Diagnostic protein quantitation of actionable targets in patient biopsies using clinical mass spectrometry

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Overview

• Characterizing multiple biomarkers in cancer patient tissue allows for personalized cancer treatment.
• Testing multiple targets by IHC or FISH, the traditional approach in oncology, is tissue and time-consuming.
• We developed a clinically-validated multiplex selected reaction monitoring (SRM) assay to simultaneously quantifies multiple biomarker proteins in FFPE tissues.
• Multiplexed SRM assay in tumor tissues ensures all patients who may benefit from targeted therapy receive optimal treatment as early as possible.
• This clinical mass spectrometry assay could conceivably quantify upwards of 100 actionable proteins in a single analysis. Currently, there are 28 analytes that are simultaneously run on our clinical menu.

Methods

• Identify peptides unique to proteins of interest

Results

LT-SRM quantitative reproducibly from archived sections

Figure 3: (A) Precision assessment for measuring HER2 level in breast cancer (red) and GEC (blue) FFPE tissues. (B) Temporal reproducibility of FFPE sections processed and analyzed using LT-SRM at two time points over one year apart (blue, GEC (n=18); red, NSCLC (n=9)).

Quantitative distribution of chemotherapy biomarkers

- HER2 Fresh section (amol/mg)
- HER2 One-year old section (amol/mg)

Figure 4: Distribution of chemotherapy targets in NSCLC.

Conclusions

• Quantitative mass spectrometry is highly specific, absolutely quantitative, and insensitive to pre-analytical variation.
• LT-SRM assay simultaneously quantifies multiple biomarker proteins in FFPE tissues and avoids the triage of the specimens for molecular screenings to ensure all patients whose cancers express clinically-actionable markers have the opportunity to receive treatment as early as possible.
• Developing and clinically validating new LT-SRM assays requires approximately 12 weeks. The OncoPlexDx platform allows for easy “plug-n-play,” multiplexing that new analytes can be added to our menu easily.

Multiplexed proteomic analysis of TNBC tissues identifies potential chemotherapy targets

Figure 6: Lack of TOPO2A may cause poor response to anthracycline-based chemotherapies. ~14% of all TNBC are positive for folate receptor alpha (FRalpha) and negative for TOPO2A (*), suggesting that these patients may respond better to folate targeted therapy than anthracycline treatment. Multiplex targeted proteomics shows that ~30% of the TNBC are positive for both FOLR1 and TOPO2A. Simultaneous assessment of biomarkers give option for combination therapies and second line therapies.

Figure 2: Liquid Tissue®-SRM (LT-SRM) workflow for analysis of proteins from FFPE tissue

• Stable isotope-labeled peptides were synthesized
• Calibration curves were constructed to establish linearity of the assay and lower limits of detection and quantitation for each protein
• Formalin-fixed, paraffin-embedded (FFPE) human tumor tissue was used for assay validation and testing

OncoPlex Diagnostics Clinical Proteomic Menu

AR, ALK, AXL, CK-5, CK-7, EGFR, ERCC1, FGFR2, FR-alpha, hENT1, HER2, HER3, KRAS, MET, MGMT, MSLN, PD-L1, p16, RON, ROS1, RRMI, SPARC, TOP01, TOPO2A, TP63, TUBB3, TTF1