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Review

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Hepatocellular carcinoma after treatment of hepatitis C with direct-acting antivirals: a critical re-appraisal

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Abstract

Soon after introducing direct-acting antiviral agents (DAAs) for chronic hepatitis C treatment, there began a debate over the possibility of hepatocellular carcinoma (HCC) after viral clearance. Although several reports suggested that the question has been answered negatively, other reports suggested the opposite. The present review presents data in favor and against the null hypothesis and analyzes the scientific background of the possible participation of DAAs in HCC development. The reasons for the discrepancy among studies are presented. These include heterogeneity of patient selection, the nature of the studies, and the tumors themselves are responsible for varying results. Exogenous factors like alcohol consumption or metabolic syndrome confound these findings and suggest the need for statistical adjustments. The need for careful attention to the statistical details is exemplified, and the significant points of almost universal agreements are identified. The conclusion is that the definitive study is impossible for ethical and scientific reasons, and the physician should not ignore even simple personal observations and screening of all patients with extensive fibrosis in HCC, irrespective of sustained virologic response, until a robust, reliable prognostic model can be invented.

Keywords: Hepatocellular carcinoma, direct-acting antivirals, incidence, recurrence

INTRODUCTION

Direct-acting antivirals (DAAs) have changed the treatment approach to chronic hepatitis C (HCV) as they achieve high sustained virological response rates (SVRs) in cirrhotic patients and prolong overall survival with few side effects[1-3].

The first therapeutic efforts of chronic HCV were based on interferon (IFN) with or without ribavirin. Even then, there was a significant risk of HCC or recurrence after SVR; a yearly incidence of 1.13% was reported[4]. DAA treatment did not change these earlier observations, and incidences between 1.5 and 3.6/100 patient-years were not uncommon[5-7], mainly in cirrhotics with substantial portal hypertension[8]. However, soon after the introduction of DAAs, a debate started on the possibility of higher-than-expected occurrence and recurrence of HCC in patients after SVR[9,10]. Many studies supporting or disputing the initial reports were published. These findings conflict, and the debate continues[11]. A meta-analysis reported that an increased effect of DAAs on HCC recurrence could not be confirmed or refuted because of the extreme heterogeneity of studies[12]. Nevertheless, a commentary on this paper declared an end to the debate, claiming that the matter was settled and DAAs are not associated with HCC recurrence[13].

In this report, we present the evidence in favor and against the increased association of DAAs with HCC and review the possible underlying mechanisms of HCC complicating viral clearance. We focus on the reasons behind the profound diversity of the results published to date and summarize the facts that are beyond dispute by almost all investigators.

In favor of increased HCC after DAAs

Observational studies

Early after the introduction of DAAs, many observational studies reported an alarmingly higher-than-expected occurrence or recurrence of previously-treated HCC after DAA administration. These were uncontrolled reports without a control arm. Nonetheless, these findings could not be ignored, and a heated debate began[9,10,14-17].

An early meta-analysis showed high HCC recurrence rates of 7.4% after six months and 47.0% after two years in untreated patients after an initial curative resection or ablation of HCC, but also extreme variability in recurrence risk and survival[18]. An uncontrolled, real-world Spanish retrospective study confirmed the initial high HCC recurrence rates[19]. Similar unexpectedly high occurrence and recurrence rates were reported in more uncontrolled prospective and retrospective studies[20-25]. A critical observation was that de novo development and HCC recurrence appeared early after DAAs administration[26]. Another essential observation was made by a large retrospective study reporting an increased observed HCC occurrence associated with the time of DAAs introduction and the presence of uncharacterized liver nodules[27]. Early HCC occurrence and increased recurrence in patients with undefined nodules after DAAs treatment was confirmed in a prospective study[28].

Interestingly, the modality used for HCC treatment was associated with the recurrence rate after DAAs. Thus, patients treated with Ttrans arterial chemoembolization (TACE) had a much greater recurrence than other modalities[29]. This observation was confirmed in a multicenter retrospective study that assessed risk factors of late HCC recurrence 24 weeks after SVR with DAAs. The five-year cumulative HCC recurrence rates for the previous curative and palliative treatment groups were 45.4% and 65.7%, respectively[30].

DAAS vs. no treatment

A higher recurrence rate after liver transplantation was reported in patients treated with DAAs than in untreated patients[31]. Increased recurrence was also reported in a large group of patients from a liver transplantation registry in a prospective cohort using untreated patients as the control arm[32]. HCC aggressivity was increased after DAAs treatment compared to treatment-naïve patients[33].

DAAs vs. IFN

Several studies compared DAAs with IFN. A Belgian retrospective study compared DAAs with IFN administration but failed to find differences because the control arm was DAAs plus IFN. Nevertheless, they confirmed an earlier development of HCC in the DAAs-only arm[34]; a retrospective controlled study with IFN as the control arm confirmed this result[35]. An initial analysis of the ANRS CO12 CirVir study showed more HCC in patients treated with DAAs than IFN; however, they attributed the difference to patient characteristics and screening protocols[36]. A large retrospective study analyzed a liver transplant registry and showed that patients during the IFN period had a reduced HCC occurrence rate compared to patients during the DAAs period[37].

Two studies assessed HCC recurrence rates in DAAs-treated patients and compared them with two control groups (IFN administration and no treatment). In the first study, there was a strong trend toward an increased HCC recurrence in transplanted patients who received DAAs before transplantation compared with viremic patients without any treatment, while no difference was observed between DAAs and IFN[38]. In contrast, another study reported a higher recurrence rate in DAAs-treated patients than in IFN-treated patients; however, no difference was found between DAAs and viremic untreated patients[39]. Controlled studies showed similar results. Higher recurrence or occurrence rates were reported in patients treated with DAAs compared with IFN treatment[40-42].

Additional studies

A prospective study found that patients treated with DAAs before radical HCC therapy had a higher and earlier recurrence rate than patients not exposed to DAAs. Recurrence rates were significantly higher in those treated with arterial chemoembolization than in curative treatment[43].

A compelling finding was reported in a prospective Italian study where an earlier occurrence was observed in sofosbuvir-based treatment compared to patients who received ribavirin as part of the treatment protocol[44]. Finally, a prospective study of genotype 3-infected patients compared four sofosbuvir-based treatments with or without ribavirin and IFN. Earlier and higher occurrence rates were observed in cirrhotics without IFN[45].

Table 1 summarizes the studies reporting a higher or earlier HCC development after DAAs administration. It should be noted that the authors of 31% of the studies declared a conflict of interest due to some support by the pharmaceutical companies.

Against an increase of HCC after DAAs

Papers supporting that DAAs did not report an increased occurrence or recurrence of HCC after broadly separating patients into three groups: Those comparing DAAs with no treatment, those comparing SVR and no SVR after DAAs, and those comparing DAAs with IFN-based treatment. There are also some observational papers without any control group for comparison.

Table 1. Studies reporting increased development of HCC after HCV viral cure

Study	Year	Type	Endpoint	Controls	Result
Reig *et al.*[9]	2016	Retrospective	Recurrence	No	Unexpected high
Rinaldi *et al.*[10]	2016	Retrospective	Recurrence	No	Unexpected high
Conti *et al.*[14]	2016	Retrospective	Occur-Recur	No	Unexpected high
Kozbial *et al.*[15]	2016	Retrospective	Occurrence	No	Unexpected high
Cardoso *et al.*[17]	2016	Retrospective	Occurrence	No	Unexpected high
Yang *et al.*[31]	2016	Retrospective	Recurrence	No treat	Higher in DAAs
Ravi *et al.*[16]	2017	Prospective	Occurrence	No	Unexpected high
Worns *et al.*[158]	2017	Systematic review	Occur-Recurr	IFN	Higher in DAAs
Bielen *et al.*[34]	2017	Retrospective	Occur-Recur	IFN	Similar occurrence Higher recurrence
Calleja *et al.*[19]	2017	Retrospective	Recurrence	No	Unexpected high
El Kassas *et al.*[32]	2018	Prospective	Recurrence	No treat	Higher in DAAs
Kwong *et al.*[37]	2018	Retrospective	Occurrence	No DAAs	Higher in DAAs
Nahon *et al.*[36]	2018	Retrospective	Occurrence	IFN	Higher in DAAs
Rinaldi *et al.*[44]	2019	Retrospective	Occurrence	SOF *vs.* SOF + Rib	Higher in SOF
Nakano *et al.*[22]	2019	Prospective	Recurrence	No	High recurrence
Lashen *et al.*[20]	2019	Retrospective	Occur-Recur	No	High Recur-Occur
Marino *et al.*[27]	2019	Retrospective	Occurrence	No	Unexpected high
Degasperi *et al.*[26]	2019	Retrospective	Occur-Recur	No	High Occur-Recur
Kuo *et al.*[39]	2020	Retrospective	Recurrence	IFN-No treat	Higher in DAAs *vs.* IFN. Similar DAAs *vs.* No treat
Lim *et al.*[38]	2020	Retrospective	Recurrence	IFN- viremic	No difference vs. IFN DAAs SVR higher than viremic
Tayyab *et al.*[45]	2020	Prospective	Occurrence	4 DAAs schemes	Better when IFN added
Fatima *et al.*[35]	2020	Retrospective	Aggressiveness	IFN	Similar but earlier HCC in DAAs
Sandgiovani *et al.*[28]	2020	Prospective	Occur-Recur	No	High Occur-Recur
Teng *et al.*[40]	2019	Retrospective	Recurrence	IFN	Higher in DAAs
Hassany *et al.*[21]	2020	Prospective	Occurrence	No	Unexpected high
Chan *et al.*[23]	2020	Retrospective	Occur-Recur	No	High Occur-Recur
Ji *et al.*[42]	2021	Prospective	Occurrence	IFN	Higher in DAAs
Fouad *et al.*[33]	2021	Retrospective	Aggressiveness	No treat	More aggressive in DAAs
Ahn *et al.*[24]	2021	Retrospective	Recurrence	No	High recurrence
Tani *et al.*[29]	2021	Retrospective	Recurrence	No	TACE higher recurrence; Similar survival recur-non recur
Kamal *et al.*[25]	2021	Retrospective	Recurrence	No	Unexpected high
Karbeyaz *et al.*[41]	2021	Retrospective	Occurrence	IFN	Higher in DAAs
Lithy *et al.*[43]	2022	Prospective	Recurrence	No DAAs	Higher in DAAs. Less after radical than palliative. Earlier in DAAs
Ogawa *et al.*[30]	2022	Retrospective	Recurrence	No	High late recurrence. Less after radical than palliative

HCC: Hepatocellular carcinoma; HCV: hepatitis C; SVRs: sustained virological response rates; IFN: interferon; DAAsL: direct-acting antiviral agents; TACE: Ttrans arterial chemoembolization.

DAAs vs. no treatment

The first direct answer to early studies supporting the lack of DAAs implication in HCC development came from two simultaneously published studies. The first was a prospective observational study reporting that treated and untreated patients had similar HCC occurrences[46]. The second study reported that the recurrence rates were similar between those treated with DAAs and those without treatment[47]. In both studies, overall survival was similar between treated and untreated patients, although treatment reduced liver complications. No differences between treated and untreated patients were reported in several

subsequent studies, usually using statistical adjustments[12,48-51]. In contrast to the two initial reports, there were studies suggesting that, in addition to similar recurrence rates, the improvement in survival was significant in the treated group[52,53].

A similar recurrence rate was reported in a large retrospective study from North America[54]. This study was heavily criticized for the high heterogeneity of patients and HCC curative treatments despite a high rate of 46% in the first year after the HCC cure[55,56].

Similar results were found when different DAAs regimens were used[57] or after transplantation on HCV-related HCC[58]. As should be expected, there were also papers reporting a reduced HCC recurrence risk after DAAs treatment, always compared to untreated patients[59,60]with[61,62] or without improved survival[63].

SVR vs. no SVR

The second group of papers compared DAAs-treated patients achieving SVR with those still viremic at the end of treatment. Many reported that SVR patients had lower hepatoma occurrence or recurrence rates. However, there was an agreement that several patients, even after SVR, developed HCC[5,64-67]. A study, however, reported that recurrence rates were similar between SVR and viremic patients, but the survival was better after SVR[68] in contrast to a previous paper where no difference in survival was found between SVR and viremic patients[69].

A notable exception is a report indicating that DAAs eradicating HCV in patients with precancerous liver lesions did not increase or decrease HCC occurrence compared with viremic patients[70].

DAAs vs. IFN

In the third group, all comparisons of occurrence between DAAs and IFN were retrospective by necessity. By contrast, recurrence studies were prospective and retrospective with a variable follow-up time, and all but two small studies showed a considerable recurrence rate of around 30% in the DAAs arm.

One study reported a lower recurrence rate in the DAAs arm than in IFN and untreated arms; however, their Kaplan-Meier curves showed no differences among the three arms[71]. Similarly, a propensity score-matched analysis found no difference in occurrence or recurrence between DAAs- and IFN-treated patients[72]. The same conclusions appeared in a meta-analysis from Australia. However, their figures reveal that occurrence and recurrence after DAAS appear earlier[73]. Strong criticism of statistical problems due to the heterogeneity of studies in this meta-analysis was also published[74]. Several additional papers agreed that there was no difference between DAAs and IFN[6,7,75-83].

A compelling finding was confirmed in several studies. Despite the insignificant difference between DAAs and IFN eradication in HCC occurrence and recurrence, the tumors appeared much earlier after DAAs than after IFN[84-87]. In addition, reports demonstrated that occurrence and recurrence are reduced after DAAs compared to IFN[88,89]. In a meta-analysis[89], three additional papers were presented showing lower recurrence rates of HCC in the DAAs group than IFN[76,82,87]; however, this finding is not justified by actual comparisons [Table 2].

Observational studies

It has been reasonably argued that this form is the only currently feasible study due to ethical and logistic reasons. It avoids many but not all statistical perplexities[90]. Nevertheless, once again, there are discrepancies among studies. All prospective observational studies compared DAAs-treated to untreated patients; some

Table 2. Studies reporting no higher development of HCC after HCV viral cure

Study	Year	Type	Endpoint	Controls	Result
Cheung *et al.*[46]	2016	Prospective	Occurrence	No treat	No difference. Mortality similar
ANRS study[47]	2016	Retrospective	Recurrence	No treat	No difference. Mortality similar
Minami *et al.*[71]	2016	Retrospective	Recurrence	IFN-No treat	No difference
Kanwal *et al.*[5]	2017	Retrospective	Occurrence	No SVR	Reduced in SVR
Wasiry *et al.*[73]	2017	Meta-analysis	Occur-Recur	IFN	No difference
Nagata *et al.*[72]	2017	Retrospective	Occur-Recur	IFN	No difference
Ikeda *et al.*[59]	2017	Retrospective	Recurrence	No treat	Better in DAAs
Li *et al.*[75]	2018	Retrospective	Occurrence	IFN	No difference
Romano *et al.*[64]	2018	Prospective	Occurrence	No SVR	SVR lower
Calvaruso *et al.*[65]	2018	Prospective	Occurrence	No SVR	SVR reduced
Innes *et al.*[86]	2018	Retrospective	Occurrence	IFN	No difference
Ioannou *et al.*[84]	2018	Retrospective	Occurrence	IFN	No difference
Singer *et al.*[88]	2018	Retrospective	Occurrence	No treat-IFN	Better than IFN
Mettke *et al.*[48]	2018	Prosp-Retro	Occurrence	No treat	No difference
Mashiba *et al.*[85]	2018	Retrospective	Recurrence	IFN	Similar, BUT earlier in DAAs
Finkelmeier *et al.*[6]	2018	Retrospective	Occurrence	IFN	No difference
Huang *et al.*[66]	2018	Meta-analysis	Occur-Recur	DAAs-No DAAs	Lower with DAAs
Mecci *et al.*[69]	2019	Retrospective	Occurrence	No SVR	No difference. Similar survival
Nishibatake *et al.*[76]	2019	Retrospective	Recurrence	IFN	No difference ($P = 0.43$)
Nagaoki *et al.*[87]	2019	Retrospective	Recurrence	IFN	No difference ($P < 0.37$)
Cabibbo *et al.*[52]	2019	Prospective	Recurrence	No treat	No difference
Carrat *et al.*[49]	2019	Prospective	Occurrence	No treat	No difference
Mun *et al.*[57]	2019	Retrospective	Occurrence	Multiple DAAs	No difference
Singal *et al.*[54]	2019	Retrospective	Recurrence	No treat	No difference
Jain *et al.*[67]	2019	Retrospective	Recurrence	No DAAs	Better after SVR No DAAs better than noSVR
Toyoda *et al.*[68]	2019	Prospective	Occurrence	No SVR	No difference
Rutledge *et al.*[7]	2019	Meta-analysis	Occurrence	IFN	No difference
Pinero *et al.*[58]	2020	Retrospective	Recurrence	No treat	Reduced in treat
Janjua *et al.*[78]	2020	Retrospective	Occurrence	IFN	No difference? DAAs 6.9/1000PY IFN 1.8/1000PY
Imai *et al.*[79]	2020	Retrospective	Recurrence	IFN	No difference
Shiha *et al.*[91]	2020	Prospective	Occurrence	No	Reduced? HCC incidence was 2.917/100PY in cirrhosis **NO**
Lin *et al.*[53]	2020	Retrospective	Recurrence	No treat	No difference
Miuma *et al.*[50]	2020	Retrospective	Recurrence	No treat	No difference
Tahata *et al.*[80]	2020	Retrospective	Recurrence	IFN	No difference
Gorgen *et al.*[77]	2020	Retrospective	Recurrence	IFN-no treat	No difference. Better than no treatment but similar mortality
Lui *et al.*[60]	2020	Meta-analysis	Recurrence	No treat	Better after SVR
Tani *et al.*[92]	2020	Prospective	Occurrence	No	Low incidence?
Hamoir *et al.*[93]	2021	Prospective	Occurrence	No	Low incidence
Wu *et al.*[81]	2021	Retrospective	Recurrence	IFN	No difference
Chi *et al.*[82]	2021	Prospective	Recurrence	IFN	No difference ($P = 0.13$)
Frazzoni[89]	2021	Meta-analysis	Recurrence	IFN	DAAs are better than IFN. DAAs early recurrence. Differences among countries
Ismail *et al.*[83]	2021	Retrospective	Recurrence LT	IFN	No difference
Ochi *et al.*[61]	2021	Retrospective	Recurrence	No treat	Better DAAs
Minami *et al.*[168]	2021	Retrospective	Occurrence	IFN	No difference
Toyoda *et al.*[68]	2021	Retrospective	Recurrence	SVR-No SVR	No difference
Sapena *et al.*[12]	2022	Retrospective	Recurrence	No treat	No difference but no firm conclusions

Chen *et al.*[63]	2022	Retrospective	Recurrence	No treat	Better in DAAs. Similar survival
Kuromatsu *et al.*[51]	2022	Retrospective	Recurrence	No treat	Reduced in treat
Ikenaga *et al.*[62]	2022	Retrospective	Recurrence	No DAAs	Better after SVR

HCC: Hepatocellular carcinoma; HCV: hepatitis C; SVRs: sustained virological response rates; DAAs: direct-acting antiviral agents; IFN: interferon.

had no control arm. Most conclusions are that they do not increase or decrease occurrence[91-93].

It should be stressed, however, that in any attempt to evaluate heterogeneous studies with complex designs, investigators should remember that a non-significant *P*-value does not mean that the null hypothesis is confirmed. As Altman and Bland put it, "absence of evidence is not evidence of absence"[94].

Table 2 summarizes the studies reporting that DAAs administration is not associated with increased development of HCC. It should be noted that the authors of 61% of the studies declared a conflict of interest due to some support by the pharmaceutical companies.

An explanation for increased HCC after DAAS

All these studies, whether positive or negative, agree that the risk of HCC development is real despite SVR. Various explanations have been proposed.

The pathogenesis of HCC in HCV infection is multifactorial. There is a fundamental difference between HCV-related HCC and other cancers. Sequential mutations of oncogenes and tumor suppressor genes are necessary for developing tumors such as colorectal cancer (Vogelstein-type). This phenomenon may or may not be necessary for HCV-related HCC, where core protein expression contributes to the induction of HCC, even without a complete set of genetic aberrations required for carcinogenesis (non-Vogelstein-type)[95]; they usually cause epigenetic alterations and dysregulation of signaling pathways. A report summarized histone modifications, DNA methylation abnormalities of 18 gene targets, and various signaling pathways[96]. A multi-gene transcription profile was identified in HCV-associated cirrhosis that may be used as a prognostic biomarker of HCC development[97-99]. Factors associated with HCC in HCV were reviewed[100,101].

DAAs treatment can remove the HCV patient serum; however, epigenetic abnormalities in cells persist despite the absence of the virus[102]. Reig *et al.* proposed an immunological explanation based on a rapid fall in antigenic load during DAAs eradication leading to hypo-responsiveness of memory T cells and, therefore, a possible breakdown of immune surveillance[103]. The finding supports this proposition that immune surveillance by $CD8^+$ T lymphocytes was reduced after HCV eradication by DAAs, possibly mediated through IL-12[104]. An analysis of immune cells before and after DAAs revealed the long-term persistence of immune exhaustion, possibly causing an irreversible defect in the immune system[105].

Epigenetic alterations are associated with an increased risk of HCC and form an "epigenetic signature" of eight genes persisting after DAAs treatment. Drugs that inhibit epigenetic modifying enzymes suppress this signature[106-108]. Epigenetic alterations regulate the expression of genes leaving the nucleotide sequence unaffected. Viral proteins are responsible for hyper and hypo-DNA methylation affecting the natural killer cell-dependent innate immune response[109,110].

Additional factors are involved in the pathogenesis of HCV-associated HCC. The gut microbiota may be causally associated with HCC through inflammation and fibrosis. HCV clearance with DAAs in patients

with cirrhosis modifies the gut microbiota but does not influence the dysregulated intestinal barrier function[111]. A compelling finding was the overexpression of human endogenous retroviruses (HERV)-H-pol and HERV-K-pol proteins that persisted after DAAs eradication of HCV, suggesting that overexpression of HERVs (which constitute 8% of the human genome) may predispose to HCC[112].

Other factors not restored in liver tissue after DAAs treatment that may contribute to HCC are the decrease of microRNA-122 and the upregulation of oncogenic transcriptomic profiles like CYR61[113-115]. Moreover, a reduction of mucosal-associated invariant T cell cytotoxicity, possibly caused by rapid phenotyping changes of these cells after DAAs treatment, attenuates their action against cancer cells. SVR achieved after DAAs does not correct the increased frequency of regulatory T cells observed in chronic HCV infection, leading to a local immunosuppressive environment inhibiting the clearance of cancer cells[116]. A detailed overview of immunological factors not restored after DAAs obtained SVR[117].

Non-immunological factors were also investigated. HCV infection causes endoplasmic reticulum stress responses, degradation of p53 in lysosomes, and dysregulation of autophagy. These abnormalities are corrected after IFN-induced HCV eradication but not after DAAs[118-120]. Given this, many HCV patients have non-alcoholic fatty liver disease features, which may be partly responsible for HCC occurrence or recurrence after SVR[121]. Neo-angiogenesis may also favor HCC development, and markers of angiogenesis, like VEGF and angiopoietin-2, either increase or persist during or after DAAs administration[122,123]. Another explanation for HCC recurrence and occurrence after DAAs was proposed after the finding that SVR achievement with DAAs was accompanied by a high prevalence of occult HCV persistence in peripheral blood mononuclear cells[124,125]. This occult persistence was not observed when ribavirin was added to the DAAs scheme[126].

In contrast, there have been reports that the development of HCC after DAAs eradication is genetically associated with a single-nucleotide polymorphism identified using genome-wide analysis[127]. Potential mechanisms for HCC recurrence after DAAs treatment have been reviewed[128-130]. It should be noted, however, that there is no direct evidence of incriminating DAAs in hepatic carcinogenesis.

Reasons for problems

Papers described the many variables that influence every attempt to clarify the problem of HCC after DAAs eradicate HCV infection. They found an extreme variation in HCC occurrence or recurrence among reported studies ranging between 0 and 7.4% for occurrence and 0% to 54.4% for recurrence[100,131].

We analyzed the explanations for discrepancies among published studies.

Heterogeneity of studies

A review of the problem noted that the heterogeneity of studies was significant irrespective of the primary outcome and pointed to the difficulty of comparisons, particularly when an untreated group is used as the control[18]. A meta-analysis reported high geographical differences in HCC development after DAAs. Recurrence rates increased when moving from Europe (23%) to the US (34%) to Egypt (37%) and Asia (33%). These studies showed extreme heterogeneity (I^2 = 84.7%)[89]. The reason for these differences is not apparent.

Most studies use simple classification based on clinical parameters to distinguish patient groups. One study reported that the development of HCC depended on the response to treatment. Other biochemical predictors were found in patients without SVR compared with those with SVR. They classified their patients

into eight phenotypes according to HCC risk. However, patients without SVR but a good liver biochemical profile were found to have similar low five-year incidence to those with SVR[132].

Other studies suggested that fibrosis and the previous tumor burden are more critical than the type of treatment for HCC occurrence or recurrence. However, estimation of the degree of fibrosis is challenging without a liver biopsy; even among cirrhotics, the degree of fibrosis is not identical. The same is true for accurately estimating the tumor burden[133]. In addition, the diagnosis of cirrhosis in most studies is based on elastography findings or indirect scores like FIB4 or APRI (with all the problems associated with these tests). Definite diagnosis based on liver biopsy is rare. The severity of cirrhosis should also be considered. Unfortunately, no accurate clinical marker can be used to that end. Albumin is not directly related to the severity of cirrhosis, and many studies show either no difference among groups or very low significance. Currently, the best indicator of the severity of cirrhosis appears to be the hepatic venous pressure gradient; unfortunately, this index is rarely, if ever, available.

Heterogeneity of HCCs

Several lines of evidence suggest that all hepatocellular carcinomas are not similar, and several publications addressed a variety of risk indicators, including genetic single-nucleotide polymorphisms[134]. The increased heterogeneity of HCCs is a problem in many other aspects of HCC and is not unique in HCC occurrence or recurrence after DAAs. Heterogeneity is present even when the Child classification is used as many studies include only Child A cirrhotics, while others include both Child B and sometimes Child C patients.

Liu *et al.* demonstrated that a viral exposure signature predicts HCC in at-risk patients with long-term follow-up for HCC development[135]. These data suggest a new approach to grading cancer risk that, combined with established parameters, could become the clue to define the absence of HCC risk[135].

The CRAFITY score, based on the estimations of CRP and alpha-fetoprotein levels, was validated as HCC biomarkers associated with survival, allowing for a much better classification of patients about to receive immunotherapy[136]. There was some criticism of the retrospective design and lack of comparisons with other available tests[137]. Nevertheless, these studies occurred during the era of individualized medicine[138].

A possible revolution is expected in the classification of HCC that will affect future clinical trials and predict the development of the tumors or the response to therapies. Most advances are due to the progress of the so-called "omics" technologies.

Immunogenomics identified six tumor subtypes with different microenvironments[139]. Transcriptomics identified three subtypes associated with three hepatocellular metabolic pathways: the WNT/β-catenin pathway, telomere maintenance, and several subtype-specific genes[140,141]. Proteomics also classified subtypes of HCC while integrating with transcriptomic data and identified two genes associated with poor survival in addition to 18 tumorigenesis genes[142,143]. Integration of data also revealed that changes caused by the MYC oncogene were implicated in vascular invasion[144]. The new molecular and genetic classifications of HCC have been reviewed[145,146].

The tumor cell population is highly heterogeneous between tumors and within a tumor. New data are also emerging from single-cell technology, allowing for more accurate classification of HCC sub-groups. Inter- and intratumoral differences can be identified using this technology to better understand HCC biology[147]. The single-cell analysis demonstrated that an upregulation of the inhibitor of apoptosis MLX interacting protein-like (MLXIPL) was related to poor survival in HCC[148]. HCC survival was also deceased in

association with a proliferating cell type identified in HCC tumors[149].

Combined data from genomics and transcriptomics allowed for classifying HCC into three subtypes, CTNNB1-mutated, proliferative, and metabolic disease-associated, with different molecular characteristics[150].

HCC molecular signatures have been rarely used in clinical decision-making as they are not widely available, and some are cumbersome. However, their generalized use will completely change our clinical perception of HCC behavior[151].

Efforts to correct for heterogeneity include statistical tests like multivariate analysis or propensity score matching to account for confounding factors. However, the exclusion of selection bias is highly doubtful, as discussed[84].

The heterogeneity of methods, patients, and tumor variability may lead to studies reporting disparate results. Nevertheless, not all discrepancies are due to bias and statistical imputation[90]. Unfortunately, there are many more problems.

Time of DAAs treatment after HCC treatment

It was suggested that the interval between the curative intervention and the initiation of DAAs treatment is essential for the recurrence of HCC[152]. Thus, the interval was significantly higher in patients without recurrence than in those with recurrence (36 months and seven months, respectively)[153]. This was true even when hepatectomy was used as the modality of complete HCC treatment before DAAs initiation[154].

An essential time association between IFN-free treatment and HCC development was reported as HCC appeared earlier in the IFN-free treated than in the IFN-treated patients[90]. This observation has been repeatedly confirmed, and most studies report an interval between 6 months and one year for the recurrence of HCC after DAAs[24,27,76,155,156].

Based on these observations, experts suggested that after curative treatment of HCC by liver resection or ablation, the initiation of DAAs should be deferred. The precise timing remains debatable[157].

The German Alliance for Liver Cancer proposed that DAAs initiation should start 6-12 months after HCC curative intervention[158]. The American Gastroenterology Association suggests that DAAs initiation can be deferred for 4-6 months after HCC curative treatment[155]. A six-month delay was recommended in another systematic review[159].

However, a study of transplanted patients with HCV-related HCC recommended that a delay should be avoided, and treatment should begin within 0-3 months after transplantation[160].

It should be expected that, with such heterogeneity of studies, conflicting results would appear. Reports have demonstrated that the delay time is not critical for HCC recurrence[161]. Another report supported this finding where there was no significant difference between those with delayed treatment and those with early administration. However, more patients (41%) with early initiation developed HCC recurrence than 30% with delayed administration. The lack of significance was obviously due to the small number of patients[26].

Time of follow-up

The evidence suggests that the follow-up time for HCC recurrence should exceed one year if late recurrence is to be identified[162]. Moreover, a report showed that the median time between SVR and the occurrence of HCC was 28.1 months, suggesting that shorter follow-up times may underestimate the occurrence rate[163]. Nevertheless, there is at least one well-documented case of HCC occurrence eight years after liver transplantation[164]. In many studies, there was a shorter duration of follow-up in DAAs-treated patients than in IFN-treated patients, which might affect the comparison of HCC occurrence; however, in most studies, the occurrence of HCC occurs earlier after DAAs.

The role of alcohol

There are several papers indicating that studies should consider ethanol consumption. HCV-related cirrhosis had a significantly lower decompensation rate than patients with HCV and alcohol consumption[165]. Moreover, primary liver cancer and alcohol were the most significant causes of death in HCV patients who achieved SVR, while high alcohol consumption was associated with an increased rate of HCC occurrence or recurrence after the achievement of SVR with DAAs treatment[5,27,28,166-168]. This observation was confirmed[169].

The role of diabetes and the metabolic syndrome

Obesity, diabetes, and metabolic syndrome are associated with increased morbidity and mortality in HCV infection[170]. These factors might influence the occurrence and recurrence rates of HCC development after SVR is achieved by IFN or DAAs treatment[5,69,171].

In two studies from Italy, diabetes was identified as a risk factor for the three-year probability of HCC occurrence or recurrence after DAAs[26,172]. Notably, the presence of obesity and metabolic syndrome has drawn more attention than those of earlier years[42,167,173]. Interestingly, increased glycation end-products were suggested as a causative factor of increased HCC incidence in diabetes[174], while a genetic component with somatic mutations in the tumor suppressor gene ACVR2A was also significantly associated with HCC[121].

A meta-analysis showed that diabetes significantly increased the risk of HCC occurrence in the entire group of HCV patients treated with DAAs; however, the risk was insignificant in patients achieving SVR[175]. The significance of non-alcoholic fatty liver disease (and alcohol) in developing HCC despite the achievement of SVR by DAAs has been suggested in reviews[130,176].

The role of HIV

The significance of HIV co-infection in HCC occurrence or recurrence is controversial. HIV co-infected patients were reported to have an earlier appearance and worse HCC outcomes[177]; however, those who achieved SVR after DAAs appeared to have a lower risk of HCC occurrence[178]. To add further confusion, no difference in the HCC incidence was reported in HIV co-infected patients[179].

The role of intravenous drug use

The role of intravenous drugs in developing HCC after HCV elimination by DAAs has not been extensively studied. However, it is essential to note that DAAs viral clearance does not protect from re-infection in intravenous drug users[117].

Concluding remarks

There is considerable discussion among researchers worldwide on several aspects of HCC management. There are two points of agreement on the question of DAAs and HCC, while one point is still debated. Agreement appears to exist on the surveillance issue and the time of DAAs introduction after the initial treatment of HCC. In contrast, the high risk of HCC occurrence or recurrence in cirrhotic patients who received DAAs remains open despite efforts to declare that the problem has been settled[117].

Screening for HCC development after DAAs viral cure

The need for careful monitoring for HCC occurrence or recurrence after DAAs is widely recognized. Studies indicate a substantial risk of death from HCC 5-10 years after DAAs eradication in patients with extensive fibrosis or cirrhosis[180,181]. Studies indicate that patients with[12,129] or without cirrhosis[68] should be followed up, as SVR obtained after DAAs reduces but does not abolish HCC occurrence or recurrence[22,36,68,179,182-184]. The risk is higher if SVR is not achieved[185].

Most reports recommend that patients after SVR achievement should be followed in a surveillance program[186-188].

All three major liver associations agree that cirrhotics should be indefinitely followed after SVR; however, there are differences in their recommendations for patients with advanced fibrosis without cirrhosis. The European and Asian associations recommend surveillance, but the American does not[189]. A large study on stage 3 fibrosis reported that most patients have a low risk of HCC after SVR; however, this was not the case for males over 55 years old[190].

Surveillance cost is a severe problem for most economies, particularly in the SARS-CoV-2 era, due to the large number of patients involved. To reduce the burden, investigators developed several prediction models[129,191,192], but none has been universally accepted.

Time of DAAs introduction after HCC treatment

The AGA Clinical Practice Update recommendation is that DAAS should be introduced after HCC curative therapy is completed and should be deferred for 4-6 months after HCC radical treatment[155]. However, most investigators agree that treatment with DAAS should be postponed for at least 6-12 months after successful treatment of HCC[11,157]. The theoretical justification for the delayed administration may be that the delay allows the immune surveillance to recover and that the verification of the treatment success is more accurate[157].

DAAs and HCC development

There have been two general opinions. The first is that the question has been solved once and for all in favor of DAAs[193]. Interestingly, some investigators changed their attitude toward their earlier observations[10,44] after reviewing new data[194]. Some investigators use strong expressions like "another nail in the coffin of the association of DAAs with HCC"[195] or claim that the story has ended[13] even though their comment was based on one study concluding that the effect of DAAs on HCC recurrence risk was inconclusive[12].

Three reviews, one by some authors[196] of the previous study[12], also agreed that the survival of HCC patients treated with DAAs is significantly improved[196-198]. However, the final issue remains unresolved, as many studies, including those that report no effect of DAAs in HCC development, found no difference in survival between treated and untreated patients [Tables 1 and 2]. The answer to this problem is critical. If survival is similar, it would be challenging to suggest further treatment after HCC cure and SVR, or as Sanduzzi-Zamparelli *et al.* point out, "until further information is available"[199].

The second opinion is that the matter has not been settled, and all available studies produce conflicting results[55,56,155,200,201].

The primary reason for such divergent views is the extreme heterogeneity of studies[131,202]. One of the problems is the presence of ribavirin in the IFN-treated groups. Ribavirin affects total DNA and RNA synthesis[203].This phenomenon may be further implicated in interpreting results, as in most studies, there is no discrimination between IFN + Rib and IFN monotherapy patients. Moreover, some papers included ribavirin in the DAAs treatment groups. Two examples come from Japan[71,168].

An observation demonstrated an interesting problem connected to the heterogeneity of studies. Retrospective studies underestimate HCC development compared to prospective studies[204]. Another reason that should be seriously considered is geographical variation[11,89].

The situation has been elegantly exemplified in a review of HCC recurrence after DAAs, where all results are available. HCC recurrence increased, decreased, or showed no difference[157]. A similar situation was demonstrated for the occurrence of HCC after DAAs[6,8,180,182].

Given the practically unresolved problems of heterogeneity, it should be stressed that all studies are based on statistical manipulations to incorporate as many confounding factors as possible. Although this is a perfectly accepted practice, it may produce precarious results. An example is revealed by a study of HCC after IFN treatment. In a multivariate analysis of risk factors, the use of illicit drugs appeared to offer strong protection against HCC development [subdistribution hazard ratio (SHR) = 0.27][205]. Moreover, patients with an SVR and viral cure have much higher overall mortality than viremic patients[205].

In another study, a misleading Forest plot line appeared in a DAAs meta-analysis of HCC occurrence. In the IFN occurrence Forest plot, the vertical line was placed correctly at 1 of the horizontal axis. In contrast, in the DAAs occurrence Forest plot, the line was not placed at 1. If one replaces the line at 1, it is evident that occurrence after DAAS is significantly increased[73].

What is the ideal study to resolve the problem?

A robust, correctly designed study is needed, including patients with comorbidities[197,206].

Such a study should be prospective, with patients carefully matched for alcohol consumption, the presence of metabolic syndrome, and diabetes, preferably after a liver biopsy. Most importantly, the selection of HCC patients for recurrence studies should be performed based on the current molecular classification of HCC and followed up for long intervals under careful surveillance. The control arms should be untreated patients (which is, by definition, unethical)[84].

Even if a study with these requirements were possible, the need for statistical adjustments would remain. Since this definitive study does not appear feasible, it is time for the debate to be halted, and the opposing attitudes should be reconciled, even though the final answer will be doubtful.

An essential observation of liver diseases was published two decades ago. The authors suggested that the 20-year survival of the scientific truth did not differ between randomized controlled trials and nonrandomized studies, while worse truth survival was observed in the meta-analyses! More importantly, the investigators found that conclusions based on suitable methodology and those based on poor methodology remained true for a similar period. This somewhat unorthodox view should be recalled when criticizing papers heavily

based on statistical adjustments [207,208].

From the clinician's point of view, it should be remembered that, when facing the individual patient, not all propensity score matches are applicable, and crude non-controlled observational data may be nearer to the clinical situation and should not be ignored. Unless a robust prognostic model is invented, all patients, even those with less extensive fibrosis, should be carefully monitored for the occurrence or recurrence of HCC after DAAs treatment.

DECLARATIONS

Authors' contributions

Designed the study and revised the final manuscript: Kouroumalis E
Performed data acquisition and wrote the first draft: Tsomidis I
Performed data analysis and interpretation and supervised the manuscript: Voumvouraki A

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