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ORIGINAL ARTICLE

Clinical and translational results from the phase II ABACO trial evaluating the activity of cabozantinib in pretreated patients with metastatic colorectal cancer

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Background: Angiogenesis is a key mechanism in metastatic colorectal cancer (mCRC). Novel agents targeting this pathway, like cabozantinib, are of great therapeutic need.

Patients and methods: We conducted an open-label, single-arm phase II trial to assess the antitumor activity of cabozantinib in patients with pretreated mCRC who had progressed following at least two prior lines of therapy. The primary endpoint was the progression-free survival (PFS) rate at 16 weeks, whereas secondary endpoints included median PFS, overall survival (OS), response rate, disease control rate, and safety. DNA- and RNA-based translational analyses were carried out on biological samples.

Results: From October 2019 to January 2023, 33 patients were treated with oral cabozantinib, 60 mg daily. The primary endpoint was met: 11/33 assessable patients (33%) were progression-free at 16 weeks. Median PFS was 2.27 months [95% confidence interval (CI) 1.71-3.65 months], median OS was 6.25 months (95% CI 3.81-10.26 months). Disease control rate was 45.5%. Cabozantinib was generally fairly tolerated. Exploratory analyses investigating the effect of clinical disease features on PFS showed no significant correlation. Comprehensive genomic profiling on 30 patients (tissues and plasma) suggested that absence of *TP53* mutations and tumor mutational burden (TMB) $\geq$4 mutations/Mb positively correlated with response (PFS $>$16 weeks). Additionally, for a subset of 18 (54.5%) patients, RNA sequencing from archival formalin-fixed paraffin-embedded samples was carried out. Molecular subtypes 4 (CMS4) was the most represented transcriptional subtype (10/18 cases). To verify if other transcriptional features were associated with treatment benefit, for each sample we computed gene set variation analysis. For epithelial-mesenchymal transition (EMT) and angiogenesis gene sets, we identified a trend toward higher scores in tumors from patients with longer PFS.

Conclusion: Within the limitations of a single-arm phase II trial, cabozantinib demonstrates both safety and antitumor activity in mCRC. The observed correlation between specific molecular features, such as EMT activation and angiogenesis, and cabozantinib activity is hypothesis-generating and warrants further investigation.

Key words: cabozantinib, mCRC, angiogenesis, EMT, chemorefractory

INTRODUCTION

Colorectal cancer (CRC) is the third most common malignancy worldwide and the second leading cause of cancer-related death.[1] Despite advances in first- and second-line therapies, including chemotherapy, targeted agents, and immunotherapy, prognosis for patients with refractory

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disease remains poor.[2] Recent advances in sequencing technologies have provided further insight into the profound complexity and molecular heterogeneity of CRC. According to the Consensus on CRC Molecular Subtypes (CMS) four biologically distinct subtypes have been identified with different sex and age of onset, localization, activation of molecular pathways, and prognosis.[3,4] However, although the translational features captured by CMS are clinically informative in principle, this knowledge cannot be immediately translated into clinical practice, because CMS has not yet been prospectively validated to stratify patients for anticancer treatments. In particular, metastatic chemorefractory tumors are enriched for mesenchymal features, such as increased angiogenesis and immune evasion, which are incorporated in the molecular subtypes 4 (CMS4) cluster.[3] Moreover, although a tumor with mesenchymal features and *RAS/BRAF* wild-type (wt) status may still benefit from treatment with anti-epidermal growth factor receptor (EGFR) agents, particularly in combination with irinotecan,[5] mesenchymal-like[6] *RAS* mutant tumors still constitute a clinical unmet need. Of note, previous attempts to treat mesenchymal-like tumors have been pursued but eventually failed to provide meaningful clinical benefit.[7] Currently, targeted options are still available for molecularly selected chemorefractory CRC, particularly if not used in earlier lines of treatment: anti-HER2 agents for *HER2*-amplified tumors, $KRAS^{G12C}$ inhibitors for $KRAS^{G12C}$ mutant tumors, anti-EGFR monoclonal antibodies for *RAS/BRAF* wt tumors, combined BRAF and EGFR blockade for *BRAF* mutant tumors, kinase inhibition for tumors with oncogenic translocations (such as *NTRK* rearrangements), besides immune checkpoint inhibition for mismatch repair deficiency and microsatellite instability (dMMR/MSI)-high tumors.[2,8] However, approved treatments for chemorefractory CRC are mostly agnostic for the molecular features and converge on the inhibition of angiogenesis, consisting of either the combination of trifluridine/tipiracil with bevacizumab or in the anti-angiogenic multi-kinase inhibitors regorafenib or fruquintinib.[2,8]

For this reason, a better understanding of the complex molecular landscape of advanced CRC would contribute to developing new treatment strategies and refining existing ones. In this context, novel agents targeting alternative molecular pathways involved in mesenchymal pathways are of clinical relevance. Cabozantinib is an oral multi-kinase inhibitor that targets multiple tyrosine kinases involved in tumor growth, angiogenesis, and resistance mechanisms, including MET, vascular endothelial growth factor receptor 2 (VEGFR2), AXL, and RET.[9] It is currently approved for the treatment of patients with medullary thyroid cancer, renal cell carcinoma, hepatocellular carcinoma, and advanced neuroendocrine tumors.[10-12] As for the other agents, cabozantinib activity on the tumor vasculature is potentially clinically relevant due to the crucial role of angiogenesis in CRC development and progression,[13] and some preclinical works suggest potential efficacy of cabozantinib in metastatic CRC (mCRC).[9] Moreover, cabozantinib activity may also intercept other features that are enriched in chemorefractory tumors. In particular, AXL and MET signaling pathways are implicated in CRC invasion and metastasis and are involved in the occurrence of drug resistance to anti-EGFR therapy.[14-17] By modulating the immune tumor microenvironment, cabozantinib may synergize with immunotherapeutic agents or EGFR-targeting monoclonal antibodies to overcome cancer cell resistance pathways.[18] Clinical experience with cabozantinib alone (AGICC 17CRC01 trial),[19] or in combination with immunotherapy (CAMILLA trial)[20] in pretreated mCRC also support its antitumor activity in this setting. The positive results of the phase III trial STELLAR-303, in which patients have been treated with zanzalintinib (a VEGF, MET, AXL, and MER inhibitor) in combination with atezolizumab in microsatellite stable (MSS) mCRC suggest immune modulation may constitute a potential mechanism elicited by pharmacological inhibition of mesenchymal pathways, although the clinical relevance of this finding needs further investigation.[21]

Here we report clinical and translational findings from the phase II ABACO study, a single center clinical trial that has been designed to assess the clinical activity and the safety profile of cabozantinib in patients with refractory mCRC, who had progressed on all available standard lines of therapy. Additionally, we have carried out a translational analysis for identifying potential genomic and transcriptional molecular determinants of response to therapy.

PATIENTS AND METHODS

Study design and participants

ABACO was a phase II, open-label, single-arm clinical trial conducted at a single academic institution (University of Campania 'Luigi Vanvitelli') in Italy (EudraCT Number: 2019-000674-28). Patients were eligible if $\geq$18 years old with histologically confirmed mCRC, measurable disease per RECIST v1.1, Eastern Cooperative Oncology Group (ECOG) performance status 0 or 1, and adequate organ function. Key inclusion criteria required prior treatment with fluoropyrimidines, oxaliplatin, irinotecan, anti-VEGF agents (bevacizumab or aflibercept), and anti-EGFR antibodies (for *RAS* wt patients). Key exclusion criteria included previous exposure to cabozantinib or other multi-kinase inhibitors (e.g. regorafenib), untreated or unstable brain metastases, concurrent oral anticoagulant/antiplatelet therapy, active infection requiring systemic treatment, or significant comorbidities. The trial was approved by the institutional review board of our study site. The study was carried out in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines and all patients provided written informed consent.

Treatment plan

Cabozantinib was administered at a starting dose of 60 mg orally once daily. Dose reductions to 40 mg and 20 mg were allowed based on toxicity. Treatment continued until

disease progression, unacceptable toxicity, withdrawal of consent, or investigator decision. Subjects were monitored for radiographic response and progression per Response Evaluation Criteria In Solid Tumors (RECIST) guidelines version 1.1, every 8 weeks. Safety was assessed on a schedule based on the date of the first dose and at a minimum every 2 weeks up to week 9, then every 4 weeks. Severity grade was defined by the National Cancer Institute (NCI) Common Terminology NCI CTCAE v. 5.0.

Endpoints

The primary endpoint was the 16-week progression-free survival (PFS) rate, defined as the proportion of patients alive and progression-free at 16 weeks. Secondary endpoints included overall survival (OS), median PFS, objective response rate (ORR), disease control rate, and safety. Exploratory endpoints included the identification of molecular correlates of response through genomic and transcriptomic profiling.

Statistical design

The study utilized a Simon two-stage design to minimize patient exposure to potentially ineffective therapy. With $\alpha = 0.05$ and power $= 90\%$, a 16-week PFS rate of $<10\%$ was considered insufficient activity, whereas $\geq 30\%$ was deemed clinically relevant. Twenty-two patients were evaluated in the first stage; if ≥ 3 remained progression-free at 16 weeks, the trial would expand to a total of 33 assessable patients. Survival curves were generated based on the Kaplan—Meier method. Statistical significance of survival curves was calculated using the log-rank test. MedCalc (version 23.1.3, MedCalc Software Ltd.) was used to generate survival curves. Univariate and multivariate Cox proportional hazards regressions, multiple logistic regression, and area under the curve (AUC) analyses were carried out using Prism (version 10.4.1, GraphPad Software LLC). A P value < 0.05 was considered statistically significant.

Genomic and transcriptional analyses

Comprehensive genomic testing was carried out on 24 tissue (72.8%) and 16 plasma (48.5%) samples, including 10 cases for which both tissue and plasma were available. Both tumor tissue and plasma samples were analyzed using FoundationOne CDx (https://www.foundationmedicine.com/test/foundationone-cdx) and FoundationOneLiquid biopsy (https://www.foundationmedicine.com/test/foundationone-liquid-cdx) platforms for next-generation sequencing-based genomic profiling.

Additionally, a total of 23 baseline formalin-fixed paraffin-embedded (FFPE) samples (69.7%) were available for transcriptomic analysis via RNA-sequencing (RNA-seq) to assess gene expression signatures and pathway activation. Total RNA was extracted from FFPE samples using RNeasy FFPE kit (Qiagen). RNA quantification and integrity were evaluated with Agilent 2100 Bioanalyzer (Agilent, Santa Clara, CA). Total RNA was used as input to the Illumina Stranded Total RNA Prep - Ligation with Ribo-Zero™ Plus (Illumina, San Diego, CA), according to the manufacturer's protocol. After library preparation, sequencing was carried out on Novaseq 6000DX (Illumina) to get paired-end 2 × 75 base pair reads. Most (18/23) samples passed the quality check for further analyses. RNA-seq reads were initially aligned to hg38 using STAR aligner included in RSEM.[22] Then, the quantification of transcripts and genes was conducted using RNA-Seq by Expectaction Maximization (RSEM)[22] as well and GENCODE v33 as gene annotation. Starting from the RSEM-derived gene results (expected counts and effective lengths), robust Fragments Per Kilobase Million (FPKM) values were subsequently calculated, using the tximport R Bioconductor package and the FPKM function included in the DESeq2 R Bioconductor package.[23] Finally, the robust FPKM values were log2-transformed and the gene expression matrix was annotated with gene names from the GENCODE annotation file.

For the heatmap, genes of interest were selected to calculate the z-scores from the log2-transformed gene expression values. The prediction of gene expression-based consensus molecular subtypes (CMS) was carried out after log2 transformation of gene expression values using CMSclassifier R guinnpackage with default parameters.[3] Starting from gene expression values, gene set variation analysis (GSVA) scores were obtained with the gsva function from the GSVA R Bioconductor package[24] for all the human hallmarks gene sets from MSigDB.[25]

RESULTS

Patients

Between October 2019 and January 2023, 35 patients were enrolled in the study, and 33 were assessable for response (2 patients were excluded in the screening phase due to clinical conditions) (Supplementary Figure S1, available at https://doi.org/10.1016/j.esmogo.2026.100303). Median age was 58 years (range 31-80 years), and 57.6% were male. Most patients had an ECOG performance status of 1 (60.6%) and a left-sided tumor (75.8%). The majority (63.6%) had already received more than two prior lines of systemic therapy (median, three lines). At baseline, 78.8% had liver metastases, and 75.8% had more than one site of metastatic disease. *RAS* mutations were present in 42.4%, and *BRAF* mutations in 6.1%. All assessable patients had MSS/proficient mismatch repair disease. Baseline characteristics are summarized in Table 1.

Efficacy outcomes

The primary endpoint was met: 11/33 assessable patients (33%) were progression-free at 16 weeks (Figure 1A). Median PFS was 2.27 months [95% confidence interval (CI) 1.71-3.65 months] (Figure 1B). Median OS was 6.25 months (95% CI 3.81-10.26 months) (Figure 1C). Figure 2 shows the best radiological response for each patient; we observed one partial response in a patient with left-sided, *RAS/BRAF* wt CRC, previously treated with three lines of therapy which included fluoropyrimidine, irinotecan, oxaliplatin, bevacizumab and cetuximab.

Table 1. Characteristics of patients

Characteristic	All participants ($N = 33$), *n* (%)
Age, years	
Median (range)	58 (31-80)
Males	19
ECOG performance status	
0	13 (39.4)
1	20 (60.6)
Sidedness	
Right-sided	8 (24.2)
Left-sided	25 (75.8)
RAS gene status	
Mutant	14 (42.4)
Wild-type	19 (57.6)
BRAF gene status	
Mutant	2 (6.1)
Wild-type	31 (93.9)
MSI/MMR status	
MSI-H/dMMR	0
MSS/pMMR	33 (100)
Prior lines of therapy	
≤2	12 (36.4)
>2	21 (63.6)
Number of metastatic sites	
1	8 (24.2)
>1	25 (75.8)
Liver metastases	
Yes	26 (78.8)

dMMR, mismatch repair deficiency; ECOG, Eastern Cooperative Oncology Group; MSI-H, high microsatellite instability; MSS, microsatellite stable; pMMR, proficient mismatch repair.

The objective response rate (ORR) and disease control rate were 3% (1/33) and 45.5% (15/33 patients), respectively. Patients achieving PFS >16 weeks are highlighted in green (Figure 2). As a non-prespecified exploratory analysis investigating the effect of age, sex, sidedness, number of previous lines or features of metastatic disease, no significant correlation with PFS for both the uni- or multivariate analysis was reported (Supplementary Figure S2, available at https://doi.org/10.1016/j.esmogo.2026.100303).

Safety and tolerability

Cabozantinib was generally fairly tolerated. The most frequent treatment-emergent adverse events (AEs) (Table 2) were fatigue (54.6%), elevated aspartate aminotransferase/alanine aminotransferase (54.6%), hand-foot syndrome (27.2%), hypothyroidism (24.2%), and diarrhea (24.2%). Grade ≥3 AEs occurred in 17% of patients. Dose reductions were required in 13/33 patients (39.4%), with three patients reaching the lowest protocol-permitted dose of 20 mg (Supplementary Figure S3, available at https://doi.org/10.1016/j.esmogo.2026.100303). Three patients discontinued treatment due to unacceptable toxicity.

Exploratory genomic and transcriptional analyses

Next, we proceeded to molecularly characterize the tumors with the aim of exploring useful biomarkers of response and/or resistance to cabozantinib. Patients were categorized based on the clinical outcome as 'responders' (PFS >16 weeks) or 'non-responders' (PFS ≤16 weeks).

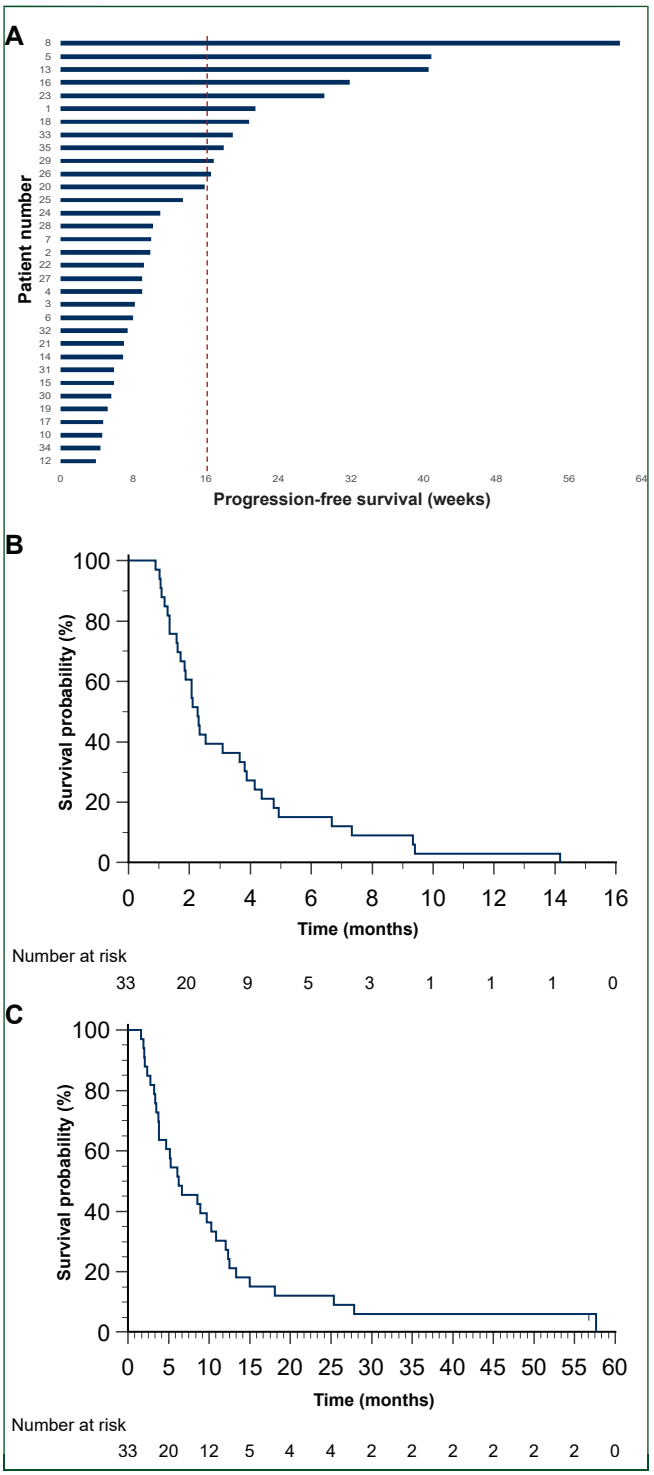


Figure 1. (A) Bar graph showing progression-free patients at 16 weeks (red line). Progression-free survival (B) and overall survival (C) in overall population.

A total of 30 patients (10 responders and 20 non-responders) had pretreatment archival FFPE tumor tissue and/or plasma samples available for comprehensive genomic profiling (CGP). Figure 2 shows the individual key mutational profiles for each patient alongside their best response, with unavailable molecular data indicated by 'X'. The oncoprint scheme representing genetic alterations

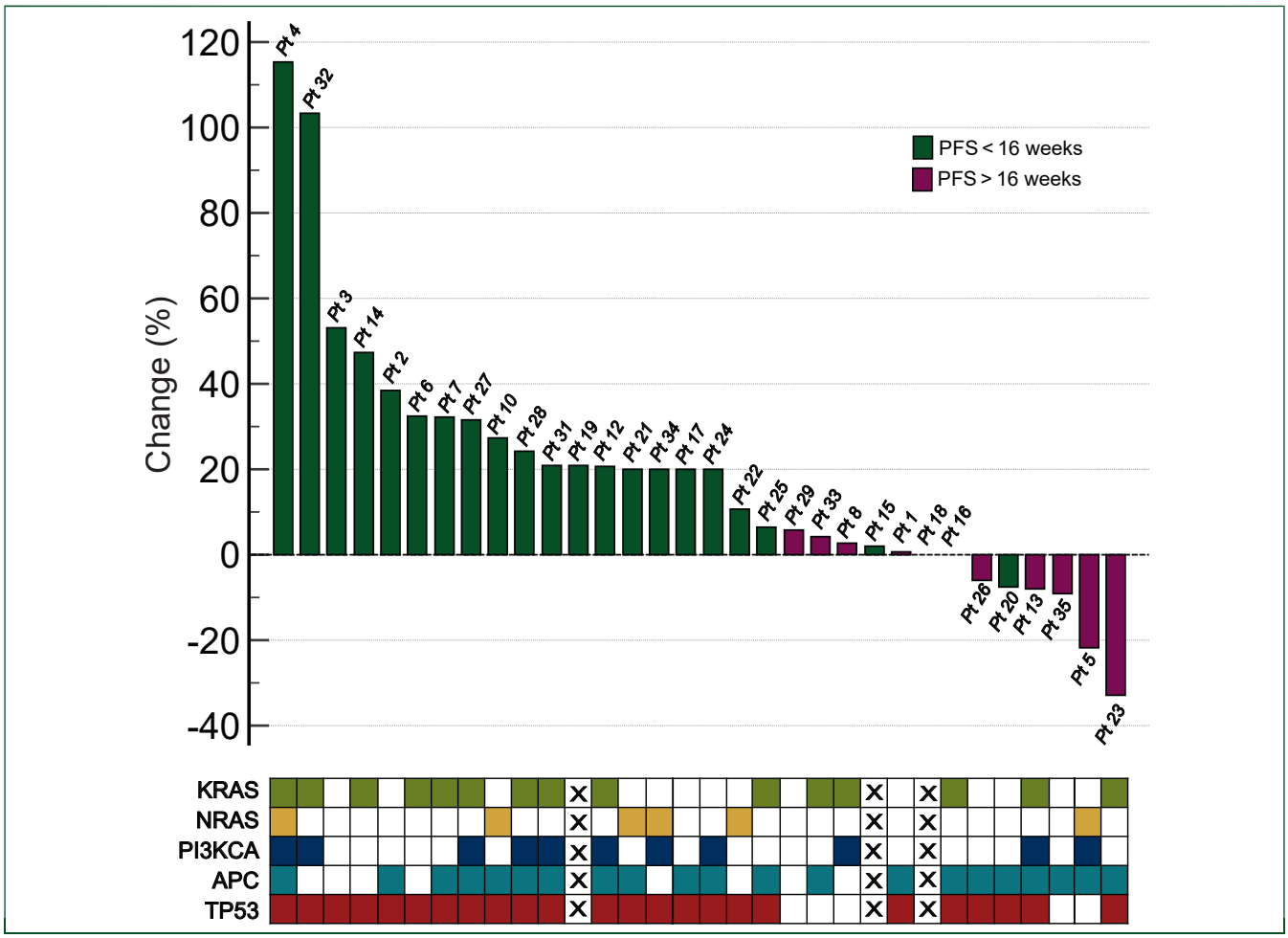


Figure 2. Waterfall plot for response of overall population. The main molecular alterations found with comprehensive genomic profiling for each patient are presented in the table. Molecular data are not available for squares with 'x'.
PFS, progression-free survival; pt, patient.

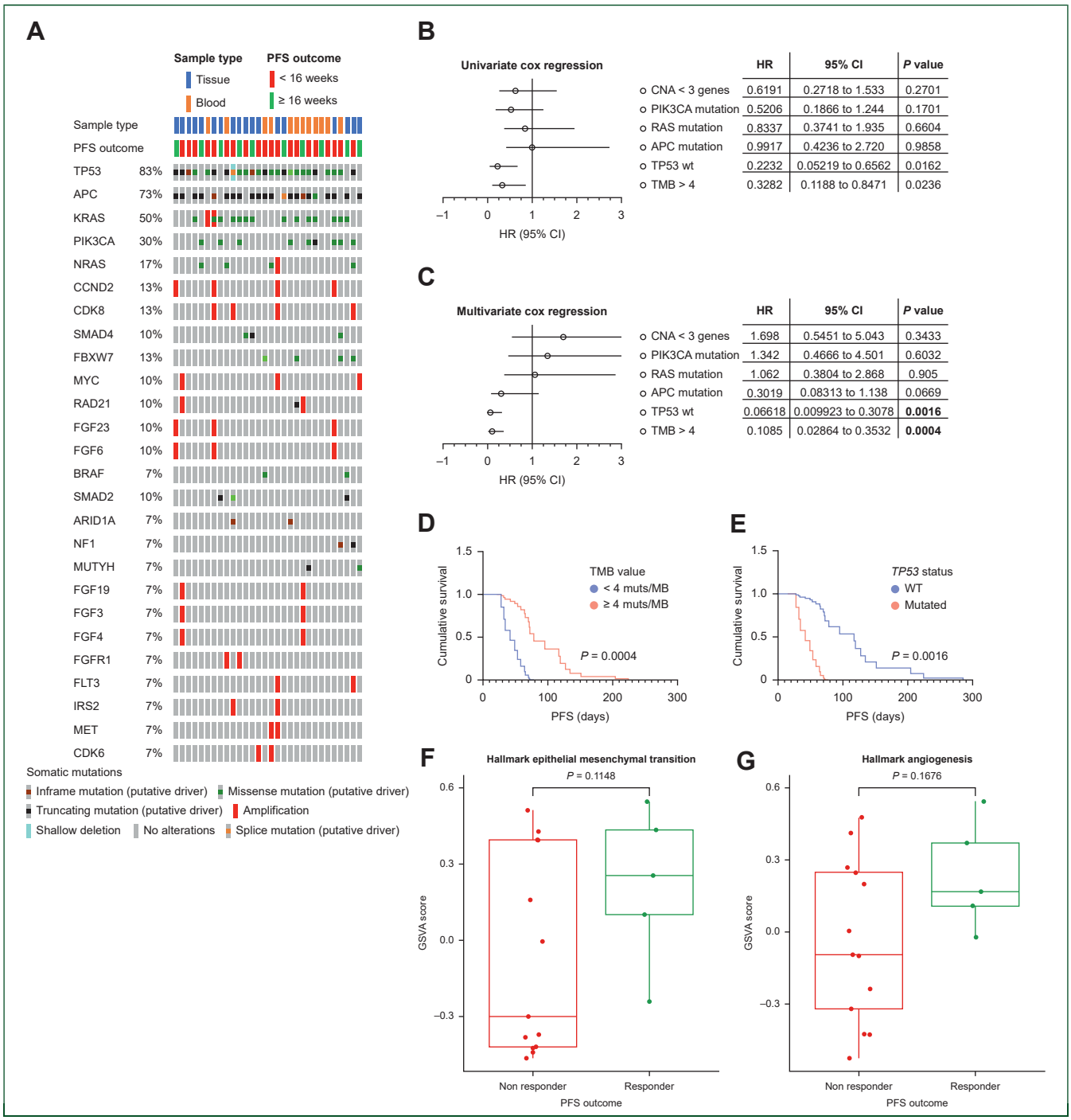
found in at least two cases alongside the PFS outcome is summarized in Figure 3A, whereas rare alterations found in only one case are collected in Supplementary Figure S4A, available at https://doi.org/10.1016/j.esmogo.2026.100303. These data provided key insights into tumor heterogeneity and potential mechanisms of resistance. However, no genetic alteration was specifically enriched in patients who benefited from the treatment as opposed to those patients who rapidly progressed.

The median value of the tumor mutational burden (TMB)was four mutations/megabase (muts/Mb) (range, 0-21 muts/Mb). Molecular alterations were predominantly found in *TP53* and *APC* genes, accounting for 83% and 73% of cases, respectively. *RAS* (*KRAS* + *NRAS*) mutations were detected in 67% of patients, while *PIK3CA* and *BRAF* mutations were present in 30% and 7% of tumors, respectively (Figure 3A). A subset of 7/30 cases presented at least three copy number alterations, mostly gene amplifications, from the CGP. Interestingly, although no significant association was found between *MET* amplifications and treatment benefit (Figure 3A), the only patient whose tumor presented *AXL* gene amplification experienced a PFS of 5 months (Supplementary Figure S4A, available at https://doi.org/10.1016/j.esmogo.2026.100303). The univariate analysis showed that the only two genomic factors captured in the panel that positively correlated with the PFS were the absence of *TP53* mutations and the TMB > median (≥4 muts/Mb) (Figure 3B). The statistical relevance of this finding was confirmed on the multivariate analysis (Figure 3C). Of note, both *TP53* mutational

Table 2. Adverse events

Adverse events	G1-G2, *n* (%)	G3-G4, *n* (%)
Hypertension	5 (15.2)	1 (3)
HFS	8 (24.2)	1 (3)
Fatigue/asthenia	15 (45.5)	3 (9.1)
Diarrhea	7 (21.2)	1 (3)
Decreased appetite	1 (3)	0
Vomiting	2 (6)	0
Nausea	5 (15.2)	0
Proteinuria	0	3 (9.1)
Anemia	2 (6)	0
Thrombocytopenia	2 (6)	0
Neutropenia	1 (3)	0
AST-ALT increased	15 (45.5)	3 (9.1)
Hypothyroidism	8 (24.2)	0

ALT, alanine aminotransferase; AST, aspartate aminotransferase; G, grade; HFS, hand-foot syndrome.



	HR	95% CI	*P* value
CNA < 3 genes	0.6191	0.2718 to 1.533	0.2701
PIK3CA mutation	0.5206	0.1866 to 1.244	0.1701
RAS mutation	0.8337	0.3741 to 1.935	0.6604
APC mutation	0.9917	0.4236 to 2.720	0.9858
TP53 wt	0.2232	0.05219 to 0.6562	0.0162
TMB > 4	0.3282	0.1188 to 0.8471	0.0236

	HR	95% CI	*P* value
CNA < 3 genes	1.698	0.5451 to 5.043	0.3433
PIK3CA mutation	1.342	0.4666 to 4.501	0.6032
RAS mutation	1.062	0.3804 to 2.868	0.905
APC mutation	0.3019	0.08313 to 1.138	0.0669
TP53 wt	0.06618	0.009923 to 0.3078	**0.0016**
TMB > 4	0.1085	0.02864 to 0.3532	**0.0004**

Figure 3. Distribution of genomic events in the overall population and correlation of response to genomic alteration and transcriptomic signatures. (A) Oncoprint scheme of frequent genetic events (present in at least two patients from the study cohort). (B and C) Univariate (B) and multivariate (C) analyses of the impact of main genomic features [copy number alterations >3; PIK3CA mutations; RAS mutations; APC mutations, TP53 mutations; tumor mutational burden (TMB) ≥4 muts/Mb on PFS]. (D and E) Estimated survival based on TMB ≥4 muts/Mb (D) and TP53 wild-type (WT) status (E) using Cox proportional hazards model. (F and G) Transcriptional signatures of epithelial-mesenchymal transition (EMT) and angiogenesis are enriched in patients who benefit from cabozantinib. Distribution of the gene set variation analysis (GSVA) scores for the EMT (F) and angiogenesis (G) hallmarks based on the PFS outcome. Statistical analysis carried out using Wilcoxon rank-sum test. Responders: cases with PFS >16 weeks; non-responders: cases with PFS <16 weeks.
CI, confidence interval; CNA, copy number alteration; HR, hazard ratio; muts/Mb, mutations/megabase; PFS, progression-free survival.

status and higher-than-median TMB were able to identify patients with different PFS values (Figure 3D and E), whereas other genomic biomarkers did not have an impact on PFS (Supplementary Figure S4B-D, available at https://doi.org/10.1016/j.esmogo.2026.100303). Interestingly, the combination of these genomic factors presented an AUC of 0.87 to identify patients benefiting from the treatment, with a positive predictive value of 77.78%, and a negative predictive value of 81.25% (Supplementary Figure S5, available at https://doi.org/10.1016/j.esmogo.2026.100303). For a subset of 16

patients (of whom 5 had positive PFS outcome), pretreatment liquid biopsy results were available. In these patients, we correlated the highest variant allele frequency (VAF) values with PFS, as previously reported.[26-28] In this subset, both Pearson's correlation and Cox regression showed a non-significant trend between the lower values of the VAF and the longer PFS time (Supplementary Figure S6A and B, available at https://doi.org/10.1016/j.esmoop.2025.106031).

Additionally, for a subset of 18 (54.5%) patients (5 responders and 13 non-responders), RNA sequencing from archival FFPE samples was carried out for translational analyses. Of note, the analyses were done on tissues collected before first-line treatment (i.e. at the moment of diagnosis) and thus capturing the transcriptional features of the tumors before the perturbations induced by therapies.[29] As expected from a cohort of mCRC patients,[3,30] CMS4 was the most represented consensus molecular subtype, characterizing 10/18 (55%) of the cases (Supplementary Figure S7A, available at https://doi.org/10.1016/j.esmogo.2026.100303), suggesting that the patient cohort enrolled in the trial is particularly enriched for tumors with poor prognosis. Interestingly, even though all patients with exceptionally long PFS fell into the CMS4 subgroup, the overall distribution of PFS values was not statistically different between CMS4 and non-CMS4 tumors, and this feature did not identify different survival groups (Supplementary Figure S7B and C, respectively, available at https://doi.org/10.1016/j.esmoop.2025.106031). However, this result should be interpreted with caution due to the small sample size. Moreover, an unsupervised clustering based on the expression of cabozantinib targets revealed no co-segregation of samples of the same clinical outcome (responders versus non-responders) (Supplementary Figure S8A, available at https://doi.org/10.1016/j.esmogo.2026.100303).

Next, in order to verify if other transcriptional features of the primary tumors were associated with treatment benefit, we computed GSVA scores using MSigDB hallmarks gene sets for each sample.[25] Interestingly, among the 10 hallmark gene sets with the highest absolute difference in GSVA score between responders and non-responders, there were the hallmark epithelial-mesenchymal transition (EMT) and angiogenesis gene sets (Supplementary Figure S8B and C, available at https://doi.org/10.1016/j.esmogo.2026.100303), that are biologically related to the molecular pathways intercepted by cabozantinib. For both these hallmark gene sets, we identified a trend toward higher GSVA scores in tumors from patients meeting the primary PFS endpoint (responders) compared with patients who progressed within 16 weeks (non-responders) (Figure 3F and G, Supplementary Figure S8C, available at https://doi.org/10.1016/j.esmogo.2026.100303). Altogether, these molecular findings suggest that tumor genomic features may impact on the benefit derived from cabozantinib, though to a lesser extent. Moreover, although the GSVA score does not allow us to discriminate responders from non-responders, the transcriptional analyses suggest that tumors with a higher EMT or angiogenesis score at baseline may potentially derive a higher benefit from cabozantinib treatment.

DISCUSSION

Angiogenesis inhibition is a cornerstone for the treatment of mCRC throughout the continuum of care, particularly in the case of tumors not harboring specific oncogenic activations that are susceptible to targeted agents.[31]

Beside the genomic features driving treatment, such as microsatellite status, or presence of *RAS* or *BRAF* mutations, recent advancements in molecular subtyping in CRC have allowed us to identify transcriptional features with potential clinical relevance, incorporated in four CMS groups.[3] In particular, mCRC with a poor response to chemotherapy and high metastatic potential are enriched for mesenchymal features, including EMT, angiogenesis activation, and immune suppression, that are recapitulated in the CMS4 cluster.[3]

However, despite the increasing ability to molecularly annotate CRC, the current treatment landscape of chemorefractory disease is still molecular-agnostic.[2] Interestingly, all approved regimens for chemorefractory CRC (regorafenib, trifluridine/tipiracil + bevacizumab, fruquintinib) rely on inhibition of angiogenesis as the main mechanism of action, for which no predictive biomarkers have been described so far.[32-34] In this framework, identifying novel agents that couple angiogenesis inhibition with suppression of other mesenchymal features would provide a novel treatment opportunity. Cabozantinib is a multi-kinase inhibitor that is able to block VEGFR2, the main angiogenesis mediator in CRC, as well as MET and AXL signaling pathways that characterize key mediators of therapeutic resistance and induction of EMT.[35,36] In order to validate the hypothesis that cabozantinib may be active in the chemorefractory setting and correlate the outcome with the molecular features of the tumors, we designed and conducted the ABACO trial. Importantly, most patients enrolled in this trial presented features of aggressive tumors, including *TP53* mutations (83%) and MAPK pathway activation (73%, consisting of either *RAS* or *BRAF* activating mutations). Nonetheless, this trial met its primary endpoint in this population of mCRC patients that have progressed on all standard lines of therapy. Indeed, one-third of patients achieved a PFS >16 weeks, and the median PFS and OS were only slightly inferior to the results of the latest phase III monotherapy studies in advanced lines. It should also be considered that the genetic characteristics of the tumors mentioned earlier also influence this aspect. No new safety signal emerged from the trial. The most frequent and severe AEs, particularly hypertransaminasemia, were effectively managed with temporary suspension of the drug and resumption at a lower dosage. Of note, clinical features such as age, sex, sidedness, and features of the metastatic disease, did not correlate with the treatment efficacy. On the other hand, from a genomic point of view, the presence of *TP53* mutations strongly correlated with worse PFS in this patient cohort, as no patient with a *TP53*-mutant tumor reached the primary endpoint. Interestingly, the patients whose tumors presented >4 muts/Mb (TMB median in our

cohort) displayed longer PFS with cabozantinib. Although this value is lower than the cut-off used to define high-TMB cancers,[37] it may still identify MSS cancers with different biological features. However, this finding merits further investigation, as previous evidence suggests that MSS CRCs with a higher TMB may instead be at a prognostic disadvantage.[38] Although not statistically significant, patients with lower VAF values on pretreatment circulating tumor DNA trended towards longer PFS times. The transcriptional findings captured by our analyses suggest some relevant considerations. Firstly, our results confirm that the most common CMS subtype in chemorefractory CRC is CMS4.[3,39] Secondly, we highlight that the transcriptional status of the tumor at baseline may retain its biological value also for treatments administered much later during the continuum of care, possibly increasing the value of archival tissue. Thirdly, we found an interesting trend between the transcriptional activation of EMT and angiogenesis pathways and the benefit derived from cabozantinib. Although the CMS4 cluster did not correlate to treatment benefit, we suggest that the transcriptional activation of the two main biological pathways inhibited by cabozantinib (EMT and angiogenesis) may effectively identify tumors with a higher chance of clinical response, worthy of being prospectively investigated in future clinical studies.

The molecular and clinical results from this trial compare favorably with the results from other trials investigating cabozantinib in advanced CRC. In particular, despite similar ORR and median PFS, treatment benefit was not limited to CRCs with a *RAS* wt status and left-sidedness, as seen in the AGICC 17CRC01 trial.[19,20] Among the 10 genetically annotated tumors which remained progression-free for >16 weeks, only 2 were *RAS/BRAF* wt. This result highlights that MAPK pathway activation does not necessarily predict absence of benefit from cabozantinib, suggesting that its use should not be limited to *RAS/BRAF* wt CRC cases.

Conversely, the ORR and the 16-week PFS rate (27.6% and 45%, respectively) are higher when cabozantinib is combined with programmed death ligand 1 (PD-L1) inhibition, as shown in the CAMILLA trial.[19,20] Moreover, given the use of archival tissue, we could not find the same correlation between response and upregulation of cabozantinib targets identified in the CAMILLA trial.

We recognize this study presents some limitations. All the molecular analyses were performed retrospectively and were not pre-specified and are thus to be conceived as hypothesis-generating. In particular, we acknowledge that the association between *TP53* mutations and outcome may represent a prognostic role of this alteration rather than a predictive effect. Moreover, all the transcriptional analyses were carried out on archival samples derived from baseline tissues, prior to the initiation of any treatment, and may not fully represent the transcriptional profile of the tumor at the moment of cabozantinib treatment.[29] Nonetheless, although this characterizes a biological limitation, its translational relevance is even broader, showing that intrinsic baseline transcriptional features potentially impact on the treatment response throughout the natural history of the disease.

Overall, this work reinforces the evidence that cabozantinib is safe and active in CRC and suggests that specific molecular features associated with biological aggressiveness of the disease, such as EMT activation and angiogenesis, may in fact predict a stronger benefit by cabozantinib. These results deserve a further prospective investigation and advocate for incorporating tumor transcriptional features in the design of future trials investigating the activity of cabozantinib alone or in combination for the treatment of chemorefractory CRC.

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DISCLOSURE

PPV served in a consulting role for Biocartis and has received speaking fees from Biocartis and Merck KgA. DC declares advisory board/consultation for Bayer, Merck KgA, Foundation Medicine; and travel support from Sanofi, Bristol Myers Squibb (BMS) and Merck KgA. SN reported receiving personal fees from Novartis; and a travel grant from Amgen. GM reported receiving honoraria from Servier, Incyte, and Pierre Fabre. EM reported serving as advisor and speaker for AstraZeneca, Eli Lilly, Servier, Sanofi Genzyme, Roche, Merck, Eisai, and Pfizer. AA reported receiving personal fees from Amgen, AstraZeneca, Merck, Sharp & Dohme (MSD), Eisai, and BMS. MGZ reported receiving honoraria for advisory board participation from GlaxoSmithKline (GSK). GC reported receiving honoraria for speaker's engagement from Roche, Seattle Genetics, Novartis, Lilly, Pfizer, Foundation Medicine, NanoString, Samsung, Celltrion, BMS, and MSD; honoraria for providing consultancy for Roche, Seattle Genetics, and NanoString; honoraria for participating on advisory board for Roche, Lilly, Pfizer, Foundation Medicine, Samsung, Celltrion, and Mylan; honoraria for writing engagement from Novartis and BMS; honoraria for participation in the Ellipsis Scientific Affairs Group; institutional research funding for conducting phase I and II clinical trials from Pfizer, Roche, Novartis, Sanofi, Celgene, Servier, Orion, AstraZeneca, Seattle Genetics, AbbVie, Tesaro, BMS, Merck Serono, MSD, Janssen-Cilag, Philogen, Bayer, Medivation, and Medimmune. NF received honoraria as a consultant and speaker from Novartis and ITM. AB received research support by

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DATA SHARING

The data that support the findings of our study are available from the corresponding author upon reasonable request.

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