HIGH DIMENSIONAL FLOW CYTOMETRY FOR COMPREHENSIVE IMMUNE MONITORING IN CLINICAL TRIALS

Dominic Gagnon, Yoav Peretz, Marylène Fortin, Claire Landry, and David Favre

ImmuNeCarta Services, 2901 Rachel Est, Suite 22, Montréal, QC, Canada, H1W 4A4

Introduction

ImmuNeCarta Services is a leading provider of services for preclinical and clinical studies related to immunology. Over the past 7 years, we have developed a broad battery of innovative assays to characterize cell populations and immune responses in the setting of infectious diseases, cancer, vaccine trials and immune-based therapies. Based in Montreal, ImmuNeCarta Services is specialized in advanced multiparametric flow-based assays performed according to GLP regulations, GCLP guidelines governed by quality Management Systems and standard operating procedures. ImmuNeCarta expertise includes the assessment of phenotypic and functional markers, the characterization of cell subset lineages, activation states, and signaling molecules, as well as the quantitative analysis of vaccine-, pathogen- or drug-specific responses based on antibody signatures, cytokine/chemokine profiles, and signaling pathways. We describe here our experience as a contract research organization providing services to the biopharmaceutical industry, in the execution of high dimensional flow cytometry analysis of subjects enrolled in Phase I/II clinical trials.

Immune Monitoring

Over the past 7 years, we have implemented assays using high throughput analytical methods with 10 to 18 flow cytometry parameters on fresh and cryopreserved human peripheral blood samples. Overall, immune monitoring of clinical trials involve study planning, assay validation, specimen handling, assay execution, monitoring, reporting, and quality review performed as per applicable GLP regulations and GCLP guidelines governed by quality systems and standard operating procedures.

Flow cytometry is based on fluorescence, fluidic and optical tools with the help of signal and image computer treatment. ImmuNeCarta Services uses 3- and 4-laser LSR II Becton Dickinson instruments. These instruments are high performance systems allowing simultaneous analysis of up to 18 colors using automated sampler in 96 well-plate format. High dimensional flow cytometry requires sensitive methods with optimal stability and reproducibility of the signal. For customized antibody panels, qualification or validation steps are necessary to address specificity, precision, accuracy, lower and upper limits and range of detection, stability and reproducibility of the analysis. The precision and accuracy of flow cytometry experiments also depends on stable application settings (CSAT beads) as well as internal and/or external quality control (QC) samples.

High throughput analysis of high dimensional flow cytometry data requires advanced software and methods. Data analysis performed at ImmuNeCarta Services relies on flow data acquisition using DIVA software (BD Biosciences), and data analysis with FlowJo (Treestar Inc.), Excel (Windows), PROSTATE (NHII, Cluster Open source, Stanford), Java TreeView (Open source), Prism (GraphPad Software) and/or other specialized software for statistical analysis and systems biology. All data are acquired and analyzed in compliance with 21 CFR Part11 to ensure quality and integrity of the raw data and its analysis. Pre-defined FlowJo templates are qualified or validated (GLP study) prior to being used throughout studies and require minimal gating adjustments that are documented accordingly.

Study Cases of Immune Monitoring

Since 2004, ImmuNeCarta has applied a broad array of innovative assays for the biopharmaceutical industry and government institutions to characterize the immune profiling of adaptive and innate immunity and the potency of immune-related drugs or vaccines in exploratory and Phase I and II clinical trials. ImmuNeCarta has recently formed a strategic alliance with Capro Proteome Inc., the leading company in proteomics and biomarker discovery (www.caprion.com) in order to integrate single-cell multiparametric flow cytometry analysis with soluble markers, serological measurements and other large datasets including genomics and proteomics.

Conclusions

Flow cytometry is a unique way to address complex cellular immunological profiling for drug development and Phase I/II clinical trials in infectious diseases, cancer, vaccine, transplantation, autoimmune disorders and related immunomodulation-based therapies. High dimensional multiparametric single-cell analysis is not only aimed to define multiple markers of different cell populations simultaneously -though helpful when clinical sample availability is limited- it is also one of very few analytical platforms that can address complex protein-based signatures (biomarkers, disease stage, etc.) and functional networks (mechanisms) from well-characterized human or animal cells at the single cell level.

The immune monitoring of Phase I to Phase III clinical trials aims to design, perform and interpret immunological data that enable industry to move vaccines, immunotherapies and drug candidates through the regulatory process (FDA, EMEA, others). High dimensional flow cytometric analysis also allows for the definition of immunological profiles that are disease and stage specific, enabling elimination of many unsuitable drug, vaccine or therapy candidates prior at the time of “m-nan” studies. This requires both a scientific expertise in immunology, physiology and pathology as well as a clear understanding of technicalities related to instruments, reagents and high dimensional data mining. As a service company for the pharmaceutical industry, ImmuNeCarta regulatory process and standardized procedures are critical to ensure data integrity and quality, especially when interpreting complex data sets to define disease stage, drug efficacy or toxicity. Overall, immune assays for diagnostic, research or biomarker discovery may impact on all aspects and stages of immune system testing, vaccine and immunotherapeutic design and development as well as drug screening. They are enablers, permitting GO/NO-GO decision-making, thus saving both time and money, enhancing safety and providing surrogates markers of clinical efficacy and/or mechanistic insights.