Conclusion: The technical as well as the biological aspects of this high throughput setup is very interesting.

References

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PP 79

Low levels of cleaved urokinase receptor in plasma from healthy individuals

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Background: The involvement of the urokinase plasminogen activator, uPA, and its cellular receptor uPAR, in cancer invasion is well-established. uPA can cleave intact uPAR, uPAR(I-III) in the linker region between domains I and II, whereby uPAR domain I (uPAR(I)) is released and the cleaved uPAR(II-III) is left on the cell surface. Cleavage of uPAR(I-III) thus reflects the activity of uPA and possibly the aggressiveness of the cancer. uPAR can be shed from the cell surface and all uPAR forms have been identified in tumor tissue and blood. The cleaved uPAR forms are strong prognostic markers in colorectal cancer (CRC) (1). In order to determine a reference interval of the uPAR forms in blood from healthy individuals, we measured the uPAR forms in plasma from 200 men and 200 women, all without registered medication and co-morbidities and with no findings by colonoscopy.
Materials and Methods: Citrate plasma samples were collected before colonoscopy. The individual uPAR forms were measured by time-resolved fluorescence immunoassays specific for the three different uPAR forms.
Results: The median age of the included individuals was 48 (21−85) years. The mean level (geometric mean, male age 60 years) of uPAR(I-III) was 36.02 pmol/L with an upper normal limit of 55.06 pmol/L. Women had 22% higher levels and the level increased by 3.8% per 10 years. The mean level of uPAR(I-III)+uPAR(II-III) was 58.74 pmol/L and the upper normal limit was 94.15 pmol/L. Women had 18% higher levels and an increase of the level by 5.6% per 10 years was found. The mean level of uPAR(I) was 12.91 pmol/L and the upper normal limit was 36.90 pmol/L. Females had 25% higher levels and an upper limit of 42.13 pmol/L. The level of uPAR(I) was independent of age. The corresponding levels measured in citrate plasma from colorectal cancer patients (1) showed that 9% of the patients had elevated levels of uPAR(I-III), 23% of uPAR(I-III)+uPAR(II-III) and 32% of uPAR(I), as compared to the normal upper limit.
Conclusion: We have determined a reference interval for the three uPAR forms in citrate plasma. Women have significantly higher levels of all uPAR forms and the levels increase slightly with age in both genders for uPAR(I-III) and uPAR(I-III)+uPAR(II-III). Comparing the normal upper limits with the levels measured in the CRC patients reveal a greater proportion of patients with elevated levels of the cleaved uPAR forms compared to intact uPAR(I-III).

References

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PP 2

Generation of a panel of "actionable" cancer genes for molecular profiling (MP) in a feasibility study of targeted and genome wide sequencing (TGWS)

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Background: Increasing identification of genetic aberrations in cancers and the growing inventory of molecularly targeted agents (MTA) with potential predictive biomarkers (BM) are driving personalized cancer medicine (PCM). However, in clinical practice few MTA have had their regulatory approval predicated on specific predictive BM. Real time MP of tumors has potential to enable PCM but validation of this approach is necessary. Development of a recognized panel of cancer genes to enhance MP prioritization in clinical trial patients is relevant. This study utilizes the knowledge of cancer drug developers (DD) and genomic scientists (GS) in generating a gene panel for MP in a feasibility study of TGWS.
Materials and Methods: A survey exploring the perceived importance of 194 genes with aberrations proven or suspected to be tumorigenic was distributed to 29 DD and GS. Respondents were asked to assign importance to each gene, based on its likelihood to impact treatment recommendations for predictive or prognostic reasons: (1) highest; (2) intermediate; (3) lowest; (4) unknown. Genes were then ranked by mean score. Genes with aberrations targeted by established or investigational agents were identified. Subgroup analyses identified significant differences in scores assigned by DD and GS using chi-square.
Results: A total of 19 (73%) invitees, 10 (53%) DD and 9 (47%) GS completed the survey. Of the 194 genes, aberrations in 58 are targeted by established or investigational agents and a further 48 are within targeted pathways. The top 10 ranked genes include EGFR, BRAF, KIT, BRCA1, BRCA2, ErbB2, KRAS, ALK, ABL-1, BCR; all have aberrations predictive of efficacy with established or investigational agents. When compared to GS, DD are more likely to assign highest priority to genes where aberrations have MTA (37% v 31%, $p = 0.036$) and less likely to identify genes as unknown (21% v 33%, $p < 0.001$).
Conclusion: The ranked gene list generated by our survey allows generation of a prioritized panel of "actionable" genes for MP. This survey demonstrates the importance of utilizing expert knowledge of both DD and GS in both design of clinical trials using MP and successful translation of cancer genomics to PCM.

PP 86

Immunohistochemistry and molecular biology of the PI3K pathway did not correlate with treatment efficacy of everolimus as second line or third line treatment of advanced endometrial carcinoma

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Background: Recent evidence suggests the particular importance of the phosphatidylinositol 3-kinase (PI3K) pathway in patients (pts) who have recurrent or metastatic endometrial cancer [Oza et al. Abstract 5009, 2011 ASCO Meeting]. Activating mutations of the PI3K pathway is common, as well as mutation/loss of the tumor suppressor gene PTEN. Thus, mTOR (mammalian target of rapamycin) is activated and pS6K is a downstream marker of mTOR activation. Everolimus is an oral rapamycin analog that selectively inhibits mTOR. In the ENDORAD trial, we evaluated everolimus as a single agent for second- or third-line treatment of endometrial cancer pts [Ray-Coquard et al. Abstract P-8046, 2010 ESMO Meeting].
Materials and Methods: In the ENDORAD trial, pts received everolimus (10 mg PO daily) until progression or toxicity and were evaluated at 3 and 6 months for response and toxicity. Among 44 pts, 36 tumor blocks, mostly from primary tumor, were available to determine whether expression of biomarkers in the mTOR pathway would predict tumor response. Correlative studies evaluating ER, PR, HER2, LKB1, PI3K, PTEN, pAKT, 4EBP1, S6K and pS6K expression by immunohistochemistry (IHC) were performed. PTEN deletion (by FISH analysis) and mutational status of K-RAS, PI3KCA, PTEN and AKT1 genes were analyzed.
Results: 12 of 34 (35%) evaluable patients had partial response or stable disease (PR, SD) according to RECIST criteria, 22 pts had disease progression. Expression of ER, PR, LKB1, PI3K, pAKT, 4EBP1, S6K and pS6K using IHC did not predict response to everolimus (respectively: 8/12, 9/12, 3/12, 9/12, 6/12, 11/12, 11/12, 8/12 for responders, and 15/22, 13/22, 3/22, 11/22, 8/22, 18/22, 21/22, 19/22 for non-responders). Neither loss of PTEN expression (8/12 for responders and 13/22 for non-responders, $p = 0.6$), nor PTEN deletion, nor PTEN mutation (5/12 for responders and 7/22 for non-responders) predict pts outcomes. 31 specimens were evaluable for K-RAS mutations (10 for responders and 21 for non-responders). None of the pts with PR or SD had K-RAS mutation, whereas 4 mutations (19%) were identified in tumors that had progressed on everolimus.
Conclusion: None of the protein from the PI3K pathway tested in this study could predict response to everolimus. Interestingly, K-RAS mutational status correlated with response to everolimus. Other studies presented at the 2011 ASCO Meeting suggested also that K-RAS mutations were associated with resistance to everolimus.

PP 39

Multi-determinants analysis of molecular alterations as predictor of resistance to cetuximab in metastatic colorectal cancer

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Background: KRAS mutations negatively affect outcome after cetuximab (CTX) in metastatic colorectal cancer (mCRC). As only 20% of KRAS wild-type (WT) patients respond it is possible that other mutations, constitutively activating the EGFR pathway, are present in the non-responding WT patients. We retrospectively correlated progression-free survival (PFS) with the mutational status of KRAS, BRAF, PIK3CA and expression of PTEN in 64 mCRC patients treated with Cetuximab, with the aim to clarifying the relative contribution of these molecular alterations to resistance.

Materials and Methods: DNA was extracted from 5 μM paraffin-embedded sections containing at least 50% of tumoral cells, derived from primary or metastatic lesions. Exon 2 of KRAS and exon 15 of BRAF were amplified by PCR and analysed by direct sequencing. PIK3CA status was analysed by Pyrosequencing. PTEN espression was analyzed by immunohistochemistry using a Dako monoclonal antibody diluted 1:100 (cut-off 5%). PFS was estimated by Kaplan–Meier method and curves were compared by log-rank test. Hazard Ratios (HR) were estimated according to Cox multiple regression model.
Results: Patient characteristics were as follows: Male/Female = 35/29; primary site: rectum/colon = 13/51. KRAS, BRAF and PI3K mutations were present in 28.1%, 21.3% and 15.1% of CRC lesions, respectively, while PTEN positivity in 34.7%. KRAS WT was associated with median trend towards a higher PFS: 5.1 (95% CI 3.3−6.2) vs 3.1 months (95% CI 2.3−5.5), $p = 0.85$. BRAF WT was associated with higher PFS: 5.1 (95% CI 2.9−6.5) vs 3.4 months (95% CI 1.4−5.1), $p < 0.01$. PI3K WT was associated with higher PFS 5.3(95% CI 3.6−6.5) vs 2.2 months (95% CI 1.1−3.3), $p < 0.01$. High PTEN expression was associated with PFS 6.7 (2.8−8.7)/3.4 (2.6−5.3) months, $p = 0.031$. Moreover, the multivariate analysis of PFS indicated BRAF and PI3K as potential independent predictors of clinical benefit: HR (95% CI) 3.14 (1.38–7.16), $p = 0.006$ and 6.68 (2.17–20.52), $p = 0.0009$, respectively.
Conclusion: Our results seem to confirm that comprehensive molecular dissection of the EGFR signaling pathways should be considered to better select mCRC patients for CTX based therapies.

PP 40

Tumor microenvironment and prognosis in breast carcinoma

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Background: Multiple lines of evidence indicate that tumor microenvironment (TME) influences the breast cancer growth and metastasis and therefore could be promising prognostic parameter. The aim of the present study was to determine the prognostic significance of immune response, by quantifying mediators and immune cells, and (lymph)angiogenesis, by quantifying (lymph)angiogenic mediators and vessels, in the primary breast tumors.
Materials and Methods: The study included 135 pN0M0 breast cancer patients, who received no adjuvant therapy. Median follow-up of the natural disease course was 107 months. Indirect quantification of TME components was done by measuring expression of 19 (lymph)angiogenic factors and cytokines on protein level, in cytosolic fraction of tumors using classical biochemical method for ER and PR, and ELISA for others, and on mRNA level, by applying RT-qPCR in RNA fraction of the tissue samples. Direct quantification of TME parameters was done on cellular level, by performing IHC staining of FFPE tissue slides, using antibodies against CD3, CD8, CD45RO, CD68, CD105 and podoplanin. IHC positively stained cells were quantified as number of cells per tissue surface, in the hotspot regions of center of tumor (CT) and invasive margin (IM), by HISTOLAB software analysis of scanned tissue slides.
Results: DFI analysis on protein level showed that high ER (>60 fmol/mg), IL8 (>120.3 pg/mg) and PAI (>4.97 pg/mg) levels in tumors were associated with unfavorable prognosis ($p = 0.003$, $p > 0.001$, $p = 0.05$), contrary to high bFGF (>64.6 pg/mg) values in tumors ($p = 0.007$). On mRNA level high IL1β (dCt < 20.3) and IL8 (dCt < 21.9) values in tumors were linked with unfavorable prognosis ($p = 0.02$, $p = 0.02$), contrary to high VEGF-A values (dCt < 12.1, $p = 0.05$). Finally, on cellular level high density of blood (but not lymphatic) vessels at IM and high density of CD8+ infiltrates both in CT and IM, indicate a good prognostic groups. Combined status of classical prognostic parameters and analyzed biomarkers improved their individual prognostic impact and provided better separation of patients into prognostic groups.
Conclusion: This study suggests that accessing ER, IL8, PAI, bFGF, IL1β, VEGF-A levels, as wells as estimating degree of vascularisation and lymphocyte infiltrates, in the primary tumors, may be important for classifying patients with pN0M0 breast carcinoma into different prognostic groups and may help the individualization of the breast cancer targeted therapy.

PP 48

TRIB3: a prognostic factor and involved in hypoxia sensitivity in breast cancer patients

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Background: Tribbles homolog 3 (TRIB3) is a hypoxia induced pseudokinase involved in the regulation of several signaling pathways involved in cell survival and/or cell stress. Here, we determined the correlation between breast cancer prognosis and TRIB3 protein levels and established the role of TRIB3 in cell survival after hypoxia and/or radiotherapy.
Materials and Methods: TRIB3 mRNA and protein were quantified in breast cancer cell lines and in a breast cancer patient cohort ($n \geqslant 95$) using qPCR and a new specific avian antibody against TRIB3. Correlation between TRIB3 mRNA and protein were investigated and in the patient cohort prognostic and predictive value of both measurements were determined. In addition, we used siRNA-mediated knockdown of TRIB3 in a colony-forming assay after hypoxia and radiotherapy.
Results: We found that TRIB3 mRNA levels did not correlate with protein levels in breast cancer cell lines neither in the human breast cancer material. We validated our earlier finding that high TRIB3 mRNA denotes a poor prognosis, but found that high TRIB3 protein levels were associated with a good prognosis in breast cancer patients. We also show that knockdown of TRIB3 resulted in an increased survival under hypoxic conditions. Furthermore, we have indications that TRIB3 is relevant for hypoxia induced cell death in cells with AKT knockdown.
Conclusion: Our results presented here indicate that these data on mRNA levels do not necessarily translate to protein, nor to function, of all genes. Whereas mRNA levels of TRIB3 are related with a poor prognosis, TRIB3 protein is associated with a good prognosis in human breast cancer patients, possibly due to the fact that TRIB3 is involved in hypoxia tolerance.

PP 47

Prediction of non-responders to chemoradiation in HPV-positive head and neck cancers by gene expression profiling

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Background: Human papillomavirus (HPV) has been shown to have a causal role in the development of Head and Neck Squamous Cell Carcinoma (HNSCC). In one study, 22% of tumors were HPV+, with 87% being of the HPV16 subtype. HPV-related HNSCC represents a distinct and well-defined pathology that is associated with a better response to radiation and more favorable prognosis. However, approximately 15% of the patients do not respond positively to radiation or chemoradiation, thus suffering unnecessary morbidity and delay to receive effective therapy.
Materials and Methods: 11 patients with laryngeal and oropharyngeal HPV+ HNSCC were included in the study, 5 of which had a complete response to treatment and 6 who were non-responders. RNA was extracted from prospectively collected, pre-treatment tumor specimens and subjected to gene expression analysis using Affymetrix Human Exon 1.0ST arrays. HPV-status was confirmed by detection of HPV16 E7 with RT-PCR.
Results: ANOVA ($p > 0.05$) and a 2-fold cutoff were used to identify 118 altered genes, including 112 genes over-expressed in the complete responders compared to the non-responders. Interestingly, in the complete responders, over-expressed were associated with T cell proliferation (PTPRC, ITGAL, IL6ST, CD3E, CORO1A) and antigen processing/presentation (HLA-F, HLA-DRA, HLA-DQA2, PSMB9, ERAP1, CD74). Utilizing Ariadne Pathway Studio to characterize the data, changes in gene expression were enriched in genes encoding proteins involved in regulating cell processes such as presentation of endogenous peptide antigen, lymphocyte adhesion, and T-cell related processes. Further, genes associated with the Gene Ontology group “response to virus” were up-regulated in complete responders.
Conclusion: The data obtained in this study suggested that differences in response to chemoradiation therapy were related to immune response prior to treatment. These data can potentially lead to an assay that can be used clinically to predict HPV+ HNSCC patients that will not benefit from chemoradiation and who may benefit from earlier surgical intervention.

PP 30

Prospective comparison of Recurrence Score, uPA/PAI-1, central grade and molecular subtyping in early breast cancer: first results from the WSG-Plan B trial (interim analysis)

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Background: For decision support regarding adjuvant chemotherapy in early breast cancer,the recurrence score (RS) multi-gene assay and invasion factors uPA/PAI-1 are included in national and international guidelines. We present first correlation analysis of RS, uPA/PAI-1 and molecular subtypes by protein expression of the first pre-planned trial, WSG Plan-B.
Materials and Methods: The Plan-B trial ($n = 2.448$) is evaluating anthracycline-free adjuvant chemotherapy ($6 \times$ TC) versus $4 \times$ EC − $4 \times$ Doc in Her 2 neg. breat cancer. RS is used as selection criterion