Optimizing Longitudinal Peripheral Blood–Based Immune Monitoring in Cancer Patients by Single-Cell Mass Cytometry

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Mass cytometry by time-of-flight (CyTOF) enables high-dimensional, quantitative profiling of millions of immune cells at single-cell resolution by multiplexing dozens of metal-tagged antibodies. This technology provides unprecedented resolution for deconvoluting the composition, abundance, and functional state of peripheral immune cells. However, the impact of longitudinal, peripheral blood–based deep immune profiling on the development of next-generation predictive and prognostic biomarkers for cancer patients remains elusive. In this study, we first assessed the resolution of the Maxpar immune profiling panel in several cohorts of cancer patients undergoing radiotherapy at our institution. Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized blood using Ficoll density gradients at baseline and at least one post-treatment time point. Deep immune profiling was performed with the 30-marker Maxpar Direct Immune Profiling Assay (MDIPA), enabling detection of 37 immune cell subsets in approximately 0.5 million cells, and was expanded by six activation/exhaustion markers (PD-1, TIGIT, CD69, LAG-3, TIM-3, NKG2A) to further characterize immune cell functional states. Data were initially processed with the automated Maxpar Pathsetter software, which identifies 37 immune populations but provides limited support for in-depth exploratory analysis. To extend these analyses and integrate the additional functional markers, we applied a Pathsetter-derived gating strategy in Cytolution, a cloud-based platform for high-dimensional CyTOF data analysis, enabling validation and refinement of Pathsetter results as well as dimensionality reduction (UMAP), clustering, and cell-type annotation. Well-characterized clinical trial cohorts served as a training set to iteratively expand and optimize the panel, which is now being applied to a large retrospective cohort of matched baseline and post-RT samples. These efforts aim to enhance the identification and functional characterization of relevant immune subpopulations and to guide the development of next-generation immune profiling tools to individualize therapeutic strategies for cancer patients, particularly those undergoing high-precision radiotherapy.