8 December 2016
EMA/103226/2017
Pharmacovigilance Risk Assessment Committee (PRAC)

Assessment report

Procedure under Article 20 of Regulation (EC) No 726/2004 resulting from pharmacovigilance data

Daklinza (daclatasvir)	EMEA/H/A-20/1438/C/3768/0016
Exviera (dasabuvir)	EMEA/H/A-20/1438/C/3837/0017
Harvoni (sofosbuvir/ledipasvir)	EMEA/H/A-20/1438/C/3850/0027
Olysio (simeprevir)	EMEA/H/A-20/1438/C/2777/0019
Sovaldi (sofosbuvir)	EMEA/H/A-20/1438/C/2798/0029
Viekirax (ombitasvir/paritaprevir/ritonavir)	EMEA/H/A-20/1438/C/3839/0018

INN/active substance: daclatasvir, dasabuvir, sofosbuvir/ledipasvir, simeprevir, sofosbuvir, ombitasvir/paritaprevir/ritonavir

Procedure No.: EMEA/H/A-20/1438

Note

Assessment report as adopted by the PRAC and considered by the CHMP with all information of a commercially confidential nature deleted.

30 Churchill Place ● Canary Wharf ● London E14 5EU ● United Kingdom
Telephone +44 (0)20 3660 6000 **Facsimile** +44 (0)20 3660 5555
Send a question via our website www.ema.europa.eu/contact

Table of contents

List of abbreviations

AASLD: American Association for the Study of Liver Diseases

ALT: alanine aminotransferase

anti-HBc: hepatitis B core antibody

anti-HBs: hepatitis B surface antibody

APRI: aspartate aminotransferase to platelet ratio

ASV: asunaprevir

CHC: chronic hepatitis C

CHMP: Committee for Medicinal Products for Human Use

DAAs: direct-acting antivirals

DCV: daclatasvir

DNA: deoxyribonucleic acid

EASL: European Association for the Study of the Liver

EBV: Epstein-Barr virus

EC: European Commission

EMA: European Medicines Agency

EU: European Union

FIB-4: high fibrosis-4

HBcAb: HBV core antibody

HBeAb: HBV e-antibody

HBeAg: HBV e-antigen

HBsAg: HBV surface antigen

HBV: hepatitis B virus

HCC: hepatocellular carcinoma

HCV: hepatitis C virus

HIV: human immunodeficiency virus

IFN: interferon

IgG: immunoglobulin G

ISG: IFN-stimulated gene

MAH: marketing authorisation holder

PEG: pegylated interferon

PegIFN(α): pegylated interferon (alfa)

PRAC: Pharmacovigilance Risk Assessment Committee

PSUR: Periodic safety update report

PT: MedDRA preferred term

RBV: ribavirin

RMP: risk management plan

RNA: ribonucleic acid

SAG: scientific advisory group

SmPC: summary of product characteristics

SOF: sofosbuvir

SVR: sustained viral response

SVR12: sustained virologic response 12 weeks after completion of treatment

1. Information on the procedure

1.1. Referral of the matter to the PRAC

Hepatitis B virus (HBV) and hepatitis C virus (HCV) co-infection is not uncommon due to overlapping transmission modes. Literature cases (Balagopal et al., 2015; Collins et al., 2015; Ende et al., 2015) described HBV viral load increase after a rapid decline of HCV viral load in patients treated with direct acting antivirals (DAA) in interferon-free regimens, and further cases have been identified in EudraVigilance. Some of the cases identified with direct acting antivirals had serious outcomes, with worsening of hepatic status and in at least one case the patient required liver transplantation.

On 9 March 2016 the European Commission (EC) initiated a procedure under Article 20 of Regulation (EC) No 726/2004, and requested the Agency to assess the above concerns and their impact on the authorised direct-acting antiviral medicinal products, namely Daklinza, Exviera, Harvoni, Olysio, Sovaldi and Viekirax. The EC requested the Agency to give its opinion under Article 20 of Regulation (EC) No 726/2004 on whether regulatory action(s) with regard to the marketing authorisation for these products is necessary.

Following the initiation of this review, results from a study (Reig et al., 2016) performed between October 2014 and December 2015 in hepatology units of four University Spanish hospitals in patients with chronic hepatitis C and previous history of hepatocellular carcinoma (HCC) treated with direct-acting antivirals suggested an unexpected early hepatocellular carcinoma recurrence in these patients. In order to allow consideration of these data in the Article 20 review, the EC decided on 14 April 2016 to extend the scope of the procedure and request the Agency to assess the risk of hepatocellular carcinoma and its impact on the benefit-risk balance of the medicinal products mentioned above.

As the above safety concerns result from the evaluation of data resulting from pharmacovigilance activities, the EC requested the opinion to be adopted by the Committee for Medicinal Products for Human Use (CHMP) on the basis of a recommendation of the Pharmacovigilance Risk Assessment Committee (PRAC).

When the referral procedure was initiated, six medicinal products including direct-acting antiviral agents had been approved in the EU, via centralised procedure: Daklinza (daclatasvir), Exviera (dasabuvir (NS5B inhibitor)), Harvoni (ledipasvir/sofosbuvir), Olysio (simeprevir), Sovaldi (sofosbuvir) and Viekirax (ombitasvir/paritaprevir/ritonavir).

Other medicinal products including direct-acting antiviral agents have since been submitted and/or approved in the EU for interferon (IFN)-free treatment of chronic hepatitis C. The applicants/MAHs of direct-acting antiviral agents not considered in this assessment report but currently authorised in the EU, or subject to a future authorisation, shall take due consideration of the scientific conclusions.

2. Scientific discussion

2.1. Introduction

The number of chronically infected patients with HCV worldwide is estimated to be about 160 million. Hepatitis B virus (HBV) and hepatitis C virus (HCV) co-infection is not uncommon due to overlapping

transmission modes. In the EU, among HCV-infected patients, the prevalence of chronic HBV coinfection varies substantially from <1% - 30% (Cacciola et al., 1999; Georgiadou et al., 2004; Crockett et al., 2005; Cardoso et al., 2013; Konstantinou et al., 2015).

The virological and immunological aspects of HBV/HCV coinfection are not fully comprehended. Although liver disease activity and progression are generally more severe in the presence of a double infection, HBV replication is often suppressed in the presence of HCV co-infection. The European Association for the Study of the Liver (EASL) recommendations on treatment of hepatitis C made reference to the potential risk of HBV reactivation during or after HCV clearance.

It is estimated that more than 200.000 patients have been treated with DAAs in the EU since the first approval of such drugs.

Direct-acting antiviral agents target specific non-structural proteins of the hepatitis C virus and result in disruption of viral replication and infection. The risk of HBV reactivation may be greater with newer HCV treatment regimens, given their increased potency against HCV and lack of anti-HBV activity.

Literature cases (Balagopal et al., 2015; Collins et al., 2015; Ende et al., 2015) described HBV viral load increase after a rapid decline of HCV viral load in patients treated with direct acting antivirals (DAA) in interferon-free regimens, and further cases have been identified in EudraVigilance. Some of the cases identified with DAAs had serious outcomes, with worsening of hepatic status and at least one case where the patient required liver transplantation.

HBV replication after starting treatment with DAAs for HCV infection is not currently described in the product information of currently authorised products and in view of the seriousness of the events, the need for intervention on HBV replication and the biological plausibility of the replication it was considered that further investigation was warranted. The current referral procedure was triggered by the European Commission (EC) to allow further investigation of the risk of hepatitis B virus replication after starting treatment with DAAs and recommend any appropriate measure to minimise the risk.

Following the initiation of this review, results from a study (Reig et al. 2016) performed between October 2014 and December 2015 in Hepatology Units of four University Spanish hospitals in patients with chronic hepatitis C and previous history of hepatocellular carcinoma (HCC) treated with DAAs suggested unexpected early HCC recurrence.

It was considered that in addition to the hepatitis B virus reactivation, the risk of hepatocellular carcinoma should also be further investigated and that consideration should be given for adequate measures to optimise the safe and effective use of these medicinal products. The European Commission therefore extended the scope of the procedure in April 2016 to allow consideration of other data to assess the risk of hepatocellular carcinoma and its impact on the benefit-risk balance for all DAAs in the treatment of chronic hepatitis C.

2.2. Clinical aspects

In its assessment, the PRAC considered all the data submitted by the MAHs, the available scientific literature and additional information from a scientific advisory group in relation to the risk of hepatitis B reactivation and to the recurrence and occurrence of hepatocellular carcinoma. This report provides an evaluation of the relevant data.

The global effectiveness of DAA regimens is high, with rates of sustained virological response (SVR) equal or above 90%, with differences related to the baseline degree of the hepatic lesion.

2.2.1. Hepatitis B reactivation

2.2.1.1. Clinical trials data

The MAHs performed a search in their clinical trial databases in order to identify subjects with possible HBV infection and events of HBV reactivation.

From the cases detected among the clinical trials with serious adverse events involving hepatic disorders, the most relevant data are the following:

- The MAH of products containing dasabuvir, ombitasvir, paritaprevir and/or ritonavir identified 38 cases with serious hepatic events and 72 subjects with Grade 3 or higher alanine aminotransferase (ALT) elevations. None had reactivation of hepatitis B.
- The MAH of products containing daclastavir identified 30 cases with serious hepatic events. Only one of them reported HBcAb positive with liver failure and death, but information about HBV DNA was not provided.
- Most of the cases that referred hepatic events did not include information on the serology of hepatitis B; where serological data were available there were very few cases with occult HBV infection.

Overall, there is limited information on hepatitis B reactivation obtainable from the completed clinical trials since chronic hepatitis B (HBsAg+) was considered an exclusion criterion in most DAA trials and the collection of data regarding full HBV serology or DNA was not mandatory in the development programme of DAAs.

2.2.1.2. Post-marketing reports

The MAHs performed cumulative searches of their safety databases of all post-marketing reports in subjects with HBV co-infection, searching for cases of hepatic events in patients with a prior history of HBV and in patients with HBV/HCV co-infection.

The search resulted in the identification of 22 cases of possible HBV reactivation. Some of these cases had serious outcomes, with worsening of hepatic status and at least one case where the patient required liver transplantation. Another 17 cases reported hepatic events but the lack of information on HBV serology and/or HBV DNA precluded an adequate assessment of HBV infection.

The reactivation generally occurred in subjects with detectable HBsAg and active HBV replication of any level, as evaluated by measurable levels of HBV-DNA, and in subjects without detectable HBsAg but with detectable anti-HBc antibody, of which a small percentage presented with variable levels of active HBV replication.

HBV reactivation was not described in subjects that were under active antiviral treatment for HBV, and was reported in only one HIV co-infected subject, who was not under active HBV treatment.

2.2.1.3. Literature review

Four cases of HBV reactivation in HBV/HCV co-infected patients shortly after initiation of sofosbuvir (SOF)-containing treatment regimens for HCV infection have been reported in the literature (Balagopal et al., 2015; Collins et al., 2015; Ende et al., 2015). Three additional literature reports of HBV reactivation in the setting of DAA HCV treatment have been published: two reports of HBV reactivation in patients receiving daclatasvir (DCV) + asunaprevir (ASV) resolved with initiation of entecavir treatment (Hayashi et al., 2016; Takayama et al., 2016), and a report of three patients who were

HBsAg+ and experienced HBV reactivation (Wang et al., 2016). The 7 published reports on HBV reactivation are summarised below in Table 1.

Table 1: Summary of published cases of HBV reactivation in the setting of DAA treatment

	HCV Tx	HBV profile	Clinical Event (Time to Onset)	HBV Tx	HCV Tx Action	Clinical outcome	Comments
HBsAg+	SOF+SMV[1]	HBeAg- DNA 2300 IU	DNA 22 million IU/mL ALT 1792 U/L (Wk 7-8)	TVD	DCed	HBV suppressed	Cirrhosis
	HVN[2]	DNA undetectable	DNA 303 IU/L (Wk 8)	No	None	Spontaneous resolution	No clinical flare
	DCV+ASV[3]	HBeAg– HBcAb+ DNA 2.5 log copies/ml	DNA 7.0 log cp/ml ALT 237 U/L (Day 43)	ETV	DCed	HBV suppressed	
	HVN, DCV+SOF, Viekira Pak[4]	3 patients	HBV reactivation unclear 1 case of liver failure (WK 4 – 10)	N/A	N/A	N/A	
HBsAg-	SOF+SMV +RBV[5]	HBcAb+ HBsAb-	Acute hepatic failure HBV 29 million (Wk 11)	TDF	N/A	Liver Transplant	NASH, Burkitt's lymphoma (presumed rituximab)
	HVN[6]	HBcAb+ HBsAb- DNA undetectable	DNA 8.9 log IU/mL HBsAg+ HBcIgM+ (2 week post-tx)	TDF	N/A	HBV suppressed	HIV on ART w/o TDF
	SOF + SMV[1]	HBcAb+ HBsAb- DNA < 20 IU/mL	HBV 11,255 IU/mL (Wk 4)	TDF	None	HBV suppressed	No clinical flare
	DCV+ ASV[7]	HBcAb– HBsAb-	Acute liver failure HBsAg + ALT 1066 U/L (5 months post-tx)	ETV	N/A	HBV DNA suppressed	Cirrhosis

HCV agents: ASV: asunepravir, DCV: daclatsavir, HVN: Harvoni (ledipasvir/sofosbuvir), SMV: simeprevir, SOF: sofosbuvir, ViekiraPak: ombitasvir, paritaprevir,ritonavir,dasabuvir; HBV agents: ETV:entecavir, TDF: Tenofovir disoproxil fumarate, TVD: Truvada (emtricitabine/tenofovir disoproxil fumarate)
Key: ALT – alanine aminotransferase; ART – antiretroviral therapy; DCed – discontinued; DNA – deoxyribonucleic acid; HBcAb – hepatitis B core antibody; HBeAg – hepatitis B e antigen; HBsAb – hepatitis B surface antibody; HBsAg – hepatitis B surface antigen; HBV – hepatitis B virus; HIV – human immunodeficiency virus; N/A – not applicable; NASH – non-alcoholic steatohepatitis; Tx – treatment;
1. {Collins et al 2015} 2. {Balagopal et al 2015} 3. {Takayama et al 2016} 4. {Wang et al 2016} 5. {Ende et al 2015} 6. {De Monte et al 2016} 7. {Hayashi et al 2016}

2.2.1.4. Mechanistic hypotheses

Various mechanisms for an interaction between HCV and HBV in co-infected patients have been proposed. According to the MAHs, the most plausible mechanism for an interaction between HCV and HBV is that actively replicating HCV promotes a host immune state that is favourable for controlling HBV replication. Alteration of interferon expression and an altered host innate immune response has been suggested to play a role in suppression of HBV replication in the presence of HCV co-infection (Balagopal et al., 2015). HCV infection is proposed to stimulate expression of interferon-stimulated genes (ISGs) in the liver with antiviral effects that are insufficient to suppress HCV replication, but may suppress HBV replication (Bigger et al., 2001). Circulating levels of interferon gamma induced protein 10 (IP-10), one of the ISGs expressed in the liver, have been shown to be significantly higher in HBV/HCV co-infected patients when HCV is the dominant virus, and IP-10 levels correlate with HCV RNA levels (Wiegand et al., 2015; Sarasin-Filipowicz et al., 2008; Zeremski et al., 2008; Reiberger et al., 2008). IP-10 levels have been shown to rapidly decline following treatment of HCV with DAAs, with a 49% decline after 1 week of treatment with DAAs (Lin et al., 2014). Therefore, an altered innate

immune response following elimination of HCV with HCV therapy may account for the observed HBV reactivation that is observed in some HBV/HCV co-infected patients, and other viruses present in a co-infected patient also controlled by the same innate immune response could potentially be reactivated following elimination of HCV infection.

Based on these assumptions, the MAHs were asked to conduct a review on any reported replication of other hepatotropic viruses, including data from post-marketing activities, clinical trials and scientific literature. This review showed that cases of infection or reactivation of hepatotropic viruses with evidence of liver involvement in patients receiving DAA therapy for HCV infection are rare in the published literature. No relevant cases were retrieved from the MAHs' clinical trials and post-marketing safety databases. The vast majority of cases identified by a broad search consisted of mucocutaneous outbreaks of latent herpes virus infections, with no findings suggestive of hepatic involvement and/or systemic disease. A small number of cases of cytomegalovirus infection were identified in immunosuppressed liver transplant patients, with no evidence of liver inflammation.

2.2.1.5. Discussion

Recent publications describe cases of HBV viral load increase after a rapid decline of HCV viral load in patients treated with direct acting antivirals in interferon-free regimens. Patients who are co-infected with HBV/HCV are known to be at higher risk of developing severe liver disease. HBV reactivation may be associated with transaminitis, and potentially impact liver function. Some of the cases identified with direct-acting antivirals had serious outcomes, with worsening of hepatic status and in at least one case the patient required liver transplantation.

The data available provide evidence that the reactivation of HBV may occur in the context of the treatment of chronic HCV active infection with any form of effective treatment in patients co-infected with HBV and HCV. The reactivation occurred mostly in subjects with detectable HBsAg and active HBV replication of any level, as evaluated by measurable levels of HBV-DNA, and may also occur in subjects without detectable HBsAg but with detectable anti-HBc antibody, of which a small percentage may also present with variable levels of active HBV replication. These data also indicates that although severe and even fatal cases of HBV reactivation have been described in the literature, reactivation of HBV replication may mostly be mild and without clinical consequences. Generally, reactivation occurred shortly after the initiation of treatment in a pattern that implies a correlation with the rapid decrease in HCV viral load which characterises the viral load dynamics with DAA.

The impact of chronic HCV infection characteristics, such as HCV genotype, viral load and histopathologic staging, on the risk of occurrence of HBV reactivation could not be clarified from the data, although it may be assumed that patients with more advanced liver disease may have a higher risk of severe clinical complications should HBV reactivation occur.

The MAHs have neither conducted nor are conducting any specific studies in order to clarify the mechanism of HBV reactivation in patients treated with DAAs. It is acknowledged that the most probable mechanism may be related to a decrease of host immune activation secondary to the sudden and significant decrease in HCV viral load, probably independently of the drug regimen to which the subject is exposed.

In conclusion, the case reports retrieved from a systematic search of the literature, MAHs' clinical and post-marketing databases indicate that HBV reactivation may occur in HCV/HBV co-infected patients when HCV replication is suddenly and profoundly inhibited by DAA therapy.

In order to minimise the risk, HBV screening should be performed in all patients before initiation of treatment. HBV/HCV co-infected patients being at risk of HBV reactivation should be monitored and

managed according to current clinical guidelines. Hepatitis B reactivation should also be considered an important identified risk in the risk management plan (RMP) for all direct-acting antivirals.

2.2.2. Hepatocellular carcinoma

The primary goal of HCV therapy is to cure the infection by achieving a sustained virological response (SVR) defined as undetectable HCV RNA 12 weeks (SVR12) or 24 weeks (SVR24) after treatment completion. The infection is cured in almost all patients who achieve an SVR. As a consequence of virological cure and the subsequent termination of hepatic necro-inflammation, HCV therapy is expected to lead to prevention of complications of HCV, including decompensated liver disease, HCC, extrahepatic disease and death.

HCV infection is a leading cause of cirrhosis and is associated with a 15- 20-fold increased risk of HCC. The annual incidence of HCC in subjects with HCV-related cirrhosis has been estimated at 1%-7% (Fattovich et al., 2004; Ferlay et al., 2015).

SVR following treatment with interferon (IFN) has been shown to reduce the risk of developing HCC (Hiramatsu et al., 2015, Morgan et al., 2010). Risk factors for the development of HCC after attaining SVR include older age, male sex, cirrhosis, liver fibrosis, diabetes, obesity, lipid metabolism disorders, glucose metabolism disorders, alcohol intake, high aspartate aminotransferase to platelet ratio (APRI) and high fibrosis-4 (FIB-4) index (Hedenstierna et al., 2016; Hiramatsu et al., 2015; Kim et al., 2016; Payance et al., 2016; Toyoda et al., 2015).

Studies have estimated HCC incidence in cirrhotic patients who achieve SVR following IFN-based antiviral therapy to be 0.5-2.0% at three years, 2.3-8.8% at five years, and 3.1%-11.1% at 10 years (Hiramatsu et al., 2015; Kim et al., 2016, Pinzone et al., 2014; Toyoda et al., 2015).

Literature data suggest that the risk of HCC and all-cause mortality is significantly reduced, but not eliminated, in cirrhotic patients who clear HCV compared to untreated patients and non-sustained virological responders (Van der Meer, 2017, Bruno 2016). However, data on patients treated with IFN-free agents are still limited.

Unexpected early HCC recurrence in patients in complete remission after treatment of HCC was reported in two retrospective studies in patients with HCV-related HCC who had achieved complete radiological response after HCC treatment and were subsequently treated with DAAs, mostly leading to SVR. Contradictory results were published, reporting a lack of evidence of an effect of DAA-based regimens on the recurrence of HCC in such patients.

In this review, the PRAC considered all available data on the recurrence of HCC. In view of the need to clearly distinguish recurrent cases of HCC from de novo cases, an analysis of de novo cases was also considered needed.

2.2.2.1. Early recurrence

Clinical trials

The development programmes for the presently approved DAAs generally had previous history or active or suspected malignancy as an exclusion criterion and data on treatment in this specific group of patients are therefore lacking. As a consequence, recurrence rate of HCC could not be established based on data from clinical trials.

Observational cohorts

Results from a study (Reig et al., 2016) performed between October 2014 and December 2015 in Hepatology Units of four University Spanish hospitals in patients with chronic hepatitis C and previous history of hepatocellular carcinoma treated with DAAs suggested unexpected early hepatocellular carcinoma recurrence. Out of 103 patients with prior HCC who received DAAs between 2014 and 2015, 58 patients with HCV infection and prior history of treated HCC had achieved complete response and lacked non-characterised nodules at the time they underwent anti-HCV treatment with oral DAAs. Patients receiving interferon were excluded, as were patients who had previously received a liver transplant, and patients with no radiological follow-up after starting DAAs. After a median follow-up time of 5.7 months, three patients died and 16 patients developed radiologic tumour recurrence, providing an estimated a recurrence rate of 27.6% (CI 95% 16.70-40.90). The authors concluded that there was an unexpected high rate of tumour recurrence associated with HCV clearance. The authors acknowledge that their findings should be considered with caution due to the low numbers of patients involved in their study and suggest that further larger studies are required to define the risk of cancer recurrence in patients treated with DAAs. The authors postulate that the HCC recurrence could be explained by a reduction in inflammatory signals (i.e., as evidenced by normalisation of transaminases) following rapid decline of HCV viral load after initiation of DAA therapy; they suggest that the resultant immune suppression could potentially favour growth of tumour clones.

Similar results were reported by Conti et al. (2016) with a recurrence rate after starting DAA treatment of 28.8% (CI 95% 17.80-42.10). These publications have led to a number of letters to editors that reflect the interest in the area and highlight the methodological challenges in studying this concern.

Data from the French ANRS cohorts on HCV patients (Pol et al., 2016), which do not support the aforementioned observations, were reviewed as part of this procedure. In this program, three independent cohorts have been followed-up: the CO12 CirVir, CO23 CUPILT and CO22 HEPATHER cohorts.

The ANRS CO12 CirVir cohort was aimed at assessing any DAA complication and registering screening as per current ASLD guidelines. In this dataset, 79 HCC patients were considered to be in remission at least 3 months following the implementation of at least one curative procedure. Thirteen patients subsequently received a DAAs-based regimen after anti-tumoral treatment. One patient (7.7%) experienced HCC recurrence after 7.1 months while 31 recurrences occurred among the remaining 66 patients (47.0%) who did not received DAAs. However, the limited number of patients with a previous HCC who were treated with DAA after complete radiological response (n=13) precludes any firm conclusion. Unfortunately, patient recruitment was stopped in 2012, which corresponds to when DAAs became available in France (Trinchet et al., 2011, 2015; Ganne-Carrie et al., 2016; Nahon et al., 2016).

ANRS CO23 CUPILT cohort comprises a very different population as it enrolled only patients that have undergone liver transplant.

The ANRS CO22 HEPATHER cohort, focused on 267 patients with a history of treated HCC prior to inclusion, among whom 189 received DAA from inclusion (DAA group), 78 did not receive DAA (untreated group). Overall, 24 recurrences of HCC were reported in 3,292 treated person-months (at a rate of 0.73/100 person-months), while 16 recurrences of HCC were reported in 2438 untreated person-months (at a rate of 0.66/100 person-months, p = 0.8756). Results do not suggest an effect of DAAs on HCC recurrence HR1.21 (CI 95% 0.62-2.34). However, HCC was not an endpoint for this observational study, so a prospective definition of early progression was not established, and data about procedures to ascertain complete radiological response before DAA treatment and timing, and

procedures to diagnose HCC after starting DAA treatment were not provided. This information is essential when assessing early HCC recurrence.

In summary, no firm conclusions could be drawn from the observational studies reviewed as they were either not designed to specifically address this issue or the number of patients studied with DAA exposure was low. The concern remains and further studies in a well-defined set of patients with a systematic approach for the diagnosis of HCC after DAA treatment are warranted.

2.2.2.2. De novo HCC

Clinical trials

Based on data from on-going and finalised clinical trials, the MAHs presented incidence rates of HCC in patients treated with DAA interferon-free regimens and DAA interferon-containing regimens focused on patients achieving SVR and stratified by disease severity.

The table below summarises the results presented:

Table 2: HCC incidence rates in patients who reached a SVR in clinical trials of DAA following DAA IFN-free and IFN-based regimens

MAH DAA	**Disease severity**	**IFN free DAA regimens**	**IFN-containing regimens**	**HCC screening method/timelines**
Abbvie Exviera (dasabuvir) Viekirax (ombitasvir /paritaprevir /ritonavir)	Non-cirrhotic	IR: **0.15** (8 cases;5365.1 PY)	IR: **0** (0 cases; 122 PY)	Ultrasonography every 6 months in cirrhotics
	Cirrhotic	IR: 0.96 (9 cases;936.6 PY)	IR: NA (0 cases;0 PY)	
	Overall	IR: 0.27 (17 cases;6301,70 PY)	IR: 0 (0 cases;122 PY)	
BMS Daklinza (daclatasvir)	Non-cirrhotic	Data not provided	Data not provided	According to investigator
	Cirrhotic	Data not provided	Data not provided	
	Overall	Data not provided	Data not provided	
Gilead Sovaldi (sofosbuvir) Harvoni (sofosbuvir/ ledipasvir)	Non-cirrhotic	IR: **0.23** (2 cases;5759 PY)	IR: **0.09** (1 case;1107.33 PY)	Investigators are expected to follow screening guidelines for HCC
	Cirrhotic Compensated	IR: **0.55** (9 cases;1625.10 PY)	IR: **0** (0 cases;5759 PY)	
	Cirrhotic Decompensated	IR: 1.67 (7 cases;418.45 PY)	IR: NA (0 cases;0 PY)	
	Overall	IR: **0.23** (18 cases;7802.55 PY)	IR: **0.08** (1 case;1266.7 PY)	
Janssen Olysio (simeprevir)	Non-cirrhotic	IR: **0** (0 cases;614.0 PY)	IR: **0.06** (2 cases;3280.6 PY)	According to the investigator
	Cirrhotic	IR: **1.70** (4 cases;235.1 PY)	IR: **1.05** (3 cases;286.1 PY)	
	Overall	IR: **0.47** (4 cases;849.1 PY)	IR: **0.14** (5 cases;3566.8 PY)	

IR: HCC cases per 100 person-year (PY)

Overall, as expected, higher incidence rates of new onset HCC were found in patients with more advanced disease, namely cirrhotic patients. Also DAA IFN-free regimens showed a higher incidence of *de novo* HCC compared to patients treated with DAA IFN-based therapies. This higher incidence was observed in more comparable groups of disease severity, i.e. presence of cirrhosis or compensated cirrhosis. However, it is notable that inclusion criteria in trials on cirrhotic patients on IFN-free

regimens were stricter on the status of liver disease (e.g., a platelet count >90,000 was required in studies of IFN-based regimens). Furthermore, general inclusion and exclusion criteria have been considerably stricter, e.g., in terms of allowed comorbidities, with IFN-based therapy, in accordance with the label and experience of IFN-based therapies. Therefore, it is recognised that the groups are not strictly comparable, even when taking cirrhosis status into account. It remains to be determined to what extent the actual population of patients included in these trials treated with IFN-containing or IFN free regimens actually differ in terms of relevant risk factors for HCC development.

Two clinical trials included non-cirrhotic patients. These studies included 122 subjects exposed to telaprevir + pegIFN/RBV for a total of 170.5 patient-years. None of these 122 subjects experienced HCC after initiating treatment during the study.

An analysis of incident HCC in subjects in the Gilead clinical database by SVR and cirrhosis status detected 36 events of HCC among 13,525 patients. About half of the events occurred in a population that did not achieve SVR, consistent with a considerably higher rate in this population. Only two events (5,5%) were reported in patients treated with IFN-containing regimens. The difference in the baseline severity of hepatic impairment is expected to have contributed significantly for this difference as no decompensated cirrhosis patients have been included in the IFN group and the number of patients with compensated cirrhosis was small. In patients with compensated cirrhosis not achieving SVR, the rates of HCC events were slightly higher for the DAA-only group (2.02% vs 1.3%). HCC incidence rates excluding decompensated cirrhosis patients (for which IFN was contraindicated) and stratifying by SVR achievement and by regimen type (IFN-free or IFN containing) were as follows:

IR (per100py)	SVR	no SVR
DAA+IFN	0,079	0,500
DAAfreeIFN	0,149	1,131

For both SVR and non SVR patients in IFN free groups the HCC incidence rate was doubled. This may reflect different baseline risks among populations. Regarding the time to occurrence of the events, the 36 emergent HCC clustered around the first few weeks to months. The limited data in IFN-free regimens did not allow a comparison between the groups.

Observational cohorts

The Hepatitis C Therapeutic Registry and Research Network (HCV-TARGET) is an MAH-sponsored ongoing prospective observational study conducted at 44 academic and 17 community medical centres in North America, Europe and Israel. It was designed to evaluate clinical and virologic outcomes of patients treated with DAAs. This study plans to enrol HCV infected patients treated with DAA therapies. A secondary goal is to serve as the core resource for important collaborative translational studies utilizing biospecimens and clinical data from diverse patient populations. In September 2016, over 6000 HCV infected patients had been enrolled in the cohort. Demographic, clinical, adverse event and virological data are collected throughout treatment and post-treatment follow-up from enrolled patients. In this cohort, 40.6% of patients are cirrhotic and 16.9% have evidence of prior decompensation. It is unknown whether this registry includes risk factors, HCC baseline and follow-up screenings (neither method, nor frequency), and if not, this registry may not allow for an evaluation of the incidence of HCC.

2.2.2.3. EudraVigilance data on hepatic cancer

Data from EudraVigilance have been provided in a review with a cut-off date 01 June 2016. It identified 266 instances of emergent events of hepatic or hepatic related neoplasms, of which 157 with

the preferred terms (PT) hepatocellular carcinoma, 58 hepatic cancer, 27 hepatic neoplasm and 9 hepatic cancer recurrent.

The usual limitations and caveats of using pharmacovigilance databases apply to this analysis, in particular the under-reporting of suspected adverse drug reactions. The classification of events as de novo, progression or recurrence was done by expert consensus for some case reports, as the information provided was not entirely clear. Furthermore, some of these case reports had incomplete information, namely in what concerns the medical history of each patient, which may have led to an underestimation of the cases of recurrence and progression.

2.2.2.4. Discussion

HCC recurrence

Data on early recurrence of HCC in subjects treated with DAA is very limited, since none of the controlled studies, or observational treatment programs sponsored by MAHs, has been designed to specifically address this issue.

Based on the recently published reviews on HCC recurrence, no firm conclusions could be drawn due to the heterogeneity of the patient population and the lack of harmonisation of diagnostic tools for HCC diagnosis and defined intervals for follow-up. Also, no existing prospective studies designed to appropriately investigate the risk were identified.

The results of the published reviews are of sufficient concern to warrant further studies to evaluate the HCC recurrence associated with DAAs and to address any uncertainties about this potential risk. The heterogeneity of this population and the lack of harmonisation of diagnostic tools for HCC diagnosis and defined intervals for follow-up are of key importance and should be taken into account in the design of further studies.

The PRAC considered that the conduct of a randomised clinical comparing early versus delayed DAA treatment in patients with a previous HCC and reaching complete radiological response would be the most informative approach, in line with the recommendations from the SAG HIV/Viral diseases. It is however acknowledged that the conduct of such clinical trial could be challenging.

The MAHs proposed to further investigate the risk through a prospective observational approach using TARGET-HCC registry. TARGET-HCC is a new cohort study prospectively collecting data according to clinical practice in patients with a previous HCC. The primary objective of this cohort study is to evaluate whether the exposure to DAA therapy contributes to a higher recurrence rate of HCC than in participants without DAA therapy. However, it is doubtful whether such approach will provide the appropriate evidence. Factors influencing the risk of HCC recurrence after a complete radiological response of a previous treated HCC are extensive, practice dependent and difficult to capture in the observational setting. The risk of recurrence is highly influenced by the stage [number, size and characteristics of the lesions (EASL HCC guideline)], specific election of treatment (surgical resection, ablation, liver transplantation or chemoembolization), anatomo-pathologic characteristics of the tumour, such as microvascular invasion, satellites and fundamentally by the frequency and method to detect recurrences, which are not standardised. Internationally accepted clinical guidelines for the management of these patients are lacking.

Any study performed according to clinical practice in order to compare the recurrence rate in patients treated immediately vs. deferred DAA treatment or vs. untreated patients will have important confounders that cannot be managed with this purely observational design. The decision to treat and when to treat patients by clinicians, when and how to search for a recurrence, and the validity of the

tests performed to assess response, may depend on a number of factors. It is therefore considered that the design as proposed by the MAHs will not be adequate to ascertain the role of DAA treatment in the development of HCC recurrences.

Any further investigation of the risk of HCC recurrence should be conducted in an homogeneous set of patients having achieved complete radiological response independently confirmed (e.g. at intermediate or high risk of recurrence after surgical resection according to pre-defined criteria; tumour assessment by CT-scan or MRI at baseline and during follow-up according to predefined timelines). Specific DAA treatment should be selected as per clinical practice. The study should assess HCC recurrence rates and pattern of recurrence, as well as time-to-onset of HCC recurrence from initiation of DAA treatment. The results should be compared to the best available dataset in terms of similarities.

Based on the above, the PRAC considered that to evaluate the incidence of HCC recurrence associated with DAAs, the MAHs of direct-acting antivirals shall perform a prospective study in a well-defined group of patients with criteria for entry and follow-up of patients. The protocol for this study should be agreed with the PRAC. The PRAC also emphasised that a joint study with all MAHs concerned was encouraged.

In addition, it is recommended that all ongoing clinical trials including HCC patients adhere to the operational methods of this initiative as far as possible in order to increase the possibility of cross-studies comparison.

***De novo* HCC**

Based on the findings of Reig and colleagues (2016), concerns on the development of *de novo* HCC in cirrhotic HCV patients treated with DAA were also raised, as these patients may harbour not yet diagnosed HCC. Clinical trial data on incidence of new on-set HCC show higher point estimates for HCC after reaching SVR with IFN-free regimens compared to IFN-containing regimens, also when stratifying by presence of cirrhosis. However, the difficulty of fully controlling confounding in this non-randomised comparison was recognised. Furthermore, follow-up during these studies was limited and it is uncertain how HCC was monitored and diagnosed. The data also support that the most important risk factor for the development of HCC in HCV infected patients, regardless of treatment with DAA is the stage of the liver disease, (status of liver function, presence of portal hypertension) (García-Tsao et al., 2010; Ripoll et al., 2009).

Studies have been recently published on the incidence of HCC with DAAs. However, the information provided is still limited due to the type of patients selected, uncompleted data in the cohort of comparison, short follow-up, different follow-up time among groups or different timing for assessing incidence (Foster et al., 2016; Cheung et al 2016; Mangia et al., 2016). In the recent American Association for the study of liver diseases (AASLD) congress (November 2016), some investigators who did not find an increased risk of HCC have reported a more aggressive than usual pattern of *de novo* HCC in cirrhotic patients (Romano et al., 2016; Renzulli et al., 2016); this issue is of concern and should also be investigated.

Further analysis is considered needed. Data from observational cohorts may be considered for this analysis, provided that the cohorts capture all the necessary data for a meaningful assessment of the concern. However, the existing cohorts HCV TARGET and ANRS HEPATHER were not designed to assess HCC incidence and it is unclear whether frequency and timing of screening (HCV TARGET) are registered or not, which would be of high relevance for assessing the incidence of HCC. In the ANRS CirVir cohort, all relevant risk factors for HCC were recorded as well as 6-monthly imaging testing (as per ASLD guidelines). However its recruitment stopped in 2012 and most of the patients were therefore treated prior to DAA treatment becoming available.

Considering the difficulties in analysing the existing data due to the potential imbalance in baseline factors, the PRAC considers that data should be collected through a prospective cohort study in compensated cirrhotic patients without history of HCC for which HCC has been ruled out by adequate image technique within a maximum of 3 months before starting DAA, and that are treated with DAAs. Risk factors patient-related and present at baseline (eg. alcohol intake, age at starting HCV treatment, diabetes), management related (HCC screening, methods for follow-up HCC and timing of follow-up), and evolutionary events (achievement of SVR, Child-Pugh status, MELD, AFP changes, alcohol intake and diabetes), among others, should be available. Such registry should carefully register at baseline and during follow-up clinical, biochemical, and imaging results, performed according with EASL current guidelines for the management of cirrhotic patients. Feasibility analysis of the use of existing registries for capturing such data to evaluate *de novo* HCC after DAAV should be provided. Should the use of existing data sources prove to not be adequate, a proposal by MAHs for a prospective collection of data should be provided.

3. Experts consultation

The scientific advisory group (SAG) HIV/Viral diseases experts were consulted on both hepatitis B reactivation and potential hepatocellular carcinoma.

Concerning the risk of hepatitis B reactivation, the experts considered that a warning on HBV reactivation during DAA treatment should only concern patients with chronic HBV/HCV coinfection, since this is the population at highest risk of HBV reactivation. They also considered appropriate to refer to clinical practice/guidelines for the management of HBV reactivation in the product information. The experts considered that the risk was sufficiently characterised.. The experts also considered that based on the available data, the replication of other hepatotropic viruses was not of concern. Finally, the experts were of the view that the risk of hepatitis B reactivation with DAAs is well known by specialists and therefore no additional specific targeted communication was deemed necessary.

With regards to HCC, the experts overall considered that the signal of early recurrence HCC would merit further evaluation and that a clinical trial would be the most powerful approach to detect and quantify the risk. The experts overall considered this approach feasible, although they acknowledged that setting up such a study may be challenging. The trial could assess the rate of early reactivation comparing early treatment with DAAs after HCC remission vs. delayed treatment. This could give an insight on whether reactivation occurs earlier than expected and if timing of DAA treatment could play a role.

The available data on HCC incidence after DAAV treatment were considered scarce, and the signal weaker than the one of early recurrence of HCC. However, considering the large population being exposed to DAAs, data on incidence of HCC after DAA should be obtained. Albeit the limitations, an observational design was considered the most suitable approach, provided that the data needed for a proper assessment would be collected: image methods for ruling out an HCC before treatment, DAA treatment and outcome, details on the patient cirrhotic status, imaging data at regular intervals, relevant concomitant medication during follow up. The group considered that historical cohorts could be used for comparison.

4. Benefit-risk balance

In its assessment, the PRAC considered all the data submitted by the MAHs, literature and additional information from a scientific advisory group in relation to the risk of hepatitis B reactivation and to the recurrence and occurrence of hepatocellular carcinoma.

Hepatitis B virus reactivation

With regards to the risk of hepatitis B reactivation, since chronic hepatitis B infection (HbsAg+) was generally considered an exclusion criterion and the collection of data regarding HBV serology and DNA was not mandatory in the development programme of DAAs agents, there is limited information on hepatitis B reactivation obtainable from the completed clinical trials. Therefore data on HBV reactivation with DAAs mostly arose post-marketing.

The available data provide evidence that the reactivation of HBV replication may occur in the context of the treatment of chronic HCV active infection with any form of effective treatment in patients co-infected with HBV and HCV. The reactivation may occur mostly in subjects with detectable HBsAg and active HBV replication of any level, as evaluated by measurable levels of HBV-DNA, and may also occur in subjects without detectable HBsAg but with detectable anti-HBc antibody, of which a small percentage may also present with variable levels of active HBV replication.

Although severe and even fatal cases of HBV reactivation have been described in the literature, the available data indicate that reactivation of HBV replication in most cases may be mild and without clinical consequences. The impact of chronic HCV infection characteristics, such as HCV genotype, viral load and histopathologic staging, on the risk of occurrence of HBV reactivation could not be clarified from the available data. It may be assumed however that patients with more advanced liver disease may have a higher risk of severe clinical complications should HBV reactivation occur. Generally, the reactivation occurred shortly after the initiation of treatment in a pattern that implies a correlation with the rapid decrease in HCV viral load which characterises the viral load dynamics with DAAs.

Overall, the PRAC was of the view that evidence exists on a risk of HBV reactivation in HBV/HCV co-infected patients treated with DAAs and therefore HBV reactivation in co-infected patients should be considered as an important identified risk which should be closely monitored through routine risk minimisation activities.

In order to minimise the risk of HBV reactivation, the PRAC recommended that all patients should be screened for HBV infection before initiation of treatment with DAAs and that patients presenting a co-infection HBV/HCV should be monitored and managed according to current clinical guidelines. The product information should reflect these recommendations and inform healthcare professionals about this risk. In addition, patients should be advised to contact their doctor if they have ever been infected with HBV as close monitoring is required.

Hepatocellular carcinoma

With regards to the review of HCC with DAAs, MAHs were requested to perform a comprehensive review of all available data from clinical trials, observational studies, spontaneous reports, and published literature of cases of HCC in patients with chronic hepatitis C after treatment with DAAs.

A study from Reig and colleagues (2016) showed a signal of HCC recurrence in patients treated with DAAs, similar results were obtained by Conti and colleagues (2016). Other published data from larger cohorts did not support the findings (Pol et al., 2016). However, these cohorts were either not designed for assessing HCC recurrence, as is the case of the ANRS CO22 HEPATHER cohort, or included a limited number of patients with a previous HCC reaching complete radiological response and subsequently treated with DAAs as in the ANRS CO12 CirVir cohort.

Overall, the PRAC considered that further studies were warranted to further characterise the risk of HCC recurrence associated with DAAs, in order to address remaining uncertainties about this potential risk and conclude on the need for any additional advice on clinical management. Taking all available data into account, the PRAC was of the view that MAHs should conduct and submit the results of a

prospective safety study using data deriving from a cohort of a well-defined group of patients, based on an agreed protocol setting out criteria for entry and follow-up of patients in terms of timing and method for screening. The protocol of this study shall be submitted to the PRAC by 15 June 2017 and the final study results by Q4 2019.

Based on the findings of Reig and colleagues (2016), concerns on the development of *de novo* HCC in cirrhotic HCV patients treated with DAA were also raised, as these patients may harbour not yet diagnosed HCC. Clinical trial data on incidence of new on-set HCC show higher point estimates for HCC after reaching SVR with IFN-free regimens compared to IFN-containing regimens, also when stratifying by presence of cirrhosis. However, the difficulty of fully controlling confounding in this non-randomised comparison was recognised. It was considered that the impact of DAAs therapies on the incidence and type of *de novo* HCC should be further investigated by the MAHs through a prospective cohort study to be conducted in HCV infected patients with compensated cirrhosis (CPT-A) without history of HCC and treated with DAAs. The research should capture prospectively the known risk factors for HCC and the periodic image testing for HCC diagnosis, according to current European clinical guidelines (EASL). A feasibility assessment of the use of existing data sources for this purpose should be submitted for PRAC assessment by 15 June 2017. Should the use of existing data sources not show feasible, a proposal for a prospective collection of data should be provided.

The PRAC was also of the view that 'emergence of hepatocellular carcinoma' and 'recurrence of hepatocellular carcinoma' should be considered as important potential risks. In addition, 'patients with previous HCC' should be considered as missing information, since this population was excluded from available clinical trials. The RMP of the relevant medicinal products will be updated accordingly.

In conclusion, the PRAC considered that the benefit-risk balance of DAAs-containing products remained favourable subject to the amendments of the terms of the marketing authorisations.

5. Risk management

The PRAC considered that 'hepatitis B reactivation' should be considered as important identified risk in the RMP for all direct-acting antivirals. In addition, 'emergence of hepatocellular carcinoma' and 'recurrence of hepatocellular carcinoma' should be included as important potential risks. 'Patients with previous HCC' should be reflected as missing information in the RMP of the DAAs, since this population was excluded from existing clinical trials.

The following ongoing and planned activities described under section 5.2 are considered relevant to better characterise these risks and should be reflected in the RMP. An updated RMP should be submitted within 3 months of adoption of CHMP opinion.

5.1. Risk minimisation activities

5.1.1. Amendments to the product information

Hepatitis B reactivation

The PRAC considered that routine risk minimisation measures in the form of amendments to the product information would be necessary in order to minimise the risk of hepatitis B reactivation associated with the use of direct-acting antivirals. These changes include amendments to section 4.4 of the summary of product characteristics (SmPC) to inform healthcare professional and patients that HBV screening should be performed in all patients before initiation of treatment and that HBV/HCV co-infected patients should be monitored and managed according to current clinical guidelines.

It is also recommended to amend section 2 of the package leaflet (PL) to advise patients to inform their doctor in case of current or previous infection with hepatitis B virus as closer monitoring is required.

Hepatocellular carcinoma

Based on the currently available data, the PRAC considered that at this stage, no amendments of the SmPC and PL were required.

5.2. Pharmacovigilance activities

The potential risk of emergence and recurrence of HCC will be addressed by the following prospective evaluations.

Recurrence of hepatocellular carcinoma

To evaluate the incidence of HCC recurrence associated with DAAs, the MAHs of direct-acting antivirals shall perform a prospective safety study in a well-defined group of patients based on an agreed protocol setting out criteria for entry and follow-up of patients in term of timing and method for HCC screening.

Recognised risk factors for recurrence and results from a standardised screening in terms of method and timing will be captured through a long term prospective safety study using data deriving from a cohort of a well-defined group of patients, based on an agreed protocol setting out criteria for entry and follow-up of patients in terms of timing and method for screening. Such results should be compared with the best available comparison group.

The protocol for this study shall be submitted to the PRAC by 15 June 2017 and the final study results by Q4 2019. A joint study to be conducted by the concerned MAHs is encouraged.

***De novo* hepatocellular carcinoma**

To estimate the impact of DAA therapies on the incidence and type of *de novo* HCC, it is considered that a prospective cohort study in HCV infected patients with compensated cirrhosis (CPT-A) without history of HCC and treated with DAAs should be conducted. The research should capture prospectively the known risk factors for HCC and the periodic image testing for HCC diagnosis, according to current European clinical guidelines (EASL). This category 3 study should be included in the risk management plan (RMP) of each DAAs and be conducted according to an agreed protocol. A feasibility assessment of the use of existing data sources for this purpose should be submitted for PRAC assessment by 15 June 2017. Should the use of existing data sources prove to be unfeasible or inadequate, a proposal by MAHs for a prospective collection of data should be provided. This may be a modification of a current registry or a new data collection.

6. Grounds for the recommendation

Whereas,

- The PRAC considered the procedure under Article 20 of Regulation (EC) No 726/2004 resulting from pharmacovigilance data for direct-acting antiviral agents (DAAs) indicated in the treatment chronic hepatitis C.
- The PRAC reviewed the totality of the data submitted in writing and during the oral explanations by the marketing authorisation holders in relation to the risk of hepatitis B reactivation and to the concerns raised following reports of hepatocellular carcinoma in patients

using DAAs, as well as the outcome of the meeting of the scientific advisory group on HIV/Viral diseases.

- Concerning HBV reactivation, the PRAC concluded that available data provide evidence of a risk of HBV reactivation in patients co-infected with HBV/HCV treated for chronic hepatitis C with DAAs. The PRAC was of the view that all patients should be screened for hepatitis B virus infection before initiation of treatment with DAAs. Patients with HBV/HCV co-infection should be monitored during and after treatment according to current clinical guidelines. The product information will include a warning to inform about the risk of hepatitis B reactivation and reflect these recommendations.
- Concerning the risk of recurrence of HCC in patients using DAAs, the PRAC considered that further data are required on the impact of DAAs treatment on the incidence of HCC recurrence. All MAHs of DAAs shall conduct a prospective safety study in a well-defined group of patients based on an agreed protocol setting out criteria for entry and follow-up. A joint study is encouraged.
- The PRAC was also of the opinion that the impact of DAAs treatment on the incidence and type of *de novo* hepatocellular carcinoma should be further investigated though a prospective cohort study in HCV infected patients with cirrhosis. A joint study is encouraged.

In view of the above, the PRAC considers that the benefit-risk balance of direct-acting antivirals remains favourable subject to the amendments to the terms of the marketing authorisations.

The PRAC, as a consequence, recommends the variation to the terms of the marketing authorisations for Daklinza, Exviera, Harvoni, Olysio, Sovaldi and Viekirax.

References

1. Balagopal A and Chloe L. Thio. 'Editorial Commentary: Another call to cure Hepatitis B' Clinical Infectious Disease, Vol. 61 (8), 2015, p. 1307-9.
2. Bigger CB, Brasky KM, Lanford RE. 'DNA microarray analysis of chimpanzee liver during acute resolving hepatitis C virus infection' Journal of Virology, Vol. 75 (15), 2001, p. 7059-66.
3. Bruno S, Di Marco V, Iavarone M, Roffi L, Crosignani A, Calvaruso V, et al. 'Survival of patients with HCV cirrhosis and sustained virologic response is similar to the general population' Journal Hepatology, Vol. 64(6), 2016 Jun, p. 1217-23.
4. Bruno S, Silini E, Crosignani A, Borzio F, Leandro G, Bono F, et al. 'Hepatitis C virus genotypes and risk of hepatocellular carcinoma in cirrhosis: a prospective study' Hepatology, Vol. 25 (3), 1997, p. 754-8.
5. Cacciola I, Pollicino T, Squadrito G, Cerenzia G, Orlando ME, Raimondo G 'Occult hepatitis B virus infection in patients with chronic hepatitis C liver disease' New England Journal of Medicine, Vol 341(1), Jul 1, 1999, p. 22-6.
6. Cardoso C, Alves AL, Augusto F, Freire R, Quintana C, Gonçalves M, Oliveira AP. 'Occult hepatitis B infection in Portuguese patients with chronic hepatitis C liver disease: prevalence and clinical significance' European Journal of Gastroenterology & Hepatology, Vol. 25(2), 2013 Feb, p. 142-6.
7. Cardoso H, Vale AM, Rodrigues S, Gonçalves R, Albuquerque A, Pereira, P 'High incidence of hepatocellular carcinoma following successful interferon-free antiviral therapy for hepatitis C associated cirrhosis', Journal of Hepatology vol. 65(5), 2016, Nov, p. 1070-1071.
8. Cheung MCM, Walker AJ, Hudson BE, Verma S, McLauchlan J, Mutimer DJ et al. 'Outcomes after successful direct-acting antiviral therapy for patients with chronic hepatitis C and decompensated cirrhosis.' Journal of Hepatology vol 65(4), 2016 Oct, p. 741-7.
9. Coilly A, Fougerou-Leurent C, de Ledinghen V, Houssel-Debry P, Duvoux C, Di Martino V et al. 'Multicentre experience using daclatasvir and sofosbuvir to treat hepatitis C recurrence - The ANRS CUPILT study'. Journal of Hepatology vol. 65(4), 2016 Oct, p. 711-8.
10. Collins JM, Raphael KL, Terry C, Cartwright EJ, Pillai A, Anania FA, et al. 'Hepatitis B Virus Reactivation During Successful Treatment of Hepatitis C Virus With Sofosbuvir and Simeprevir' Clinical Infectious Diseases vol. 61(8), 16-Jun-2015, p. 1304-1306.
11. Conti F, Buonfiglioli F, Scuteri A, Crespi C, Bolondi L, Caraceni P et al. 'Early occurrence and recurrence of hepatocellular carcinoma in HCV-related cirrhosis treated with direct-acting antivirals' Journal of Hepatology vol. 65(4), 2016 Oct, p. 727-33.
12. Crockett SD, Keeffe EB. 'Natural history and treatment of hepatitis B virus and hepatitis C virus coinfection' Annals of Clinical Microbiology Antimicrobials, 2005 Sep 13; p. 4-13.
13. Ende AR, Kim NH, Yeh MM, Harper J, Landis CS. 'Fulminant hepatitis B reactivation leading to liver transplantation in a patient with chronic hepatitis C treated with simeprevir and sofosbuvir: a case report' Journal of medical case reports, 2015, p. 9-64.
14. Fattovich G, Stroffolini T, Zagni I, Donato F 'Hepatocellular carcinoma in cirrhosis: Incidence and risk factors' Gastroenterology Vol. 127 (5 Suppl 1), 2004, p. S35-50.

15. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. 'Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012' Internal Journal of Cancer vol. 136 (5), 2015, p. E359-86.

16. Foster GR, Irving WL, Cheung MC, Walker AJ, Hudson BE, Verma S, et al. 'Impact of direct acting antiviral therapy in patients with chronic hepatitis C and decompensated cirrhosis.' Journal of Hepatology vol. 64, 2016, p. 1224-123.

17. Garcia-Tsao G, Friedman S, Iredale J, Pinzani M. 'Now there are many (stages) where before there was one: In search of a pathophysiological classification of cirrhosis' Hepatology vol. 51(4), 2010 Apr, p. 1445-9.

18. Georgiadou SP, Zachou K, Rigopoulou E, Liaskos C, Mina P, Gerovasilis F et al. 'Occult hepatitis B virus infection in Greek patients with chronic hepatitis C and in patients with diverse non-viral hepatic diseases' Journal of Viral Hepatitis vol. 11(4), 2004, p. 358–365.

19. Hayashi K, Ishigami M, Ishizu Y, Kuzuya T, Honda T, Nishimura D, et al. 'A case of acute hepatitis B in a chronic hepatitis C patient after daclatasvir and asunaprevir combination therapy: hepatitis B virus reactivation or acute self-limited hepatitis?, Clinical Journal of Gastroenterology 9 (4), 2016, p. 252-6.

20. Hedenstierna M, Nangarhari A, Weiland O, Aleman S. 'The Presence of Diabetes Mellitus is a Strong Risk Factor Hepatocellular Carcinoma in Hepatitis C Infected Patients with Advanced Fibrosis or Cirrhosis Who Have Achieved Sustained Virological Response' Journal of Hepatology, European Association for the Study of the Liver (EASL), Vol. 64 (2) Suppl., 2016, p. S620.

21. Hiramatsu N, Oze T, Takehara T. 'Suppression of hepatocellular carcinoma development in hepatitis C patients given interferon-based antiviral therapy' Hepatology Research vol. 45 (2), 2015, p. 152-61.

22. Kim YN, Lee SJ, Cho YK, Song BC. 'FIB-4 can predict HCC development in CHC patients with SVR to interferon-based combination therapy' [Abstract P-0064]. 25th Annual Conference of the Asian Pacific Association for the Study of the Liver (APASL), 2016 20-24 February, Tokyo, Japan.

23. Konstantinou D, Deutsch M. 'The spectrum of HBV/HCV coinfection: epidemiology, clinical characteristics, viral interactions and management' Annals of gastroenterology: quarterly publication of the Hellenic Society of Gastroenterology vol. 28 (2), 2015, p. 221-8.

24. Lin JC, Habersetzer F, Rodriguez-Torres M, Afdhal N, Lawitz EJ, Paulson MS, et al. 'Interferon gamma-induced protein 10 kinetics in treatment-naive versus treatment experienced patients receiving interferon-free therapy for hepatitis C virus infection: implications for the innate immune response' The Journal of Infectious Diseases vol. 210(12), 2014, p. 1881-5.

25. Mangia A, Arleo A, Copetti M, Miscio M, Piazzolla V, Santoro R, et al. 'The combination of daclatasvir and sofosbuvir for curing genotype 2 patients who cannot tolerate ribavirin' Liver International vol. 36(7), 2016, p. 971-6.

26. Morgan TR, Ghany MG, Kim HY, Snow KK, Shiffman ML, De Santo JL, et al. 'Outcome of sustained virological responders with histologically advanced chronic hepatitis C' Hepatology vol. 52 (3), 2010, p. 833-44.

27. Nahon P, Bourcier V, Layese R, Audureau E, Cagnot C, Marcellin P, et. al. 'Eradication of Hepatitis C virus infection in Patients with cirrhosis reduces risk of Liver and non-liver complications' Gastroenterology, 2016 Sep 15., doi: 10.1053/j.gastro.2016.09.009. [Epub ahead of print]

28. Payance A, Goutte N, Clair E, Paradis V, Bouattour M, Vullierme MP, et al. 'Risk Factors and Characteristics of Hepatocellular Carcinoma Occurring in HCV Cirrhotic Patients After Sustained Virological Response' [Abstract THU-041], European Association for the Study of the Liver (EASL); 2016 13-17 April; Barcelona, Spain.

29. Pinzone MR, Zanghi AM, Rapisarda L, D'Agata V, Benanti F, Sparta D, et al. 'Cirrhotic patients are still at risk of developing hepatocellular carcinoma despite Interferon-induced sustained virological response' European review for medical and pharmacological sciences vol. 18(2 Suppl), 2014, p. 11-5.

30. Pol S. 'Lack of evidence of an effect of direct-acting antivirals on the recurrence of hepatocellular carcinoma: data from three ARNS cohorts' Journal of Hepatology, vol. 65(4), 2016, p. 734-40.

31. Reiberger T, Aberle JH, Kundi M, Kohrgruber N, Rieger A, Gangl A, et al. 'IP-10 correlates with hepatitis C viral load, hepatic inflammation and fibrosis and predicts hepatitis C virus relapse or non-response in HIV-HCV coinfection' Antiviral Therapy vol. 13 (8), 2008, p. 965-76.

32. Reig M, Marino Z, Perello C, Inarrairaegui M, Ribeiro A, Lens S, et al. 'Unexpected early tumor recurrence in patients with hepatitis C virus -related hepatocellular carcinoma undergoing interferon-free therapy' Journal of Hepatology vol. 65, 2016, p. 719-726.

33. Renzulli M, Golfieri R; Bologna Liver Oncology Group (BLOG). 'Proposal of a New Diagnostic Algorithm for Hepatocellular Carcinoma Based on the Japanese Guidelines but Adapted to the Western World for Patients Under Surveillance for Chronic Liver Disease' Journal of Gastroenterology and Hepatology vol. 31(1), 2016 , p. 69-80.

34. Ripoll C, Groszmann RJ, Garcia-Tsao G, Bosch J, Grace N, Burroughs A et al. 'Hepatic venous pressure gradient predicts development of hepatocellular carcinoma independently of severity of cirrhosis.' Journal of Hepatology vol. 50(5), 2009 May, p. 923-8.

35. Romano A, Piovesan S, Anastassopoulos G, et al. 'Incidence and pattern of "*de novo*" hepatocellular carcinoma in HCV patients treated with oral DAAs.' Presented at: AASLD Liver Cancer Meeting; Boston, Massachusetts, November 11-15, 2016.

36. Sarasin-Filipowicz M, Oakeley EJ, Duong FH, Christen V, Terracciano L, Filipowicz W, et al. 'Interferon signaling and treatment outcome in chronic hepatitis C' Proceedings of the National Academy of Science of United States of America, vol. 105(19), 2008, p. 7034-9.

37. Seike M, Honda K, Oriba J, Endo M., Yoshihara M., Iwao M. et al. 'The impact of α-fetoprotein level during interferon-free treatment of hepatitis C virus' Hepatology vol. 62 (1 Suppl.), 2015, p. 1311A.

38. Takayama H, Sato T, Ikeda F, Fujiki S. 'Reactivation of hepatitis B virus during interferon-free therapy with daclatasvir and asunaprevir in patient with hepatitis B virus/hepatitis C virus co-infection' Hepatology Research vol. 46 (5), 2016, p. 489-91.

39. Toyoda H, Kumada T, Tada T, Kiriyama S, Tanikawa M, Hisanaga Y, et al. 'Risk factors of hepatocellular carcinoma development in non-cirrhotic patients with sustained virologic response for chronic hepatitis C virus infection' Journal of Gastroenterology and Hepatology vol. 30 (7), 2015, p. 1183-9.

40. Trinchet J-C, Bourcier V, Chaffaud C, Ait Ahmed M, Allan S, Marcellin P et al. 'Complications and competing risks of death in compensated viral cirrhosis (ANRS C012 CIRVIR prospective cohort). Hepatology vol. 62(3), 2015, p. 737-50.

41. Van der Meer AJ, Veldt BJ, Feld JJ, Wedemeyer H, Dufour JF, Lammert F, et al. 'Association between sustained virological response and all-cause mortality among patients with chronic hepatitis C and advanced hepatic fibrosis'. JAMA vol. 308 (24), 2012, p. 2584-93.

42. Wang C, Ji D, Chen J, et al. 'Hepatitis B reactivation in CHC Chinese treated with pan-oral DAAs-a prospective study' Hepatology International vol. 10 (Suppl. 1), 2016, p. S51.

43. Wiegand SB, Jaroszewicz J, Potthoff A, Honer Zu Siederdissen C, Maasoumy B, et al. 'Dominance of hepatitis C virus (HCV) is associated with lower quantitative hepatitis B surface antigen and higher serum interferon-gamma-induced protein 10 levels in HBV/HCV-coinfected patients' Clinical Microbiology and Infection vol. 21(7), 2015, p. 710 e1-9.

44. Zeremski M, Petrovic LM, Chiriboga L, Brown QB, Yee HT, Kinkhabwala M, et al. 'Intrahepatic levels of CXCR3-associated chemokines correlate with liver inflammation and fibrosis in chronic hepatitis C' Hepatology vol. 48 (5), 2008, p. 1440-50.