WHITE PAPER | NOVEMBER 2021

A World of Information

FLOW CYTOMETRY ADVANTAGES IN IMMUNOTHERAPY CLINICAL TRIALS

Essential technology for precision medicine

More than three decades ago, scientists at the National Cancer Institute began exploring ways to fight cancer using the patient's immune system. Their work formed the basis of what is now a promising cancer treatment that doesn't require surgery or radiation.

Patients provide blood samples for clinical trial sponsors to develop and deliver these emerging immunotherapies. Each sample they give is precious, as they all provide a wealth of biological data that contributes to therapy development. To ensure samples arrive at the lab intact, robust sample transportation and global logistics are critical. The deep scientific expertise housed within those labs ensures the highest-quality analysis and reporting.

A central lab with a global footprint provides both the efficient transportation arrangement and scientific rigor required to unlock the valuable information contained within patients' biological samples. Flow cytometry (FCM) is the primary method scientists use to monitor immune responses in clinical trials, primarily because of its power, speed, and ability to provide a comprehensive view of a disease's macro-environment.

With an increased focus on personalized medicine, including immunotherapy, there's high demand for FCM. It's used primarily to monitor changes in immune cell phenotype, to monitor therapeutic cells, and to detect and monitor abnormal cells. The advantages of FCM over other methods include:

Comprehensive data: Using a single tube of blood, researchers obtain information on immune cell subsets, memory phenotype, activation, regulatory exhaustion, and more.

High throughput: Flow cytometers can measure more than 35,000 events per second (and counting).

ABOUT THE AUTHORS

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Nithianandan came to Cerba Research to head the flow cytometry unit in 2019, after 20 years in industry and academia developing expertise in biomarker discovery, immunology, and regulatory affairs. Most recently, he was a lead scientist in global flow cytometry at Covance.

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Ishita brings more than 15 years of clinical and diagnostic industry experience to Cerba Research. Her scientific expertise includes infectious diseases and genetics, with extensive experience in microbiology, virology, and molecular biology. She is also a New York State certified Medical Technologist and certified IRB professional.

Multiparameter: Current technology can measure 40 or more cellular markers simultaneously.

Quantifiable results: Researchers can obtain relative percentages and learn the absolute count and number of receptors in a cell.

Statistically powerful: FCM's high-parameter capabilities produce powerful data in a short time frame.

The information obtained from FCM informs diagnosis, treatment, and monitoring of residual or relapsed disease. While much of its use centers on oncology, researchers also use it for immunology, rare disease, and infectious disease research.

Researchers turn to FCM for the following:

Immunophenotyping to detect the absence or presence of antigens without compromising sensitivity. The most common applications include identifying immune cells, detecting and analyzing subsets or statuses of immune cells, and detecting leukemic cells and therapeutic cells. Immunophenotyping panels for blood and bone marrow aspirate (BMA) are suitable for patient follow-up and assessing recovery and remission in lymphoma, myeloma, and leukemia clinical trials.

Report characteristics of cell populations with high sensitivity, with frequencies as low as 1 in 10,000 cells, which is key for detecting minimum/measurable residual disease (MRD).

Characterize and/or quantify proteins, cellular activation, proliferation, exhaustion, and apoptosis status.

Cell cycle analysis to determine each phase of the cell cycle.

Drug occupancy of cellular target markers.

Flow Cytometry in Biomarker Research

Flow cytometry has become invaluable for biomarker research and development because it provides such highly detailed information on single cells in a heterogeneous population. FCM provides the data to help researchers identify potential biomarkers to better understand why certain patients develop resistance to a therapy. This helps sponsors identify patients for clinical trials, as well as helps clinicians devise patient-specific treatment plans for participants.

In addition to identifying new biomarkers, FCM assays accurately and reproducibly measure biomarkers to ensure consistency of measurement both within and between patients. FCM data are used to make clinical decisions around safety, efficacy, and pharmacodynamics. More complex panels provide information for exploratory assessment in clinical trials.

The Importance of Validation

All biomarkers must undergo advanced validation to ensure data are interpretable within multiple time points, within large patient cohorts, and tested at different locations around the world. Data coming out of the validation provide guidance for interpreting clinical data.

A Fit-for-Purpose Approach

To ensure the quality of FCM assay performance in preclinical and clinical applications, several scientific organizations developed recommendations for FCM instrumentation and method validation. Cerba Research and other laboratories used these recommendations as well as GLP and GcLP guidelines to develop strategies to demonstrate a method is fit for purpose within the context of use.[2]

Under this approach:

- The data must be reliable and accurate (Fit).
- The data are used for decision-making during drug development (Purpose).
- The validation requirements are specific to the stage of drug development, with consideration toward the intended use of the biomarker data and any applicable regulatory requirements (Fit-for-Purpose).

With a standardized process such as fit-for-purpose, sponsors have the validation data they need to present to regulatory authorities to show validations were performed. The FDA and/or other regulatory agencies typically request validation reports to evaluate the assay performance for the intended purpose.[2,3]

Fit-for-purpose method validation starts with assay development or optimization and moves to validation and implementation. Sponsors should review the data to assess whether the assay needs any type of change or improvement for future expansion of the trial, and if so, the cycle begins again.

Validation parameters for new assays include assessments of the following:

- Precision
- Inter-operator variability
- Inter-instrument variability
- Sensitivity
- Stability
- Post-stained stability (for larger labs)
- Carry-over (especially for high-sensitivity assays)

When assays transfer to another lab, these parameters are assessed again, with the exception of sensitivity and stability. This ensures consistency among instrument settings and other parameters.

Emerging Trends in Flow Cytometry

While early flow cytometers could only measure one or two characteristics, or parameters, of a cell, current technology can measure 40 or more parameters. High-parameter flow cytometers use spectral detection to detect up to 50 parameters on a single cell simultaneously. These customizable tools detect multiple cell subsets in a single panel, maximizing the amount of data obtained from each sample. These instruments promise improved data quality over conventional instruments, as well as cost and time savings.

We expect to see a dramatic increase in the use of high-parameter flow cytometry over the next few years, particularly for exploratory assays. High-parameter devices allow researchers to assess various combinations of biomarkers using a smaller sample.

As instrumentation has advanced, so has the chemistry, including developments in reagents and fluorochromes. Tools for data analysis — a necessary component for high-parameter flow cytometry due to the increase in possible subsets — are also emerging.[4]

Flow Cytometry in Global Clinical Trials: Points to Consider

When planning an immunotherapy trial, consider the following aspects of FCM assay development and validation to make sure you receive all the data needed to evaluate your therapy and monitor disease.

Time: Fresh blood and BMA samples must be sent to the labs within the stability period (about 72 hours) and analyzed the same day. This quick turnaround maintains sample integrity and reduces the risk of data loss. Particularly for early research, when speed is critical, scientific teams must also analyze and review data in a short time.

Sample: Fresh whole blood and BMA remain industry standards. However, some projects do require processed whole blood so researchers can store cells for longer periods or perform batch testing over several time points. In these situations, the lab will isolate peripheral blood mononuclear cells (PBMCs) from whole blood and freeze them for later testing. These blood cells consist of lymphocytes (T, B, NK cells), monocytes, and dendritic cells. These blood cells are a critical component in the immune system for fighting infection and adapting to intruders.

While it's easier to obtain blood and BMA, samples have limited stability and therefore must be processed right away. PBMCs can be frozen and stored for later use. However, using PBMCs adds an additional processing step to FCM, which adds to turnaround time as well as generates additional cost. Blood and BMA are more cost-effective and tend to align well with other samples drawn for the clinical research project.

Whether to use PBMCs depends on budget, timeline, and the intended use of the data. Some populations of interest may not be present after PBMC processing. Scientists at central labs can assist with developing and validating proper assays.

Data Quality: After method validation, FCM scientists should review the data to make sure it meets all the sponsors' needs. When clinical trials begin, centralized data analysis and standardization among labs help ensure consistent quality throughout. Data from all the labs are reviewed by FCM scientists to monitor for quality and accuracy in analysis.

Global Capabilities: A central lab with a global network of FCM scientists offers several advantages for immunotherapy developers. A harmonized network with standardized equipment and methods, as well as central analysis, means data remain consistent no matter how large or global the study. The lab runs all experiments and tests in the same manner, using the same instrument settings and the same reporting format.

With FCM scientists based in multiple locations, experts can work across multiple time zones, which helps improve turnaround time. A point of contact in the sponsors' time zone allows for easier, more efficient communication.

Partnering with a lab that offers a global footprint also provides assurance in an environment that remains uncertain. Kits and samples can dispatch from local hubs to sites within the same continent or country, lessening customs requirements and bypassing the need for a costly premium carrier. When sponsors need to screen or randomize patients right away, they can be sure the lab will receive samples on time despite natural disasters or other disruptions.

Conclusion

In precision medicine, every tube of blood reveals a patient's complete story. Flow cytometry allows researchers to read every word of that story in an instant. When choosing a central lab to analyze those samples, partner with an organization that has the expertise, technology, and facilities to accurately and consistently convey all the information needed. A central lab with a global footprint ensures every story remains intact, giving sponsors the data they need to develop life-changing therapies for people who need them.

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About Cerba Research

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Cerba Research is part of Cerba HealthCare, a leading player in medical diagnosis.

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