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Original Article

Clinical impact of *TP53* functional mutations in patients with metastatic colorectal cancer treated with bevacizumab and chemotherapy

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Abstract

Background: Clinical and experimental studies indicate that the tumor protein p53 (*TP53*) gene loss of function due to missense mutations (MMs) may confer sensitivity to anti-angiogenics. This effect seems to be linked to cross-talk mechanisms among *TP53*, vascular endothelial growth factor (VEGF), and VEGF receptors. We investigated whether specific *TP53* MMs are associated with clinical outcomes of patients with metastatic colorectal cancer (mCRC) treated with first-line chemotherapy plus Bevacizumab. The study focused on *KRAS*-mutated, liver-only mCRC cases as a homogeneous subgroup that may represent a relevant setting for exploring this association.

Materials and methods: MMs were identified on primary tumors. MMs were classified by mutant-specific residual transcriptional activity scores ($TP53_{RTAS}$) as transcriptionally inactive ($TP53_{inactive}$ = $TP53_{RTAS}$ 0%) or active ($TP53_{active}$ = $TP53_{RTAS}$ ≥ 1%) and used for stratifying patients in progression-free survival (PFS), response rate, and overall survival (OS) analyses.

Results: The study population consisted of 62 patients. MMs were found in 39 cases (62%) with 16 having $TP53_{inactive}$ and 23 $TP53_{active}$ MMs. Patients with $TP53_{inactive}$ MMs showed better PFS in comparison with the remaining groups (wild-type and $TP53_{active}$). This effect was retained in the multivariate model. A similar clinical impact was observed in the OS analysis. There was a significant difference in the overall response rate and rate of post-treatment resection of liver metastases between the $TP53_{inactive}$ and the wild-type or $TP53_{active}$ MMs cases.

Conclusions: Specific *TP53* MMs may identify sub-groups of patients who benefit from Bevacizumab-based systemic therapy and these findings could lead to novel tailored treatment strategies in this setting.

Key words: *TP53*; missense mutation; colorectal cancer; Bevacizumab; liver metastasis.

Implications for practice

TP53 missense mutations may identify sub-groups of patients with metastatic colorectal cancer who benefit from anti-angiogenics.

Introduction

Recent clinical findings in advanced cancer patients have shown improved survival outcomes with inhibitors of the vascular endothelial growth factor (VEGF) and VEGF receptor (VEGFR) mostly in tumors harboring a *TP53* mutation.[1-5] In gastric adenocarcinomas, we found improved survival in metastatic patients treated with ramucirumab/chemotherapy in the presence of a p53 loss of function (LOF) mutation.[6] Notably, *VEGF-A* mRNA expression levels were significantly increased in the *TP53* LOF mutation cases.[6] This finding parallels previous analyses in cancer tissues, which showed significant increases in VEGF expression levels in the presence

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of *TP53* mutations.[7-9] Indeed, several experimental and preclinical studies have recently indicated the molecular background underlying the role of *TP53* in the control of tumor VEGF. The *VEGF* promoter displays a highly conserved and functional p53-binding site and the p53 tumor suppressor downregulates VEGF expression.[3] In experimental cancer models, the interaction between loss of *TP53* and hypoxia inducible factor 1 or transcription factor E2F1 (E2F1) caused VEGF up-regulation.[10-12] In addition, mutant *TP53* can up-regulate the transcription of VEGF receptor 2 (VEGFR2) by promoter remodeling.[13] Over the last decades, *TP53* has been generally considered an unfavorable predictive or prognostic factor in translational cancer studies. However, this novel functional background could support the hypothesis that carriers of *TP53* mutations may benefit from anti-VEGF therapy.

Bevacizumab is a humanized anti-VEGF monoclonal IgG_1 antibody, which is approved for the treatment of several cancer types. Bevacizumab acts by selectively binding circulating VEGF, thereby inhibiting the binding of VEGF to its cell surface receptors, VEGFR-1 and VEGFR-2. It is currently employed in the systemic therapy of patients with metastatic colorectal cancer coupled with chemotherapy. Colorectal cancer represents the cancer entity with the highest prevalence of p53 mutations, with a prevalence ranging from 35% up to 70% of colorectal carcinomas harboring p53 mutations. In contrast to other tumor suppressor genes, which are predominantly altered by truncating mutations, approximately 90% of p53 mutations are missense mutations, meaning that the full-length protein with a single amino acid change is expressed.[14] As a consequence, somatic *TP53* missense mutations cannot be considered a homogeneous group inducing an on/off LOF effect since they may display a large gradient of functional consequences.[1] However, with the development of novel techniques of functional analyses, *TP53* missense mutations can now be classified for clinical purposes by considering their residual activity by functional assays.[15]

We investigated the possible clinical impact of *TP53* missense mutations in *KRAS*-mutated metastatic colorectal cancer patients in first-line chemotherapy plus Bevacizumab. The liver-only metastatic disease was deemed of particular interest considering the opportunity to study a homogeneous group of patients in a relevant clinical setting where translational information may improve treatment strategies including surgery for metastatic disease.

Materials and methods

The retrospective cohort study was implemented from consecutive metastatic colorectal cancer cases who were treated with first-line systemic therapy at the 3 Institutions of the Azienda Sanitaria Territoriale 1, Marche Nord, Italy (AST1) between 2018 and 2022.

Patients were considered eligible for study inclusion if they had received standard staging procedures with whole body computed tomography scan coupled, when indicated, with nuclear magnetic resonance or positron emission tomography. They had to be evaluated by the local multidisciplinary team conference and they had to be judged with unresectable colorectal cancer liver metastases (synchronous or metachronous) without extrahepatic disease. Liver metastases were considered non-resectable[16] in the case of expected failure of achieving a complete (R0) resection of all lesions in one single surgical procedure (excluding pre-operative hypertrophy procedures of the future liver remnant) by surgical resection alone (excluding additional thermal destruction of metastasis: radiofrequency ablation/microwave ablation), leaving a minimum remnant liver volume of 30% in normal livers or 40% in compromised livers (chemotherapy pretreated, cirrhotic).

Furthermore, the study inclusion required the availability of primary tumor tissue samples, the presence of a *KRAS* mutation (thus excluding therapy with anti-epidermal growth factor receptor, EGFR), the adoption of a 5-fluorouracil/Irinotecan (FOLFIRI) chemotherapy regimen coupled with Bevacizumab and adequate follow-up information. Carriers of *TP53* non-missense mutations were excluded. According to standardized clinical practices at the three AST1 participating institutions, re-staging procedures during systemic therapy were performed every 3 months. Treatment outcomes were evaluated in routine liver surgery and multi-disciplinary meetings with a dedicated radiologist. Liver-only metastatic disease allowed for the monitoring of the response of defined and measurable single-organ parenchymal lesions, with adherence to the RECIST criteria.

The study was performed in accordance with the reporting recommendations for tumor marker prognostic (REMARK) guidelines.[17] The study was performed in accordance with the International Conference on Harmonization Good Clinical Practice Guidelines, the Declaration of Helsinki (1996) and approved by the local Ethics Committee (CERM, Comitato Etico Regionale delle Marche) on December 15, 2022 (Protocol code no. 2022_364).

TP53 analysis

A sample of 4–6 10-µm sections from formalin-fixed paraffin-embedded specimens was obtained from patient tumors and matched with normal tissues. Before cutting sections for total nucleic acid isolation, an additional slide was prepared for hematoxylin-eosin staining and the pathologists identified representative areas with an almost complete representation of tumor infiltration. Tissues were micro-dissected and placed in a 1.5 mL reaction tube containing 1 mL xylene to remove paraffin. According to the manufacturer's instructions, DNA was extracted using the RecoverAll™ Multi-Sample RNA/DNA Isolation Workflow (Invitrogen™ by Thermo Fisher). DNA concentration and purity were measured using the NanoDrop 1000 spectrophotometer (Nanodrop Technologies). Next-generation sequencing and *TP53* analysis were performed as previously reported.[6]

Each *TP53* missense mutation was assigned a residual transcriptional activity score ($TP53_{RTAS}$) according to the results of a site-directed mutagenesis technique and yeast-based functional assay.[18,19] The $TP53_{RTAS}$ represents the median transcriptional activity value measured across eight different p53-responsive promoter elements (p21, Mdm2, Bax, 14-3-3, p53AIP1, GADD45, Noxa, and p53R2), that share the characteristic of being direct target genes of p53. This panel has been adopted in previous studies[6,19] and it displays a spectrum of p53-dependant regulation: cell growth arrest (p21, Gadd45 14-3-3); apoptosis (p53AIP1, BAX, NOXA); DNA repair (p53R2) TP53 auto-regulatory feedback loop (MdM2).

$TP53_{RTAS}$ provides positive values, which may exceed 100% activity in the case of uncommon hyper-active *TP53* missense mutations. Based on these functional scores, *TP53* missense mutations were then divided into 2 distinct groups: $TP53_{RTAS}$ = 1% (when $TP53_{RTAS} \geq 1\%$) and $TP53_{RTAS}$ = 0% (when $TP53_{RTAS}$ values between 0.1% and 0.9%, values were

rounded to the nearest whole number). This categorization denotes a clear distinction between a transcriptionally inactive group ($TP53_{inactive}$ =$TP53_{RTAS}$ 0%) vs a transcriptionally active group ($TP53_{active}$ =$TP53_{RTAS}$ ≥ 1%). The distribution of *TP53* gain-of-function (GOF) missense mutations[20] was analyzed in the $TP53_{inactive}$ and $TP53_{inactive}$ groups. The dominant negative effect (DNE) and DNA damage repair function (DDR) were analyzed in the $TP53_{active}$ and $TP53_{inactive}$ groups with assigned values as measured previously.[21]

Statistical analysis

The primary endpoint of the study was the progression-free survival (PFS) analysis in carriers of $TP53_{inactive}$ vs $TP53_{active}$ missense mutations. With 50 events and assuming a 25% prevalence of $TP53_{inactive}$ mutations, the scenario for sample size estimation would allow the detection of a 60% reduced risk of death with a power of 80% and a 2-sided type I error of 5%. PFS was calculated from the date of the first cycle of the first-line therapy to the date of progression or death. Tumor response was assessed every 12 weeks until disease progression. The Kaplan–Meier method was used to estimate survival curves and the log-rank test was used to compare survival times between groups. A multivariate Cox proportional hazards model was adopted to adjust it according to clinical and pathological features. Contingency tables were analyzed by the Chi-square test. For DNE and DDR analyses, *Z*-scores were plotted and statistical significance was determined using Mann-Whitney *U* tests. All reported *P*-values were 2-sided, and CIs were at the 95% level. A *P*-value < .05 was considered statistically significant. Survival analyses were performed using MedCalc™ for Windows, version 15.0 (MedCalc Software, Ostend, Belgium).

Results

The study population consisted of 62 liver-only metastatic colorectal cancer patients who underwent first-line systemic therapy with 5-fluorouracil and irinotecan in the FOLFIRI regimen. All cases were carriers of a *KRAS* mutation. A *TP53* missense mutation was found in 39 patients, while 23 were *TP53* wild-type. As shown in Table 1, no significant difference was observed in the distribution of clinical and pathological features between *TP53* mutated and *TP53* wild-type cases.

Table 1. Characteristics of the 62 patients and distribution according to the *TP53* status.

Variable	Number of patients (%)			*P*-value
	Total	*TP53* mutated	*TP53* Wild-type	
Age				
>65 years	20 (32)	10 (50)	10 (43)	0.1
≤65 years	42 (68)	29 (69)	13 (77)	
Gender				
Male	32 (56)	23 (59)	9 (39)	0.1
Female	30 (44)	16 (41)	14 (61)	
Primary tumor location				
Left colon	36 (52)	24 (61)	12 (52)	0.5
Right colon	26 (48)	15 (39)	11 (48)	
Primary tumor resection				
No	12 (20)	7 (18)	5 (22)	0.7
Yes	50 (80)	32 (82)	18 (78)	
Mucinous histology				
No	53 (85)	34 (87)	19 (83)	0.7
Yes	9 (15)	5 (13)	4(17)	
ECOG-PS				
0	47 (76)	31 (79)	16 (69)	0.5
1	15 (24)	8 (21)	7 (31)	

Abbreviations: ECOG-PS, Eastern Cooperative Group Performance Status.

Characterization of *TP53* missense mutations

In Table 2, the residual transcriptional activity scores for each *TP53* missense mutation are reported. The range of the RTAS median value was 0%-18.1%. Accordingly, 16 patients were classified as carriers of a $TP53_{inactive}$ missense mutation and 23 patients were classified as carriers of a $TP53_{active}$ missense mutation. The distribution of GOF missense mutations was balanced between the two groups, with 7 and 8 carriers of GOF *TP53* missense mutation in the $TP53_{inactive}$ and $TP53_{active}$ groups, respectively (Table 2). As shown in Figure 1, DNE was significantly higher in $TP53_{active}$ carriers than in $TP53_{inactive}$ carriers, while DDR activity was comparable between $TP53_{inactive}$ and $TP53_{active}$ groups.

Progression-free survival analysis

Figure 2 shows Kaplan-Meier survival plots displaying PFS in carriers of *TP53* missense mutations versus *TP53* wild-type cases (A) and in carriers of $TP53_{inactive}$ or $TP53_{active}$ missense mutations versus *TP53* wild-type cases (Figure 2B). Considering carriers of *TP53* missense mutations as a whole (Figure 2A), no significant difference was observed. In patients with *TP53* wild-type, the median PFS was 16.7 months (95%CI, 13.7-18.0 months), while carriers of *TP53* missense mutations showed a median PFS of 15 months (95%CI, 10.2-17.0 months). A significant difference in PFS became evident after the dichotomization of *TP53* missense mutation carriers (Figure 2B) with 22.3 months (95%CI, 9.0-28.7 months) median PFS time in the $TP53_{inactive}$ group and 12 months (95%CI, 9.0-15.0 months) median PFS time in the $TP53_{active}$ group.

The multivariate model included: age (>65 years vs ≤65 years), gender (male vs female), primary tumor colorectal location (right vs left), primary tumor resection (yes vs no), liver metastasectomy with clear margins (yes vs no), Eastern Cooperative Group performance status (1 vs 0), mucinous histology (present vs absent) and *TP53* status ($TP53_{inactive}$ vs $TP53_{active}$ and wt). Among these factors, $TP53_{inactive}$ status (0.35 hazard ratio with 0.14-0.88 CI, *P* = .02) and liver metastasectomy with clear margins (0.41 hazard ratio with 95% CI, 0.17-0.98, *P* = .04) retained the favorable effect on PFS.

Tumor response and liver surgery

Forty patients (64.5%) showed complete or partial responses and the remaining 22 patients (35.5%) showed stable disease or disease progression. Liver metastasectomy was carried out in 19 patients. The distribution of tumor responses and liver surgery procedures was analyzed according to the *TP53* status. As shown in Table 3, carriers of $TP53_{inactive}$ missense

Table 2. Observed *TP53* missense mutations with RTAS values.

Missense mutation	*Frequency (*N*)	RTAS median
p.C135Y	1	6.6
p.C176Y	2	3.2
p.C242S	2	0
p.D259V	1	10.8
p.E285K	1	15.8
p.F270C	1	0
p.G245S	2	0
p.H178P	2	7.1
p.H193D	1	1.3
p.H193P	2	11.8
p.L130F	1	18.0
p.P151H	2	7.8
p.R175H**	8	9.2
p.R213L	2	0
p.R248Q**	5	0
p.R273C	2	0.4
p.R273H**	2	0
p.R280S	1	11.9
p.V157L	1	18.1
p.V274L	1	4.4

*One patient was the carrier of 2 *TP53* missense mutations.
**Gain-of-Function (GOF) missense mutation.
Abbreviations: RTAS, residual transcriptional activity scores.

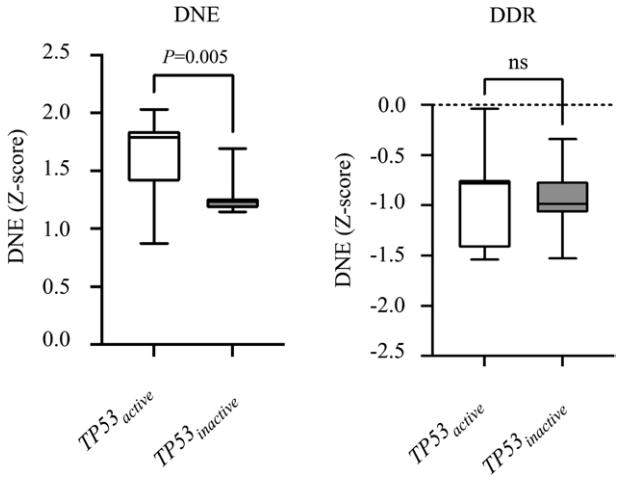

Figure 1. Comparison of dominant negative effect (DNE) and DNA damage repair function (DDR) in carriers of $TP53_{inactive}$ missense mutations and $TP53_{active}$ missense mutations.

mutations were more likely to attain an objective response (P = .03). This positive effect on tumor shrinkage seems to result in a significantly higher frequency of liver metastasectomies in $TP53_{inactive}$ missense mutation carriers (P = .02).

Overall survival analysis

Figure 3 shows Kaplan-Meier survival plots displaying the overall survival (OS) in carriers of *TP53* missense mutations versus patients with *TP53* wild-type status (Figure 3A) and in carriers of $TP53_{inactive}$ or $TP53_{active}$ missense mutations vs patients with *TP53* wild-type status (Figure 3B).

In *TP53* wild-type cases, the median PFS was 25.5 months (95%CI, 17.7-32.5 months), whereas carriers of *TP53* missense mutations exhibited a median PFS of 26 months (95%CI, 18.2-33.4 months). The distinction among *TP53* missense mutations (Figure 3B) showed a significant impact on OS. Median OS times were 35 months (95%CI, 21.5-38.5 months) in carriers of $TP53_{inactive}$ missense mutations, 25.5 months (95%CI, 22.6-29.7 months) in patients with *TP53* wild-type status, and 24.9 months (95%CI, 19.7-29.3 months) in carriers of $TP53_{active}$ missense mutations.

Discussion

Over half of newly diagnosed patients with colorectal cancer develop liver metastases. In liver-only metastatic colorectal cancer, about 75% of cases are diagnosed with liver metastases not amenable to upfront resection. In these cases, and in the presence of *KRAS* mutations that confer resistance to EGFR inhibitors, systemic therapy with Bevacizumab plus chemotherapy is the standard of care.[22] Nevertheless, the overall treatment strategy could consider the surgical approach in responsive and prognostically favorable sub-groups treated with first-line systemic therapy.[23] Unfortunately, despite intense efforts, no predictive biomarker has been identified yet that would optimize the personalized use of Bevacizumab and the management of patients with an oligometastatic disease.[23] In the present study, patients with liver-only metastatic colorectal cancer and with tumors harboring inactivating *TP53* missense mutations showed favorable PFS, tumor responsiveness and increased rates of liver metastasectomy after systemic therapy with Bevacizumab plus chemotherapy. Our clinical findings add to those recently reported in suggesting a favorable role of *TP53* mutations on the efficacy of inhibitors of VEGF-stimulated angiogenesis.[1-6] Prior to these findings, translational studies focusing on tumor genetic features and clinical outcomes of metastatic colorectal cancer patients treated with Bevacizumab did not report significant associations with *TP53* mutational status.[24-27] The discrepancy between these data may be explained in light of the expanding information on *TP53* missense mutations, their characteristics and their classification for clinical purposes.

TP53 missense mutations constitute the majority of *TP53* mutations in colorectal cancer, but our findings demonstrate that they can no longer be considered a homogeneous group, especially for translational clinical analyses.[28] In the present study, no significant association with PFS was found when the 39 carriers of *TP53* missense mutations were analyzed as a whole. However, the sub-group of 16 carriers (41%) with $TP53_{inactive}$ missense mutations showed a positive association with PFS and other study outcomes.

GOF *TP53* missense mutations are considered to confer a more aggressive phenotype.[20] However, in this study population, they were equally distributed between $TP53_{inactive}$ and $TP53_{active}$ missense mutation carriers. *TP53* missense mutations with DNE were more frequent among $TP53_{active}$ carriers. Strong evidence suggests that DNA damage (ie, chemotherapy-induced) may determine the DNE.[29] According to experimental data,[30] DNE may have caused a predominant apoptosis-suppressive phenotype in this group.

In the $TP53_{inactive}$ group, several mechanisms may have led to an anti-VEGF-sensitive phenotype. Experimental and pre-clinical data have elucidated the cross-talk mechanisms among *TP53*, VEGF, and VEGF receptors.[9-13] LOF

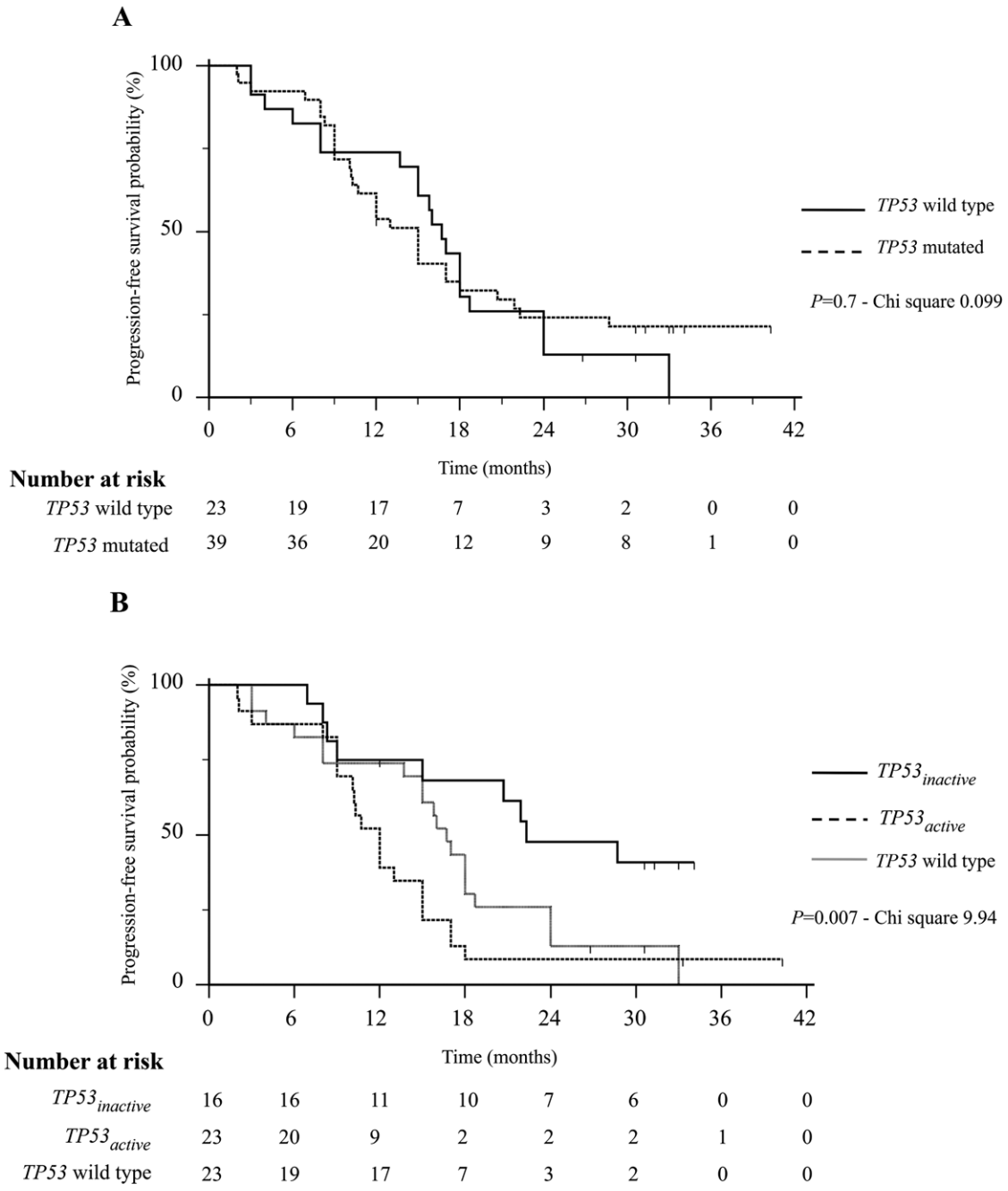

Figure 2. Kaplan-Meier survival plots for PFS in carriers of *TP53* missense mutations vs patients with *TP53* wild-type status (A) and in carriers of $TP53_{inactive}$ or $TP53_{active}$ missense mutations vs patients with *TP53* wild-type status (B).

Table 3. Distribution of responses and liver surgery according to the *TP53* status.

Variable	Number of patients (%)			*P*-value
	Total	$TP53_{inactive}$	$TP53_{active}$ and *TP53*wt	
Objective responses				
CR plus PR	40 (64.5)	14 (87.5)	26 (56)	0.03
SD plus PD	22 (35.5)	2 (12.5)	20 (44)	
Liver Metastasectomy				
Yes	19 (30.5)	9 (56)	10 (21)	0.02
No	43 (68.5)	7 (44)	36 (79)	

Abbreviations: CR, complete response; PD, disease progression; PR, partial response; RTAS, residual transcriptional activity scores; SD, stable disease; wt, wild-type.

TP53 missense mutations were found to be associated with VEGF up-regulation,[6-8] which in turn may sensitize tumors to anti-angiogenic exposure.[1-6] However, additional mechanisms may link $TP53_{inactive}$ missense mutation to positive anti-tumor effects of anti-angiogenics.

In response to DNA damaging agents, cells activate a checkpoint signaling pathway downstream of Ataxia-telangiectasia Mutated (ATM)/ATM- and Rad3-related (ATR) using effectors Checkpoint Kinase 1 (Chk1)/Checkpoint Kinase 2 (Chk2) and a more recently identified signaling cascade through p38/Mitogen-activated protein kinase-activated protein kinase 2 (MK2) to arrest cell cycle progression and repair DNA. The ability of cells to activate cell cycle checkpoints prevents progression into vulnerable phases of the cell cycle, leading to

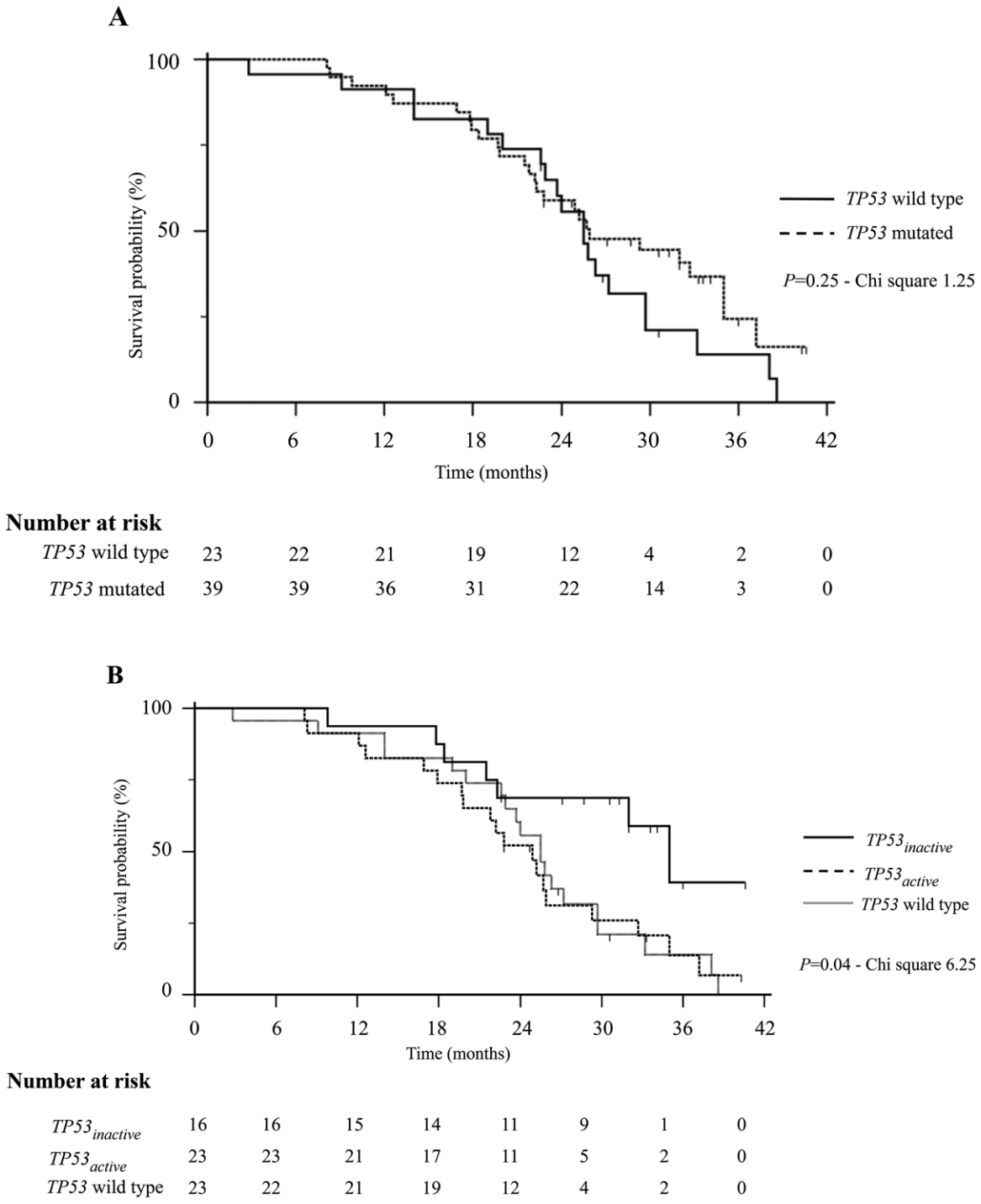

Figure 3. Kaplan-Meier survival plots for OS in carriers of *TP53* missense mutations versus patients with *TP53* wild-type status (A) and in carriers of $TP53_{inactive}$ or $TP53_{active}$ missense mutations vs patients with *TP53* wild-type status (B).

chemoresistance. It has been demonstrated that cancer cells with *TP53* LOF mutations must activate alternative pathways to maintain critical cell cycle checkpoints.[31] One of these is the p38/MK2/Cell-Division Cycle 25C (CDC25C)/CyclinB/ Cell-Division Cycle 2 (Cdc2) signaling cascade, which allows cells to repair DNA at G2 prior to entering M. Blocking this signaling pathway completely abrogates the checkpoint in cells lacking p53[29]. Mallen et al[32] analyzed *TP53* mutations in 250 endometrial cancer patients in the 3-arm randomized GOG/NRG/86P study. Similar to our study, the longest PFS (19.6 months) was found in patients with *TP53* LOF mutations treated with bevacizumab plus chemotherapy, compared to 12.2 months of patients with *TP53* wild-type status and 10.6 months of patients with other *TP53* mutations. Using cell models, Mallen et al[32] described a mechanism of synthetic lethality resulting from the ability of agents such as bevacizumab, which blocks signaling downstream of tyrosine kinases, to abrogate cell cycle checkpoints in the absence of p53.

Our study population consisted of patients whose tumors were positive for *KRAS* mutations; therefore, the coexistence

of the *KRAS* and *TP53* mutations is an additional feature that deserves consideration.[33] Bevacizumab may display a favorable selection pressure with a decrease in *KRAS* mutant cells, which might reflect the efficacy of bevacizumab to specifically eradicate the *KRAS* mutant population, leading to an increased prevalence of *KRAS* wild-type cells.[34] Intriguingly, Hendricks et al[35] described a case of the rapid response of a patient with metastatic colorectal cancer with *Adenomatous Polyposis Coli/TP53/KRAS* mutations to FOLFIRI and Bevacizumab combination chemotherapy. In this report,[35] the authors found the *TP53 Arg282Trp* (R282W) missense mutation, which can be classified among the $TP53_{inactive}$ missense mutations. In a biological perspective, *KRAS* signaling could influence several molecular pathways, which may ultimately favor the use of VEGF inhibitors. *KRAS* impacts the remodeling of the cancer microenvironment, including angiogenesis,[36] and VEGF itself was found to be up-regulated by *KRAS* signaling.[37-40] Also, VEGF inhibition may counteract the immunosuppressive status[41] induced by molecular pathways under *TP53/KRAS* co-mutations control.[42] Ferroptosis is an iron-dependent mode of regulated cell death caused by unrestricted lipid peroxidation and subsequent plasma membrane rupture. *KRAS*-driven and *TP53* mutated cancer cell lines seemed to be selectively sensitive to ferroptosis inducers among which are anti-angiogenic compounds.[43] Therefore, the overall molecular background may provide the rationale for a positive role of bevacizumab in the treatment of patients with metastatic colorectal carcinomas with $TP53_{inactive}$ and *KRAS* mutations.

Our findings must also be evaluated in the context of current knowledge on genetic profiling of colorectal carcinomas of patients treated with resection of liver metastases. Translational studies showed poor survival outcomes of these patients when tumors were positive for *TP53* and/or *KRAS* mutations.[44] Considering the aforementioned biological aspects, these unfavorable prognostic features, however, may provide targets and vulnerabilities for modern chemotherapy and biologic agents such as the anti-VEGF Bevacizumab.

Conclusions

Our study shows a possible favorable effect of the anti-VEGF Bevacizumab in a setting of oligo-metastatic colorectal cancer patients with $TP53_{inactive}$ and *KRAS* mutations, supporting a precision oncology approach to metastatic colorectal cancer treatment. Limitations to this study that deserve further investigation are the clinical impact of partially active *TP53* missense mutations, the use of alternative $TP53_{RTAS}$ cutoffs, as well as alternative functional characterizations of *TP53* missense mutations. Here, we focused only on the *TP53* missense mutations analysis, but the p53 expression by immunohistochemistry and its consistency with the presence of *TP53* mutations could be considered in future studies. Lastly, it could be explored whether increased chemosensitivity in the *TP53* inactive group may contribute to clinical differences after liver metastasectomy.

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Author contributions

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Conflicts of interest

The authors declare no competing interests.

Data availability

The data underlying this article cannot be shared publicly. The data will be shared on reasonable request to the corresponding author.

References

1. Said R, Hong DS, Warneke CL, et al. P53 mutations in advanced cancers: clinical characteristics, outcomes, and correlation between progression-free survival and bevacizumab-containing therapy. *Oncotarget*. 2013;4(5):705-714. https://doi.org/10.18632/oncotarget.974
2. Wheler JJ, Janku F, Naing A, et al. TP53 alterations correlate with response to VEGF/VEGFR inhibitors: implications for targeted therapeutics. *Mol Cancer Ther*. 2016;15:2475-2485. https://doi.org/10.1158/1535-7163.MCT-16-0196
3. Hsu H-C, You J-F, Chen S-J, et al. TP53 DNA binding domain mutations predict progression-free survival of bevacizumab therapy in metastatic colorectal cancer. *Cancers (Basel)*. 2019;11:1079. https://doi.org/10.3390/cancers11081079
4. Leslie KK, Filiaci VL, Mallen AR, et al. Mutated p53 portends improvement in outcomes when bevacizumab is combined with chemotherapy in advanced/recurrent endometrial cancer: An NRG Oncology study. *Gynecol Oncol*. 2021;161:113-121. https://doi.org/10.1016/j.ygyno.2021.01.025
5. Thiel KW, Devor EJ, Filiaci VL, et al. TP53 sequencing and p53 immunohistochemistry predict outcomes when bevacizumab is added to frontline chemotherapy in endometrial cancer: an NRG Oncology/Gynecologic Oncology Group Study. *J Clin Oncol*. 2022;40:3289-3300. https://doi.org/10.1200/JCO.21.02506
6. Graziano F, Fischer NW, Bagaloni I, et al. TP53 mutation analysis in gastric cancer and clinical outcomes of patients with metastatic disease treated with ramucirumab/paclitaxel or standard chemotherapy. *Cancers (Basel)*. 2020;12:2049. https://doi.org/10.3390/cancers12082049
7. Schwaederlé M, Lazar V, Validire P, et al. VEGF-A expression correlates with TP53 mutations in non-small cell lung cancer: implications

for antiangiogenesis therapy. *Cancer Res*. 2015;75:1187-1190. https://doi.org/10.1158/0008-5472.CAN-14-2305
8. Li AM, Boichard A, Kurzrock R. Mutated TP53 is a marker of increased VEGF expression: analysis of 7,525 pan-cancer tissues. *Cancer Biol Ther*. 2020;21:95-100. https://doi.org/10.1080/15384047.2019.1665956
9. Joshi H, Bhanot G, Børresen-Dale A-L, Kristensen V. Potential tumorigenic programs associated with TP53 mutation status reveal role of VEGF pathway. *Br J Cancer*. 2012;107:1722-1728. https://doi.org/10.1038/bjc.2012.461
10. Ravi R, Mookerjee B, Bhujwalla ZM, et al. Regulation of tumor angiogenesis by p53-induced degradation of hypoxia-inducible factor 1alpha. *Genes Dev*. 2000;14:34-44.
11. Hayashi Y, Tsujii M, Kodama T, et al. p53 functional deficiency in human colon cancer cells promotes fibroblast-mediated angiogenesis and tumor growth. *Carcinogenesis*. 2016;37:972-984. https://doi.org/10.1093/carcin/bgw085
12. Qin G, Kishore R, Dolan CM, et al. Cell cycle regulator E2F1 modulates angiogenesis via p53-dependent transcriptional control of VEGF. *Proc Natl Acad Sci U S A*. 2006;103:11015-11020. https://doi.org/10.1073/pnas.0509533103
13. Pfister NT, Fomin V, Regunath K, et al. Mutant p53 cooperates with the SWI/SNF chromatin remodeling complex to regulate VEGFR2 in breast cancer cells. *Genes Dev*. 2015;29:1298-1315. https://doi.org/10.1101/gad.263202.115
14. Liebl MC, Hofmann TG. The role of p53 signaling in colorectal cancer. *Cancers (Basel)*. 2021;13:2125. https://doi.org/10.3390/cancers13092125
15. Camplejohn RS, Rutherford J. p53 functional assays: detecting p53 mutations in both the germline and in sporadic tumours. *Cell Prolif*. 2001;34:1-14. https://doi.org/10.1046/j.1365-2184.2001.00193.x
16. Huiskens J, van Gulik TM, van Lienden KP, et al. Treatment strategies in colorectal cancer patients with initially unresectable liver-only metastases, a study protocol of the randomised phase 3 CAIRO5 study of the Dutch Colorectal Cancer Group (DCCG). *BMC Cancer*. 2015;15:365. https://doi.org/10.1186/s12885-015-1323-9
17. McShane LM, Altman DG, Sauerbrei W, et al; Statistics Subcommittee of the NCI-EORTC Working Group on Cancer Diagnostics. Reporting recommendations for tumor marker prognostic studies. *J Clin Oncol*. 2005;23:9067-9072. https://doi.org/10.1200/JCO.2004.01.0454
18. Kato S, Han S-Y, Liu W, et al. Understanding the function-structure and function-mutation relationships of p53 tumor suppressor protein by high-resolution missense mutation analysis. *Proc Natl Acad Sci U S A*. 2003;100:8424-8429. https://doi.org/10.1073/pnas.1431692100
19. Fischer NW, Prodeus A, Gariépy J. Survival in males with glioma and gastric adenocarcinoma correlates with mutant p53 residual transcriptional activity. *JCI Insight*. 2018;3:e121364. https://doi.org/10.1172/jci.insight.121364
20. Pan M, Jiang C, Tse P, et al. TP53 gain-of-function and non-gain-of-function mutations are differentially associated with sidedness-dependent prognosis in metastatic colorectal cancer. *J Clin Oncol*. 2022;40:171-179. https://doi.org/10.1200/JCO.21.02014
21. Giacomelli AO, Yang X, Lintner RE, et al. Mutational processes shape the landscape of TP53 mutations in human cancer. *Nat Genet*. 2018;50:1381-1387. https://doi.org/10.1038/s41588-018-0204-y
22. Garcia J, Hurwitz HI, Sandler AB, et al. Bevacizumab (Avastin®) in cancer treatment: a review of 15 years of clinical experience and future outlook. *Cancer Treat Rev*. 2020;86:102017. https://doi.org/10.1016/j.ctrv.2020.102017
23. Miller ED, Hitchcock KE, Romesser PB. Oligometastatic colorectal cancer: a review of definitions and patient selection for local therapies. *J Gastrointest Cancer*. 2023;54:1116-1127. https://doi.org/10.1007/s12029-022-00900-5
24. Ince WL, Jubb AM, Holden SN, et al. Association of k-ras, b-raf, and p53 status with the treatment effect of bevacizumab. *J Natl Cancer Inst*. 2005;97:981-989. https://doi.org/10.1093/jnci/dji174
25. De Bruijn MT, Raats DAE, Tol J, et al. Combined KRAS and TP53 mutation status is not predictive in CAPOX-treated metastatic colorectal cancer. *Anticancer Res*. 2011;31:1379-1385.
26. Kara O, Duman BB, Kara B, et al. Analysis of PTEN, VEGF, HER2 and P53 status in determining colorectal cancer benefit from bevacizumab therapy. *Asian Pac J Cancer Prev*. 2012;13:6397-6401. https://doi.org/10.7314/apjcp.2012.13.12.6397
27. Huemer F, Thaler J, Piringer G, et al. Sidedness and TP53 mutations impact OS in anti-EGFR but not anti-VEGF treated mCRC - an analysis of the KRAS registry of the AGMT (Arbeitsgemeinschaft Medikamentöse Tumortherapie). *BMC Cancer*. 2018;18:11. https://doi.org/10.1186/s12885-017-3955-4
28. Kennedy MC, Lowe SW. Mutant p53: it's not all one and the same. *Cell Death Differ*. 2022;29:983-987. https://doi.org/10.1038/s41418-022-00989-y
29. Gencel-Augusto J, Lozano G. p53 tetramerization: at the center of the dominant-negative effect of mutant p53. *Genes Dev*. 2020;34:1128-1146. https://doi.org/10.1101/gad.340976.120
30. Aurelio ON, Kong XT, Gupta S, Stanbridge EJ. p53 mutants have selective dominant-negative effects on apoptosis but not growth arrest in human cancer cell lines. *Mol Cell Biol*. 2000;20:770-778. https://doi.org/10.1128/MCB.20.3.770-778.2000
31. Meng X, Dizon DS, Yang S, et al. Strategies for molecularly enhanced chemotherapy to achieve synthetic lethality in endometrial tumors with mutant p53. *Obstet Gynecol Int*. 2013;2013:828165. https://doi.org/10.1155/2013/828165
32. Mallen AR, Filiaci VL, Levine DA, et al. Evidence for synthetic lethality between bevacizumab and chemotherapy in advanced, p53 null endometrial cancers. *Gynecol Oncol*. 2018;149:29-30. https://doi.org/10.1016/j.ygyno.2018.04.069
33. Zhou M, Yu P, Qu J, et al. Efficacy of bevacizumab in the first-line treatment of patients with RAS mutations metastatic colorectal cancer: a systematic review and network meta-analysis. *Cell Physiol Biochem*. 2016;40:361-369. https://doi.org/10.1159/000452551
34. Nicolazzo C, Belardinilli F, Vestri A, et al. RAS mutation conversion in bevacizumab-treated metastatic colorectal cancer patients: a liquid biopsy based study. *Cancers (Basel)*. 2022;14:802. https://doi.org/10.3390/cancers14030802
35. Hendricks A, Rosenstiel P, Hinz S, et al. Rapid response of stage IV colorectal cancer with APC/TP53/KRAS mutations to FOLFIRI and Bevacizumab combination chemotherapy: a case report of use of liquid biopsy. *BMC Med Genet*. 2020;21:3. https://doi.org/10.1186/s12881-019-0941-5
36. Dias Carvalho P, Guimarães CF, Cardoso AP, et al. KRAS oncogenic signaling extends beyond cancer cells to orchestrate the microenvironment. *Cancer Res*. 2018;78:7-14. https://doi.org/10.1158/0008-5472.CAN-17-2084
37. Rak J, Mitsuhashi Y, Bayko L, et al. Mutant ras oncogenes upregulate VEGF/VPF expression: implications for induction and inhibition of tumor angiogenesis. *Cancer Res*. 1995;55:4575-4580.
38. Okada F, Rak JW, Croix BS, et al. Impact of oncogenes in tumor angiogenesis: mutant K-ras up-regulation of vascular endothelial growth factor/vascular permeability factor is necessary, but not sufficient for tumorigenicity of human colorectal carcinoma cells. *Proc Natl Acad Sci U S A*. 1998;95:3609-3614. https://doi.org/10.1073/pnas.95.7.3609
39. Zeng M, Kikuchi H, Pino MS, Chung DC. Hypoxia activates the K-ras proto-oncogene to stimulate angiogenesis and inhibit apoptosis in colon cancer cells. *PLoS One*. 2010;5:e10966. https://doi.org/10.1371/journal.pone.0010966
40. Zhang X, Gaspard JP, Chung DC. Regulation of vascular endothelial growth factor by the Wnt and K-ras pathways in colonic neoplasia. *Cancer Res*. 2001;61:6050-6054.

41. Ribatti D. Immunosuppressive effects of vascular endothelial growth factor. *Oncol Lett*. 2022;24:369. https://doi.org/10.3892/ol.2022.13489
42. Dosch AR, Chatila WK, Ban Y, et al. Ras-p53 genomic cooperativity as a model to investigate mechanisms of innate immune regulation in gastrointestinal cancers. *Oncotarget*. 2021;12:2104-2110. https://doi.org/10.18632/oncotarget.27983
43. Nie Q, Hu Y, Yu X, Li X, Fang X. Induction and application of ferroptosis in cancer therapy. *Cancer Cell Int*. 2022;22:12. https://doi.org/10.1186/s12935-021-02366-0
44. Rehman AH, Jones RP, Poston G. Prognostic and predictive markers in liver limited stage IV colorectal cancer. *Eur J Surg Oncol*. 2019;45:2251-2256. https://doi.org/10.1016/j.ejso.2019.06.038