if an experimental therapy induces a favorable result, such as eliciting a potent anti-tumor response or quelling destructive pro-inflammatory responses associated with an autoimmune disease. Alternatively, flow cytometry stimulation assays can show if an experimental drug or biologic does not adequately activate the appropriate cells or triggers a destructive and potentially harmful reaction

Flow cytometry cell stimulation assays can be developed in different ways. One approach uses the therapeutic biologic or drug to stimulate immune cells in vitro and measure how this stimulation activates different biomarkers. Alternatively, an activation assay can start by treating cells with a drug or biologic and then stimulating cells with a mitogen, which is a type of molecule that stimulates immune cells non-specifically. This type of assay can tell scientists if a biologic or drug broadly alters or skews the function of different immune cells and can reveal shifts in the immune response that may be too subtle to be otherwise detected.

Scientists gain valuable insights into how drugs and biologics alter immune responses by using flow cytometry stimulation assays. These results are extremely valuable for identifying strong candidates during preclinical development and for beginning to understand how these experimental molecules function.

Achieving Activation

Many immune system cells are in a state of restful surveillance in a healthy body. They are searching for indications of foreign pathogens or abnormal cells, and these signals trigger certain immune cell types to be activated. This activation state transforms certain immune cells, like natural killer cells or macrophages, into cells that can destroy pathogens or tumor cells and can alert other types of immune cells to respond. Flow cytometry activation assays are powerful tools for measuring if an experimental drug or biologic exerts any activation effects on different immune cell types. These assays are similar to in vitro stimulation assays but will measure markers of activation, which may include changes in the overall size and shape of a given cell type or differences in the expression of surface markers or intracellular proteins.

Flow cytometry activation assays are a powerful tool for studying certain immune cell subsets, and activation of these cells by experimental drugs or biologics can be an indication of potential antimicrobial or antitumor activity.

Purity Power

Cell sorting is a technique that allows users to sort cells into discrete populations. It works by the same principles as flow cytometry. Cells can be stained with any custom combination of fluorescent dyes and then isolated into a relatively pure population. This technique is particularly powerful for preclinical research because many new therapies, especially for cancer, use purified immune cells like dendritic cells or T cells as forms of treatment. Researchers can study purified cell populations in vitro to identify potential cell therapy candidates and can also study these cells in animal models to better understand underlying immunological mechanisms.

Sorted cells are rarely 100% pure, however preclinical studies can help establish cell sorting protocols that isolate a cell population to a level that is both functional and practical for downstream clinical applications. Many applications of purified cells still function well with a less than perfectly pure cell population, and the preclinical phase of research is the best time to "sort" out your sorting parameters and purity needs.

How Does It Work? - Flow Cytometry and MOA

The path from bench to bedside for a drug or biologic requires that researchers understand how the candidate molecule works. Flow cytometry is indispensable for working out how these molecules work through mechanisms of action (MOA) assays.

Many biologics use some sort of therapeutic antibody or antibody conjugate that bind to target cells to exert their effects. But antibody-based therapies can work by different MOAs. Flow cytometry can be used to define the MOA, especially for well-defined antibody-mediated mechanisms including antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent cell-mediated phagocytosis (ADCP), complement-dependent cytotoxicity (CDC), and trogocytosis. ADCC is an MOA by which antibodies bind to a target cell and serve as markers for cytotoxic cells like T cells and natural killer cells to recognize and destroy via cytotoxic mechanisms. ADCP is a similar MOA that involves antibodies binding to target cells, after which cells like macrophages recognize the antibody-coated targets and destroy them by phagocytosis. CDC is another antibody-driven MOA by which molecules of the complement system bind to antibody-coated targets and mediate destruction. Trogocytosis is a different type of MOA by which T cells, B cells or NK cells bind to antigen-presenting cells and extract and express surface molecules to their own cell membrane.

Flow cytometry can be used to efficiently and precisely define these different MOAs and can also be used to develop new protocols for other novel MOAs, thus affirming once again the power of flow cytometry in clinical development.

Flow Cytometry's Preclinical Power

Flow cytometry is an unrivaled tool for preclinical development of drugs and biologics, especially in the areas of inflammation, autoimmunity, cancer, and infectious disease. No other tools gives researchers access to so many parameters of the immune system, and many assays used in preclinical development can be adapted to clinical studies.

Flow cytometry is instrumental in identifying strong biologic or drug candidates, gauging their safety and efficacy, and defining their MOA. The growing class of immunology-based drugs affirms that flow cytometry will be a powerful preclinical development tool for years to come.

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Quantifying Biological Response Through Cytometry