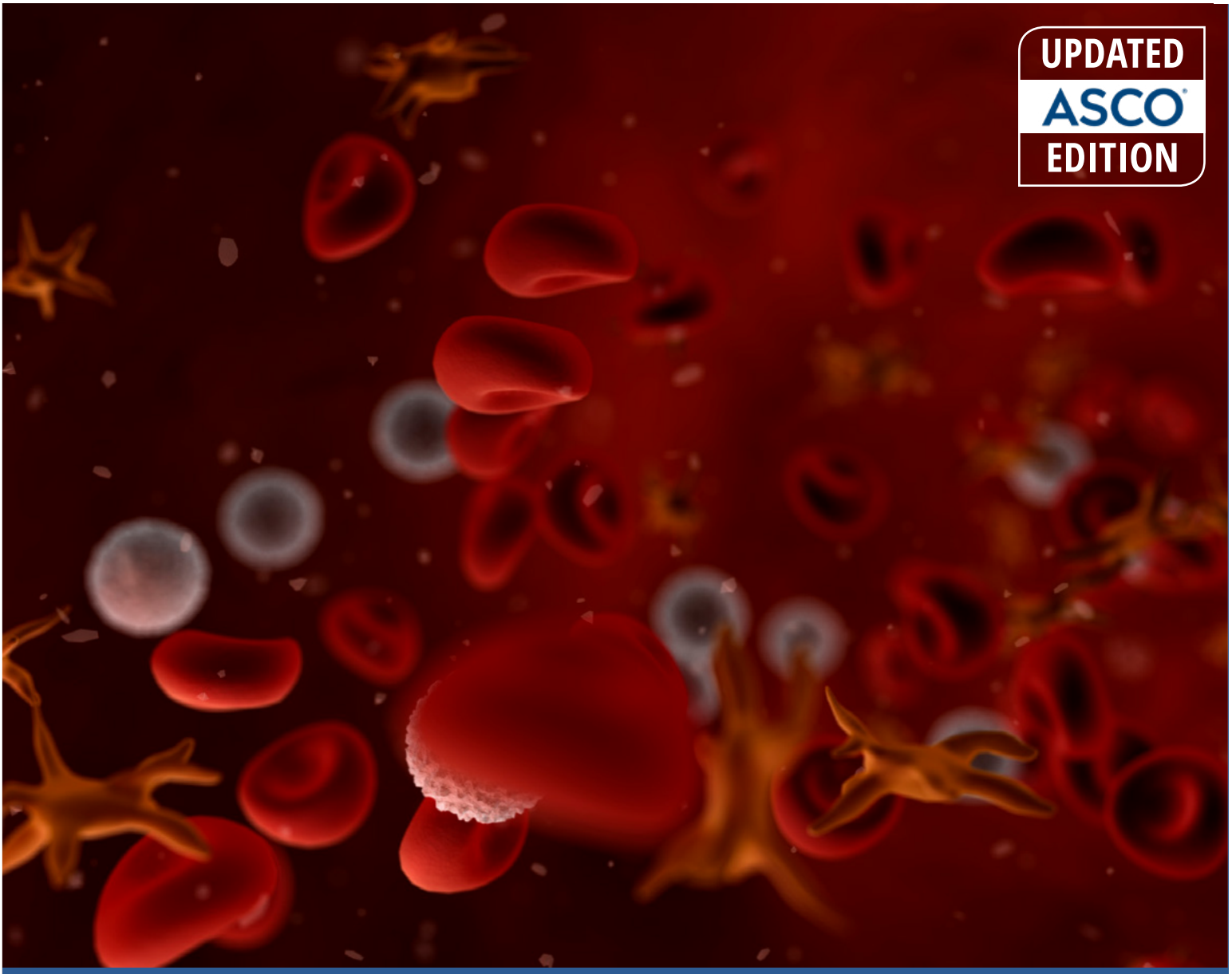


FLOW CYTOMETRY FOR CLINICAL TRIALS

Assay Development, Validation, Harmonization, and Clinical Data Acquisition

Brought to you by: FlowMetric™

Many novel drug and biologic therapies under development target aspects of the immune system, and this trend has made flow cytometry an essential tool for monitoring immune responses during clinical trials. Flow cytometry can measure multiple cellular characteristics simultaneously, which makes it an "assay of choice" for profiling cell phenotypes or measuring production of immune molecules, and it can also be used to characterize signature biomarkers in a variety of situations.

Unlike basic research, flow cytometry assays for clinical trials must fulfill regulatory requirements including instrument and method validation. Most assays used in clinical settings are quasi-quantitative, although in some situations, truly quantitative measures can be made. Many aspects of a flow cytometry assay must be considered, such as how samples will be collected, transported, or stored, what the composition of a fluorescent antibody panel will be, and how data will be analyzed across multiple patients and over the course of the trial.[1]

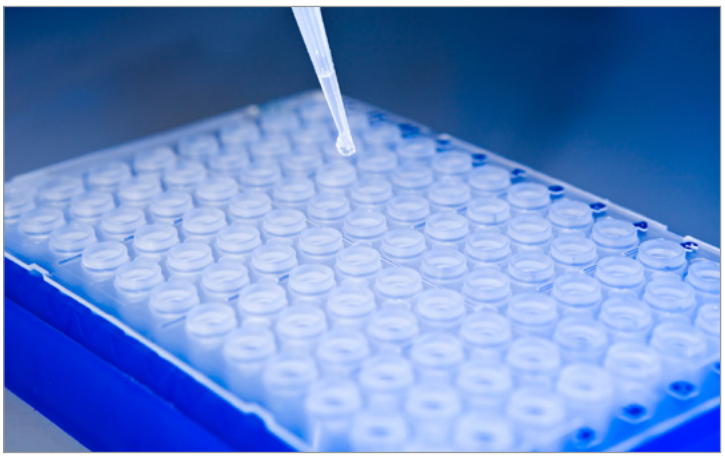
If you are considering using a flow cytometry assay for your next pre-clinical research project or clinical trial, consider consulting with an expert in clinical flow cytometry as you plan your project. This white paper gives an overview of points to consider when implementing a clinical trial flow cytometry assay from project inception to data collection

[1] *Maecker HT, McCoy JP Jr, and the FOCIS Human Immunophenotyping Consortium. A model for harmonizing flow cytometry in clinical trials. Nat Immunol. 2010. 11(11):975-8.*

Planning and Pilot Experiments

High quality data for clinical trials can be obtained consistently using flow cytometry, but none of these data are usable if the study is improperly designed. Flow cytometry allows you to examine immune cell subsets by staining them with fluorescently-conjugated antibodies that bind to specific proteins on the cell surface or within the cell.

Dozens of unique immune cells have been characterized and can be measured by flow cytometry, including multiple types of T cells and B cells, as well as myeloid cells such as monocytes and dendritic cells. These different cell types can all be detected in the blood but are found at varying frequencies such that some cells are extremely rare in the circulation. It is critical to know the expected frequencies of your cells of interest as a trial flow cytometry assay is planned. If these cells are being measured from blood samples, this information will be critical for determining the amount of blood needed.



Pilot assays may be needed to determine the frequency of cell subsets under experimental conditions. Different types of blood collection tubes may also need to be evaluated in order to ascertain the best type of tube and cell handling processes for the optimal cell subset yield. Experts in developing flow cytometry assays, such as a specialized flow cytometry Contract Research Organizations (CROs), can serve as valuable guides during this early phase.

Sample Storage

Flow cytometry analysis can be undermined before cells are even stained, if your samples are not stored under appropriate conditions. Incorrect storage can result in all the cells being dead and essentially unsuitable for flow cytometry analysis. Some types of immune cells can only be measured from fresh samples, whereas other samples can be frozen under specific cryopreservation conditions and stained at a later time for flow cytometry. Pilot experiments and discussion with experts are essential to determining the optimal storage conditions for samples set aside for clinical trial flow cytometry experiments.

The Flow Cytometer

The flow cytometer is the instrument at the heart of flow cytometry analysis. Lasers directed at the flow stream excite fluorochrome-conjugated antibodies bound to the cells at specific wavelengths. This excitation causes electrons from the fluorescent molecules to move to a higher energy state briefly then return to its ground state whereby it emits photons at a higher wavelength. Photons pass through filters so as to be detected at a specific wavelength by a sensor called a photomultiplier tube (PMT), which converts this signal into voltage that is reported as an event. Computer software is used to analyze the events and represent them as data measuring different cell parameters including size, shape, granularity, and frequency of different antibody-labeled cells. Dozens of flow cytometers exist with different lasers and emission detectors that allow use of multiple antibodies that span the visible and ultraviolet light spectra. Each cytometer has a unique configuration of excitation and emission parameters, and this must be considered when selecting antibodies for a staining panel.

Flow cytometers used for clinical trial studies are required to undergo regular instrument validation to assure that the cytometer is reliable and precise. Instrument validation is also critical if multiple instruments are being used in different sites for a multi-site clinical trial to assure standardization in data acquisition parameters.

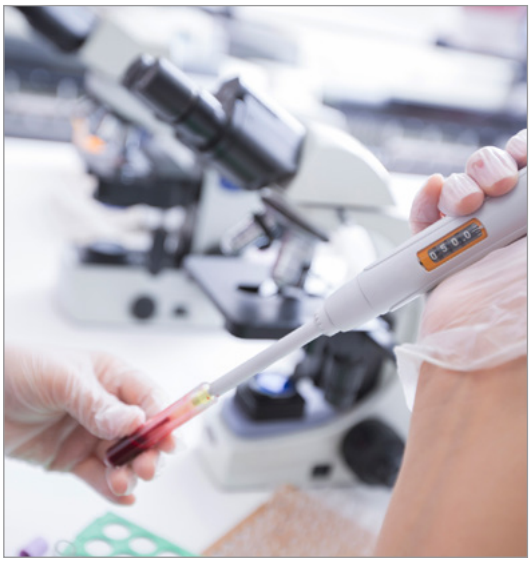
Assay Design

Clinical flow cytometry assays may be run several hundred times during the course of a multi-year study, so assay design is critical during the planning phase. The bulk of hands-on assay work typically includes isolating cells from a given sample, stimulating cells with specific molecules, and staining them with fluorescent antibodies. Many variations of protocols exist for flow cytometry that measure intracellular cytokines, cell surface marker expression, receptor occupancy, and cell cycle analysis, however, these protocols must be customized and validated for a clinical flow cytometry assay.

Elements of assay design include determining if samples will be processed and run in tubes or 96-well plates, how many samples can be run in a single experiment, and what reagents are needed, including specific proteins or cell stimulation molecules. Specific cell stimulation reagents may need to come from a single batch lot in order to ensure minimal lot-to-lot variability over the length of the clinical trial. Other logistical issues should be considered, including the feasibility of overnight incubations, and whether or not cells should be treated with a cell fixative.

Panel Design

The antibody panel for cell staining is one of the most fundamental parts of a clinical trial flow cytometry experiment. Multiple fluorescent antibodies can be used in a single assay, and the use of an antibody panel is a key advantage of flow cytometry because it facilitates the identification of multiple cell subsets within a single sample.



Each flow cytometer has unique technical specifications, and the optics system dictates which fluorescent wavelengths can be excited and detected by the machine. It is critical to know the optical parameters of the flow cytometer being used for a clinical flow assay as you design an antibody panel, in order to assure that the fluorochromes can be detected by the cytometer.

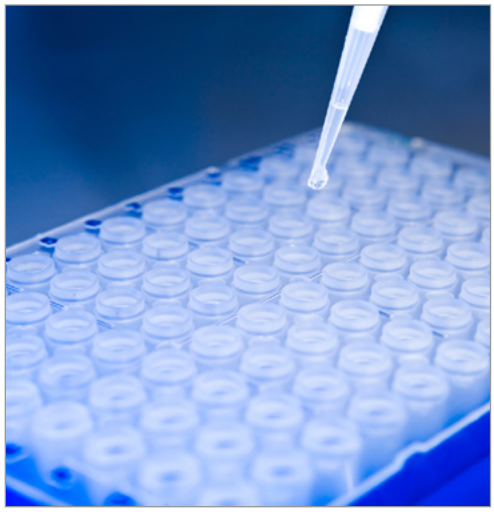
Selection of fluorochromes is dependent on the density and stability of the marker it is detecting. Some fluorochromes are “brighter” and are better suited for detecting biomarkers on cells that may be less abundant. Pilot experiments are essential to selecting the best fluorescent antibodies for a staining panel, and optimization experiments should include antibody titrations to assure that the correct concentration of staining antibody is used to avoid off-target staining with too much antibody or weak signal with too little antibody.

Validation, Standardization, and Harmonization

For clinical trial flow cytometry assays, validation follows the assay design and development phases. The FDA considers flow cytometry to be an analytical

method, and as such, must meet specific criteria for analytical methods. Validation verifies that an assay is appropriate for its intended use and can meet specific criteria for stability, accuracy, precision, specificity, detection limit, limit of quantitation, linearity and range, ruggedness, and robustness.

Validation assays and assays performed with clinical trial samples and must be done under rigorous GLP/GCP (good laboratory/clinical practice) conditions in order to satisfy regulatory requirements. CROs that specialize in validation or clinical trial flow cytometry can be critical partners for clinical researchers unfamiliar with validation protocols or in need of GLP/GCP-compliant laboratory conditions.



Following validation, a standardized assay can be developed in which users are trained to follow a precise protocol using specific reagents. Standardized protocols work well for clinical trials that depend on a single site for flow cytometry assays. Alternatively, multi-site trials can use assay harmonization to assure quality of data and enable comparison of data sets from multiple sites.[2] Harmonization involves coordinating protocols from individual laboratories with standard operating procedures and assay performance benchmarks, and using proficiency panels as a way to identify and control for inter-laboratory variables.

Consider the scope of your clinical trial to determine how and where your validated assay will be used in order to assure the quality and reliability of your data.

[2] *van der Burg SH, Kalos M, Gouttefangeas C, Janetzki S, Ottensmeier C, Welters MJ, Romero P, Britten CM, Hoos A. Harmonization of immune biomarker assays for clinical studies. 2011. Sci Transl Med. 3(108):108ps44.*

Therapeutic Areas

Immune-based therapeutics are the driving force behind many cancer therapies that have been approved recently or are under development. Many of these therapies are monoclonal antibodies that target specific immune molecules and harness the power of an individual's immune system to destroy tumor cells. These immune-modulating therapies require monitoring by flow cytometry in order to assure that the immune response is effective at targeting tumor without causing excessive collateral damage. As such flow cytometry assays are essential to clinical trials as well as monitoring disease in patients under treatment. This requires a close relationship with the laboratory performing the trial cytometry and that performing the standard clinical evaluation in a hospital-based lab. Both pieces of information are often required.

Clinically-focused flow cytometry is also critical to other major fields of clinical immunology research including autoimmunity, immunodeficiencies, infectious diseases, and transplantation. Better and more precise therapeutic options are needed in all of these fields to improve patient outcomes and reduce side effects of currently available treatment options.

Clinically-focused flow cytometry is becoming an indispensable tool for clinical research across biomedicine. Partnering with clinical flow cytometry experts is critical to assuring that your flow cytometry data is valid, reliable, and can be used to make informed decisions about candidate therapies.

With the spotlight on immuno-oncology, over 5,600 active drugs in development and a total of 6,500 trials in progress, flow cytometry is poised as a very versatile tool in aiding in the advance of these studies bringing cutting-edge therapies to patients in need. Current research includes adoptive cell transfer of genetically engineered T cells that express a chimeric antigen receptor (CAR) that specifically recognizes a tumor antigen.

These CAR T cells have been associated with anti-tumor activity in patients with certain types of leukemia13 and lymphoma14 and may become a standard treatment in the future. Flow cytometry assays are critical to these studies at all phases of development. FlowMetric has implemented a scientific approach that helps drive successful immune-oncology clinical trial studies. This approach utilizes multiple assays that can look at specific mechanisms of action in the areas of T Cell Exhaustion or the role of checkpoint inhibitors utilizing methods that incorporate cell surface, intracellular immunophenotyping as well receptor occupancy assays to determine specific mechanisms of action for targeted therapeutics. These approaches can help to shed light on a better understanding of a patient's immune profile and status in response to tumor treatment.

Immuno-oncology is changing the nature of cancer treatment and new therapies will be coming to market in the coming years. Flow cytometry is a powerful and versatile tool for IO research and development and will be essential to the advancement of future therapies.

FlowMetric™

Quantifying Biological Response Through Cytometry