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

Wei-Li Liao, Fabiola Cecchi, Adele Blackler, Sheeno Thyparambil, Eunkyung An, Zhichang Yang, Kathleen Bengali, Alexi Drilea, Joseph Reilly, Marlene Darfler, David Krizman, Jon Burrows, Todd Hembrough

OncoPlex Diagnostics Inc., Rockville, MD; NantOmics LLC, Culver City, CA

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

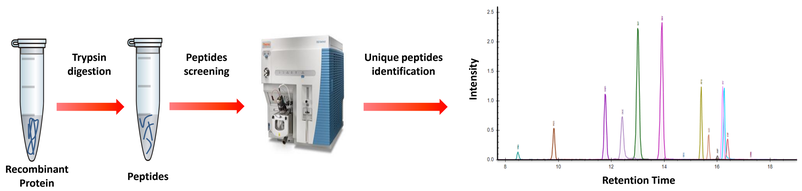


Figure 1: Schematic view of assay development

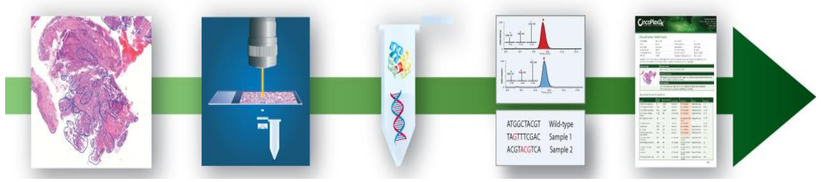


Figure 2: Liquid Tissue®-SRM (LT-SRM) workflow for analysis of proteins from FFPE tissue

- 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, RRM1, SPARC, TOPO1, TOPO2A, TP63, TUBB3, TTF1

Results

LT-SRM quantitative reproducibly from archived sections

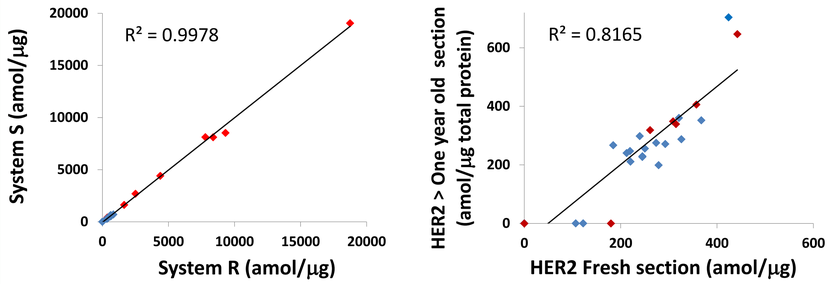
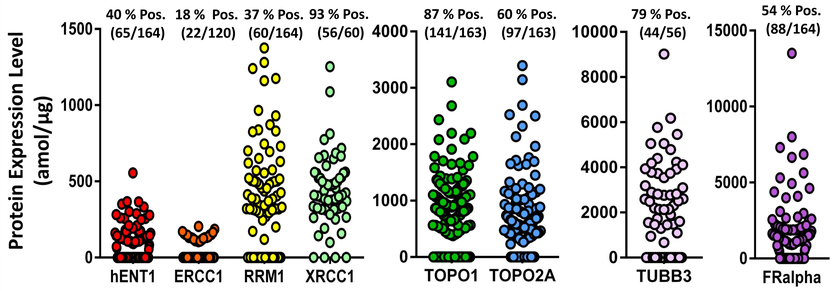


Figure 3: (A) Precision assessment for measuring HER2 level in breast cancer (red) and GEC (blue) FFPE tissues. (B) Temporal reproducibility of FFPE sections processed and analyzed using LT-SRM at two time points over one year apart (blue, GEC (n=18); red, NSCLC (n=9)).

Quantitative distribution of chemotherapy biomarkers



Percentile	hENT1	ERCC1	RRM1	XRCC1	TOPO1	TOPO2A	TUBB3	FRalpha
Minimum	53	104	120	100	380	231	669	468
25 % Percentile	108	119	332	334	725	501	1957	1024
Median	136	132	481	464	925	675	2792	1412
75% Percentile	186	154	684	554	1238	1107	4067	2125
Maximum	555	205	1375	1253	3105	3395	9017	13500

Figure 4: Distribution of chemotherapy targets in NSCLC.

Multiplexed proteomic analysis of NSCLC identifies multiple rare actionable targets

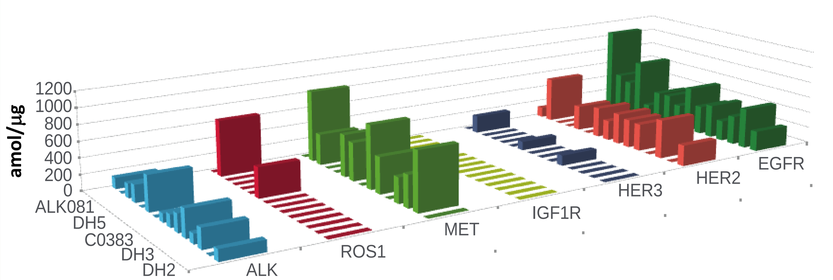


Figure 5: Multiplex proteomic analysis allows for the simultaneous assessment of low-frequency tumor drivers and biomarkers for targeted therapies in NSCLC, including ALK or ROS1 protein from translocation positive cases.

Multiplexed proteomic analysis of TNBC tissues identifies potential chemotherapy targets

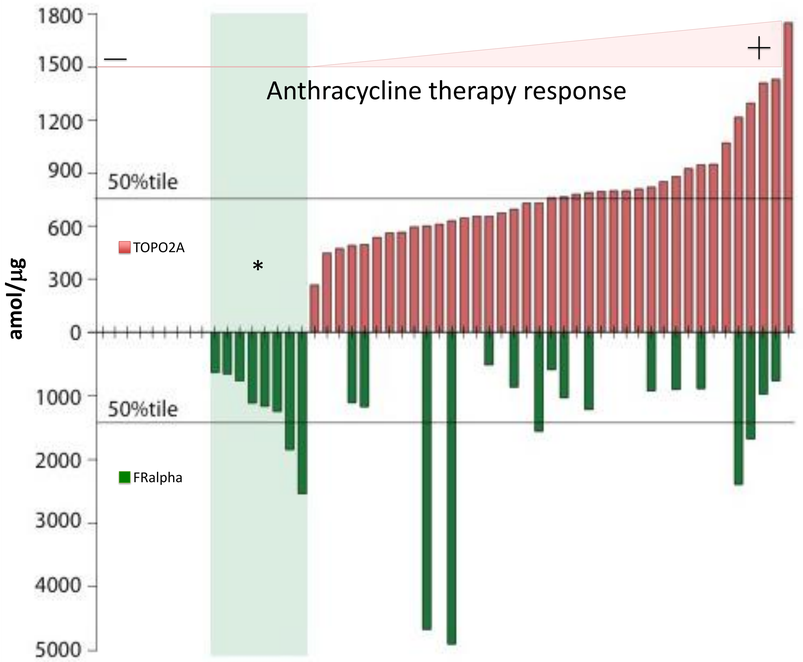


Figure 6: Lack of TOPO2A may cause poor response to anthracycline-based chemotherapies. ~14% of all TNBC are positive for folate receptor alpha (FRalpha) and negative for TOPO2A (*), suggesting that these patients may respond better to folate targeted therapy than anthracycline treatment. Multiplex targeted 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.

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 testings 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.