

## BRIEF REPORT

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# Feasibility, safety, and outcome of second-line nivolumab/bevacizumab in liver transplant patients with recurrent hepatocellular carcinoma

To the editor,

Liver transplantation (LT) is the only therapeutic option for both HCC and cirrhosis. Despite careful patient selection, HCC-R\_LT occurs in as much as 20%, and is often characterized by an aggressive clinical course and high mortality. We therefore designed a proof-of-concept study aimed to analyze the safety of combination therapy with nivolumab and bevacizumab in patients with HCC-R\_LT, whose tumor progressed in most cases while on sorafenib therapy. In particular, we focused on the risk of rejection and overall survival (OS).

## METHODS

We evaluated the feasibility and safety of nivolumab (240 mg every 14 d) and bevacizumab (5 mg/kg every 14 d) in HCC-R\_LT. Candidate patients underwent hepatic, renal, and cardiac function tests (at baseline and after each infusion), echocardiography, and total-body CT (at baseline and every 3 months unless otherwise indicated). Bevacizumab was added if nivolumab was well tolerated and no rejection occurred after the first 2 nivolumab infusions. Patients unsuitable for nivolumab/bevacizumab were treated with regorafenib (160 mg orally once daily for 3 weeks out of each 4-week cycle). Adverse events (AE) were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) v.5.0.

The biological aggressiveness of the tumor at transplantation and the plausibility of antiangiogenic therapy were evaluated as indicated in Supplementary Methods, <http://links.lww.com/LVT/A334>.

## Endpoints and statistics

Efficacy assessments were made using Response Evaluation Criteria in Solid Tumors 1.1. Outcome endpoints were defined as stable disease  $\geq 6$  months, partial response, complete response or progressive disease (ie,  $\geq 20\%$  increase in tumor size against a known baseline lesion or new intrahepatic or extrahepatic lesions in the liver, lymph nodes, lungs, and bones). Overall survival (OS) was evaluated by the Kaplan–Meier method after the initiation of second-line therapy. Statistical analysis was performed with IBM SPSS Statistics for Macintosh, Version 28.0.

## Declaration of ethical approval

Combination therapy received nominal authorization after a formal review of the health records of each patient by the county off-label drug Committee [NOP]. Informed written consent was obtained from each subject. The study complied with good clinical practice guidelines, the Declaration of Helsinki and Istanbul, and the applicable local laws.

## RESULTS

Between February 2018 and September 2021, 22 patients with HCC-R\_LT were consecutively referred to the Gastroenterology Unit of Azienda Ospedaliero-Universitaria di Modena, Italy, after a median period of 14.5 months (range, 4–106 mo) from LT. Demographic, biochemical, clinical data, pretransplant downstaging

**Abbreviations:** AE, Adverse events; AFP, alpha-fetoprotein; BSC, best supportive care; HCC-R\_LT, HCC recurrence after LT; LT, Liver transplantation; OS, overall survival.

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interventions, HCC characteristics at transplant, and histochemical staining for Angiopoietin-2 and VEGF\_A are reported in Supplementary Table 1, <http://links.lww.com/LVT/A335> and Supplementary Figure 1, <http://links.lww.com/LVT/A333>.

Of the 22 patients, 4 (18.1%) underwent best supportive care (BSC) alone (all for Eastern Cooperative Oncology Group Performance Status =3). The other 18 (81.8%) started treatment with sorafenib.

Seventeen/18 (94.4%) progressed after a median of 6 months. Twelve/17 (70.6%) were switched to regorafenib, not being eligible for Nivolumab or Bevacizumab for the following: Nivolumab, hyperthyroidism  $n=2$ ; cardiac ejection fraction  $<45\%$   $n=2$ ; Bevacizumab: low neutrophils count  $n=3$ ; arterial hypertension on treatment  $n=2$ , and chronic heart failure  $n=1$ . The other 5 patients (29.4%) received nivolumab as initial treatment. One patient received only 1 infusion. Three weeks later, bilirubin levels increased, liver and lung tumoral invasion rapidly progressed, and massive neoplastic ascites developed together with acute kidney insufficiency. The patient died 2 weeks later.

All the other 4 patients tolerated nivolumab well. One patient experienced after the first infusion G2 bilirubin and G3 aspartate aminotransferase increase. A moderate-to-severe rejection was present in the liver biopsy (Figure 1, A). First, Methylprednisolone (1 g/day) for 3 days, then prednisone 50 mg/day for

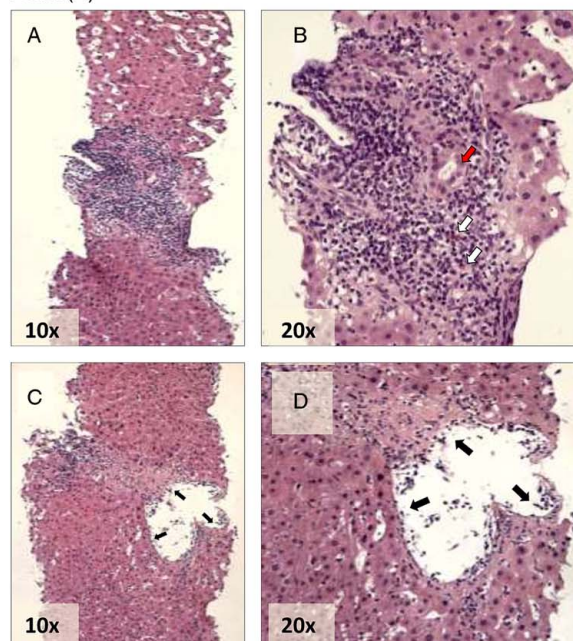
1 week tapered and discontinued after 3 weeks resolved the clinical event. Nivolumab was continued without recurrence of rejection. Once stable on nivolumab and with no evident AE, the 4 patients started bevacizumab.

Stable disease at the hepatic, lung, and lymph node levels was achieved in all 4 patients. Two patients with bone metastasis experienced progression after 8 and 10 months, respectively, from starting nivolumab/bevacizumab. Both patients underwent 2 courses of radiotherapy 5 months apart, obtaining satisfactory pain control.

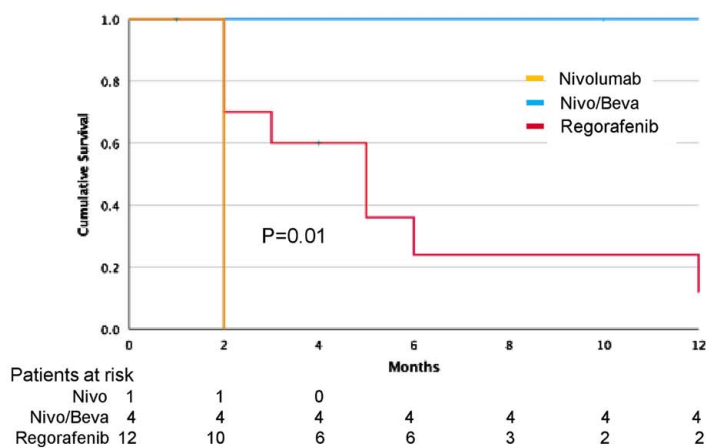
All 4 patients are still on combination therapy: the mean treatment period has been so far  $13.4 \pm 5.1$  months (median 16 mo). OS from the initiation of sorafenib has been  $26.5 \pm 10.4$  for patients on nivolumab/bevacizumab versus  $9.5 \pm 5.5$  for those on regorafenib ( $p=0.02$ ). The survival of patients in BSC has been  $5.5 \pm 5.2$  months. OS from the initiation of second-line therapy has been  $16.0 \pm 4.5$  months for patients on nivolumab/bevacizumab versus  $5.8 \pm 6.1$  months for those on regorafenib ( $p=0.01$ ) (Figure 1, B; Table 1).

Two/18 patients on sorafenib experienced G1 diarrhea and 2 patients experienced G1 palmar-plantar erythrodysesthesia. After the switch to regorafenib, 3/12 had G2 diarrhea and 1 had G1 fatigue. With nivolumab/bevacizumab, apart from the patient who developed moderate–severe liver rejection after

Panel (A)



Panel (B)



**FIGURE 1** (A) Liver biopsy performed 10 days after the first nivolumab infusion. HE staining shows prominent portal mixed inflammation with interface activity ([A],  $\times 10$ , [B]  $\times 20$ ) and eosinophil infiltrate (B, white arrows). There is a presence of cytoplasmic vacuolization of the duct epithelium consistent with bile duct damage ([B], red arrow). In [C ( $\times 10$ )] and [D ( $\times 20$ )], a representative example of subendothelial lymphocytic inflammation with lifting up of the endothelium compatible with endothelitis is shown (black arrows). (B), Kaplan–Meier curve for the survival of patients with recurrence of HCC after liver transplant after starting second-line therapy with Nivolumab/Bevacizumab (blue line), or Regorafenib (red line). Differences in survival were compared by the log-rank test. The orange line indicates the patient, who received only 1 Nivolumab infusion but died of rapid tumoral progression.

**TABLE 1** Clinical characteristics of patients at the time of HCC recurrence after LT divided by different types of second-line treatment (nivolumab, nivolumab/bevacizumab, or regorafenib)

Features	Nivolumab (n = 1)	Nivo/Beva (n = 4)	Regorafenib (n = 12)	p <sup>a</sup>
Age at LT (mean, SD)	51	56.7 ± 8.5	60.1 ± 9.1	0.52
Age at recurrence (mean, SD)	53	58.5 ± 9.2	62.3 ± 8.9	0.48
Sex (male/female)	0/1	3/1	11/2	0.66
Blood type				
A	1	1 (20.0)	5 (38.4)	0.49
B	–	–	2 (15.3)	–
AB	–	–	2 (15.3)	–
0	–	3 (60.0)	4 (30.7)	–
Etiology of Liver disease, n (%)				
HCV	1	1 (25.0)	6 (46.2)	0.60
HBV	–	1 (25.0)	3 (25.0)	–
Other causes	–	2 (50.0)	3 (25.0)	–
Bilirubin (mg/dL) (mean, SD)	0.9	0.5 ± 0.2	0.5 ± 0.1	0.94
Albumin (g/dL)(mean, SD)	4.1	3.9 ± 0.6	3.7 ± 0.4	0.68
Creatinine (mg/dL) (mean, SD)	0.7	0.9 ± 0.2	1.0 ± 0.2	0.31
INR	1.0	1.0 ± 0.2	1.0 ± .01	0.86
AFP (ng/mL) (mean, SD)	53	25 ± 35	149 ± 403	0.56
Edmondson-Steiner grading, n (%)				
1	–	–	1 (8.3)	0.83
2	1	2 (50.0)	5 (41.7)	–
3	–	2 (50.0)	6 (50.0)	–
Presence of viable tumor at explant, n (%)	0	3 (75.0)	10 (76.9)	0.32
Post-transplant complications, n (%)				
Biliary stenosis	0	2 (50.0)	3 (23.0)	0.81
Re-transplant	0	1 (25.0)	1 (7.6)	–
Immunosuppression at recurrence, n (%)				
Everolimus	0	1 (25.0)	6 (50.0)	0.40
Sirolimus	1	2 (50.0)	2 (16.7)	–
Tacrolimus	0	1 (25.0)	4 (33.3)	–
Tumor burden at recurrence (volume, mm <sup>3</sup> )				
Liver	1,677	827 ± 319	1053 ± 1152	0.83
Lung	3,350	5481 ± 4271	3116 ± 4869	0.41
Lymph node	29,300	7195 ± 6481	10,221 ± 11,685	0.74
Bone	18,392	5598 ± 7280	8350 ± 10,677	0.79
Adrenal gland	–	–	2567	–
Tumor burden at progression, before starting second-line therapy (volume, mm <sup>3</sup> )				
Liver	1,593	1139 ± 585	1203 ± 1394	0.94
Lung	6,688	11,917 ± 14,868	10,497 ± 20,423	0.91
Lymph node	13,346	16,279 ± 21,455	16,033 ± 34,392	0.99
Bone	50,240	31,081 ± 21,395	14,527 ± 12,372	0.31
Adrenal gland	–	5558 ± 1423	10,321 ± 5606	0.43
Treatments performed after HCC recurrence before systemic therapy, n (%)				
Surgery	–	–	–	0.65
Lung resection	1	–	4 (30.7)	–
Adrenal gland removal	–	1 (25.0)	2 (15.3)	–
Hepatic hilar lymph node resection	–	1 (25.0)	–	–

TABLE 1. (continued)

Features	Nivolumab (n = 1)	Nivo/Beva (n = 4)	Regorafenib (n = 12)	p <sup>a</sup>
Cutaneous metastasis removal	–	–	1 (7.6)	–
Locoregional Treatments	–	–	1 (7.6)	–
None	–	2 (25.0)	5 (38.4)	–
Timing of events (months, mean ± SD)				
Recurrence-free survival	10	21.5 ± 25.8	25.7 ± 24.1	0.77
Interval from recurrence to sorafenib	12	3.0 ± 1.4	3.8 ± 8.0	0.89
Time between sorafenib initiation and discontinuation for progression	2	7.5 ± 1.9	6.7 ± 3.1	0.68
Time from progression to nivo/beva or regorafenib initiation	2	2.5 ± 0.7	2.0 ± 1.0	0.59
Time from nivo/beva or regorafenib to death or last visit	2	16.0 ± 4.5	5.8 ± 6.1	0.01

Note: The patient who received a single infusion of Nivolumab is reported individually.

<sup>a</sup>The significance is referred to the comparison between nivolumab/bevacizumab and regorafenib.

Abbreviations: AFP indicates alpha-fetoprotein; Beva, bevacizumab; BSC, Best supportive care; Nivo, nivolumab.

the first nivolumab infusion, the only AE were G1 alanine-aminotransferase increase in 1 patient with nivolumab and G1 proteinuria in another after bevacizumab, not requiring active intervention apart from temporary dose reduction.

## DISCUSSION

HCC recurrence after LT represents a dramatic occurrence, only moderately influenced by tyrosine kinase inhibitors.<sup>[1,2]</sup> Indeed, the survival figures reported in the literature indicate an 18-month median survival in sorafenib responders<sup>[2]</sup> and 13 months in regorafenib-treated sorafenib failures,<sup>[3]</sup> with an OS less than 3 months for those on BSC.<sup>[1]</sup> Despite increased tumor stabilization and improved survival obtained in non-LT patients with immune checkpoint inhibitors and bevacizumab,<sup>[3]</sup> LT patients are excluded from experimental protocols for concerns over the potential interaction between anti-cancer and immunosuppressive drugs. Although preliminary, our data show that the combination of nivolumab/bevacizumab can achieve a stabilization of the disease in the liver and in the lungs, with a gain in survival for patients who have failed sorafenib and would otherwise have only been amenable to BSC or regorafenib. The switch to regorafenib in 12 patients not eligible to nivolumab/bevacizumab was associated with significantly lower survival rates. This is even more relevant because of the notable biological aggressiveness of HCC-R\_LT, demonstrated by the extremely high angiopoietin-2 expression, especially in hepatic sinusoidal endothelia.<sup>[4]</sup> VEGF was expressed at a much lower level in tumor tissue, with elevated expression in the surrounding nontumoral tissue.<sup>[5]</sup> This suggests that the relevant clinical effect of bevacizumab could be mediated through an indirect effect on the surrounding nontumoral tissues, preventing further VEGF-mediated signaling.

A limitation of this observational study resides in the low number of enrolled patients. The results show that the combination of nivolumab/bevacizumab is feasible for LT patients, with few and manageable AE. The favorable results observed in terms of survival suggest that a larger and controlled study could be pursued. Bone metastasis remains an active and unsolved problem, indicating the need for alternative approaches.

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## CONFLICT OF INTEREST

The authors have no conflicts to report.

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