Session PO.CH03.01 - Proteomics and Biomarker Discovery

5128 / 11 - Quantitative mass spectrometry of HER2 protein levels reveals high variability within HER2 IHC grades

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Presenter/Authors

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Disclosures

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Abstract

About 15% of breast cancers are HER2 over-expressing (HER2 IHC 3+ or IHC 2+/ISH+)), but another 45% have low levels of HER2 (HER2 IHC 2+/ISH- or IHC 1+), and these patients are not currently approved for treatment with trastuzumab. Recently, a new HER2 ADC, DS-8201 showed anti-tumor activity, not only in patients with HER2 over-expressing breast cancer but also in HER2 low expressing tumors in whom to date, there are no effective anti-HER2 therapies indicated. FDA-approved HER2 in vitro diagnostic tests have recognized several limitations including effects of pre-analytical variable (fixation affects antibody sensitivity), limited dynamic range of chromogen-based IHC, and subjectivity in interpretation of the HER2 score. Additionally, the cut-off values (percentage of cells to be positive) defining HER2 positive have been changing over time. Therefore, more accurate, sensitive, precise and objective assays to better identify patients who may benefit from anti-Her2 treatment therapies (e.g DS-8201) are needed. To address this gap, we evaluated upcoming technologies targeted MS and QRT-PCR and aim to compare expression with current diagnostic HER2 tests in FFPE samples

Using selected reaction monitoring mass spectrometry (SRM-MS), we quantified proteins from formalin-fixed, paraffin-embedded tissue samples that had been classified as HER2 0, 1+, 2+ or 3+ by IHC (n=107). HER2 protein concentration measured by SRM-MS was compared between patients in different HER2 IHC classifications using an ANOVA, adjusting for multiple comparisons.

HER2 concentration (measured by SRM-MS) was progressively increased according to HER2 IHC grouping (i.e. lowest concentration in HER2 0 samples, highest in HER2 3+ samples). HER2 levels were significantly elevated in 2+ vs. 0 (2.2-fold increase, $p < 0.05$) samples, and trended higher in 2+ vs. 1+ (1.6-fold increase, $p = 0.07$) and in 1+ vs. 0 (1.4-fold increase, $p = 0.17$) samples. About 73% of samples scored as IHC0 had detectable Her2 by SRM-MS (from 168 to 623 amol/µg). Among HER2 IHC 0 samples, ~15% (7/47) had HER2 concentrations above the median levels for the 1+ group. Similarly, 19% (3/16) 1+ samples had HER2 levels above the median for the 2+ group. About 20% of samples co-expressed either ERBB1 and/or ERBB3. Simultaneously from FFPE sections we quantified protein level of payload response and resistance markers (MDR, MRP1, Topo1 and SLFN11).

We used an objective multiplex non-antibody-based method to quantify multiple targets from FFPE tissue. SRM-MS revealed a range of HER2 expression over 100 orders of magnitude and identify markers of payload

response or resistance in the same assay. The differences seen in payload markers expression could affect therapeutic efficacy and may suggest differing responses to Her2-targeted ADC, depending on tumor biology. Multiplexed quantitative MS could be used to accurately predict which patients will derive the most benefit from Her2-ADC therapy based on the specific biology of their tumor. These studies are ongoing.