Development of a Quantitative Gastroesophageal Cancer SRM assay for Use in FFPE Tumor Tissues

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Introduction

The objective of this study is to develop a multiplexed mass spectrometry based quantitative assay for gastroesophageal carcinoma (GEC) utilizing Liquid Tissue - Selected Reaction Monitoring (SRM).

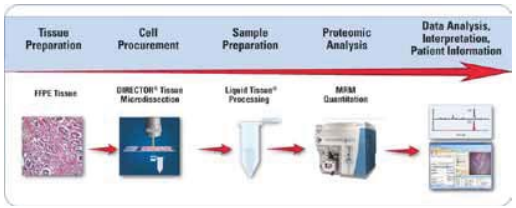
Aberrant over-expression of receptor tyrosine kinases, including the MET, HER2, FGFR, and IGFR families along with other critical downstream oncogenic mediators including KRAS, BRAF, PI3 Kinase and cSRC are known drivers of gastroesophageal cancer (GEC). Expression level of individual oncogene proteins subdivides the disease into distinct molecular subsets and inter-patient tumor heterogeneity suggests that an expedient and reliable protein expression profiling will provide vital information to better personalize cancer care. Moreover, intra-patient tumor heterogeneity from primary tumor to metastatic disease is likely to influence biomarker prediction of response to specific targeted agents.

We preclinically validated the GEC-multplexed SRM assay on cell lines. Representative proteins of interest for oncology (IGF-1R, Her2 and EGFR) were analyzed from parallel processed fixed and unfixed cells using ELISA, ECL and SRM and they demonstrate very high concordance with antibody based analysis.

In a cohort of 137 GEC tumors, many including primary and metastatic tumor tissues, we measured expression level of multiple oncogene proteins from small tissue samples.

GEC-plex Protein Markers:

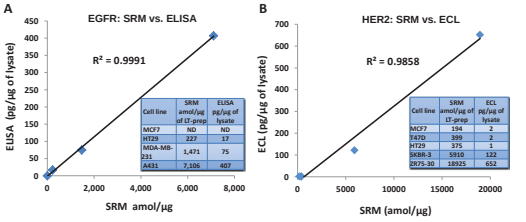
HER2, cMet, Ron, FGFR2, KRAS, SPARC



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

Technical Validation and Correlation of SRM Assay

SRM Analysis is Highly Concordant with Antibody-based Approaches



Cell line	SRM amol/µg of LT-prep	ELISA pg/µg of lysate
MCF7	ND	ND
HT29	227	17
MDA-MB-231	1,471	75
A431	7,106	407

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 1. Comparison of Liquid Tissue–SRM and ELISA or ECL in the quantification of EGFR or HER2 in cell lines. (A) EGFR expression in cells using either SRM (FFPE) or ELISA (Fresh). (B) HER2 expression in cells using either SRM (FFPE) or ECL (Fresh). There is a very high degree of concordance between SRM and ELISA for quantifying EGFR (R^2 = 0.9991) and SRM and ECL for HER2 (R^2 = 0.9858). Table lists cell lines used and raw data.

Preclinical Validation of GEC SRM Assay in Cell Lines

	cMet		Ron_Out		EGFR		HER2		HER3		FGFR2	
	Average	SD	Average	SD	Average	SD	Average	SD	Average	SD	Average	SD
KatoIII	1054.50	39.69	736.50	15.90	867.83	48.95	544.72	82.20	ND		19300	989
HT29	708.83	12.91	262.38	51.06	429.77	31.80	1232.17	81.01	174.00	36.60	ND	
PC3	705.17	19.78	ND		1209.17	48.86	ND		ND		ND	
A431	591.67	20.31	297.20	8.63	11788.33	710.38	521.72	115.85	178.60	8.90	ND	
SNU-16	493.92	4.29	273.78	22.38	970.00	59.98	417.53	6.54	ND		8150	1150
Colo205	ND		315.63	0.95	202.25	13.04	642.83	53.85	ND		ND	
ZR75-30	ND		ND		ND		14590.00	420.74	386.20	27.50	ND	
MCF7	ND		ND		ND		325.85	36.98	385.78	70.77	ND	

Table 1. Eight tumor cell lines from multiple indications were analyzed by SRM to quantitate the expression of cMet, Ron, EGFR, HER2, HER3 and FGFR2. Analytes were quantitated in triplicate 1 µg injections.

Analysis of GEC Multiplex in FFPE Clinical Tissues

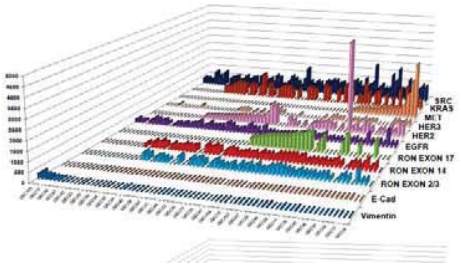


Figure 2. GEC FFPE tissue expression for each peptide within the GEC-Plex as a multivariate analysis, sorted by MET expression from low to high, left to right.

Correlation of GEC SRM with IHC and FISH

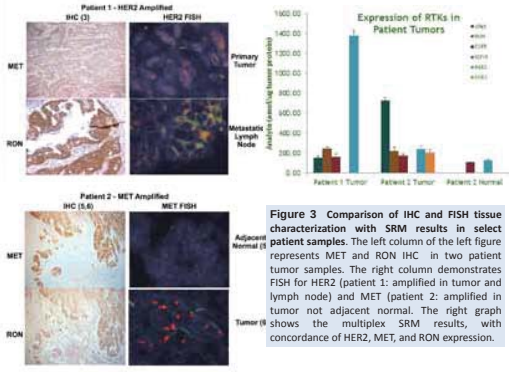


Figure 3 Comparison of IHC and FISH tissue characterization with SRM results in select patient samples. The left column of the left figure represents MET and RON IHC in two patient tumor samples. The right column demonstrates FISH for HER2 (patient 1: amplified in tumor and lymph node) and MET (patient 2: amplified in tumor not adjacent normal. The right graph shows the multiplex SRM results, with concordance of HER2, MET, and RON expression.

Correlation of HER2 SRM and FISH in GEC Cells

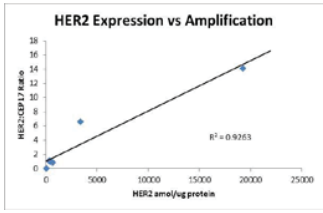


Figure 4.11 GEC tumor derived cell lines were subjected to HER2 FISH analysis or SRM analysis. Correlation of gene amplification in this small cohort was >92%. This cohort includes diploid, high polyploid and amplified samples.

Expression of HER2 in GEC Tissues

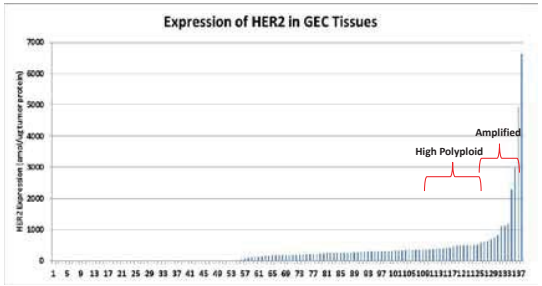


Figure 5. 137 human GEC tissue samples were processed from FFPE sections for the GEC-multiplex. Measureable HER2 expression is seen in ~60% of tumor samples. All samples are being assess by FISH and IHC. Proposed HER2 SRM cutoffs are based on preclinical comparison of HER2 SRM and FISH.

Conclusions

- Liquid Tissue SRM analysis of primary and metastatic colorectal tumors allows for a uniquely specific and quantitative method of measure as many as 20 key oncology targets in multiplex from a single small FFPE tumor sample.
- In advanced GEC tumors, quantitation of high levels of HER2 protein is predictive of trastuzumab response, while elevated cMet levels are required for inclusion in clinical trials. Both assays will be offered as CLIA LDTs in Q1.

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