

# Impact of direct-acting antiviral agents on the development of hepatocellular carcinoma: evidence and pathophysiological issues

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## Abstract

Hepatitis C virus (HCV) infection is currently one of the main causes of cirrhosis and hepatocellular carcinoma (HCC) at a global level. Recently, a new generation of direct-acting antiviral agents (DAAs) has entered the HCV treatment landscape, providing impressively high rates of sustained virological response (SVR), and is expected to lead to an eventual decrease in HCV-related cirrhosis, liver transplantation and mortality. However, during the first years of their use, several studies reported a possible correlation between DAA treatment and an increased risk of HCC. Following the publication of larger prospective studies, the risk of *de novo* HCC occurrence has clearly been proven to be lower after the achievement of SVR, regardless of antiviral treatment. On the other hand, the risk of HCC recurrence following treatment with DAAs is debatable; existing data remain controversial, possibly because of the lack of large, well designed cohorts with more homogeneous patient populations. With regard to the pathophysiology behind the above observations, especially in patients with previous HCC history, HCC development could possibly be favored by the changes in the immunological milieu and the different cellular behavior after eradication of HCV infection with DAA treatment.

**Keywords** Hepatocellular carcinoma, direct acting antivirals, hepatitis C, cirrhosis, interferon

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## Introduction

Chronic infection with hepatitis C virus (HCV) affects more than 70 million individuals worldwide [1]. Progression of liver disease to advanced fibrosis or cirrhosis due to chronic HCV infection is a risk factor for the development of hepatocellular carcinoma (HCC), with an annual rate ranging from 1-7% [2]. The prognosis of HCC remains poor, because it has usually reached an advanced stage by the time of diagnosis. HCC is considered as the third leading cause of cancer death in the world and it is estimated to be responsible for approximately 1 million deaths per year [3].

HCV incidence has shown a declining trend in recent years. This is mainly due to decreased exposure to HCV-related risk factors, which include injected drug use and

contaminated blood product transfusion [4]. This declining course was interrupted by a rise of HCV incidence among people who inject drugs [5]. On the other hand, we are now in the era of interferon (IFN)-free anti-HCV treatments, since the development of many very potent direct-acting antiviral agents (DAAs), which provide impressively high rates (>95%) of sustained virological response (SVR) [6].

The expected wide use of this new generation of antiviral treatments makes it difficult to predict the incidence of HCV-related cancer in the future. The prediction is even more difficult since other morbidities such as non-alcoholic fatty liver disease or non-alcoholic steatohepatitis, obesity, and diabetes are on the increase as independent risk factors for the development of HCC [7]. Moreover, it must be taken into consideration that there is a long interval, 2-8 decades, from the initial HCV infection to the development of HCC [8]. During this long period, many pathological processes take place, including chronic inflammation, hepatocyte death, regeneration, fibrosis, and cirrhosis [9]. Nowadays, the residual damage and its surveillance after the successful treatment of chronic HCV infection are not the only issues. There are also some concerns about the role of DAAs in the recurrence or development of HCC after the achievement of a virological response.

The aim of this article is to review published data on the risk of HCC occurrence or recurrence following DAA therapy and provide a literature update on the underlying immunological mechanisms.

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## The impact of IFN treatments on HCC occurrence

According to previous studies focusing on the effects of IFN treatment, the eradication of HCV reduces the long-term incidence of HCC while halting the progression of disease, thus preventing the clinical complications associated with chronic viral infection [10,11]. In 1995 Nishiguchi *et al* [12] showed that patients with HCV-related cirrhosis who achieved SVR with IFN treatment had a lower risk of HCC development. Since then, other studies with long-term follow up, such as the HALT-C trial (Hepatitis C Antiviral Long-term Treatment against Cirrhosis) [13], the CO-PILOT (Colchicine versus Peginterferon alfa 2b Long-term Therapy) [14], the EPIC3 (Evaluation of PegIntron in Control of Hepatitis C Cirrhosis) [15,16], and a trial by the Swedish Hepatitis C Group [17], have confirmed that patients who achieved SVR had a lower incidence of HCC compared to non-responders. However, patients older than 65 years old and those with advanced liver fibrosis or cirrhosis are at higher risk [18]. The long-term risk of developing HCC persists in cirrhotic patients for up to 8-10 years, especially in the presence of comorbidities such as diabetes [17,19].

## The impact of DAAs on HCC *de novo* occurrence and recurrence

In the era of IFN-free regimens, HCV patients can receive treatment regardless of HCV genotype, fibrosis stage or even the presence of severe comorbidities [20]. However, concerns were raised when two studies reported both increased rates of *de novo* HCC occurrence and unexpectedly high rates of recurrence in patients who cleared HCV after IFN-free DAA therapy [21,22]. Conti *et al* in a retrospective cohort study from Italy, observed a significant increase (28.81%) in the early recurrence of HCC in 59 HCV patients who had SVR after DAA treatment and had been previously treated for HCC, either with curative regimens (resection and radiofrequency ablation) or with potentially non-curative treatment (transcatheter arterial chemoembolization [TACE]) [21]. Moreover, Reig *et al* investigated the benefits of DAAs in a cohort of 58 patients with a prior history of HCC and complete response to heterogeneous types of treatment, such as resection, ablation or chemoembolization. After a median follow up of 5.7 months, 16 of 58 (28%) patients exhibited HCC recurrence [22].

Since then, several studies have been conducted with a view to reevaluating the role of DAAs and the established general assumption that HCV eradication minimizes the risk of HCC development. The ANRS collaborative study group on HCC elaborated the results of 3 prospective multicenter cohort studies in France (ANRS CO22 HEPATHER, CO12 CIRVIR, and CO23 CUPILT). This analysis, which included more than 6000 patients treated with DAAs, did not support an increased risk of HCC recurrence after DAA therapy [23]. However, in contrast to both previous studies, the ANRS register enrolled only HCC patients treated with curative procedures, including

patients who received orthotopic liver transplantation (OLT), but excluded HCC patients treated with TACE [23].

Yang *et al* investigated pre- and post-OLT outcomes in patients with HCV-associated HCC treated with DAAs and compared them to those of untreated patients. Unexpectedly, a trend toward a higher risk of HCC recurrence was reported in patients who had received pre-OLT DAA treatment (5/18, 27.8%) compared to the risk in untreated patients (6/63, 9.5%) [24]. Nevertheless, it should be taken into account that most of the studies showing an increased risk for HCC recurrence (Table 1) were retrospective and included relatively small numbers of patients with quite heterogeneous characteristics. Two recent prospective studies by Cabibbo *et al* and Kassar *et al* [26,30], which included a relatively small number of patients, yielded conflicting results and cannot provide a clear conclusion. Based on the above, large, prospective cohorts with adequately homogeneous patient populations, in terms of HCC staging system, treatment strategy and evaluation of HCC response before DAA administration, are needed.

Apart from the reports of higher HCC recurrence rates, a higher rate of *de novo* HCC appearance has also been described. In a report from a single US tertiary center, 9 of 66 HCV cirrhotic patients who received DAAs developed *de novo* HCC over a period of 6 months following the end of therapy [31]. The same trend appeared in patients with negative history for HCC who showed *de novo* occurrence (9 of 285 patients; 3.16%) during a follow-up period of 24 weeks after DAA therapy in the abovementioned study by Conti [21].

On the other hand, a recent retrospective analysis of 22,500 DAA-treated HCV patients from a US Veteran Cohort has shown that, compared to patients without SVR, those with SVR had a significantly lower risk of HCC (0.90 vs. 3.45 HCC/100 patient years; adjusted hazard ratio, 0.28, 95% confidence interval 0.22-0.36). However, in the same analysis, cirrhotic patients who achieved SVR had higher HCC rates compared to the general population [32]. The most recent study by Ioannou *et al* further supports the opinion that DAA-induced SVR is not associated with an increased risk for HCC. According to a retrospective analysis of 62,354 patients who received anti-HCV treatment from 1999 to 2015 (IFN, IFN+DAAs, or DAAs only), the achievement of SVR led to a significant reduction in HCC development, regardless of the type of regimen. More specifically, DAA-induced SVR resulted in a 71% reduction in HCC risk [33]. In summary, in terms of DAA-related *de novo* HCC occurrence, all retrospective studies [32,33,36,37,39] investigating this hypothesis, apart from 2 smaller studies by Ravi *et al* and Conti *et al* [21,31] (Table 2), as well as two recent large, prospective cohorts by Calvaruso *et al* and Romano *et al* [34,35], clearly show that there is no increased risk of HCC following HCV eradication with DAA regimens. Furthermore, SVR seems to have a protective effect against HCC development, regardless of the type of antiviral treatment.

Although there is no convincing explanation for the reported “better” outcomes in IFN-containing regimens, we should emphasize the obvious bias that characterizes studies focusing on DAA treatment, since HCV patients with severely

**Table 1** Studies evaluating the risk of HCC recurrence after DAA therapy

| Author [ref.]             | Objective      | Type of study | Patient population  | Median follow up                    | HCC incidence  | Comments   |
|---------------------------|----------------|---------------|---|-------------------------------------|--|--|
| Reig <i>et al</i> [22]    | HCC recurrence | Retrospective | 58 all DAA treated  | 5.7 months post DAA initiation      | 28% HCC recurrence   | All pts had HCC with complete response after resection, RFA, TACE  |
| ANRS [23]                 | HCC recurrence | Prospective   | HEPATHER:<br>267 pts with HCC<br>189 received DAA treatment<br><br>CirVir:<br>79 pts with HCC history in total:<br>13 DAA-treated | 20 months post-DAA initiation       | HEPATHER:<br>0.73/100 person-months in DAA treated<br>vs.<br>0.66/100 person-months in non-treated<br>(P=0.8756)<br><br>CirVir :<br>1.11/100 person-months in DAA treated<br>vs.<br>1.73/100 person-months in non-treated<br>(P=0.748) | All pts had a history of previously treated HCC (resection, ablation, LT)<br><br>All pts had cirrhosis with history of treated HCC (resection, RFA)  |
| Yang <i>et al</i> [24]    | HCC recurrence | NA            | CUPLIT: 314 Liver transplant (LT) due to HCC<br>All had DAAs  | 70 months post-LT                   | CUPLIT:<br>2.2% in DAA treated LT recipients<br><br>27.8% (5/18) in DAA treated LT recipients<br>vs.<br>9.5% (6/63) in non-treated LT recipients<br>(P=0.06)   | All pts received DAAs following LT for HCC<br><br>81/112 had LT<br>Baseline MELD score lower and mean size of largest HCC smaller in pts receiving pre-LT DAAs vs. pts not receiving pre-LT DAAs |
| Calleja <i>et al</i> [25] | HCC recurrence | Retrospective | 70 DAA treated pts with complete response to therapy for previous HCC   | 20 months after HCC treatment       | 12.9% within 6 months after DAA treatment<br>30% within 12 months after DAA treatment  | 46.7% of patients were cirrhotic   |
| Cabibbo <i>et al</i> [26] | HCC recurrence | Prospective   | 143 DAA treated with complete response to therapy for previous HCC  | Mean: 9.1 months post DAA treatment | 12% (6 m)<br>26.6% (12 months)<br>29.1% (18 months) after DAA treatment  | Previous HCC treatment: resection, ablation, LT  |
| Minami <i>et al</i> [27]  | HCC recurrence | NA            | 1191 pts with a history of treated HCC :<br>27 had DAAs<br>38 had IFN<br>861 untreated for HCV                                    | 1.3 years after DAA initiation      | 21.1% (12 months) in DAA treated<br>26.3% in IFN treated<br>vs.<br>31% in untreated<br>(P=0.101)   | Previous HCC treatment: RFA  |

(Contd...)

Table 1 (Continued)

| Author [ref.]             | Objective      | Type of study | Patient population   | Median follow up                                 | HCC incidence   | Comments                                       |
|---------------------------|----------------|---------------|--|--|---|--|
| Ikeda <i>et al</i> [28]   | HCC recurrence | Retrospective | After HCC treatment 89 DAA-treated 89 not treated                | 20.7 months                                      | 18.1% (1st yr) 28.8% (2nd yr) in DAA treated vs. 21.8% (1st yr) 46.5% (2nd yr) in untreated (P=0.003) | Previous HCC treatment: surgery and RFA        |
| Adhoute <i>et al</i> [29] | HCC recurrence | Retrospective | 22 DAA treated and 49 non treated patients with HCC history      | 19 m post-DAA treatment, 68 m post-HCC treatment | 41% in DAA treated vs. 35% in non-treated (P=0.7904)  | Previous HCC treatment: LT, surgery, RFA, TACE |
| Kassas <i>et al</i> [30]  | HCC recurrence | Prospective   | 53 DAA treated 63 non treated following successful HCC treatment | 2 years after complete HCC response              | 37.7% in DAA treated vs. 25.4% in non-treated   | Previous HCC treatment: local ablation         |

DAA, direct-acting antiviral agents; HCC, hepatocellular carcinoma; IFN, interferon; LT, liver transplantation; MELD, model for end-stage liver disease; RFA, radiofrequency ablation; SVR, sustained virological response; TACE, transcatheter arterial chemoembolization

impaired liver function or other significant comorbidities who received DAAs would not have been qualified to receive IFN treatment.

The clinical impact of HCV eradication is more relevant in patients with compensated cirrhosis, who have not developed clinically significant portal hypertension, liver decompensation or HCC [10,11]. HCV eradication leads to elimination of the inflammatory status and the subsequent up-modulation of liver regeneration, which could lead to a progression of precancerous lesions into malignant cell clones [40,41]. Experimental studies have shown that the oxidative damage induced by chronic inflammatory status causes DNA double-strand breaks (DSBs), leading to cell cycle arrest, DNA repair and apoptosis [42,43]. Under the replicative stress following the elimination of HCV, some of the impaired cells containing DSBs could reenter the replicative cycle with increased genomic instability, thus facilitating tumorigenesis [42]. Similar scenarios have been postulated in patients who had surgery for HCC. It has been suggested that, considering the stress after ischemia reperfusion, the subsequent liver regeneration promotes acceleration of liver tumor growth or even carcinogenesis after resection [44].

### The role of the immune system

IFN treatment for HCV is based on anti-inflammatory and immunomodulatory mechanisms that inhibit viral replication, leading to virus elimination in a substantial number of patients [45]. The possible alterations in the immune system after clearance of the virus with the DAAs and how they may affect the conventional cancer immunity cycle are still unknown.

### HCV-related T-cell exhaustion

In chronic HCV infection, the continuous antigen stimulation and the impaired CD4<sup>+</sup> T cells can lead to exhaustion and/or deletion of pathogen-specific CD8<sup>+</sup> T-cell clones [46]. This leads to a loss of immune control during chronic infection. Initially, the effector T cells lose their proliferative capacity and fail to develop into memory CD8<sup>+</sup> T cells. Subsequently, they present with impaired cytokine production and ultimately low IFN $\gamma$  production [47]. The "exhausted" T cells are a heterogeneous population of virus-specific CD8 T cells that go through a maturation process [48,49]. At least two distinct virus-specific CD8 T-cell subpopulations are included, the T-bet<sup>hi</sup>PD-1<sup>int</sup> CD8 T cells, representing the progenitor cells that increase in response to persisting antigen stimulation, leading to progressive differentiation to the Eomes<sup>hi</sup>PD-1<sup>hi</sup> CD8 T cells, the terminal progeny, which do not have replicative ability and persist over the long-term during a chronic infection (Fig. 1) [50]. This phenomenon of antigen stimulation, required for their continued survival, is referred as antigen addiction [49,50]. At the more advanced stages, the above process leads to a markedly diminished number of progenitor cells compared

Table 2 Studies evaluating risk of *de novo* HCC occurrence after DAA therapy

| Author [ref.]               | Objective                   | Type of study | Patient Population   | Median follow up                              | HCC incidence  | Comments   |
|-----------------------------|-----------------------------|---------------|--|---|--|--|
| Conti <i>et al</i> [21]     | HCC occurrence & recurrence | Retrospective | 285 no HCC history<br>59 HCC history   | 24 weeks post-DAA treatment                   | 3.16% <i>de novo</i> HCC<br>28.81% HCC recurrence  | All pts with cirrhosis who received DAAs<br><br>Treatment for HCC: resection, ablation, TACE   |
| Ravi <i>et al</i> [31]      | HCC occurrence              | Retrospective | 66 DAA treated   | 6 months post-DAA treatment                   | 9.1%<br>vs.<br>Historical rate 3-5%/yr in cirrhotics   | All patients were cirrhotic  |
| Kanwal <i>et al</i> [32]    | HCC occurrence              | Retrospective | 22,500 who received DAAs   | 22,963 person-years post-DAA treatment        | SVR: 0.9 per 100 pt-yrs<br>Non-SVR: 3.45 per 100 pt-yrs (P<0.001)  | 39% had cirrhosis<br>In SVR pts: HCC<br>1.82 vs. 0.34/100 person-years in pts with or without cirrhosis                                  |
| Ioannou <i>et al</i> [33]   | HCC occurrence              | Retrospective | 62,354 in total:<br>21,948 DAA-treated<br>35,871 IFN-only regimens<br>4,535 DAA+IFN regimens | 6.1 years                                     | IN SVR Pts:<br>0.92/100 pt-yrs in DAAs<br>0.28/100 pt-yrs in IFN-only<br>0.6/100 pt-yrs in DAA+IFN treated   | SVR was associated with a significantly decreased risk of HCC irrespective of the antiviral treatment                                    |
| Calvaruso <i>et al</i> [34] | HCC occurrence              | Prospective   | 2,249 DAA-treated cirrhotic patients   | 14 months                                     | Child-Pugh A<br>SVR: 2.1%<br>Non-SVR: 6.6%<br>Child Pugh B<br>SVR: 7.8%<br>Non-SVR: 12.4% (P<0.001)  | Absence of SVR independently associated with increased risk for HCC (hazard ratio, 3.40; 95% confidence interval 1.89-6.12; P<0.001).    |
| Romano <i>et al</i> [35]    | HCC occurrence              | Prospective   | 3,917 DAA-treated patients   | 536.2±197.6 days after start of DAA treatment | 0.97×100 pts/yr in DAA treated<br>vs.<br>1.18×100 pts/yr in the whole cohort of cirrhotic pts  | 70% of DAA treated patients were cirrhotic   |
| Li <i>et al</i> [36]        | HCC occurrence              | Retrospective | 5,834 DAA treated<br>3,534 IFN treated<br>8468 untreated                                     | 396 days                                      | In cirrhotic pts with SVR:<br>22.8 per 1000 person-yrs in DAA-treated<br>21.2 per 1000 person-yrs in IFN-treated<br>45.31/1000 person-yrs in untreated cirrhotic pts | Both groups of treated persons had significantly lower probability of HCC development compared to untreated persons (log-rank, P=0.0004) |

(Contd...)

Table 2 (Continued)

| Author [ref.]             | Objective                     | Type of study | Patient Population  | Median follow up                                 | HCC incidence  | Comments   |
|---------------------------|-------------------------------|---------------|---|--|--|--|
| Nagata <i>et al</i> [37]  | HCC occurrence and recurrence | Retrospective | 752 received DAAs<br>1145 received IFN  | 1.8 years for DAA-treated<br>6.8 for IFN-treated | 3-yr recurrence incidence:<br>1.4% in DAA<br>3.3% in IFN<br>(P=0.49)<br>5-yr recurrence incidence: 45.1% in DAA<br>54.2% in IFN<br>(P=0.54)                              | Fibrosis stage $\geq$ F3=23% in IFN-treated $\geq$ F3=33% in DAA-treated   |
| Ogawa <i>et al</i> [38]   | HCC occurrence And recurrence | NA            | 1675 received sofosbuvir-based regimens<br>1523 without previous HCC<br>152 with previous treated HCC | 17 months  | 1-yr cumulative rate of <i>de novo</i> HCC: 0.4% in non-cirrhotic vs. 4.9% in cirrhotic (P<0.001).<br>HCC recurrence: 6.5% in non-cirrhotic 23.1% in cirrhotic (P=0.023) | The cumulative <i>de novo</i> HCC incidence of non-cirrhotic vs. cirrhotic was significantly lower (P<0.001)<br>The cumulative HCC recurrence of non-cirrhotic vs. cirrhotic was significantly lower (P=0.023) |
| Nagaoki <i>et al</i> [39] | HCC occurrence                | Retrospective | 154 treated with daclatasvir/asunaprevir and 244 IFN-treated  | 23 months post-DAA treatment                     | HCC rate at 1, 3 and 5 yrs:<br>In DAAs:<br>0.6%, 9% and 9% respectively<br>In IFN-treated: 0.4%, 3% and 5% respectively<br>(P=0.053)                                     | Liver fibrosis was more severe in the DAA group compared with the IFN group  |

DAA, direct-acting antiviral agents; HCC, hepatocellular carcinoma; IFN, interferon; SVR, sustained virological response; TACE, transcatheter arterial chemoembolization

to the terminally differentiated T cells, a situation that may lead to a loss of immune control in chronic infections [48]. Characteristically, in their differentiation process, they express high levels of numerous inhibitory receptors (PD-1, LAG-3, CD160, 2B4, TIM-3, CTLA-4). However, the blockade of PD-1 can induce specific “revitalization” of the T-bet<sup>hi</sup>PD-1<sup>int</sup>CD8 T cells [51]. All the above indicate the complexity of the mechanisms that lead gradually to CD8 T-cell exhaustion during chronic infection, while it is still unknown whether or how current treatments could reverse the process.

### HCV-related dysfunctional CD4 T cell response

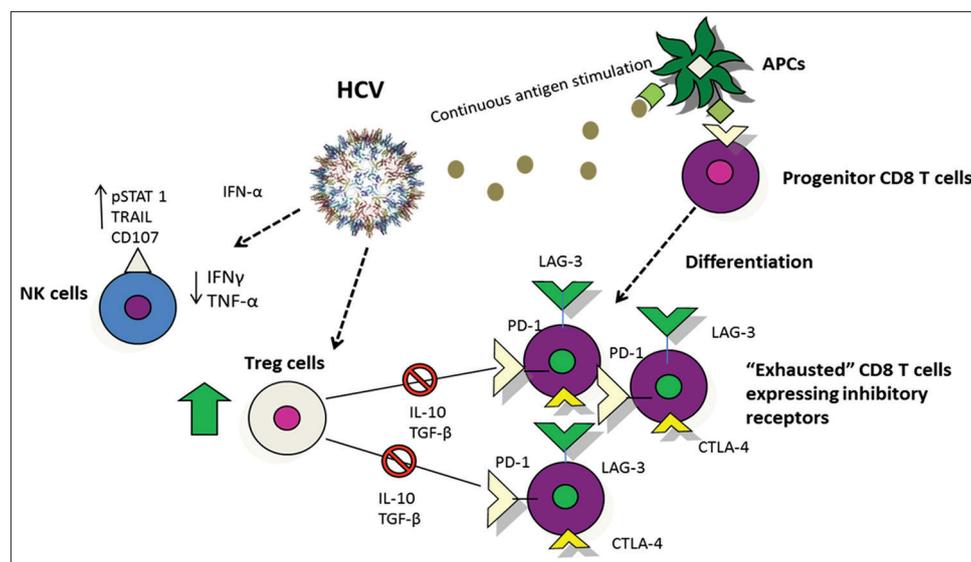
Patients with chronic hepatitis develop a lack of effective CD4 responses combined with a progressive expansion of regulatory T cells (Treg) (Fig. 1) and a reduction in interleukin (IL)-17-expressing T cells (Th17 cells) [52]. This change in the Treg/Th17 ratio leads to an impaired capability to eliminate the HCV virus, due to the suppression of HCV-specific CD8<sup>+</sup> T cells through increased IL-10 production [53]. Moreover, the affected balance between Treg and Th17 cells could generally impact the process and outcome of autoimmune and inflammatory pathways [53], including immunological defense versus development of malignancy.

### The impact of HCV treatment on immune response

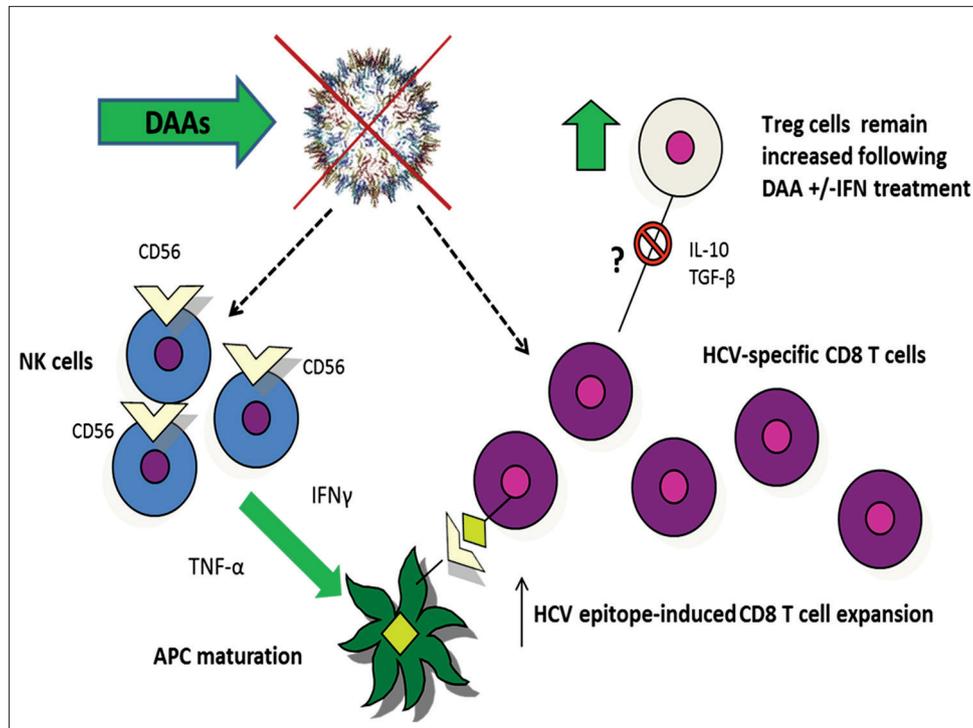
IFN treatment has been shown to partially reinforce the recovery of the impaired immune response, resulting in a post-treatment decline in circulating and liver-infiltrating

Treg cells [54]. In this context, it should be noted that the slower kinetics of virus elimination, in parallel with the immune modulatory and anti-proliferative properties of IFN, could contribute to a more gradual restoration of post-SVR immune function [55]. In contrast, there is the hypothesis that swift HCV eradication with DAAs could have a presumably destabilizing effect on the immune reconstitution in the liver microenvironment, caused by rapid reduction of viral load and leading to impaired surveillance of HCC occurrence or recurrence [55]. A recent study by Langhans *et al* examined the levels of Tregs before therapy and at different time points during and after successful treatment with sofosbuvir plus IFN, or with IFN-free regimens [56]. The population of Tregs, increased at baseline because of chronic HCV infection, did not normalize regardless of treatment type, while the Tregs activation status remained high even 1 year after successful treatment. Based on this observation, all DAA treatments, including those combined with IFN, failed to restore Treg activity to normal levels following HCV elimination [56]. Persistence of negative modulation by Treg cells may negatively contribute to immune reconstitution, even long after HCV has been cured, with an unknown impact on the process of carcinogenesis.

Nevertheless, according to Serti *et al* [57], successful HCV clearance with DAAs can rectify abnormal natural killer (NK)-cell activity due to chronic HCV infection. Specifically, it has been reported that antiviral treatment with daclatasvir/asunaprevir caused the normalization of NK-cell cytotoxic effector functions by altering the type of cytokine production as soon as the second week of treatment (Fig. 2). As a result, suppressed production of IFN $\gamma$  and tumor necrosis factor- $\alpha$  by NK cells returned to normal, while the number of cytotoxic CD107 NK cells decreased. In total, these alterations following DAA treatment represent the reset of NK cells to their original phenotype and activity as observed before chronic HCV



**Figure 1** Immunological effects of chronic hepatitis C virus (HCV) infection: NK cells show abnormal activity with suppressed cytokine production, CD8 T-cell exhaustion expressing mainly inhibitory receptors, and increased Treg cells causing suppression of HCV-specific CD8 T cells  
HCV, hepatitis C virus; NK cells, natural killer cells; Treg, regulatory T cells; TNF- $\alpha$ , tumor necrosis factor alpha; IL-10, interleukin-10; TGF- $\beta$ , transforming growth factor beta; IFN- $\gamma$ , interferon gamma; APCs, antigen presenting cells



**Figure 2** Impact of DAA-induced HCV clearance on immune functions: restoration of NK cell activity with increased IFN $\gamma$  and TNF- $\alpha$  production, leading to APC maturation and proliferation of HCV-specific CD8 $^+$  T cells. No impact on Treg population is seen following DAA treatment +/- interferon

APC, antigen-presenting cell; DAAs, direct-acting antiviral agents; HCV, hepatitis C virus; IFN, interferon; IL, interleukin; TGF, transforming growth factor; Treg, regulatory T cells; TNF- $\alpha$ , tumor necrosis factor alpha

infection [57]. This is further supported by a recent study specifically showing an increased frequency of CD56 NK cells in the peripheral blood of 31 HCV patients at the end of treatment and following achievement of SVR with DAAs [58]. The observed normalization of innate immune activity after successful DAA treatment could contribute to a more effective immunological defense against HCC. Moreover, patients who achieved SVR with DAAs were found to have more HCV-specific CD8 $^+$  T cells post-treatment compared to patients who failed. Apart from the frequency of HCV-specific CD8 $^+$  cells, the number of positive responses after HCV-specific peptide stimulation increased in patients with SVR between baseline and follow-up week 24 [59]. These results, although based on a limited number of patients (n=51), imply that CD8 $^+$  T-cell dysfunction caused by ongoing viral replication could be reversible shortly after DAA-induced HCV clearance.

To recapitulate, given the previous studies that correlated DAAs with higher HCC rates, it has been presumed that the fast-driven HCV clearance could provoke an abrupt cessation of antigen stimulation, causing a rapid imbalance in immune control and affecting HCC immunological surveillance, especially in cases of advanced liver disease or with previous HCC history. However, this mechanism is purely speculative and there are no robust published data to prove it. Although DAA treatment seems to have no significant impact on the

HCV-related increased number of Treg cells, even after SVR [56], it seems to restore NK and HCV-specific CD8 $^+$  T-cell function [57-59]. Therefore, the exact status of the complex immunological equilibrium following HCV clearance and its impact on HCC development need to be further studied before we can draw safe conclusions regarding the specific role of DAAs in hepatocarcinogenesis.

### Biomarkers

So far, a novel diagnostic biomarker to detect early HCC does not exist.  $\alpha$ -Fetoprotein is no longer recommended by the American Association for the Study of Liver Diseases' guidelines for surveillance or diagnosis of HCC [60]. Molecules such as vascular endothelial growth factor (VEGF), angiopoietin-2 or c-Kit, micro-RNAs etc., have been used as predictors of outcome in patients with advanced hepatocellular carcinoma [61,6]. In a recent study it was shown that DAA administration induced an early increase in serum VEGF level, which returned to the pre-treatment levels after the end of therapy [63]. The possibility that this finding may represent a mechanism that could possibly lead to accelerated growth of undetected HCC after DAA administration still needs to be clarified.

## Concluding remarks

Recently, raised concern regarding an increased risk of either *de novo* development of HCC or HCC recurrence after treatment with DAAs has been under discussion. Questions have also been raised regarding the optimal timing of HCV treatment in patients with HCC. Importantly, most recent studies including large numbers of patients confirm that DAA-induced SVR decreases the risk of *de novo* occurrence of HCC, as expected after the elimination of HCV. However, patients with previous HCC should be carefully investigated to confirm complete HCC remission before starting, and close follow up should be performed after DAA treatment. Further research is certainly needed to elucidate the changes in the immunological milieu and affected cellular behavior after eradication of HCV infection with DAA treatment.

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