Unmet needs in the post-DAA era: the risk and molecular mechanisms of hepatocellular carcinoma after HCV eradication

Short running title: HCC after HCV eradication

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Abstract

Hepatitis C virus (HCV) is one of the major etiologies of hepatocellular carcinoma with approximately 30% of HCC being due to HCV infection worldwide. HCV eradication by antivirals greatly reduces the risk of HCC; nevertheless, HCC remains to occur in CHC patients who have achieved a sustained virological response (SVR). The proportion of post-SVR HCC among newly diagnosed HCC patients is increasing in the DAA era and might be due to preexisting inflammatory and fibrotic liver background, immune dysregulation between host and virus interaction, as well as host epigenetic scar, genetic predispositions and alternations. By means of applying surrogate markers and adopting risk stratification, HCC surveillance should be consistently performed in high-risk populations. In this review, we discuss the possible molecular mechanism, risk factors and surveillance strategy for HCC development after HCV eradication.

Keywords: HCV; HCC; SVR; genetic; epigenetic; surveillance
Introduction

An estimated 60 million people are infected with hepatitis C virus (HCV). Chronic hepatitis C (CHC) is a public health threat globally since 10–20% will develop liver complications including decompensated cirrhosis and hepatocellular carcinoma (HCC).[1] HCC is the fifth most common cancer and the second most common cause of cancer death with approximately 30% of HCC being due to HCV infection.[2] The endorsement of the World Health Assembly and declaration of viral hepatitis elimination by 2030 set by the World Health Organization has facilitated HCV management in recent years. With the great innovation of direct-acting antivirals (DAAs), nearly 10 million CHC patients were treated with DAAs between 2015 and 2019.[3] It is anticipated that a significantly increased number of CHC patients will be in the post-curative status in the following decades, and how to deal with such post-SVR CHC patients is of great importance.

HCV eradication by antivirals greatly reduces the risk of HCC with up to 70% of HCC risk being reduced either by interferon-based regimens[4] or DAAs [5] Nevertheless, HCC is still found in CHC patients who have achieved a sustained virological response (SVR). The annual incidence of HCC after HCV eradication ranges from 0.6% to 4.9% [6], of which the risk did not differ between those who received interferon-based regimens or DAAs after adjusting potential confounders.[7]
However, unlike in the interferon era when more elderly patients with advanced liver disease were relatively contraindicated for antivirals, more CHC patients who possess the two HCC risk factors have been cured by DAAs.\[7\] As a consequence, the proportion of post-SVR HCC among newly diagnosed HCC patients is increasing in the DAA era; for example, in comparison to HCV-viremic HCC and non-HCV HCC, the percentage of post-HCV SVR HCC increased from 3% between years 2009-2012 to 16% between the years 2017 and 2019 in a Japanese study.\[8\] Taken collectively, it is critical to address the topic of post-HCV SVR HCC due to the increasing health burden year-by-year. In this review, we discuss the possible molecular mechanism, risk factors and surveillance strategy for HCC development after HCV eradication.

\textbf{Screening targets and surveillance strategies}

The recommendation of the surveillance target population is based on cost-effective analysis. Screening the population with an annual incidence of 1.5% or greater has been generally acceptable in the past.\[9\]; A recent study has shown the incremental cost-effectiveness ratio (ICER) would be $50,000 per quality-adjusted life-year (QALY), a traditional willingness-to-pay threshold, if the incidence is less than 1.32%.\[10\] Another study took the costs of surveillance harms into consideration by simulating 1 million patients with compensated cirrhosis. Biannual
surveillance with ultrasonography plus alpha-fetoprotein (AFP) would be cost-effective for an HCC incidence rate >0.4% provided by surveillance adherence >19.5% if the willingness-to-pay threshold was set at $100,000.\textsuperscript{[11]} It should be noted that the cost-effective analyses were based on the Markov model, but a prospective interventional study is lacking; furthermore, the analyses did not consider benefits or costs of emerging HCC treatment modalities as well. Importantly, the willingness-to-pay threshold is largely dependent on the support of the healthcare system, which tends to vary among regions. To this end, it is difficult to make a conclusion in defining the best candidates for post-SVR surveillance if the decision merely relies on cost-effective judgments.

All international societies agree that cirrhotic patients should receive HCC surveillance although different recommendations are being suggested according to different regional consensus (Table 1).\textsuperscript{[6, 12-15]} The Asian Pacific Association for the Study of the Liver (APASL) recommends screening all SVR patients with sonography and tumor markers including AFP, protein Induced by Vitamin K Absence or Antagonist-II (PIVKA-II) and AFP-L3. For patients with fibrotic stage 0-2, surveillance should be performed every 6 months for the first two years then annually. For patients with fibrotic stages 3-4, surveillance should be performed every 6 months. The European Association for the Study of the Liver (EASL) recommends screening patients with fibrotic stages 3-4 every 6
months. EASL does not recommend using tumor markers as the screening tool due to potential false positivity. By contrast, the recent American Association for the Study of Liver Diseases (AASLD) guideline suggests screening cirrhotic patients every 6 months by sonography in addition to the tumor marker, AFP. The Taiwan Association for the Study of the Liver (TASL) has more stringent recommendations, which suggests screening for fibrotic stage 0-1 (F0-1) with HCC risk factors and fibrotic stage 2 every 6-12 months and fibrotic stage 3-4 every 3-6 months. [6, 12-15]

A controversy exists whether patients with F3 could be discharged [10] or should receive regular post-SVR HCC surveillance since fibrosis regression after HCV eradication would also decrease HCC risk. [16] A meta-analysis showed that the incidence of HCC after HCV eradication by DAAs was 2.99 per 100 person-years and 0.47 per 100 person-years in cirrhotic and non-cirrhotic patients respectively. For patients with F3, the incidence of HCC was 0.63 per 100 person-years. Based on the relatively low incidence of HCC, the authors concluded that screening HCC for F3 patients was not warranted. [17] Notably, the definition of each fibrotic stage was not universal across studies and misclassification would exist. APASL did not mention the definition of each fibrotic stage, and only EASL defined F3 by histology or liver stiffness measurement (transient elastography 10-13 kPa, Aixplorer 9-13 kPa or Acoustic Radiation Force Impulse 1.6-2.17 m/s). [18]
Accordingly, the management of SVR patients with advanced fibrosis should be individualized in terms of local healthcare policies.

For patients with mild fibrosis, those with comorbidities or ongoing risk behaviors (alcohol use, diabetes [DM], obesity) shall be kept for HCC surveillance.\(^{[12]}\) DM is well-recognized as the oncogenic factor of HCC through the mechanism of the hyperinsulinemia-related PI3K/AKT/mTOR-signaling pathway, oxidative stress and chronic inflammation.\(^{[19]}\) Pre-DM status has even been reported to carry a higher risk of HCC than normoglycemic status in SVR patients with mild fibrosis.\(^{[20]}\) Meanwhile, metabolic dysfunction (MS) has been shown to increase HCC risk in SVR patients with advanced fibrosis. Patients with MS, in particular with the existence of DM, had a 3.03-fold risk of HCC compared to those without MS.\(^{[21]}\) As increased body weight and hepatic steatosis have been reported after HCV eradication \(^{[22]}\), the concurrence of steatotic liver disease should be incorporated with HCV infection with respect to holistic care in the post-SVR era \(^{[23]}\) since it might be an independent risk factor for HCC after HCV eradication.\(^{[24, 25]}\)

**How long should post-SVR HCC surveillance be maintained?**

A study using a microsimulation model suggested that rather than lifelong monitoring, screening for post-SVR HCC is cost-effective up to age 70 in those with cirrhosis and up to age 60 in those
with stable advanced fibrosis.\textsuperscript{[26]} The aforementioned meta-analysis indicated a pooled HCC incidence after SVR in patients with cirrhosis was very high (2.99/100 person-years) but would decline as time went by after HCV eradication.\textsuperscript{[17]} For example, the incidence of HCC was 6.17% for studies with a follow-up period less than 1 year and decreased to 1.83% for studies with a follow-up period greater than 3 years. Notably, HCC risk remains and persists up to decades after HCV eradication.\textsuperscript{[27, 28]} All the three regional guidelines suggest post-SVR surveillance should be maintained indefinitely for the recommended target populations.\textsuperscript{[6, 12, 13]}

**Risk factors and predictors of post-SVR HCC**

Plenty of risk factors or surrogate markers have been identified to predict post-SVR HCC.\textsuperscript{[15]} As mentioned earlier, liver cirrhosis per se is the major risk factor predictive of post-SVR HCC. Liver fibrosis would augment after HCV eradication, and post-treatment liver fibrotic change could be more accurate than the pre-treatment status in predicting HCC.\textsuperscript{[16]} Of the non-cirrhotic patients, several surrogate markers/predictors have been reported, which could be briefly divided into fibrosis-related (age, platelet count, aspartate aminotransferase [AST] to platelet ratio index [APRI], fibrosis-4 index [FIB-4], AST/alanine aminotransferase [ALT] ratio, albumin) or non-fibrosis-related (DM, HCV genotype 3, AFP and gamma-glutamyl transferase level).\textsuperscript{[20, 27-33]}
Developing an HCC prediction model by weighing and combining individual risk factors may help to promote risk stratification (Table 2). A web-based, model-guided strategy has been developed to facilitate HCC screening, of which age, platelet count, AST/ALT ratio and albumin level were the four major determinants. A dynamic transient elastography-based model has also been created to identify very low risk-patients, which helps to avoid unnecessary surveillance. An algorithm that combines age liver stiffness measurement, alcohol consumption, albumin and AFP in SVR patients with advanced liver chronic disease successfully stratified HCC risk, while an artificial intelligence-based prediction model using the recurrent neural network of age, sex, race, HCV genotype and 24 laboratory tests has proven to be more accurate than using the traditional regression model. A decision-tree algorithm combining gene score including TAS1R3, FOSL1 and ABCA3 and FIB-4 has also been created to predict post-SVR HCC. Nevertheless, regarding all these contributions to the field, the “black box” of artificial intelligence-based study outcomes awaits further validation in clinical practice.

**HCC recurrence after achieving SVR**

Unlike CHC patients who received interferon-based therapy, an increased HCC recurrent risk was postulated in the early era of DAAs. A large-scale study did not suggest a higher HCC recurrence
rate in DAA-treated patients compared to those untreated. Following this, pooled analysis also did not reveal a higher HCC recurrence risk. Owing to the heterogeneity of patient characteristics and varying follow-up periods among published papers, Sapena et al. conducted an individual patient-data-based meta-analysis from 21 studies and found that the HCC recurrence rate did not differ between DAA-exposed (14.75 per 100 person-years) and -unexposed (23.21 per 100 person-years) patients after a median follow-up period of 15 months. The result highlights the fact that the recurrence rate remained high even after HCV eradication, and CHC patients with curative HCC should receive close follow-up after achieving SVR. APASL recommended following SVR patients with HCC history every 4 months.

**Genetic polymorphism and somatic mutation signatures post SVR**

HCC is a multifactorial disease that is the result of genetic and epigenetic alterations, followed by the process of selection. Recent extensive sequencing of liver cancer samples identified genomic signatures and driver genes associated with HCC. However, the genomic profile of liver genomes after SVR is currently poorly defined. A lower frequency of mutated ARID genes in HCV-SVR as compared to HCV-positive tumors was found; mutations in this gene are specifically
induced in HCV-related HCC\textsuperscript{[49]}. In contrast, mutations in the KEAP1 and PREX2 genes were more frequently identified in HCV-SVR samples as compared to HCV-positive samples\textsuperscript{[48]}, and were previously reported in HCC\textsuperscript{[50]}. We have summarized the mechanisms of post-SVR HCC in revised Table 3 of the revised version.

These mutations result in resistance to oxidative stress while inducing metabolic transformation of cancer cells\textsuperscript{[51]}, and therefore, could be valid therapeutic and prognostic markers for HCC post-curation.

Recent findings show significant differences between HCV-related HCC following treatment with DAAs vs. IFN. In SVR patients, TP53 mutations as well as genomic abnormalities were significantly more frequent in DAA-treated as compared to IFN-treated patients\textsuperscript{[48]}, suggesting that mutations in TP53 result in genomic instability after SVR by DAAs and might serve as prognostic markers for post-cured HCC specifically after DAAs treatment. In contrast, in IFN-treated patients, a higher activation of the PI3K/AKT/mTOR pathway was observed and was associated with tumor aggressiveness and invasive phenotypes\textsuperscript{[48]}.

In addition to somatic mutations, several recent genome-wide association studies (GWAS) have shown high prevalence of alleles in specific loci in HCV-related HCC cases, which bear potential as
markers for predisposition for HCC, also after SVR. For example, the 5’ flanking region of MHC class I chain-related A (MICA) (6p21.33) in the major histocompatibility complex (MHC) of class I region \(^{52,53}\), and single nucleotide polymorphisms (SNPs) in the HLA-DQB1 locus \(^{54}\), are associated with HCC. In SVR patients, a variant of the gene toloid-like 1 gene (TLL1) on chromosome 4 (rs17047200), associated with the TGFβ signaling pathway, was suggested as a marker of increased risk for HCC \(^{55}\). Recently, a genetic risk score associated with hepatic steatosis including patatin-like phospholipase domain-containing protein 3 (PNPLA3), transmembrane 6 superfamily member 2 (TM6SF2), membrane bound O-acyltransferase domain-containing 7 (MBOAT7), and glucokinase regulator (GCKR) was reported to be related to HCC development in cirrhotic patients \(^{56}\). Consequently, hepatic fat might be a prognostic marker for HCC development in patients cured by DAAs and a target for chemoprevention. Polymorphism in interferon-λ3 (formerly known as interleukin-28B [IL28B]) was also found to be associated with increased risk for HCC pre- and post-SVR \(^{57}\).

To summarize, genetic predictors for HCC, either genetic variants or somatic mutations in cirrhotic patients, could enable stratification of cured patients for personalized HCC monitoring. Since gene expression profiles encompass both the epigenetic landscape and genomic aberrations, more
Multi-omics studies are required to elucidate the relationship between the two and their relative contributions to cancer progression.

**Epigenetics and gene expression scar post SVR**

Epigenetics is the study of heritable events occurring in the genome that determine chromatin structure but not in the DNA sequence, including post-translation histone modifications (PTMs), DNA methylation and RNA-based mechanisms, and affects transcription programs \[^{[58, 59]}\]. These modifications may shift between active and silent states, resulting in activation or repression of gene expression \[^{[60]}\]. They depend on the activity of specific enzymes such as histone acetyltransferase (HAT), Gcn5/PCAF and p300/CMAP, histone deacetylase (HDACs) and histone methyltransferase enzymes (HMTs) \[^{[61, 62]}\].

Viruses can impose epigenetic changes that alter host transcription programs, thereby promoting their own propagation, and may contribute to cancer occurrence. We and others have recently shown that the altered epigenetic state associated with HCV infection persists, to some extent, even after cure following DAAs and IFN-based treatments \[^{[63-67]}\]. This observation is consistent in various HCV infection models, including immortalized human liver and hepatoma cells, a human liver chimeric mouse model, and post-SVR human liver samples. In cell culture, HCV–induced
changes in active chromatin markers H3K4Me3 and H3K9Ac and silent chromatin marker H3K9Me3 were associated with altered expression of genes involved in cancer-related pathways. A positive correlation was observed between chromatin modification and gene expression in HCV-infected cells, both before and after virus eradication by DAAs [63]. This persistent epigenetic imprint was recapitulated in pre- and post-SVR human liver samples, even years after virus eradication by DAAs. Interestingly, our data indicate more reversion of both RNA and epigenetic marker levels following IFN-based compared to DAAs-based treatment, which is in agreement with higher risk for HCC development in DAAs vs. IFN-cured patients reported in several publications [68-71]. Hamdane et al. showed that chronic HCV infection induces genome-wide changes in active histone modification of H3K27Ac following SVR with DAA or IFN therapies, many of which persisted after HCV cure, depending on the liver fibrosis stage. These changes were found to be partly induced by direct HCV–hepatocyte interactions, as demonstrated in a HCV-infected human liver chimeric mouse model that did not develop inflammation or liver fibrosis. Collectively, the data demonstrated that both direct virus-mediated and indirect inflammation and fibrosis-mediated mechanisms contribute to the epigenetic changes in HCV-infected patients that are imprinted after cure with DAA [64]. Jühling et al. found that both HCV- and non-alcoholic
steatohepatitis (NASH)-related HCC have common epigenetic alterations, with a positive correlation between the epigenetic alteration in H3K27ac and transcriptomics, which mostly did not reverse after cure [65].

Imprint of global changes in HCV-induced DNA methylation has also been reported recently, where HCV-infected Hu1545-immortalized hepatocyte cells showed significant changes in DNA methylation that correlated with oncogenic gene expression after IFN- and DAAs-based treatments. The activated pathways were associated with disease development and HCC. Interestingly, IFN treatment in the absence of active HCV induced a similar epigenetic scar. Moreover, HCV was shown to persistently suppress innate immune pathways, including TLR3 activity, via epigenetic changes [66]. The effect of DNA methylation on transcription factors (TFs) that regulate gene expression was also recently evaluated pre- and post-SVR, which remain dysregulated after HCV eradication [67].

HCV infection induces changes in host cell epigenetics and gene expression related to pathways that may be required for the virus life cycle but which also contribute to carcinogenesis. Gene signatures associated with increased risk for HCC were found to intersect with epigenetic and gene expression scars [63, 64, 67]. These persistently altered genes and pathways are summarized in
Table 3. Overall, these data all point to the involvement of the epigenetic scar in post-SVR hepatocarcinogenesis.

**Immune scars and immunosurveillance post SVR**

The nature of the immune response induced by HCV infection determines the outcome of infection, i.e., whether it resolves or progresses to chronic infection, and contributes to HCC development. Since complete viral elimination by DAAs is possible, HCV infection is a unique model to study the effect of infection and its eradication on immune responses and clinical outcomes. Due to the residual risk of liver diseases and HCC after cure with DAAs, it is important to understand whether the HCV-induced alterations in the immune response return to normal after viral eradication. Indeed, recent studies have reported that the altered characteristics and functions of various immune cells in chronic HCV infections persist to some extent as an immunological imprint after cure with DAAs; accordingly, the elimination of HCV by DAAs and its influence on the immune response could affect the development of hepatocarcinogenesis.

Overall, the innate immune response following HCV cure is only partially restored. A decrease in ISGs expression and type I IFN response in peripheral blood mononuclear cells (PBMCs) in the liver was observed after DAAs treatment, therefore resulting in a weaker antitumor-immune state.
and contributing to hepatocarcinogenesis\textsuperscript{[72-75]}. In patients with acute or chronic infection following virus elimination by DAAs treatment\textsuperscript{[76, 77]}, and spontaneously resolved HCV infections\textsuperscript{[78]}, cytokines levels were decreased but still not returned to normal range. Yet, most of these studies were conducted within several months after treatment, and longer follow-up studies are still required.

NK cells play an important role in the innate anti-HCV immune response\textsuperscript{[79]}, but are damaged in chronic HCV infections\textsuperscript{[80, 81]}. Following HCV eradication by DAAs, some of the damaged phenotypes and functions of NK cells were reversed\textsuperscript{[82-84]}, while some functions persisted, such as decreased intra-individual NK cell diversity\textsuperscript{[85]}. NK cells also bear antitumor activity, and their frequency is associated with HCC recurrence-free survival\textsuperscript{[86]}. Downregulation of NK group 2D (NKG2D), which is important for NK cell antitumor activity, has been reported in association with HCC occurrence and recurrence post-DAA therapy\textsuperscript{[87, 88]}. In addition to NK cells, unique innate-like T-cells such as γδ T-cells and mucosa-associated invariant T (MAIT) cells were found to be impaired pre- and post-cure of chronic HCV infections\textsuperscript{[89-92]} and may underlie pathologies and HCC development post-cure\textsuperscript{[93, 94]}.

An imprinted adaptive immune response was also observed after HCV cure. Effective CD8+ and
CD4+ T cell responses were associated with HCV clearance, while weak and exhausted responses were associated with chronic infection \[95\]. Persistent induction of T cells in chronic infection leads to T-cell exhaustion, which was only partially restored after cure with DAA- and IFN-based treatments \[96-99\]. Specifically, mitochondrial function \[96\] and transcription programs \[100\] of exhausted HCV-specific CD8+ T-cells were not fully recovered following cure, and were associated with a distinct epigenetic signature \[97, 101-103\], and post-cure HCC development \[104\]. Furthermore, CD4+ T cells remained impaired in chronic HCV infections after cure with DAAs \[105\].

The levels of circulating regulatory T cells (T regs) remain persistently high long after HCV cure with DAAs \[106, 107\], and increase with HCC progression \[108\], suggesting that high T regs levels after DAAs treatment may be related to post-cure HCC. T regs count correlate with myeloid-derived suppressor cell (MDSC) counts, which are increased in chronic HCV patients and remain high following cure with DAAs \[109\].

To summarize, although some functions of the innate and adaptive immune responses associated with HCV infections are normalized after HCV cure with DAAs, many persist after cure. These may lead to a pro-cancerous environment that may contribute to post-cure HCC. Understanding the molecular mechanisms that impact these immunological scars may set foundations for their
prevention or reversion, though, for example, epigenetic drugs that revert the epigenetic scar in immune cells.

**Proteomic and metabolomic prognostic markers post SVR**

The changes of the levels of cytokines and chemokines after achieving SVR have been proposed as predictors of HCC. Lu et al. showed that downregulation of members of the TNF superfamily, including TNF-α and TNF-like weak inducer of apoptosis (TWEAK), increased the risk of HCC development [110]. A strong prediction model for post-SVR HCC treated with either DAA or IFN included fibrosis-4 index (FIB-4), hemoglobin A1c, and levels of TNF-α and TWEAK. Increase in TNF-α levels are associated with increased hepatic inflammation and HCC risk, suggesting that its pretreatment concentration predicts post-SVR HCC risk and an association between its persistent high expression after SVR and the development of HCC [111]. The sharp decline in TNF-α after cure may impair immune surveillance and inhibit antitumor response. Moreover, a correlation between high pretreatment serum levels of 12 immune mediators and post-treatment HCC development was identified [111, 112], as well as high levels of IL-13 and IL-4 [113].

Circulating protein biomarkers of HCC such as AFP have also been suggested as prognostic markers. More specifically, higher pre- and post-treatment levels of AFP were associated with HCC
development \cite{24, 114-116}. However, the accuracy in particular the sensitivity of AFP is an issue \cite{117}.

Other circulating protein biomarkers including wisteria floribunda agglutinin-positive Mac-2-binding protein (WFA+ M2BP) \cite{116}, serum sphingolipids\cite{118}, VEGF and angiopoietin-2 (ANGPT2) \cite{119, 120} also associate with post-curative HCV related HCC occurrence. A large cohort study found that the MICA A allele and high serum MICA (sMICA) levels correlated with HCC development, but only in cirrhotic non-SVR patients \cite{121}. A follow-up study found its levels to be lower and to gradually decline in non-HCC compared to HCC patients, and higher sMICA levels that gradually increased in post-SVR HCC, but only in MICA GG and not A allele carriers \cite{122}.

Circulating microRNA (miRNA) profiles have also been suggested as biomarkers for HCC development. MiR-3197 was identified as a potential prognostic marker for HCC risk during DAA treatment \cite{123}. Circulating miRNA levels of members of the Let-7 family were associated with fibrosis progression, were downregulated in HCV infection and lower in patients who developed HCC after SVR compared to those without HCC. This may be related to the antitumor activity of Let-7, which downregulates chronic inflammation \cite{124}.

**Modulation of risk gene signature with targeting agents for HCC post SVR**
Identification of potentially reversible HCV-related alterations in epigenetics and gene expression might contribute to efforts to reduce risk of HCC post-SVR. The discovery of druggable targets requires the elucidation of molecular mechanisms that drive these altered signatures. Recently, specific molecular pathways were identified as inducers of the epigenetic state dysregulated by HCV and as potential targets for HCC chemoprevention. Nakagawa et al. studied the pan-etiology PLS gene predictive of risk for HCC both before and after SVR and identified the pro-fibrosis lysophosphatidic acid (LPA) pathway as a potential chemoprevention target. Inhibition of this pathway by inhibitors AM063 and AM095 resulted in reversal of the altered expression of gene signature after SVR and fibrosis attenuation and prevented HCC development in animal and in vitro models \[125\]. Another potential druggable target is the EGFR, which is a cofactor for HCV entry into cells and is also activated by HCV and contributes to HCC development \[126-128\]. The EGFR inhibitor erlotinib induced reversion of the PLS genes altered expression and prevented progression of cirrhosis and HCC in animal models \[126\]. We found that erlotinib reversed gene expression and epigenetic signatures after HCV cure with DAAs \[63\]. In addition, the unfolded protein response (UPR) that is activated by HCV has been reported to contribute to HCV-induced epigenetic and transcriptional alterations; treatment with the UPR inhibitor BAPTA partially reversed this effect \[65\].

Targeting epigenetic enzymes as a genome-wide approach was recently demonstrated to efficiently reverse the epigenetic and gene expression signatures associated with HCV infection. We showed that the histone acetyl transferase (HAT) p300/CBP inhibitor C646 reversed the persistent changes in H3K9Ac induced by HCV and the associated gene expression signature \[63\].
A panel of inhibitors targeting epigenetic enzymes, including HATs, bromodomain-containing proteins 3/4 (BRD3/4), mixed-lineage leukemia protein/WD repeat domain 5 (MLL/WDR5) complexes and HDACs, reversed the altered expression of the PLS genes in an HCV-infected cell culture model [65]. In addition, the authors observed common gene expression patterns between the HCC etiologies HCV and NASH, and the reversion of this signature following treatment with the BRD4 inhibitor as well as inhibition of cancer progression and liver inflammation in a NASH mouse model [65]. Further studies are urgently needed to identify additional druggable targets for prevention of liver disease and HCC development both pre- and post-SVR.

Chemoprevention of post-SVR HCC

Cigarette smoking is demonstrated as the risk of liver fibrogenesis and hepatocarcinogenesis, and smoking cessation may decrease the risk of HCC.[129] Whether quitting smoking reduces post-SVR HCC in CHC patients remains to be explored. Albeit DM is a risk factor for HCC, metformin use seemed to play a protective role in CHC-related HCC.[130] Tsai et al. enrolled 7,249 Taiwanese CHC patients who achieved SVR, and the 5-year cumulative HCC incidence was 10.9% in diabetic non-metformin users and 2.6% in diabetic metformin users, compared to 3.0% in individuals without DM. Diabetic patients without metformin use had a 2.83-fold risk of HCC compared to non-diabetic patients, whereas the risk of HCC in diabetic patients who used metformin had reduced
and had similar risk of HCC as with non-diabetic patients.\textsuperscript{[131]} Recent meta-analysis and pooled data has showed that statin and aspirin might reduce HCC risk as a whole \textsuperscript{[132, 133]}, and while the role of aspirin in preventing post-SVR HCC in CHC is elusive. A Taiwanese cohort has demonstrated the chemopreventive effect of statin in reducing HCC risk in SVR patients.\textsuperscript{[131]}

**Impact of antiviral therapy on HCV viremic HCC patients**

Earlier reports have indicated an inferior SVR rate in HCC patients who received DAAs. A meta-analysis including 49 studies has shown a lower SVR rate of 73.1 % in active HCC patients compared to that of 92.6% in inactive patients and of 93.3 % in non-HCC patients.\textsuperscript{[134]} Notably, much of the data came from the reports using suboptimal regimens in the early DAA era. With the current standard of care regimens, sofosbuvir/velpatasvir and glecaprevir/pibrentasvir used in two nationwide studies from Taiwan have validated similar treatment efficacy among patients with/without inactive or active HCC.\textsuperscript{[135, 136]} HCC is no longer an unfavorable factor associated with treatment failure with the application of more potent DAAs. Imperatively, long-term survival would be better for the viremic HCC receiving subsequent HCV eradication compared to the persistently viremic HCC.\textsuperscript{[137, 138]} A postulation is that HCC patients might benefit from viral eradication in terms of more preserved liver function for salvaging anti-cancer treatments once
the patients encounter primary HCC treatment failure. The result suggests that it is better-late-than-never to treat HCV. CHC patients with active HCC should be treated with DAAs aggressively unless a short life expectancy due to HCC is anticipated.

Lastly, HCV eradication significantly reduce hepatic vein pressure gradient. Recompensation occurs in a substantial proportion of decompensated patients. The benefit of HCC risk reduction in decompensated patients during the recovery of liver function reserve is controversial. The conduction of a prospective treated-versus-untreated controlled trial is unethical and impractical. Recently a meta-analysis including 4 retrospective studies have demonstrated a marginal benefit of 26% HCC risk reductions (95% CI: 0.52, 1.00; \( p = 0.05 \)) in DAA treated decompensated patients compared to untreated control.\[139\]

In conclusion, post-SVR HCC remains as occurring in a subset of CHC patients due to preexisting inflammatory and fibrotic liver background, immune dysregulation as well as host epigenetic scar, genetic predispositions and alternations (Figure 1). There are remaining unmet needs in post-SVR HCCV surveillance and management (Table 4). By means of applying surrogate markers and adopting risk stratification, HCC surveillance should be consistently performed in high-risk populations.
**Conflict of Interests**

Ming-Lung Yu

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62. Balasubramanyam K, Swaminathan V, Ranganathan A, Kundu TK. Small molecule modulators


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134. Ji F, Yeo YH, Wei MT, Ogawa E, Enomoto M, Lee DH, et al. Sustained virologic response to


<table>
<thead>
<tr>
<th></th>
<th>APASL</th>
<th>EASL</th>
<th>AASLD</th>
<th>TASL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Target population</td>
<td>All patients (C2)</td>
<td>F3, F4 (A1)</td>
<td>F4 (B2)</td>
<td>All patients (B1)</td>
</tr>
<tr>
<td>Screening for patients with mild fibrosis with comorbidities</td>
<td>Yes (A1)</td>
<td>Yes (A1)</td>
<td>No (B2)</td>
<td>Yes (B1)</td>
</tr>
<tr>
<td>Screen Interval</td>
<td>F0-2: every 6 months for 2 years, then every 12 months F3-4: every 6 months (C2)</td>
<td>Every 6 months indefinitely (A1)</td>
<td>Every 6 months indefinitely (A1)</td>
<td>F0-1 with HCC risk factors* and F2: every 6-12 months (B1) F3-4: every 3-6 months (A1)</td>
</tr>
<tr>
<td>Modality</td>
<td>Sonography+ tumor markers (AFP, PIVKA-II, AFP-L3) (A1)</td>
<td>Sonography (B1)</td>
<td>Sonography with AFP (B1)</td>
<td>N/A</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Regimen</th>
<th>Parameter</th>
<th>Accuracy/discrimination power</th>
<th>Note</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN; IFN plus DAA; DAA</td>
<td>Major determinant: age, platelet count, AST/VALT ratio and albumin level. Minor determinant: sex, race-ethnicity, HCV genotype, body mass index, hemoglobin and AFP</td>
<td>Gonen and Heller’s $\kappa$-statistic 0.70-0.77</td>
<td>excellent correlation in patients with cirrhosis/SVR; no cirrhosis/no SVR; and no cirrhosis/SVR, and moderate correlation in patients with cirrhosis/no SVR</td>
<td>33</td>
</tr>
<tr>
<td>DAA</td>
<td>Baseline LSM, 1-year delta-LSM and albumin</td>
<td>Harrell’s C: 0.77</td>
<td>Predict patients with very low risk of HCC to avoid unnecessary surveillance</td>
<td>34</td>
</tr>
<tr>
<td>DAA</td>
<td>Age, LSM, alcohol consumption, albumin and AFP</td>
<td>Bootstrapped AUC 0.67-0.80</td>
<td>Stratify HCC risk in patients with compensated advanced chronic liver disease</td>
<td>35</td>
</tr>
<tr>
<td>IFN; DAA</td>
<td>FIB-4 and gene score including post-treatment TAS1R3, FOSL1 and ABCA3</td>
<td>AUC 0.91 in the gene score and 0.95 in the nomogram</td>
<td>Decision-tree-based algorithms based on genetic alternations and clinical profile</td>
<td>37</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Molecular mechanisms</th>
<th>Characteristics</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Genomic factors</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Somatic mutations</td>
<td>ARID: Lower frequency of mutations in HCV-SVR as compared to HCV-positive tumors</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>PREX2, KEAP1: More frequently identified in HCV-SVR samples as compared to HCV-positive samples and were previously reported in HCC</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>TP53: More frequent in DAA-treated as compared to IFN-treated patients</td>
<td>48</td>
</tr>
<tr>
<td><strong>Genetic predisposition</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MICA</td>
<td>Associated with reduced counts of NK and CD8+ T cells. Marker for predisposition for HCC.</td>
<td>52</td>
</tr>
<tr>
<td>TLL1</td>
<td>Associated with TGFβ signaling pathway. A variant is a marker for increases risk for HCC post SVR.</td>
<td>55</td>
</tr>
<tr>
<td>Genetic risk score:</td>
<td>PNPLA3, TM6SF2, MBOAT7, GCKR: Specific variants are associated with predisposition for HCC pre and post SVR</td>
<td>56</td>
</tr>
<tr>
<td>IFNL3</td>
<td>Polymorphism is associated with increases risk for HCC pre and post SVR.</td>
<td>57</td>
</tr>
<tr>
<td><strong>Epigenetics and gene expression</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytoskeleton, epithelial—</td>
<td>Genes and pathways persistently altered pre and post SVR by</td>
<td>63-65</td>
</tr>
</tbody>
</table>
mesenchymal transition, WNT, Development, Immune response, B-Raf, NGF, mTOR/MAPK, Lipid metabolism, TNFα, G2M checkpoint, cell cycle, phosphoinositide 3-kinase, Akt, Oncogenes (FGFR1, CCND2, MLLT3, MAML2) and Tumor suppressor genes (FANCC, TSC2)

TLR3 and innate immune response genes, TFs (RXRA, KLF4, RUNX1, and RORA) Genes and TF persistently altered pre and post SVR by epigenetic dysregulation, identified by DNA methylation markers and associated with HCC development.

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**Immune scar and immunosurveillance**

**Innate immunity**

ISGs expression and type I IFN response Decreased after DAAs treatment; reduce immune surveillance and increase risk for HCC post SVR. Partially persistent after SVR and spontaneously resolved infections.
<table>
<thead>
<tr>
<th>Immune Component</th>
<th>Description</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>NK cells</td>
<td>Damage of NK functions partially persist after SVR: decreased intra-individual NK cell diversity, decreased of NK group 2D (NKG2D), associated with HCC occurrence and recurrence post DAA.</td>
<td>77-88</td>
</tr>
<tr>
<td>Innate-like T-cells (γδ T-cells MAIT)</td>
<td>Impaired pre and post cure of chronic and acute HCV infections, associated with HCC development post cure.</td>
<td>89-92</td>
</tr>
<tr>
<td>HCV-specific CD8 + T-cells</td>
<td>Partial restoration of CD8 + T-cells exhaustion post cure with DAAs and IFN-based treatments, impaired mitochondrial function, transcription program, epigenetic signature and TF post cure.</td>
<td>97,101-103</td>
</tr>
<tr>
<td>Adaptive immunity</td>
<td>CD4+ T cells</td>
<td>Impaired in chronic HCV infections post cure by DAAs.</td>
</tr>
<tr>
<td></td>
<td>T regs</td>
<td>Remain persistently high long after HCV cure by DAAs, related to post cure HCC.</td>
</tr>
<tr>
<td>Prognostic markers</td>
<td>Cytokines and chemokines biomarkers</td>
<td>Downregulation after SVR increased the risk of HCC development.</td>
</tr>
<tr>
<td></td>
<td>TNF-α and TWEAK</td>
<td>Prediction model for post SVR HCC.</td>
</tr>
<tr>
<td></td>
<td>FIB-4 with HbA1c, TNF-α and TWEAK</td>
<td>Decline may affect immune</td>
</tr>
</tbody>
</table>
surveillance and inhibit antitumor response. Persistent high expression after SVR is involved in HCC.

### Protein biomarkers

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Description</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIG, IL22, TRAIL, APRIL, VEGF, I3, TWEAK, SCF, IL21</td>
<td>High serum levels predict de novo HCC development post cure.</td>
<td>111,112</td>
</tr>
<tr>
<td>IL-13 and IL-4</td>
<td>High serum levels predict HCC development post cure.</td>
<td>113</td>
</tr>
<tr>
<td>AFP</td>
<td>Higher levels pre and post treatment associated with HCC after SVR by DAAs.</td>
<td>117</td>
</tr>
<tr>
<td>Albumin and platelets</td>
<td>High levels associated with risk of HCC following SVR by DAAs.</td>
<td>33,35</td>
</tr>
<tr>
<td>WFA and M2BP</td>
<td>Biomarkers associated with increased risk for de novo HCC post SVR by DAAs.</td>
<td>216,118</td>
</tr>
<tr>
<td>Sphingolipids</td>
<td>Increased circulating levels associated with occurrence and recurrence of HCC development after DAA treatment.</td>
<td>119,120</td>
</tr>
<tr>
<td>VEGF and ANGPT2</td>
<td>High serum levels correlated with HCC development, but only in cirrhotic non-SVR patients.</td>
<td>121,122</td>
</tr>
</tbody>
</table>

### microRNA

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Description</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MiR-3197</td>
<td>Lower levels identified as a potential prognostic marker for HCC risk during DAA treatment.</td>
<td>123</td>
</tr>
<tr>
<td>Let-7 family</td>
<td>Lower levels in patients who developed HCC after SVR compared to those without HCC.</td>
<td>124</td>
</tr>
</tbody>
</table>

### Chemoprevention
<table>
<thead>
<tr>
<th>Inhibition of</th>
<th>LPA</th>
<th>Inhibitors AM063 and AM095 reverse the altered expression of gene signature after SVR and fibrosis attenuation and prevented HCC development.</th>
</tr>
</thead>
<tbody>
<tr>
<td>signaling pathways</td>
<td>EGFR</td>
<td>Erlotinib reversed HCV-induced gene expression and epigenetic signatures after HCV cure with DAAs.</td>
</tr>
<tr>
<td></td>
<td>UPR</td>
<td>BAPTA reversed HCV-induced gene expression and epigenetic signatures after HCV cure with DAAs.</td>
</tr>
<tr>
<td>Inhibition of</td>
<td>HATs</td>
<td>Inhibitors reversed HCV-induced gene expression and epigenetic signatures after HCV cure with DAAs.</td>
</tr>
<tr>
<td>epigenetic modifiers</td>
<td>BRD3/4 MLL/WDR5 inhibitors, HDACs</td>
<td></td>
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</table>
Table 4. Unmet needs for the post-SVR HCC

- Identify the target population for surveillance on the cost-effective basis
- Define the screen interval and duration after achieving SVR
- Adopt the precise screening tools including image modalities and biomarkers
- Marginal benefit of HCC risk reduction in patients with decompensated liver cirrhosis
- Predict the high risk population based on the pathophysiological mechanisms
- Construct a precision-medicine guided strategy that incorporates clinical and molecular surrogates
Figure 1. Scheme of molecular mechanisms of hepatocellular carcinoma after HCV eradication. Development of HCC pre and post SVR is related to impaired immune response and immune surveillance, epigenetic and gene expression alterations and genomic factors. Identified persistent mechanisms that remain impaired after HCV SVR and prognostic markers for HCC risk post SVR and HCC chemoprevention targets are shown.