Three Best Poster Abstracts – Oral Presentations

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Levels of amphiregulin and epiregulin expression correlate with overall survival in colorectal cancer treated with cetuximab

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Background: To date, there is no validated marker of response to cetuximab (CTX) in colorectal cancer (CRC). A recent microarray study has shown high mRNA expression of the EGFR ligands amphiregulin (AREG) and epiregulin (EREG) in metastases of CRC responding to monotherapy CTX. We measured AREG and EREG mRNA expression levels in primary CRC of patients treated with cetuximab and irinotecan and correlated expression levels with response and survival.
Methods: We measured AREG and EREG mRNA expression by real-time quantitative RT-PCR (TaqMan) on FFPE tumor samples from patients with irinotecan-resistant metastatic CRC treated with CTX and irinotecan. Gross dissection to isolate tumor tissue was performed. Relative expression levels were calculated using the ÄCt method in which average values of duplicate reactions were compared, with GAPDH serving as the internal reference. As cut-off value to distinguish high from low expression, we used the median of all ÄCT values of 73 samples. RECIST criteria for tumor response were used. Differences in response rates between patients with low and high ligand expression were evaluated by means of a two-sided Fisher's Exact test. The overall survival (OS) was estimated by the Kaplan–Meier method and compared between groups with use of the log-rank test.
Results: For 54 patients, we correlated ligand expression with response. Of the 20 responders (complete/partial response), 16 (80%) had a high AREG and 19 (95%) a high EREG mRNA expression level. Of the 34 non-responders (stable/progressive disease), only 6 (17.6%) had a high AREG and only 13 (38.2%) a high EREG expression level ($p < 0.0001$; responders vs. non-responders). For 73 patients, we correlated ligand expression with OS. Patients with tumors with high AREG expression (39/73) had a median OS of 43.29 weeks (95% CI [36.12-50.46]) compared to 19.57 weeks (95% CI [10.78-28.36]) in the low expression group ($p < 0.0001$). Patients with tumors with high EREG expression (34/73) had a median OS of 42.14 weeks (95% CI [34.62-49.66]) compared to 24.30 weeks (95% CI [21.36-27.24]) in the low expression group ($p = 0.006$).
Conclusions: We demonstrate that high levels of amphiregulin and epiregulin mRNA expression, as evaluated by real-time quantitative RT-PCR on FFPE tumor samples, are associated with objective response and significantly longer overall survival in patients with metastatic CRC treated with cetuximab in combination with irinotecan.

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Systematic validation of novel breast cancer progression-associated biomarkers via high-throughput antibody generation and application of tissue microarray technology: an initial report

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Background: Omic-based discovery approaches, such as DNA microarray-based gene expression profiling, have provided powerful tools for biomarker identification. The limited availability of antibodies is a key barrier to the application of tissue microarrays (TMAs) for biomarker identification. To circumvent this issue, the Swedish Human Proteome Resource (SHPR; www.proteinatlas.org) is using a high-throughput method to generate affinity-purified, mono-specific antibodies against all non-redundant human proteins. Here, we provide an initial report in relation to use of SHPR-derived antibodies generated against a collection of candidate breast cancer progression-associated biomarkers.
Methods: We applied a novel bioinformatic technique to in-house, and publicly available DNA microarray datasets relating to breast cancer invasion and metastasis and identified a cohort of several hundred candidate progression-associated biomarkers. Of these genes, 137 targets were then selected for antibody production within the SHPR. In February 2007, an initial 32 antibodies were released for extended analysis. Antibodies were screened via Western blot (WB) analysis and immunohistochemistry (IHC) against a range of normal (48) and tumor tissues (20), as well as cultured cells (66), represented on TMAs. Highly optimised antibodies were screened against a TMA constructed from a cohort of 524 consecutive breast cancer cases diagnosed between 1988 and 1992. The median age was 65 years and median follow-up time regarding disease-specific and overall survival was 11 years. All invasive TNM stages were represented within the cohort.
Results: One quarter of the 32 initial antibodies show differential staining between normal and breast cancer tissue. PDZK1, an estrogen-responsive gene associated with good prognosis at the transcript level in breast tumors showed discrete banding at the predicted molecular weight (57 kDa) in estrogen receptor (ER)-positive breast tumor cell lines. The differential expression of PDZK1 protein between ER+ and ER− cell lines was also confirmed by IHC. Expression of PDZK1 protein was associated with improved breast cancer-specific survival ($p = 0.0247$), ER positivity ($p = 0.041$) and low grade ($p = 0.002$).
Conclusions: We have developed a comprehensive biomarker pathway that extends from discovery through to validation on TMA and is yielding clinically relevant biomarkers.