



The Role of p53 Expression in Patients with *RAS/BRAF* Wild-Type Metastatic Colorectal Cancer Receiving Irinotecan and Cetuximab as Later Line Treatment

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Abstract

Background Preclinical and clinical data indicate that p53 expression might modulate the activity of the epidermal growth factor receptor (EGFR), influencing response/resistance to anti-EGFR monoclonal antibodies. However, the association between p53 status and clinical outcome has not been clarified yet.

Objective In our study, we evaluated the role of p53 expression in patients with *RAS/BRAF* wild-type metastatic colorectal cancer (mCRC) receiving irinotecan/cetuximab in an exploratory and a validation cohort.

Patients and Methods p53 expression was analysed in patients with *RAS/BRAF* wild-type mCRC receiving second-line or third-line irinotecan/cetuximab. Survival distribution was assessed by the Kaplan–Meier method, while the log-rank test was used for survival comparison.

Results Among 120 patients with *RAS/BRAF* wild-type mCRC included in our analysis, 52 (59%) and 19 (59%) patients showed p53 overexpression in the exploratory and validation cohort, respectively. In the exploratory cohort, low p53 expression was correlated with better median progression-free survival (hazard ratio 0.39; $p < 0.0001$), median overall survival (hazard ratio: 0.23; $p < 0.0001$) and response rate ($p < 0.0001$). These results were confirmed by data of the validation cohort where we observed better median progression-free survival (hazard ratio: 0.48; $p = 0.0399$), median overall survival (hazard ratio: 0.26; $p = 0.0027$) and response rate ($p = 0.0007$) in patients with p53 normal expression mCRC.

Conclusions In our study, p53 overexpression was associated with anti-EGFR treatment resistance in patients with *RAS/BRAF* WT mCRC, as confirmed in a validation cohort. Larger studies are needed to validate the role of p53 and investigate EGFR cross-talk in these patients.

1 Introduction

The transmembrane tyrosine kinase epidermal growth factor receptor (EGFR or HER1, ErbB1) belongs to the ErbB family along with ErbB-2, ErbB-3 and ErbB-4a and is a key driver of cell proliferation, survival, adhesion, migration and differentiation [1–3]. Deregulation of the EGFR pathway was observed in several malignancies such as colorectal, non-small cell lung, breast, ovary, renal, head and neck,

pancreatic, prostate, cervical and bladder cancer [1, 4, 5]. In this setting, EGFR overexpression/upregulation and hyperactivation might be responsible for the promotion of tumour cell growth, resistance to apoptosis, synthesis of angiogenic and growth factors and metastatic spread. As a consequence, EGFR blockade plays an established role as an anti-cancer strategy, especially in colorectal cancer (CRC), non-small cell lung cancer, and head and neck tumours [6–14]. Presently, the anti-EGFR monoclonal antibodies cetuximab and panitumumab represent the cornerstone of treatment for *RAS* wild-type (WT) metastatic CRC (mCRC) [15, 16].

Unfortunately, not all patients with *RAS* WT mCRC are sensitive to EGFR-targeting agents and resistance to these drugs is still an open issue [17–21]. Several potential mechanisms for the lack of efficacy of anti-EGFR have been

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Key Points

TP53 status may affect the response to anti-epidermal growth factor receptor (EGFR) therapies as a consequence of p53 activity on the EGFR promoter, and *TP53* mutation is often associated with its overexpression.

Our analysis suggests a predictive/prognostic role of abnormal p53 expression in patients with metastatic colorectal cancer treated with anti-EGFR therapy.

The finding of p53 overexpression as a resistance mechanism to anti-EGFR therapies represents a further step toward personalised treatment.

investigated beyond *N-RAS* and *K-RAS* mutational status, such as *BRAF* V600E mutation [22–26], *PI3KCA* exon 20 mutation, *PTEN* loss [22, 27–29], Stat3 and Akt phosphorylation [22, 30–32], *HER2* amplification [22, 33–36], *HER3* expression [37], IGF1R activation [22, 38, 39], *MET* amplification [22, 40], altered vascular endothelial growth factor/vascular endothelial growth factor receptor expression [22, 41], *EGFR* gene copy number alteration and *EGFR* methylation [42, 43], but for most of these no definitive data are available.

Among the potential mechanisms of resistance, data suggest that *TP53* mutations might play a role in tumour cell sensitivity to anti-EGFR antibodies. In fact, recent studies have suggested that activation of the EGFR pathway leads to malignant transformation only if the p53 protein is inactivated [44].

The p53 protein, which is encoded by the tumour suppressor gene p53 (*TP53*) located in chromosome 17p, is one of the most important tumour suppressors. Acting as a zinc-containing transcription factor [45–49], it regulates downstream genes involved in DNA repair, cell-cycle arrest and apoptosis [50–54]. More specifically, in the event of cellular stress signals such as genotoxic damage, oncogene activation, hypoxia or loss of intercellular adhesion, p53 takes on an active tetrameric form and stops cell-cycle progression and induces DNA repair, senescence and cell death through apoptosis to preserve the genomic integrity. Consequently, p53 has a crucial role in the regulation of cell proliferation and cancer development inhibition; for its pivotal role in protection from tumour development, it has been defined as “the guardian of the genome” [54–61].

Mutations or deletions of the *TP53* gene can be found in nearly 50% of human cancers and they lead to an impaired tumour suppressor function [62]. Tumours harbouring a *TP53* mutation are usually characterised by genomic instability and poor prognosis [54, 63, 64]. Moreover, it was

shown that in p53 mutants, the loss of WT p53 function is also associated with the gain of new oncogenic roles promoting cancer, metastasis, inhibition of apoptosis and drug resistance [64–67].

Mutations in *TP53* usually occur in 40–60% of patients with CRC. There are well-known logistical difficulties and resource limitations associated with direct sequencing of the *TP53* gene. Therefore, most studies have used immunohistochemistry to detect mutant p53, with the assumption that a mutation is often associated with its overexpression. However, the lack of expression is generally indicative of WT *TP53*. Only in limited cases, a complete loss of p53 expression is associated with truncating mutations and loss of heterozygosity (LOH). Several trials documented conflicting results regarding the association between p53 overexpression and clinical outcome [68–71].

The predictive function of *TP53* mutations in patients with mCRC treated with targeted therapies has not been established so far. However, some evidence suggests that *TP53* status may affect the response to anti-EGFR therapies as a consequence of the activity of p53 on the promoter of the EGFR [72–77]. However, findings from previous analyses investigating the role of p53 in this setting were contradictory. Based on these considerations, we designed our study with the aim to assess the predictive/prognostic role of p53 abnormal expression in patients with mCRC treated with anti-EGFR therapy by using a validation cohort.

2 Methods

2.1 Patients and Methods

Patients with *RAS* and *BRAF* WT mCRC treated with second-line or third-line irinotecan and cetuximab were included in our retrospective analysis. Between May 2011 and May 2018, subjects treated at the University of Cagliari were included in the exploratory cohort, while subjects treated at Istituto Oncologico Veneto constituted the validation cohort. Tumour samples were retrospectively tested for p53 immunohistochemical (IHC) expression with the aim of evaluating the correlation with clinical outcome in terms of overall survival (OS), progression-free survival (PFS), response rate (RR) and disease control rate (DCR).

The analysis of p53 expression were performed on formalin-fixed and paraffin-embedded tumour samples for all patients using an anti-p53 antibody (DO7, prediluted; Leica Biosystems, Newcastle upon Tyne, UK) and the Bond Polymer Refine Detection Kit (Leica Biosystems) on a BOND-MAX automated IHC stainer (Leica Biosystems), as instructed by the manufacturer. The cut-off

point for p53 expression was set as 30%, as previously described. In detail, if 30% or more of the malignant nuclei were positive, the tumour was scored as positive, while if fewer than 30% of the nuclei were stained, the tumour was scored as negative (Fig. 1) [78–80]. For study purposes, right-sided and left-sided colorectal primary tumours were defined as proximal or distal to the splenic flexure.

2.2 Statistical Analysis

Statistical analysis was performed with the MedCalc Statistical Software Version 14.10.2 (MedCalc Software bvba, Ostend, Belgium; <http://www.medcalc.org>; 2014). The association between categorical variables was estimated by the Fisher exact test for categorical binomial variables or by the chi-square test in all other instances. Survival probability over time was estimated by the Kaplan–Meier method. Significant differences in the probability of survival between the strata were evaluated by the log-rank test. The independent role of variables that were statistically significant at a univariate analysis was assessed with a logistic regression analysis.

Overall survival was defined as the time interval between the date of the beginning of cetuximab/irinotecan treatment to death or the last follow-up visit for patients who were lost at follow-up. Progression-free survival was defined as the interval between the date of the beginning of cetuximab/irinotecan treatment to death, first sign of clinical progression or the last follow-up visit for patients who were lost at follow-up.

Response rate was defined as the percentage of patients who achieved a partial or complete response to treatment according to RECIST Version 1.1. Disease control rate was defined as the percentage of patients with stable disease or partial/complete response to treatment.

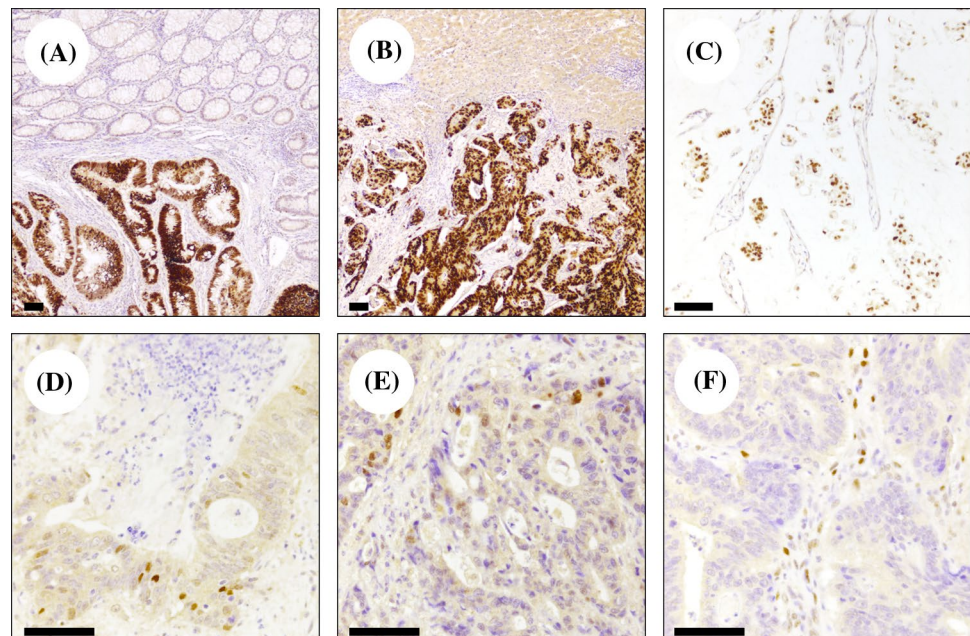
Based on the results from the 88 patients of the exploratory cohort, we tried to validate the findings in a validation cohort. Then, we identified the validation group sample size according to good/poor prognosis group ratio and survival analysis, from the exploratory cohort. To validate the difference in terms of a 12-month rate among poor prognosis patients (around 15%) and good prognosis patients (around 70%), as we obtained in the exploratory cohort (assuming a probability alpha of 0.05 and a beta of 0.10), with a two group ratio of 0.7, the required sample size would have been 32 patients (13 + 19), using a “comparison of proportion test”.

3 Results

3.1 Patient Characteristics

Globally, 120 patients with *RAS/BRAF* WT mCRC were included in our analysis, 88 in the exploratory cohort, treated at the University of Cagliari and 32 in the validation cohort, treated at Istituto Oncologico Veneto, between May 2011 and May 2018. Patient baseline characteristics are detailed in Tables 1 and 2.

Fig. 1 Representative p53 immunohistochemical staining in *RAS/BRAF* wild-type colorectal adenocarcinomas. Diffuse overexpression in a primary colorectal carcinoma (a) and in a hepatic metastatic tumour (b); note the absence of p53 immunostaining in the surrounding normal colon mucosa and hepatic parenchyma. c A p53 overexpressing mucinous colorectal adenocarcinoma. d, e Heterogeneous and low p53 expression in two cases of primary colorectal adenocarcinoma. f A complete loss of p53 expression; note the presence of positive stromal cells that are used as positive controls of the reaction (original magnifications $\times 5$, $\times 10$ and $\times 20$; scale bars 100 μm)



In the exploratory cohort as well as in the validation cohort, p53 IHC low expression was 41% (36/88 and 13/32 patients, respectively). p53 IHC overexpression was found in the 59% of patients in both cohorts (52/88 patients in the exploratory cohort and 19/32 patients in the validation cohort).

In the exploratory cohort, 59% of the patients had a left-sided colorectal cancer, 46% of them had p53 low expression and 54% p53 overexpression. Conversely, among patients with right-sided CRC, 30% had p53 low expression and 70% had p53 overexpression ($p = 0.15$).

The left colon site was the most common in the validation group with 62% of patients. In this subgroup of patients, 48% had p53 low expression and 52% had p53 overexpression. Conversely, among patients with right-sided CRC, 14% had p53 low expression and 86% had p53 overexpression ($p = 0.114$).

3.2 Clinical Outcomes

At a median follow-up of 10.5 months (95% confidence interval [CI] 9.0–13.7), in the exploratory cohort, we observed a median PFS (mPFS) of 6 months (95% CI 3.8–18.0) and a median OS (mOS) of 10.0 months (95% CI 9.0–13.6). Median PFS was 8.0 months (95% CI 6.9–18.0) vs 3.0 months (95% CI 2.9–11.1) in patients non-overexpressing p53 and in patients overexpressing p53, respectively (hazard ratio [HR]: 0.39; $p < 0.0001$). Median OS was 19.5 months (95% CI 17.0–30.5) vs 8 months (95% CI 5.9–9.8) in patients non-overexpressing p53 and in patients overexpressing p53, respectively; (HR: 0.23; $p < 0.0001$) (Fig. 2). Overall RR was 33%. Response rate was 58% vs 15% in patients with p53 non-overexpressing tumours and in patients with p53 overexpressing tumours, respectively ($p < 0.0001$). Overall DCR was 59%. Disease control rate was 86% vs 40% in patients with p53 non-overexpressing

Table 1 Patient characteristics in the exploratory and validation cohorts

<i>N</i>	Global population 120	Exploratory cohort 88	Validation cohort 32
Age, years, median (range)	62 (37–79)	64 (37–79)	60 (49–66)
Sex, <i>n</i> (%)			
M	89 (74)	69 (78)	20 (62)
F	31 (26)	19 (22)	12 (38)
ECOG PS, <i>n</i> (%)			
0–1	105 (88)	76 (86)	29 (90)
≥2	15 (12)	12 (14)	3 (10)
Primary tumour location, <i>n</i> (%)			
Left	72 (60)	52 (59)	20 (62)
Right	48 (40)	36 (41)	12 (38)
Time of metastatic disease, <i>n</i> (%)			
Metachronous	45 (37)	35 (39)	10 (32)
Synchronous	75 (63)	53 (61)	22 (68)
Anti-EGFR treatment line, <i>n</i> (%)			
II	76 (63)	56 (64)	20 (62)
III	44 (37)	32 (34)	12 (38)
Median OS, months	11.8	10	15.8
Median PFS, months	6	6	7
Best response to treatment, <i>n</i> (%)			
CR	–	–	–
PR	40 (33)	29 (33)	11 (34)
SD	39 (33)	23 (26)	16 (50)
PD	41 (34)	36 (41)	5 (15)
p53 IHC, <i>n</i> (%)			
p53 over expression	71 (59)	52 (59)	19 (59)
p53 low expression	49 (41)	36 (41)	13 (41)

CR complete response, ECOG Eastern Cooperative Oncology Group, F female, IHC immunohistochemistry, M male, OS overall survival, PD progression disease, PFS progression-free survival, PR partial response, SD stable disease

Table 2 Patient baseline characteristics according to p53 expression

	Overall population	Left sided	Right sided
Exploratory cohort			
Total, <i>n</i> (%)	88	52 (59%)	36 (41)
p53 overexpressed	52 (59)	33 (54%)	19 (70)
p53 low expression	36 (41)	28 (46)	8 (30)
Validation cohort			
Total, <i>n</i> (%)	32	20 (62)	12 (38)
p53 overexpressed	19 (59)	13 (52)	6 (86%)
p53 low expression	13 (41)	12 (48)	1 (14)

tumours and in patients with p53 overexpressing tumours, respectively ($p < 0.0001$) (Table 3).

These results were confirmed in the validation cohort, where we observed an mPFS of 7 months (95% CI 4.6–12.0) and an mOS of 15.8 months (95% CI 9.5–28.0). Response rate was 34%, and DCR was 84%. Response rate was 69% in patients with low p53 and 11% in patients with abnormal p53 ($p = 0.0007$); DCR was 100% vs 74% for patients with low p53 and abnormal p53 tumours, respectively ($p = 0.0475$) (Table 3). Median OS was 18 months in patients with p53 low expression (95% CI 13.6–28.0) vs 11.46 months in patients with p53 overexpression (95% CI 8.2–18.0; HR: 0.26; $p = 0.0027$) and mPFS was 8.1 months in patients with low p53 (95% CI 6.5–12.0) vs 5.8 months in patients with abnormal p53 (95% CI 3.0–11.4; HR: 0.48; $p = 0.0399$) (Fig. 3).

Regarding the tumour sidedness, patients with left-sided CRC had better outcomes than patients with right-sided CRC in the exploratory cohort, with an mOS of 12.5 months (95% CI 9.9–19.0) vs 8 months (95% CI 5.9–9.8), respectively (HR: 0.5; $p = 0.0232$). Finally, while not statistically significant, patients with left-sided CRC had better outcomes than patients with right-sided CRC in the validation cohort with 16.0 months (95% CI 13.5–28) vs 8.9 months (95% CI 3.4–9.0), respectively (HR: 0.3; $p = 0.067$) (Fig. 4).

Therefore, p53 expression and sidedness showed a statistically significant correlation with OS at univariate analysis in both cohorts. At the multivariate analysis, in the exploratory cohort, p53 expression (Exp (b) 4.5750, $p < 0.0001$) and primary tumour location (Exp (b) 2.0820, $p = 0.0078$) maintained an independent role. In the validation cohort, only p53 expression (Exp (b) 5.0367, $p = 0.0050$) maintained an independent role.

Furthermore, to validate the prognostic role and the predictive effect of p53 in patients with *RAS/BRAF* WT mCRC treated with anti-EGFR, we analysed the survival data from the first-line palliative therapy in both cohorts. At a median follow-up of 25.1 months (95% CI 20.5–28.5), in the exploratory cohort, a significant difference in mOS was found between patients non-overexpressing p53 and patients overexpressing p53 [30.2 months (95% CI 26.9–47.9) vs 17.1 (95% CI 13.8–44.9), $p < 0.0001$, respectively]. These results were confirmed in the validation cohort with an mOS of 34.7 months (95% CI 25.8–40) vs 22.5 (95% CI 17–34), $p = 0.0003$ (Fig. 5). Finally, a non-statistically significant trend towards better mPFS was found in patients

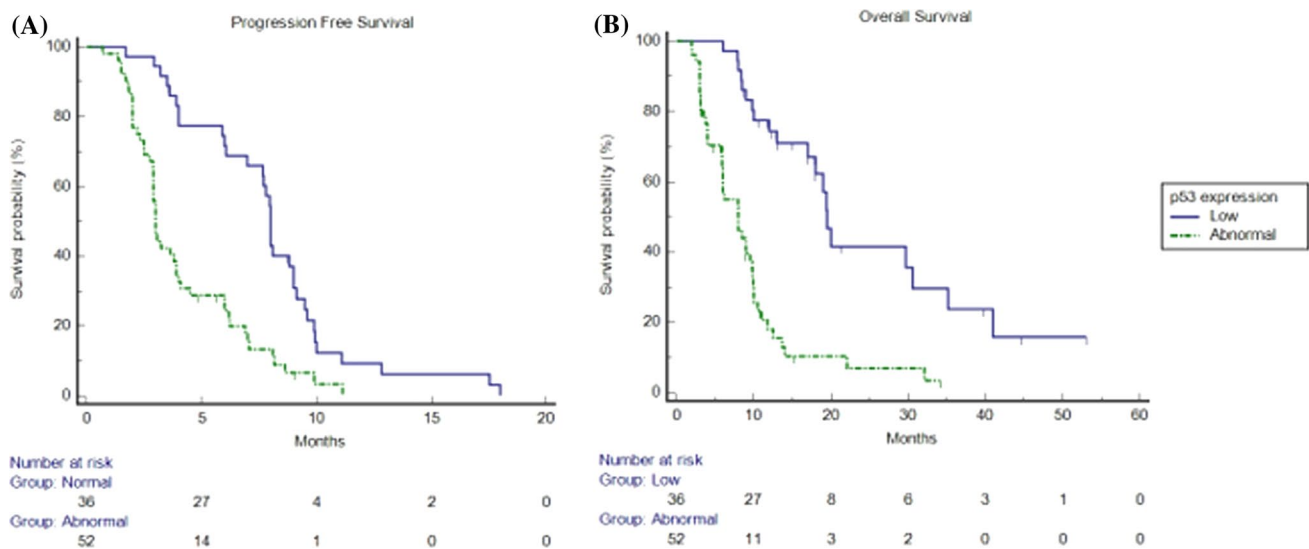


Fig. 2 Kaplan–Meier progression-free survival and overall survival in patients overexpressing p53 (dotted line) and in patients non-overexpressing p53 (continuous line) in the exploratory cohort. **a** Median progression-free survival was 8.0 months (95% confidence interval [CI] 6.9–18.0) vs 3.0 months (95% CI 2.9–11.1) in patients overex-

pressing p53 and in patients non-overexpressing p53, respectively (hazard ratio 0.39; $p < 0.0001$). **b** Median overall survival was 19.5 months (95% CI 17.0–30.5) vs 8 months (95% CI 5.9–9.8) in patients overexpressing p53 and in patients non-overexpressing p53, respectively (hazard ratio: 0.23; $p < 0.0001$)

Table 3 Response in patients of the exploratory and validation cohorts in overall population, in non-overexpressing p-53 tumours and in overexpressing p53 tumours

	Overall population	Overexpressing p53	Non-over-expressing p53
Exploratory cohort, <i>n</i> (%)			
ORR	29 (33)	8 (15)	21 (58)
DCR	52 (59)	21 (40)	31 (86)
Validation cohort, <i>n</i> (%)			
ORR	11 (34)	2 (11)	9 (69)
DCR	27 (84)	14 (74)	13 (100)

DCR disease control rate, ORR overall response rate

non-overexpressing p53 vs patients overexpressing p53 in the exploratory cohort [11 months (95% CI 10.6–20.9) vs 8 months (95% CI 7.9–20), $p = 0.135$] respectively] and the validation cohort [13.1 months (95% CI 11.8–20.4) vs 11.7 months (95% CI 8–18.9), $p = 0.1933$], respectively] (data not shown).

4 Discussion and Conclusions

The emergence of drug resistance represents the major limitation to the development and use of molecularly targeted cancer therapies. In this scenario, only a few studies tried to identify the prognostic and the predictive role of p53 in patients treated with anti-EGFR therapies.

Preclinical data suggest that both WT and mutant *TP53* can modulate EGFR expression in various tumours. Ludes-Meyers et al. showed that WT and mutant *TP53* use different mechanisms to activate EGFR transcription. They identified the binding site for WT *TP53* in an EGFR promoter gene-specific sequence and observed that the p53 DNA-binding capacity seemed to be related to this gene transcription activation; furthermore, this sequence appeared to be the same target site of positive and negative regulators of EGFR promoter activity. Conversely, mutant *TP53* did not require the WT p53-binding site for EGFR promoter transactivation but the oligomerisation domain after recruitment by other transcription factors. As mutant p53 is often overexpressed in tumour cells, this could lead to strong and persistent activation of growth factor genes, such as high EGFR levels and consequent aberrant cell proliferation [72–76]. A further study demonstrated that the loss of p53 in normal human keratinocytes is responsible for increased EGFR expression by a mechanism involving YY1 and Sp1 and does not require p53 binding to the EGFR promoter [77]. In summary, preclinical findings show that p53 modulates EGFR promoter activity both directly by DNA binding and indirectly through other transcription factors.

Other clinical studies evaluated the co-expression of mutant p53 and EGFR mutations/overexpression in different tumours, suggesting a correlation with poor prognosis and a lack of response to tyrosine kinase inhibitors, chemotherapy and radiotherapy in non-small cell lung cancer, head and neck, and hepatocellular malignancies [81–84].

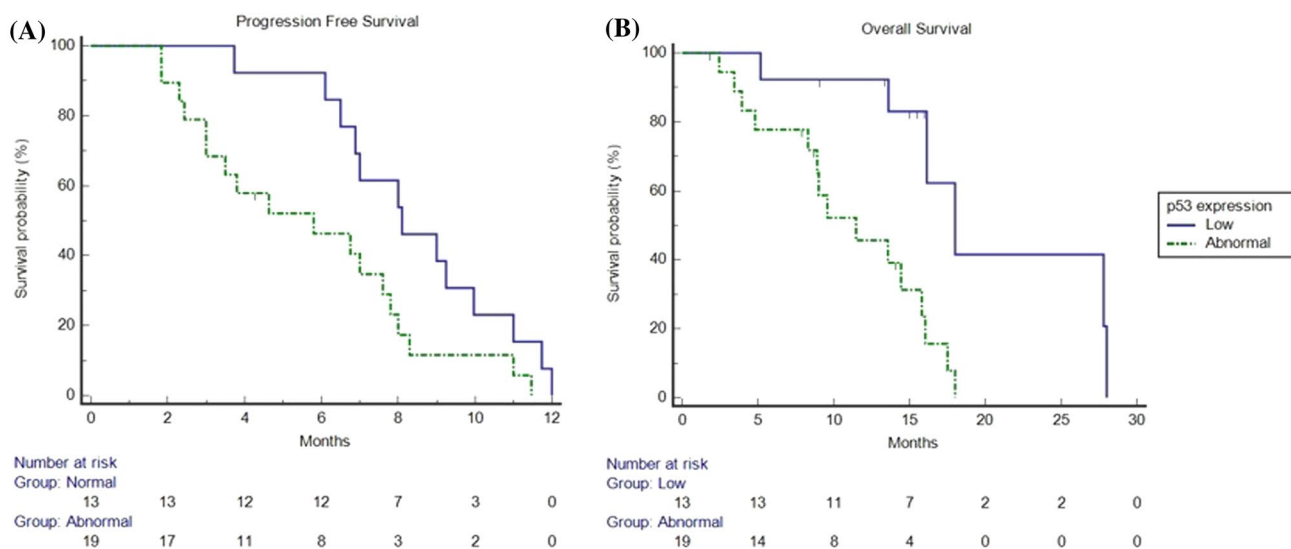


Fig. 3 Kaplan–Meier progression-free survival and overall survival in patients overexpressing p53 (dotted line) and in patients non-overexpressing p53 (continuous line) in the validation cohort. **a** Median progression-free survival was 8.1 months (95% confidence interval [CI] 6.5–12.0) in patients non-overexpressing p53 vs 5.8 months

(95% CI 3.0–11.4) in patients overexpressing p53 (hazard ratio: 0.48; $p = 0.0399$). **b** Median overall survival was 18 months (95% CI 13.6–28.0) in patients non-overexpressing p53 vs 11.4 months (95% CI 8.2–18.0) in patients overexpressing p53 (hazard ratio: 0.26; $p = 0.0027$)

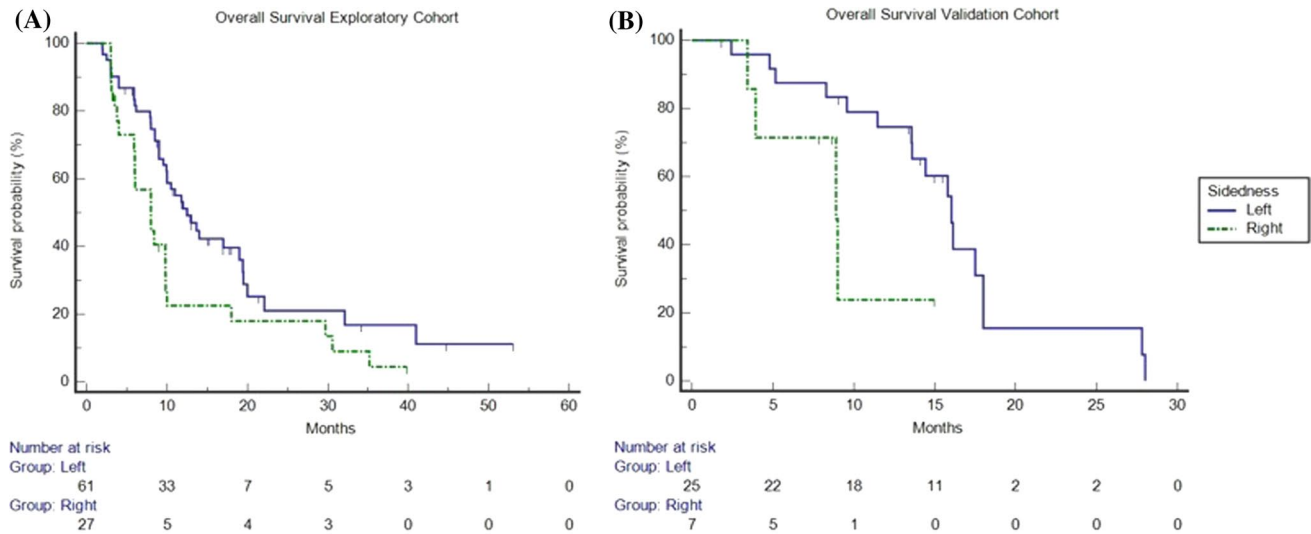


Fig. 4 Kaplan–Meier overall survival in patients with right-sided colorectal cancer [CRC] (dotted line) and in patients with left-sided CRC (continuous line) in the exploratory and validation cohorts. **a** In the exploratory cohort, median overall survival was 12.5 months (95% confidence interval [CI] 9.9–19.0) vs 8 months (95% CI 5.9–9.8) in patients with left-sided CRC and in patients with right-sided CRC, respectively (hazard ratio: 0.5; $p = 0.0232$). **b** In the validation

cohort, median overall survival in patients with right-sided CRC (dotted/green line) and in patients with left-sided CRC (continuous/blue line) is shown. Median overall survival was 16.0 months (95% CI 13.5–28.0) vs 8.9 months (95% CI 3.4–9.0) in patients with left-sided CRC and in patients with right-sided CRC, respectively (hazard ratio: 0.37; $p = 0.0670$)

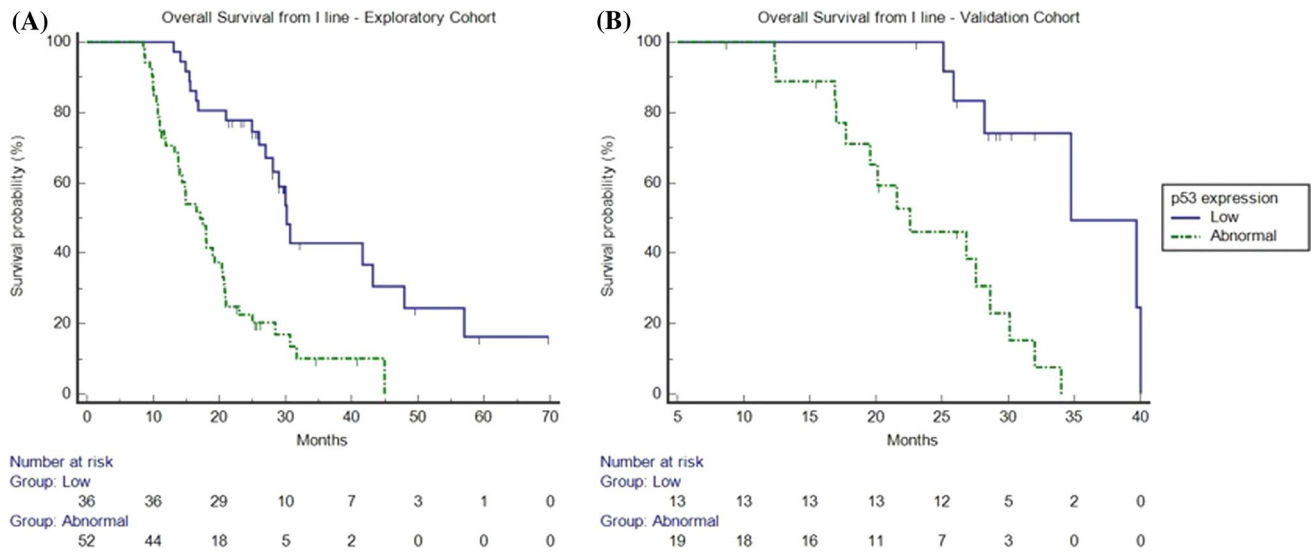


Fig. 5 Kaplan–Meier overall survival from first-line palliative therapy in patients overexpressing p53 (dotted line) and in patients non-overexpressing p53 (continuous line) in the explorative (**a**) and the validation cohort (**b**). **a** Median overall survival was 30.2 months (95% confidence interval [CI] 26.9–47.9) vs 17.1 (95% CI 13.8–44.9) in patients with low p53 expression and in patients overexpressing

p53, respectively (hazard ratio: 0.28; $p < 0.0001$) in the exploratory cohort. **b** Median overall survival was 34.7 months (95% CI 25.8–40) vs 22.5 (95% CI 17–34) in patients with low p53 expression and in patients overexpressing p53, respectively (hazard ratio: 0.17; $p = 0.0003$) in the validation cohort

However, the clinical role of *TP53* mutational status in CRC is still controversial. Several studies showed that patients with a tumour harbouring a *TP53* mutation have a significantly poorer outcome than patients with WT *TP53*

tumours. Moreover, p53 overexpression has a negative prognostic impact. Conversely, other findings failed to demonstrate a correlation between p53 overexpression and clinical outcome in patients with CRC [81–83]. Theodoropoulos

et al. retrospectively evaluated the relationship between p53 and EGFR expression assessing the correlation with clinical/histological prognostic factors and the impact on prognosis or survival in 164 patients with CRC with at least a 5-year follow-up. Overexpression of EGFR and p53 were significantly associated with an advanced T stage, suggesting that both proteins cause a growth advantage in deep invasion and they are a late event in CRC carcinogenesis [85].

In contrast with previous findings, Shyhmin et al. revealed that WT *TP53* may enhance sensitivity to EGFR inhibitors and radiation through cell-cycle arrest, apoptosis and DNA damage repair, in CRC and non-small cell lung cancer [86]. The other two studies showed that *TP53* mutations may be predictive of cetuximab sensitivity and *TP53* genotyping could be useful to optimise the selection of patients with mCRC who should benefit from cetuximab-based chemotherapy [87, 88].

Of note, most pathologists do not have access to *TP53* sequencing, and therefore, they use p53 immunohistochemistry as a surrogate for *TP53* mutational analysis. Immunohistochemistry is quick, easy to perform and less expensive than sequencing. Hence, p53 immunohistochemistry represents a feasible biomarker already in use in the clinical setting. On this basis, our study aimed to evaluate whether the p53 abnormal expression could influence the clinical outcome of a patient with *RAS/BRAF* WT mCRC, treated with anti-EGFR.

Globally, our analysis suggests that p53 overexpression may predict resistance to anti-EGFR treatment in patients with mCRC. In the exploratory cohort, a significant benefit in terms of all clinical outcomes (OS, PFS and RR) was observed in p53 WT patients, compared with those showing p53 over-expression. Furthermore, the p53 low expression status was more frequent in left-sided tumours. These results were confirmed in the validation cohort.

Furthermore, we analysed survival data from the first palliative therapy, which did not contain anti-EGFR antibodies. This analysis showed a significant benefit in terms of OS in p53 low expression patients compared with those showing p53 over-expression, in both cohorts. Conversely, no statistically difference in terms of PFS was observed in patients non-overexpressing p53 vs patients overexpressing p53 in the exploratory and validation cohorts. These data confirmed the prognostic role of p53 expression and suggested the absence of a predictive impact to response to therapies that do not contain anti-EGFR.

In the era of precision medicine, p53 analysis could improve the selection of patients who could benefit from anti-EGFR therapy. The identification of the optimal treatment strategy is successfully provided by a better knowledge of the mCRC heterogeneous nature. Therefore, the finding of p53 overexpression as a resistance mechanism

to anti-EGFR therapies represents a further step toward personalised treatment.

The limitations of our study are mainly related to its retrospective nature. In particular, this latter feature and the absence of a control arm without EGFR therapy do not allow us to clearly demonstrate that p53 overexpression is associated with anti-EGFR resistance. Still, our analysis suggests a possible predictive p53 role in this setting. In future studies, it would also be interesting to investigate the association between the *TP53* mutation, p53 IHC expression and clinical outcomes to confirm the use of p53 IHC, which is quick, easy to perform and inexpensive, as a surrogate for *TP53* mutational analysis.

Although retrospective, we believe that our analysis could provide a relevant addition to the biological picture underlying the mechanism of resistance to anti-EGFR monoclonal antibodies in patients with mCRC. Further prospective studies, with a control arm without anti-EGFR therapy, will be needed to validate the prognostic and predictive effect of p53 expression in this setting, to better refine the molecular profile of patients more likely to benefit from this crucial treatment strategy.

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Declarations

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Conflict of interest Pina Ziranu, Eleonora Lai, Marta Schirripa, Marco Puzzone, Mara Persano, Andrea Pretta, Giada Munari, Nicole Liscia, Valeria Pusceddu, Fotios Loupakis, Laura Demurtas, Michela Libertini, Stefano Mariani, Marco Migliari, Marco Dubois, Riccardo Giampieri, Giovanni Sotgiu, Angelo Paolo Dei Tos, Sara Lonardi, Alberto Zaniboni, Matteo Fassan and Mario Scartozzi have no conflicts of interest that are directly relevant to the content of this article.

Ethics approval This study was performed in accordance with the study protocol, the ethical principles stated in the Declaration of Helsinki as well as those indicated in the International Conference on Harmonization (ICH) Note for Guidance on Good Clinical Practice (GCP; ICH E6, 1995), and all applicable regulatory requirements.

Consent to participate All patients signed a written informed consent before study entry. Adequate information was given to eligible patients by the principal investigator or co-investigators at each participating centre and in accordance with local regulations. The declaration of informed consent was personally signed and dated by the subject, and by the investigator/person designated by the investigator to conduct the informed consent discussion.

Consent for publication Patients signed an informed consent regarding the publication of their data.

Availability of data and material The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Code availability Not applicable.

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