

# Non-alcoholic steatohepatitis and risk of hepatocellular carcinoma

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## Abstract

The emergence of non-alcoholic fatty liver disease (NAFLD) as the leading chronic liver disease worldwide raises some concerns. In particular, NAFLD is closely tied to sedentary lifestyle habits and associated with other metabolic diseases, such as obesity and diabetes. At the end of the disease spectrum, non-alcoholic steatohepatitis (NASH) may progress to cirrhosis and hepatocellular carcinoma (HCC), representing a serious health problem to modern society. Recently, an increasing number of HCC cases originating from this progressive disease spectrum have been identified, with different levels of severity and complications. Updating the current guidelines by placing a bigger focus on this emerging cause and highlighting some of its unique features is necessary. Since, the drivers of the disease are complex and multifactorial, in order to improve future outcomes, having a better understanding of NASH progression into HCC may be helpful. The risks that can promote disease progression and currently available management strategies employed to monitor and treat NASH-related HCC make up the bulk of this review.

**Keywords:** Non-alcoholic fatty liver disease; Non-alcoholic steatohepatitis; Liver disease; Hepatocellular carcinoma; Insulin resistance; Oxidative stress; Metabolic associated fatty liver disease

## Introduction

Despite non-alcoholic fatty liver disease (NAFLD) affecting a great number of people (about one-quarter of the global population), only a small quantity of patients progresses beyond the initial stages of the disease into more severe conditions, with the onset of end-stage liver disease affecting less than 13% of NAFLD patients.<sup>[1]</sup> These stages are a reflection of the disease spectrum, characterized by specific histological signs, that range from non-alcoholic fatty liver (NAFL), to non-alcoholic steatohepatitis (NASH), to fibrosis and then cirrhosis.<sup>[2]</sup> Following this 'exacerbation path', after cirrhosis, patients develop hepatocellular carcinoma (HCC) and end-stage liver disease. Despite affecting a large portion of the population worldwide, we do not fully understand all factors contributing to disease progression, as well as the impact these factors have on clinical outcomes.

NASH is defined by an accumulation of fat in hepatocytes (>5%) together with measurable signs of cell injury (hepatocyte ballooning and lobular inflammation) that are detected through histological examination. However, the fact that these histological changes to the normal liver cells

can also be observed in alcoholic hepatitis, and that a liver biopsy is required for a definite diagnosis, indicates that the current diagnostic methodology has some limitations.<sup>[3-9]</sup>

Recently, there has been a push towards updating the definition of NAFLD to a more accurate reflection of currently available knowledge. MAFLD, or metabolic-associated fatty liver disease, was proposed to an international panel of experts as an alternative nomenclature for NAFLD, where a consensus was reached regarding adopting this change, due to its many advantages such as positive diagnostic criteria, capturing the full spectrum of the disease (instead of just NASH and simple steatosis), as well as a concrete framework that includes other metabolic causes of fatty liver disease.<sup>[10,11]</sup> The diagnostic criteria of MAFLD are based on the presence of hepatic steatosis plus any of the next three criteria: elevated levels of body mass index (BMI), diabetes mellitus type 2, and evidence of metabolic dysregulation.<sup>[11,12]</sup>

Obesity carries a negative impact in NASH patients, with elevated adiposity levels, on average, being associated with increased severity of fibrosis and inflammation. Likewise, diabetes (in particular type 2 diabetes), has been identified

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as a contributor to the development of NAFLD. Similarly, NAFLD being associated with an increased risk of diabetes has also been shown to be true.<sup>[13]</sup> As for HCC, it is more frequently associated with risk factors such as age (> 65 years old), diabetes, and metabolic syndrome.<sup>[14-16]</sup>

This review aims to discuss the pathogenesis, disease progression, and current management of NASH-related HCC, which is a pertinent topic, now that NAFLD has become the most prevalent chronic liver disease in the world.

## Pathogenesis

The path from NAFLD to HCC is underscored by multiple factors. From the simple accumulation of fat in hepatocytes to the more serious state of necroinflammation and fibrosis, it is currently understood that the foundation of all complications lies in the metabolic disturbance of NAFLD patients.<sup>[17]</sup> This section will explore the role of multiple factors in NAFLD development, such as insulin resistance, lipotoxicity, oxidative stress, and DNA damage response.<sup>[17]</sup>

### Insulin resistance

Insulin resistance represents a growing problem that is exacerbated by the modern, western-influenced lifestyle and diet, and as the name states, implies a decrease in the effectiveness of the role that insulin molecules play in the regulation of glucose metabolism. Additionally, insulin resistance interferes in the metabolism of glucose, ketones, and lipids, generating an excess of free fatty acids (FFAs) that interfere with the normal function of the mitochondria in hepatocytes, playing a key role in the progression from NAFLD to HCC.<sup>[18,19]</sup> Initially, it was established that reduced insulin activation, together with lipid buildup, was associated with interference to the tricarboxylic acid (TCA) cycle, as well as, with increased production of reactive oxygen species (ROS).<sup>[18]</sup> In NASH patients, the production of ROS is almost doubled through a persistent increase in activity by the TCA cycle, despite suboptimal  $\beta$ -oxidation, insufficient lipid esterification, and impaired ketogenesis.<sup>[18,20,21]</sup> However, due to the adaptive capabilities of the hepatocytes to regulate their metabolism and to compensate for an initial disturbance (caused by increased FFAs and insulin resistance), changes in the TCA cycle may not be easily detected.<sup>[21]</sup> The problems caused by an increase in ROS, addressed in more detail in the oxidative stress segment, include inflammation, fibrogenesis, and DNA damage. Therefore, highlighting the importance of diabetes and insulin resistance in predicting more advanced stages of the disease, such as cirrhosis and HCC.<sup>[22-24]</sup>

### Lipotoxicity

Following the changes introduced by the elevated levels of circulating FFAs, we can start to observe a change in the normal metabolic pathway, namely lipotoxicity. Lipotoxicity<sup>[25]</sup> is a phenomenon that occurs when elevated levels of free fatty acids interfere with the glucose

metabolism pathway, which in turn, increases the production of toxic byproducts and the likelihood of tumor formation in the liver.<sup>[26]</sup> Due to the properties of liver tissue, namely its regenerative capabilities and its role in filtering the blood, the impact that NASH (a condition characterized by constant inflammation and, subsequently, fibrotic changes) has, is somewhat difficult to quantify. These properties also complicate the measurement of the impact on cellular DNA and, by consequence, its role in causing HCC. HCC cells show an upregulation of genes promoting *de novo* lipogenesis (a trait that is more predominant in cancer cells than in normal cells, which prefer to obtain fatty acids from circulation),<sup>[27,28]</sup> working together with the already accumulated lipids from steatosis, increasing ROS production, causing further oxidative stress in NASH patients, and, leading to HCC cells that adapt to the new lipotoxic environment (also known as lipid metabolic reprogramming<sup>[26]</sup>). Due to the elevated energy requirements of tumor cells and the need to maintain high-energy metabolism, HCC cells can adopt a lipid-dependent metabolism, particularly when located in fatty liver tissue, which is abundant in lipids.<sup>[29]</sup> Understanding this process can provide an explanation to the limitation of current anti-angiogenesis medication (e.g., sorafenib and lenvatinib), as well as propose additional options for the treatment of NASH and prevent its progression to HCC.<sup>[20,26]</sup>

### Oxidative stress

Oxidative stress is a syndrome that occurs when there is an increase in the production of free radicals (e.g. ROS) or cytotoxic oxidants (e.g. hydrogen peroxide), that damage tissues and cellular components due to unregulated oxidation.<sup>[30]</sup> This not only leads to hepatocyte apoptosis and consequently inflammation (due to immune response), but also fibrogenesis as well as DNA damage, as evidenced by studies reporting that NASH patients suffer more oxidative insults to hepatocytes than other diseases affecting the liver, particularly in patients having both NASH and HCC.<sup>[22,23]</sup> Normal physiological processes in the body produce a manageable sum of free radicals, the best example being ROS, which is a byproduct of aerobic respiration as part of adenosine triphosphate synthesis in the mitochondria. However, NAFLD patients have excessive free fatty acids being metabolized by hepatic mitochondria, leading to incomplete  $\beta$ -oxidation, impaired ketogenesis, and overproduction of ROS.<sup>[18,20,21]</sup> This saturation of ROS, together with inflammation, seems to be more often associated with oncological outcomes, observed not only in NAFLD patients but also in hepatitis patients.<sup>[24,31-34]</sup> Additionally, ROS production isn't limited to byproducts of aerobic respiration, since macrophages and neutrophils are capable of employing ROS for their cytotoxic properties.<sup>[35]</sup> Mitochondrial dysfunction can be interpreted as a response to the unhealthy environment faced by the hepatocytes in a fatty liver. The elevated amount of (FFAs) that the mitochondria have to process require an adjustment to the regular energy production mechanism, furthermore, the response that the hepatocytes have towards apoptosis, necrosis, and inflammation motivates mitochondria to undergo mutations that promote cell viability. So mutations that affect the

development of insulin resistance, rate of hepatic fat build-up, and promote metabolic reprogramming (e.g. impaired ketogenesis and increased glycolysis while in the presence of oxygen) will increase the risk of NASH-related HCC.<sup>[36]</sup>

### DNA damage response

Regarding DNA damage response, several studies have shown that carcinogenesis might not only appear as a consequence of a mutation caused by direct damage to the DNA but also as a response to the damage that is misinterpreted by anti-cancer mechanisms (e.g., ROS triggering ATM [a protein kinase that activates tumor suppression], causing apoptosis of non-cancer cells).<sup>[37-40]</sup> Various mouse models investigating the liver also compared DNA repair enzymes with the impact of oxidation on DNA, proving an inverse relationship between the two, inferring that genetic inheritance or acquired genetic characteristics of DNA repair enzymes, that differ between individuals, may be the underlying cause of variations of susceptibility to HCC and worsening of NAFLD.<sup>[37,41]</sup> Genetic predisposition to hepatic fat accumulation in the form of single nucleotide polymorphisms (SNPs) also represents a significant risk regarding disease progression to HCC.<sup>[42,43]</sup> Lastly, overexpression of DNA repair mechanisms (e.g., DNA-dependent protein kinase) that can occur when there is a greater accumulation of DNA damage (possibly triggered by an increased sensitivity of these repair mechanisms as a response), has been correlated with a worse survival rate.<sup>[17,44,45]</sup>

### Disease progression and risk factors

#### Fibrosis

Well-designed longitudinal cohorts featuring multiple liver biopsies reveal a more complex progression from NAFLD, to NASH, and fibrosis than the common linear sequence of events. Regarding fibrosis, for example, the time required to advance from one stage to the next requires almost double the amount of time for NAFLD patients when compared to NASH patients (14 and 7 years, respectively). This is of particular notice since fibrosis remains the most significant factor for predicting mortality in NAFLD patients, even when adjusting for confounding factors.<sup>[2,46-48]</sup> Accumulation of fibrotic tissue is problematic, being the most important step toward cirrhosis and HCC.<sup>[49]</sup> These bands of collagen and fibrotic changes are formed as part of the normal healing process, but without being restricted by natural degradation rates, or with continuous injury, this tissue accumulates, increasing in density and entanglement as a result, between cell colonies, and interfering with liver architecture.<sup>[50]</sup> This leads to the isolation of hepatocytes into nodules, separated by these bands, reducing blood flow and disrupting normal liver function. The cells in charge of producing this fibrotic tissue are named hepatic stellate cells, which go from a standby state (quiescent) to actively secreting tumor growth factor- $\beta$ , producing collagen and establishing an extracellular matrix.<sup>[51-54]</sup> Monitoring these changes can allow physicians to

establish an accurate prediction of disease progression rate and assess the level of severity, particularly, when compared with liver steatosis and inflammation measurement, which are easily influenced by changes (i.e., medication or lifestyle intervention).<sup>[55,56]</sup>

### Genetic factors

Many investigations during this past decade were dedicated to identifying (SNPs) or other genetic markers that demonstrate susceptibility to NAFLD, NASH, and HCC. Some examples of studied SNPs are phospholipase domain-containing 3 (*PNPLA3*),<sup>[57-59]</sup> transmembrane 6 superfamily member 2 (*TM6SF2*),<sup>[58,60]</sup> 17 $\beta$ -Hydroxysteroid dehydrogenase 13 (*HSD17B13*)<sup>[61,62]</sup> and membrane-bound O-acyltransferase domain containing 7 (*MBOAT7*).<sup>[63,64]</sup> Among these, *PNPLA3* has the most concrete correlation with fibrosis and fatty hepatocytes, based on histology grading.<sup>[65,66]</sup> *PNPLA3*, *TM6SF2*, and *MBOAT7* represent genetic variations related to fat build-up in liver cells while an inhibited *HSD17B13* variant was associated with a reduced risk of NAFL to NASH.<sup>[42]</sup> Alteration to the standard lipid metabolism can promote metaplastic changes in hepatocytes.<sup>[67]</sup> Due to the quick nature of tumor growth in cancer, the aggregated mass often outgrows vascularization, resulting in a low oxygen environment.<sup>[68]</sup> To overcome this cause of cell death, the tumor will modify its genetic expression (e.g. upregulating HIF-2 $\alpha$  [*EPAS1*] gene, which is a part of a family of genes of key importance to hypoxic environment response) which in turn, can increase its lethality, resistance to treatment and expanded metastasis regions.<sup>[69-74]</sup> HIF-2 $\alpha$ , due to its role in promoting disease progression in a subset of patients with both steatosis and HCC, could be a valuable target for the treatment of NAFLD-related HCC.<sup>[75]</sup> Desterke *et al* released a study that attempted to identify all genes involved in NASH progression, as well as genes/proteins involved in the transition from NASH to HCC, using data-mining and text-mining, finding 25 genes for NASH development and 44 genes/proteins in the advancement from NASH to HCC.<sup>[76]</sup> Initially, to find out which genes were relevant for the study, the authors searched for genes that were expressed distinctly between NASH patients and obese patients with healthy livers. Of the 25 genes found for NASH, 22 were upregulated and three were downregulated (when compared with healthy obese patients). Following this step, patients were then organized into three subsets: healthy obese, NAFL + obese, and NASH groups. Furthermore, the gene *YWHAZ*<sup>[77]</sup> was responsible for leading a pre-cancerous pathway according to the analysis results.<sup>[76]</sup> This study, not only narrowed down the list of lipid-related genes that are associated with disease progression from NAFL to HCC but also, for the first time, organized these genes together with their roles and interactions mapped out in a “canonical pathway”.<sup>[76]</sup> The pathway clarifies the role that these genes have in increasing the synthesis of triglycerides, cholesterol, and *de novo* fatty acids; in promoting inflammatory processes and chemo-attraction; higher FFA accumulation due to the disruption of the mitochondria and its beta-oxidation; and, faster development of insulin resistance and cancer progression.<sup>[76]</sup> To prove these findings, an additional stratified test was performed, using new patients subdivided into healthy

controls, NAFL and NASH. Three genes were selected to test for inflammation and cancer progression (*YWHAZ*, *SMPD2*, *CCL2*), and the other three genes (*FASN*, *CIDEA*, *VLDLR*) were selected for testing the metabolism and formation of lipid droplets. The results displayed significantly higher activity in the NASH group than in the other two groups.<sup>[76]</sup> Besides the mentioned 25 genes involved in the progression from NAFL to NASH, 5 additional genes had a role in cancer development. So to comprehend the roles these 30 genes play in the progression from NASH to HCC, datasets from Gene Expression Omnibus (GEO),<sup>[78]</sup> in the National Center for Biotechnology Information, and from The Cancer Genome Atlas project consortium<sup>[79]</sup> were selected to perform an analysis.<sup>[76]</sup> 13 genes had roles related to lipid metabolism and accumulation, as well as inflammation and cancer processes. The later genes were observed in increased quantities in both cirrhotic and HCC patients, with a marked decrease in lipid accumulation genes, which might be related to the metabolic alterations that liver neoplastic cells undergo, using the stored fat for energy production.<sup>[69]</sup> 5 genes in particular (*DGAT1*, *FASN*, *YWHAZ*, *LPL*, and *IRS2*) were identified as upregulated in over 4% of HCC patients, indicating a less optimistic prognosis, with increased possibilities of a relapse. From data-mining studies like this, we can conclude that patients identified with the genes specified in this study possess an increased risk of disease progression since the pathway established ascertains a connection to most processes associated with steatosis, inflammation, insulin resistance, and HCC.<sup>[76]</sup> Wu *et al* found that tumor suppressor Zinc fingers and homeoboxes 2 has a key role in regulating hepatic fat homeostasis, both in healthy and neoplastic cells, observed in the changes resulting from alterations in the expression of this gene.<sup>[80]</sup> Fujiwara *et al*<sup>[81]</sup> tested that downregulating carnitine palmitoyltransferase 2 not only allowed HCC to resist lipotoxic effects but also increase the generation of hepatic cancer cells. Other mutations types of mutation, such as telomerase reverse transcriptase (*TERT*) promoter mutations are additional risk factors for HCC since overexpression of telomerase is observed in 90% of human tumors.<sup>[82,83]</sup> These occur frequently in HCC patients (from 60% to 90%), with a few studies using *TERT* mutation as a therapeutic target with little success.<sup>[84]</sup> However, Akuta *et al*<sup>[85]</sup> have proven the feasibility of *TERT* promoter mutation (*TERT* C228T) in diagnosing primary HCC, which performed better than alpha-fetoprotein (AFP). Liver cancer diagnosis in family members can be correlated with an increased probability of developing HCC, with both inherited and acquired factors promoting carcinogenesis.<sup>[86,87]</sup> In addition to determining how different ethnicities have different inherent susceptibilities, NAFLD inheritability has been discussed in multiple twin studies.<sup>[88-91]</sup> Furthermore, a study performed on an ethnically diverse cohort, comparing Hispanic, Caucasian, and African Americans found that a higher prevalence of NAFLD manifests in Hispanics and is the least prevalent in African Americans.<sup>[92]</sup> A possible explanation for this distribution could lie in the metabolism of fat, wherein Western cohorts, it was verified that NAFLD patients, who were ethnically African American, have lower levels of TGs and higher high-density lipoprotein cholesterol when compared with that of Hispanics and Caucasians.<sup>[92]</sup>

### Hormonal factors

Besides the often discussed metabolic causes of NAFLD, endocrine diseases (commonly responsible for hormonal imbalance) and non-pathological conditions, like aging and menopause, seem to have a clear impact on the disease prevalence and outcome severity.

Sex has long been identified as an independent risk factor for the development of NAFLD and is addressed as such in multiple studies, acknowledging that male patients have a higher risk of developing NAFLD and have a more rapid progression to more advanced stages of the disease when compared to female patients. Furthermore, HCC also has a higher onset in males than females.<sup>[56,93]</sup> However, this comparison is often performed without distinguishing menopause status, which represents a shift in the hormonal balance of women. When comparing pre-menopausal with post-menopausal women, the latter have a higher prevalence of NAFLD. Additionally, women that undergo HRT (hormone replacement therapy) seem to have lower NAFLD prevalence when compared with women that didn't, suggesting the possibility that these hormones might have a protective effect in NAFLD.<sup>[94-98]</sup> Regarding HCC, a study that analyzed the protective effect of sex in overall survival in 39,345 patients, observed that this effect was more predominant in women between the ages of 18 to 44.<sup>[99]</sup> This implies that improving the current guidelines concerning sex as a risk factor might provide a more accurate assessment of disease prevalence and risk outcome.

Thyroid hormone levels can also have a protective effect in NAFLD, as observed in studies relating hyperthyroidism with lower NAFLD prevalence. Hypothyroidism has been identified in multiple studies as having an increased risk of prevalent NAFLD, however, several studies seem to have conflicting results regarding which thyroid hormones are relevant (subclinical type *vs.* overt type hypothyroidism), which hormone levels are significant, and some publications conclude that there is no correlation to NAFLD prevalence with either type.<sup>[100-104]</sup> The current lack of consensus reflects that there is room for further investigation, and the role that thyroid function has in risk prediction should not be ignored. Regarding HCC, a study performed by Pinter *et al* found that elevated free tetraiodothyronine (T4) was associated with a poor survival outcome while increased levels of thyroid-stimulating hormone (TSH) were correlated with bigger tumors (despite being unrelated to survival).<sup>[105]</sup> Sahin *et al* observed that elevated free triiodothyronine (T3) was associated with HCC progression.<sup>[106]</sup> As such, thyroid function can be considered a potential risk factor for NAFLD, as well as a possible marker to measure disease progression in HCC.

### Lifestyle choices

Of all risk factors known to affect the disease progression of NASH, routine lifestyle behavior is expected to have one of the most important impacts on outcome and severity. A correlation was observed between NAFLD and living locations in proximity to a large variety of food options,

consumption of processed foods, unbalanced diets leaning heavily towards high meat/fat to fresh fruit ratio, and little regular exercise.<sup>[107-112]</sup> Although the strength of such observations might vary in different countries, there is enough evidence to establish a strong association between an unhealthy lifestyle and the presence of NAFLD.<sup>[91]</sup>

### Epidemiological studies

Studies documenting prevalence data of NAFLD-related HCC are less rigorous. A relative level of selection bias seems to be applied in available data, particularly when access to large databases is not possible.<sup>[113-116]</sup> Cryptogenic cirrhosis shows many similarities with NASH-related cirrhosis (leading to an underestimation of NAFLD), and with an increased awareness of the disease, it could inflate the growing curve representing the number of patients diagnosed every year for the past decades.<sup>[115,117]</sup>

A United States study collected information on 170,540 adult patients from a transplant waitlist database.<sup>[118]</sup> This data was collected over a span of 15 years (2002–2017), and 17% of the patients were listed with HCC, and of those patients, roughly 16% had no discernible etiology. However, 24,431 patients had identifiable causes, of which, 2520 patients (10.3%) were identified as having NASH as the cause of HCC. This study features a very large sample size of NASH-HCC patients, and it allows us to find an approximate proportion of NASH within the varied etiologies of HCC, in an American population.<sup>[118]</sup> Information regarding the distribution of NASH-related HCC patients is not easily available, particularly in such a large sample size.

Other publications that feature extensive data regarding the epidemiology of NASH in the context of hepatocellular carcinoma focus more extensively on NASH-related cirrhosis rather than HCC,<sup>[119]</sup> identifying NASH as the third most common risk factor for liver cirrhosis.<sup>[120]</sup> Since cirrhosis is a valuable factor in increased mortality of liver patients, both findings implicate the importance that NASH has not only as an earlier predictor of mortality risk, but its role in the cause of mortality as well.

NASH-related HCC also fuels debate and discord regarding its monitoring and management. Since the progressive nature of this metabolic disease has already been established, the need to closely monitor patients can have a very important impact on management and early intervention. On the other hand, since the number of NAFLD patients that end up developing HCC is quite low in proportion, it can be argued that repetitive monitoring might be costly and devoid of practicality, that unless the liver reaches the stage of cirrhosis, monitoring for HCC is possibly premature.<sup>[121]</sup> However, documented reports of significant correlation between non-cirrhotic steatosis/steatohepatitis patients and HCC have been established as well, showing that NASH alone could progress to HCC, contradicting the idea that pre-cirrhosis monitoring is premature.<sup>[122-124]</sup> Sahil *et al*<sup>[125]</sup> reports that, in the absence of cirrhosis, NAFLD and metabolic syndrome are the most common causes of HCC, when compared with viral hepatitis and other causes. In NAFLD-related HCC

patients, this study showed that around one-third (34.6%), had no evidence of cirrhosis. However, in this study, there was no assessment of the level of threat implied by non-cirrhotic NAFLD-related HCC, and the benefits that screening non-cirrhotic NAFLD patients will have in treatment and disease outcome when weighed against the added cost (caused by the large increase in the number of patients to screen). A different study observed that, frequently, tumor size found in non-cirrhotic HCC patients appeared to be larger.<sup>[124]</sup> This could be a result of late detection of the tumor, due to its asymptomatic nature, which could be an argument for more aggressive screening. This hypothesis is also presented by Dasari *et al*<sup>[126]</sup> when investigating the overall survival post-surgery of non-cirrhotic and cirrhotic HCC patients. Regarding liver transplantation, its viability as a treatment option is limited by very high recurrence rates (50%), organ donor shortage, rejection or immunosuppressant complications, cost, and possible surgery complications.<sup>[126]</sup>

Further studies are required in order to establish a more reasonable guideline, improve the current diagnostic/monitoring methods, and develop a more thorough understanding of the mechanisms that dictate neoplastic transformation of hepatocytes. When comparing the severity of HCC between NAFLD patients and viral hepatitis patients, the lifespan of NAFLD patients was significantly reduced, while those with NAFLD-related HCC carried a worse outcome in terms of mortality rate.<sup>[121]</sup>

### Disease management

Disease progression in NAFLD patients is relatively slow, with most patients spending years without any symptoms. This added to the fact that the main preventative measure of progression is introducing changes to lifestyle habits, and on a consistent basis to make a significant difference, which can be highly dependent on the patient motivation to maintain such a healthy lifestyle. The management of NAFLD/NASH-related HCC is not yet addressed by any of the major guidelines, this is mainly due to the fact that when the consensus was established regarding HCC management, its main cause was viral hepatitis<sup>[17,127,128]</sup> and that no approved pharmacologic agents can be directed for NASH patients. Comparatively, viral hepatitis is much simpler to manage, unlike NASH, where it usually requires targeting other associated complications and comorbidities. As mentioned above, current evidence suggests a difference in the outcome between NAFLD-related HCC and HCC of other etiologies.<sup>[121]</sup>

Studies regarding the establishment of a stage-based approach to diagnosis and treatment of NAFLD, as well as the introduction of novel, noninvasive biomarkers to correctly identify the histological progress of the disease, have been performed.<sup>[129-132]</sup> From lipid signatures specific to NASH to biomarkers detecting advanced fibrosis, and recommending, for example, the introduction of pharmacological treatment during the intermediate stage, screening for HCC and esophageal varices at the late stage of NASH, and lifestyle interventions and diet regimen control across all stages.<sup>[129,132-134]</sup> Beyond laboratory tests,

imaging technology has also been a focus when tackling the noninvasive diagnosis and staging of NAFLD.<sup>[49,131]</sup>

Abdominal ultrasound (US) examination is a common test used to screen and monitor HCC, particularly if the patient liver has already shown signs of fibrotic tissue, with follow-up examinations being repeated in periods between 4 and 6 months. According to the American Association for the Study of Liver Diseases guideline,<sup>[128]</sup> computer tomography (CT) or magnetic resonance imaging should be used for further verification for HCC if abnormal US and AFP levels were found in severe fibrosis patients (stages 3–4). Confirming the diagnosis of NASH, however, still requires performing a liver biopsy, which remains the diagnostic ‘gold-standard’, despite its invasiveness and cost.<sup>[117,135,136]</sup> Abdominal US often lacks sensitivity, as ultrasound waves are influenced by large amounts of abdominal fat, which may compromise their accuracy, particularly in patients with very elevated (BMI).

Furthermore, since the results obtained can vary depending on the experience of the operator, US accuracy in detecting NASH becomes less rigorous.<sup>[127,137]</sup> Cost-effectiveness is very important when discussing the feasibility of monitoring techniques, particularly in diseases like NASH, that are complex in management as well as requiring long-term follow-ups. Therefore, studies aiming to document factors that can increase accuracy, improve risk assessment and reduce cost, and therapeutic targets are needed.<sup>[17]</sup>

Certain cancer-targeted biomarkers such as circulating tumor DNA, or extracellular vesicles,<sup>[138,139]</sup> showed potential screening capabilities for HCC, and research related to telomere shortening has found indications of the dysfunction of hepatocytes, generation of fibrosis, and interference in the metabolic pathway of lipids, all factors related to disease progression risks.<sup>[17,140]</sup> Despite liver biopsy being the ‘gold standard’ of NAFLD and NASH diagnosis, when possible, HCC should be screened by imaging techniques. While liver biopsy still has the highest diagnostic accuracy, HCC is easy to notice under contrast-enhanced imaging when compared to NAFLD, but more importantly, the invasiveness of the biopsy introduces a risk of spreading the cancer cells and bleeding, while risking incorrect assessment due to sampling location.<sup>[141,142]</sup> Future progress will likely phase out biopsies and expensive imaging tests, in favor of targeted cellular components, such as proteomic and genomic measurements, and cost-effective serum exams.

## Treatment

Treatment strategy guidelines for HCC traditionally follow the format of matching treatment options to disease stages, like the Barcelona Clinic Liver Cancer guidelines, however, studies that have more flexible approaches to treatments, where administering therapy from the next stage of the disease concurrently with the current stages’ treatment have provided different advantages and disadvantages to the established stage hierarchy system.<sup>[143]</sup> Furthermore, changes to the current therapy methods will be reliant on either the development of new strategies or an increase in the feasibility of existing ones.

Immunotherapy has untapped potential but its reliability has still not been proven and it is still being tested in clinical trials.<sup>[144]</sup> Meanwhile, the greatest progress made in HCC management has been the improvement in the diagnostic methods and screening, that allow for the established treatment methods to have a greater impact on survivability by treating the tumor in earlier stages, since HCC is a type of cancer that was conventionally identified in later stages of the disease.<sup>[145]</sup>

Established treatment methods for NAFLD-related HCC can be divided into three categories: lifestyle changes, surgery, and pharmacology. Lifestyle changes (mainly diet control and frequent exercise) have a positive effect on slowing or reversing NASH progression, but once HCC is confirmed, more significant measures need to be adopted. Further study on the impact that behavioral changes have in NAFLD-related HCC patients is necessary.

Surgical options for HCC patients include liver resection, ablation, chemoembolization, radioembolization, and liver transplantation. The liver resection is limited to smaller-scaled tumors as a general rule, but the thresholds for operable size and the number of tumors can vary in different countries, which in accordance with EASL–EORTC guidelines, can reach 5 year survival rates between 60% and 80%.<sup>[127,146,147]</sup> Thermal ablation is mainly used in early HCC and embolization can be used from early to advanced HCC.<sup>[148]</sup> Radiofrequency ablation (RFA) has been thoroughly researched, and its performance grants 5-year survival rates of about 40% to 70% by itself, however, combination with other methods (e.g. chemoembolization) has been investigated as well.<sup>[127,149]</sup> Transcatheter arterial chemoembolization (TACE) plus CT-guided (RFA) has produced very good results, with an overall survival rate of 96.1%, 76.7%, and 41.3%, for 1, 3 and 5 years, respectively. However, it has yet to be validated in Western centers.<sup>[150,151]</sup> Liver transplant carries fewer risks for the patient when compared with resection, and may sometimes help treat the underlying cause for liver disease.<sup>[152]</sup> The Milan criteria, featuring over 17 thousand patients, established a 5-year survival rate ranging between 65% and 78%.<sup>[153]</sup> However, the main disadvantages of transplantation are donor shortage and possible immune response/rejection.<sup>[154]</sup>

Drug treatment for end-stage HCC patients can be subcategorized into first-line and second-line drugs. First-line includes two tyrosine kinase inhibitors (TKIs), sorafenib<sup>[155]</sup> (established) and lenvatinib<sup>[156]</sup> (novel), with a life expectancy increase of about 10 weeks. Additionally, atezolizumab and bevacizumab have been administered as a combination therapy in a Phase III study, obtained FDA approval, with ‘overall survival’ numbers superior to sorafenib.<sup>[157,158]</sup>

Second-line drugs include regorafenib (TKI),<sup>[159]</sup> cabozantinib (TKI),<sup>[160]</sup> and ramucirumab (anti-angiogenic),<sup>[148]</sup> with an average increase of life expectancy of 3.6, 10.2, and 8.5 months, respectively.<sup>[161]</sup> Ramucirumab is a phase III trial drug that presents an alternative to sorafenib, being effective in patients with AFP levels over 400, but no difference in efficacy. Nivolumab<sup>[162]</sup> on the other hand,

belongs to the novel immunotherapy category, with an overall response rate of 20%, currently in phase III clinical trial, performing significantly better than sofenib.<sup>[163,164]</sup>

## Conclusion

The continuous rise in prevalence of NAFLD patients worldwide warrants the need to coordinate appropriate healthcare measures. However, the lack of disruptive symptoms renders its early development relatively unnoticed by those affected, and thus conducive to the progression of simple steatosis to NASH and/or fibrosis, and ultimately evolving into HCC. The role played by lipotoxicity, insulin resistance and mitochondrial activity reveal a possible route of pharmacology research for NASH management. However, the mechanism underlying the neoplastic change among NAFLD patients is poorly understood; therefore, future studies are suggested to focus on NASH-related HCC investigations.

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## Conflicts of interest

None.

## References

- White DL, Kanwal F, El-Serag HB. Association between nonalcoholic fatty liver disease and risk for hepatocellular cancer, based on systematic review. *Clin Gastroenterol Hepatol* 2012;10:1342–1359. e1342. doi: 10.1016/j.cgh.2012.10.001.
- Singh S, Allen AM, Wang Z, Prokop LJ, Murad MH, Loomba R. Fibrosis progression in nonalcoholic fatty liver vs nonalcoholic steatohepatitis: a systematic review and meta-analysis of paired-biopsy studies. *Clin Gastroenterol Hepatol* 2015;13:643–654. e641–649; quiz e639–640. doi: 10.1016/j.cgh.2014.04.014.
- Hashimoto E, Taniai M, Tokushige K. Characteristics and diagnosis of NAFLD/NASH. *J Gastroenterol Hepatol* 2013;28 (Suppl 4):64–70. doi: 10.1111/jgh.12271.
- Ludwig J, Viggiano TR, McGill DB, Oh BJ. Nonalcoholic steatohepatitis: Mayo Clinic experiences with a hitherto unnamed disease. *Mayo Clin Proc* 1980;55:434–438.
- Sanyal AJ. AGA technical review on nonalcoholic fatty liver disease. *Gastroenterology* 2002;123:1705–1725. doi: 10.1053/gast.2002.36572.
- Neuschwander-Tetri BA, Caldwell SH. Nonalcoholic steatohepatitis: summary of an AASLD Single Topic Conference. *Hepatology* 2003;37:1202–1219. doi: 10.1053/jhep.2003.50193.
- Farrell GC, Chitturi S, Lau GK, Sollano JD. Guidelines for the assessment and management of non-alcoholic fatty liver disease in the Asia-Pacific region: executive summary. *J Gastroenterol Hepatol* 2007;22:775–777. doi: 10.1111/j.1440-1746.2007.05002.x.
- Ratziu V, Bellentani S, Cortez-Pinto H, Day C, Marchesini G. A position statement on NAFLD/NASH based on the EASL 2009 special conference. *J Hepatol* 2010;53:372–384. doi: 10.1016/j.jhep.2010.04.008.
- Chalasanani N, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, *et al.* The diagnosis and management of non-alcoholic fatty liver disease: practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. *Hepatology* 2012;55:2005–2023. doi: 10.1002/hep.25762.
- Eslam M, Sanyal AJ, George J. MAFLD: a consensus-driven proposed nomenclature for metabolic associated fatty liver disease. *Gastroenterology* 2020;158:1999–2014. e1991. doi: 10.1053/j.gastro.2019.11.312.
- Eslam M, Newsome PN, Sarin SK, Anstee QM, Targher G, Romero-Gomez M, *et al.* A new definition for metabolic dysfunction-associated fatty liver disease: an international expert consensus statement. *J Hepatol* 2020;73:202–209. doi: 10.1016/j.jhep.2020.03.039.
- Zheng KI, Fan JG, Shi JP, Wong VW, Eslam M, George J, *et al.* From NAFLD to MAFLD: a “redefining” moment for fatty liver disease. *Chin Med J (Engl)* 2020;133:2271–2273. doi: 10.1097/cm9.0000000000000981.
- Ballestri S, Zona S, Targher G, Romagnoli D, Baldelli E, Nascimbeni F, *et al.* Nonalcoholic fatty liver disease is associated with an almost twofold increased risk of incident type 2 diabetes and metabolic syndrome. Evidence from a systematic review and meta-analysis. *J Gastroenterol Hepatol* 2016;31:936–944. doi: 10.1111/jgh.13264.
- Leung JC, Loong TC, Wei JL, Wong GL, Chan AW, Choi PC, *et al.* Histological severity and clinical outcomes of nonalcoholic fatty liver disease in nonobese patients. *Hepatology* 2017;65:54–64. doi: 10.1002/hep.28697.
- Wang Y, Wang B, Shen F, Fan J, Cao H. Body mass index and risk of primary liver cancer: a meta-analysis of prospective studies. *Oncologist* 2012;17:1461–1468. doi: 10.1634/theoncologist.2012-0066.
- Fan JG, Wei L, Zhuang H. Guidelines of prevention and treatment of nonalcoholic fatty liver disease (2018, China). *J Dig Dis* 2019;20:163–173. doi: 10.1111/1751-2980.12685.
- Anstee QM, Reeves HL, Kotsiliti E, Govaere O, Heikenwalder M. From NASH to HCC: current concepts and future challenges. *Nat Rev Gastroenterol Hepatol* 2019;16:411–428. doi: 10.1038/s41575-019-0145-7.
- Satapati S, Sunny NE, Kucejova B, Fu X, He TT, Méndez-Lucas A, *et al.* Elevated TCA cycle function in the pathology of diet-induced hepatic insulin resistance and fatty liver. *J Lipid Res* 2012;53:1080–1092. doi: 10.1194/jlr.M023382.
- Noureddin M, Rinella ME. Nonalcoholic fatty liver disease, diabetes, obesity, and hepatocellular carcinoma. *Clin Liver Dis* 2015;19:361–379. doi: 10.1016/j.cld.2015.01.012.
- Sunny NE, Bril F, Cusi K. Mitochondrial adaptation in nonalcoholic fatty liver disease: novel mechanisms and treatment strategies. *Trends Endocrinol Metab* 2017;28:250–260. doi: 10.1016/j.tem.2016.11.006.
- Patterson RE, Kalavalapalli S, Williams CM, Nautiyal M, Mathew JT, Martinez J, *et al.* Lipotoxicity in steatohepatitis occurs despite an increase in tricarboxylic acid cycle activity. *Am J Physiol Endocrinol Metab* 2016;310:E484–494. doi: 10.1152/ajpendo.00492.2015.
- Seki S, Kitada T, Yamada T, Sakaguchi H, Nakatani K, Wakasa K. In situ detection of lipid peroxidation and oxidative DNA damage in non-alcoholic fatty liver diseases. *J Hepatol* 2002;37:56–62. doi: 10.1016/s0168-8278(02)00073-9.
- Tanaka S, Miyanishi K, Kobune M, Kawano Y, Hoki T, Kubo T, *et al.* Increased hepatic oxidative DNA damage in patients with nonalcoholic steatohepatitis who develop hepatocellular carcinoma. *J Gastroenterol* 2013;48:1249–1258. doi: 10.1007/s00535-012-0739-0.
- Egea J, Fabregat I, Frapart YM, Ghezzi P, Görlach A, Kietzmann T, *et al.* European contribution to the study of ROS: a summary of the findings and prospects for the future from the COST action BM1203 (EU-ROS). *Redox Biol* 2017;13:94–162. doi: 10.1016/j.redox.2017.05.007.
- For Farlex Partner Medical Dictionary: lipotoxicity. (n.d.) Farlex Partner Medical Dictionary. (2012). Retrieved February 19 2021 from <https://medical-dictionary.thefreedictionary.com/lipotoxicity>.
- Nakagawa H, Hayata Y, Kawamura S, Yamada T, Fujiwara N, Koike K. Lipid metabolic reprogramming in hepatocellular carcinoma. *Cancers (Basel)* 2018;10:447. doi: 10.3390/cancers10110447.
- Budhu A, Roessler S, Zhao X, Yu Z, Forgues M, Ji J, *et al.* Integrated metabolite and gene expression profiles identify lipid biomarkers associated with progression of hepatocellular carcinoma and patient outcomes. *Gastroenterology* 2013;144:1066–1075. e1061. doi: 10.1053/j.gastro.2013.01.054.

28. Björnson E, Mukhopadhyay B, Asplund A, Pristovsek N, Cinar R, Romeo S, *et al.* Stratification of hepatocellular carcinoma patients based on acetate utilization. *Cell Rep* 2015;13:2014–2026. doi: 10.1016/j.celrep.2015.10.045.
29. Iwamoto H, Abe M, Yang Y, Cui D, Seki T, Nakamura M, *et al.* Cancer lipid metabolism confers antiangiogenic drug resistance. *Cell Metab* 2018;28:104–117. e105. doi: 10.1016/j.cmet.2018.05.005.
30. For Miller-Keane Encyclopedia: oxidative stress. (n.d.) Miller-Keane Encyclopedia and Dictionary of Medicine, Nursing, and Allied Health, Seventh Edition. (2003). Retrieved February 19 2021 from <https://medical-dictionary.thefreedictionary.com/oxidative+stress>.
31. Wiseman H, Halliwell B. Damage to DNA by reactive oxygen and nitrogen species: role in inflammatory disease and progression to cancer. *Biochem J* 1996;313:17–29. doi: 10.1042/bj3130017.
32. Kuper H, Adami HO, Trichopoulos D. Infections as a major preventable cause of human cancer. *J Intern Med* 2000;248:171–183. doi: 10.1046/j.1365-2796.2000.00742.x.
33. Ohnishi S, Ma N, Thanan R, Pinlaor S, Hammam O, Murata M, *et al.* DNA damage in inflammation-related carcinogenesis and cancer stem cells. *Oxid Med Cell Longev* 2013;2013:387014. doi: 10.1155/2013/387014.
34. Ivanov AV, Valuev-Ellistov VT, Tyurina DA, Ivanova ON, Kochetkov SN, Bartosch B, *et al.* Oxidative stress, a trigger of hepatitis C and B virus-induced liver carcinogenesis. *Oncotarget* 2017;8:3895–3932. doi: 10.18632/oncotarget.13904.
35. Wilson CL, Jurk D, Fullard N, Banks P, Page A, Luli S, *et al.* NF(B1 is a suppressor of neutrophil-driven hepatocellular carcinoma. *Nat Commun* 2015;6:6818. doi: 10.1038/ncomms7818.
36. Longo M, Paolini E, Meroni M, Dongiovanni P. Remodeling of mitochondrial plasticity: the key switch from NAFLD/NASH to HCC. *Int J Mol Sci* 2021;22:4173. doi: 10.3390/ijms22084173.
37. Daugherty EK, Balmus G, Al Saei A, Moore ES, Abi Abdallah D, Rogers AB, *et al.* The DNA damage checkpoint protein ATM promotes hepatocellular apoptosis and fibrosis in a mouse model of non-alcoholic fatty liver disease. *Cell Cycle* 2012;11:1918–1928. doi: 10.4161/cc.20259.
38. McKinnon PJ. ATM and the molecular pathogenesis of ataxia telangiectasia. *Annu Rev Pathol* 2012;7:303–321. doi: 10.1146/annurev-pathol-011811-132509. PMID22035194.
39. Ditch S, Paull TT. The ATM protein kinase and cellular redox signaling: beyond the DNA damage response. *Trends Biochem Sci* 2012;37:15–22. doi: 10.1016/j.tibs.2011.10.002.
40. Guo Z, Kozlov S, Lavin MF, Person MD, Paull TT. ATM activation by oxidative stress. *Science* 2010;330:517–521. doi: 10.1126/science.1192912.
41. Gao D, Wei C, Chen L, Huang J, Yang S, Diehl AM. Oxidative DNA damage and DNA repair enzyme expression are inversely related in murine models of fatty liver disease. *Am J Physiol Gastrointest Liver Physiol* 2004;287:G1070–1077. doi: 10.1152/ajpgi.00228.2004.
42. Bianco C, Jamialahmadi O, Pelusi S, Baselli G, Dongiovanni P, Zanoni I, *et al.* Non-invasive stratification of hepatocellular carcinoma risk in non-alcoholic fatty liver using polygenic risk scores. *J Hepatol* 2021;74:775–782. doi: 10.1016/j.jhep.2020.11.024.
43. Trépo E, Valenti L. Update on NAFLD genetics: from new variants to the clinic. *J Hepatol* 2020;72:1196–1209. doi: 10.1016/j.jhep.2020.02.020.
44. Cornell L, Munck JM, Alsinet C, Villanueva A, Ogle L, Willoughby CE, *et al.* DNA-PK-A candidate driver of hepatocarcinogenesis and tissue biomarker that predicts response to treatment and survival. *Clin Cancer Res* 2015;21:925–933. doi: 10.1158/1078-0432.Ccr-14-0842.
45. Evert M, Frau M, Tomasi ML, Latte G, Simile MM, Seddaiu MA, *et al.* Deregulation of DNA-dependent protein kinase catalytic subunit contributes to human hepatocarcinogenesis development and has a putative prognostic value. *Br J Cancer* 2013;109:2654–2664. doi: 10.1038/bjc.2013.606.
46. McPherson S, Hardy T, Henderson E, Burt AD, Day CP, Anstee QM. Evidence of NAFLD progression from steatosis to fibrosing-steatohepatitis using paired biopsies: implications for prognosis and clinical management. *J Hepatol* 2015;62:1148–1155. doi: 10.1016/j.jhep.2014.11.034.
47. Angulo P, Kleiner DE, Dam-Larsen S, Adams LA, Björnsson ES, Charatcharoenwithaya P, *et al.* Liver fibrosis, but no other histologic features, is associated with long-term outcomes of patients with nonalcoholic fatty liver disease. *Gastroenterology* 2015;149:389–397. e310. doi: 10.1053/j.gastro.2015.04.043.
48. Taylor RS, Taylor RJ, Bayliss S, Hagström H, Nasr P, Schattenberg JM, *et al.* Association between fibrosis stage and outcomes of patients with nonalcoholic fatty liver disease: a systematic review and meta-analysis. *Gastroenterology* 2020;158:1611–1625. e1612. doi: 10.1053/j.gastro.2020.01.043.
49. Rios RS, Zheng KI, Targher G, Byrne CD, Zheng MH. Non-invasive fibrosis assessment in non-alcoholic fatty liver disease. *Chin Med J* 2020;133:2743–2745. doi: 10.1097/cm9.0000000000000989.
50. Pedersen JS, Bendtsen F, Moller S. Management of cirrhotic ascites. *Ther Adv Chronic Dis* 2015;6:124–137. doi: 10.1177/2040622315580069.
51. Friedman SL, Roll FJ, Boyles J, Bissell DM. Hepatic lipocytes: the principal collagen-producing cells of normal rat liver. *Proc Natl Acad Sci USA* 1985;82:8681–8685. doi: 10.1073/pnas.82.24.8681.
52. Friedman SL. Hepatic stellate cells: protean, multifunctional, and enigmatic cells of the liver. *Physiol Rev* 2008;88:125–172. doi: 10.1152/physrev.00013.2007.
53. Mederacke I, Hsu CC, Troeger JS, Huebener P, Mu X, Dapito DH, *et al.* Fate tracing reveals hepatic stellate cells as dominant contributors to liver fibrosis independent of its aetiology. *Nat Commun* 2013;4:2823. doi: 10.1038/ncomms3823.
54. Wells RG, Schwabe RF. Origin and function of myofibroblasts in the liver. *Semin Liver Dis* 2015;35:97–106. doi: 10.1055/s-0035-1550061.
55. Prashanth M, Ganesh HK, Vima MV, John M, Bandgar T, Joshi SR, *et al.* Prevalence of nonalcoholic fatty liver disease in patients with type 2 diabetes mellitus. *J Assoc Physicians India* 2009;57:205–210.
56. Vilar-Gomez E, Calzadilla-Bertot L, Wai-Sun Wong V, Castellanos M, Aller-de la Fuente R, Metwally M, *et al.* Fibrosis severity as a determinant of cause-specific mortality in patients with advanced nonalcoholic fatty liver disease: a multi-national cohort study. *Gastroenterology* 2018;155:443–457. e417. doi: 10.1053/j.gastro.2018.04.034.
57. Romeo S, Kozlitina J, Xing C, Pertsemlidis A, Cox D, Pennacchio LA, *et al.* Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. *Nat Genet* 2008;40:1461–1465. doi: 10.1038/ng.257.
58. Speliotes EK, Yerges-Armstrong LM, Wu J, Hernaez R, Kim LJ, Palmer CD, *et al.* Genome-wide association analysis identifies variants associated with nonalcoholic fatty liver disease that have distinct effects on metabolic traits. *PLoS Genet* 2011;7:e1001324. doi: 10.1371/journal.pgen.1001324.
59. Liu WY, Zheng KI, Pan XY, Ma HL, Zhu PW, Wu XX, *et al.* Effect of PNPLA3 polymorphism on diagnostic performance of various noninvasive markers for diagnosing and staging nonalcoholic fatty liver disease. *J Gastroenterol Hepatol* 2020;35:1057–1064. doi: 10.1111/jgh.14894.
60. Liu YL, Reeves HL, Burt AD, Tiniakos D, McPherson S, Leathart JB, *et al.* TM6SF2 rs58542926 influences hepatic fibrosis progression in patients with non-alcoholic fatty liver disease. *Nat Commun* 2014;5:4309. doi: 10.1038/ncomms5309.
61. Chambers JC, Zhang W, Sehmi J, Li X, Wass MN, Van der Harst P, *et al.* Genome-wide association study identifies loci influencing concentrations of liver enzymes in plasma. *Nature Genetics* 2011;43:1131–1138. doi: 10.1038/ng.970.
62. Abul-Husn NS, Cheng X, Li AH, Xin Y, Schurmann C, Stevis P, *et al.* A protein-truncating HSD17B13 variant and protection from chronic liver disease. *N Engl J Med* 2018;378:1096–1106. doi: 10.1056/NEJMoa1712191.
63. Donati B, Dongiovanni P, Romeo S, Meroni M, McCain M, Miele L, *et al.* MBOAT7 rs641738 variant and hepatocellular carcinoma in non-cirrhotic individuals. *Sci Rep* 2017;7:4492. doi: 10.1038/s41598-017-04991-0.
64. Pelusi S, Baselli G, Pietrelli A, Dongiovanni P, Donati B, McCain MV, *et al.* Rare pathogenic variants predispose to hepatocellular carcinoma in nonalcoholic fatty liver disease. *Sci Rep* 2019;9:3682. doi: 10.1038/s41598-019-39998-2.



65. Anstee QM, Seth D, Day CP. Genetic factors that affect risk of alcoholic and nonalcoholic fatty liver disease. *Gastroenterology* 2016;150:1728–1744. e1727. doi.org/10.1053/j.gastro.2016.01.037.
66. Anstee QM, Darlay R, Cockell S, Meroni M, Govaere O, Tiniakos D, *et al.* Genome-wide association study of non-alcoholic fatty liver and steatohepatitis in a histologically characterised cohort☆. *J Hepatol* 2020;73:505–515. doi: 10.1016/j.jhep.2020.04.003.
67. Currie E, Schulze A, Zechner R, Walther TC, Farese RV Jr. Cellular fatty acid metabolism and cancer. *Cell Metab* 2013;18:153–161. doi: 10.1016/j.cmet.2013.05.017.
68. Bertout JA, Patel SA, Simon MC. The impact of O<sub>2</sub> availability on human cancer. *Nat Rev Cancer* 2008;8:967–975. doi: 10.1038/nrc2540.
69. Rankin EB, Rha J, Selak MA, Unger TL, Keith B, Liu Q, *et al.* Hypoxia-inducible factor 2 regulates hepatic lipid metabolism. *Mol Cell Biol* 2009;29:4527–4538. doi: 10.1128/mcb.00200-09.
70. Qu A, Taylor M, Xue X, Matsubara T, Metzger D, Chambon P, *et al.* Hypoxia-inducible transcription factor 2 promotes steatohepatitis through augmenting lipid accumulation, inflammation, and fibrosis. *Hepatology* 2011;54:472–483. doi: 10.1002/hep.24400.
71. Cao R, Zhao X, Li S, Zhou H, Chen W, Ren L, *et al.* Hypoxia induces dysregulation of lipid metabolism in HepG2 cells via activation of HIF-2 $\alpha$ . *Cell Physiol Biochem* 2014;34:1427–1441. doi: 10.1159/000366348.
72. Lin Q, Yun Z. The hypoxia-inducible factor pathway in adipocytes: the role of HIF-2 in adipose inflammation and hypertrophic cardiomyopathy. *Front Endocrinol (Lausanne)* 2015;6:39. doi: 10.3389/fendo.2015.00039.
73. Gilkes DM, Semenza GL, Wirtz D. Hypoxia and the extracellular matrix: drivers of tumour metastasis. *Nat Rev Cancer* 2014;14:430–439. doi: 10.1038/nrc3726.
74. Hlouschek J, Ritter V, Wirsdörfer F, Klein D, Jendrossek V, Matschke J. Targeting SLC25A10 alleviates improved antioxidant capacity and associated radioresistance of cancer cells induced by chronic-cycling hypoxia. *Cancer Lett* 2018;439:24–38. doi: 10.1016/j.canlet.2018.09.002.
75. Chen J, Chen J, Huang J, Li Z, Gong Y, Zou B, *et al.* HIF-2( upregulation mediated by hypoxia promotes NAFLD-HCC progression by activating lipid synthesis via the PI3K-AKT-mTOR pathway. *Aging (Albany NY)* 2019;11:10839–10860. doi: 10.18632/aging.102488.
76. Desterke C, Chiappini F. Lipid related genes altered in NASH connect inflammation in liver pathogenesis progression to HCC: a canonical pathway. *Int J Mol Sci* 2019;20:5594. doi: 10.3390/ijms20225594.
77. Zhao JF, Zhao Q, Hu H, Liao JZ, Lin JS, Xia C, *et al.* The ASH1-miR-375-YWHAZ signaling axis regulates tumor properties in hepatocellular carcinoma. *Mol Ther Nucleic Acids* 2018;11:538–553. doi: 10.1016/j.omtn.2018.04.007.
78. Mas VR, Maluf DG, Archer KJ, Yanek K, Kong X, Kulik L, *et al.* Genes involved in viral carcinogenesis and tumor initiation in hepatitis C virus-induced hepatocellular carcinoma. *Mol Med* 2009;15:85–94. doi: 10.2119/molmed.2008.00110.
79. Sanchez-Vega F, Mina M, Armenia J, Chatila WK, Luna A, La KC, *et al.* oncogenic signaling pathways in the cancer genome atlas. *Cell* 2018;173:321–337. e310. doi: 10.1016/j.cell.2018.03.035.
80. Wu Z, Ma H, Wang L, Song X, Zhang J, Liu W, *et al.* Tumor suppressor ZHX2 inhibits NAFLD-HCC progression via blocking LPL-mediated lipid uptake. *Cell Death Differ* 2020;27:1693–1708. doi: 10.1038/s41418-019-0453-z.
81. Fujiwara N, Nakagawa H, Enooku K, Kudo Y, Hayata Y, Nakatsuka T, *et al.* CPT2 downregulation adapts HCC to lipid-rich environment and promotes carcinogenesis via acylcarnitine accumulation in obesity. *Gut* 2018;67:1493–1504. doi: 10.1136/gutjnl-2017-315193.
82. Zucman-Rossi J, Villanueva A, Nault JC, Llovet JM. Genetic landscape and biomarkers of hepatocellular carcinoma. *Gastroenterology* 2015;149:1226–1239. e1224. doi: 10.1053/j.gastro.2015.05.061.
83. Lee DD, Leão R, Komosa M, Gallo M, Zhang CH, Lipman T, *et al.* DNA hypermethylation within TERT promoter upregulates TERT expression in cancer. *J Clin Invest* 2019;129:223–229. doi: 10.1172/jci121303.
84. Khemlina G, Ikeda S, Kurzrock R. The biology of Hepatocellular carcinoma: implications for genomic and immune therapies. *Mol Cancer* 2017;16:149. doi: 10.1186/s12943-017-0712-x.
85. Akuta N, Kawamura Y, Kobayashi M, Arase Y, Saitoh S, Fujiyama S, *et al.* TERT promoter mutation in serum cell-free DNA is a diagnostic marker of primary hepatocellular carcinoma in patients with nonalcoholic fatty liver disease. *Oncology* 2021;99:114–123. doi: 10.1159/000510366.
86. Turati F, Edefonti V, Talamini R, Ferraroni M, Malvezzi M, Bravi F, *et al.* Family history of liver cancer and hepatocellular carcinoma. *Hepatology* 2012;55:1416–1425. doi: 10.1002/hep.24794.
87. Nahon P, Zucman-Rossi J. Single nucleotide polymorphisms and risk of hepatocellular carcinoma in cirrhosis. *J Hepatol* 2012;57:663–674. doi: 10.1016/j.jhep.2012.02.035.
88. Loomba R, Schork N, Chen CH, Bettencourt R, Bhatt A, Ang B, *et al.* Heritability of hepatic fibrosis and steatosis based on a prospective twin study. *Gastroenterology* 2015;149:1784–1793. doi: 10.1053/j.gastro.2015.08.011.
89. Schwimmer JB, Cledon MA, Lavine JE, Salem R, Campbell N, Schork NJ, *et al.* Heritability of nonalcoholic fatty liver disease. *Gastroenterology* 2009;136:1585–1592. doi: 10.1053/j.gastro.2009.01.050.
90. Cui J, Chen CH, Lo MT, Schork N, Bettencourt R, Gonzalez MP, *et al.* Shared genetic effects between hepatic steatosis and fibrosis: a prospective twin study. *Hepatology* 2016;64:1547–1558. doi: 10.1002/hep.28674.
91. Younossi Z, Anstee QM, Marietti M, Hardy T, Henry L, Eslam M, *et al.* Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. *Nat Rev Gastroenterol Hepatol* 2018;15:11–20. doi: 10.1038/nrgastro.2017.109.
92. Anstee QM, Day CP. The genetics of NAFLD. *Nat Rev Gastroenterol Hepatol* 2013;10:645–655. doi: 10.1038/nrgastro.2013.182.
93. Villa E. Role of estrogen in liver cancer. *Womens Health (Lond)* 2008;4:41–50. doi: 10.2217/17455057.4.1.41.
94. Lonardo A, Nascimbeni F, Ballestri S, Fairweather D, Win S, Than TA, *et al.* Sex differences in nonalcoholic fatty liver disease: state of the art and identification of research gaps. *Hepatology* 2019;70:1457–1469. doi: 10.1002/hep.30626.
95. Park SH, Jeon WK, Kim SH, Kim HJ, Park DI, Cho YK, *et al.* Prevalence and risk factors of non-alcoholic fatty liver disease among Korean adults. *J Gastroenterol Hepatol* 2006;21:138–143. doi: 10.1111/j.1440-1746.2005.04086.x.
96. Long MT, Pedley A, Massaro JM, Hoffmann U, Ma J, Loomba R, *et al.* A simple clinical model predicts incident hepatic steatosis in a community-based cohort: the Framingham Heart Study. *Liver Int* 2018;38:1495–1503. doi: 10.1111/liv.13709.
97. Wang Z, Xu M, Hu Z, Hultström M, Lai E. Sex-specific prevalence of fatty liver disease and associated metabolic factors in Wuhan, south central China. *Eur J Gastroenterol Hepatol* 2014;26:1015–1021. doi: 10.1097/meg.0000000000000151.
98. Eguchi Y, Hyogo H, Ono M, Mizuta T, Ono N, Fujimoto K, *et al.* Prevalence and associated metabolic factors of nonalcoholic fatty liver disease in the general population from 2009 to 2010 in Japan: a multicenter large retrospective study. *J Gastroenterol* 2012;47:586–595. doi: 10.1007/s00535-012-0533-z.
99. Yang D, Hanna DL, Usher J, LoCoco J, Chaudhari P, Lenz HJ, *et al.* Impact of sex on the survival of patients with hepatocellular carcinoma: a Surveillance, Epidemiology, and End Results analysis. *Cancer* 2014;120:3707–3716. doi: 10.1002/cncr.28912.
100. Liangpunsakul S, Chalasani N. Is hypothyroidism a risk factor for non-alcoholic steatohepatitis? *J Clin Gastroenterol* 2003;37:340–343. doi: 10.1097/00004836-200310000-00014.
101. Ittermann T, Haring R, Wallaschofski H, Baumeister SE, Nauck M, Dörr M, *et al.* Inverse association between serum free thyroxine levels and hepatic steatosis: results from the Study of Health in Pomerania. *Thyroid* 2012;22:568–574. doi: 10.1089/thy.2011.0279.
102. Carulli L, Ballestri S, Lonardo A, Lami F, Violi E, Losi L, *et al.* Is nonalcoholic steatohepatitis associated with a high-normal thyroid stimulating hormone level and lower cholesterol levels? *Intern Emerg Med* 2013;8:297–305. doi: 10.1007/s11739-011-0609-4.
103. Parikh P, Phadke A, Sawant P. Prevalence of hypothyroidism in nonalcoholic fatty liver disease in patients attending a tertiary

- hospital in western India. *Indian J Gastroenterol* 2015;34:169–173. doi: 10.1007/s12664-015-0541-z.
104. Lee KW, Bang KB, Rhee EJ, Kwon HJ, Lee MY, Cho YK. Impact of hypothyroidism on the development of non-alcoholic fatty liver disease: a 4-year retrospective cohort study. *Clin Mol Hepatol* 2015;21:372–378. doi: 10.3350/cmh.2015.21.4.372.
  105. Pinter M, Haupt L, Hucke F, Bota S, Bucsecs T, Trauner M, *et al.* The impact of thyroid hormones on patients with hepatocellular carcinoma. *PLoS one* 2017;12:e0181878–e1181878. doi: 10.1371/journal.pone.0181878.
  106. Sahin T, Oral A, Turker F, Kocak E. Can hypothyroidism be a protective factor for hepatocellular carcinoma in cirrhosis? *Medicine* 2020;99:e19492–e119492. doi: 10.1097/MD.00000000000019492.
  107. Leslie T, Pawloski L, Kallman-Price J, Escheik C, Hossain N, Fang Y, *et al.* Survey of health status, nutrition and geography of food selection of chronic liver disease patients. *Ann Hepatol* 2014;13:533–540.
  108. Kim CH, Kallman JB, Bai C, Pawloski L, Gewa C, Arsalla A, *et al.* Nutritional assessments of patients with non-alcoholic fatty liver disease. *Obes Surg* 2010;20:154–160. doi: 10.1007/s11695-008-9549-0.
  109. McCarthy EM, Rinella ME. The role of diet and nutrient composition in nonalcoholic fatty liver disease. *J Acad Nutr Diet* 2012;112:401–409. doi: 10.1016/j.jada.2011.10.007.
  110. Gerber L, Otgonsuren M, Mishra A, Escheik C, Biredinc A, Stepanova M, *et al.* Non-alcoholic fatty liver disease (NAFLD) is associated with low level of physical activity: a population-based study. *Aliment Pharmacol Ther* 2012;36:772–781. doi: 10.1111/apt.12038.
  111. Hallsworth K, Thoma C, Moore S, Ploetz T, Anstee QM, Taylor R, *et al.* Non-alcoholic fatty liver disease is associated with higher levels of objectively measured sedentary behaviour and lower levels of physical activity than matched healthy controls. *Frontline Gastroenterol* 2015;6:44–51. doi: 10.1136/flgastro-2014-100432.
  112. Keating SE, George J, Johnson NA. The benefits of exercise for patients with non-alcoholic fatty liver disease. *Expert Rev Gastroenterol Hepatol* 2015;9:1247–1250. doi: 10.1586/17474124.2015.1075392.
  113. Park JW, Chen M, Colombo M, Roberts LR, Schwartz M, Chen PJ, *et al.* Global patterns of hepatocellular carcinoma management from diagnosis to death: the BRIDGE Study. *Liver Int* 2015;35:2155–2166. doi: 10.1111/liv.12818.
  114. Younossi ZM, Otgonsuren M, Henry L, Venkatesan C, Mishra A, Erario M, *et al.* Association of nonalcoholic fatty liver disease (NAFLD) with hepatocellular carcinoma (HCC) in the United States from 2004 to 2009. *Hepatology* 2015;62:1723–1730. doi: 10.1002/hep.28123.
  115. Wong RJ, Cheung R, Ahmed A. Nonalcoholic steatohepatitis is the most rapidly growing indication for liver transplantation in patients with hepatocellular carcinoma in the U.S. *Hepatology* 2014;59:2188–2195. doi: 10.1002/hep.26986.
  116. Tateishi R, Okanoue T, Fujiwara N, Okita K, Kiyosawa K, Omata M, *et al.* Clinical characteristics, treatment, and prognosis of non-B, non-C hepatocellular carcinoma: a large retrospective multicenter cohort study. *J Gastroenterol* 2015;50:350–360. doi: 10.1007/s00535-014-0973-8.
  117. Dyson J, Jaques B, Chattopadhyay D, Lochan R, Graham J, Das D, *et al.* Hepatocellular cancer: the impact of obesity, type 2 diabetes and a multidisciplinary team. *J Hepatol* 2014;60:110–117. doi: 10.1016/j.jhep.2013.08.011.
  118. Younossi Z, Stepanova M, Ong JP, Jacobson IM, Bugianesi E, Duseja A, *et al.* Nonalcoholic steatohepatitis is the fastest growing cause of hepatocellular carcinoma in liver transplant candidates. *Clin Gastroenterol Hepatol* 2019;17:748–755. e743. doi: 10.1016/j.cgh.2018.05.057.
  119. Mak L-Y, Cruz-Ramón V, Chinchilla-López P, Torres HA, LoConte NK, Rice JP, *et al.* Global epidemiology, prevention, and management of hepatocellular carcinoma. *Am Soc Clin Oncol Educ Book* 2018;262–279. doi: 10.1200/edbk\_200939.
  120. Schütte K, Kipper M, Kahl S, Bornschein J, Götze T, Adolf D, *et al.* Clinical characteristics and time trends in etiology of hepatocellular cancer in Germany. *Digestion* 2013;87:147–159. doi: 10.1159/000346743.
  121. Wong SW, Ting YW, Chan WK. Epidemiology of non-alcoholic fatty liver disease-related hepatocellular carcinoma and its implications. *JGH Open* 2018;2:235–241. doi: 10.1002/jgh3.12070.
  122. Alexander J, Torbenson M, Wu TT, Yeh MM. Non-alcoholic fatty liver disease contributes to hepatocarcinogenesis in non-cirrhotic liver: a clinical and pathological study. *J Gastroenterol Hepatol* 2013;28:848–854. doi: 10.1111/jgh.12116.
  123. Ertle J, Dechêne A, Sowa JP, Penndorf V, Herzer K, Kaiser G, *et al.* Non-alcoholic fatty liver disease progresses to hepatocellular carcinoma in the absence of apparent cirrhosis. *Int J Cancer* 2011;128:2436–2443. doi: 10.1002/ijc.25797.
  124. Schütte K, Schulz C, Poranzke J, Antweiler K, Bornschein J, Bretschneider T, *et al.* Characterization and prognosis of patients with hepatocellular carcinoma (HCC) in the non-cirrhotic liver. *BMC Gastroenterol* 2014;14:117. doi: 10.1186/1471-230x-14-117.
  125. Mittal S, El-Serag HB, Sada YH, Kanwal F, Duan Z, Temple S, *et al.* Hepatocellular carcinoma in the absence of cirrhosis in united states veterans is associated with nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol* 2016;14:124–131. e121. doi: 10.1016/j.cgh.2015.07.019.
  126. Dasari BV, Kamarajah SK, Hodson J, Pawlik TM, Vauthey JN, Ma YT, *et al.* Development and validation of a risk score to predict the overall survival following surgical resection of hepatocellular carcinoma in non-cirrhotic liver. *HPB (Oxford)* 2020;22:383–390. doi: 10.1016/j.hpb.2019.07.007.
  127. EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. *J Hepatol* 2012;56:908–943. doi: 10.1016/j.jhep.2011.12.001.
  128. Heimbach JK, Kulik LM, Finn RS, Sirlin CB, Abecassis MM, Roberts LR, *et al.* AASLD guidelines for the treatment of hepatocellular carcinoma. *Hepatology* 2018;67:358–380. doi: 10.1002/hep.29086.
  129. Rinella ME, Sanyal AJ. Management of NAFLD: a stage-based approach. *Nat Rev Gastroenterol Hepatol* 2016;13:196–205. doi: 10.1038/nrgastro.2016.3.
  130. Jennison E, Patel J, Scorletti E, Byrne CD. Diagnosis and management of non-alcoholic fatty liver disease. *Postgrad Med J* 2019;95:314–322. doi: 10.1136/postgradmedj-2018-136316.
  131. Castera L, Friedrich-Rust M, Loomba R. Noninvasive assessment of liver disease in patients with nonalcoholic fatty liver disease. *Gastroenterology* 2019;156:1264–1281. e1264. doi: 10.1053/j.gastro.2018.12.036.
  132. Vilar-Gomez E, Chalasani N. Non-invasive assessment of non-alcoholic fatty liver disease: clinical prediction rules and blood-based biomarkers. *J Hepatol* 2018;68:305–315. doi: 10.1016/j.jhep.2017.11.013.
  133. Chiappini F, Desterke C, Bertrand-Michel J, Guettier C, Le Naour F. Hepatic and serum lipid signatures specific to nonalcoholic steatohepatitis in murine models. *Sci Rep* 2016;6:31587. doi: 10.1038/srep31587.
  134. Chiappini F, Coilly A, Kadar H, Gual P, Tran A, Desterke C, *et al.* Metabolism dysregulation induces a specific lipid signature of nonalcoholic steatohepatitis in patients. *Sci Rep* 2017;7:46658. doi: 10.1038/srep46658.
  135. Singal AG, Yopp AC, Gupta S, Skinner CS, Halm EA, Okolo E, *et al.* Failure rates in the hepatocellular carcinoma surveillance process. *Cancer Prev Res (Phila)* 2012;5:1124–1130. doi: 10.1158/1940-6207.Ccr-12-0046.
  136. Piscaglia F, Svegliati-Baroni G, Barchetti A, Pecorelli A, Marinelli S, Tiribelli C, *et al.* Clinical patterns of hepatocellular carcinoma in nonalcoholic fatty liver disease: a multicenter prospective study. *Hepatology* 2016;63:827–838. doi: 10.1002/hep.28368.
  137. Della Corte C, Colombo M. Surveillance for hepatocellular carcinoma. *Semin Oncol* 2012;39:384–398. doi: 10.1053/j.seminoncol.2012.05.002.
  138. Yáñez-Mó M, Siljander PR, Andreu Z, Zavec AB, Borràs FE, Buzas EI, *et al.* Biological properties of extracellular vesicles and their physiological functions. *J Extracell Vesicles* 2015;4:27066. doi: 10.3402/jev.v4.27066.
  139. Hirsova P, Ibrahim SH, Verma VK, Morton LA, Shah VH, LaRusso NF, *et al.* Extracellular vesicles in liver pathobiology: small particles with big impact. *Hepatology* 2016;64:2219–2233. doi: 10.1002/hep.28814.
  140. Sheka AC, Adeyi O, Thompson J, Hameed B, Crawford PA, Ikramuddin S. Nonalcoholic steatohepatitis: a review. *Jama* 2020;323:1175–1183. doi: 10.1001/jama.2020.2298.

141. Sherman M, Bruix J. Biopsy for liver cancer: how to balance research needs with evidence-based clinical practice. *Hepatology* 2015;61:433–436. doi: 10.1002/hep.27563.
142. Friemel J, Rechsteiner M, Frick L, Böhm F, Struckmann K, Egger M, *et al.* Intratumor heterogeneity in hepatocellular carcinoma. *Clin Cancer Res* 2015;21:1951–1961. doi: 10.1158/1078-0432.Ccr-14-0122.
143. Vitale A, Trevisani F, Farinati F, Cillo U. Treatment of hepatocellular carcinoma in the precision medicine era: from treatment stage migration to therapeutic hierarchy. *Hepatology* 2020;72:2206–2218. doi: 10.1002/hep.31187.
144. Forner A, Da Fonseca LG, Díaz-González Á, Sanduzzi-Zamparelli M, Reig M, Bruix J. Controversies in the management of hepatocellular carcinoma. *JHEP Rep* 2019;1:17–29. doi: 10.1016/j.jhepr.2019.02.003.
145. Lin S, Hoffmann K, Schemmer P. Treatment of hepatocellular carcinoma: a systematic review. *Liver Cancer* 2012;1:144–158. doi: 10.1159/000343828.
146. Bruix J, Sherman M. Management of hepatocellular carcinoma: an update. *Hepatology* 2011;53:1020–1022. doi: 10.1002/hep.24199.
147. Han KH, Kudo M, Ye SL, Choi JY, Poon RT, Seong J, *et al.* Asian consensus workshop report: expert consensus guideline for the management of intermediate and advanced hepatocellular carcinoma in Asia. *Oncology* 2011;81 (Suppl 1):158–164. doi: 10.1159/000333280.
148. Xie D-Y, Ren Z-G, Zhou J, Fan J, Gao Q. 2019 Chinese clinical guidelines for the management of hepatocellular carcinoma: updates and insights. *Hepatobiliary Surg Nutr* 2020;9:452–463. doi: 10.21037/hbsn-20-480.
149. Omata M, Tateishi R, Yoshida H, Shiina S. Treatment of hepatocellular carcinoma by percutaneous tumor ablation methods: ethanol injection therapy and radiofrequency ablation. *Gastroenterology* 2004;127:S159–166. doi: 10.1053/j.gastro.2004.09.030.
150. Pan T, Mu L-W, Wu C, Wu X-Q, Xie Q-K, Li X-S, *et al.* Comparison of combined transcatheter arterial chemoembolization and CT-guided radiofrequency ablation with surgical resection in patients with hepatocellular carcinoma within the up-to-seven criteria: a multicenter case-matched study. *J Cancer* 2017;8:3506–3513. doi: 10.7150/jca.19964.
151. Lencioni R, Cioni D, Crocetti L, Franchini C, Pina CD, Lera J, *et al.* Early-stage hepatocellular carcinoma in patients with cirrhosis: long-term results of percutaneous image-guided radiofrequency ablation. *Radiology* 2005;234:961–967. doi: 10.1148/radiol.2343040350.
152. Lurje I, Czigan Z, Bednarsch J, Roderburg C, Isfort P, Neumann UP, *et al.* Treatment strategies for hepatocellular carcinoma - a multidisciplinary approach. *Int J Mol Sci* 2019;20:1465. doi: 10.3390/ijms20061465.
153. Mazzaferro V, Bhoori S, Sposito C, Bongini M, Langer M, Miceli R, *et al.* Milan criteria in liver transplantation for hepatocellular carcinoma: an evidence-based analysis of 15 years of experience. *Liver Transpl* 2011;17 (Suppl 2):S44–S57. doi: 10.1002/lt.22365.
154. Verna EC, Patel YA, Aggarwal A, Desai AP, Frenette C, Pillai AA, *et al.* Liver transplantation for hepatocellular carcinoma: management after the transplant. *Am J Transplant* 2020;20:333–347. doi: 10.1111/ajt.15697.
155. Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, *et al.* Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* 2008;359:378–390. doi: 10.1056/NEJMoa0708857.
156. Kudo M, Finn RS, Qin S, Han KH, Ikeda K, Piscaglia F, *et al.* Lenvatinib versus sorafenib in first-line treatment of patients with unresectable hepatocellular carcinoma: a randomised phase 3 non-inferiority trial. *Lancet* 2018;391:1163–1173. doi: 10.1016/S0140-6736(18)30207-1.
157. Casak SJ, Donoghue M, Fashoyin-Aje L, Jiang X, Rodriguez L, Shen YL, *et al.* FDA Approval summary: atezolizumab plus bevacizumab for the treatment of patients with advanced unresectable or metastatic hepatocellular carcinoma. *Clin Cancer Res* 2021;27:1836–1841. doi: 10.1158/1078-0432.Ccr-20-3407.
158. Hack SP, Spahn J, Chen M, Cheng AL, Kaseb A, Kudo M, *et al.* IMbrave 050: a Phase III trial of atezolizumab plus bevacizumab in high-risk hepatocellular carcinoma after curative resection or ablation. *Future Oncol* 2020;16:975–989. doi: 10.2217/fo-2020-0162.
159. Bruix J, Qin S, Merle P, Granito A, Huang YH, Bodoky G, *et al.* Regorafenib for patients with hepatocellular carcinoma who progressed on sorafenib treatment (RESORCE): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet* 2017;389:56–66. doi: 10.1016/s0140-6736(16)32453-9.
160. Abou-Alfa GK, Meyer T, Cheng AL, El-Khoueiry AB, Rimassa L, Ryoo BY, *et al.* Cabozantinib in patients with advanced and progressing hepatocellular carcinoma. *N Engl J Med* 2018;379:54–63. doi: 10.1056/NEJMoa1717002.
161. Montironi C, Montal R, Llovet JM. new drugs effective in the systemic treatment of hepatocellular carcinoma. *Clin Liver Dis* 2019;14:56–61. doi: https://doi.org/10.1002/cld.796.
162. Chiew Woon L, Joycelyn Jie Xin L, Su Pin C. Nivolumab for the treatment of hepatocellular carcinoma. *Expert Opin Biol Ther* 2020;20:687–693. doi: 10.1080/14712598.2020.1749593.
163. El-Khoueiry AB, Sangro B, Yau T, Crocenzi TS, Kudo M, Hsu C, *et al.* Nivolumab in patients with advanced hepatocellular carcinoma (CheckMate 040): an open-label, non-comparative, phase 1/2 dose escalation and expansion trial. *Lancet* 2017;389:2492–2502. doi: 10.1016/S0140-6736(17)31046-2.
164. El Dika I, Makki I, Abou-Alfa GK. Hepatocellular carcinoma, novel therapies on the horizon. *Chin Clin Oncol* 2021;10:12. doi: 10.21037/cco-20-113.

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