DMD Fast Forward. Published on October 27, 2014 as DOI: 10.1124/dmd.114.060798
This article has not been copyedited and formatted. The final version may differ from this version.

Mass cytometry: a highly multiplexed single cell technology for advancing drug development

Authors: Kondala R. Atkuri, Jeffrey C. Stevens and Hendrik Neubert

Affiliation: Pharmacokinetic, Dynamics and Metabolism, New Biological Entities, Pfizer, Andover, MA 01810

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 19, 2024

DMD Fast Forward. Published on October 27, 2014 as DOI: 10.1124/dmd.114.060798
This article has not been copyedited and formatted. The final version may differ from this version.

Running Title: Mass cytometry in drug development

Corresponding author's mailing address:

Kondala R. Atkuri

Address: G2002-21, 1 Burtt Road, Andover, MA 01810

Telephone: 978.247.1765

Email: Kondala.atkuri@pfizer.com

No. of text pages: 32

No of tables: 1

No of references: 50

Word count in abstract: 249

Word count in review: 7298

Abbreviations:

ABC – antibody bound per cell; CyTOF – Cytometry by Time-of-Flight; DOTA – 1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetraacetic acid; FLAME- flow analysis with automated multivariate estimation; FLOCK – FLOW clustering without k; ICP-MS – inductively coupled plasma – mass spectrometry; ICS- intracellular cytokine staining; IdU – iodo-2-deoxyuridine; PCA – principal component analysis; PE – Phycoerythrin; PBMC – peripheral blood mononuclear cells; RF- radiofrequency; SamSPECTRAL –

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 19, 2024

DMD Fast Forward. Published on October 27, 2014 as DOI: 10.1124/dmd.114.060798
This article has not been copyedited and formatted. The final version may differ from this version.

similarity matrix and spectral clustering algorithm ; SPADE – spanning tree progression of density normalized events; TOF – time of flight

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 19, 2024

DMD Fast Forward. Published on October 27, 2014 as DOI: 10.1124/dmd.114.060798
This article has not been copyedited and formatted. The final version may differ from this version.

Abstract

Advanced single cell analysis technologies (e.g. Mass Cytometry) that help in multiplexing cellular measurements in limited volumes of primary samples are critical in bridging the discovery efforts to successful drug approval. Mass cytometry is the state-of-the-art technology in multi-parametric single-cell analysis. Mass cytometers (also known as CyTOF or Cytometry by Time-of-Flight) combine the cellular analysis principles of traditional fluorescence-based flow cytometry with the selectivity and quantitative power of Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). Standard flow cytometry is limited in the number of parameters that can be measured due to the overlap in signal when detecting the fluorescently labeled antibodies. Mass cytometry uses antibodies tagged to stable isotopes of rare earth metals, requiring minimal signal compensation between the different metal tags. This unique feature enables researchers to seamlessly multiplex up to 40 independent measurements on single cells. In this perspective we first present an overview of mass cytometry and compare it with traditional flow cytometry. We then discuss the emerging and potential applications of CyTOF technology in the pharmaceutical industry that include quantitative and qualititative deep profiling of immune cells and their applications in assessing drug immunogenicity, extensive mapping of signaling networks in single cells, cell surface receptor quantification and multiplexed internalization kinetics, multiplexing sample analysis by barcoding, and establishing cell ontologies based on phenotype and/or function. We end with a discussion on the anticipated impact of this technology on drug development lifecycle with special emphasis on the utility of mass cytometry in deciphering the drug's pharmacokinetics and pharmacodynamics relationship.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 19, 2024

DMD Fast Forward. Published on October 27, 2014 as DOI: 10.1124/dmd.114.060798
This article has not been copyedited and formatted. The final version may differ from this version.

Introduction

Single cell cytometry technologies (flow cytometry and image cytometry) have substantially impacted drug discovery and development for the past few decades. Extensive use of cell sorters and analyzers have not only enabled cloning or sorting pure populations of cells (cell lines, primary cells and more recently stem cells), they have also enabled researchers to measure the effects of a drug at the single cell level and thus better understand its mechanism of action.

While, until recently, the application of cytometry assays in drug development had largely been limited to targeted measurements of one or a handful of diagnostic markers on single cells, the precision medicine era is necessitating a more comprehensive multiplexed “single cell omics” approach for the discovery and monitoring of cellular biomarkers to track disease, stratify patients or assess the success of therapy. For example, detailed phenotyping combined with identification of potential signaling nodes that constitute the intended and unintended targets of drugs have been successfully employed in drug screens (Zhang et al., 2009; Ellinger et al., 2014) and clinical studies with signaling inhibitors such as anti-cancer drugs (Guerrouahen et al., 2009; Tinhofer et al., 2012). As new drugs are developed for targeted therapy of patients stratified using a precision medicine approach, advances in multiplexed single cell assay technology will be instrumental during the discovery and development phases of drug development. The invention of mass cytometry has opened up new possibilities for such highly multiplexed assays on single cells.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 19, 2024

DMD Fast Forward. Published on October 27, 2014 as DOI: 10.1124/dmd.114.060798
This article has not been copyedited and formatted. The final version may differ from this version.

Although still in its early stages, mass cytometry has already initiated a transformation of single cell analysis in both academia and the pharmaceutical industry alike. The initial ground-breaking publications on mass cytometry underscored its utility and amenability for highly multiplexed assays to measure cellular phenotype and functional markers (e.g., intracellular cytokine assays and phospho-signaling fingerprinting of immune cells). Mass cytometry is increasingly being recognized as a significant step forward in single cell profiling that has the potential to accelerate biomarker discovery throughout drug development.

Mass cytometers are essentially inductively coupled plasma – mass spectrometers (ICP-MS) that have been adapted for single cell analysis. Traditionally, ICP-MS has been used for elemental analysis with a high degree of sensitivity and can detect metals at concentrations as low as one part per trillion (one in 10^{12}). In biology and biological industries, ICP-MS is commonly used to quantify toxic metal contaminants, metal containing biofactors and proteins, or in the study of metal transport, metallo-drugs and metal speciation.

The use of ICP-MS in biological assays was first demonstrated in a study that used conjugated gold clusters to quantify endocytic low-density lipoprotein in cultured cells (Andreu et al., 1998). Adaptation of ICP-MS to an immuno-assay format was demonstrated later by Baranov *et al* who showed the feasibility of using antibodies tagged to either gold (Au) or lanthanide metal tags in analyzing immune complexes (Baranov et al., 2002; Ornatsky et al., 2006). The same investigators further developed cell based immune-assays coupled to ICP-MS; a technology that formed the basis for the invention of the first mass cytometer (Ornatsky et al., 2008a; Ornatsky et al.,

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 19, 2024

DMD Fast Forward. Published on October 27, 2014 as DOI: 10.1124/dmd.114.060798
This article has not been copyedited and formatted. The final version may differ from this version.

2008b). In 2010, Ornatsky *et al.* described their ground breaking invention of the mass cytometer and demonstrated its capabilities for highly multiplexed analysis of cellular markers (Ornatsky et al., 2010).

In this brief perspective we describe the principles and instrumentation behind the CyTOF technology. Subsequently, we will discuss emerging applications in biomarker research in the pharmaceutical industry in the context of the anticipated impact on drug development.

Mass cytometry: principles and instrumentation

CyTOF is essentially an ICP-MS with a time of flight detector that is designed for simultaneous quantitative measurements on single cells or bead-based immune-detection assays. CyTOF uses antibodies that are linked to rare earth metals (referred to as tags in this review). The metal tags serve the same function as fluorophores in traditional fluorescence-based flow cytometry. The current state-of-the-art mass cytometer has the capability to measure up to 100 different stable isotopes of rare earth metals, although the current availability of these tags in high purity limits the usage to around 40 different rare earth metal tags.

CyTOF can be broadly divided into three main functional units – sample introduction, ICP combustion region and TOF (MS detector) (Figure 1). A detailed description of the instrument has been discussed in several reviews published previously (Bandura et al., 2009; Ornatsky et al., 2010; Tanner et al., 2013).

Sample Introduction. The sample introduction system performs three main functions; introduction of the cell suspension, pneumatic nebulization of the cell suspension, and

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 19, 2024

DMD Fast Forward. Published on October 27, 2014 as DOI: 10.1124/dmd.114.060798
This article has not been copyedited and formatted. The final version may differ from this version.

evaporation of water from the droplets containing the cells before they are introduced into the plasma. Cells can be introduced either manually or via an autosampler. Upon injection, a narrow capillary tube delivers the cells to a nebulizer wherein argon gas-based pneumatic nebulization converts the cell suspension into a fine spray of water droplets and releases the droplets (containing cells) into a heated chamber called the spray chamber. The argon gas in the spray chamber (known as make-up gas) carries the nebulized cells through the heated spray chamber and into the plasma. During transit in the heated spray chamber, the water in the cell droplets is evaporated by the combined action of argon gas and heat. The distal end of the spray chamber is connected to a RF generator/ spark plug combination to together generate the plasma. Only about 30-40% of nebulized cells make their way into the plasma while the remaining cells are non-specifically lost on the walls of the spray chamber. This process results in a 30-40% efficiency of analysis of the injected cell samples.

ICP-MS. Inductively coupled plasma is initiated by the spark plug which generates ions in the argon gas. The electrons released by the initial spark are accelerated back-and-forth by the alternating magnetic fields generated by the RF coil. The resulting collisions between the electrons and the argon atoms create further ionization finally resulting in sustained plasma which is maintained by a continuous supply of argon flow from the torch body. The cells are delivered concentrically into the plasma core which is maintained at a temperature of 7000K and at atmospheric pressure.

Upon entering the plasma, the cells are completely vaporized and atomized. The resulting ion cloud exists at the other end of the plasma and expands at diffusion controlled rates on its way to the sampler cone. As the ion cloud passes through three

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 19, 2024

DMD Fast Forward. Published on October 27, 2014 as DOI: 10.1124/dmd.114.060798
This article has not been copyedited and formatted. The final version may differ from this version.

cones of the sampler, skimmer and reducer, the ions are moved through progressively higher levels of vacuum generated between the cones. The ion cloud is then passed through an ion optics to filter out the neutral and negatively charged ions. The filtered circular beam of ion cloud is thus enriched in positively charged ions and is then passed through an ion guide that converts a circular beam of ion cloud into a beam compatible with the rectangular slit of the orthogonal-acceleration reflectron time of flight analyzer. This ion guide consists of two rf-quadrupoles and is tuned to a low mass cutoff (m/z = 80) to filter out the abundant low mass ions generated from metals and other elements found naturally in cells. Thus, the filtration results in an ion cloud which is considerably less dense than the parent ion cloud which can be manipulated and focused more efficiently. Furthermore, the filtering enables the ICP-MS to be configured to a time-of-flight (TOF) mass analyzer.

TOF and data processing. In the TOF chamber, ions are accelerated at a fixed potential. As a result the ions travel at speeds proportional to the square root of their masses. In essence, low mass ions travel faster than high mass ions. The ions then impinge on a sheet beam in the detector. Subsequently, ion counts are converted to digital signals using an 8-bit 1GHz analog-to-digital converter. The detector is set to measure m/z in the range of 80-200 at a 1 ns sampling resolution.

The ion cloud derived from a cell or bead is measured in 13-15 segments, each with a duration of 20 µs. The total duration of a typical cell-derived ion cloud is 200-300 µs and is measured as “event length”. Events longer than 300 µs are “gated” out of the analysis as doublets (doublets are un-separated ion clouds arising from highly concentrated cell suspensions). The event length feature is useful in identifying cellular or bead events

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 19, 2024

DMD Fast Forward. Published on October 27, 2014 as DOI: 10.1124/dmd.114.060798
This article has not been copyedited and formatted. The final version may differ from this version.

from background and to discriminate between single events and doublets or cell aggregates. The data can be saved either as text files or the standard flow cytometry FCS 3.0 format that can be analyzed by any standard flow cytometry data analysis software.

Data normalization and analysis. Since CyTOF lacks the light scatter (forward and side scatter) measurements for cells, a combination of event length and DNA stain is used to identify cellular events from debris and to distinguish single cells from doublets or aggregates. Typically cells are stained with two DNA stains; the first DNA stain is a viability stain while the second DNA stain (added at the end of the staining protocol) is used to distinguish cellular events from debris and for discriminating single events from doublets or cell aggregates. The cells can be stained with either a Rhodium-based (Darzynkiewicz et al., 2010) (Ornatsky et al., 2008b; Darzynkiewicz, 2010) or Ir-based DNA intercalator (Ornatsky et al., 2008b; Tinhofer et al., 2012), or a platinum-containing compound such as cisplatin (Ellinger et al., 2014). The Rh- and Ir-DNA intercalators stain by reaching pseudo-equilibrium and neither reach saturation nor are fixable and hence tend to leach out of the cells after staining. In contrast, cisplatin is a fast labeling DNA stain and is fixable (Ellinger et al., 2014).

Continuous operation of the machine leads to signal drift primarily due to changes in plasma temperature and buildup of cellular material in the nebulizer and on the cones. In addition, regular cleaning and maintenance can also lead to day-to-day variation in performance characteristics. To control for signal variation, data normalization beads (typically two metal or four metal calibration beads) are spiked into each sample before sample acquisition. Since these beads are stable over long periods of time, they serve

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 19, 2024

DMD Fast Forward. Published on October 27, 2014 as DOI: 10.1124/dmd.114.060798
This article has not been copyedited and formatted. The final version may differ from this version.

as internal standards to correct for signal drift either over several hours of continuous operation or to correct day-to-day variation. Data is normalized using the “data normalization algorithm” (Finck et al., 2013) available as part of the mass cytometer software or as a free downloadable stand-alone utility. This feature is particularly useful in longitudinal studies wherein sample acquisition and analysis is performed over an extended period of time.

Traditional flow cytometry data analysis software such as FlowJo™ and FCSExpress™ can be used to analyze data generated from mass cytometers. These software programs are particularly useful for user-guided data analysis. However, detailed data analysis of CyTOF data by the manual gating of a series of bidirectional plots could be a daunting task and could compromise identification of new cell phenotypes or functional markers. To address this limitation, several computer guided data analysis packages [e.g., Cytobank (Guerrouahen et al., 2009; Zhang et al., 2009), SPADE (Qiu et al., 2011; Chattopadhyay et al., 2014), viSNE (Amir el et al., 2013), PCA (Newell et al., 2012; Amir el et al., 2013), FLAME (Guilliams et al., 2014), SamSpectral (Wong et al., 2012), FLOCK (Nimmerjahn and Ravetch, 2006), and RchyOptimyx (Aghaeepour et al., 2012)] are available that enable unbiased data analysis in multi-dimensional space. These software are capable of reducing multi-dimensional data into two dimensions without loss of single cell resolution and can be used to identify cell populations, analyze heterogeneity in cell composition and quantify functional responses at the single cell level (Finck et al., 2013). A majority of the above software can either be used in a fully automated mode or in a semi-automated mode with user input. Establishment

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 19, 2024

DMD Fast Forward. Published on October 27, 2014 as DOI: 10.1124/dmd.114.060798
This article has not been copyedited and formatted. The final version may differ from this version.

of the mass cytometer technology and the associated data analysis packages calls for a significant investment in infrastructure and personnel training.

Applications of mass cytometry in PK/PD and biomarker discovery

Adoption of flow cytometry by the pharmaceutical industry in the late 20th century revolutionized drug discovery efforts (Nolan et al., 1999). Wide-spread application of flow cytometry to small molecule drug and biologics research enabled the development of single cell functional assays (Chang et al., 2010), cell line differentiation and sorting, biomarker discovery (Kantor, 2002), assessment of drug toxicity (Lappin and Black, 2003) and pharmacokinetic/pharmacodynamic drug profiles (Dieterlen et al., 2011; Vafadari et al., 2012). As pharmaceutical research is becoming increasingly focused on the mechanism of drug-target interactions and its resulting pharmacology, the importance of cytometry techniques that enable single cell analysis cannot be overstated. The introduction of mass cytometry constitutes a significant advance in multiplexing phenotypic, signaling and functional biomarkers in single cells. The ability to simultaneously measure up to 40 different cellular measures and the advantage to perform multiplex measurement on limited samples volumes makes mass cytometry uniquely suited to contemporary drug development workflows. In this section we present a few examples of emerging and potential applications of mass cytometry in drug discovery.

Deep phenotyping. Phenotyping refers to measurements that identify constituent cellular subsets in complex mixtures of cells (e.g., peripheral blood preparation, splenocytes, bone marrow aspirates, single cell preparation of tissues or tumors) by

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 19, 2024

DMD Fast Forward. Published on October 27, 2014 as DOI: 10.1124/dmd.114.060798
This article has not been copyedited and formatted. The final version may differ from this version.

measuring the expression pattern of cell surface markers (i.e., proteins or their glycosylation patterns) using antibodies conjugated to fluorophores or metal tags. The presence or absence of a protein, its post-translational modifications (e.g., glycosylation) or degree of expression on the cell surface can be used to identify unique cell subsets.

The ability to multiplex up to 40 cellular subset markers in mass cytometry, without a requirement for compensation for overlap in fluorescence signals as needed in conventional flow cytometry, makes mass cytometry an ideal technology to deeply phenotype cells in complex cell populations. This feature was elegantly demonstrated by the seminal paper by Bendall *et al* in which the investigators characterized human bone marrow cells using antibody cocktails that consisted of 13 cell surface subseting markers in combination with 18 intracellular functional markers (Bendall et al., 2011). In a more recent study Horowitz *et al* demonstrated the multiplexing power of mass cytometry to characterize human NK cell subsets (Horowitz et al., 2013). This study revealed that the phenotypic diversity of NK inhibitory receptors (largely controlled by genetics) and activating receptor expression (largely regulated by exposure to environmental cues) produces 6000-30,000 distinct subsets of NK cells in a given individual. This number can be upwards of greater than 100,000 distinct subsets in a given donor panel, a result that was heavily underappreciated before the advent of mass cytometry.

Since typical flow cytometry assays employ 3-8 markers with more advanced labs frequently multiplexing up to 12-14, a study for deep phenotyping often requires staining aliquots of samples with multiple cocktails of antibodies. This approach can also pose

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 19, 2024

DMD Fast Forward. Published on October 27, 2014 as DOI: 10.1124/dmd.114.060798
This article has not been copyedited and formatted. The final version may differ from this version.

challenges in clinical studies where samples are often limiting in volume. Thus the ability to multiplex up to 40 different markers makes mass cytometry ideally suited for phenotypic characterization of pre-clinical and clinical samples in limited sample volumes.

Cellular barcoding and sample multiplexing. Cell barcoding in cytometric analyses was first developed for fluorescence based flow cytometry (Krutzik et al., 2008; Krutzik et al., 2011). The technique involves barcoding cells with a unique combination of amine-reactive fluorescent dyes that are not used as fluorescent tags on antibodies. Since each cell population has a unique fluorescent barcode, the different cell populations can be mixed together, and stained with a cocktail of fluorescently tagged antibodies in a single tube. Using a combination of different intensities of three different fluorescent amine reactive dyes, 62-216 unique barcodes can be generated providing sufficient barcode choices to label each well of a 96-well plate (Krutzik et al., 2008).

Bodenmiller *et al* extended the barcoding technique to mass cytometry by developing amine reactive DOTA polymer based metal-chelating reagents (Bodenmiller et al., 2012). Using seven metals in a binary fashion, the investigators generated 128 unique metal based barcodes to uniquely label each well of a 96 well plate. The wells were subsequently combined into one tube for further sample processing and data collection. The resultant data was deconvoluted to yield results for 96 distinct sample treatments.

Cell barcoding offers several advantages for high throughput assays including a dramatic reduction in data acquisition time, ~100-fold reduction in antibody volumes, and minimizing experimental variation introduced by sample handling (Krutzik et al.,

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 19, 2024

DMD Fast Forward. Published on October 27, 2014 as DOI: 10.1124/dmd.114.060798
This article has not been copyedited and formatted. The final version may differ from this version.

2008). Considering the application to the drug discovery to development continuum, this technique can be combined with functional assays (e.g., phospho-signaling and intracellular cytokine assays) to enable fast and efficient screening of drugs during discovery and extended to multiplexing assays in longitudinal clinical studies. Since current mass cytometers can multiplex up to 40 different metals tags, dedicating a few channels for cell barcoding still leaves upwards of 30 different channels for cellular phenotypic and functional markers.

Functional assays

A. Cell signaling state characterization. Antigen- or cytokine-induced phospho-signaling pathways (Hale and Nolan, 2006; Perez and Nolan, 2006) have been used as sensitive and early markers of cell activation in clinical studies to characterize cancers (Lim et al., 2012), measure intended targets (Strumberg et al., 2005; Crump et al., 2010; Brandwein et al., 2011; Pallis et al., 2012) and assess the off-target effects of drugs (Powers et al., 2011). However, it is being increasingly recognized that a full appreciation of the nature and extent of cellular activation can be best assessed by simultaneously mapping several intersecting signaling pathways in single cells (Bonilla et al., 2008). To date, phospho-protein analysis by flow cytometry has been the tool of choice and may continue to be the choice for targeted phospho-protein measurements in clinical trials. However, the limited numbers of fluorophores available and the incompatibility of protein fluorophores to alcohol based permeabilization in phospho-protein assays limit the multiplexing ability to simultaneously measure multiple phosphorylation targets. In contrast, the metal tags used in mass cytometry are not affected by alcohol based permeabilization reagents making mass cytometry more

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 19, 2024

DMD Fast Forward. Published on October 27, 2014 as DOI: 10.1124/dmd.114.060798
This article has not been copyedited and formatted. The final version may differ from this version.

compatible with highly multiplexed phospho-signaling assays in early drug discovery, biomarker development and assessing biologics immunogenicity.

Several studies have highlighted the versatility of mass cytometry to analyze the signaling state of immune cells. In one study the investigators analyzed the signaling state of normal human bone marrow cells in response to external cytokine stimuli in the presence or absence of signaling inhibitors (Bendall et al., 2011) while in a different study the same investigators used cell barcoding strategies to perform pharmacological measurements of kinase activation and inhibition in cell lines upon exposure to cytokine stimuli in the presence or absence of kinase inhibitors (Bodenmiller et al., 2012). In a recent Immunological Genome Project (ImmGen) collaborative study the investigators utilized the multiplexing power of mass cytometry to combine several cell surface markers with fifteen markers of the cell signaling state across fifteen time points to elucidate the transcriptional landscape of αβ T cell differentiation (Darzynkiewicz, 2012). The success of these studies was singularly dependent on their ability to perform high-dimensional measurements on single cells which required multiplexing upwards of 20-30 independent cellular measurements. Furthermore, clinical trials often necessitate adoption of technology that can multiplex upwards of 20-30 markers on limited sample volumes. Thus the high number of simultaneous cellular measurements, the ability to multiplex samples by cell barcoding and virtual absence of the need for compensation make mass cytometry uniquely suited for kinetic or single time-point assessment of the signaling state of cells.

B. Intracellular cytokine analysis. Intracellular cytokine assays are widely used as functional markers of immune activation in assessing; a) disease state, b) therapeutic

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 19, 2024

DMD Fast Forward. Published on October 27, 2014 as DOI: 10.1124/dmd.114.060798
This article has not been copyedited and formatted. The final version may differ from this version.

response, c) drug immunogenicity, d) response to vaccination or e) autoimmune disease status. Several studies have established the necessity to use multi-parameter (>9 color) flow panels to accurately identify the cellular and functional phenotype of immune cells (De Rosa et al., 2001; Roederer et al., 2004). While multi-color flow cytometry panels may continue to be commonly employed for ICS assays worldwide, the sample limitations and technical expertise required to run complex multi-color flow cytometry experiments can be challenging (McLaughlin et al., 2008a; McLaughlin et al., 2008b). Even the use of pre-standardized panels can only allow for the measurement of not more than 3 cytokines in a panel when combined with several surface markers (Maecker, 2009). The higher capacity of multiplexing and virtual absence of fluorescence style compensation in mass spectrometry makes it an attractive alternative in preclinical and clinical studies that aim to get maximum biomarker measurements on limited sample volumes. A well designed mass cytometry panel that includes a comprehensive set of surface phenotyping markers, activation markers and cytokines would be immensely beneficial in not only assessing the primary endpoints of the study but would also enable early detection of undesirable immune cell activation and cytokine secretion in response to therapy.

C. Cell cycle analysis. Cell cycle analysis is a useful tool in measuring cell activation, malignancy or as a marker for cell health. Cell cycling assays by flow cytometry have traditionally used DNA and RNA intercalating dyes (e.g., propidium iodide, 7-AAD, pyronin Y) in combination with bromodeoxyuridine assays that mark replicating cells (Darzynkiewicz, 2010; Darzynkiewicz et al., 2010). A recent study adapted mass cytometry to analyze stages of the cell cycle in both cell lines and

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 19, 2024

DMD Fast Forward. Published on October 27, 2014 as DOI: 10.1124/dmd.114.060798
This article has not been copyedited and formatted. The final version may differ from this version.

mitogen stimulated PBMC, and demonstrated the feasibility of cell cycle analysis by mass cytometry (Behbehani et al., 2012). This study combined stage-specific cell cycle protein markers (e.g., cyclins, retinoblastoma proteins, and phospho-histones) with DNA dyes (Ir-based DNA intercalators and iodine deoxyuridine) to identify individual stages of the cell cycle. While this strategy opens new possibilities for simultaneous assessment of the cell cycle and detailed cellular phenotype in complex mixtures of cells (e.g., blood from cancer patients), further optimization of mass cytometry techniques is required to achieve the depth of cell cycle analysis currently available using flow cytometry protocols (Darzynkiewicz, 2012).

D. Receptor quantification (ABC). Quantification of cell surface receptor or protein levels (measured as antibodies bound per cell, ABC) is central to theoretical modeling and simulation of drug pharmacokinetics and phamacodynamics. Often *ex vivo* measurements on representative cell lines or primary cells from healthy donors are used to predict the kinetics of drug binding to the intended target, for example a monoclonal antibody directed against a membrane bound receptor, and the subsequent internalization. Currently several flow cytometry based assays are available for the quantification of cell surface antigens of which the Quantibrite method is most widely used. This bead method requires an anti-target antibody conjugated to phycoerythin (PE) at a ratio of one PE molecule per molecule of antibody. Often this requires custom conjugation of therapeutic antibodies to PE and subsequent purification of desired conjugates which results in low yields of the conjugated antibody.

Since mass cytometers can be quantitative, in theory the measurement of receptors on the cell surface can be achieved by direct staining with metal conjugated antibodies

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 19, 2024

DMD Fast Forward. Published on October 27, 2014 as DOI: 10.1124/dmd.114.060798
This article has not been copyedited and formatted. The final version may differ from this version.

without the need for standard beads or conjugation at a pre-determined ratio of metal tag to antibody (e.g. PE needs to be conjugated 1:1 to the staining antibody). Once the ratio of metal bound per antibody molecule is accurately determined using traditional ICP-MS, ABC can be calculated from the staining intensity on cells and the transmission coefficients for the metal tag in the mass cytometer, a number which is determined prior to the ABC measurement.

Wang *et al* compared traditional flow cytometry and mass cytometry based methods to quantify the CD4 number (ABC) of T cells from live or cryopreserved human PBMC. The ABC for CD4 obtained by mass cytometry was consistent with the number obtained with Quantibrite bead-based flow cytometric methods, thus demonstrating the utility of mass cytometry in receptor number quantification. While ABC determination by Quantibrite bead-based methods does not allow for the simultaneous measurement of multiple markers in a single assay, in contrast, with mass cytometry every metal channel can theoretically be used for ABC measurements independent of the other metal tags. This allows for the multiplexing of ABC quantification against either single or multiple receptors in a complex mixture of cells.

Potential role of mass cytometry in drug development

Mass cytometry has numerous emerging and potential applications in various stages of drug research. These assays include drug screening, deep phenotyping, biomarker discovery and pharmacodynamic marker discovery. All assays on the mass cytometer require cells to be fixed as cells are introduced into the machine in water to minimize salt buildup on cones. While this feature is advantageous in minimizing exposure to

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 19, 2024

DMD Fast Forward. Published on October 27, 2014 as DOI: 10.1124/dmd.114.060798
This article has not been copyedited and formatted. The final version may differ from this version.

biologically hazardous material it precludes assays that require use of live cells. Thus while flow cytometry can be used to assay metabolites such as reactive oxygen species or calcium signaling, such assays cannot be currently performed on a mass cytometer.

Discovery and preclinical phase: Currently flow cytometry is widely employed in screening compounds for hits in high throughput systems via cell lines with reporter proteins, sorting (cloning) cells during cell line development, and functional assays and biomarker discovery. While flow cytometry will continue to be used for targeted screening within a compound library, mass cytometry is anticipated to play a major role in elucidating the mechanism of action of a lead compound selected after a prescreen for potential therapeutic benefit. For example, in screening for kinase inhibitors, mass cytometry could be used to more fully elucidate the pharmacology of the inhibitors by measuring the kinetic parameters of the primary target and simultaneously measuring the off-target effects of the inhibitors on other kinases as well as pathway changes up or downstream of the target. In other words, mass cytometry can enable researchers to create a kinase inhibition fingerprint of the potential therapeutic which can be play a major role in go or no-go decisions during early drug research. Furthermore, mass cytometers also allow multiplexing signaling assays with deep phenotyping and other functional assays and thus enable researchers to conduct the preclinical assessment on complex mixtures of primary cells isolated from blood or other tissues. This is expected to become a key contribution to translational pharmacology efforts as the therapeutic and target transition into clinical development.

Clinical phase of drug development: As the lead therapeutic enters clinical trials the focus often shifts to measuring defined biomarkers of therapeutic efficacy, disease

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 19, 2024

DMD Fast Forward. Published on October 27, 2014 as DOI: 10.1124/dmd.114.060798
This article has not been copyedited and formatted. The final version may differ from this version.

severity or unintended effects of the therapeutic. The ability to multiplex over 40 cellular measurements gives mass cytometry a unique advantage in measuring the multiple biomarkers in limited sample volumes. Deep phenotyping combined with functional assays such as intracellular cytokine assays and basal levels of critical phospho-proteins levels (e.g., transcription factors such as STATs, NF-kB) linked to disease or cellular dysfunction can be used in patient stratification to identify target populations for therapy.

In essence, mass cytometry is anticipated to accelerate discovery of PD markers for dose optimization, toxicological markers to assess drug safety, and biomarkers of disease severity and therapeutic efficacy in proof of concept studies. Successful application of mass cytometry in drug development will require a thorough comparison with current standard technologies, rigorous validation of biomarkers assays, ultimately leading to an expected, increased adoption in pharmaceutical industry.

Flow cytometry or mass cytometry: which one to choose and when

Mass cytometry is in its infancy and is yet to be extensively adopted for use in drug development. The establishment of mass cytometers entails significant investment in infrastructure and training of personnel for operation and maintenance of the instrument. Although the workflow for sample preparation and staining is very similar to the workflow currently used in flow cytometry assays, optimization of workflows is still required to achieve high quality results.

A majority of commonly used antibodies for phenotyping, intracellular cytokine and phospho-signaling assays are commercially available as ready-to-use metal conjugated

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 19, 2024

DMD Fast Forward. Published on October 27, 2014 as DOI: 10.1124/dmd.114.060798
This article has not been copyedited and formatted. The final version may differ from this version.

antibodies. These are available either as stand-alone reagents or as pre-formulated kits (eleven marker mouse phenotyping kit, seventeen-marker human phenotyping kit and an eleven marker T cell deep phenotyping kit) that can be either used independently or further combined to yield a >30 marker cocktail. In addition, commercially available antibody labeling kits provide the flexibility to generate custom metal-conjugated antibodies. Almost all antibodies validated for use in flow cytometry can be used in mass cytometry although the staining indices (a function of efficiency of conjugation and the sensitivity of the instrument) could be dramatically different between the two technologies. This can limit the use of certain antibody-metal conjugates in mass cytometers while their fluorescently conjugated counterparts function well in flow assays (Chattopadhyay et al., 2014). Thus, development of an antibody panel for the mass cytometer requires significant time and effort in reagent standardization and optimization.

We hold the opinion that flow cytometry and mass cytometry are complementary technologies each with unique advantages and limitations (see Table 1). The choice of the technology depends on several factors including degree of multiplexing desired, whether the assay requires analysis of live cells or fixed cells, downstream workflows using sorted cells, availability of appropriately tagged reagents (fluorophore tagged or metal tagged), sample size, desired speed of acquisition, access to instrumentation, and availability of expertise for data analysis. For clinical studies, the study design, single or multi-site data acquisition, and GLP certification of instrumentation would further influence the choice of technology.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 19, 2024

DMD Fast Forward. Published on October 27, 2014 as DOI: 10.1124/dmd.114.060798
This article has not been copyedited and formatted. The final version may differ from this version.

Mass cytometry promises to impact the mechanistic study of biologics, pre-clinical assessment of drug toxicity and unwanted immunogenicity, phospho-signaling analysis and biomarker discovery for the biopharmaceutical industry. Based on early publications and the high level of interest in this technology in academia and industry alike, we project an increasing role for mass cytometers in drug development in the near future. Early adoption, rigorous validation and an open collaborative communication between academia and industry was instrumental in advancing instrumentation, reagent availability and assay development for flow cytometry. We believe mass cytometry would also benefit from a similar approach.

Authors Contributions:

Wrote or contributed to the writing of the manuscript: Atkuri, KR, Stevens JC, and Neubert, Hendrik

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 19, 2024

DMD Fast Forward. Published on October 27, 2014 as DOI: 10.1124/dmd.114.060798
This article has not been copyedited and formatted. The final version may differ from this version.

References

Aghaeepour N, Jalali A, O'Neill K, Chattopadhyay PK, Roederer M, Hoos HH, and Brinkman RR (2012) RchyOptimyx: cellular hierarchy optimization for flow cytometry. *Cytometry Part A : the journal of the International Society for Analytical Cytology* **81:**1022-1030.

Amir el AD, Davis KL, Tadmor MD, Simonds EF, Levine JH, Bendall SC, Shenfeld DK, Krishnaswamy S, Nolan GP, and Pe'er D (2013) viSNE enables visualization of high dimensional single-cell data and reveals phenotypic heterogeneity of leukemia. *Nature biotechnology* **31:**545-552.

Andreu EJ, Martin de Llano JJ, Moreno I, and Knecht E (1998) A rapid procedure suitable to assess quantitatively the endocytosis of colloidal gold and its conjugates in cultured cells. *The journal of histochemistry and cytochemistry : official journal of the Histochemistry Society* **46:**1199-1201.

Bandura DR, Baranov VI, Ornatsky OI, Antonov A, Kinach R, Lou X, Pavlov S, Vorobiev S, Dick JE, and Tanner SD (2009) Mass cytometry: technique for real time single cell multitarget immunoassay based on inductively coupled plasma time-of-flight mass spectrometry. *Analytical chemistry* **81:**6813-6822.

Baranov VI, Quinn Z, Bandura DR, and Tanner SD (2002) A sensitive and quantitative element-tagged immunoassay with ICPMS detection. *Analytical chemistry* **74:**1629-1636.

Behbehani GK, Bendall SC, Clutter MR, Fantl WJ, and Nolan GP (2012) Single-cell mass cytometry adapted to measurements of the cell cycle. *Cytometry Part A : the journal of the International Society for Analytical Cytology* **81:**552-566.

Bendall SC, Simonds EF, Qiu P, Amir el AD, Krutzik PO, Finck R, Bruggner RV, Melamed R, Trejo A, Ornatsky OI, Balderas RS, Plevritis SK, Sachs K, Pe'er D, Tanner SD, and Nolan GP (2011) Single-cell mass cytometry of differential immune and drug responses across a human hematopoietic continuum. *Science* **332:**687-696.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 19, 2024

DMD Fast Forward. Published on October 27, 2014 as DOI: 10.1124/dmd.114.060798
This article has not been copyedited and formatted. The final version may differ from this version.

Bodenmiller B, Zunder ER, Finck R, Chen TJ, Savig ES, Bruggner RV, Simonds EF, Bendall SC, Sachs K, Krutzik PO, and Nolan GP (2012) Multiplexed mass cytometry profiling of cellular states perturbed by small-molecule regulators. *Nature biotechnology* **30:**858-867.

Bonilla L, Means G, Lee K, and Patterson S (2008) The evolution of tools for protein phosphorylation site analysis: from discovery to clinical application. *BioTechniques* **44:**671-679.

Brandwein JM, Hedley DW, Chow S, Schimmer AD, Yee KW, Schuh AC, Gupta V, Xu W, Kamel-Reid S, and Minden MD (2011) A phase I/II study of imatinib plus reinduction therapy for c-kit-positive relapsed/refractory acute myeloid leukemia: inhibition of Akt activation correlates with complete response. *Leukemia* **25:**945-952.

Chang RL, Yeh CH, and Albitar M (2010) Quantification of intracellular proteins and monitoring therapy using flow cytometry. *Current drug targets* **11:**994-999.

Chattopadhyay PK, Gierahn TM, Roederer M, and Love JC (2014) Single-cell technologies for monitoring immune systems. *Nature immunology* **15:**128-135.

Crump M, Hedley D, Kamel-Reid S, Leber B, Wells R, Brandwein J, Buckstein R, Kassis J, Minden M, Matthews J, Robinson S, Turner R, McIntosh L, Eisenhauer E, and Seymour L (2010) A randomized phase I clinical and biologic study of two schedules of sorafenib in patients with myelodysplastic syndrome or acute myeloid leukemia: a NCIC (National Cancer Institute of Canada) Clinical Trials Group Study. *Leukemia & lymphoma* **51:**252-260.

Darzynkiewicz Z (2010) Critical aspects in analysis of cellular DNA content. *Current protocols in cytometry / editorial board, J Paul Robinson, managing editor [et al]* **Chapter 7:**Unit7 2.

Darzynkiewicz Z (2012) Cycling into future: mass cytometry for the cell-cycle analysis. *Cytometry Part A : the journal of the International Society for Analytical Cytology* **81:**546-548.

Darzynkiewicz Z, Halicka HD, and Zhao H (2010) Analysis of cellular DNA content by flow and laser scanning cytometry. *Advances in experimental medicine and biology* **676:**137-147.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 19, 2024

DMD Fast Forward. Published on October 27, 2014 as DOI: 10.1124/dmd.114.060798
This article has not been copyedited and formatted. The final version may differ from this version.

De Rosa SC, Herzenberg LA, Herzenberg LA, and Roederer M (2001) 11-color, 13-parameter flow cytometry: identification of human naive T cells by phenotype, function, and T-cell receptor diversity. *Nature medicine* **7:**245-248.

Dieterlen MT, Eberhardt K, Tarnok A, Bittner HB, and Barten MJ (2011) Flow cytometry-based pharmacodynamic monitoring after organ transplantation. *Methods in cell biology* **103:**267-284.

Ellinger B, Silber J, Prashar A, Landskron J, Weber J, Rehermann S, Muller FJ, Smith S, Wrigley S, Tasken K, Gribbon P, Labes A, and Imhoff JF (2014) A phenotypic screening approach to identify anticancer compounds derived from marine fungi. *Assay and drug development technologies* **12:**162-175.

Finck R, Simonds EF, Jager A, Krishnaswamy S, Sachs K, Fantl W, Pe'er D, Nolan GP, and Bendall SC (2013) Normalization of mass cytometry data with bead standards. *Cytometry Part A : the journal of the International Society for Analytical Cytology* **83:**483-494.

Guerrouahen BS, Wieder E, Blanchard EG, Lee FY, Aplenc R, and Corey SJ (2009) Flow cytometric determination of Src phosphorylation in pediatric patients treated with dasatinib. *Pediatric blood & cancer* **53:**1132-1135.

Guilliams M, Bruhns P, Saeys Y, Hammad H, and Lambrecht BN (2014) The function of Fcgamma receptors in dendritic cells and macrophages. *Nature reviews Immunology* **14:**94-108.

Hale MB and Nolan GP (2006) Phospho-specific flow cytometry: intersection of immunology and biochemistry at the single-cell level. *Current opinion in molecular therapeutics* **8:**215-224.

Horowitz A, Strauss-Albee DM, Leipold M, Kubo J, Nemat-Gorgani N, Dogan OC, Dekker CL, Mackey S, Maecker H, Swan GE, Davis MM, Norman PJ, Guethlein LA, Desai M, Parham P, and Blish CA (2013) Genetic and environmental determinants of human NK cell diversity revealed by mass cytometry. *Science translational medicine* **5:**208ra145.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 19, 2024

DMD Fast Forward. Published on October 27, 2014 as DOI: 10.1124/dmd.114.060798
This article has not been copyedited and formatted. The final version may differ from this version.

Kantor AB (2002) Comprehensive phenotyping and biological marker discovery. *Disease markers* **18:**91-97.

Krutzik PO, Clutter MR, Trejo A, and Nolan GP (2011) Fluorescent cell barcoding for multiplex flow cytometry. *Current protocols in cytometry / editorial board, J Paul Robinson, managing editor [et al]* **Chapter 6:**Unit 6 31.

Krutzik PO, Crane JM, Clutter MR, and Nolan GP (2008) High-content single-cell drug screening with phosphospecific flow cytometry. *Nature chemical biology* **4:**132-142.

Lappin PB and Black LE (2003) Immune modulator studies in primates: the utility of flow cytometry and immunohistochemistry in the identification and characterization of immunotoxicity. *Toxicologic pathology* **31 Suppl:**111-118.

Lim SM, Hwang JW, Bae SK, Bae SH, Ahn JB, Rha SY, Roh JK, Chung HC, and Shin SJ (2012) Quantitative analysis of ERK signaling inhibition in colon cancer cell lines using phospho-specific flow cytometry. *Analytical and quantitative cytology and histology / the International Academy of Cytology [and] American Society of Cytology* **34:**309-316.

Maecker HT (2009) Multiparameter flow cytometry monitoring of T cell responses. *Methods in molecular biology* **485:**375-391.

McLaughlin BE, Baumgarth N, Bigos M, Roederer M, De Rosa SC, Altman JD, Nixon DF, Ottinger J, Li J, Beckett L, Shacklett BL, Evans TG, and Asmuth DM (2008a) Nine-color flow cytometry for accurate measurement of T cell subsets and cytokine responses. Part II: Panel performance across different instrument platforms. *Cytometry Part A : the journal of the International Society for Analytical Cytology* **73:**411-420.

McLaughlin BE, Baumgarth N, Bigos M, Roederer M, De Rosa SC, Altman JD, Nixon DF, Ottinger J, Oxford C, Evans TG, and Asmuth DM (2008b) Nine-color flow cytometry for accurate measurement of T

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 19, 2024

DMD Fast Forward. Published on October 27, 2014 as DOI: 10.1124/dmd.114.060798
This article has not been copyedited and formatted. The final version may differ from this version.

cell subsets and cytokine responses. Part I: Panel design by an empiric approach. *Cytometry Part A : the journal of the International Society for Analytical Cytology* **73:**400-410.

Newell EW, Sigal N, Bendall SC, Nolan GP, and Davis MM (2012) Cytometry by time-of-flight shows combinatorial cytokine expression and virus-specific cell niches within a continuum of CD8+ T cell phenotypes. *Immunity* **36:**142-152.

Nimmerjahn F and Ravetch JV (2006) Fcgamma receptors: old friends and new family members. *Immunity* **24:**19-28.

Nolan JP, Lauer S, Prossnitz ER, and Sklar LA (1999) Flow cytometry: a versatile tool for all phases of drug discovery. *Drug discovery today* **4:**173-180.

Ornatsky O, Bandura D, Baranov V, Nitz M, Winnik MA, and Tanner S (2010) Highly multiparametric analysis by mass cytometry. *Journal of immunological methods* **361:**1-20.

Ornatsky O, Baranov VI, Bandura DR, Tanner SD, and Dick J (2006) Multiple cellular antigen detection by ICP-MS. *Journal of immunological methods* **308:**68-76.

Ornatsky OI, Kinach R, Bandura DR, Lou X, Tanner SD, Baranov VI, Nitz M, and Winnik MA (2008a) Development of analytical methods for multiplex bio-assay with inductively coupled plasma mass spectrometry. *Journal of analytical atomic spectrometry* **23:**463-469.

Ornatsky OI, Lou X, Nitz M, Schafer S, Sheldrick WS, Baranov VI, Bandura DR, and Tanner SD (2008b) Study of cell antigens and intracellular DNA by identification of element-containing labels and metallointercalators using inductively coupled plasma mass spectrometry. *Analytical chemistry* **80:**2539-2547.

Pallis M, Abdul-Aziz A, Burrows F, Seedhouse C, Grundy M, and Russell N (2012) The multi-kinase inhibitor TG02 overcomes signalling activation by survival factors to deplete MCL1 and XIAP and induce cell death in primary acute myeloid leukaemia cells. *British journal of haematology* **159:**191-203.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 19, 2024

DMD Fast Forward. Published on October 27, 2014 as DOI: 10.1124/dmd.114.060798
This article has not been copyedited and formatted. The final version may differ from this version.

Perez OD and Nolan GP (2006) Phospho-proteomic immune analysis by flow cytometry: from mechanism to translational medicine at the single-cell level. *Immunological reviews* **210:**208-228.

Powers JJ, Dubovsky JA, Epling-Burnette PK, Moscinski L, Zhang L, Mustjoki S, Sotomayor EM, and Pinilla-Ibarz JA (2011) A molecular and functional analysis of large granular lymphocyte expansions in patients with chronic myelogenous leukemia treated with tyrosine kinase inhibitors. *Leukemia & lymphoma* **52:**668-679.

Qiu P, Simonds EF, Bendall SC, Gibbs KD, Jr., Bruggner RV, Linderman MD, Sachs K, Nolan GP, and Plevritis SK (2011) Extracting a cellular hierarchy from high-dimensional cytometry data with SPADE. *Nature biotechnology* **29:**886-891.

Roederer M, Brenchley JM, Betts MR, and De Rosa SC (2004) Flow cytometric analysis of vaccine responses: how many colors are enough? *Clinical immunology* **110:**199-205.

Strumberg D, Richly H, Hilger RA, Schleucher N, Korfee S, Tewes M, Faghih M, Brendel E, Voliotis D, Haase CG, Schwartz B, Awada A, Voigtmann R, Scheulen ME, and Seeber S (2005) Phase I clinical and pharmacokinetic study of the Novel Raf kinase and vascular endothelial growth factor receptor inhibitor BAY 43-9006 in patients with advanced refractory solid tumors. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **23:**965-972.

Tanner SD, Baranov VI, Ornatsky OI, Bandura DR, and George TC (2013) An introduction to mass cytometry: fundamentals and applications. *Cancer immunology, immunotherapy : CII* **62:**955-965.

Tinhofer I, Hristozova T, Stromberger C, Keilhoiz U, and Budach V (2012) Monitoring of circulating tumor cells and their expression of EGFR/phospho-EGFR during combined radiotherapy regimens in locally advanced squamous cell carcinoma of the head and neck. *International journal of radiation oncology, biology, physics* **83:**e685-690.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 19, 2024

DMD Fast Forward. Published on October 27, 2014 as DOI: 10.1124/dmd.114.060798
This article has not been copyedited and formatted. The final version may differ from this version.

Vafadari R, Weimar W, and Baan CC (2012) Phosphospecific flow cytometry for pharmacodynamic drug monitoring: analysis of the JAK-STAT signaling pathway. *Clinica chimica acta; international journal of clinical chemistry* **413:**1398-1405.

Wong KL, Yeap WH, Tai JJ, Ong SM, Dang TM, and Wong SC (2012) The three human monocyte subsets: implications for health and disease. *Immunologic research* **53:**41-57.

Zhang J, Yang PL, and Gray NS (2009) Targeting cancer with small molecule kinase inhibitors. *Nature reviews Cancer* **9:**28-39.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 19, 2024

DMD Fast Forward. Published on October 27, 2014 as DOI: 10.1124/dmd.114.060798
This article has not been copyedited and formatted. The final version may differ from this version.

Figure 1: General workflow of cell analysis in a mass cytometer. The cells are introduced into the nebulizer via a narrow capillary. As the cells exit from the nebulizer they are converted to a fine spray of droplets which are then carried into the plasma where they are completely atomized and ionized. The resulting ion cloud is filtered and selected for positive ions of mass range 80-200 and measured in a TOF chamber. The data is then converted to .fcs format and analyzed using traditional flow cytometry software. Figure reproduced from Bendall et al., Trends Immunol 2012 (Elsevier license number 3440260897505)

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 19, 2024

DMD Fast Forward. Published on October 27, 2014 as DOI: 10.1124/dmd.114.060798
This article has not been copyedited and formatted. The final version may differ from this version.

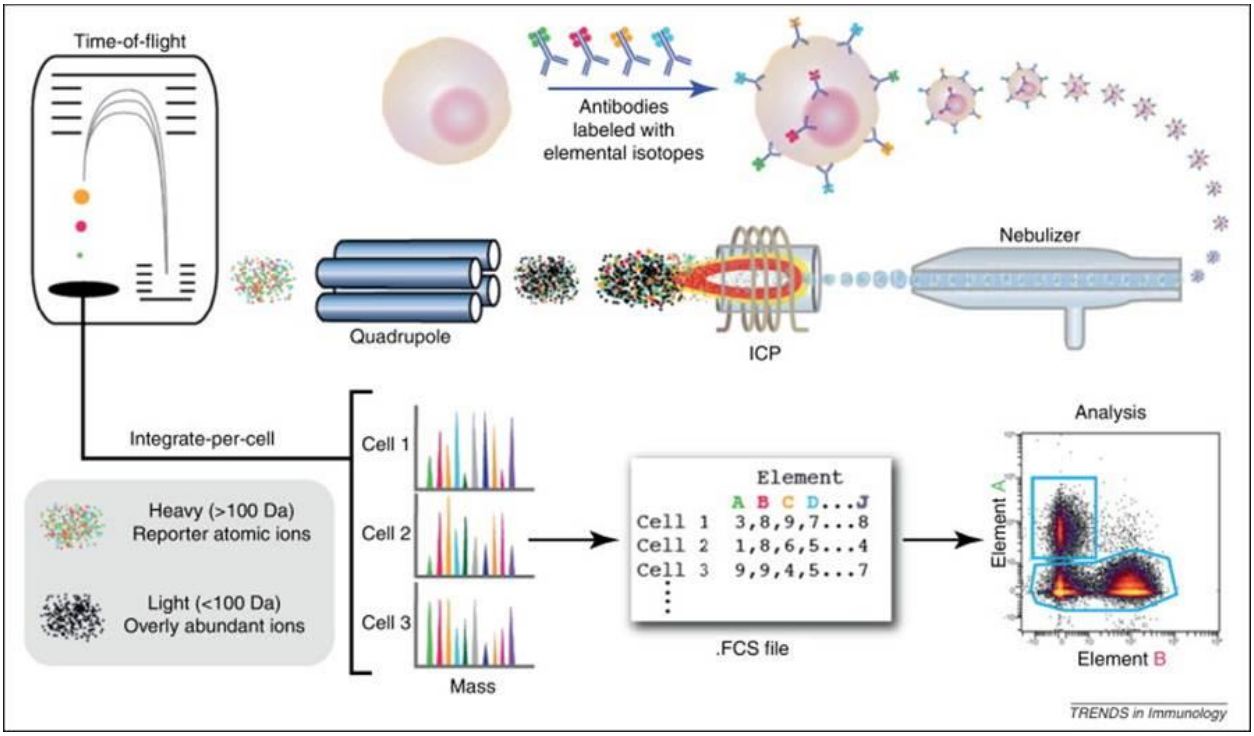
Table 1. Comparison of features of mass cytometry and flow cytometry

Feature	**Flow Cytometry**	**Mass Cytometry**
No. of biomeasures that can be multiplexed	3-18 parameters	Up to 40 parameters (the instrument is capable of multiplexing 100 biomeasures
Compensation	Fluorescence compensation absolutely required	No fluorescence style compensation required. However, correction for isotopic impurities in metal tags and oxides is needed
Status of cells	Both live and fixed cells can be analyzed	Cell are required to be fixed prior to analysis
Cell Sorting	Yes (in sorters)	No
Efficiency of analysis of injected samples	>95%	30-40% of injected cells are analyzed
Speed of acquisition	Up to 60000 cells/sec	Up to 1000 cells/sec
High-throughput compatible	Yes (with a 96 well format)	Yes (with a 96 well format)

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 19, 2024

DMD Fast Forward. Published on October 27, 2014 as DOI: 10.1124/dmd.114.060798
This article has not been copyedited and formatted. The final version may differ from this version.

Figure 1



Downloaded from dmd.aspetjournals.org at ASPET Journals on April 19, 2024