PATIENT STRATIFICATION USING CLINICAL PROTEOMICS – VALIDATED MULTIPLEXED MRM ASSAYS TO QUANTIFY HER2 AND OTHER BIOMARKERS IN CLINICAL FFPE TISSUES

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Background The advent of precision oncology has led a shift towards biomarker-driven clinical trial designs and molecular profiling of individual patients. Identification of patients with the target biomarker profile may be useful in guiding patient selection as an enrichment strategy for clinical trials. Targeted multiple reaction monitoring reaction mass spectrometry (MRM-MS) analysis for multiplexed quantitation of biomarker proteins in FFPE tissue provides direct, more accurate and precise quantitation over current ‘gold standard’ immunohistochemistry (IHC) methods. However, MRM-MS has not yet been broadly applied to clinical trials. In this study, we demonstrate the systematic development, optimization and analytical validation of quantitative, multiplexed MRM-MS assays for robust biomarker quantification in clinical FFPE tissues, including sample analysis under GCLP. Results from an MRM panel targeting 8 clinically relevant biomarker proteins will also be shown, including the measured HER2 levels in FFPE breast tumors classified by IHC as 0, 1+, 2+ or 3+. MRM-MS biomarker panels were developed and optimized for multiplexed quantitation of ≤12 proteins, in which unique peptides derived from each target protein were monitored as a surrogate measure of protein levels. Tumor regions from FFPE tissue sections were dissected using laser capture or microdissection, solubilized, digested with trypsin to generate peptides for analysis, spiked with fixed levels of stable isotope labeled (SIL) peptide standards, and analyzed by MRM-MS. Analytical validation was performed per NCI CPTAC guidelines, including response curves, assay repeatability, selectivity, stability, and reproducibility of endogenous detection. Clinical performance was assessed using commercially sourced FFPE-tumor tissues, including a cohort of breast tumor tissues with a wide range of HER2 expression. Results Assay performance results were protein/peptide dependent, with sensitivity in the low pg/μg total protein range. For HER2, assay linearity was demonstrated over 2.5 to 3 orders of magnitude, with a precision and accuracy of <15% over 3 independent runs. In sample analysis, the MRM-MS was sufficiently sensitive to detect HER2 in 1 μg total protein from FFPE breast tumor classified by IHC as negative (0). Conclusions GCLP-compliant quantitative multiplexed large-scale clinical analysis of protein biomarkers by MRM-MS in FFPE tissue is feasible and enables precise and accurate quantitation of proteins when IHC methods are unsuitable or unavailable. Data can be used for patient stratification, optimization of treatment outcomes, drug resistance prediction, and to support clinical development of novel therapies.

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