Quantification of MET Expression Using Mass Spectrometry (MS): Assay Precision and Stability in FFPE Tumor Tissue

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Overview

- **Overexpression of MET in gastroesophageal cancer (GEC) is associated with poor prognosis and potentially predictive of anti-MET therapy. Immunohistochemistry (IHC) is currently used to determine patients eligibility for ongoing MET-specific trials.**
- **However, IHC suffers from antibody non-specificity, lack of quantitative resolution, and, when quantifying multiple proteins, inefficient use of scarce tissue.**
- **Successful MET IHC is hampered by antigenic instability in FFPE sections, limiting its utility to recently cut FFPE sections.**
- **We developed a clinically-validated multiplex MS assay to measure MET protein level in FFPE GEC tissue.**
- **We are running the assay in a CLIA-certified, CAP-accredited laboratory to concurrently assess protein expression levels for MET and other diagnostic and potentially targetable biomarkers, e.g. EGFR, HER2, HER3, RON, KRAS, IGF1R, and PD-L1.**

Methods

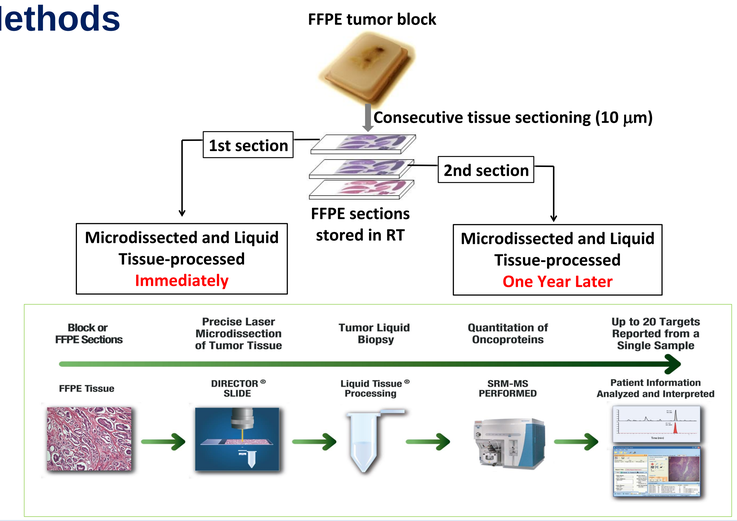

Figure 1: Liquid Tissue®-SRM workflow for analysis of proteins from FFPE tissue.

Analytical Performance of MET Assay

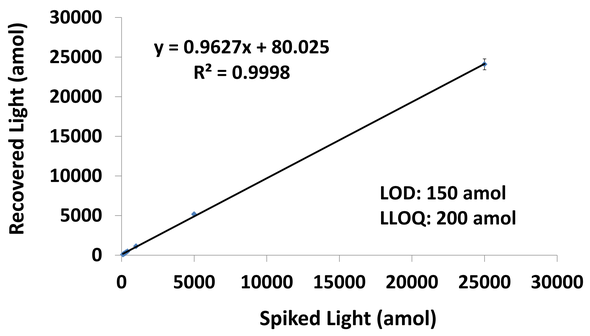

Figure 2: Calibration curve of MET in eukaryotic cell matrix. The calibration curve was built by adding various concentrations (eight non-zero points from 150 amol-25,000 amol) of unlabeled (light) synthetic MET peptide into a matrix obtained from formalin-fixed SKBR3 cells containing 5 fmol of isotopically-labeled MET peptide.

Results

SRM Assay is Highly Concordant with Antibody-based ECL Assay

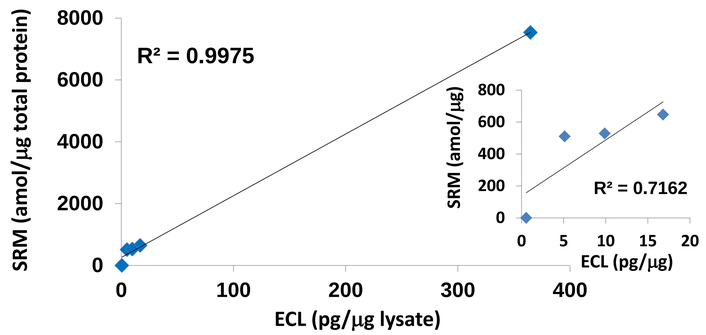

Figure 3. Comparison of MET levels measured in five cell lines using SRM and ECL immunoassay. Overall there was good correlation of the measurements provided by LT-SRM and ECL (R^2=0.9975 when all 5 cell lines were compared and R^2=0.7162 when the four cell lines containing the lowest concentration of MET were compared as shown in inset).

SRM Quantitative Reproducibly from Archival FFPE Sections

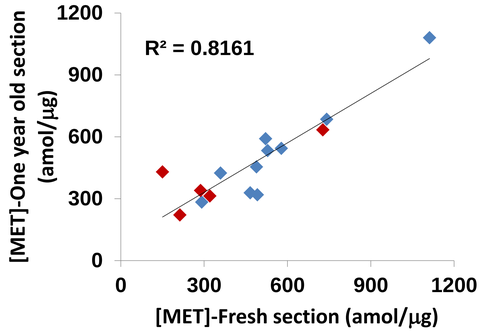

Figure 4. Temporal reproducibility of FFPE tissues processed and analyzed using LT-SRM over one year apart. The R^2 between these two groups of samples was 0.8161 demonstrating that the LT-SRM process provides reproducible results for archival FFPE sections up to 13 months prior to analysis. Blue square: NSCLC tumors (N=10). Red square: GEC tumors (N=5).

SRM Measurement is Highly Concordant with *MET* Copy Number or *MET/CEP7* Ratio

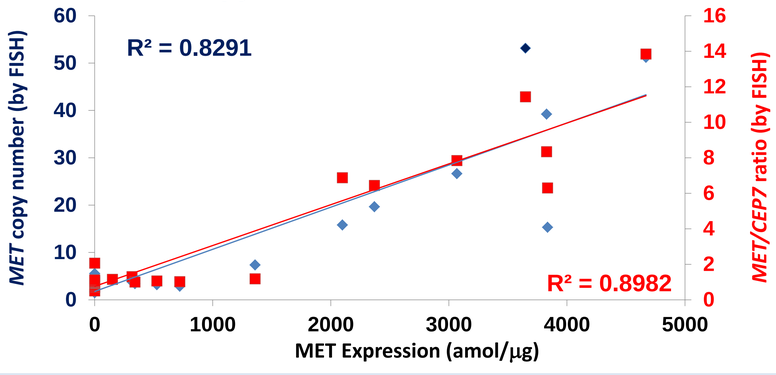

Figure 5. Comparison of MET protein level and *MET* gene copy number in GEC tissues (N=30). The MET SRM result is plotted against *MET* GCN (blue) or *MET:CEP7* ratio (Red). The R^2 between the two sets of measurements were 0.8291 when SRM was compared to *MET* copy number per nucleus and 0.8982 when SRM and *MET:CEP7* ratio were compared.

Quantitation of MET in Clinical FFPE GEC Tumors

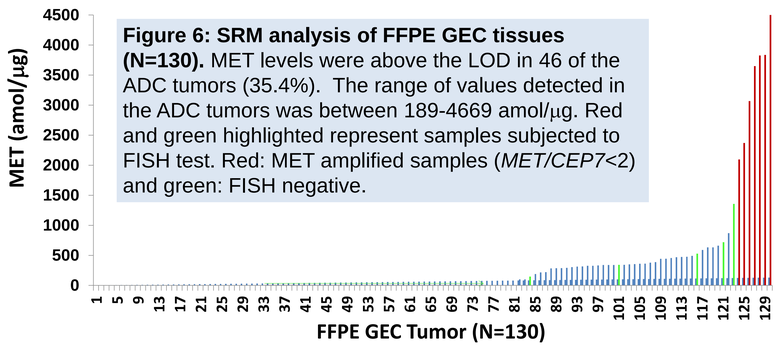

Figure 6: SRM analysis of FFPE GEC tissues (N=130). MET levels were above the LOD in 46 of the ADC tumors (35.4%). The range of values detected in the ADC tumors was between 189-4669 amol/μg. Red and green highlighted represent samples subjected to FISH test. Red: MET amplified samples (*MET/CEP7*<2) and green: FISH negative.

SRM analysis of FFPE GEC tumors (N=130)			
	MET SRM (amol/μg)		
	ND (<LOD)	150-1500	>1500
Sample#	84	39	7
Percentage	64.6%	30%	5.4%

SRM vs. FISH (N=30)	MET SRM (amol/μg)		
	ND	150-1500	>1500
Sample#	17	6	7
MET amplified (MET/CEP7≥2)	0	0	7
Percentage positive	0%	0%	100%

Table 1: Summary for MET expression in GEC tumors (N=130). Upper table shows that 5.4% of GEC have MET level >1500 amol (7/130). Using this value (>1500 amol/μg) as the cut off, the MET assay reliably detected MET amplified GEC tumors with 100% sensitivity and 100% specificity (shown in lower table).

Multiplexed Analysis Allows Better Characterization of Tumors

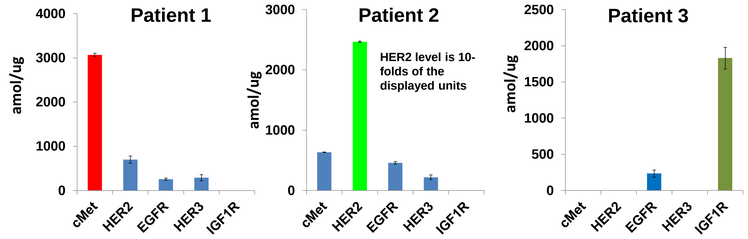

Figure 7: Expression levels of multiple "actionable" biomarkers in three selected GEC tissues. Multiplex SRM assay maximizes information in limited tissue, leading to a better personalized patient care.

Conclusions

- **We have developed a mass spectrometry-based assay to measure the absolute level of MET in clinical FFPE tumor tissues with high level of specificity and temporal stability, and quick turn around time (5 days from time of tissue receiving).**
- **The SRM assay is able to detect MET amplified samples with high sensitivity and specificity as compared to FISH.**
- **The ability to concurrently quantify MET and other relevant proteins represents a novel clinical tool for efficient tumor expression profiling, potentially leading to better informed therapeutic decisions for patients with GEC.**