Results: 86 patients were included. M/F = 44/42, median age = 67 (41−78), median number of previous lines of chemotherapy = 2 (1−5). In the subgroup of 52 (60%) *KRAS* wt patients, *BRAF* mutation was associated with a trend toward lower response rate (RR 1/10, 10% vs 12/42, 29%; OR: 3.86 [95%CI: 0.44−33.88], p = 0.224) and with significantly shorter PFS (HR: 2.33 [95%CI: 1.12−4.84], p = 0.023) and OS (HR: 3.51 [95%CI:1.55−7.98], p = 0.003). *KRAS* wt patients with higher AR expression showed a trend toward better RR (OR: 0.94 [95%CI: 0.88−1.02], p = 0.119) and PFS (HR: 0.971 [95%CI:0.938−1.005], p = 0.095) that translated into significantly longer OS (HR: 0.950 [95%CI: 0.907−0.995], p = 0.030. A strong association between *BRAF* mutations and lower AR levels was found both in the overall population (t-test; p = 0.0005) and in *KRAS* wt subgroup (t-test; p = 0.0023). In the subgroup of 40 (47%) *KRAS* and *BRAF* wt patients AR expression did not predict RR (OR: 0.969 [95%CI: 0.898−1.046], p = 0.422) nor PFS (HR: 0.983 [95%CI: 0.948−1.019], p = 0.345) nor OS (HR: 0.968 [95%CI: 0.924−1.014], p = 0.175).
In *KRAS* wt subgroup, at the multivariate analysis *BRAF* mutation retained its predictive value in terms of both PFS (HR: 2.577 [95%CI: 1.103−6.022], p = 0.029) and OS (HR: 3.472 [95%CI: 1.417−8.506], p = 0.007), while AR expression did not predict PFS (HR: 0.982 [95%CI: 0.947−1.018], p = 0.320) nor OS (HR: 0.968 [95%CI: 0.924−1.014], p = 0.17).
Conclusions: *KRAS* and *BRAF* mutations are confirmed as predictors of resistance to cetuximab plus irinotecan. The significant association between *BRAF* mutations and lower AR expression suggests that decreasing levels of AR expression may be an epiphenomenon of *BRAF* mutations. Future studies of potential predictors of benefit should take into account their possible overlap.

6114 POSTER

K-ras and B-raf mutation analysis has clinical value in stage III colon carcinoma

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Background: Mutations in the k-ras pathway have been widely studied in metastatic colon carcinoma due to their value as predictive markers of response to anti-epithelial growth factor receptor therapy. However the value of disruption of this pathway in other stages of colon carcinoma remains unknown.
Our aim is to study the clinical value of mutations in the *k-ras* and *b-raf* genes in a well defined and clinically homogeneous group of stage III colon carcinoma patients.
Patients and Methods: 213 patients with stage III disease treated with surgery followed by 5-FU based adjuvant therapy were selected. DNA was isolated from selected areas of paraffin material, after determination of percentage of tumoral cells. K-ras mutations in codons 12 and 13 were determined by sequencing. The V600E mutation in the B-raf gene was studied by real time PCR with specific probes for the mutated and the wild type allele. MSI status was determined by typing the BAT 26 marker which is positive in 99% of MSI positive Caucasian patients.
Results: Median age of the group was 64 years (30−83), median follow up was 47 months (4–133). 56.8% of the patients was male and 52.6% had a right sided tumor. 76.4% of the patients had less than 4 positive lymph nodes at diagnosis and 73.7% had a T_3 tumor. 14% was MSI positive, 19.5% had a mutation in the *b-raf* gene and 35% had a mutation in the *k-ras* gene. Mutations in the *b-raf* and *k-ras* genes were mutually exclusive. There was a significant relationship between B-raf mutation and MSI positive tumors (p < 0.0001) and between B-raf mutation and right sided disease (p < 0.0001). In our group the presence of a mutation in the *k-ras* gene significantly correlated with developing a distant metastasis or local recidive during follow-up (p = 0.009).
In a multivariate survival analysis adjusting for known prognostic factors like lymph node status, T status, age, gender, tumor location, MSI, B-raf and K-ras mutations; the V600E mutation in B-raf was an independent factor significantly predicting a worse overall survival (p = 0.006 95% CI (0.21−0.78)). K-ras mutations was an independent factor predicting shorter disease free survival (p = 0.028 95% CI (0.34−0.94)).
Conclusion: We conclude that mutation analysis of the K-ras pathway is a useful clinical tool to predict overall survival and disease free survival in stage III colon carcinoma patients.

6115 POSTER

VEGF gene polymorphisms in the prediction of benefit from first-line FOLFIRI plus bevacizumab (BV) in metastatic colorectal cancer (mCRC) patients (pts)

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Background: Addition of BV to first-line irinotecan plus 5FU improves PFS and OS of mCRC pts. Meanwhile, the anti-VEGF causes specific toxicities and increases costs of treatment. At the same time, not all pts derive an equal benefit from the VEGF inhibitor. So far, molecular predictors of BV efficacy have not yet been identified. Specific *VEGF* polymorphisms may affect gene transcription, thus indirectly influencing efficacy of BV.
Materials and Methods: Peripheral blood samples for genomic DNA extraction were collected from consecutive mCRC pts receiving FOLFIRI plus BV as first-line treatment (BV-group). *VEGF* −2578A/C, −460C/T, +405C/G, +936C/T polymorphisms were analysed by means of PCR-RFLP. One-hundred-seven pts, treated with FOLFIRI alone, served as historical control group.
Results: One-hundred-eleven pts were included in the BV-group. M/F = 57/54, median age = 63 (34−82), Köhne score (low/intermediate/high/data missing) = 57/39/12/3. Sixty-nine out of 111 pts achieved response (RR = 62%). Median PFS (mPFS) and median OS (mOS) were 10.2 and 22.2 months, respectively. *VEGF* −460C/C, C/T and T/T allelic variants were found in 20%, 54% and 26% of pts, respectively. −460 T allele demonstrated shorter PFS and OS with an additive effect of each T allele (PFS: HR = 2.65, [1.49−6.62], p = 0.003; OS: 2.47, [0.91−7.66], p = 0.074). −460C/C pts achieved significantly longer PFS and OS in comparison to pts carrying at least one T allele (mPFS: 12.8 vs 9.8 months; HR = 0.48 [0.28−0.85], p = 0.012; mOS: 27.3 vs 20.5 months; HR = 0.38 [0.19−0.94], p = 0.034). In the control group mPFS and mOS were 8.2 and 20.6 months; −460C/C, C/T and T/T variants were found in 23%, 52% and 25% of pts, respectively; there was no significant association with PFS or OS. Other investigated polymorphisms did not affect outcome neither in BV-group nor in the control group.
Conclusions: At our knowledge this is the first report of a pharmacogenetic determinant of improved PFS and OS for mCRC pts treated with first-line BV-containing therapy. The observation that *VEGF* −460C/T variants did not influence the outcome in the control group led to hypothesize a predictive other than a prognostic role for such genetic signature. These preliminary data deserve investigation in prospective, randomized, validating trials.

6116 POSTER

Epidermal growth factor receptor (EGFR) expression in stage II-III colon carcinoma (CC) – nine years of follow-up

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Background: Epidermal growth factor receptor (EGFR) is a protoncogen that is found overexpressed in colorectal carcinomas and it correlates with a worse prognosis. The aim was to describe EGFR overexpression patterns in non-metastatic CC and to correlate these data with follow-up.
Methods: We analyzed a series of 194 CC. Inclusion criteria were: a) resected primary adenocarcinoma; b) curative surgery; c) pT3 N0−2 M0 without progression during the first 6 months post surgery; d) minimum follow-up over 8 years. EGFR overexpression was analyzed by immunohistochemistry (IHC) using the Dako PharmaDx kit (Glostrup, Denmark). As positive control the Dako slides and a bloc cell of A431-AAM cells were used. Presence of cytoplasmic and membrane patterns (intensity 1(+), 2(+) and 3(+)) were evaluated as well as the percentage of positive cells. Statistical analysis: association between qualitative variables was analyzed by Fisher's exact test. Disease-free and overall survival distributions were estimated by the Kaplan-Meier method and were analyzed with the log rank test. All *P* values are from two-sided statistical tests.

Results: Characteristics of the patients (pts) were as follows: mean age: 71 years (range: 28−92); sex: 92 male and 102 female; location: 80 right colon and 114 left colon; Follow-up: mean: 9.4 years. Disease free survival: St2A: 77.23%, St3B: 57.89%, St3C: 45.45%.
IHC: 170 cases were positive (87.62%) and were classified as follows: staining was cytoplasmic predominance in 86 cases (44.32%) and 84 (43.29%) with membrane predominance.
Disease free survival according EGFR status was: Negative and Cytoplasmic positivity cases: St2A: 77%, St3B: 73.68%, St3C: 52.9%. Membrane positivity: St2A: 77.5%, St3B: 42.1%, St3C: 37.5%. Only those patients with tumors harboring membrane positivity (intensity 1(+), 2(+) and 3 (+)) had more probability to present metastasis ($p < 0.05$). No significant differences were found between negative and cytoplasmic staining.
Conclusions: EGFR overexpression evaluated as a membrane pattern is related with metastatic development in colonic carcinomas. This worse prognostic is maintained over 9 years after resection, and affects only stage 3.
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6117 POSTER

Circulating endothelial cells (CECs) and FDG-PET for early prediction of response in high-risk locally advanced rectal cancer (HR-LARC) patients (pts) treated with two different schedules of bevacizumab (BEV) in combination with preoperative chemo-radiotherapy (CT-RT)

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Background: Vascular endothelial growth factor (VEGF) has a crucial role in tumor angiogenesis; its inhibition leads to the normalization of tumor vessels, increases tumor oxygenation and drug delivery. However, the clinical benefits of current anti-VEGF treatments have thus far been rather moderate, stimulating interest in developing more effective ways to combine anti-VEGF drugs and CT, and in identification of predictive biomarkers of clinical benefit. We have previously shown that pre-operative oxaliplatin (OXA), raltitrexed (RTX), fluorouracil (5FU), and folinic acid (LFA) during pelvic RT yelded a high rate of complete (TRG1) or subtotal (TRG2) tumor regression in HR-LARC. Therefore, we planned to add BEV, a MoAb against VEGF, to primary CH-RT in two different schedules, to evaluate the relevance of BEV timing during CT and RT. Changes of CECs and glucose metabolism evaluated by flow cytometry and FDG-PET were used as early surrogate markers of tumor response.
Methods: 28 pts (cT4, cN+, cT3$\leqslant$5 cm from the anal verge and/or +ve CRM, M1 resectable/initially unresectable) received 3 biweekly courses of OXA (100 mg/m^2)/RTX (2.5 mg/m^2) on day 1, and 5FU (800 mg/m^2)/LFA 250 mg/m^2) on day 2 during pelvic RT (45 Gy). In schedule A (16 pts) BEV (5 mg/kg) was given biweekly from day −14 for 4 courses, while in schedule B (12 pts) it was given from day −4 for 2 courses. Toxicity was graded with NCI-CTC version3. According to the Simon's two-stage design, assuming a hypothesis of 50% TRG1 (α error = 0.05, β error = 0.20), at least 6/16 TRG1 should be obtained to continue pts accrual.
Results: No death occurred. As in the previous phase II study without BEV, grade 3/4 neutropenia was the most common adverse event with schedule A (7 pts, 44%), but it was lower with schedule B (2 pts, 17%). After the 1st course of CT, a significantly greater reduction of CECs levels (median, −78% *vs* −29%, $p < 0.05$), and of tumor metabolic volume (median, −78% *vs* −50%, $p < 0.05$) was observed with schedule B compared to schedule A. Moreover, we observed different CECs kinetics with schedule B compared with schedule A. So far, all but one pt (because of refusal) in schedule A, and 11 pts in schedule B have proceeded to surgery. In the schedule A, only 2 (12%) pts obtained a TRG1, while the number of TRG1 required by the statistical design has already been reached in schedule B (6 cases, 55%).
Conclusion: Our data suggest the relevance of BEV schedule to optimize the feasibility and efficacy of the combination treatment, and the potential role of CECs and FDG-PET as early predictive markers of tumor response.

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The prognostic value of tissue inhibitor of metalloproteinases-1 (TIMP-1) in metastatic colorectal cancer treated with third-line cetuximab-irinotecan

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Background: KRAS wildtype (wt) status is essential to the effect of treatment with EGFR inhibitor cetuximab, but still the overall response rate is less than 40%. Consequently the majority of patients will suffer from substantial side effects and no apparent benefit. TIMP-1 is a glycoprotein, which regulates activity of matrix metalloproteinases and may consequently play a prominent role in tumour behaviour. TIMP-1 has shown a promising potential as biomarker in colorectal cancer (CRC), and recent results have demonstrated a relation between TIMP-1 and EGFR. The aim of the present study was to investigate the clinical value of plasma TIMP-1 in patients with KRAS wt metastatic CRC treated with cetuximab and irinotecan.
Materials and Methods: Patients with KRASwt chemotherapy resistant mCRC submitted for third-line therapy with cetuximab (initial 400 mg/m^2 followed by weekly 250 mg/m^2)/irinotecan (350 mg/m^2 q3w) were prospectively included in a biomarker study. Pre-treatment blood samples were collected and plasma TIMP-1 was measured by a validated in-house ELISA assay. Response was classified according to RECIST. Survival data were analysed by the Kaplan-Meier method and log-rank testing.
Results: 51 patients were included and the overall response rate was 35%. The median plasma TIMP-1 level was 282 ng/ml (range 75–948) and used as cut-off level for statistical analysis. There was no correlation between pre-treatment patient characteristics and TIMP-1 levels. However, the median baseline plasma TIMP-1 levels were significantly higher in patients with early progression compared to patients who achieved disease control, 349 ng/ml (233–398 95%CI) and 215 ng/ml (155–289 95%CI), respectively, $p = 0.03$. This difference translated into a longer PFS in patients with low plasma TIMP-1 levels;7.7 months (5.3−9.2 95%CI) compared to 2.8 months (2.3−6.5 95%CI), respectively, ($p = 0.056$). Furthermore, a significantly different median OS of 11.5 months (8.6−17.3 95%CI) versus 5.3 months (3.9−10.4 95%CI) was demonstrated, and the HR was 1.83 (1.02−3.28), p = 0−03.
Conclusion: Despite the obvious limitations of a small sample size the present results suggest that KRASwt patients with a low pre-treatment plasma TIMP-1 level are more likely to benefit from third-line treatment with cetuximab/irinotecan. Further analyses in larger studies are warranted.

6119 POSTER

Triple mutational testing for response to EGFR inhibitor treatment with Cetuximab and Irinotecan in metastatic colorectal cancer

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Background: A major fraction of KRAS wild-type patients are non-responders to EGFR inhibitor treatment. Investigations of supplementary predictive factors are therefore highly relevant. Mutations in the other genes coding for the RAS-RAF-MAPK pathway have been identified and may also determine primary resistance to EGFR inhibition. As supplement to KRAS analysis, BRAF and PIK3CA mutations may account for additional non-responders. A few previously published studies have combined data from patients treated with different EGFR containing regimes and different treatment lines. We investigated the predictive and prognostic value of these mutations in a uniform material of third-line treatment with combination therapy CETIRI.
Materials and Methods: 88 patients with mCRC were prospectively included. All patients were previously exposed to 5FU, irinotecan and oxaliplatin and progressed on treatment. Treatment consisted of CETIRI (irinotecan (350 mg/m^2 q3w) and cetuximab (400 mg/m^2 loading dose followed by weekly 250 mg/m^2)). Response was evaluated according to RECIST. Following tumour DNA purification, mutational analyses were performed on tumour tissue by commercially available KRAS, BRAF and PIK3CA kits. Survival analysis was performed by Kaplan Mayer plot with log rank testing.
Results: A total of 88 patients had DNA available for triple mutational testing. The median number of cycles was 6 (range 1−18), and 21% achieved a partial response. Forty patients (45%) harboured KRAS mutations, 3 (3.4%) BRAF and 14 (16%) PIK3CA. All were non-responders,