

ANALYTICAL CHARACTERIZATION OF A MULTIPLEX PROXIMITY EXTENSION ASSAY PANEL MEASURING 45 CYTOKINES IN PLASMA FROM SUBJECTS WITH NSCLC

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Background Cytokines are core regulators of immune system processes, driving the differentiation, proliferation, and function of immune cells. With many therapeutic strategies focused on leveraging the host immune system, or components thereof, precise quantification of circulating biomarkers has become essential to describe their pharmacodynamic and mechanistic effects. To support these efforts, multiplexed immunoassay panels have been developed, enabling broad spectrum measurement of cytokine profiles. In this study, we characterized the analytical performance of one such panel, based on proximity extension assay (PEA) technology, to measure 45 cytokines in plasma.

Methods Plasma samples (n = 27) from subjects with non-small cell lung cancer (NSCLC), along with age-matched samples (n = 6) from normal subjects were analyzed with the Olink Cytokine 48 kit. Samples were thawed and processed according to manufacturer instructions for incubation, extension, and detection on the Olink Signature Q100 instrument. Selected samples were run in quadruplicate across runs and instruments.

Results Of the 45 cytokines in the PEA panel, 41 had values consistently (detected in >75% of samples) above the assay limit of detection (LOD). When compared to the normal human plasma, 26 cytokines were significantly elevated (p-value <0.05) in plasma from NSCLC subjects, including those known to be associated both with poor (e.g., IL-1 β , IL-6) and good prognosis (e.g., IFN- γ). QC samples (from the screening set) were evaluated with multiple replicates and runs. At mid- and high- levels of cytokines, the median intra-assay CV across all assays was 6.2% which increased to 7.7% in inter-run analysis. Inter-run concordance plots showed that even for low abundance cytokines (IFN- γ), with a 6-fold change (0.2 to 1.2 pg/ml), the correlation was high (>0.94).

Conclusions The results in this study highlight the technical performance of a PEA based multiplex cytokine analysis and demonstrate that it can be effectively deployed to screen complex cytokine profiles to identify clinically relevant changes in low abundant cytokines.

<http://dx.doi.org/10.1136/jitc-2023-SITC2023.0035>