SAFETY AND TOXICITY STUDIES USING FLOW CYTOMETRY

For GLP and non-GLP-Compliant Applications

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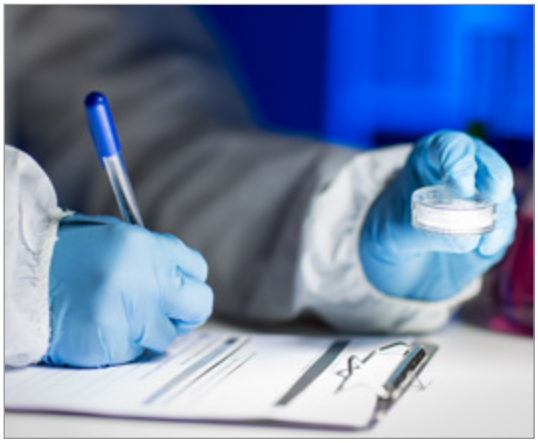
Flow cytometry is a widely used platform in drug discovery research because it is rapid, customizable and can yield a wide range of data that can inform drug and biologic pipeline decisions. The power of flow cytometry is based on its ability to measure specific features of individual cells at a scale of millions of cells per run. This technology depends on cells being stained with fluorescently labeled antibodies that bind to specific molecules on cells. A flow cytometer houses lasers that can excite these fluorescent markers, and this excitation is followed by an emission of photons, which are detected and translated into semi-quantitative data that shows how abundant a marker is on each cell. Customized panels of fluorescent antibodies are able to discern different cell types, like B cells or T cells, or even subsets of cells like cytotoxic T cells and memory T cells. Flow cytometry assays can also be used to measure cell functions such as phagocytosis or antibody dependent cellular cytotoxicity (ADCC).

Flow cytometry has moved beyond the basic research lab and is being used for preclinical development of immunomodulatory drugs and biologics. These candidates typically undergo safety and toxicology analysis to assess suitability for human use. At present, flow cytometry-based safety and toxicology assays are used in tandem with traditional assays and can be performed under good laboratory practice (GLP) conditions or can be used for non-GLP safety and toxicology assessments. This white paper provides

an overview of how flow cytometry-based assays can be used to your safety and toxicity analysis and provide you with information critical to informing preclinical development decisions.

GLP versus Non-GLP-Compliant Assays

GLP compliance is a broad set of laboratory standards that include how studies are planned, performed, monitored, reported and archived. Any investigator studying a drug or biologic that may eventually be considered for use in humans will have to consider when and how to include GLP compliant assays in their research program. Researchers will need to satisfy regulatory requirements, such as the FDA's Investigational New Drug (IND) application, in order to receive regulatory approval for the use of a drug or biologic in humans . These regulatory requirements typically include use of GLP compliant assays in order to assure the safety of the product that may be tested in humans.



Non-GLP compliant toxicity and safety assays are ideal during preclinical studies after the lead selection stage. Flow cytometry-based approaches can be used to narrow down promising drug or biologic candidates and can sort out less promising leads before moving into the more costly and time-consuming animal model testing. These assays can profile biological responses to a candidate in a number of blood cells simultaneously and can provide mechanistic insight as well as identify potentially undesirable responses.

[1] *https://www.fda.gov/Drugs/DevelopmentApprovalProcess/HowDrugsareDevelopedandApproved/ApprovalApplications/InvestigationalNewDrugINDApplication/*

GLP-Compliant Flow Cytometry Assays

Instrumentation Considerations

GLP compliance of an assay goes beyond the actual protocol being carried out. The handling and storage of samples must be carefully documented and every piece of equipment used for sample analysis must undergo instrument validation. This is a critical aspect of GLP-compliant flow cytometry assays, as you may need to have a dedicated flow cytometer for running GLP assays. Flow cytometers have complex and delicate fluidics and optics systems, and instrument validation assures that systems are functioning properly and meeting specific parameters. For GLP compliance, instrument validation procedures must be written and carried out at predetermined times, and specific GLP-compliant records must be correctly maintained and archived.

Sample Considerations

The sample type will determine several aspects of a GLP-compliant assay, particularly how samples are stored and how an assay is validated. Assays using a purified fraction of cells such a peripheral blood mononuclear cells can rely on cryopreservation of samples, which offers users greater flexibility as to when samples are used for flow cytometry. In addition, a collection of stored control samples can be used for assay validation and as control samples throughout a given study. In contrast, whole blood cannot be cryopreserved or stored for long periods of time, so assays using whole blood are under greater time constraints analysis must be done soon after sample collection. Moreover, assay validation must be handled differently to address changing sources of whole blood control samples.

Assay Validation

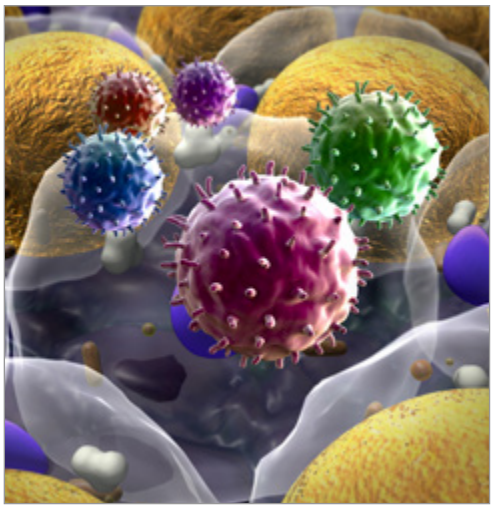
Assay validation evaluates the reliability and overall performance of a particular assay. Flow cytometry assays are highly customizable, but validation assures an assay's performance by assessing precision, robustness, sample stability, assay specificity and inter-sample variability. Validation can be used to determine the limit of detection, repeatability, and specificity of a given assay, as well as long term stability of a given sample. The rigorous validation process can be time consuming and many researchers work with assay validation experts, such as contract research organizations, who have experience with developing test scripts for validation of semi-quantitative assays like flow cytometry. Validation experts are also familiar with the extensive documentation associated with assay validation, which is critical for the development of a GLP-compliant assay.

Studies between Species

Flow cytometry assays have been developed for use across numerous species, including mouse, rat, primate and human. In fact, many species-specific products are commercially available, so custom fluorescent antibody

staining panels can be developed for most standard animal models used in preclinical studies. The flexibility and convenience of these species-specific reagents can be a challenge if you are developing a GLP-compliant flow cytometry assay for later use in a clinical trial. Many antibodies that bind to lineage markers used for immunophenotyping, like CD3 and CD4, are cross-reactive between non-human primates and humans, whereas few antibodies used to detect rodent targets can be used to stain human cells. As you develop different flow cytometry staining panels for non-GLP and GLP-compliant assays, consider how panels used for mouse samples can be translated for use in human clinical samples. This may require the development of novel antibodies or may require the use of different biomarkers.

Examples of Safety Assessments



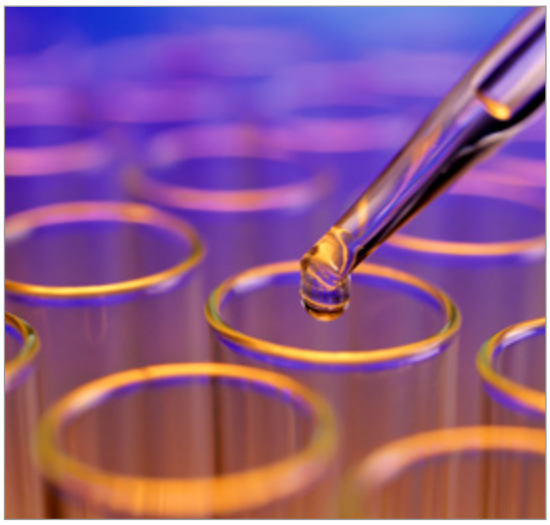
Flow cytometry is especially well-suited for monitoring immune cells in peripheral blood that can be treated with experimental compounds in vitro and then analyzed for effects on immune responses. These assays can be used to measure efficacy by demonstrating that an experimental biologic or drug is specifically engaging a cellular target and causing a biological response. Experimental treatments may promote expansion or destruction of specific cell types, which can be measured using immunophenotyping protocols. Flow cytometry can also measure changes in biomarkers on different cell types, such as changes in expression of specific cytokines, chemokines or transcription factors. These biomarker changes may correlate with desirable or undesirable immune responses, such as a cytokine storm, and biomarker assays can be used for assessing the further development of candidate drugs

or can be used as a GLP-compliant assay to monitor immune responses in animal models or in patients participating in clinical trials. 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.

Examples of Toxicology Assessments

Flow cytometry is being used more frequently for toxicological screening of drug and biologics candidates, especially therapeutic antibodies. These assays are used in parallel with other standard in vitro toxicological screening assays or to inform animal model studies. Flow cytometry studies can be used to assess maximum tolerated dose, or measure pharmacokinetic and pharmacodynamic



2 *Lynch CM, Hart BW, Grewal IS. Practical considerations for nonclinical safety evaluation of therapeutic monoclonal antibodies. mAbs. 2009;1(1):2-11.*

3 *tewart JJ, Green CL, Jones N, Liang M, Xu Y, Wilkins, DEC, Moulard M, Czechowska K, Lanham D, McCloskey TW, Ferbas, J, van der Strate, BWA, Högerkorp C, Wyant T, Lackey A, Litwin V. Role of Receptor Occupancy Assays by Flow Cytometry in Drug Development. Cytometry Part B 2016; 90B: 110–116.*

properties of an experimental drug in vitro or in animal models. Receptor occupancy (RO) assays measure binding of a molecule or drug to a receptor expressed on a specific cell. This quantitative assay can be adapted to a flow cytometry platform and can be part of the pharmacodynamic profiling of therapeutic candidates. Flow cytometry can be used to monitor genotoxic effects of experimental drugs and can be used in tandem with other technologies like high-throughput sequencing.

Mechanistic Insights

Flow cytometry assays can be valuable tool for gaining an understanding of mechanisms used by experimental drugs or biologics. Non-GLP safety assessments can include mechanistic investigations, such as measuring cytotoxic cell degranulation , . Antibody-dependent mechanisms, such as receptor blockade, apoptosis induction, and antibody dependent cellular cytotoxicity, can also be measured using flow cytometry. Any of these assays can be validated and used as a GLP-compliant assay if the mechanistic readout is critical to monitoring clinical effects.

Conclusion

Flow cytometry has moved to the forefront of preclinical and clinical development protocols as immunomodulatory drugs and biologics have flooded research pipelines. Consider using a flow cytometry based assay in your preclinical studies or as a GLP-compliant assay

4 *Zaritskaya L, Shurin MR, Sayers TJ, Malyguine AM. New flow cytometric assays for monitoring cell-mediated cytotoxicity. Expert Review of Vaccines. 2010. 9(6):601-616*

5 *Betts MR1, Brenchley JM, Price DA, De Rosa SC, Douek DC, Roederer M, Koup RA. Sensitive and viable identification of antigen-specific CD8+ T cells by a flow cytometric assay for degranulation. 2003. J Immunol Methods. Oct 1; 281(1-2):65-78*

Quantifying Biological Response
Through Cytometry