## *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.

**6008** ORAL

**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.

This difference in the time patterns of recurrence and impact of AT may ultimately explain why MMR is predictive of AT benefit.

**6010** ORAL

**A three-arm phase III randomized trial of FOLFOX-4 vs. FOLFOX-4 plus bevacizumab vs. XELOX plus bevacizumab in the adjuvant treatment of patients with stage III or high-risk stage II colon cancer: results of the interim safety analysis of the AVANT trial**

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**Background:** Bevacizumab (Bev) and capecitabine (Cap) are established drugs for patients (pts) with metastatic colorectal cancer (mCRC). The AVANT trial is evaluating the efficacy and safety of Bev in combination with either intermittent Cap plus oxaliplatin (XELOX+Bev) or fluorouracil/leucovorin with oxaliplatin (FOLFOX-4+Bev) vs. FOLFOX-4 in the adjuvant treatment of pts with stage III or high-risk stage II colon cancer.
**Materials and Methods:** Pts were randomized to receive 12 cycles (q2 weeks) of FOLFOX-4 (Arm A), 12 cycles (q2 weeks) of FOLFOX-4+Bev (Arm B) or 8 cycles (q3 weeks) of XELOX+Bev (Arm C) followed by a further 8 cycles (q3 weeks) of Bev in Arms B and C (1 year of total Bev duration). Primary objective is to show superiority of Arm B or Arm C vs. Arm A in pts with stage III colon cancer in terms of disease-free survival (DFS). An interim safety analysis was planned 6 months after the last randomized pt ended treatment.
**Results:** Between December 2004 and June 2007, 3451 pts were randomized (stage III/high-risk stage II: 2867/573). Arm A, 955/192; Arm B, 960/194; Arm C, 952/187. Treatment arms were well balanced for disease stage, age, ECOG status and ethnicity. Median duration of oxaliplatin-containing chemotherapy was 5.3, 5.2 and 4.9 months, respectively, and median duration of Bev treatment was 10.6 months (Arm B) and 10.4 months (Arm C). Main toxicities of interest for Bev are shown in the table. All-cause mortality within 60 days of treatment start was 2 (0.2%) pts in Arm A, 4 (0.3%) in Arm B and 6 (0.5%) in Arm C. Number of deaths not due to colon cancer within 28 days after last drug administration were: Arm A, 8 (0.7%); Arm B, 4 (0.3%); Arm C, 10 (0.9%).
**Conclusions:** Bev plus fluoropyrimidine/oxaliplatin combination is safe in the adjuvant treatment of colon cancer pts. The adverse event profile is comparable to the safety profile in mCRC and in the NSABP C-08 trial (ASCO 2008–2009).

Table. Grade 3−5 AEs within 6 months of last treatment

| No. (%) of pts | Arm A FOLFOX-4* n = 1126 | Arm B FOLFOX-4 + Bev** n = 1145 | Arm C XELOX + Bev** n = 1135 |
|---|---|---|---|
| Venous thrombotic events | 62 (5.5) | 95 (8.3) | 52 (4.6) |
| Hypertension | 12 (1.1) | 119 (10.4) | 109 (9.6) |
| Arterial thrombotic events | 11 (1.0) | 18 (1.6) | 17 (1.5) |
| Bleeding/haemorrhage | 7 (0.6) | 14 (1.2) | 5 (0.4) |
| Wound healing complications | 4 (0.4) | 3 (0.3) | 5 (0.4) |
| Abscess/fistula | 3 (0.3) | 16 (1.4) | 9 (0.8) |
| Gastrointestinal perforations | 1 (0.1) | 8 (0.7) | 2 (0.2) |
| Proteinuria | 1 (0.1) | 11 (1.0) | 11 (1.0) |

*planned treatment duration 5.5 months; ** planned treatment duration 11 months.

**6011** ORAL

**Calcium and magnesium (Ca/Mg) infusions to reduce oxaliplatin-induced neurotoxicity and outcome in advanced colorectal cancer (ACC) patients (pts) treated with oxaliplatin- and cetuximab-based therapy**

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**Background:** Peripheral neurotoxicity is a potentially invalidating side effect of oxaliplatin treatment. Ca/Mg infusions are frequently used to prevent this toxicity, but the relationship with outcome is still controversial. Hypomagnesemia (hypoMg) is a frequent side effect of treatment with cetuximab (an epidermal growth factor receptor monoclonal antibody) and is associated with response to this agent. We assessed the effect of Ca/Mg infusions on toxicity and outcome.
**Materials and Methods:** 755 previously untreated ACC pts received capecitabine, oxaliplatin (to a maximum of 6 cycles) and bevacizumab (CB) or the same combination with the addition of cetuximab (CBC) in a phase III randomized trial (CAIRO2 study of the Dutch Colorectal Cancer Group, Tol et al., N Engl J Med 2009). Pts were divided into 2 groups: group I received Ca/Mg infusions at their first treatment cycle, group II did not. Progression-free survival (PFS), overall survival (OS), response rate (RR), and toxicity (NCI-CTC v.3.0) were assessed per treatment arm in these 2 groups and calculated using a Cox-proportional hazards model and Chi-square analysis.
**Results:** 732 pts were evaluable for these analyses. Group I consisted of 552 patients (75%), 269 in the CB arm and 283 in the CBC arm, of which 369 (67%) received Ca/Mg at all 6 cycles oxaliplatin. In group II, 133 out of 180 pts (74%) did not receive Ca/Mg during subsequent cycles. Baseline characteristics were comparable between group I and II. The incidence of peripheral neurotoxicity (⩾grade 2) was comparable in group I and group II (39% vs 42%; p = 0.49) in the CB and in the CBC arm. The incidence of all grade hypoMg also did not differ between groups (28% vs 24%; p = 0.32) in both treatment arms. The median PFS (95% confidence interval [CI]) in the CB arm was 10.6 (9.4−12.6) months in group I and 10.7 (9.0−12.7) months in group II (p = 0.54). In the CBC arm the median PFS was 9.2 (8.2−10.3) months in group I and 11.2 (8.6−12.6) months in group II (p = 0.15). The median OS (95% CI) was also comparable between group I and II in both the CB arm (20.0 [17.1−25.4] vs 20.4 [16.7−27.8] months; p = 0.68) and the CBC arm (18.9 [16.2−21.5] vs 20.6 [17.6−25.2] months; p = 0.20). The RR was 35% in group I vs 40% in group II in the CB arm (p = 0.45), and 36% vs 49% in the CB arm (p = 0.06).
**Conclusions:** Ca/Mg infusions were not correlated with a decreased incidence of peripheral neurotoxicity in pts treated with capecitabine, oxaliplatin and bevacizumab with or without cetuximab. No statistically significant differences in outcome were observed based on Ca/Mg infusions.

**6012** ORAL

**The FIRIS study; A Phase III trial of 5-FU/l-leucovorin/irinotecan (FOLFIRI) versus irinotecan/S-1 (IRIS) as 2nd-line chemotherapy for metastatic colorectal cancer (mCRC) [FIRIS study group]**

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**Background:** Several phase II studies of irinotecan (IRI) plus S-1 combination therapy (IRIS) conducted in Japan have shown promising efficacy and safety for mCRC, suggesting the potential to replace FOLFIRI. We conducted a randomized phase III trial to demonstrate the