Driving Clinical Trials Forward: The Benefits of Establishing a Strategic Partnership for Flow Cytometry

PEER REVIEWED

Targeted therapies for use in clinical studies, such as those in immuno-oncology (I-O), are the future of pharmaceuticals. Critically assessing the efficacy and safety of a targeted molecule in I-O trials is essential. Flow cytometry is a sophisticated technology that can provide specific information on how biologics and other targeted therapies interact with living cells on a cellular level in clinical trials.

With laser technology and single cell analysis, flow cytometry precisely measures how specifically and efficiently a targeted therapy binds to its intended cellular receptor. In this way, flow cytometry can measure the proportion of available target receptors to which a drug candidate binds. This assessment may correlate with a study drug's therapeutic potential and clinical efficacy. Flow cytometry can also determine how treated cells or test subjects are responding to treatment by measuring the presence of other molecules in biological matrices, such as blood or tissue samples. These capabilities make flow cytometric assays invaluable for characterising and evaluating preclinical research, demonstrating proof-of-concept, and monitoring patient outcomes in clinical trials.

Many standardised flow cytometric assays have been validated for common tests, and may be off-the-shelf, for example quantifying specific intracellular biomarkers, cytokines and immune cells such as T, B and natural killer lymphocytes. However, detecting the presence of molecules, such as bi-specific monoclonal antibodies or checkpoint inhibitors, studied in a number of I-O trials, that target specific receptors, requires custom flow cytometric assays specific to these molecular targets.

In addition, global studies are now common and, regardless of where and when they are done, require an infrastructure of laboratories capable of ensuring comparable results for study tests, including those for flow cytometric assays. Global capabilities require significant investment to create and maintain, not only in sample collection and transportation monitoring, but also in expertise and quality control experience. Given the cost, time and expertise needed to develop and qualify robust flow cytometric capabilities internally, many sponsors find strategic partnering the best solution. A partner with the scientific expertise, global resources and mission commitment required to effectively develop and uniformly execute flow cytometry can help drive successful clinical trials forward, while increasing the overall efficiency of a pharmaceutical sponsor's drug development programme.

This article outlines best practices in developing and implementing flow cytometric assays for clinical study samples, as well as the benefits of adopting a strategic partner to perform these complex assays.

A Customised Approach to Flow Cytometry

Customised assays used in research and early clinical trials are major factors in improving targeted therapy development efficiency. Because of the specificity of these customised assays, they can powerfully predict the performance potential of targeted compounds '*in vivo*', and precisely measure performance in clinical trials. Three types of custom flow cytometric assays and how they can be used to predict and assess targeted therapy performance are detailed below.

1. **Receptor occupancy assays** – The potential effectiveness of drugs and biologics can be assessed using receptor occupancy (RO) assays, which measure how efficiently the compound binds to cell surface receptors. The classic approach to RO cytometric assays is to develop two monoclonal antibodies that bind to a target protein: one that is non-competitive, meaning it binds to all target receptors even if they are already occupied by the test compound; and one that is competitive, meaning it only binds to target receptors not occupied by the test compound. Both antibodies are tagged with different fluorochromes.

   When added to a cell sample that has been treated with the test compound, the non-competitive antibody attaches to all target receptors, and these can be counted using flow cytometry to determine the total number of potential receptor positive cells in the sample. The competitive antibody binds only to unoccupied target receptors and dividing this number by the number of total target receptors reveals the proportion of receptors bound by the test compound (see Figure 1).

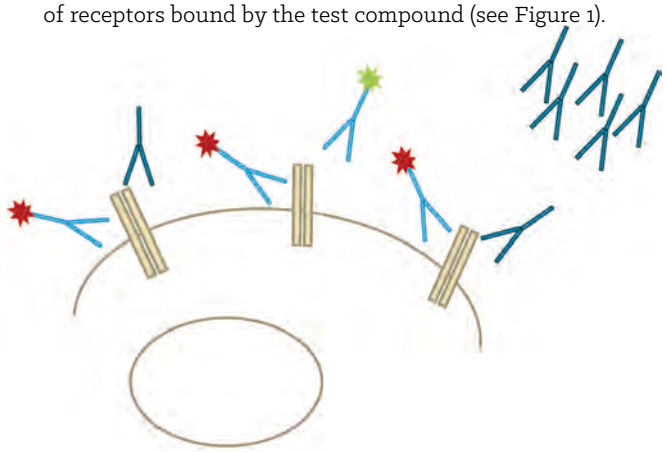


Figure 1. Determination of Receptor Occupancy Using Competitive and Non-Competitive Antibodies. A cell membrane receptor serves as target for two different antibodies, one that is non-competitive (✳) and one that is competitive (✳) for the epitope recognised by the test drug (λ). A saturating dose of unconjugated test drug can be used as a pretreatment to bind all the target epitopes and determine maximal binding. Ideally, this binding is similar to that obtained with the non-competitive antibody.

   An alternate method involves sequentially treating the test sample with tagged competitive antibodies and antibodies that bind to the target compound bound to receptors, directly measuring bound and unbound receptors (see Figure 2). In general, the higher the proportion of receptors bound, the greater the potential efficacy of the compound.

   Running RO tests on samples treated with different concentrations and exposure times in drug development and preclinical trials can generate useful pharmacodynamic information on which compounds are likely to be successful. Flow cytometric RO tests in clinical trials can be used to

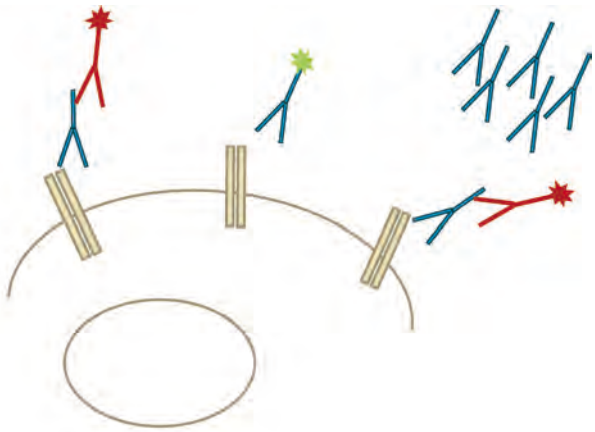


Figure 2. Receptor Occupancy Assay Experimental Design Using A Direct Detection Approach. A cell membrane receptor serves as target for an antibody that is competitive ( ) for the epitope recognised by the test drug ( ). Wherever free target epitopes exist, competitive antibody will bind. Unconjugated bound study drug can be detected with a secondary antibody ( ). A saturating dose of unconjugated test drug can be used as a pretreatment to bind all target epitopes and determine maximal binding. Free versus bound receptor is quantitated and percent receptor occupancy is determined.

inform go/no-go decisions in early clinical trials and determine dosage levels in later trials to assess clinical effectiveness. An analysis of clinical trials using flow cytometry between 2006 and 2018 found that these methods were most often used in Phase I, I / II, and Phase II studies. Since every target of a test compound is receptor-specific, a customised RO assay is required for every compound tested.

2. **Immunophenotyping assays** – Binding a targeted compound to one cell type may trigger intracellular reactions that affect other immune cells and the immune response, which can be potentially dangerous. Therefore, immunophenotyping assays must be run concurrently with RO tests to detect these effects.

   Immunophenotyping involves identifying a range of markers for immune activity in blood or tissue samples. These include the presence and quantification of sample characteristics including white blood cell types, T cells and B cells with specific biomarkers. Like RO assays, these tests use antibodies tagged with fluorescent molecules that bind to specific molecular targets, allowing cells to be identified and counted by type. Many of these assays are standard and commonly used, though custom assays for identifying specific antigens may also be required.

3. **Functional assays** – Functional assays seek to characterise not only the presence of specific immune cells and factors, but also how they are affected by a candidate compound. Subjects are treated with the compound, and flow cytometry is used to assess changes before and after stimulation in immune cell subpopulations. For example, intracellular cytokine production can be measured, which can provide an indication of magnitude of response. This gives additional insight into the pharmacodynamics and potential efficacy and side-effects of candidate compounds.

Taken together, these three types of flow cytometric assays are powerful tools for developing targeted therapies. However, they do entail highly technical processes and clinical experience which are presented below.

Best Practices for Developing and Implementing Flow Cytometric Assays in Clinical Development Programmes

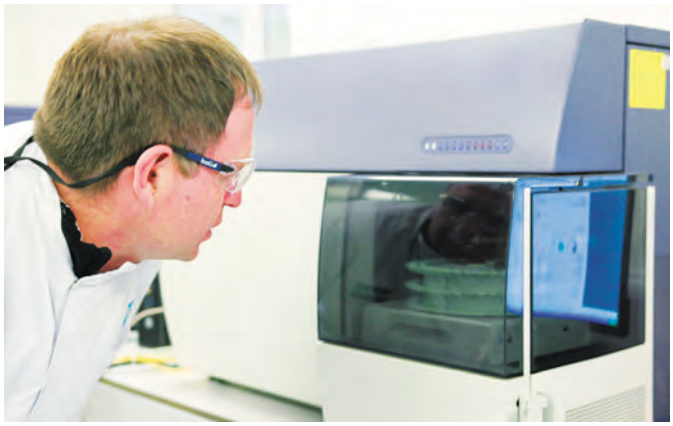
Due to the complexity and highly technical nature of developing custom flow cytometric assays and performing standardised assays, many sponsors choose to outsource these functions to experts. Regardless of whether assay design and execution are outsourced or undertaken internally, a close working partnership between product researchers, clinical study design and operations, regulatory and flow cytometry experts is essential for success.

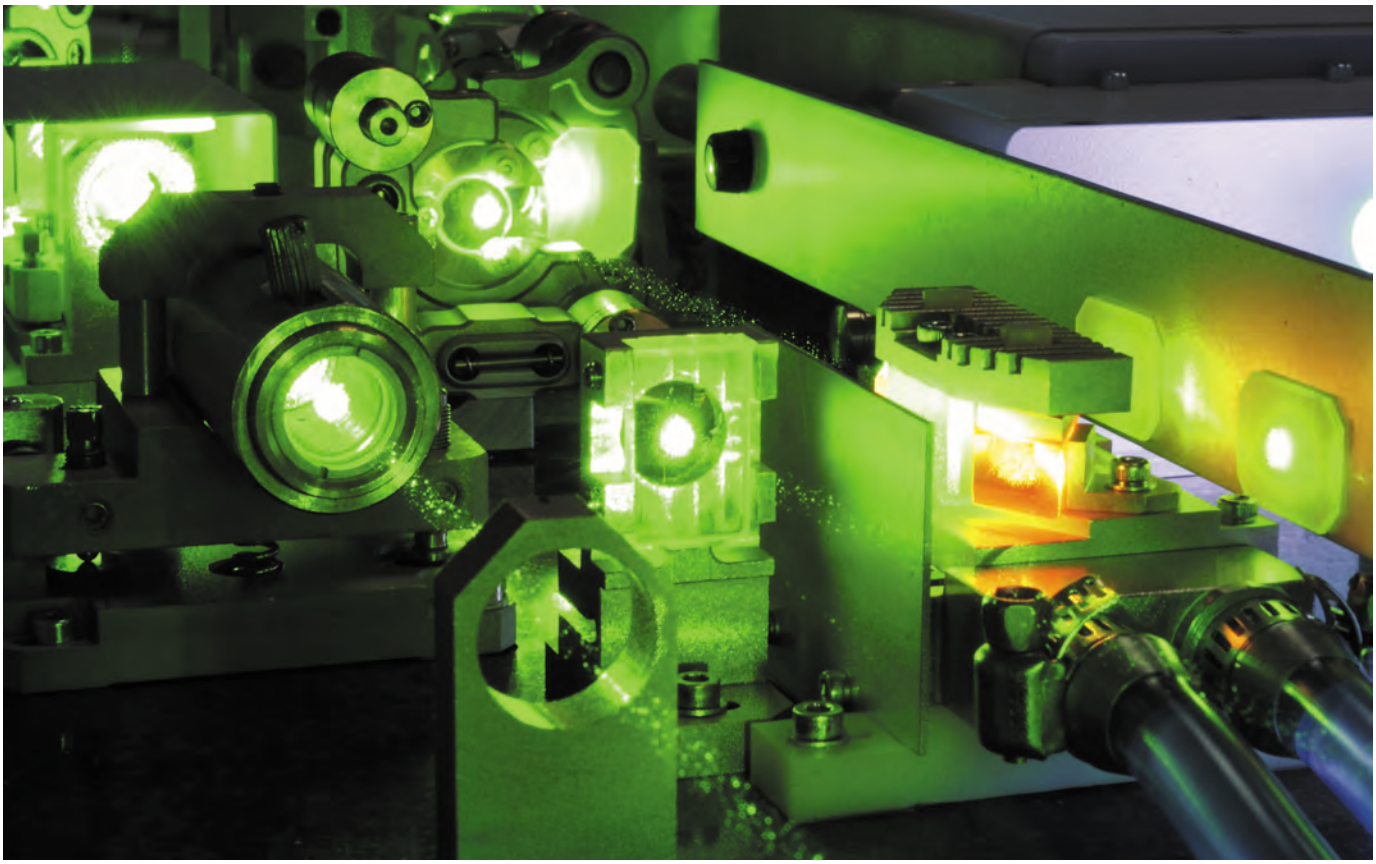
Communication, early, often and ongoing – Open communication about the goals, methods and technical details of an assay programme between developers and test experts is critical to matching assay approaches to specific study needs. Whether the need is for consistent execution of a panel of previously determined standardised assays; refinement, validation and execution of an existing custom assay; or conceptualising and developing new custom assays, establishing early on a common understanding of the requirements for the assay makes the entire process easier and more efficient.

Because clinical development programmes are iterative, with details and goals shifting as results and evidence accumulate, ongoing communication and collaboration are needed to ensure that assays address changing needs. For example, as a compound advances through clinical trials, adjustments to the assay protocol or interpretation framework are required when differences exist between the health of populations used to develop the assay and the disease indication of the study population. Also various state and national guidelines, typically those promulgated by the US Food and Drug Administration and the New York State Department of Health, must be considered depending on whether the assay will be used for research only, clinical trials or clinical treatment.

For portfolios with multiple products, it is helpful to take a strategic communication approach throughout all product development stages. This can ensure that tests run during early development provide the necessary information to inform clinical trial design, and inform decisions about which candidates or programmes have the best chance of success.

Robust and use-appropriate assay method validation process – Demonstrating the scientific integrity of a study, not to mention winning regulatory approval, requires the presentation of methodologically and statistically reliable clinical evidence. Because flow cytometric assays provide much of the evidence supporting product development, test programmes must be designed to ensure they address relevant research and clinical questions in a way that will pass scientific and statistical scrutiny. It is also necessary to ensure assays are characterised for the intended use of the results, such as for exploratory, clinical diagnostic for patient management during a trial, or as a companion diagnostic test for drug treatment.





Keep in mind that the evidence threshold differs in the development process, according to the context of use for an assay, with its stringency increasing as patient risk increases. For example, assays with exploratory endpoints may be validated for use across laboratories and study sites with an initial demonstration of 20 to 30 per cent variation in precision for standard cell samples.

At the other end of the patient risk spectrum, however, flow cytometric assay requirements are more stringent. Any assay used to support patient treatment decisions, whether in clinical trials or *in vitro* diagnostic tests for monitoring clinical treatment, must undergo a more rigorous assessment across test laboratories. In these cases, the observed variation in results from an assay should be lower, as achieved by using increased sample sizes and post-collection time points.

For all flow cytometric methods to be implemented in clinical trials, validation processes should include:

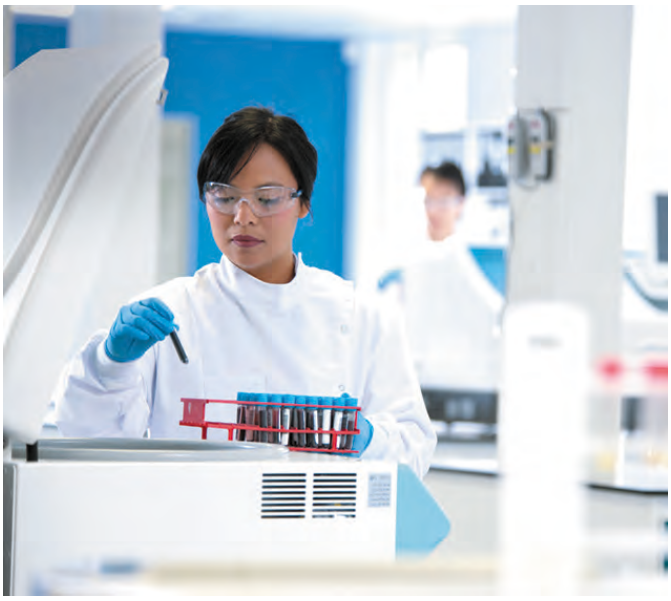
- **A detailed plan** including experiment outlines and goals, such as a timeline and specific types of cells or phenotypes that will be identified; intended assay use and any associated acceptance criteria; descriptions of assay methods employed; and any equipment, reagents, test cell lines or other standardised supplies needed.
- **Quantitative assessments** of assay characteristics, including feasibility for measuring theoretically relevant variables; biologic variability across sample sources when needed; precision of measurement; stability of samples and consistency of results over time and across sites; and statistical validation of results.
- **Documentation** of all processes, methods, instrumentation and materials sufficient to reproduce results.

Note that determining an appropriate assay for a particular clinical application is as much an art as it is a science. Experts with extensive experience developing, optimising and validating complex cell-based methods that can troubleshoot issues as they arise are an invaluable asset for moving development programmes and clinical trials forward.

Robust data reporting, analysis and validation process – Similarly, demonstrating assay data integrity and significance are essential for showing study scientific integrity and passing regulatory muster. Details of data collection methods, raw data results and statistical frameworks, assumptions and calculations should be outlined to ensure reliability, and developed and documented to ensure they meet requirements.

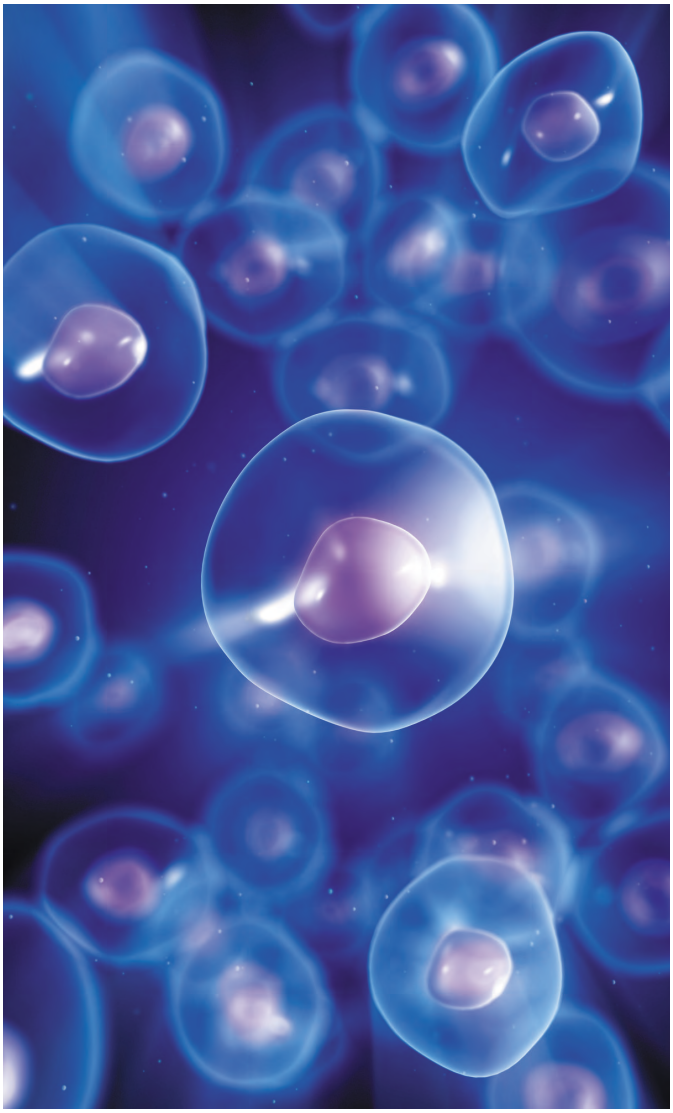
Quality control – Documenting quality control is critical for demonstrating assay reliability. Aspects that must be documented include:

- **Process quality control.** This includes ensuring appropriate sample collection, timely transportation, and any necessary temperature or other environmental control required to ensure sample integrity. It also includes ensuring processes for preparing and analysing samples are uniform at laboratories around the world.
- **Fluorescence quality control.** This entails ensuring that fluorescent tags necessary for identifying cells or cell-associated biomarkers are consistent over time and across instruments, whether in the same laboratory or globally, and that antibodies for attaching them to targets are specific and reliable.
- **Customised assay quality control.** Ensures that assays and processes – such as any special cell line, sample preparation and quantitative or qualitative evidence – required to measure or demonstrate a particular effect are developed, and scientifically and statistically validated as needed.



Benefits of Adopting a Strategic Flow Cytometry Partnership

Ensuring that the highly technical aspects of flow cytometric assays are suitable for specific development purposes is both valuable and difficult – particularly for sponsors with limited experience developing targeted therapies. Because many sponsors do not have the ability to develop custom flow cytometric assays in house, a strategic relationship with an experienced partner can assist sponsors to capitalise on the benefits of flow cytometry. Partners build on scientist-to-scientist relationships and have established regional laboratories and sample handling capabilities for supporting basic research and global clinical trials. Moreover, these partnerships can aid in developing high-value products at lower cost and in less time than developing a comparable internal capability.



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ICON's R&D Department, located in Farmingdale, NY, develops and validates fit-for-purpose flow cytometry-based biomarker assays and qualifies client developed assays for use in clinical trials. The scientific team, with over 75 years of combined experience, works closely with ICON partners to provide complex flow cytometric assays that reduce cost, enhance quality and expedite drug development.

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