Research Article

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Unexpected high rate of early tumor recurrence in patients with HCV-related HCC undergoing interferon-free therapy☆

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Background & Aims: The success of direct-acting antivirals (DAA) against hepatitis C is a major breakthrough in hepatology. Until now, however, there are very few data on the effect of hepatitis C virus (HCV) eradication in patients who have already developed hepatocellular carcinoma.

Methods: The study included patients with HCV infection and prior history of treated hepatocellular carcinoma who achieved complete response and lacked 'non-characterized nodules' at the time they underwent anti-HCV treatment with all-oral DAAs in 4 hospitals. Patients receiving interferon as part of the antiviral regimen were excluded. The baseline characteristics, laboratory and radiologic tumor response were registered in all patients before starting antiviral therapy and during the follow-up according to the clinical practice policy.

Results: Between 2014 and 2015, 103 patients with prior hepatocellular carcinoma received DAA, 58 of them met the inclusion criteria. After a median follow-up of 5.7 months, 3 patients died and 16 developed radiologic tumor recurrence (27.6%). The pattern of recurrence was: intrahepatic growth (3 patients), new intrahepatic lesion (1 nodule in 5 patients, up to 3 nodules less or equal to 3 cm in 4 cases and multifocal in one patient) and infiltrative ill-defined hepatocellular carcinoma and/or extra-hepatic lesions in 3 patients.

Conclusions: Our data show an unexpected high rate and pattern of tumor recurrence coinciding with HCV clearance and, although based in a very small cohort of patients, should be taken as a note of caution and prime a large scale assessment that exceeds the individual investigators capacity.

Lay summary: High rate of cancer recurrence after DAA treatment in patients with prior hepatocellular carcinoma. Disruption of immune surveillance may facilitate the emergence of metastatic clones.

Keywords: Immune responses; HCV viral kinetics; Interferon-free regimen; Direct-acting antivirals; Hepatocellular carcinoma; Tumor recurrence.

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Abbreviations: HCV, hepatitis C virus; DAA, direct-acting antivirals; SVR, sustained virological response; MELD, model for end-stage liver disease; HCC, hepatocellular carcinoma; BCLC, Barcelona Clinic Liver Cancer group; LOD, limit of detection; EOT, end of treatment; SVR12, sustained virological response 12 weeks after treatment interruption; SVR4, sustained virological response 4 weeks after treatment interruption; MR, magnetic resonance; CT, computed tomography; TACE, transarterial chemoembolization; P25-P75, percentile 25-75; IFN, interferon; SOF, sofosbuvir; SMV, simeprevir; DCV, daclatasvir; RBV, ribavirin; STORM, adjuvant sorafenib for hepatocellular carcinoma after resection or ablation; HALT-C, the hepatitis C long-term treatment against cirrhosis (HALT-C) trial; EPIC, Evaluation of pegIntron in control of hepatitis C cirrhosis (EPIC) 3 program; MRD, minimal residual disease; ISGs, interferon stimulated genes.

Introduction

Treatment of hepatitis C virus (HCV) infection has experienced a major advancement with the advent of the new direct-acting antivirals (DAA). Current HCV infection cure rates exceed 90% and this has occurred in a very short time. Different studies have shown rates of sustained virological response (SVR) around 95–97% in compensated cirrhosis [1–3] and 85–90% in patients with more advanced liver disease including those awaiting liver transplantation [4,5]. More importantly, these high efficacy results have been shown to be also reproducible in large real-life cohorts [6–8] reporting improvements in disease severity (Child-Pugh score, MELD) in some patients early after treatment.

Cancer

This clinical reality has raised several expectations: i) the evolution of infected patients into cirrhosis and the need of transplant would decrease sharply in one decade; ii) the incidence of liver cancer would also decrease as a result of the abrogation of the chronic inflammation related to viral infection, ultimately leading to cirrhosis and oncogenic damage. These predictions are all well grounded on a population basis, but in some specific populations, the impact of viral infection cure with sudden changes in the relationship between inflammatory status and immune stimulation may induce the emergence of events that were totally unpredicted.

This may be the case in patients with HCV-related hepatocellular carcinoma (HCC) that have been successfully treated for their cancer and, later on received effective antiviral therapy. We started the treatment of such patients when approval was granted for the indication (2014). Due to the large number of cases attending in the liver cancer unit (BCLC) and the viral hepatitis unit, we detected some unfortunate patients in whom antiviral therapy and HCV eradication was followed by the detection of HCC recurrence. The recognition of more cases with recurrent disease with a clear-cut temporal association between antiviral therapy and HCC recurrence, prompted us to carefully review the clinical experience that was part of a prospective health plan in viral hepatitis, and to involve other groups in such an effort, who had the same clinical experience and concerns. This is particularly relevant since data from real-life antiviral experience with DAA [6–8] regarding HCC incidence or HCC outcomes are absent or heterogeneous, and do not allow extraction of any clear recommendation. Indeed, the indications on antiviral therapy for virus-related HCC patients remains incomplete in most clinical guidelines [9].

In this study we expose the findings from a well-defined population of patients with chronic HCV infection and HCC that were treated with DAA after reaching complete tumor response after treatment for their tumor.

Patients and methods

Patient evaluation

The study included patients with HCV infection and complete radiologic response after a prior history of HCC treated by ablation, resection or chemoembolization between 13th October 2014 and 15th December 2015, and who had received treatment with all-oral DAA in four Spanish referral hospitals (Hospital Clinic de Barcelona, Hospital Universitario Puerta de Hierro, Clínica Universidad de Navarra and Hospital Universitario Central de Asturias). Cases were identified in each hospital registry and their follow-up ended in February, 2016.

The inclusion criteria were: 1) HCC diagnosed by pathology or by non-invasive criteria according to American Association for the Study of Liver Diseases (AASLD) guidelines, 2) HCC should have been treated prior to DAA by resection, ablation or chemoembolization, 3) complete radiologic response (absence of residual tumor or complete necrosis according to European Association for the Study of the Liver (EASL) criteria) as well as absence of 'non-characterized nodules' at imaging confirmed before starting DAA; 4) treatment with an all-oral DAA combination, and 5) at least one tumor status assessment after starting antiviral therapy. The patients with 'non-characterized nodules' (nodules detected as lesions <10 mm irrespective of their dynamic pattern or non-specific vascular images detected during the arterial phase of dynamic imaging) were excluded as they may represent either new HCC sites that had not reached the phase to allow them to be registered as such [10] or any other entity such as benign regerative nodules [11].

Exclusion criteria were: 1) prior history of liver transplantation; 2) patients with treated HCC but without radiologic complete response and/or presence of 'non-characterized nodules' before starting DAA; 3) patients receiving interferon (IFN) as part of the antiviral regimen.

Hepatitis C treatment

Antiviral therapy and treatment duration (12/24 weeks) was indicated in each patient according to the viral genotype/subtype and the severity of liver disease, in accordance with the current international guidelines [9]. HCV-RNA quantification was assessed by real-time PCR, with a limit of detection (LOD) of 15 IU/ml.

Patients were followed-up monthly for clinical and laboratory evaluation during antiviral treatment. Virological response to DAA-based treatment was assessed by quantitative HCV-RNA at week 4 (for adherence purposes), at the end of treatment (EOT) and at 4 and 12 weeks after the EOT, to confirm SVR. SVR12 was defined as undetectable HCV-RNA at week 12 after the end of therapy (either by completion of the therapy, discontinuation due to adverse events, or liver transplantation). In the remaining patients without a complete 12-weeks follow-up, the final virological status at the time of the analysis was reported. Virological failures and early discontinuations of therapy due to adverse events were also registered.

Clinical and radiological follow-up

The baseline characteristics, laboratory and radiologic tumor response were registered in all patients before starting antiviral therapy. Follow-up included clinical, laboratory data and radiological tumor assessment according to clinical practice.

The follow-up policy for HCC patients who achieve complete radiologic response after TACE is to perform imaging with a magnetic resonance (MR) or computed tomography (CT) every 6 months. In patients treated by ablation a contrast-ultrasound is done at months 1 and 3; a MR or CT is performed every 6 months thereafter. Finally, dynamic CT or MR every 6 months is carried out in resected patients.

Time points for radiology evaluation of tumor status

We registered 3 time periods for each patient in order to expose the time relationship between HCC treatment and achievement of complete response, the initiation of DAA, and the length of follow-up until HCC recurrence or last imaging follow-up without recurrence. The first time period corresponds to 'time between HCC treatment and last assessment of complete response by imaging'. It reflects the interval between HCC treatment and the date of the last radiologic evaluation (which confirmed the complete response in each patient) prior to DAA therapy. The second time period - 'Time window between last complete response assessment and DAA initiation' - reflects the time between the date of the last radiologic confirmation of complete response, and the start date of DAA. The last time period – 'time for HCC evolution after starting DAA' - reflects the time between the date of the first dose of DAA and the date of radiologic tumor progression or the last radiologic evaluation during follow-up in those patients without radiologic HCC progression. Finally, we also registered the patients' status (alive/death) at the end of follow-up.

Statistical analysis

Quantitative variables were expressed as median and range or percentile 25-75 (P25-75); the categorical variables as count number and proportions. The last date for data collection was 19th February 2016. All calculations were done with SPSS package version 23 (SPSS Inc., Chicago, IL).

Results

Baseline characteristics of patients

Between October 2014 and December 2015, 103 HCC patients with prior HCC and a HCV infection received treatment with DAA. A total of 98 patients had a history of HCC treatment before starting DAA. Eighty-six of them were confirmed to have a complete radiologic response following the validated EASL criteria that take into account tumor necrosis. To avoid confounders because of HCC understaging, 8 out of the 86 with complete radiologic response were excluded because the existence of 'non-characterized nodules' that could represent malignant sites which may become apparent during follow-up. In addition, and in accordance with the selection criteria, we excluded 11 addi-

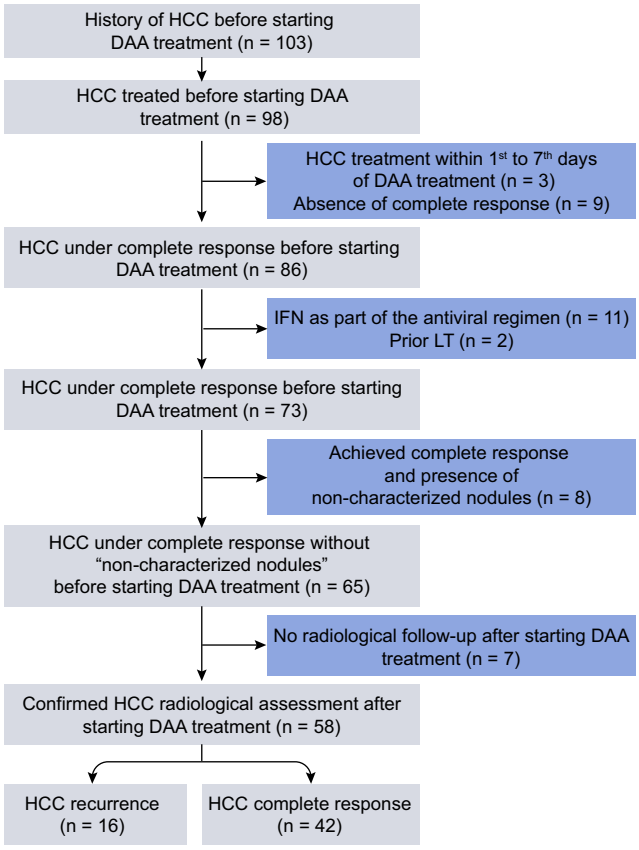

Fig. 1. Flowchart of the study.

tional patients because they received an IFN-based treatment, and 2 due to prior history of liver transplantation. Moreover, we excluded 7 extra patients post data collection in February as they still did not have a radiologic control after DAA. Finally, 58 patients met the inclusion criteria and constituted the target population of this study (Fig. 1).

Baseline characteristics at the time of starting HCV antiviral therapy and data about DAA combinations and treatment duration are depicted in Table 1. All but 3 patients were cirrhotic (91% Child-Pugh A), and when HCC was diagnosed and treated, they corresponded to BCLC stage 0 in 16 cases and BCLC stage A in 42 cases.

Virological response

At the time of this analysis, 40 patients had already reached the 12-week follow-up period, and 39 had achieved SVR12. One patient infected with genotype 1b and treated with sofosbuvir (SOF), simeprevir (SMV) and RBV for 12 weeks experienced a virological failure (relapse), accounting for a per protocol SVR12 rate of 97.5% (39/40). Of the remaining 18 patients, 3 are still on treatment, 11 patients have already finalized therapy (all of them have achieved undetectable HCV-RNA) and 3 additional patients have reached SVR at week 4 after therapy (SVR4). One patient had to prematurely discontinuate therapy at week 20 due to an episode of incarcerated umbilical hernia; the patient died because of postoperative liver failure.

Table 1. Baseline characteristics of the whole cohort.

	Total cohort (n = 58)
Age, median [range] (yr)	66.3 [45-83]
Gender, (M/F), n (%)	40 (69)/18 (31)
Non-cirrhosis/cirrhosis, n (%)	3 (5.2)/55 (94.8)
Child-Pugh, A/B/C, n (%)	50 (91)/3 (5.4)/2 (3.6)
BCLC stage, 0/A, n (%)	16 (27.6)/42 (72.4)
AST, median [range] (IU/L)	82.5 [23-433]
ALT, median [range] (IU/L)	85 [28-487]
AP, median [range] (IU/L)	104.5 [39-357]
GGT, median [range] (IU/L)	74 [21-1181]
PT, median [range] (%)	76.5 [12.60-100]
Bilirubin, median [range] (mg/dl)	1.00 [0.30-6.00]
Albumin, median [range] (g/L)	40 [20-50]
Creatinine, median [range] (mg/dl)	0.75 [0.40-2.37]
Haemoglobin, median [range] (g/dl)	14.1 [8.00-18.50]
Platelets, median [range] (x10^9/L)	101 [33-229]
AFP, median [range] (ng/ml)*	11.45 [1-369]
HCV genotype, n (%)	
GT1a	8 (13.8)
GT1b	45 (77.6)
GT3	2 (3.4)
GT4	3 (5.2)
Naïve/treatment experienced	29 (50)/29 (50)
Previous triple therapy (PR + DAA)**	6 (20.6)
HCV-RNA (Log_{10}) (IU/ml)	6.08 (3.11-6.92)
DAA combination, n (%)	
SOF/LDV	21 (36.2)
3D	15 (25.9)
SOF/SMV	15 (25.9)
SOF/DCV	6 (10.3)
SMV/DCV	1 (1.7)
Use of RBV, n (%)	48 (82.8)
Treatment duration 12 wk/24 wk, n (%)	44 (75.9)/14 (24.1)
HCC treatment before DAA, n (%)	
Resection	20 (34.5)
Ablation	32 (55.2)
TACE	6 (10.3)

M, male; F, female; BCLC, Barcelona Clinic Liver Cancer; AST, aspartate aminotransferase; ALT, alanine aminotransferase; AP, alkaline phosphatase; GGT, gammaglutamyl transpeptidase; PT, prothrombin time; AFP, alpha-fetoprotein; HCV, hepatitis C virus; GT, genotype; PR, pegylated-interferon plus ribavirin; DAA, direct-acting antivirals; KPa, kilopascals; SOF, sofosbuvir; LDV, ledipasvir; 3D, 3-drug combination paritaprevir/ritonavir/ombitasvir plus dasabuvir; SMV, simeprevir; DCV, daclatasvir; RBV, ribavirin; w, weeks; HCC, hepatocellular carcinoma; TACE, transarterial chemoembolization.
*Absent in 26 patients; **2 patients had received telaprevir, 2 patients boceprevir, and 2 patients sofosbuvir, in combination with PR.

Hepatocellular carcinoma evolution after starting DAA

The overall median follow-up time after DAA was 5.7 months (0.4–14.6). At that time, 55 patients were alive, 3 patients died and 16 developed radiologic tumor recurrence (27.6%); median time from DAA start to recurrence was 3.5 months (1.1–8). The median radiologic follow-up time after starting DAA in the 42 patients without HCC recurrence was 5.7 months (0.4–14.6) (Fig. 2, and Table 2 and Supplementary Table 2). Eighteen out of 42 patients without HCC recurrence (46.6%) had data from a single imaging follow-up, and remain on the surveillance schedule described above.

The median time between HCC treatment and start of DAA was 11.2 months (percentile (P) 25-P75: 3.6–23.2). One single patient started DAA after 1 month of computed radiography

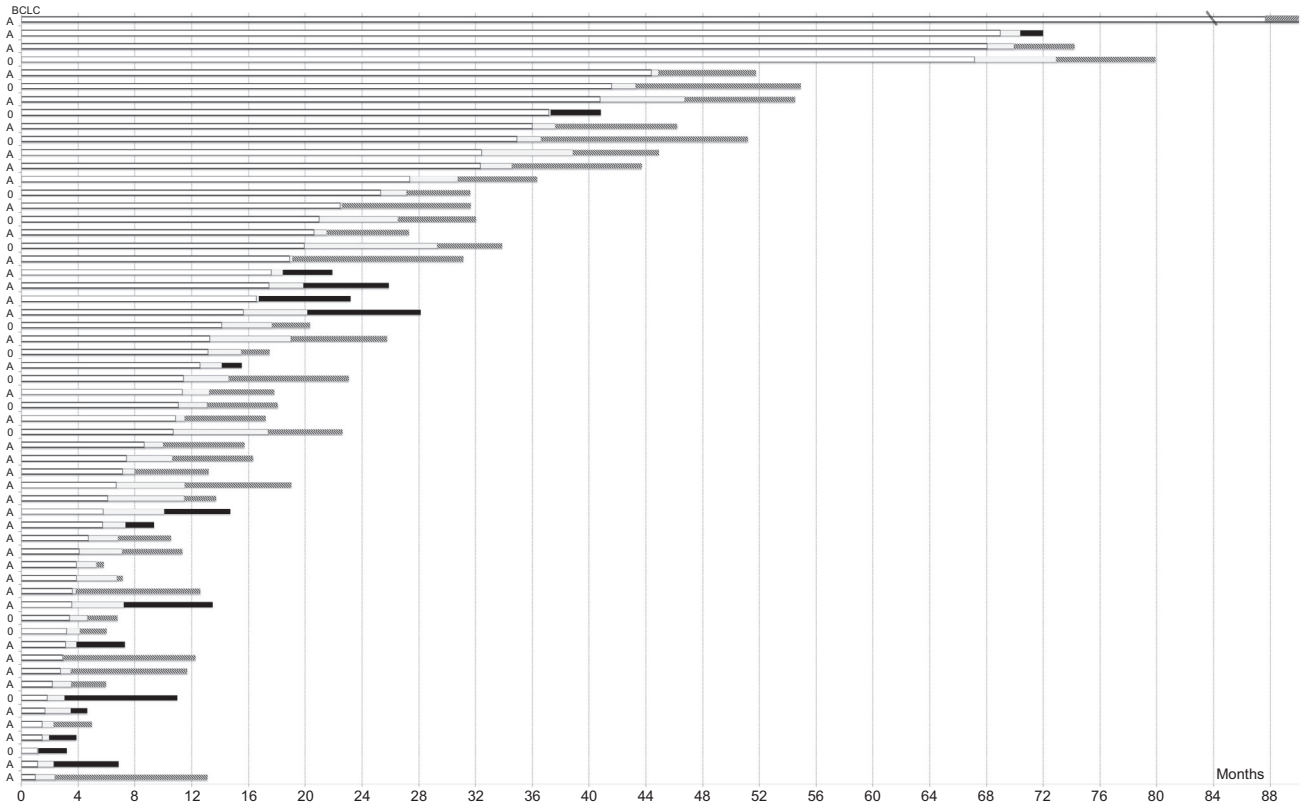

Fig. 2. Time points for radiology evaluation of tumor status. The evolution of each patient is described in 3 time periods: white boxes reflect 'time between HCC treatment and last assessment of HCC complete radiologic response by imaging'. It shows the interval between HCC treatment and the date of the last radiologic evaluation (which confirmed the complete radiologic response in each patient) prior to DAA therapy. b) Grey boxes represent the second time period, 'time window between last complete radiologic response assessment and DAA initiation', reflecting the time between the date of the last radiologic confirmation of complete radiologic response, and the start date of DAA. The last time period depicts 'HCC evolution after starting DAA', depends on the outcome of the patients: black boxes indicate the time between the date of the first dose of DAA and the date of radiologic tumor progression; gridded boxes indicate the time until the last radiologic evaluation during follow-up in patients without radiologic tumor progression.

Table 2. Liver function and tumor-related variables of patients with HCC recurrence at the three relevant time points of the study.

	At time of HCC treatment			At time of starting DAA		At time of HCC recurrence after DAA	
Patient	PS	Child-Pugh	BCLC	PS	Child-Pugh	PS	Child-Pugh
1	0	5	A (one nodule)	0	5	0	5
2	0	6	A (one nodule)	0	8	2	8
3	0	6	0	0	5	0	5
4	0	6	A (one nodule)	0	5	0	6
6	0	n.a.*	A (one nodule)	0	n.a.*	0	n.a.*
7	0	5	A (one nodule)	0	5	0	5
8	0	6	A (≤3 nodules and ≤3 cm)	0	6	0	6
9	0	5	A (one nodule)	0	5	0	5
10	0	6	A (one nodule)	0	6	0	5
11	0	5	A (one nodule)	0	5	0	5
12	0	5	A (≤3 nodules and ≤3 cm)	0	5	0	5
13	0	5	A (one nodule)	0	5	0	5
14	0	5	0	0	7	3	7
15	0	7	A (one nodule)	0	10	0	12
16	0	6	0	0	6	0	6

HCC, hepatocellular carcinoma; DAA, direct-acting antivirals; PS, performance status; BCLC, Barcelona Clinic Liver Cancer; n.a., not available.
*Non-cirrhotic patient.

(CR) assessment and was free of recurrence after 10.7 months of follow-up. This time period was longer than the 3 months in the rest of the cohort. The median time from the last radiologic confirmation of complete response before starting antiviral therapy and the DAA start day was 1.7 months (P25-P75: 0.85–3.42) in the whole cohort and 1.3 months (P25-P75: 0.6–2.3) in the 16 patients who developed recurrence.

Table 2 describes the liver function and tumor-related variables of the patients with recurrence at the three relevant time points of the study. The characteristics of the patients

Table 3. Baseline characteristics and outcome of the 16 patients with hepatocellular recurrence.

Patient	Treatment of HCC before DAA	Risk profile at pathology*	At time of starting DAA		At the time of HCC recurrence		HCC treatment	Status at the end of follow-up
			BCLC	AFP (ng/dl)	Pattern of progression	AFP (ng/dl)		
1	Resection	Low risk	A	91	NIH (one nodule)	912	Resection	Alive
2	Resection	Low risk	A	18	NIH (multinodular)	42	BSC	Dead
3	Resection	Low risk	0	2.3	NIH (one nodule)	1271	Resection	Alive
4	Resection	Low risk	A	12	NIH (≤3 nodules ≤3 cm)	5	Ablation	Alive
5	Resection	Low risk	A	4.2	NIH (≤3 nodules ≤3 cm)	2.1	OLT	Alive
6	Resection	High risk	A	1	NIH (one nodule)	112	Ablation	Alive
7	Resection	High risk	A	8	NIH (one nodule)	6	OLT	Alive
8	Ablation	n.a.	A	38	NIH (infiltrative) + NEH**	21,184	Sorafenib	Alive
9	Ablation	n.a.	A	66.2	IHG	7.9	Ablation	Alive
10	Ablation	n.a.	A	3	NIH (infiltrative) ***	n.a.	BSC	Alive
11	Ablation	n.a.	A	21.2	IHG	10.2	Ablation	Alive
12	Ablation	n.a.	A	6.7	NIH (one nodule)	3.8	OLT	Alive
13	Ablation	n.a.	A	14	IHG	5	Ablation	Alive
14	Ablation	n.a.	0	369	NIH (infiltrative) + NEH	n.a.	BSC	Alive
15	Ablation	n.a.	A	5	NIH (≤3 nodules ≤3 cm)	8	OLT	Alive
16	Ablation	n.a.	0	26	NIH (≤3 nodules ≤3 cm) ****	26	Ablation	Alive

BCLC, Barcelona Clinic Liver Cancer; CHC, carcinoma hepatocellular; DAA, direct-acting antivirals; TACE, transarterial chemoembolization; IHG, intrahepatic growth; EHG, extra-hepatic growth; NIH, new intrahepatic lesion; NEH, new extra-hepatic lesion and/or vascular invasion; OLT, orthotropic liver transplantation; BSC, best supportive care; AFP, alpha-fetoprotein; n.a., not available.

*Low risk, patients without microvascular invasion and satellites; High risk, patients with microvascular invasion or satellites in pathology; **Portal vein thrombosis; ***The patient presented an infiltrative HCC and developed early tumor progression with biliary tract invasion; ****Early tumor progression, the patient received TACE and the last radiologic evaluation describes a 10 cm HCC with macrovascular invasion.

without recurrence are given in Supplementary Table 2. The results in the subgroup of patients with CR after TACE are shown in Supplementary Table 3. No recurrence has been registered in them.

Tumor recurrence was registered in 3 out of the 16 BCLC stage 0 patients and in 13 out of the 42 BCLC stage A patients. The HCC treatment, the BCLC stage at the time of starting DAA, the pattern of radiologic recurrence and its treatment, as well as the status at the end of follow-up of the 16 patients with recurrence are summarized in Table 3. The radiologic recurrence was registered before finishing the HCV treatment in 8 of these patients.

The pattern of recurrence was heterogeneous: 3 patients developed intrahepatic growth that in 10 cases had a nodular profile (one nodule in 5 of them, up to 3 nodules less or equal to 3 cm in 4 cases, and multifocal in one patient), while 3 patients developed infiltrative ill-defined HCC and/or extra-hepatic lesions. The extent of recurrence impeded any effective therapy in 3 cases that received best supportive care (Table 3).

Subgroup analysis of patients with short time span between HCC treatment and DAA therapy

Seventeen (29.3%) patients started DAA treatment with a 'time between HCC treatment and last assessment of complete response by imaging' less than 4 months. Seven of these 17 patients (41.17%) developed radiologic tumor progression (2 BCLC stage 0 and 5 BCLC stage A at the time of HCC therapy). The pattern of recurrence in this subgroup of patients was: local recurrence in 2 patients, new intrahepatic lesions in 5 (one nodule in 4 patients and up to ⩽3 nodules less or equal 3 cm in 1 case).

Subgroup analysis of patients treated by surgical resection

Twenty patients of the cohort were resected patients who were free of recurrence at the time of DAA treatment evaluation. Only 4 of them presented high risk of recurrence according to the pathology of the resected tumor (2 patients with microvascular invasion and satellites, 1 patient with microvascular invasion and 1 with satellites). Two (50%) out of these high risk patients presented recurrence, while 5 (31%) out of the 16 in the low risk strata did so.

Survival of the whole cohort

At the end of follow-up, three patients had died (12.4, 9.4 and 5.4 months after DAA treatment initiation). One of them developed coincidental radiologic tumor progression with performance status and liver function deterioration (Performance status 2 and Child-Pugh stage B, 8 points) and the other 2 presented complete response but developed cirrhosis complications during the DAA treatment.

Discussion

Until recently, therapeutic eradication of chronic HCV infection has required the use of injectable IFN formulations, which are associated with a significant number of side effects and suboptimal efficacy in terms of viral eradication. The current scenario with several available combinations of DAA has completely changed the landscape of HCV therapy. While these represented a major breakthrough because of the high efficacy and optimal safety profile, there is still a need to collect further information about the evolution of patients after viral cure. This is especially relevant in specific populations that have not been included in the pivotal trials. One relevant niche of patients is that of those with HCC who have been successfully treated of their tumor and are treated with DAA afterwards.

The present data raise a concern about the benefits of DAA-based antiviral therapy in such subgroup. We describe a surprisingly high recurrence rate as compared to the already known incidence in patients with successfully treated HCC. Our cohort

includes patients with very early HCC (single tumor <2 cm) or early HCC with a low to moderate risk of recurrence. Only 6 patients were under complete response after TACE that could be considered of higher risk, but no recurrence has been detected in this subgroup (Supplementary Table 3). Importantly, all patients in our cohort had confirmation of complete response by imaging prior to antiviral therapy and a follow-up imaging schedule as conventional in our group. Hence, our data do not suffer from a potential flaw because of a more intense screening. Furthermore, in order to sense if the recurrence rate is higher than expected, we scrutinized several data sources. We dissected our prospective study after surgical resection, the prospective database of small HCC treated by ablation and finally and the database of the double blind placebo controlled STORM trial [14] that tested the efficacy of sorafenib to prevent recurrence after surgical resection or ablation. In the latter, complete response prior to randomization was confirmed by central review and follow-up images were obtained at regular intervals and evaluated by an independent panel of expert radiologists. Hence, STORM provides the best data set to establish comparisons with our cohort while matching for tumor burden. In that sense, it is worth mentioning that the STORM did not include patients with solitary HCC <2 cm if pathology would not disclose a profile linked to high recurrence risk. Furthermore, since the follow-up of our treated patients was not as long as in the STORM trial, we have specifically assessed the probability of recurrence within the first 4 months of achieving the complete response rather than comparing the entire Kaplan-Meier curves. The recurrence rate in these datasets analysed is consistently lower than the figure we have observed. The actuarial probability of recurrence in our contemporary ablation cohort for small HCC (unpublished data) is 2.45% (4/163) at 4 months and 27.6% (45/163) at 12 months, and the recurrence rate in our recent surgical study [13] to test indication of liver transplant because of recurrence risk at 4 months in high risk patients and in low risk patients is 13.5% and 3.8%, respectively. If we concentrate in the subgroup of 17 patients with a limited time ($\leqslant$4 months) between HCC treatment, complete response verification and DAA treatment initiation (very similar target population as compared to STORM [14]) the recurrence rate in our cohort (41.2%) is also sharply higher than the reported in STORM, 21.5% according to the independent assessment and 17.6% according to investigator assessment in STORM [14]. The same difference is observed when stratifying for other parameters such as Child-Pugh stage, recurrence risk profile at pathology and specific DAA agent received. Obviously, with the limited number of cases in each stratum, the comparisons are not robust enough and should be considered with caution. However, the global figures should raise a concern and we feel that it is mandatory to engage a worldwide effort to unequivocally define the risk of cancer recurrence in this specific population.

The key question is to envision the mechanism that could explain the development of cancer recurrence at a higher rate than expected. Indeed, the expectation by all experts in the field was that HCV cure would result in a reduction of recurrence or metachronic cancer development. As known, recurrence after initial complete response may be due to dissemination of cells prior to treatment and to the emergence of new oncogenic clones within the underlying cirrhotic liver that has already received the genetic damage [15]. No validated criteria are available for this distinction and the frequent use of time of appearance (within 1–2 years *vs.* beyond 2 years) is arbitrary and not validated [12]. High risk of dissemination [13,16] is evidenced by the detection of microscopic vascular invasion or satellites, whereas sustained inflammation with persistent liver damage is the predictor of metachronic tumors. However, cancer dissemination and development of metastatic nests that are recognised by imaging is not a mere mechanical process [17]. Malignant cell spread is almost universal in all neoplasms but not all spread results in clinically relevant metastasis. Cancer cells have been described in bone marrow of patients classified as cancer-free, and these do not always result in significant recurrence. The metastatic machinery is highly complex and involves tissue invasion, cell detachment from the stroma with anchor free survival while circulating in blood or lymph, nesting in a distant tissue/organ, and ultimately, uncontrolled growth, intense angiogenesis to finally become detectable, first by imaging and clinically afterwards [17]. In our cohort study we have been surprised by the close time association between DAA treatment and recurrence recognition by imaging, thus suggesting a sudden increase in cell proliferation without the counterbalance of immune induced cell death. Although a direct enhancing effect of DAA on tumor cell growth cannot be totally discarded it is highly unlikely. In fact, an HIV protease inhibitor such as indinavir has been shown to exert antitumor activity in animal models [18] and ribavirin has shown growth inhibitory effects in various cancer cell lines [19].

As mentioned, one of the most important partners in allowing or preventing metastatic cell survival and growth is immune cancer surveillance [20]. If immune surveillance and its proper balance are key to prevent or fight against an effective metastatic process, it is likely that disruption of the immune surveillance system plays a key role in the recognition of HCC recurrence. Immune surveillance is the result of several factors that are the consequence of the relationship between the personal inflammatory/immune phenotype and the modification of the inflammation process that is in place during viral infection and its modification by effective therapy. Thus, inflammatory status with heterogeneous activation of stromal cells and lymphocyte recruitment is sure responsible for the delay, or even complete abrogation, of the growth of clones of cells that may have nested away from the primary tumor. In the setting of DAA-based antiviral therapy, there is an extremely fast inhibition of HCV production. We should take into account that around 10^{12}–10^{13} virions are produced every day from an infected liver and with the current treatment regimens, HCV-RNA becomes undetectable in only a few days or weeks after treatment initiation. This is almost constantly accompanied by a reduction of inflammation signals (i.e., normalization of transaminases). Abrupt resolution of a chronic inflammatory state (such as chronic hepatitis C) may disturb the baseline status and abolish the immune "brake" to tumor progression. This change may allow the tumor clones to progress and be recognised as a recurrence. Indeed, there is evidence that the resolution of some types of inflammatory responses, such those triggered by some respiratory viruses, are followed by a prolonged period of immune suppression [21,22]. The role of fast viral eradication has also been raised by Hofnagle [23] in a recent editorial about the potential development of lactic acidosis while under DAA therapy. If this hypothesis is correct, why should this not have been observed after viral eradication by IFN-based regimes? No signal of alarm has been identified in all studies assessing the development of HCC or its recurrence after achiev-

ing viral cure. Indeed, all data favour a reduced HCC risk after HCV eradication through IFN-based regimes, and suggestions for a beneficial effect of chronic IFN administration have been raised [24–26]. The HALT-C [27] and the EPIC [28] trials testing this possibility were negative, but what is obvious is that no suggestion for increased cancer development was identified. The potential difference between prior IFN-based regimes may relay in the fact that the kinetics of viral suppression and associated inflammation are sharply different with DAA therapy when compared with IFN-based regimes. HCV eradication occurs in the first days after therapy for DAA and it takes longer with prior regimes. Furthermore, the use of IFN may have secured the immune cancer control benefits induced by IFN. As an example, IFNs are known to exert an antiproliferative effect by prolongation of all phases of cell cycle, have extrinsic effects on tumors by regulation of angiogenesis and, moreover, can regulate the activity of almost all immune cell types (including macrophages, dendritic cells, B cells, T cells and innate lymphocytes), creating a well-orchestrated immune response against infectious and malignant diseases. Indeed, it seems that IFN therapy is more effective at targeting disseminated cancer cells and minimal residual disease (MRD) before they form large proliferative metastases, suggesting that promoting antitumor immunity, rather than an antiproliferative response, is the likely mechanism of action [29].

The effect of DAA on the host immune status has recently been addressed in two studies. In the first, Serti *et al.* [30] describe the reconstitution of innate immunity by DAA therapy. In the second, Meissner *et al.* demonstrated that HCV clearance achieved during treatment with sofosbuvir and ribavirin is accompanied by hepatic downregulation of type II and III IFNs, their receptors, and interferon stimulated genes (ISGs) [31]. This might have a negative impact on immune cancer control. In the same study, the authors show that HCV patients able to reestablish IFN homeostasis by the EOT with SOF/RBV may be more likely to achieve a SVR, whereas patients who fail to restore homeostasis may be more prone to viral relapse. In a similar way, recent data regarding hepatitis B virus reactivation during DAA therapy supports the derangement of immune surveillance [32,33].

It is important to recall that the personal pro-inflammatory phenotype with a more or less intense inflammatory reactivity varies across humans and that the time gap between cell nesting in a distant site and nodule growth until radiology recognition will take a variable period of time.

If our concerns are confirmed by other groups, our clinical description raises a note of caution and should trigger a more ambitious pharmacovigilance action to be undertaken by all partners involved in the topic: official agencies, biomedical industry and investigators. It will become of paramount importance to scrutinize the follow-up events of patients enrolled in all prior investigations irrespective of a prior diagnosis of HCC. Frequently, the practicalities of the trials end at the time of the primary endpoint evaluation; the post-study follow-up may be done by phone and probably, do not intentionally collect cancer events. In the absence of a specific interest, such reports may have been classified as merely unfortunate coincidence and not related to the study drugs themselves. However, if the disruption of the immune surveillance system is associated with the deregulation of dormant or preclinical clones of malignant cells of any kind, careful revision of the post-study evolution of treated patients with or without HCC may disclose higher than expected cancer events at any site. It is known that younger individuals are at less risk of having suffered oncogenic hits leading to subclinical malignancy, but beyond 50–60 years the probability of cancer increases and this would become a target population on which to concentrate.

Effective treatment for HCV has been a major achievement in medicine and a long awaited goal in hepatology. Now that the available agents offer a major hope for current and future patients, we may face a drawback that may change these predictions in specific groups of patients. As mentioned, our data should be taken as a note of caution and prime a large scale assessment to confirm (or not) our findings that exceed the individual investigators capacity.

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Conflict of interest

MR: Advisory boards, conferences, travel grants from Bayer; ZM: Speaker fees from Abbvie, Gilead, Janssen; MI: Conferences, travel grants from Bayer; SL: Speaker fees from Abbvie, Gilead, MSD, Janssen; Advisor for Gilead and Janssen; MV: Advisory boards, conferences, travel grants from Bayer; BS: Speaker and/or consulting fees from Astra Zeneca, Bayer Healthcare, BMS, BTG, Medimmune, Novartis, Onxeo, and Sirtex; JLC: Speaker and consultant: MSD, Gilead, Abbvie, Janssen; XF: Unrestricted Grant Support from Abbvie and Gilead. Advisor for Abbvie, Gilead and Janssen; JB: Consultancy for Gilead, Abbvie, Kowa, Bayer, BTG, Arqule, Terumo, BMS, Boehringer Ingelheim, Kowa, Novartis, OSI, Roche and Onxeo. CP, AD, RV, AD and AR declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

Authors contributions

MR: concept and design, database, analysis writing of article; ZM: concept and design, database, analysis writing of article; CP: database, analysis writing of article; MI: database, analysis writing of article; AR: database, analysis writing of article; SL: database, analysis writing of article; AD: analysis writing of article; RV: database, analysis writing of article; AD: database, analysis writing of article; MV: database, analysis writing of article; BS: analysis writing of article; JLC: analysis writing of article; XF: concept and design, analysis writing of article; JB: concept and design, analysis writing of article.

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Supplementary data

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