Mass Spectrometry Based Quantitative Analysis of the HER Family receptors in FFPE Breast Cancer Tissue

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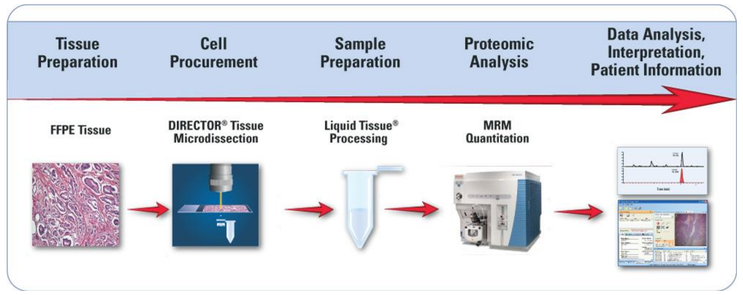
Introduction

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

The human EGF receptor family (HER's) consists of two clinically validated drug targets (EGFR and HER2), a third (HER3) currently under investigation for its possible role in the acquisition of multidrug resistance and a fourth (HER4), the role of which is still matter of debate. Drugs inhibiting EGFR or HER2 show significant antitumor activity in the clinic, however, the acquisition of resistance is a hallmark of these and most other targeted therapies. In the case of EGFR and HER2 targets, one of the emerging resistance mechanisms is the co-expression of HER3. Indeed, recent reports show that inhibition of the PI3K pathway leads to upregulation of HER3, and subsequent resistance.

In order to address these issues, we developed a panel of quantitative mass spectrometric (MS) assays to measure the levels of EGFR, HER2, HER3 and other clinically relevant targets in FFPE breast cancer tissue. Assays performance was preclinically validated on 10 different formalin fixed cell lines, then tested on a cohort of 30 HER2+ breast cancer tissues.



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

SRM Assay Technical Validation

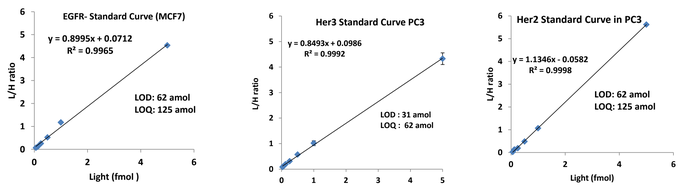
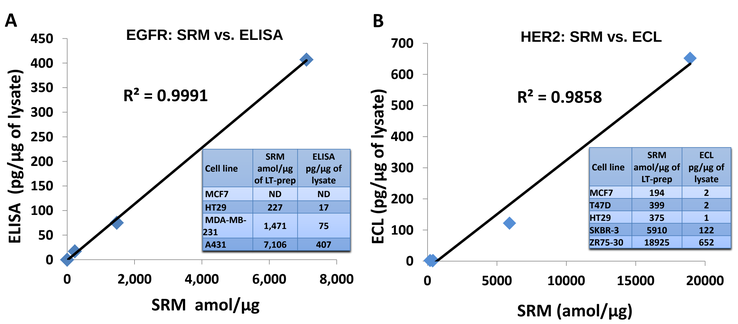


Figure 1. Technical validation of SRM assays for EGFR, HER2 and HER3. Stable isotope labeled control peptides were diluted against light peptides to define the LLOD, LLOQ, accuracy, range and precision of the assay in a eukaryotic *Pfu* matrix.

SRM Analysis is Concordant with ELISA and ECL



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

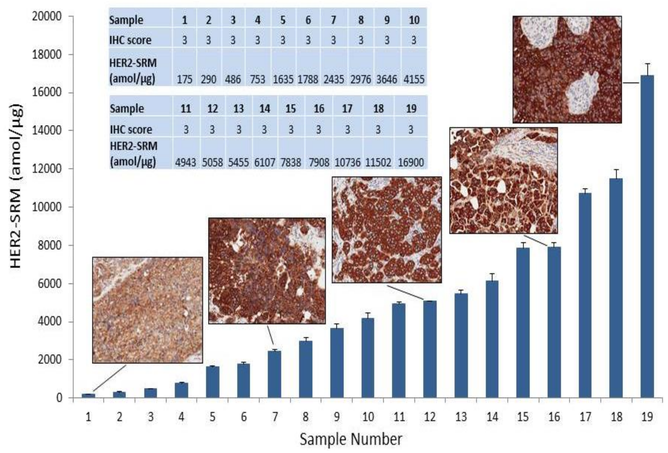
Figure 2. Comparison of Liquid Tissue–SRM and ELISA or ECL in the quantification of EGFR or HER2 in cell lines. (A) EGFR expression in cells using either SRM (FFPE) or ELISA (Fresh). (B) HER2 expression in cells using either SRM (FFPE) or ECL (Fresh). There is a very high degree of concordance between 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 Pan-HER SRM Assay in Cell Lines

		Her3 (amol/µg)			EGFR (amol/µg)			Her2 (amol/µg)		
Cell Line	Type	Avg	SD	CV	Average	SD	CV	Average	SD	CV
HCC827	NSCLC	104.0	27.0	26.0	8213.3	426.8	5.2	0	0	0
A431	Epidermoid	178.6	8.9	5.0	7911.7	165.9	2.1	529.7	50.4	9.5
MDA231	Breast	0	0	0	1661.2	160.4	9.7	0	0	0
PC3	Prostate	0	0	0	977.9	110.5	11.3	0	0	0
HT29	Colorectal	174.0	36.6	21.0	422.7	42.3	10.0	1437.9	83.5	5.8
SkBr3	Breast	146.1	33.3	22.8	371.9	32.3	8.7	7717.5	69.5	0.9
Colo205	Colorectal	0	0	0	211.5	27.0	12.8	524.1	48.5	9.3
T47D	Breast	198.1	35.3	17.8	0	0	0	697.7	113.8	16.3
Zr75-30	Breast	386.2	27.5	7.1	0	0	0	16693.8	912.2	5.5
MCF7	Breast	309.2	23.0	7.4	0	0	0	0	0	0

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

HER2 Analysis of OHN Breast Cancer Tissues vs. IHC



Sample	1	2	3	4	5	6	7	8	9	10
IHC score	3	3	3	3	3	3	3	3	3	3
HER2-SRM (amol/µg)	175	290	486	753	1635	1788	2435	2976	3646	4155

Sample	11	12	13	14	15	16	17	18	19
IHC score	3	3	3	3	3	3	3	3	3
HER2-SRM (amol/µg)	4943	5058	5455	6107	7838	7908	10736	11502	16900

Figure 3 – Analysis of HER2 expression levels among 19 HER2 3+ IHC tissues. There is a 100 fold range of expression based on SRM, where the low expressers correspond to non-amplified HER2 expression based on cell line expression. HER2 IHC was confirmed by HerceptTest in a central lab.

HER2 Analysis of VHIO Breast Cancer Tissues

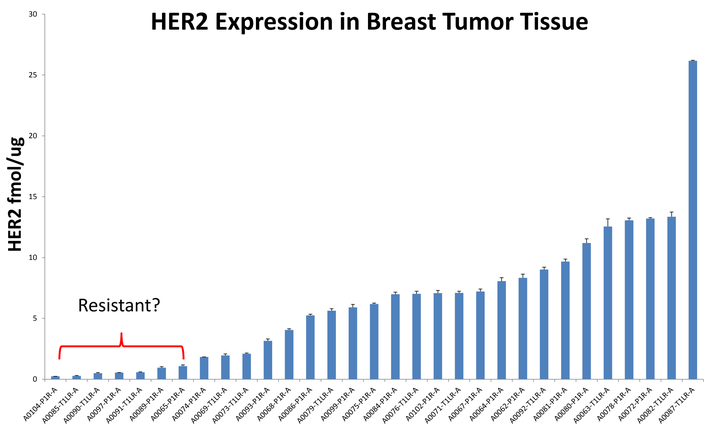


Figure 4. – Confirmation of HER2 expression by SRM in a second cohort of clinical samples. Again, a 100-fold range of expression is seen. At least five samples diagnosed as amplified based on IHC show low-normal expression of HER2. These patients would be predicted to be unresponsive to trastuzumab.

Initial Outcome Data from VHIO Breast Cancer Tissues

Total HER2	Therapy	Clinical Benefit		
0.28	Blinded	Not available (HER2 neg?)	94-8687	A0085-T1LR-A
0.5	Blinded	no response	98-30623A	A0090-T1LR-A
0.55	Blinded	no response	00-9815B	A0097-P1R-A
0.58	Blinded	no response	99-10215A	A0091-T1LR-A
0.95	Blinded		98-27571A2	A0089-P1R-A
1.08	Blinded		08-17559A4	A0065-P1R-A
1.83	Blinded		09-30233A1	A0074-P1R-A
1.96	Blinded		08-28185A1	A0069-T1LR-A
2.1	Blinded	PR	0928397A2	A0073-T1LR-A
3.16	Blinded	PR	99-9938A3	A0093-P1R-A
4.05	Blinded		08-24450A2	A0068-P1R-A
5.25	Blinded		95-11155R4	A0086-P1R-A
5.46	Blinded		11-2992A3	A0079-T1LR-A
5.91	Blinded	no response	01-19858A1	A0099-P1R-A
6.19	Blinded		09-501575A5	A0075-P1R-A
6.99	Blinded	SD > 6 months	94-12960	A0084-P1R-A
7.02	Blinded		10-10891A5	A0076-T1LR-A
7.08	Blinded	PR	03-15268A4	A0102-P1R-A
7.1	Blinded		09-6125A1	A0071-T1LR-A
7.21	Blinded		08-24500A1	A0067-P1R-A
8.07	Blinded	Progression	08-7581A1	A0064-P1R-A
8.34	Blinded		08-861A3	A0062-P1R-A
9.01	Blinded		99-27460A	A0092-T1LR-A
9.67	Blinded		92-10361A3	A0081-P1R-A
11.2	Blinded		11-3417A1	A0080-P1R-A
12.55	Blinded		08-2418A4	A0063-T1LR-A
13.06	Blinded	PR	11-499A6	A0078-P1R-A
13.2	Blinded	Progression	09-27304A3	A0072-P1R-A
13.35	Blinded	CR	93-11666R4	A0082-T1LR-A
26.17	Blinded	SD > 6 months	98-22969A1	A0087-T1LR-A

Figure 5. Clinical outcome, where available, on the 30 HER2+ tumor samples. Three of the five tumors expressing <1fmol/ug of HER2 protein were resistant to trastuzumab treatment. These data suggest that HER2 analysis by IHC is inadequate for the diagnosis of a subset of patients.

Pan HER Analysis of Breast Cancer Tissues

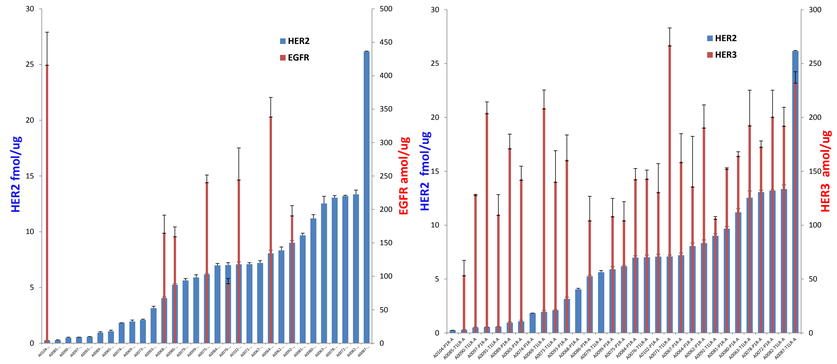


Figure 6. 30 breast cancer tissue samples from HER2+ patients were analyzed to measure expression of HER2, HER3 and EGFR. HER2 is shown on the left axes in fmol/ug of tumor protein. HER3 and EGFR are shown on the right axes in amol/ug of tumor protein.

Conclusions

- Liquid Tissue SRM analysis of HER2 amplified breast cancer tumor tissues allows for specific and quantitative method to multiplex analysis of pan-HER receptors.
- Patients diagnosed as HER2 amplified by IHC (IHC3+) were shown to have at least a 100fold range of expression when this quatitative method was used. Many actually express low levels of HER2 proteins. These patients would be expected to be nonresponsive to trastuzumab. Indeed in a 30 patient cohort from VHIO 3 of the lowest expressing patients where response data were available were confirmed non-responsive to trastuzumab.
- Demonstration of quantitation of other HER receptors from the same cell milieu is a major step forward for understanding their contributions to the tumor's cellular biochemistry in terms of both growth/response and resistance. It may help to establish cutoffs for HER family drugs.
- A larger study with complete outcome data is being designed to assess the utility of measuring HER family protein levels to predict trastuzumab response/resistance.

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