Quantification of HER2 from Gastroesophageal Cancer (GEC) FFPE Tissue by Mass Spectrometry (MS)

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Overview

- **Trastuzumab has a survival benefit in HER2 positive GEC. Two companion diagnostics, IHC and FISH, are currently used to test HER2 status to determine patients' eligibility for the treatment.**
- **However, both IHC and FISH have limitations. IHC is semi-quantitative, subjective, and sensitive to antigen instability in FFPE; FISH is laborious, expensive, and subjective. Moreover these are low throughput assays.**
- **We developed a clinically-validated multiplex MS assay and evaluated our MS platform on GEC FFPE tissues for HER2 status compared to IHC and FISH.**
- **We are running the assay in a CLIA-certified, CAP-accredited laboratory to concurrently assess protein expression levels for HER2 and other diagnostic and potentially targetable biomarkers, e.g. EGFR, HER3, MET, RON, KRAS, IGF1R, and PD-L1.**

Methods

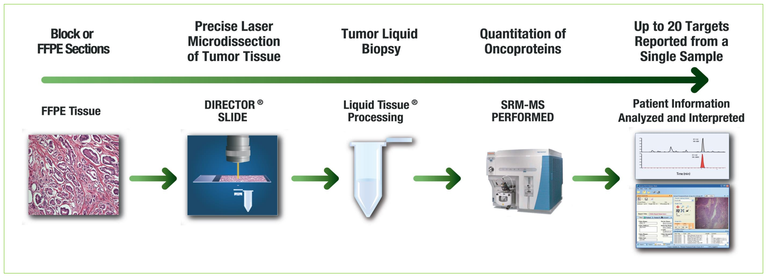


Figure 1: Liquid Tissue®-SRM workflow for analysis of proteins from FFPE tissue.

Analytical Performance of HER2 Assay

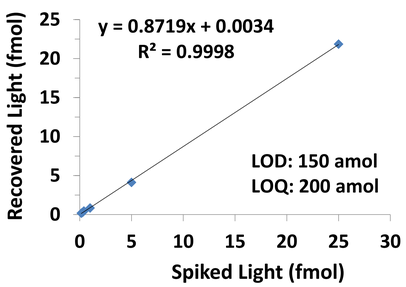
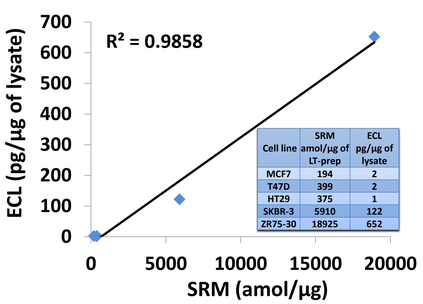


Figure 2: Calibration curve of HER2 in eukaryotic cell matrix. The calibration curve was built by adding various concentrations of unlabeled (light) synthetic HER2 peptide (eight non-zero points ranging from 150 amol to 25,000 amol) into formalin-fixed PC3 cell lysates containing 5 fmol of isotopically-labeled HER2 peptide.

Correlation of SRM Assay with ECL



Cell line	SRM amol/µg of LT-prep	ECL pg/µg of lysate
MCF7	194	2
T47D	399	2
HT29	375	1
SKBR-3	5910	122
ZR75-30	18925	652

Figure 3. Comparison of HER2 levels measured in five cell lines using SRM and ECL immunoassay. There is high correlation of the measurements provided by SRM and ECL (R^2=0.9858). Table lists cell lines information and the raw data.

Results

SRM Quantitative Reproducibly from Archival FFPE Sections

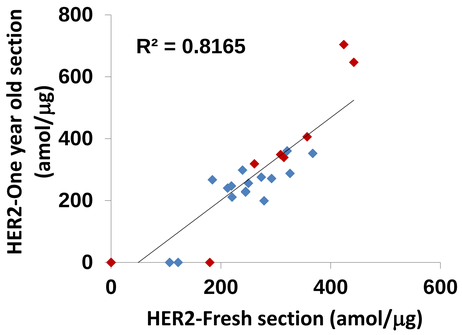


Figure 4. Temporal reproducibility of FFPE tissues processed and analyzed using LT-SRM over one year apart. The R^2 between these two groups (13 months apart) of samples was 0.8165 demonstrating that the LT-SRM process provides reproducible results for archival FFPE sections. Red: GEC tumors (N=18). Blue: NSCLC (N=8).

HER2 SRM Measurement is Highly Concordant with *FISH (MET/CEP17)* Ratio in GEC Cell Lines

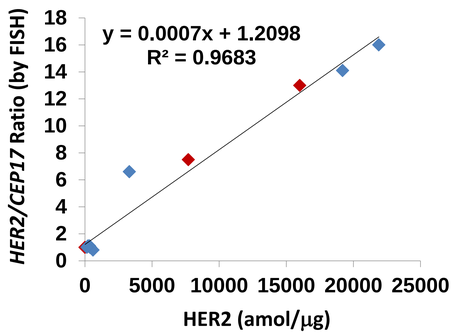


Figure 5. Correlation of HER2 SRM and FISH in GEC cell lines and reference breast cancer cell lines. The HER2 SRM result is plotted against *HER2:CEP17* ratio. The R^2 between the two sets of measurements were 0.9683 in a cohort of 10 GEC cell lines (blue) and 3 reference breast cancer cell lines (red).

ASCO *HER2* FISH Interpretation:
***HER2/CEP17* ≥ 2.0 = FISH+**
Based on the SRM/FISH correlation curve: y=0.0007x+1.2098
When y=2 (*HER2/CEP17* ≥ 2.0 = FISH+), x=1128.9 (amol/µg)

Based on the equation, If *Her2/CEP17* ratio of two or greater is indicative of gene amplification, the corresponding protein concentration would be about 1128.9 amol/µg. Data from a pure cell line might not be directly equated to a clinical sample due to the existence of additional non-tumor cellular material. However, the linear relationship between the protein concentration measured by SRM and the *HER2/CEP17* ratio established by FISH is reasonably expected in tumor samples.

Quantitation of HER2 in Clinical FFPE GEC Tumors

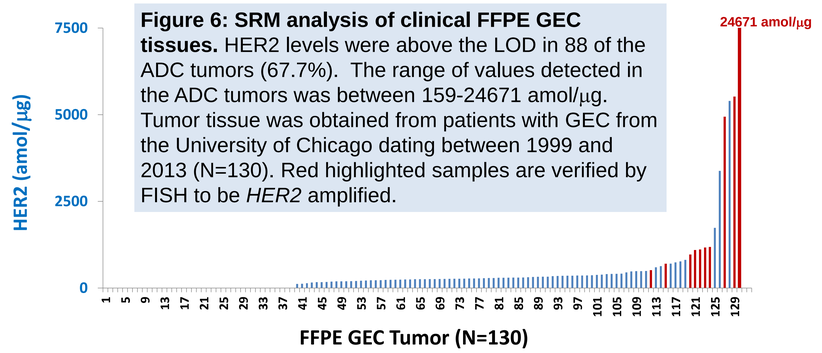


Figure 6: SRM analysis of clinical FFPE GEC tissues. HER2 levels were above the LOD in 88 of the ADC tumors (67.7%). The range of values detected in the ADC tumors was between 159-24671 amol/µg. Tumor tissue was obtained from patients with GEC from the University of Chicago dating between 1999 and 2013 (N=130). Red highlighted samples are verified by FISH to be *HER2* amplified.

Correlation of HER2 SRM with FISH and IHC in Clinical FFPE GEC Tumor

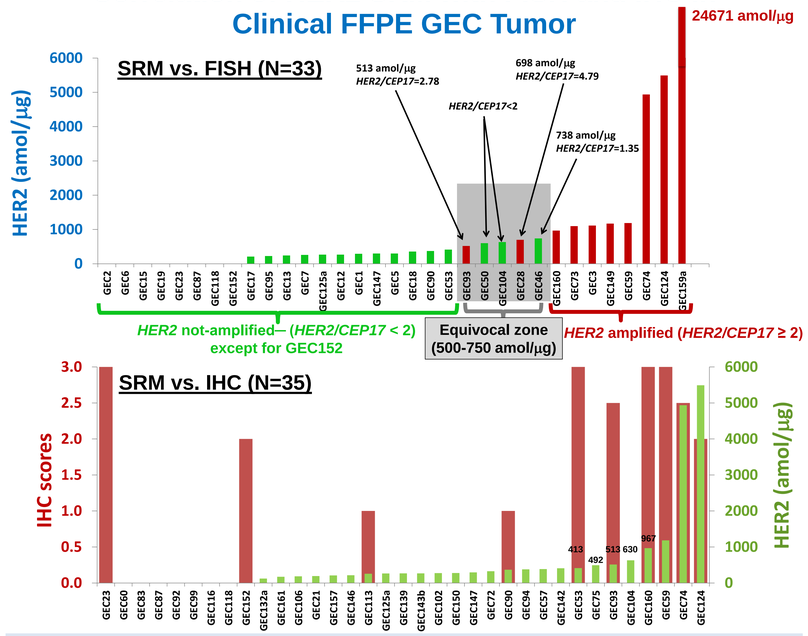


Figure 7: SRM/FISH and SRM/IHC analysis of GEC tissues. Upper graph shows the correlation between SRM and FISH on GEC tumors (N=33). *HER2* is considered amplified if *HER2/CEP17* is >2 by FISH. Lower graph shows the correlation between SRM and IHC on a subset of GEC tumor set (N=35). The data show that HER2 overexpression by SRM is more closely correlated with FISH *HER2* status than IHC HER2 score.

Sample Characterization

SRM analysis of FFPE GEC tumors (N=130)			
	HER2 SRM (amol/µg)		
	<500	500-750	>750
Sample#	111	6	13
Percentage	85.4%	4.6%	10%

SRM vs. FISH (N=33)	HER2 SRM (amol/µg)		
	<500	500-750	>750
Sample#	20	5	8
HER2 amplified (HER2/CEP17≥2)	1	2	8
Percentage	5%	40%	100%

SRM vs. IHC (N=35)	HER2 SRM (amol/µg)		
	500	500-750	>750
Sample#	29	2	4
IHC HER2 2+ or 3+	4	1	4
Percentage	14%	50%	100%

Table 1: GEC sample characterization and HER2 expression. Upper left table: 10% showed HER2>750amol/µg (13/130) and all samples in this category were confirmed to be *HER2* amplified (FISH) or overexpressed (HER2 2+ or 3+).

Conclusions

- **We have developed a quantitative assay to measure HER2 levels in FFPE tissue with high degree of specificity, sensitivity and temporal stability.**
- **The *HER2/CEP17* FISH ratio is linear with the level of SRM HER2(R^2 0.9683).**
- **HER2 expression (any level) was seen in 67.7% of GEC cases.**
- **10% (13/130) of samples were >750amol/µg → and these all were *HER2* amplified by FISH. Our SRM/IHC/FISH correlation results suggest that HER2 overexpression determined by SRM is more closely correlated with FISH *HER2* status than IHC HER2 score.**
- **Correlation of SRM HER2 level and clinical outcome with anti-HER2 therapy is ongoing, in comparison to parallel IHC and FISH scoring.**
- **The ability to concurrently quantify HER2 and other relevant proteins via mutiplex SRM testing represents a novel clinical tool for efficient and expedient tumor expression profiling for clinical application.**

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