Conclusions: Analytical performance validation shows compatibility of this oncodiagnostic device with its intended use. The results of the multi-centre clinical performance evaluation demonstrated that the *MLL* Fusion*Chip*™ gave reproducible and reliable results in a range of clinical laboratories, and provided accurate results when compared with those obtained by more conventional methods. Further studies are necessary to evaluate the clinical utility of the molecular classification of acute leukemia, and whether this tool will facilitate optimised use of molecular targeted-based therapeutics for the different *MLL* partners, based on their unique molecular targets.

586 POSTER

Response assessment classification: effect of multiple measurement criteria and parameters

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Background: To evaluate the response assessment classication in patients with metastatic oesophageal cancer using unidimensional and bidimensional criteria. For unidimensional criteria the impact of short axis, rather than long axis measurement will also be assessed. Tumor eccentricity, a new parameter, of response and change was also assessed.
Material and Methods: 22 patients with metastatic oesophageal cancer involved in a phase II trial were included in this study. Ninety-three lesions were assessed at baseline and followed on serial CT scans. Response assessment was calculated with unidimensional and bidimensional tumor measurements. To measure the eccentricity of tumor shape (the degree of divergence from a perfect sphere), a new parameter, "EF", was calculated (EF = $\sqrt{}$) where LPD = largest perpendicular diameter, and MD = maximal diameter).
Results: There was a 27.3% disagreement rate in the best overall response categorization between unidimensional and bidimensional measurements. The average change in lesion EF was 0.45 for patients with agreement and 0.8 for patients with disagreement between unidimensional and bidimensional measurements. This difference was statistically significant ($p<0.001$). By utilizing the short axis for lymph node measurement there was no disagreement between bidimensional and unidimensional short axis measurement.
Conclusion: There is a significant difference in response assessment between both measurements methods which may be due in part to the change in eccentricity of tumors measured over time with EF. The greater the change in eccentricity the greater the discordance. The short axis measurement better predicts the tumor response when compared to the bidimensional response. This factor could be critical to the assessment of overall tumor response on any therapy.

587 POSTER

Development of proximity based assay to detect and quantify erbB (or Her) receptor dimerization in formalin fixed-paraffin embedded tissue sections

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The EGFR, ErbB2, ErbB3 and ErbB4 are members of the Type I receptor tyrosine receptor kinase family (also known as Her or ErbB family). Overexpression of these receptors found in a number of cancers (e.g. breast, colon, ovarian, Lung) has aggressive phenotype with poor prognosis. However, recent clinical trials have shown that overexpression of erbB receptors alone is not sufficient to predict patient response. A thorough analysis of the activation status in the erbB pathway will likely achieve better prognosis.
Immunohistochemistry (IHC) is the most commonly employed method used to evaluate the expression of receptors in formalin fixed-paraffin embedded (FFPE) clinical samples. Although IHC provides valuable information about the relative level of expression and subcellular localization of a particular target, it is not quantitative. The scoring of results is also very subjective and prone to error among independent observers. Consequently, there is a need to develop assays to circumvent these issues. Here, we report the development of novel proximity based assays to detect and quantify various Her dimers in formalin fixed-paraffin embedded (FFPE) samples. In this assay, the sample was first deparaffinized and rehydrated by regular xylene/ethanol/water protocols. After antigen retrieval, the sample was incubated with a mixture of erbB specific antibodies conjugated either with reporter etag ™ or a chemical scissor. The reporter etag were then released based on its proximity to the scissors. The released etags were separated by capillary gel electrophoresis and quantified by etag-informer ™ software. Assays were developed to quantify the levels of EGFR/EGFR, EGFR/Her2, Her2/Her2 and Her2/Her3 homo- and hetero-dimers in the FFPE sample. Tublin was used as an internal reference control for the total cellular content. The assays were first developed using ligand (heregulin or EGF) stimulated tumor cell line pellets in FFPE sections. The assays were then used to detect and quantify Her dimers in various xenograft models and clinical patient tissue samples. The data demonstrated the simultaneous detection and quantification of Her receptor expression, dimerization and phosphorylation in a single tissue section. Inter- and intra-assay reproducibility was 8–20% (n=8). The validity of the detection and quantification of Her dimers was independently confirmed by etag assay analysis of the Her dimers in FFPE sections and cell lysates from the same samples.
We conclude that the etag assays are simple, sensitive and provide a quantitative assessment of various Her dimers from the same sample. They can be used to determine the activation status of erbB/Her receptor in clinical sample for the correlation with disease prognosis or response to targeted therapies.

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Prevalence of erbB/Her dimerization in breast cancer

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Background: Epidermal growth factor receptors (EGFRs) and signaling pathways activated by these receptors have been implicated in the development of breast cancer. The EGFR family includes, human EGFR-1 (Her1), human EGFR-2 (Her2), human EGFR-3 (Her3), and human EGFR-4 (Her4). It is well established that ligands like EGF and HRG bind to the extracellular region of the EGFR monomers and promote receptor dimerization. Receptor dimerization leads to increased tyrosine kinase activity resulting in uncontrolled cell proliferation and inhibition of apoptosis. Determining the dimerization patterns in breast cancer may provide useful information for the treatment of breast cancer. Hence, we have developed eTag ™-multiplexed assays, to detect and quantify the different types of erbB/Her dimers in breast cancer tissues.
Materials and Method: We have analyzed 61 snap-frozen human breast tissues comprising of 31 ductal or lobular carcinoma samples and 30 normal samples. Of these, 8 tumors and 8 normal breast tissues were matched with the same donor. Using the proximity-based multiplexed eTag assays, we determined the dimerization profiles in these tissues.
Results: ErbB/Her dimerization was detected only in tumor tissues but not in normal breast tissues, whether matched with the same donor or not. Out of the 31 tumor samples analyzed, Her1/2 dimers were detected in 19 tumor samples while 24 tumor samples had Her-2/3 dimers. We also found that all tumor samples had higher Her-2 levels compared to normal breast samples. In addition, we detected Her-2/2 homodimers in 23 out of 31 tumor samples. Our quantitative dimerization assays showed the presence of different amounts of Her-1/2 and/or Her-2/3 and/or Her-2/2 dimers in different breast cancer tissues of either ductal or lobular types.
Conclusion: As erbB/Her dimerization levels are associated with activation status of the receptor, eTag technology can be a valuable prognostic tool both, in stratifying patients with breast cancer for targeted therapy as well as for assessing the activation state of the receptor during the course of patient treatment. These assays represent the first quantitative methods that can provide receptor activation signatures for the erbB/Her family.

589 POSTER

Gene expression profiling defines new molecular classes and predicts response to adjuvant anthracycline-based treatment in breast cancer patients: development of a biochip to predict prognosis and improve clinical management of breast cancer

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Background: The significant genetic heterogeneity amongst breast cancer patients continues to be one of the primary obstacles to effective clinical diagnosis and management. Recent advances in microarray technology have contributed to enhanced understanding of the underlying diverse molecular mechanisms that drive tumorigenesis in individual patients, and emerging technologies based on gene expression profiling (GEP) may provide clinically useful tools to improve the standard of care in breast cancer. However, the translation of large-scale GEP technologies from the research to clinical setting has yet to be achieved. In this study, we describe the development of the Breast Cancer Profile*Chip* (BCPC), a device based on GEP for molecular characterization and management of breast cancer.