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Navigating disease phenotypes – A multidimensional single-cell resolution compass leads the way

Marc Beyer[1,6], Kristian Händler[2], Patrick Günther[2], Kevin Baßler[2], Thomas Ulas[2], Matthias Becker[2], Kathrin Klee[2], Joachim L. Schultze[2,3,6] and Andreas Schlitzer[4,5,6]

Abstract

Cellular phenotyping, in particular immune cell phenotyping, has become an integral part of personalized and stratified medicine approaches in order to facilitate classification of patient cohorts according to proteomic, transcriptomic or genomic information, with the ultimate goal to increase treatment efficiency and outcome. However, choosing the optimal and most informative phenotyping approach to discover novel and predictive biomarkers for patient cohorts has become a major challenge and greatly hampers knowledge gain to successfully develop and tailor new and existing therapies to suitable patient collectives [1]. Recent technological innovations, such as single-cell proteomics (Mass Cytometry) and single-cell transcriptomics have become available which possess the power to measure thousands of features for thousands to millions of cells in parallel, thereby allowing the deep characterization of complex cellular networks in homeostasis as well as perturbations under disease conditions [2]. These multidimensional approaches dramatically accelerate the discovery of novel biomarkers for disease prediction and progression within personalized medicine approaches. These approaches now allow for the characterization of small amounts of patient material both on the protein and on the transcriptome level to allow for an unbiased, high-dimensional, and bioinformatically supported systems biology approach which enables discovery, design and implementation of novel biomarkers into the clinical routine in a rapid fashion. In this review, we will discuss the available technologies and recent applications and scientific advances enabled by these technologies highlighting our view of how to integrate these technologies into translational research to achieve a more reliable, more rapid and better informed approach to molecular phenotyping ultimately achieving the level of knowledge needed to implement personalized medicine approaches for a wider patient base.

Addresses

[1] German Center for Neurodegenerative Diseases (DZNE), 53127 Bonn, Germany

[2] Genomics and Immunoregulation, LIMES-Institute, University of Bonn, 53115 Bonn, Germany

[3] Platform for Single Cell Genomics and Epigenomics (PRECISE) at the German Center for Neurodegenerative Diseases and the University of Bonn, Germany

[4] Myeloid Cell Biology, LIMES-Institute, University of Bonn, 53115 Bonn, Germany

[5] Agency for Science, Technology and Research (A*STAR), Singapore Immunology Network (SIgN), Singapore

Corresponding author: Schultze, Joachim L; Beyer, Marc; Schlitzer, Andreas (marc.beyer@dzne.de), (j.schultze@uni-bonn.de), (andreas.schlitzer@uni-bonn.de)

[6] Authors contributed equally.

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Introduction

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However, choosing the optimal and most informative phenotyping approach to discover novel and predictive biomarkers for patient cohorts has become a major challenge and greatly hampers knowledge gain to successfully develop and tailor new and existing therapies to suitable patient collectives [1].

Recent technological innovations, such as single-cell proteomics (Mass Cytometry) and single-cell transcriptomics have become available which possess the power to measure thousands of features for thousands to millions of cells in parallel, thereby allowing the deep characterization of complex cellular networks in homeostasis as well as perturbations under disease conditions [2]. These multidimensional approaches dramatically accelerate the discovery of novel biomarkers for disease prediction and progression within

personalized medicine approaches. These approaches now allow for the characterization of small amounts of patient material both on the protein and on the transcriptome level to allow for an unbiased, high-dimensional, and bioinformatically supported systems biology approach which enables discovery, design and implementation of novel biomarkers into the clinical routine in a rapid fashion.

In this review, we will discuss the available technologies and recent applications and scientific advances enabled by these technologies highlighting our view of how to integrate these technologies into translational research to achieve a more reliable, more rapid and better informed approach to molecular phenotyping ultimately achieving the level of knowledge needed to implement personalized medicine approaches for a wider patient base.

Advances in protein based biomarker discovery

Traditional low dimensional approaches using technologies such as flow cytometry only had limited success in determining new prognostic biomarkers in complex immune-driven diseases such as rheumatic diseases, systemic lupus erythematosus and inflammatory diseases. This is caused by several factors, including the high complexity of the disease etiology as well as the activation states, which are difficult to deconvolute. Finally, manifestation, progression and severity of disease is driven by inflammatory infiltrates, which are constituted of a variety of different cell types. Complete description of these complex immune cell continuums has proven difficult as conventional technologies, such as flow cytometry, are limited to 12 parameters on a routine basis and the choice of markers is therefore highly biased and investigator-dependent, making the discovery of novel biomarker candidates difficult without detailed *a priori* information [3–5]. Recently, protein-level phenotyping has gained a novel level of complexity as Mass Cytometry and next generation flow cytometry instruments became available. Mass Cytometry, a technology relying on antibodies coupled to stable metal isotopes instead of fluorochromes faithfully detects up to 40 antigens with single-cell resolution [6,7]. Initial studies have utilized this technology to shed light on the complexity and heterogeneity of several important cell populations in both mouse and human during health and disease, illustrating how Mass Cytometry can help to unravel novel cellular subsets and their functionality. Early efforts analyzed the complexity of mouse and human bone marrow paired with a novel way of reducing complexity within these high-dimensional data sets [7,8]. Following this, Becher et al. provided a fine-grained mapping of the murine myeloid cell universe to provide a global myeloid cell framework across eight lymphoid and non-lymphoid tissues to understand heterogeneity and functional diversification between the different tissues analyzed [9,10]. Additionally, applying systems biology principles to the phenotyping approach, Spitzer et al. used Mass Cytometry coupled to a dedicated network analysis approach to understand the dynamics of the steady state immune system. They achieved this by producing so-called scaffold maps as a reference map across a diverse range of homeostatic processes in the human and mouse immune system [11]. Following this seminal paper, this approach was applied to investigate the contribution of systemic immunity in the response elicited by cancer immunotherapy in a mouse model of triple negative breast cancer treated with anti-PD1 antibodies [12]. There the authors showed that a specific systemic immune response is crucial for mounting a sustained localized anti-tumor response utilizing their global phenotyping approach.

As Mass Cytometry-based phenotyping is already applicable to very low cell numbers, it quickly made its way into a pre-clinical human setting [13]. Several landmark studies have applied Mass Cytometry as a proof of principle to human peripheral blood and the human NK (natural killer) cell repertoire [7,14]. Additionally, one focus of recent efforts using Mass Cytometry is to better understand the complexity and multidimensionality of homeostatic processes. To this end, human $CD4^+$ and $CD8^+$ T cells, B cells and innate lymphoid cells (ILC) have been studied in detail [15–18].

Wong et al. investigated the trafficking properties of T cells across eight different human tissues alongside their cytokine production to understand the organization and early functional polarization of different T cell subsets. This revealed a complex tissue-dependent picture of T cell functionality which will help to understand heterogeneity of tissue-specific T cell responses [15,16]. Coupling of Mass Cytometry to a powerful bioinformatics toolbox can inform us about homeostatic processes as illustrated by Pe'er et al., investigating human B cell lymphopoesis. In their work they recapitulated human B cell development from early hematopoietic stem cells towards mature B cells, highlighting developmental checkpoints alongside crucial developmental cytokine requirements [17].

The body's pre-polarized lymphoid rapid response force consisting of ILCs was characterized using Mass Cytometry to understand how their distribution and phenotype differs in various organs and across pathological conditions, such as lung or colorectal cancers. There the authors revealed tissue, activation and disease specific ILC subpopulations, similar to what has been shown in the mouse. That paved the way to study the mechanistic underpinnings of such regulation in