Accepted Manuscript

Incidence of Hepatocellular Carcinoma in Patients with HCV-associated Cirrhosis Treated with Direct-Acting Antiviral Agents

Vincenza Calvaruso, Giuseppe Cabibbo, Irene Cacciola, Salvatore Petta, Salvatore Madonia, Alessandro Bellia, Fabio Tinè, Marco Distefano, Anna Licata, Lydia Giannitrapani, Tullio Prestileo, Giovanni Mazzola, Maria Antonietta Di Rosolini, Licia Larocca, Gaetano Bertino, Antonio Digiacomo, Francesco Benanti, Luigi Guarneri, Alfonso Averna, Carmelo Iacobello, Antonio Magro, Ignazio Scalisi, Fabio Cartabellotta, Francesca Savalli, Marco Barbara, Antonio Davì, Maurizio Russello, Gaetano Scifo, Giovanni Squadrito, Calogero Cammà, Giovanni Raimondo, Antonio Craxì, Vito Di Marco

PII: S0016-5085(18)30441-4
DOI: 10.1053/j.gastro.2018.04.008
Reference: YGAST 61836

To appear in: *Gastroenterology*
Accepted Date: 8 April 2018

Please cite this article as: Calvaruso V, Cabibbo G, Cacciola I, Petta S, Madonia S, Bellia A, Tinè F, Distefano M, Licata A, Giannitrapani L, Prestileo T, Mazzola G, Di Rosolini MA, Larocca L, Bertino G, Digiacomo A, Benanti F, Guarneri L, Averna A, Iacobello C, Magro A, Scalisi I, Cartabellotta F, Savalli F, Barbara M, Davì A, Russello M, Scifo G, Squadrito G, Cammà C, Raimondo G, Craxì A, Di Marco V, on behalf of Rete Sicilia Selezione Terapia - HCV (RESIST-HCV), Incidence of Hepatocellular Carcinoma in Patients with HCV-associated Cirrhosis Treated with Direct-Acting Antiviral Agents, *Gastroenterology* (2018), doi: 10.1053/j.gastro.2018.04.008.

TITLE: Incidence of Hepatocellular Carcinoma in Patients with HCV-associated Cirrhosis Treated with Direct-Acting Antiviral Agents

Short Title: HCC occurrence in HCV cirrhosis treated with DAA

AUTHORS: Vincenza Calvaruso[1], Giuseppe Cabibbo[1], Irene Cacciola[2], Salvatore Petta[1], Salvatore Madonia[3], Alessandro Bellia[4], Fabio Tinè[5], Marco Distefano[6], Anna Licata[7], Lydia Giannitrapani[7], Tullio Prestileo[8], Giovanni Mazzola[9], Maria Antonietta Di Rosolini[10], Licia Larocca[11], Gaetano Bertino[12], Antonio Digiacomo[13], Francesco Benanti [14], Luigi Guarneri[15], Alfonso Averna[16], Carmelo Iacobello[17], Antonio Magro[18], Ignazio Scalisi[19], Fabio Cartabellotta[20], Francesca Savalli[21], Marco Barbara[1], Antonio Davì[10], Maurizio Russello[4], Gaetano Scifo[6], Giovanni Squadrito[2], Calogero Cammà[1], Giovanni Raimondo[2], Antonio Craxì[1], Vito Di Marco[1] on behalf of Rete Sicilia Selezione Terapia - HCV (RESIST-HCV)

INSTITUTIONS: [1]Sezione di Gastroenterologia e Epatologia, Dipartimento Biomedico di Medicina Interna e Specialistica (Di.Bi.M.I.S.), University of Palermo; [2]UOC Epatologia Clinica e Biomolecolare; AOUP G. Martino, Dipartimento di Medicina Interna e Sperimentale, University of Messina; [3]UOC Medicina Interna, AO Villa Sofia-Cervello, Palermo; [4]UOS Epatologia, ARNAS Garibaldi-Nesima, Catania; [5] UOC Gastroenterologia, AO Villa Sofia-Cervello, Palermo; [6]UOC Malattie Infettive, Ospedale Vittorio Emanuele di Siracusa, ASP Siracusa; [7]UOC Medicina Interna, AOUP Paolo Giaccone, Palermo; [8]UOC Malattie Infettive, ARNAS Civico-Di Cristina-Benefratelli, Palermo; [9]UOC Malattie Infettive, Azienda Ospedaliera Universitaria Paolo Giaccone, Palermo; [10]UOC Malattie Infettive, Ospedale di Modica; ASP Ragusa; [11]UOC Malattie infettive, AOUP G. Rodolico, Catania; [12]UOC Medicina Interna, AOUP G. Rodolico, Catania; [13]UOC Medicina Interna, Ospedale

di Comiso, ASP Ragusa; [14]UOC Malattie Infettive, ARNAS Garibaldi-Nesima, Catania; [15]UOC Malattie Infettive, Ospedale di Enna, ASP Enna; [16]UOC Malattie Infettive, Ospedale di Caltanissetta, ASP Caltanissetta; [17]UOC Malattie Infettive, AO Cannizzaro, Catania; [18]UOC Medicina Interna, Ospedale di Agrigento, ASP Agrigento; [19]UOC Medicina Interna, Ospedale di Mazzara del Vallo, ASP, Trapani; [20]UOC Medicina Interna, Ospedale Buccheri La Ferla, Palermo; [21]UOC Malattie Infettive, Ospedale di Trapani, ASP Trapani.

CORRESPONDING AUTHOR: Vito Di Marco, Sezione di Gastroenterologia & Epatologia, Dipartimento Biomedico di Medicina Interna e Specialistica, Università di Palermo, Piazza delle Cliniche 2, 90127 Palermo, Italy. E-mail: vito.dimarco@unipa.it

Word count: 4,366

Number of figures: 4

Number of supplementary figures: 4

Number of tables: 3.

Number of supplementary table: 5

ABBREVIATIONS:

HCC, Hepatocellular carcinoma; SVR, sustained virological response; HCV, hepatitis C Virus; DAA, Direct Antiviral Agents; US, Ultrasonography; EGS, Ephofagealgastroscopy.

Keywords: hepatitis C Virus (HCV), Cirrhosis, Direct Antiviral Agents (DAAs), Sustained Virological Response (SVR), Hepatocellular Carcinoma (HCC),

DISCLOSURES:

Marco Distefano: participated in advisory board for Abbvie. Gaetano Scifo: participated in advisory board for Abbvie. Vincenza Calvaruso: participated in advisory board for Abbvie. Salvatore Petta: participated in advisory board for Abbvie. Giuseppe Cabibbo: participated in advisory board for Bayer. Vito Di Marco: research support from Abbvie, BMS, Gilead,

Merck/MSD. Participated in advisory boards for Abbvie, BMS; MSD/Merck. Antonio Craxì: Research support from Abbvie, BMS, Gilead, Merck/MSD, Intercept, provided consultancy, speakers bureau and participated in advisory boards for Abbvie, BMS, Gilead, MSD/Merck. Giovanni Raimondo: Participated in advisory boards for Abbvie, BMS, Gilead, MSD/Merck. Calogero Cammà: participated in advisory board for MSD/Merck. The other authors have no disclosures to declare

FINANCIAL SUPPORT: The RESIST-HCV is funded by unrestricted grants from Gilead, MSD, Abbvie and BMS.

AUTHOR CONTRIBUTIONS: V.C. (analysis and interpretation of data; drafting of the manuscript; statistical analysis, critical revision of the manuscript for important intellectual content) G.C. (acquisition of data, critical revision of the manuscript for important intellectual content), I.C. (acquisition of data), S.P. (acquisition of data; critical revision of the manuscript for important intellectual content), S.M. (acquisition of data), A.B (acquisition of data), F.T. (acquisition of data), M.D. (acquisition of data), L.G. (acquisition of data), T.P. (acquisition of data), G.M. (acquisition of data) MR.D.R. (acquisition of data), L.L (acquisition of data), A.A. (acquisition of data), A.D. (acquisition of data) M.G. (acquisition of data), L.G. (acquisition of data), A.A (acquisition of data), C.I. (acquisition of data), A.M. (acquisition of data), I.S. (acquisition of data), F.C. (administrative, technical, or material support; acquisition of data), F.S. (acquisition of data), M.B. (statistical analysis), A.D. (acquisition of data), M.R. (acquisition of data), G.S. (acquisition of data), G.S. (acquisition of data), C.C. (study concept and design; analysis and interpretation of data; statistical analysis; critical revision of the manuscript for important intellectual content) G.R. (study concept and design; critical revision of the manuscript for important intellectual content) A.C. (study concept and design; analysis and interpretation of data;

critical revision of the manuscript for important intellectual content), V.DM. (study concept and design; analysis and interpretation of data; drafting of the manuscript; study supervision)

ABSTRACT

Background & Aims: Studies have produced conflicting results of the incidence of hepatocellular carcinoma (HCC) in patients with in hepatitis C virus (HCV)-associated cirrhosis treated with direct-acting antivirals (DAAs). Data from clinics are needed to accurately assess the occurrence rate of HCC in patients with cirrhosis in the real world.

Methods: We collected data from a large prospective study of 2249 consecutive patients (mean age, 65.4 years and 56.9% male) with HCV-associated cirrhosis (90.5% Child-Pugh class A, 9.5% Child-Pugh class B) treated with DAAs from March 2015 through July 2016 at 22 academic and community liver centers in Sicily, Italy. HCC occurrence was evaluated by Kaplan-Meier curves. Cox regression analysis was used to identify variables associated with HCC development.

Results: A sustained virologic response (SVR) was achieved by 2140 patients (95.2% total; 95.9% of Child Pugh class A and 88.3% of Child Pugh class B patients (*P*<.001). Seventy-eight patients (3.5%) developed HCC during a mean follow-up of 14 months (range, 6–24 months). At 1 year after DAA exposure, HCC developed in 2.1% of Child-Pugh class A patients with an SVR and 6.6% of patients with no SVR; HCC developed in 7.8% of Child-Pugh class B patients with an SVR and 12.4% of patients with no SVR (*P*<.001 by log-rank test). Albumin level below 3.5 g/dL (hazard ratio,1.77; 95% CI, 1.12–2.82; *P*=.015), platelets count below $120x10^9$/L (hazard ratio, 3.89; 95% CI, 2.11–7.15; *P*<.001), and absence of SVR (hazard ratio, 3.40; 95% CI,1.89–6.12; *P*<.001) were independently associated increased risk for HCC. The mean interval in time from DAA exposure to an HCC diagnosis was 9.8 months (range, 2–22 months) and did not differ significantly between patients with (n=64, 9.2 months) and without an SVR (n=14, 12.0 months) (*P*=.11). A higher proportion of patients with an SVR had a single HCC lesion

(78% vs 50% without an SVR; *P*=.009) or HCC lesion less than 3 cm (58% vs 28% without an SVR; *P*=.07).

Conclusions: In an analysis of data from a large prospective study of patients with HCV-associated compensated or decompensated cirrhosis, we found that SVR to DAA treatment to reduce the incidence of HCC over a mean follow-up of 14 months.

Keywords: RESIST-HCV, liver cancer risk, reduction, sofosbuvir

INTRODUCTION

Soon after its discovery, hepatitis C virus (HCV) was identified as an independent risk factor for the development of hepatocellular carcinoma (HCC), especially in patients with cirrhosis, (1). Older age, male gender, and active hepatic necro-inflammation were also widely acknowledged as independent risk factors for HCC, regardless of the etiology of chronic liver disease (2,3).

Because no regimen has a proven antifibrotic effect, only drugs acting on the etiology of liver damage can stop the progression of liver fibrosis and, thus, indirectly reduce the risk of hepatic decompensation and of HCC (4). Etiological treatment of patients with chronic HCV infection has progressed massively since the days of IFN-based regimens, in terms of effective viral clearance and of applicability. Until 2015, IFN-based therapy had limited indications; its effectiveness varied according to degree of fibrosis, stage of liver disease, viral genotype, and presence of co-morbidity (5,6). Despite these limitations, large cohorts of patients with compensated cirrhosis treated with IFN-based regimens and followed-up over the long term showed substantially reduced rates of disease decompensation, HCC incidence, and mortality, when a sustained virological response (SVR) was obtained (7-10). Unfortunately, the low SVR rate in these cohorts (<40%) and the limited applicability of IFN-based therapy for patients with advanced or decompensated liver disease reduced the relevance of these outcomes.

New regimens with direct-acting antiviral (DAA) agents have changed both the spectrum and the scope of treatment, all patients being amenable to viral eradication at any stage of liver disease. SVR rates to currently available combinations exceed 95% in real-life practice. Based on the results of trials (11,12), the U.S. Food and Drug Administration (FDA) and European Medicines Agency (EMA) approved DAA for all patients with chronic HCV liver disease, regardless of age, disease stage, and viral

genotypes. Practice guidelines from AASLD (13) and EASL (14) recommend treating patients with compensated or decompensated cirrhosis with priority in order to reduce short-term complications.

While a reduction of deaths due to improved liver function is reported in a retrospective analysis of a large cohort of patients with advanced liver fibrosis treated with DAA (15), data on the risk and modality of HCC occurrence in patients with cirrhosis are discordant (16-20); the first observational studies triggered a debate on the early incidence of HCC (21,22). This uncertainty could affect clinical practice; hence the safety of therapy in cirrhosis patients needs to be assessed in extensive, prospective, real-life cohort studies.

We report the outcome of a large prospective cohort of patients with HCV cirrhosis and no previous diagnosis of HCC, in order to assess the *de novo* occurrence of HCC, to identify risk factors associated with its occurrence, and to characterize the presentation of HCC according to HCV response to DAA.

PATIENTS AND METHODS

RESIST-HCV (Rete Sicilia Selezione Terapia – HCV) is a web-based regional database that includes data on all patients with HCV liver disease treated with DAA since March 2015 in 22 academic and community liver centres in Sicily. Registration in the RESIST HCV network and recording of baseline data are mandatory to for prescription of treatment. The database includes information on reported criteria for diagnosis, staging of liver disease, baseline virological evaluation, DAA regimens, adverse events (AEs), and treatment outcomes.

The database records the timing of virological and instrumental tests (US and EGS) performed before the start of DAA, as well as virological and clinical outcomes observed

during treatment and follow-up. Patients are considered drop-outs if they did not have SVR or did not present for at least 2 consecutive clinical controls.

According to Agenzia Italiana del Farmaco (AIFA) criteria, the diagnosis of cirrhosis is established if at least one of the following clinical characteristics is present: previous liver biopsy with stage 4 fibrosis (by METAVIR score), presence of esophageal and/or gastric varices at esophageal gastroscopy (EGS), liver stiffness >12 KPa (by Fibroscan), platelet count <100 x 10^9/L. Child-Pugh score was used to indicate functional class of cirrhosis.

DAA regimens available for treatment of cirrhosis from March 2015 to March 2017 were: Sofosbuvir (SOF) + Ribavirin (RBV); Sofosbuvir plus Ledipasvir (SOF/LDV) ± RBV; Ombitasvir/Paritaprevir/Ritonavir (OBV/PTV/r) + Dasabuvir (DSV) ± RBV; Ombitasvir/Paritaprevir/Ritonavir (OBV/PTV/r) ± RBV; Sofosbuvir plus Daclatasvir (SOF/DCL) ± RBV; Sofosbuvir + Simeprevir (SOF/SIM) ± RBV. Physicians at each centre included in RESIST-HCV established the patient's treatment regimen and use of ribavirin.

Regional Heath Authority required a control of serum HCV-RNA 12 wk after the end of therapy (SVR_{12}). HCC surveillance was performed with US every 6–12 mo during and after antiviral treatment. When a focal lesion was identified in liver by US, HCC should be confirmed by imaging (CT and/or MR) and/or biopsy, as suggested by guidelines (23).

Four expert clinical monitors together physicians of 22 RESIST-HCV centres performed recording and verification of virological and clinical outcomes. We analyzed the records of all patients with cirrhosis treated with DAA and consecutively included in the RESIST-HCV database from March 1, 2015 to November 30– 2016 and who were available for SVR_{12} at the time of analysis (February 28, 2017). Patients with previous diagnosis of HCC, previous liver transplant (LT), or on LT waiting list were excluded. Patients who withdrew from therapy, those not evaluable for SVR, and patients who had not undergone ≥1 US after the start of DDA were not assessed for outcomes.

STATISTICAL ANALYSIS

To evaluate the early occurrence of HCC, we included all patients who received a complete regimen of DAA treatment and were evaluated for SVR in the analysis. Data were analyzed using the R statistical computing environment (version 3.4) Data for continuous variables are presented as mean and SD or as median and range, and data for categorical variables are presented as frequency and percentage.

Differences between continuous data were analyzed by Student's t test. Chi-squared tests with Yates' continuity correction were used for dichotomous or categorical variables.

HCC occurrence rates were evaluated by means of Kaplan-Meier estimators, and differences among groups of patients were tested using log-rank (Mantel-Cox) tests.

Univariate and multivariate Cox regression analysis was used to identify baseline variables such as age, sex, diagnosis of diabetes, bilirubin, albumin, INR, platelet values, Child-Pugh class, and SVR associated with the development of HCC.

Variables with a threshold value of <0.10 at univariate analysis were included in the multivariate model, and variables in the final model with P <0.05 were considered statistically significant. Results are expressed as adjusted hazard ratio (HR) with 95% confidence interval (CI).

RESULTS

Patient characteristics

From March 2015 to July 2016, 4,440 patients included in the RESIST-HCV database started DAA therapy; 2,877 of them had clinical criteria for diagnosis of cirrhosis. Of these patients, 273 (9.5%) were excluded from analysis because they had a previous diagnosis of HCC, had received a LT or were on the LT waiting list; 138 (4.8%) patients were not

included in the analysis because they did not have an SVR assessment; 217 (7.5%) were excluded by analysis because they had undergone ≥1 US after DAA (Supplementary Figure 1).

Analyses were performed on 2,249 consecutive adult patients with HCV cirrhosis who completed a full course of antiviral therapy with a DAA regimen (Supplementary Table 1), had available SVR data, and underwent US surveillance after treatment. The baseline characteristics of patients are reported in Table 1. The mean age of patients was 65.4 yr, and 56.9% of them were males. The most frequent HCV genotype was 1b (71.4%), and 1,267 (56.3%) patients had previously been unsuccessfully treated with IFN-based therapy. At the start of DAA, 2,035 patients (90.5%) were classified as Child-Pugh class A and 214 patients (9.5%) were classified as Child-Pugh class B. Among 1,562 patients with available EGS, 808 (51.7%) had esophageal varices. Mean platelet count was 128.7 $x10^9$/L; mean albumin, bilirubin, and INR values were 3.8 g/dL, 1.1 mg/dL, and 1.1, respectively. Only a small proportion of patients had co-infection with hepatitis B virus (HBV; 1.4%) or human immunodeficiency virus (HIV; 2.8%).

Virological response to DAA

SVR_{12} was achieved in 2,140 patients (95.2%), with a significant difference between patients in Child-Pugh class A and patients in Child-Pugh class B (95.9% vs. 88.3% respectively, P<0.001).

Disease outcomes: One-hundred and twenty-four patients (5.5%) from this cohort experienced liver disease complication during follow-up. Seventy-eight patients (3.4%) received a diagnosis of HCC, 32 patients (1.4%) developed ascites, 10 patients (0.5%) experienced EPS, and 4 patients (0.2%) had variceal bleeding.

Occurrence of HCC

All patients had an US scan before therapy and the exam dates recorded in the RESIST-HCV database. The mean time between last US and the start of DAA was 5.2 mo (range, 1-36). A total of 1,564 patients (69.6%) performed the US in the last 6 mo before the start of DAA; 580 patients (25.8%) performed US between 6 and 12 mo before the start of DAA; 105 patients (4.6%) performed US >12 mo before the start of DAA.

Seventy-eight patients (3.4%) received a diagnosis of HCC during the observation period (mean, 14 mo; range 6-24).

The overall rate of HCC occurrence was 3% (64/2,140) in SVR patients and 12.8% (14/109) in patients without SVR (p<0.001). By Kaplan-Meier analysis, the overall cumulative rate of HCC at 1 yr was 2.9%, with rates of 2.6% for the 2,140 SVR patients and 8% for the 109 patients without SVR (p<0.001 by log-rank test) (Figure 1, Supplementary Table 2). The analysis of HCC incidence according to Child-Pugh class and SVR status is shown in Figure 2. In Child-Pugh class A, the cumulative rates of HCC at 1 yr were 2.1% in SVR patients and 6.6% in patients without SVR, while patients in Child-Pugh class B who obtained SVR had a cumulative risk of developing HCC at 1 yr of 7.8% in SVR patients and 12.4% in patients who did not obtained SVR (P<0.001 by log-rank test).

A similar rate of HCC incidence was observed in the subgroup of 1,564 patients who performed the US control <6 mo before therapy (Supplementary Figure 2, Supplementary Table 3). When we evaluated the subgroup of 2,035 Child-Pugh A patients, we observed a HCC rate slightly lower than that observed for the cohort overall; however, the correlation with SVR was maintained. (Supplementary Figure 3, Supplementary Table 4).

Seventeen patients (0.76%) died during the observation period. Of these, 4 patients were

in the group of 109 patients without SVR, and 13 patients were among the 2,140 patients who achieved SVR (P=0.008). Seven out of 78 patients (9%) who received the diagnosis of HCC (6 for liver failure and 1 for gastrointestinal bleeding) and 10 patients (0.5%) in the group of 2,171 patients without HCC diagnoses (1 for liver-related causes and 9 for non–liver-related causes) died during the observation period (P<0.001).

B. Risk factors associated with HCC

We analyzed the clinical characteristics of patients who developed HCC in comparison with patients without HCC (Table 2). Univariate Cox regression analysis showed that male gender (HR: 1.77, P=0.02), INR (HR: 2.40, P=0.005), albumin (HR: 0.33, P<0.001) and bilirubin values (HR 1.67, p<0.001), platelet count (HR:0.99, P<0.001), duration of DAA regimen (HR: 1.04, P=0.02), and absence of SVR (HR: 4.02, P<0.001) were associated with HCC. There was no correlation between the incidence of HCC and presence of diabetes.

Multivariate Cox regression analysis showed that albumin value (HR: 0.52, CI 95%: 0.31-0.86, P=0.01), platelet count (HR:0.99, CI 95%: 0.99-1.00, P=0.01), and absence of SVR (HR: 2.88, CI 95%: 1.57-5.29, P=0.001) were independently associated with higher risk of HCC development. We thus categorized the two continuous variables, albumin and platelets, according to median value and inserted them in the multivariate model (Table 3). Albumin <3.5 g/dL (HR, 1.77; 95% CI, 1.12-2.82; P=0.01), platelets <120 x 10^9/L (HR, 3.89; 95% CI, 2.11–7.15; P<0.001), and absence of SVR (HR, 3.40; 95% CI, 1.89–6.12; P<0.001) were independently associated with an increased cumulative risk of HCC. The area under the ROC curve of the model generated by the three variables in predicting HCC at 12 mo was 0.714 (Supplementary Figure 4)

Cox multivariate analysis was used to assess the crude rate of HCC at the end of follow-up among risk classes (Figure 3). The same classes of risk obtained from the multivariate

model were used to analyze the predicted cumulative incidence of HCC (Figure 4). The results confirmed that SVR patients with good albumin and PLT values had low risk for development of HCC at 1 yr (0.8%). This risk increased to 2.8% in patients with both high albumin and PLT, even without SVR. Similar risk for HCC (3%) is predicted for SVR patients with albumin or PLT <3.5 g/dL and $120x10^9$/L, respectively. SVR patients with low albumin and PLT values had predicted risk of developing HCC at 1 yr of 5.6%. Patients without SVR and one unfavorable factor had risk of 10.2% and 18.2%, respectively.

The classes identified with albumin and PLT values also differed with respect to other clinical features such as age, Fibroscan values, presence of EV, INR, and bilirubin values used to confirm the progressive severity of liver disease (Supplementary Table 5).

Clinical features of HCC during observation

We assessed the interval between most recent US and initiation of DAA treatment, interval from start of DAA to diagnosis with HCC, tumor number and size of HCC nodules, presence of portal thrombosis and Barcelona Clinic Liver Cancer (BCLC) class in 78 patients diagnosed with HCC during the observation period (Supplementary Table 4). The interval between the last US and the start of DAA was similar in patients who developed HCC and those who did not develop HCC (5.4 vs. 5.2 mo). Seven patients (8%) received a diagnosis of HCC on DAA therapy; mean time from last US to start of DAA in this group of patients was 4.8 mo (range, 2-8). Six out of 7 patients had a single HCC nodule, and only 2 patients had tumor size >3 cm. Two patients presented with portal thrombosis at the time of diagnosis with HCC; 5 patients were BCLC class A; 5 patients received loco-regional therapy; 1 patient was added to the waiting list for LT. All concluded the DAA regimen and achieved SVR after diagnosis with HCC.

The remaining 71 patients (92%) received the diagnosis of HCC after the end of DAA. In

this group, the interval between US surveillance and start of DAA was 5.5 mo (range, 2-19)

Mean interval from start of DAA exposure to HCC diagnosis was 9.8 mo (range, 2-22 mo); this measure was similar in patients with vs. without SVR (9.2 vs. 12.0 mo; P=0.11).

Regarding the clinical pattern of HCC, patients with SVR were more likely to have a single HCC (50/64;78% vs. 7/14;50%), P=0.009) and HCC <3 cm (37/64;58% vs. 4/14;28.6%, P=0.07), compared with patients who did not achieve SVR. The rate of neoplastic portal thrombosis was similar in patients with vs. without SVR (10/64; 15.6% vs. 3/14; 21.4%; P=0.6).

At the time of HCC diagnosis, 57 patients (73%) were BCLC Stage A, 9 (11.5%) were Stage B, and 12 (15.4%) were class C. Fifty out of 64 patients with SVR and 6 out of 14 patients without SVR were classified as BCLC stage A (78.5% vs. 42.8%, P=0.01).

DISCUSSION

Widespread availability of DAA has enormously increased the number of HCV patients achieving SVR, including those with advanced or decompensated HCV cirrhosis. Immediate evidence of the exceptional efficacy and tolerability of these regimens has not allowed for the design of RCTs with untreated control groups of patients, which are necessary to demonstrate the ultimate impact on clinical outcomes of liver disease. The lack of RCTs may be partially addressed by the assessment of large real-life cohorts. However, such studies may be flawed by recruitment bias, retrospective analysis, and the inaccuracy of clinical goals scheduled at the start of treatment. In addition, the assumption that SVR may be considered a safe surrogate marker for clinical efficacy of DAA treatment at all stages of liver disease remains to be proven (24).

These uncertainties have fuelled a debate on two issues: a) the risk for and modality of HCC in cirrhotic patients treated with DAA (17-22); b) the effects of new antiviral drugs on the risk of developing liver disease events and liver-related mortality (15).

Given the difficulty of designing and carrying out RCTs in patients treated with DAA, well-designed cohort studies are useful because they can be used to measure the frequency of clinical outcomes and can provide evidence for the association between disease outcomes and risk factors or predictive factors. RESIST-HCV is a complex model for the management of DAA therapy, which is based on the appropriateness of diagnosis, the effectiveness of DAA regimens and the evaluation of disease outcomes. The real-time recording of clinical data on the web platform allows a prospective assessment of all patients. Our analysis included only patients with diagnosis of cirrhosis based on histological evidence of F4 METAVIR stage or on reliable clinical indexes (presence of EV, low platelets, liver stiffness >12 kPa). The majority of patients in our cohort performed TE before starting DAA treated, as recommended by the American Gastroenterology Association Technical Guidelines. Therefore, a liver stiffness cut-off of 12.5 (± 1) kPa would correctly identify >85% of patients with liver cirrhosis (24). In about one-third of patients, diagnosis of cirrhosis was confirmed by the presence of EV at the time of EGS surveillance (25). Although the diagnosis of cirrhosis was based on platelet count in only 11 patients, platelet count is a validated serum test for diagnosis of advanced fibrosis or cirrhosis (26). Mean age of our patient cohort was considerably higher than that of previous cohorts treated with IFN-based therapy. Moreover, many patients had significant chronic co-morbidities.

The first strength of our study was that all patients had undergone US surveillance prior the start of DAA (mean duration, 5.2 mo), and only 4.6% of patients had US control

>12 mo before the start of therapy. The interval between the last US and the start of DAA was similar in patients that developed HCC and other patients. When performed according to the guidelines, US surveillance (23) significantly reduced the risk of misdiagnosed HCC after initiation of DAA. All patients continued US surveillance in adherence to guidelines (23) during treatment as well as follow-up; it was therefore possible to establish the timing of diagnosis with HCC. Finally, we followed patients with and without SVR. Laboratory data were used to distinguish patients with Child-Pugh class A and class B cirrhosis. These data allowed us to differentiate the risk of HCC development according to the response to antiviral therapy and the stage of cirrhosis. Only 78 patients (3.4%) developed HCC during mean observation of 14 mo from the start of DAA treatment; the overall cumulative rate of HCC at 1 yr was 2.9%.

The incidence of de novo HCC was significantly different in patients with vs. without SVR and in Child-Pugh class A vs. Child-Pugh class B patients. In addition to the SVR, two other clinical variables were used to classify the risk of developing HCC. Patients with albumin values >3.5 g/dL and PLT >120 $x10^9$/L have low risk of developing HCC. Approximately 40% of patients of our cohort were in the low-risk class and showed a predicted cumulative incidence of HCC per year <1%. On the other hand, 14.4% of SVR patients who had a more advanced disease profile with low albumin and PLT values. Clinical evidence of portal hypertension predicted incidence per year of comorbid HCC (up to 7.3%).

These data indicate that in the heterogeneous population of cirrhotic patients that achieved SVR after DAA therapy, the risk of developing HCC should be assessed in relation to disease stage. Over 90% of the 887 patients in our cohort belonging to the lowest risk group (SVR with normal values of albumin and PLT) had a Child-Pugh score of 5, and only 1 out of 4 had esophageal varices. These patients had predicted cumulative

incidence of HCC per year <1%, similar to that observed in patients treated with IFN-based therapy (27). Most studies including only patients with Child-Pugh A class cirrhosis in patients aged <60 yr, reported a rate of HCC per 100 persons per year of 1% among patients achieving SVR (7, 28-34). Patients included in risk groups 2 and 3 often have portal hypertension (EV rates >50%), advanced age (mean age >65 yr), and compromised liver function. For these reasons, they cannot be compared with patients included in cohorts treated with IFN-based therapy. As evidenced by studies on the natural history of cirrhosis (3,35), these patients have a greater risk of developing decompensation, HCC, and death due to liver conditions. Our data show that SVR patients in the intermediate risk group had a predicted cumulative incidence of HCC per year of 3.4%; this risk increased to 7.3% per year in the high-risk SVR group.

The grading of risk of developing HCC in relation to clinical stage of disease is only partially addressed by published studies (27). In fact, previous authors have pointed out that the incidence of HCC among patients with cirrhosis may depend on epidemiological and clinical factors, including mechanisms for detection, schedule of HCC screening, and timing and extent of follow-up. The only study published as a full paper (16) analyzed patients with and without HCC before initiation of treatment with DAA. The study was retrospective and did not indicate the time of US pre-therapy. The results of three other studies (17,19,20) are reported in letters to the editor and therefore do not analytically report the patient's clinical features and the methods used for data analysis. Finally, 5 studies (18,36-39) are still in the form of abstracts reported in scientific meetings, and no analytical data are available to properly evaluate the results. However, analysis of a retrospective cohort study of 22,500 patients treated with DAA in 129 Veterans Health Administration hospitals in 2015 reported an annual incidence of HCC after SVR of 1.82% in patients with diagnosis of cirrhosis, but this study did not report the stage of cirrhosis in

patients who achieved SVR (40).

Our data conclusively show that the rate of *de novo* HCC was not higher than expected, in cirrhotic patients who achieved SVR after DAA, considering the stage of disease. We are convinced that in the coming year, hepatologists should carefully evaluate the stage of liver disease in patients treated with DAA to avoid selection bias and errors in evaluating the results of their studies.

Finally, we evaluated the HCC pattern. Recently, Romano et al (18) observed an unusual HCC pattern during and after DAA. While 61% of patients developed single small HCC nodules or 2-3 small HCC nodules, the remaining 39% were diagnosed with larger or aggressive, often infiltrative, tumors. This unusual pattern was more frequent during rather than after DAA treatment, in patients who did not achieve SVR. In our prospective study, we were able to evaluate the time of the last US and the time since the start of DAA and diagnosis of HCC, the number and the size of HCC nodules, the presence of portal thrombosis, and BCLC class. We therefore chose to consider the start of therapy as the starting point for observation. The 7 patients who received a diagnosis of HCC on DAA therapy had a mean interval from last US to the start of DAA of 4.8 mo. Therefore, the risk of failing to diagnose HCC at the start of therapy was particularly low. None of these patients had a multinodular or aggressive HCC pattern; all obtained SVR, and six received adequate therapy for HCC. Overall, patients with SVR were more likely to have single HCC or HCC <3 cm, compared with patients who did not achieve SVR. Finally, patients with SVR were more likely than patients without SVR to be classified as BCLC stage A.

Our data do not support the hypothesis that HCC that develops during DAA or early follow-up is more aggressive and more difficult to treat with available therapies (21,22). We do not agree with the hypothesis that profound immunological and molecular changes in the liver microenvironment following abrupt interruption of HCV replication may increase the

risk of early HCC (41,42).

With the availability of a broad spectrum of DAA with high efficacy and tolerability, antiviral therapy should reach a rate of SVR close to 100%. Physicians will be able to eradicate HCV infection in all patients, but they must be able to inform patients about the risks for liver disease progression and complications. To give patients the correct information, the physicians must correctly grade disease stage before starting the therapy and suggest appropriate clinical follow-up (43).

We are aware that our study has some limitations. The main limitation was the short follow-up after SVR. The short observation time may have amplified the difference in the number of events in different classes of cirrhosis. Patients without SVR patients may be those with more advanced liver disease, who have greater likelihood of developing HCC. Therefore, longer follow-up is required to evaluate the risk for HCC, especially in patients with portal hypertension or co-morbidities such as diabetes or metabolic syndrome. However, we believe that the observation period used for this study was adequate to demonstrate that the risk of developing HCC does not increase during early follow-up after HCV clearance.

One other limitation of this study is the potential heterogeneity among clinical centers participating in RESIST, in terms of competence in the diagnosis of HCC. We believe this limitation has been overcome through the application of radiological or histological guidelines for the diagnosis of HCC and through adequate monitoring. Moreover, moderate heterogeneity is an intrinsic characteristic of all studies that include a high number of centers.

In conclusion, our prospective observational study confirms that the early benefit of viral eradication in HCV cirrhosis persists throughout all stages of cirrhosis. The occurrence of HCC is significantly reduced in patients with compensated cirrhosis without

signs of portal hypertension and normal liver function. In patients with advanced disease, eradication of HCV infection reduces the risk of developing HCC. Long-term observation of our cohort and of other similar groups of treated patients will determine the ultimate treatment benefit. Meanwhile, DAA treatment must be guaranteed to all patients with cirrhosis at any functional stage, considering the residual and unavoidable risk of HCC after viral eradication.

PARTECIPANTS TO RESIST-HCV

PALERMO AOUP Paolo Giaccone, Palermo: UOC di Gastroenterologia e Epatologia (A. Craxì, V. Di Marco, C. Cammà, V. Calvaruso, S. Petta, G. Cabbibbo); UOC di Malattie Infettive (P. Colletti, G. Mazzola, A. Cascio); UOC di Medicina Interna (G. Montalto, A. Licata, L. Giannitrapani); ARNAS Civico-Di Cristina-Benefratelli, Palermo: UOC di Malattie Infettive (T. Prestileo, F. Di Lorenzo, A. Sanfilippo, A. Ficalora) AO Villa Sofia-Cervello, Palermo: UOC di Medicina Interna (S. Madonia); UOC di Gastroenterologia (F. Tinè, G. Malizia, F. Latteri, M. Maida); Ospedale Buccheri La Ferla, Palermo UOC di Medicina Interna (F. Cartabellotta, R. Vassallo)

MESSINA AUOP G Martino, Messina: UOC di Epatologia Clinica e Biomolecolare (I. Cacciola; G. Caccamo, S. Maimone, C. Saitta, G. Squadrito, G. Raimondo) AO Papardo-Piemonte, Messina. UOC di Malattie Infettive (L. Mondello, A. Smedile, S. D'Andrea)

CATANIA AOUP Vittorio Emanuele, Catania: UOC di Medicina Interna e d'Urgenza (G. Bertino, A.L. Ardiri, E. Frazzetto, G. Rigano) UOC di Malattie Infettive (A. Montineri, L. N. Larocca,) ARNAS Garibaldi-Nesima, Catania: U.S.C. di Malattie Infettive (B. Cacopardo, F. Benanti), U.S.D. di Epatologia (M. Russello, R. Benigno, A. Bellia) A.O. Cannizzaro, Catania U.O.C. Malattie Infettive (C. Iacobello)

RAGUSA ASP di Ragusa, U.O.C. di Malattie Infettive Ospedale di Modica (A. Davì, MA Di Rosolini) UOC Medicina Interna Ospedale di Comiso (A. Digiacomo, G. Fuduli)

SIRACUSA ASP di Siracusa, UOC Malattie Infettive Ospedale di Siracusa (G. Scifo, M Distefano)

TRAPANI ASP di Trapani. UOC Malattie Infettive Ospedale di Trapani (V. Portelli. F. Savalli) UOC Medicina Interna Ospedale di Mazzara e Castelvetrano (I. Scalici, G. Gioia)

AGRIGENTO ASP di Agrigento. U.O.C. Medicina Interna Ospedale di Agrigento (A. Magro, G. Alaimo, MR. Alinovi)

CALTANISSETTA ASP di Caltanissetta, UOC Malattie Infettive Ospedale di Caltanissetta(A. Salvo, A. Averna, F. Lomonaco).

ENNA ASP di Enna; UOC Malattie Infettive Ospedale di Enna (L. Guarneri F. Maffeo,E. Falzone, F. Pulvirenti)

Legend of figures

Figure 1. Overall cumulative rate of HCC according with SVR status. ($p < 0.001$ by log-rank test).

Figure 2. Analysis of HCC incidence according with Child-Pugh class and SVR status.

Figure 3: The crude rate of HCC at the end of follow up in different classes of risk

Figure 4. The predicted cumulative incidence of HCC in the classes of risk by the multivariate model.

REFERENCES

1. Simonetti RG, Cammà C, Fiorello F et al. Hepatitis C virus infection as a risk factor for hepatocellular carcinoma in patients with cirrhosis. A case-control study. Ann Intern Med. 1992;116:97-102.

2. Fattovich G, Stroffolini T, Zagni I, Donato F. Hepatocellular carcinoma in cirrhosis: incidence and risk factors. Gastroenterology. 2004 Nov;127:S35-50.

3. D'Amico G, Pasta L, Morabito A et al. Competing risks and prognostic stages of cirrhosis: a 25-year inception cohort study of 494 patients. Aliment Pharmacol Ther. 2014;39:1180-93.

4. Trautwein C, Friedman SL, Schuppan D, Pinzani M. Hepatic fibrosis: Concept to treatment. J Hepatol. 2015, 62:S15-24.

5. Kohli A, Shaffer A, Sherman A, Kottilil S. Treatment of hepatitis C: a systematic review. JAMA. 2014;312:631-40.

6. van der Meer AJ, Wedemeyer H, Feld JJ et al. Is there sufficient evidence to recommend antiviral therapy in hepatitis C? J Hepatol. 2014;60:191-6.

7. Di Marco V, Calvaruso V, Ferraro D et al. Effects of Eradicating Hepatitis C Virus Infection in Patients With Cirrhosis Differ WithStage of Portal Hypertension. Gastroenterology. 2016;151:130-139.

8. Bruno S, Di Marco V, Iavarone M et al. Survival of patients with HCV cirrhosis and sustained virologic response is similar to the general population. J Hepatol. 2016 Jun;64:1217-23.

9. van der Meer AJ, Veldt BJ, Feld JJ, et al. Association between sustained virological response and all-cause mortality among patients with chronic hepatitis C and advanced hepatic fibrosis. JAMA. 2012;308:2584-93.

10. Janjua NZ, Chong M, Kuo M et Long-term effect of sustained virological response on hepatocellular carcinoma in patients with hepatitis C in Canada. J Hepatol; 66: 504-513.

11. Falade-Nwulia O, Suarez-Cuervo C et al. Oral Direct-Acting Agent Therapy for Hepatitis C Virus Infection: A Systematic Review. Ann Intern Med. 2017;166:637-648.

12. Majumdar A, Kitson MT, Roberts SK. Systematic review: current concepts and challenges for the direct-acting antiviral era in hepatitis C cirrhosis. Aliment Pharmacol Ther. 2016;43:1276-92.

13. European Association for the Study of the Liver. EASL Recommendations on Treatment of Hepatitis C. J Hepatol 2016;66: 153–194

14. AASLD/IDSA HCV Guidance Panel. Hepatitis C guidance: AASLD-IDSA recommendations for testing, managing, and treating adults infected with hepatitis C virus. Hepatology. 2015;62:932-54.

15. Backus LI, Belperio PS, Shahoumian TA, Mole LA. Impact of Sustained Virologic Response with Direct-Acting Antiviral Treatment on Mortality in Patients with Advanced Liver Disease. Hepatology. 2017 Jul 27. [Epub ahead of print]

16. Conti F, Buonfiglioli F, Scuteri A et al. Early occurrence and recurrence of hepatocellular carcinoma in HCV-related cirrhosis treated with direct acting antivirals. J Hepatology 2016;65:727-733.

17. Kozbial K, Moser S, Schwarzer R et al. Unexpected high incidence of hepatocellular carcinoma in cirrhotic patients with sustained virologic response following interferon-free direct-acting antiviral treatment. J Hepatol 2016;65:856-858.

18. Romano A, Capra F, Piovesan S et al. Incidence and pattern of" de novo" hepatocellular carcinoma in HCV patients treated with oral DAAs. Hepatology 2016;64: 10A.

19. Zeng Q-L, Li Z-Q, Liang H-X et al. Unexpected high incidence of hepatocellular carcinoma in patients with hepatitis C in the era of DAAs: Too alarming? J Hepatol 2016;65:1068-1069.

20. Cardoso H, Vale AM, Rodrigues S et. High incidence of hepatocellular carcinoma following successful interferon-free antiviral therapy for hepatitis C associated cirrhosis. J Hepatol 2016;65:1070-1071.

21. Alberti A, Piovesan S. Increased incidence of liver cancer after successful DAA treatment of chronic hepatitis C: Fact or fiction? Liver Int. 2017;37:802-808.

22. Reig M, Boix L, Bruix J. The impact of direct antiviral agents on the development and recurrence of hepatocellular carcinoma. Liver Int. 2017;37 Suppl 1:136-139.

23. Bruix J, Reig M, Sherman M. Evidence-Based Diagnosis, Staging, and Treatment of Patients With Hepatocellular Carcinoma. Gastroenterology. 2016;150:835-53.

24. Singh S, Muir AJ, Dieterich DT, Falck-Ytter YT. American Gastroenterological Association Institute Technical Review on the Role of Elastography in Chronic Liver Diseases. Gastroenterology. 2017;152:1544-1577.

25. de Franchis R; Baveno VI Faculty. Expanding consensus in portal hypertension: Report of the Baveno VI Consensus Workshop: Stratifying risk and individualizing care for portal hypertension. J Hepatol. 2015;63:743-52.

26. Procopet B, Berzigotti A. Diagnosis of cirrhosis and portal hypertension: imaging, non-invasive markers of fibrosis and liver biopsy. Gastroenterol Rep (Oxf). 2017;5:79-89.

27. Waziry R, Hajarizadeh B, Grebely J et al. Hepatocellular carcinoma risk following direct-acting antiviral HCV therapy: A systematic review, meta-analyses, and meta-regression. J Hepatol. 2017 Aug 9. [Epub ahead of print]

28. D'Ambrosio R, Aghemo A, Rumi M et al. The course of esophageal varices in patients with hepatitis C cirrhosis responding to interferon/ribavirin therapy. Antivir Ther

2011;16:677.

29. Hung CH, Lee CM, Lu SN, et al. Long-term effect of interferon alpha-2b plus ribavirin therapy on incidence of hepatocellular carcinoma in patients with hepatitis C virus-related cirrhosis. J Viral Hepat 2006;13:409-414.

30. Fernandez-Rodriguez CM, Alonso S, Martinez SM et al. Peginterferon plus ribavirin and sustained virological response in HCVrelated cirrhosis: outcomes and factors predicting response. Am J Gastroenterol 2010;105:2164-2172.

31. Velosa J, Serejo F, Marinho R, Nunes J, Gloria H. Eradication of hepatitis C virus reduces the risk of hepatocellular carcinoma in patients with compensated cirrhosis. Dig Dis Sci 2011;56:1853-1861.

32. Aleman S, Rahbin N, Weiland O et al. A risk for hepatocellular carcinoma persists long-term after sustained virologic response in patients with hepatitis C-associated liver cirrhosis. Clin Infect Dis 2013;57:230-236.

33. Mallet V, Gilgenkrantz H, Serpaggi et al. Brief communication: the relationship of regression of cirrhosis to outcome in chronic hepatitis C. Ann Intern Med 2008;149:399-403.

34. Nahon P, Bourcier V, Layese R et al. Eradication of hepatitis C virus infection in patients with cirrhosis reduces risk of liver and non-liver complications. Gastroenterology 2017;152:142-156.

35. Waziry R, Grebely J, Amin J et al. Trends in hepatocellular carcinoma among people with HBV or HCV notification in Australia (2000-2014). J Hepatol 2016;65:1086-1093.

36. Carrat F. Clinical outcomes in HCV-infected patients treated with direct acting antivirals-18 month post-treatment follow-up in the french anrs CO22 hepather cohort study. J Hepatol 2016;1:S215.

37. Affronti A, Ju M, Catt J et al. Successful hepatitis C treatment in advanced cirrhosis with DAA reduces HCC incidence. Hepatology 2016;64:475A-476A.

38. Muir AJ, Buti M, Nahass R et al. Long-term follow-up of patients with chronic HCV infection and compensated or decompensated cirrhosis following treatment with sofosbuvir-based regimens. Hepatology 2016:437A-438A.

39. Rinaldi L, Di Francia R, Coppola N et al. Hepatocellular carcinoma in HCV cirrhosis after viral clearance with direct acting antiviral therapy: preliminary evidence and possible meanings. WCRJ 2016;3:e748.

40. Kanwal F, Kramer J, Asch SM et al. Risk of Hepatocellular Cancer in HCV Patients Treated With Direct Acting Antiviral Agents. Gastroenterology. 2017;153 :996-1005.

41. Llovet JM, Villanueva A. Liver cancer: Effect of HCV clearance with direct-acting antiviral agents on HCC. Nat Rev Gastroenterol Hepatol. 2016;13:561-562.

42. Debes JD, Janssen HL, Boonstra A. Hepatitis C treatment and liver cancer recurrence: cause for concern? Lancet Gastroenterol Hepatol. 2017;2:78-80.

43. Jacobson IM, Lim JK, Fried MW. American Gastroenterological Association Institute Clinical Practice Update-Expert Review: Care of Patients Who Have Achieved a Sustained Virologic Response After Antiviral Therapy for Chronic Hepatitis C Infection. Gastroenterology. 2017;152:1578-1587.

Variables	2,249 patients
Age (years, mean, SD)	65.4 ± 10.7
BMI (mean, SD)	26.0 ± 4.6
Gender (males, %)	1,280 (56.9%)
ALT (IU/L, mean, SD)	89.0 ± 59.7
Platelets (x 10^9/L)	128.7 ± 64.7
INR (mean, SD)	1.1. ± 0.2
Bilirubin (mg/dL, mean,SD)	1.1 ± 0.6
Albumin (g/dL, mean, SD)	3.8 ± 0.5
HCV genotypes 1b 1a 2 3 4 other	1,606 (71.4) 191 (8.5) 202 (9.0) 154 (6.8) 91 (4.0) 5 (0.2)
Criteria for diagnosis of cirrhosis (%): - Transient Elastography ≥ 12 kPa - Esophageal Varices at EGS - METAVIR stage 4 fibrosis at liver biopsy - PLT < 100 x 10^9/L.	 1,366 (60.7) 808 (35.6) 64 (2.8) 11 (0.5)
TE values (kPa, mean, SD)	22.4 ± 11.9
Child-Pugh score A B	2,035 (90.5) 214 (9.5)
Presence of esophageal varices* (%)	808/1,562 (51.7)
Months from US to DAAs start (mean, range)	5.1 (1-36)
Diabetes (%)	672 (29.9)
Previous IFN-based therapy • Naive (%) • Experienced (%)	 982 (43.7) 1,267 (56.3)

* 1,562 patients with endoscopy

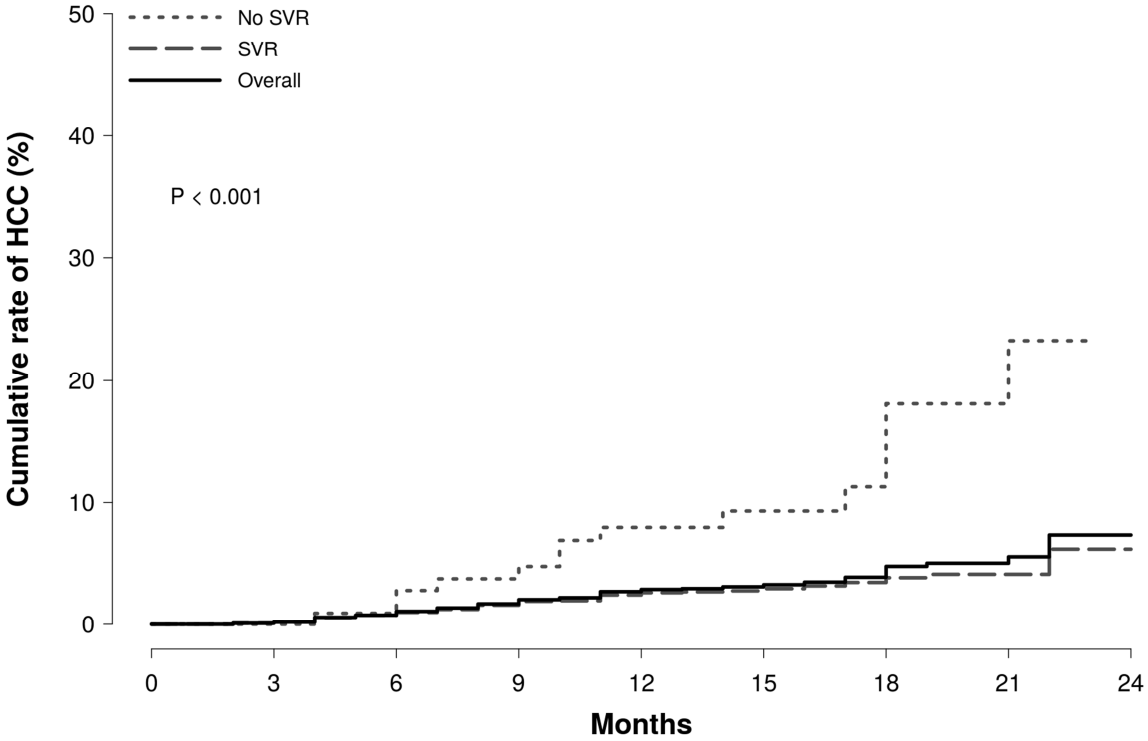
Table 1: Baseline clinical and virological features of 2,249 patients with HCV cirrhosis treated with DAAs

	Univariate Cox's regression				Multivariate Cox's regression	
	No HCC (2,171 pts)	**HCC (78 pts)**	**HR (95% CI)**	**p**	**Adjusted HR (95%CI)**	**p**
Age (years)	65.3 ± 10.4	66.2 ± 10.6	1.01 0.99 - 1.03	0.35		
Gender (male)	1,225 (56.4)	55 (70.5)	1.77 1.09 - 2.88	0.022	1.49 0.91 - 2.44	0.11
BMI	26.0 ± 3.9	26.1 ± 3.9	1.00 0.95 1.06	0.90		
Diabetes (%)	650 (29.9)	22 (28.2)	0.94 0.58 - 1.54	0.81		
INR	1.1 ± 0.2	1.2 ± 0.2	2.40 1.31 - 4.39	0.005	1.37 0.55 - 3.41	0.49
Bilirubin (mg/dl)	1.0 ± 0.7	1.4 ± 0.9	1.67 1.36 - 2.05	<0.001	1.25 0.97 – 1.62	0.08
Albumin (g/dl):	3.8 ± 0.6	3.5 ± 0.6	0.33 0.21 - 0.52	<0.001	0.52 0.31 – 0.86	0.010
Platelets (x 10^3)	129.9 ± 72.3	93.9 ± 66.6	0.99 0.98 - 0.99	<0.001	0.99 0.98 – 0.99	0.011
Weeks of therapy	17.2 ± 6.0	19.2 ± 5.8	1.04 1.01 - 1.08	0.022	1.02 0.98 – 1.06	0.38
No SVR 12 (%)	95 (4.4)	14 (17.9)	4.02 2.25 – 7.17	<0.001	2.88 1.57 – 5.29	0.001

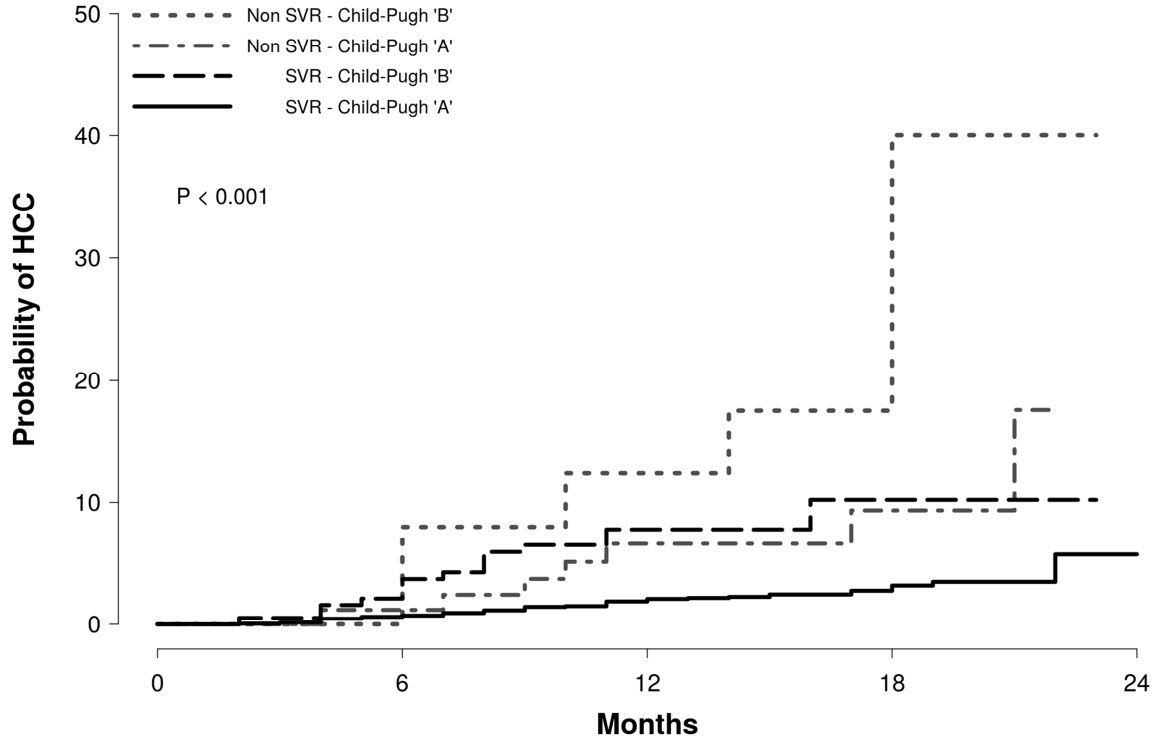
Table 2. Risk factors for HCC occurrence by Cox multivariate model

Multivariate Cox's regression				
		HR	HR (95% CI)	p
Albumin (mg/dl):	≥ 3.5 < 3.5	1.77	1.12 - 2.82	0.015
Platelets (x10^3/dL)	≥ 120 < 120	3.97	2.23 - 7.09	< 0.001
SVR 12 No SVR 12		3.40	1.89 - 6.12	< 0.001

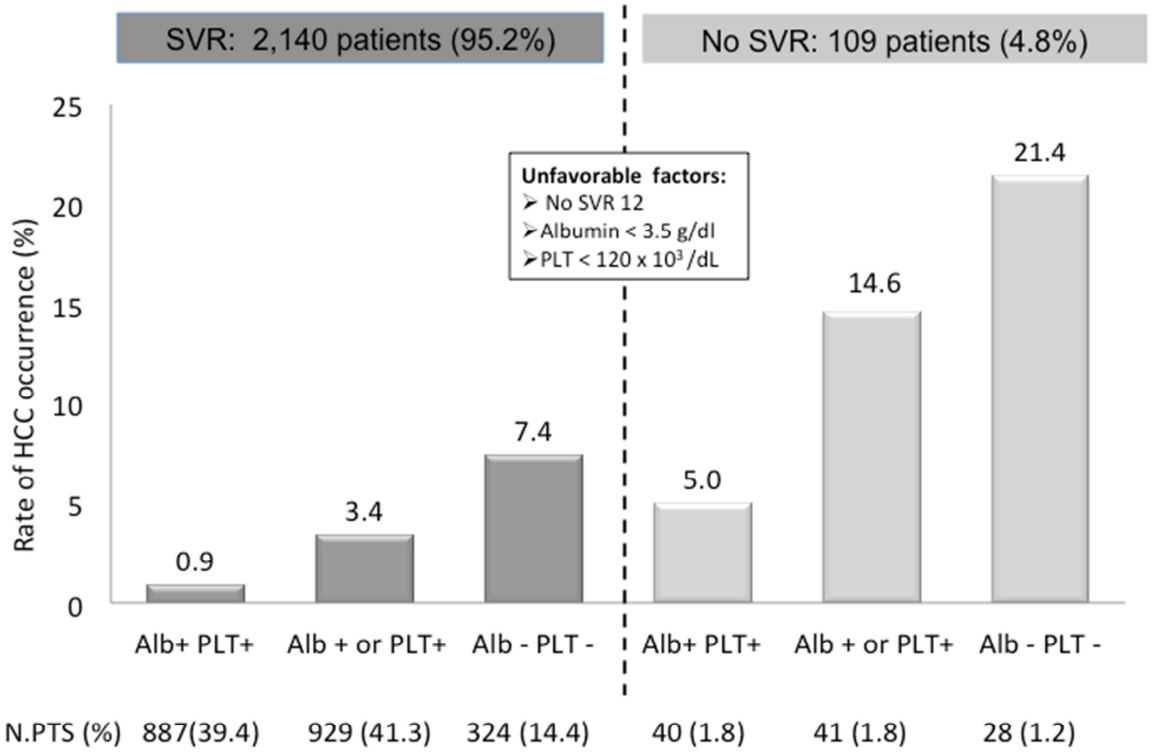
Table 3. Risk factors for HCC occurrence by Cox multivariate model

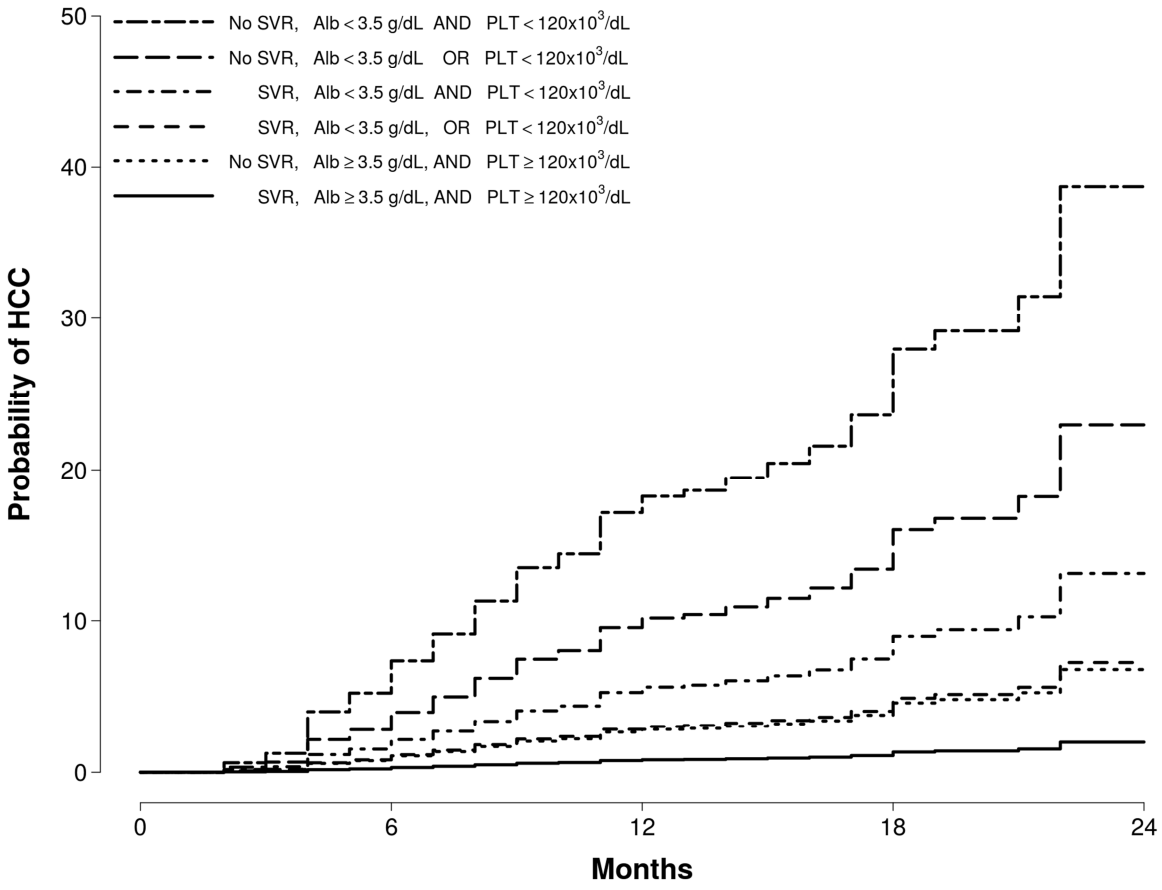


No SVR	109	107	80	39	
SVR	2140	2111	1545	503	8
Overall	2249	2218	1625	542	8



Non SVR - B	25	25	18	11	2
Non SVR - A	84	82	62	28	6
SVR - B	189	184	132	42	2
SVR - A	1951	1927	1413	461	8





	Genotype 1a (191 pts)	Genotype 1b (1,606 pts)	Genotype 2 (202 pts)	Genotype 3 (154 pts)	Genotype 4 (91 pts)
SOF+RBV 12 wks	1	3	115		
SOF+RBV 24 wks	2	10	44	24	1
SOF/LDV 12 wks	10	66			1
SOF/LDV+RBV 12 wks	32	141			10
SOF/LDV 24 wks	45	446			33
SOF/LDV+RBV 24 wks	9	38			2
SOF/SIM 12 wks	6	52			3
SOF/SIM+RBV 12 wks	26	249			10
SOF/SIM24 wks	-	3			
SOF/SIM+RBV 24 wks	-	2			
SOF+DCV 12 wks	-	7	40	6	3
SOF/DCV+RBV 12 wks	6	48		1	6
SOF/DCV 24 wks	19	82	3	57	6
SOF/DCV+RBV 24 wks	1	30		66	
OBV/PTV/r/DSV 12 wks	3	169			
OBV/PTV/r/DSV + RBV12 wks	12	265			
OBV/PTV/r/DSV 24 wks	2	3			
OBV/PTV/r/DSV + RBV 24 wks	17	1			
OBV/PTV/r + RBV 12 wks	-	-			5
OBV/PTV/r + RBV 24 wks	-	-			13

Supplementary table 1. DAA regimens received by patients

Supplementary table 2: Cumulative incidence of HCC by Kaplan-Meier analysis

	Overall (2,249 patients)			SVR (2,140 patients)			No SVR (109 patients)		
Time (months)	Number patients at risk	Cumulative events	rate	Number patients at risk	Cumulative events	rate	Number patients at risk	Cumulative events	rate
6	2,218	24	1.1%	2,111	21	1.0 %	107	3	2.8
12	1,625	59	2.9%	1,545	51	2.6 %	80	8	8.0
18	542	74	4.7%	503	61	3.8 %	39	13	18.1
24	8	78	7.4%	8	64	6.1 %	0	14	23.2

	Overall (1,564 patients)			SVR (1,485 patients)			No SVR (79 patients)		
Time (Months)	Number patients at risk	Cumulative events	rate	Number patients at risk	Cumulative events	rate	Number patients at risk	Cumulative events	rate
0	1564	0	0	1485	0	0	79	0	0
6	1543	15	1.0%	1465	13	0.9%	78	2	2.5%
12	1113	35	2.4%	1057	28	2.0%	56	7	9.7%
18	374	46	4.5%	349	34	3.1%	25	12	24.7%
24	5	50	8.2%	0	37	6.4%	0	13	31.0%

Supplementary table 3: Cumulative incidence of HCC by Kaplan-Meier analysis in 1,564 patients with US control performed within 6 months before the start of DAAs.

	Overall (2,035 patients)			SVR (1,951 patients)			No SVR (84 patients)		
Time (Months)	Number patients at risk	Cumulative events	rate	Number patients at risk	Cumulative events	rate	Number patients at risk	Cumulative events	rate
0	2035	0	0	1951	0	0	84	0	0
6	2009	15	0.7%	1927	14	0.7	82	1	1.2%
12	1475	42	2.3%	1413	37	2.1%	62	5	6.6%
18	489	51	3.5%	461	45	3.2%	28	6	9.4%
24	8	55	6.4%	8	48	5.7%	0	7	17.6%

Supplementary table 4: Cumulative incidence of HCC by Kaplan-Meier analysis in 2,035 patients with Child Pugh A cirrhosis

Response to DAAs	Class of risk	Number of patients (%)	Age (year; mean, SD)	Liver Stiffness by fibroscan (*) (kPa; mean; SD)	Patients with EV (**) (number, %)	Albumin (g/dL;mean, SD	Bilirubin (mg/dL; mean, SD)	INR (mean, SD)	Platelet (x 10^9 /L;mean, SD)
SVR (2,140; 95.2%)	Alb + PLT+	887 (39.4)	64.6 ±10.8	19.0 ± 9.2	182/492 (36.9)	4.02± 0.32	0.88 ± 0.51	1.05 ± 0.10	178.6± 53.5
	Alb + or PLT +	929 (40.5)	65.6 ±10.2	24.8± 12.8	388/721 (53.8)	3.81 ± 0.42	1.09 ± 0.56	1.12 ± 0.21	100 .3± 45.0
	Alb - PLT -	324 (14.5)	67.8 ±10.2	26.7± 12.4	210/297 (70.8)	3.15 ± 0.25	1.37 ± 0.70	1.21 ± 0.29	75.5± 24.5
No SVR (109; 4.8%)	Alb + PLT+	40 (1.6)	61.6 ±12.2	21.6 ± 8.3	10/24 (41.6)	3.91± 0.32	1.06 ± 0.71	1.02± 0.14	204.2 ± 103. 0
	Alb + or PLT +	41 (1.9)	63.2 ±12.6	32.8± 16.2	19/30 (62.5)	3.82 ± 0.47	1.36 ± 0.77	1.13 ± 0.28	84.2± 54.1
	Alb - PLT -	32 (1.3)	64.6 ±10.5	28.7± 14.4	19/25 (75)	3.00 ± 0.21	1.80 ± 1.10	1.26 ± 0.32	62.2± 22.2

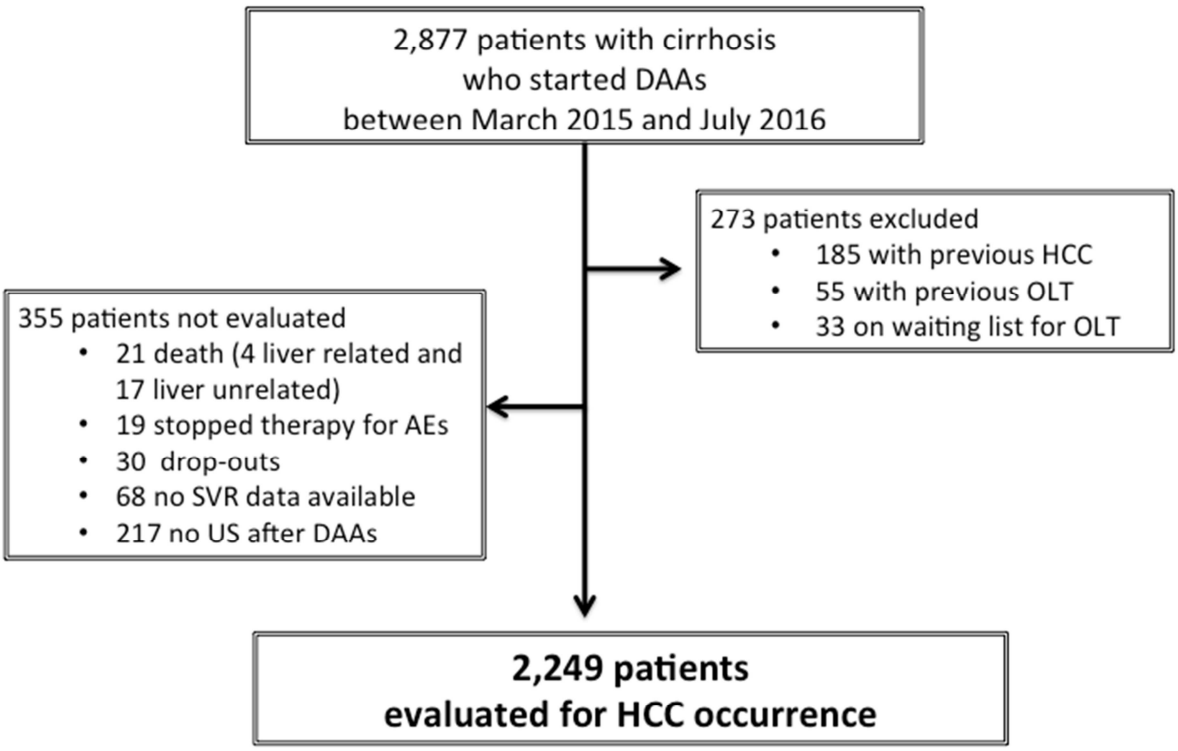
Supplementary table 5: Clinical features of patient's classes with different risk to develop HCC
(*) The Liver Stiffness by fibroscan was performed in 1,695 patients
(**) The EGS was performed in 1,484 patients

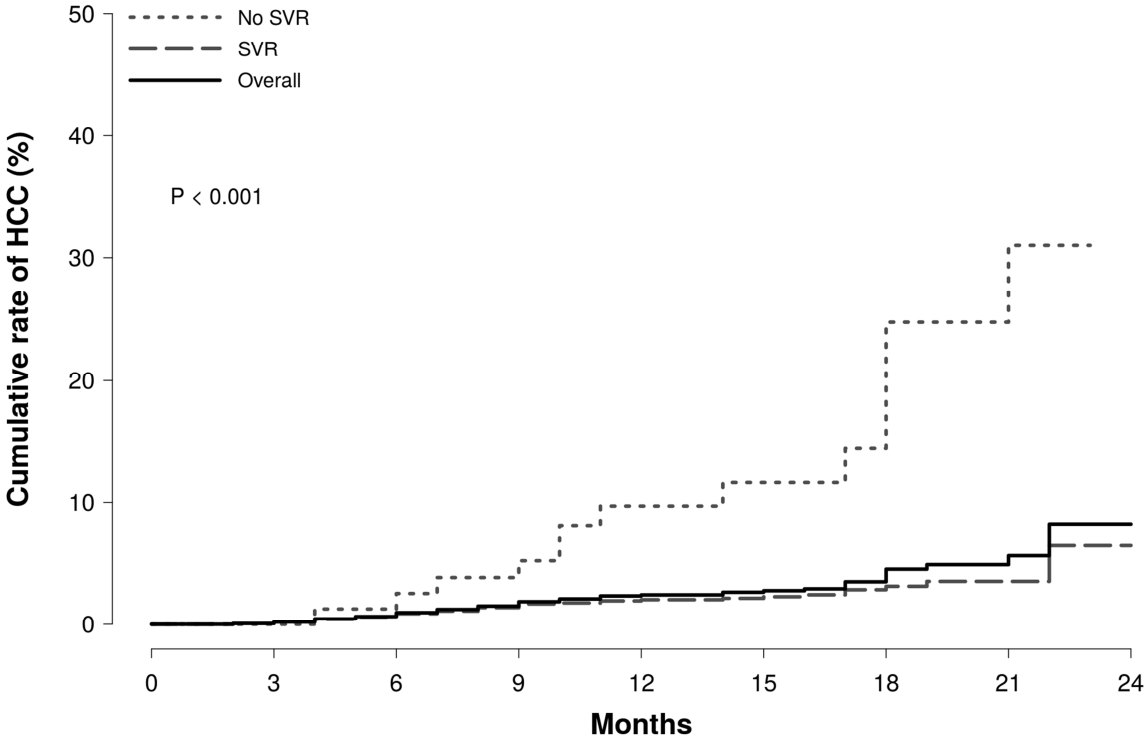
Supplementary figure 1. The flow-chart of the RESIST – HCV cohort

Supplementary figure 2. Overall cumulative rate of HCC according with SVR status in 1,564 patients with an US performed within 6 months before therapy. ($p < 0.001$ by log-rank test).

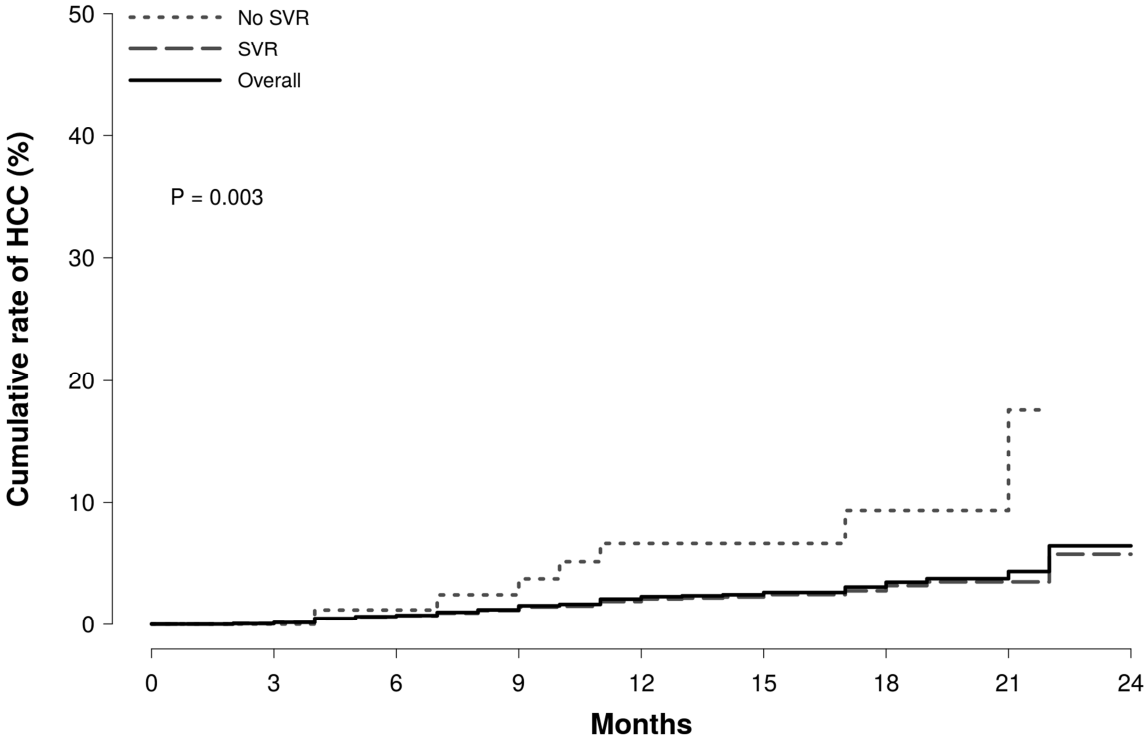
Supplementary figure 3. Overall cumulative rate of HCC according with SVR status. ($p < 0.001$ by log-rank test) in the 2035 patients with Child-Pugh score A.

Supplementary figure 4. Area under the ROC of of prediction of HCC in DAA treated patients with cirrhosis





No SVR	79	78	56	25	
SVR	1485	1465	1057	349	5
Overall	1564	1543	1113	374	5



No SVR	84	82	62	28	
SVR	1951	1927	1413	461	8
Overall	2035	2009	1475	489	8