Oral presentations (Tue, 22 Sep, 09:00–10:45)
Gastro-intestinal malignancies – Colorectal cancer II

6007 ORAL

Combining clinical factors with a genomic signature (ColoPrint) for the prognosis prediction of stage II and III colon cancer patients

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Background: Recently, microarray analysis has shown great promise in predicting outcome and treatment response of individual patients. Histological factors (e.g. grade) and single molecular markers like microsatellite instability (MSI) or K-ras mutation may provide an additional mean of characterizing colorectal tumors.
Methods: We earlier described the development of a prognostic gene signature (ColoPrint) in a training set of 188 patients with stage I, II and III colorectal cancer (CRC) using gene expression data from Agilent 44K oligonucleotide arrays. The signature was validated in samples from an independent cohort of 178 stage II and III CRC patients and in *in-silico* datasets (n = 322). Mutation analysis by sequencing was performed on all samples using mRNA as starting material (KRAS: codons 12, 13 and 61; PI3KCA: exons 9 and 20; BRAF exon 15). For 124 patients, the microsatellite stability status was measured by IHC of the MLH1 and MSH6 gene product. Uni- and multi-variate analyses are used to evaluate the significance of risk stratification of the prognostic profile in relation to existing molecular clinic-pathological parameters.
Results: In the validation cohort of 178 stage II and III CRC patients, the ColoPrint signature classified 61% of the patients as low-risk and 39% as high-risk. The low- versus high-risk patients showed a significant difference in DMFS (Distant Metastasis-Free Survival) with a HR of 3.2 (P = 8.5e-4). Five-year DMFS rates were 89% (95CI, 83–95%) for low-risk and 62% (95CI, 50–77%) for high-risk patients. KRas, PI3K and BRaf mutations were present in 31%, 12% and 10%, respectively. In total, 48% of all patients had <1 activating mutations. The mutation status had no prognostic power in this patient cohort.
In a multivariate analysis, the prognostic signature remained the most prognostic factor with a HR of 2.95 (p = 0.015). The signature showed a significant performance within stage II (P = 0.0058) and III (P = 0.036) patients separately. Patients with MSI (20/124) had a high frequency of B-Raf mutation (50%) and were mainly ColoPrint low risk (86%). Loss of expression of the MLH1 gene was strongly correlated with MSI status (p < 0.0001).
Conclusion: The ColoPrint signature is able to predict the prognosis of stage II and III CRC patients. The combination of the genomic signature with additional clinical factors might further improve the identification of patients who are most likely to benefit from adjuvant chemotherapy.

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Stroma production within the primary tumor correlates with poor survival for stage I-II colon cancer patients

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Background: Recent models on metastatic invasion focus on the tumor-"host" interface, in particular the role of the stromal tissue. The biological meaning of the stromal compartments are thought to be part of the process of wound healing, but there is also strong emphasis that CAF's (cancer-associated fibroblasts) are important promotors for tumor growth and progression. Assuming these models are correct we anticipated that changes in the proportion of stroma in the primary tumor could reflect progression. We therefore investigated if the amount of intra-tumor stroma could be applied as a candidate marker to identify patients for adjuvant therapy.
Methods: In a first study we have investigated the proportion of intra-tumor stroma, on heamatoxylin-eosin (H&E) stained histological sections in a set of 122 patients (stage I-III) and distinguished between patients with a high amount of stroma (stroma-high) and patients with less stroma (stroma-low). The second study is based on stage I-II patients only, a subgroup of patients who might benefit from adjuvant therapy. We have analyzed 135 stage I-II colon cancer patients for the proportion of tumor related stroma and for TGFβ-R2, SMAD4 and β-catenin, markers involved in pathways related to stromal production and epithelial-to-mesenchymal transition (EMT).
Results: The first study showed five-year survival rates for stroma-high versus stroma-low of respectively for OS: 15.2% and 73.0% and for DFS: 12.1% and 67.4% (OS p < 0.0001, HZ 3.73; DFS p < 0.0001, HZ 4.18). In a multivariate Cox regression analysis, the amount of stroma remained an independent variable when adjusted for either stage or for tumor status and lymph-node status (OS: p < 0.001, OS: p < 0.001).
For the second study of 136 analyzed patients 35 (25.7%) patients were stroma-high and 101 (74.3%) stroma-low. Significant differences in survival were observed between the two groups, with stroma-high patients showing poor survival (OS p < 0.0001, HZ 2.59; DFS p = 0.0002, HZ 2.31).
A high-risk group was identified with stroma-high and SMAD4 loss (OS p = 0.008, HZ 7.98, CI 4.12–15.44, DFS p = 0.005, HZ 6.57, CI 3.43–12.56); 12 of 14 (85.7%) patients died within 3 years. In a logistic-regression analysis a high proportion of stroma and SMAD4 loss were strongly related (HZ 5.42, CI 2.13–13.82, p < 0.001).
Conclusions: Conventional haematoxylin-eosin stained tumor slides contain more prognostic information than previously fathomed. This can be unleashed by assessing the tumor-stroma ratio. The combination of analyzing the tumor-stroma ratio and staining for SMAD4 results in an independent parameter for confident prediction of clinical outcome. It should be considered to implement this parameter in standard pathological reports in addition to theTNM classification.

6009 ORAL

Time-dependent patterns of treatment effect and failure as an explanation for the predictive role of deficient mismatch repair (dMMR) in stage II and III colon cancer

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Background: In stage II and III colon cancer, dMMR has been confirmed as a prognostic marker of favorable outcome and a predictive marker for lack of benefit from 5FU-based adjuvant chemotherapy (AT). In an effort to better understand the relationship between MMR status, treatment benefit, relapse, and survival, additional analyses were performed. Specifically, do patients (pts) with dMMR and proficient MMR (pMMR) tumors differ in the time-pattern of recurrence, and in the nature and duration of AT benefit?
Methods: The dataset of 1027 pts from randomized clinical trials of 5-FU based AT versus surgery alone (S) assembled for the recent dMMR confirmatory report (Sargent ASCO 2008) was analyzed. Tumors from stage II (n = 530) and III (n = 497) pts were assayed for MMR status by MSI or IHC. Time-dependent patterns of recurrence (TTR), disease free survival (DFS), and overall survival (OS) were analyzed by MMR status and treatment received.

MMR status	Annualized Risk for Recurrence (%)							
	Year 0–2		Years 2–4		Years 4–6		Years 6–8	
	S	AT	S	AT	S	AT	S	AT
dMMR	7.1	8.5	4.0	6.1	1.4	4.0	0.5	2.3
pMMR	16.8	8.5	10.4	6.6	5.4	4.0	2.3	1.2

Results: Over the 8-year study period, in patients treated with S alone, dMMR pts maintained a consistent advantage in the clinical endpoints TTR, DFS, OS compared to pMMR pts (see Table). dMMR pts treated with S alone recurred at a 2–3 fold lower rate than pMMR pts in particular during the first 4 years after treatment. dMMR patients recur less overall, without a trend towards later recurrences. 5-FU based AT provided pMMR pts a pronounced reduction in the risk of recurrence within the first-four years of follow-up that is not observed in dMMR pts, in whom AT was not associated with a reduced recurrence risk. Importantly, this AT benefit persists in pMMR pts with few long-term events. Finally, prognosis is poor (median survival ~1 year) following disease recurrence and is independent of MMR status (p = 0.87 in S pts, p = 0.52 in AT pts).
Conclusions: The favorable prognosis and low recurrence risk in dMMR pts is consistent and maintained throughout the natural course of the disease. In particular, in dMMR pts, the recurrence risk is low in all years following surgery, and no benefit from AT is observed at any point. Conversely, pMMR pts have an increased risk of recurrence during the first four years post-surgery that is significantly reduced with 5-FU based AT.