Hepatocellular carcinoma

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Abstract

Hepatocellular carcinoma (HCC) represents approximately 90% of all cases of primary liver cancer, which is the second leading cause of cancer related deaths globally and has an incidence of 850,000 new cases per year. The main risk factors for developing HCC are well-known and include infection with hepatitis B and C viruses, alcohol intake and ingestion of the fungal metabolite aflatoxin B1. Nonetheless, knowledge is emerging regarding additional risk factors such as non-alcoholic steatohepatitis. Advances in the understanding of the molecular pathogenesis of HCC led to identification of critical driver mutations, however the most prevalent of these are not yet druggable targets. The molecular classification of HCC is not established, and the Barcelona-Clinic-Liver Cancer Classification is the main clinical algorithm for the stratification of patients according to prognosis and treatment allocation. Surveillance programmes enable detection of early-stage tumours that are amenable to curative therapies — resection, liver transplantation or local ablation. At more-developed stages, only chemoembolization (for intermediate HCC) and sorafenib (for advanced HCC) have shown survival benefits. There are major unmet needs in HCC management that might be addressed through discovery of new therapies and their combinations for use in the adjuvant setting and for intermediate and advanced stage disease, biomarkers for therapy stratification, patient-tailored strategies targeting driver mutations and/or activating signalling cascades and validated measurements of quality of life. Recent failures in testing systemic drugs for intermediate and advanced stages have pointed towards a refinement in trial design and defining novel approaches.

[H1] Introduction

Liver cancer is a major health problem, with more than 850,000 cases annually worldwide[1]. This neoplasm is currently the second leading cause of cancer-related death globally, a figure that is on the rise[2]. Among all primary liver cancers, hepatocellular carcinoma (HCC) is the most common neoplasm, accounting for 90% of cases[1,3–12]. Various risk factors for HCC development are well-defined, such cirrhosis (chronic liver damage caused by fibrosis), hepatitis B virus (HBV) infection, hepatitis C virus (HCV) infection, alcohol abuse and metabolic syndrome[3]. Other co-factors, such as tobacco inhalation and aflatoxin B1 (a fungal carcinogen present in food supplies associated with mutations in the tumour suppressor gene *TP53*) intake are well-characterized contributors to HCC (**Figure 1**)[1,3–10]. Recent discoveries have pointed to infection with adeno-associated virus 2 (AAV2) as a novel cause of the disease, particularly in individuals without cirrhosis[13]. Primary prevention of HCC through HBV vaccination has been demonstrated. Similarly, in patients with chronic infection, effective anti-viral therapies against HBV and HCV that produce sustained virological responses are associated with a profound decrease in HCC incidence. Guidelines recommend surveillance by using ultrasonography every 6 months in at risk-populations.

Over the past decade, there has been an improvement in the understanding of the molecular pathogenesis of the disease[14]. Genomic analysis has provided a clear picture of the main drivers responsible for tumour initiation and progression. Each HCC has an average of 40 genomic aberrations among which few are considered drivers. Common mutations affect telomere maintenance (mutations in telomere reverse transcriptase (*TERT*)), Wnt pathway activation (mutations in catenin beta 1 (*CTNNB1*)), inactivation of cellular tumour antigen p53 (p53, encoded by *TP53*), chromatin remodelling (mutations in AT-rich interaction domain 1A *(ARID1A*)), Ras signalling, mammalian target of rapamycin (mTOR) signalling and oxidative stress pathway activation. Only a handful of these drivers are currently druggable targets, such as amplification of fibroblast growth factor 19 (*FGF19*).

Classification of HCC is not based on the TNM system, as opposed to the majority of neoplasms, but on the Barcelona-Clinic-Liver Cancer (BCLC) Classification, which is endorsed by European and American clinical practice guidelines[3,15,16]. This staging system defines five prognostic subclasses and allocates specific treatments for each stage. Five treatments can extend the life expectancy of patients with HCC: surgical resection, liver transplantation, radiofrequency ablation, chemoembolization and the multikinase inhibitor sorafenib[3]. Around 40% of patients (early stages 0 and A) are eligible for potentially curative therapies — resection, transplantation or local ablation — which can provide median survival rates of 60 months and beyond, in contrast with an historical natural history survival of 36 months. For patients with more-advanced disease, only two treatments have demonstrated survival advantages in the setting of randomized-controlled trials (RCTs). Patients at intermediate stage (stage B) with preserved liver function benefit from chemoembolization[17,18] and have an estimated median survival of 26 months. Patients at advanced stage (stage C) benefit from systemic sorafenib, which extends survival by approximately 3 months (from 8 to 11 months) and represents the standard of care in this setting[19]. During the past years, several studies have tested therapies in the adjuvant setting, in combination or alternatives to chemoembolization, and alternative systemic first-line and second-line treatments.

This Primer provides an overview and up-dated summary of the current knowledge on the epidemiology, pathogenesis and treatment of HCC. We describe current prevention, evidence-based standards of care and novel therapies emerging in light of the understanding of the pathogenesis of HCC, such as proof-of-concept studies and immunotherapy.

[H1] Epidemiology

Worldwide, liver cancer is the sixth most common cancer (approximately 850,000 new cases each year), and the second leading cause of cancer-related deaths (approximately 800,000 per year) (**Figure 1**)[1,3–10]. 85-90% of all primary liver cancers are HCC. Unlike other human

malignancies, the risk factors for HCC are well established (**Table 1**). Indeed, HCC is common in patients with advanced hepatic fibrosis or cirrhosis due to chronic liver disease, and in particular with liver damage caused by HBV and HCV infection and unhealthy alcohol use.

The worldwide incidence of HCC and chronic viral hepatitis parallel each other. HBV is a DNA virus that can cause insertional mutagenesis. In the context of chronic HBV infection, HCC usually occurs on a background of cirrhosis (up to 85% in selected studies)[20]. Moreover, in patients with chronic HCV infection, which is caused by an RNA virus, HCC rarely occurs in the absence of advanced hepatic fibrosis or cirrhosis[21]. The highest incidence rates of HCC are in Asia and sub-Saharan Africa owing to the high prevalence of HBV infection (**Figure 2**)[1]. In Africa, aflatoxin B1 appears to be synergistic with HBV in causing HCC[22]. This synergy is a likely explanation for the earlier onset of HCC in this continent compared with the rest of the world. In North America, Japan and Europe, HCV is the leading cause of HCC. Other causes of cirrhosis associated with HCC include unhealthy alcohol use, nonalcoholic steatohepatitis (NASH), alpha-1 antitrypsin deficiency and hemochromatosis[23]. By contrast, HCC is less common in cirrhosis that is caused by autoimmune hepatitis, Wilson's disease and cholestatic liver disorders.

Along with viral hepatitis and cirrhosis, other factors can contribute to disease risk. HCC has a strong gender predilection, being threefold more common in men than women[24] (**Figure 2**)[1]**.** Most patients with HCC are over 45 years of age, except in sub-Saharan Africa, given the latency between the onset of virus-mediated liver inflammation and the development of cirrhosis. Unidentified host, viral and environmental interactions are likely responsible for the lower age of onset in Africa. The association between tobacco use and HCC, even in the presence of HBV or HCV, has been inconsistent. An emerging cause of HCC is the metabolic syndrome due to diabetes and obesity, and the associated liver disease nonalcoholic fatty liver disease (NAFLD) and NASH[25]. NAFLD or NASH might be — along with chronic HBV infection — the exception to the rule that HCC is always associated with advanced hepatic fibrosis or cirrhosis. A recent study that needs careful verification suggests that approximately 40% of patients with HCC and NAFLD or NASH might not have cirrhosis[25].

As discussed in greater detail below, HCC is one of the cancers for which prevention is possible. HBV vaccination has been shown to reduce the incidence of HCC in populations with a high prevalence of HBV[26]. Antiviral therapy for HBV with nucleotide and nucleoside analogues reduces, but does not eliminate, the risk of HCC in treated cohorts of patients[27]. Likewise, anti-viral therapy with interferon might reduce the risk of HCC in patients with HCV infection that is associated chronic liver disease[28]; however, this effect will need to be re-assessed with non-interferon based direct acting antiviral drugs that are now approved for practice. Finally, statin use and coffee consumption are associated with a reduced risk of

HCC in population studies[29]. In the future, potent liver directed antifibrotic therapies might also reduce the risk of developing HCC.

[H1] Mechanisms/pathophysiology

[H2]Molecular alterations and drivers

[H3] Early molecular alterations during hepatocarcinogenesis.

HCC development is a complex multistep process that usually occurs in the context of liver cirrhosis and is related to the diversity of aetiologies of the underlying liver disease. The natural history of HCC in cirrhosis follows a sequence of events starting with the successive development of pre-cancerous cirrhotic nodules with low grade dysplasia, called low-grade dysplastic nodules (LGDN). This is followed by high-grade dysplastic nodules (HGDN) that can transform into early-stage HCC (stages 0 and A) and progress into more advanced HCC (stages B and C). Malignant transformation into HCC can originate from various cell types including mature hepatocyte and stem or progenitor cells[30].

Similar to other epithelial solid tumours, HCC is the result of the accumulation of somatic genomic alterations in passenger and driver cancer genes. As previously defined[31], a cancer driver would be a cell-autonomous or non–cell-autonomous alteration that contributes to tumour evolution at any stage — including initiation, progression, metastasis and resistance to therapy — by promoting a variety of functions including proliferation, survival, invasion or immune evasion. In each HCC nodule, a mean number of 40 functional somatic alterations are accumulated in coding regions and consequently each tumour is the result of a unique combination of genetic alterations mixed together with epigenetic modifications[32,33]. This general observation underlines the complexity of hepatocarcinogenesis and the huge diversity of HCC (**Table 2; Figure 3**)[33–38]**.** However, the fact that genomic alterations are not accumulated randomly suggests that several pathways can cooperate to promote oncogenesis and that some of them can be related to specific risk factors[33].

Molecular markers identified to discriminate early-stage HCC from the pre-cancerous nodules (LGDN and HGDN) have provided some clues about the early steps of carcinogenesis that occurs in the context of cirrhosis[39–43]. On the basis of these markers, the mechanism of hepatocyte malignant transformation has been shown to include Wnt/β-catenin pathway activation, re-expression of fetal genes, deregulation of protein folding machinery and the response to oxidative stress. Moreover, several lines of evidence have shown that telomere maintenance and the telomerase complex that controls the nucleotide TTAGGG, a repeated sequence at the end of chromosomes, play a major part in initiation and promotion of HCC in cirrhosis[44,45]. First, mice deficient of telomerase RNA component (*Terc*), which codes for

the catalytic unit of the telomerase complex, showed short telomeres and developed cirrhosis followed by HCC[46]. Second, constitutive inactivating mutations in *TERT*, which encodes the telomerase reverse transcriptase, are associated with an increased risk of cirrhosis in humans[47–49]. However, progression to HCC involves a second step, with telomerase reactivation required to promote liver carcinogenesis and to allow uncontrolled hepatocyte proliferation (the 'telomerase switch')[46,50]. In humans, *TERT* is not expressed in normal hepatocytes, but becomes re-expressed early during hepatocarcinogenesis in LGDN and particularly HGDN[42,51]. In these lesions, telomerase re-expression is related to the occurrence of point mutations at two hotspots in the *TERT* promoter. These alterations are the most frequent recurrent somatic mutations identified in LGDN (6%), HGDN (20 %) and HCC (60%)(**Figure 3**)[33–38]. Together, these results suggest that *TERT* promoter mutations are oncogenic and, in most cases of HCC (>90%), telomerase activation is selected during malignant transformation and tumour progression in cirrhotic and non-cirrhotic livers[34,37]. Within this paradigm, *TERT* promoter activation is required at an early step of transformation in order to bypass the replicative senescence of cirrhotic hepatocytes. By contrast, acquisition of genomic diversity appears to be a late event in liver carcinogenesis[14].

Viral infections by HCV and HBV are also associated with early molecular alterations involved in malignant transformation through the induction of chronic inflammation, the expression of viral proteins and the viral life cycle[52,53] (**Figure 3**)[33–38]. Specific molecular alterations are frequently related to HBV infection that can induce mutagenesis by insertion of viral DNA into major driver genes of hepatocarcinogenesis[54]. The most frequent insertions of HBV DNA in hepatocytes occur within the *TERT* promoter and activate telomerase and other oncogenes including lysine (K)-specific methyltransferase 2B (*KMT2B*, also called *MLL4*), cyclin E1 (*CCNE1*) and SUMO1/sentrin specific peptidase 5 (*SENP5*)[55]. In cooperation with HBV infection, exposure to aflatoxin B1 , which is common in subtropical regions, induces DNA adducts and the occurrence of frequent mutations, in particular within *TP53*, that have a specific nucleotide signature[22,33]. Recently, deep sequencing analysis revealed another DNA virus — AAV2 — that is related to a frequent harmless infection in the general population and causes insertional mutagenesis in rare cases of HCC[13]. AAV2 insertions were mainly identified in HCC that had developed in normal liver tissues, without other classical risk factors. Viral insertions are found within normal and cancerous cells, and in the tumour genome normally occur within typical oncogenes such as *TERT*, cyclin A2 (*CCNA2*), *CCNE1*, tumour necrosis factor superfamily member 10 (*TNFSF10*) and *KMT2B*.

[H3] Cancer drivers in progressed HCC.

Several pathways and processes have been implicated in HCC progression (**Table 2**). First, telomere maintenance contributes to the evasion of cellular senescence. As previously mentioned, telomerase is overexpressed in 90% of HCC and this is overexpression is related to *TERT* promoter mutations in 60% of cases and to gene amplification in 5% of the cases[33,34,38]. The two hotspots of mutations are located at nucleotide positions -124 and -146 upstream of the ATG. Mutations at both sites can create a new binding site recognized by a transcription factor inducing *TERT* mRNA expression.

Second, the Wnt/β-catenin pathway is frequently activated in HCC through *CTNNB1* mutations that activate β-catenin (11-37% HCC cases), particularly in patients without HBV infection and well-differentiated tumours[56,57]. Inactivating mutations or deletions are also frequently identified in axin 1 (*AXIN1,* 10% of HCC cases) or more rarely in adenomatous polyposis coli (*APC*, 1-2% of HCCs) and zinc and ring finger 3 (*ZNRF3*, 3% of HCCs). All of these mutations result in activation of the Wnt/β-catenin pathway[33,38].

Third, inactivation of p53 and alterations of cell cycle are major defects in HCC, particularly in cases related to HBV infection. In this context, *TP53* mutations are the most frequent alterations with a specific hotspot of mutation (R249S) in patients with aflatoxin B1 exposure[22,33,38,58]. Inactivation of the retinoblastoma pathway is also frequently observed through retinoblastoma 1 (*RB1*) mutations (3-8% of HCCs) or cyclin-dependent kinase inhibitor 2A deletions (*CDKN2A*, 2-12% of HCCs). Most of these molecular defects are associated with poor prognosis and could contribute to a more-aggressive phenotype[33,35,59].

Fourth, chromatin remodelling complexes and epigenetic regulators are frequently altered in HCC. These alterations include mutations in the BRG1- or HRBM-associated factors (BAF) and polybromo-associated BAF (PBAF) chromatin complex (*ARID1A* mutations in 4-17% of cases and *ARID2* mutation in 3-18% of cases) or in the histone methylation writer family (*KMT2* – *MLL* genes mutated in 2-4% of cases), which can also be modified by HBV insertions in *KMT2B* (*MLL4*) (10% of cases)[33,38,55]. Recently, H3K9 modifier histone-lysine N-methyltransferase SETDB1 was identified as over-expressed in HCC[60,61]. SETDB1 over-expression promotes cancer cell growth via p53 methylation and is associated with tumour aggressiveness and poor prognosis. Interestingly, DNA methylation is globally altered in HCC and aberrant modifications are associated with prognosis[62] or HBV infection[63].

Fifth, the Ras/Raf/MAP and the PI3K/AKT-mTOR pathways are frequently activated in HCC. These changes are caused by amplification a region that includes fibroblast growth factor 3 (*FGF3*), *FGF4* and *FGF19* in approximately 5% of tumours, and can also be related to inactivating mutations in tuberous sclerosis 1 (*TSC1*) or *TSC2* (3-8% of cases), or in phosphatase and tensin homolog (*PTEN*) (1-3% of cases). Ribosomal protein S6 kinase, 90kDa, polypeptide 3 (*RPS6KA3*) mutations that cause inactivation of ribosomal protein S6

kinase alpha-3 (also called RSK2) (5-9% cases) lead to an activation of ras/MAPK signalling[32,33], whereas mutations that activate Ras proteins themselves (KRAS, HRAS, NRAS of BRAF) are rarely identified (<1% of cases). However, additional mechanisms of pathway activation remain to be identified.

Finally, the oxidative stress pathway is constitutively activated in HCC owing to mutations that activate nuclear factor erythroid 2-related factor 2 (*NFE2L2*) or that inactivate kelch-like ECH-associated protein 1 (*KEAP1*) in 5-15% of HCC cases. Interestingly, these observations suggest that *NFE2L2* can protect from HCC occurrence during the development of chronic liver disease but its constitutive activation can also contribute to tumour progression at a late stage of tumour progression[32,64].

DNA amplifications are also associated with HCC. The most common high-level amplifications in HCC are in chromosome regions 11q13 and 6p21 (5–10% of cases)[32,35,65,66]. Cyclin D1 (*CCND1*) and *FGF19* are bona fide oncogenes in 11q13 and represent potential therapeutic targets[66]. Similarly, high-level gains of 6p21 that contain more than four copies of vascular endothelial growth factor A (*VEGFA*) have been identified in 4–8% of HCC cases[65]. *VEGFA* amplification induces both neoangiogenesis and tumour proliferation resulting from the induction of macrophage-mediated hepatocyte growth factor secretion[67]. Whether these amplifications represent targetable oncogenic addiction loops in HCC remains to be elucidated by the testing of selective molecules in clinical trials.

Modelling of HCC development in mice is a very useful approach for achieving better understanding of the mechanisms of hepatocarcinogenesis and for testing for new therapies. For drug screening, HCC cell lines or primary tumours xenografted in immuno-deficient mice are easy to test for drug response. In more sophisticated models, candidate oncogenes or tumour suppressor genes can be genetically modified in classical transgenic or knockout animal models. More recently, siRNA[68] and CRISPR/Cas9[69] techniques have emerged as powerful methods to generate tumours in mice and to subsequently test for drug response.

[H2] Molecular classes

Genomic studies have revealed molecular subclasses of HCC (reviewed in[14])[65,70–73]. Two main molecular classes, each representing approximately 50% of patients, have been identified: proliferative and non-proliferative HCC[65,71,73]. The proliferative subclass is enriched by activation of Ras, mTOR, and insulin-like growth factor (IGF) signalling and *FGF19* amplification, and is associated with HBV-related aetiologies and poor outcomes[65,71,72,74]. Some authors have proposed that there are two subtypes of the proliferative class: the Wnt/transforming growth factor β (TGF-β) group and the progenitor-cell group. The

progenitor cell group is enriched in progenitor cell markers, such as epithelial cell adhesion molecule (EpCAM), and the overexpression of α-fetoprotein[65,71,72,74,75]. By contrast, the non-proliferative subclass is more heterogeneous, but there is still a clear subtype characterized by *CTNNB1* mutations that are associated with alcohol-related and HCV-related HCCs[75]. Direct translation of molecular HCC subclasses into clinical management is yet to be achieved.

[H2] Role of the microenvironment

[H3] Chronic inflammation.

An altered microenvironment is now perceived to be a key enabling characteristic of cancer and is known to participate in all stages of malignant progression, from the initial transformation phases, through invasion and all the way to metastasis[76,77]. Pathologists have long recognized that some tumours are densely infiltrated by cells of both the innate and adaptive arms of the immune system and thereby mirror inflammatory conditions arising in non-neoplastic tissues[76,78]. HCC is a prototypical inflammation associated cancer, with approximately 90% of HCC burden associated with prolonged hepatitis due to viral hepatitis, excessive alcohol intake or NAFLD-NASH. This indicates that the immune microenvironment plays pivotal parts in the pathogenesis of this disease[79]. Interestingly, in fully developed HCCs, the presence of immune infiltrates is associated with a better prognosis, likely owing to more effective anti- tumour immunity[80]. Remarkably, an unbiased screen of gene expression patterns in HCC revealed that activation of the key innate inflammatory signalling mediators nuclear factor of κ light polypeptide gene enhancer in B-cells (NF-κB), epidermal growth factor (EGF) and interleukin-6 (IL6) in the liver parenchyma, but not in tumour cells, is associated with poor prognosis[81]. Furthermore, as noted above, genomic screens of HCC also did not reveal mutations that activate inflammatory signalling pathways. Thus, it seems that the inflamed liver promotes the seminal HCC cells at early phases of development[82].

Multiple cell types interact with hepatocytes in the chronically inflamed liver. These include among others macrophages, stellate cells, endothelial cells and lymphocytes; all of which were shown in certain mouse models to favour tumour growth. Importantly, although the liver is envisaged as a metabolic organ, it maintains a uniquely tolerant immune system, which his necessary to prevent the induction of immunity against multiple antigens and immunostimulatory molecules, such as gut-derived nutrients and microbiota derived signals which constantly flood the liver via the portal system. Understanding this unique hepatic immune system is likely important in the context of the complex interaction between malignant hepatocytes and the liver immune system[83,84].

The mechanisms through which immune cells promote growth of early-stage HCC are beginning to be elucidated. Multiple experimental models have substantiated that secretion of various cytokines by immune cells can change function of the interacting hepatocyte, rendering it less sensitive to intracellular tumour suppressor pathways (and possibly also to extracellular ones such as anti-tumour adaptive immune responses). An example is secretion of TNF by macrophages in the chronic inflammatory hepatic infiltrate, which activates the NF-κB pathway in hepatocytes, rendering the latter less sensitive to apoptosis and thus promoting carcinogenesis[85].Two important inflammatory signalling pathways that are activated by inflammatory cytokines in chronically inflamed livers and promote HCC are the NF-κB and JAK-STAT pathways[86]. Similarly, multiple molecules that are secreted by microenvironmental constituents have been shown to promote hepatocyte growth; these include TNF, lymphotoxin-α, lymphotoxin-β[86], IL-6[87] and hepatocyte growth factor (HGF)[88]. The ability of inflammatory cells to produce potentially mutagenic reactive oxygen species (ROS) and reactive nitrogen species (RNS) is considered by many to underlie some of their pro- tumourigenic activity[89]; yet this must still be substantiated by rigorous testing in animal models. However, it is clear that the increased hepatocyte proliferation that occurs in chronic hepatitis can potentiate DNA damage induced mutations[90].

[H3] Fibrosis.

Hepatic stellate cells normally reside in the liver sinusoids and have multiple roles in hepatic homeostasis including retinoid storage, immunomodulation, liver regeneration and vasoregulation. Importantly, upon liver injury they are the primary cellular mediators of hepatic fibrosis and cirrhosis, which are strongly associated with HCC[79]. The presence of a stellate cell gene expression signature is a poor prognosis indicator in human HCC[91] and overexpression of platelet-derived growth factor C (PDGFC) in the mouse liver induces stellate cell activation and hepatic fibrosis followed by HCC[92]**.** Taken together, these data indicate that hepatic fibrosis and cirrhosis have a causative role in HCC. The mechanisms through which activated stellate cells could drive HCC progression include cytokine secretion, angiogenesis promotion, protumorigenic extracellular matrix components, increased tissue stiffness and immunosuppression; yet the human relevance of each of these mechanisms and its relative contribution remain to be elucidated[93]. In summary, chronic liver inflammation generates a pathologic microenvironment, infiltrated by adaptive and innate immune cells and stellate cells, together producing a pathological milieu composed of collagen, multiple extracellular matrix proteins, growth factors and cytokines which can form a protumorigenic stroma.

[H3] Targeting the microenvironment in HCC.

Because the tumour microenvironment plays a pivotal part in the natural history of HCC, there is a strong rationale for modulating the dynamic cross talk between hepatocytes and the stroma as treatment for this disease[79], in particular in HCC prevention. Reversing liver fibrosis is already feasible in patients with chronic liver disease using antifibrotic therapies[94]. Conceivably, targeting various immune cells is also achievable, as is the targeting of specific molecules which are present in the pro tumourigenic hepatic microenvironment. Despite these possibilities, improved knowledge of the relative contribution of the different cells and molecules composing the chronically inflamed hepatic network to HCC pathogenesis, and elucidation of the network's hierarchy, is needed for planning potent preventive or therapeutic schemes. Such developments will entail better understanding of the complex natures of the interactions that take place in chronically inflamed human livers, in concert with meticulous testing of their functional relevance in animal models recapitulating the different aetiologies of hepatic inflammation (viral, metabolic and other). The ideal preventive treatment should convert a pro-tumourigenic microenvironment into an anti-tumourigenic one.

[H1] Diagnosis, screening and prevention

[H2] Prevention

[H3] Vaccination.

The most effective approach for preventing HCC is prevention of the underlying liver disease, the best example of which is hepatitis B vaccination. Universal vaccination against hepatitis B would be expected to reduce HCC incidence, and this has indeed been demonstrated[26,95]. The first evidence of the ability of the HBV vaccine to reduce the incidence of HCC came from Taiwan after the introduction of universal neonatal vaccination in 1984. This programme was associated with a reduction in the incidence of childhood HCC from 0.7 per 100,000 individuals to 0.36 per 100,000 individuals between 1981 and 1994 ($P<0.01$)[95]. As the vaccinated cohort aged, the decrease in HCC incidence was carried through to adolescents and is now being detected in young adults[26].

However, there are currently approximately 400 million adults who are infected with hepatitis B. Not all of those with hepatitis B are at equal risk of developing HCC. Various risk factors are well known, including hepatitis B viral load, male sex, older age and active liver disease[96–103]. For those who have active disease and who receive treatment for hepatitis B, the risk of HCC is reduced, although not eliminated[104]. This has been demonstrated in a single RCT using the antiviral medication lamivudine[105], a study that unfortunately did not have HCC incidence as an endpoint. However, similar results have now been obtained in cohort studies using various methods to match treated and untreated populations, including historical controls[106].

Similarly, treatment and eradication of hepatitis C results in a decreased HCC incidence[21]. This has only been documented in cohort studies. There are no RCTs comparing HCC incidence in treated patients compared with untreated patients. Such a study would not be ethical. However, cohort studies have documented that the HCC incidence in those successfully treated for hepatitis C is lower than in historical untreated controls[107–110]. Until recently, standard treatment of hepatitis C was based on interferon, and sustained virologic responses in patients with hepatitis C cirrhosis have been associated with a substantial reduced risk in HCC development (hazard ratio (HR) 0.23, confidence interval (CI) 0.16-0.35)[111]. It is too early to ascertain the effect of new direct acting antiviral (DAA) drugs for HCV – that achieve sustained virological responses in 90% of cases- on the occurrence of HCC[112]. If one extrapolates from the interferon era to the current DAA therapy setting, as a greater proportion of individuals who are infected with HCV are treated, the overall hepatitis burden, and the absolute number of patients with hepatitis who become at risk for HCC, will decrease. However, the overall effect of DAA therapy will be modest because most individuals with HCV remain undiagnosed, and treatment penetrance is limited owing to socioeconomic factors. Assuming that one-third of patients with HCV come to medical attention and achieve 90% sustained virologic responses with DAA agents, the burden of HCC in the United Stated might be reduced by 10-15% over the next decade[113,114].

[H3] Chemoprevention.

There are several putative chemopreventative agents that have been proposed to reduce HCC incidence in at-risk populations, including statins and metformin[115]. The data on metformin are becoming more robust, as demonstrated by a meta-analysis that suggested metformin decreases the risk of HCC in people with diabetes[116]. Moreover, a study has shown that metformin may be safely continued in people with diabetes who also have cirrhosis[117]. Nonetheless, the evidence for the ability of metformin to reduce HCC risk is not strong enough to recommend using it in at-risk patients, and prospective studies testing this agent are required.

[H3] Lifestyle modification.

Whether abstinence from alcohol in those with alcoholic liver disease decreases the incidence of HCC is not yet known. Of course, primary prevention by counselling against unhealthy use would reduce the incidence of alcohol associated cirrhosis and the attendant risk of HCC. Similarly, for those with NAFLD who successfully lose weight or otherwise control their disease, there is no information as to whether HCC incidence is reduced[113]. Thus, in summary, prevention of HCC in large part depends on prevention or treatment of the underlying liver disease.

[H2] Surveillance for HCC

When HCC causes symptoms, the disease is most often in an advanced stage, and not amenable to potentially curative treatment. Death usually ensues within a few months. However, HCC has a prolonged sub-clinical course that provides the opportunity for early detection. Early-stage HCC lesions are small and frequently curable, often by minimally invasive methods. These considerations have led to the development of protocols for surveillance for HCC in patients at risk for this cancer (**Table 1**)**.** Surveillance, however, remains controversial since the only RCT that demonstrated decreased mortality was probably statistically incorrectly analyzed[118]. Nonetheless, there is a wealth of evidence of lesser quality, including cohort studies with comparisons between screened and unscreened populations, and demonstration that cure is more likely with HCC that is detected by surveillance compared with cases that are diagnosed once symptoms have become apparent[119–124]. Several cost-efficacy analyses have also demonstrated benefits of surveillance[125–137]. Furthermore, in considering the potential benefits from surveillance compared with the potential harms it is clear that benefits outweigh harms. Benefits include early detection and potential cure of HCC. Harms include some additional investigations and potentially some unnecessary interventions. However, as discussed below, a diagnostic algorithm has been developed that was designed to minimize false-positives, and thereby reduce the likelihood of harm. More recently, there have been a number of risk scores developed to improve identification of the at-risk population. None have yet received widespread application.

The techniques for surveillance are also controversial. There is no question that ultrasound should be part of the algorithm, but the use of biomarkers remains controversial. There is some suggestion that biomarkers improve early detection[138–144], but there is as yet no evidence that this leads to improved cure rates compared with ultrasound alone. Most importantly, the usual serum biomarkers that are used — α-fetoprotein (AFP), des-γ carboxyprothrombin (DCP) and the L3 fraction of AFP (AFP-L3) — are all more frequently associated with advanced-stage disease than early-stage disease and would therefore be theoretically unsuitable for detection of early disease[138–144]. Among these, AFP is the most widely used and this still remains an area of controversy.

The ideal surveillance interval for at-risk patients (**Table 1**) according to guidelines is 6 months[3,23,145–148]. Studies have demonstrated that a 3-month interval does not enhance outcomes[146], and survival is lower with 12 month than with 6 month intervals[145].

[H2] Diagnosis

Patients enrolled in a surveillance programme are diagnosed by identification of a new liver nodule on abdominal ultrasound, and diagnostic confirmation using either non-invasive

criteria or biopsy. These patients are generally asymptomatic and have early stage HCC. Conversely, patients diagnosed outside surveillance usually present at advanced stages with large symptomatic tumours and/or portal vein invasion. Symptoms include malaise, weight loss, anorexia, abdominal discomfort or signs related to advanced liver dysfunction. Diagnosis can be made by non-invasive (radiological) or invasive (biopsy) approaches. Radiological diagnosis is achieved with a high degree of confidence if the lesion is found in a patient with cirrhosis. Using contrast imaging, the lesion shows the radiological hallmarks of HCC, which are hypervascularity in the arterial phase of a contrast study (CT or MRI) and a decreased signal compared with the rest of the liver in the venous and/or delayed phases of the study[149]. When these typical features are observed the diagnosis is confirmed and a biopsy is not necessary[3,15,150,151]. Latest generation CT and/or MRI following reported protocols are recommended[152]. MRI with liver specific contrast agents might help in the diagnosis of HCC, but the specificity of these agents is still suboptimal.

The caveat of the non-invasive radiological criteria is that this algorithm only applies to those who have an elevated risk of HCC. A biopsy is required for patients who do not have any special risks for HCC, for the most part patients without cirrhosis. The recommended algorithm for investigation of lesions in at-risk patients is as follows[3,15]: For nodules < 1 cm in size, ultrasound follow-up at 3 months is recommended. For lesions > 1 cm, the radiological hallmarks of HCC define diagnosis. If the radiology is not typical in at least one of two imaging techniques (CT and MRI), a liver biopsy is recommended[3,15]. Of note, this accepted practice puts the assessment of HCC at a disadvantage compared with most cancers since tumour tissue for molecular studies would not be routinely obtained in clinical practice. Recent guidelines recommend obtaining tissue samples in the setting of all research studies in HCC[3].

Diagnostic difficulty occurs mainly with early-stage HCC lesions, in which the radiological appearance might be atypical, necessitating a biopsy. However, biopsy is not an ideal gold standard, because of variation introduced by sampling and complications. The risk of complications, such as tumour seeding and bleeding, after liver biopsy is less than 3%[153]. Although the sensitivity of liver biopsies ranges between 70-90% for all tumour sizes[3], small lesions might be missed, giving a false-negative result. In addition, in early-stage HCC, morphological changes may be minimal compared with dysplastic hepatocytes, making the diagnosis uncertain[154]. In this setting, the use of special stains may help to resolve diagnostic uncertainties. For example, HCC cells expresses glypican 3, glutamine synthetase, heat shock protein 70 and clathrin heavy chain[155–157]. Positive staining for two of these four markers is highly specific for HCC. In addition, comparison of the tumour biopsy to biopsy of the surrounding non-tumourous liver may be helpful in highlighting the early features of malignancy. Patients with suspicious lesions in whom a diagnosis cannot be confirmed on a biopsy should be closely monitored, and might even require a second biopsy.

[H1] Management

[H2] Overview of evidence-based management and staging systems

The BCLC staging system provides an easy-to-use algorithm that links tumour stages with treatment allocation policies based on evidence (**Figure 4**)[3,158,159]. Treatments are classified as radical therapies with potential to cure HCC — such as surgical resection, liver transplantation or percutaneous ablation — or palliative therapies which are aimed at improving survival —chemoembolization and sorafenib[3]. Treatment allocation for standard of care follows the levels of evidence defined by the National Cancer Institute, which rely on strengths of study design and end points (**Table 3**). Controversy regarding HCC staging systems remains. In fact, alternative staging or scoring systems have been proposed, such as the Hong Kong classification[160], the Cancer of the Liver Italian Program (CLIP) score[161], the TNM system[162] and the Japan Integrated Staging (JIS) score[163]. None of these systems has acquired global consensus. Scoring systems, such as the CLIP, are not widely use since they do not incorporate treatment allocation to distinct prognostic stages, whereas others are specifically applied in Asia (the Hong Kong system and the JIS).

[H3] Early-stage disease.

Surgical resection is the first-line option for patients with early-stage HCC (BCLC 0 or A) with solitary tumours, and confers 5-year survival rates of 70%[3,15,164,165]. Introduction of restrictive selection of candidates for resection — patients with single nodules, absence of portal hypertension and well-preserved liver function — along with anatomic resection — in which tissue removal is performed in-line with the location of functional segments of the liver — has minimized complications and reduced recurrence[3,15,164,166]. Metastases and *de novo* HCC account for 70% 5-year recurrence after resection [167], and no adjuvant therapies are able to prevent this complication[168]. Adoptive immunotherapy[169] and the vitamin A derivatives acyclic retinoids[170] were reported as effective treatments in this setting; however, these trials were conducted in small study populations and the results have not been reproduced.

Liver transplantation is the best first-line option for patients with tumours within Milan criteria (defined as a single tumour <5 cm in size or three nodules <3 cm in size without vascular invasion) (BCLC A) that are unsuitable for resection[3,171]. These criteria have been independently validated internationally and have been adopted by guidelines[3,15,165] and Liver Transplant Units. Nonetheless, some studies have pointed to the fact that moderate expansion of Milan criteria might lead to acceptable and competitive long-term outcomes[172–174]. Local ablation using radiofrequency has been proposed as an optimal

alternative to resection in patients with a single tumour of <2 cm in size who are unsuitable for transplantation[16,175], but no RCTs specifically addressing this issue have been reported. On the other hand, in patients with early tumours (BCLC 0 or A) who are not suitable for surgery, local ablation is the standard of care with 5-year survival rates of 50–70%[3].

[H3] Intermediate-stage disease.

Patients with intermediate-stage HCC (BCLC B) are characterized by multinodular disease, preserved liver function and the absence of tumour-related symptoms, vascular invasion and extrahepatic spread. Chemoembolization in the form of transcatheter arterial chemoembolization (TACE) — a minimally invasive technique that combines local delivery of beads to restrict tumour blood supply with local administration of chemotherapy — is recommended based on results from RCTs and one meta-analysis of pooled data[17,18,176]. Improvements in the selection of candidates, supra-selective embolization – selective blockade of the artery feeding the tumour that minimizes collateral hepatic toxicity — and improvements in embolization devices (drug-eluting beads) have led to current median survival times of 26 months[177], and beyond 40 months in referral centres[178]. Alternative embolization strategies, such as radioembolization using beads coated with yttrium-90 (Y-90) — an isotope that emits short-range β radiation — are effective and have a favourable safety profile[179], but only well-designed, properly powered RCTs will determine the therapeutic niche of this intervention.

[H3] Advanced and end-stage disease.

The Sorafenib HCC Assessment Randomized Protocol (SHARP) trial demonstrated that sorafenib, a multiple tyrosine kinase inhibitor, was able to substantially increase survival in patients with advanced-stage HCC (BCLC C) from 7.9 months to 10.7 months (HR 0.69)[19]. The beneficial effects of sorafenib occur regardless of HCC aetiology, as was validated in Asian patients who were infected with HBV[180]. On the basis of these data, sorafenib became the standard of care in this setting. Eight RCTs testing alternative systemic molecular treatments reported since the SHARP trial have not shown survival benefits in HCC. These findings can be partially explained by the specific toxicity of some agents owing to the underlying liver cirrhosis, the high molecular heterogeneity of HCC and a relative resistance to conventional chemotherapy[181]. Patients with end-stage disease (BCLC D) should be considered for nutritional and psychological support and proper management of pain, but are not candidates for entering clinical trials.

[H2] Surgical therapies

Surgery is the treatment of choice for most patients with early-stage HCC, with 5-year survival in appropriately selected cases exceeding 70%; its role in more-advanced HCC is more controversial[3,165]. The large majority of patients with HCC have underlying liver disease, and both the location and extent of the tumour and the status of the non-malignant tumour liver tissue must be considered in the choice of surgical procedure. The prognosis of chronic liver disease is commonly assessed using the Child-Pugh score, which uses five clinical measures — total bilirubin, serum albumin, prothrombin time, ascites severity and hepatic encephalopathy grade — to classify patients into one of three groups (A-C) of predicted survival rates. In brief, Child-Pugh's A reflects well-preserved liver function, Child's B moderate liver dysfunction with a median life expectancy of approximately 3 years and Child C severe liver dysfunction with life expectancy of approximately 1 year[182].

[H3] Resection.

Patients with a single technically resectable HCC without macrovascular invasion, with preserved liver function (Child-Pugh class A with bilirubin < 1 mg per dl) and no portal hypertension are optimal candidates for partial hepatectomy, and experience low (1-2%) perioperative mortality[15,164–166](**Table 3; Figure 4**)[3,158,159]. Limited, yet anatomical, resection is preferred when possible in order to spare uninvolved liver parenchyma and to remove satellite tumours that result from local vascular invasion[183]; when major (≥ 3 segment) resection is required, preliminary portal embolization[184] or lobar radioembolization[185] is performed in some centres to induce growth of the future liver remnant. Over a period of 4-6 weeks following portal embolization, the volume of the contralateral liver lobe typically increases by 20-25%, thereby decreasing the risk of postoperative hepatic insufficiency. These practical approaches have not yet been adopted by guidelines. With improving technology and experience, laparoscopic resection is increasingly employed with improved early outcomes[186], and percutaneous thermal ablation has become an acceptable alternative for accessible tumours < 2cm[175]. Resection is often applied outside of guidelines for patients with multifocal HCC or portal hypertension, particularly in Asia where availability of transplantation is limited, albeit with decreased 5-year survival (50-60% compared with > 70% in optimal candidates)[187].

Recurrence of HCC in the liver is common after resection (up to 70% at 5 years)[165,188], because the remaining liver is both the most common site of metastasis of the primary HCC, and is at risk for developing *de novo* HCC. With careful follow-up, recurrence can often be treated effectively by repeat resection[189], thermal ablation or liver transplantation[190] with resultant long-term survival. There is no proven adjuvant therapy for HCC resection; small trials have produced positive results for retinoids[170], immunotherapy[169] and I-131 lipiodol[191],

but these have not been confirmed in larger studies, and a large RCT including more than 1000 patients of sorafenib in this setting showed no benefit[168].

[H3] Transplantation.

Liver transplantation is indicated for patients with early-stage HCC who fall within Milan criteria and who are not candidates for partial hepatectomy[3,15,171]. For these patients, transplantation yields a 5-year survival rate of 70% with a recurrence rate of approximately 10%, and 10-year survival rates > 50%[192](**Table 3; Figure 4**)[3,158,159]**.** Transplantation offers the appeal of removing unrecognized intrahepatic metastases and essentially eliminating the risk of *de novo* tumour development, but this benefit is offset by higher perioperative and late non- tumour-related mortality such that post-transplant survival, at least to 5 years, is not substantially better than after resection[188]. Donor organ scarcity varies geographically. To the extent that patients must wait for organs, drop-out from the waiting list owing to tumour progression reduces transplant survival on an intention-to-treat basis[166], and loco-regional treatment to prevent progression while waiting is an integral part of the process[193] . Living donor transplantation is a way for patients with suitable donors to avoid the risk of waiting list drop-out; results are comparable to those achieved with deceased donors[194]. The mTOR inhibitors sirolimus or everolimus are often added to the immunosuppressive regimen[195]. However, their effectiveness as anti-tumour therapies to prevent recurrence-free-survival after transplantation failed in a recent RCT[196,197].

The Milan criteria are widely used as the basis for transplant eligibility, and adherence to them yields good post-transplant survival. Increasingly, however, the benefit provided by transplant compared with the alternatives (for example, resection and loco-regional therapies), as opposed to the absolute survival rate, is being considered in discussions about donor organ allocation; basing allocation on benefit of transplant rather than on absolute survival could lead to different choices of transplant candidates. For example, the benefit of transplant for a patient with single HCC within Milan criteria as compared with resection may be considerably less than the benefit of transplant for multinodular HCC beyond Milan criteria as compared to chemoembolization, even though the absolute survival after transplantation in the second scenario is lower[198]. Several cohorts have explored the implication of extending Milan criteria for transplantation in HCC[199]. Extended criteria such as the University of California San Francisco (UCSF)[200] and Up-to-7[172] proposals have been reported to yield survival rates similar to the Milan criteria, as has down-staging of HCC beyond the Milan criteria by loco-regional treatment[201]. Selection criteria for extended indications based on genomic information have reported good outcomes[175]. The reproducibility of these proposals on a large scale awaits confirmation.

Postsurgical outcomes are dependent on the nature of the underlying liver disease. Historically, results have been better in HBV-related HCC compared with HCV-related HCC[188] because viral control has been possible for HBV, leading some authors to propose more liberal surgical guidelines for patients with HBV-related HCC[160].There is good reason to expect that with the recent development of effective HCV treatment[202], improved results of both resection and transplant might lead to a broadening of the accepted indications for surgical treatment in patients with hepatitis C.

[H2] Loco-regional therapies

[H3] Percutaneous ablation.

Percutaneous and intraarterial therapies are usually performed by interventional radiologists and are the mainstay of the treatment of patients with early-stage and intermediate-stage HCC (BCLC 0-B) who are not candidates for surgery (**Table 3**)**.** Percutaneous tumour ablation involves the insertion of a needle through the skin to access the inside of a tumour. This approach can be used to inject agents that induce tumour cell killing (usually absolute ethanol) or to insert a probe that delivers energy that induces a deleterious increase in temperature (radiofrequency, microwave or laser)[203]. Tumour size (≤ 3 cm), number (≤ 3 tumours) and location (accessible with ultrasound guidance) limit the applicability of percutaneous ablation[204].

Several RCTs have demonstrated a significant benefit of radiofrequency ablation compared with percutaneous ethanol injection in terms of complete response rate (absence of contrast uptake within the treated lesion in the arterial phase of CT or MRI) and time to recurrence[205,206]. As a result, radiofrequency is the standard ablative therapy at early stages of the disease since it provides better results than ethanol[205] (**Figure 4**)[3,158,159]. Ethanol injection is nevertheless a valuable option for tumours located near the large hepatic vessels and bile ducts, or in centers with limited access to technology. Five-year survival rates after radiofrequency ablation averages 60%[207]. Although tumour progression or relapse is higher after percutaneous ablation than after liver resection, the long-term outcomes are similar and ablation has been proposed as first-line therapy for tumours that are < 2 cm in size[16].

[H3] Intraarterial therapy.

The most commonly used intraarterial therapy is TACE, which involves the sequential injection into one or more branches of the hepatic artery of chemotherapeutic drugs (doxorubicin, mitomycin C, cisplatin or combinations) loaded to the particles or emulsified in Lipiodol (an oily contrast agent that is selectively retained in the HCC nodules) and embolizing particles that interrupt blood flow[208,209]. The result is the induction of acute ischaemic necrosis and eventually a prolonged exposure of tumour cells to the drugs. TACE is

nowadays usually performed 'on demand', with patients being evaluated every 6-8 weeks with contrast-enhanced CT or MRI and additional selective TACE sessions performed only if active tumour areas are found[210]. The use of the more costly drug-eluting particles results in a simpler and more standardized procedure that increases tumour response rates but does not improve survival[211].

Strong scientific evidence makes TACE the standard of care for patients with large or multiple tumours (BCLC B) or those small tumours that cannot be resected or percutaneously ablated (BCLC A unsuitable for surgery or local ablation), if the patient has a preserved liver function, no cancer-related symptoms and no vascular invasion or extrahepatic spread[3]. Two RCTs [17,176] and one meta-analysis of pooled data established a significant benefit of TACE versus supportive care or suboptimal therapies (tamoxifen and oral 5--fluorouracil (5-FU) in this patient population[18] (**Figure 4**)[3,158,159]. Median survival after TACE ranges from 16-45 months in the early stage (BCLC 0-A), 15.6-26.3 months in intermediate stage (BCLC B) and 6.8-13.6 in the advanced stage (BCLC C)[177,212]. The largest RCT ever reported for TACE describes a median survival duration of 26 months for patients with intermediate-stage HCC[177]. Combination strategies with TACE and systemic therapies (brivanib or sorafenib) have not resulted in clinical benefit[177,213]. A different meta-analysis that included the combination of different loco-regional therapies (TACE or radiofrequency ablation in the active arms) has questioned the benefits of TACE[214]. The use of TACE alone or in combination with sorafenib for patients with advanced-stage HCC is not supported by scientific evidence or recommended by guidelines[3,23].

Alternatives to TACE include Y90-radioembolization, which uses much smaller embolizing particles that are injected into the hepatic arteries to provide selective internal irradiation of the tumours[179,215]. Evidence of survival benefit for radioembolization has not yet been proven in the setting of RCTs compared with the standard of care, which is TACE in intermediate-stage HCC and sorfenib in advanced-stage HCC. This technique is usually applied to those patients that are not good candidates for TACE because of large tumour burden, vascular invasion or progression to prior TACE[216]. A second alternative to TACE is bland transarterial embolization (TAE), which involves occluding the tumour blood supply using microbeads without simultaneous administration of chemotherapeutic agents. TAE has provided lower response rates compared with TACE in RCTs[217], and a meta-analysis showed that it provides suboptimal survival compared with TACE[18]. Finally, hepatic arterial infusion chemotherapy — a catheter-based procedure for the long-term administration of agents directly into the liver — is frequently used in Japan to treat patients who are poor candidates for TACE, however there is no scientific evidence supporting this approach.

[H2] Systemic therapies and future treatment approaches

More than 100 RCTs have been reported testing chemotherapy or other types of systemic therapies in HCC, but only one drug, sorafenib, has proven survival advantages[19,180,218]. Treatment with systemic chemotherapy and anti-oestrogen therapies has been shown to be ineffective in HCC[18]. Systemic chemotherapy with doxorubicin, PIAF regime (platinum, interferon, doxorubicin and 5-FU)[181] and FOLFOX regime[219] lacked survival advantages and was accompanied in some instances with important toxicity. Treatment with sorafenib is associated with manageable adverse events (diarrhoea and skin reactions on the hands and feet) and an absolute increase in median survival of 3 months[19]. Subsequent studies revealed a stable benefit of this drug in all regions of the world and in all HCC aetiologies[220], an in recent trials sorafenib consistently showed a median survival of approximately 10 months. Unfortunately, no predictive biomarkers of responsiveness to sorafenib have been identified[221]. Although sorafenib is in a unique position of primacy in the management of HCC, it has some restrictions in the target population (not indicated in patients with poor liver function or in the adjuvant setting) and in the understanding of the mechanism of action. The efficacy of sorafenib probably results from a balance between targeting cancer cells and the microenvironment by blocking multiple kinases — up to 40, including vascular endothelial growth factor receptor (VEGFR), platelet-derived growth factor receptor (PDGFR), mast/stem cell growth factor receptor (c-Kit) and/or serine/threonine-protein kinase B-raf (BRAF)[222]. Interestingly, this wide blockade does not induce liver damage or other life-threatening complications.

The main characteristics of the SHARP trial have been adopted by guidelines of trial design[223] and replicated by almost all subsequent studies testing molecular therapies in HCC[218]. This seminal study enrolled patients with well-preserved liver function (as indicated by Child-Pugh A class), with advanced disease (BCLC C) or those with intermediate stage disease (BCLC B) that progressed following TACE, and defined overall survival as the primary end point (**Table 5**). Nonetheless, none of the therapies tested after the SHARP trial, including brivanib (a fibroblast growth factor receptor (FGFR) and VEGFR inhibitor)[224], sunitinib (a c-Kit, VEGFR and PDGFR inhibitor)[225], linifanib (a VEGFR and PDGFR inhibitor)[226], erlotinib (an epidermal growth factor receptor (EGFR) inhibitor)[227] in the first line-setting and brivanib[228], everolimus (an mTOR inhibitor)[229] and ramucirumab (a VEGFR2 inhibitor)[230] in the second-line setting have demonstrated survival benefits in patients with HCC**.** The reasons for the disappointing Phase III clinical trial results are reviewed elsewhere[231] and include a marginal anti-tumour potency, liver toxicity, flaws in trial design and lack of biomarker-based enrichment. Randomized Phase II studies are recommended prior to conducting pivotal Phase III trials. Phase II studies are essential for identifying signals of efficacy, futility or toxicity, and might prevent the devotion of resources to Phase III trials that test therapies with marginal chances of success. Overall survival is the mainstay end-point for Phase III studies. Although time-to-progression remains relevant in Phase II studies[223], recent results pointing to a lack of correlation between TTP and OS[225,226,228] suggest assessing

simultaneously other end-points such as objective response using the modified Response Evaluation Criteria In Solid Tumours (mRECIST)[231,232]. Regarding biomarkers, none of the Phase III studies were enriched by biomarker analysis, which could have indicated effectiveness of the drug in selected subpopulations.

Most of the drugs currently being tested in Phase III trials are antiangiogenic agents, cell cycle inhibitors, receptor tyrosine kinase inhibitors and checkpoint inhibitors (**Table 4; Figure 5**). These broad spectrum compounds are tested in HCC in 'all comers', such as levantinib (a VEGFR2 and VEGFR3 inhibitor) for first-line treatment and regorafenib (multikinase inhibitor, including a VEGFR2 and angiopoietin-1 receptor (TIE2) inhibitor) or cabozantinib (a VEGFR and hepatocyte growth factor receptor (MET) inhibitor). However, the fact that many agents in Phase III trials failed with non-biomarker enriched populations is currently being counteracted by a more precise approach. Primarily, Phase II proof-of-concept studies that test drugs blocking potential oncogenic addiction loops and Phase II and III studies using biomarker-based trial enrichment strategies to define activation of signalling pathways in HCC subgroups are currently on-going. For instance, genomic studies defined a human HCC subclass characterized by TGF-β signalling activation[233] that is associated with an aggressive phenotype[74]. The small molecule galunisertib blocks TGF-β signalling and has been tested in Phase II as a single agent or in combination with sorafenib[234]. Similarly, overexpression and/or amplification of *FGF19* is characteristic of 10-20% of cases of HCC[65,66]. New generation FGFR-specific kinase inhibitors have been tested in pre-clinical models demonstrating that *FGF19* amplification is a predictive biomarker of response[235]. More recently, BLU9931, a highly specific FGFR-4 inhibitor, demonstrated pre-clinical activity in patient derived xenografts, providing the rationale for exploring this compound in phase I or II clinical trials[236]. Finally, universal activation of the RAS/MAPK axis is common in patients with advanced HCC[237]. First attempts to block this pathway with mitogen-activated protein kinase kinase (MEK) inhibitors (selumetinib) have failed in detecting significant objective radiological tumour responses[238]. The Assessing BAY86-9766 Plus Sorafenib for the Treatment of Liver Cancer (BASIL) trial tested refametinib (a MEK1-2 inhibitor) in advanced HCC, and results indicated that *RAS* mutations could be a potential biomarker of treatment response [239].

Alternatively, recent data has emerged pointing towards biomarker-driven selection of candidates in testing drugs in Phase III trials. This has been the case for ramucirumab, which is currently in Phase III for second-line treatment of patients with advanced HCC and AFP > 400 ng per mL, based on subgroups analysis data[229]. Similarly, tivantinib, a tubulin and MET inhibitor[240], is currently being tested in Phase III only in MET-positive patients in a second-line setting. Activation of MET is estimated to occur in approximately 50% of these patients.

A similar drug, cabozantinib , is being tested as a second-line therapy in an all-comers after preliminary positive effect in clinical studies[241].

Immune checkpoint inhibitors have been approved by regulatory agencies owing to their considerable activity in patients with advanced-stage melanoma and lung cancer[242]. The rationale for an immunological approach to treat HCC has been proposed for years. Indirect evidence includes the pivotal role that the immune system has in the development of chronic liver disease and HCC, dendritic cell-based approaches showing certain anti- tumour activity[243,244] and occasional reports of cases of spontaneous remission. Although pilot studies with the cytotoxic T-lymphocyte protein 4 (CTLA-4) blocking antibody tremelimumab did not produce signals of efficay[245], a recent phase I-II trial with the programmed cell death protein 1 (PD-1) inhibitor nivolumab showed a manageable adverse event profile and produced durable responses in patients with HCC patients who had tumours that were resistant to sorafenib[246]. Phase III trials have been designed to test this drug. Finally, two alternative types of molecular-based therapies are currently being explored in early clinical research studies: epigenetic modifying therapies[247,248] and microRNAs[249].

Molecular targeted therapies are associated with acquired drug resistance. The most common traits of acquired resistance mechanisms are the persistent activation of the oncogenic target itself owing to secondary mutations — for example, mutation of *EGFR* (T790M) in gefitinib/erlotinib-resistant patients with non-small-cell lung cancer[250] — or acquired mutations in alternative drivers of the pathway — such as mutations in genes encoding MAPKs in vemurafenib-resistant BRAF melanoma[251]. Acquired resistance to sorafenib in HCC has mostly been explored in experimental models. Several mechanisms have been implicated, including activation of MAPK14 signalling [68], enrichment of tumour-initiating cells and re-activation of IGF/FGF signalling[252].

[H1] Quality of life

Unsurprisingly, as liver disease and HCC tumour progresses quality of life (QOL) suffers. The purpose of measuring QOL should be to compare outcomes between treatment arms, even if one is a placebo. There is little agreement as to the best method of measuring QOL in HCC research. Although general instruments are in use, there are two that are specific to HCC. These are the European Organisation for Research and Treatment of Cancer Quality of Live Questionnaire (EORTC QLQ) 18[253,254] and the Functional Assessment of Cancer Therapy-Hepatobiliary (FACT-Hep)[255] questionnaire. Both of these tools are derivatives of more general QOL questionnaires for cancer and have been validated externally, but there is little indication that they are measuring the same thing, or that they are comparable.

There is very little literature on QOL in HCC. Most studies report changes in QOL in single arm studies, and very few studies have designed primarily to compare two different treatments in patients with similar stage disease. For example, one study compared QOL after resection with QOL following radiofrequency ablation[255]. As expected, QOL was much better after radiofrequency ablation than after resection, and remained superior up to 36 months post-treatment. In addition, QOL following radioembolization has been compared with TACE[256]. In this study, there was no overall difference in QOL between the two groups, but the sample size was small. Despite the lack of statistically significant differences, in the TACE group QOL was decreased at 2 and 4 weeks, whereas in the radioembolization group some aspects of QOL actually improved. However, in this study the patients who underwent radioembolization had more advanced disease than those who underwent TACE, so the results are not directly comparable. Finally, the SHARP trial demonstrating survival benefits of sorafenib compared to placebo also tested time-to-symptomatic progression — as measured by the Functional Assessment of Cancer Therapy–Hepatobiliary Symptom Index 8 (FHSI8) — as co-primary end-point. The negative results of this end-point contrasted with the survival benefits obtained by sorafenib, thus challenging the accuracy the tool used[19].

[H1] Outlook

[H2] Global disease burden

The global burden of HCC is increasing and considerable challenges are ahead for improving the understanding and treatment of this complex disease. The main unmet medical needs for HCC are summarized in **Box 1**. Considering that HBV and HCV infection are the main risk factors for HCC development, it can be presumed that the implementation of new more effective anti-viral therapies might decrease the incidence of HCC on a global scale in the following decade. Antiviral therapies approved for HCV infection achieve sustained viral clearance in more than 90% of cases[202,257], and well-established anti-HBV therapies lead to undetectable viral titres (circulating HBV-DNA) in most patients. Nonetheless, the fact that the risk factors can be eliminated (efficacy), does not always translate into global improvements (effectiveness) owing to suboptimal implementation of treatments in underdeveloped areas and other complex reasons. Similarly, despite that fact that surveillance is cost-effective in HCC, the global implementation of such programmes is still suboptimal and is estimated to engage <50% of the target population in the West. Public health policies encouraging the implementation of such programmes in well-defined populations should lead to an increase in early tumour detection, and hence survival benefits. Finally, in parallel with advances in the treatment of viral hepatitis, other aetiologies of HCC are emerging, particularly NASH-related HCC which is associated with obesity and diabetes. The effect of these unfolding risk factors on HCC burden remains to be elucidated, but might counterbalance the decreases expected with HCV control.

[H2] Drug and biomarker discovery

High-throughput genomic studies reporting gene sequencing of large cohorts have already established the main oncogenic drivers of HCC. However, most of these drivers, such as the *TERT* promoter, *TP53* and *CTNNB1*, have not proven to be druggable and as such understanding of their role in HCC has not translated into improving the management of the disease. Drug discovery targeting these complex proteins and regulatory mechanisms should represent a major breakthrough in HCC research[258]. On the other hand, the identification of driver mutations or amplifications in relevant genes — such as *FGF19*, *CCND1* and *VEGF* — has not yet translated into proof-of-concept early clinical trials based on biomarkers. Understanding the targets of the microenvironment in tumour progression and response to therapies has been an area clearly underexplored[79,231], considering the clinical relevance of this compartment in the risk of tumour development, prognosis and immunomodulation[76–79]. In addition, despite results reporting preclinical testing of drugs in genetically modified models or patient-derived xenografts, clinically relevant models recapitulating the spectrum of human disease are still suboptimal.

[H2] Disease management

[H3] Standard therapy.

Only five treatments are recommended by the European Association for the Study of the Liver (EASL) and the American Association for the Study of Liver Diseases (AASLD) guidelines for HCC management (**Table 3; Figure 4**)[3,158,159]. Several other treatments have been tested in RCTs since the last successful therapy (sorafenib) was approved, and all of these studies produced negative or inconclusive results. In early HCC, no adjuvant therapy has shown efficacy[168] and this represents an important unmet medical need. In addition, since the advent of radiofrequency as the mainstream of local ablation, no other proposed approach, such as microwave, has led to a major control of the disease. Chembomebolization remains the sole proven effective therapy in intermediate HCC. None of the combination therapies with chemoembolization have shown additive outcome advantages. Phase III trials testing alternative therapies, such as radioemebolization, are awaited. Indications beyond guidelines for resection[259] and TACE are widely applied in clinical practice, particularly in Asia[260], and this represents an important challenge that needs to be addressed with robust and well-designed studies.

[H3] Progress in treating advanced disease.

Management of advanced HCC has attracted major attention during the past decade. Trials testing systemic chemotherapy have been almost abandoned and the few that were conducted showed disappointing results[219]. Sorafenib approval represented a breakthrough,

challenged the concept of advanced HCC as a cancer that is not druggable[19] and paved the way for testing novel drugs. What has been unexpected is the failure of all molecular agents tested afterward sorafenib[224–230]. As a consequence, this major cause of cancer-related death remains as one of the few solid tumours with only one systemic therapy available. New avenues are currently being explored to overcome this situation. Among the drugs currently being tested in Phase II and Phase III trials (**Table 4; Figure 5**), some are exploring novel areas that led to major advancements in cancer management, such as immune checkpoint inhibition[261]. Others are targeting specific subpopulations of HCC patients based on biomarkers identified in phase I/II studies, such as tivantinib for MET-positive patients[240], or ramucirumab for those patients progressing on sorafenib with an AFP of >400 ng per mL[230]. Finally, a few studies are exploring targeting specific tumour driver genes, which are those responsible for 'oncogenic addiction'. Oncogene addiction is defined as the state of dependency of tumour cells on a given driver. Since solid tumours are expected to share 4-8 drivers per tumour, the addiction to one specific oncogene confers status of primacy to this target[262]. Thus, clinically relevant anti-tumour responses are achieved when targeting those drivers, as is the case for inhibiting the effects of antiplastic lymphoma receptor tyrosine kinase (*ALK*) fusion rearrangement with crizotinib in patients with non-small-cell lung cancer[263]. Trial design in HCC should follow specific recommendations that are provided in **Table 5.**

[H3] The future of precision medicine for HCC.

HCC is one of the few cancers where non-invasive radiological criteria suffice for the diagnosis of the disease in patients with an underlying risk factor (cirrhosis)[3,15]. This clinical approach conflicts with the concept of precision medicine, which is based on the administration of selective therapies targeting molecular alterations relevant to tumour progression in a given individual. In order to implement precision medicine in HCC, a tumour biopsy is required and, as such, routine biopsy has now been adopted by guidelines for clinical trials in HCC research[3]. The reliability of this strategy is based on the assumption that one tumour biopsy suffices for recapitulating the molecular information found in the whole neoplasm. This concept is currently debatable in oncology owing to evidence of inter-tumoural and intra-tumoural heterogeneity in all malignancies, including in HCC[31,264]. This observation prompts an important question: can we rely upon a single biopsy for decision-making or should we obtain multiple biopsies, even though this more thorough approach appears clinically impractical?

To explore heterogeneity we need to understand the concept of trunk, branch and passenger mutations[31,265]. Trunk mutations occur at the onset of the disease and are potent transforming drivers present in all cells of a given tumour at early stages[31]. Conversely,

branch mutations develop late in the natural history of the tumours or as result of acquired resistance under the pressure of therapies and thus are only present in a subgroup of tumour cells. Finally passenger mutations, the most common type of mutation, are of marginal relevance in terms of cell transformation, progression or dissemination, but they might be helpful in defining the clonality of tumours or their immunogenicity. Therefore, as reported in solid cancers such as non-small-cell cancer[266], breast cancer and melanoma, molecular therapies that achieve good tumour response and survival benefits can do so by targeting trunk mutations that have been identified with a single biopsy. Conversely, heterogeneity might be cumbersome when branch mutations have a more dominant role, for instance at very advanced stages[266] or in the setting of acquired resistance[265]. In these instances, liquid biopsy —checking either tumour DNA and mRNA in cell-free plasma or circulating tumour cells — emerges as the most promising alternative for the molecular monitoring of tumour progression and relapse[267,268]. Recent reports point to the benefits of liquid biopsy in recapitulating tissue trunk driver mutations[269] and in capturing unique branch subclonal mutations acquired under treatment pressure[270]. Finally, heterogeneity has more important therapeutic implications for molecular agents than for immune check-point inhibitors.

Figure 1. The global burden of hepatocellular carcinoma. Incidence of hepatocellular carcinoma (HCC) according to data from Globocan 2012[1,3–9]. ASR, Age-standardised rate per 100,000 (Modified from[10]).

Figure 2. Liver cancer incidence according to region and sex. Figures reflect age-standardized rate per 100,000 inhabitants. (Adapted from[1].)

Figure 3. Cancer progression and driver genes. Major recurrent molecular defects observed early in liver carcinogenesis. *TERT* promoter mutations are common early events identified in most of HCC that develops in a cirrhotic liver. Other mechanisms are specifically related to risk factors: HBV and AAV2 viral infections induce insertional mutagenesis that recurrently targets oncogenes. Also, hepatocellular adenoma, a rare benign liver tumour occurring most frequently in women who take oral contraception, can transform into HCC with sequential accumulation of *CTNNB1* and *TERT* promoter mutations with or without STAT3 activation[33–38]. AAV2, adeno-associated virus 2; CCN, cyclin; CTNNB1, catenin β 1; FRK, fyn-related Src family tyrosine kinase; GNAS, GNAS complex locus; HBV, hepatitis B virus; HCA, hepatocellular adenoma; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HNF1A, HNF1 homeobox A; JAK1, Janus kinase 1; KMT2B (also called MLL4), lysine (K)-specific methyltransferase 4; IL6ST, interleukin 6 signal transducer; STAT3, signal transducer and activator of transcription 3 (acute-phase response factor); TERT, telomere reverse transcriptase.

Figure 4: BCLC staging system and therapeutic strategy. Classification comprises five stages that select the best candidates for the best therapies currently available. Patients with asymptomatic early tumours (stages 0–A) are candidates for radical therapies (resection, transplantation or local ablation). Asymptomatic patients with multinodular HCC (stage B) are suitable for transcatheter arterial chemoembolization (TACE), whereas patients with advanced symptomatic tumours and/or an invasive tumoural pattern (stage C) are candidates to receive sorafenib. End-stage disease (stage D) includes patients with poor prognosis that should be treated by best supportive care. *Patients in Child-Pugh class C should be first considered for liver transplantation. **Treatment stage migration: Consider the next efficacious treatment in the algorithm when previous therapies fail. BCLC, Barcelona Clinic Liver Cancer; DDLT, deceased donor liver transplantation; EASL, European Association for the Study of Liver Disease; EORTC, European Organisation for Research and Treatment of Cancer; GRADE, grading of recommendations assessment, development and evaluation; HCC, hepatocellular carcinoma; LDLT, living donor liver transplantation; PEI, percutaneous ethanol injection; RF, radiofrequency ablation; TACE, transcatheter arterial chemoembolization; OS, overall survival; (Modified from[3,158,159]).

Figure 5. Molecular targeted therapies for HCC and their target signalling pathways. Summary of treatments tested in Phase II-III clinical trials. Orange receptors have tyrosine kinase activity and red receptors have serine/threonine kinase activity. Green boxes contain drugs with positive Phase III studies, red boxes contain drugs with negative results from Phase III trials and drugs in grey boxes have been tested in phase II studies. AA3R, adenosine A(3) receptor; AKT, protein kinase B; c-Kit, mast/stem cell growth factor receptor; AR, androgen receptor; CDK, cyclin dependent kinase; CTLA-4, cytotoxic T-lymphocyte protein 4; SDF1, stromal cell-derived factor 1; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; ERK, mitogen activated protein kinase kinase kinase; HDAC, histone deacetylase; HGF, hepatocyte growth factor;MEK, mitogen-activated protein kinase kinase; MET, hepatocyte growth factor receptor; mTOR, mammalian target of rapamycin; MYC, myc proto-oncogene protein; PD-1, programmed cell death protein 1; PD-L1, programmed cell death protein 1 ligand 1; PDGF, platelet-derived growth factor; PDGFR, platelet-derived growth factor receptor; PI3K, phosphatidylinositol-4,5-bisphosphate 3-kinase; PLK1, serine/threonine-protein kinase PLK1; RAF, Raf family protein; RAS, Ras family protein; STAT3, signal transducer and activator of transcription 3; TAF, tumour-associated fibroblast; TAM: tumour-associated macrophage; TGFβ; transforming growth factor beta; TGFβR1, transforming growth factor beta receptor type-1; TIE2, angiopoietin-1 receptor; VEGF, vascular endothelial growth factor.

References

1. Torre, L. *et al.* Global cancer statistics, 2012. *CA Cancer J Clin* **65,** 87–108 (2015).

2. Global, regional, and national age–sex specific all-cause and cause-specific mortality for 240 causes of death, 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet* **385,** 117–71 (2014).

3. EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. *J. Hepatol.* **56,** 908–43 (2012).

4. Liu, J. & Fan, D. Hepatitis B in China. *Lancet* **369,** 1582–1583 (2007).

5. Mohd Hanafiah, K., Groeger, J., Flaxman, A. D. & Wiersma, S. T. Global epidemiology of hepatitis C virus infection: new estimates of age-specific antibody to HCV seroprevalence. *Hepatology* **57,** 1333–42 (2013).

6. Mohamoud, Y. A., Mumtaz, G. R., Riome, S., Miller, D. & Abu-Raddad, L. J. The epidemiology of hepatitis C virus in Egypt: a systematic review and data synthesis. *BMC Infect. Dis.* **13,** 288 (2013).

7. El-Serag, H. B. Hepatocellular carcinoma. *N. Engl. J. Med.* **365,** 1118–27 (2011).

8. Hyattsville, M. Health, United States, 2014: With Special Feature on Adults Aged 55–64. *Natl. Cent. Heal. Stat.* (2015).

9. Omer, R. E. *et al.* Population-attributable risk of dietary aflatoxins and hepatitis B virus infection with respect to hepatocellular carcinoma. *Nutr. Cancer* **48,** 15–21 (2004).

10. Laursen, L. A preventable cancer. *Nature* **516,** S2–3 (2014).

11. Sartorius, K., Sartorius, B., Aldous, C., Govender, P. S. & Madiba, T. E. Global and country underestimation of hepatocellular carcinoma (HCC) in 2012 and its implications. *Cancer Epidemiol.* **39,** 284–290 (2015).

12. Ferlay, J. *et al.* Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int. J. Cancer* **136,** E359–86 (2015).

13. Nault, J.-C. *et al.* Recurrent AAV2-related insertional mutagenesis in human hepatocellular carcinomas. *Nat. Genet.* **10,** 1187–93 (2015).

14. Zucman-Rossi, J., Villanueva, A., Nault, J.-C. & Llovet, J. M. The genetic landscape and biomarkers of hepatocellular carcinoma. *Gastroenterology* **5,** 1226–1239 (2015).

15. Bruix, J. & Sherman, M. Management of hepatocellular carcinoma: An update. *Hepatology* **53,** 1020–1022 (2011).

16. Forner, A., Llovet, J. M. & Bruix, J. Hepatocellular carcinoma. *Lancet* **379,** 1245–1255 (2012).

17. Llovet, J. M. *et al.* Arterial embolisation or chemoembolisation versus symptomatic treatment in patients with unresectable hepatocellular carcinoma: a randomised controlled trial. *Lancet* **359,** 1734–9 (2002).

18. Llovet, J. M. & Bruix, J. Systematic review of randomized trials for unresectable hepatocellular carcinoma: Chemoembolization improves survival. *Hepatology* **37,** 429–442 (2003).

19. Llovet, J. M. *et al.* Sorafenib in advanced hepatocellular carcinoma. *N. Engl. J. Med.* **359,** 378–90 (2008).

20. Yang, J. D. *et al.* Cirrhosis is present in most patients with hepatitis B and hepatocellular carcinoma. *Clin. Gastroenterol. Hepatol.* **9,** 64–70 (2011).

21. Lok, A. S. *et al.* Incidence of hepatocellular carcinoma and associated risk factors in hepatitis C-related advanced liver disease. *Gastroenterology* **136,** 138–48 (2009).

22. Hsu, I. C. *et al.* Mutational hotspot in the p53 gene in human hepatocellular carcinomas. *Nature* **350,** 427–428 (1991).

23. Bruix, J. & Sherman, M. Management of hepatocellular carcinoma. *Hepatology* **42,** 1208–1236 (2005).

24. Yang, D. *et al.* Impact of sex on the survival of patients with hepatocellular carcinoma: a Surveillance, Epidemiology, and End Results analysis. *Cancer* **120,** 3707–3716 (2014).

25. Mittal, S. *et al.* Temporal trends of nonalcoholic fatty liver disease-related hepatocellular carcinoma in the veteran affairs population. *Clin. Gastroenterol. Hepatol.* **13,** 594–601 e1 (2015).

26. Chang, M. H. *et al.* Decreased incidence of hepatocellular carcinoma in hepatitis B vaccinees: a 20-year follow-up study. *J. Natl. Cancer Inst.* **101,** 1348–1355 (2009).

27. Wong, G. L. *et al.* Entecavir treatment reduces hepatic events and deaths in chronic hepatitis B patients with liver cirrhosis. *Hepatology* **58,** 1537–1547 (2013).

28. Singal, A. G., Volk, M. L., Jensen, D., Di Bisceglie, A. M. & Schoenfeld, P. S. A sustained viral response is associated with reduced liver-related morbidity and mortality in patients with hepatitis C virus. *Clin. Gastroenterol. Hepatol.* **8,** 280–8, 288 e1 (2010).

29. Singh, S., Singh, P. P., Singh, A. G., Murad, M. H. & Sanchez, W. Statins are associated with a reduced risk of hepatocellular cancer: a systematic review and meta-analysis. *Gastroenterology* **144,** 323–332 (2013).

30. Marquardt, J. U., Andersen, J. B. & Thorgeirsson, S. S. Functional and genetic deconstruction of the cellular origin in liver cancer. *Nat. Rev. Cancer* **15,** 653–67 (2015).

31. Alizadeh, A. A. *et al.* Toward understanding and exploiting tumor heterogeneity. *Nat. Med.* **21,** 846–853 (2015).

32. Guichard, C. *et al.* Integrated analysis of somatic mutations and focal copy-number changes identifies key genes and pathways in hepatocellular carcinoma. *Nat. Genet.* **44,** 694–8 (2012).

33. Schulze, K. *et al.* Exome sequencing of hepatocellular carcinomas identifies new mutational signatures and potential therapeutic targets. *Nat. Genet.* **47,** (2015).

34. Nault, J. C. *et al.* High frequency of telomerase reverse-transcriptase promoter somatic mutations in hepatocellular carcinoma and preneoplastic lesions. *Nat. Commun.* **4,** 2218 (2013).

35. Ahn, S.-M. *et al.* Genomic portrait of resectable hepatocellular carcinomas: implications of RB1 and FGF19 aberrations for patient stratification. *Hepatology* **60,** 1972–82 (2014).

36. Nault, J. C. *et al.* Telomerase reverse transcriptase promoter mutation is an early somatic genetic alteration in the transformation of premalignant nodules in hepatocellular carcinoma on cirrhosis. *Hepatology* **60,** 1983–92 (2014).

37. Pilati, C. *et al.* Genomic profiling of hepatocellular adenomas reveals recurrent FRK-activating mutations and the mechanisms of malignant transformation. *Cancer Cell* **25,** 428–41 (2014).

38. Totoki, Y. *et al.* Trans-ancestry mutational landscape of hepatocellular carcinoma genomes. *Nat. Genet.* **46,** 1267–73 (2014).

39. Di Tommaso, L. *et al.* Diagnostic value of HSP70, glypican 3, and glutamine synthetase in hepatocellular nodules in cirrhosis. *Hepatology* **45,** 725–34 (2007).

40. Di Tommaso, L. *et al.* The application of markers (HSP70 GPC3 and GS) in liver biopsies is useful for detection of hepatocellular carcinoma. *J. Hepatol.* **50,** 746–54 (2009).

41. Llovet, J. M. *et al.* A molecular signature to discriminate dysplastic nodules from early hepatocellular carcinoma in HCV cirrhosis. *Gastroenterology* **131,** 1758–67 (2006).

42. Wurmbach, E. *et al.* Genome-wide molecular profiles of HCV-induced dysplasia and hepatocellular carcinoma. *Hepatology* **45,** 938–47 (2007).

43. Paradis, V. *et al.* Molecular profiling of hepatocellular carcinomas (HCC) using a large-scale real-time RT-PCR approach: determination of a molecular diagnostic index. *Am. J. Pathol.* **163,** 733–41 (2003).

44. Günes, C. & Rudolph, K. L. The role of telomeres in stem cells and cancer. *Cell* **152,** 390–3 (2013).

45. Satyanarayana, A. *et al.* Mitogen stimulation cooperates with telomere shortening to activate DNA damage responses and senescence signaling. *Mol. Cell. Biol.* **24,** 5459–74 (2004).

46. Farazi, P. A. *et al.* Differential impact of telomere dysfunction on initiation and progression of hepatocellular carcinoma. *Cancer Res.* **63,** 5021–7 (2003).

47. Hartmann, D. *et al.* Telomerase gene mutations are associated with cirrhosis formation. *Hepatology* **53,** 1608–17 (2011).

48. Calado, R. T. *et al.* Constitutional telomerase mutations are genetic risk factors for cirrhosis. *Hepatology* **53,** 1600–7 (2011).

49. Rudolph, K. L., Chang, S., Millard, M., Schreiber-Agus, N. & DePinho, R. A. Inhibition of experimental liver cirrhosis in mice by telomerase gene delivery. *Science* **287,** 1253–8 (2000).

50. Lechel, A. *et al.* Telomerase deletion limits progression of p53-mutant hepatocellular carcinoma with short telomeres in chronic liver disease. *Gastroenterology* **132,** 1465–75 (2007).

51. Kotoula, V. *et al.* Expression of human telomerase reverse transcriptase in regenerative and precancerous lesions of cirrhotic livers. *Liver* **22,** 57–69 (2002).

52. Bartosch, B., Thimme, R., Blum, H. E. & Zoulim, F. Hepatitis C virus-induced hepatocarcinogenesis. *J. Hepatol.* **51,** 810–20 (2009).

53. Neuveut, C., Wei, Y. & Buendia, M. A. Mechanisms of HBV-related hepatocarcinogenesis. *J. Hepatol.* **52,** 594–604 (2010).

54. Wang, J., Chenivesse, X., Henglein, B. & Bréchot, C. Hepatitis B virus integration in a cyclin A gene in a hepatocellular carcinoma. *Nature* **343,** 555–7 (1990).

55. Sung, W.-K. *et al.* Genome-wide survey of recurrent HBV integration in hepatocellular carcinoma. *Nat. Genet.* **44,** 765–9 (2012).

56. De La Coste, A. *et al.* Somatic mutations of the beta-catenin gene are frequent in mouse and human hepatocellular carcinomas. *Proc. Natl. Acad. Sci. U. S. A.* **95,** 8847–51 (1998).

57. Audard, V. *et al.* Cholestasis is a marker for hepatocellular carcinomas displaying beta-catenin mutations. *J. Pathol.* **212,** 345–52 (2007).

58. Bressac, B., Kew, M., Wands, J. & Ozturk, M. Selective G to T mutations of p53 gene in hepatocellular carcinoma from southern Africa. *Nature* **350,** 429–31 (1991).

59. Amaddeo, G. *et al.* Integration of tumour and viral genomic characterizations in HBV-related hepatocellular carcinomas. *Gut* **64,** 820–9 (2015).

60. Fei, Q. *et al.* Histone methyltransferase SETDB1 regulates liver cancer cell growth through methylation of p53. *Nat. Commun.* **6,** 8651 (2015).

61. Wong, C.-M. *et al.* Up-regulation of histone methyltransferase SETDB1 by multiple mechanisms in hepatocellular carcinoma promotes cancer metastasis. *Hepatology* (2015).

62. Villanueva, A. *et al.* DNA methylation-based prognosis and epidrivers in hepatocellular carcinoma. *Hepatology* **61,** 1945–56 (2015).

63. Herceg, Z. & Paliwal, A. Epigenetic mechanisms in hepatocellular carcinoma: how environmental factors influence the epigenome. *Mutat. Res.* **727,** 55–61 (2011).

64. Sporn, M. B. & Liby, K. T. NRF2 and cancer: the good, the bad and the importance of context. *Nat. Rev. Cancer* **12,** 564–71 (2012).

65. Chiang, D. Y. *et al.* Focal gains of VEGFA and molecular classification of hepatocellular carcinoma. *Cancer Res.* **68,** 6779–6788 (2008).

66. Sawey, E. T. *et al.* Identification of a therapeutic strategy targeting amplified FGF19 in liver cancer by Oncogenomic screening. *Cancer Cell* **19,** 347–58 (2011).

67. Horwitz, E. *et al.* Human and mouse VEGFA-amplified hepatocellular carcinomas are highly sensitive to Sorafenib treatment. *Cancer Discov.* **4,** 730–743 (2014).

68. Rudalska, R. *et al.* In vivo RNAi screening identifies a mechanism of sorafenib resistance in liver cancer. *Nat. Med.* **20,** 1138–46 (2014).

69. Weber, J. *et al.* CRISPR/Cas9 somatic multiplex-mutagenesis for high-throughput functional cancer genomics in mice. *Proc. Natl. Acad. Sci. U. S. A.* **112,** 13982–7 (2015).

70. Hoshida, Y. *et al.* Molecular classification and novel targets in hepatocellular carcinoma: recent advancements. *Semin. Liver Dis.* **30,** 35–51 (2010).

71. Boyault, S. *et al.* Transcriptome classification of HCC is related to gene alterations and to new therapeutic targets. *Hepatology* **45,** 42–52 (2007).

72. Lee, J.-S. *et al.* Classification and prediction of survival in hepatocellular carcinoma by gene expression profiling. *Hepatology* **40,** 667–76 (2004).

73. Lee, J.-S. *et al.* A novel prognostic subtype of human hepatocellular carcinoma derived from hepatic progenitor cells. *Nat. Med.* **12,** 410–6 (2006).

74. Hoshida, Y. *et al.* Integrative transcriptome analysis reveals common molecular subclasses of human hepatocellular carcinoma. *Cancer Res.* **69,** 7385–92 (2009).

75. Lachenmayer, A. *et al.* Wnt-pathway activation in two molecular classes of hepatocellular carcinoma and experimental modulation by sorafenib. *Clin. Cancer Res.* **18,** 4997–5007 (2012).

76. Hanahan, D. & Weinberg, R. A. Hallmarks of cancer: the next generation. *Cell* **144,** 646–674 (2011).

77. Grivennikov, S. I., Greten, F. R. & Karin, M. Immunity, inflammation, and cancer. *Cell* **140,** 883–899 (2010).

78. Dvorak, H. F. Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing. *N Engl J Med* **315,** 1650–1659 (1986).

79. Hernandez-Gea, V., Toffanin, S., Friedman, S. L. & Llovet, J. M. Role of the microenvironment in the pathogenesis and treatment of hepatocellular carcinoma. *Gastroenterology* **144,** 512–527 (2013).

80. Wada, Y., Nakashima, O., Kutami, R., Yamamoto, O. & Kojiro, M. Clinicopathological study on hepatocellular carcinoma with lymphocytic infiltration. *Hepatology* **27,** 407–414 (1998).

81. Hoshida, Y. *et al.* Gene expression in fixed tissues and outcome in hepatocellular carcinoma. *N Engl J Med* **359,** 1995–2004 (2008).

82. Finkin, S. *et al.* Ectopic lymphoid structures function as microniches for tumor progenitor cells in hepatocellular carcinoma. *Nat. Immunol.* **16,** 1235–44 (2015).

83. Crispe, I. N. The liver as a lymphoid organ. *Annu Rev Immunol* **27,** 147–163 (2009).

84. Thomson, A. W. & Knolle, P. A. Antigen-presenting cell function in the tolerogenic liver environment. *Nat Rev Immunol* **10,** 753–766 (2010).

85. Pikarsky, E. *et al.* NF-kappaB functions as a tumour promoter in inflammation-associated cancer. *Nature* **431,** 461–6 (2004).

86. Bauer, J. *et al.* Lymphotoxin, NF-kB, and cancer: the dark side of cytokines. *Dig Dis* **30,** 453–468 (2012).

87. Taniguchi, K. & Karin, M. IL-6 and related cytokines as the critical lynchpins between inflammation and cancer. *Semin Immunol* **26,** 54–74 (2014).

88. LeCouter, J. *et al.* Angiogenesis-independent endothelial protection of liver: role of VEGFR-1. *Science (80-.).* **299,** 890–893 (2003).

89. Hussain, S. P., Hofseth, L. J. & Harris, C. C. Radical causes of cancer. *Nat Rev Cancer* **3,** 276–285 (2003).

90. Kiraly, O., Gong, G., Olipitz, W., Muthupalani, S. & Engelward, B. P. Inflammation-induced cell proliferation potentiates DNA damage-induced mutations in vivo. *PLoS Genet* **11,** e1004901 (2015).

91. Zhang, D. Y. *et al.* A hepatic stellate cell gene expression signature associated with outcomes in hepatitis C cirrhosis and hepatocellular carcinoma after curative resection. *Gut* (2015).

92. Campbell, J. S. *et al.* Platelet-derived growth factor C induces liver fibrosis, steatosis, and hepatocellular carcinoma. *Proc. Natl. Acad. Sci. U. S. A.* **102,** 3389–94 (2005).

93. Dapito, D. H. & Schwabe, R. F. *Hepatic Stellate Cells and Liver Cancer*. *Stellate Cells in Health and Disease* (Elsevier, 2015).

94. Lee, Y. A., Wallace, M. C. & Friedman, S. L. Pathobiology of liver fibrosis: a translational success story. *Gut* **64,** 830–41 (2015).

95. Chang, M. H. *et al.* Universal hepatitis B vaccination in Taiwan and the incidence of hepatocellular carcinoma in children. Taiwan Childhood Hepatoma Study Group. *N. Engl. J. Med.* **336,** 1855–9 (1997).

96. Yuen, M.-F. *et al.* Independent risk factors and predictive score for the development of hepatocellular carcinoma in chronic hepatitis B. *J. Hepatol.* **50,** 80–8 (2009).

97. Wong, V. W.-S. *et al.* Clinical scoring system to predict hepatocellular carcinoma in chronic hepatitis B carriers. *J. Clin. Oncol.* **28,** 1660–5 (2010).

98. Wong, G. L.-H. *et al.* Liver stiffness-based optimization of hepatocellular carcinoma risk score in patients with chronic hepatitis B. *J. Hepatol.* **60,** 339–45 (2014).

99. Chen, C.-J. Risk of Hepatocellular Carcinoma Across a Biological Gradient of Serum Hepatitis B Virus DNA Level. *JAMA* **295,** 65 (2006).

100. Yang, H.-I. *et al.* Nomograms for risk of hepatocellular carcinoma in patients with chronic hepatitis B virus infection. *J. Clin. Oncol.* **28,** 2437–44 (2010).

101. Yang, H.-I. *et al.* Risk estimation for hepatocellular carcinoma in chronic hepatitis B (REACH-B): development and validation of a predictive score. *Lancet. Oncol.* **12,** 568–74 (2011).

102. Lee, M.-H. *et al.* Prediction models of long-term cirrhosis and hepatocellular carcinoma risk in chronic hepatitis B patients: risk scores integrating host and virus profiles. *Hepatology* **58,** 546–54 (2013).

103. Wong, G. L.-H. *et al.* Accuracy of risk scores for patients with chronic hepatitis B receiving entecavir treatment. *Gastroenterology* **144,** 933–44 (2013).

104. Arends, P. *et al.* Entecavir treatment does not eliminate the risk of hepatocellular carcinoma in chronic hepatitis B: limited role for risk scores in Caucasians. *Gut* **64,** 1289–95 (2015).

105. Liaw, Y.-F. *et al.* Lamivudine for patients with chronic hepatitis B and advanced liver disease. *N. Engl. J. Med.* **351,** 1521–31 (2004).

106. Papatheodoridis, G. V *et al.* Incidence and predictors of hepatocellular carcinoma in Caucasian chronic hepatitis B patients receiving entecavir or tenofovir. *J. Hepatol.* **62,** 363–70 (2015).

107. Ogawa, E. *et al.* Efficacy of pegylated interferon alpha-2b and ribavirin treatment on the risk of hepatocellular carcinoma in patients with chronic hepatitis C: a prospective, multicenter study. *J. Hepatol.* **58,** 495–501 (2013).

108. Harada, N. *et al.* Risk factors for hepatocellular carcinoma in hepatitis C patients with normal alanine aminotransferase treated with pegylated interferon and ribavirin. *J. Viral Hepat.* **21,** 357–65 (2014).

109. Dohmen, K. *et al.* The incidence and risk factors for the development of hepatocellular carcinoma after peginterferon plus ribavirin therapy for chronic hepatitis C. *Hepatogastroenterology.* **60,** 2034–8 (2013).

110. Van der Meer, A. J. *et al.* Association between sustained virological response and all-cause mortality among patients with chronic hepatitis C and advanced hepatic fibrosis. *JAMA* **308,** 2584–93 (2012).

111. Morgan, R. L. *et al.* Eradication of hepatitis C virus infection and the development of hepatocellular carcinoma: a meta-analysis of observational studies. *Ann. Intern. Med.* **158,** 329–37 (2013).

112. Foster, G. R. *et al.* Sofosbuvir and Velpatasvir for HCV Genotype 2 and 3 Infection. *N. Engl. J. Med.* **373,** 2608–17 (2015).

113. Singal, A. G. & El-Serag, H. B. Hepatocellular Carcinoma from Epidemiology to Prevention: Translating Knowledge into Practice. *Clin. Gastroenterol. Hepatol.* **13,** 2140–51 (2015).

114. Younossi, Z. M. *et al.* The impact of hepatitis C burden: an evidence-based approach. *Aliment. Pharmacol. Ther.* **39,** 518–31 (2014).

115. Bosch, J. & Forns, X. Therapy. Statins and liver disease: from concern to 'wonder' drugs? *Nat. Rev. Gastroenterol. Hepatol.* **12,** 320–1 (2015).

116. Zhang, H., Gao, C., Fang, L., Zhao, H.-C. & Yao, S.-K. Metformin and reduced risk of hepatocellular carcinoma in diabetic patients: a meta-analysis. *Scand. J. Gastroenterol.* **48,** 78–87 (2013).

117. Zhang, X. *et al.* Continuation of metformin use after a diagnosis of cirrhosis significantly improves survival of patients with diabetes. *Hepatology* **60,** 2008–16 (2014).

118. Zhang, B.-H., Yang, B.-H. & Tang, Z.-Y. Randomized controlled trial of screening for hepatocellular carcinoma. *J. Cancer Res. Clin. Oncol.* **130,** 417–22 (2004).

119. Yeh, Y.-P. *et al.* Evaluation of abdominal ultrasonography mass screening for hepatocellular carcinoma in Taiwan. *Hepatology* **59,** 1840–9 (2014).

120. Tong, M. J., Sun, H.-E., Hsien, C. & Lu, D. S. K. Surveillance for hepatocellular carcinoma improves survival in Asian-American patients with hepatitis B: results from a community-based clinic. *Dig. Dis. Sci.* **55,** 826–35 (2010).

121. Tanaka, H. *et al.* Surveillance of hepatocellular carcinoma in patients with hepatitis C virus infection may improve patient survival. *Liver Int.* **26,** 543–51 (2006).

122. Wong, G. L.-H. *et al.* Surveillance programme for hepatocellular carcinoma improves the survival of patients with chronic viral hepatitis. *Liver Int.* **28,** 79–87 (2008).

123. Taura, N. *et al.* Clinical benefits of hepatocellular carcinoma surveillance: a single-center, hospital-based study. *Oncol. Rep.* **14,** 999–1003 (2005).

124. Chan, A. C. Y. *et al.* Changing paradigm in the management of hepatocellular carcinoma improves the survival benefit of early detection by screening. *Ann. Surg.* **247,** 666–73 (2008).

125. Nouso, K. *et al.* Cost-effectiveness of the surveillance program of hepatocellular carcinoma depends on the medical circumstances. *J. Gastroenterol. Hepatol.* **23,** 437–44 (2008).

126. Lin, O. S., Keeffe, E. B., Sanders, G. D. & Owens, D. K. Cost-effectiveness of screening for hepatocellular carcinoma in patients with cirrhosis due to chronic hepatitis C. *Aliment. Pharmacol. Ther.* **19,** 1159–72 (2004).

127. Arguedas, M. R., Chen, V. K., Eloubeidi, M. A. & Fallon, M. B. Screening for hepatocellular carcinoma in patients with hepatitis C cirrhosis: a cost-utility analysis. *Am. J. Gastroenterol.* **98,** 679–90 (2003).

128. Andersson, K. L., Salomon, J. A., Goldie, S. J. & Chung, R. T. Cost effectiveness of alternative surveillance strategies for hepatocellular carcinoma in patients with cirrhosis. *Clin. Gastroenterol. Hepatol.* **6,** 1418–24 (2008).

129. Cucchetti, A. *et al.* Cost-effectiveness of semi-annual surveillance for hepatocellular carcinoma in cirrhotic patients of the Italian Liver Cancer population. *J. Hepatol.* **56,** 1089–96 (2012).

130. Sarasin, F. P., Giostra, E. & Hadengue, A. Cost-effectiveness of screening for detection of small hepatocellular carcinoma in western patients with Child-Pugh class A cirrhosis. *Am. J. Med.* **101,** 422–34 (1996).

131. Thompson Coon, J. *et al.* Surveillance of cirrhosis for hepatocellular carcinoma: systematic review and economic analysis. *Health Technol. Assess.* **11,** 1–206 (2007).

132. Saab, S. *et al.* Hepatocellular carcinoma screening in patients waiting for liver transplantation: a decision analytic model. *Liver Transpl.* **9,** 672–81 (2003).

133. Naugler, W. E. & Sonnenberg, A. Survival and cost-effectiveness analysis of competing strategies in the management of small hepatocellular carcinoma. *Liver Transpl.* **16,** 1186–94 (2010).

134. Thompson Coon, J. *et al.* Surveillance of cirrhosis for hepatocellular carcinoma: a cost-utility analysis. *Br. J. Cancer* **98,** 1166–75 (2008).

135. Patel, D., Terrault, N. A., Yao, F. Y., Bass, N. M. & Ladabaum, U. Cost-effectiveness of hepatocellular carcinoma surveillance in patients with hepatitis C virus-related cirrhosis. *Clin. Gastroenterol. Hepatol.* **3,** 75–84 (2005).

136. Kang, J. Y., Lee, T. P., Yap, I. & Lun, K. C. Analysis of cost-effectiveness of different strategies for hepatocellular carcinoma screening in hepatitis B virus carriers. *J. Gastroenterol. Hepatol.* **7,** 463–8 (1992).

137. Shih, S. T.-F., Crowley, S. & Sheu, J.-C. Cost-effectiveness analysis of a two-stage screening intervention for hepatocellular carcinoma in Taiwan. *J. Formos. Med. Assoc.* **109,** 39–55 (2010).

138. Marrero, J. A. *et al.* Alpha-fetoprotein, des-gamma carboxyprothrombin, and lectin-bound alpha-fetoprotein in early hepatocellular carcinoma. *Gastroenterology* **137,** 110–8 (2009).

139. Asaoka, Y. *et al.* Frequency of and predictive factors for vascular invasion after radiofrequency ablation for hepatocellular carcinoma. *PLoS One* **9,** e111662 (2014).

140. Kudo, A. *et al.* Does the preoperative alpha-fetoprotein predict the recurrence and mortality after hepatectomy for hepatocellular carcinoma without macrovascular invasion in patients with normal liver function? *Hepatol. Res.* **44,** E437–46 (2014).

141. Shirabe, K. *et al.* New scoring system for prediction of microvascular invasion in patients with hepatocellular carcinoma. *Liver Int.* **34,** 937–41 (2014).

142. Park, H. *et al.* Clinical usefulness of double biomarkers AFP and PIVKA-II for subdividing prognostic groups in locally advanced hepatocellular carcinoma. *Liver Int.* **34,** 313–21 (2014).

143. Nakazawa, T. *et al.* Early increase in α-fetoprotein for predicting unfavorable clinical outcomes in patients with advanced hepatocellular carcinoma treated with sorafenib. *Eur. J. Gastroenterol. Hepatol.* **25,** 683–9 (2013).

144. Park, W.-H. *et al.* Clinical utility of des-γ-carboxyprothrombin kinetics as a complement to radiologic response in patients with hepatocellular carcinoma undergoing transarterial chemoembolization. *J. Vasc. Interv. Radiol.* **23,** 927–36 (2012).

145. Han, K.-H. *et al.* Survival of hepatocellular carcinoma patients may be improved in surveillance interval not more than 6 months compared with more than 6 months: a 15-year prospective study. *J. Clin. Gastroenterol.* **47,** 538–44 (2013).

146. Trinchet, J.-C. *et al.* Ultrasonographic surveillance of hepatocellular carcinoma in cirrhosis: a randomized trial comparing 3- and 6-month periodicities. *Hepatology* **54,** 1987–97 (2011).

147. Santi, V. *et al.* Semiannual surveillance is superior to annual surveillance for the detection of early hepatocellular carcinoma and patient survival. *J. Hepatol.* **53,** 291–7 (2010).

148. Wang, J.-H. *et al.* Hepatocellular carcinoma surveillance at 4- vs. 12-month intervals for patients with chronic viral hepatitis: a randomized study in community. *Am. J. Gastroenterol.* **108,** 416–24 (2013).

149. Mitchell, D. G., Bruix, J., Sherman, M. & Sirlin, C. B. LI-RADS (Liver Imaging Reporting and Data System): summary, discussion, and consensus of the LI-RADS Management Working Group and future directions. *Hepatology* **61,** 1056–65 (2015).

150. Khalili, K. *et al.* Optimization of imaging diagnosis of 1-2 cm hepatocellular carcinoma: an analysis of diagnostic performance and resource utilization. *J. Hepatol.* **54,** 723–8 (2011).

151. Sangiovanni, A. *et al.* The diagnostic and economic impact of contrast imaging techniques in the diagnosis of small hepatocellular carcinoma in cirrhosis. *Gut* **59,** 638–44 (2010).

152. Lencioni, R., Cioni, D., Della Pina, C., Crocetti, L. & Bartolozzi, C. Imaging diagnosis. *Semin. Liver Dis.* **25,** 162–70 (2005).

153. Silva, M. A. *et al.* Needle track seeding following biopsy of liver lesions in the diagnosis of hepatocellular cancer: a systematic review and meta-analysis. *Gut* **57,** 1592–6 (2008).

154. Pathologic diagnosis of early hepatocellular carcinoma: a report of the international consensus group for hepatocellular neoplasia. *Hepatology* **49,** 658–64 (2009).

155. Libbrecht, L. *et al.* Glypican-3 expression distinguishes small hepatocellular carcinomas from cirrhosis, dysplastic nodules, and focal nodular hyperplasia-like nodules. *Am. J. Surg. Pathol.* **30,** 1405–11 (2006).

156. Tremosini, S. *et al.* Prospective validation of an immunohistochemical panel (glypican 3, heat shock protein 70 and glutamine synthetase) in liver biopsies for diagnosis of very early hepatocellular carcinoma. *Gut* **61,** 1481–7 (2012).

157. Di Tommaso, L. *et al.* Diagnostic accuracy of clathrin heavy chain staining in a marker panel for the diagnosis of small hepatocellular carcinoma. *Hepatology* **53,** 1549–1557 (2011).

158. Bruix, J., Han, K., Gores, G., Llovet, J. M. & Mazzaferro, V. Liver cancer: Approaching a personalized care. *J. Hepatol.* **62,** 144–156 (2015).

159. Llovet, J. M., Brú, C. & Bruix, J. Prognosis of hepatocellular carcinoma: the BCLC staging classification. *Semin. Liver Dis.* **19,** 329–38 (1999).

160. Yau, T. *et al.* Development of Hong Kong Liver Cancer staging system with treatment stratification for patients with hepatocellular carcinoma. *Gastroenterology* **146,** 1691–700.e3 (2014).

161. A new prognostic system for hepatocellular carcinoma: a retrospective study of 435 patients: the Cancer of the Liver Italian Program (CLIP) investigators. *Hepatology* **28,** 751–5 (1998).

162. Sobin, L. H. & Compton, C. C. TNM seventh edition: what's new, what's changed: communication from the International Union Against Cancer and the American Joint Committee on Cancer. *Cancer* **116,** 5336–9 (2010).

163. Kudo, M., Chung, H. & Osaki, Y. Prognostic staging system for hepatocellular carcinoma (CLIP score): its value and limitations, and a proposal for a new staging system, the Japan Integrated Staging Score (JIS score). *J. Gastroenterol.* **38,** 207–15 (2003).

164. Bruix, J. *et al.* Surgical resection of hepatocellular carcinoma in cirrhotic patients: prognostic value of preoperative portal pressure. *Gastroenterology* **111,** 1018–22 (1996).

165. Llovet, J. M., Schwartz, M. & Mazzaferro, V. Resection and Liver Transplantation for Hepatocellular Carcinoma. *Semin Liver Dis* **25,** 181–200 (2005).

166. Llovet, J. M., Fuster, J. & Bruix, J. Intention-to-treat analysis of surgical treatment for early hepatocellular carcinoma: resection versus transplantation. *Hepatology* **30,** 1434–1440 (1999).

167. Imamura, H. *et al.* Risk factors contributing to early and late phase intrahepatic recurrence of hepatocellular carcinoma after hepatectomy. *J. Hepatol.* **38,** 200–7 (2003).

168. Bruix, J. *et al.* Adjuvant sorafenib for hepatocellular carcinoma after resection or ablation (STORM): a phase 3, randomised, double-blind, placebo-controlled trial. *Lancet. Oncol.* **16,** 1344–54 (2015).

169. Takayama, T. *et al.* Adoptive immunotherapy to lower postsurgical recurrence rates of hepatocellular carcinoma: a randomised trial. *Lancet* **356,** 802–7 (2000).

170. Muto, Y. *et al.* Prevention of second primary tumors by an acyclic retinoid, polyprenoic acid, in patients with hepatocellular carcinoma. Hepatoma Prevention Study Group. *N. Engl. J. Med.* **334,** 1561–1567 (1996).

171. Mazzaferro, V. *et al.* Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. *N. Engl. J. Med.* **334,** 693–9 (1996).

172. Mazzaferro, V. *et al.* Predicting survival after liver transplantation in patients with hepatocellular carcinoma beyond the Milan criteria: a retrospective, exploratory analysis. *Lancet. Oncol.* **10,** 35–43 (2009).

173. Toso, C. *et al.* Total tumor volume and alpha-fetoprotein for selection of transplant candidates with hepatocellular carcinoma: A prospective validation. *Hepatology* **62,** 158–65 (2015).

174. Miltiadous, O. *et al.* Progenitor cell markers predict outcome of patients with Hepatocellular Carcinoma beyond Milan criteria undergoing liver transplantation. *J. Hepatol.* (2015).

175. Livraghi, T. *et al.* Sustained complete response and complications rates after radiofrequency ablation of very early hepatocellular carcinoma in cirrhosis: Is resection still the treatment of choice? *Hepatology* **47,** 82–89 (2008).

176. Lo, C.-M. *et al.* Randomized controlled trial of transarterial lipiodol chemoembolization for unresectable hepatocellular carcinoma. *Hepatology* **35,** 1164–71 (2002).

177. Kudo, M. *et al.* Brivanib as adjuvant therapy to transarterial chemoembolization in patients with hepatocellular carcinoma: A randomized phase III trial. *Hepatology* **60,** 1697–707 (2014).

178. Burrel, M. *et al.* Survival of patients with hepatocellular carcinoma treated by transarterial chemoembolisation (TACE) using Drug Eluting Beads. Implications for clinical practice and trial design. *J. Hepatol.* **56,** 1330–5 (2012).

179. Salem, R. *et al.* Radioembolization results in longer time-to-progression and reduced toxicity compared with chemoembolization in patients with hepatocellular carcinoma. *Gastroenterology* **140,** 497–507.e2 (2011).

180. Cheng, A.-L. *et al.* Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomised, double-blind, placebo-controlled trial. *Lancet Oncol.* **10,** 25–34 (2009).

181. Yeo, W. *et al.* A randomized phase III study of doxorubicin versus cisplatin/interferon alpha-2b/doxorubicin/fluorouracil (PIAF) combination chemotherapy for unresectable hepatocellular carcinoma. *J. Natl. Cancer Inst.* **97,** 1532–8 (2005).

182. D'Amico, G., Garcia-Tsao, G. & Pagliaro, L. Natural history and prognostic indicators of survival in cirrhosis: A systematic review of 118 studies. *J. Hepatol.* **44,** 217–231 (2006).

183. Eguchi, S. *et al.* Comparison of the outcomes between an anatomical subsegmentectomy and a non-anatomical minor hepatectomy for single hepatocellular carcinomas based on a Japanese nationwide survey. *Surgery* **143,** 469–75 (2008).

184. Palavecino, M. *et al.* Major hepatic resection for hepatocellular carcinoma with or without portal vein embolization: Perioperative outcome and survival. *Surgery* **145,** 399–405 (2009).

185. Vouche, M. *et al.* Radiation lobectomy: time-dependent analysis of future liver remnant volume in unresectable liver cancer as a bridge to resection. *J. Hepatol.* **59,** 1029–36 (2013).

186. Cherqui, D. Laparoscopic liver resection: A new paradigm in the management of hepatocellular carcinoma? *J. Hepatol.* **63,** 540–2 (2015).

187. Ishizawa, T. *et al.* Neither Multiple Tumors Nor Portal Hypertension Are Surgical Contraindications for Hepatocellular Carcinoma. *Gastroenterology* **134,** 1908–1916 (2008).

188. Franssen, B. *et al.* Differences in surgical outcomes between hepatitis B- and hepatitis C-related hepatocellular carcinoma: a retrospective analysis of a single North American center. *Ann. Surg.* **260,** 650–6; discussion 656–8 (2014).

189. Roayaie, S., Bassi, D., Tarchi, P., Labow, D. & Schwartz, M. Second hepatic resection for recurrent hepatocellular cancer: a Western experience. *J. Hepatol.* **55,** 346–50 (2011).

190. Fuks, D. *et al.* Benefit of initial resection of hepatocellular carcinoma followed by transplantation in case of recurrence: an intention-to-treat analysis. *Hepatology* **55,** 132–40 (2012).

191. Lau, W. Y. *et al.* Adjuvant intra-arterial iodine-131-labelled lipiodol for resectable hepatocellular carcinoma: a prospective randomised trial. *Lancet (London, England)* **353,** 797–801 (1999).

192. Clavien, P.-A. *et al.* Recommendations for liver transplantation for hepatocellular carcinoma: an international consensus conference report. *Lancet. Oncol.* **13,** e11–22 (2012).

193. Lesurtel, M., Müllhaupt, B., Pestalozzi, B. C., Pfammatter, T. & Clavien, P.-A. Transarterial chemoembolization as a bridge to liver transplantation for

hepatocellular carcinoma: an evidence-based analysis. *Am. J. Transplant* **6,** 2644–50 (2006).

194. Kulik, L. M. *et al.* Outcomes of living and deceased donor liver transplant recipients with hepatocellular carcinoma: results of the A2ALL cohort. *Am. J. Transplant* **12,** 2997–3007 (2012).

195. Toso, C., Merani, S., Bigam, D. L., Shapiro, A. M. J. & Kneteman, N. M. Sirolimus-based immunosuppression is associated with increased survival after liver transplantation for hepatocellular carcinoma. *Hepatology* **51,** 1237–43 (2010).

196. Schnitzbauer, A. A. *et al.* A prospective randomised, open-labeled, trial comparing sirolimus-containing versus mTOR-inhibitor-free immunosuppression in patients undergoing liver transplantation for hepatocellular carcinoma. *BMC Cancer* **10,** 190 (2010).

197. Geissler, E. K. *et al.* Sirolimus Use in Liver Transplant Recipients With Hepatocellular Carcinoma: A Randomized, Multicenter, Open-Label Phase 3 Trial. *Transplantation* (2015).

198. Vitale, A. *et al.* Barcelona Clinic Liver Cancer staging and transplant survival benefit for patients with hepatocellular carcinoma: a multicentre, cohort study. *Lancet. Oncol.* **12,** 654–62 (2011).

199. Menon, K. V, Hakeem, A. R. & Heaton, N. D. Review article: liver transplantation for hepatocellular carcinoma - a critical appraisal of the current worldwide listing criteria. *Aliment. Pharmacol. Ther.* **40,** 893–902 (2014).

200. Yao, F. Y. *et al.* Liver transplantation for hepatocellular carcinoma: expansion of the tumor size limits does not adversely impact survival. *Hepatology* **33,** 1394–403 (2001).

201. Yao, F. Y. *et al.* Excellent outcome following down-staging of hepatocellular carcinoma prior to liver transplantation: an intention-to-treat analysis. *Hepatology* **48,** 819–27 (2008).

202. Kowdley, K. V *et al.* Ledipasvir and sofosbuvir for 8 or 12 weeks for chronic HCV without cirrhosis. *N. Engl. J. Med.* **370,** 1879–88 (2014).

203. Ahmed, M. *et al.* Image-guided tumor ablation: standardization of terminology and reporting criteria--a 10-year update. *Radiology* **273,** 241–60 (2014).

204. Lencioni, R. & Crocetti, L. Image-guided ablation for hepatocellular carcinoma. *Recent Results Cancer Res* **190,** 181–94 (2013).

205. Cho YK, Kim JK, Kim MY, Rhim H, H. J. Systematic review of randomized trials for hepatocellular carcinoma treated with percutaneous ablation therapies. *Hepatology* **49,** 453–9 (2009).

206. Orlando, A., Leandro, G., Olivo, M., Andriulli, A. & Cottone, M. Radiofrequency Thermal Ablation vs. Percutaneous Ethanol Injection for Small Hepatocellular Carcinoma in Cirrhosis: Meta-Analysis of Randomized Controlled Trials. *Am. J. Gastroenterol.* **104,** 514–524 (2009).

207. Shiina, S. *et al.* Radiofrequency ablation for hepatocellular carcinoma: 10-year outcome and prognostic factors. *Am. J. Gastroenterol.* **107,** 569–77; quiz 578 (2012).

208. Salem, R. *et al.* Research reporting standards for radioembolization of hepatic malignancies. *J. Vasc. Interv. Radiol.* **22,** 265–78 (2011).

209. Bruix, J., Sala, M. & Llovet, J. M. Chemoembolization for hepatocellular carcinoma. *Gastroenterology* **127,** S179–88 (2004).

210. Raoul, J.-L. *et al.* Evolving strategies for the management of intermediate-stage hepatocellular carcinoma: available evidence and expert opinion on the use of transarterial chemoembolization. *Cancer Treat. Rev.* **37,** 212–20 (2011).

211. Golfieri, R. *et al.* Randomised controlled trial of doxorubicin-eluting beads vs conventional chemoembolisation for hepatocellular carcinoma. *Br. J. Cancer* **111,** 255–64 (2014).

212. Sangro, B. & Salem, R. Transarterial chemoembolization and radioembolization. *Semin. Liver Dis.* **34,** 435–43 (2014).

213. Lencioni, R. *et al.* Sorafenib or Placebo plus TACE with Doxorubicin-Eluting Beads for Intermediate-Stage HCC: Phase II, Randomized, Doubled-Blind SPACE Trial. *J Hepatol* **In press,** (2015).

214. Oliveri, R. S., Wetterslev, J. & Gluud, C. Transarterial (chemo)embolisation for unresectable hepatocellular carcinoma. *Cochrane database Syst. Rev.* CD004787 (2011).

215. Sangro, B., Iñarrairaegui, M. & Bilbao, J. I. Radioembolization for hepatocellular carcinoma. *J. Hepatol.* **56,** 464–73 (2012).

216. Sangro, B. *et al.* Survival after yttrium-90 resin microsphere radioembolization of hepatocellular carcinoma across Barcelona clinic liver cancer stages: a European evaluation. *Hepatology* **54,** 868–78 (2011).

217. Meyer, T. *et al.* A randomised phase II/III trial of 3-weekly cisplatin-based sequential transarterial chemoembolisation vs embolisation alone for hepatocellular carcinoma. *Br. J. Cancer* **108,** 1252–9 (2013).

218. Llovet, J. M., Villanueva, A., Lachenmayer, A. & Finn, R. S. Advances in targeted therapies for hepatocellular carcinoma in the genomic era. *Nat. Rev. Clin. Oncol.* (2015).

219. Qin, S. *et al.* Randomized, multicenter, open-label study of oxaliplatin plus fluorouracil/leucovorin versus doxorubicin as palliative chemotherapy in patients with advanced hepatocellular carcinoma from Asia. *J. Clin. Oncol.* **31,** 3501–8 (2013).

220. Bruix, J. *et al.* Efficacy and safety of sorafenib in patients with advanced hepatocellular carcinoma: subanalyses of a phase III trial. *J. Hepatol.* **57,** 821–9 (2012).

221. Llovet, J. M. *et al.* Plasma biomarkers as predictors of outcome in patients with advanced hepatocellular carcinoma. *Clin. Cancer Res.* **18,** 2290–300 (2012).

222. Wilhelm, S. M. *et al.* Preclinical overview of sorafenib, a multikinase inhibitor that targets both Raf and VEGF and PDGF receptor tyrosine kinase signaling. *Mol. Cancer Ther.* **7,** 3129–3140 (2008).

223. Llovet, J. M. *et al.* Design and endpoints of clinical trials in hepatocellular carcinoma. *J. Natl. Cancer Inst.* **100,** 698–711 (2008).

224. Johnson, P. J. *et al.* Brivanib versus sorafenib as first-line therapy in patients with unresectable, advanced hepatocellular carcinoma: results from the randomized phase III BRISK-FL study. *J. Clin. Oncol.* **31,** 3517–24 (2013).

225. Cheng, A.-L. *et al.* Sunitinib versus sorafenib in advanced hepatocellular cancer: results of a randomized phase III trial. *J. Clin. Oncol.* **31,** 4067–75 (2013).

226. Cainap, C. *et al.* Linifanib versus Sorafenib in patients with advanced hepatocellular carcinoma: results of a randomized phase III trial. *J. Clin. Oncol.* **33,** 172–9 (2015).

227. Zhu, A. X. *et al.* SEARCH: a phase III, randomized, double-blind, placebo-controlled trial of sorafenib plus erlotinib in patients with advanced hepatocellular carcinoma. *J. Clin. Oncol.* **33,** 559–66 (2015).

228. Llovet, J. M. *et al.* Brivanib in patients with advanced hepatocellular carcinoma who were intolerant to sorafenib or for whom sorafenib failed: results from the randomized phase III BRISK-PS study. *J. Clin. Oncol.* **31,** 3509–3516 (2013).

229. Zhu, A. X. *et al.* Effect of everolimus on survival in advanced hepatocellular carcinoma after failure of sorafenib: the EVOLVE-1 randomized clinical trial. *JAMA* **312,** 57–67 (2014).

230. Zhu, A. X. *et al.* Ramucirumab versus placebo as second-line treatment in patients with advanced hepatocellular carcinoma following first-line therapy with sorafenib (REACH): a randomised, double-blind, multicentre, phase 3 trial. *Lancet Oncol.* 859–870 (2015).

231. Llovet, J. M. & Hernandez-Gea, V. Hepatocellular carcinoma: reasons for phase III failure and novel perspectives on trial design. *Clin. Cancer Res.* **20,** 2072–9 (2014).

232. Lencioni, R. & Llovet, J. Modified RECIST (mRECIST) Assessment for Hepatocellular Carcinoma. *Semin. Liver Dis.* **30,** 052–060 (2010).

233. Coulouarn, C., Factor, V. M. & Thorgeirsson, S. S. Transforming growth factor-beta gene expression signature in mouse hepatocytes predicts clinical outcome in human cancer. *Hepatology* **47,** 2059–67 (2008).

234. Faivre, S. J., Santoro, A. & Kelley, R. K. A phase 2 study of a novel transforming growth factor-beta (TGF-β 1) receptor I kinase inhibitor, LY2157299 monohydrate (LY), in patients with advanced hepatocellular carcinoma. *J. Clin. Oncol* **32,** LBA173 (2014).

235. Finn, R. S. Abstract 3858: Gains in FGF19 are predictive of response to the fibroblast growth factor receptor (FGFR) small molecule tyrosine kinase inhibitor BGJ 398 in vitro. *Cancer Res.* **72,** 3858 (2012).

236. Hagel, M. *et al.* First Selective Small Molecule Inhibitor of FGFR4 for the Treatment of Hepatocellular Carcinomas with an Activated FGFR4 Signaling Pathway. *Cancer Discov.* **5,** 424–37 (2015).

237. Calvisi, D. F. *et al.* Ubiquitous Activation of Ras and Jak/Stat Pathways in Human HCC. *Gastroenterology* **130,** 1117–1128 (2006).

238. O'Neil, B. H. *et al.* Phase II study of the mitogen-activated protein kinase 1/2 inhibitor selumetinib in patients with advanced hepatocellular carcinoma. *J. Clin. Oncol.* **29,** 2350–6 (2011).

239. Lim, H. Y. *et al.* A phase II study of the efficacy and safety of the combination therapy of the MEK inhibitor refametinib (BAY 86-9766) plus sorafenib for Asian patients with unresectable hepatocellular carcinoma. *Clin. Cancer Res.* **20,** 5976–85 (2014).

240. Goyal, L., Muzumdar, M. D. & Zhu, A. X. Targeting the HGF/c-MET pathway in hepatocellular carcinoma. *Clin. Cancer Res.* **19,** 2310–8 (2013).

241. Xiang, Q. *et al.* Cabozantinib suppresses tumor growth and metastasis in hepatocellular carcinoma by a dual blockade of VEGFR2 and MET. *Clin. Cancer Res.* **20,** 2959–2970 (2014).

242. Robert, C. *et al.* Nivolumab in Previously Untreated Melanoma without BRAF Mutation. *N. Engl. J. Med.* **372,** 141116004513004 (2014).

243. Butterfield, L. H. *et al.* A phase I/II trial testing immunization of hepatocellular carcinoma patients with dendritic cells pulsed with four alpha-fetoprotein peptides. *Clin. Cancer Res.* **12,** 2817–25 (2006).

244. Palmer, D. H. *et al.* A phase II study of adoptive immunotherapy using dendritic cells pulsed with tumor lysate in patients with hepatocellular carcinoma. *Hepatology* **49,** 124–32 (2009).

245. Sangro, B. *et al.* A clinical trial of CTLA-4 blockade with tremelimumab in patients with hepatocellular carcinoma and chronic hepatitis C. *J. Hepatol.* **59,** 81–8 (2013).

246. El-Khoueiry, A. *et al.* Phase I/II safety and antitumor activity of nivolumab in patients with advanced hepatocellular carcinoma (HCC): CA209-040. *J Clin Oncol* **33,** (suppl; abstr LBA101) (2015).

247. Yeo, W. *et al.* Epigenetic therapy using belinostat for patients with unresectable hepatocellular carcinoma: a multicenter phase I/II study with biomarker and pharmacokinetic analysis of tumors from patients in the Mayo Phase II Consortium and the Cancer Therapeutics Res. *J. Clin. Oncol.* **30,** 3361–7 (2012).

248. Bitzer, M. *et al.* Efficacy, safety, tolerability, and PK of the HDAC inhibitor resminostat in sorafenib-refractory hepatocellular carcinoma (HCC): Phase II SHELTER study. *J Clin Oncol* **30,** (Suppl;abst 4115) (2012).

249. https://clinicaltrials.gov/ct2/show/NCT01507168. A Study of RO5137382 (GC33) in Patients With Advanced or Metastatic Hepatocellular Carcinoma. *US Natl. Libr. Med.* (2015).

250. Pao, W. *et al.* Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain. *PLoS Med.* **2,** e73 (2005).

251. Van Allen, E. M. *et al.* The genetic landscape of clinical resistance to RAF inhibition in metastatic melanoma. *Cancer Discov.* **4,** 94–109 (2014).

252. Tovar, V. *et al.* Tumor initiating cells and IGF/FGF signaling contribute to sorafenib resistance in hepatocellular carcinoma. *Gut* **In press,** (2015).

253. Chie, W.-C. *et al.* International cross-cultural field validation of an European Organization for Research and Treatment of Cancer questionnaire module for patients with primary liver cancer, the European Organization for Research and Treatment of Cancer quality-of-life ques. *Hepatology* **55,** 1122–9 (2012).

254. Blazeby, J. M. *et al.* Development of a questionnaire module to supplement the EORTC QLQ-C30 to assess quality of life in patients with hepatocellular carcinoma, the EORTC QLQ-HCC18. *Eur. J. Cancer* **40,** 2439–44 (2004).

255. Huang, G. *et al.* Quality of life after surgical resection compared with radiofrequency ablation for small hepatocellular carcinomas. *Br. J. Surg.* **101,** 1006–15 (2014).

256. Salem, R. *et al.* Increased quality of life among hepatocellular carcinoma patients treated with radioembolization, compared with chemoembolization. *Clin. Gastroenterol. Hepatol.* **11,** 1358–1365.e1 (2013).

257. Afdhal, N. *et al.* Ledipasvir and sofosbuvir for untreated HCV genotype 1 infection. *N. Engl. J. Med.* **370,** 1889–98 (2014).

258. Kahn, M. Can we safely target the WNT pathway? *Nat. Rev. Drug Discov.* **13,** 513–32 (2014).

259. Roayaie, S. *et al.* The role of hepatic resection in the treatment of hepatocellular cancer. *Hepatology* **62,** 440–51 (2015).

260. Park, J.-W. *et al.* Global patterns of hepatocellular carcinoma management from diagnosis to death: the BRIDGE Study. *Liver Int.* **35,** 2155–2166 (2015).

261. Robert, C. *et al.* Pembrolizumab versus Ipilimumab in Advanced Melanoma. *N. Engl. J. Med.* **372,** 2521–32 (2015).

262. Weinstein, I. B. Cancer. Addiction to oncogenes--the Achilles heal of cancer. *Science* **297,** 63–4 (2002).

263. Shaw, A. T. *et al.* Crizotinib in ROS1 -Rearranged Non–Small-Cell Lung Cancer. *N. Engl. J. Med.* **371,** 1963–71 (2014).

264. Nault, J.-C. & Villanueva, A. Intratumor molecular and phenotypic diversity in hepatocellular carcinoma. *Clin. Cancer Res.* **21,** 1786–8 (2015).

265. McGranahan, N. & Swanton, C. Biological and Therapeutic Impact of Intratumor Heterogeneity in Cancer Evolution. *Cancer Cell* **27,** 15–26 (2015).

266. Gridelli, C. et al. Non-small-cell lung cancer. *Nat. Rev. Dis. Prim.* **1,** 1–16 (2015).

267. Tabernero, J. *et al.* Analysis of circulating DNA and protein biomarkers to predict the clinical activity of regorafenib and assess prognosis in patients with metastatic colorectal cancer: a retrospective, exploratory analysis of the CORRECT trial. *Lancet Oncol.* **16,** 937–48 (2015).

268. Crowley, E., Di Nicolantonio, F., Loupakis, F. & Bardelli, A. Liquid biopsy: monitoring cancer-genetics in the blood. *Nat. Rev. Clin. Oncol.* **10,** 472–84 (2013).

269. Thierry, A. R. *et al.* Clinical validation of the detection of KRAS and BRAF mutations from circulating tumor DNA. *Nat. Med.* **20,** 430–5 (2014).

270. Thress, K. S. *et al.* Acquired EGFR C797S mutation mediates resistance to AZD9291 in non-small cell lung cancer harboring EGFR T790M. *Nat. Med.* **21,** 560–2 (2015).

Annotated References

EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. *J. Hepatol.* **56,** 908–43 (2012).

This article outlines the european consensus guidelines for the management for HCC, including definition of treatment allocation criteria according to evidence

Llovet, J. M. *et al.* Arterial embolisation or chemoembolisation versus symptomatic treatment in patients with unresectable hepatocellular carcinoma: a randomised controlled trial. *Lancet* **359,** 1734–9 (2002).

This paper reports a positive RCT supporting the use of TACE in intermediate HCC

Llovet, J. M. *et al.* Sorafenib in advanced hepatocellular carcinoma. *N. Engl. J. Med.* **359,** 378–90 (2008).

This paper details a positive RCT supporting the use of sorafenib in advanced HCC . It is the first RCT demosntarting survival benefits for a systemic drug in HCC. The study provided the rationale for approval of this drug in the management of HCC

Schulze, K. *et al.* Exome sequencing of hepatocellular carcinomas identifies new mutational signatures and potential therapeutic targets. *Nat. Genet.* **47,** (2015).

This is the largest study reporting whole exome sequencing in a cohort of HCC patients from the West

Totoki, Y. *et al.* Trans-ancestry mutational landscape of hepatocellular carcinoma genomes. *Nat. Genet.* **46,** 1267–73 (2014).

This is the largest study reporting whole exome sequencing in a cohort of HCC patients from the Asia

Hoshida, Y. *et al.* Gene expression in fixed tissues and outcome in hepatocellular carcinoma. *N Engl J Med* **359,** 1995–2004 (2008).

This is the first manuscript to define the importance of the 'cancer field effect' in the prognosis of HCC patients after resection

Chang, M. H. *et al.* Universal hepatitis B vaccination in Taiwan and the incidence of hepatocellular carcinoma in children. Taiwan Childhood Hepatoma Study Group. *N. Engl. J. Med.* **336,** 1855–9 (1997).

This study demostrates the effect of universal HBV vaccination in decreasing the incidence of HCC in Taiwan

Mazzaferro, V. *et al.* Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. *N. Engl. J. Med.* **334,** 693–9 (1996).

This paper defines the 'Milan criteria' for selection of candidates for liver transplantation. These criteria are currrently adopted by most transplant units globally

Lo, C.-M. *et al.* Randomized controlled trial of transarterial lipiodol chemoembolization for unresectable hepatocellular carcinoma. *Hepatology* **35,** 1164–71 (2002).

This RCT demosntrated the efficacy of TACE in patients with intermediate HCC

Clavien, P.-A. *et al.* Recommendations for liver transplantation for hepatocellular carcinoma: an international consensus conference report. *Lancet. Oncol.* **13,** e11–22 (2012).

This publication outlines consensus guidelines for the management of HCC with liver transplantation

Llovet, J. M. *et al.* Design and endpoints of clinical trials in hepatocellular carcinoma. *J. Natl. Cancer Inst.* **100,** 698–711 (2008).

This paper describes consensus guidelines of the design of clinical trials in HCC