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No increased risk of hepatocellular carcinoma after eradication of hepatitis C virus by direct-acting antivirals, compared with interferon-based therapy

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Abstract: It is well-known that sustained virological response (SVR) by interferon (IFN)-based therapy against hepatitis C virus (HCV) infection reduced the incidence of hepatocellular carcinoma (HCC). However, whether IFN-free direct-acting antivirals reduce the risk of HCC is controversial. Therefore, this study aims to compare the incidence of HCC after the achievement of SVR between sofosbuvir combined with ledipasvir (SOF/LDV) and simeprevir with pegylated interferon plus ribavirin (Sim+IFN). Japanese patients with HCV infection (genotype 1) who achieved SVR between January 2013 and December 2014 by SOF/LDV (NCT01975675, n = 320) or Sim+IFN (000015933, n = 289) therapy in two nationwide, multicenter, phase III studies were prospectively monitored for the development of HCC by ultrasonography for 5 years after the end of treatment (EOT). No HCC was detected before the treatment. HCC was detected in 9 and 7 patients in the SOF/LDV and the Sim+IFN group in 5 years, respectively. The cumulative incidences of HCC rates 1, 3, and 5 years after EOT were similar between the two groups (1.5%, 2.7%, and 3.2% for the SOF/LDV and 1.8%, 2.8%, and 3.0% for the Sim+IFN group, respectively). No HCC was developed 3.5 years after EOT. Interestingly, a retrospective careful review of imaging taken before therapy revealed hepatic nodules in 50% of HCC patients, suggesting HCC was pre-existed before therapy. In conclusion, we could not find any differences in the incidence of HCC after the HCV eradication between the two therapeutic regimens, suggesting no enhancement of HCC development by DAA.

***Keywords*:** hepatitis C virus, direct-acting antivirals, interferon

Introduction

Hepatitis C virus (HCV) infection is a leading cause of cirrhosis, liver failure, and hepatocellular carcinoma (HCC) (*1,2*). Particularly among cirrhotic patients with HCV infection, the annual incidence of HCC is 3% to 7% (*3,4*). Therefore, one of the goals for the treatment is the eradication of HCV and the prevention of HCC. Interferon (IFN) has long been used for anti-HCV therapy, and achievement of sustained virological response (SVR) by IFN-based therapy such as IFN monotherapy (*5-7*) or pegylated-interferon plus ribavirin (Peg-IFN/RBV) therapy (*8*) significantly reduced the incidence of HCC in patients with chronic HCV infection.

Direct-acting antivirals (DAA) that selectively inhibit HCV proteins such as nonstructural protein (NS) 3/4A protease, NS5A, and NS5B polymerase, have been approved for the treatment of chronic hepatitis C. In Japan, a protease inhibitor with Peg-IFN/RBV regimen has been firstly introduced for patients with chronic hepatitis C in 2013 and its SVR rate in the IFN-naïve patients was about 80-90% (*9*). However, there are no reports on whether a protease inhibitor with Peg-IFN/RBV reduced the long-term risk of HCC development.

Meanwhile, an IFN-free DAA regimen such as sofosbuvir combined with ledipasvir (SOF/LDV) was approved for the treatment of genotype 1 HCV infection in 2015 in Japan, which showed a tremendous high SVR rate (*10*). However, concerns were raised from two studies that reported the increased rates of *de novo* HCC occurrence and high rates of recurrence in patients who eradicated HCV by IFN-free DAA therapy (*11,12*). Recent large prospective studies showed a reduction in the incidence of HCC after SVR in patients treated with IFN-free DAA therapy (*13-15*), but these observation periods were not long enough to evaluate the long-term risk of HCC. A similar risk of HCC development after HCV eradication has been reported between IFN-free DAA and Peg-IFN/RBV therapy, but the observation periods of IFN-free DAA therapy was not long enough to evaluate the long-term risk of HCC as well (*16,17*).

Therefore, we extended two different nationwide, multicenter prospective cohort studies after the end of treatments (EOT) and compared the long-term incidence of HCC after SVR.

Materials and Methods

Study design and patient population

We have two nationwide, multicenter, phase III prospective studies for HCV treatment such as simeprevir with Peg-IFN/RBV (Sim+IFN, 000015933) and SOF/LDV (*clinicaltrials.gov* identifier NCT01975675) (*10*) in Japanese patients with HCV infection. In these two studies, eligible patients had been 20 years of age or higher with genotype 1 HCV infection with serum HCV RNA levels of 5 log10 IU/mL or higher and creatinine clearance levels of 1.0 mL/s or higher (Cockcroft-Gault equation). Patients with hepatic decompensation (as shown by the presence of ascites, encephalopathy, or a history of variceal hemorrhage), bodyweight less than 40 kg, or coinfection with hepatitis B virus or human immunodeficiency virus had been excluded. The standard treatment of Sim+IFN was as follows: Simeprevir (Sovriad®, 100 mg, once-daily, Janssen Tokyo, Japan) was co-administered with RBV (Copegasys®, 400–600 mg, twice a day, Chugai, Tokyo, Japan), and Peg-IFNα-2a (Pegasys®, 180 μg, Chugai, Tokyo, Japan) was injected weekly for 12 weeks, followed by additional Peg-IFN/RBV treatment for 12 weeks. The SOF/LDV group received SOF/LDV once a day (Harvony®, Gilead Sciences, Tokyo, Japan) for 12 weeks. No participants had used DAA before this cohort.

We extended the above-mentioned two prospective studies to monitor the incidence of HCC after EOT. Then, we retrospectively reviewed 653 consecutive patients with genotype 1 HCV infection who achieved SVR by SOF/LDV (n = 338) or Sim+IFN (n = 315) at 19 hospitals between January 2013 and December 2014. Among them, 18 and 26 patients in the SOF/LDV and the Sim+IFN group, respectively, rejected participation in this follow-up study and we excluded them from this cohort (Figure S1, *https://www.globalhealthmedicine.com/site/supplementaldata.html?ID=54*). Therefore, 320 and 289 patients in the SOF/LDV and the Sim+IFN group, respectively, were prospectively monitored for HCC development after EOT by ultrasonography (US) at least every 3 months, and the incidence of HCC between the two groups was compared. Contrast-enhanced computed tomography (CT), or gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid-enhanced magnetic resonance imaging (EOB-MRI) was used on demand.

Written informed consent was obtained from each participant. This study was conducted under provisions of the 1975 Declaration of Helsinki and approved by the Institutional Ethics Committee of the National Center for Global Health and Medicine (NCGM-A-001649) and each hospital participating in the studies.

Clinical and laboratory assessments

Clinical and laboratory assessments were performed before treatment. Alpha-fetoprotein (AFP) and des-γ-carboxy prothrombin (DCP) were used as tumor markers of HCC. Serum HCV RNA levels were measured using a COBAS 135 TaqMan HCV test (Roche Diagnostics, Tokyo, Japan). Its detection limit was 1.2 log IU/mL. HCV genotype was determined by sequence determination of the 5' non-structural region of the HCV genome, followed by phylogenetic analysis. Fibrosis-4 (FIB-4) index was used as a surrogate

marker for liver fibrosis. A formula of FIB-4 index was age (years) × aspartate aminotransferase (AST) [IU/L] / (platelet count [10^9/L] × (alanine aminotransferase, ALT [IU/L])1/2). A liver biopsy was done if necessary. We considered a FIB-4 index ≥ 3.25 and/or FibroScan® score ≥ 12.5 kPa, and/or fibrosis staging ≥ F3 as advanced fibrosis.

The presence of type 2 diabetes mellitus (T2DM) was determined based on fasting blood glucose levels > 126 mg/dL, hemoglobin A1c (HbA1c) > 6.5%, or by the use of anti-diabetic agents. The presence of steatosis was recognized as a marked increase in hepatic echogenicity, poor penetration of the posterior segment of the right lobe of the liver, and poor or no visualization of the hepatic vessels and diaphragm by the US. Patients with a hepatic injury who consumed more than 60 g of alcohol per day were classified as having alcoholic liver disease.

HCC surveillance

HCC surveillance was done using the combination of tumor markers (AFP and DCP) and the US at least every 3 months after EOT for 5 years in all participants. CT and/or EOB-MRI were used at the discretion of the attending physician. The diagnosis of HCC was based on the hypervascular staining pattern of the arterial phase and the hypovascular staining pattern of the portal phase and was confirmed by dynamic CT, EOB-MRI, or target biopsy if necessary.

Statistical analysis

Continuous variables were analyzed using the Mann-Whitney *U*-test. Categorical variables were compared using the chi-square or Fisher exact test. The incidence of HCC was calculated by the Kaplan-Meier method, and differences between the groups were assessed by the log-rank test. A p-value < 0.05 was considered statistically significant.

Results

Patients characteristics

The clinical characteristics of the 609 patients before anti-viral therapy are shown in Table 1. Serum AFP levels in the SOF/LDV group were significantly higher than those in the Sim+IFN group (11.9 and 7.4 ng/mL, p = 0.0045). Serum creatinine levels (average 0.74 *vs.* 0.68 mg/dL) and serum HCV RNA levels (average 6.6 *vs.* 6.3 mg/dL) were significantly lower in the Sim+IFN group. However, there were no differences in their ages, genders, body mass index (BMI), prior anti-viral therapies, hepatic biochemical data, or the FIB-4 index before therapy between the two groups. The presence of T2DM, fatty liver, or alcohol inhabit was similarly observed. A total of 30.5% of patients showed a FIB-4 index value > 3.25, indicating advanced fibrosis at baseline (31% for the SOF/LDV and 34% for the Sim+IFN groups, respectively). Before starting anti-viral therapy, all patients have performed the abdominal US, but 89 patients (14.6%) have performed CT and/or EOB-MRI within 4 months before the treatments (16% for the SOF/LDV and 14% for the Sim+IFN groups, respectively).

Follow-up and tumor development

The median observation periods of the SOF/LDV and Sim+IFN were 56.1 (6-60) and 52.3 (6-60) months, respectively. The follow-up rates were similar between the two groups 1, 3, and 5 years after EOT (Figure 1, 98%, 92%, and 89% for the SOF/LDV and 96%, 85%, and 81% for the Sim+IFN group, respectively). HCC was developed in 9 and 7 patients in the SOF/LDV and the Sim+IFN groups, respectively. There were no differences in the cumulative rates of HCC development between the two groups (Figure 1, 1, 3, and 5 years after EOT: 1.5%, 2.7%, and 3.2% for the SOF/LDV and 1.8%, 2.8%, and 3.0% for the Sim+IFN group, respectively.).

Propensity score matching analysis

To overcome the bias due to differences in the

Table 1. Comparison of clinical characteristics in different therapeutic groups before anti-viral therapy

Characteristics	SOF/LDV	Sim+IFN	p value
Number of patients	320	289	N.S.
Age, mean (range)	59.4 (28-80)	60.1 (20-78)	N.S.
Age ≥ 65 year, *n* (%)	108 (33)	107 (37)	N.S.
Male, *n* (%)	132 (41)	134 (46)	N.S.
BMI, kg/m^2 mean (range)	23.3 (16-36)	23.0 (17-34)	N.S.
Treatment for Naïve, *n* (%)	165 (52)	141 (49)	N.S.
Albumin g/dL mean (SD)	4.2 (0.3)	4.1 (0.4)	N.S.
AST, IU/L mean (SD)	55 (37)	51 (37)	N.S.
ALT, IU/L mean (SD)	59 (45)	57 (50)	N.S.
γ-GTP, IU/L mean (SD)	47 (46)	44 (45)	N.S.
Creatinine mg/dL (SD)	0.74 (0.15)	0.68 (0.17)	< 0.001
WBC, (×10^2/mm^3) (SD)	45.8 (14.0)	44.7 (18.2)	N.S.
Hb, (g/dL) (SD)	14.0 (1.3)	13.9 (1.3)	N.S.
Platelet, ×10^4/μL mean (SD)	17.6 (6.3)	16.7 (5.6)	N.S.
HCV *RNA*, logIU/L mean (SD)	6.6 (0.5)	6.3 (1.0)	< 0.001
FIB-4 index, mean (SD)	2.90 (2.00)	2.90 (1.80)	N.S.
FIB-4 index > 3.25, *n* (%)	99 (31)	97 (34)	N.S.
AFP, ng/mL mean (SD)	11.9 (22.0)	7.4 (14.0)	0.0045
Diabetes, *n* (%)	26 (8)	33 (11)	N.S.
Alcohol (> 60 g/day), *n* (%)	40 (12)	43 (15)	N.S.
Fatty liver, *n* (%)	45 (14)	38 (13)	N.S.
Image (US/CT/MRI)	320/35/15	289/27/12	N.S.

SOF/LDV, sofosbuvir combined with ledipasvir; Sim-IFN, simeprevir with pegylated interferon plus rivabirin; BMI, body mass index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GTP, gamma glutamyltransferase; WBC, white blood cell counts; Hb, hemoglobin concentration; HCV, hepatitis C virus; AFP, alpha-fetoprotein; US, ultrasonography; CT, contrast-enhanced computed tomography; MRI, Gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid enhanced magnetic resonance imaging; N.S., not significant.

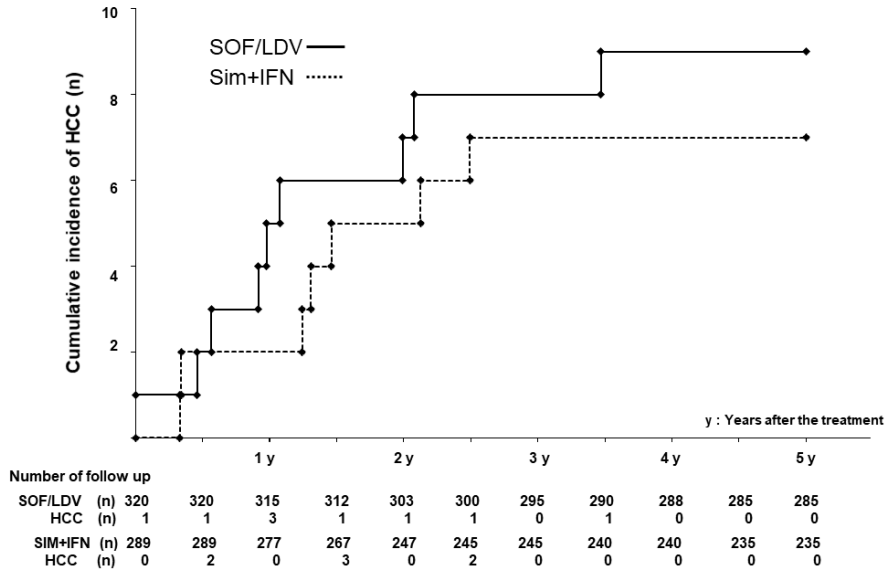

Figure 1. Kaplan-Meier curves of cumulative incidence of hepatocellular carcinoma after eradication of HCV according to the different therapies. SOF/LDV, sofosbuvir combined with ledipasvir; Sim+IFN, simeprevir with pegylated-interferon plus ribavirin.

distributions of covariates between the two groups, one-to-one matches were created using propensity score analysis. Variables entered in the propensity model included their ages, genders, BMI, prior treatments, serum albumin, ALT, AST, γ-glutamyl transpeptidase (γ-GTP), HCV-RNA, AFP levels, white blood cell counts, hemoglobulin levels, platelet counts, FIB-4 index, the presence of T2DM, alcohol habits and fatty liver. The model was used to obtain a one-to-one match by using the nearest-neighbor matching method in 206 patients with SOF/LDV and 206 patients with Sim-IFN therapies, resulting in a sample size of more than 70% patients per cohort (Table S1, *https://www.globalhealthmedicine.com/site/supplementaldata.html?ID=54*). Propensity score matching analysis did not find any differences in the incidence of HCC ($n = 3$ each) between the two groups.

Risk factors before anti-viral therapy for the development of HCC in patients treated with SOF/LDV and Sim+IFN

In the SOF/LDV group, older ages, male gender, lower platelet counts, higher FIB-4 index, and higher serum AFP level were observed in patients who developed HCC, compared with those who did not ($p < 0.001$, Table 2). Meanwhile, in the Sim+IFN group, older ages were the only factors that were significantly observed in patients with HCC, compared with those without HCC ($p < 0.05$, Table 3). There were no differences in the clinical characteristics of patients who developed HCC in different treatment regimens before anti-viral therapy (Table 4).

Table 2. Comparison of clinical characteristics in patients who developed HCC or not in the SOF/LDV group before anti-viral therapy

Characteristics	Non-HCC	HCC	*p* value
Number of patients	311	9	N.S.
Age, mean (range)	59 (28-80)	66 (57-75)	< 0.05
Age ≥ 65 year, *n* (%)	98 (31)	4 (44)	N.S.
Male, *n* (%)	124 (39)	8 (89)	< 0.001
BMI, kg/m^2 mean (range)	23.2 (16-36)	23.7 (22-27)	N.S.
Treatment for Naïve, *n* (%)	161 (52)	4 (44)	N.S.
Albumin g/dL mean (SD)	4.2 (0.4)	4.1 (0.2)	N.S.
AST, IU/L mean (SD)	55 (37)	64 (32)	N.S.
ALT, IU/L mean (SD)	59 (45)	61 (43)	N.S.
γ-GTP, IU/L mean (SD)	44 (45)	68 (31)	N.S.
Platelet, ×10^4/μL mean (SD)	17.8 (6.3)	10.2 (2.7)	< 0.001
FIB-4 index, mean (SD)	2.83 (1.90)	5.90 (2.35)	< 0.001
FIB-4 index > 3.25, *n* (%)	91 (29)	8 (89)	< 0.001
AFP, ng/mL mean (SD)	11.3 (21.0)	34.0 (38.0)	< 0.001
Diabetes, *n* (%)	25 (8)	1 (11)	N.S.
Alcohol (> 60 g/day), *n* (%)	37 (12)	2 (22)	N.S.
Fatty liver, *n* (%)	43 (14)	2 (22)	N.S.

SOF/LDV, sofosbuvir combined with ledipasvir; HCC, hepatocellular carcinoma; BMI, body mass index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GTP, gamma glutamyltransferase; AFP, alpha-fetoprotein; N.S., not significant.

Clinical characteristics of HCC patients in each therapeutic group

Clinical background before anti-viral therapy and the detailed information on HCC at detection are shown in Table 5 and Table 6, respectively. All patients who developed HCC in the SOF/LDV group ($n = 9$) had advanced fibrosis (FibroScan ≥ 12.5 kPa and/or FIB-4 index ≥ 3.25 and/or fibrosis staging ≥ 3). Meanwhile,

Table 3. Comparison of clinical characteristics in patients who developed HCC or not in the Sim+IFN group before anti-viral therapy

Characteristics	Non-HCC	HCC	*p* value
Number of patients	282	7	N.S.
Age, mean (range)	60 (20-78)	68 (58-75)	< 0.05
Age ≥ 65 year, *n* (%)	101 (36)	5 (71)	< 0.05
Male, *n* (%)	133 (47)	3 (42)	N.S.
BMI, kg/m^2 mean (range)	23.0 (17-34)	22.9 (20-27)	N.S.
Treatment for Naïve, *n* (%)	133 (47)	5 (71)	N.S.
Albumin g/dL mean (SD)	4.1 (0.4)	4.2 (0.2)	N.S.
AST, IU/L mean (SD)	51 (38)	62 (32)	N.S.
ALT, IU/L mean (SD)	57 (50)	64 (38)	N.S.
γ-GTP, IU/L mean (SD)	44 (46)	36 (17)	N.S.
Platelet, ×10^4/μL mean (SD)	16.7 (5.6)	14.3 (4.5)	N.S.
FIB-4 index, mean (SD)	2.86 (1.80)	4.13 (2.10)	N.S.
FIB-4 index > 3.25, *n* (%)	93 (33)	4 (57)	N.S.
AFP, ng/mL mean (SD)	7.3 (14.0)	10.1 (9.7)	N.S.
Diabetes, *n* (%)	32 (11)	1 (14)	N.S.
Alcohol (> 60 g/day), *n* (%)	42 (15)	1 (14)	N.S.
Fatty liver, *n* (%)	45 (16)	0 (0)	N.S.

Sim+IFN, simeprevir with pegylated interferon plus ribavirin; HCC, hepatocellular carcinoma; BMI, body mass index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GTP, gamma glutamyltransferase; AFP, alpha-fetoprotein; N.S., not significant.

Table 4. Comparison of clinical characteristics in patients who developed HCC in the different therapeutic groups before anti-viral therapy

Characteristics	SOF/LDV	Sim+IFN	*p* value
Number of patients	9	7	N.S.
Age, mean (range)	66 (57-75)	68 (58-75)	N.S.
Age ≥ 65 year, *n* (%)	4 (44)	5 (71)	N.S.
Male, *n* (%)	8 (89)	3 (42)	N.S.
BMI, kg/m^2 mean (range)	23.7 (22-27)	22.9 (20-27)	N.S.
Treatment for Naïve, *n* (%)	4 (44)	5 (71)	N.S.
Albumin g/dL mean (SD)	4.1 (0.2)	4.2 (0.2)	N.S.
AST, IU/L mean (SD)	64 (32)	62 (32)	N.S.
ALT, IU/L mean (SD)	61 (43)	64 (38)	N.S.
γ-GTP, IU/L mean (SD)	68 (31)	36 (17)	N.S.
Platelet, ×10^4/μL mean (SD)	10.2 (2.7)	14.3 (4.5)	N.S.
FIB-4 index, mean (SD)	5.90 (2.35)	4.13 (2.10)	N.S.
FIB-4 index > 3.25, *n* (%)	8 (89)	4 (57)	N.S.
AFP, ng/mL mean (SD)	34.0 (38.0)	10.1 (9.7)	N.S.
Diabetes, *n* (%)	1 (11)	1 (14)	N.S.
Alcohol (> 60 g/day), *n* (%)	2 (22)	1 (14)	N.S.
Fatty liver, *n* (%)	2 (22)	0 (0)	N.S.

SOF/LDV, sofosbuvir combined with ledipasvir; Sim+IFN, simeprevir with pegylated interferon plus rivabirin; HCC, hepatocellular carcinoma: BMI, body mass index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GTP, gamma glutamyltransferase; AFP, alpha-fetoprotein; *N.S.*, not significant.

5 out of 7 patients in the Sim+IFN group had advanced fibrosis, but the other two (Sim+IFN-2 and Sim+IFN-7) showed high BMI and/or DM and alcohol habits (Table 5). Fourteen (88%, except for SOF/LDV-9 and Sim+IFN-7) patients developed HCC within 2.5 years after EOT while no one developed HCC 3.5 years after EOT (Table 6).

Therapeutic regimens and prognosis of HCC

All patients who developed HCC were successfully treated and survived during follow-up (Table 6). No recurrences of HCC were observed in patients whose HCC was detected at a very early stage (BCLC 0, *n* = 11), regardless of HCC therapeutic strategies. Meanwhile, all patients in the SOF/LDV group whose HCC was detected at BCLC A (*n* = 4) underwent radiofrequency ablation (RFA), in all of whom HCC recurred within 1 year after RFA, but were successfully treated by further RFA. The Sim+IFN-5 (75-year-old male) who was detected HCC at BCLC B despite repeated US every 3 months was treated by transcatheter arterial chemoembolization, which needed further treatment for HCC recurrence.

Discussion

This study compared the relative long-term risk of HCC developments after eradication of HCV between the SOF/LDV and the Sim+IFN groups. We found no differences in the cumulative incidence of HCC between the two groups for at least 5 years.

The achievement of SVR by IFN-based therapy was associated with a significant reduction in HCC development (*5-7*). HCV proteins exert a direct carcinogenic effect by deregulating the host cell cycle checkpoints and increasing the immune-mediated oxidative stress, which leads to DNA mutations frequently in the liver cells (*18*). Necro-inflammatory activity and its process is a contributor to a wide variety of neoplasm (*19*). Thus, HCV clearance by DAA should theoretically reduce the risk of HCC development as seen in IFN-based therapy (*5-7*). Indeed, DAA contributed to a significant reduction of HCC incidence as well as improved the SVR rate, compared with patients with non-SVR (*13-15*,*20*).

However, unexpected data suggest the potential increased risk of *de novo* and recurrent HCC after DAA therapy (*11,12*). Cases with the rapid growth of HCC were observed during DAA (*21*), which raised the question of whether DAA could enhance hepatic carcinogenesis. The mechanism underlying this association has been hypothesized that abrupt hepatic downregulation of type II and III IFN by HCV clearance might hurt immune cancer control (*22*). An experimental study has shown that double-stranded DNA breaks induced by chronic inflammation lead to genomic instability, which accelerates carcinogenesis by liver regeneration (*23*). Therefore, whether DAA enhances hepatic carcinogenesis or not has been controversial.

Kanwal recently reported the relative long-term incidence of HCC in patients who eradicated HCV by DAA, which revealed that the cumulative rate of 1 and 3 year risks of HCC were 1.1% and 2.8%, respectively, in a nationwide, prospective cohort (*24*). Meanwhile, Nahon P, *et al.* (*14*) and Innes H, *et al.*

Table 5. Clinical characteristics of patients who developed HCC in the SOF/LDV (*n* = 9) and the Sim+IFN group (*n* = 7) before anti-viral therapy

Groups	Age	Sex	Prior treatment	BMI (kg/m2)	LSM (kPa) or Staging	FIB-4 index	ALT (IU/L)	DM	Alcohol	Fatty liver
SOF/LDV-1	70	M	Naïve	23.7	12.5	7.18	34	-	-	-
SOF/LDV-2	75	M	Naïve	23.5	24.5	5.79	42	+	-	+
SOF/LDV-3	71	M	PegIFN/RBV	25.3	26.7	5.81	160	-	-	-
SOF/LDV-4	63	M	PegIFN/RBV	27.1	38.6	4.12	41	-	-	+
SOF/LDV-5	63	M	PegIFN/RBV	22.2	F3	8.26	77	-	+	-
SOF/LDV-6	57	F	PegIFN/RBV	22.3	22.3	10.05	75	-	-	-
SOF/LDV-7	62	M	PegIFN/RBV	25.3	28.4	5.64	25	-	-	-
SOF/LDV-8	73	M	Naïve	23.7	11.8	3.75	40	-	-	-
SOF/LDV-9	59	M	PegIFN/RBV	23.0	F3	2.46	40	-	+	-
Sim+IFN-1	68	F	Naïve	22.7	F3	5.76	74	-	-	-
Sim+IFN-2	74	F	PegIFN/RBV	26.4	6.5	2.69	21	-	-	-
Sim+IFN-3	73	F	Naïve	21.3	F2	4.48	25	-	-	-
Sim+IFN-4	64	F	PegIFN/RBV	23.4	F3	7.80	104	-	-	-
Sim+IFN-5	75	M	Naïve	20.0	F1	3.76	106	-	-	-
Sim+IFN-6	66	M	Naïve	20.2	F3	2.78	108	-	-	-
Sim+IFN-7	58	M	Naïve	26.6	F1	1.66	30	+	+	-

SOF/LDV, sofosbuvir combined with ledipasvir; Sim-IFN, Simeprevir with pegylated interferon plus rivabirin; M, male; F, female; PegIFN/RBV, pegylated interferon plus ribavirin; BMI, body mass index; LSM, liver stiffness measurement; Staging, fibrosis staging by liver biopsy; ALT, alanine aminotransferase; DM, diabetes mellitus.

Table 6. Clinical characteristics at detection of patients who developed HCC in the SOF/LDV (*n* = 9) and the Sim+IFN groups (*n* = 7)

Groups	Detection from EOT (days)	US	CT	MRI	AFP (ng/mL)	AFP-L3 (%)	DCP (mAU/mL)	BCLC stage	CPT score	Therapy	Recurrence	Alive at 5 years after EOT
SOF/LDV-1	0	○	–	○	41.1	3.2	13	0	A	RFA	No	Yes
SOF/LDV-2	168	○	○	○	4.7	< 0.5	19	0	A	Surgery	No	Yes
SOF/LDV-3	207	○	○	–	14.1	20.7	17	A	A	RFA	Yes	Yes
SOF/LDV-4	334	○	○	○	4.1	< 0.5	17	A	A	RFA	Yes	Yes
SOF/LDV-5	357	○	○	○	4.1	< 0.5	14	0	A	Surgery	No	Yes
SOF/LDV-6	393	○	○	○	375.0	2.4	57	A	A	RFA	Yes	Yes
SOF/LDV-7	727	○	–	○	3.6	< 0.5	23	A	A	RFA	Yes	Yes
SOF/LDV-8	759	○	–	○	4.0	< 0.5	< 10	0	A	RFA	No	Yes
SOF/LDV-9	1,267	×	○	○	2.9	< 0.5	12	0	A	Surgery	No	Yes
Sim+IFN-1	121	○	–	○	7.0	< 0.5	23	0	A	RFA	No	Yes
Sim+IFN-2	125	○	○	○	3.7	< 0.5	66	0	A	RFA	No	Yes
Sim+IFN-3	454	○	○	–	2.0	< 0.5	20	0	A	Surgery	No	Yes
Sim+IFN-4	478	○	○	○	25.6	24.1	353	0	A	Surgery	No	Yes
Sim+IFN-5	533	○	○	○	59.2	75.9	754	B	A	TACE	Yes	Yes
Sim+IFN-6	776	○	–	○	3.8	43.9	47	0	A	Surgery	No	Yes
Sim+IFN-7	943	×	○	○	2.5	< 0.5	14	0	A	Surgery	No	Yes

SOF/LDV, sofosbuvir combined with ledipasvir; Sim+IFN, simeprevir with pegylated interferon plus rivabirin; EOT, end of treatments; BCLC, Barcelona Clinic Liver Cancer Score; CPT, Child-Pugh score; –, not tested; ○, detected; ×, not detected; US, ultrasonography; CT: contrast-enhanced computed tomography; MRI: Gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid enhanced magnetic resonance imaging; AFP, alpha-fetoprotein; DCP, des-γ-carboxy prothrombin, RFA, radiofrequency ablation; TACE, transcatheter arterial chemoembolization.

(*25*) showed a higher incidence of HCC following SVR by DAA therapy, compared with IFN-based therapy, but these were dependent on the baseline characteristics of patients such as their ages and liver function. Comparison between DAA and IFN-based therapy in HCC incidence after HCV eradication was reported to be similar (*16,17*). However, the median observation periods of these two reports were 23 (4-78) and 21.6 (1.2–92.4) months, respectively, which were not long enough to evaluate the long-term risk of HCC occurrence. Therefore, we conducted the current study. Indeed, the median observation period of our SOF/LDV group was significantly longer (56.1 months) than those previous reports and we found no differences in the risk of HCC between the SOF/LDV group and the Sim+IFN group (1, 3, and 5 years risks of HCC were 1.5%, 2.7%, and 3.2% for the SOF/LDV, and 1.8%, 2.8% and 3.0% for the Sim+IFN, respectively), which were the similar incidence of HCC as Kanwal F, *et al.* reported (*23*). These accumulating pieces of evidence suggest that

the HCC risk after SVR might be similar regardless of treatment regimens although higher SVR rates, fewer side effects, and higher costs are observed in the DAA therapy (*10,26*) than those in the IFN monotherapy (*5-7*) or the Sim+IFN therapy (*9*).

In this cohort, 88% (14/16) of patients developed HCC within 2.5 years after EOT. Therefore, we hypothesized that pre-existing HCC before DAA therapy could develop during therapy or after EOT although all patients were confirmed to be no HCC by pre-treatment screening. To confirm this hypothesis, we retrospectively reviewed the abdominal imaging of patients who developed HCC (n = 16), which were taken before anti-viral therapy. Surprisingly, a retrospective review with location information of HCC was able to recognize hepatic nodules in eight patients (50%) by at least one imaging of US, CT, or EOB-MRI (Table S2, Figure S2, *https://www.globalhealthmedicine.com/site/supplementaldata.html?ID=54*) although no imaging showed typical characteristics of HCC such as early enhancement or washout at the portal phase. These results partially support the notion that DAA did not induce hepatic carcinogenesis, but HCC was pre-existing before therapy (*27*). Mariño Z, *et al.* (*28*) reported the presence of non-characterized nodules before DAA was associated with a 3 times greater risk of HCC development. Therefore, screening of the early stage of HCC by other imaging modalities such as EOB-MRI before therapy could reduce the incidence of HCC after SVR since no lesions were detected by the US alone at baseline in our study and EOB-MRI is superior to detecting early HCC (*29,30*).

It is well known that advanced fibrosis is one of the risk factors for HCC after the achievement of SVR (*18*), but no differences in the FIB-4 index were observed between the DAA group and the Sim+IFN group (Table 1). However, all patients who developed HCC in the DAA group showed advanced liver fibrosis before anti-viral therapy (Tables 3). Meanwhile, several other factors such as older age, male gender, high serum AFP levels, and presence of T2DM, obesity, steatosis, and alcohol abuse were associated with long-term risk of HCC after SVR by DAA (*12,16*). Indeed, in the current study, two patients without advanced fibrosis (Sim+IFN-2 and Sim+IFN-7) had metabolic disorders. Therefore, environmental factors, as well as liver fibrosis, should be considered for a careful HCC survey. In general, higher AFP levels represent the presence of severer inflammation in the liver, which suggested that patients with a higher risk of HCC development were included in the DAA group. Alternatively, to our knowledge, we cannot find any reports regarding serum creatine levels and HCC development in patients with chronic hepatitis C. Therefore, the significant differences in the serum creatine levels between the DAA and the Sim+IFN group may not affect our results. HCV genotype 1 is one of the well-known risk factors for HCC (*31*). Since all enrolled patients had genotype 1b, the conclusion from this study should be limited to patients with genotype 1.

Results from our study must be interpreted in light of other limitations. First, this is not a randomized study. However, these two studies were prospectively done in similar periods. Therefore, the bias between the two groups might be small. Second, the sample size was relatively small and the follow-up periods were not long enough for HCC surveillance. We had planned to follow these patients longer but lost patients during follow-up partially because of COVID-2019. Third, there were many missing data, especially after EOT. If not, we could show predictive factors for HCC development from laboratory data. Forth, enhanced CT and/or EOB-MRI for the pre-treatment screening were done in only 15% of patients. If not, we could clearly show no pre-existing HCC before therapy. Fifth, the majority of included patients did not have advanced fibrosis, which may result in a low incidence of HCC in this cohort. Similar incidence of HCC in the two therapeutic regimens could be shown more clearly when we used more patients with advanced fibrosis. The conclusion of this study should be mainly limited to chronic hepatitis without severe fibrosis.

In conclusion, we could not find any differences in the incidences of HCC after eradication of HCV between the SOF/LDV and the Sim+IFN therapy by two nationwide, prospective, multicenter studies. However, we should remind that HCC develops from undetectable pre-existing nodules before therapy. Therefore, we should follow the patients after EOT for at least 3.5 years until useful clinical predictive markers for HCC would be settled in the future.

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References

1. Thomas DL, Seeff LB. Natural history of hepatitis C. Clin Liver Dis. 2005; 9:383-398.
2. Niederau C, Lange S, Heintges T, Erhardt A, Buschkamp M, Hürter D, Nawrocki M, Kruska L, Hensel F, Petry W, Häussinger D. Prognosis of chronic hepatitis C: results of a large, prospective cohort study. Hepatology. 1998; 28:1687-1695.

3. European Association for the Study of the Liver; European Organization for Research and Treatment of Cancer. EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. J Hepatol. 2012; 56:908-943.
4. Lok AS, Seef LB, Morgan TR, di Bisceglie AM, Sterling RK, Curto TM, Everson GT, Lindsay KL, Lee WM, Bonkovsky HL, Dienstag JL, Ghany MG, Morishima C, Goodman ZD; HALT-C Trial Group. Incidence of hepatocellular carcinoma and associated risk factors in hepatitis C-related advanced liver disease. Gastroenterology. 2009; 136:138-148.
5. Nishiguchi S, Kuroki T, Nakatani S, Morimoto H, Takeda T, Nakajima S, Shiomi S, Seki S, Kobayashi K, Otani S. Randomized trial of effects of interferon-alpha on incidence of hepatocellular carcinoma in chronic active hepatitis C with cirrhosis. Lancet. 1995; 346:1051-1055.
6. Yoshida H, Shiratori Y, Moriyama M, *et al.* Interferon therapy reduces the risk for hepatocellular carcinoma: national surveillance program of cirrhotic and non-cirrhotic patients with chronic hepatitis C in Japan. IHIT Study Group. Inhibition of Hepatocarcinogenesis by Interferon Therapy. Ann Intern Med. 1999; 131:174-181.
7. Camma C, Giunta M, Andreone P, Craxi A. Interferon and prevention of hepatocellular carcinoma in viral cirrhosis: an evidence-based approach. J Hepatol. 2001; 34:593-602.
8. Ogawa E, Furusyo N, Kajiwara E, *et al.* Efficacy of pegylated interferon alpha-2b and ribavirin treatment on the risk of hepatocellular carcinoma in patients with chronic hepatitis C: A prospective, multicenter study. J Hepatol. 2013; 58:495-501.
9. Takehara T. Simeprevir for the treatment of chronic hepatitis C genotype 1 infection. Expert Rev Anti Infect Ther. 2014; 12:909-917.
10. Mizokami M, Yokosuka O, Takehara T, *et al.* Ledipasvir and sofosbuvir fixed-dose combination with and without ribavirin for 12 weeks in treatment-naive and previously treated Japanese patients with genotype 1 hepatitis C: an open-label, randomized, phase 3 trial. Lancet Infect Dis. 2015; 15:645-653.
11. Conti F, Buonfiglioli F, Scuteri A, Crespi C, Bolondi L, Caraceni P, Foschi FG, Lenzi M, Mazzella G, Verucchi G, Andreone P, Brillanti S. Early occurrence and recurrence of hepatocellular carcinoma in HCV-related cirrhosis treated with direct-acting antivirals. J Hepatol. 2016; 65:727-733.
12. Reig M, Boix L, Mariño Z, Torres F, Forns X, Bruix J. Liver cancer emergence associated with antiviral treatment: An immune surveillance failure? Semin Liver Dis. 2017; 37:109-118.
13. Calvaruso V, Cabibbo G, Cacciola I, *et al.* Incidence of hepatocellular carcinoma in patients with HCV-associated cirrhosis treated with direct-acting antiviral agents. Gastroenterology. 2018; 155:411-421.e4.
14. Nahon P, Layese R, Bourcier V, *et al.* Incidence of hepatocellular carcinoma after direct antiviral therapy for HCV in patients with cirrhosis included in surveillance programs. Gastroenterology. 2018; 155:1436-1450.e6.
15. Carrat F, Fontaine H, Dorival C, *et al.* Clinical outcomes in patients with chronic hepatitis C after direct-acting antiviral treatment: a prospective cohort study. Lancet. 2019; 393:1453-1464.
16. Nagaoki Y, Imamura M, Aikata H, *et al.* The risks of hepatocellular carcinoma development after HCV eradication are similar between patients treated with peg-interferon plus ribavirin and direct-acting antiviral therapy. PLoS One. 2017; 12:e0182710.
17. Nagata H, Nakagawa M, Asahina Y, *et al.* Effect of interferon-based and -free therapy on early occurrence and recurrence of hepatocellular carcinoma in chronic hepatitis C. J Hepatol. 2017; 67:933-939.
18. Lemons SM, McGiven DR. Is hepatitis C virus carcinogenic? Gastroenterology. 2012; 12:1274-1278.
19. Karin M, Greten FR. NF-kappaB: linking inflammation and immunity to cancer development and progression. Nat Rev Immunol. 2005; 5:749-759.
20. Kanwal F, Kramer J, Asch SM, Chayanupatkul M, Cao Y, El-Serag HB. Risk of hepatocellular cancer in HCV patients treated with direct-acting antiviral agents. Gastroenterology. 2017; 153:996-1005.e1.
21. Nakao Y, Hashimoto S, Abiru S, Komori A, Yamasaki K, Nagaoka S, Saeki A, Bekki S, Kugiyama Y, Kuroki T, Ito M, Nakao K, Yatsuhashi H. Rapidly growing, moderately differentiated HCC: A clinicopathological characteristic of HCC occurrence after IFN-free DAA therapy? J Hepatol. 2018; 68:854-855.
22. Meissner EG, Wu D, Osinusi A, *et al.* Endogenous intrahepatic IFNs and association with IFN-free HCV treatment outcome. J Clin Invest. 2014; 124:3352-3363.
23. Barash H, R Gross E, Edrei Y, Ella E, Israel A, Cohen I, Corchia N, Ben-Moshe T, Pappo O, Pikarsky E, Goldenberg D, Shiloh Y, Galun E, Abramovitch R. Accelerated carcinogenesis following liver regeneration is associated with chronic inflammation-induced double-strand DNA breaks. Proceed Natl Acad Sci USA. 2010; 107:2207-2212.
24. Kanwal F, Kramer JR, Asch SM, Cao Y, Li L, El-Serag HB. Long-term risk of hepatocellular carcinoma in HCV patients treated with direct-acting antiviral agents. Hepatology. 2020; 71:44-55.
25. Innes H, Barclay ST, Hayes PC, Fraser A, Dillon JF, Stanley A, Bathgate A, McDonald SA, Goldberg D, Valerio H, Fox R, Kennedy N, Bramley P, Hutchinson SJ. The risk of hepatocellular carcinoma in cirrhotic patients with hepatitis C and sustained viral response: role of the treatment regimen. J Hepatol. 2018; 68:646-654.
26. Lim SG, Aghemmo A, Chen PJ, *et al.* Management of hepatitis C virus infection in the Asia-Pacific region: an update. Lancet Gastroenterol Hepatol. 2017; 2:52-62.
27. Romano A, Angeli P, Piovesan S, *et al.* Newly diagnosed hepatocellular carcinoma in patients with advanced hepatitis C treated with DAAs: A prospective population study. J Hepatol. 2018; 69:345-352.
28. Mariño Z, Darnell A, Lens S, *et al.* Time association between hepatitis C therapy and hepatocellular carcinoma emergence in cirrhosis: Relevance of non-characterized nodules. J Hepatol. 2019; 70:874-884.
29. Tamada T, Korenaga M, Yamamoto A, Higaki A, Kanki A, Nishina S, Hino K, Ito K. Assessment of clinical and magnetic resonance imaging features of de novo hypervascular hepatocellular carcinoma using gadoxetic acid-enhanced magnetic resonance imaging. Hepatol Res. 2017; 47:E152-E160.
30. Morimoto N, Miura K, Watanabe S, Tsukui M, Takaoka Y, Nomoto H, Murayama K, Hirosawa T, Goka R, Kunitomo N, Nakamura H, Sugimoto H, Isoda N, Yamamoto H. Usefulness of Gd-EOB-DTPA-enhanced MRI for evaluating the potential for early development of

hepatocellular carcinoma after HCV eradication by direct-acting antiviral treatment. J Rural Med. 2019; 14:78-86.
31. Silini E, Bottelli R, Asti M, Bruno S, Candusso ME, Brambilla S, Bono F, Iamoni G, Tinelli C, Mondelli MU, Ido G. Hepatitis C virus genotypes and risk of hepatocellular carcinoma in cirrhosis: A case-control study. Gastroenterology. 1996; 111:199-205.

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