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Mass spectrometry-based platform for rapid biomarker assay development and validation (peptide multiple reaction monitoring (pMRM))

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The use of biomarkers in drug discovery and development is considered a key solution to the decreased productivity and increased costs in the pharmaceutical industry. The role of biomarkers spans all aspects of drug discovery and development. Biomarkers are essential for the realization of personalized medicine and provide the critical link in translational medicine (bench to bedside research). The commercially available assays for biomarkers will not support all these objectives. Currently, high-quality biomarker assays exist for less than 500 proteins (across all species), a fraction (1-2%) of the total number of proteins encoded by the genomes of key species (example human and rodent). In recent years a mass spectrometry-based approach to protein quantitation known as peptide multiple reaction monitoring (pMRM) has emerged as a promising platform for protein biomarker assays. Quantitation is achieved using surrogate peptides generated from an enzymatic digest of the native protein in a biological sample. The application LC-Multiple Reaction Monitoring mass spectrometry (LC-MRM/MS) technology enables the quantitation of the surrogate peptide in the digested biological sample. The stoichometric relationship between the peptide and the native protein can be used to confer the protein level in a given sample. Ultimately the use of an isotope labeled internal standard peptide yields absolute quantitation data. The primary objective of this workflow is to significantly decrease the cost and timeline for assay development and biomarker validation. pMRM features include the following: High specificity for targeted proteins including post-translational modifications and isoforms; Multiplexing capabilities of greater than 20 proteins in a single assay; Absolute or relative quantification based on mass spectrometry; Not dependent on affinity reagents (no antibodies required); Applicable to pre-clinical and clinical samples; Small amount of sample required and Protein indices to drive the development of accurate, precise and robust protein biomarker assays. Several case studies will be presented utilizing this mass spectrometry-based method and the newly developed biomarker workflow that is synergistic with the approach.

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Directed proteomics of DNA-binding proteins

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The common difficulty with any enrichment technique is developing a method that can be widely applicable and yet still extract a small subset of the proteome. We discuss a high-throughput MALDI-MS method that uses DNA to enrich for transcriptions factors while simultaneously removing interfering sample components. This was accomplished by using a polyvinylpyrrolidone coated MALDI plate which has the unique advantage of enrichment, desalting, digestion and characterization on a single platform. Protein as well as DNA can be analyzed directly from the support. This approach positively identified five transcription factors from nuclear extract using the hTERT promoter using MS/MS of a discrete set of tryptic peptides. On-target enrichment coupled to MALDI-TOF-MS has shown to be fast, sensitive and highly reproducible with low redundancy and can be applied to any protein that binds DNA including those with low affinities.

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