GLOBAL SCIENCE LIBRARY

REVIEW

Frontiers in Molecular Medicine and Therapeutics

Hepatocellular carcinoma: Causes, putative biomarkers, and current therapies

Marwah Al-Aloosi[1] and Michael J. Bouchard[2*]

[1]Graduate Program in Cancer Biology, Graduate School of Biomedical Sciences and Profession Studies, College of Medicine, Drexel University, USA
[2]Department of Biochemistry and Molecular Biology, College of Medicine, Drexel University, USA

Abstract

Recent advances in molecular diagnostic technologies continue to facilitate the discovery of diverse cellular signaling pathways that are involved in the development and progression of hepatocellular carcinoma (HCC). HCC accounted for 30,000 cancer-related deaths in the United States (U.S.) in 2020. In the era of personalized medicine that has led to improved therapies for many cancers, the efficacy of current anti-HCC therapies remains modest at best. Although significant advances in basic science research have identified alterations of cellular, genetic, and epigenetic signals that influence HCC development, and some of these have led to the development of anti-HCC therapies, overall, HCC cures continue to be elusive. Moreover, tumor-related changes in the microbiome and the tumor microenvironment have been identified and have revealed multiple potential pathways that can either aid in diagnosing HCC or serve as new therapeutic targets. However, even here, there is a need to translate these observations into a clinical outcome, and HCC typically has a poor prognosis.

In any clinical scenario, the clinical oncologist starts by identifying risk factors, followed by using diagnostic tools, including testing for specific biomarkers, to help diagnose the stage of the disease, which aids in selecting the best treatment options to improve the patient's prognosis. In this review, we will follow a similar pathway by first providing a description of HCC risk factors, biomarkers, and diagnostic tools and then discussing treatment options. Rather than provide a historical review, we will focus on the most current HCC biomarkers and treatments that have been published and will integrate the results of recent basic science research on cellular signals that are altered in HCC, especially where these have contributed to novel HCC therapies.

Introduction

According to GLOBOCAN, ~905,677 new patients were diagnosed with, and ~ 830,180 patients died of HCC in 2020 [1]. The worldwide male to female ratio is 4:1, and the average age of presentation globally is ~70 years [2]. In developing countries and Asia, a chronic Hepatitis B virus (HBV) infection is the most common cause of HCC; whereas, in the U.S., a chronic Hepatitis C virus (HCV) infection is a more common cause of HCC [3]. In addition to geographic differences in the incidence of chronic HBV or HCV infections, geographic differences in the incidence of HCC have also been linked to variations in the presence of other risk factors which include chronic alcohol consumption, liver cirrhosis, non-alcoholic fatty liver disease, obesity, smoking, and aflatoxin exposure [2,3]. Globally, HCC is most prevalent in Asia followed by North America and Europe [4]. In the U.S., the age of HCC diagnosis is ~60 years, with the highest incidence in Asians. Although the incidence is much lower in Americans of African descent in the U.S., the age of presentation is around 44 years [5].

HCC is currently ranked as the sixth most common malignant tumor and the fourth leading cause of cancer-related death worldwide [6]. Surveillance, Epidemiology, and End Results (SEER) data (2009-2015) regarding the 5-year survival rate for the patients diagnosed with HCC showed that there is a 33% 5-year survival rate for patients with localized disease as compared with only 2% for patients with disseminated disease [7]. Despite comprehensive, population-based HCC screening programs in some countries that have enhanced the early HCC detection rate, most of the patients still present to the clinic at advanced stage [4]. Current treatment options for limited stage HCC consist of surgical liver resection and local ablative therapy provided that the patient has a good hepatic reserve. In contrast, the 5-year HCC recurrence rate of 70% in patients with limited stage HCC but inadequate liver function who are not suitable candidates for localized therapies makes liver transplantation a superior treatment option for these patients [8]. For patients with advanced HCC, the combination therapies of anti-angiogenic drugs plus immune check point inhibitors have improved the response rate and delayed tumor progression, but the benefit is still limited to a small subset of HCC patients [9]. The high mortality of patients with HCC, and its limited treatment response, supports the need for a more comprehensive understanding of the molecular pathways that affect HCC development and continued collaboration between scientists in basic research and clinicians to translate discoveries at the bench to HCC therapies. Although we provide some historical context, a summary of all identified risk factors and how these could be linked to HCC initiation, progression, and metastasis, as well as a description of all potential biomarkers and therapies, is beyond the scope of this review. Instead, our focus will be on recent discoveries, and we will present information in a similar order as the work-up of a clinical HCC vignette.

Risk factors

Although the exact cause of HCC is still unknown, data provided by epidemiological studies and genomic studies have defined several risk factors that play a role in the development of HCC. Chronic infection with HBV or HCV, chronic alcohol consumption, liver cirrhosis, obesity, Non-Alcoholic Fatty Liver

Table 1: *A list of some of the risk factors and how they are thought to contribute to HCC development*

Risk Factors	The proposed mechanism of the risk factors to HCC development	Reference
HBV	-Indirect immune-mediated destruction of HBV-infected hepatocytes and associated inflammation, fibrosis, and cirrhosis. -Direct :(HBx) integration within the genome - HBx-LINE 1.	[10,12-14,21,76]
HCV	-Direct (viral protein). -Indirect (Liver Cirrhosis). -Bystander effect.	[23-27,30-36]
Chronic Alcohol Consumption	-Directly (via genotoxicity). -Indirectly (cirrhosis).	[37-42]
Obesity	-TNF-a activates JNK pathway and NF-kB. -IL-6 activates STAT 3 pathway. -Leptin activates JAK 2/STAT3 and PI-3K/Akt pathways.	[46,47,50-52]
NASH	-(TGFβ) increases the levels of circulating IgA. -TNF-a activates JNK pathway.	
NAFLD	-Leptin activates JAK 2/STAT3 and PI-3K/Akt pathways. -(TGFβ) increases the levels of circulating IgA.	
Aflatoxin	-Formation of (AFB1-FABY) adduct. -ROS generation.	[71,73-75]

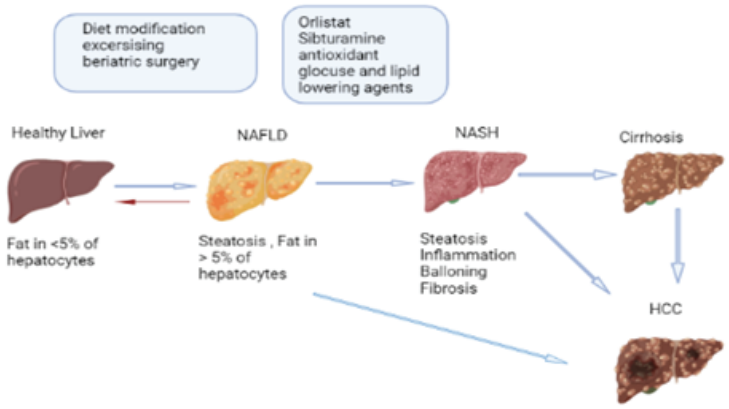


Figure 1: Pathophysiological stages of HCC development

Disease (NAFLD), and nonalcoholic steatohepatitis (NASH) are among those risk factors [10]. Fungal mycotoxins, especially aflatoxin, are also reported to contribute to HCC pathogenesis [11]. Here we review these risk factors and how they are thought to contribute to HCC development.

Hepatitis B virus

HBV is a hepatotropic virus that contains a 3.2 Kb circular DNA genome and is thought to promote hepatic carcinogenesis both directly and indirectly. The indirect mechanisms include immune-mediated destruction of HBV-infected hepatocytes and associated inflammation that can cause fibrosis, cirrhosis, and eventually HCC. Direct mechanisms of HBV-mediated HCC are thought to include consequences of HBV protein activities, such as activities of the HBx protein. HBx expression stimulates HBV replication and can modify cellular signaling pathway such as those that regulate cellular proliferation, cell metabolism, and cell survival signals which could promote carcinogenesis [10,12-14]. The HBV genome is also found inserted into the host genome in some HCCs, and this insertion could alter expression or activities of oncogenic factors and may provide another mechanism for the development of HBV associated HCC. Numerous studies that have mapped HBV integration sites have shown that integration is likely random [12-14]. Some studies have identified common themes such as integration in the vicinity of oncogenes, although this does not contradict the notion that integration is random but that integration in the vicinity of an oncogene could provide a survival advantage of a cell. For example, in a study that involved whole-genome sequencing of HCC tumors derived from 88 Chinese individuals (81 HBV-positive and 7 HBV-negative) [15], the HBV genome was found integrated in telomerase reverse transcriptase (TERT),which encodes for telomerase [16], (18 of 76)), mixed lineage leukemia4 (MLL4), which encodes for proteins that act as mono methyltransferases at enhancers [17] (9 of 76), or cyclin E gene (CCNE), which encodes for cyclin E that accumulates at the G1–S transition of the cell cycle [18] (4 0f 76) accounting

for 40.8% of the HCC samples. This recurrent integration was associated with an alteration in protein expression that could potentially contribute to HCC development [15]. Two other studies showed a recurrent HBV integration at the TERT promoter (35.8% of the studied HBV-related HCC cases) [19] and HBV-MLL4 integration in 18% of HBV-related HCC Chinese patients [20]. Finally, one study described the result of integration of HBV near the normally transcriptionally silent long interspersed nuclear elements (LINEs) that generated a hybrid long non-coding RNA that includes a portion of the HBx sequence. This fusion, HBx-LINE 1, was shown to influence HCC development by activating β-catenin/Wnt signaling, a hallmark in HCC tumorigenesis. HBx-Line 1 expression was associated with shorter survival in HCC patients [21].

Hepatitis C virus

A chronic HCV infection is another major risk factor for HCC; HCV-associated HCC is more common in Western countries. The WHO (World Health Organization) considers chronic HCV infection and associated diseases as a global health crisis, and it was estimated that ~ 3% of the world's population is infected with HCV. Infection with HCV can lead to chronic inflammation, liver cirrhosis, and HCC. Moreover, the combination of HCV infection with other risk factors (e.g., HBV infection, obesity, and HIV infection) can significantly increase the risk for HCC development [22]. Three mechanisms have been proposed for the development of HCV-associated HCC. The first mechanism involves effects of HCV proteins that alter hepatocyte signaling pathways involved in survival, metabolism, migration, and transformation, and thus, directly inducing tumorigenesis. TERT, p53, Rb, angiogenesis, Wnt/B-catenin and RTKs are examples of the cellular pathways that are altered in HCV-related HCC [23-27]. The second mechanism is indirect and linked to the induction of liver cirrhosis that results from long-term injury to hepatocytes due to HCV infection. The persistence of this injury forces hepatocytes to adapt alternative physiological and pathological pathways to resist injury. These pathways often overlap with stress-induced pathways caused by chronic alcohol consumption, obesity, and oxidative stress causing additional inflammation, fibrosis, and cell death that can contribute the increased risk for HCC that is observed when HCV infection is combined with these other risk factors. Cirrhosis is one form of the cellular responses to long term stress in the liver [28,29]. In a study conducted by Aydin et al., chronic HCV infection was shown to increase both endoplasmic reticulum (ER) and oxidative stress leading to activation of nuclear factor erythroid 2-related factor 2 (NRF2) that could be involved in the development of HCV-related HCC. NRF2 is an activator of STAT3, which is a member of STAT family that is known to be involved in inflammation and tumorigenesis [30]. A third interesting mechanism underlying the development of HCV-associated HCC that has been proposed is the bystander effect, which was thought to mimic to the bystander effect commonly linked to radiation therapy. In the radiation-therapy associated bystander effect, the irradiated cells use Gap Junction Intercellular Communication (GJIP) to spread cargo containing ROS, DNA molecules, and cellular proteins from damaged cells to surrounding healthy cells. Gap junctions are cell—cell junctions that connect two adjacent cells and have channels made mainly by Connexin family protein which facilitate the intercellular exchange. Several studies showed that HCV uses the GJIP to enhance the survival of surrounding uninfected hepatocytes (Bystander cells) through spreading cargos that can alter the signaling pathways in the bystander cells and hence promotes the tumorigenic transformation of these cells. It could also help explain the development of HCC in HCV-cured patients where the anti-viral therapy could clear the infection but could not relieve the infection-induced stress in hepatocytes [31-36]. On the other hand, some studies showed that Gap junctions might act as "conditional" tumor suppressors through inhibiting cell migration, and Connexin upregulation was found to inhibit hepatocarcinogenesis [32,36].

Chronic alcohol consumption

One of the major risk factors for HCC development is chronic alcohol consumption, and alcohol was listed by the WHO as a Group 1 carcinogen. Chronic alcohol consumption accounts for 32-45% of HCC in Italy [37], and Europe has the highest alcohol consumption globally [38]. Chronic alcohol consumption can work simultaneously with other risk factors (e.g., chronic HBV infection or chronic HCV infection) to augment the risk of carcinogenesis by increasing free radical generation while dampening the hepatic defense mechanisms [37,39,40]. Chronic Alcohol consumption can induce hepatocarcinogenesis either directly (via genotoxicity) or indirectly (cirrhosis) [39]. The proposed mechanism for the direct genotoxic effects of alcohol involves the induction of cytochrome (CYP)2E1, acetaldehyde formation, immunological disruption, and activation of angiogenesis. Chronic alcohol consumption (> 10 years) elevates expression of CYP2E1, hepatic acetaldehyde, oxidative stress, and ROS generation. ROS form harmful adducts with proteins, DNA, cell-membrane lipids in hepatocytes. Alcohol-mediated elevation of free radicals can also impact the DNA of peripheral liver-specific lymphocytes causing DNA fragmentation that can interfere with lymphocyte functions [37,38,40,41]. Indirect mechanisms of alcohol-associated HCC are mediated by induction of liver cirrhosis that culminates into HCC, and many studies have confirmed a linear relationship between chronic alcohol consumption, liver cirrhosis, and HCC [38,39,42].

Non-alcoholic fatty liver disease

Recently, much attention has been directed towards obesity, nonalcoholic fatty liver disease (NAFLD), and nonalcoholic steatohepatitis (NASH), the progressive form of NAFLD, as causative factors for HCC. In the U.S., it was reported that HCC-related deaths were 1.5-2 times higher in men with Body Mass Index (BMI) > 35kg/m^2 than in men with normal BMI (18.5-25 kg/m2) [43], while in UK, HCC-related death was 4.5 times higher in obese men compared to men with normal weight [44]. On the other hand, NAFLD accounts for 59% of HCC cases in the U.S., and it is currently considered the most common HCC causative factor in the U.S. [45,46]. Despite initially neglecting to consider the effects of NASH on HCC development, around 116 HCC cases in NASH patients were registered between 2004-2015 in the U.S. [47]. The proportion of patients with NASH and HCC increased 7.7-fold from 2002-2016, and the prevalence of HCC with NASH increased 11.8-fold during the same period [48]. Obesity is linked to the accumulation of lipids in hepatocytes, initially causing NAFLD, which can eventually become NASH. The lipid accumulation (mainly triglycerides) in liver interferes with the normal hepatic glucose and fatty acid metabolism resulting in severe hepatic diseases [49]. Lipid accumulation in hepatocytes also induces macrophage recruitment and the release of cytokines such as tumor necrosis factor alpha (TNFα) and interleukin 6 (IL 6). TNF α promotes inflammation, cell proliferation (through activation of JNK (Jun N terminal kinase)) and apoptosis (through interaction with NF-kB). IL6, on the other hand, enhances cell growth and proliferation through activation of STAT 3. Both TNFα and IL6, and activation of JNK signaling, contribute to HCC development from NASH [46,47,50]. In obesity, there is also

an increased level of Leptin and a decreased level of adiponectin, and both are involved in NAFLD progression to HCC. Leptin was reported to cause insulin resistance, liver fibrosis, angiogenesis, and activation of several cellular factors such as JAK 2/STAT3 and PI-3K/Akt [46,51]. PI3K/Akt signaling is involved in cell growth, proliferation, and survival [47]. Cumulatively, the above studies showed that obesity, NASH, and NAFLD share common molecular effects and that NASH and NAFDL mostly arise within the context of obesity. Another inflammatory mediator of liver fibrosis is transforming growth factor β (TGFβ), an immunosuppressive cytokine that stimulates the switching of B-cells expressing IgM into B cells expressing IgA. It was reported that patients with NAFLD or NASH with liver fibrosis have higher levels of circulating IgA than patients without fibrosis. B cells expressing IgA+ contribute to HCC development by inhibiting the activation of cytotoxic CD8+ T lymphocytes (CTL) in human and mouse NASH-affected livers, and thus, can mask anti-tumor immunity [52]. Finally, although there has been considerable focus on the association between inflammation, NAFLD, and NASH, some recent studies have shown that 50% of HCC cases developed in the context of NASH without liver cirrhosis [53-55], and that HCC might develop in patients with simple steatosis without the need to pass through stages of NASH, inflammation and fibrosis [54,56,57].

Management of NAFLD and NASH

Since NAFLD is linked to obesity and insulin resistance, and only a minority of the cases with NAFLD progress to NASH, which can lead to liver cirrhosis and HCC, treatment options for NAFLD target the pathophysiological pathway that include adiponectin, leptin, TNF-α, and interleukins [58,59]. Patients with NAFLD are at an increased risk for developing cardiovascular disease [60], and angiogenesis plays an important role in the development of NASH [61]. Therefore, the treatment options for NAFLD aim to prevent NASH development, decrease the risk of cardiovascular disease, and ensure better quality of life for the patients to reduce the NAFLD-associated liver mortality rate [59]. The first option that carries the highest potential for treating NAFLD is weight loss by dietary modification, exercising, or bariatric surgery. Weight loss has been shown in several studies to induce both biochemical and histological improvement in patients with NAFLD [62-64]. Moreover, weight loss assisted by FDA- approved medications such as Orlistat and Sibutramine have been shown to improve the outcome in patients with NASH [65-67]. Antioxidants, lipid- and serum- glucose lowering agents have also been investigated in several studies as therapeutic options for NAFLD and NASH [59,68,69], and showed promising results.

Mycotoxins

In 1965, the mycotoxin (Aflatoxin) was mentioned for the first time. The United Nations Food and Agriculture Organization and the WHO reported that the mold and fungal growth contaminate about one quarter of the world's crops. Unfortunately, data regarding mycotoxicosis in developing countries are limited [11]. The main route for mycotoxicosis is via oral ingestion, but it can be inhaled or directly affect the skin [70]. However, the degree of mycotoxicosis is time and dosage dependent: an extended exposure time with a high dose of a mycotoxin is necessary to seriously affect human health [11]. Aflatoxin, and mainly Aflatoxin B1 (AFB1) is one of the most potent hepatic carcinogens in mammals, and it was listed as a Group I carcinogen by the International Agency for Research on Cancer (IARC) [71]. AFB1, which is mainly produced by Aspergillus flavus and Aspergillus parasiticus, affects the liver and has is strongly correlated with the development of HCC [72]. AFB1 is metabolized by the liver cytochrome p450 system to produce aflatoxin-8,9-exo-epoxide. Aflatoxin-8,9-exo-epoxide can react with proteins, RNA, DNA and p53 and change the structure or characteristics of these molecules. For example, the binding of Aflatoxin-8,9-exo-epoxide to DNA results in the generation of 8,9 dihydro-8-(N7 guanyl)-9-hydroxy AFB1 adduct (AFB1-N7-Gua), which in turn, is converted to the more stable AFB1-formamidopyrimidine (AFB1-FABY) adduct. This adduct causes transversion of guanine (G) to thymine (T), which is mutagenic if it was inserted in the cell genome and over time, can cause malignant transformation [71,73,74]. In a study involved the aflatoxin-B1 and P53 mutations, it was found that CYP1A2 and CYP3A4 both contribute to the formation of AFB1-induced P53 mutations that give cells a growth advantage and promotes carcinogenesis [74]. Moreover, aflatoxins can directly affect the GI health by changing the composition of the gut microbiota, disrupting the intestinal epithelial barrier, inhibiting cell growth, and inducing genetic damage by generating reactive oxygen species (ROS) that damage both the DNA and the plasma membrane of the enterocytes [75].

Liver cancer initiation and progression

Hepatocarcinogenesis is a multi-step process in which the normal hepatocytes are transformed to form preneoplastic lesions which then progress to dysplastic lesions and finally to frank HCC [77]. In response to chronic stressors that can stimulate cell death pathways such as apoptosis, cells often adapt by activating cellular survival mechanisms that may lead to apoptotic resistance which also may provide the cells a selective survival advantage. Continued survival and proliferation of these cells can eventually result in transformation, tumor formation, including new blood vessels formation, and as malignancy progresses, invasiveness and metastasis of the transformed cells. These cells often also develop mechanisms to suppress the activity of the cytotoxic T cells in an attempt to escape the immune surveillance and destruction of tumor cells [78]. It is now well established that tumors contain cancer stem cells (CSC), which is a population of cells in tumors that is characterized by autonomous proliferation and self-renewal. CSCs are known to be major contributors to HCC development and progression. CSCs, which are present in most tumors, are often resistance to chemotherapy and radiation, and when present at high levels, result in higher relapse rates [79]. HCC cell lines and primary HCC tumors often contain a subpopulation of cells that express CD133, an established CSC marker [80]. It is now clear that genetic, epigenetic, and cellular signal-transduction pathway alterations, as well as the disruptions to the microenvironment, play a role in HCC initiation and progression. In this section, we summarize common genetic, epigenetic, and molecular alterations identified in HCC (readers are referred to Setshedi et al., Dhanasekaran et al. and Niu et al. for a more comprehensive reviews) and then focus on some recent findings that provide additional support for the role of these genetic, epigenetic, and molecular alterations in HCC.

Genomic instability

The application of genome-wide association studies (GWAS) and next-generation sequencing (NGS) have revealed alterations in several genes and related pathways in HCC. Genomic instability, single-nucleotide polymorphisms (SNPs), and somatic mutations all play a major role in the process of hepatocarcinogenesis. Genomic instability (defined as a high frequency of mutations within the genome) in tumors can be classified into chromosomal instability (CIN) and microsatellite instability (MSI) [81]. Several

SNPs are reported to be associated with HCC. However, results of studies of SNP involvement in HCC are inconsistent, requiring further studies, and SNPs involvement in HCC will not be described here.

CIN, which is a hallmark of human cancers [82], is common in HCC [83]. CIN could be either numerical (the gain or loss of whole chromosomes leading to aneuploidy), or structural (the gain or loss, translocation, inversion, amplification, deletion and/or allelic loss [loss of heterozygosity (LOH)] of part of a chromosome) [84]. In HCC, common examples of structural CIN include gain in chromosomes (1q, 5p, 6p, 7q, 8q, 17q, and 20q) or loss in chromosomes (1p, 4q, 6q, 8p, 9p, 13q, 14q, 16p-q, 17p, 21p-q, and 22q) [81,85]. LOH in HCC found frequently at (1p, 4q, 5q, 8p, 8q, 9p, 10q, 11p, 13q, 14q, 16q, 17p, and 22q). High levels of AFP, an established HCC biomarker (see below), HCC high grades, and HCC poor prognosis were found to be related to LOH at different loci in HCC [86]. DNA copy number alterations can be used to identify driver genes, provide prognostic and predictive biomarkers, and serve as potential therapeutic target [81]. For example, the genes that encode for cyclin D1 (CCND1), which regulate cell proliferation [87], and fibroblast growth factor 19 (FGF19), which regulates various metabolic process including activation of mTOR signaling [88], are among the most highly amplified genes in HCC and have been validated as oncogenic drivers of HCC. Moreover, DNA copy number alterations of the genes that encode B-cell CLL/lymphoma 9 (BCL9), which is a transcriptional co activator of the Wnt/β-catenin signaling, and metadherin (MTDH), which interacts with NF-KB and elicits the expression of the downstream genes that are involved in cell migration and invasion [89], have been linked to advanced stage and aggressive types of HCC [90].

MSI, although initially reported to be a rare event in HCC, especially as compared to other cancers, is now known to be at least 3 times higher in cirrhotic livers than normal livers with equal incidence between cirrhotic livers and HCCs. MSI was reported to occur at a later stage than LOH in HCC. Therefore, MSI was suggested to be linked to progression of late stages of liver cirrhosis to HCC [91]. Other studies have shown that MSI can correlate with tumor recurrence, high grade tumors, portal vein involvement, and poor prognosis of HCC [81].

Epigenetic alterations

Epigenetic alterations, such as modifications to DNA and histones, have been found in HCC. For example, methylation of histone H3 lysine 4 (H3K4me3), which can enhance transcription of certain genes, is significantly elevated in HCC and correlates with aggressive HCC phenotypes and 3.5 higher mortality rate [92]. Moreover, the silencing of tumor suppressor gene expression by hypermethylation of their transcription promoter regions and activation of oncogene transcription by hypomethylation of their transcription promoters occur frequently in HCC [78]. The genes encoding suppressor of cytokine signaling 1 (SOCS1), which are negative feedback inhibitors of the JAK/STAT cytokine signaling pathway, and adenosis polyposis coli (APC), which is a tumor suppressor gene that encodes a large protein with multiple cellular functions and interactions, including signal transduction in the Wnt-signaling pathway , are examples of genes that are inactivated by DNA hypermethylation in HCC [93,94]. It is noteworthy that both HCV and HBV infections can change the DNA methylation pattern in HCC [94,95]. HCV infection is known to result in DNA hypermethylation of certain genes (e.g., HCV-induced hypermethylation of the Gadd45β promote; Gadd45β plays central roles in the cellular response to genotoxic stress) [95]. HBV, via its HBx protein, can either induce DNA hypermethylation to suppress transcription of certain genes (e.g., HBV-induced hypermethylation of APC) [96], or induce DNA acetylation and activate of other genes (e.g., HBx-induced activation of co-activators of the CREB-binding protein CBP–P300 complex leading to upregulated transcription of the IL-8 gene) [97].

Alterations in cell signal transduction pathways

Somatic mutations in oncogenes and/or tumor suppressor genes, as well as alterations in various cell signal transduction pathways, have been shown to play a role in HCC development [10]. For example, the activation of the PI3K/AKT pathway was found in many tumors including HCC and plays a role in cell survival and proliferation [47]. PI3K activates AKT, which is a lipid second messenger that phosphorylates various intracellular proteins, including mTOR. The activation of mTOR causes cell proliferation and BAD inhibition. BAD induces cell apoptotic resistance [98,99]. Other signaling pathways that were reported in HCC: VEGF/VEGFR that contributes to HCC hypervascularity, insulin-like growth factors (IGF) which promotes HCC development and progression, EGFR, and c-Met that are related to aggressive HCC phenotypes, and Ras-mitogen-activated protein kinase (MAPK), ERK, and JAK/STAT pathways that are crucial mediators of HCC initiation and progression via promoting cellular proliferation and survival [10,47,78,98]. The large number of factors and pathways that have been linked to HCC prevent a comprehensive review here, and only those most identified are discussed.

TERT

Telomerase (TERT) is the enzyme that is responsible for maintaining the length of DNA telomeres [100]. The most frequent driver mutations in HCC were reported to be mutations of the telomerase reverse transcriptase (TERT) promoter, which increase the expression of telomerase and occurs in 40-65% of HCC cases [10,100,101]. Under normal conditions, telomerase is not expressed in somatic cells due to silencing of the TERT transcriptional promoter; however, in many cancer cells the TERT transcriptional promoter is derepressed and this contributes to tumorigenesis by facilitating indefinite cancer cell proliferative [102,103]. Both chronic inflammation and TERT promoter loss of function mutations contribute to liver cirrhosis [104], while in the case of HCC, the activating mutations of (TERT) promoter render allow for indefinite replication of the cancer cells as well as resistance to apoptosis, thus contributing to HCC development [78].

TP53

TP53 is a tumor suppressor gene that plays a critical role in cell response to DNA damage, and loss of its expression or function has been linked to the develop of many types of cancer [105]. TP53 inactivating mutations are found in 18 – 50% of HCCs [106]. TP53 was reported to contribute to HCC development particularly in patients with HBV and exposed to high levels of aflatoxins [107]. Aflatoxin causes transversion of G:C to T:A at codon 249 resulting in TP53 mutation [78]. TP53 is correlated with HCC poor prognosis and lower OS [78,81,106].

Wnt/β-catenin

Mutations in Wnt/β-Catenin have been identified in 20-40% of HCC cases [108]. Wnt/β-catenin signaling is known to regulate cellular migration and proliferation by promoting Epithelial to Mesenchymal Transition (EMT). This pathway can be activated

by either gain of function mutations in CTNNB1, which encodes for β-catenin, and have been found in 11%–37% of HCCs or by inactivating mutations in AXIN1, which encodes for AXIN1 protein that interacts with APC, β-catenin, and other proteins, have been found in 5%–15% of HCCs [10]. Mutations in Wnt/β-catenin were found to be higher in cases of alcohol- or HCV-induced HCC than in other HCCs [10,81,108].

NR1D2

A recent study by Tong et al. showed that a component of the circadian clock, namely the nuclear hormone receptor, nuclear receptor subfamily 1 group D member 2 (NR1D2) also promotes EMT in HCC via activation of β–Catenin. NR1D2 knockdown in HCC cell lines led to inhibition of β-catenin and reduced tumor cell migration and invasion [6].

EZH2

Elevation of Enhancer of Zeste 2 (EZH2), a chromatin-modifying protein that mediates epigenetic silencing, was found to be correlated with high levels of metastasis and poor prognosis of HCC through activation of Wnt signaling by silencing expression of Wnt antagonists, including the growth-suppressive AXIN2, NKD1, PPP2R2B, PRICKLE1, and SFRP5 [109] Kim et al. reported that the extensive EZH2 occupancy at promoters marked by either acetylation of H3K27 or trimethylation of H3K27, leading to gene activation or repression, respectively [110].

ARIDs

The chromatin remodeling proteins ARID1A and ARID2 were also found to be mutated in HCC samples. ARID1A mutations were found in 4%–17% of HCCs, and ARID2 mutations were found in 3%-18% of HCCs [10,81,111]. Moreover, cross talk between mutations in ARID1A and mutations in CTNNB1 or mutations in AXIN1 was suggested, which might explain Wnt effects on chromatin remodeling in hepatocarcinogenesis [81,112].

Hedgehog

Hedgehog genes encode signaling molecules that are involved in patterning processes during embryogenesis [113]. Hedgehog proteins are thought to contribute to HCC development by promoting cellular adhesions, enhancing migration and invasiveness, increasing expression and activity of matrix metalloproteinases-2 and 9 (MMP-2 and MMP-9), and ERK-induced activation of MMP-9 [114,115]. Hedgehog was also found to potentiate radiation-induced liver fibrosis [116].

DERL 1

DERL 1 is one member of DERL family that consists of DERL 1,2, and 3. The DERL1 protein is located in the ER and involved in degrading misfolded proteins [117-119]. DERL 1 was found to be overexpressed in up to 75% of HCCs and is mainly associated with tumors > 5 cm or those with lymph node metastasis. DERL1 was suggested to be involved in HCC progression and as a predictor for HCC metastasis. DERL1 was shown to exert its activity via both Akt and ERK pathways. DERL1 overexpression promotes cell proliferation and migration, while decreasing the number of apoptotic events in HCC cell lines [120].

CD276

Another protein that contributes to HCC development via activation of PI3K/AKT pathway is CD 276, which is an endothelial marker for the pathogenic angiogenesis [119]. CD 276 belongs to B7 superfamily, and it has an important role in both innate and T-cell mediated immunity [121,122]. CD276 was found to be overexpressed in a number of tumors, including HCC and to correlate with tumor aggressiveness and poor prognosis [123]. CD276 was shown to promote cell migration and invasiveness by increasing the expression and the activity of (MMPs) and inducing vascular mimicry (VM) formation via upregulation of MMPs and expression of E-cadherins and laminin in HCC, which ultimately leads to extracellular matrix remodeling and neoangiogenesis. CD276 was shown to work upstream of Akt and to stimulate VM formation via activation of the PI3K/Akt/MMPs pathway; whether this is the only mechanism for VM formation in HCC requires further studies [119].

CCND1

The CCND1 gene encodes Cyclin D1, a regulator of cell cycle progression and the PI3K/AKT pathway, is often overexpressed in HCC. Cyclin D1 is expressed in the G1 phase of the cell cycle and is involved stimulating progression from G1 to the S phase of the cell cycle. Therefore, cyclin D1 overexpression can cause uncontrolled cellular division, and can also alter transcription of certain genes, translation of mRNAs, and even affect protein structure by affecting post-translation modification of proteins [87,124]. Cyclin D1 was found to be overexpressed in HCC, as well as in many other tumors. Although cyclin D1 regulation of cell proliferation involves numerous effects, a recent study showed that in HCC, the effect of cyclin D1 on cell proliferation as included activation of the PI3K/Akt pathway. In this study, silencing of cyclin D1 expression led to suppressed the PI3K/Akt pathway, inhibited cellular growth, and promoted apoptotic events [125].

ADAM17

A disintegrin and metalloproteinase metallopeptidase domain 17 (ADAM17) is a proteolytic enzyme that cleaves the Notch receptor. The Notch pathway is important during embryogenesis, liver repair and regeneration. The Notch receptor family is composed of 4 receptors, and their role in HCC is still incompletely understood [78]. While G-protein-coupled receptor 50 (GPR50) was overexpressed in HCC and correlated with HCC progression, a study conducted by Saha et al. 2020, showed that GPR50 knockdown inactivates the Notch signaling pathway in a ligand-independent manner by interaction with ADAM17, of which expression is positively correlated with GPR50 overexpression in hepatocytes. The authors also reported that GPR50 regulates the transcription and translation of ADAM17 through the AKT/specificity protein-1 (SP1) which is a transcription factor that binds to ADAM17 promoter region [126].

The gut microbiome

The anatomical connection between the intestine and the liver via the portal vein makes the liver the largest target affected by gut microbiota [127]. Bile acids produced by the liver also affect the gut microbiota, and any pathological decrease in bile acid secretion contributes to bacterial overgrowth, inflammation-induced disruption of the gut barrier, and a leaky gut. Both the disrupted gut barrier and gut dysbiosis were reported to occur at early stages of chronic liver diseases and contribute to the development and progression of HCC [127,128]. Several metagenomic studies demonstrated that alterations in gut microbiota in liver cirrhosis, which is a major risk factor for HCC, result from either impaired gut motility that leads to bacterial overgrowth and a leaky gut, or from bacterial translocation (for example, the invasion of the microbiota from mouth to the gut in liver cirrhosis) [127,129,130]. Another set of studies also showed a link between HBV infection,

HCV infection, and alcohol consumption with the gut dysbiosis and HCC development [131]. Moreover, Toll-like receptor (TLR)-4-dependent innate immunity is a major mediator of the immune response to either an HBV or HCV infection [132]. TLR4 is activated by lipopolysaccharides (LPS), a component of bacterial cell membranes that are also endotoxins. LPS level are elevated in HBV related HCC cases [131], and LPS can also be elevated by prolonged alcohol consumption which disrupts the intestinal barrier [133]. LPS can be transported to the liver through the portal vein and interact with TLR4 and induce TNF-α expression in Kupffer cells leading to cytokine release and inflammation, which can eventually cause liver fibrosis and HCC development [131,132,134].

Bile acid

The cross talk between metabolites and host cells has an important role in regulating the immune system and affects disease development and response to treatment [135]. The interaction between the host cells and the gut microbiota can affect the production of several metabolites including bile acid. Bile acid, which is produced by hepatocytes and can be metabolized by the intestinal bacteria, can modulate the composition of the gut microbiota through the antimicrobial activity of bile acids [136]. Bile acid acts as a ligand for a number of receptors such as farnesoid X receptor (FXR), which are nuclear receptors that regulates bile acid synthesis, conjugation, and transport, as well as lipid and glucose metabolism [137], and G-protein-coupled bile acid receptors (TGR5), which are involved in energy homeostasis, bile acid homeostasis, and glucose metabolism [138]. There is a growing evidence that bile acids have a role in hepatocarcinogenesis [136]. While bile acid binding to TGR5 plays a role in colorectal inflammatory diseases, the decreased FXR signaling induced by hepatic inflammation inhibits the bile acid transport and enhances hepatic accumulation of bile acid resulting in prolonged inflammation, DNA damage, apoptosis, and HCC development [139-141].

HCC molecular classification

The BCLC (Barcelona Clinic Liver Cancer) staging and TNM (Tumor-Node-Metastasis) are among the most widely used classification systems for HCC. The Barcelona Clinic Liver Cancer (BCLC) system was originally proposed in 1999, and is used for both prognostic and therapeutic purposes in HCC [142]. The system defines 5 HCC stages: very early, early, intermediate, advanced, and terminal. The stages are based on variables such as: tumor burden, Child-Pugh class (a scoring system which is designed to predict mortality in patients with cirrhosis by using five clinical and laboratory criteria to categorize patients: serum bilirubin, serum albumin, ascites, neurological disorder, and clinical nutrition status) [143], clinical status, and Eastern Cooperative Group Performance Status [ECOG PS],which is the assessment of level of function and capability of self-care used for oncology patients [144]. BCLC-A includes very early and early HCC patients, BCLC-B includes HCC patients with intermediate stage, and BCLC-C involves the patients with advanced stage (patients with one or more poor tumor variables like: ECOG PS 1 or 2, macrovascular invasion (MVI), and extrahepatic spread (lymph node involvement or distant metastases), and Child-Pugh class A or B) [145]. This system has been validated, updated, and adapted by the American Association for the Study of Liver Diseases (AASLD), the American Gastroenterology Association (AGA), the European Association for the Study of Liver (EASL), and the European Organization for Research and Treatment of Cancer (EORTC) [146,147]. A difficulty in using this staging system is in how to categorize BCLC-B and BCLC-C patients with overlapping clinical features that can affect therapeutic decisions [148]. To this end, several subclassifications for intermediate stage B and advanced stage C HCC were proposed by merging different criteria such as tumor burden, Child-Pugh score, and performance status to define the best treatment option for each subclass [149-151]. However, due to the patient-HCC heterogeneity, none of the subclassifications has yet been incorporated into the practice guidelines, and there is a need for a valid subclassification system [148]. A potential problem with current staging systems is that they rely on clinical aspects of the disease and do not consider genomic or molecular data [152].

The advances in molecular and genomic data allowed their utilization for classifying many tumors. Breast cancer, is the best example in which the molecular classification provides a substantial aid in treatment decision [153], and there is an urgent need for molecular classification systems for HCCs. To this end, subclassifications, combinations of the staging systems, as well as novel molecular classifications have been proposed to predict prognosis and aid in HCC management. Here, we will list some of them.

Molecular-based prognostic classification

Ke et al, identified two prognosis-associated molecular HCC subtypes: type 1, which is the better prognosis type and type 2, which is the poor prognosis type. The subtypes are based on the expression of genes that are associated with overall survival (OS) and were based on analysis of data from 371 HCC patients' available in The Cancer Genome Atlas (TCGA). A total of 774 significant OS-related genes were identified, the expression profiles of these genes in the 371 HCC patients were used to define subtype 1 and 2. Longer median survival time for molecular subtype 1 than for molecular subtype 2 was observed. The two molecular subtypes also showed significant differences in several clinical outcomes such as the degree of tumor differentiation, TNM stage, vascular invasion, as well as serum AFP level. Using Prediction Analysis for Microarrays (PAM) and Significance Analysis of Microarray (SAM) analyses, 337 subtype-specific signature genes were identified, with 56 signature genes for molecular subtype 1 and 281 signature genes for molecular subtype 2. The authors reported that several "metabolism" associated pathways were significantly enriched for subtype 1 signature genes, while numerous pathways linked to the "cell cycle" (mainly the mitotic phase) were observed for subtype 2 signature genes. Moreover, while the expressions of signature genes of subtype 1 were significantly downregulated in HCC tissues, which reflects more intact hepatocyte metabolism and better clinical outcome, the expressions of signature genes of subtype 2 were significantly upregulated in HCC tissues, potentially contributing to uncontrolled cell proliferation, as compared to adjacent normal tissues. The subtype 2 HCCs, the poorer prognostic subtype, exhibited higher stage, poorer differentiation, and more frequent macrovascular invasion, which are clinical features that are consistent with the aggressive phenotype of the tumor. The authors suggested that the application of this classification will help predict HCC prognosis [152].

Molecular network-based stratification

Another molecular stratification of HCC was proposed by Bidkhori et al. using transcriptomics data, genome-scale metabolic networks, and network topology/controllability analysis. The authors identified three subtypes, named iHCC1 (low grade) to iHCC3 (high grade), that have significant changes in various metabolic, signaling, and survival pathways that correlate with subtype specific genomic, transcriptomic, and proteomic differences. These three subtypes were classified according to

differences in the level of expression of key genes that regulate pathways related to survival such as kynurenine metabolism, WNT/β-catenin–associated lipid metabolism, and PI3K/AKT/mTOR signaling. 8 to 28 genes that are specific to each subtype and contribute to controlling the metabolic network were identified. The three iHCCs have 18 metabolic "stratifying genes" that are highly expressed by one group but not the others. For the low-grade iHCC1 tumors with the highest patient survival, these tumors showed a high degree of inflammation, as well as high level of amino acid, fatty acid, and pyruvate metabolism. 56% of the patients were obese or overweight, which is consistent with the findings that iHCC1 stratifying genes were upregulated in obesity. The hallmark of the iHCC2 group is the upregulation of β-catenin along with its targets GLUL gene, encodes the glutamine synthetase, and the glutamate transporters, and solute carrier family 1 member 2 (SLC1A2) gene, as well as lower survival rate as compared with iHCC1. β-catenin regulates expression of acetaldehyde dehydrogenases, activates detoxifying pathways, and controls mitochondrial fatty acid oxidation and the tricarboxylic acid (TCA) cycle that protects the liver against alcohol induced - injury. Therefore, iHCC2 exhibits high fatty acid oxidation and low fatty acid biosynthesis and were mostly associated with alcohol-induced HCC. Finally, the iHCC3 group had the lowest survival and aggressive tumors with a high rate of metastasis. The aggressive clinical features correlated with overexpression of hypoxia-regulated genes (e.g., TGF-β, HIF1-α, and NF-κB), a strong Warburg effect, high epithelial-to-mesenchymal transition, and PI3K/AKT/mTOR activation. Moreover, this group also showed significant overexpression of asparagine synthetase that increases metastatic potential. Using transcriptomics analysis in HepG2 cells culture at different oxygen levels, iHCC3s were shown to have high expression metabolic controlling and stratifying genes compared to iHCC1 and 2 and these genes were upregulated by hypoxia. The results of these studies suggest that mechanistic differences between HCC subtypes might foster the development of HCC subtype-specific treatment strategies [154].

Transcriptomic molecular signatures

Initially, four different transcriptomic-based molecular classification systems for HCC were separately described and applied by Lee et al, Hoshida et al, Boyault et al, and Chiang et al [155-158]. Genome-wide gene-expression profiling was used to investigate the potential roles of specific genes and molecular pathways in hepatocarcinogenesis and to develop the molecular classification systems. However, these classification systems also considered HCC etiology and specific patient populations. The diverse HCC-etiological factors, the differences in the clinical presentations, and the correlation of the gene-expression patterns with specific molecular events affect the molecular heterogeneity of the HCC pathogenesis and posed a question of whether these molecular classifications can be applied across different patient populations [159]. Therefore, subsequent meta-analyses of these 4 systems broadly categorized HCC into either "proliferative and non-proliferative", or "progressive and less progressive" [160,161]. Such global framework allows HCC-molecular classification that is based on chromosomal alterations and works across HCC patients with different backgrounds and tumor-related etiologies. The "proliferative/progressive" category is characterized by mitogenic and stem cell-like properties, HBV infection, poor tumor features such as high AFP, poor differentiation, vascular invasion, and poor prognosis. The "non-proliferative/less progressive" is heterogeneous with ambiguous clinical and pathological features [160,162].

Finally, a combination of molecular and immunological classification was proposed by Shimada and colleagues, in which the HCC is categorized into 3 major subtypes: MS1, the mitogenic and stem cell-like tumors with chromosomal instability "progressive/proliferative" subtype, and the MS2 and MS3 subtypes which subclassifies the "non proliferative/less progressive" subtypes. MS2 are CTNNB1-mutated tumors in which Wnt/β-Catenin pathway is activated. The tumors exhibit an immunosuppressive phenotype with a lower number of regulatory T (Treg) cells than MS1. On the other hand, MS3 tumors are metabolic disease-associated tumors that mostly developed in patients with obesity, diabetes, or NASH that are associated inflammation and ROS generation. MS3 tumors are further subdivided into immunogenic (i) and non-immunogenic (n) MS3i and MS3n subclasses. Activated M1 and M2 macrophages infiltrated MS3i tumor tissues [162]. Therefore, MS3i tumors are considered "hot tumors" and generally have a favorable prognosis and high response to immune check point inhibitors [163].

Diagnostic and prognostic biomarkers

Although measuring the level of AFP combined with abdominal ultrasound was a part of HCC screening for high-risk population, AFP has been removed from several international HCC screening guidelines because it was shown to not have adequate sensitivity and specificity. However, removal of AFP as a diagnostic tool for HCC screening has been highly controversial, which explains the persistence of AFP use for surveillance in many Asian countries [164].

Here, we will describe the most common studied HCC biomarkers, excluding AFP, as well as some recently discovered HCC biomarkers. Although some of the biomarkers that we will describe have low sensitivity or specificity, it has been suggested that the use of combinations of biomarkers with low sensitivity or specificity scores could improve their clinical application [165]. More extensive descriptions of HCC biomarkers can also be found in a series of excellent reviews on this topic [165-167].

AFP-L3

AFP-L3 is a glycoform of AFP that has strong binding affinity for Lens culinaris agglutinin (LCA), which recognizes sequences containing α-linked mannose residues. AFP-L3 showed diagnostic HCC specificity of nearly 92%; however, its 37% sensitivity score and reduced diagnostic ability when AFP-L3 is <20 ng/ml have limited its clinical use [168]. A high-sensitive AFP-L3 (hs-AFP-L3) assay has been generated using chip affinity-based electrophoresis and showed an improved sensitivity score that could overcome the low diagnostic value of AFP-L3 [167].

DCP

Des-γ-carboxyprothrombin (DCP), an abnormal prothrombin that is produced by a defective, vitamin K-induced posttranslational carboxylation of prothrombin, was elevated in HCC cases and promotes HCC cell proliferation [167,169]. Initially, DCP was not validated to be used as an independent HCC biomarker, but its later combination with AFP and AFP-L3 has improved its sensitivity and specificity scores, especially in HCC patients with an AFP level <10 ng/ml. This combination is used as a part of HCC diagnosis in some Eastern guidelines [166].

GCP-3

Glypican-3 (GCP-3) is a cell-surface heparin-sulfate proteoglycan that belongs to the glypican family and has an important role in

cell proliferation [167]. Moreover, the presence of its NH2-soluble fraction in serum enables its use as a serologic biomarker because it can be easily detected in the sera of HCC patients and its level was shown to be higher in HCC patients than in healthy individuals or patients with cirrhosis. Although assessment of GCP-3 levels is better than AFP in differentiating small HCCs from cirrhosis, its combination with assessment of AFP showed higher diagnostic sensitivity for small HCC lesions [170]. When the level of GCP-3 was also used in combination with assessing the levels of heat shock protein (HSP)70 and Glutamine Synthetase (GS), which are highly expressed in HCC and show a positive correlation between the increment of their levels and the grade of HCC (from precancerous to advanced HCC), the combined analysis improved the diagnostic ability to differentiate cirrhotic nodules from early HCC. Therefore, the European Association for the Study of the Liver and European Organization for Research and Treatment of Cancer (EASL-EORTC) joint guidelines now recommends the use of GCP3, either alone or in combination with HSP70 and GS, to differentiate early HCC from cirrhotic nodules [166,167].

Putative but currently not approved HCC biomarkers

Another set of HCC biomarker that have been studied but not yet approved for clinical use include: CK 19, which might predicts poor prognosis; Golgi protein 73 (GP73), a potential diagnostic HCC marker; Osteopontin (OPN), which is overexpressed in > 30 types of malignant tumors including HCC; squamous cell carcinoma antigen (SCCA), which might predict response to treatment in HCC patients; and Annexin A2, which was suggested as a serological biomarker for the diagnosis and prognosis of HCC, but has not yet been incorporated into clinical practice. It is interesting to note that the combination of CK19 and GPC3 showed better diagnostic sensitivity than GPC3 alone and that GP73, OPN, SCCA, and Annexin A2 demonstrated higher diagnostic power when combined with AFP [165-167].

HOXA9

Homeobox protein Hox-A9 is encoded by HOXA9 gene, a member of the HOX genes family. Members of this family control precise spatial and temporal regulation during embryogenesis as well as overall body shape. HOXA9 is overexpressed in many cancers. Although HOX9 overexpression was not detected in HCC, hypermethylation of HOXA9 was discovered in HCC samples that were assessed by quantitative methylation-specific PCR (qMSP). This hypermethylation showed 73.3% sensitivity and 97.1% specificity in HCC diagnosis [165].

EFABP

The Epidermal fatty acid-binding protein (EFABP), a member of the FABP family that acts as a soluble FA-binding protein and is uniquely expressed in epidermal cells [171], was found to be overexpressed in many tumors, including HCC [172]. A recent study showed that EFABP overexpression in HCC is positively associated with high-grade tumors, vascular invasion, and a higher TNM stage. Kaplan–Meier analysis revealed that the patients with high EFABP expression had significantly worse overall survival, worse disease-free survival, and a higher recurrence rate. It was reported that EFABP contributes to HCC development and progression via promoting the EMT transition, upregulating the matrix metalloproteinase 9 expression, and increasing angiogenesis. EFABP could be a potential HCC prognostic biomarker because EFABP high expression is an independent risk factor for overall survival, disease-free survival, and HCC recurrence. EFABP may also be a potential therapeutic target to prevent accumulation of fat in the liver of patients with NAFDL or NASH [173].

CKAP4

Another protein that showed a potential diagnostic role in detection of HCC is the Cytoskeleton-Associated Protein 4 (CKAP4), a non-glycosylated and reversibly palmitoylated type II trans-membrane protein that is a cell surface receptor of the plasminogen activator and surfactant protein A [174]. CKAP4 overexpression was shown to decrease both the proliferative and invasion of HCC by suppressing the activation of epithelial growth factor receptor (EGFR) signaling [175]. However, one study showed that CKAP4 was shown to be significantly elevated in some HCCs, and that the combination of CKAP4 and AFP detection showed better diagnostic performance than either one of them alone. However, this was a retrospective study, and further

Table 2: A list of some of the novel HCC biomarkers

Biomarker	Type of Biomarker	Clinical application	Reference
Epidermal fatty acid-binding protein (EFABP)	FA binding protein	Potential HCC prognostic biomarker	[173]
Cytoskeleton-Associated Protein 4 (CKAP4)	trans-membrane protein & a cell surface receptor	the combination with AFP showed better diagnostic performance than either one of them alone	[176]
Endocan	Endothelial-specific molecule	the combination of Endocan, VEGF, and AFP can be a potential diagnostic biomarker for HCC, and can predict HCC mortality	[179]
D-dimer	Fibrinolysis associated protein	potential biomarker to predict PVT risk in HCC	[181]
TSH	Thyroid Stimulating Hormone	Potential prognostic biomarker	[182]
Betaine and Propionyl carnitine	Tissue metabolites	Differentiating HCC from nonmalignant liver disease	[188]
Combination of: (methionine, proline, ornithine, pimelyl carnitine, and octanoyl carnitine)	Tissue metabolites	Potential HCC diagnostic biomarker	[189]
Oleic acid, Octanoic (caprylic) acid and Glycine	Tissue metabolites	differentiating HCC cases among HCV-cirrhotic patients	[194]

studies to confirm the biomarker value of CKAP4 are still required [176].

Endocan

Endocan or endothelial-specific molecule-1 is a soluble proteoglycan that is produced and secreted by activated vascular endothelial cells, including tumor endothelial cells [177]. Endocan production is increased by angiogenic factors, TNFα and IL-β [178]. A study conducted by Youssef et al. showed that the combination of Endocan, VEGF, and AFP can be a potential diagnostic biomarker for HCC and can predict HCC mortality. A total of 195 patients with CHC were divided into HCV cirrhotic patients with HCC and HCV cirrhotic patients without HCC. Endocan had higher sensitivity and specificity in diagnosing HCC than either AFP or VEGF. The mean serum endocan levels were higher among HCC patients than the non-HCC patients. A statistically significant positive correlation between serum endocan level and poor tumor features such as large, numerous focal lesions and, most commonly, a child Pugh C score. The OS survival of HCC patients with high levels of VEGF and endocan (>4,000 pg/mL) were significantly lower than that of patients with low levels [179].

D-Dimer

Portal Vein Thrombosis (PVT) is a major complication and poor prognostic factor that affects the treatment decisions and options for HCC [180]. D-dimer levels in HCC patients with PVT were shown to be significantly higher than in HCC patients without PVT. D-dimer, is produced by endogenous fibrinolysis by the action of activated thrombin on fibrinogen; therefore, D-dimer levels increase with increased vascular fibrinolysis. This suggests that D-dimer levels can be a potential biomarker to predict PVT risk in HCC and can serve as a cheaper noninvasive alternative to US [181].

TSH

The high recurrence rate of HCC following surgical resection inspired some researchers to explore for biomarkers that could predict recurrence. One potential biomarker that was investigated was Thyroid Stimulating Hormone (TSH) [182]. The rationale supporting the use of TSH level as a novel predictor is that hypothyroidism patients (TSH 5μIU/mL) have 2-fold risk of HCC [183]. Moreover, some studies confirmed the presence of TSH/TSHR (TSH receptors) in human liver and the TSH/TSHR overexpression is correlated with poor prognosis in patients with HCC [184]. As a result, this study confirmed that higher TSH levels is correlated with shorter progression period and the combination of high TSH levels with BCLC C-D predicts shorter PFS in HCC patients. The prognostic model consists of BCLC stage, presence of PVT, AFP level, and TSH level; this gives the model more accuracy than other models to predict the tumor recurrence [182].

The metabolome as biomarkers

The modified metabolism of the cancer cells, such as the Warburg effect, has been the focus of research for many decades [185]. Tissue metabolic profiling reflects the metabolic milieu of the tissue or organ, whereas serum metabolomic profiling gives a general idea about an organism's entire metabolic system. Metabolic profiling, also referred to a metabolomics, could identify metabolic biomarkers in the metabolome of tissue and serum in response to hepatocarcinogenesis, and these could also serve as new HCC prognostic, therapeutic, or follow up biomarkers [186,187].

In a study conducted by Huang et.al using liquid chromatography-mass spectrometry (LC/MS), the metabolic characteristics of HCC tissues and their effects on surrounding and distant non-cancerous tissues were evaluated. The study showed a decrease in TCA-related metabolites and an increase in glycolysis related metabolites. The authors concluded that betaine and propionyl carnitine can be potential diagnostic biomarkers for HCC and are better than AFP for differentiating HCC from nonmalignant liver diseases [188].

Another panel of metabolites that showed higher accuracy than AFP in distinguishing HCC from cirrhosis was related to defects in ammonia recycling, the urea cycle, and amino acid metabolism. This panel consists of methionine, proline, ornithine, pimelylcarnitine, and octanoylcarnitine, which were significantly elevated in HCC, and their combined elevation can act as a potential diagnostic HCC biomarker. A positive correlation between the severity of HCC and the levels of methionine, ornithine, and proline was also demonstrated. Therefore, this panel of metabolites may provide a high diagnostic value for HCC [189].

Some studies demonstrated the correlation between the HCC etiology and the expression of certain metabolites. For example, Sun et al. identified a set of metabolites that can be used as non-invasive diagnostic biomarkers in HBV-related HCC patients using LC-MS based metabolomic assays. These assays were performed on plasma samples from treatment-naïve HBV-infected HCC patients including data sets for gender- and age-matched AFP–ve(<7 ng/ml) HCC and AFP+ve HCC subjects, as well as other HCC groups, HBV-infected individuals without HCC, and healthy individuals. The authors identified 8 metabolites that had a significant discriminatory power between HBV-related HCC and healthy controls. These metabolites were associated with protein and sphingolipid metabolism and play an important role in hepatocarcinogenesis. Lipid metabolism was significantly different among the HBV-related HCC, HBV-infected, and healthy control groups [190]. These results are consistent with a previous studies demonstrating that the hypoxic environment associated with HCC growth makes the cancer cells increase lipid metabolism to adapt to this environment [191]. Supporting this notion, Sun et al. showed that increased cellular lipid metabolism and consumption resulted in decreased expression levels of lipid metabolites in the plasma of HCC samples [190]. Moreover, the activation of lipid metabolism promotes the generation of the anti-apoptotic protein, human phosphatidylethanolamine binding protein 4 (hPEBP4), which activates PI3K/Akt/mTOR signaling to promote tumorigenesis [192]. Sun et al. also demonstrated that there were higher HBV DNA levels in the AFP+ve HCC group [190]. Interestingly, the HBV HBx protein enhances the expression of AFP, and AFP, in turn, suppresses expression of the tumor suppressor gene growth arrest and DNA damage 45a (GADD45a) protein, which has decreased expression in HBV-related HCC [193]. Therefore, these lipid metabolites, such as sphingolipids, might serve as potential biomarkers in diagnosing HBV-related HCC [190].

A similar study was conducted in HCV-infected patients and identified 5 metabolites that exhibited a strong discriminatory power between HCV-associated HCC and HCV-associated cirrhosis. These metabolites were octanoic acid (caprylic acid) and decanoic acid (capric acid), medium-chain saturated fatty acids; oleic acid, a monounsaturated fatty acid; oxalic acid, a saturated dicarboxylic acid; and glycine, a glucogenic amino acid. Data analysis demonstrated that oleic acid, octanoic acid, and glycine had higher positive predictive value than AFP in differentiating HCV-related HCC from HCV-related cirrhosis [194]. These

findings are supported by the notion that HCV dysregulation of lipid metabolism, including liponeogenesis, which supplies a lipid rich environment for HCV replication, causes lipid accumulation in the liver [195]. It is important to note, however, that there is discrepancy in the findings of studies assessing fatty acid metabolism in HCC and how fatty acid metabolism is altered in carcinogenesis. While some studies showed upregulation of de novo fatty acid synthesis, others showed increased fatty acid oxidation [196].

The microbiome as a biomarker

There is a growing appreciation for the role of the gut microbiota in tumorigenesis, and a study conducted by Ren et al. demonstrated that microbial biomarkers can potentially be used to diagnose HCC, even in advanced stages, and to differentiate early HCC from cirrhosis. 419 fecal samples were collected and analyzed using 16S rRNA Miseq sequencing (a sequencer that can perform DNA sequencing, and data analysis in a single run [197]). 30 Operational Taxonomy Units (OTUs) were identified and showed a significant power to differentiate early HCC from non-cancerous lesions. The lipopolysaccharide producing genera were increased in early HCC compared to healthy controls. While microbial diversity decreased from healthy individuals to cirrhosis, it increased from cirrhosis to early HCC with cirrhosis. Since the study involved populations from different regions with different gut microbiomes, the results demonstrated that the same general features are shared among microbial dysbiosis signatures of HCC [198]. These results are similar to the results obtained from the colorectal carcinoma-related gut microbiota [199].

Biomarkers of the immunotherapy response

The increasing role of immunotherapy in treating several cancer types makes it a very promising therapy, but the response rate is still limited to a subset of patients [200,201]. This might be related to the tumor features and/or the complexity of the tumor microenvironment. Several studies showed that directly targeting the tumor with treatments such as chemotherapy or radiation therapy and/or indirectly targeting the microenvironment by altering factors such as the gut microbiome or the cytokine milieu can result in higher responses to immunotherapy. Therefore, several biomarkers, albeit weak, have been validated to predict the immunogenicity of the tumor and its microenvironment including PD-L1 expression, tumor mutational load, and DNA mismatch repair (MMR) deficiency [202]. In a recent study, additional predictors of responsiveness to anti-PD-1 immunotherapy were identified: the associations with T cell receptor (TCR) diversity and/or clonality, host HLA genotype, a favorable gut microbiome, and the body mass index (mediated by leptin) [203].

Lung Immune Prognostic Index (LIPI)

An interesting prognostic model, lung immune prognostic index (LIPI), was suggested by Mezquita and his colleagues. The model uses a combination of a derived neutrophil-to-lymphocyte ratio (dNLR) $\geq$ 3 and a pretreatment lactate dehydrogenase (LDH) level $\geq$ the upper limit of normal (ULN) to predict prognosis for patients with non-small-cell lung cancer (NSCLC) treated with immune checkpoint inhibitors (ICIs) [204]. This prognostic model has been successfully used in tumors other than NSCLC [205]. Another analysis has shown its potential to be applied in patients with advanced HCC treated with PD-1 inhibitors. The results demonstrated significantly higher OS and PFS in HCC patients treated with PD-1 inhibitors who have good LIPI (dNLR < 3 and LDH normal) as compared to those with intermediate/poor LIPI (dNLR $\geq$ 3 or/and LDH $\geq$ ULN) [206].

MicroRNAs(miRNAs)

miRNAs are small, (19-20) nucleotide , highly conserved, single-stranded, noncoding RNA molecules that represents 1-5% of the human genome [207]. In the cell, miRNAs play a significant role in posttranscriptional regulation of up to 60% of the protein-coding genes [208]. MiRNAs can be found in blood as free molecules or bound to proteins. The combination of PCR and bioinformatics studies has identified several miRNAs that are upregulated in HCC and represent promising biomarkers for HCC diagnosis, prognosis, and treatment [165,166,209].

MiRNAs bind the complementary target mRNA by base pairing and inhibit protein production either by inhibiting mRNA translation or by degrading the complementary mRNAs [10,207]. MiRNAs are involved in several cellular pathways that regulate immune responses, metabolism, and apoptosis, to name a few [208,210]. Dysregulated levels of miRNAs are found in numerous cancers [210] and were reported to contribute to tumorigenesis [208]. In liver cancer, miRNAs are involved in cancer initiation, progression, and angiogenesis and can mediate responses to treatments [207]. Several studies have reported that miRNA-21, miRNA-221, and miRNA-222 are upregulated in liver cancers, while the expression of miRNA-122-a, miRNA-145, miRNA-199-a, and miRNA-223 is downregulated in HCC as compared to normal tissue [211]. MiRNA-122 constitutes up to 52% of the human hepatic miRNAs and plays an important role in hepatocyte differentiation. Decreased miRNA-122 levels were correlated with poor prognosis in HCC patients due to hepatic phenotype repression [208]. MiRNA-122 also interacts with the HCV genome and contributes to HCV stability and disease development [212]. MiRNA-122 is upregulated in HCV-induced HCC suggesting that it plays a different role than in non-HCV induced HCCs [213]. Miravirsen, a miRNA-122 inhibitor, was the first miRNA-targeted drug that was introduced into the clinic [208]. Miravirsen acts by sequestering miRNA-122, and it was reported to be safe drug due to very few off-target effects and short term use [214]. As compared to HCV-infection, HBV infection was found to be correlated with a decreased level of miRNA-122. One suggested mechanism is that HBV mRNA contains numerous miRNA-122 binding sites that sponge miRNA-122 resulting in lower available miRNA-122. Although the mechanism is still undetermined, many studies have reported that mi-RNA 122 interferes with HBV replication, protein expression, and RNA transcription and that HBV viral load is conversely correlated with the level of miRNA-122 [215-217]. Many in-vivo experiments showed that miRNA-122 restoration has reversed HCC tumorigenesis in human and mouse hepatoma cell lines, which makes miRNA-122 restoration a potential therapeutic strategy to prevent HBV-induced HCCs [208,218,219]. Similarly, some studies showed that the level of miRNA-122 is reduced when HCC-miRNA 122 expressing cells are treated with Sorafenib and that miRNA-122 restoration increases HCC sensitivity to Sorafenib and Doxorubicin treatment, suggesting that miRNA-122 mimetics might be used alone or in combination with chemo/targeted therapy for treating HCC patients [220,221]. Additionally, miRNA combinations, for example panel of 3 miRNAs (miR92-3p, miR-107, and miR-3126-5p) have been shown to be promising biomarkers for early HCC detection and diagnosis, to have higher sensitivity than AFP, and to better detect AFP-negative HCC [222-225].

Exosomes

Exosomes are membranous vesicles released from the cells into the extracellular space and are formed by the fusion of intracellular

multivesicular bodies with the plasma membrane. Exosomes are composed of a membrane lipid bilayer and can contain DNA, proteins, mRNAs, or miRNAs according to their source. Exosomes can be found in either biological body fluids, such as urine, blood, and saliva, or secreted from certain types of cells including B lymphocytes, T cells, mast cells, dendritic cells, tumor cells, endothelial cells, and mesenchymal stem cells [211]. Exosomes are considered as an additional mechanism of signaling between cells by delivering their content from one cell to another [226]. Exosomes carry proteins referred to as the "exosome marker" that are cell-specific and reflect the phenotype of the cell of origin. The constituents of the exosomes, for example the protein, lipids, and nucleic acid contents correlate with the physiological state of the cell of origin [227].

The combination of the difficulties in degrading the proteins, DNA, and RNA content of exosomes owing to the protection by the bilipid layer, the ease of extracting exosomes from the biological body fluids, and the growing role of miRNA in cancer detection, diagnosis, and protection, render the exosomes applicable targets for treating many cancers, including HCC [211,228,229]. Manipulation of exosomes and miRNAs have been shown to improve the response to chemotherapeutic agents in HCC [230]; however their role in immunotherapy in HCC still needs to be investigated [211].

DNA methylation

The results of one study found that DNA methylation mostly affects six hub genes and their pathways. These include, Mitotic Arrest Deficient 2 Like 1 (MAD2L1), Cell Division Cycle 20 (CDC20), CCNB1, CCND1, Androgen receptor (AR) and Estrogen receptor 1(ESR1). Pathway analysis showed that patients with high expression of MAD2L1, CDC20, and CCNB1 and low expression of CCND1, AR, and ESR1 had shorter overall survival. MAD2L1 and CDC20, two mitotic checkpoint genes, and CCNB1, a G2/M-phase checkpoint regulator, may all be abnormally methylated genes in HCC that regulate cell cycle and proliferation and are correlated with tumor aggressiveness, recurrence, and poor prognosis. CCND1, a regulator of G1 to S phase progression [231]; AR, a steroid hormone receptor that contributes to human hepatocarcinogenesis by stimulating cell growth [232]; and ESR1, a transcription factor which regulates cell cycle, cell proliferation, apoptosis, and inflammation [233]; are correlated with poor HCC prognosis, hepatocarcinogenesis , and metastasis. Therefore, the results of this study suggested that these genes and their methylation can serve as a new diagnostic and prognostic biomarker for HCC. Because DNA methylation can be reversible, the identification of aberrantly methylated oncogenes and tumor suppressor genes can have potential therapeutic applications in the treatment of HCC [234]. Zebularine, a DNA methyltransferase inhibitor, blocked cell proliferation and induced apoptosis in HepG2 cells [235]. 5-aza-2'-deoxycytidine (DAC), another DNA methyltransferase inhibitor, was shown to inhibit telomerase activity, induce reactivation of p16 by demethylation of its promoter, and repress c-Myc expression in HCC cell lines. DAC also synergized with cisplatin to induce apoptosis in HCC cell lines [236].

Diagnostic tools

According to several practice guidelines, the major diagnostic methods for HCC are Ultrasonography (US), Computed Tomography (Ct)-scan, and Magnetic Resonance Imaging (MRI) [237]. This section is a transition from the research side to the clinical side in which we will list some of the advances that allow better detection rates using US and MRI. We will also discuss the evolving role of PET-SCAN in HCC diagnosis.

Ultrasonography (US)

US is a major diagnostic method for HCC around the world due to its availability, low cost, and non-invasive nature. But US findings can be confounded by other factors, like the expertise of the examiner, the nature of the liver disease, and the body's nature variation from one patient to another. The US sensitivity for detecting any HCC lesion is about 84% as shown by a meta-analysis of cohort of US studies, with significantly lower (47%) for small HCC lesions [238,239]. Because cirrhosis is a major risk factor for developing HCC and US showed a key role in HCC early detection in patients with cirrhotic livers, the international guidelines have implemented US as a method for early HCC detection and surveillance. The safety of this method (no radiation exposure) as compared to CT-scan and MRI, as well as its low price, has made US a very convenient method [238-240]. Moreover, the use of blood flow dynamics color Doppler and contrast-enhanced US have increased the sensitivity and specificity for early HCC detection [239]. More recently, the American Association for the Study of Liver Diseases (AASLD) has incorporated alpha-fetoprotein (AFP) with Liver Imaging Reporting and Data System (LI-RADS) for HCC surveillance and has defined two risk groups for which the surveillance is highly recommended. The first risk group includes patients with liver cirrhosis plus one of the following risk factors: chronic HBV infection, chronic HCV infection, chronic alcohol consumption, nonalcoholic steatohepatitis, stage 4 primary biliary cholangitis, genetic hemochromatosis, alpha-1-antitrypsin deficiency, or other cirrhosis-causative factors. The second risk group includes patients without cirrhosis but at high risk of developing HCC; these include: Asian male hepatitis B carriers ≥40 years old, Asian female hepatitis B carriers ≥50 years old, Hepatitis B carriers with family history of HCC, African/North American black individuals with hepatitis B who are ≥20 years old. The LI-RAD system classifies the liver lesions from definitely benign (LR-1) to intermediate probability (LR-3) to definite HCC (LR-5) , and the HCC diagnosis probability is based on a combination of major and minor criteria, including arterial enhancement, delayed washout, enhancing capsule, and threshold growth [238]. Depending on HCC lesion size, the patterns of internal echoes range from hyperechoic pattern 12–38% to hypoechoic pattern 23–54% to mosaic pattern 17–38% [239]. In healthy people, 75% of the liver blood supply come from the portal vein, whereas a nodule in the liver or an HCC tumor that originates in the context of liver cirrhosis mainly receives an arterial blood supply [240]. The artifacts that are produced by tissue motion during US can be now reduced by using a new technique developed by Canon Medical Systems, Otawara, Japan called superb microvascular imaging. Using this technique can differentiate between the low-velocity capillary blood flow and tissue motion [239].

Sonazoid is a second-generation US contrast agent that is a lipid-stabilized suspension of perfluoro butane gas microbubbles, and it is metabolized via the lungs, which make it safe for patients with renal dysfunction or allergy [239]. Sono Vue is another second-generation contrast agent micro bubble with Sulphur- hexafluoride gas and a palmitic acid shell [240]. CEUS (Contrast-enhanced ultrasound) using Sonazoid or Sono Vue has significantly aided in HCC detection and tumor grade determination, and it can be also used for US-guided ablative therapy [239,240]. The limitation of Sono Vue CEUS is that it cannot be used for surveillance because

it cannot evaluate more than one lesion at a time due to the short arterial phase period; Sonazoid does not have this limitation [240].

CT-scan and MRI are not recommended for HCC surveillance due to radiation exposure, but they represent an important HCC diagnostic tool. In the following section, we will summarize some of the advancements in MRI HCC-detection protocols.

MRI

Diffusion Weighted Imaging (DWI) MRI is the routine MRI protocol used in HCC evaluation that depends on the diffusion properties of water molecules in biological tissues [241]. This protocol also supplies information about cellular density of the tissues, the microvascular invasion (MVI), as well as the degree of tissue necrosis in response to therapy [242]. DWI when combined with Gd-EOB-DTPA, Gadolinium-based hepatobiliary contrast agents, can assess the presence of MVI by assessing tumor size, tumor margin, peritumor enhancement, and peritumor hypointensity during the hepatobiliary phase of the MRI. The presence of MVI affects the surgical choice in HCC treatment [243]. Moreover, the dynamic contrast-enhanced (DCE)-perfusion MRI is used to assess angiogenesis development in HCC through quantitative assessment of several parameters including the volume transfer constant (Ktrans). Ktrans is correlated with vascular endothelial growth factor (VEGF) expression, which is higher in the high grade HCC than in low grade HCC [242]. DCE-MRI can be used to assess the response to anti-angiogenic drugs and to predict the patient's survival [244].

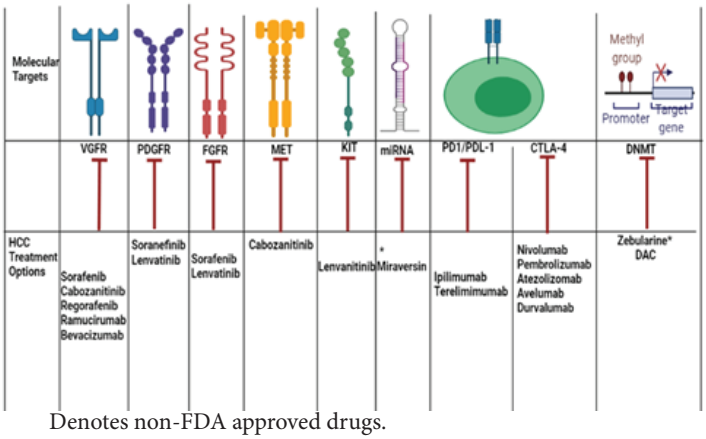
A recent MRI technique is the Mass Spectrometry or Nuclear Magnetic Resonance spectroscopy (MRS) which depends on the magnetic properties of essential atomic nuclei like protons (1 H) or carbon-13 (13C) to assess metabolite concentrations in the tissues [245]. This technique is useful in differentiating HCC arising from non-alcoholic fatty liver disease (NAFLD) from HCC arising from cirrhosis depending on the level of Glutamine Synthetase (GS) and aromatic amino acid levels, respectively [246]. Despite all the advantages of MRI in HCC diagnosis, it can be limited by difficulties in positioning the patient, generation of artifacts, and poor image quality [242].

FDG-PET

Although fluorodeoxyglucose (FDG)-positron emission tomography (FDG-PET) has a major diagnostic utility in general oncology, its initial use in HCC diagnosis was very limited [247]. However, there is a growing evidence that FDG-PET is an important tool for HCC evaluation. Low survival of HCC patients, high risk of recurrence, high grade tumors, and tumors with MVI correlated with enhanced FDG uptake, and FDG-PET can predict the risk of recurrence following hepatectomy [248]. A recent study by Yoh et al, suggested a novel combination of Standardized Uptake Value (SUV) ratio of the PDG-PET and albumin–bilirubin grade to assess the hepatic functional reserve, which is the most important predictor of OS following hepatectomy [249]. Another study done by Park et al. showed an improved OS with resection margins > 1 cm in HCC patients with PET positive lesions and not in PET negative lesions. Moreover, PET-SCAN can be used to select patients who will benefit from surgical resections vs the patients who need a liver transplant [250]. Although chemotherapy is the standard of care for patients with locally advanced HCC, some patients might benefit from locoregional treatments. Therefore, Rhee et al. proposed a combination of AFP with SUV max to characterize the high-risk patients (SUVmax >4.825 + AFP >550 ng/ml) who might benefit from systemic therapy and low risk patients who would benefit more from the locally aggressive treatment options [251]. Additionally, FDG-PET can be used for tumor necrosis assessment following HCC locoregional therapies [248].

Radiomics and radiogenomics

A recent field that combines imaging techniques with tumor biology is called "radio genomics". Radiogenomics can predict the molecular profile of the tumor from cross-sectional imaging and expands the ability of the radiologists to correlate quantitative and qualitative data obtained from imaging with the genetic profile of the patient such as gene expression patterns, gene mutations, and other genome-related characteristics [252]. For example, Wnt/β-catenin activation is associated with lower enhancement ratio on diffusion-weight imaging of HCC. Radiogenomic correlations are more informative than correlations with anatomy or histology alone. Therefore, this field aids in providing a more personalized management for the cancer patient. However, this is different from "radiation genomics" in which the scientists study the effects of radiation therapy on the genome [253]. Radiomics, on the other hand, correlates the image texture features (e.g., mean and SD, mean of positive pixels) with the clinical data (e.g., PFS, OS), and thus plays a role as a noninvasive prognostic tool [254]. The role of radiogenomics and radiomics in HCC has been studied and validated in a number of studies and demonstrated promising



- Denotes non-FDA approved drugs.

Figure 2: Some of the HCC cellular targets and their directed treatment options

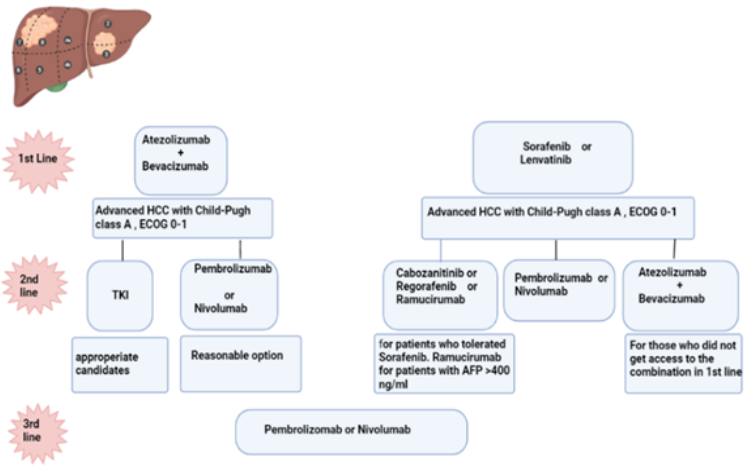


Figure 3: 2021 ASCO guidelines for selecting available treatment options for advanced HCC

results. Primary studies showed the potential use of radiogenomics and radiomics for therapeutic and prognostic stratification in HCC; however, further studies are warranted [253].

Treatment options

The treatment options for HCC depend on several factors that are either tumor related, such as the number, size, and location of the tumor lesions, or patient related, such as the presence of cirrhosis, the patient's performance status, the operative risk, and whether the stage of the disease is early or advanced. Although hepatectomy and liver transplantation often represent the best surgical strategies for HCC, liver transplantation is only suitable for ~5% of HCC patients, and hepatectomy is complicated by substantial risk of local recurrence (~75%) within 5 years [255,256]. Microvascular Invasion (MVI) is an independent risk factor for postoperative HCC recurrence [257]. MVI is defined as the microscopic presence of cancer tumor cells in any hepatic branch of the portal vein [258]. MVI determines the eligibility for liver transplant, the possibility of anatomical liver resection, and the resection margin [257].

Ethanol injection, RFA, cryotherapy, and transcatheter arterial chemoembolization (TACE) are local ablative procedures that are commonly used to treat HCC patients who are not surgical candidates. These procedures as well as systemic therapy can also serve as a bridge to transplant therapy by delaying HCC progression [255,256,259]. The hypervascular nature of HCC tumors justifies the use of TACE and other procedures that are directed at the hepatic artery [260]. However, there is high HCC recurrence following TACE, which was proposed to be related to the shedding of tumor cells into the blood stream during the procedure [261]. Circulating tumor cells (CTCs) are considered to be an important predictive marker for tumor recurrence in breast and colorectal cancers [262], but the role of CTCs in HCC recurrence remain undefined.

For patients with very advanced disease and/or those who are ineligible for liver transplant, and/or not surgical candidates, systemic therapy can be used either as a palliative therapy or in an attempt to convert unresectable lesions to resectable ones [255,256]. Different classes of systematic therapies are approved for HCC treatment (i.e., chemotherapy, TKIs, and immune checkpoint inhibitors). HCC systemic therapies using tyrosine kinase inhibitors (TKIs) that target VEGF/VEGFR overexpression [260], which upregulates angiogenesis in tumors [178], can also overcome problems associated with the hypervascular nature of HCC [260]. However, systemic therapy is complicated by either the development of resistance or the accumulation of somatic mutations and chromosomal aberrations that play a key role in HCC initiation and progression and cannot be targeted by current treatment options [263,264].

In this section, we will review some recent updates in the field of HCC management.

Local ablative therapies and surgical resection

To determine whether CTCs affect TACE-association HCC recurrence, Fang et al. assessed the levels of CTCs in the peripheral blood and right atrium of HCC patients before and after TACE and compared these levels with time to progression to local recurrence or appearance of a new lesion. This study showed that prior to TACE, there was significantly higher numbers of CTCs in the right atrial blood than in peripheral blood, but the numbers of CTCs in both sites did not change after TACE. Therefore, this study demonstrated that intraoperative tumor-cell shedding did not affect the time to recurrence [265].

Deng at el. recently proposed a nomogram to predict the preoperative risk of MVI in HCC patients. This nomogram uses a combination of tumor size, preoperative AFP level, and neutrophil-to-lymphocyte ratio (NLR) and has sensitivity (61.64%), specificity (71.53%) and positive predictive value (64.13%). The cutoff value of predicted probability for MVI was set to 0.44, with low-risk patients defined as having a value for predicted probability as 0.44 or less. The clinical application of this nomogram helps determine which patients will receive liver transplant and guides surgeons in the choice of the best surgical option [266].

Systemic therapies

In this section, we will discuss some of widely used FDA approved TKIs and immune checkpoint inhibitors.

Tyrosine Kinase Inhibitors (TKI)

Sorafenib was the first TKI approved by the U.S FDA as the

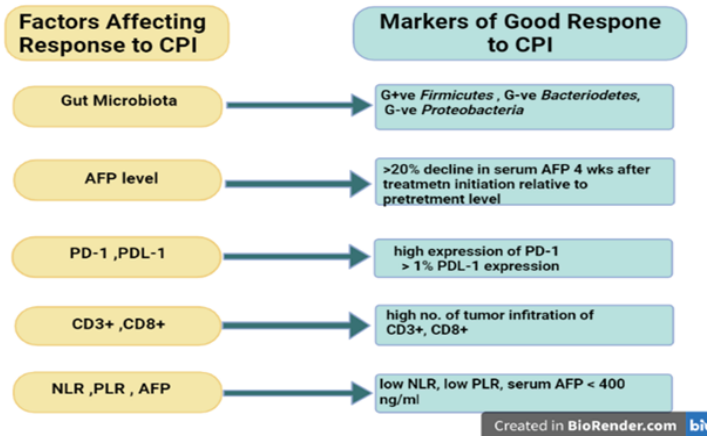


Figure 4: *Factors affecting response to check point inhibitors(CPI).*

first line treatment for advanced HCC [260]. The SHARP trial showed that Sorafenib treatment resulted in three months median survival benefit and an improved progression free survival for HCC patients [267]. Lenvatinib showed a noninferior median survival and could replace Sorafenib. The side effects associated with both drugs include hypertension, weight loss, and palmar-plantar erythrodysesthesia but are manageable [260]. The 3 trials that tested the efficacy of TKIs were: the RESORCE trial, which tested Regorafenib, a multitarget TKI; the CELESTIAL trial, which tested Cabozantinib, a TKI targeting mainly cMET and VEGFR2; and the REACH-2 trial, which tested Ramucirumab, an anti-VEGFR2 antibody. The three trials demonstrated efficacy of these TKIs as second-line treatments for patients who progressed on Sorafenib. All three agents showed a significant progression-free survival benefit versus placebo. Only Regorafenib and Cabozantinib demonstrated a significant overall survival benefit versus placebo. A subgroup analysis of the REACH-2 trial showed that Ramucirumab activity was limited to patients with baseline AFP levels > 400 ng/mL [260,268,269].

Checkpoint Inhibitors (CPI)

The current checkpoint inhibitors (CPI) that are approved by the FDA are: cytotoxic T lymphocyte–associated protein 4 (CTLA-4) inhibitors (Ipilimumab and Tremelimumab), anti–programmed cell death protein 1 (anti-PD-1) agents (Nivolumab and Pembrolizumab), and anti–programmed death-ligand 1 (PD-L1) agents (Atezolizumab, Avelumab, and Durvalumab). The lack of significant improvement of outcome, OS, and PFS in phase 3 trials to date impedes the use of CPIs as single agents in the first- or second-line setting. Therefore, multiple clinical trials have investigated the combination of CPIs with other agents in an attempt to modulate the tumor microenvironment and improve the treatment efficacy and tumor response [270]. In the IMbrave 150 phase 3 trial, Atezolizumab plus Bevacizumab showed a significant improvement in median PFS (6.8 vs. 4.3 months) and a significant improvement in OS at 12 months (67.2% vs 54.6%) as compared to Sorafenib for 1st line treatment with grade 3/4 adverse events (57% vs 55% for Sorafenib) [271]. A subgroup analysis showed that OS benefit was higher in patients who were ECOG PS 1, hepatitis B positive, hepatitis C positive, Barcelona clinic liver cancer stage C, and/or alpha-fetoprotein < 400 ng/mL, and in patients without extrahepatic spread or macroscopic vascular invasion [260]. The rationale for this combination of Atezolizumab plus Bevacizumab was based on the fact that VEGF overexpression precludes cytotoxic T lymphocyte recruitment, inhibits their proliferation, and blocks their function [272]. Therefore, treatment with anti-VEGF/VEGFR (Bevacizumab) favors the tumor response to Atezolizumab via increasing the tumor access of cytotoxic T cells [273]. Finally, the high complete response rate and overall survival showed by CheckMate 40 trial led to an accelerated approval by U.S FDA for the combination of Nivolumab plus Ipilimumab for patients with advanced HCC previously treated with Sorafenib. Treatment-related adverse events were manageable and consistent with other tumor types treated by immunotherapy. This combination is the first approved combined immunotherapy for second line treatment [274].

Factors that affect response to CPIs

The gut microbiota has been shown to affect the immunotherapy response in melanoma, SCLC, colorectal carcinoma, and other cancer, but its effects on HCC immunotherapy was reported for the first time by Zheng et al [275]. Eight HCC patients with BCLC stage C disease receiving anti-PD-1 antibodies every 3 weeks as second line after progression on Sorafenib were enrolled in this study. The researchers classified the patients as responders and non-responders based on radiological evaluation according to Response Evaluation Criteria in Solid Tumors (RECIST 1.1). Using metagenomic sequencing, the changes in the gut microbiota were followed by collecting fecal samples at different time points. This study revealed that there was no severe drug dysbiosis before starting the treatment in both groups, and the fecal samples were dominated by the Gram-positive Firmicutes, Gram-negative Bacteroidetes, and Gram-negative Proteobacteria, which resembled the findings from healthy adults. However, as the treatment proceeded, the microbiota composition in non-responders sequentially changed but remained relatively unchanged in responders. This study also showed that oral administration of Bifidobacterium, or any other prebiotics, can improve the response to anti-PD-1 agents in non-responders. This study demonstrated that the shift in microbiota composition relative to immunotherapy treatment in HCC patients can be used for disease monitoring and changing treatment strategies [275].

The association between the post-treatment level of serum AFP and response to immune checkpoint inhibitors has also been explored. In a study by Shao et al., 60 patients with advanced HCC

who were receiving immunotherapy and had a pretreatment AFP level > 20 ng/ml were evaluated for early AFP response, which was defined as a >20% decline in serum AFP levels within the first 4 weeks of treatment initiation relative to pretreatment levels. The study demonstrated that OS and PFS were significantly higher in early AFP responders as compared to early AFP nonresponders [276].

Another analysis involving the CheckMate 040 patients explored the association of PD-1 and PD-L1 expression, biomarkers of inflammation, and inflammatory gene signatures with response to Nivolumab monotherapy and survival in patients with advanced HCC. The results of this study showed that high PD-L1 expression on ≥1% of tumor cells was associated with improved OS. High PD-1 expression was associated with an improved response to Nivolumab and an improved OS. Moreover, patients with a high number of tumor-infiltrating CD3+, CD8+ T-cells showed an improved response to Nivolumab. This study also showed that the expression of a number of the inflammatory signature genes, the interferon gamma signature, and the T-cell exhaustion signature were associated with enhanced clinical response to Nivolumab and increased OS. The inflammatory signature genes can also be used to select patients who might benefit from treatment with Nivolumab. Finally, patients with low NLR, low PLR, and AFP <400 ng/ml had better prognosis and exhibited better response to Nivolumab [277].

Ongoing clinical trials and promising treatment modalities

There are several ongoing clinical trials worldwide that aim to test either the efficacy of new HCC treatment modalities or improve the response rate of the already existing options. In this section, we will describe some of the ongoing clinical trials that show promising results.

In a study conducted by Ni et al 2019, involving patients with advanced HCC, the researchers compared the efficacy and safety of a combination of transarterial chemoembolization and Sorafenib (TACE-S) with microwave ablation (TACE-S-MWA) to transarterial chemoembolization and Sorafenib (TACE-S). The study inclusion criteria were (a) age of 18–75 years, (b) ECOG performance status score of no more than 2, (c) Child–Pugh class A or B (d) BCLC stage C HCC, (e) less than five HCC lesions that were not greater than 10.0 cm in maximum diameter, (f) no history of receiving liver transplantation or surgical resection including interventional treatments (e.g., radiofrequency ablation, cryoablation or percutaneous ethanol injection), and (h) no severe coagulation abnormalities. Sorafenib treatment was administered at a standard dose of 400 mg twice daily. The authors reported a significantly higher OS and Time to Progression (TTP) for the patients treated with (TACE-S-MWA) as compared to (TACE-S). The adverse events were comparable between the two groups. These results suggest that the TACE-S-MWA combination is safe and effective, but larger studies are required to validate the results [278].

(LEAP-002) is an ongoing phase 3 clinical trial evaluating the combination of Lenvatinib plus Pembrolizumab as a first-line treatment of advanced HCC. Phase 1 showed a response rate of 46% [260].

The high recurrence rate of HCC following surgery, and the promising results of CPIs inspired Kaseb et al. to study the efficacy of Nivolumab plus Ipilimumab perioperative administration for HCC patients with resectable tumors. This phase 2 trial compared perioperative administration of Nivolumab to Nivolumab plus Ipilimumab followed by all patients continuing adjuvant immunotherapy for 2 years. The first interim analysis showed that the combination demonstrated a pathological complete response rate of 37.5%, no grade 3/4 adverse events, and the surgery was performed on time. This trial is ongoing [279].

COSMIC-312 is an international, open label, phase 3 trial that compares Cabozantinib plus/minus Atezolizumab versus Sorafenib as first line treatment for advanced HCC, and it is planned at 200 sites worldwide. Eligibility criteria include age ≥18 years, BCLC stage B or C, Child-Pugh A, ECOG PS 0 or 1, and measurable disease per RECIST 1.1. The results regarding OS and PFS as well as safety are currently unknown [280]. For more details about ongoing clinical trials , the readers are referred to (Journal of Clinical Oncology ;Volume 38, Issue 36) [281].

There are some ongoing trials evaluating the use of radiation in HCC management. The growing role of radiotherapy and CPIs in treating many cancers has motivated many researchers to explore this combination in treating HCC patients. The synergistic effect of radiation and immunotherapy is due to the “abscopal effect”, which is an innate and adaptive immune mediated phenomenon in which there is a remission of the tumor outside the irradiated field. Radiotherapy facilitates the efflux of CD8+T-cells, which slow the tumor growth, and the release of other inflammatory cytokines that recruit cytotoxic T-cells to the tumor site. CPIs are believed to potentiate the “abscopal effect” by blocking PD-1 and CTLA4, negative regulators of cytotoxic T cells; therefore, CPIs augment the immune response and reduce resistance to radiation therapy [282,283]. Although there are several ongoing trials that evaluate the combination of radiotherapy with immunotherapy, most of these trials are using Stereotactic Body Radiotherapy (SBRT) or Selective Internal RT (SIRT). However, there is an ongoing experimental study that evaluates safety and efficacy of the combination of Durvalumab plus Tremelimumab plus External Beam Radiotherapy (EBRT) for treating patients with advanced HCC who have progressed on Sorafenib and are immunotherapy naïve. The trial ends by October 2025 [284].

Finally, DOSISPHERE-01 is a phase 2 trial comparing standard dosimetry with a goal to deliver 120±20Gy to the treated volume to personalized dosimetry arm with a goal to deliver at least 205Gy to the target lesion using 90Y loaded microsphere SIRT (radioembolization) in unresectable HCC patients with at least one tumor ≥7cm. The personalized dosimetry treatment demonstrated higher response and OS than the standard dosimetry with fewer grade 3 adverse events [285].

Additional ongoing clinical trials testing the combination of immunotherapy with ablative procedures or radiation therapy are listed in (Table 3) [284].

Conclusions

HCC is a global issue. The dismal prognosis of HCC is largely related to the heterogeneity of the disease. Although the recent advances in the field of HCC diagnosis and management offer some hope, the treatment benefits are limited to a small subset of HCC patients. Therefore, In the era of personalized medicine, there is a critical need for a valid molecular HCC classification system that helps the clinician in treatment selection. Moreover, a deeper understanding of the genetic and epigenetic alterations that are peculiar for each HCC patient will provide further help. With the substantial number of systemic therapies, the clinicians need either an effective drug that hits a universal target among HCC

Table 3: A list of some of ongoing clinical trials testing the combination of immunotherapy plus ablative or radiation therapies (Note: adapted from: Jordan, A. C. & Wu, J. Immunotherapy in hepatocellular carcinoma: Combination strategies. World Journal of Meta-Analysis 8, 190-209 (2020).

Summary of The Trial	Phase of The Trial	Primary End Point	Secondary End Point	Indication	Reference
Pembrolizumab combined with RFA /MWA or brachytherapy	II	ORR	TTR/ Recurrence free survival/OS/ Incidence and severity of adverse events/ Identification of molecular biomarkers in tumor tissue and blood samples	First-line for Early Stage HCC.	NCT03753659 (IMMULAB)
Nivolumab plus TACE	II	ORR	PFS/TTP/OS/OR/TTFS/ QoL/ Incidence of Treatment Emergent Adverse Events	First-line for intermediate stage HCC.	NCT03572582 (IMMUTACE)
Pembrolizumab plus TACE	I/II	Safety and Tolerability	PFSR/ The efficacy of pembrolizumab following TACE.	First-line	NCT03397654 (PETAL)
Durvalumab plus tremelimumab compared to Durvalumab plus tremelimumab+ RFA/ Cryo/TACE	II	PFS	Safety	Second-line for advanced stage HCC(BCLC Stage B/C) or Biliary Tract Carcinomas(BCT).	NCT02821754
Durvalumab plus tremelimumab plus DEB-TACE	II	ORR	PFS/OS/Tumor response/ drug-related toxicities	The Effect of CTLA-4/PD-L1 Blockade Following Drug-eluting Bead Transarterial Chemoembolization (DEB-TACE) in Patients with Intermediate Stage of HCC Using Durvalumab (MEDI4736) and Tremelimumab	NCT03638141
Durvalumab, bevacizumab and tremelimumab compared to Durvalumab, bevacizumab and tremelimumab + TACE	II	PFS in patients with advanced HCC BCLC stage B/ patients with BTC and HCC BCLC stage C	BOR/OS/Safety	Second-line for advanced HCC& BCT.	(NCT03937830)
Durvalumab plus tremelimumab and radiation	II	BOR	OS/Disease Control Rate/ PFS/TTP/Duration of Response/ Treatment Related Adverse Events	Second-line for HCC or BCT.	NCT03482102
Pembrolizumab plus SBRT	II	ORR	PFS/OS/ Response rate in non-irradiated tumor lesions	for advanced HCC patients as Second-line after Sorafenib.	NCT03316872
Nivolumab+SBRT compared to Nivolumab+ Ipilimumab+SBRT	I	Safety	ORR/TTP/OS/ Number of long-term adverse events/ Rate of disease control/ Rate of local control of the SBRT treated lesion	SBRT Followed by Nivolumab or Ipilimumab With Nivolumab in Unresectable Hepatocellular Carcinoma	NCT03203304

patients with minimal side effects, or they have to tailor the drug choice according to the genetic profile for each patient. In either case, there is a need for more extensive treatment options. Finally, a noninvasive biomarker(s) (prognostic, therapeutic or follow-up) that has very high specificity and sensitivity will dramatically facilitate the treatment decision making.

Future considerations

- Implementation of HCC surveillance programs will facilitate early detection and decrease HCC-related mortality.
- The increasing incidence of obesity and its correlation with NAFLD and NASH, needs more serious awareness programs about the obesity associated risk of HCC plus the

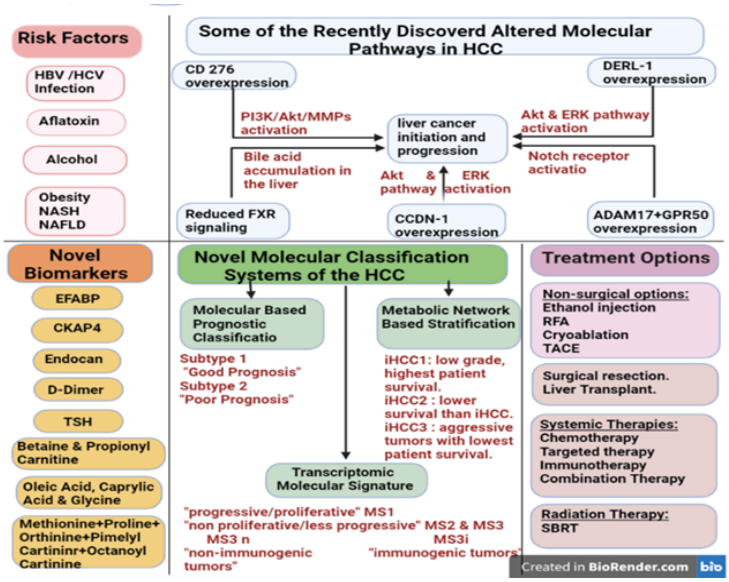


Figure 5: *A summary diagram of the recent developments in the field of HCC .*

implementation of wight loss strategies for individuals who are at high risk.

- There is a need for more and larger clinical trials using recently developed treatment options that can efficiently eradicate HCV and HBV infections and decrease the risk of developing HCC.
- Identification and clinical implementation of new noninvasive biomarkers will save time and money and will help in both early detection of HCC and in differentiating HCC from non-HCC lesions with higher sensitivity and specificity.
- There is a need for more adequate tissue-based diagnosis of HCC that will help tailor treatment options for each patient depending on the etiology and histology of the tumor and the genetic profile of the patient. This strategy could decrease treatment-related off target effects and reduce the costs.
- Since HCC that develops in the background of HBV infection, HCV infection and alcohol abuse mostly affects people with low income, and due to the high cost of the currently available HCC-treatment options , there should be universal strategies to ensure an equal access to available treatments across the world.
- Development of drug resistance might be delayed by using either lower drug doses or a combination of drugs that have different mechanisms of action.
- The results of key ongoing clinical trials will allow physicians to have a sequence for HCC treatment options; for example, the sequence of immunotherapy and TKI agents, the use of immunotherapy combinations, or immunotherapy combinations with other types of treatments.

References

1. Sung, H. *et al.* Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* 2021; 71: 209-249.
2. Janevska D, Chaloska-Ivanova V, Janevski V. Hepatocellular Carcinoma: Risk Factors, Diagnosis and Treatment. *Open Access Maced J Med Sci.* 2015; 3: 732-736.
3. Liver Cancer Risk Factors. *American Cancer Society* (April 2019).
4. Tejeda-Maldonado J, García-Juárez I, Aguirre-Valadez J, González-Aguirre A, Vilatobá-Chapa M, et al. Diagnosis and treatment of hepatocellular carcinoma: An update. *World J Hepatol.* 2015; 7: 362-376.
5. Kennedy K, Graham SM, Arora N, Shuhart MC, Kim HN. Hepatocellular carcinoma among US and non-US-born patients with chronic hepatitis B: Risk factors and age at diagnosis. *PLoS One.* 2018; 13: e0204031-e0204031.
6. Tong, H, Liu X, Li T, Qiu W, Peng C, et al. NR1D2 Accelerates Hepatocellular Carcinoma Progression by Driving the Epithelial-to-Mesenchymal Transition. *Onco Targets Ther.* 2020; 13: 3931-3942.
7. Liver Cancer Survival Rates. *American Cancer Society* (January 2020).
8. Wege H, Li J, Ittrich H. Treatment Lines in Hepatocellular Carcinoma. *Visc Med.* 2019; 35: 266-272.
9. Hilmi M, et al. Angiogenesis and immune checkpoint inhibitors as therapies for hepatocellular carcinoma: current knowledge and future research directions. *Journal for ImmunoTherapy of Cancer.* 2019; 7: 333.
10. Dhanasekaran R, Nault JC, Roberts LR, Zucman-Rossi J. Genomic Medicine and Implications for Hepatocellular Carcinoma Prevention and Therapy. *Gastroenterology.* 2019; 156: 492-509.
11. Liew WP, Mohd-Redzwan S. Mycotoxin: Its Impact on Gut Health and Microbiota. *Front Cell Infect Microbiol.* 2018; 8: 60.
12. Koshy R, Koch S, von Loringhoven AF, Kahmann R, Murray K, et al. Integration of hepatitis B virus DNA: evidence for integration in the single-stranded gap. *Cell.* 1983; 34: 215-223.
13. Brechot C, Pourcel C, Louise A, Rain B, Tiollais P. Presence of integrated hepatitis B virus DNA sequences in cellular DNA of human hepatocellular carcinoma. *Nature.* 1980; 286: 533-535.
14. Feitelson MA, Lee J. Hepatitis B virus integration, fragile sites, and hepatocarcinogenesis. *Cancer Lett.* 2007; 252: 157-170.
15. Sung WK, Zheng H, Li S, Chen R, Liu X, et al. Genome-wide survey of recurrent HBV integration in hepatocellular carcinoma. *Nat Genet.* 2012; 44: 765-769.
16. Dahse R, Fiedler W, Ernst G. Telomeres and telomerase: biological and clinical importance. *Clin Chem.* 1997; 43: 708-714.

17. Sze CC, Shilatifard A. MLL3/MLL4/COMPASS Family on Epigenetic Regulation of Enhancer Function and Cancer. *Cold Spring Harb Perspect Med.* 2016; 6: a026427.
18. Cassia R, Moreno-Bueno G, Rodríguez-Perales S, Hardisson D, Cigudosa JC, et al. Cyclin E gene (CCNE) amplification and hCDC4 mutations in endometrial carcinoma. *J Pathol.* 2003; 201: 589-595.
19. Sze KM, Ho DW, Chiu YT, Tsui YM, Chan LK, et al. Hepatitis B Virus-Telomerase Reverse Transcriptase Promoter Integration Harnesses Host ELF4, Resulting in Telomerase Reverse Transcriptase Gene Transcription in Hepatocellular Carcinoma. *Hepatology*. 2020; 73: 23-40.
20. Dong H, Zhang L, Qian Z, Zhu X, Zhu G, et al. Identification of HBV-MLL4 Integration and Its Molecular Basis in Chinese Hepatocellular Carcinoma. *PLoS One.* 2015; 10: e0123175.
21. Lau CC, Sun T, Ching AK, He M, Li JW, et al. Viral-human chimeric transcript predisposes risk to liver cancer development and progression. *Cancer Cell.* 2014; 25: 335-349.
22. Vescovo T, Refolo G, Vitagliano G, Fimia GM, Piacentini M. Molecular mechanisms of hepatitis C virus–induced hepatocellular carcinoma. *Clin Microbiol Infect.* 2016; 22: 853-861.
23. Donaires FS, Scatena NF, Alves-Paiva RM, Podlevsky JD, Logeswaran D, et al. Telomere biology and telomerase mutations in cirrhotic patients with hepatocellular carcinoma. *PLoS One.* 2017; 12: e0183287.
24. Mitchell JK, Midkiff BR, Israelow B, Evans MJ, Lanford RE, et al. Hepatitis C Virus Indirectly Disrupts DNA Damage-Induced p53 Responses by Activating Protein Kinase R. *mBio.* 2017; 8: e00121-17.
25. 25 Munakata T, Nakamura M, Liang Y, Li K, Lemon SM. Down-regulation of the retinoblastoma tumor suppressor by the hepatitis C virus NS5B RNA-dependent RNA polymerase. *Proc Natl Acad Sci U S A.* 2005; 102: 18159-18164.
26. Novo E, Povero D, Busletta C, Paternostro C, di Bonzo LV, et al. The biphasic nature of hypoxia-induced directional migration of activated human hepatic stellate cells. *J Pathol.* 2012; 226: 588-597.
27. Whittaker S, Marais R, Zhu AX. The role of signaling pathways in the development and treatment of hepatocellular carcinoma. *Oncogene.* 2010; 29: 4989-5005.
28. Grivennikov SI, Greten FR, Karin M. Immunity, inflammation, and cancer. *Cell.* 2010; 140: 883-899.
29. Nakagawa H, Maeda S, Yoshida H, Tateishi R, Masuzaki R, et al. Serum IL-6 levels and the risk for hepatocarcinogenesis in chronic hepatitis C patients: an analysis based on gender differences. *Int J Cancer.* 2009; 125: 2264-2269.
30. Aydin Y, Kurt R, Song K, Lin D, Osman H, et al. Hepatic Stress Response in HCV Infection Promotes STAT3-Mediated Inhibition of HNF4A-miR-122 Feedback Loop in Liver Fibrosis and Cancer Progression. *Cancers (Basel).* 2019; 11: 1407.
31. Dash S, Aydin Y, Widmer KE, Nayak L. Hepatocellular Carcinoma Mechanisms Associated with Chronic HCV Infection and the Impact of Direct-Acting Antiviral Treatment. *J Hepatocell Carcinoma.* 2020; 7: 45-76.
32. Naus CC, Laird DW. Implications and challenges of connexin connections to cancer. *Nat Rev Cancer.* 2010; 10: 435-441.
33. Vinken M, Henkens T, De Rop E, Fraczek J, Vanhaecke T, et al. Biology and pathobiology of gap junctional channels in hepatocytes. *Hepatology.* 2008; 47: 1077-1088.
34. Verma N, Tiku AB. Significance and nature of bystander responses induced by various agents. *Mutat Res Rev Mutat Res.* 2017; 773: 104-121.
35. Kofahi HM, Taylor NG, Hirasawa K, Grant MD, Russell RS. Hepatitis C Virus Infection of Cultured Human Hepatoma Cells Causes Apoptosis and Pyroptosis in Both Infected and Bystander Cells. *Sci Rep.* 2016; 6: 37433.
36. Evert M, Ott T, Temme A, Willecke K, Dombrowski F. Morphology and morphometric investigation of hepatocellular preneoplastic lesions and neoplasms in connexin32-deficient mice. *Carcinogenesis.* 2002; 23: 697-703.
37. Testino G, Leone S, Borro P. Alcohol and hepatocellular carcinoma: a review and a point of view. *World J Gastroenterol.* 2014; 20: 15943-15954.
38. Ramadori P, Cubero FJ, Liedtk, C, Trautwein C, Nevzorova YA. Alcohol and Hepatocellular Carcinoma: Adding Fuel to the Flame. *Cancers (Basel).* 2017; 9: 130.
39. Donato F, Tagger A, Gelatti U, Parrinello G, Boffetta P, et al. Alcohol and Hepatocellular Carcinoma: The Effect of Lifetime Intake and Hepatitis Virus Infections in Men and Women. *Am J Epidemiol.* 2002; 155: 323-331.
40. Ganne-Carrié N, Nahon P. Hepatocellular carcinoma in the setting of alcohol-related liver disease. *J Hepatol.* 2019; 70: 284-293.
41. Niemelä O, Parkkila S, Pasanen M, Iimuro Y, Bradford B, et al. Early alcoholic liver injury: formation of protein adducts with acetaldehyde and lipid peroxidation products, and expression of CYP2E1 and CYP3A. *Alcohol Clin Exp Res.* 1998; 22: 2118-2124.
42. Matsushita H, Takaki A. Alcohol and hepatocellular carcinoma. *BMJ Open Gastroenterology.* 2019; 6: e000260.
43. Calle EE, Rodriguez C, Walker-Thurmond K, Thun MJ. Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. *N Engl J Med.* 2003; 348: 1625-1638.
44. Gupta A, Das A, Majumder K, Arora N, Mayo HG, *et al.* Obesity is Independently Associated With Increased Risk of Hepatocellular Cancer-related Mortality: A Systematic Review and Meta-Analysis. *Am J Clin Oncol.* 2018; 41: 874-881.
45. Pennisi G, Celsa C, Giammanco A, Spatola F, Petta S. The Burden of Hepatocellular Carcinoma in Non-Alcoholic Fatty Liver Disease: Screening Issue and Future Perspectives. *Int J Mol Sci.* 2019; 20: 5613.
46. Baffy G. Hepatocellular Carcinoma in Non-alcoholic Fatty Liver Disease: Epidemiology, Pathogenesis, and Prevention. *J Clin Transl Hepatol.* 2013; 1: 131-137.
47. Marengo A, Rosso C, Bugianesi E. Liver Cancer: Connections with Obesity, Fatty Liver, and Cirrhosis. *Annu Rev Med.* 2016; 67: 103-117.
48. Negro F. Natural history of NASH and HCC. *Liver Int.* 2020; 40: 72-76.
49. Dhamija E, Paul SB, Kedia S. Non-alcoholic fatty liver disease associated with hepatocellular carcinoma: An increasing concern. *Indian J Med Res.* 2019; 149: 9-17.
50. Shoelson SE, Herrero L, Naaz A. Obesity, inflammation, and insulin resistance. *Gastroenterology.* 2007; 132: 2169-2180.
51. Sharma D, Wang J, Fu PP, Sharma S, Nagalingam A, et al. Adiponectin antagonizes the oncogenic actions of leptin in hepatocellular carcinogenesis. *Hepatology.* 2010; 52: 1713-1722.
52. Shalapour S, Lin XJ, Bastian IN, Brain J, Burt AD, et al. Inflammation-induced IgA+ cells dismantle anti-liver cancer immunity. *Nature.* 2017; 551: 340-345.
53. 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.

54. Grohmann M, Wiede F, Dodd GT, Gurzov EN, Ooi GJ, et al. Obesity Drives STAT-1-Dependent NASH and STAT-3-Dependent HCC. *Cell.* 2018; 175: 1289-1306.e1220.
55. Takuma Y, Nouso K. Nonalcoholic steatohepatitis-associated hepatocellular carcinoma: our case series and literature review. *World J Gastroenterol.* 2010; 16: 1436-1441.
56. Paradis V, Zalinski S, Chelbi E, Guedj N, Degos F, et al. Hepatocellular carcinomas in patients with metabolic syndrome often develop without significant liver fibrosis: a pathological analysis. *Hepatology.* 2009; 49: 851-859.
57. Rahman R, Hammoud GM, Almashhrawi AA, Ahmed KT, Ibdah JA. Primary hepatocellular carcinoma and metabolic syndrome: An update. *World J Gastrointest Oncol.* 2013; 5: 186-194.
58. Estep JM, Baranova A, Hossain N, Elariny H, Ankrah K, et al. Expression of cytokine signaling genes in morbidly obese patients with non-alcoholic steatohepatitis and hepatic fibrosis. *Obes Surg.* 2009; 19: 617-624.
59. Lam B, Younossi ZM. Treatment options for nonalcoholic fatty liver disease. *Therapeutic advances in gastroenterology.* 2010; 3: 121-137.
60. Tana C, Ballestri S, Ricci F, Di Vincenzo A, Ticinesi A, et al. Cardiovascular Risk in Non-Alcoholic Fatty Liver Disease: Mechanisms and Therapeutic Implications. *Int J Environ Res Public Health.* 2019; 16: 3104.
61. Lefere S, Van de Velde F, Hoorens A, Raevens S, Van Campenhout S, et al. Angiopoietin-2 Promotes Pathological Angiogenesis and Is a Therapeutic Target in Murine Nonalcoholic Fatty Liver Disease. *Hepatology.* 2019; 69: 1087-1104.
62. Suzuki A, Lindor K, St Saver J, Lymp J, Mendes F, et al. Effect of changes on body weight and lifestyle in nonalcoholic fatty liver disease. *J Hepatol.* 2005; 43: 1060-1066.
63. Tendler D, Lin S, Yancy WS Jr, Mavropoulos J, Sylvestre P, et al. The effect of a low-carbohydrate, ketogenic diet on nonalcoholic fatty liver disease: a pilot study. *Dig Dis Sci.* 2007; 52: 589-593.
64. Furuya CK Jr, de Oliveira CP, de Mello ES, Faintuch J, Raskovski A, et al. Effects of bariatric surgery on nonalcoholic fatty liver disease: preliminary findings after 2 years. *J Gastroenterol Hepatol.* 2007; 22: 510-514.
65. Hussein O, Grosovski M, Schlesinger S, Szvalb S, Assy N. Orlistat reverse fatty infiltration and improves hepatic fibrosis in obese patients with nonalcoholic steatohepatitis (NASH). *Dig Dis Sci.* 2007; 52: 2512-2519.
66. Harrison SA, Fincke C, Helinski D, Torgerson S, Hayashi P. A pilot study of orlistat treatment in obese, non-alcoholic steatohepatitis patients. *Aliment Pharmacol Ther.* 2004; 20: 623-628.
67. Sabuncu T, Nazligul Y, Karaoglanoglu M, Ucar E, Kilic FB. The effects of sibutramine and orlistat on the ultrasonographic findings, insulin resistance and liver enzyme levels in obese patients with non-alcoholic steatohepatitis. *Rom J Gastroenterol.* 2003; 12: 189-192.
68. Sharma, B. & John, S. in *StatPearls* (StatPearls Publishing Copyright © 2021, StatPearls Publishing LLC., 2021).
69. Hasegawa T, Yoneda M, Nakamura K, Makino I, Terano A. Plasma transforming growth factor-beta1 level and efficacy of alpha-tocopherol in patients with non-alcoholic steatohepatitis: a pilot study. *Aliment Pharmacol Ther.* 2001; 15: 1667-1672.
70. Peraica M, Domijan AM. Contamination of food with mycotoxins and human health. *Arh Hig Rada Toksikol.* 2001; 52: 23-35.
71. Muhammad I, Sun X, Wang H, Li W, Wang X, et al. Curcumin Successfully Inhibited the Computationally Identified CYP2A6 Enzyme-Mediated Bioactivation of Aflatoxin B1 in Arbor Acres broiler. *Front Pharmacol.* 2017; 8: 143.
72. Galarza-Seeber R, Latorre JD, Bielke LR, Kuttappan VA, Wolfenden AD, et al. Leaky Gut and Mycotoxins: Aflatoxin B1 Does Not Increase Gut Permeability in Broiler Chickens. *Front Vet Sci.* 2016; 3: 10-10.
73. Kew MC. Aflatoxins as a cause of hepatocellular carcinoma. *J Gastrointestin Liver Dis.* 2013; 22: 305-310.
74. Macé K, Aguilar F, Wang JS, Vautravers P, Gómez-Lechón M, et al. Aflatoxin B1-induced DNA adduct formation and p53 mutations in CYP450-expressing human liver cell lines. *Carcinogenesis.* 1997; 18: 1291-1297.
75. Broom L. Mycotoxins and the intestine. *Animal Nutrition.* 2015; 1: 262-265.
76. Dhanasekaran R, Venkatesh SK, Torbenson MS, Roberts LR. Clinical implications of basic research in hepatocellular carcinoma. *J Hepatol.* 2016; 64: 736-745.
77. Rinninella E, Zocco MA, De Gaetano A, Iezzi R, Campanale M, et al. From small nodule to overt HCC: a multistep process of carcinogenesis as seen during surveillance. *Eur Rev Med Pharmacol Sci.* 2012; 16: 1292-1294.
78. Setshedi M, Andersson M, Kgatle MM, Roberts LR. Molecular and cellular oncogenic mechanisms in hepatocellular carcinoma. *S Afr Med J.* 2018; 108: 41-46.
79. Clarke MF, Dick JE, Dirks PB, Eaves CJ, Jamieson CH, et al. Cancer stem cells--perspectives on current status and future directions: AACR Workshop on cancer stem cells. *Cancer Res.* 2006; 66: 9339-9344.
80. Yin S, Li J, Hu C, Chen X, Yao M, et al. CD133 positive hepatocellular carcinoma cells possess high capacity for tumorigenicity. *Int J Cancer.* 2007; 120: 1444-1450.
81. Niu ZS, Niu XJ, Wang WH. Genetic alterations in hepatocellular carcinoma: An update. *World J Gastroenterol.* 2016; 22: 9069-9095.
82. Lee JK, Choi YL, Kwon M, Park PJ. Mechanisms and Consequences of Cancer Genome Instability: Lessons from Genome Sequencing Studies. *Annu Rev Pathol.* 2016; 11: 283-312.
83. Eferl R, Trauner M. Chromosomal instability in HCC: a key function for checkpoint kinase 2. *Gut.* 2018; 67: 204-205.
84. Giam M, Rancati G. Aneuploidy and chromosomal instability in cancer: a jackpot to chaos. *Cell Div.* 2015; 10: 3-3.
85. Kan Z, Zheng H, Liu X, Li S, Barber TD, et al. Whole-genome sequencing identifies recurrent mutations in hepatocellular carcinoma. *Genome Res.* 2013; 23: 1422-1433.
86. Zhang SH, Cong WM, Xian ZH, Wu MC. Clinicopathological significance of loss of heterozygosity and microsatellite instability in hepatocellular carcinoma in China. *World J Gastroenterol.* 2005; 11: 3034-3039.
87. Kim JK, Diehl JA. Nuclear cyclin D1: an oncogenic driver in human cancer. *J Cell Physiol.* 2009; 220: 292-296.
88. Liu Y, Cao M, Cai Y, Li X, Zhao C, et al. Dissecting the Role of the FGF19-FGFR4 Signaling Pathway in Cancer Development and Progression. *Front Cell Dev Biol.* 2020; 8: 95.
89. Dhiman G, Srivastava N, Goyal M, Rakha E, Lothion-Roy J, et al. Metadherin: A Therapeutic Target in Multiple Cancers. *Front Oncol.* 2019; 9: 349.
90. Wang K, Lim HY, Shi S, Lee J, Deng S, et al. Genomic landscape of copy number aberrations enables the identification of oncogenic drivers in hepatocellular carcinoma. *Hepatology.* 2013; 58: 706-717.
91. Kondo Y, Kanai Y, Sakamoto M, Mizokami M, Ueda R, et al. Genetic instability and aberrant DNA methylation in chronic hepatitis and cirrhosis—A comprehensive study of loss of heterozygosity and

microsatellite instability at 39 loci and DNA hypermethylation on 8 CpG islands in microdissected specimens from patients with hepatocellular carcinoma. *Hepatology.* 2000; 32: 970-979.

92. He C, Xu J, Zhang J, Xie D, Ye H, et al. High expression of trimethylated histone H3 lysine 4 is associated with poor prognosis in hepatocellular carcinoma. *Hum Pathol.* 2012; 43: 1425-1435.
93. Zhang X, Wang J, Cheng J, Ding S, Li M, et al. An integrated analysis of SOCS1 down-regulation in HBV infection-related hepatocellular carcinoma. *J Viral Hepat.* 2014; 21: 264-271.
94. Csepregi A, Röcken C, Hoffmann J, Gu P, Saliger S, *et al.* APC promoter methylation and protein expression in hepatocellular carcinoma. *J Cancer Res Clin Oncol.* 2008; 134: 579-589.
95. Higgs MR, Lerat H, Pawlotsky JM. Downregulation of Gadd45beta expression by hepatitis C virus leads to defective cell cycle arrest. *Cancer Res.* 2010; 70: 4901-4911.
96. Zhang C, et al. Association between gene methylation and HBV infection in hepatocellular carcinoma: A meta-analysis. *J Cancer.* 2019; 10: 6457-6465.
97. Cougot D, Wu Y, Cairo S, Caramel J, Renard CA, et al. The hepatitis B virus X protein functionally interacts with CREB-binding protein/p300 in the regulation of CREB-mediated transcription. *J Biol Chem.* 2007; 282: 4277-4287.
98. Meguro M, Mizuguchi T, Kawamoto M, Hirata K. The Molecular Pathogenesis and Clinical Implications of Hepatocellular Carcinoma. *Int J Hepatol.* 2011; 2011: 818672.
99. Avila MA, Berasain C, Sangro B, Prieto J. New therapies for hepatocellular carcinoma. *Oncogene* 2006; 26: 3866-3884.
100. Zvereva MI, Shcherbakova DM, Dontsova OA. Telomerase: structure, functions, and activity regulation. *Biochemistry (Mosc).* 2010; 75: 1563-1583.
101. Nault JC, Mallet M, Pilati C, Calderaro J, Bioulac-Sage P, et al. High frequency of telomerase reverse-transcriptase promoter somatic mutations in hepatocellular carcinoma and preneoplastic lesions. *Nat Commun.* 2013; 4: 2218.
102. Li Y, Tergaonkar V. Telomerase reactivation in cancers: Mechanisms that govern transcriptional activation of the wild-type vs. mutant TERT promoters. *Transcription.* 2016; 7: 44-49.
103. Hornsby PJ. Telomerase and the aging process. *Exp Gerontol.* 2007; 42: 575-581.
104. Calado RT, Brudno J, Mehta P, Kovacs JJ, Wu C, et al. Constitutional telomerase mutations are genetic risk factors for cirrhosis. *Hepatology.* 2011; 53: 1600-1607.
105. Aubrey BJ, Strasser A, Kelly GL. Tumor-Suppressor Functions of the TP53 Pathway. *Cold Spring Harb Perspect Med.* 2016; 6: a026062.
106. Cleary SP, Jeck WR, Zhao X, Chen K, Selitsky SR, et al. Identification of driver genes in hepatocellular carcinoma by exome sequencing. *Hepatology.* 2013; 58: 1693-1702.
107. Kirk GD, Lesi OA, Mendy M, Szymañska K, Whittle H, et al. 249 ser TP53 mutation in plasma DNA, hepatitis B viral infection, and risk of hepatocellular carcinoma. *Oncogene.* 2005; 24: 5858-5867.
108. Waly Raphael S, Yangde Z, Yuxiang C. Hepatocellular carcinoma: focus on different aspects of management. *ISRN Oncol.* 2012; 2012: 421673.
109. Cheng AS, Lau SS, Chen Y, Kondo Y, Li MS, et al. EZH2-mediated concordant repression of Wnt antagonists promotes β-catenin-dependent hepatocarcinogenesis. *Cancer Res.* 2011; 71: 4028-4039.
110. Kim J, Lee Y, Lu X, Song B, Fong KW, *et al.* Polycomb- and Methylation-Independent Roles of EZH2 as a Transcription Activator. *Cell Rep.* 2018; 25: 2808-2820.e2804.
111. Fujimoto A, Totoki Y, Abe T, Boroevich KA, Hosoda F, et al. Whole-genome sequencing of liver cancers identifies etiological influences on mutation patterns and recurrent mutations in chromatin regulators. *Nat Genet.* 2012; 44: 760-764.
112. Amaddeo G, Guichard C, Imbeaud S, Zucman-Rossi J. Next-generation sequencing identified new oncogenes and tumor suppressor genes in human hepatic tumors. *Oncoimmunology.* 2012; 1: 1612-1613.
113. Heussler HS, Suri M. Sonic hedgehog. *Mol Pathol.* 2003; 56: 129-131.
114. Efroni S, Meerzaman D, Schaefer CF, Greenblum S, Soo-Lyu M, et al. Systems analysis utilising pathway interactions identifies sonic hedgehog pathway as a primary biomarker and oncogenic target in hepatocellular carcinoma. *IET Syst Biol.* 2013; 7: 243-251.
115. Lu Jt, Zhao Wd, He W, Wei W. Hedgehog signaling pathway mediates invasion and metastasis of hepatocellular carcinoma via ERK pathway. *Acta Pharmacol Sin.* 2012; 33: 691-700.
116. Kabarriti R, Guha C. Hedgehog signaling and radiation induced liver injury: a delicate balance. *Hepatol Int.* 2014; 8: 316-320.
117. Chen CY, Malchus NS, Hehn B, Stelzer W, Avci D, et al. Signal peptide peptidase functions in ERAD to cleave the unfolded protein response regulator XBP1u. *EMBO J.* 2014; 33: 2492-2506.
118. Eshraghi A, Dixon SD, Tamilselvam B, Kim EJ, Gargi A, et al. Cytolethal distending toxins require components of the ER-associated degradation pathway for host cell entry. *PLoS Pathog.* 2014; 10: e1004295.
119. Cheng R, Wang B, Cai XR, Chen ZS, Du Q, et al. CD276 Promotes Vasculogenic Mimicry Formation in Hepatocellular Carcinoma via the PI3K/AKT/MMPs Pathway. *Onco Targets Ther.* 2020; 13: 11485-11498.
120. Fan J, Tian L, Huang S, Zhang J, Zhao B. Derlin-1 Promotes the Progression of Human Hepatocellular Carcinoma via the Activation of AKT Pathway. *Onco Targets Ther.* 2020; 13: 5407-5417.
121. Greenwald RJ, Freeman GJ, Sharpe AH. The B7 family revisited. *Annu Rev Immunol.* 2005; 23: 515-548.
122. Picarda E, Ohaegbulam KC, Zang X. Molecular Pathways: Targeting B7-H3 (CD276) for Human Cancer Immunotherapy. *Clin Cancer Res.* 2016; 22: 3425-3431.
123. Dong P, Xiong Y, Yue J, Hanley SJB, Watari H. B7H3 As a Promoter of Metastasis and Promising Therapeutic Target. *Front Oncol.* 2018; 8: 264.
124. Ao R, Zhang DR, Du YQ, Wang Y. Expression and significance of Pin1, β-catenin and cyclin D1 in hepatocellular carcinoma. *Mol Med Rep.* 2014; 10: 1893-1898.
125. Yan W, Cheng L, Zhang D. Ultrasound-Targeted Microbubble Destruction Mediated si-CyclinD1 Inhibits the Development of Hepatocellular Carcinoma via Suppression of PI3K/AKT Signaling Pathway. *Cancer Manag Res.* 2020; 12: 10829-10839.
126. Saha SK, Choi HY, Yang GM, Biswas PK, Kim K, et al. GPR50 Promotes Hepatocellular Carcinoma Progression via the Notch Signaling Pathway through Direct Interaction with ADAM17. *Mol Ther Oncolytics.* 2020; 17: 332-349.
127. Yu LX, Schwabe RF. The gut microbiome and liver cancer: mechanisms and clinical translation. *Nat Rev Gastroenterol Hepatol.* 2017; 14: 527-539.
128. Boursier J, Mueller O, Barret M, Machado M, Fizanne L, et al. The severity of nonalcoholic fatty liver disease is associated with gut dysbiosis and shift in the metabolic function of the gut microbiota. *Hepatology.* 2016; 63: 764-775.
129. Qin N, Yang F, Li A, Prifti E, Chen Y, et al. Alterations of the human gut microbiome in liver cirrhosis. *Nature.* 2014; 513: 59-64.

130. Chen Y, Yang F, Lu H, Wang B, Chen Y, et al. Characterization of fecal microbial communities in patients with liver cirrhosis. *Hepatology.* 2011; 54: 562-572.

131. Gupta H, Youn GS, Shin MJ, Suk KT. Role of Gut Microbiota in Hepatocarcinogenesis. *Microorganisms.* 2019; 7: 121.

132. Yang J, Li M, Zheng QC. Emerging role of Toll-like receptor 4 in hepatocellular carcinoma. *J Hepatocell Carcinoma.* 2015; 2: 11-17.

133. Rao RK, Seth A, Sheth P. Recent Advances in Alcoholic Liver Disease I. Role of intestinal permeability and endotoxemia in alcoholic liver disease. *Am J Physiol Gastrointest Liver Physiol.* 2004; 286: G881-G884.

134. Trebicka J, Krag A, Gansweid S, Appenrodt B, Schiedermaier P, et al. Endotoxin and tumor necrosis factor-receptor levels in portal and hepatic vein of patients with alcoholic liver cirrhosis receiving elective transjugular intrahepatic portosystemic shunt. *Eur J Gastroenterol Hepatol.* 2011; 23: 1218-1225.

135. Iida N, Dzutsev A, Stewart CA, Smith L, Bouladoux N, et al. Commensal bacteria control cancer response to therapy by modulating the tumor microenvironment. *Science.* 2013; 342: 967-970.

136. Schnabl B, Brenner DA. Interactions between the intestinal microbiome and liver diseases. *Gastroenterology.* 2014; 146: 1513-1524.

137. Claudel T, Staels B, Kuipers F. The Farnesoid X receptor: a molecular link between bile acid and lipid and glucose metabolism. *Arterioscler Thromb Vasc Biol.* 2005; 25: 2020-2030.

138. Guo C, Chen WD, Wang YD. TGR5, Not Only a Metabolic Regulator. *Front Physiol.* 2016; 7: 646-646.

139. Jia W, Xie G, Jia W. Bile acid-microbiota crosstalk in gastrointestinal inflammation and carcinogenesis. *Nat Rev Gastroenterol Hepatol.* 2018; 15: 111-128.

140. Chiang JYL, Ferrell JM. Bile Acid Metabolism in Liver Pathobiology. *Gene Expr.* 2018; 18: 71-87.

141. Jena PK, et al. Dysregulated bile acid receptor-mediated signaling and IL-17A induction are implicated in diet-associated hepatic health and cognitive function. *Biomarker Research.* 2020; 8: 59.

142. Forner A, Reig ME, de Lope CR, Bruix J. Current strategy for staging and treatment: the BCLC update and future prospects. *Semin Liver Dis.* 2010; 30: 61-74.

143. Tsoris A, Marlar CA. in *StatPearls* (StatPearls Publishing Copyright © 2021, StatPearls Publishing LLC., 2021).

144. Azam F, Latif MF, Farooq A, Tirmazy SH, AlShahrani S, et al. Performance Status Assessment by Using ECOG (Eastern Cooperative Oncology Group) Score for Cancer Patients by Oncology Healthcare Professionals. *Case Rep Oncol.* 2019; 12: 728-736.

145. Forner A, Reig M, Bruix J. Hepatocellular carcinoma. *Lancet.* 2018; 391: 1301-1314.

146. Bruix J, Sherman M. Management of hepatocellular carcinoma: an update. *Hepatology.* 2011; 53: 1020-1022.

147. EASL Clinical Practice Guidelines: Management of hepatocellular carcinoma. *J Hepatol.* 2018; 69: 182-236.

148. Golfieri R, Bargellini I, Spreafico C, Trevisani F. Patients with Barcelona Clinic Liver Cancer Stages B and C Hepatocellular Carcinoma: Time for a Subclassification. *Liver Cancer.* 2019; 8: 78-91.

149. Bolondi L, Burroughs A, Dufour JF, Galle PR, Mazzaferro V, et al. Heterogeneity of patients with intermediate (BCLC B) Hepatocellular Carcinoma: proposal for a subclassification to facilitate treatment decisions. *Semin Liver Dis.* 2012; 32: 348-359.

150. Jun CH, Yoon JH, Cho E, Shin SS, Cho SB, et al. Barcelona clinic liver cancer-stage C hepatocellular carcinoma: A novel approach to subclassification and treatment. *Medicine (Baltimore).* 2017; 96: e6745.

151. Golfieri R, Mosconi C, Cappelli A, Giampalma E, Galaverni MC, et al. Efficacy of radioembolization according to tumor morphology and portal vein thrombosis in intermediate-advanced hepatocellular carcinoma. *Future Oncol.* 2015; 11: 3133-3142.

152. Ke K, Chen G, Cai Z, Huang Y, Zhao B, et al. Evaluation and prediction of hepatocellular carcinoma prognosis based on molecular classification. *Cancer Manag Res.* 2018; 10: 5291-5302.

153. Villanueva A, Toffanin S, Llovet JM. Linking molecular classification of hepatocellular carcinoma and personalized medicine: preliminary steps. *Curr Opin Oncol.* 2008; 20: 444-453.

154. Bidkhori G, Benfeitas R, Klevstig M, Zhang C, Nielsen J, et al. Metabolic network-based stratification of hepatocellular carcinoma reveals three distinct tumor subtypes. *Proc Natl Acad Sci U S A.* 2018; 115: E11874-E11883.

155. Hoshida Y, Nijman SM, Kobayashi M, Chan JA, Brunet JP, et al. Integrative transcriptome analysis reveals common molecular subclasses of human hepatocellular carcinoma. *Cancer Res.* 2009; 69: 7385-7392.

156. Boyault S, Rickman DS, de Reyniès A, Balabaud C, Rebouissou S, et al. Transcriptome classification of HCC is related to gene alterations and to new therapeutic targets. *Hepatology.* 2007; 45: 42-52.

157. Chiang DY, Villanueva A, Hoshida Y, Peix J, Newell P, et al. Focal gains of VEGFA and molecular classification of hepatocellular carcinoma. *Cancer Res.* 2008; 68: 6779-6788.

158. Lee JS, Chu IS, Heo J, Calvisi DF, Sun Z, et al. Classification and prediction of survival in hepatocellular carcinoma by gene expression profiling. *Hepatology.* 2004; 40: 667-676.

159. Hoshida Y, Toffanin S, Lachenmayer A, Villanueva A, Minguez B, et al. Molecular classification and novel targets in hepatocellular carcinoma: recent advancements. *Semin Liver Dis.* 2010; 30: 35-51.

160. Zucman-Rossi J, Villanueva A, Nault JC, Llovet JM. Genetic Landscape and Biomarkers of Hepatocellular Carcinoma. *Gastroenterology.* 2015; 149: 1226-1239.e1224.

161. Llovet JM, Villanueva A, Lachenmayer A, Finn RS. Advances in targeted therapies for hepatocellular carcinoma in the genomic era. *Nat Rev Clin Oncol.* 2015; 12: 408-424.

162. Shimada S, Mogushi K, Akiyama Y, Furuyama T, Watanabe S, et al. Comprehensive molecular and immunological characterization of hepatocellular carcinoma. *EBioMedicine.* 2019; 40: 457-470.

163. Duan Q, Zhang H, Zheng J, Zhang L. Turning Cold into Hot: Firing up the Tumor Microenvironment. *Trends Cancer.* 2020; 6: 605-618.

164. Song PP, Xia JF, Inagaki Y, Hasegawa K, Sakamoto Y, et al. Controversies regarding and perspectives on clinical utility of biomarkers in hepatocellular carcinoma. *World J Gastroenterol.* 2016; 22: 262-274.

165. Reichl P, Mikulits W. Accuracy of novel diagnostic biomarkers for hepatocellular carcinoma: An update for clinicians (Review). *Oncol Rep.* 2016; 36: 613-625.

166. Zacharakis G, Aleid A, Aldossari KK. New and old biomarkers of hepatocellular carcinoma. *Hepatoma Research.* 2018; 4: 65.

167. Lou J, Zhang L, Lv S, Zhang C, Jiang S. Biomarkers for Hepatocellular Carcinoma. *Biomarkers in Cancer.* 2017; 9: 1179299X16684640.

168. Sterling RK, Jeffers L, Gordon F, Venook AP, Reddy KR, et al. Utility of Lens culinaris agglutinin-reactive fraction of alpha-fetoprotein and des-gamma-carboxy prothrombin, alone or in combination, as biomarkers for hepatocellular carcinoma. *Clin Gastroenterol*

Hepatol. 2009; 7: 104-113.

169. Naraki T, Kohno N, Saito H, Fujimoto Y, Ohhira M, et al. gamma-Carboxyglutamic acid content of hepatocellular carcinoma-associated des-gamma-carboxy prothrombin. *Biochim Biophys Acta.* 2002; 1586: 287-298.
170. Tangkijvanich P, Chanmee T, Komtong S, Mahachai V, Wisedopas N, et al. Diagnostic role of serum glypican-3 in differentiating hepatocellular carcinoma from non-malignant chronic liver disease and other liver cancers. *J Gastroenterol Hepatol.* 2010; 25: 129-137.
171. Furuhashi M, Ogura M, Matsumoto M, Yuda S, Muranaka A, et al. Serum FABP5 concentration is a potential biomarker for residual risk of atherosclerosis in relation to cholesterol efflux from macrophages. *Sci Rep.* 2017; 7: 217.
172. Wang S, Gao H, Zhang J, Ye X. [Stable isotope labeling and parallel reaction monitoring-based proteomic quantification for biomarker screening and validation of hepatocellular carcinoma]. *Se Pu.* 2017; 35: 934-940.
173. Lu JB, Cai SH, Pan YH, Yun JP. Altered epidermal fatty acid-binding protein expression in hepatocellular carcinoma predicts unfavorable outcomes. *Cancer Manag Res.* 2018; 10: 6275-6284.
174. Yanagita K, Nagashio R, Jiang SX, Kuchitsu Y, Hachimura K, et al. Cytoskeleton-Associated Protein 4 Is a Novel Serodiagnostic Marker for Lung Cancer. *Am J Pathol.* 2018; 188: 1328-1333.
175. Li SX, Liu LJ, Dong LW, Shi HG, Pan YF, et al. CKAP4 inhibited growth and metastasis of hepatocellular carcinoma through regulating EGFR signaling. *Tumour Biol.* 2014; 35: 7999-8005.
176. Wang Y, Yu W, He M, Huang Y, Wang M, et al. Serum cytoskeleton-associated protein 4 as a biomarker for the diagnosis of hepatocellular carcinoma. *OncoTargets and therapy.* 2018; 12: 359-364.
177. Abid MR, Yi X, Yano K, Shih SC, Aird WC. Vascular endocan is preferentially expressed in tumor endothelium. *Microvasc Res.* 2006; 72: 136-145.
178. Rennel E, Mellberg S, Dimberg A, Petersson L, Botling J, et al. Endocan is a VEGF-A and PI3K regulated gene with increased expression in human renal cancer. *Exp Cell Res.* 2007; 313: 1285-1294.
179. Youssef AA, Issa HA, Omar MZ, Behiry EG, Elfallah AA, et al. Serum human endothelial cell-specific molecule-1 (endocan) and vascular endothelial growth factor in cirrhotic HCV patients with hepatocellular carcinoma as predictors of mortality. *Clin Exp Gastroenterol.* 2018; 11: 431-438.
180. Malaguarnera G, Catania VE, Francaviglia A, Malaguarnera M, Drago F, et al. Lipoprotein(a) in patients with hepatocellular carcinoma and portal vein thrombosis. *Aging Clin Exp Res.* 2017; 29: 185-190.
181. Malaguarnera M, Latteri S, Bertino G, Madeddu R, Catania VE, et al. D-dimer plasmatic levels as a marker for diagnosis and prognosis of hepatocellular carcinoma patients with portal vein thrombosis. *Clin Exp Gastroenterol.* 2018; 11: 373-380.
182. Yu L, Liu X, Jiang Y, Wang X, Wang X, et al. Use of a Novel Thyroid-Stimulating Hormone Model for Predicting the Progression of Hepatocellular Carcinoma. *OncoTargets ther.* 2020; 13: 11421-11431.
183. Reddy A, Dash C, Leerapun A, Mettler TA, Stadheim LM, et al. Hypothyroidism: a possible risk factor for liver cancer in patients with no known underlying cause of liver disease. *Clin Gastroenterol Hepatol.* 2007; 5: 118-123.
184. Shih YL, Huang YH, Lin KH, Chu YD, Yeh CT. Identification of Functional Thyroid Stimulating Hormone Receptor and TSHR Gene Mutations in Hepatocellular Carcinoma. *Anticancer Res.* 2018; 38: 2793-2802.
185. DeBerardinis RJ, Chandel NS. We need to talk about the Warburg effect. *Nat Metab.* 2020; 2: 127-129.
186. Christofk HR, Vander Heiden MG, Harris MH, Ramanathan A, Gerszten RE, et al. The M2 splice isoform of pyruvate kinase is important for cancer metabolism and tumour growth. *Nature.* 2008; 452: 230-233.
187. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell.* 2011; 144: 646-674.
188. Huang Q, Tan Y, Yin P, Ye G, Gao P, et al. Metabolic characterization of hepatocellular carcinoma using nontargeted tissue metabolomics. *Cancer Res.* 2013; 73: 4992-5002.
189. Kim DJ, Cho EJ, Yu KS, Jang IJ, Yoon JH, et al. Comprehensive Metabolomic Search for Biomarkers to Differentiate Early Stage Hepatocellular Carcinoma from Cirrhosis. *Cancers (Basel).* 2019; 11: 1497.
190. Sun J, et al. Metabolomic Profiles for HBV Related Hepatocellular Carcinoma Including Alpha-Fetoproteins Positive and Negative Subtypes. *Front Oncol.* 2019; 9: 1069-1069.
191. Balaban S, Lee LS, Schreuder M, Hoy AJ. Obesity and cancer progression: is there a role of fatty acid metabolism? *Biomed Res Int.* 2015; 2015: 274585.
192. Yu G, Huang B, Chen G, Mi Y. Phosphatidylethanolamine-binding protein 4 promotes lung cancer cells proliferation and invasion via PI3K/Akt/mTOR axis. *J Thorac Dis.* 2015; 7: 1806-1816.
193. Zhang C, Chen X, Liu H, Li H, Jiang W, et al. Alpha fetoprotein mediates HBx induced carcinogenesis in the hepatocyte cytoplasm. *Int J Cancer.* 2015; 137: 1818-1829.
194. Nomair AM, Madkour MA, Shamseya MM, Elsheredy HG, Shokr A. Profiling of plasma metabolomics in patients with hepatitis C-related liver cirrhosis and hepatocellular carcinoma. *Clin Exp Hepatol.* 2019; 5: 317-326.
195. Syed GH, Amako Y, Siddiqui A. Hepatitis C virus hijacks host lipid metabolism. *Trends Endocrinol Metab.* 2010; 21: 33-40.
196. Currie E, Schulze ., Zechner R, Walther TC, Farese RV Jr. Cellular fatty acid metabolism and cancer. *Cell Metab.* 2013; 18: 153-161.
197. Ravi RK, Walton K, Khosroheidari M. MiSeq: A Next Generation Sequencing Platform for Genomic Analysis. *Methods Mol Biol.* 2018; 1706: 223-232.
198. Ren Z, et al. Gut microbiome analysis as a tool towards targeted non-invasive biomarkers for early hepatocellular carcinoma. *Gut.* 2019; 68: 1014-1023.
199. Yu J, Feng Q, Wong SH, Zhang D, Liang QY, et al. Metagenomic analysis of faecal microbiome as a tool towards targeted non-invasive biomarkers for colorectal cancer. *Gut.* 2017; 66: 70-78.
200. Lu J, Lee-Gabel L, Nadeau MC, Ferencz TM, Soefje SA. Clinical evaluation of compounds targeting PD-1/PD-L1 pathway for cancer immunotherapy. *J Oncol Pharm Pract.* 2015; 21: 451-467.
201. Yun S, Vincelette ND, Green MR, Wahner Hendrickson AE, Abraham I. Targeting immune checkpoints in unresectable metastatic cutaneous melanoma: a systematic review and meta-analysis of anti-CTLA-4 and anti-PD-1 agents trials. *Cancer Med.* 2016; 5: 1481-1491.
202. Duffy MJ, Crown J. Biomarkers for Predicting Response to Immunotherapy with Immune Checkpoint Inhibitors in Cancer Patients. *Clin Chem.* 2019; 65: 1228-1238.
203. Murciano-Goroff YR, Warner AB, Wolchok JD. The future of cancer immunotherapy: microenvironment-targeting combinations. *Cell Res.* 2020; 30: 507-519.
204. Mezquita L, Auclin E, Ferrara R, Charrier M, Remon J, et al. Association of the Lung Immune Prognostic Index With Immune

Checkpoint Inhibitor Outcomes in Patients With Advanced Non-Small Cell Lung Cancer. *JAMA Oncol.* 2018; 4: 351-357.

205. Meyers DE, Stukalin I, Vallerand IA, Lewinson RT, Suo A, et al. The Lung Immune Prognostic Index Discriminates Survival Outcomes in Patients with Solid Tumors Treated with Immune Checkpoint Inhibitors. *Cancers (Basel).* 2019; 11: 1713.
206. Chen S, Huang Z, Jia W, Tao H, Zhang S, et al. Association of the Pretreatment Lung Immune Prognostic Index with Survival Outcomes in Advanced Hepatocellular Carcinoma Patients Treated with PD-1 Inhibitors. *J Hepatocell Carcinoma.* 2020; 7: 289-299.
207. Macfarlane LA, Murphy PR. MicroRNA: Biogenesis, Function and Role in Cancer. *Curr Genomics.* 2010; 11: 537-561.
208. Bandiera S, Pfeffer S, Baumert TF, Zeisel MB. miR-122--a key factor and therapeutic target in liver disease. *J Hepatol.* 2015; 62: 448-457.
209. Tutar L, Tutar E, Tutar Y. MicroRNAs and cancer; an overview. *Curr Pharm Biotechnol.* 2014; 15: 430-437.
210. Calin GA, Sevignani C, Dumitru CD, Hyslop T, Noch E, et al. Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. *Proc Natl Acad Sci U S A.* 2004; 101: 2999-3004.
211. Pan JH, Zhou H, Zhao XX, Ding H, Li W, et al. Role of exosomes and exosomal microRNAs in hepatocellular carcinoma: Potential in diagnosis and antitumour treatments (Review). *Int J Mol Med.* 2018; 41: 1809-1816.
212. Janssen HL, Reesink HW, Lawitz EJ, Zeuzem S, Rodriguez-Torres M, et al. Treatment of HCV infection by targeting microRNA. *N Engl J Med.* 2013; 368: 1685-1694.
213. Varnholt H, Drebber U, Schulze F, Wedemeyer I, Schirmacher P, et al. MicroRNA gene expression profile of hepatitis C virus-associated hepatocellular carcinoma. *Hepatology.* 2008; 47: 1223-1232.
214. Janssen HL, Reesink HW, Lawitz EJ, Zeuzem S, Rodriguez-Torres M, et al. Treatment of HCV Infection by Targeting MicroRNA. *N Engl J Med.* 2013; 368: 1685-1694.
215. Chen Y, Shen A, Rider PJ, Yu Y, Wu K, et al. A liver-specific microRNA binds to a highly conserved RNA sequence of hepatitis B virus and negatively regulates viral gene expression and replication. *FASEB J.* 2011; 25: 4511-4521.
216. Wang S, Qiu L, Yan X, Jin W, Wang Y, et al. Loss of microRNA 122 expression in patients with hepatitis B enhances hepatitis B virus replication through cyclin G1-modulated P53 activity. *Hepatology.* 2012; 55: 730-741.
217. Li C, Wang Y, Wang S, Wu B, Hao J, et al. Hepatitis B virus mRNA-mediated miR-122 inhibition upregulates PTTG1-binding protein, which promotes hepatocellular carcinoma tumor growth and cell invasion. *J Virol.* 2013; 87: 2193-2205.
218. Hsu SH, Wang B, Kota J, Yu J, Costinean S, et al. Essential metabolic, anti-inflammatory, and anti-tumorigenic functions of miR-122 in liver. *J Clin Invest.* 2012; 122: 2871-2883.
219. Tsai WC, Hsu SD, Hsu CS, Lai TC, Chen SJ, et al. MicroRNA-122 plays a critical role in liver homeostasis and hepatocarcinogenesis. *J Clin Invest.* 2012; 122: 2884-2897.
220. Bai S, Nasser MW, Wang B, Hsu SH, Datta J, et al. MicroRNA-122 inhibits tumorigenic properties of hepatocellular carcinoma cells and sensitizes these cells to sorafenib. *J Biol Chem.* 2009; 284: 32015-32027.
221. Fornari F, Gramantieri L, Giovannini C, Veronese A, Ferracin M, et al. MiR-122/cyclin G1 interaction modulates p53 activity and affects doxorubicin sensitivity of human hepatocarcinoma cells. *Cancer Res.* 2009; 69: 5761-5767.
222. Zhou J, Yu L, Gao X, Hu J, Wang J, et al. Plasma microRNA panel to diagnose hepatitis B virus-related hepatocellular carcinoma. *J Clin Oncol.* 2011; 29: 4781-4788.
223. Ali HEA, Abdel Hameed R, Effat H, Ahmed EK, Atef AA, et al. Circulating microRNAs panel as a diagnostic tool for discrimination of HCV-associated hepatocellular carcinoma. *Clin Res Hepatol Gastroenterol.* 2017; 41: e51-e62.
224. Zhang Y, Li T, Qiu Y, Zhang T, Guo P, et al. Serum microRNA panel for early diagnosis of the onset of hepatocellular carcinoma. *Medicine (Baltimore).* 2017; 96: e5642.
225. Ji J, et al. A miRNA Combination as Promising Biomarker for Hepatocellular Carcinoma Diagnosis: A Study Based on Bioinformatics Analysis. *J Cancer.* 2018; 9: 3435-3446.
226. Ludwig AK, Giebel B. Exosomes: small vesicles participating in intercellular communication. *Int J Biochem Cell Biol.* 2012; 44: 11-15.
227. Sung S, Kim J, Jung Y. Liver-Derived Exosomes and Their Implications in Liver Pathobiology. *Int J Mol Sci.* 2018; 19: 3715.
228. Li Y, Xiang GM, Liu LL, Liu C, Liu F, et al. Assessment of endogenous reference gene suitability for serum exosomal microRNA expression analysis in liver carcinoma resection studies. *Mol Med Rep.* 2015; 12: 4683-4691.
229. Skog J, Würdinger T, van Rijn S, Meijer DH, Gainche L, et al. Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. *Nat Cell Biol.* 2008; 10: 1470-1476.
230. Qu Z, Wu J, Wu J, Luo D, Jiang C, et al. Exosomes derived from HCC cells induce sorafenib resistance in hepatocellular carcinoma both in vivo and in vitro. *J Exp Clin Cancer Res.* 2016; 35: 159.
231. Karimkhanloo H, Mohammadi-Yeganeh S, Ahsani Z, Paryan M. Bioinformatics prediction and experimental validation of microRNA-20a targeting Cyclin D1 in hepatocellular carcinoma. *Tumour Biol.* 2017; 39: 1010428317698361.
232. Zhang H, Li XX, Yang Y, Zhang Y, Wang HY, et al. Significance and mechanism of androgen receptor overexpression and androgen receptor/mechanistic target of rapamycin cross-talk in hepatocellular carcinoma. *Hepatology.* 2018; 67: 2271-2286.
233. Heldring N, Pike A, Andersson S, Matthews J, Cheng G, et al. Estrogen receptors: how do they signal and what are their targets. *Physiol Rev.* 2007; 87: 905-931.
234. Fan G, et al. DNA methylation biomarkers for hepatocellular carcinoma. *Cancer Cell Int.* 2018; 18: 140-140.
235. Nakamura K, Aizawa K, Nakabayashi K, Kato N, Yamauchi J, et al. DNA methyltransferase inhibitor zebularine inhibits human hepatic carcinoma cells proliferation and induces apoptosis. *PLoS One.* 2013; 8: e54036.
236. Tao SF, Zhang CS, Guo XL, Xu Y, Zhang SS, et al. Anti-tumor effect of 5-aza-2'-deoxycytidine by inhibiting telomerase activity in hepatocellular carcinoma cells. *World J Gastroenterol.* 2012; 18: 2334-2343.
237. Bialecki ES, Di Bisceglie AM. Diagnosis of hepatocellular carcinoma. *HPB (Oxford).* 2005; 7: 26-34.
238. Jane Lim AGS. Surveillance and Diagnosis of Hepatocellular Carcinoma. *Clin Liver Dis (Hoboken).* 2019; 13.
239. Tanaka H. Current role of ultrasound in the diagnosis of hepatocellular carcinoma. *J Med Ultrason (2001).* 2020; 47: 239-255.
240. Giorgio A, Research H. Contrast-enhanced ultrasound with sulphur-hexafluoride in diagnosis of early HCC in cirrhosis. 2019; 5.
241. Inchingolo R, De Gaetano AM, Curione D, Ciresa M, Miele L, et al. Role of diffusion-weighted imaging, apparent diffusion coefficient and correlation with hepatobiliary phase findings in the

differentiation of hepatocellular carcinoma from dysplastic nodules in cirrhotic liver. *Eur Radiol.* 2015; 25: 1087-1096.

242. Suardi N, et al. A Recent Short Review in Non-Invasive Magnetic Resonance Imaging on Assessment of HCC Stages: MRI Findings and Pathological Diagnosis. *Journal of Gastroenterology and Hepatology Research.* 2020; 9: 3113-3123.
243. Huang M, Liao B, Xu P, Cai H, Huang K, et al. Prediction of Microvascular Invasion in Hepatocellular Carcinoma: Preoperative Gd-EOB-DTPA-Dynamic Enhanced MRI and Histopathological Correlation. *Contrast Media Mol Imaging.* 2018; 2018: 9674565.
244. Chen BB, Hsu CY, Yu CW, Liang PC, Hsu C, et al. Early perfusion changes within 1 week of systemic treatment measured by dynamic contrast-enhanced MRI may predict survival in patients with advanced hepatocellular carcinoma. *Eur Radiol.* 2017; 27: 3069-3079.
245. ter Voert EG, Heijmen L, van Laarhoven HW, Heerschap A. In vivo magnetic resonance spectroscopy of liver tumors and metastases. *World J Gastroenterol.* 2011; 17: 5133-5149.
246. Teilhet C, Morvan D, Joubert-Zakeyh J, Biesse AS, Pereira B, et al. Specificities of Human Hepatocellular Carcinoma Developed on Non-Alcoholic Fatty Liver Disease in Absence of Cirrhosis Revealed by Tissue Extracts ^{1}H-NMR Spectroscopy. *Metabolites.* 2017; 7: 49.
247. Donswijk ML, Hess S, Mulders T, Lam MG. [18F]Fluorodeoxyglucose PET/Computed Tomography in Gastrointestinal Malignancies. *PET Clin.* 2014; 9: 421-441.
248. Kornberg A, Friess H. 18F-fludeoxyglucose positron emission tomography for diagnosis of HCC: implications for therapeutic strategy in curative and non-curative approaches. *Therap Adv Gastroenterol.* 2019; 12: 1756284819836205.
249. Yoh T, Seo S, Ogiso S, Kawai T, Okuda Y, et al. Proposal of a New Preoperative Prognostic Model for Solitary Hepatocellular Carcinoma Incorporating (18)F-FDG-PET Imaging with the ALBI Grade. *Ann Surg Oncol.* 2018; 25: 542-549.
250. Park JH, Kim DH, Kim SH, Kim MY, Baik SK, et al. The Clinical Implications of Liver Resection Margin Size in Patients with Hepatocellular Carcinoma in Terms of Positron Emission Tomography Positivity. *World J Surg.* 2018; 42: 1514-1522.
251. Rhee WJ, Hwang SH, Byun HK, Yun M, Han KH, et al. Risk stratification for locally advanced hepatocellular carcinoma using pretreatment alpha-foetoprotein and (18) F-fluoro-2-deoxyglucose positron emission tomography. *Liver Int.* 2017; 37: 592-599.
252. Pinker K, Shitano F, Sala E, Do RK, Young RJ, et al. Background, current role, and potential applications of radiogenomics. *J Magn Reson Imaging.* 2018; 47: 604-620.
253. Saini A, Breen I, Pershad Y, Naidu S, Knuttinen MG, et al. Radiogenomics and Radiomics in Liver Cancers. *Diagnostics (Basel).* 2019; 9: 4.
254. Kiryu S, Akai H, Nojima M, Hasegawa K, Shinkawa H, et al. Impact of hepatocellular carcinoma heterogeneity on computed tomography as a prognostic indicator. *Sci Rep.* 2017; 7: 12689.
255. Cicalese L. Hepatocellular Carcinoma (HCC) Treatment & Management. *MedScape.* 2020;
256. Raza A, Sood GK. Hepatocellular carcinoma review: current treatment, and evidence-based medicine. *World J Gastroenterol.* 2014; 20: 4115-4127.
257. Rodríguez-Perálvarez M, Luong TV, Andreana L, Meyer T, Dhillon AP, et al. A Systematic Review of Microvascular Invasion in Hepatocellular Carcinoma: Diagnostic and Prognostic Variability. *Ann Surg Oncol.* 2013; 20: 325-339.
258. Zhou J, Sun HC, Wang Z, Cong WM, Wang JH, et al. Guidelines for Diagnosis and Treatment of Primary Liver Cancer in China (2017 Edition). *Liver Cancer.* 2018; 7: 235-260.
259. Llovet JM, Real MI, Montaña X, Planas R, Coll S, et al. Arterial embolisation or chemoembolisation versus symptomatic treatment in patients with unresectable hepatocellular carcinoma: a randomised controlled trial. *Lancet.* 2002; 359: 1734-1739.
260. Philip MA, a PA. Treatment of Hepatocellular Cancer and the Emerging Role of Immunotherapy. *ASCO Daily News.* 2020;
261. Weitz J. Does intraoperative tumor cell dissemination matter? *J Am Coll Surg.* 2007; 205: S31-S33.
262. Hauch S, Zimmermann S, Lankiewicz S, Zieglschmid V, Böcher O, et al. The clinical significance of circulating tumour cells in breast cancer and colorectal cancer patients. *Anticancer Res.* 2007; 27: 1337-1341.
263. Schulze K, Nault JC, Villanueva A. Genetic profiling of hepatocellular carcinoma using next-generation sequencing. *J Hepatol.* 2016; 65: 1031-1042.
264. Zehir A, Benayed R, Shah RH, Syed A, Middha S, et al. Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients. *Nat Med.* 2017; 23: 703-713.
265. Fang ZT, Zhang W, Wang GZ, Zhou B, Yang GW, et al. Circulating tumor cells in the central and peripheral venous compartment - assessing hematogenous dissemination after transarterial chemoembolization of hepatocellular carcinoma. *Onco Targets Ther.* 2014; 7: 1311-1318.
266. Deng G, Yao L, Zeng F, Xiao L, Wang Z. Nomogram For Preoperative Prediction Of Microvascular Invasion Risk In Hepatocellular Carcinoma. *Cancer Manag Res.* 2019; 11: 9037-9045.
267. Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, et al. Sorafenib in Advanced Hepatocellular Carcinoma. *N Engl J Med.* 2008; 359: 378-390.
268. Stenger M. Systemic Therapy in the First- and Second-Line Treatment of Advanced Hepatocellular Carcinoma: Review and Meta-analysis. *The ASCO Post.* 2020;
269. De Luca E, Marino D, Di Maio M. Ramucirumab, A Second-Line Option For Patients With Hepatocellular Carcinoma: A Review Of The Evidence. *Cancer Manag Res.* 2020; 12: 3721-3729.
270. Huppert LA, Gordan JD, Kelley RK. Checkpoint Inhibitors for the Treatment of Advanced Hepatocellular Carcinoma. *Clin Liver Dis (Hoboken).* 2020; 15: 53-58.
271. Finn RS, Qin S, Ikeda M, Galle PR, Ducreux M, et al. Atezolizumab plus Bevacizumab in Unresectable Hepatocellular Carcinoma. *N Engl J Med.* 2020; 382: 1894-1905.
272. Voron T, Colussi O, Marcheteau E, Pernot S, Nizard M, et al. VEGF-A modulates expression of inhibitory checkpoints on CD8+ T cells in tumors. *J Exp Med.* 2015; 212: 139-148.
273. Fukumura D, Kloepper J, Amoozgar Z, Duda DG, Jain RK. Enhancing cancer immunotherapy using antiangiogenics: opportunities and challenges. *Nature Reviews Clinical Oncology.* 2018; 15: 325-340.
274. Yau T, Kang YK, Kim TY, El-Khoueiry AB, Santoro A, et al. Efficacy and Safety of Nivolumab Plus Ipilimumab in Patients With Advanced Hepatocellular Carcinoma Previously Treated With Sorafenib: The CheckMate 040 Randomized Clinical Trial. *JAMA Oncol.* 2020; 6: e204564.
275. Zheng Y, Wang T, Tu X, Huang Y, Zhang H, et al. Gut microbiome affects the response to anti-PD-1 immunotherapy in patients with hepatocellular carcinoma. *J Immunother Cancer.* 2019; 7: 193.
276. Shao YY, Liu TH, Hsu C, Lu LC, Shen YC, et al. Early alpha-foetoprotein response associated with treatment efficacy of immune checkpoint inhibitors for advanced hepatocellular carcinoma. *Liver Int.* 2019; 39: 2184-2189.

277. Sangro B, Melero I, Wadhawan S, Finn RS, Abou-Alfa GK, et al. Association of inflammatory biomarkers with clinical outcomes in nivolumab-treated patients with advanced hepatocellular carcinoma. *J Hepatol.* 2020; 73: 1460-1469.

278. Ni JY, Sun HL, Luo JH, Jiang XY, Chen D, et al. Transarterial Chemoembolization and Sorafenib Combined with Microwave Ablation for Advanced Primary Hepatocellular Carcinoma: A Preliminary Investigation of Safety and Efficacy. *Cancer Manag Res.* 2019; 11: 9939-9950.

279. Kaseb AO, Pestana RC, Vence LM, Blando JM, Singh S, et al. Randomized, open-label, perioperative phase II study evaluating nivolumab alone versus nivolumab plus ipilimumab in patients with resectable HCC. *Journal of Clinical Oncology.* 2019; 37: 185-185.

280. Kelley RK, Cheng AL, Braiteh FS, Park JW, Benzaghou F, et al. Phase 3 (COSMIC-312) study of cabozantinib (C) in combination with atezolizumab (A) versus sorafenib (S) in patients (pts) with advanced hepatocellular carcinoma (aHCC) who have not received previous systemic anticancer therapy. *Journal of Clinical Oncology.* 2019; 37: TPS4157-TPS4157.

281. Gordan JD, Kennedy EB, Abou-Alfa GK, Beg MS, Brower ST, et al. Systemic Therapy for Advanced Hepatocellular Carcinoma: ASCO Guideline. *J Clin Oncol.* 2020; 38: 4317-4345.

282. Kalbasi A, June CH, Haas N, Vapiwala,N. Radiation and immunotherapy: a synergistic combination. *J Clin Invest.* 2013; 123: 2756-2763.

283. 283 Park B, Yee C, Lee KM. The effect of radiation on the immune response to cancers. *Int J Mol Sci.* 2014; 15: 927-943.

284. Jordan AC, Wu J. Immunotherapy in hepatocellular carcinoma: Combination strategies. *World Journal of Meta-Analysis.* 2020; 8: 190-209.

285. Garin E, et al. Major impact of personalized dosimetry using 90Y loaded glass microspheres SIRT in HCC: Final overall survival analysis of a multicenter randomized phase II study (DOSISPHERE-01). *Journal of Clinical Oncology.* 2020; 38: 516-516.

***Correspondence:** Michael J. Bouchard, PhD, Director, Division of Biomedical Science Programs, Graduate School of Biomedical Sciences and Professional Studies, College of Medicine, Drexel University, Mail Stop 497, New College Building, Room 11301, 245 N. 15th Street, Philadelphia, PA 19102, USA, Tel: 267.359.2328, E-mail: mjb93@drexel.edu

Rec: 24 Sep 2021; **Acc**: 21 Oct 2021; **Pub**: 24 Oct 2021

Front Mol Med Therap. 2021;1(1):104

DOI: 10.36879/FMMT.21.000104