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.

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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.