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Review

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Direct-acting antivirals and hepatocellular carcinoma occurrence and recurrence in hepatitis C virus-related liver cirrhosis: fact or fiction

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Abstract

Since the widespread adoption of new direct-acting antiviral agents (DAAs), the approach to hepatitis C virus (HCV) infection has changed profoundly as almost all patients can be cured regardless of the stage of their liver disease. On the other hand, there are a few conflicting reports on the risk of hepatocellular carcinoma (HCC) occurring and recurring in patients given DAA-based therapy. The present review focuses on the latest and most relevant literature providing evidence on the occurrence and recurrence of HCC after HCV antiviral treatment with the new DAAs. Retaining the distinction between HCC occurrence and recurrence, we also discuss its patterns of presentation and speculate on the possible pathogenic mechanisms. We offer our personal viewpoints on this important issue, which has kept clinicians second-guessing in real-world clinical practice, when dealing with HCV eradication in the setting of advanced liver disease in this interferon-free era.

Keywords: Hepatitis C virus, direct-acting antiviral agent, occurrence, recurrence, hepatocellular carcinoma and liver cirrhosis

INTRODUCTION

The development of safe and effective treatments for hepatitis C virus (HCV) infection has been a major concern for hepatologists in the last few decades. The era of interferon (IFN)-based treatment regimens was plagued with frequent, severe adverse events necessitating a strict follow-up and prompt management of

complications, while sustained virological response (SVR) rates were often not very high. The prescription of IFN was also constrained by disease stage and a patient's comorbidities, making any attempt to eradicate HCV unfeasible in the case of end-stage disease.

The last few years have seen a major step forward in the treatment of HCV with the introduction of all-oral therapies. Direct-acting antiviral agents (DAAs) have since revolutionized the management of HCV patients, achieving high eradication rates with an excellent safety profile[1]. The restrictions that IFN treatments imposed have been consigned to history and SVR rates have consistently exceeded 90%, regardless of the chosen antiviral schedule[2-5]. These unexpected, striking results led to the assumption that HCV could be virtually eradicated and on a global level, interrupting the natural history of the disease without incurring any severe side effects. In fact, it has been demonstrated that liver function can improve after the virus has been eradicated, with a significant reduction in the hepatic venous pressure gradient in most patients[6-8]. This improvement has prompted the delisting of some patients with advanced disease[9,10], but the greatest benefit is seen in cases of well-compensated cirrhosis with no clinically significant portal hypertension[11].

Bearing in mind that chronic HCV infection is one of the main risk factors for the onset of hepatocellular carcinoma (HCC), lower rates of the latter's occurrence/recurrence were expected in the HCV-eradicated population, whatever the stage of their liver disease was. Accordingly as previously seen for patients successfully treated with IFN[12], the incidence rate of HCC dropped from 7.2/1000 person-years among patients with no SVR to 1.1/1000 person-years among those who achieved a SVR, as recently reported by Janjua *et al.*[13]. The widespread use of DAAs is clinically and economically cost-effective for society in the short and long term, justifying the provision of this treatment for patients with all stages of liver disease[14-17]. In fact, current treatment guidelines issued by the European and American Associations for the study of the liver recommend a timely treatment for all infected patients in order to prevent not only hepatic but also extrahepatic HCV-related complications[18-20], and thereby contain tumor-unrelated mortality[21].

Solid data on the long-term outcome of cirrhotic patients treated with these new regimens are still unavailable, however, and DAA registration trials did not distinguish between patients with and without a history of HCC[3,22-34]. Patients with HCC were treated with DAAs regardless of any presence of concomitant or prior HCC, or any other cancer. Great concern was raised, however, by the unexpected report from Reig *et al.*[35] of a presumable time-related association between DAA treatment and recurrent HCC with an aggressive pattern. This prompted several groups to analyze their results in an effort to establish whether this red flag raised on DAAs was justifiable.

To date, controversial data have emerged on HCC occurrence/recurrence after HCV eradication with IFN-free treatment regimens. Most reports come from single-center, often retrospective, observational studies, with differences in patients' characteristics and length of follow-up [Tables 1 and 2]. The picture consequently remains unclear for now, with a marked heterogeneity, even in terms of the control groups considered: some authors compared DAA-treated patients with those treated with IFN-based regimens; others compared them with untreated patients; and retrospective cohorts belonging to different eras were often involved. This might be partly attributable to several factors. For a start, HCC onset was not an endpoint in the initial DAA registration studies. Secondly, trials included patients with both chronic hepatitis C (CHC) and cirrhosis, with or without a history of HCC, and usually with a follow-up after treatment too short for the purpose of assessing HCC onset or recurrence. The incidence of HCC is also difficult to compare between patients given DAAs as opposed to IFN-based regimens because the former group also includes patients with decompensated cirrhosis, who are at higher risk of developing HCC[36]. The present review summarizes all the latest and most relevant literature regarding this issue, providing evidence on the occurrence and recurrence of HCC after HCV antiviral treatment with the new DAAs. Retaining the distinction between HCC occurrence and recurrence, we also provide a distinction between those studies comparing DAAs and no treatment, and

Table 1. Hepatocellular carcinoma occurrence after direct-acting antiviral agents treatment

Authors, year	Type of study	Patients	Cirrhotic patients (%)	Child-Pugh	%	Control group	Follow-up (median)	HCC occurrence rates	Time between DAA treatment and HCC occurrence
Conti *et al*.[37], 2016	Retrospective	285	100	CTPA CTPB	88.7 11.3	-	6 mo	3.16%	NA
Cheung *et al*.[40], 2016	Prospective	377	100	CTPA CTPB CTPC	17.2 72.7 10.1	Untreated	15 mo	4%	NA
Foster *et al*.[2], 2016	Prospective	467	87.5	CTPA CTPB CTPC	17.4 72.6 10	Untreated	6 mo	5.4%	NA
Zeng *et al*.[96], 2016	NA (letter)	31	100	NA			15 mo	0%	NA
Kozbial *et al*.[50], 2016	NA (letter)	195	100	NA	NA		12 mo	6.6%	NA
Kobayashi *et al*.[45], 2017	Retrospective	77	NA	NA		IFN-based	48 mo	3- and 5-year cumulative 1.3% and 3%, *vs.* 1% and 2.2% in controls (*P* = NS)	NA
Romano *et al*.[42], 2018	Prospective	2279	85.7	CTPA CTPB	91 9		7.4 mo	1-year cumulative 2.1	NA
Cardoso *et al*.[51], 2016	Retrospective (letter)	54	100	-	-	-	12 mo	7.4%	7.6 mo after HCV-RNA undetectability
Affronti *et al*.[47], 2016	Retrospective (abstract)	105	100	CTP > 7	80%	Relapse after IFN-free	15 mo	1-year cumulative 4.4% (*P* < 0.002 *vs.* controls)	NA
Muir *et al*.[41], 2016	Prospective	859	100%	NA	-	-	12 mo	1%	NA
Buonfiglioli *et al*.[39], 2016	Prospective (abs)	285	100	NA	-		6 mo	3.2%	NA
Carrat[97], 2016	Prospective (abs)	2156	63	NA	-	-	18 mo	4.3%	NA
Ji *et al*.[44], 2017	Prospective (Abs)	165	NA	NA	-	IFN-RBV	14 mo	Not different between groups	NA
Innes *et al*.[98], 2017	Retrospective (Abs)	570	100	NA	-	IFN-based	22 mo	7% (not different between groups)	NA
Calvaruso *et al*.[99], 2017	Retrospective (Abs)	3447	77.8	CTPA CTPB	68 9.2	-	8.5 mo	1.44% overall 1.69% in CTPA 4.37% in CTPB	NA
Issachar *et al*.[100], 2017	Retrospective (Abs)	273	NA		-	-	15 mo	2.1%	NA
Bielen *et al*.[48], 2017	Retrospective (Abs)	332	NA	CHC + CTPA	100	-	-	1.5%	NA
Waziry *et al*.[61], 2017	Metanalysis	9 studies	100	NA	-	IFN-based studies	-	RR 0.68, 95% CI 0.18-2.55, *P* = 0.5)	NA
Ioannou *et al*.[49], 2017	Retrospective	26483	16.8	-	-	IFN based cohort	72 mo	3.8% in IFN-FREE + IFN 2% in IFN-FREE only	NA
Nagaoki *et al*.[43], 2017	Retrospective	154	NA	NA		IFN based cohort	23 mo	Cumulative 1 and 5-year: 0.6% and 9% (*P* = ns *vs.* control group)	22 mo after end of treatment
Nagata *et al*.[46], 2017	Retrospective	669	NA	NA	-	IFN-based cohort	20 mo	Cumulative 3-year: 1.4% (*P* = 0.49 *vs.* controls)	NA

Kanwal *et al*.[101], 2017	Retrospective	22500	39	NA	-	Relapse after SVR		Cumulative 1-year - Overall 1.18% - SVR 0.9% - Relapse 3.4%	5.2 mo in SVR patients vs. 6.1 mo in non NA SVR, after end of treatment
Ogata *et al*.[102], 2017	Retrospective	1170	NA	NA	-	-	1.3 years	1.9%	0.5 years after end of treatment
Deterding *et al*.[103], 2017	Retrospective	863	100	CTPA CTPB CTPC	69.9 13.7 1.7	-	-	1.4%	NA
Finkelmeier *et al*.[104], 2018	Retrospective	819	32.8	CTPA CTPB CTPC	78 19 3	IFN-based cohort	8.8 mo	3.1% *vs.* 5.4 in controls (*P* = NS)	312 days after end of treatment
Li *et al*.[53], 2018	Retrospective	5834	19.9%	NA	-	IFN-based cohort	-	0.86% (22.8 per 1000 person year; *P* = NS *vs.* IFN)	NA
Romano *et al*.[42], 2018	Prospective	3917	75.5	CTPA CTPB	80.7 11.9	Untreated	17.4 mo	1.4% overall - 0.42% in F3 patients - 1.88% in cirrhotics	31.8 w after treatment start

HCC: hepatocellular carcinoma; DAA: direct-acting antiviral agent; NA: not available; NS: not significant; IFN: interferon; CTP: Child-Turcotte-Pugh; SVR: sustained virological response; CHC: chronic viral hepatitis; RBV: ribavirin

those ones including a control group of patients treated with IFN. Finally, we discuss HCC patterns of presentation, speculating on the possible pathogenic mechanisms.

HCC OCCURRENCE

DAAs *vs.* no treatment

Conti *et al*.[37] retrospectively analyzed the occurrence of HCC in compensated patients with cirrhosis with no history of liver cancer, who achieved a SVR after IFN-free treatment regimens. They found an HCC occurrence rate of 3.1% within 6 months after treatment, which was higher than what was previously observed in the natural history of untreated HCV-related cirrhosis[38]. These preliminary findings were confirmed in a subsequent publication by the same authors[39]. The occurrence of HCC after IFN-free treatment was again similar to the rate seen in untreated patients in another prospective English study by Cheung *et al*.[40] in patients with decompensated disease. It is worth noting that most of these new cancers were diagnosed within the first 3 months of therapy, which might mean that the cancer was already there when the antiviral treatment was started. These two studies were underpowered due to a short follow-up, and might well have underestimated the true incidence of HCC, but data coming from studies with a longer follow-up substantially confirmed these results[41]. In particular, our experience comes from a large sample of patients treated at several centers in northern Italy with a median follow-up of 17.4 months[42]. During this period, the HCC occurrence rate in the sub cohort of cirrhotic patients was much the same as (or even lower than) expected without antiviral therapy. The incidence of HCC significantly dropped after the first year in both Child-Turcotte-Pugh (CTP)-A and CTP-B patients (Mantel-Cox test, $P = 0.00008$). The reason for this is unclear, but it might relate to a greater reduction in intrahepatic inflammation in the longer term after stopping the antiviral therapy. Foster *et al*.[2] prospectively compared the outcome of 467 patients treated with DAAs in the UK in 2014 with a group of untreated cirrhotic patients finding no difference in HCC occurrence rates within 6 months. Even though the incidence found in the English cohort was almost twice as high as in the Italian study by Conti *et al*.[37], we have to consider that the patients were much more severely decompensated.

It seems that the SVR obtained with DAAs does not substantially change the natural incidence of HCC in cirrhotic patients, in the short to medium term at least. Patients already in the advanced fibrotic stage before

Table 2. Hepatocellular carcinoma recurrence after direct-acting antiviral agents treatment

Authors, year	Type of study	Patients	Cirrhotic patients (%)	Child-Pugh	%	Control group	Follow-up (median)	HCC recurrence rates	Time between last HCC treatment and DAA start	Time between DAA treatment and HCC recurrence
Reig *et al*.[35], 2016	Retrospective	58	94.8	CTPA CTPB CTPC	91 5.4 3.6	-	5.7 mo	28%	11.2 mo (8.7 mo in patients with recurrence, 15 mo in those without)	3.5 mo after DAA start
Conti *et al*.[37], 2016	Retrospective	59	100	CTPA CTPB	88.7 11.3	-	6 mo	28.8%	376 days (446 days in patients with recurrence, 360 days in those without)	NA
ANRS collaborative study group on hepatocellular carcinoma[56], 2016	Retrospective HEPATHER cohort	189	85	NA	-	Untreated	20 mo	0.73/100 person-month (P = 0.87 *vs.* controls)	NA	NA
ANRS collaborative study group on hepatocellular carcinoma[56], 2016	Retrospective CirVir cohort	13	100	CTPA	100	Untreated	59 mo	1.1/100 person-month (P = 0.75 *vs*. controls)	Included patients were considered to be in remission at least 3 mo following implementation of at least one curative procedure	37.1 mo (one patient)
Cheung *et al*.[40], 2016	Prospective	29	100	CTPA CTPB CTPC	17.2 72.7 10.1	-	15 mo	6.9%	NA	20 w and 26 w after treatment start (two patients)
Zavaglia *et al*.[57], 2017	Retrospective (letter)	31	100	CTPA CTPB	81 19	-	8 mo	3.2%	19.3 mo (1.7 mo since last assessment)	8 mo (one patient)
Petta *et al*.[59], 2017	Retrospective	58	94.8	CTPA CTPB CTPC	91 5 4	IFN based cohort	18 mo	Cumulative 1 and 5-year: 12.9% and 39.1% (P NS *vs.* controls)	NA	NA
Cabibbo *et al*.[58], 2017	Prospective	143	100	CTPA CTPB	86 14	Untreated	8.7 mo	6-,12-,18-month recurrence: 12%, 26.6%, 29.1%. No differences in terms of time to recurrence with untreated patients	NA	NA
Ikeda *et al*.[105], 2017	Restrospective	177	NA	NA	-	Untreated	20.7 mo	Recurrence rates at 1st and 2nd year were 18.1 and 25.0% in pts with DAA therapy and 21.8 and 46.5% in those without DAAs, (P = 0.003)	10.7 mo	NA

HCC: hepatocellula carcinoma; DAA: direct-acting antiviral agent; NA: not available; NS: not significant; IFN: interferon; CTP: Child-Turcotte-Pugh; SVR: sustained virological response; CHC: chronic viral hepatitis; RBV: ribavirin

Table 3. Supposed immunological derangements induced by direct-acting antiviral agents viral eradication

Reduced homing of leucocytes towards the liver (HCC-specific and non-specific CD8+ T cells)
Normalization of the NK-cell compartment
Decreased TRAIL-expression
Enhanced proliferation of few isolated malignant cells already present at treatment starting
Lack of continuous IFN-stimulation in the liver
Changes in miR-122 levels
Increase in serum vascular endothelial growth factor with increased liver cancer angiogenesis
More aggressive immunologic pattern already present, before the immune changes due to DAAs occur
Partial reversion of histone modifications induced by chronic HCV infection

HCC: hepatocellula carcinoma; NK: natural killer; TRAIL: tumor necrosis factor-related apoptosis-inducing ligand; IFN: interferon; DAA: direct-acting antiviral agent; HCV: hepatitis C virus

HCV eradication carried the same neoplastic risk afterwards. The benefits of eradicating the virus, as opposed to leaving cirrhotic patients untreated, would probably emerge more clearly in the much longer term, when the effects of having put a stop to the continuous virus-related damage would become more evident.

DAAs *vs.* IFN-based regimens

Some authors compared DAA-treated patients with those given IFN to see whether the novel, rapid virus-eradicating mechanism was able to modulate HCC occurrence as much as previous treatments had done. But the two populations being compared not only belonged to different historical periods, but also happened to differ considerably because of the greater accessibility of today's new drugs. The comparison was also further undermined by the excessively broad inclusion criteria used in most of such studies (in which both patient groups had CHC or cirrhosis, for instance).

Nagaoki *et al.*[43] investigated HCC occurrence after daclatasvir/asunaprevir treatment in 154 patients with CHC or cirrhosis. After appropriate propensity score matching analysis with a historical cohort of 244 patients treated with IFN-based regimens, the cumulative HCC incidence at 1, 3 and 5 years was respectively 0.6%, 9% and 9% for the DAA group, and 0.4%, 3% and 5% for the IFN group ($P = 0.053$).

The above findings were confirmed in another two Asian cohorts from China[44] and Japan[45]. On multivariate analysis, the risk of HCC onset was significantly associated with alcohol abuse as a cofactor, but not with IFN-based as opposed to IFN-free antiviral treatment, after adjusting for age, gender and baseline cirrhotic status.

Nagata *et al.*[46] retrospectively compared large cohorts of patients treated with IFN-based *vs.* DAA regimens in Japan who had CHC or cirrhosis with no history of HCC, but no sub analysis of cirrhotics alone was available. When the authors used propensity score analysis to reduce the bias of different follow-ups after achieving a SVR (6.8 *vs.* 1.8 years, respectively), they found the 3-year cumulative occurrence rate of HCC was similar in the two groups (3.3% *vs.* 1.4%, $P = 0.49$). Notably, the cumulative incidence of HCC was significantly lower for patients achieving a SVR in both groups so that SVR after IFN-free regimens was likewise associated with a lower rate of HCC development as in the previously-mentioned study by Cheung *et al.*[40] and even in the cohort described by Affronti *et al.*[47] which had a great percentage of decompensated patients. In a retrospective multicenter analysis involving 15 centers in Belgium, Bielen *et al.*[48] found that early HCC occurrence rates were similar in patients with CHC or compensated cirrhosis treated with DAAs, with or without IFN (DAAs plus IFN *vs.* DAAs alone: 3.6% *vs.* 1.1%). Lastly, Ioannou *et al.*[49] found SVR associated with a significant reduction in HCC developing in HCV-related cirrhosis compared with situations when the treatment failed, whatever the type of treatment (IFN, DAA, or combinations of both), after the mean 6.1 years of follow-up.

Conversely, Kozbial *et al.*[50] and Cardoso *et al.*[51] reported their experiences, from Austria and Portugal respectively, of an unexpectedly high incidence of HCC, which however remained isolated reports. Additionally, the whole cohorts' baseline characteristics were unavailable and patients with F3 fibrosis were included, making it impossible to compare these results with other studies.

After the initial rush to report on small populations with short follow-ups, some longer-term studies on larger cohorts have begun to emerge. One recent study using real-world data found DAA-based HCV treatment unassociated with any increased risk of incident liver cancer. This risk even seemed to be lower than in either untreated or IFN-treated patients, suggesting that the benefits of DAA treatment will become more apparent with time. In the study by Singer *et al.*[52], after adjusting for gender, age and disease stage, DAA treatment was associated with a significantly lower risk of liver cancer by comparison with no treatment (adjusted hazard ratio (HR) = 0.84, 95% CI: 0.73-0.96), or IFN-based treatment in the pre-DAA era (HR = 0.69, 95% CI: 0.59-0.81). Using the Electronically Retrieved Cohort of HCV-Infected Veterans database, Li *et al.*[53] found that, among the cirrhotics with a SVR, neither the HCC incidence rate nor HCC-free survival differed significantly between the DAA and IFN groups (P = 0.78; and log-rank, P = 0.17). Both treated groups had a significantly lower probability of developing HCC than the untreated group (log-rank, P = 0.0004).

In short, though extremely heterogeneous, the above-mentioned studies seem to suggest (with the exception of a few isolated cases) that, regardless of how it is achieved, a SVR lowers the likelihood of HCC, albeit to a different degree depending on the stage of liver disease. Once a SVR has been achieved, it seems that comorbidities and lifestyle begin to have a major role, and should therefore be taken into account.

Taking another perspective, Cucchetti *et al.*[21] estimated the influence of DAA regimens on patient mortality using a Markov model. They found DAA-based antiviral treatment associated to a drastic reduction in mortality unrelated to cancer before any onset of HCC, with only a slight increment in the HCC occurrence rate. The 20-year mortality due to causes other than HCC dropped by 21.9% in patients without varices, and by 27.5% in those with varices. Thus, assuming the cancer risk remains unchanged, the larger number of survivors generated a longer lifetime risk of developing HCC.

HCC RECURRENCE

DAAs *vs.* no treatment

The first warning came from a Spanish multicenter study[35] showing a high rate of HCC recurrence in compensated cirrhotic patients considered to be in oncological remission after undergoing resection (34.5%), ablation (55.2%), and trans arterial chemoembolization (10.3%), and receiving DAA treatment, with a median follow-up of 5.7 months. This finding was supported by the same authors' comparisons of HCC recurrence rates, after surgical resection in one prospective study (in which HCC recurrence rates at 4 months were 13.5% in high-risk patients, and 3.8% in low-risk cases), and after ablation in another prospective series (unpublished data) (2.45% at 4 months and 27.6% at 12 months), as well as in the double-blind, placebo-controlled STORM trial[54]. Interestingly, the highest rate of recurrence (41.17%) was seen in patients with a short interval (< 4 months) between HCC treatment and latest imaging assessment of complete response. In this regard, Cammà *et al.*[55] analyzed the data in the Reig study, and showed that the probability of HCC recurrence during the first 6 months after starting DAAs was twice as high for patients with a shorter interval between their HCC treatment and their latest assessment of complete response compared with patients with longer intervals (< 15%). This may mean that the high early tumor recurrence rate described by Reig and coworkers was driven largely by individual cases, including those initially observed, initiated on DAAs shortly after being treated for HCC.

The paper by Reig *et al.*[35] was not the only one to suggest that particular attention should be paid to managing patients with HCC receiving DAA treatment. Conti *et al.*[37] confirmed that DAA-induced HCV eradica-

tion does not reduce HCC recurrence in the short term. The design of this latter study was very similar to the Spanish one. The crude HCC recurrence rate was almost identical to the one reported by Reig *et al.*[35], but the follow-up after starting DAA was longer, thus leading to a lower time-based incidence.

Here again, however, the study design did not allow for the HCC recurrence rate to be defined as higher, lower or the same as expected in the natural history of the disease.

Other studies analyzing different populations did not confirm such a high risk of HCC recurrence following DAA-based therapy. The largest study concerned a French multicenter cohort[56] in which two different groups of pre-transplant patients were prospectively followed up: the HCC recurrence rates were similar in DAA-treated and untreated patients.

A rather similar low rate of HCC recurrence in cirrhotic patients treated with DAAs was also reported by Zavaglia *et al.*[57] along with Cheung *et al.*[40] reporting respectively on compensated and decompensated cirrhotic patients.

Similarly, Cabibbo *et al.*[58] found 6-, 12- and 18-month recurrence rates comparable with the figures reported in the literature for untreated patients, and the time to recurrence was much the same too. A history of HCC recurrence and tumor size emerged as two independent risk factors, and the authors suggested they be used to stratify patients by risk of early HCC recurrence.

Besides the few initial alarming studies, in which probably the HCC recurrence just happened to coincide with their antiviral treatment follow-up, it is becoming clear that DAAs do not substantially change the risk of recurrence in patients with advanced liver disease and previous history of HCC. Indeed, in these patients, in which the neoplastic process did already take place, the risk of recurrence remains high, appearing not be influenced by viral eradication. It is possible in fact that, contrariwise to patients without any previous tumoral history, the process, once triggered by active viral replication on a cirrhotic ground, becomes independent from the replication status so that recurrence rates and timings remain unmodified after treatment being modulated by HCC characteristics instead.

DAAs *vs.* IFN-based regimens

The experience of the Italian Liver Cancer Group, recently reported by Petta *et al.*[59], demonstrated that both IFN-based and IFN-free HCV clearance result in longer times to tumor recurrence in patients with HCC radically treated with either resection or ablation, with no significant difference between the two virus eradication treatments.

In a European multicenter study by Kolly *et al.*[60], the time elapsing between HCC treatment and DAA initiation emerged as a predictor of recurrence, in line with the analysis by Cammà *et al.*[55].

Obtaining a SVR with DAAs (as opposed to IFN-based treatment or no treatment) does not seem to enhance the risk of HCC recurrence, which appears to be better predicted, again by other tumor- and patient-related variables. That said, studies on cancer recurrence should always report the tumors' baseline characteristics, the type of treatment administered, and the time elapsing before starting DAA to enable results to be interpreted correctly. In a meta-analysis, Waziry *et al.*[61] recently confirmed the uncertainty of correlating HCC occurrence or recurrence with DAAs: they found no evidence for a correlation between IFN-free regimens and HCC development, and confounders such as a shorter mean follow-up or older age emerged as potential biases influencing the studies that sounded the alarm. The authors confirmed that HCV eradication reduces the risk of HCC in patients who achieve a SVR, while older age, advanced cirrhosis, and worse baseline patient features were independent predictors of HCC onset in the DAA-treated population, which helps to

explain their apparently higher risk (3.1 *vs*. 1.1/100 per years). In another meta-analysis conducted on 24 studies[62], the factors associated with recurrent HCC included a history of HCC recurrence, and a shorter interval between HCC complete response and DAA initiation. This led the authors to recommend delaying DAA treatment for at least 6 months after HCC treatment, thus enabling a longer immune surveillance of existing microscopic HCC clones. Delaying DAA treatment could also allow more time to assess HCC treatment response, thereby minimizing the chances of misclassification bias. Such a delay was merely a precautionary (not evidence-based) suggestion, said the authors, that might be adopted in clinical practice while we wait for this HCC-DAA issue to be solved. Even though we still need more long-term evidences to disconfirm the possible role of DAA-mediated viral eradication in enhancing HCC recurrence, which is supported also by the lack of those immune-modulating properties held by IFN, current available evidences are not supporting this hypothesis. To help further evidences clarify this issue, clinicians should always document correct assessment of response after HCC treatments, possibly shortly before DAA start, and estimate recurrence risk on tumors' features and patients' related risk factors. Additionally, when comparing DAAs-treated patients with those treated with IFN, adjustments for disease stages should always be conducted as baseline risks have different reference ranges.

HCC PATTERN

Occurrence

Nakao *et al*.[63] investigated the pattern of *de novo* HCC, reporting 6 cases of pathologically-confirmed HCC in patients with a SVR after treatment with DAAs. All these patients' tumors were single nodules, moderately differentiated and growing rapidly: these unconventional features (when compared with previous series) might overlap with the unexpected early tumor recurrence as described by Reig *et al*.[35]. In our own experience, with the northern Italian cohort[42], we found what seemed to be a more aggressive pattern of HCC presentation: among 16 patients developing HCC (29.1% of the sample), 8 (14.5%) presented with multiple nodules of various size, 8 (14.5%) with an infiltrative diffuse HCC, 6 (10.9%) with portal thrombosis, and 4 (7.2%) with extrahepatic metastases. Given the clinical importance of these findings, Renzulli *et al*.[64] aimed specifically to examine the radiological features of microvascular invasion (MVI) in a retrospective analysis of 344 consecutive patients with HCV-related cirrhosis treated with DAAs and followed up for 48-74 weeks. After DAA treatment, HCC developed in 29 patients (11/29, 38% multi-nodular); forty-one HCC nodules were detected (27 of them recurrent), with imaging suggestive of MVI in 29/41 (70.7%) nodules, even in 17/29 (58.6%) nodules 10-20 mm in diameter. On the other hand, MVI was only present in 17/51 (33.3%) of the HCC nodules developing before any DAA treatment ($P = 0.0007$). These surprising data come from different cohorts and cannot be attributed simply to a lack of surveillance, because patients were strictly followed up. That said, it is important to remember that all these alarming findings came from small cohorts, and often from retrospective single-center experiences. The picture they paint contrasts with the report on the large historical French cohort[65], in which cancer presented as a single nodule in 69.6% of cases, as 2 or 3 nodules in 19.8%, and was infiltrative or with more than 3 nodules in only 10.8%.

Recurrence

Reig *et al*.[35] reported not only on a higher incidence of HCC recurrence, but also on a possibly more aggressive neoplastic pattern in recurrences after DAA treatment: 25% of the recurrences in the original Spanish cohort were multi-nodular, and 20% of them had an infiltrative pattern, despite the fact that the majority of the HCCs included in this analysis were at low risk of recurrence (judging from nodule size, Barcelona Clinic Liver Cancer stage, and histopathology of the resected tumor in patients who had surgery). In the previously-mentioned study by Cabibbo *et al*.[58], the pattern of recurrence varied: 28 patients developed intrahepatic growths, and 24 of them had a nodular profile, while 5 (one with MVI) developed infiltrative HCC. None of the patients developed extrahepatic metastases.

Very little information is available regarding the characteristics of recurrent tumors, however, so that it is almost impossible to draw any conclusions.

On the other hand, albeit in a very different setting, we were able to compare the histopathological features of HCC on livers explanted from a small cohort of patients transplanted at our center who were treated with DAAs while listed for a transplant with active HCC, and having HCC bridging treatments at the same time. We found no histopathological differences in median number and total volume of HCC nodules, tumor differentiation or MVI[66] vis-à-vis a contemporary untreated cohort involving patients and tumors with comparable baseline characteristics.

POSSIBLE PATHOGENIC MECHANISMS

Another particular issue that came to light with the first "warning report" concerns the possibility of HCV clearance from the liver, and the consequent impairment of the local immunological microenvironment, having an impact on HCC biology [Table 3]. Chronic stimulation of antigens against HCV infection contributes to virus-specific CD8+ T-cell exhaustion, continuative activation of host-mediated liver inflammation (driven partly by endogenous IFNs), and altered innate immune cell populations[67,68]. IFN can modulate both innate and adaptive immune system. Different cells and pathways can be involved, including but not limited to inhibition of CD4+ and CD8+ T-cell proliferation, direct activation of natural killer (NK) cells, and suppression of IL-12 production by monocytes[63]. The immune "restoration" that follows IFN-based antiviral therapy, together with eradication of the virus, was considered to be the pathophysiological explanation for the decrease incidence of HCC in patients achieving SVR with IFN-based antiviral therapies. There has been speculation that the mechanism by which patients with cirrhosis on IFN-free treatment might experience a higher HCC rate could relate to a reduced tumor-specific immune surveillance, particularly as concerns the HCC-specific CD8+ T-cells. In fact, DAA-induced HCV eradication could lead to a rapid decline in HCV-specific and non-specific T-cells from the liver, with a reduced homing of leukocytes towards the liver. This weaker infiltration by lymphocytes has been shown to correlate with a higher risk of HCC recurrence[69-73]. Debes *et al*.[74] recently speculated on other potential mechanisms of immune derangement during DAA therapy, involving NK-cell activity and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) expression. These immune mediators have consistently been found increased during HCV infection, potentially blocking HCC cell proliferation while attempting to contain the virus[75,76]. The demonstrated DAA-induced normalization of the NK-cell compartment, together with the decrease in TRAIL receptor 2 expression, could therefore be responsible for a less efficient immune surveillance, and thus favor HCC recurrence. Whether or not the high HCC recurrence rate could be due to radiologically-undetectable tumors growing rapidly remains to be seen, however. Cheung *et al*.[40] suggested that the rapid recurrence of HCC noted in DAA-treated patients might be prompted by the enhanced proliferation of a few isolated malignant cells already present when the treatment started giving rise to a rapid tumor growth, rather than by any *de novo* clone development. Indeed, the regeneration mechanisms activated by the rapid cure of inflammation, and differences in the immunological environment compared with IFN-based treatments could be responsible for the unrestricted growth of precancerous lesions or small malignant cell clones[50]. The lack of any continuous IFN stimulation in the liver after eradication of the virus probably has a significant impact on intrahepatic immune responses too, giving rise to a less efficient neoplastic surveillance and enhancing neoplastic cell proliferation after an SVR has been achieved[77,78]. The peculiarity of the warning in the Spanish report lies in the timing of HCC recurrences, which peaked during antiviral treatment and soon afterwards: this prompted several groups to investigate molecular changes occurring during this particular time frame. For instance, miR-122 concentrations were found to correlate with virus-induced liver inflammation and HCV-RNA levels, and serum levels decreased in patients with a SVR treated with a 12-week course of paritaprevir/ritonavir + dasabuvir or ombitasvir[79]. MiR-122 has a central role in suppressing viral replication and it reduces tumorigenesis, angiogenesis and intrahepatic metastasis[80], serving as a marker of disease status and response to therapy[81-83]. During the first two weeks of the DAA treatment, changes in miR-122 levels were similar across genotypes, and comparable with or without ribavirin. Interestingly, miR-122 remained below the baseline levels throughout post-treatment week-12 in patients who subsequently achieved

a SVR, whereas they began to return to baseline levels after the second week of treatment in patients who did not[79]. Villani *et al.*[84] also observed an early rise in serum levels of vascular endothelial growth factor (VEGF) and a change in the inflammatory pattern 4 weeks after initiating DAA treatment, suggesting an increased liver cancer angiogenesis and tumor growth during this time. These changes returned to normal after the end of treatment, however. In this regard, Faillaci *et al.*[85] recently reported the result of a prospective study that confirmed the role of VEGF pathways in HCC occurrence and recurrence. In a cohort of 183 patients with cirrhosis, 14/28 (50.0%) with previous HCC recurred while 21/155 (13.5%) developed *de novo* HCC. DAA therapy was associated with a significant increase of VEGF expression and this was significantly correlated with an increased rate of HCC occurrence/recurrence in "high-risk" patients. These patients were characterized by a baseline elevated and abnormal activation in liver tissues of neo-angiogenetic pathways, as shown by increased level of angiopoietin-2. From a clinical perspective, they presented a greater severity of baseline liver disease, as shown by higher portal collateralization and liver fibrosis scores. VEGF increased during DAA therapy, remaining elevated during follow-up, and significantly correlated with serum angiopoietin-2. Furthermore, angiopoietin-2 expression in the primary HCC or in cirrhotic tissue before DAAs was independently related with risk of HCC recurrence [odds ratio (OR), 1.137; 95% CI, 1.044-1.137; $P = 0.003$] or occurrence (OR, 1.604; 95% CI, 1.080-2.382; $P = 0.019$).

Debes *et al.*[86] found 12 different soluble tumor markers (out of 22 tested, including markers of apoptosis, cytokines and growth factors) that were significantly higher before DAA treatment in patients who developed *de novo* HCC than in matched controls who did not. This raises the possibility of patients who eventually develop HCC already having a more aggressive immunological pattern, even before any immune changes due to DAAs occur, suggesting that the immune profile modulating HCC growth is attributed more to patients' prior individual characteristics than to changes induced by DAA-mediated HCV clearance. Epigenetic effects could be affected by DAA-mediated HCV eradication too. It was recently found that the marked changes in histone methylation induced by chronic HCV infection were only partially reversed by eradicating the virus with DAAs[87]. An abnormal transcription could contribute to driving HCC tumorigenesis, alongside the other mechanisms already discussed. What is clear is that immune system plays a crucial role in terms of neoplastic surveillance[88]. Trying to translate this concept into clinical practice, different prognostic scores that include also immunological variables have been conceived and successfully implemented in different types of tumor, including HCC. In this regard, systemic immune-inflammation index, neutrophil to lymphocyte ratio, platelet to lymphocyte ratio, aspartate aminotransferase-lymphocyte ratio index (ALRI), and albumin/bilirubin score (ALBI) have been shown to predict both survival and recurrence risk in patients with HCC[89-91]. Recently, Casadei Gardini *et al.*[92] tested the applicability of these scores in the specific setting of HCC recurrence after DAA, showing that ALBI and ALRI scores are promising practical tools able to stratify the risk of HCC development/recurrence in DAA treated patients. More particularly, at multivariate analysis, increase in ALBI grade ($P = 0.038$, HR = 2.35, 95% CI: 1.05-5.25) and ALRI ($P = 0.008$, HR = 1.05, 95% CI: 1.01-1.09) were independently associated with HCC development and recurrence, respectively. Importantly, risk of recurrence was adjusted for the time from HCC treatment.

CONCLUSION

In summary, the available data are not consistent enough to judge the risk of HCC occurrence/recurrence after IFN-free HCV eradication treatment. To better understand the reportedly diverse rates of liver cancer development, several factors should be taken into account, such as the achievement of a SVR, and the severity of liver dysfunction (the higher the Child-Pugh score, the higher the risk of HCC). We should consider patients' comorbidities and lifestyles too, particularly factors that might have an additional procarcinogenic potential, such as diabetes, smoking, alcohol abuse, and so on. Another important issue in assessing HCC recurrence risk is the time frame between tumor eradication and starting DAA treatment: this is not always taken into account, but it could well help to explain the contrasting results between different studies. Large cohort studies and meta-analyses suggest that there is no direct correlation between these two events. Addi-

tionally, more recent studies are starting to exclude early occurrences/recurrences from risk analyses as they could be an expression of undetected clones rather than induced by DAAs-mediated eradications, overestimating the real post-treatment incidences. Besides, the rates of new-onset HCC appear more homogeneous than the recurrence rates across the various studies, indicating that DAAs do not modify the incidence of HCC in the short term after HCV eradication. The populations investigated differed considerably in many aspects, however, and baseline characteristics were not always available, making data comparisons difficult. The very discordant HCC recurrence rates between different studies mean that, for the time being, it is virtually impossible to draw any useful conclusions.

Given the uncertainty surrounding the HCC occurrence and recurrence rates, despite their having been widely investigated in many different settings, specific pathogenic studies are still needed to demonstrate the link, if any, between DAA-mediated virus eradication and liver carcinogenesis, especially if an aggressive pattern is confirmed in HCCs occurring or recurring afterwards. Sudden changes prompted by DAAs in a chronically-inflamed liver might disrupt its anti-tumor response, but we still have too little evidence to attempt to see the whole picture. Hopefully, further translational studies will shed light on who are those patients that should be considered at high-risk of developing HCC recurrence, giving clinicians new biomarker or clinical scores that can help them in the clinical practice.

Our strategy for now is to eradicate HCV in early-stage disease, to rule out any HCC before starting antiviral treatment, and then to strictly follow up cirrhotic patients after they have achieved a SVR (based on the current EASL-EORTC clinical practice guidelines for HCC surveillance)[93]. A strict follow-up is especially necessary in certain settings, such as patients on the waiting list for liver transplantation. Until further studies prove otherwise, we prefer not to delay antiviral treatment in well-compensated cirrhotic patients in order to avoid further liver deterioration and extrahepatic complications of HCV.

Dedicated, long-term prospective randomized interventional studies with proper controls are much needed to clarify this important issue, but the numerous variables involved in HCC occurrences and recurrences, and differences in screening protocols (often involving operator-dependent procedures, different timings, and no proper control groups) will make this a challenge. Significant changes in HCV epidemiology are also to be expected in the near future, as the virus will be virtually eradicated in the early stages of the infection. This will lead to a drastic reduction in cases of HCV-related end-stage liver disease, and correlated HCC[94], meaning that the question of whether or not to treat patients' HCV because of any associated risk of HCC will become largely irrelevant.

Randomizing patients for such intervention would not be ethical, given the clearly-demonstrated benefits of DAA therapy in patients with cirrhosis. This includes those with decompensated disease, who are at highest risk of HCC, who also gain from the additional chance of being delisted for transplantation, and thus allowing organs to be allocated to others[9,95].

DECLARATIONS

Authors' contributions

Bibliographical search, drafting of the manuscript, approval of the final version: Zanetto A, Shalaby S, Ferrarese A

Bibliographical research: Ferrarese A, Becchetti C, Sciarrone S, Germani G, Senzolo M, Gambato M, Russo FP

Critical revision of the manuscript and final approval: Burra P

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