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Original Article

TP53 Mutational Status and Prediction of Benefit from Adjuvant 5-Fluorouracil in Stage III Colon Cancer Patients

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ABSTRACT

We investigated the hypothesis that the varying treatment efficacy of adjuvant 5-fluorouracil (5FU) in stage III colon cancer is linked to the *TP53* mutational status.

ABCSG-90 was a prospective randomized trial in which effect of adjuvant 5FU was studied in stage III colon cancer patients. Tumor material of 70% of these patients (389/572) was available for analysis of the biomarker *TP53* using a *TP53*-gene-specific Sanger sequencing protocol.

Median follow-up was 88 months. *TP53* mutation frequency was 33%. A significant interaction between *TP53* status, outcomes and nodal category was found (P = 0.0095). In the N1 category, *TP53* wildtype patients had significantly better overall survival than *TP53* mutated (81.0% vs. 62.0% overall survival at 5 years; HR = 2.131; 95% CI: 1.344–3.378; P = 0.0010). In the N2 category, the *TP53* status did not affect survival (P = 0.4992). In *TP53* wildtype patients, the prognostic significance of N category was significantly enhanced (P = 0.0002). In *TP53* mutated patients, survival curves of N1 and N2 patients overlapped and nodal category was no longer prognostic.

The biomarker *TP53* independently predicted effect of adjuvant 5FU in N1 colon cancer patients. *TP53* was not predictive in N2 patients, in whom 5FU is known to have no effect.

1. Introduction

Adjuvant chemotherapy is recommended for all patients with lymph-node positive colon cancer (Gill et al., 2004; Greene et al., 2002a). For decades, 5-fluorouracil (5FU) was the only drug demonstrating meaningful activity in colorectal cancer and is still used in any modern adjuvant chemotherapy regimens for colon cancer (Chau and Cunningham, 2006). At the beginning of this century, oxaliplatin, irinotecan, capecitabine, cetuximab, and bevacizumab became available. But the major, dramatic progress promised by new biological and targeted agents failed to emerge (Brenner et al., 2014; Cassidy et al., 2008; Saltz et al., 2008; Tol et al., 2009; Hecht et al., 2009). Still no drug shows greater single agent activity than 5FU. But it is general knowledge that treatment efficacy varies substantially among 5FU-treated patients of similar stage and baseline risk factors. The question why remained unanswered although it is burning since the early days of 5FU.

5FU induces DNA-damage in rapidly growing cells. It is generally accepted that wild type *TP53* is responsible for apoptosis induction in response to DNA-damage. In more than 30% of colon cancers *TP53* is mutated and apoptosis cannot be induced. However, clinical reports about the role of the *TP53* status for response to adjuvant chemotherapy

Abbreviations: 5FU, 5-fluorouracil; ABCSG, Austrian Breast and Colorectal Cancer Study Group; LEV, levamisole; INF, interferon; FFPE, formalin-fixed paraffin-embedded; HR, hazard ratios; CI, confidence intervals.

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in colon cancer are not consistent (Elsaleh et al., 2001; Russo et al., 2005; Petersen et al., 2001; Ahmed et al., 2008; Bleeker et al., 2001).

The aim of this study was to evaluate whether and how the *TP53* mutational status is linked to the varying efficacy of 5FU treatment in stage III colon cancer. For this purpose, we performed a retrospective biomarker analysis in a prospective randomized cohort of stage III colon cancer patients treated with adjuvant 5FU. The prospective randomized trial (ABCSG-90) was initially reported by the Austrian Breast and Colorectal Cancer Study Group (ABCSG) in 2005 (Schippinger et al., 2005).

2. Materials and Methods

2.1. Patient Cohort

Between 1991 and 1999, 572 stage III colon cancer patients were recruited for ABCSG-90 in order to investigate the influence of adding levamisole (LEV) and/or interferon (INF) alpha to adjuvant fluorouracil. In 2005 the results of ABCSG-90 were published. It was reported that neither LEV nor INF improved the efficacy of adjuvant 5FU in stage III colon cancer (Schippinger et al., 2005). Patient inclusion criteria were histologically proven lymph node positive carcinoma of the colon (stage III), age between 18 and 80 and curative resection. Rectal cancers were excluded.

2.2. Tumor Material

Formalin-fixed paraffin-embedded (FFPE) tissue samples of the surgical specimens from 389 patients were available for the retrospective *TP53* biomarker analysis, corresponding to 70% of the original 572 study patients. The 389 analyzed patients were equally distributed between the randomized treatment arms of ABCSG-90.

2.3. Ethics

The retrospective biomarker analysis was approved by the local ethics committee of the Medical University of Vienna (EK 761/2008).

2.4. TP53 Sequencing

The mutational status of *TP53* was assessed using DNA extracts from archived FFPE material from the surgical specimens. *TP53*-gene-specific Sanger sequencing was used for gene analysis. (MARK53gssx6, p53-gene-specific sequencing kit; Mark53 Ltd.; www.mark53.com; Vienna). Genetic variations in the *TP53* gene were reported and interpreted according to recommendations of the Human Genome Variation Society (www.hgvs.org) (den Dunnen and Antonarakis, 2000).

The reference genomic sequence used for *TP53* was NM_000546.5 and U94788 (NCBI GenBank).

The functional activity of *TP53* mutations was classified based on the overall transcriptional activity on eight different promoters as measured in yeast assays by Kato et al. (Kato et al., 2003; Petitjean et al., 2007) : *TP53* mutants considered to have a median transcriptional activity >75% were classified as wildtype for the calculation. A median transcriptional activity of <75% was considered partially functional and was classified as mutant for calculation.

2.5. Statistical Methods

Continuous data are described by median, minimum, and maximum. Categorical data are described with absolute and relative frequencies. A chi-square test was used to assess for group differences for binary and nominal variables. For ordinal variables, a trend chi-square test was used. In the case of sparse data, Fisher's Exact test or exact chi-square tests were used.

The primary outcome measure was overall survival. Overall survival is defined as the time between the date of randomization until death from any cause or censored at last time known to be alive. Median follow-up time was calculated using the Kaplan–Meier method, in which deaths are censored for further follow-up. Survival probabilities were estimated by Kaplan–Meier graphs, and group differences were assessed by log-rank test. A Cox regression model was used to assess group differences by hazard ratios (HR) and corresponding 95% confidence intervals (CI). A Cox regression model was used to assess interactions between prognostic factors and *TP53* mutation status. In the case of a significant interaction, separate subgroup analyses were performed. Multiple Cox regression models were fitted to assess the impact of *TP53* on survival above that of standard prognostic factors.

Statistical calculations were performed with SAS® (SAS Institute Inc., Cary, NC, USA). All p-values are two-sided, and $P \le 0.05$ was considered statistically significant.

Colon cancers were classified according to the UICC/AJCC staging system (N1 = metastasis in one to three regional lymph nodes; N2 = metastasis to four or more regional lymph nodes) (Greene et al., 2002b). No rectal cancers were included.

3. Results

TP53 sequencing analysis was successful in all 389 specimens. *TP53* mutations were detected in 129 patients (33%). Mutations are characterized in Table 1. Three mutants had a median transcriptional activity >75% and were classified as wildtype for the calculation. Six mutants were only partially functional and were classified as mutant for calculation. Four mutations, though located in the introns, had a predicted effect on splicing and were classified as mutant (NNsplice 0.9, HSF_V2.3) (Petitjean et al., 2007). Four silent mutations had a predicted effect on splicing and were classified as mutant. Seven patients had two mutations in the *TP53* gene.

The prognostic covariates are shown in Table 2. The frequency of *TP53* mutations did not differ between nodal status (N1, N2; P = 0.187), tumor stages (IIIA, IIIB, IIIC; P = 0.445), or between the treatment arms of the ABCSG-90 (fluorouracil, fluorouracil + LEV, fluorouracil + INF, fluorouracil + LEV + INF; P = 0.148).

Median age was 63.3 years (min 20.2 and max 79.2 years). Median follow-up time was 88 months. The five-year overall survival of the analyzed 389 patients was 71.2%, which is comparable to five-year overall survival reported for the original cohort of the ABCSG-90, which included 572 patients.

3.1. Nodal Category Specific Interaction Between TP53 Status and Survival

Overall, *TP53* wildtype patients performed better than *TP53* mutant patients, but this difference was not significant (P = 0.0640). A significant interaction between *TP53* status and lymph node categories was seen in Cox regression models (P = 0.0095) and justified the examination of the effect of *TP53* status in the nodal categories N1 versus N2 separately.

In N1 patients, the five-year overall survival was 81.0% for *TP53* wildtype patients compared to 62.0% in *TP53* mutant patients (HR = 2.131 (95 % CI: 1.344–3.378); P = 0.0010) (Fig. 1A). *In N2 patients*, the *TP53* status did not affect the overall survival (P = 0.4992). The five-year overall survival was 66.3% for *TP53* wildtype patients and 69.3% for mutant patients (HR = 0.834 (95% CI: 0.493–1.412)) (Fig. 1B).

After adjustment for other prognostic factors, including sex, age as continuous variable, grading, therapy, and tumor location, the *TP53* mutational status remained a significant factor for overall survival in N1 patients (HR = 1.886 (95% CI: 1.172–3.036) P = 0.0090). In N2 patients, *TP53* remained a non-significant factor for overall survival.

As expected, N1 patients had a significant better survival than N2 patients (P = 0.0117) (Fig. 2). *In TP53 wildtype patients*, discrimination between favorable and poor survival prognosis by nodal category was improved (P = 0.0002) (Fig. 3A). *In TP53 mutant patients*, survival curves of N1 and N2 patients overlapped (P = 0.6393) and nodal category was no longer prognostic in *TP53* mutant patients (Fig. 3B).

Table 1
TP53 mutations.

Type of *TP53* mutation[a]	Nr. of patients with that mutation	Exon
c.102dupC p.(Pro36fs)	1	4
c.216dupC p.(Val73fs)	1	4
c.326T>C p.(Phe109Ser)	1	4
c.332T>G p.(Leu111Arg)	1	4
c.338T>G p.(Phe113Cys)	1	4
c.341T>A p.(Leu114Ter)	1	4
c.375G>A p.?	1	4
c.380C>T p.(Ser127Phe)	1	5
c.402T>A p.(Phe134Leu)	1	5
c.404G>T p.(Cys135Phe)	1	5
c.405C>G p.(Cys135Trp)	1	5
c.406C>T p.(Gln136Ter)	1	5
c.413C>T p.(Ala138Val)	1	5
c.452C>A p.(Pro151His)	1	5
c.454_466del p.(Pro152fs)	1	5
c.455C>T p.(Pro152Leu)	1	5
c.470T>G p.(Val157Gly)	1	5
c.475G>C p.(Ala159Pro)	1	5
c.485T>G p.(Ile162Ser)	1	5
c.493C>T p.(Gln165Ter)	1	5
c.514_524del p.(Val172fs)	1	5
c.517G>A p.(Val173Met)	1	5
c.524G>A p.(Arg175His)	10	5
c.527G>T p.(Cys176Phe)	1	5
c.536A>C p.(His179Pro)	1	5
c.553delA p.(Ser185fs)	1	5
c.559 + 1G>T p.?	1	Intron 5
c.571_573delCCT p.(Pro191del)	1	6
c.576_577delinsCA p.[(Gln192His(;)His193Asn)]	1	6
c.578A>G p.(His193Arg)	1	6
c.578A>T p.(His193Leu)	1	6
c.580C>T p.(Leu194Phe)	1	6
c.583A>T p.(Ile195Phe)	1	6
c.586C>T p.(Arg196Ter)	1	6
c.612_613insA p.(Tyr205fs)	1	6
c.626_627delGA p.(Arg209fs)	1	6
c.637C>T p.(Arg213Ter)	9	6
c.638G>A p.(Arg213Gln)	1	6
c.641A>G p.(His214Arg)	1	6
c.646G>A p.(Val216Met)	1	6
c.650_658del p.(Val217_Tyr220delinsAsp)	1	6
c.672 + 1G>C p.?	1	Intron 6
c.672 + 1G>T p.?	1	Intron 6
c.700T>G p.(Tyr234Asp)	1	7
c.701A>G p.(Tyr234Cys)	1	7
c.706T>G p.(Tyr236Asp)	1	7
c.707A>C p.(Tyr236Ser)	1	7
c.711G>T p.(Met237Ile)	1	7
c.712T>C p.(Cys238Arg)	1	7
c.713G>A p.(Cys238Tyr)	1	7
c.716A>G p.(Asn239Ser)	1	7
c.723delC p.(Cys242fs)	1	7
c.726C>T p.?	2	7
c.733G>A p.(Gly245Ser)	2	7
c.737T>C p.(Met246Thr)	1	7
c.742C>T p.(Arg248Trp)	3	7
c.743G>A p.(Arg248Gln)	5	7
c.746G>T p.(Arg249Met)	2	7
c.747G>T p.(Arg249Ser)	1	7
c.750delC p.(251Ilefs)	1	7
c.770T>C p.(Leu257Pro)	1	7
c.772G>A p.(Glu258Lys)	1	7
c.773A>G p.(Glu258Gly)	1	7
c.782_782 + 1delGG p.?	1	7
c.794T>C p.(Leu265Pro)	1	8
c.799C>A p.?	1	8
c.800G>A p.(Arg267Gln)	2	8
c.809T>C p.(Phe270Ser)	1	8
c.811G>A p.(Glu271Lys)	1	8
c.814G>A p.(Val272Met)	3	8
c.814G>C p.(Val272Leu)	1	8
c.817C>T p.(Arg273Cys)	3	8
c.818G>A p.(Arg273His)	7	8
c.824G>A p.(Cys275Tyr)	1	8
c.832C>T p.(Pro278Ser)	1	8
c.833C>T p.(Pro278Leu)	1	8
c.844C>T p.(Arg282Trp)	3	8
c.851_852delCA p.(Thr284fs)	1	8
c.856G>A p.(Glu286Lys)	1	8
c.880delG p.(Glu294fs)	1	8
c.892G>T p.(Glu298Ter)	1	8
c.916C>T p.(Arg306Ter)	6	8
c.920-2A>G p.?	1	Intron 8
c.920-7_923delTTCCTAGCACT p.?	1	9/Intron 8
c.1009C>T p.(Arg337Cys)	1	10
c.1024C>T p.(Arg342Ter)	1	10
c.1025_1041dup17 p.(Leu348fs)	1	10
c.1027G>A p.(Glu343Lys)	1	10
c.1045G>T p.(Glu349Ter)	1	10
c.274C>T p.(Pro92Ser)[b]	1	4
c.548C>T p.(Ser183Leu)[b]	1	5
c.629A>G p.(Asn210Ser)[b]	1	6
c.277C>T p.(=)[c]	1	4
c.291C>T p.(=)[c]	1	4
c.525C>A p.(=)[c]	1	5
c.624C>T p.(=)[c]	1	6
c.699C>T p.(=)[c]	1	7
c.717C>T p.(=)[c]	1	7
c.1014C>T p.(=)[c]	1	10
c.1023C>T p.(=)[c]	1	10
c.1098C>T p.(=)[c]	1	10

[a] Mutations are reported according to Recommendations of the Human Genome Variation Society (www.hgvs.org).
[b] Mutations were considered and calculated as wildtype due to transactivation activity >75%.
[c] Mutations were considered and calculated as wildtype as they were characterized as silent mutations without affecting transcription, splicing or translation.

3.2. Tumor-stage Specific Interaction of TP53 Status and Survival

The Cox regression models consistently showed a significant interaction between *TP53* status and traditional tumor stage ($P = 0.0374$). Due to this significant interaction, the effect of *TP53* status was also investigated separately in the different tumor stages (IIIA, IIIB, and IIIC).

Table 2
Prognostic covariates.

Cohorts:	Study cohort n = 389	*TP53* wildtype n = 260	*TP53* mutant n = 129	P-value
Stage IIIA	32	23 (71.9%)	9 (28.1%)	0.445
Stage IIIB	191	120 (62.8%)	71 (37.2%)	
Stage IIIC	166	117 (70.5%)	49 (29.5%)	
N1 (1-3)	223	143 (64.1%)	80 (35.9%)	0.1878
N2 (≥4)	166	117 (70.5%)	49 (29.5%)	
Female	190	132 (69.5%)	58 (30.5%)	0.2806
Male	199	128 (64.3%)	71 (35.7%)	
age	63.3	62.1 (20.2–79.1)	65.1 (32.8–79.2)	0.0062
Tumor grade				
G1-G2	262	169 (64.5%)	93 (35.5%)	0.1751
G3	126	90 (71.4%)	36 (28.6%)	
Location				
Proximal	190	135 (71.1%)	55 (28.9%)	0.0845
Distal	199	125 (62.8%)	74 (37.2%)	
Treatment arms				
5FU	94	57 (60.6%)	37 (39.4%)	0.1482
5FU/interferon	102	76 (74.5%)	26 (25.5%)	
5FU/levamisol	91	63 (69.2%)	28 (30.8%)	
5FU/interferon/levamisol	102	64 (62.7%)	38 (37.3%)	

Age is given as median (minimum–maximum), other categorical variables are described with absolute frequencies and percentages.

In stage IIIA, the five-year survival for patients with wildtype *TP53* was 91.3% versus 77.8% for *TP53* mutant stage IIIA patients. The HR was 3.008 (95% CI: 0.598–15.128; P = 0.1609). Statistical significance was not reached due to the small number of stage IIIA patients (n = 32) and the small number of events (n = 6) in this subgroup.

In stage IIIB, the five-year survival for *TP53* wildtype patients was 79.0% versus 60.1% for *TP53* mutant patients (HR = 2.014 (95% CI: 1.246–3.258); P = 0.0036).

Stage IIIC was identical to N2, with no differential benefit in relation to *TP53* status.

Factors evaluated in a *multiple Cox regression model* included sex, age, grading, therapy, and tumor location. After adjustment for the prognostic factors, the effect of *TP53* on survival increased for stage IIIA patients (HR = 8.775 (95% CI: 0.608–126.619); *P* = 0.1108), although this was still non-significant due to the small numbers of patients and events as mentioned above. For stage IIIB patients, the effect of *TP53* on survival remained significant (HR = 1.731 (95% CI: 1.048–2.859); P = 0.0321). For stage IIIC patients, *TP53* still had no effect on survival (HR = 1.023 (95% CI: 0.584–1.791); P = 0.9379).

4. Discussion

Here we report the presence of a significant interaction between *TP53* status and survival in stage III colon cancer patients treated with adjuvant 5FU. We found that adjuvant 5FU resulted in a marked survival benefit in *TP53* wildtype N1 patients, while N1 patients with mutant *TP53* performed as bad as N2 patients. The *TP53* status independently predicted the effect of adjuvant 5FU on survival in N1 patients while there was no prediction of 5FU effect in N2 patients.

Colon cancer patients analyzed in this study were treated in the 1990s, when adjuvant 5FU was the standard of care in stage III colon cancer. In the meantime it was recognized that adjuvant 5FU alone is too week as chemotherapy at least for patients with advanced stage of disease (N2 disease). Our results reflect this clinical experience on a molecular level: *TP53 predicted* the effect of 5FU in N1 patients but not in N2 patients, in whom we do not await a therapeutic effect from 5FU alone. It makes sense that in the absence of a therapeutic effect, a predictive marker does not predict an effect.

Moreover, the here reported results support the hypothesis that *TP53* is not a prognostic but purely predictive type of biomarker (Pilat et al., 2015). A purely predictive marker is considered to affect survival only in the presence of a treatment. In the absence of a treatment or a treatment effect, a pure predictive marker will not interfere with survival (Pilat et al., 2015; Akiyama et al., 2014). In N2 patients *TP53* was not predictive for an effect of 5FU most likely because there is no effect of 5FU in these advanced patients.

TP53 has been intensively studied in colorectal cancer. Some currently unexplained findings of meta-analyses appear in a new light

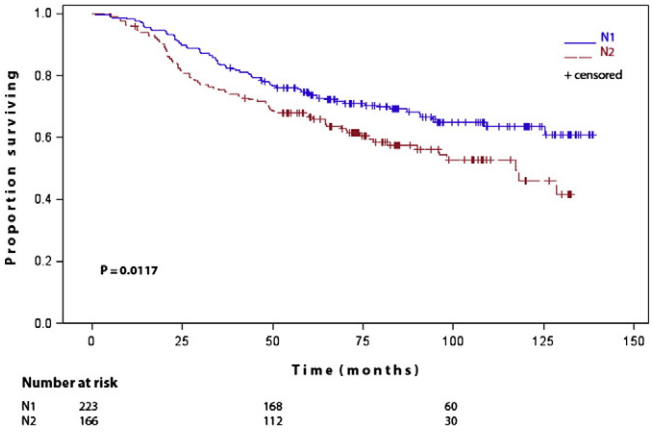

Fig. 2. Kaplan–Maier estimates of cumultative survival of N1 patients versus N2 patients.

based upon our findings. For instance, it has been reported that alterations in the *TP53* gene have little or no influence on survival in patients treated with surgery alone, but were associated with worse survival for patients treated with chemotherapy (Iacopetta, 2003). This again supports our observation that *TP53* is not a prognostic but a predictive biomarker, influencing survival only in the presence of effective chemotherapy, as mentioned above. Another meta-analysis including more than 18,000 patients was published 2005, when 5FU was standard adjuvant chemotherapy (Munro et al., 2005). The analysis found "the adverse impact of abnormal *TP53* to be greater in colon cancer patients with a lower baseline risk of dying". This is consistent with our finding that *TP53* was predictive for the effect of 5FU in N1 patients but not in N2 patients.

The traditional T and N classification, originally developed in the 1950s, still provides the most important prognostic information in colon cancer and is guiding treatment decisions (Brenner et al., 2014; Denoix and Schwartz, 1959). But in stage III colon cancer, prognostic inconsistency is a well-known phenomenon. By contrast to stage II, stage III patients usually are treated with adjuvant chemotherapy. Our IIIA/B patients with wildtype *TP53* status experienced a marked benefit from adjuvant 5FU, resulting in survival rates, which are comparable to those of stage II colon cancer patients (Greene et al., 2002b; Gunderson et al., 2010). A positive treatment effect occurred in *TP53* wild type patients with stage IIIA/B but not IIIC as well as in wild type N1 but not in N2 patients. Therefore the prognostic significance of the N category shown in Fig. 2 appeared to be enhanced in *TP53* wild type patients as demonstrated in Fig. 3A. Our *TP53* mutated stage IIIA/B or N1 patients however, did as bad as our stage IIIC or N2 patients. Thus a negative treatment effect occurred in *TP53* mutated N1 patients but not in N2 patients. Therefore N category was no longer prognostic in

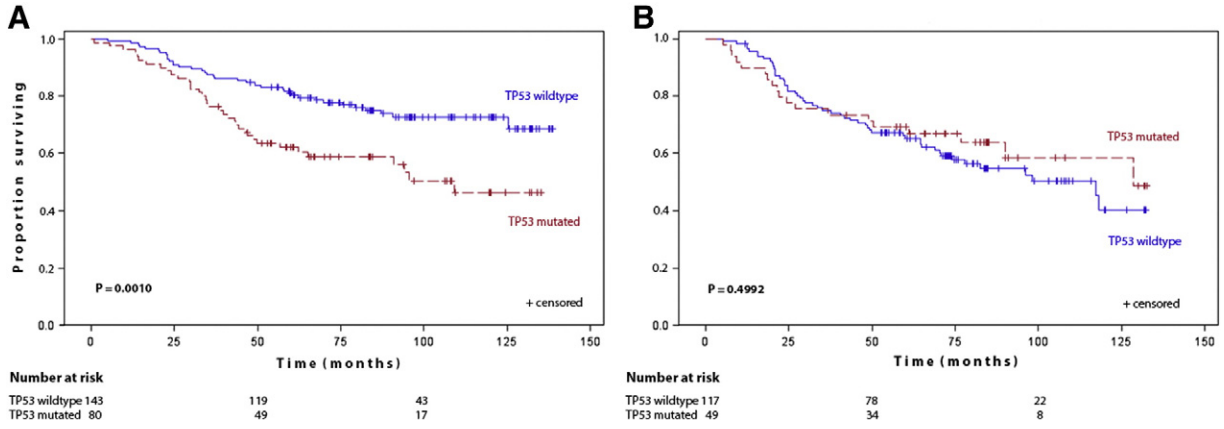

Fig. 1. (A,B): Kaplan–Maier estimates of cumultative survival by *TP53* status (A) in N1 patients (B) in N2 patients.

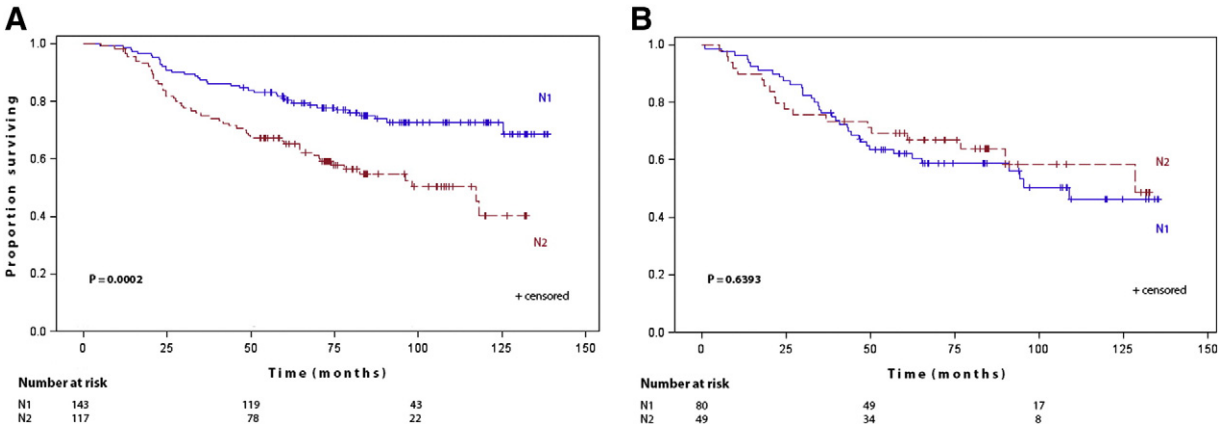

Fig. 3. (A,B): Kaplan–Maier estimates of cumultative survival by lymphnode category (A) in *TP53* wildtype patients (B) in *TP53* mutant patients.

TP53 mutated patients which represent 33% of stage III patients. We suggest that the *TP53* marker status is linked to varying treatment effects, which are likely to cause prognostic inconsistency in low risk stage III colon cancer.

Biomarker research is complex in that proof of the clinical utility of a biomarker is crucially linked to the analytical validity of the respective biomarker-test. *TP53* has not yet been recommended for clinical use as a marker. Thus demonstration of the validity of *TP53* tests was not top of the list (Petersen et al., 2001; Munro et al., 2005; Roth et al., 2012). Even though immunohistochemistry has been recognized as an insufficient method for indicating the presence of *TP53* mutations, and even though sequencing has been generally accepted as the 'gold standard' for investigating the *TP53* status, methodology is still far from being standardized. Various sequencing technologies and protocols are in use worldwide, and there is evidence for dramatic variations in the sensitivity and specificity of these methods (Petitjean et al., 2007; Edlund et al., 2012; Soussi et al., 2006).

In recent years, we have identified numerous pitfalls while sequencing the *TP53* gene. We believe that they arise from the special characteristics of the *TP53* gene itself and its mutations, as well as from technical conditions such as FFPE material, low copy numbers of tumor DNA in biopsy material, or from the presence of normal cells in tumor material, to list only a few. Over the years, we developed a p53-gene-specific sequencing protocol to addressing these and other conditions in a stepwise fashion. We believe that this was a crucial step that allowed us to uncover the here reported interaction between *TP53* and chemotherapy effect. Meanwhile, we proceeded to validating this standardized, *TP53* gene-specific sequencing protocol in prospective biomarker trials (www.ClinicalTrials.gov; PANCHO NCT00525200, PART NCT02140723).

ABCSG-90 was a prospective randomized adjuvant colon cancer trial investigating the influence of adding levamisole (LEV) and/or interferon (INF) alpha to adjuvant fluorouracil. Tumor material from only 70% of patients from the ABCSG-90 was available for *TP53* analysis in the here reported study. Thus study limitations may arise from incomplete analysis of the ABCSG-90 cohort as well as from the treatment combinations, which were investigated in ABCSG-90. However, we believe that random patient selection as well as different treatment effects is unlikely to bias our results: The ABCSG-90 reported no difference in survival among the investigated treatment arms. The patients included in the retrospective biomarker analysis were distributed equally among treatment arms as demonstrated in Table 2. The *TP53* mutations were also distributed equally among treatment arms as well as among tumor stages and lymph node categories. And finally the five-year overall survival of ABCSG-90 was comparable to the smaller cohort analyzed for the biomarker.

In conclusion, *TP53* was found to be a strong, independent marker for predicting the effect of adjuvant 5FU chemotherapy in lymph node positive colon cancer patients.

We found that adjuvant 5FU resulted in a marked survival benefit in *TP53* wildtype N1 patients, while *TP53* mutated N1 patients performed as bad as N2 patients. The *TP53* status independently predicted the effect of adjuvant 5FU on survival in N1 patients while there was no prediction of 5FU effect in N2 patients. The significant interaction between *TP53* status, nodal category and treatment effect offers an explanation for the known prognostic inconsistency in stage III colon cancer patients. Further studies are needed to validate these findings.

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Conflict of Interest Statement

Leadership position, associate: Daniela Kandioler (Mark53 Ltd).
Employment: Sonja Kappel (half time) (Mark53 Ltd).
Consultant or Advisory Role: Daniela Kandioler (uncompensated) (academic sponsored clinical *TP53* trials).
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