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, IGFR1, 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) to formalin-fixed PC3 cell lysates containing 5 fmol of isotypically-labeled HER2 peptide.

Correlation of SRM Assay with ECL

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 (R²=0.9858). Table lists cell lines information and the raw data.

Figure 4: Temporal reproducibility of FFPE tissues processed and analyzed using LT-SRM over one year apart. The R² 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).

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 R² 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).

Figure 6: SRM analysis of clinical FFPE GEC tumors. 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 were verified by FISH to be HER2 amplified.

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 >2 by FISH. Lower graph shows the correlation between SRM and IHC on a subset of GEC tumor set (N=45). The data show that HER2 overexpression by SRM is more closely correlated with FISH HER2 status than IHC HER2 score.

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(R²=0.9683).
- HER2 expression (any level) was seen in 67.7% of GEC cases.
- 10% (13/130) of samples were >750amol/µg and all these 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 multiplex SRM testing represents a novel clinical tool for efficient and expedient tumor expression profiling for clinical application.