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Topoisomerase 1(*TOP1*) gene copy number in stage III colorectal cancer patients and its relation to prognosis

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ABSTRACT

Purpose: A Topoisomerase 1 (Top1) poison is frequently included in the treatment regimens for metastatic colorectal cancer (mCRC). However, no predictive biomarkers for Top1 poisons are available. We here report a study on the *TOP1* gene copy number in CRC patients and its association with patient prognosis and tumor cell proliferation.
Experimental design: The study included *TOP1* and CEN-20 fluorescence *in situ* hybridization (FISH) analyses on formalin fixed paraffin embedded (FFPE) tissue sections from 154 stage III CRC chemonaïve patients. The frequencies of aberration in the *TOP1* gene copy number, the CEN-20 copy number and the *TOP1*/CEN-20 ratio were analyzed and associated with overall survival (OS), time to recurrence (TTR) and in a subgroup analysis of rectal cancer patients only with time to local recurrence (LR in RC). Moreover, the *TOP1* and CEN-20 copy numbers were correlated with the tumor Ki67 proliferation index.
Results: 35.7% of the tumors had an increased *TOP1* copy number above 4*n* gene copies per cell and 28.6% and 9.7% had a *TOP1*/CEN-20 ratio $\geq$1.5 or $\geq$2.0, respectively. The *TOP1* copy number and the *TOP1*/CEN-20 ratios were separately added into multivariate analyses as continuous variables, in which also age, gender, primary tumor location and Ki67 status were added as covariates. In contrast to the *TOP1*/CEN-20 ratio, the *TOP1* copy number was significantly associated with OS (HR: 0.62; 95% CI: 0.42–0.90; $p = 0.01$). Neither the *TOP1* copy number nor the ratio was significantly associated with TTR and only the *TOP1*/CEN-20

Abbreviations: CEN-20, centromere 20; CC, colon cancer; CI, confidence interval; CRC, colorectal cancer; DFS, disease free survival; 5FU, 5-fluorouracil; FFPE, formalin-fixed paraffin-embedded; FISH, fluorescence *in situ* hybridization; GISTIC, Genomic Identification of Significant Targets in Cancer; HR, hazard ratio; Ki67, Antigen KI-67; LR in RC, local recurrence in rectal cancer; mCRC, metastatic CRC; PFS, progression free survival; OS, overall survival; REMARK, REporting recommendations for tumor MARKer prognostic studies; RC, rectal cancer; SN38, 7-Ethyl-10-hydroxy-camptothecin; SD, standard deviation; TTR, time to recurrence; Top1, DNA Topoisomerase 1 protein; *TOP1*, DNA Topoisomerase 1 gene; Top2α, DNA Topoisomerase II alpha protein; *TOP2A*, DNA Topoisomerase 2 gene.

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ratio was significantly associated with LR in RC (HR: 0.25; 95% CI: 0.08–0.83; $p = 0.02$). No significant correlation was found between the *TOP1* copy number and proliferation, while a weak and inverse correlation between the CEN-20 copy number and proliferation was observed.

Conclusions: This study showed that increased *TOP1* gene copy numbers are frequent findings in cancer cells in stage III CRC tumors but unrelated to the proliferative status of the tumors. The association with prognosis is important to consider when planning and analyzing future studies investigating *TOP1* as a potential predictive biomarker for Top1 poisons.

1. Introduction

In early as well as late stage colorectal cancer (CRC) there is a need to identify and validate predictive markers, which will allow for a personalized treatment approach where the individual patient upfront receives the treatment with the highest likelihood of a beneficial effect.

Most predictive biomarkers also carry a prognostic value, which may disturb the interpretation of the clinical value of such biomarkers (Nielsen and Brunner, 2011). One good example of this is the gene for topoisomerase IIα (Top2α), *TOP2A*. Breast cancer patients with *TOP2A* gene aberrations (amplifications or deletions) have a significantly worse prognosis than patients with normal *TOP2A* copy numbers (Knoop et al., 2005). At the same time, these patients have an increased likelihood of obtaining a beneficial effect from treatment with Top2α poisons. For example, treating *TOP2A* gene aberrated breast cancer patients with adjuvant chemotherapy containing an anthracycline (Top2α poison) will significantly increase disease free survival (DFS) and overall survival (OS) of these patients and the *TOP2A* aberrant patients will now have DFS and OS comparable to *TOP2A* normal patients treated with an anthracycline (Knoop et al., 2005; Di et al., 2011; Nielsen and Brunner, 2011). However, without a control group receiving non-anthracycline containing chemotherapy, the predictive value of the *TOP2A* copy number determinations for anthracycline treatment could be overlooked (Tubbs et al., 2009; Harris et al., 2009; Nielsen and Brunner, 2011).

Topoisomerase 1 (Top1) has recently been suggested as a predictive biomarker for the effect of irinotecan, a Top1 poison frequently used in combination therapy of metastatic CRC (mCRC) (Braun et al., 2008). In one study (Braun et al., 2008), the authors reported a significant association between high Top1 immunoreactivity as determined in formalin-fixed paraffin-embedded (FFPE) sections from the primary tumor and a benefit (i.e. longer progression free survival (PFS)) for patients receiving irinotecan or oxaliplatin based chemotherapy but not for patients receiving 5-fluorouracil (5FU) alone. In a retrospective case control study (Kostopoulos et al., 2009) investigating Top1 immunoreactivity in FFPE sections from Dukes' stage B and C CRC tumors receiving adjuvant treatment with 5FU or 5FU plus irinotecan, the investigators reported that patients with Top1 high protein expressing tumors had improved OS. However, because of short follow-up time on patients receiving adjuvant 5FU plus irinotecan, the study had limitations in differentiating between the potential prognostic and/or a predictive value of Top1 (Kostopoulos et al., 2009). The authors raised the question whether high Top1 protein expression was a favorable prognostic marker by itself, or whether the prognosis could be further improved by adding irinotecan to patients with Top1 high expression tumors in the adjuvant treatment of CRC.

Reproducible quantification of protein levels in FFPE tissue may be difficult to obtain, due to variation in the handling of samples and justify the search for alternative methods to establish relationships between Top1 and the clinical response to irinotecan. Counting gene copy numbers with fluorescence *in situ* hybridization (FISH) technology is appealing, since DNA is more stable and is not as sensitive to the preanalytical sampling, handling, processing and storage of the samples, as is protein. Furthermore, the gene signals are easily quantified on a cell-to-cell basis.

We recently reported on *TOP1* FISH analyses on 50 FFPE primary CRC tissues and 10 human CRC cell lines (Romer et al., 2012). A reference probe representing centromere 20 (CEN-20) was also included. More than 80% of the clinical samples (CRC stage III) demonstrated an increased *TOP1* gene copy number as compared with the mean *TOP1* gene copy number + 3 standard deviations (SD) in unaffected colorectal mucosa. Approximately 2/3 of the samples had an increased *TOP1*/CEN-20 ratio as compared with the non-affected mucosa. Furthermore, our study on cell lines suggested an association between *TOP1* gene copy number or the *TOP1*/CEN-20 ratio with sensitivity to the Top1 poison irinotecan (SN38), but not to oxaliplatin.

However, in order to bring *TOP1* copy number determination forward as a predictive biomarker for Irinotecan treatment a number of additional analyses have to be performed. We here report on a study that aims to answer three questions: 1) Can the high frequency of *TOP1* gene aberrations found in our previous study (Romer et al., 2012) be confirmed in a larger CRC patient population, since a certain frequency is required to make it clinically relevant as a predictive biomarker for irinotecan treatment? 2) Does the *TOP1* copy number associate with patient prognosis? i.e. the course of the disease in untreated patients, since as mentioned above, a prognostic component of a biomarker requires a control group in order to study its predictive value, and 3) Is there an association between *TOP1* copy number and cancer cell proliferation? This is important because *TOP1* gene copy numbers in tumors may be overestimated if more cells are in the