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FLOW CYTOMETRY: ITS ROLE IN CLINICAL TRIAL BIOMARKERS: Q&A WITH MEDPACE SCIENTISTS

What are the reasons a pharma/biotech sponsor would need to use flow cytometry in a clinical trial?

The human immune system is a complex, comprehensive defense system. While the system's intensity and scope are fine-tuned at the "sweet spot" where pathogen clearance and autoimmunity are balanced, the functionality of this system is based on interactions at the cellular level. Therefore, scrutinizing the immune system at the level of a single cell is a valuable approach when it comes to human disease clinical trials.

While there are many ways to assess a cell's property and function, flow cytometry can readily handle tens of thousands of cells and simultaneously address surface and intracellular marker expressions for up to 10-20 molecules.

Several ways that modern flow cytometry can uniquely aid clinical trials' different endpoints include:

1. Facilitating the PK effect of therapeutic cells, such as CAR-T and CAR-B therapies
2. Studying the body's response to a drug by scrutinizing surface expression or intracellular functions on or in targeted cells
3. Enumerating and characterizing immune cells rapidly to evaluate the general function of our immune system, with a typical turnaround time of 24h to 48h post receipt
4. Providing informative data for drug efficacy and safety evaluation through reliable measurement of receptor occupancy (RO)

In clinical trials involving engraftment and minimal residual disease (MRD), flow cytometry has gained popularity due to its protein-level analysis and multi-dimensional capacity with a quick turnaround time. As a single-cell-based technique, flow cytometry allows for the analysis of expression networks, differentiation hierarchies, and interactions between molecules and cell types using Boolean gating and association analysis.

BD FACSLyric, located at Medpace Central Laboratory in Cincinnati

How can flow cytometry testing be employed as a biomarker tool to benefit a clinical trial?

Modern flow cytometry can tackle 10-40 biomarkers within a single cell simultaneously without technical barriers. Typical data from a single subject contains 0.5-1 million events, which provide sufficient functional sensitivity (LLOQ, linearity) for a given subpopulation of leukocyte, including rare populations such as CD34+ progenitors and memory effector pathogenic T cells.

Flow cytometry has unique value in identifying and characterizing rare tumor cells in circulation and in the affected tissue or organ. For engraftment evaluation, flow cytometry stands out as the fastest and perhaps easiest method to assess successful engraftment at different time points post-transplant.

Cross-comparison to prior treatment time points is easy, especially with the aid of quantification beads. When drugs work on a target organ that is protected from routine assessment (e.g., brain tissues protected by the blood–brain barrier), flow cytometry can assess the same biomarker expressed in circulating cells, serving as a peripheral surrogate biomarker for PK/PD studies and *in vivo* efficacy monitoring.

How would flow cytometry be used in PK and CAR-T cell therapy studies?

Within the past decade, chimeric antigen receptor (CAR) T cell therapy has become the most prominent technology in the onco-hematology field. In this regimen, autologous T cells are obtained from the patient's blood and genetically engineered to express a surface receptor that binds to its specific target, which is often a surface CD marker on malignant blood tumor cells (e.g., B-cell blasts in lymphocyte leukemia). Following this targeted ligation, the CAR-T cell will destroy the target tumor cells with its natural onboard weaponry. To date, several CAR-T and CAR-B therapies have been approved by the FDA and can be extended to the management of non-Hodgkin lymphomas.

With molecular biology technology advancing quickly, CAR-T technology represents an innovative therapy for blood tumors and is becoming increasingly available for other types of cancers. As an *in vivo* therapeutic agent, CAR-T cells are subjected to standard PK and PD studies during their first-in-human trials (Phase I). For the PK arm of a study, flow cytometry is one of the few methods capable of tracking the turnover and bioavailability of CAR-T cells in tissue and in circulation. For the PD arm, flow cytometry can readily assess the clinical efficacy of CAR-T therapy by quantitative enumeration and immunotyping on target cells at the protein level because many of these therapies were designed against malignant leukocytes (e.g., B-cell blasts).

How can flow cytometry aid a humanized antibody therapy study ("biologics" therapy)?

Biological products are potential therapeutics that are produced by a living organism. Examples include antibodies, recombinant proteins, vaccines, serum/blood components, tissue, or somatic cells. In immune therapies, biologics often refer to recombinant humanized antibodies against a known and well-studied epitope expressed on targeted pathogenic cells. Biologics are typically administered into circulation to reach the specific target where their functions are needed, making them an effective immunotherapy.

Unlike traditional drugs that are chemically synthesized with known structure, most biologics possess complex secondary and tertiary structures that are not easily identifiable or chronologically characterized by HPLC-MS methods. Fortunately, generating an "anti-idiotype" antibody that recognizes the therapeutic antibody is a standard practice in the immunology field. Some are designed to bind directly to the antibody, while others target the antibody-epitope complex. In either case, these idiotype-specific antibodies can be fluorescently conjugated, allowing them to be employed to track receptor occupancy and target efficacy at different time points once the biologic is infused into the body.

With tissue dissociation, the technique can be employed to assess the PD of the treatment antibody in the tissue (e.g., solid tumor or lung capillary vessel endothelium). If a single cell suspension is available, flow cytometry can assess all these parameters at the single cell expression level. These features can generate key data supporting a successful Phase I trial and provide dosage information for the subsequent Phase II.

What is the relationship between modern immunology and flow cytometry?

Flow cytometry is a "single cell" analysis with multi-dimensional data from each cell recorded and analyzed. Likewise, our immune system functions with a single leukocyte as the basic bioactive unit.

Immune cells are well differentiated cells with a myriad of terminal functions associated with their intracellular and surface expression. There are at least 20 types of immune cells with distinct but well-orchestrated functionality, which can be mechanistically investigated by interrogating their surface "CD" marker expression and intracellular cytokine/chemokine expressions (e.g., interleukins, chemokines). Studying these biomarkers is important to understand how the immune system works as well as how efficiently it functions given the relationship between disease onset and investigational therapeutics.

The immune system does not function by itself. Often, lymphocytes communicate with non-immune cells bidirectionally through cell surface receptors to maximize the body's defense.

Therefore, comprehending the immune system's biomarker profile can also shed light on the cross talk between the immune (hematopoietic) system and the non-hematopoietic system. In clinical trials (both Phase I and II), this cellular insight could be essential in comprehending how an immune-modulating drug works at cellular and molecular levels.

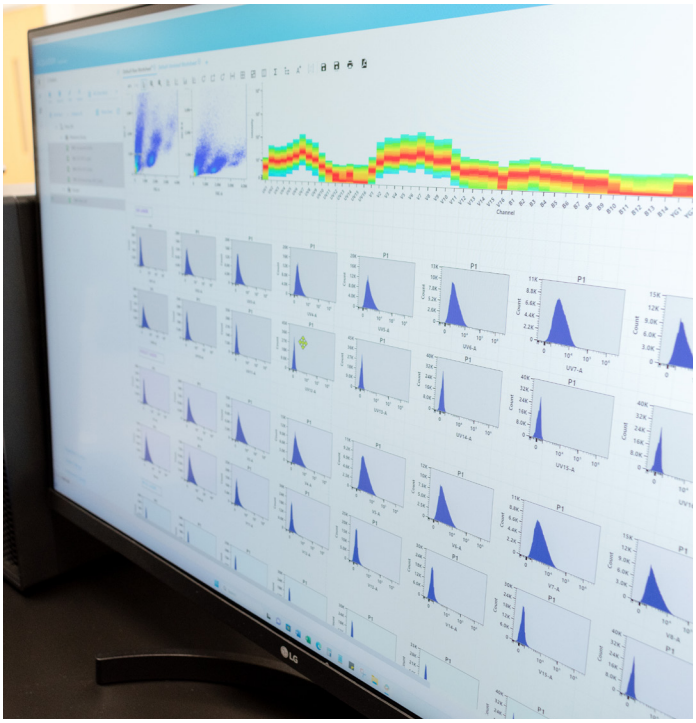
Finally, flow cytometry can readily quantify target effects of therapeutics ranging from small molecules (e.g., receptor activation inhibitor compounds) to biologics (e.g., therapeutic antibodies), rendering receptor activation assessment and occupancy measurements more straightforward and cost effective than other methodologies. Receptor occupancy is becoming increasingly important in the development of biological-based therapeutic agents. Flow cytometry receptor assays can qualitatively and/or quantitatively assess the binding of a therapeutic agent to its cell surface target.

How do you view the field trends of flow cytometry development in the next decade, for both IVD and RUO assays?

So far, the flow cytometry IVD market does not utilize a panel of more than 6 colors (e.g., the 6-color "TBNK" blood lymphocyte enumeration assay) because the FDA currently defines >6 colors as RUO. Of note, Becton Dickinson's FDA 510(k) cleared flow cytometer BD FACSLyric™ has a polychromatic channel capacity of >10 colors. As the clinical field demands more complex flow assays, some FDA-cleared cytometers are being upgraded to 10 to 12 colors while retaining their IVD status.

However, more color channels also mean more complex validation processes and challenges to find routine quality controls (QCs) for each marker of interest. Eventually, the application of technical advance is solely dependent on clinical need or requirements from physicians and diagnostic labs.

For RUO assays, the field is advancing quickly. When combined with the advances in molecular biology (particularly those involving next generation sequencing), flow cytometry can offer powerful tools for novel discoveries. Collaboration between clinical flow cytometry and a molecular diagnostic lab is exemplified by surface-marker-based sorting. Flow cytometry offers the unique capacity to isolate specific cell subsets rapidly and with great purity, which can then be processed using molecular techniques. Multiple leading flow cytometer manufacturers offer 4-5 laser equipped, >20 color capacity flow cytometers. While having 20 colors in a reaction tube may generate its own difficulties, some experiments demand this many colors and >15 color are becoming increasingly popular, which may become the research standard in 2-3 years.

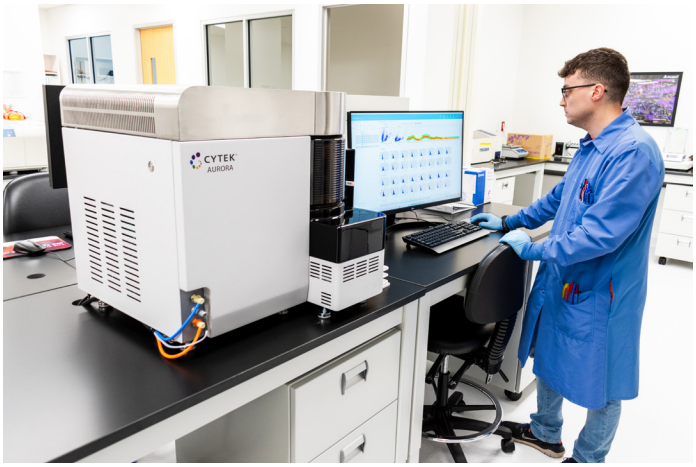


What are some of the advancements in flow cytometry?

Spectral cytometry has emerged as the comprehensive solution to the limitations of multiparametric flow cytometry. From an immunological viewpoint, the number of desired phenotypic markers can range from less than 5 individual subsets to more than 30 in a single assay. With the advent of spectral cytometry, population resolution is an ever-increasing target and is likely to reach over 100 within the next 5 years. In addition, due to the combined merits of flow cytometry and fluorescence microscopy, imaging flow cytometry has become an established tool for cell analysis in diverse biomedical fields. Capabilities that are not possible with traditional flow cytometry are addressed by imaging flow cytometry. It provides quantitative image data of every event (e.g., cells, cell clusters, debris), allowing the morphometric characterization of single cells in large heterogeneous populations and further advancing our understanding of cellular heterogeneity.

FLOW CYTOMETRY AT MEDPACE

Medpace has an experienced flow cytometry team overseen by PhD-level scientists with over 50 years of team cumulative experience designing, analyzing, and interpreting multicolor flow cytometry assays. In addition to those in-house validated, ready-to-go panels (e.g., TBNK, T-Cell, B Cell, NK Cell, Dendritic Cell, Monocyte, Stem/Progenitor Panel, and PNH), we also offer study-specific fully customized panels with capacity for more than 20 colors. Whether a study requires the development of a custom panel or the transfer of a method, we have the experience to quickly validate and implement flow cytometry testing. Medpace has harmonized global flow cytometry capabilities at the US, Belgium, and Singapore laboratories.



Cytek Aurora, located at Medpace Cental Laboratory in Cincinnati

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