J Chin Med Assoc

ORIGINAL ARTICLE

# Analyzing risk factors and developing a stratification system for hepatocellular carcinoma recurrence after interferon-free direct-acting antiviral therapy in chronic hepatitis C patients

**Chih-Hsuan Luan[a], Pin-Shuo Su[a], Chi-Jen Chu[a,b,*], Chung-Chi Lin[b,c], Chien-Wei Su[b,d], Jiing-Chyuan Luo[a,b], I-Cheng Lee[a,b], Chen-Ta Chi[a,b], Shou-Dong Lee[b,e], Yuan-Jen Wang[b,c], Fa-Yauh Lee[a,b], Yi-Hsiang Huang[b,c], Ming-Chih Hou[a,b]**

*[a]Division of Gastroenterology and Hepatology, Department of Medicine, Taipei Veterans General Hospital, Taipei, Taiwan, ROC; [b]Faculty of Medicine, National Yang Ming Chiao Tung University, Taipei, Taiwan, ROC; [c]Healthcare and Services Center, Taipei Veterans General Hospital, Taipei, Taiwan, ROC; [d]Division of General Medicine, Department of Medicine, Taipei Veterans General Hospital, Taipei, Taiwan, ROC; [e]Division of Gastroenterology, Department of Medicine, Cheng Hsin General Hospital, Taipei, Taiwan, ROC*

## Abstract

**Background:** The introduction of direct-acting antiviral agents (DAAs) has revolutionized the therapeutic landscape of chronic hepatitis C (CHC), however real-world data on the risk factors of hepatocellular carcinoma (HCC) recurrence following DAA treatment in CHC-HCC patients are limited in Taiwan. We aimed to evaluate the therapeutic efficacy of DAAs in Taiwanese patients with prior hepatitis C virus (HCV)-induced HCC and identify the posttreatment risk factors for HCC recurrence.

**Methods:** Between January 2017 and August 2021, 208 CHC-HCC patients underwent DAA treatment at Taipei Veterans General Hospital. Among them, 94 patients met the inclusion criteria (Barcelona clinic liver cancer [BCLC] stage 0/A after treatment with complete radiological response) for analysis. Comprehensive demographic, clinical, and laboratory data were collected before and after DAA treatment. The primary outcome was HCC recurrence post-DAA treatment, and independent variables were assessed using multivariate Cox proportional hazards models.

**Results:** The mean age of the enrolled patients was 75.9 ± 8.9 years; 44.7% were male, and 94.7% were Child-Pugh class A. Before DAA treatment, 31.9% experienced HCC recurrence. The median follow-up after DAA treatment was 22.1 months (interquartile range, 8.6-35.9 months). After treatment, 95.7% of the patients achieved a sustained virological response ($SVR_{12}$), but HCC recurrence occurred in 54.3%. Cumulative HCC recurrence rates after treatment were 31.1% at 1 year, 57.3% at 3 years, and 68.5% at up to 5.69 years. Multivariate analysis revealed that prior HCC recurrence before DAA treatment (hazard ratio [HR] = 3.15, $p = 0.001$), no $SVR_{12}$ after treatment (HR = 6.829, $p = 0.016$), 12-week posttreatment alpha-fetoprotein (AFP) level >10 ng/mL (HR = 2.34, $p = 0.036$), and BCLC A3 lesions (two or three nodules without any tumor exceeding 3 cm) (HR = 2.31, $p = 0.039$) were independent risk factors for HCC recurrence. We further developed a risk stratification system based on these significant independent factors.

**Conclusion:** This investigation underscores the critical influence of factors such as prior HCC recurrence, successful attainment of $SVR_{12}$, posttreatment AFP level, and specific tumor characteristics in determining the risk of HCC recurrence after treatment with DAAs. Our proposed innovative risk stratification system may not only contribute to enhanced personalized care but also holds the potential to optimize treatment outcomes.

**Keywords:** Alpha-fetoprotein; Chronic hepatitis C; Hepatocellular carcinoma; Recurrence

** Address correspondence. Dr. Chi-Jen Chu, Division of Gastroenterology and Hepatology, Department of Internal Medicine, Taipei Veterans General Hospital, 201, Section 2, Shi-Pai Road, Taipei 112, Taiwan, ROC. E-mail address: cjchu@vghtpe.gov.tw (C.-J. Chu).*

*Conflicts of interest: Dr. Chien-Wei Su, Dr. Fa-Yauh Lee, Dr. Yi-Hsiang Huang, and Dr. Ming-Chih Hou, editorial board members at Journal of the Chinese Medical Association, have no roles in the peer review process of or decision to publish this article. The other authors declare that they have no conflicts of interest related to the subject matter or materials discussed in this article.*

*Journal of Chinese Medical Association. (2024) 87: 357-368.*

*Received September 10, 2023; accepted December 15, 2023.*

*doi: 10.1097/JCMA.0000000000001051*



## 1. INTRODUCTION

Chronic infection with hepatitis C virus (HCV) is a well-established risk factor for hepatocellular carcinoma (HCC), which ranks among the most prevalent and lethal malignancies worldwide.[1] HCV triggers liver inflammation, leading to tissue necrosis, fibrosis, and cellular regeneration, thus fostering genetic mutations that drive neoplastic transformation. Moreover, HCV has direct carcinogenic potential, inducing pro-oncogenic effects within infected cells through oxidative stress, host cell checkpoint dysregulation, and deoxyribonucleic acid (DNA) damage.[2,3] In addition, an estimated 10% to 20% of patients develop liver cirrhosis over a span of 20 to 30 years following HCV infection, with an annual hepatic decompensation risk of 3% to 6%, and an annual HCC risk of 1% to 5%.[4]

Detecting the onset of HCC is often hampered by the absence of clinical symptoms and physical indicators, resulting in late-stage diagnosis and unfavorable prognosis. Despite the availability of many therapeutic avenues for HCC, the primary curative approaches continue to be surgical liver resection, local radiofrequency ablation, and liver transplantation. Nevertheless, even after successful curative interventions, the HCC recurrence rate can be as high as 70%, thereby contributing to significant long-term mortality.[5–7] To address this issue, effective antiviral therapy has emerged as the most promising strategy to prevent both the onset and recurrence of HCC in patients with chronic hepatitis C (CHC). Notably, studies from the interferon (IFN) era revealed that achieving a sustained virological response (SVR) could significantly reduce the risk of HCC occurrence and recurrence compared to not achieving an SVR.[8]

The landscape of CHC treatment was revolutionized with the advent of IFN-free, all-oral direct-acting antivirals (DAAs). DAAs have exhibited superior safety profiles and shorter treatment duration, even for advanced liver disease stages, including decompensated cirrhosis, yielding a remarkable SVR rate exceeding 95%.[9–12] Although two studies published in 2016 raised concerns about the high risk of HCC occurrence and recurrence after DAA therapies,[13,14] most recent studies have not supported that DAA therapy increases the risk of HCC recurrence.[15–22] In addition, a meta-analysis of six studies encompassing 1105 patients who received DAAs vs 1912 controls either untreated or undergoing peg-IFN-α-based regimens with follow-up ranging from 1.25 to 4 years concluded that DAA therapy was correlated with a substantial 64% decrease in HCC recurrence compared to untreated controls.[23]

With regards to prognosticators for HCC recurrence among DAA-treated patients, previous studies have identified factors such as DAA treatment response, duration between HCC treatment and initiating DAA treatment, tumor characteristics including size and number of nodules, history of multiple HCC treatments, alpha-fetoprotein (AFP) level, and Child-Pugh score.[16,17,21,24–27] However, limited real-world data are currently available on the risk factors, particularly tumor characteristics, associated with tumor recurrence in CHC-HCC patients post all-oral DAA treatment in Taiwan. Therefore, this study aimed to evaluate the therapeutic efficacy of DAAs in Taiwanese patients with prior HCV-induced HCC and investigate the independent risk factors of HCC recurrence following DAA therapy. To ensure homogeneity, we exclusively included patients with early HCC (Barcelona clinic liver cancer [BCLC] stages 0 and A) who were successfully treated without radiological evidence of residual tumors before starting DAA treatment. In addition, we developed a risk stratification system based on the significant independent factors identified in our analysis to estimate the cumulative HCC recurrence rate after DAA treatment.

## 2. METHODS

### 2.1. Study design and patient population

This retrospective, single-center study enrolled a total of 208 consecutive adult (≥18 years) patients with CHC-HCC who underwent all-oral DAA treatment at our hospital between January 2017 and August 2021. CHC was defined as the presence of detectable HCV antibodies (anti-HCV) (Abbott HCV EIA 2.0; Abbott Laboratories, Abbott Park, IL) and quantifiable serum HCV ribonucleic acid (RNA) (Cobas TaqMan HCV Test version 2.0; Roche Diagnostics GmbH, Mannheim, Germany; lower limit of quantification: 15 IU/mL) for a minimum of 6 months. The inclusion criteria were (1) HCC diagnosis at first occurrence, established either through pathology or noninvasive criteria according to guidelines from the American Association for the Study of Liver Disease (AASLD)[28]; (2) HCC before DAA exposure classified as BCLA stage 0 or A[29]; (3) attainment of a complete radiological response (CRR) via any form of anti-HCC therapy, in line with the modified Response Evaluation Criteria in Solid Tumor (mRECIST) criteria, as evidenced by the absence of residual tumors or complete necrosis assessed with dynamic computed tomography (CT) or magnetic resonance imaging (MRI) 1 to 2 months after HCC treatment, and subsequent 3 to 6-month follow-up[30]; and (4) verification of CRR status by at least one dynamic CT/MRI assessment conducted within 3 months before or after initiating DAA treatment. The exclusion criteria were (1) undergoing liver transplantation; (2) a follow-up duration of less than 6 months; (3) advanced stage HCC (BCLC stage B, C, D); and (4) active HCC, treated HCC lesions lacking CRR, or the presence of uncharacterized nodules within 3 months before or after commencing DAA treatment. Finally, 94 patients were included for statistical analysis. This study was granted approval by the Institutional Review Board of Taipei Veterans General Hospital and adhered to the principles of the Declaration of Helsinki and the International Conference on Harmonization for Good Clinical Practice. All patients provided written informed consent before participation. The case collection process is depicted in Fig. 1.

For each enrolled patient, the following demographic characteristics and clinical parameters were recorded before initiating DAA treatment: sex, body mass index, age, history of previous antiviral treatment, liver fibrosis status, signs of portal hypertension, details and stage of HCC, previous HCC treatment modality, and recurrence history. Standard laboratory techniques were used to collect pre- and post-DAA therapy laboratory data for subsequent statistical analysis.

### 2.2. DAA treatment protocol

In this study, selection of the DAA regimen was based on clinical decisions by physicians, taking into account factors such as HCV genotype/subtype, viral load, patient attributes, drug-drug interaction profiles, and evolving criteria for reimbursement by the National Health Insurance Administration of the Ministry of Health and Welfare.[31,32] Given the hepatotoxicity risks associated with some NS3/4A protease inhibitors, guidelines recommend pairing a nucleotide NS5B polymerase inhibitor with an NS5A inhibitor as the standard regimen for patients with decompensated cirrhosis.[31,32] The HCV genotype was determined using a commercially available assay (Cobas HCV GT; Roche Diagnostics GmbH). Before the initiation of DAA therapy, a comprehensive review of the patients' regular medications was performed to assess potential drug-drug interactions. Medications with the potential for drug-drug interactions were either discontinued, substituted with alternative drugs, or initiated at the lowest dose with

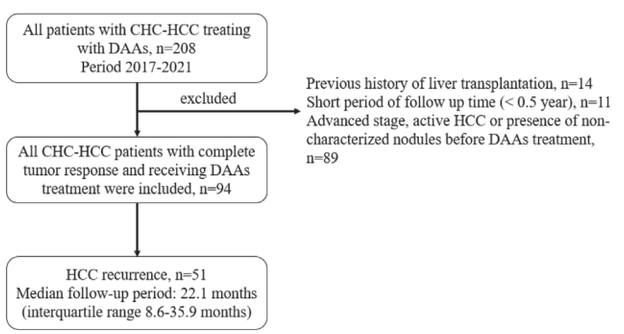


**Fig. 1** Flowchart of case collection. CHC = chronic hepatitis C; DAA = direct-acting antiviral; HCC = hepatocellular carcinoma.

regular monitoring by physicians.[33] During the treatment period, the patients were evaluated by physicians at weeks 1 and 2, followed by bi-weekly assessments (or more frequent evaluations in cases of adverse events) until completion of therapy. SVR at 12 weeks ($SVR_{12}$) was defined as undetectable HCV RNA (≤15 IU/mL) at the end of DAA treatment and again 12 weeks after completing therapy. Patients without $SVR_{12}$ data were considered not to have achieved $SVR_{12}$.

### 2.3. Follow-up post-DAA treatment and HCC recurrence surveillance

All of the included patients underwent periodic imaging follow-up to detect HCC recurrence, in accordance with international guidelines. AFP levels were measured every 3 months, and dynamic CT/MRI imaging was generally performed every 3 to 6 months for patients achieving CRR within 2 years. Patients who achieved CRR after more than 2 years of prior HCC treatment underwent ultrasonography or dynamic CT/MRI every 4 to 6 months. When a suspected HCC lesion was detected in the liver through ultrasonography, further imaging studies such as dynamic CT/MRI and/or biopsy were arranged for confirmation. HCC recurrence was diagnosed by histology or characteristic imaging findings observed in contrast-enhanced CT/MRI, showing hyper-enhancement in the arterial phase and a washout pattern in the portal or delayed phases.

### 2.4. Primary and secondary endpoints

The primary endpoint was the cumulative incidence of HCC recurrence after initiating DAA treatment. The date of HCC diagnosis, determined through typical imaging such as CT/MRI, was used for cumulative failure curve analysis. To explore the influence of tumor characteristics on post-DAA HCC recurrence, patients with BCLC stage A HCC were categorized into three subgroups: (A1) a single tumor sized 2 to 5 cm; (A2) a single tumor larger than 5 cm; and (A3) two or three nodules, none exceeding 3 cm in size. The secondary endpoints encompassed the efficacy of DAA treatment in the included patients and identification of independent baseline and 12-week post-DAA treatment variables capable of predicting HCC recurrence, including the DAA treatment response ($SVR_{12}$).

### 2.5. Definitions

The fibrosis-4 (FIB-4) score is a noninvasive scoring system used to estimate the degree of hepatic fibrosis through laboratory test data. An FIB-4 score >3.25 has been shown to have a positive predictive value of 82.1% for confirming advanced fibrosis (F3-F4) with a specificity of 98.2%.[34] In this study, advanced fibrosis (F3-4) was defined as the presence of cirrhosis-related clinical or radiological manifestations, along with evidence of portal hypertension such as esophageal or gastric varices, or a FIB-4 score >3.25.

The albumin-bilirubin (ALBI) score/grade serves as a measure of liver function. It was originally devised as a prognostic factor for HCC patients, and has since gained widespread recognition. The ALBI score is calculated as: ($\log_{10}$ bilirubin [μmol/L] × 0.66) + (albumin [g/L] × −0.0852). An ALBI score ≤−2.60 is defined as ALBI grade 1; a score >−2.60 to ≤−1.39 as ALBI grade 2; and a score >−1.39 as ALBI grade 3. The ALBI score/grade offers an objective measure, capable of detecting subtle changes in liver function compared to the Child-Pugh or model for end-stage liver disease (MELD) scores. The ALBI score/grade has been shown to be a good predictor of survival in patients with various etiologies of nonmalignant liver diseases.[35]

### 2.6. Statistical analysis

All statistical analyses were conducted using SPSS version 23.0 (SPSS Inc., Chicago, IL). Demographic and clinical parameters before initiating DAA treatment were presented as means, SDs, or percentages as appropriate. Categorical variables were analyzed using the chi-squared test or Fisher exact test. Continuous variables were analyzed using independent *t* tests or the Mann-Whitney *U* test. Paired *t* tests were used to compare sequential changes in laboratory data before and after DAA therapy. Kaplan-Meier survival and cumulative incidence curves were compared using the log-rank test. Multivariable Cox regression analysis (hazard ratios [HRs] and 95% CI estimates) was used to assess the risk of HCC recurrence at any point during follow-up, incorporating baseline and 12-week post-DAA treatment variables. A statistically significant result was defined as $p < 0.05$. The duration of follow-up was calculated from the initiation of DAA treatment to death, the last visit, or the final abdominal image follow-up, as applicable.

## 3. RESULTS

### 3.1. Clinical characteristics of the enrolled patients

A total of 208 patients with CHC and a history of HCC who underwent DAA treatment were initially enrolled. Of these patients, 114 were subsequently excluded according to the exclusion criteria, and the remaining 94 patients were included in the final analysis (Fig. 1). The mean age of the included patients was 75.9 ± 8.9 years, and 42 (44.7%) were male. Eighteen (19.1%) patients had failed previous pegylated IFN therapy. The median baseline HCV RNA level was 5.92 $\log_{10}$ IU/mL (interquartile range [IQR] = 5.09-6.52 $\log_{10}$ IU/mL). The distribution of HCV genotypes was as follows: 1.1% genotype 1a, 67.0% genotype 1b, 26.6% genotype 2, 1.1% genotype 3, 2.1% genotype 6, and 2.1% unclassified genotype. According to the predefined criteria, 66 (70.2%) patients were classified as having advanced (F3-4) fibrosis, and five (5.3%) were diagnosed with decompensated (CTP class B-C) cirrhosis. About the type of DAAs used, 61.7% of the patients were treated with sofosbuvir-based regimens. Detailed information about the DAA regimens is summarized in Table 1. The median follow-up period after initiating DAA treatment was 22.1 months (IQR = 8.6-35.9 months).

### 3.2. HCC characteristics before initiating DAA treatment and previous treatment history

Before the commencement of DAA therapy, the HCC characteristics of the 94 patients were as follows: 17.0% were classified as BCLC stage 0 (ie, single tumor ≤2 cm); 46.8% as BCLC stage A with a single tumor of 2 to 5 cm in diameter (A1); 13.8% as BCLC stage A with a single tumor ≥5 cm in diameter (A2); 11.7% as BCLC stage A with two or three nodules (A3); and 10.7% could not be categorized due to insufficient data. Before initiating DAA treatment, 31.9% of the patients had a history of HCC recurrence after initial treatment. The distribution of the first HCC treatment was as follows: 55.3% surgical treatment, 31.9% radiofrequency ablation, and 12.8% transcatheter arterial chemoembolization (TACE). About the last HCC treatment to achieve CRR, 48.9% underwent surgery, 36.2% received radiofrequency ablation, 13.8% underwent TACE, and 1.1% underwent stereotactic body radiation therapy (Table 2). The median time from the initial HCC diagnosis to DAA treatment was 23.4 months (IQR = 3.5-67.4 months), and the median time from last HCC treatment with CRR to initiating DAA treatment (treatment durability) was 7.8 months (IQR = 2.0-31.3 months).

### 3.3. Virological response

Following the initiation of DAA therapy, only one patient did not complete the full course due to personal reasons, and consequently did not achieve $SVR_{12}$. All patients who completed DAA treatment achieved viral clearance (HCV RNA <15 IU/mL) at the end of the treatment. Posttreatment follow-up revealed that

**Table 1**

**Baseline clinical characteristics of the study population**

| Characteristics | Patients (n = 94) |
|---|---|
| Age, y, mean ± SD | 75.9 ± 8.9 |
| Male sex, n (%) | 42 (44.7%) |
| Genotype 1, n (%) | |
| 1a | 1 (1.1%) |
| 1b | 63 (67%) |
| 2 | 25 (26.6%) |
| 3 | 1 (1.1%) |
| 6 | 2 (2.1%) |
| Unclassified | 2 (2.1%) |
| HCV RNA, IU/mL | |
| ≥1,000,000, IU/mL, n (%) | 49 (52.1%) |
| <1,000,000, IU/mL, n (%) | 45 (47.9%) |
| Decompensated (CTP class B-C) cirrhosis (%) | 5 (5.3%) |
| Previous IFN failure, n (%) | 18 (19.1%) |
| Diabetes mellitus, n (%) | 28 (29.8%) |
| Body mass index, mean ± SD | 24.78 ± 3.69 |
| Coinfected with HBV, n (%) | 3 (3.2%) |
| Advanced fibrosis (F3-F4), n (%) | 66 (70.2%) |
| Presence of EV/GV | 16 (17%) |
| DAAs regimen | |
| PrOD | 16 (17.0%) |
| DCV + ASV | 9 (9.6%) |
| SOF + RBV | 13 (13.8%) |
| SOF + DCV | 2 (2.1%) |
| SOF + LDV | 31 (33.0%) |
| SOF + VEL | 12 (12.8%) |
| EBR + GZR | 2 (2.1%) |
| GLE + PIB | 9 (9.6%) |

ASV = asunaprevir; CTP = Child-Turcotte-Pugh; DAAs = direct-acting antivirals; DCV = daclatasvir; EBR = elbasvir; EV = esophageal varices; GLE = glecaprevir; GV = gastric varices; GZR = grazoprevir; HBV = hepatitis B virus; IFN = interferon; LDV = ledipasvir; PIB = pibrentasvir; PrOD = paritaprevir/ritonavir, ombitasvir, and dasabuvir; RBV = ribavirin; SOF = sofosbuvir; VEL = velpatasvir.

three patients had virologic relapse at 12 weeks after completing DAA treatment. Intent-to-treat analysis demonstrated an $SVR_{12}$ rate of 95.7% (90/94 patients). Among the four patients who did not achieve $SVR_{12}$, one received the daclatasvir + asunaprevir regimen, and the other three received the sofosbuvir + ribavirin regimen. The $SVR_{12}$ rate was 100% (21/21) in the pangenotype regimen and 94.5% (69/73) in the genotype-specific regimen ($p = 0.572$).

### 3.4. Laboratory data before and 12 weeks after DAA treatment

Laboratory data of the patients before and 12 weeks after DAA treatment are summarized in Table 3. The median alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels at 12 weeks after DAA treatment ($EOT_{12}$) were 19 and 25 IU/L, respectively, and the median levels of albumin, total bilirubin, and platelet count at 12 weeks after DAA were 4.1 g/dL, 0.83 mg/dL, and 131 × 1000/μL, respectively. Based on these data, the distribution of ALBI grades was as follows: grade 1 (56.4%), grade 2 (41.5%), and grade 3 (2.1%). Compared to pretreatment values, significant declines in AST ($p < 0.001$), ALT ($p < 0.001$), and FIB-4 score ($p < 0.001$) were observed after DAA therapy. In addition, significant increases in albumin ($p < 0.001$) and hemoglobin ($p = 0.002$) were noted after DAA therapy. Due to the potential effect of hepatitis on laboratory values, $EOT_{12}$ laboratory data were used to investigate the risk of future HCC recurrence in the analysis.

**Table 2**

**HCC characteristics before the initiation of DAAs**

| Characteristics | Patients (n = 94) |
|---|---|
| HCC morphology before DAA initiation by BCLC stage, n (%) | |
| Single tumor ≤2 cm (0) | 16 (17.0%) |
| Single tumor 2-5 cm (A1) | 44 (46.8%) |
| Single tumor >5 cm (A2) | 13 (13.8%) |
| Two or three nodules, each ≤3 cm (A3) | 11 (11.7%) |
| Insufficient information to classify | 10 (10.7%) |
| Previous HCC recurrence, n (%) | 30 (31.9%) |
| HCC treatment required to achieve CRR, n (%) | |
| 1 | 64 (68.1%) |
| 2 | 12 (12.8%) |
| ≥3 | 18 (19.1%) |
| First HCC treatment, n (%) | |
| Surgery | 52 (55.3%) |
| RFA | 30 (31.9%) |
| TACE | 12 (12.8%) |
| Time interval from initial HCC diagnosis to DAA treatment, n (%) | |
| ≤12 mo | 42 (44.7%) |
| 12-24 mo | 5 (5.3%) |
| 24-48 mo | 16 (17.0%) |
| 48-72 mo | 9 (9.6%) |
| >72 mo | 22 (23.4%) |
| Last HCC treatment, n (%) | |
| Surgery | 46 (48.9%) |
| RFA | 34 (36.2%) |
| TACE | 13 (13.8%) |
| Other (SBRT) | 1 (1.1%) |
| Time interval from HCC CRR to DAA treatment, n (%) | |
| ≤6 mo | 45 (47.9%) |
| 6-12 mo | 10 (10.6%) |
| 12-24 mo | 9 (9.6%) |
| 24-36 mo | 11 (11.7%) |
| >36 mo | 19 (20.2%) |

BCLC = Barcelona clinic liver cancer; CRR = complete radiological response; DAAs = direct-acting antivirals; HCC = hepatocellular carcinoma; RFA = radiofrequency ablation; SBRT = stereotactic body radiation therapy; TACE = transcatheter arterial chemoembolization.

### 3.5. Cumulative incidence of HCC recurrence and comparison of characteristics

Over a median follow-up period of 22.1 months (IQR = 8.6-35.9 months) after DAA treatment, HCC recurred in 51 (54.3%) of the study patients. Recurrence occurred adjacent to the site of the previously treated (ablated, operated, or embolized) tumors in 18 (35.3%) of these 51 patients. Seven of these 18 (38.9%) patients had recurrence within 1 year after the last HCC treatment, whereas 11 (61.1%) had recurrence more than 1 year after the last treatment. The cumulative incidence rates of HCC recurrence after initiating DAA treatment were 31.1% at 1 year, 42.5% at 2 years, 57.3% at 3 years, 60.6% at 4 years, and 68.5% at 5.7 years (Fig. 2). The median time to HCC recurrence from initiating DAA treatment was 9.6 months (IQR = 5.8-22.6 months). Comparing the characteristics of the patients with and without recurrent HCC, those who developed HCC recurrence were significantly more likely to have an $EOT_{12}$ AFP level >10 ng/mL (19.6% vs 4.7%, $p = 0.03$) (Table 4). Furthermore, the patients with a history of recurrent HCC before initiating DAA treatment ($p = 0.003$) and those with BCLC A3 lesions (two or three nodules without any tumor exceeding 3 cm, $p = 0.044$) were more likely to experience HCC recurrence after DAA treatment (Table 4).

**Table 3**

**Laboratory data before and 12 wk after DAA treatment of the enrolled patients (n = 94)**

| Characteristics | Before DAA treatment | 12 wk post-DAA treatment | *p* (paired) |
|---|---|---|---|
| Mean white cell count (×1000/μL) | 5.1 ± 1.7 | 5.4 ± 1.8 | 0.084 |
| Mean hemoglobin, g/dL | 12.4 ± 1.9 | 12.8 ± 1.9 | 0.002 |
| Mean platelet count (×1000/μL) | 140 ± 59 | 134 ± 51 | 0.173 |
| Mean prothrombin time, s | 11.9 ± 2.0 | 12.0 ± 1.9 | 0.810 |
| Mean albumin, g/dL | 3.8 ± 0.5 | 4.0 ± 0.5 | <0.001 |
| Mean AST, U/L | 79 ± 74 | 34 ± 43 | <0.001 |
| Mean ALT, U/L | 75 ± 67 | 28 ± 48 | <0.001 |
| Mean total bilirubin, mg/dL | 0.82 ± 0.42 | 0.86 ± 0.42 | 0.244 |
| Mean creatinine, mg/dL | 1.12 ± 1.06 | 1.21 ± 1.25 | 0.138 |
| Mean alpha-fetoprotein, ng/mL | 51.5 ± 153.3 | 241.6 ± 1513.9 | 0.219 |
| Mean FIB-4 score | 5.96 ± 4.66 | 4.43 ± 2.96 | <0.001 |
| ALBI grade 1, n (%) | 46 (48.9%) | 53 (56.4%) | 0.179 |

ALBI = albumin-bilirubin; ALT = alanine aminotransferase; AST = aspartate aminotransferase; DAA = direct-acting antiviral; FIB-4 = fibrosis-4.

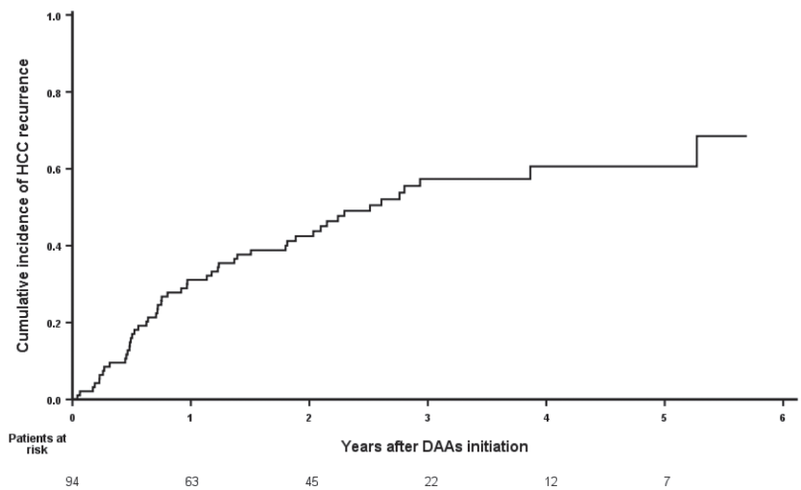


**Fig. 2** The overall cumulative incidence of HCC recurrence after initiating DAA treatment was 31.1% at 1 y, 42.5% at 2 y, 57.3% at 3 y, 60.6% at 4 y, and 68.5% at 5.7 y, respectively. DAA = direct-acting antiviral; HCC = hepatocellular carcinoma.

### 3.6. Independent factors associated with HCC recurrence

Building on the differences in characteristics between the patients with and without HCC recurrence, continuous variables were transformed into categorical data for Cox regression analysis. In univariate analysis, the variables associated with HCC recurrence were failure to achieve $SVR_{12}$ after DAA treatment, $EOT_{12}$ AFP >10 ng/mL, BCLC A3 lesions, last HCC treatment with CRR being TACE, and history of previous recurrence before DAA treatment (Table 5). Because TACE is the standard treatment option for BCLC A3 lesions, to avoid a dilutional effect, only the variable of BCLC A3 lesions was selected into multivariate analysis. A multivariable Cox model was then used to identify variables independently associated with the primary endpoint, and the results were as follows: previous tumor recurrence before DAA treatment (HR = 3.15, 95% CI, 1.63-6.11, $p$ = 0.001), failure to achieve $SVR_{12}$ after DAA treatment (HR = 6.829, 95% CI, 1.42-32.83, $p$ = 0.016), $EOT_{12}$ AFP >10 ng/mL (HR = 2.34, 95% CI, 1.06-5.17, $p$ = 0.036), and multiple tumor nodules (BCLC A3 lesions) compared to single nodules (BCLC 0, A1, A2) (HR = 2.31, 95% CI, 1.05-5.09, $p$ = 0.039) (Table 5).

### 3.7. Comparison of cumulative rates of HCC recurrence by independent factors

The influence of tumor characteristics on the risk of HCC recurrence is demonstrated in Fig. 3A. All patients (100%) with BCLC A3 lesions (two or three nodules without any tumor exceeding 3 cm) experienced HCC recurrence post-DAA treatment within a follow-up period of up to 2.8 years. Conversely, the cumulative incidence of recurrent HCC was 66.2% for BCLC 0-A2 lesions at up to 5.7 years ($p$ = 0.012) (Fig. 3A). The impact of previous HCC treatment on the risk of HCC recurrence post-DAA treatment was evident. The cumulative incidence of HCC recurrence was notably higher among the patients with prior tumor recurrence before initiating DAA treatment compared to those without such recurrence (83.3% vs 62.3%, $p$ = 0.001) (Fig. 3B). We also found that $EOT_{12}$ AFP >10 ng/mL, but not baseline AFP >10 ng/mL, was independently associated with HCC recurrence post-DAA treatment, and the patients with $EOT_{12}$ AFP >10 ng/mL had a higher rate of HCC recurrence compared to those with $EOT_{12}$ AFP ≤10 ng/mL (87.5% vs 63.9%, $p$ = 0.002) (Fig. 3C).

### 3.8. Risk stratification system for estimating HCC recurrence

Utilizing the results of the multivariate analysis, we devised a risk stratification system by integrating BCLC stage, $EOT_{12}$ AFP level, history of previous HCC recurrence, and achieving $SVR_{12}$ after DAA treatment. BCLC A3 lesions were assigned a score of 1, and BCLC 0-A2 lesions were assigned a score of 0. An $EOT_{12}$ AFP value ≥10 ng/mL was scored as 1, and <10 ng/mL was scored as 0. A history of previous recurrence before

**Table 4**

**Comparison of the characteristics between the patients with or without HCC recurrence post-DAA treatment**

| Characteristics | No HCC recurrence (n = 43) | HCC recurrence (n = 51) | *p* |
|---|---|---|---|
| Mean age, y | 75.6 ± 8.4 | 76.3 ± 9.4 | 0.709 |
| Male sex, % | 18 (41.9%) | 24 (47.1%) | 0.614 |
| Mean BMI, kg/m$^2$ | 24.6 ± 4.0 | 25.0 ± 3.4 | 0.611 |
| HCV RNA ≥1,000,000, IU/mL (%) | 21 (48.8%) | 24 (47.1%) | 0.863 |
| HCV genotype 1, % | 31 (73.8%) | 33 (66.0%) | 0.417 |
| Previous IFN failure, % | 8 (18.6%) | 10 (19.6%) | 0.902 |
| Diabetes mellitus, % | 11 (25.6%) | 17 (33.3%) | 0.413 |
| SOF-based DAAs, % | 27 (62.8%) | 31 (60.8%) | 0.842 |
| $SVR_{12}$ rate by DAAs, % | 43 (100%) | 47 (92.2%) | 0.082 |
| Advanced fibrosis (F3-F4), % | 28 (65.1%) | 38 (74.5%) | 0.321 |
| Decompensated (CTP class B-C) cirrhosis, % | 3 (7.0%) | 2 (3.9%) | 0.511 |
| Mean $EOT_{12}$ albumin, g/dL | 4.04 ± 0.44 | 3.98 ± 0.47 | 0.500 |
| Mean $EOT_{12}$ AST, U/L | 35.2 ± 60.9 | 33.1 ± 18.1 | 0.815 |
| Mean $EOT_{12}$ ALT, U/L | 28.8 ± 67.9 | 27.5 ± 19.6 | 0.893 |
| Mean $EOT_{12}$ total bilirubin, mg/dL | 0.82 ± 0.43 | 0.90 ± 0.40 | 0.336 |
| $EOT_{12}$ AFP >10 ng/mL (%) | 2 (4.7%) | 10 (19.6%) | **0.03** |
| Mean $EOT_{12}$ WBC (×1000/μL) | 5.49 ± 1.72 | 2.29 ± 1.84 | 0.600 |
| Mean $EOT_{12}$ Hgb, g/dL | 12.7 ± 1.7 | 12.9 ± 2.1 | 0.484 |
| Mean $EOT_{12}$ platelet count (×1000/μL) | 140 ± 59 | 129 ± 43 | 0.324 |
| Mean $EOT_{12}$ prothrombin time, s | 11.8 ± 1.5 | 15.2 ± 22.2 | 0.329 |
| $EOT_{12}$ ALBI grade, % | | | 0.250 |
| 1 | 27 (62.8%) | 26 (51.0%) | |
| 2/3 | 16 (37.2%) | 25 (49.0%) | |
| BCLC stage before DAAs, n (%) | | | **0.044** |
| 0/A1/A2 | 37 (86.0%) | 36 (70.6%) | |
| A3 | 2 (4.7%) | 9 (17.6%) | |
| Unclassified | 4 (9.3%) | 6 (11.8%) | |
| Prior HCC recurrence, n (%) | 7 (16.3%) | 23 (45.1%) | **0.003** |
| Last HCC treatment, n (%) | | | 0.113 |
| Surgery | 26 (60.5%) | 20 (39.2%) | |
| RFA | 14 (32.6%) | 20 (39.2%) | |
| TACE | 3 (7.0%) | 10 (19.6%) | |
| Other (SBRT) | 0 (0%) | 1 (2.0%) | |
| The mean time interval from HCC CRR to DAA treatment, mo | 25.2 ± 36.1 | 21.3 ± 34.8 | 0.838 |

The bold value merely indicates that the p-value is statistically significant, but it is not necessary; it can also be presented without bold.

ALBI = albumin-bilirubin; ALT = alanine aminotransferase; AST = aspartate aminotransferase; BCLC = Barcelona clinic liver cancer; BMI = body mass index; CRR = complete radiological response; CTP = Child-Turcotte-Pugh; DAA = direct-acting antiviral; $EOT_{12}$ = 12 wk post end of DAAs treatment; HCC = hepatocellular carcinoma; HCV = hepatitis C virus; Hgb = Hemoglobin; IFN = interferon; RFA = radiofrequency ablation; SBRT = stereotactic body radiation therapy; SOF = sofosbuvir; TACE = transcatheter arterial chemoembolization; WBC = white blood cell.

DAA treatment was scored as 1, and no previous recurrence was scored as 0. Not achieving $SVR_{12}$ was assigned a score of 1, and achieving $SVR_{12}$ was assigned a score of 0. The total score thus ranged from 0 to 4. Among the 94 patients, 52.1% had a score of 0 (low-risk group), 36.2% had a score of 1 (medium-risk group), and 11.7% had a score exceeding 2 (high-risk group). Fig. 4 illustrates the cumulative incidence of HCC among these three groups. The log-rank test confirmed a statistically significant difference in cumulative HCC incidence between these groups ($p < 0.001$). The incidence rates of HCC at 1, 2, 3, 4, and 5 years after initiating DAA treatment in the low-risk group were 12.3%, 23.0%, 32.0%, 32.0%, and 32.0%, respectively, compared to 48.1%, 60.5%, 81.5%, 87.7%, and 87.7% in the medium-risk group. In the high-risk group, the rates were 63.6%, 72.9%, and 86.4% at 1, 2, and 2.49 years, respectively (Fig. 4).

### 3.9. Dynamic changes in AFP value before and after DAA therapy and the predictive value on recurrent HCC

Using a cutoff value of 10 ng/mL, the patients were categorized into three groups based on dynamic changes in AFP values before and after DAA treatment. Notably, none of the patients experienced a transition from low (≤10 ng/mL) to high (>10 ng/mL) AFP values after DAA treatment. Intriguingly, patients with consistently high AFP values (>10 ng/mL) before and after DAA treatment had the highest risk of HCC recurrence (Fig. 5). In this group, the cumulative incidence of HCC recurrence was 66.7% at 1 year, 75.0% at 2 years, 75% at 3 years, and 87.5% at 4 years, and the difference was significant compared to the other two groups ($p < 0.01$). Conversely, patients whose AFP values declined from high (>10 ng/mL) to low (≤10 ng/mL) after DAA treatment had the lowest incidence of HCC recurrence, with a cumulative incidence rate of 14.3% at 1 year, 29.5% at 2 years, and 40.4% at 3 years (Fig. 5).

## 4. DISCUSSION

The development of highly effective, IFN-free, all-oral DAAs has revolutionized HCV treatment, offering high rates of SVR in a very short time, broader candidacy even for patients with decompensated cirrhosis, and very few side effects compared to IFN-based therapy.[9–12] In addition, a substantially lower risk of liver-related events has been reported in patients with CHC successfully treated with DAAs.[36] Consequently, clinicians

**Table 5**

**Cox regression analysis for independent predictors of HCC recurrence**

| | Univariate | | | Multivariate | | |
|---|---|---|---|---|---|---|
| **Parameter** | **HR** | **95% CI** | ***p*** | **HR** | **95% CI** | ***p*** |
| Age, y | | | | | | |
| ≥65 vs <65 | 1.244 | 0.490-3.160 | 0.646 | | | |
| Sex | | | | | | |
| Female vs male | 0.881 | 0.508-1.528 | 0.652 | | | |
| HCV RNA, IU/mL | | | | | | |
| ≥1,000,000 vs <1,000,000 | 0.974 | 0.561-1.689 | 0.924 | | | |
| Genotype | | | | | | |
| Type non-1 vs type 1a + 1b | 1.497 | 0.828-2.708 | 0.182 | | | |
| Previous IFN failure | | | | | | |
| Yes vs no | 0.771 | 0.375-1.586 | 0.479 | | | |
| Child-Pugh class | | | | | | |
| B + C vs A | 0.748 | 0.181-3.088 | 0.688 | | | |
| Advanced fibrosis (F3-F4) | | | | | | |
| Yes vs no | 1.271 | 0.675-2.394 | 0.457 | | | |
| Presence of EV/GV | | | | | | |
| Yes vs no | 1.448 | 0.741-2.829 | 0.278 | | | |
| Diabetes mellitus | | | | | | |
| Yes vs no | 1.149 | 0.641-2.057 | 0.641 | | | |
| BMI, kg/m$^2$ | | | | | | |
| ≥25 vs <25 | 0.878 | 0.500-1.543 | 0.652 | | | |
| HBsAg (+) | | | | | | |
| Yes vs no | 0.997 | 0.239-4.156 | 0.996 | | | |
| $SVR_{12}$ by DAAs | | | | | | |
| No vs yes | 3.219 | 1.139-9.120 | **0.028** | 6.829 | 1.420-32.834 | **0.016** |
| $EOT_{12}$ ALBI grade | | | | | | |
| 2 + 3 vs 1 | 1.381 | 0.811-2.351 | 0.234 | | | |
| Hb, g/dL | | | | | | |
| <12 vs ≥12 | 0.798 | 0.418-1.526 | 0.495 | | | |
| PLT (×10$^9$/L) | | | | | | |
| <100 vs ≥100 | 1.522 | 0.839-2.761 | 0.167 | | | |
| PT | | | | | | |
| >12 vs ≤12 | 1.332 | 0.724-2.449 | 0.357 | | | |
| Albumin, g/dL | | | | | | |
| ≤3.5 vs >3.5 | 1.763 | 0.825-3.764 | 0.143 | | | |
| AST, U/L | | | | | | |
| ≥40 vs <40 | 1.252 | 0.625-2.506 | 0.526 | | | |
| ALT, U/L | | | | | | |
| ≥40 vs <40 | 2.036 | 0.987-4.199 | 0.054 | 0.984 | 0.371-2.613 | 0.974 |
| Bilirubin, mg/dL | | | | | | |
| ≥1.0 vs <1.0 | 1.510 | 0.856-2.662 | 0.155 | | | |
| Baseline AFP, ng/mL | | | | | | |
| >10 vs ≤10 | 0.985 | 0.554-1.750 | 0.958 | | | |
| $EOT_{12}$ AFP, ng/mL | | | | | | |
| >10 vs ≤10 | 2.934 | 1.160-5.896 | **0.003** | 2.339 | 1.057-5.172 | **0.036** |
| BCLC before DAAs | | | | | | |
| 0 | Ref | | | | | |
| A1 | 1.048 | 0.445-2.467 | 0.915 | | | |
| A2 | 1.366 | 0.476-3.916 | 0.562 | | | |
| A3 | 2.810 | 1.041-7.580 | **0.041** | | | |
| BCLC before DAAs | | | | | | |
| 0/A1/A2 | Ref | | | | | |
| A3 | 2.589 | 1.229-5.452 | **0.012** | 2.307 | 1.045-5.093 | **0.039** |
| First HCC treatment | | | | | | |
| TACE vs others | 1.610 | 0.755-3.435 | 0.218 | | | |
| Last HCC treatment | | | | | | |
| TACE vs others | 2.354 | 1.170-4.735 | **0.016** | | | |
| Previous recurrence | | | | | | |
| Yes vs no | 2.605 | 1.490-4.556 | **0.001** | 3.151 | 1.625-6.112 | **0.001** |
| Time interval from HCC | | | | | | |
| ≤1 vs >1 y | 1.023 | 0.581-1.803 | 0.937 | | | |
| CRR to DAA treatment | | | | | | |
| ≤0.5 vs >0.5 y | 0.864 | 0.495-1.506 | 0.605 | | | |

The bold value merely indicates that the p-value is statistically significant, but it is not necessary; it can also be presented without bold.

AFP = alpha-fetoprotein; ALBI = albumin-bilirubin; ALT = alanine aminotransferase; AST = aspartate aminotransferase; BCLC = Barcelona clinic liver cancer; BMI = body mass index; CRR = complete radiological response; DAAs = direct-acting antivirals; $EOT_{12}$ = 12 wk post end of DAAs treatment; EV = esophageal varices; GV = gastric varices; HBsAg = hepatitis B surface antigen; HCC = hepatocellular carcinoma; HCV = hepatitis C virus; Hgb = Hemoglobin; HR = hazard ratio; IFN = interferon; PLT = platelet; PT = prothrombin time; $SVR_{12}$ = sustained virological response; TACE = transcatheter arterial chemoembolization.

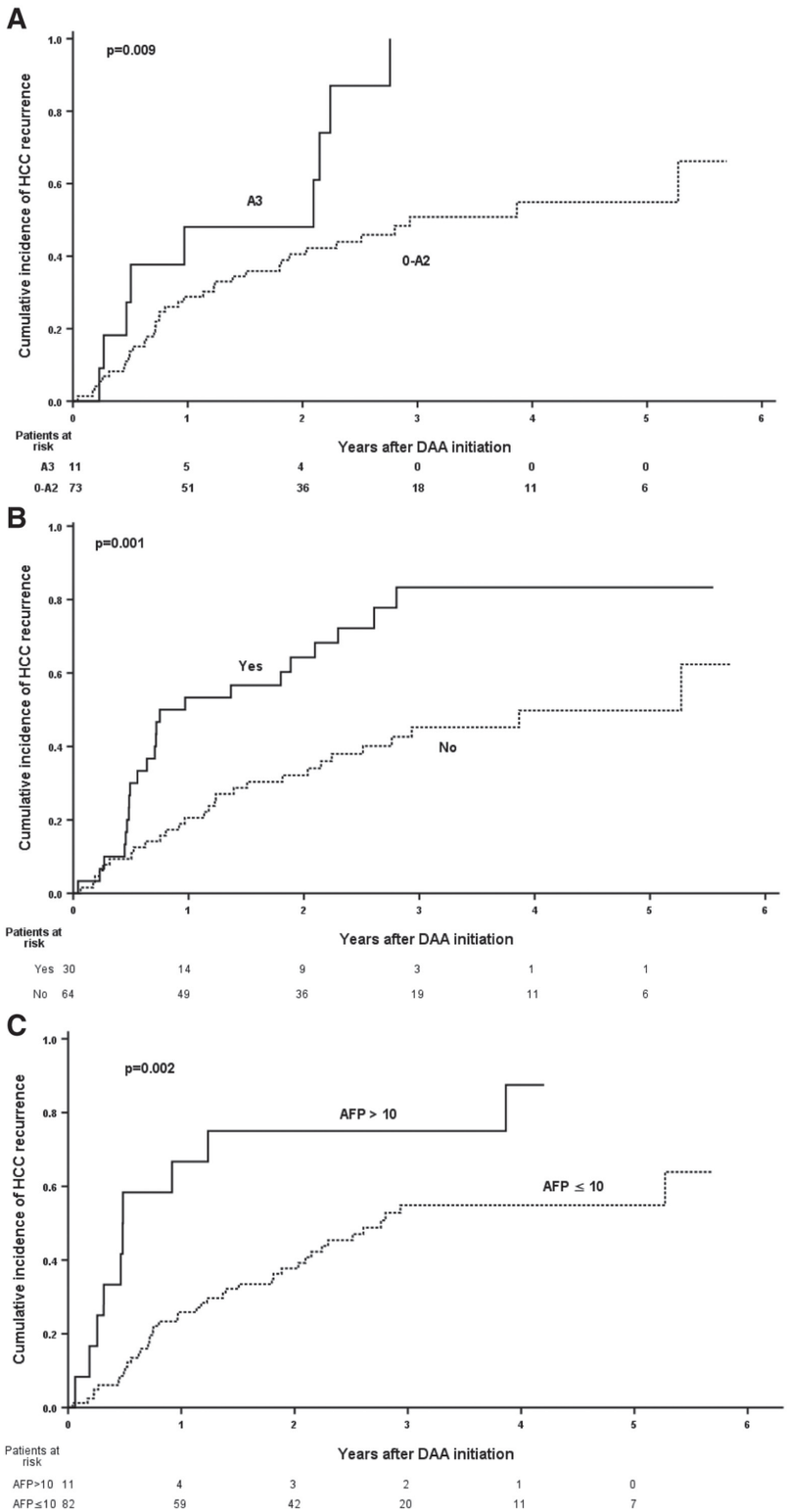


**Fig. 3** Comparison of cumulative rates of HCC recurrence by independent factors. A, The cumulative incidence of HCC recurrence according to BCLC stage: A3: 100% up to 2.8 y vs 0-A2: 66.2% (95% CI, 55.1-77.3) up to 5.7 y. B, The cumulative incidence of HCC recurrence according to previous HCC recurrence: yes: 83.3% (95% CI, 75.3-91.3) vs no: 62.3% (95% CI, 50.0-74.6) up to 5.7 y. C, The cumulative incidence of HCC recurrence according to $EOT_{12}$ AFP: >10 ng/mL: 87.5% (95% CI, 76.7-98.3) up to 4.2 y vs ≤10: 63.9% (95% CI, 54.4-73.4) up to 5.7 y. AFP = alpha-fetoprotein; BCLC = Barcelona clinic liver cancer; DAA = direct-acting antiviral; $EOT_{12}$ = 12 wk post end of DAAs treatment; HCC = hepatocellular carcinoma.

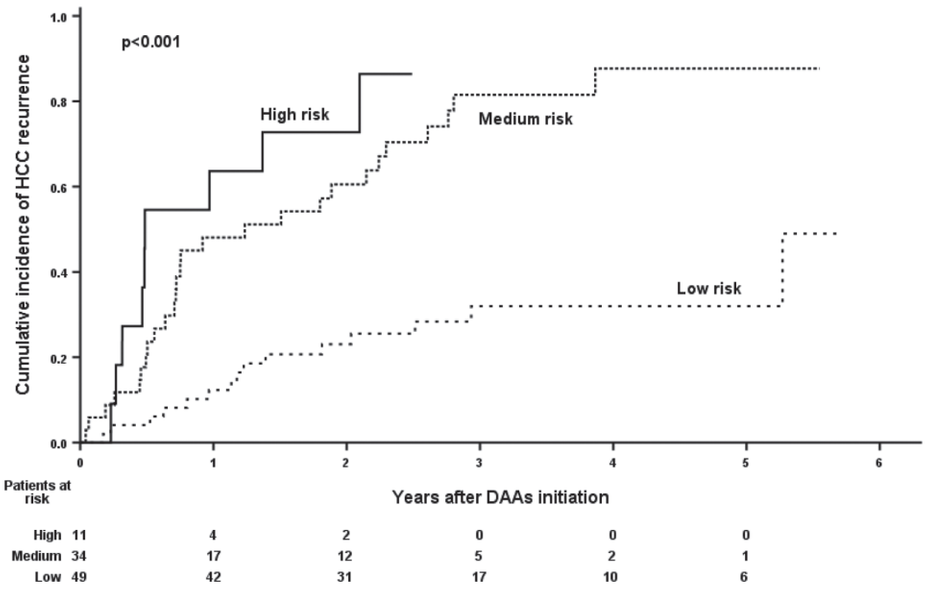


**Fig. 4** The cumulative incidence of HCC recurrence stratified by the scoring system: high risk (≥2): 86.4% (95% CI, 74.7-98.1) up to 2.49 y vs medium risk (1): 87.7% (95% CI, 80.8-94.6) vs low risk (0): 49.0% (95% CI, 33.3-64.7) up to 5.7 y. DAAs = direct-acting antivirals; HCC = hepatocellular carcinoma.

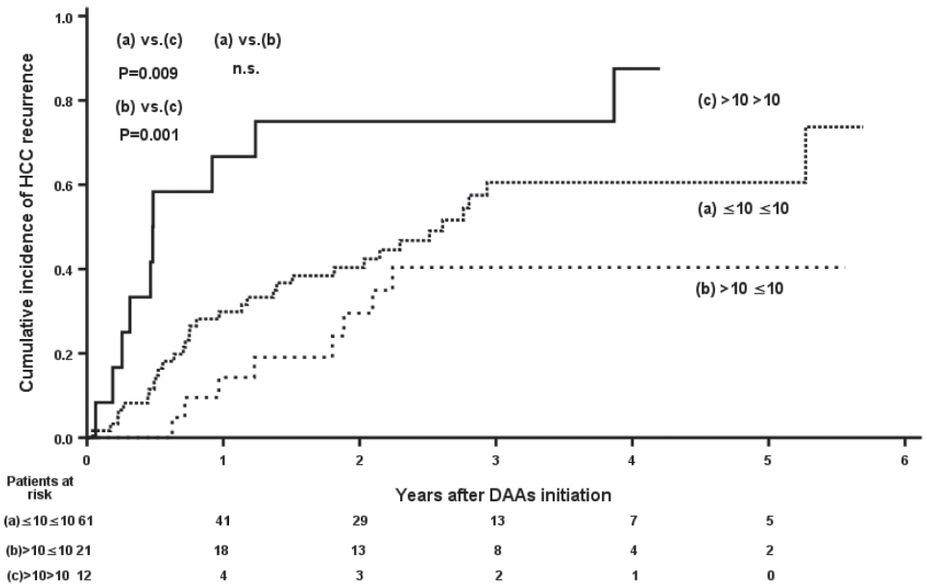


**Fig. 5** The dynamic changes in AFP level before and after DAA therapy and the predictive value on the recurrence of HCC. Patients with persistently high AFP values (>10 ng/mL) before and after DAA treatment were associated with the highest risk of HCC recurrence. AFP = alpha-fetoprotein; DAA = direct-acting antiviral; HCC = hepatocellular carcinoma.

and patients have high hopes for DAAs in terms of reducing the incidence and recurrence rates of HCC, and the number of related clinical studies continues to increase. However, results about the impact of DAAs on tumor recurrence in patients with HCV-HCC post-curative treatment have been conflicting.[13–22] This may be due to heterogeneity and discrepancies among the previous studies, including different study designs and patient populations, tumor characteristics, the absence of a control group, and inconsistent follow-up times. Several meta-analyses have reported significant benefits from DAA therapy in reducing recurrence and improving overall survival of HCV-related HCC patients compared with no intervention.[23,37]

Several studies have suggested that patients with active HCC are more resistant to DAAs,[38,39] and poor penetration of DAAs caused by HCC or previous HCC treatment and impaired immunological control have been proposed as possible mechanisms.[40–43] However, a previous study reported that the efficacy of DAA treatment was not impaired in a cohort of cirrhotic patients with successfully treated HCC.[44] Concordantly, the overall $SVR_{12}$ rate in the current study was high (95.7%), suggesting that a satisfactory response can be achieved with DAA treatment for CHC patients with successfully treated HCC. In the current study, the $SVR_{12}$ rate was 100% in the patients who received the more potent pan-genotype regimen, and all four

patients who did not achieve $SVR_{12}$ received a genotype-specific regimen, indicating that the selection of the DAA regimen may be an important consideration. The difference in treatment success rate between the two regimen groups did not reach significance, however this may have been due to the substantial disparity in the number of patients in the two groups, as well as the limited occurrence of treatment failure.

Many studies have reported the beneficial effects of DAAs on long-term outcomes in CHC patients with successfully treated HCC.[22,45–47] For example, a prospective study by Cabibbo et al[45] demonstrated that $SVR_{12}$ after DAA treatment was associated with significantly reduced HCC recurrence (HR = 0.25, 95% CI, 0.11-0.57, $p < 0.001$), hepatic decompensation (HR = 0.12, 95% CI, 0.02-0.38, $p = 0.02$) and mortality (HR = 0.02, 95% CI, 0.00-0.19, $p < 0.001$). In line with these findings, all four patients who did not achieve $SVR_{12}$ in our study experienced HCC recurrence during follow-up, and not achieving $SVR_{12}$ after DAA treatment was an independent factor contributing to tumor recurrence. Although we cannot draw definitive conclusions about the effect of reducing recurrence based on our findings alone, achieving $SVR_{12}$ in successfully treated HCC-CHC patients in the early stage of liver fibrosis may suggest favorable long-term outcomes, as also supported by other recent studies.[48,49] The improvement in functional liver reserve after successful DAA therapy allows patients the opportunity to receive further antitumor therapy, leading to improved overall survival. Concordantly, a meta-analysis by Liu et al[37] focusing on the role of DAAs in patients with HCV-HCC post antitumor therapy with CRR concluded that compared with non-responders, patients treated with DAAs with $SVR_{12}$ had a greater reduction in tumor recurrence (HR = 0.37, 95% CI, 0.16-0.84, $p = 0.017$) and better overall survival (HR = 0.17, 95% CI, 0.06-0.50, $p = 0.001$).

The appropriate timing of initiating DAA treatment in patients with HCV-related HCC who have undergone antitumor therapy is also a crucial topic. Concerns about the benefits of DAAs in patients with HCC were raised by Reig et al,[13] who observed a surprisingly high rate of early HCC recurrence after post-curative DAA therapy. To further investigate this issue, Tsai et al[50] reanalyzed the data and found that the recurrence rate was significantly higher in patients with a time lag of within 4 months (54.6%) compared to those with a time lag of more than 4 months (21.3%). In addition, in a large multicenter study, Singal et al[20] compared patients treated with DAAs to untreated controls, and suggested that tumor recurrence could be associated with the timing of DAA therapy. Specifically, they reported that HCC recurred less in patients who delayed DAA treatment by more than 6 months after CRR (HR = 0.56, 95% CI, 0.22-1.38), although this difference was not statistically significant. In addition, several studies have reported that patients treated with DAAs <12 months following HCC treatment had higher HCC recurrence rates.[51,52] It is possible that studies showing high HCC recurrence rates in patients treated with DAAs shortly after HCC treatment may have been affected by selection bias, as they may have failed to detect small HCC nodules due to incomplete treatment before initiating DAAs. In the current study, the median time from the last HCC treatment with CRR to initiating DAA treatment was 7.8 months. To minimize selection bias, we only enrolled patients with early-stage (BCLC 0/A) HCC with confirmed CRR status through dynamic CT/MRI. In addition, we excluded patients with non-characterized nodules within 3 months before or after starting DAA therapy. With these selection strategies, we found that the time lag from CRR to DAA treatment was not an independent factor for HCC recurrence posttreatment. However, given the opportunity to achieve long-term preservation of liver function with DAA therapy, patients in clinical practice may be reluctant to delay starting treatment for 6 to 12 months to verify the treatment response after HCC is treated with curative intent.

Several mechanisms for intrahepatic HCC recurrence after curative treatment have been proposed. HCC recurrence has been classified as "early" or "late" in previous studies, with a 2-year cutoff from treatment. "Early recurrence" has been suggested to be related to primary tumor characteristics including microscopic metastases, whereas "late recurrence" is thought to be driven by the underlying liver cirrhosis and its carcinogenic properties.[53] In our study, the factors independently associated with HCC recurrence, apart from achieving $SVR_{12}$, were a history of previous HCC recurrence, $EOT_{12}$ AFP level >10 ng/mL, and multi-nodularity (BCLC A3 lesions), and the median follow-up period after initiating DAA treatment was 22.1 months. Based on the relatively short follow-up period, it is conceivable that risk factors for recurrent HCC are closely related to baseline tumor characteristics and microscopic metastatic lesions. Our findings suggest that heterogeneity exists even among patients classified as BCLC stage A, and that multi-nodularity may be a significant determinant of HCC recurrence after DAA treatment. The clinical significance of multi-nodularity has been reported in several previous studies.[52,54] In line with other reports,[17,26,27,54] we also found that prior HCC recurrence was associated with HCC recurrence after DAA treatment, which is biologically reasonable as it reflects the aggressiveness of tumor behavior in patients with a history of recurrence. Large-scale studies with long-term follow-up are warranted to investigate the impact of different severities of fibrosis, cirrhosis, or ALBI grade on HCC recurrence, especially at more than 2 years after DAA therapy.

AFP is the most commonly used diagnostic marker for HCC. In our study, pretreatment AFP level did not appear to be a significant risk factor for HCC recurrence. However, an $EOT_{12}$ AFP level >10 ng/mL was independently associated with tumor recurrence post-DAA treatment. Similarly, previous studies have suggested that AFP level at the completion of antiviral therapy may be an important predictor of future HCC recurrence.[25,27,55] A crucial finding in our study is that the dynamic changes in AFP values before and after DAA therapy were strongly correlated with HCC recurrence. Interestingly, the patients whose AFP values remained persistently high (>10 ng/mL) before and after DAA treatment had the highest risk of HCC recurrence. Conversely, the patients whose AFP levels declined from high (>10 ng/mL) to low (≤10 ng/mL) after DAA treatment had the lowest incidence of HCC recurrence. It is possible that DAA treatment can reduce the elevated AFP levels attributed to the hepatitis activity of CHC, making the monitoring of carcinogenic properties easier. Thus, we suggest that measuring AFP levels before and after DAA treatment can serve as a useful tool for monitoring HCC recurrence after DAA treatment. However, of note, 42 (44.7%) patients in the current study who developed HCC had consistently low (<10 ng/mL) AFP levels during the study period. Therefore, incorporating alternative tumor markers such as protein induced by vitamin K absence-II (PIVKA-II) in the analysis may be helpful to improve predictive accuracy, although further studies are warranted to verify this hypothesis.

Our results showed that the probability of tumor recurrence remained high in the included patients, all of whom had early-stage HCV-HCC (BCLC stage 0/A) with CRR after treatment with DAAs. The cumulative incidence of HCC recurrence after initiating DAA treatment was 31.1% at 1 year, 42.5% at 2 years, 57.3% at 3 years, 60.6% at 4 years, and 68.5% at up to 5.7 years. These rates are even higher than during the IFN era, which may be because older, comorbidity-prone, and advanced liver disease patients are more likely to be treated with DAAs.

As the pathogenesis of recurrent HCC may be multifactorial, the best approach for risk stratification is to combine independent risk factors into an algorithm. Our proposed risk stratification system included four independent factors: previous tumor recurrence before DAA treatment, failure to achieve $SVR_{12}$, $EOT_{12}$ AFP >10 ng/mL, and multiple tumor nodules (BCLC A3 lesions). The cumulative rate of HCC recurrence was high (1 year: 63.6%, 2 years: 72.9%, and 86.4% up to 2.49 years) in the patients with at least two risk factors. In comparison, the 1-, 2-, 3-, 4-, and 5-year incidence rates of HCC recurrence in the patients without any of these four risk factors were 12.3%, 23.0%, 32.0%, 32.0%, and 32.0%, respectively. The identification of risk factors for HCC recurrence is mandatory to define which patient subgroups may benefit less from DAA treatment. Moreover, more intensive (every 3-4 months) monitoring of HCC recurrence using dynamic studies such as CT/MRI should be arranged for patients with risk factors.

There are several limitations to this study. First, its retrospective design and the inclusion of data from only one tertiary center in Taiwan may limit the generalizability of the findings. Second, the sample size was not large, as we aimed to have a more homogeneous study population with verified CRR status. Third, the median follow-up time after DAA treatment was approximately 22 months, making it challenging to identify risk factors for late recurrence of HCC post-DAA treatment. Fourth, even though our analysis included numerous factors, unmeasured or unmeasurable factors could still potentially have impacted our results.

In conclusion, our real-world data showed that achieving $SVR_{12}$ post-DAA treatment was significantly associated with a reduction in tumor recurrence in CHC patients with early HCC who had been successfully treated and achieved CRR. However, even with a high $SVR_{12}$ rate, HCC recurrence after DAA treatment was relatively common. Based on a median follow-up period of about 22 months after DAA treatment, we identified that previous recurrence, multinodularity, and $EOT_{12}$ AFP >10 ng/mL or dynamic AFP changes were major risk factors for HCC recurrence. The valuable insights gained from our study can be used to stratify the risk of HCC recurrence and guide the development of individualized surveillance protocols.

## ACKNOWLEDGMENTS

This study was supported in part by Taipei Veterans General Hospital (V105C-068), Szu-Yuan Research Foundation of Internal Medicine (108-033, 109-020, 110-016, 111-008, and 112-017), and Research Foundation of Digestive Medicine (112-07).

We thank Miss Yu-Ling Chou and Miss Chiao-Yu Chang for patient follow-up and clinical data collection.

## REFERENCES

1. El-Serag HB. Hepatocellular carcinoma. *N Engl J Med* 2011;365:1118–27.
2. Lemon SM, McGivern DR. Is hepatitis C virus carcinogenic? *Gastroenterology* 2012;142:1274–8.
3. Vescovo T, Refolo G, Vitagliano G, Fimia GM, Piacentini M. Molecular mechanisms of hepatitis C virus-induced hepatocellular carcinoma. *Clin Microbiol Infect* 2016;22:853–61.
4. Westbrook RH, Dusheiko G. Natural history of hepatitis C. *J Hepatol* 2014;61(Suppl 1):S58–68.
5. Hasegawa K, Kokudo N, Makuuchi M, Izumi N, Ichida T, Kudo M, et al. Comparison of resection and ablation for hepatocellular carcinoma: a cohort study based on a Japanese nationwide survey. *J Hepatol* 2013;58:724–9.
6. Roayaie S, Obeidat K, Sposito C, Mariani L, Bhoori S, Pellegrinelli A, et al. Resection of hepatocellular cancer ≤2 cm: results from two Western centers. *Hepatology* 2013;57:1426–35.
7. Tampaki M, Papatheodoridis GV, Cholongitas E. Intrahepatic recurrence of hepatocellular carcinoma after resection: an update. *Clin J Gastroenterol* 2021;14:699–713.
8. Morgan RL, Baack B, Smith BD, Yartel A, Pitasi M, Falck-Ytter Y. Eradication of hepatitis C virus infection and the development of hepatocellular carcinoma: a meta-analysis of observational studies. *Ann Intern Med* 2013;158:329–37.
9. Manns M, Samuel D, Gane EJ, Mutimer D, McCaughan G, Buti M, et al; SOLAR-2 Investigators. Ledipasvir and sofosbuvir plus ribavirin in patients with genotype 1 or 4 hepatitis C virus infection and advanced liver disease: a multicentre, open-label, randomised, phase 2 trial. *Lancet Infect Dis* 2016;16:685–97.
10. Charlton M, Everson GT, Flamm SL, Kumar P, Landis C, Brown RS, Jr, et al; SOLAR-1 Investigators. Ledipasvir and sofosbuvir plus ribavirin for treatment of HCV infection in patients with advanced liver disease. *Gastroenterology* 2015;149:649–59.
11. Curry MP, O'Leary JG, Bzowej N, Muir AJ, Korenblat KM, Fenkel JM, et al; ASTRAL-4 Investigators. Sofosbuvir and velpatasvir for HCV in patients with decompensated cirrhosis. *N Engl J Med* 2015;373:2618–28.
12. Su PS, Wu SH, Chu CJ, Su CW, Lin CC, Lee SD, et al. Sofosbuvir-based antiviral therapy provided highly treatment efficacy, safety, and good tolerability for Taiwanese chronic hepatitis C patients with decompensated cirrhosis. *J Chin Med Assoc* 2022;85:152–9.
13. Reig M, Mariño Z, Perelló C, Iñarrairaegui M, Ribeiro A, Lens S, et al. Unexpected high rate of early tumor recurrence in patients with HCV-related HCC undergoing interferon-free therapy. *J Hepatol* 2016;65:719–26.
14. 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. *J Hepatol* 2016;65:727–33.
15. ANRS Collaborative Study Group on Hepatocellular Carcinoma (ANRS CO22 HEPATHER, CO12 CirVir and CO23 CUPILT Cohorts). Lack of evidence of an effect of direct-acting antivirals on the recurrence of hepatocellular carcinoma: data from three ANRS cohorts. *J Hepatol* 2016;65:734–40.
16. Nagata H, Nakagawa M, Asahina Y, Sato A, Asano Y, Tsunoda T, et al; Ochanomizu Liver Conference Study Group. Effect of interferon-based and -free therapy on early occurrence and recurrence of hepatocellular carcinoma in chronic hepatitis C. *J Hepatol* 2017;67:933–9.
17. Cabibbo G, Petta S, Calvaruso V, Cacciola I, Cannavò MR, Madonia S, et al; Rete Sicilia Selezione Terapia-HCV (RESIST-HCV). Is early recurrence of hepatocellular carcinoma in HCV cirrhotic patients affected by treatment with direct-acting antivirals? *Aliment Pharmacol Ther* 2017;46:688–95.
18. Waziry R, Hajarizadeh B, Grebely J, Amin J, Law M, Danta M, et al. Hepatocellular carcinoma risk following direct-acting antiviral HCV therapy: a systematic review, meta-analyses, and meta-regression. *J Hepatol* 2017;67:1204–12.
19. Ikeda K, Kawamura Y, Kobayashi M, Kominami Y, Fujiyama S, Sezaki H, et al. Direct-acting antivirals decreased tumor recurrence after initial treatment of hepatitis C virus-related hepatocellular carcinoma. *Dig Dis Sci* 2017;62:2932–42.
20. Singal AG, Rich NE, Mehta N, Branch A, Pillai A, Hoteit M, et al. Direct-acting antiviral therapy not associated with recurrence of hepatocellular carcinoma in a multicenter North American cohort study. *Gastroenterology* 2019;156:1683–92.e1.
21. Ochi H, Hiraoka A, Hirooka M, Koizumi Y, Amano M, Azemoto N, et al. Direct-acting antivirals improve survival and recurrence rates after treatment of hepatocellular carcinoma within the Milan criteria. *J Gastroenterol* 2021;56:90–100.
22. Wu KC, Lee IC, Chi CT, Lei HJ, Chau GY, Yeh YC, et al. Comparable benefits of HCV eradication by direct acting antivirals and interferon-based therapy in patients with hepatocellular carcinoma undergoing surgical resection. *Am J Cancer Res* 2021;11:5526–42.
23. Lui FH, Moosvi Z, Patel A, Hussain S, Duong A, Duong J, et al. Decreased risk of hepatocellular carcinoma recurrence with direct-acting antivirals compared with no treatment for hepatitis C: a meta-analysis. *Ann Gastroenterol* 2020;33:293–8.
24. El Kassas M, Funk AL, Salaheldin M, Shimakawa Y, Eltabbakh M, Jean K, et al. Increased recurrence rates of hepatocellular carcinoma after DAA therapy in a hepatitis C infected Egyptian cohort: a comparative analysis. *J Viral Hepat* 2018;25:623–30.
25. Mashiba T, Joko K, Kurosaki M, Ochi H, Osaki Y, Kojima Y, et al. Does interferon-free direct-acting antiviral therapy for hepatitis C after curative treatment for hepatocellular carcinoma lead to unexpected recurrences of HCC? *PLoS One* 2018;13:e0194704.

26. Nakano M, Koga H, Ide T, Kuromatsu R, Hashimoto S, Yatsuhashi H, et al. Predictors of hepatocellular carcinoma recurrence associated with the use of direct-acting antiviral agent therapy for hepatitis C virus after curative treatment: a prospective multicenter cohort study. *Cancer Med* 2019;8:2646–53.
27. Ogawa E, Nakamuta M, Furusyo N, Kajiwara E, Dohmen K, Kawano A, et al; Kyushu University Liver Disease Study (KULDS) Group. Long-term assessment of recurrence of hepatocellular carcinoma in patients with chronic hepatitis C after viral cure by direct-acting antivirals. *J Gastroenterol Hepatol* 2022;37:190–9.
28. Marrero JA, Kulik LM, Sirlin CB, Zhu AX, Finn RS, Abecassis MM, et al. Diagnosis, staging, and management of hepatocellular carcinoma: 2018 practice guidance by the American Association for the Study of Liver Diseases. *Hepatology* 2018;68:723–50.
29. Reig M, Forner A, Rimola J, Ferrer-Fàbrega J, Burrel M, Garcia-Criado A, et al. BCLC strategy for prognosis prediction and treatment recommendation: the 2022 update. *J Hepatol* 2022;76:681–93.
30. Lencioni R, Llovet JM. Modified RECIST (mRECIST) assessment for hepatocellular carcinoma. *Semin Liver Dis* 2010;30:52–60.
31. European Association for the Study of the Liver. EASL recommendations on treatment of hepatitis C 2018. *J Hepatol* 2018;69:461–511.
32. AASLD-IDSA HCV Guidance Panel. Hepatitis C Guidance 2018 Update: AASLD-IDSA recommendations for testing, managing, and treating hepatitis C virus infection. *Clin Infect Dis* 2018;67:1477–92.
33. Garrison KL, German P, Mogalian E, Mathias A. The drug-drug interaction potential of antiviral agents for the treatment of chronic hepatitis C infection. *Drug Metab Dispos* 2018;46:1212–25.
34. Vallet-Pichard A, Mallet V, Nalpas B, Verkarre V, Nalpas A, Dhalluin-Venier V, et al. FIB-4: an inexpensive and accurate marker of fibrosis in HCV infection comparison with liver biopsy and fibrotest. *Hepatology* 2007;46:32–6.
35. Toyoda H, Johnson PJ. The ALBI score: from liver function in patients with HCC to a general measure of liver function. *JHEP Rep* 2022;4:100557.
36. Calvaruso V, Craxì A. Hepatic benefits of HCV cure. *J Hepatol* 2020;73:1548–56.
37. Liu H, Yang XL, Dong ZR, Chen ZQ, Hong JG, Wang DX, et al. Clinical benefits of direct-acting antivirals therapy in hepatitis C virus patients with hepatocellular carcinoma: a systematic review and meta-analysis. *J Gastroenterol Hepatol* 2022;37:1654–65.
38. Beste LA, Green PK, Berry K, Kogut MJ, Allison SK, Ioannou GN. Effectiveness of hepatitis C antiviral treatment in a USA cohort of veteran patients with hepatocellular carcinoma. *J Hepatol* 2017;67:32–9.
39. Prenner SB, VanWagner LB, Flamm SL, Salem R, Lewandowski RJ, Kulik L. Hepatocellular carcinoma decreases the chance of successful hepatitis C virus therapy with direct-acting antivirals. *J Hepatol* 2017;66:1173–81.
40. Soria A, Fabbiani M, Lapadula G, Gori A. Unexpected viral relapses in hepatitis C virus-infected patients diagnosed with hepatocellular carcinoma during treatment with direct-acting antivirals. *Hepatology* 2017;66:992–4.
41. Furihata T, Matsumoto S, Fu Z, Tsubota A, Sun Y, Matsumoto S, et al. Different interaction profiles of direct-acting anti-hepatitis C virus agents with human organic anion transporting polypeptides. *Antimicrob Agents Chemother* 2014;58:4555–64.
42. Wlcek K, Svoboda M, Riha J, Zakaria S, Olszewski U, Dvorak Z, et al. The analysis of organic anion transporting polypeptide (OATP) mRNA and protein patterns in primary and metastatic liver cancer. *Cancer Biol Ther* 2011;11:801–11.
43. Riaz A, Awais R, Salem R. Side effects of yttrium-90 radioembolization. *Front Oncol* 2014;4:198.
44. Persico M, Aglitti A, Aghemo A, Rendina A, Lleo A, Ciancio A, et al. High efficacy of direct-acting anti-viral agents in hepatitis C virus-infected cirrhotic patients with successfully treated hepatocellular carcinoma. *Aliment Pharmacol Ther* 2018;47:1705–12.
45. Cabibbo G, Celsa C, Calvaruso V, Petta S, Cacciola I, Cannavò MR, et al. Direct-acting antivirals after successful treatment of early hepatocellular carcinoma improve survival in HCV-cirrhotic patients. *J Hepatol* 2019;71:265–73.
46. Watanabe T, Tokumoto Y, Joko K, Michitaka K, Horiike N, Tanaka Y, et al. AFP and eGFR are related to early and late recurrence of HCC following antiviral therapy. *BMC Cancer* 2021;21:699.
47. Lleo A, Aglitti A, Aghemo A, Maisonneuve P, Bruno S, Persico M; Collaborators. Predictors of hepatocellular carcinoma in HCV cirrhotic patients treated with direct acting antivirals. *Dig Liver Dis* 2019;51:310–7.
48. Kuromatsu R, Ide T, Okamura S, Noda Y, Kamachi N, Nakano M, et al. Hepatitis C virus elimination using direct acting antivirals after the radical cure of hepatocellular carcinoma suppresses the recurrence of the cancer. *Cancers* 2022;14:2295.
49. Ahn YH, Lee H, Han JE, Cho HJ, Cheong JY, Park B, et al. Effect of direct-acting antivirals for hepatitis C virus-related hepatocellular carcinoma recurrence and death after curative treatment. *J Liver Cancer* 2022;22:125–35.
50. Tsai PC, Huang CF, Yu ML. Unexpected early tumor recurrence in patients with hepatitis C virus-related hepatocellular carcinoma undergoing interferon-free therapy: issue of the interval between HCC treatment and antiviral therapy. *J Hepatol* 2017;66:464.
51. Ahn YH, Lee H, Kim DY, Lee HW, Yu SJ, Cho YY, et al. Independent risk factors for hepatocellular carcinoma recurrence after direct-acting antiviral therapy in patients with chronic hepatitis C. *Gut Liver* 2021;15:410–9.
52. Ogawa E, Furusyo N, Nomura H, Dohmen K, Higashi N, Takahashi K, et al; Kyushu University Liver Disease Study (KULDS) Group. Short-term risk of hepatocellular carcinoma after hepatitis C virus eradication following direct-acting anti-viral treatment. *Aliment Pharmacol Ther* 2018;47:104–13.
53. Gao X, Zhan M, Wang L, Ding Y, Niu J. Timing of DAA initiation after curative treatment and its relationship with the recurrence of HCV-related HCC. *J Hepatocell Carcinoma* 2020;7:347–60.
54. Sapena V, Enea M, Torres F, Celsa C, Rios J, Rizzo GEM, et al. Hepatocellular carcinoma recurrence after direct-acting antiviral therapy: an individual patient data meta-analysis. *Gut* 2022;71:593–604.
55. Tani J, Senoh T, Moriya A, Ogawa C, Deguchi A, Sakamoto T, et al. Long-term outcomes and evaluation of hepatocellular carcinoma recurrence after hepatitis C virus eradication by direct-acting antiviral treatment: all Kagawa liver disease group (AKLDG) study. *Cancers (Basel)* 2021;13:2257.