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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 need to use flow cytometry in a clinical trial?

The human immune system is a complex comprehensive defense system. While the intensity and scope of the system is fine-tuned at the right "sweet spot" where pathogen clearance and autoimmunity are well balanced, the functionality of this system is based on interactions at cellular level. Therefore, scrutinizing the immune system at single cell level is an approach that cannot be bypassed when it comes to human disease clinical trials.

While there are many ways to assess a cell's property and function, there is no methodology, other than flow cytometry, that can readily handle tens of thousands of cells, while simultaneously addressing surface and intracellular expressions for up to 10-20 molecules.

There are several ways that modern flow cytometry can uniquely aid clinical trials' different endpoints:

1. Facilitate the PK effect of therapeutic cells, such as CAR-T and CAR-B therapy.
2. Study the body's response to investigating drug by scrutinizing surface expression or intracellular functions on/in targeted cells.
3. Enumerate and characterize the immune cells in a rapid fashion to evaluate the general function of our immune system, with a typical turnaround time.
4. Provide informative data for drug efficacy and safety evaluation through reliable measurement of receptor occupancy (RO).

In some clinical trials involving engraftment and minimal residue disease (MRD), flow cytometry has become a popular trend of the field due to its protein level analysis and multi-dimensional capacity with a quick turnaround time. Since flow cytometry is a single-cell-based analysis, the expression network, differentiation hierarchy, and interaction among different molecules and cell types, can be analyzed by 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 any technical barrier. Typical data format from a single subject contains 0.5-2 million events, resulting in sufficient functional sensitivity (LLOQ, linearity) for a given sub population of leukocyte, even for those rare populations such as CD34+ progenitors and memory effector pathogenic T cells.

Flow cytometry has a unique value in identifying and characterizing rare tumor cells in circulation and in the affected tissue/organ. In the case of engraftment evaluation, flow cytometry stands out as the fastest and perhaps easiest method to assess the percentage of successful engraft at different time points, by a readily quantifiable assay at protein level.

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

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

Chimeric antigen receptor (CAR) T-cell therapy becomes the most well-known technology in onco-hematology field gaining huge amount of popularity in the past decade. In this regimen, autologous T cells are obtained from blood and are genetically engineered to express a surface receptor binding to its specific target, usually a surface CD marker on malignant blood tumor cells (E.g., B blast in lymphocyte leukemia). Following this targeted ligation, the T cell will destroy the target tumor cells with it is nature onboard weaponry. To date, several CAR-T/B therapies have been approved by FDA and can be extended to the management of non-Hodgkin lymphomas.

With the molecular biology technology advancing fast, CAR-T technology represents an innovative therapy for blood tumor and becomes increasingly available to other type 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 a PK arm of a study, flow cytometry is almost the only method able to track the turnover and bioavailability of CAR-T cells in the tissue and circulation. For a PD arm, since many of these CAR-T therapies were designed against malignant leukocytes (e.g., B cell blast), flow cytometry can readily assess the clinical efficacy of CAR-T therapy by quantitative enumeration and immunotyping on target cells at protein level.

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

Biological products are potential therapeutics that is produced by a living organism. Examples include antibodies, recombinant proteins, vaccines, serum/blood components, tissue, or somatic cells. Often, when it comes to immune therapies, biologics typically refer to recombinant humanized antibodies against a known and well-studied epitope expressed on targeted pathogenic cells. As an innovative yet effective trend in the immunotherapy field, biologics are typically administrated into the circulation and can reach to the organ where their functions are needed.

In contrast to a traditional drug that are chemically synthesized with known structure, most biologics possess complex secondary and tertiary structures that are not as easily identifiable or chronologically characterized by HPLC-MS method. Fortunately, it is a standard technology in the immunology field to generate an "anti-idiotype" antibody that recognizes the treatment antibody. Some are designed to bind to the antibody itself; others are designed to bind to the antibody-epitope complex. Either way, this idiotype specific antibody can be fluorescently conjugated; hence, it can be employed to track the 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 above-mentioned parameters at single cell expression level. As one can imagine, these features would generate key data supporting a successful Phase I trial providing dosage information to the subsequent Phase II. Moreover, this approach will substantiate to understand the molecular mechanism regarding how the biologics works at the borderline of the immune non-immune systems crosstalk.

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 single leukocyte as the basic bioactive unit. Therefore, immunology and flow cytometry are always like "peas-and-carrots combo" coupled together in most studies.

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 quite distinct but well-orchestrated functionality, which can be mechanistically investigated by interrogating their surface "CD" marker expression and intracellular

cytokine/chemokine expressions (e.g., the interleukins, chemokines). Studying these immunocyte biomarkers are important in understanding how immune system works, and how efficient it functions considering the relationship between the disease onset and investigational therapeutics.

The immune system does not function by itself. Often, immunocytes communicate with non-immune cells bidirectionally to maximize the body defense via their surface receptor profiles. 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 a clinical trial (both Phase I and II), this cellular insight could be an essential approach to comprehend how an immune-modulating drug would work at cellular and molecular levels.

Finally, flow cytometry can readily quantify target effects of a therapeutic, either as a small molecule (e.g., receptor activation inhibitor compound) or as a biologic (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 development of biological based therapeutic agents. The qualitative and/or quantitative assessment of the binding of a therapeutic agent to its cell surface target can be expressed by flow cytometry receptor occupancy assays.

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

Thus far, the flow 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, two of the BD's FDA 510(k) cleared flow cytometers (CANTO and LYRIC) have polychromatic channel capacity >10 colors. Therefore, with more complicated flow assays needed by the clinical field, this may change soon with some FDA-cleared cytometers readily upgraded to 10- 12 colors potentially still maintaining the IVD status.

However, one needs to keep in mind that more color channels also mean the validation process and routine quality controls would be more complicated and need to synchronize with this capacity upgrade. Eventually, the application of technical advance is solely depending on the need from the clinical field or the requirements from the physicians and diagnostic labs.

Does a specific test for a given disease need a 12-color panel to assess? This is a question more to the clinical management goal and diagnostic need than to a clinical flow cytometry lab, as technically there should be no barrier. And we also need to keep in mind that there will be certain degree of "inertia" from the currently IVD flow kit consisting of much less colors, as these are well accepted assays already adopted in many institutions as routine assessment for different disorders.

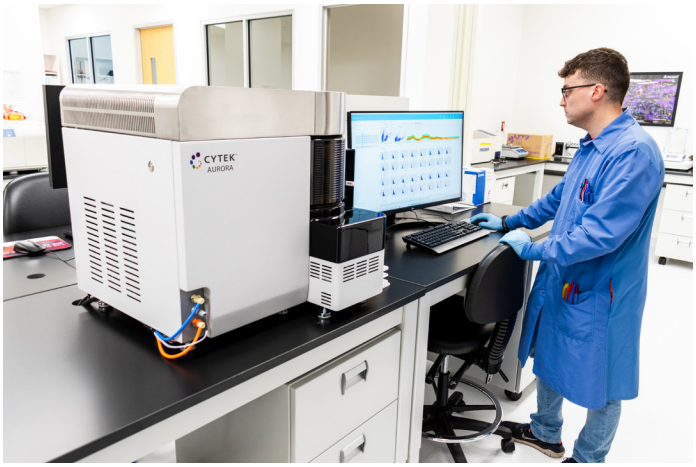
For RUO, the field is advancing rather fast coupled with the rapid pace of modern molecular biology pipelines, particularly surrounding the next generation sequencing. The collaboration between clinical flow cytometry and a molecular diagnostic lab will be concatenated by surface-marker-based sorting — a unique capacity only offered by flow cytometry. The flagship machine from multiple providers/lead companies are already equipped with 4-5 lasers and >20 color capacities. While having 20 conjugated antibodies in on reaction tube may generate its own difficulties some experiments demand these many channels and >15 channels are becoming more and more popular, which may even become the research standard in 2-3 years.

What are some of the advancements in flow cytometry?

Spectral cytometry emerges as the one stop solution to the limitations of polychromatic flow cytometry. From an immunological viewpoint, this is termed the number of phenotypic markers desired, which might range from just a couple to 30–70 individual subsets of cells. Currently, in a single assay, around 30 subsets can be defined by polychromatic cytometry, and so far, we have about 40 for spectral cytometry. This is an ever-increasing target and is likely to reach over 100 within the next 5 years. In addition, imaging flow cytometry, by advantage of the combined merits of flow cytometry and fluorescence microscopy, has become an established tool for cell analysis in diverse biomedical fields. Capabilities that are not possible with traditional flow cytometry are seamlessly 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 10 years of 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, PNH, CFSE Proliferation Assay and Cytokine Panel), we also offer study-specific full customized panels with capacity for more than 20 colors. Whether a custom panel needs to be developed or a method needs to be transferred to our laboratory to support a global clinical trial, we have the experience to quickly validate and implement the flow cytometry testing. Medpace has global flow cytometry capabilities at the US, Belgium, and Singapore laboratories.



Cytek Aurora, located at Medpace Cental Laboratory in Cincinnati

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