

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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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 folic acid (LFA) during pelvic RT yielded 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≤5cm from the anal verge and/or +ve CRM, M1 resectable/initially unresectable) received 3 biweekly courses of OXA (100 mg/m²)/RTX (2.5 mg/m²) on day 1, and 5FU (800 mg/m²)/LFA 250 mg/m² 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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POSTER

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/m² followed by weekly 250 mg/m²)/irinotecan (350 mg/m² 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.

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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/m² q3w) and cetuximab (400 mg/m² loading dose followed by weekly 250 mg/m²). 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,