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Regorafenib-Avelumab Combination in Patients with Microsatellite Stable Colorectal Cancer (REGOMUNE): A Single-arm, Open-label, Phase II Trial

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ABSTRACT

Purpose: Regorafenib is synergistic with immune checkpoint inhibition in colorectal cancer preclinical models.

Patients and Methods: This was a single-arm, multicentric phase II trial. Regorafenib was given 3 weeks on/1 week off, 160 mg every day; avelumab 10 mg/kg i.v. was given every 2 weeks, beginning at cycle 1, day 15 until progression or unacceptable toxicity. The primary endpoint was the confirmed objective response rate under treatment, as per RECIST 1.1. The secondary endpoints included a 1-year nonprogression rate, progression-free survival (PFS), and overall survival (OS), safety and biomarkers studies performed on sequential tumor samples obtained at baseline and at cycle 2 day 1.

Results: Forty-eight patients were enrolled in four centers. Forty-three were assessable for efficacy after central radiological review. Best response was stable disease for 23 patients (53.5%) and progressive disease for 17 patients (39.5%). The median PFS and OS were 3.6 months [95% confidence interval (CI), 1.8–5.4] and 10.8 months (95% CI, 5.9–NA), respectively. The most common grade 3 or 4 adverse events were palmar-plantar erythrodysesthesia syndrome ($n = 14$, 30%), hypertension ($n = 11$, 23%), and diarrhea ($n = 6$, 13%). High baseline infiltration by tumor-associated macrophages was significantly associated with adverse PFS (1.8 vs. 3.7 months; $P = 0.002$) and OS (3.7 months vs. not reached; $P = 0.002$). Increased tumor infiltration by $CD8^+$ T cells at cycle 2, day 1 as compared with baseline was significantly associated with better outcome.

Conclusions: The combination of regorafenib + avelumab mobilizes antitumor immunity in a subset of patients with microsatellite stable colorectal cancer. Computational pathology through quantification of immune cell infiltration may improve patient selection for further studies investigating this approach.

Introduction

Colorectal cancer remains one of the leading causes of cancer-related deaths worldwide (1). In the metastatic setting, the standard of care for patients who are not candidate for surgical procedures is based on palliative fluorouracil-based chemotherapy regimens associated with agents targeting angiogenesis or the epidermal growth factor receptor (2).

Regorafenib, a small molecule targeting several protein kinases involved in tumor angiogenesis, is the sole targeted therapy approved for the management of patients with metastatic colorectal cancer who have failed standard chemotherapies and have no other treatment options. This approval was based on the result of a pivotal randomized study which showed that patients who received regorafenib in addition to supportive care experienced longer progression-free survival (PFS; median of 2 vs. 1.7 months) and overall survival (OS) than those who received placebo (median of 6.4 vs. 5 months), despite an objective response rate which was only 1% (3). This clinical benefit appears as modest and, therefore, new therapeutic strategies are needed to improve outcome of patients with chemo-refractory colorectal cancer.

The development of immune checkpoint inhibitors has dramatically changed the landscape of treatment of several cancers, in particular, malignant melanoma and non–small cell lung cancer. However, in colorectal cancer, only a subset of patients with mismatch repair–deficient or microsatellite instability-high (MSI-H) tumors are likely to respond to treatment with immune checkpoint inhibitors when used as single agent (4, 5).

There are several lines of evidence indicating that targeting VEGF and its receptor (VEGFR) may be synergistic with immune checkpoint inhibition in human tumors (6, 7). For instance, inhibition of VEGFR has been shown to inhibit proliferation of regulatory T cells in patients with colorectal cancer (8). Furthermore, VEGF-A, which is abundant in the tumor microenvironment of human tumors, has been shown to upregulate the expression of programmed cell death 1 (PD-1), which plays a crucial role in $CD8^+$ T-cell exhaustion (9).

Besides targeting VEGFR, regorafenib inhibits CSF1R, a tyrosine kinase receptor that is involved in macrophage proliferation (10). Interestingly, regorafenib was shown to reduce immunosuppressive macrophage infiltration and to synergize with anti–PD-1 inhibition in

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Translational Relevance

Preclinical and translational studies have suggested a synergistic activity of antiangiogenic agents, such as regorafenib, with anti-programmed cell death 1/programmed cell death ligand 1 mAbs in solid tumors, including models of aggressive colorectal cancer. To our knowledge, our study is the first phase II study investigating a combination of antiangiogenic therapy and immune checkpoint inhibitor in patients with advanced microsatellite stable (MSS) colorectal cancer. Our results show that regorafenib combined with avelumab, mobilizes antitumor immunity in a subset of MSS colorectal cancers and may warrant further investigation in selected patients.

a preclinical model of microsatellite stable (MSS) colorectal cancer (11, 12). In such models, regorafenib consistently reduced tumor-infiltrating macrophages and anti–PD-1 treatment was associated with elevated IFNγ levels, indicative of enhanced T-cell activation.

Therefore, we hypothesized that combining regorafenib with anti–PD-1/anti-programmed cell death ligand 1 (PD-L1) antibodies may be associated with significant clinical benefit in patients with MSS metastatic colorectal cancer who have failed on previous standard chemotherapy regimens.

Avelumab is the first anti–PD-L1 antibody to have been approved to be used in combination with an antiangiogenic agent for the treatment of solid tumors. This approval was based on the result of a randomized, multicenter, open-label trial of avelumab plus axitinib which enrolled in 886 patients with untreated advanced renal cell carcinoma regardless of tumor PD-L1 expression (13). Patients were randomized to receive either avelumab 10 mg/kg intravenous infusion every 2 weeks in combination with axitinib 5 mg twice daily orally or sunitinib 50 mg once daily orally for 4 weeks, followed by 2 weeks off until radiographic progression or unacceptable toxicity. The results of this study showed a significant improvement in PFS and OS, confirming the potential for combining immune checkpoint inhibitor with antiangiogenic agents for the treatment of solid tumors.

We report here the clinical and biomarker results of a phase II study investigating the combination of regorafenib plus avelumab in MSS metastatic colorectal cancer.

Patients and Methods

Study design and participants

REGOMUNE was a single-arm, multicenter phase II basket study for which patients were recruited from four French sites. In the colorectal cancer cohort, patients were eligible if they were aged at least 18 years and had histologically proven MSS advanced or metastatic colorectal cancer; Eastern Cooperative Oncology Group performance status of 0–1; measurable disease according to RECIST 1.1 (14); at least one previous line of systemic treatment; and adequate hematologic, renal, metabolic, and hepatic functions (see study protocol for a full list of eligibility criteria, Supplementary Data S4). Blood test included assessment of blood cell count, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, albumin, bilirubin, lipase, creatinine phosphokinase, coagulation test, creatinine, and urea nitrogen. Main exclusion criteria included previous treatment with avelumab or regorafenib, anti–PD-1, anti–PD-L1, anti–PD-L2, anti-CD137, or anti-cytotoxic T-lymphocyte–associated antigen-4 antibody, and are detailed in the protocol. The REGOMUNE study was conducted in accordance with the Declaration of Helsinki. As required by French regulations, the protocol was approved by a Central Institutional Review Board (Comité de Protection des Personnes Sud-Est II, Lyon, France) that reviewed the appropriateness of the clinical trial protocol, as well as the risks and benefits to study participants. All patients provided written informed consent.

Procedures

After an assessment of eligibility, patients received regorafenib 160 mg per day on a 3 weeks on/1 week off schedule, in cycles of 28 days. Avelumab treatment began on day 15 cycle 1, by intravenous infusion once every 2 weeks at the dose of 10 mg/kg. Treatment was continued until disease progression, unacceptable toxicity, investigator's decision to discontinue, or withdrawal of patient consent. Participants were monitored for adverse events at every follow-up assessment. Adverse events were graded according to NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0. Laboratory assessments were done at baseline, and every 2 weeks afterward until treatment discontinuation. Regorafenib dose modifications to manage adverse events were allowed (see study protocol). The dose of regorafenib could be reduced to 120 mg and then to 80 mg. Dose interruptions were allowed on the basis of the clinical situation. Patients requiring a delay of >4 weeks since the last dose of regorafenib had to permanently discontinue regorafenib, but could continue avelumab if it was considered appropriate. No dose reduction of avelumab was allowed. Dose interruptions were allowed on the basis of the severity of immune-related adverse events. Patient requiring two or more consecutive cancellations of avelumab injection had to permanently discontinue avelumab and were allowed to continue regorafenib. Tumor lesions were assessed according to RECIST version 1.1 at baseline (within 4 weeks prior to cycle 1 day 1), and every 8 weeks until disease progression or the start of another treatment. Tumor samples were collected at baseline and cycle 2 day 1 for all consenting patients to assess the impact of treatment on tumor microenvironment and to identify potential biomarkers associated with outcome.

Outcomes

The primary endpoint was the 6-month objective response rate under treatment defined as the proportion of patients with objective response (confirmed or unconfirmed) under treatment based on adapted RECIST 1.1 after centralized radiological review.

Secondary objectives included best overall response, objective response rate at 6 months, 6-month progression-free rate, 1-year PFS, 1-year OS, and safety. Best overall response was defined as the best response across all timepoints. PFS was defined as the time from study treatment initiation to the first occurrence of disease progression or death from any cause. OS was defined as the time from study treatment initiation to death from any cause. Safety was graded as per the common toxicity criteria from the NCI CTCAE v5.0.

Statistical analysis

The study was based on a Bayesian adaptive phase II design approach following an adaptive trial design. The primary endpoint was the 6-month objective response rate. The probability of success was estimated from a beta-binomial model (15). This model was based on a binary variable (success or failure) following a binomial distribution (n, π), where n is the number of observed patients and π is an unknown and random probability of success on a fixed number of Bernoulli trials (beta-distribution).

Initial parameters of the model needed to be prespecified (the prior distribution represents the knowledge of the nonprogression

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probability prior to observing the data). Successive results observed were then used to update and refine the distribution, generating the so-called posterior distribution. In the absence of a strong idea about the response rates to be observed, a noninformative prior distribution [beta (1, 1)] was considered. Maximal response probability threshold and minimal response probability threshold were defined as 20% versus 5%, respectively. Maximum sample size was set at 50 patients. The analysis of the primary endpoint was carried out sequentially, with interim analyses planned after 16-week follow-up for the first 10 patients and then every five patients.

At each interim analysis, stopping rules recommended to stop the trial for inefficacy [if there was a high predictive probability (≥80%) that the objective response rate was lower or equal to 5%, the minimal response probability threshold) or efficacy [if there was a high predictive probability (≥80%) that the objective response rate was higher or equal to 20%, the maximal response probability threshold].

The efficacy population included all participants who met the eligibility criteria and who received at least one complete or two incomplete treatment cycles. All enrolled patients who initiated the study treatment were included in the safety analysis.

The median follow-up was calculated using the reverse Kaplan–Meier method. Survival endpoints were described using the Kaplan–Meier method as described previously (16). Data for patients who were alive and event free were censored at the date of the last follow-up. Quantitative variables were described using the median and range. Qualitative variables were described using frequency, rates, and 95% confidence interval (95% CI, binomial law). Estimated parameters were reported with two-sided 95% CIs. *P* values less than 0.05 (typically ≤0.05) were statistically significant. Statistical analyses were performed using SAS software (version 9.4). This study was registered with ClinicalTrials.gov number, NCT03475953.

Tumor mutational burden analysis

For DNA extraction, two punch cores of 1 mm of formalin-fixed, paraffin-embedded (FFPE) tissue were deparaffinized and processed using the Maxwell RSC FFPE PLUS DNA Kit, ref: AS1720

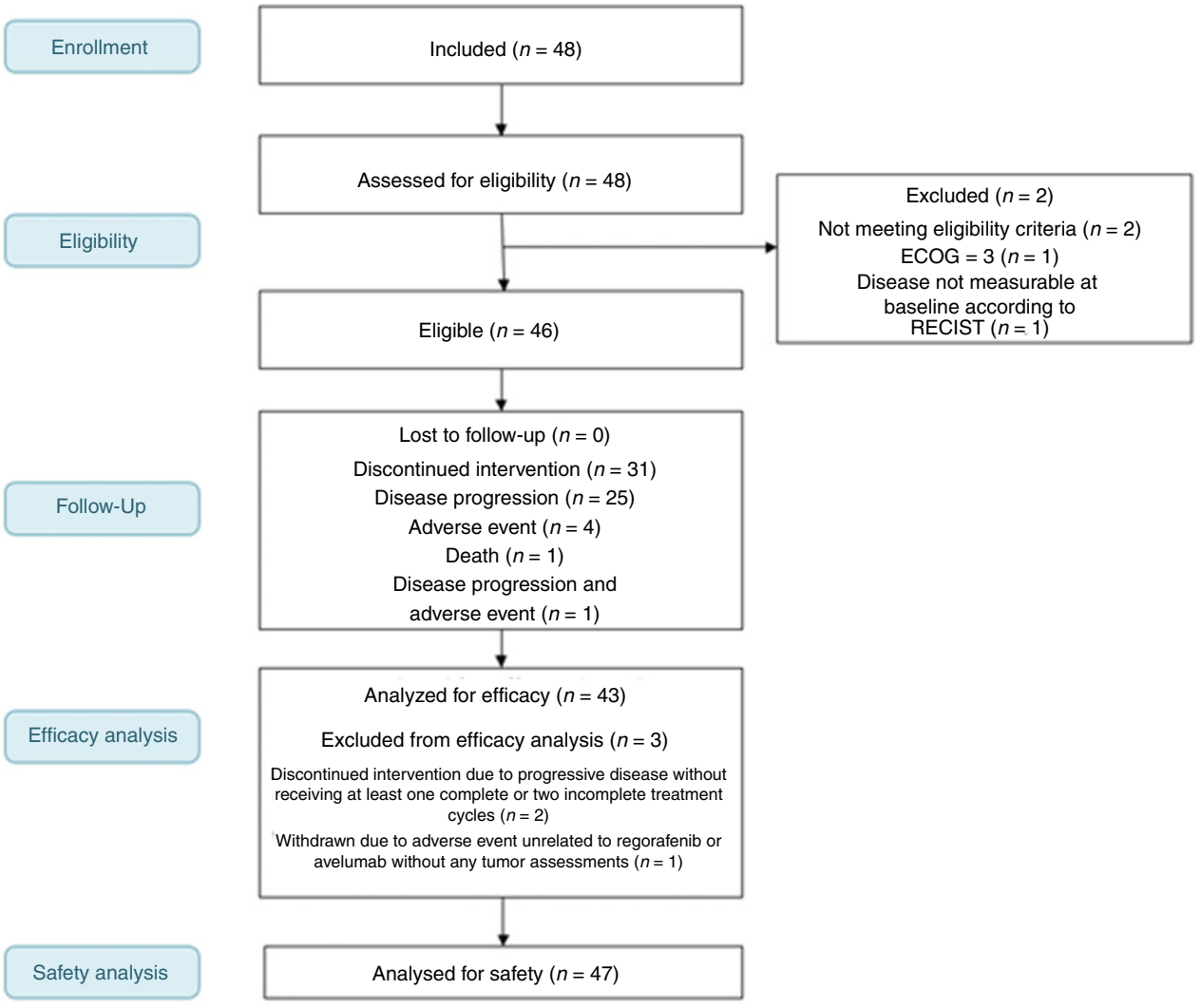

Figure 1.
Flow chart of patients included in the REGOMUNE study.

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(Promega), according to the manufacturer's instructions. Extracted DNA was pretreated with Uracil-DNA Glycosylase (heat labile, ref: 78310100UN, Thermo Fisher Scientific) to eliminate deamination artifacts. Tumor mutational burden (TMB) was assessed using a 409-gene targeted next-generation sequencing assay that detects variants in all coding regions (Oncomine Tumor Mutation Load Assay, Chef-ready Library Preparation, ref: A37910, Thermo Fisher Scientific) as described previously (17). For library preparation, 5–20 ng of DNA was used, depending on the availability of input material. The libraries were prepared on the Ion Chef instrument, following the manufacturer's instructions, and quantified on TapeStation HSD1000 (Agilent Technologies).

Sequencing runs were planned on the Torrent Suite Software v5.10 and libraries were diluted to 50 pm, combined in batches of eight libraries, loaded on an Ion 540 chip using the Ion Chef Instrument, and sequenced on an Ion S5XL Instrument (Thermo Fisher Scientific). Raw data were processed automatically on the Torrent Server and aligned to the hg19 reference genome. An average coverage of 560 reads (325×–721×) was obtained per sample, with 96.5% (93.2%–97.5%) read uniformity. Sequencing data were then uploaded in BAM format to the Ion Reporter analysis server for tumor mutational load score calculation and variant calling. Variant detection and TMB calculation were performed on Ion Reporter analysis software v5.10 (IR) using the Oncomine Tumor Mutation Load w2.0 workflow and TMB algorithm v2.5. The default limit of detection was set at 5% allelic frequency. Germline variants were filtered automatically by cross-referencing with UCSC common SNPs, ExAC, 1000 Genomes, and 5000 Exomes databases. Somatic variants in homopolymer stretches longer than 7 bp were also excluded.

Tissue sample analysis

Tumor biopsies were collected at baseline and at day 1 of cycle 2. These samples were analyzed to characterize the impact of regorafenib combined with avelumab on tumor microenvironment and to identify potential predictive biomarkers of clinical benefit. Immunohistofluorescence analysis was performed on the automated Ventana Discovery XT Staining Platform (Ventana Medical Systems). Slides of tumor tissue were deparaffinized in xylene and hydrated in serial alcohol solutions. Antigen retrieval was performed by heat-induced epitope retrieval method using standard CC1 (tris-based buffer) pH 8 (Ventana Medical Systems). The slides were incubated with the following primary antibodies: anti-CD8 (clone C8/144B, Dako, dilution 1/25), anti-CD163 (clone 10D6, Leica, dilution 1/100eme), anti-PDL1 (clone QR1, Diagomics, dilution 1/100), and anti-Pan Keratine (clone AE1/AE3/PCK26, Roche, ready-to-use). Bound primary antibodies were detected using either OmniMap anti-Ms or Rb-horseradish peroxidase with Opal Detection Kit (Akoya Bioscience). The slides were counterstained with spectral DAPI (PerkinElmer) and cover slipped. Stained slides were imaged on the Multispectral Slide Analysis System (Vectra Polans, Perkin Elmer) and analyzed in Inform Image Analysis Software (PerkinElmer, version 2.4.1) to segment tissue into tumor and stroma and to phenotype cells. $CD163^+$ cell density was calculated in the tumor compartment and patients were classified as "high infiltrated" if they exhibited a tumor macrophage cell density superior to 175 cells per mm^2. To determine this optimal cut-off point, the surv_cutpoint function from the R package "survminer" (v 0.4.7) was used. This function uses the maximally selected rank statistics from the "maxstat" R package. Log rank *P* values and HRs were computed using, respectively, the surv_fit function from "survminer" R package and the coxph function from "survival" R package.

Role of the funding source

The study was sponsored by Institut Bergonié, Comprehensive Cancer Center (Bordeaux, France). The data were collected with the sponsor data management system and were analyzed and interpreted by representatives of the sponsor in collaboration with the investigators. S. Cousin, C. Cantarel, C. Bellera, and A. Italiano had access to the raw data. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Data sharing

Individual participant data that underlie the results reported in this article will be available after deidentification upon publication and up to 6 years after article publication to researchers who provide a methodologically sound proposal. Requests should be sent to the corresponding author.

Table 1. Patient characteristics.

	Eligible patients for safety *N* = 47
Median age (years, range)	62 (26–83)
Gender	
Female	12 (26%)
Male	35 (74%)
Performance status ECOG	
0	28 (60%)
1	19 (40%)
KRAS gene mutation	
Yes	30 (64%)
No	17 (36%)
BRAF gene mutation	
Yes	3 (6%)
No	38 (81%)
Unknown	6 (13%)
Number of metastatic sites	
Single	8 (17%)
Multiple	39 (83%)
Metastatic sites	
Lung	37 (79%)
Liver	35 (75%)
Peritoneum	15 (32%)
Node	14 (30%)
Other	9 (19%)
Previous chemotherapy treatment	
Fluoropyrimidine	47 (100%)
Oxaliplatin	44 (93.6%)
Irinotecan	44 (93.6%)
Previous targeted biological treatment	
None	3 (7%)
Any (anti-VEGF[a] or anti-EGFR[b] or both)	44 (94%)
Anti-VEGF but not anti-EGFR	26 (60%)
Anti-EGFR but not anti-VEGF	3 (7%)
Anti-VEGF and anti-EGFR	15 (35%)
Previous lines of treatment for advanced disease	
0[c]	1 (2%)
1	5 (11%)
2	14 (30%)
≥2	27 (59%)

Abbreviation: ECOG, Eastern Cooperative Oncology Group.
[a]Bevacizumab.
[b]Cetuximab or panitumumab.
[c]This patient received prior systemic therapy in the neoadjuvant/adjuvant setting.

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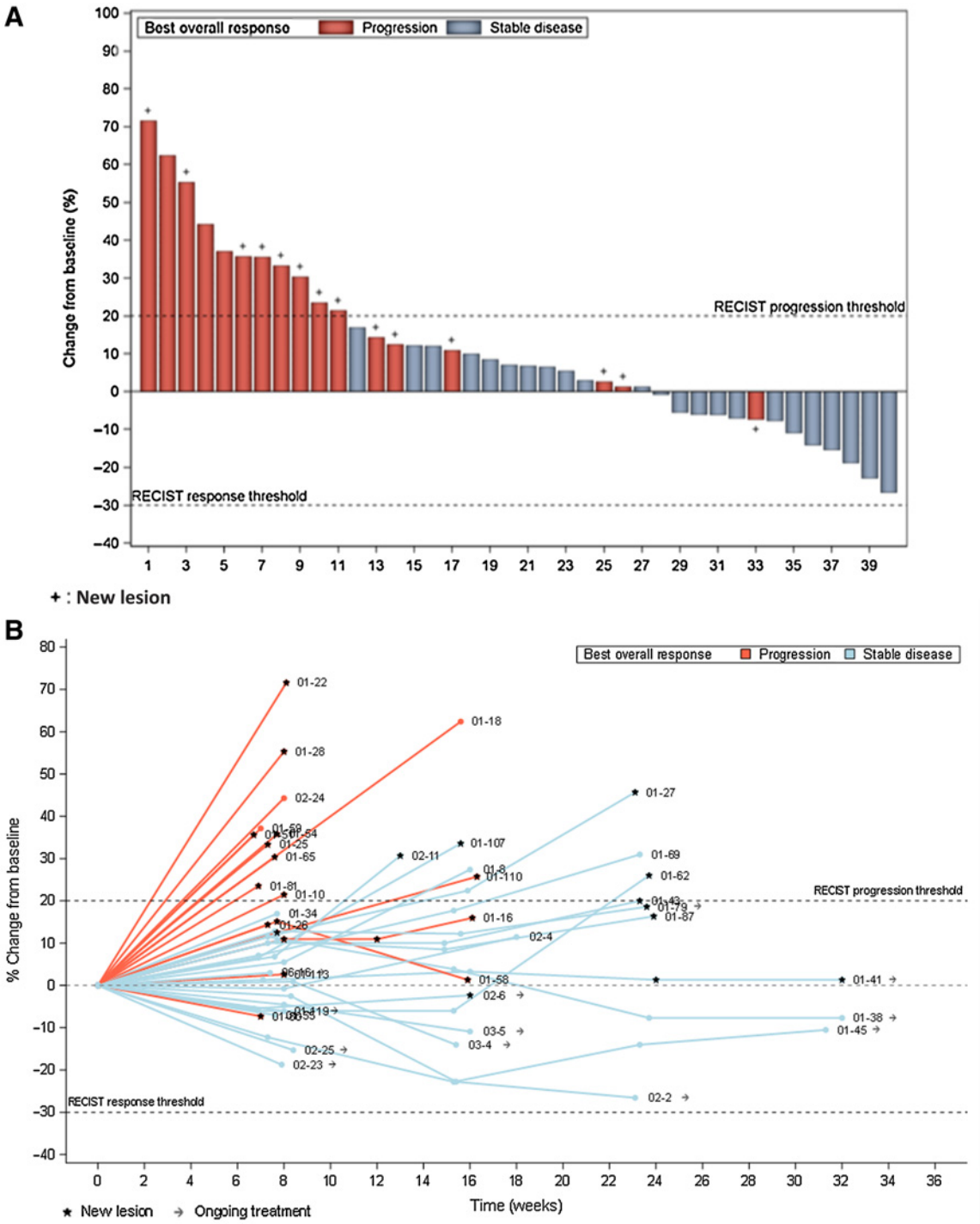

Figure 2.

Waterfall plot (**A**) and spider plot (**B**) of best overall response in patients with MSS colorectal cancer treated with regorafenib plus avelumab ($n = 40$, response based on central review assessment according to RECIST 1.1). Note: three patients were not evaluable for RECIST response: two patients discontinued study because of adverse events before the planned tumor evaluation, and one patient discontinued study due to early progressive disease (**Fig. 1**). These three patients were classified as "not evaluable" as per RECIST 1.1. Despite tumor shrinkage, one patient (ID = 33) had progressive disease as best overall response. This patient had a single tumor assessment during treatment; although shrinkage of target lesion was observed, a new lesion was identified. He was as such classified as progressive disease as per RECIST 1.1.

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Results

Between November 28, 2018 and October 4, 2019, 48 patients were recruited to the study. Forty-six patients were eligible, and 43 were eligible and assessable for the efficacy endpoint. Three patients were not eligible for the efficacy assessment due to protocol deviations (**Fig. 1**). Characteristics of the patients are summarized in **Table 1**. The median age was 62 years (range, 26–83), and 26% of the patients were women. Ninety-eight percent of the patients had already received systemic treatment for advanced disease, with a median of three (range, 0–7) previous lines.

In the efficacy population and after a median follow-up of 7.2 months (95% CI, 6.4–8.1), 29 (67%) were still alive, with 12 (28%) still under treatment.

Among the 43 patients who were eligible and assessable for efficacy, three patients were not evaluable for RECIST response: one patient discontinued study because of early progression, and two patients discontinued study because of toxicity before planned tumor evaluation.

No patient achieved an objective response (6-month objective response rate, 0%) and as such, primary efficacy criterion was not reached. Regarding best overall response as per RECIST, 23 (54%) patients showed stable disease, including 12 (28%) with tumor shrinkage (range from −0.8% to −26.6%; **Fig. 2**). Seventeen patients (40%) had progressive disease.

Median PFS and OS were 3.6 months (95% CI, 1.8–5.4) and 10.8 months (95% CI, 5.9–NA; **Fig. 3**), respectively.

Forty-seven patients received at least one dose of regorafenib and/or avelumab and were, therefore, evaluated for safety. Treatment was generally well tolerated. Treatment-related adverse events and laboratory abnormalities that were reported in more than 5% of patients for grade 1–2 and any for grade 3 and 4 are shown in **Table 2**. The most common clinical treatment-related adverse events were fatigue, anorexia, palmar-plantar erythrodysesthesia syndrome, mucositis, dysphonia, diarrhea, and infusion-related reaction. As expected, the most common treatment-related laboratory abnormalities were transaminitis and thyroid-stimulating hormone (TSH) increase. At least one serious adverse event was reported in 22 patients (47%). Thirty-five (75%) and 33 (70%) patients experienced treatment modifications with regorafenib (25 patients with temporary discontinuation, 14 patients with dose reduction, and six patients with permanent discontinuation) and avelumab (including five with permanent discontinuation), respectively, because of a drug-related adverse event. No patient died from drug-related toxicity.

Overall, baseline tumor samples and paired biopsies (baseline and cycle 2 day 1 biopsies) were available for 24 and 15 patients, respectively. By analyzing paired tumor biopsies, we observed a significant increase in CD8 T-cell infiltration in nine of 15 (60%) cases, respectively (Supplementary Fig. S2). Patients with increased infiltration by $CD8^+$ T cell at cycle 2 day 1 compared with baseline had significantly better PFS (3.7 vs. 2.3 months; $P = 0.035$) and OS (not reached vs. 4.3 months; $P = 0.03$; Supplementary Fig. S2).

PD-L1 expression was >10% on tumor cells in six of 24 (25%) cases. PD-L1 status was neither correlated with PFS nor with OS. Given the crucial role of tumor-associated macrophages in progression of colorectal cancer, we quantified them on pretreatment samples and correlated their abundance with PFS and OS. Interestingly, we found that high level of tumor-infiltrating M2 macrophages at baseline was significantly associated with adverse outcome (PFS, 1.8 vs. 3.7 months; $P = 0.002$ and OS, 3.7 months vs. not reached; $P = 0.002$; **Fig. 4**).

Baseline TMB was available for 22 patients. TMB was low (<10 mutations/megabase) or high (≥10 mutations/megabase) in 17 (77%) and 5 (23%) patients, respectively. No statistically significant difference in PFS and OS was seen according to TMB status.

Discussion

Several clinical trials are currently ongoing to investigate the potential therapeutic role of antiangiogenic therapy combined with immune checkpoint inhibitors in solid tumors (6, 7). Such combinations have already been assessed with success in renal cell carcinomas as illustrated by the recent approval of pembrolizumab plus axitinib and avelumab plus axitinib in the first-line setting for patients with advanced disease (13, 18).

This is the first report of a phase II study investigating the combination of antiangiogenic agent with anti–PD-L1 in patients with MSS colorectal cancer. Thanks to our comprehensive analysis of tumor samples, our results suggest that this combination may have an impact on the tumor microenvironment of patients with colorectal cancer. Tumor-associated macrophages play a crucial role in colorectal cancer tumorigenesis by promoting angiogenesis and metastasis due to its ability to secrete VEGF (19). Tumor-associated macrophages are dependent on CSF1R kinase activity for proliferation and

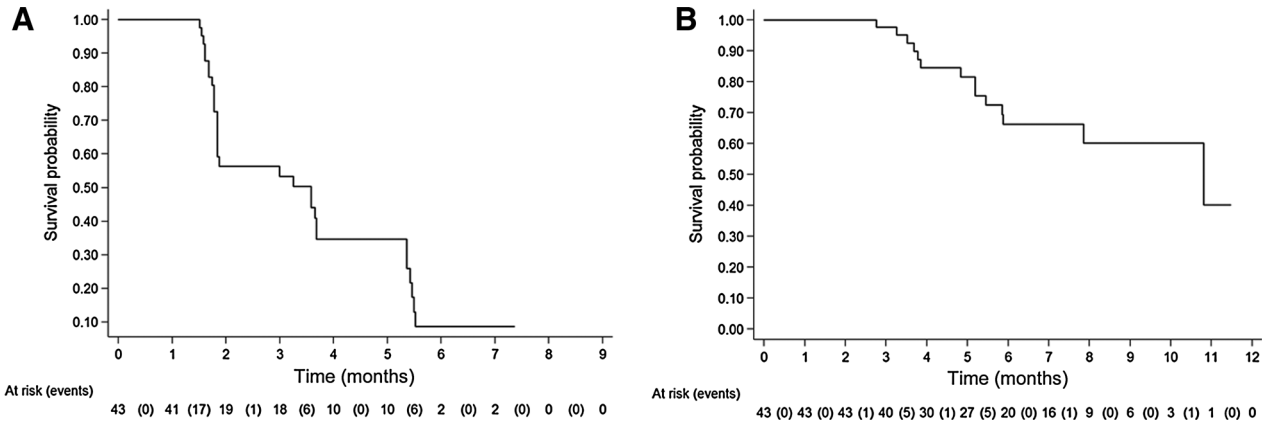

Figure 3.
Kaplan–Meier curves of PFS (**A**) and OS (**B**) in patients with MSS colorectal cancer treated with regorafenib and avelumab (43 patients were eligible for efficacy analysis).

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Table 2. Treatment-related adverse events during the treatment period in ≥5% of patients ($N = 47$).

Adverse event	Grade 1–2	Grade 3	Grade 4
Fatigue	29 (62%)	3 (6%)	
Anorexia	27 (57%)		
Palmo-plantar erythrodysesthesia	21 (45%)		
14 (30%)			
Oral mucositis	19 (40%)	2 (4%)	
Dysphonia	18 (38%)		
Diarrhea	17 (36%)	6 (13%)	
Infusion-related reaction	17 (36%)		
AST and/or ALT increased	14 (30%)	4 (9%)	2 (4%)
Blood bilirubin increase	13 (28%)	1 (2%)	1 (2%)
Alkaline phosphatase and/or GGT increased	13 (28%)		
Hypothyroidism/TSH increased	12 (26%)		
Hypertension	9 (19%)	11 (23%)	
Myalgia	8 (17%)		
Lipase increase	7 (15%)	2 (4%)	
Dry skin	5 (11%)	1 (2%)	
Nausea	5 (11%)		
Muscle cramp	5 (11%)		
Hypophosphatemia	5 (11%)		
Proteinuria	5 (11%)		
Maculopapular rash	4 (9%)	4 (9%)	
Fever	4 (9%)	1 (2%)	
Platelet count decreased	4 (9%)	1 (2%)	
CPK increase	4 (9%)		
Lymphocyte count decrease	4 (9%)		
Weight loss	4 (9%)		
Arthralgia	4 (9%)		
Multiforme erythema	4 (9%)		
Skin other	3 (6%)	3 (6%)	
Dry mouth	3 (6%)		
Constipation	3 (6%)		
Hemorrhoids	3 (6%)		
Hyperkeratosis	3 (6%)		
Neutrophil count decrease	1 (2%)	2 (4%)	
Injection site reaction	1 (2%)	1 (2%)	
Immune system disorder, other		2 (4%)	
General disorder and administration site, other		1 (2%)	
Hyponatremia		1 (2%)	
Arthritis		1 (2%)	

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CPK, creatine phosphokinase; GGT, gamma-glutamyl transferase.

differentiation. Several studies performed in immunocompetent colorectal cancer models have demonstrated that agents targeting CSF1R (including regorafenib) exhibited synergistic effects with anti–PD-1/PD-L1 antibodies in blocking tumor growth and affording immunomodulatory activity (11, 12, 20). Interestingly, in our study, patients with the lowest level of macrophage infiltration at baseline had the best outcome suggesting the potential role of tumor-associated macrophage quantification as a biomarker to select patients who are more likely to benefit from this approach.

We also observed a significant increase in CD8 T-cell infiltration in 60% of analyzed cases and this change was associated with improved outcome. Interestingly, a recent assessment of pre- to posttreatment changes in 20 patients with localized MSS colorectal cancer treated with immune checkpoint inhibitors in the neoadjuvant setting also showed significant increases in $CD8^+$ T cell, which was more pronounced in patients with good pathologic response (21). Our results suggest that immune checkpoint inhibition leads to immune activation also in metastatic MSS colorectal cancer.

PD-L1 expression is considered as an important biomarker to guide treatment with immune checkpoint inhibitors in several solid tumors, such as non–small cell lung cancer, head and neck cancer, or gastric cancer. In our study, PD-L1 status was neither correlated with PFS nor with OS. This result is in line with those of the neoadjuvant study mentioned above. In that study, PD-L1 expression was not correlated with the rate of pathologic response (21). Several studies have shown that the proportion PD-L1 cases is significantly higher in MSI-H colorectal cancer than in MSS colorectal cancer (22, 23). However, even in MSI-H colorectal cancer, PD-L1 expression is not clearly associated with clinical outcome (22). Altogether, these data indicate that PD-L1 expression status may not represent a reliable biomarker in colorectal cancer to guide immune checkpoint therapy.

The main limitation of this study is its nonrandomized design. Indeed, by minimizing many sources of potential bias, randomized, controlled clinical trials provide the most robust information about the effects of investigational drugs. However, with a median PFS and a median OS of 3.6 and 10.8 months, respectively, the results of our study compared favorably with the median PFS of 1.9 months and of OS of 6.4 months observed with regorafenib used as a single agent in the pivotal study which lead to its approval (3). However, only a well-conducted randomized controlled trial will provide the most valid estimates of the relative efficacy of regorafenib combined with avelumab versus regorafenib alone. Interestingly, we did not observe the same range of activity as compared with that in the REGONIVO study, the results of which were recently reported. This study, which investigated the combination of regorafenib and nivolumab in digestive tumors, enrolled 24 Japanese patients with metastatic MSS colorectal cancer. The objective response rate was 33% (95% CI, 15.6%–55.3%) and the median PFS was 6.3 months. Several explanations can explain these differences of outcome with the results of our study. Some of them are related to the methodology of the REGONIVO study, which was a phase IB study with an expansion cohort (24). The primary objective of the study was, therefore, to evaluate the safety of the combination. It is, therefore, likely that patients were hyperselected. For instance, only 50% of patients ($n = 12$) had target lesions in the liver, which is a proportion significantly lower than observed in routine practice, as well as in our study. It is well established that the liver is characterized by a tumor-permissive immune microenvironment (25, 26). In the REGONIVO study, all the objective responses but one, were observed in patients with extra-liver target lesions. Moreover, sample size was not calculated to allow an estimation of the efficacy rates with a reasonable CI and imaging data were not submitted to blinded review. Interestingly, as observed in our study, PD-L1 status was not significantly correlated with outcome, but no other analysis of tumor microenvironment was reported by the authors.

Most of the patients required dose reductions of regorafenib due to adverse events. However, this proportion was not different to that observed in clinical studies investigating regorafenib as single agent in colorectal cancer. In the CORRECT study (as well as in the CONCUR study which included Asian patients), up to 54% of patients had grade 3 or 4 treatment-related adverse events that needed some dose reduction (3, 27). Of note, patients included in the CORRECT study were more heavily pretreated, with 3% of patients who received only one prior line of therapy versus 11% in the REGOMUNE study. The

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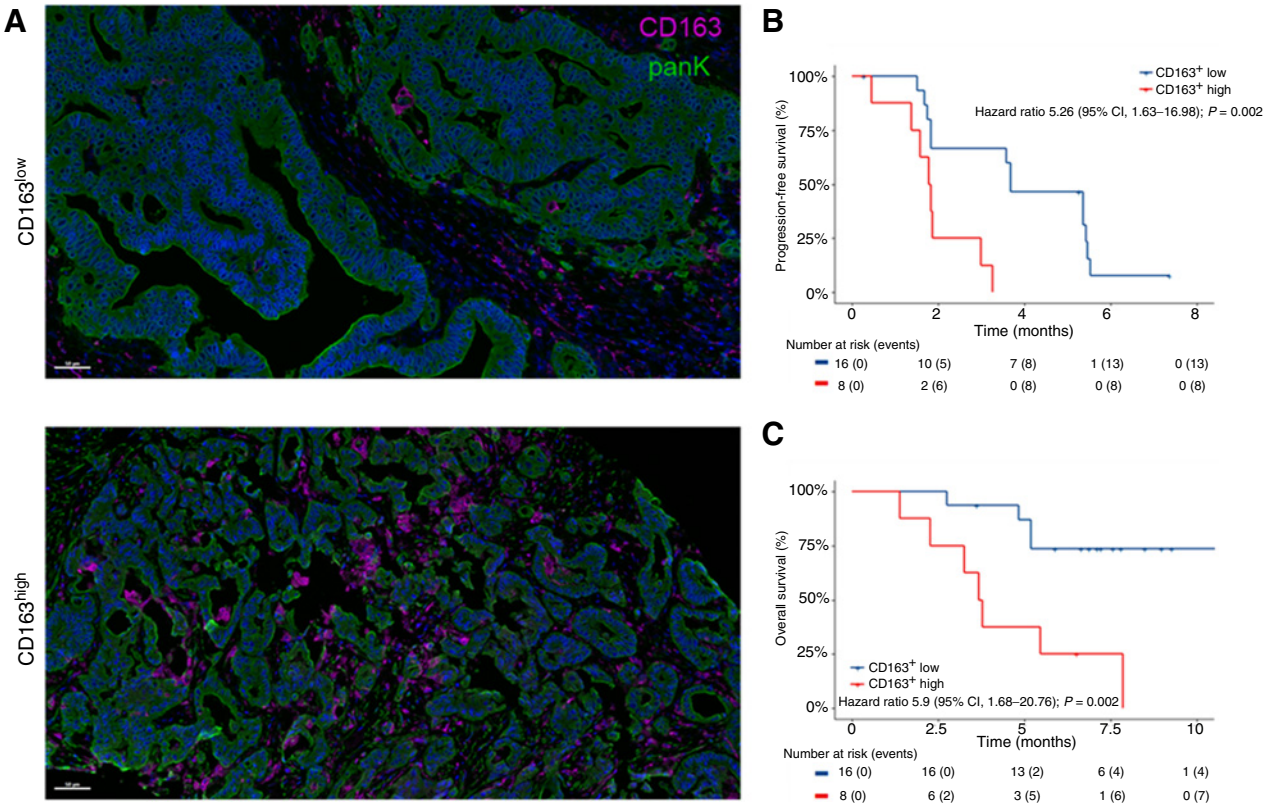

Figure 4.

Density of CD163$^+$ macrophages within the tumor is predictive of outcome in patients with cancer treated with regorafenib and avelumab. **A,** The presence of CD163$^+$ macrophages within the tumor was calculated in all tumor samples collected before treatment initiation and was based on a multiplexed immune-histofluorescence staining combining panKeratin (panK, green) and CD163 (purple) markers. Slide digitization and image analysis were then performed to calculate CD163$^+$ cells density within the tumor lesion. Image on top illustrates a patient displaying a low level of CD163 infiltration, while the image at the bottom is representative of a case with high CD163$^+$ cells density. Kaplan–Meier curves of PFS (**B**) and OS (**C**) in a cohort of 24 patients with MSS colorectal cancer treated with regorafenib and avelumab according to the density of intratumoral CD163$^+$ macrophages.

safety profile observed in our study did not show any signal in favor of an increase of toxicity of regorafenib or avelumab in comparison with their use as single agent. The optimal dose of antiangiogenic agent to combine with immune checkpoint inhibition remains a question of debate. Indeed, inhibiting the VEGF-mediated immunosuppression and angiogenesis may be potentially achieved by using alternative strategies. One of them could be careful calibration of VEGF inhibition to inhibit angiogenesis while avoiding deleterious pruning and hypoxia (28). Recent studies performed in mouse models have shown that the impact of angiogenesis inhibition on tumor microenvironment is correlated with the degree of this inhibition. For instance, low dose of anti-VEGFR2 antibody or of TNFα was associated with increased effector T-cell infiltration and a more immune stimulatory (M1) macrophage phenotype compared with higher doses of these agents (29, 30). Interestingly, a recent open-label trial which included 116 evaluable patients with metastatic colorectal cancer showed that a regorafenib dose-escalation strategy (starting dose 80 mg/day with weekly escalation in a 40 mg increment to 160 mg/day if no significant drug-related adverse events occurred) may represent an alternative approach for optimizing regorafenib dosing with comparable activity and lower incidence of adverse events, in comparison with a standard dose strategy (160 mg/day) for 21 days of a 28-day cycle (31).

Overall, our results showing modest efficacy indicate that further efforts are needed to establish successful immunotherapy strategies for a devastating disease that causes more than one million deaths each year worldwide. A new cohort in the REGOMUNE study will explore the combination of regorafenib with avelumab in patients with colorectal cancer selected on the basis of the tumor-associated macrophages infiltration level.

Authors' Disclosures

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Disclaimer

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Authors' Contributions

S. Cousin: Data curation, investigation, writing–original draft, writing–review and editing. **C. Cantarel:** Data curation, formal analysis, writing–review and editing. **J.-P. Guegan:** Data curation, formal analysis, writing–review and editing. **C. Gomez-Roca:** Investigation, writing–review and editing. **J.-P. Metges:** Investigation. **A. Adenis:** Investigation, writing–review and editing. **S. Pernot:** Investigation, writing–review and editing. **C. Bellera:** Conceptualization, formal analysis, methodology, writing–review and editing. **M. Kind:** Data curation, formal analysis, writing–review and editing. **C. Auzanneau:** Resources. **F. Le Loarer:** Resources. **I. Soubeyran:** Resources, formal analysis. **A. Bessede:** Conceptualization, formal analysis, validation, methodology, writing–review and editing. **A. Italiano:** Conceptualization, data curation, supervision, funding acquisition, investigation, methodology, writing–original draft, project administration, writing–review and editing.

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References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018;68:394–424.
2. Benson AB, Venook AP, Al-Hawary MM, Cederquist L, Chen Y-J, Ciombor KK, et al. NCCN guidelines insights: colon cancer, version 2.2018. J Natl Compr Canc Netw 2018;16:359–69.
3. Grothey A, Van Cutsem E, Sobrero A, Siena S, Falcone A, Ychou M, et al. Regorafenib monotherapy for previously treated metastatic colorectal cancer (CORRECT): an international, multicentre, randomised, placebo-controlled, phase 3 trial. Lancet 2013;381:303–12.
4. Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, et al. PD-1 blockade in tumors with mismatch-repair deficiency. N Engl J Med 2015;372:2509–20.
5. Andre T, Shiu K-K, Kim TW, Jensen BW, Jensen LH, Punt CJA, et al. Pembrolizumab versus chemotherapy for microsatellite instability-high/mismatch repair deficient metastatic colorectal cancer: the phase 3 KEYNOTE-177 study. J Clin Oncol 38:18s, 2020 (suppl; abstr LBA4).
6. Yi M, Jiao D, Qin S, Chu Q, Wu K, Li A. Synergistic effect of immune checkpoint blockade and anti-angiogenesis in cancer treatment. Mol Cancer 2019;18:60.
7. Rahma OE, Hodi FS. The intersection between tumor angiogenesis and immune suppression. Clin Cancer Res 2019;25:5449–57.
8. Terme M, Pernot S, Marcheteau E, Sandoval F, Benhamouda N, Colussi O, et al. VEGFA-VEGFR pathway blockade inhibits tumor-induced regulatory T-cell proliferation in colorectal cancer. Cancer Res 2013;73:539–49.
9. Voron T, Colussi O, Marcheteau E, Pernot S, Nizard M, Pointet A-L, et al. VEGF-A modulates expression of inhibitory checkpoints on CD8+ T cells in tumors. J Exp Med 2015;212:139–48.
10. Eso Y, Marusawa H. Novel approaches for molecular targeted therapy against hepatocellular carcinoma. Hepatol Res 2018;48:597–607.
11. Abou-Elkacem L, Arns S, Brix G, Gremse F, Zopf D, Kiessling F, et al. Regorafenib inhibits growth, angiogenesis, and metastasis in a highly aggressive, orthotopic colon cancer model. Mol Cancer Ther 2013;12:1322–31.
12. Hoff S, Grünewald S, Röse L, Zopf D. Immunomodulation by regorafenib alone and in combination with anti PD1 antibody on murine models of colorectal cancer. Ann Oncol 2017;28:v403–27.
13. Motzer RJ, Penkov K, Haanen J, Rini B, Albiges L, Campbell MT, et al. Avelumab plus axitinib versus sunitinib for advanced renal-cell carcinoma. N Engl J Med 2019;380:1103–15.
14. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer 2009;45:228–47.
15. Zohar S, Teramukai S, Zhou Y. Bayesian design and conduct of phase II single-arm clinical trials with binary outcomes: a tutorial. Contemp Clin Trials 2008;29:608–16.
16. Italiano A, Mir O, Mathoulin-Pelissier S, Penel N, Piperno-Neumann S, Bompas E, et al. Cabozantinib in patients with advanced Ewing sarcoma or osteosarcoma (CABONE): a multicentre, single-arm, phase 2 trial. Lancet Oncol 2020;21:446–55.
17. Alborelli I, Leonards K, Rothschild SI, Leuenberger LP, Savic Prince S, Mertz KD, et al. Tumor mutational burden assessed by targeted NGS predicts clinical benefit from immune checkpoint inhibitors in non-small cell lung cancer. J Pathol 2020;250:19–29.
18. Rini BI, Plimack ER, Stus V, Gafanov R, Hawkins R, Nosov D, et al. Pembrolizumab plus axitinib versus sunitinib for advanced renal-cell carcinoma. N Engl J Med 2019;380:1116–27.
19. Yahaya MAF, Lila MAM, Ismail S, Zainol M, Afizan N. Tumour-associated macrophages (TAMs) in colon cancer and how to reeducate them. J Immunol Res 2019;2019:2368249.
20. Smith BD, Leary CB, Lu W-P, Kaufman MD, Flynn DL. The highly specific CSF1R inhibitor DCC-3014 exhibits immunomodulatory and anti-invasive activities in cancer models. [abstract]. In: Proceedings of the 107th Annual Meeting of the American Association for Cancer Research; 2016 Apr 16–20; New Orleans, LA. Philadelphia (PA): AACR; 2016. Abstract 4889.
21. Chalabi M, Fanchi LF, Dijkstra KK, Van den Berg JG, Aalbers AG, Sikorska K, et al. Neoadjuvant immunotherapy leads to pathological responses in MMR-proficient and MMR-deficient early-stage colon cancers. Nat Med 2020;26:566–76.
22. Gatalica Z, Snyder C, Maney T, Ghazalpour A, Holterman DA, Xiao N, et al. Programmed cell death 1 (PD-1) and its ligand (PD-L1) in common cancers and their correlation with molecular cancer type. Cancer Epidemiol Biomarkers Prev 2014;23:2965–70.
23. Zhao P, Li L, Jiang X, Li Q. Mismatch repair deficiency/microsatellite instability-high as a predictor for anti-PD-1/PD-L1 immunotherapy efficacy. J Hematol Oncol 2019;12:54.
24. Fukuoka S, Hara H, Takahashi N, Kojima T, Kawazoe A, Asayama M, et al. Regorafenib plus nivolumab in patients with advanced gastric or colorectal cancer: an open-label, dose-escalation, and dose-expansion phase Ib trial (REGONIVO, EPOC1603). J Clin Oncol 2020;38:2053–61.
25. Lee J, Mehdizadeh S, Tsai K, Algazi A, Rosenblum M, Daud A, et al. Immunological insights into liver metastasis associated resistance to checkpoint blockade cancer immunotherapy. J Immunol 2018;200:122.26.
26. Brodt P. Role of the microenvironment in liver metastasis: from pre- to prometastatic niches. Clin Cancer Res 2016;22:5971–82.
27. Li J, Qin S, Xu R, Yau TC, Ma B, Pan H, et al. Regorafenib plus best supportive care versus placebo plus best supportive care in Asian patients with previously treated metastatic colorectal cancer (CONCUR): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet Oncol 2015;16:619–29.
28. Hato T, Zhu AX, Duda DG. Rationally combining anti-VEGF therapy with checkpoint inhibitors in hepatocellular carcinoma. Immunotherapy 2016;8:299–313.
29. Huang Y, Yuan J, Righi E, Kamoun WS, Ancukiewicz M, Nezivar J, et al. Vascular normalizing doses of antiangiogenic treatment reprogram the immunosuppressive tumor microenvironment and enhance immunotherapy. Proc Natl Acad Sci U S A 2012;109:17561–66.
30. Johansson A, Hamzah J, Payne CJ, Ganss R. Tumor-targeted TNFalpha stabilizes tumor vessels and enhances active immunotherapy. Proc Natl Acad Sci U S A 2012;109:7841–46.
31. Bekaii-Saab TS, Ou FS, Ahn DH, Boland PM, Ciombor KK, Heying EN, et al. Regorafenib dose-optimisation in patients with refractory metastatic colorectal cancer (ReDOS): a randomised, multicentre, open-label, phase 2 study. Lancet Oncol 2019;20:1070–82.

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Regorafenib-Avelumab Combination in Patients with Microsatellite Stable Colorectal Cancer (REGOMUNE): A Single-arm, Open-label, Phase II Trial

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