Overview

- Trastuzumab had a survival benefit in HER2 positive GEC. Two companion diagnostics, IHC and FISH, are currently used to test HER2 status to determine patient’s 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 (selected reaction monitoring – SRM) on GEC FFPE tissues for HER2 status evaluation compared to IHC and FISH, along with multivariate analysis of other oncogenic protein expression levels including Met-SRM, Egfr-SRM, Her3-SRM, Her2-SRM.

Methods

A preliminary HER2 SRM was validated in archived tissues from FFPE and liquid biopsies of patients with GEC cell lines and tissues, respectively. The total ion chromatogram for the light and heavy isotope labeled peptides, with b) the transition ion list and transition names for each peptide. The SRM quantitative reproducibility from archived sections. The precision assessment and temporal reproducibility of FFPE sections processed and analyzed using LT-SRM at two time points over one year apart. Blue: GEC (n=15), red: NSCLC (n=5).

Results

Quantification of HER2 in Cell Lines & Correlation with Amplification

HER2 expression in Gastroesophageal Cancer (GEC) FFPE Tissue using Mass Spectrometry (MS) and correlation with HER2 gene amplification.

HER2 expression was measured in GEC FFPE tissues (n=139) ranging <150 amol/ug to 1500 amol/ug at 99% quantitation. The two red lines (1 and 2) represent the HER2 expression falling into this equivocal range (n=9/54, 16%). (A) Comparison of HER2 gene expression levels using LT-SRM, FISH and IHC. (B) Correlation of HER2 expression levels with clinical receptor status (left, IHC2+ versus SRM). Both assays resulted in an equivocal zone in overlapping patient’s expression levels.

Conclusions

- HER2-SRM is a quantitative assay in clinical FFPE tissues with high specificity and sensitivity.
- The HER2/CPEP7 FISH ratio is linear with the level of HER2-SRM, particularly when adjusting for HER2 FISH heterogeneity and Her3-SRM and Met-SRM.
- HER2 expression (any level) was seen in 71.2% of GEC cases. 10.1% (14/139) of samples had HER2 >750 amol/ug all were HER2 FISH amplified (with an observed wide expression range within FISH+; IHC3+ cases).
- SRM/IHC/FISH correlation results suggested that HER2 overexpression determined by SRM is more closely correlated with FISH HER2 status than IHC HER2 score.
- EquivalencyHER2-SRM (450-750 amol/ug) occurred only 9.4% (13/139) vs IHC2+ 36% (44/122), with better FISH+ PPV.
- Correlation of SRM HER2 level to clinical outcome on anti-HER2 therapy is ongoing, compared to IHC and FISH scoring.
- The ability to concurrently multiplex HER2 and other relevant proteins via SRM testing represents a refined clinical tool for efficient/ expedient tumor expression profiling for clinical application.