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 mass spectrometry (MRM-MS) analysis for multiplexed quantitation of biomarker proteins in FFPE tissue provides direct, more accurate and precise quantification 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 quantitation 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+.

Methods 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 macrodissection, solubilized, digested with trypsin to generate peptides for analysis, spiked with unique peptides derived from each target protein. These peptides may potentially serve as targets for diagnostic or therapeutic applications. Using flow cytometry, immune sera showed strong antibody-binding against B16 (parental cell line of B78 without GD2 expression). These sera were then used on a Nimble Therapeutics’ peptide-array (either whole proteome or a curated list of ~650 proteins) to determine specific antibody-binding sites, and data were analyzed using a dynamic programming method that scans adjacent peptides to determine whether a peptide is bound by antibodies. Proteins were selected if peptides were bound using immune sera but not bound with the sera from naïve or non-responding tumor-bearing mice.

Results Multiple proteins were selectively identified by immune sera that were not detected by sera from naïve or non-responding tumor-bearing mice. When focusing on the whole proteome data, thousands of peptides were targeted by 2 or more mice and exhibited strong antibody binding only by immune sera. We also identified a few proteins that elicited an immune response in the naïve mouse sera that showed a significantly stronger signal in the immune sera of the same mice indicating that the cancer and/or the received therapy strengthened the immune response to these proteins.

Conclusions We are able to detect selective antibody binding to immune sera. However, we are continuing to refine our analytical methods and are further investigating the identified proteins. These peptides may potentially serve as targets for antibody-based or cellular therapies. In addition, we are examining whether some of the identified tumor-specific endogenous antibodies might be used as biomarkers to predict response to our ISV regimen and potentially other immunotherapy treatments.

REFERENCE

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