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Review

Systems immune monitoring in cancer therapy

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Abstract Treatments that successfully modulate anti-cancer immunity have significantly improved outcomes for advanced stage malignancies and sparked intense study of the cellular mechanisms governing therapy response and resistance. These responses are governed by an evolving milieu of cancer and immune cell subpopulations that can be a rich source of biomarkers and biological insight, but it is only recently that research tools have developed to comprehensively characterize this level of cellular complexity. Mass cytometry is particularly well suited to tracking cells in complex tissues because >35 measurements can be made on each of hundreds of thousands of cells per sample, allowing all cells detected in a sample to be characterized for cell type, signalling activity, and functional outcome. This review focuses on mass cytometry as an example of systems level characterization of cancer and immune cells in human tissues, including blood, bone marrow, lymph nodes, and primary tumours. This review also discusses the state of the art in single cell tumour immunology, including tissue collection, technical and biological quality controls, computational analysis, and integration of different experimental and clinical data types. *Ex vivo* analysis of human tumour cells complements both *in vivo* monitoring, which generally measures far fewer features or lacks single cell resolution, and laboratory models, which incur cell type losses, signalling alterations, and genomic changes during establishment. Mass cytometry is on the leading edge of a new generation of cytomic tools that work with small tissue samples, such as a fine needle aspirates or blood draws, to monitor changes in rare or unexpected cell subsets during cancer therapy. This approach holds great promise for dissecting cellular microenvironments, monitoring how treatments affect tissues, revealing cellular biomarkers and effector mechanisms, and

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creating new treatments that productively engage the immune system to fight cancer and other diseases.

1. Introduction

The immune system is a complex network comprising localized and specialized tissue sites connected by circulating immune cells. Traditional immunological techniques and approaches have provided a depth of knowledge within each compartment, but struggle to comprehensively dissect the network and its interactions as a whole. In addition to system-wide complexity, each cell subset is itself a 'system within a system', possessing its own hierarchies and heterogeneity. As cancer and immune system cells compete in a complex and continuously evolving cycle [1], understanding the complex rules governing anti-cancer immune responses poses a challenge. Multiple subsets of immune cells are implicated as promoters or inhibitors of the anti-tumour immune responses [2,3]. To dissect and predict anti-cancer immune responses, it is crucial to not only monitor the cellular milieu of peripheral blood, tumour sites, and draining lymph nodes but also monitor the cell surface molecules responsible for cell–cell interactions, the deep immunophenotype of cell subsets of special interest, and intracellular signalling events including post-translational protein modifications, proliferation, cytokine production, and other functional capabilities (Fig. 1).

2. Keeping track of complex immune networks

2.1. Milieu

Each step of the cancer-immunity cycle includes the potential for competition between effector and regulatory cells, and nearly every immune cell subset has been implicated in the anti-cancer immune response [8]. Dendritic cells presenting tumour antigen are required to activate a specific anti-cancer adaptive immune response [1,9]. Effectors like $CD8^+$ and $CD4^+$ T cells, NK cells, and tumour specific antibodies participate in direct killing of tumour cells [10–13]. Controlling these effectors are cells and signalling mechanisms that can check or attenuate immune responses, including regulatory and suppressive cells arising from the T cell [14], myeloid [15], and B cell lineages [16]. These effector and regulatory cells are diverse in phenotype and variable in abundance [17]. While some cell subsets comprise a substantial proportion of the total leukocyte pool (e.g.

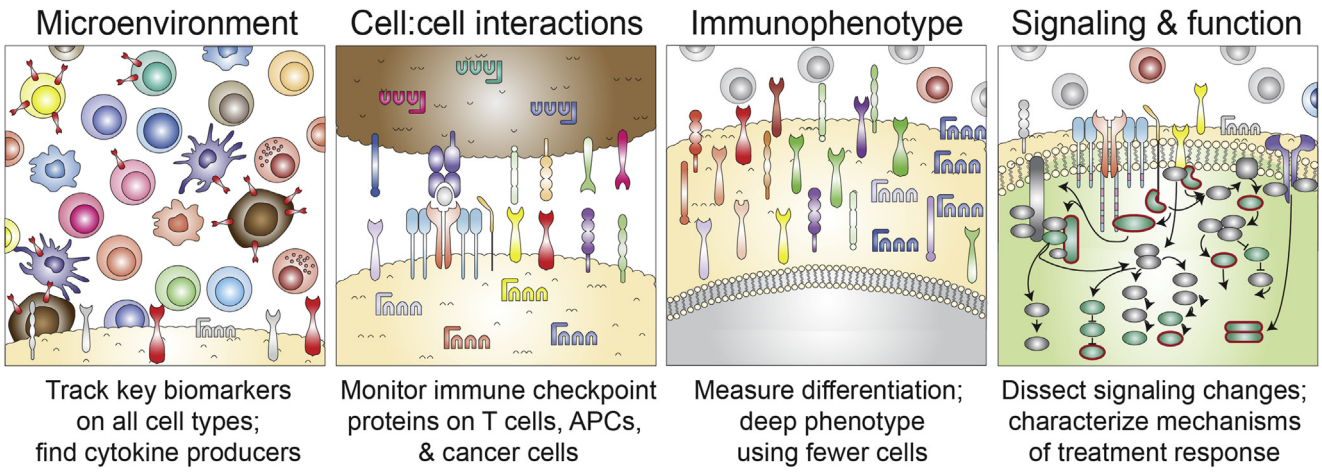

Fig. 1. Focal single cell areas in systems cancer immunology. Mass cytometry and other multidimensional single cell tools can be focused to resolve key biomarkers and mechanisms at different layers of cellular interaction. Most commonly, mass cytometry is used to provide cytomic resolution, meaning that all the different cell types present in a tissue are quantified and phenotyped. As this can generally be achieved with 10 markers on a typical mass cytometry panel, this leaves at least 25 mass channels available for detection of cell interaction markers, immunophenotype, and intracellular signalling [4]. As nearly any cellular property can now be quantified at the single cell level [5], multidimensional cytometry enables biomarkers with complex expression patterns that can vary with cell type and activation state—such as PD-L1 [6]—to be broadly monitored. Another advantage of cytomic approaches is that cells with unusual and unexpected phenotypes present in a patient's tissue sample do not escape detection due to expert bias or overly focused analysis strategies. These advantages of mass cytometry address ongoing needs in cancer and immune biomarker development [7].