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.

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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.

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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.

Methods: 220 patients with poor prognosis, were selected from Institut Paoli-Calmettes (IPC) and separated into independent identification (n=159) and validation (n=55) datasets. All tissues were analyzed on nylon cDNA microarrays (Discovery*Chip*) containing 9000 genes. Signatures for phenotypic markers and predictive gene signatures for response to anthracyclines were calculated on a training set (n=159) by t-test with moderate correction for multiple variable testing. The number of discriminatory genes was optimized using a leave-one-out approach. All analyses were performed with Profile*Software* ™ Corporate (Ipsogen). Signatures were identified, then validated for ER, PR, HER2/neu, and EGFR on 1 or 2 independent datasests (IPC, n=55; Centre Léon Bérard, n=110). Gene signatures were subsequently cross-validated at both the RNA and protein level by RQ-PCR and IHC. Sensitivity and specificity for all gene signatures were calculated in comparison to standard histopathological, IHC, biochemical/ligand binding, and/or fluorescence *in-situ* hybridization (FISH) techniques.
Results: Identified signatures comprised of 30 to 150 genes were transferred to the BCPC, a biochip containing 900 cDNA's. An amplification method based on linear PCR was developed to ensure clinical feasibility of the BCPC. Expression profiles are determined with 1 μg total RNA with high reproducibility (mean CV =5%), and automated quantification and analyses performed with the integrated software Profile*Software* Cancer. All derived gene signatures are quantitative, sensitive, and highly reproducible (mean CV = 5%).
Conclusions: These results collectively demonstrate that gene expression profiling can be utilized to enhance molecular classification of breast cancer, and may provide clinically valuable information to augment existing pathological analysis. Further, the transfer of gene expression profiling results to clinical tools such as the BCPC may contribute to improved breast cancer management. Additional studies are required to evaluate the clinical utility of the BCPC.

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Reduction of EGFR/Her-1 dimerization and phosphorylation in colorectal tumors and normal skin correlates with clinical response to Tarceva

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Background: Evaluation of the normal skin from Tarceva-treated patients enrolled in a phase I clinical trial had previously shown reduction of EGFR phosphorylation following treatment, via immunohistochemistry. In addition to using receptor phosphorylation as an activation indicator, we have sought to measure EGFR homodimerization and determine if it can be used as a pharmacodynamic marker of Tarceva activity in solid tumors and skin.
Materials and Methods: Tumor samples of liver metastasis of colorectal cancer and the corresponding normal skin samples were collected before the Tarceva treatment and 7 days after the initial administration in a phase II clinical trial. Of the 18 patients analyzed, 7 achieved disease stabilization (SD) and the rest suffered from disease progression (PD). Formalin-fixed paraffin-embedded (FFPE) sections from tumor or skin biopsies were analyzed with proximity-based multiplexed eTag assays for the levels of EGFR expression, receptor homodimerization and phosphorylation. The changes in the activation status were then compared with the clinical outcomes of the patients.
Results: The majority of tumor samples showed some decrease in the EGFR activation status as measured by receptor homodimerization and phosphorylaton. It was remarkable that one patient with SD showed complete ablation of EGFR/EGFR homodimerization and phosphorylation in the tumor sample and 83% reduction of EGFR phosphorylation in the skin biopsy. Overall, patients with higher levels of reduction were more likely to have SD and conversely, patients with lower levels of reductions were more likely to have PD. We categorized the reduction of Her1 phosphorylation in both skin and tumor biopsies, and the reduction of EGFR/EGFR homodimerization in tumor biopsies, as "High" and "Low" by applying a cut-off value. The correlation of "High" reduction levels of EGFR phosphorylation in skin biopsies with SD showed a sensitivity of 85.7% and specificity of 100% (p=0.0004, n=18). The "High" reduction levels of EGFR phosphorylation in tumor biopsies correlated with SD with a sensitivity of 100% and specificity of 88.9% (p=0.007, n=13). Interestingly, the "High" reduction levels of EGFR/EGFR homodimerization in tumor biopsies also correlated with SD with a sensitivity of 100% and specificity of 66.7% (p=0.049, n=13).
Conclusions: The correlation of the reduction in EGFR phosphorylation in skin biopsies as well as in tumor samples with clinical response has no precedence for Tarceva or other EGFR-TKI (tyrosine kinase inhibitor). The detection of reduction of EGFR/EGFR homodimerization in the Tarceva-responsive tumors has, to our knowledge, also not been reported before. Such quantitative analysis will help validate the on-target effects of the drug in humans, and presents a feasible surrogate marker approach for monitoring the response in tumor or skin biopsies.

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p53 mutation with transdominance activity is an independent prognostic factor for endometrial cancer

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Mutation of p53 tumor suppressor gene plays a key role in the carcinogenesis and progression of many different malignancies including endometrial cancer. In order to elucidate the importance of the type of p3 mutation and its resulting biological function in endometrial cancer, we organized the German-Japan Collaborative Study Group for Endometrial Cancer in 2001 and have collected 92 RNA samples of endometrial carcinoma, of which 49 were from German patient and 43 were from Japanese patients. We surveyed transdominance of p53 mutations and analyzed its correlation to patients survival. p53 mutation was found in 26 out of 92 tumors (28.3%). The 26 mutations consisted of 20 missense mutations, 1 in frame deleion, and 5 null mutations. Regarding transdominance of p53, 11 (10.9%) tumors had recessive mutation and 15 (14.9%) tumors had dominant negative mutation. Histologic subtype of tumors consisted of 78 endometrioid tumors and 14 non-endometrioid (mainly paillary serous carcinoma) tumors. FIGO stage distribution was 65 stage I/II and 27 stage III/IV. Depth of myometrial invasion was <serosa in 80 tumors and ⩾serosa in 12 tumors. Differentiation of tumors consisted of 31 Grade 1, 36 Grade 2, and 25 Grade3. There was no significant difference in distribution of these variables between Japan and German patients. Kaplan-Meier analysis revealed that FIGO stage, histologic type, grade, myometrial invasion (up to serosa), and p53 transdominace were significatly related to patients survival. Differece of the insitute had no association with survival. Using Cox regression analysis, we found that myometrial invasion (p<0.0001) and p53 transdominance (p=0.0002) were independent prognositic factors for endometria cancer. Further analysis including only advanced stage III/IV endometrial cancers, myometrial invasion (Hazard ratio=6.1, p=0.016) and p53 trancedominance (Hazard ratio=12.8, p=0.001) were independently related to survival. Prognosis for advanced stage edometrial cancer is poor even treated with combined modality of surgery plus radiation or chemotherapy. The results of this translational research has shown that dominant negative p53 mutation is quite a strong prognostic predictor and this protein may bea reasonable target of molecular targeting therapy for endometrial cancer.

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Maspin gene expression is a prognostic factor in non-small cell lung cancer

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Background: Maspin is a member of the serpin (serine protease inhibitor) superfamily, which was originally isolated from normal human mammary epithelial cells by subtractive hybridization, and it has been shown to have tumor suppressor activity attributable to the inhibition of breast cancer cell motility, invasion and metastases. Although this gene is generally deemed to be a tumor suppressor gene, the function is still controversial. Regarding lung cancer, few reports have been published, so we assessed Maspin gene expression and its clinical significance in non-small cell lung cancer (NSCLC).
Material and methods: Total RNA was extracted from primary lung cancer tissues obtained from 55 patients with pathologic (p-) stage I-VI NSCLC operated at Kyoto University Hospital between 1996 and 1998. Real-time PCR was performed using the LightCycler thermal cycler system (Roche Diagnostics Japan, Tokyo, Japan) following the manufacturer's protocol. Expression level of Maspin gene was normalized and represented as the ratio of Maspin mRNA value to GAPDH mRNA. Tumor tissues obtained from 55 NSCLC patients were reviewed to assess the correlation between Maspin mRNA expression and the clinicopathological features. The Stat View 5.0 statistical software package was used for all statistical analyses.
Results: No significant correlation was revealed between Maspin mRNA expression and age, sex, performance status (PS), grade of tumor differentiation, or p-stage. Maspin mRNA expression in squamous cell carcinoma was significantly higher than that in adenocarcinoma (p=0.011). Five-year survival rates of Maspin-high patients and Maspin-low patients were 67.7% and 41.4%, respectively, demonstrating a significant favorable prognosis of Maspin-high patients (log-rank, p=0.042). A multivariate analysis confirmed that Maspin-high expression was an independent and significant factor to predict a favorable prognosis (p=0.031).
Conclusions: In lung cancer, the function of Maspin gene may be a tumor suppressor gene as like as in breast cancer, and it has a potential to be a favorable prognostic factor in post-operative lung cancer patients.