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The Road Ahead: Implementing Mass Cytometry in Clinical Studies, One Cell at a Time

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Disease in humans involves a complicated interplay of pathology expressed on each individual's unique genetic and phenotypic backdrop. The exponential growth of genomic, transcriptomic, and proteomic single-cell technologies provides unprecedented opportunities to capture and understand this complexity. Mass cytometry in particular—a flow cytometry platform that enables simultaneous measurement of over 50 parameters per cell (1–3)—holds significant promise to identify molecular signatures that underlie clinical outcomes (4,5), to help monitor disease progression (6), and to predict therapeutic responses (7,8). However, as the dimensionality of mass cytometry datasets increases, the development of appropriate computational approaches and standardized protocols becomes paramount (9). In this special issue published jointly with *Cytometry A* (10), we explore the necessary steps to transform mass cytometry from a technological tour-de-force to a valuable clinical platform. We highlight six studies that illustrate recent progress. The manuscripts by Yao et al. (11), Corneau et al. (12), and Strauss-Albee et al. (13) emphasize the utility of mass cytometry for identifying cell subsets that capture patient-specific disease attributes in the fields of pulmonology, neonatology, and virology. The studies by Abraham et al. (14) and Vendrame et al. (15) apply visualization and statistical tools in novel combinations to interpret high-dimensional data, while the manuscript by Leelatian et al. (16) describes a standardized protocol to derive isolated cancer cells from solid tissue for analysis by mass cytometry.

Yao et al. (11) demonstrate the utility of multiparameter profiling of airway inflammatory cells in patients with cystic fibrosis (CF) to better understand the complex interactions between an individual's immune state and disease severity. Using a novel assay for the mass cytometry analysis of airway inflammatory cells, the authors identified significant differences in the frequency of immune cell subsets in sputum collected from patients with CF, patients with asthma, and healthy controls. Interestingly, within each group substantial

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variability was observed when stimulating airway monocytes with lipopolysaccharide. These findings provide the basis for future work relating patient-specific signatures of airway inflammatory cells to disease progression and severity.

Strauss-Albee et al. (13) focus on the deep immune profiling of umbilical cord blood samples to better define the natural killer (NK) cell repertoire and functional capacity of newborns. The authors provide a comprehensive overview of neonatal NK cell subsets and their functional attributes. Such characterization is important to better understand disease processes that engage the innate immune system of neonates who are uniquely vulnerable to infections and are unable to mount an efficient immune response to vaccination.

In a third example emphasizing a translational application of mass cytometry, Corneau et al. (12) examine the cell cycle of CD4+ T cells in the setting of HIV infection. Using a combination of cell cycle, differentiation, activation and exhaustion markers, they provide a more nuanced view of the effects of HIV infection on CD4+ T cell cycling and question the traditional definitions of “resting” CD4+ T cells. These results have potential clinical implications, as resting CD4+ T cells are a reservoir for HIV infection difficult to target with commonly used treatments for HIV.

Visualization of multiple cellular attributes across many cell subsets and robust statistical interpretation of high dimensional data remain critical challenges in implementing mass cytometry in clinical studies. The studies by Vendrame et al. (15) and Abraham et al. (14) illustrate these points. Vendrame et al. leverage the distinct strengths of existing computational approaches (i.e., viSNE (17) and citrus (18)] combined with custom-made statistical tools (i.e., correspondence analysis and Friedman–Rafsky significance test) to extensively explore the effects of cytokines on human NK cell phenotype and function. Abraham et al. (14) introduce a radial visualization method called RADVIS, as an elegant solution allowing for an intuitive examination of the multidimensional data. This study comprehensively profiles the immune-modifying effects of glucocorticoid receptor agonists, which are represented as a radial framework readily reflecting differences between samples. These interesting computational approaches build on the growing number of tools that are necessary for the interpretation of high dimensional flow cytometry data (19).

While mass cytometry has predominantly been utilized to study immune cells, this technology can be extended to any tissue amenable to single cell dissociation. The development of standard single-cell preparation techniques for mass cytometry analysis is therefore a critical step towards the widespread implementation of high-parameter flow cytometry in clinical studies. Leelatian et al. (15) precisely define optimal experimental conditions to dissociate solid tumors into isolated cells for mass cytometry analysis. Their approach expands the breadth of clinical applications for mass cytometry and demonstrates the ability to assay anatomically privileged microenvironments that are critical to cancer immune surveillance and oncogenesis.

Together, these highlighted manuscripts introduce new methodological standards and state-of-the-art analytical tools that will advance the clinical application of mass cytometry. Importantly, they demonstrate how the analysis, even in relatively small numbers of patients,

can generate rich datasets that are highly informative with respect to an individual patient. The exquisite resolution afforded by mass cytometry to characterize the cellular expression of a disease in individual patients is perhaps the most exciting prospect. Combined with other omics profiling approaches characterizing specific disease states, single-cell analysis with mass cytometry will likely provide new molecular metrics critical in advancing the field of precision medicine. To do this, future efforts should focus on multiplying the implementation of mass cytometry assays in rigorously designed clinical studies of precisely phenotyped patient populations, developing computational tools to visualize and interpret the mass cytometry dataset, and assembling the statistical framework to link the high-dimensional data to relevant clinical outcomes. The six studies in this issue of cytometry B pave the road towards these goals.

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