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Safety and Efficacy of Low-Dose Regorafenib Combined with Nivolumab in Patients with Advanced HCC in Progression Beyond Two or More Lines of Tyrosine Kinase Inhibitors: Case Reports and Short Review of the Literature

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ABSTRACT

Background and Aims: A few clinical studies have suggested that low doses of anti-angiogenic molecules might enhance the therapeutic effects of anti-PD1 in HCC and may improve their tolerance. Here, we present case reports of patients treated with low-dose regorafenib combined with nivolumab after the failure of at least two lines of oral chemotherapy.

Design: We report 12 observations of patients with advanced HCC in progression after at least two lines of tyrosine kinase inhibitor (TKI) therapy who received regorafenib (starting with 80 mg/day) combined with nivolumab (3 mg/kg/14 days/IV) until progression or unacceptable toxicity.The safety based on NCI-CTCAE 4 and the clinical efficacy (progression free survival, overall response rate, disease control rate, time to treatment response and median duration of response) were retropectively assessed.

Results: Median age was 68 years (IQR [64; 71.5]), and 75% had BCLC-C. The median follow-up was 18.8 months (IQR [11.5; 23.4]) and the median duration of treatment was 10 months (IQR [3.8 ;17]). Because of the frequent early adverse events associated with regorafenib, this treatment remained at an initial dose of 80 mg/day in all patients. Of note, most grade III adverse events (AEs) occurred later after a median duration of combined treatment of 10.5 months (IQR), were also mainly due to regorafenib, but allowed the pursuit of nivolumab alone [5,11,5]. The therapeutic combination led to a partial response in three patients (30 %) and a stable disease in two patients (20 %), corresponding to a disease control rate of 50% at 12 months in the 10 patients who were trated more than 2 months. The median TTR was 2 months (IQR) [1-9]. The median duration of disease control and response was 22 (IQR [21; 24]) and 24 months (IQR), respectively [23-25].

Conclusion: Combination of low-dose regorafenib and nivolumab might be associated with an interesting disease control rate and manageable safety profile in patients with progressive advanced HCC beyond the second-line treatment with tyrosine kinase inhibitors.

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Introduction

Hepatocellular carcinoma (HCC) is the sixth most common cancer worldwide, with 782 000 new cases/year [1,2]. For several years, systemic treatment of advanced or metastatic HCC has been based on the use of tyrosine kinase inhibitors (TKI) [3-5]. Immunotherapy alone using nivolumab and pembrolizumab was assessed with promising results in phase I/II but failed to show a benefit in terms

of overall survival in the phase III trials CHECKMATE 459 and KEYNOTE 240, respectively [6,7]. Only a minority of patients are initially responsive to immune checkpoint blockade, partly because of the low number of infiltrating lymphocytes in the tumor microenvironment . Furthermore, preclinical studies have shown that in addition to their antitumor effects, anti-angiogenic drugs can reprogram the tumor bed from an immunosuppressive to an immunosuppressive microenvironment [8-10]. Moreover, a few clinical studies have suggested that even a low dose of oral anti-angiogenic molecule (TKI) might have immunomodulatory activity and enhance the effects of anti-PD1 [10-12]. Regorafenib, an oral multi-kinase inhibitor which blocks the activity of protein kinases involved in angiogenesis, oncogenesis, metastasis and tumour immunity (through modification on the tumor microenvironment) has been approved in second line of the treatment of HCC after failure of sorafenib (RESORCE) [13]. It has a distinct molecular target profile and has more potent pharmacological molecular effects than sorafenib against the following receptors: VEGFR, which inhibits JAK1/2-STAT1 and MAPK signaling, which could increase PDL1 expression in tumors and increase intratumoral CD8+ T-cell infiltration by normalizing the tumor vasculature and improving the efficacy of the PD-1 antibody [1-3]. Nivolumab is an IgG4 monoclonal human antibody directed against programmed cells death 1 (PD-1) receptor checkpoint inhibitor, a cell surface protein encoded by the PDCD1 gene and mainly expressed on the surface of activated T cells [7-14].

We report here 12 patients with advanced HCC in progression after > 2 lines of tyrosine kinase inhibitor and who were not eligible in France before December 2021 to the combination of atezolizumab and bevacizumab and who had the possibility to receive regorafenib combined with nivolumab in the absence of validated treatment in 3rd and 4th line and because of good performance status and no liver failure.

Methods

Patient Population

The clinical data of 12 patients with advanced HCC (B or C of the Barcelona Clinic Liver Cancer Classification BCLC) in progression after two or more lines of tyrosine kinase inhibitors (TKI) such as sorafenib, regorafenib, cabozantinib, and lenvatinib were retrospectively analyzed. HCC was diagnosed according to the diagnostic criteria of the American Association for the Study of Liver Disease (15,16). The clinical characteristics of the 12 patients are summarized in Table 1. The Eastern Cooperative Oncology Group Performance Status (ECOG PFS) was 0-2 and liver function was preserved (Child-Pugh score < B7 and MELD < 10). All patients presented with tumor progression after at least two previous lines of TKI alone (including three patients treated with cabozantib) and thus were not eligible to receive the combination of atezolizumab and bevacizumab in France at that time.

Table 1: Demographic and clinical characteristics of patients receiving Regorafenib Plus Nivolumab at baseline

	HCC n=12
Median year (IQR)	68 (64;71.5)
Range year <65 > 65	 25% 75%
sex -no.(%) Male Female	 75% 25%
ECOG performance status score-no (%) 0 1 2	 67% 25% 8%
Chronic liver disease, no(%) Cirrhosis	92% (n=11) 55% (n=6)
Child Pugh classification-no./ total no (%) n=6 A5 A6	 5 (83%) 1 (17%)
Etiology (n=11) Viral Non-viral	 36.5% 63.5%
Bodyweight, Kg <65 >65	 33% 67%
ALBI range at baseline	-2.62 [-2.8;2.17]
ALBI score at baseline 1 2 3	 50% (n=6) 34% (n=4) 16 (n=2)
Biochemical parameters at baseline Albumin (µmol/l) Bilirubin (µmol/l) Platelets (Ga/l)	 39.5[37.3;40] 14.3[10.4;21] 176[133;247]
Barcelona clinic cancer stage (%) B C	 25% 75%
Serum Alpha-foetoprotein level, ng/ml, no(%) <400 >400	 67% 33%
Unilobar/Bilobar	0% / 100%
Number of nodules < 3 >3	 34% (n=4) 66% (n=8)
Maximal size (cm) < 3 3< …< 5 > 5	 25% (n=3) 34% (n=4) 16% (n=2)
Presence of macrovascular invasion (%) Macroscopic portal vein invasion	 8% (n=1)
Extrahepatic sites, no (%) n=8 1 2 >3	 50% (n=4) 37.5% (n=3) 12.5% (n=1)

Macroscopic portal vein invasion, extrahepatic spread or both, no (%	75 % (n=9)
Prior local therapy for hepatocellular carcinoma (before TKI) - no.(%) Surgery Radio frequency Radiothérapy Chemoembolisation Radioembolization	 75% 17% 0% 50% 0%
Number lines of previous systemic TKI treatment no (%) 2 >3	 67% 33%
Systemic TKI treatment received (%) sorafenib lenvatinib regorafenib cabozantinib	 92% 25% 84% 25%
Median time to progression under 1st line (months) IQR	7(4.5;7)
Median time to progression under 2nd line (months) IQR	8(5;12)
Median time to progression under 3rd line (months) IQR	6(5;8)

Treatment

These patients received a combination of regorafenib (starting at 80 mg/day) and nivolumab. The combination of low-dose regorafenib and nivolumab was based on previous clinical trial data to improve the tolerance of the combination, considering the stage of the tumoral disease and liver function. This proposition was validated by two multidisciplinary expert meetings, and written informed consent was obtained from all patients in the absence of validated therapeutic alternatives. All of them, except one, had previously received regorafenib as a single agent at 160 mg/day with a relatively poor tolerance. Five patients were still receiving regorafenib and presented with tumor progression before nivolumab administration. Briefly, regorafenib was started, re-started, or maintained in respectively one, six and five patients, at a reduced dose of 80 mg/day to optimize tolerance and was combined with nivolumab at 3 mg/kg/14days/IV until progression and/or toxicity. End Points and Clinical Assessments.

Treatment safety was assessed according to the Common Terminology Criteria for Adverse Events (NCI-CTCAE 4). Efficacy was assessed by overall survival, progression-free survival (PFS), objective response rate (ORR), time to response (TTR), disease control rate (DCR), and duration of response (DOR) in patients who remained under the combined treatment for at least 2 months. A CT scan was performed every two months under the combined treatment until disease progression or unacceptable toxicity. The tumor response was evaluated using mRECIST and RECIST version 1.1. respectively. Duration of response (DOR) was defined as the time between the initial response to therapy and subsequent disease progression.

Pathological and Immunohistochemical Analysis of Tumoral Tissue

The Immunohistochemistry was performed on serial paraffin sections after deparaffinization and antigen restoration by heating in citrate buffer pH6 using a Bond Max automat (Leica Biosystems) based on the labeled streptavidin-biotin method (LSAB). The antibodies used were CD20 (B lymphocytes), CD3 (T lymphocytes), CD4 (helper T cells), CD8 (cytotoxic T cells), CD57 (natural killer cells), CD138 (plasma cells), CD163 (macrophages), anti-PD1 (NAT105, Abcam), and anti-PD-L1 (clone E1L3N, Cell Signaling Technology). The immunohistochemical staining results were analyzed by two observers on digital slides. Semi-quantitative scoring was performed to evaluate the relative percentage of CD20-, CD3-, CD57-, CD138 and CD163 positive-cells of intra- and peritumoral immune cells (total = 100%). Intra-tumoral CD3-positive cells (T lymphocytes) and CD8-positive cells (cytotoxic T cells) were quantified more precisely using the QuPath software platform (17). The results are expressed as the number of cells per 100 tumor cells. CD8/CD3 ratio was calculated as the ratio of CD8/100 tumor cells to CD3/100 tumor cells. PD-L1 expression was assessed in both tumor and intratumoral immune cells. Tumor cell positivity was defined as partial or complete membrane staining. Results were expressed as the tumor proportion score (TPS) corresponding to the percentage of viable tumor cells positive for PD-L1, and the combined positive score (CPS) as the number of PD-L1 positive cells (tumor cells, lymphocytes, macrophages)/ total number of viable tumor cells x100)(18). Aggregates of PD-L1 + macrophages were counted per mm2. PD1 expression in the immune cells was semi-quantitatively assessed (0 ++).

Statistical Analysis

Qualitative variables were described as numbers and percentages and quantitative variables as medians with ranges. Statistical analysis was intended for treatment. Statistical analysis was performed using the open access website BiostaTGV (Institut Pierre Louis UMR S 1136), which uses the free R software. Fisher's exact test was used to compare tumor subtypes, and Student's t-test was used to compare immune cell infiltrates.

Results

Baseline Characteristics

The main patient characteristics are summarized in Table 1. All patients were classed within the Child-Pugh score A5-A6, and most of them were also classified ALBI score 1 (50%) or 2 (34 %). In addition, most of these patients presented with an HCC with relatively aggressive tumors (maximal size > 30 mm in 50 %, MVI in 8 %, extrahepatic disease or portal vein invasion 75 %, AFP > 400 ng/ml in 33 %) The median follow-up was 18.8 months (IQR [11.5; 23.4]) and the median duration of treatment was 10 months (IQR) [3,8,17]. Of note, 10/12 patients received the combined treatment for at least 2 months.

Safety

All patients receiving the therapeutic combination presented early adverse events (AEs), mainly grades I-II (Table 2, 3). These were mostly related to regorafenib and were initially easily manageable without discontinuation or dose adjustment. The most common AEs were elevation of transaminase activity from 1,5 upper limit of the normal range (Uln) to 5 Uln in 13%, hand-foot skin reaction in 11,5%, proteinuria in 10%, fatigue in 7%, thyroid dysfunction in 5%, and arterial hypertension in 4% of patients. This observation has justified to maintain an initial dose of 80 mg/day for regorafenib treatment. Serious grade III AEs related to regorafenib (15%) (i.e., proteinuria, arthralgia, hand-foot skin reaction, hepatic cytolysis) occurred after a median time of 10.5 months under the combined treatment in five patients (IQR) [5,11,5]. Regorafenib was definitively discontinued in 4 out of these 5 patients and the nivolumab was continued alone for a

median time of 11 months after regorafenib withdrawal in one patient (IQR) [5,5,12,4]. Adverse events related to nivolumab occurred in three patients (25%) after a median time of 1 month (IQR) [1,3] one patient developed grade III cytolysis related to immune-related hepatitis proven by liver biopsy, a second patient had grade III rhizomelic pseudo-polyarthritis and a third patient had grade III hypophysitis. All the patients recovered completely after transient corticosteroid treatment. Two of them discontinued; therefore, early and definitively, both regorafenib and nivolumab and one remained even under immunotherapy alone together with oral hydrocortisone because of the controlled hypothysitis. No grade IV or V AEs related to regorafenib or nivolumab were observed. Fifty percent of patients (n=6) had cirrhosis with very good liver function before treatment (i.e. Child Pugh score A) and the ALBI score was 2 in 34 % and 3 in 16 % (Table 1). Jaundice occurred in 16% of patients (n=2) but no ascites, encephalopathy or gastrointestinal hemorrhage was reported. In addition, no treatment was discontinued owing to hepatic decompensation. Finally, a slight change in the ALBI score under combined treatment was observed in 50% of patients (n=6) : Four patients moved from Albi 1 to 2, one from Albi 2 to 3 and one from Albi 2 to Albi 1.

Table 2: Adverse Events

Tolerance in all patients	HCC n=12
Patients with an adverse event from any cause	100 %
Grade 3 ou 4 event related to regorafenib related to nivolumab	19% 70% 30%
Median time to grade 3 AEs related to regorafenib related to nivolumab	 10 ([5;11.5]) 2 ([2;4.5]
Grade 4-5 events	0
Adverse event leading to withdrawal regorafenib - nivolumab resumption of treatment	 25% (n=3) 33% (n=1)
Adverse event leading to dose modification or interruption of any drug dose interruption of any treatment dose interruption of regorafenib	 72% (9) 58% (7)

Table 3: Most commun Treatment-related AEs with Regorafenib Plus Nivolumab (n=12)

Type of AEs	Any grade	Grade 1	Grade 2	Grade 3
Hypertension	2	1	1	0
Diarrhea	2	1	1	0
constipation	1	1	0	0
nausea	1	1	0	0
Fatigue	4	1	3	0
Biological cardiac disorder	4	3	1	0
Decreased appetite	1	0	1	0
Thyroïdis disorders	3	3	0	0
Hand foot skin reaction	6	2	3	1
Weight decrease	2	1	1	0
AST increased	7	4	1	2
Hepatitis	1	0	0	1
Rheumatism arthritis	2	0	1	1
Proteinuria	5	0	1	4
Rash	2	1	1	0
Pruritus	2	2	0	0
Abdominal pain	1	1	0	0
Alopecia	1	0	0	1
neutropenia	1	1	0	0
hypophysitis	1	0	0	1
Arteritis	1	0	0	1
Hepatic decompensation	2	0	2	0

Clinical Efficacy

Twelve patients were treated with regorafenib and nivolumab; however, only 10 patients received treatment for at least 2 months. The 2 other patients presented with severe AEs related to nivolumab after only two perfusions (i.e., hepatitis and PPR) (Figure 1). Overall survival and progression-free survival were 92%, 84%, and 66% at 3, 6, and 12 months, respectively. Before the discontinuation of regorafenib (after a median time of 10.5 months), the DCR was 50% (with an objective partial response rate of 30% and 20% of stable disease) (Figure 1; Table 4a, 4b). Of note, one patient had pseudo-progression after the first month of combined treatment (cycle 1) and thereafter presented a response after two months while the combined treatment was maintained because of a clinical benefit (cycle 2) (Figure 2, Iconography 1-3). At 18 months, the DCR was 30%; two patients had a partial response (iconography 1) and one patient had stable disease (Figure 3). Among them, two patients maintained a partial response to nivolumab alone and 1 had stable disease after restarting regorafenib because of progression under nivolumab alone without relapse of grade III AEs associated with regorafenib. The median time to response was 2.4 months (IQR) [3-8]. The median duration of disease control and response was 22 (IQR) and 24 months (IQR), respectively [21-25]. Seven patients (70%) progressed after a median time of four months under the combined treatment [2.5;9] and 3 patients had late progression after 9, 13, and 15 months, respectively, after regorafenib was discontinued.

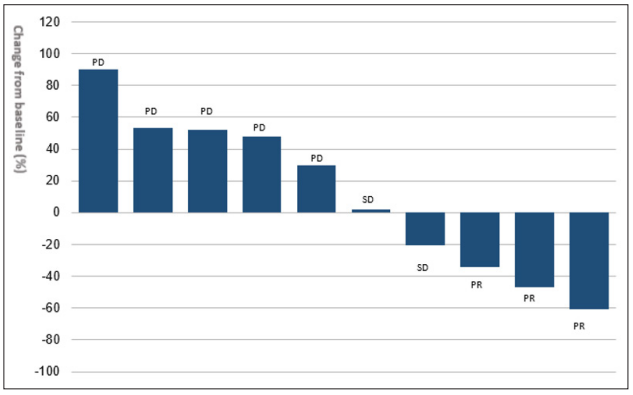


Figure 1: Percentage change from baseline in sums of diameters of target lesions by RECIST1.1 at 12 months (PD=progression disease, SD=stable disease, PR=partial response)

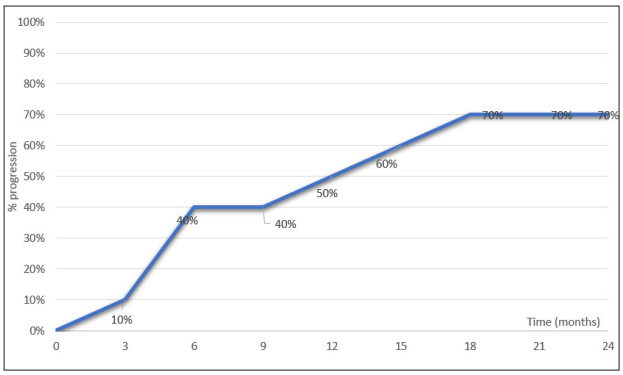


Figure 2: Progression under regorafenib + nivolumab over time (n=10)

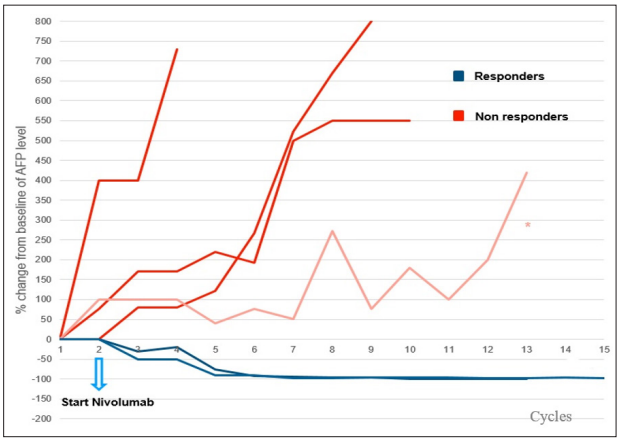


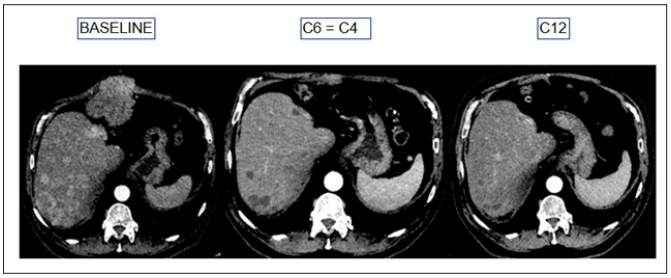
Figure 3: Percentage change from baseline of AFP level
* Delayed progression after 5 months (10 cycles) under combined treatment

Table 4a :Outcomes at 6 and 12 months

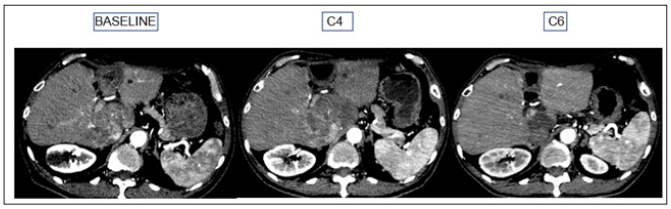
Response in patients receiving the treatment for at least 2 months (10/12)	HCC n=10 6 moths	HCC n=10 12 months
Confirmed objective response - no. (%) Complete response-no (%) Partial response - no (%)	40% 0% 40%	30% 0 % 30%
Stable disease - no (%)	20%	20%
Disease control rate - no.(%)	60%	50%
Progressive disease - no (%)	40%	50%

Table 4b: Outcomes at 18 months

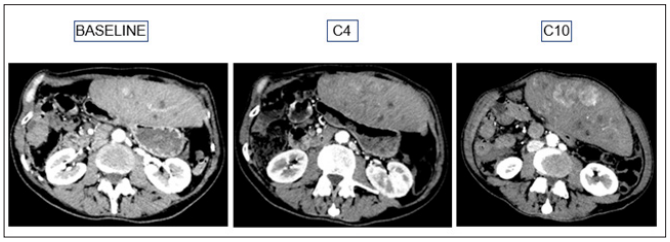
Response in all patients	HCC n=12
Confirmed objective response - no. (%) Complete response-no (%) Partial response - no (%)	20% 0 100%
Stable disease - no (%)	10%
Disease control rate - no.(%)	30%
Progressive disease - no (%)	70%
Median time to progression (months)	3 [3;9]
Median time control disease (months)	22 [21;24]
Ongoing objective response at data cut off – no(%)	30%



Iconography 1



Iconography 2



Iconography 3

Prognosis/ Predictive Factor

Six patients had a high baseline AFP level of > 400 ng/ml. Two were responders, and four progressed under combined treatment from the start. Among them, the marker decreased in two responders after cycle two of the combined treatment. In contrast, AFP levels increased in all four non-responder patients (Supplement Figure 3).

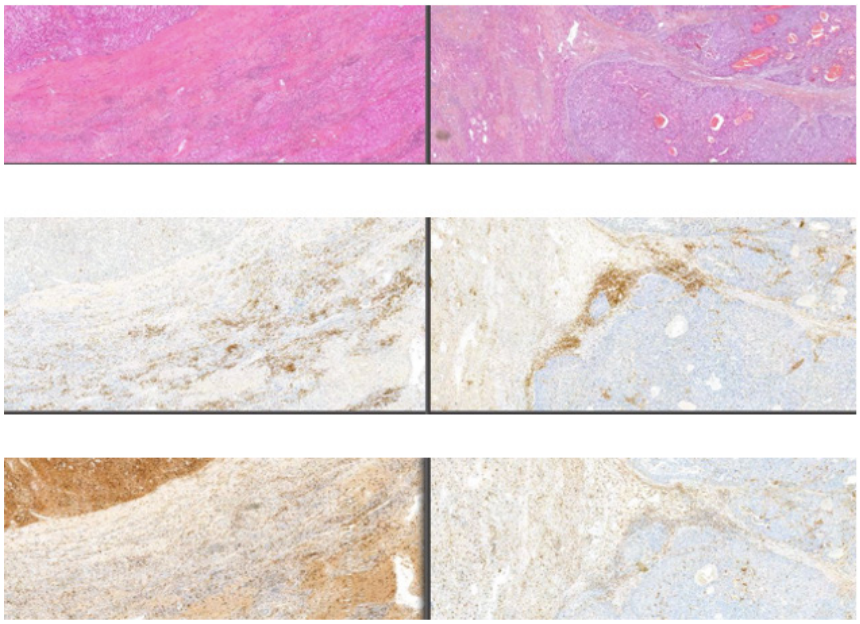
Histological and Immunohistochemical Results

Tumor specimens were available for five responders (patients 1-5) and five non-responders (patients 6–10). Among them, several (2 to 4) subsequent tumor biopsies were available in two responders and four non-responders. In all cases, the histological features of the tumors remained unchanged over time after the previous TKI treatments. Biopsies of a tumoral lesion (peritoneum = 1, liver =1) were also obtained after treatment with regorafenib and nivolumab in two patients (one responder and one non-responder). Comparative global analysis of the immune-inflammatory microenvironment between responder and non-responder patients, performed on the most recent liver biopsy specimens obtained between 22 and 108 months before the combined treatment, did not show any difference (Table 5). However, peritumoral immune cell infiltrates were more abundant in non-responders (p=0.048), with a higher proportion of CD3+ lymphocytes (p=0.03) and lower proportion of CD163+ macrophages (p=0.02). The peritoneal biopsy obtained under treatment in one of the responder patients (patient 1) showed a complete pathological response associated with a dramatically increased number of CD3 and CD8 lymphocytes within the tumor. In contrast the liver tumor biopsy obtained under treatment in one of the non-responder patients (patient 8) showed a decreased number of CD3 (but not of CD8 lymphocytes) within the tumor compared with the pre-treatment specimen (39 CD3 /6 CD8 for 100 tumor cells before treatment and 32 CD3/ 6 CD8 after treatment) together with an increased proportion of CD3 lymphocytes (60% versus 30%) in

the peritumoral infiltrates (Iconography 4).

Table 5: Summary of histological and immunohistochemical results

Tumor response	Hepatocellular carcinoma subtype	Intratumoral inflammation (semiquantitative)	Intratumoral macrophages (% of inflammatory infiltrate)	Lymphoid nodules	Intratumoral lymphocytes			PD-L1		PD-L1 positive macrophage aggregate s/mm²	PD1 (semiquantitative)	Peritumoral inflammation (semiquantitative)	Peritumoral macrophages (% of inflammatory infiltrate)	Peritumoral CD3+ lymphocytes (% of inflammatory infiltrate)	Peritumoral CD8+ lymphocytes (% of CD3+ lymphocytes)
					CD3+/100 tumor cells	CD8+/ 100 tumor cells	CD8/CD3 ratio (%)	TPS	CPS						
Responder	Conventional and steatohepatitic	++	50	presence	11.7	7.6	65	0	<1	0.12	++	+	60	38	65
Responder	Conventional	+	70	absence	11,2	3.2	29	0	2	0.4	rare	+	35	40	55
Responder	Conventional	+	95	absence	0.4	0.3	75	0	<1	0.08	0	+	50	30	70
Responder	Conventional	+	70	absence	10,6	7.9	75	0	<1	0.06	rare	+	50	35	60
Responder	Steatohepatitic	+	85	absence	3.6	0.51	14	0	3	0.13	rare	+	75	20	3
Non responder	Conventional	+	60	absence	2,1	0.7	33	0	<1	0.06	+	+	40	40	70
Non responder	Conventional	+++	30	absence	54.3	34.1	63	0	6,8	8.25	+++	++	35	60	75
Non responder	Steatohepatitic	++	50	presence	38.9	32.5	83	5	7	0.75	++	++	20	60	50
Non responder	Steatohepatitic	++	70	presence	20.4	10.4	51	0	13	0.37	++	++	40	35	60
Non responder	Steatohepatitic	+++	60	presence	3.7	2.1	57	1	2	0.47	+	++	15	50	40
	p=0,52	p=0,28	p=0,09	p=0.52	p=0.18	p=0.17	p=0.71	p=0.28	p=0.10	p=0.31		p=0,048	p=0.02	p=0.03	p=0.56
	Fisher exact test	Fisher exact test	Student *t* test	Fisher exact test	Student *t* test	Student *t* test	Student *t* test	Student *t* test	Student *t* test	Student *t* test		Fisher exact test	Student *t* test	Student *t* test	Student *t* test



Iconography 4: Responder (left) vs non responder (right)

Discussion

In these case reports, we retrospectively analyzed the clinical outcomes of heavily treated patients (> 2 lines of TKI) with tumor progression who were not eligible for atezolizumab and bevacizumab treatment. We observed encouraging results in terms of clinical efficacy and safety, with a PFS of 92%, 84%, and 66% at 3, 6, and 12 months, respectively. Anti-angiogenic drugs based on TKI and ramucirumab (an inhibitor of VEGF-R) are currently the only systemic treatments possible in HCC after failure of atezolizumab and bevacizumab [3-5,13,19-21]. Otherwise, pembrolizumab failed to show a statistical benefit compared with placebo after sorafenib treatment [6]. To date, after possible first-line treatment (atezolizumab + bevacizumab or, in some cases, sorafenib or lenvatinib), regorafenib or cabozantinib have been proposed by the European guidelines, but only cabozantinib may be used beyond the second-line treatment since the CELESTIAL trial [4,15]. However, this phase III trial that included 27% of patients after failure of two systemic TKI treatments did not show a convincing benefit of cabozantinib in third line (HR 0.9 95%CI:0.63-1.29). Three patients in our study were in this setting and presented with tumor progression under cabozantinib (second- or third line). Interestingly, one of these patients presented with an objective response during the 22 months of combined treatment.

In the absence of a randomized controlled trial after first-line treatment, real-world practice studies have suggested that TKI followed by immunotherapy may be the best therapeutic sequence in terms of PFS and OS [22–24]. In addition to its anti-tumor effects, regorafenib has pharmacological molecular effects against the VEGFR receptor and may inhibit JAK1/2-STAT1 and MAPK signaling [1-3]. These effects may increase both PDL1 expression in tumors and intratumoral CD8+ T-cell infiltration by normalizing the tumor vasculature, thereby improving the efficacy of PD-1 antibodies, such as nivolumab [12,25,26].

We report here that the combination of regorafenib at a low dose and nivolumab may have promising results, with a disease control rate at 12 months of 50% and at 18 months of 25% with 2 PR and 1 SD. More interestingly, we noted a long duration of disease control of 22 months (IQR [21;24]) and disease response at 24 months (IQR) [23- 25].To date, no controlled randomized study has reported the results and benefits of combined treatment using TKI and immunotherapy in HCC patients previously treated. A few Chinese retrospective studies have evaluated the combination treatment of regorafenib and immunotherapy (nivolumab and sintilimumab) as second-line therapy, with interesting results in terms of clinical efficacy [27,28]. For example, Huang et al. reported the safety and treatment response of regorafenib combined with sintilimab (anti-PDL1) compared to regorafenib alone, with a benefit for the combined treatment in terms of efficacy with an acceptable tolerance [29]. One hundred and thirteen patients were included (58 received sintilimab plus regorafenib and 55 received regorafenib alone) with better OS (13.4 vs 9.9 months), ORR (36.2% vs. 16.4%), and PFS (5.6 vs 4 months) in the combination group receiving sintilimab + regorafenib. Another retrospective study also suggested a better ORR and DCR with regorafenib combined with ICIs compared to regorafenib alone.

In addition, small retrospective studies have suggested that sub-maximal doses of anti-angiogenic drugs could indeed enhance the effect of immunotherapy by modifying the microenvironment [8]. Furthermore, in a multicenter retrospective study conducted by Huang et al., the mean daily regorafenib dose was 109 mg/day [29]. Moreover, in the phase Ib trial in patients with MSS gastric and colorectal cancer, a lower dose of regorafenib (80 mg versus 120 mg/day) combined with nivolumab showed better tolerability and less discontinuation or dose adjustment without impairment of the response rate [30]. Finally, other combination of immunotherapy with regorafenib also confirmed a better tolerability with a low dose of this drug (80 mg/day) without decrease of efficacy compared with higher dose of regorafenib (120 or 160 mg/day) [31,32]. Unfortunately, the final results of the efficacy of the GOING study are still pending and urgently needed to precisely determine the impact of regorafenib dose adjustment in terms of efficacy in this setting [33].

The combination of low-dose regorafenib and nivolumab appeared to be safe with mostly grade I-II AEs related to regorafenib, similar to each of these drugs used alone (CHECKMATE and RESORCE), and was easy to manage without dose adjustment. Preliminary data in the GOING study combining regorafenib and nivolumab also reported a manageable safety profile when used as second-line treatment after progression under TKI (sorafenib or after atezolizumab plus bevacizumab). However, 30 % of grade III -IV AEs probably due to the high dose of regorafenib (160 mg/day) with discontinuation of the treatment in 2% patients because of AEs related to the treatment (GOING study BCLC group). Finally, a recent review by Song et al. confirmed a good safety profile for several combinations of ICIs and anti-angiogenic drugs used in different cancers (34).Here, 36% of patients (n=4) treated with the combination had cirrhosis with Child-Pugh A5 in three patients and A6 in one patient. Among them, we did not report more cases of hepatic decompensation due to the combination in favor of a good safety profile. Two patients discontinued nivolumab after one month because of grade 3 immune-related AEs. Five patients discontinued regorafenib after a median duration of 10.5 months (IQR) because of severe disease with complete reversibility [5,11,5]. Subsequently, nivolumab was continued alone: 3 of these 5 patients had progression and 2 patients had a partial response after a median time of nivolumab alone for 13 months (IQR) [12,5,13,5]. One of these three patients could restart regorafenib treatment after a discontinuation of 3 months without relapse of AEs associated with regorafenib, and he presented with stabilization of the tumor (for up to 20 months). These results strongly suggest a synergistic effect between regorafenib and ICI. This hypothesis was supported by a recent review by Song et al., which summarized the understanding and clinical development of combination therapy with immune checkpoint inhibitors and anti-angiogenic strategies [34]. In particular, the mechanisms may include the enrichment of antigen-presenting cells in the tumor microenvironment, together with a safety profile and efficacy depending on the dose and the selection of the anti-angiogenic molecule.

AFP > 400 ng/ml, tumor burden, portal invasion, ALBI score, and Child Pugh B are reported to be negative prognostic factors for response to immunotherapy and/or TKI in HCC [19,35]. Encouraging results were observed, although most of the tumors had an aggressive pattern in the present study (Table 1: bilobar HCC in 100 %, > 4 HCC lesions in 66 %, size > 50 mm in 50 %, and extrahepatic disease in 66 %). In addition, 42% (n=5) had a high level of AFP (> 400ng/ml) and a decrease in serum AFP > 50%, which was not observed with regorafenib alone but only with the combined treatment, was associated with an objective tumor response in our patients. Unfortunately, no histological biomarkers usually considered to be predictive of a response to immunotherapy were observed in our patients (i.e., high expression of PD-1 and PD-L1, active interferon gamma signaling, and high infiltration of immune cells). The absence of predictive factors in this study is certainly due to the small number of patients. However, we observed that a high number of peritumoral immune cells with an increased proportion of CD3+ lymphocytes were present in all non-responders but not in the responders, suggesting that these peritumoral lymphocytes are probably unable to penetrate the tumoral tissue. Interestingly and according to this observation, a tumor infiltration with CD3+ lymphocytes has been validated as a pejorative prognostic factor of response to immunotherapy in other cancers and has been included in the colorectal cancer immune-score [36].

Conclusion

This observation suggests that the combination of regorafenib and nivolumab may offer an interesting and prolonged disease control rate with good tolerance in patients with progressive HCC after more than two lines of oral chemotherapy and who were not eligible for the combination of atezolizumab and bevacizumab. Finally, these results merit further validation in prospective randomized studies with more precise identification of histo-immunological factors predictive of the response to this combined treatment.

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Conflict of Interest
BAYER

References

1. Konyn P, Ahmed A, Kim D (2021) Current epidemiology in hepatocellular carcinoma. Expert Rev Gastroenterol Hepatol 15: 1295-307.
2. McGlynn KA, Petrick JL, El-Serag HB (2021) Epidemiology of Hepatocellular Carcinoma. Hepatology 73: 4-13.
3. Kudo M, Finn RS, Qin S, Han KH, Ikeda K, et al. (2018) Lenvatinib versus sorafenib in first-line treatment of patients with unresectable hepatocellular carcinoma: a randomised phase 3 non-inferiority trial. The Lancet 391: 1163-1173.
4. Abou-Alfa GK, Meyer T, Cheng AL, El-Khoueiry AB, Rimassa L, et al. (2018) Cabozantinib in Patients with Advanced and Progressing Hepatocellular Carcinoma. N Engl J Med 379: 54-63.
5. Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, et al. (2008) Sorafenib in Advanced Hepatocellular Carcinoma. N Engl J Med 359: 378-390.
6. Zhu AX, Finn RS, Edeline J, Cattan S, Ogasawara S, et al. (2018) Pembrolizumab in patients with advanced hepatocellular carcinoma previously treated with sorafenib (KEYNOTE-224): a non-randomised, open-label phase 2 trial. Lancet Oncol 19: 940-952.
7. Yau T, Park JW, Finn RS, Cheng AL, Mathurin P, et al. (2022) Nivolumab versus sorafenib in advanced hepatocellular carcinoma (CheckMate 459): a randomised, multicentre, open-label, phase 3 trial. Lancet Oncol janv 23: 77-90.
8. Huang Y, Yuan J, Righi E, Kamoun WS, Ancukiewicz M, et al. (2012) Vascular normalizing doses of antiangiogenic treatment reprogram the immunosuppressive tumor microenvironment and enhance immunotherapy. Proc Natl Acad Sci 109: 17561-17566.
9. Voron T, Colussi O, Marcheteau E, Pernot S, Nizard M, et al. (2015) VEGF-A modulates expression of inhibitory checkpoints on CD8+ T cells in tumors. J Exp Med 212: 139-148.
10. Scirocchi F, Napoletano C, Pace A, Rahimi Koshkaki H, Di Filippo A, et al. (2021) Immunogenic Cell Death and Immunomodulatory Effects of Cabozantinib. Front Oncol 11: 755433.
11. Zhao S, Ren S, Jiang T, Zhu B, Li X, et al. (2019) Low-Dose Apatinib Optimizes Tumor Microenvironment and Potentiates Antitumor Effect of PD-1/PD-L1 Blockade in Lung Cancer. Cancer Immunol Res 7: 630-643.
12. Liu J, Tao H, Yuan T, Li J, Li J, et al. (2022) Immunomodulatory effects of regorafenib: Enhancing the efficacy of anti-PD-1/PD-L1 therapy. Front Immunol 13: 992611.
13. Bruix J, Qin S, Merle P, Granito A, Huang YH, et al. (2017) Regorafenib for patients with hepatocellular carcinoma who progressed on sorafenib treatment (RESORCE): a randomised, double-blind, placebo-controlled, phase 3 trial. The Lancet 389: 56-66.
14. El-Khoueiry AB, Sangro B, Yau T, Crocenzi TS, Kudo M, et al. (2017) Nivolumab in patients with advanced hepatocellular carcinoma (CheckMate 040): an open-label, non-comparative, phase 1/2 dose escalation and expansion trial. Lancet Lond Engl 389: 2492-2502.
15. Galle PR, Forner A, Llovet JM, Mazzaferro V, Piscaglia F, et al. (2018) EASL Clinical Practice Guidelines: Management of hepatocellular carcinoma. J Hepatol. juill 69: 182-236.
16. Singal AG, Llovet JM, Yarchoan M, Mehta N, Heimbach JK, et al. (2023) AASLD Practice Guidance on prevention, diagnosis, and treatment of hepatocellular carcinoma. Hepatology 78: 1922.
17. Bankhead P, Loughrey MB, Fernández JA, Dombrowski Y, McArt DG, et al. (2017) QuPath: Open source software for digital pathology image analysis. Sci Rep 7: 16878.
18. de Ruiter EJ, Mulder FJ, Koomen BM, Speel EJ, van den Hout MFCM, et al. (2021) Comparison of three PD-L1 immunohistochemical assays in head and neck squamous cell carcinoma (HNSCC). Mod Pathol. juin 34: 1125-1132.
19. Reig M, Forner A, Rimola J, Ferrer-Fàbrega J, Burrel M, et al. (2022) BCLC strategy for prognosis prediction and treatment recommendation: The 2022 update. J Hepatol mars 76: 681-693.
20. Zhu AX, Kang YK, Yen CJ, Finn RS, Galle PR, et al. (2019) Ramucirumab after sorafenib in patients with advanced hepatocellular carcinoma and increased α-fetoprotein concentrations (REACH-2): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet Oncol 20: 282-296.
21. Finn RS, Qin S, Ikeda M, Galle PR, Ducreux M, et al. (2020) Atezolizumab plus Bevacizumab in Unresectable Hepatocellular Carcinoma. N Engl J Med 382: 1894-1905.
22. Cabibbo G, Reig M, Celsa C, Torres F, Battaglia S, et al. (2022) First-Line Immune Checkpoint Inhibitor-Based Sequential Therapies for Advanced Hepatocellular Carcinoma: Rationale for Future Trials. Liver Cancer 11: 75-84.
23. Persano M, Rimini M, Tada T, Suda G, Shimose S, et al. (2023) Sequential therapies after atezolizumab plus bevacizumab or lenvatinib first-line treatments in hepatocellular carcinoma patients. Eur J Cancer. août 189: 112933.
24. Cabibbo G, Celsa C, Enea M, Battaglia S, Rizzo GEM, et al. (2020) Optimizing Sequential Systemic Therapies for Advanced Hepatocellular Carcinoma: A Decision Analysis. Cancers 12: 2132.
25. Granito A, Marinelli S, Forgione A, Renzulli M, Benevento F, et al. (2021) Regorafenib Combined with Other Systemic Therapies: Exploring Promising Therapeutic Combinations in HCC. J Hepatocell Carcinoma 8: 477-492.
26. Shigeta K, Matsui A, Kikuchi H, Klein S, Mamessier E, et al. (2020) Regorafenib combined with PD1 blockade increases CD8 T-cell infiltration by inducing CXCL10 expression in hepatocellular carcinoma. J Immunother Cancer 8: 001435.
27. Yoo C, Park JW, Kim YJ, Kim DY, Yu SJ, et al. (2019) Multicenter retrospective analysis of the safety and efficacy of regorafenib after progression on sorafenib in Korean patients with hepatocellular carcinoma. Invest New Drugs 37: 567-572.
28. Li J, Jia Y, Shao C, Li Y, Song J (2023) Clinical Efficacy and Safety of an Immune Checkpoint Inhibitor in Combination with Regorafenib Therapy as Second-Line Regimen for Patients with Unresectable Hepatocellular Carcinoma. Ther Clin Risk Manag 19: 329-339.
29. Huang J, Guo Y, Huang W, Hong X, Quan Y, et al. (2022) Regorafenib Combined with PD-1 Blockade Immunotherapy versus Regorafenib as Second-Line Treatment for Advanced Hepatocellular Carcinoma: A Multicenter Retrospective Study. J Hepatocell Carcinoma 9: 157-170.

30. Fukuoka S, Hara H, Takahashi N, Kojima T, Kawazoe A, et al. (2020) 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 Off J Am Soc Clin Oncol 38: 2053-2061.
31. Chen B, Zhao H, Huang J, Lv H, Xu W, et al. (2022) Efficacy of regorafenib combined with PD-1 inhibitors in elderly patients with advanced metastatic colorectal cancer. BMC Geriatr 22: 987.
32. Wang C, Chevalier D, Saluja J, Sandhu J, Lau C, et al. (2020) Regorafenib and Nivolumab or Pembrolizumab Combination and Circulating Tumor DNA Response Assessment in Refractory Microsatellite Stable Colorectal Cancer. The Oncologist. août 25: 1188-1194.
33. Early nivolumab addition to regorafenib in patients with hepatocellular carcinoma progressing under first-line therapy (GOING trial), interim analysis and safety profile. | Journal of Clinical Oncologyhttps://ascopubs.org/doi/abs/10.1200/JCO.2022.40.4_suppl.428
34. Song Y, Fu Y, Xie Q, Zhu B, Wang J, et al. (2020) Anti-angiogenic Agents in Combination With Immune Checkpoint Inhibitors: A Promising Strategy for Cancer Treatment. Front Immunol 11: 1956.
35. Mlecnik B, Lugli A, Bindea G, Marliot F, Bifulco C, et al. (2023) Multicenter International Study of the Consensus Immunoscore for the Prediction of Relapse and Survival in Early-Stage Colon Cancer. Cancers 15: 418.