Hepatocellular carcinoma (HCC) is the third most common cause of cancer-related death worldwide and, owing to changes in the prevalence of the two major risk factors, hepatitis B virus and hepatitis C virus, its overall incidence remains alarmingly high in the developing world and is steadily rising across most of the developed world. Early diagnosis remains the key to effective treatment and there have been recent advances in both the diagnosis and therapy of HCC, which have made important impacts on the disease. This review outlines the epidemiological trends, risk factors, diagnostic developments and novel therapeutics for HCC, both in the developing and developed world.

**Epidemiology**

Hepatocellular carcinoma (HCC) is the most common primary liver cancer, the fifth most common cancer and the third most common global cause of cancer-related deaths [1–3]. In 2000, there were 564,000 new cases and 549,000 deaths from HCC worldwide, indicating the devastating prognosis of this tumor. The incidence of HCC is rising across the world with few exceptions. There is a distinct geographical variation in the incidence of this tumor, with 81% of cases occurring in the developing world and 54% of these occurring in China (Figure 1). The highest incidence rates are in western and central Africa and East and South-East Asia. The incidence of HCC in the developed world, is comparatively low, apart from high rates in Japan, but there has been a steady overall increase across most Western nations over the last two decades [4–7].

**HCC in the developing world**

The majority of HCCs occur in the developing world and, of these, over half occur in China, where the age-standardized incidence rate in 1999 was 35.2 cases per 100,000 individuals for men and 13.3 per 100,000 individuals for women. The incidence rate is even higher in Mongolia (99 per 100,000 individuals) and Korea (49 per 100,000 individuals). This reflects the high prevalence of hepatitis B virus (HBV) carriage in these areas, which is the major worldwide risk factor for HCC. In China and other parts of Asia, HBV infection is usually acquired vertically from mother to child, whereas sibling–sibling transmission in infancy is the more common route in Africa [8]. Encouragingly, between 1978–1982 and 1993–1997, decreases in incidence were reported among Chinese populations in Hong Kong, Shanghai and Singapore. This reduction in HCC rates is almost certainly due to a widespread HBV vaccination program, resulting in the reduced burden of chronic HBV carriage [9].

Very high incidence rates for HCC are also seen in western and central Africa. Incidence rates of greater than 20 per 100,000 of the population are found in Nigeria, Gambia, Guinea (Conakry) and Mali. Similarly high rates may exist in other African countries that have not been systematically surveyed for the disease. In Nigeria, some studies have suggested that the prevalence of hepatitis B surface antigen (HBSAg) can be as high as 15–30% of the population in some areas [10,11]. A study that was conducted in 1992 in a tertiary health center
in Nigeria, concluded that, of all liver-related causes of death, HCC accounted for 42.5% and was the most common reason for cancer diagnosis among admissions to medical wards. In the early 1990s, HCC was the most common cause of cancer mortality in middle-aged and elderly Nigerians.

The high rate of HCC within Africa is mostly due to HBV prevalence, but a significant part is also due to aflatoxin (AF) B1, an Aspergillus fungal toxin that contaminates foodstuffs. Epidemiological surveys have shown that high HCC incidence and high AF intake correspond to areas where HBV is endemic, suggesting an additive risk effect of AF on those already infected with HBV [12].

**HCC in the developed world**

Within the developed world, there is marked variation of HCC incidence due to a number of factors. Most developed-world countries display a low-to-intermediate rate of HCC incidence. The exception is Japan where, between 1975 and 2002, deaths from liver cancer tripled from 10,000 to 35,000. In Japan, hepatitis C virus (HCV) causes 80% of all HCCs. The current epidemic in Japan is thought to have originated from the use of unscreened blood transfusions in the 1970–1980s. Surveillance and HBV vaccination programs are resulting in a plateau of cases of HCC, but significant decreases in incidence are yet to be observed [13,14].

In northern Europe, the USA, Canada and Australia, HCC incidence rates remain low (UK: 2.2 males per 100,000, 1.1 females per 100,000; Canada: 3.2 males per 100,000, 1.1 females per 100,000, and Australia: 3.6 males per 100,000, one female per 100,000) [2,3]. However, there has been a doubling of incidence rates in the USA from 1.4 per 100,000 in 1976–1980 to 2.4 per 100,000 in 1991–1995, reflecting the increasing prevalence of HCV-induced liver disease contracted from unscreened blood transfusions and intravenous drug use from previous decades. Similar changes are reported from the UK and Australia (Figure 2) [5,7,15]. However, recent surveys have shown decreases in HCC incidence in some European countries within certain groups. In particular, women and young individuals have displayed a decrease in incidence. This may be a sign of the end of the ‘cohort’ effect of HCV infection, contracted in the 1960s and 1970s in these groups [16].

**Gender**

For almost all countries, males have higher rates of HCC incidence than females, usually with a ratio of 2:1. Higher discrepancies are seen in some European states, for example, Switzerland (male:female: 4:1) and Italy (male:female: 5:1). In the developing world, the rates are more equal (China [3:1], Gambia [2.8:1] and Zimbabwe [2.4:1]).
The reasons for this discrepancy are likely to be multifactorial, in part owing to higher rates of HBV and HCV infection in the male population, but also social factors, such as higher alcohol intake and obesity in men. However, testosterone levels have been shown to correlate with HCC risk, so there is probably also an innate risk for males, based on hormonal profiles [17]. Interestingly, in mouse models of carcinogen-induced HCC, male mice were more likely to develop HCC compared with females. IL-6 is thought to be implicated, as IL-6 ablation abolished the gender differences in hepatocarcinogenesis [18]. In addition, recent studies have highlighted gender disparity of HCC. Liu and colleagues found that blocking the translation and expression of estrogen receptor-α with microRNA stimulated the proliferation of hepatoma cells [19]. HBx, a carcinogenic protein derived from the HBV genome, also appears to have a role and has been found to enhance the transcriptional activity of the androgen receptor and of androgen receptor-responsive gene expression [20,21].

Age
Reflecting etiological factors, the age of patients diagnosed with HCC varies between sexes and geographical regions. In most regions, female incidence rates peak 5 years later in age compared with males. The change of incidence appears to be affecting the aging population most profoundly. UK age-specific tumor incidence has risen from 13.95 to 18.64 per 100,000 population and mortality from 10.24 to 15.72 per 100,000 population in the 75-years-and-over age group between 1979 and 2004, the greatest rate of increase of any age group [7]. This rise may partially be explained by the increasing burden of HCV amongst the aging population contracted between the 1960s and 1970s.

Risk factors
The major risk factors for HCC are the presence of cirrhosis, and HBV/HCV infection. Other factors, such as AF and nonalcoholic steatohepatitis (NASH), are important and prevalent in certain areas of the world (Figure 3).

Cirrhosis
Cirrhosis is defined as the histological development of regenerative nodules, surrounded by fibrous bands, in response to chronic liver injury. Complications of cirrhosis include HCC and portal hypertension. The majority of patients with HCC have underlying cirrhosis. In an autopsy series from Italy and Japan, the prevalence of cirrhosis in patients with HCC was between 80 and 90% [22,23]. With the exception of HBV and AF, all other etiological risks for HCC are associated with cirrhosis. Furthermore, in the USA and parts of Europe, the mortality rate of HCC incidence is increasing, but the rate of mortality due to non-HCC complications of cirrhosis is decreasing or static [24]. This would suggest that the improved management of the complications of cirrhosis has reduced the mortality of patients with cirrhosis, allowing an increased percentage of them to survive long enough to develop HCC. In a longitudinal study of Italian patients with compensated cirrhosis between 1986 and 1996, it was observed that 71% of liver-related deaths were due to HCC [25].

Although the risk of HCC is increased with cirrhosis regardless of the etiology, certain causative factors carry a higher risk than others. HCV-associated cirrhosis carries the highest risk, resulting in a 5-year cumulative incidence of 17% in many European countries and in the USA, and 30% in Japan. HBV-associated cirrhosis results in a cumulative incidence of 15% in endemic areas and 10% in developed Western nations. Interestingly, hereditary hemochromatosis-associated cirrhosis is associated with the next highest 5-year reported cumulative incidence of 21%, although this may be subject to selection bias [24]. Patients with advanced primary biliary cirrhosis (PBC) have a 4% 5-year risk of developing HCC [24]. The stage of cirrhosis also appears to be an independent risk factor for HCC risk. An Italian study of 313 patients with cirrhosis, found that an entry Child–Turcotte–Pugh (CTP) stage B or C was associated with a threefold increased risk of HCC compared with patients with CTP stage A [26]. A similar
Hepatitis B virus

Worldwide, HBV is the most prevalent risk factor for HCC. Currently, there are estimated to be 300 million carriers of the HBV in the world, and HCC may develop in as many as 25% of them. Unlike most risk factors for HCC, HBV can be hepatocarcinogenic in both noncirrhotic and cirrhotic liver disease. In 1981, the seminal work of Beasley and colleagues first exposed the link between HBV and HCC by recording the incidence of HCC in Taiwanese HBsAg-positive patients (495 per 100,000 per year), compared with those who were HBsAg negative (five per 100,000 per year). Furthermore, patients with HBV-associated cirrhosis were found to have a 1000-fold greater risk of developing HCC, compared with HBsAg-negative individuals [28]. Many studies of HCC risk in those with chronic HBV infection have been conducted in East Asian countries, where most patients acquire HBV vertically. It appears that the probability of acquiring HCC increases with the severity of liver disease. In Japan, the mean interval between HBV infection and occurrence of HCC is 50 years. As most people are infected at birth, HBV cirrhosis usually develops earlier than in Western Europe or North America.

Few adequate studies have been performed in Europe or North America to address the issue of the incidence of HCC in individuals who are HBsAg positive. However, in a cohort of 350 HBsAg-positive Western European patients with compensated cirrhosis, the 5-year cumulative incidence of HCC was 6% [29]. A retrospective analysis of European patients with HBV-related cirrhosis found the 5-year incidence of HCC to be 9%, irrespective of HBsAg or HBV DNA status at the time of diagnosis of cirrhosis [30].

Hepatocellular carcinoma has been the first human cancer amenable to prevention using mass-vaccination programs. The Taiwanese HBV mass-vaccination program has considerably reduced the rate of HBsAg carriage in children and adolescents and, as a result, reduced the incidence of childhood HCC (0.7 per 100,000 children in 1981–1986, 0.57 in 1986–1990 and 0.36 in 1990–1994). A significant decrease in HCC incidence in adults was also observed 30–40 years later [31,32].

The mechanisms of carcinogenesis in HBV infection have been studied extensively and a major factor is chronic necroinflammation with subsequent fibrosis and hepatocyte proliferation. However, HCC may occur in HBsAg carriers without significant background liver fibrosis or cirrhosis. Therefore, it is reasonable to consider that, apart from host factors, viral factors are likely to be involved in HBV-related hepatocarcinogenesis. HBV may encode viral proteins that contribute to carcinogenesis. An example is HBx, a gene that encodes a protein with multiple functions, including gene transcription, protein degradation, apoptosis and signaling pathways. Its critical role in malignant transformation has been demonstrated in HBx-overexpressing transgenic mice [33]. Certain mutations in the viral genome, particularly of the core promoter, also correlate with HCC risk. Examples of these include BCP T1762/A1764, T1653, V1753, V1766 and V1768 [32,34,35]. Viral load also appears to correlate with increased mortality from HCC. Yang and colleagues observed an increased risk of HCC dependent on viral load with an adjusted odds risk ratio of 6 in patients with HBV DNA levels greater than 13.0 pg/ml [36]. Furthermore, in a cohort study of 820 chronic HBV patients over 76.8 months, viral load was found to be a significant risk factor for HCC and the authors suggest its use in a risk-based score for HCC [37]. Large cohort and observational studies from Taiwan have observed a linear relationship between HBV viral load and the risk for HCC [38–41]. HBV genotype and associated mutations also appear important. In Asia, HBV genotype C is more commonly associated with more-severe liver disease and HCC compared with genotype B [40,41]. By contrast, in Western Europe and North America, genotype D is associated with severe liver disease and higher incidence of HCC compared with genotype A [32].

Different viral factors may predispose to HCC in young people and those without cirrhosis. Studies from Taiwan have found that HBV genotype B is of higher prevalence in patients with HCC under the age of 50 years [32]. Liu and colleagues observed that male gender, the BCP T1672/A1764 mutation and viral load greater than 10^5 copies/ml were independently associated with the risk of HCC development in the absence of cirrhosis [32]. Recently, basal core promoter mutations and pre-S deletion of HBV have been found to be associated with the progression of liver disease and HCC in HBV carriers [32,42–45].

Viral suppression, through the use of lamivudine, has been shown to reduce the incidence of HCC in chronic HBV-infected patients. Liaw and colleagues, in a placebo comparison study, observed that patients treated with lamivudine had 3.5% less incidence of HCC at 32 months compared with placebo [46].
Hepatitis C virus

Hepatitis C virus is the most important risk factor for HCC in Western Europe, North America and Japan. Epidemiological studies from these areas have shown up to 70% of patients with HCV have anti-HCV antibodies in their serum. According to WHO estimates, HCV global prevalence is 2%, representing 123 million infected individuals [47].

Almost all HCV-associated HCCs arise in patients with cirrhosis. However, the prevalence of HCC in HCV-associated cirrhosis is higher than in cirrhosis caused by nonviral etiologies. The risk of HCC correlates with advancing hepatic inflammation and fibrosis. Carriers of HCV display a low risk of HCC; those with underlying chronic hepatitis carry a 1.2–1.7% per annum risk and those with cirrhosis display a 1.4–2.5% per annum risk, although some groups have reported a lower incidence of HCC in those patients having HCV without cirrhosis [48–51]. The highest incidence rates for cirrhosis and HCC were observed in HCV-contaminated blood recipients (14 and 1 per 1000 person-years for cirrhosis and HCC, respectively) and in hemophiliacs (5 and 0.7 per 1000 person-years, respectively) [6].

The development of HCC has been shown to occur decades after the initial infection. In an American and Japanese study, HCC development took an average of 28 and 29 years gestation, respectively [52]. Continuous inflammation and hepatocyte regeneration may lead to chromosomal damage and initiate hepatic carcinogenesis [51]. A few cases of HCC developing in the noncirrhotic HCV-infected livers have been reported [53], suggesting a direct mutagenic effect of the virus, although the exact mechanism is disputed. A transgenic mouse model of HCV infection, whereby HCV proteins are expressed only in the liver, promotes the development of HCC in older animals. In these animals, there is no background cirrhosis or inflammation, suggesting that carcinogenesis is solely due to HCV proteins. Possible candidates include the core protein, which interacts with TNF receptors, increasing oxidative stress and pro-apoptotic signaling [54]. Certain studies implicate genotype 1b as carrying a higher risk, but other studies refute this theory [55,51,56].

Sustained viral suppression from successful antiviral therapy may reduce the risk of HCC in HCV-infected patients, but the evidence is weak, since it comes mostly from nonrandomized or observational studies. By the time cirrhosis has become established, the success of antiviral therapy has already diminished and any benefit therapy may have on hepatocarcinogenesis is debatable.

Viral coinfection

Follow-up studies have shown that patients coinfected with HCV and HBV have a higher risk of developing HCC than those with a single infection [51,57]. The same applies for those individuals with HBV and hepatitis D virus coinfection. It is, therefore, recommended that patients infected with HCV should be vaccinated against HBV. A recent study of HCC in HIV–HCV coinfected patients also indicated a more rapid development of HCC [58].

Nonalcoholic fatty liver disease, obesity & diabetes mellitus

Nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH – where there is biopsy-proven hepatic inflammation) have been implicated as risk factors for HCC. As part of the ‘metabolic syndrome’, these conditions often occur concurrently with obesity and diabetes mellitus (DM), and teasing out the individual contribution to the risk of each factor is, therefore, difficult. Studies from the USA have shown that no cause for HCC was identifiable in 30–40% of cases in the presence of cryptogenic cirrhosis, and it is likely that these cases were associated with NAFLD [6]. The rate of cryptogenic cirrhosis-associated HCC is probably lower in countries where obesity is less prevalent. A recent retrospective cohort study of 641 HCC cases showed that 44% of patients were women. This illustrates that NAFLD/NASH, similar to other HCC risk factors, promotes HCC through advancing liver disease and cirrhosis. However, the authors commented that it was interesting that a low grade of histological activity and low-level aminotransferase were risk factors, whereas in HCV-associated HCC, ongoing inflammation is a risk. The authors of this study hypothesize that as NAFLD/NASH patients develop cirrhosis, characteristic features of NASH, including necroinflammation and steatosis, disappear. It may, therefore, be that a histological low grade of activity is observationally associated as a risk factor for HCC.

Convincing data on the risk of obesity and DM have been gathered, particularly from the USA. In a prospective cohort study of 900,000 individuals spanning 16 years, liver cancer mortality rates were 4.5-times higher among men with a high BMI of 35–40 kg/m² compared with those with a normal BMI [61]. Similar cohort studies from Sweden and Denmark found a two- to three-fold increased relative risk in the obese compared with individuals with a normal BMI [62,63].

In cohort studies, DM has been found to represent a risk for HCC, probably through the development of NASH and cirrhosis. A study of HCC incidence in a large cohort of American veteran affairs patients indicated that HCC incidence doubled among patients with DM and was higher among those with a longer duration of follow-up, suggesting that duration of DM may be important [64].

The burgeoning epidemic of obesity worldwide will probably make NAFLD/NASH-associated HCC a prevalent problem. However, a recent retrospective study of patients with NAFLD/NASH-associated HCC undergoing liver transplant, observed good survival outcomes at 2.5 years (88% survival) highlighting NASH-associated HCC as a treatable condition [65].
Aflatoxin B1
Aflatoxin B1 is a mycotoxin produced by a fungus of the *Aspergillus* spp. These moulds are ubiquitous in nature and contaminate a number of staple foodstuffs (e.g., grain, corn, peanuts and legumes) in tropical and subtropical regions. Epidemiological studies have shown a strong correlation between the dietary intake of AFB1 and incidence of HCC [12,66]. However, it has been observed that areas of high HCC incidence and high AF intake also correspond to areas where HBV is endemic. HBV and AFB1 are, therefore, likely to interact synergistically, increasing the risk of HCC development [12,66].

At the molecular level, once ingested, AFB1 is metabolized to AFB1-exo-8, 9-epoxide, which can bind to DNA and may cause hepatocarcinogenesis through a characteristic inactivating mutation of the third base codon 249 of the p53 tumor-suppressor gene found in 30–60% of HCC tumors in AF-endemic areas [67,68].

Alcohol
Alcohol has never been proven to be directly mutagenic on hepatocytes; rather, the risk for HCC development arises through the establishment of alcohol-induced cirrhosis, which, in common with any underlying cause of end-stage liver disease, carries a higher risk for the development of this type of liver tumor. Abstinence does not appear to affect HCC risk once cirrhosis is established. However, alcohol has been proved to act synergistically with other risk factors, linearly increasing the risk of HCC in other liver disease etiologies, such as in those infected with viral hepatitis [69].

Inherited liver disorders
Hereditary hemochromatosis is the most common inherited risk factor for HCC. Most studies have studied the risk of those homozygous for the C282Y mutation and very little information is available on those heterozygous for this condition. Risk, as with most other factors, requires the presence of cirrhosis. A Swedish population-based study revealed a 1.7-fold increase in the incidence of HCC among 1800 – mostly homozygous – patients with hereditary hemochromatosis [6]. Those with *HFE* homozygous mutations have been found to be at a decreased risk of HCC [70,71]. HCC may also develop in other disorders, which are complicated by the development of cirrhosis, such as Wilson’s disease, α1-antitrypsin deficiency and type 1 glycogen-storage disease, but this is a rare occurrence.

Diagnosis & surveillance
The clinical symptoms and signs of HCC are often absent in the early stages, or nonspecific when associated with end-stage liver disease. Hepatic bruist, present in 10–20% of cases, may indicate an underlying HCC. However, most cases occur in patients with established cirrhosis and may cause hepatic decompensation but equally, may lie silent in those with compensated liver disease. In developing countries, medical advice is often sought late in the disease and, as a result, may present with end-stage signs of a palpable hepatic mass, hemoperitoneum or decompensation with portal hypertensive complications. Subsequently, diagnosis of HCC is typically made by hepatic ultrasound imaging in combination with serum α-fetoprotein (AFP) levels [72]. Population-based studies have shown mortality and morbidity benefit from surveillance for HCC in at-risk populations. A Chinese study using AFP and liver ultrasound surveillance showed a 3-year cumulative survival rate of 36% for surveyed patients and 0% for unsurveyed patients (p < 0.0001) [73]. Through international consensus, a diagnostic and surveillance algorithm for HCC has been developed and most recently presented in the American Association for the Study of Liver Disease 2005 guidelines [74–76]. Surveillance for HCC should be performed using ultrasound at 6–12-month intervals and AFP should not be used unless ultrasound is unavailable. Nodules of less than 1 cm should be followed up at 3–6-month intervals, and should no change in size occur over 2 years, routine surveillance can be reinstated. Nodules of 1–2 cm should be investigated with dynamic imaging studies: CT scan, contrast ultrasound or MRI. If, in both studies, the lesion shows typical features of HCC (hypervascular with washout in the portal/venous phase), then it should be treated as HCC. If features are not characteristic of HCC, the lesion should be biopsied, accepting the 1–3% risk of potential tumor seeding. If the lesion is greater than 2 cm and shows typical features of HCC, or if the serum AFP is greater than 200 ng/ml, then biopsy is not required to confirm the diagnosis. Biopsies should be reviewed by expert pathologists and, if negative, a high level of suspicion should be maintained and ultrasound surveillance should occur at 3–6-month intervals.

Serum markers
α-fetoprotein
Serum AFP is a fetal glycoprotein, synthesized primarily by the embryonic liver, cells of the vitelline sac and by the fetal intestinal tract in the first trimester of pregnancy. Thereafter, the serum concentration of AFP declines rapidly and is usually undetectable in adults [72]. AFP was first described by Abelev and colleagues in the 1960s and has been utilized as a marker for HCC for many years since the first quantitative serum assays were established in the 1970s by Ruoshlati and Seppala. However, not all HCCs secrete AFP. A review of several studies of AFP for HCC surveillance found that it had a sensitivity of 39–65%, a specificity of 76–94% and a positive predictive value of 9–50% [77]. The cut-off level of AFP was important in determining the diagnostic power. A cut-off value of 20 ng/ml resulted in a sensitivity of 64% and a specificity of 91% [78], while a cut-off value of 400 ng/ml resulted in a sensitivity of 17% and specificity of 99% [79]. Values of over 400 ng/ml are generally considered diagnostic of HCC, although only approximately 20% of patients with HCC display values this high. Indeed, only 30% of patients with HCC have been found to have values greater than 100 ng/ml [77,72]. The sensitivity and specificity of AFP are partly dependent on the prevalence of HCC in the target population. Patients with chronic viral hepatitis may display a raised AFP with no HCC during viral flares, although there are conflicting studies that observe a high sensitivity for nonviral HCCs [72]. For example, Trevisani and colleagues found that an AFP elevation in noninfected patients could be more
indicative of HCC compared with infected patients [79]. In a study of 290 Chinese patients with chronic HBV, 44 were found to have elevated serum AFP levels (>20 ng/ml). Of these, only six patients (13%) had HCC. The remaining 38 patients had elevated serum AFP either due to viral flares or an unknown cause [80].

Serum AFP levels, to some extent, may correlate with prognosis. A large multicenter study of over 1000 patients with HCC recorded that 18% had a serum AFP level of greater than 400 ng/ml and that these patients had a poorer survival rate [81].

Serum AFP is a widely used marker of HCC. However, it remains insensitive and nonspecific in certain circumstances and is, therefore, imperfect, particularly in the detection of early, small cancers.

Des-γ-carboxyprothrombin

Des-γ-carboxyprothrombin (DCP), also known as prothrombin, induced by vitamin K absence II, is an abnormal prothrombin protein produced as a result of an acquired defect in the post-translational carboxylation of the prothrombin precursor in malignant cells. It is also found in patients on warfarin therapy, or with vitamin K deficiency. The defect in carboxylation has been attributed to defective gene expression of γ-carboxylase in HCC patients [82]. Serum DCP was found to have a HCC diagnostic sensitivity and specificity of 48–62% and 81–98%, respectively, in several large case-control studies [82,83]. DCP has been reported to be more sensitive and specific than AFP, especially in East Asian countries and North America [72]. The discrepancy between results has been attributed to both racial and etiological factors [72]. A study comparing the performance characteristics of AFP, DCP and Lens culinaris agglutinin-reactive AFP in the diagnosis of HCC observed that DCP was significantly better than the other markers in differentiating HCC from cirrhosis, with a sensitivity of 86% and a specificity of 93% [84]. However, tumor size can affect the sensitivity and specificity of DCP in detecting HCC. According to a study by Nakamura and colleagues, the efficacy of DCP was lower than that of AFP in the diagnosis of small tumors, although higher than that of AFP for large tumors [85].

Several studies have also shown that DCP can be a useful indicator of vascular invasion of HCC and in monitoring the recurrence of HCC after treatment [86–89]. A recent study of HCC tumor DCP expression found that high expression correlated with high serum DCP levels, tumor size and portal vein infiltration and, in those patients with a low serum level but high tumor-expression level of DCP, predicted early recurrence [90].

Lens culinaris agglutinin-reactive AFP

Lens culinaris agglutinin-reactive AFP (AFP-L3), a variant of AFP, is the main glycoform of AFP in the serum of patients with HCC and can be detected in approximately a third of patients with small HCCs (<3 cm) when cut-off levels of 10–15% are used. At a cut-off level of 15%, AFP-L3 displays a sensitivity of 75–96.9% and specificity of 90–92% [91,92]. However, these studies are limited to East Asian HCC populations with raised serum AFP levels. Furthermore, within these studies, higher AFP-L3 levels correlated with poorly differentiated tumors, compared with well-differentiated tumors. Some studies have also reported that AFP-L3 acts as a marker of clearance of HCC after treatment and as a predictor of recurrence [88].

Combination tumor marker serology may prove beneficial. In a recent study conducted in a North American population, AFP-L3 and DCP levels had a higher correlation with an absence of HCC, as well as a higher specificity and negative predictive value, than total serum AFP levels [93].

α-L-fucosidase

α-L-fucosidase (AFU) is a glycosidase found in cellular lysosomes and, although its activity can be detected in healthy individuals, increased activity is found in patients with HCC. The sensitivity and specificity of AFU for diagnosing HCC compares favorably to other serum markers: 81.5–81.7% and 70.7 to 85.4%, respectively [94–96]. AFU activity also correlated to tumor size in these studies. In an Egyptian study, compared with AFP, AFU displayed a higher sensitivity (81.8 vs 68.2%) but a lower specificity (55 vs 75%). In this study, the combined use of AFP and AFU improved HCC detection from 68.2 to 88.6% [97]. However, it should be noted that prolonged storage of samples affects enzyme activity over time [98], and levels may be raised in other forms of cancer, limiting its usefulness in the clinical setting [72].

Proteomic & metabonomic biomarker discovery

Hepatocellular carcinoma has been subject to numerous recent studies using novel technologies to identify protein and metabolite biomarkers of the disease. Proteomic approaches have included SELDI-TOF and iTRAQ protein-chip technology [99,72]. In a study using SELDI-TOF MS in comparison to established serological HCC biomarkers (i.e., AFP, AFP-L3 and DCP) to distinguish serum of patients with HCC from those with HCV-associated cirrhosis, SELDI-TOF had a sensitivity and specificity of 79 and 80%, respectively, which was superior to the established serum biomarkers [100]. Geng and colleagues identified a diagnosis pattern using SELDI-TOF MS, which could distinguish HCC patient serum with 92% sensitivity and 100% specificity [101]. In this study, seven peptides or proteins were identified as possible distinguishing factors: galanin-related peptide, pro-neuregulin-4 protein, small inducible cytokine A15 precursor, 9-kDa protein, CSL-zincfinger protein 1, mitochondrial hinge protein and actin-related protein. In a tissue-based study, Chaerkady and colleagues, using iTRAQ technology, reported a number of proteins under- and over-expressed in HCC compared with normal adjacent tissue [102].

The study of metabolite derangements, or ‘metabonomics’, has been applied to HCC with some success using proton magnetic resonance spectroscopy (1H MRS). Hydrogen is the most abundant atom in living organisms and, using high magnetic fields of greater than 10,000-times stronger than the Earth’s magnetic field, clearly-defined metabolite peaks of small molecules (<2 kDa) can be obtained. There have been two recent tissue-based studies of HCC using in vitro 1H MRS. Yang and colleagues, using magic-angle spinning in vitro tissue 1H MRS, observed that, compared with normal adjacent liver tissue, HCC tissue had elevated levels of lactate, glutamate, glycine, leucine,
alanine, choline metabolites and phospholipid synthesis, but reduced levels of triglycerides, glucose and glycogen [103]. These results suggest that alteration of energy metabolism coupled with changes in the tricarboxylic acid cycle and high cell membrane turnover are dominant in HCC biochemistry. In addition, using in vitro tissue 1H MRS and a statistical classification strategy [104], Soper and coworkers were able to distinguish normal liver tissue from HCC and cirrhotic tissue with an accuracy of 98 and 92%, respectively.

**Radiological diagnosis of HCC**

Despite technical improvements in all modes of HCC diagnostic imaging, difficulties remain in accurately identifying small (≤2 cm) lesions on the background of cirrhosis in all imaging modalities.

**B-mode ultrasound**

B-mode ultrasound is the most common HCC surveillance and diagnostic imaging modality owing to its low cost, ease of use and low risk. Using B-mode ultrasound, HCC can be either hypoechoic, if a small lesion, or heterogeneous, as is often the case with larger lesions, owing to necrosis and fibrosis [105]. Limitations arise owing mostly to the technical experience of the practitioner, the presence of cirrhosis and the body habitus of the patient. If the operator is experienced, B-mode ultrasound can detect 80–95% of lesions 3–5 cm in diameter and 60–80% of lesions 1 cm in diameter [105,106]. In a retrospective study of 200 patients with end-stage liver failure requiring transplantation, ultrasound displayed a sensitivity of 75% for large lesions (>5 cm), but for small lesions (1–5 cm), the sensitivity ranged from 13.6 to 50%, according to the tumor diameter [107].

**Contrast-enhanced ultrasound**

Perfluorocarbon-based microbubble ultrasound contrast agents have allowed the real-time imaging of liver lesions. The technique is safe and the agent is not nephrotoxic. Since the blood supply to a typical HCC is arterially rather than portal venous supplied, contrast-enhanced ultrasound (CEUS) can readily identify tumors. Early intense tumoral enhancement is seen, followed by a ‘wash-out’ and hypoechoic period in the portal venous phase. Moreover, it has been observed that the more differentiated a tumor, the more gradually it will wash out [108–110]. Recently, it was reported that CEUS and multidetector CT were used to accurately identify small liver nodules (≤2 cm) in patients with cirrhosis.

**Computed tomography**

Computed tomography with contrast agents can identify HCC, based on early arterial enhancement and portal venous washout and hypodensity. In addition, the tumor can be staged, and metastatic involvement identified. Limitations of the technique include difficulty in resolution of small lesions (<2 cm) and allergic and nephrotoxic risks of contrast agents. Multiphase helical CT (MPCT) is deemed the imaging technique of choice for the detection and staging of HCC [112,113]. Spiral CT has been assessed for the diagnosis of HCC in several studies. A study of 41 patients who underwent liver transplantation within 100 days of imaging, observed that spiral CT displayed a sensitivity and specificity for HCC of 80 and 96%, respectively [114]. A study to evaluate the efficacy of contrast-enhanced helical CT (CECT) and CEUS found that CECT had a sensitivity, specificity and diagnostic accuracy of 80.4, 97.9 and 88.4%, respectively. There was no significant difference between CEUS and CECT in detecting small (1–2 cm) hepatic nodules [115]. Finally, the development of multidetector row helical CT (MDCT) with superior resolution, has improved the detection of small liver lesions. A study comparing MDCT with gadolinium-enhanced MRI found that the sensitivity for small HCC detection was 97.5–97.6% using MDCT and 90.7–94.7% using MRI. Furthermore, the sensitivity of detecting minute HCCs (≤1 cm) using MDCT was 90–95% [116].

**Magnetic resonance imaging**

Hepatocellular carcinoma appears hyperintense on T2-weighted MRI images and variable on T1-weighted images. Following gadolinium contrast injection, HCC usually exhibits typical early enhancement on arterial phase and wash-out on delayed imaging with relative hypointensity [105]. Diffusion-weighted MRI may show HCC lesions as high intensity on a low-intensity liver parenchyma background. In a study comparing MRI with CT and ultrasound measurements of serum AFP levels in patients undergoing liver transplantation, MRI was found to be more accurate than other modalities in tumor detection [117]. In a further study of patients awaiting transplantation, unenhanced MRI performed better than spiral CT in HCC detection, but was poor in detecting small (≤1 cm) lesions. The presence of cirrhosis or ascites appears to negatively affect the prediction power of MRI [118]. The use of superparamagnetic iron oxide particles in combination with gadolinium-enhanced imaging of HCC has been found to be more accurate in tumor detection than no or single-contrast imaging. Recently, there have been advances in the sensitivity of MRI using newer contrast agents. Marin and colleagues found a higher sensitivity for detection of HCC using combined interpretation of dynamic and hepatobiliary phase MRI compared with multiphasic multidetector CT.

**Positron emission tomography**

Positron emission tomography is of limited value in the diagnosis of HCC. 18F-fluorodeoxyglucose (18F-FDG) is the most widely used PET radiopharmaceutical in oncological imaging. Hepatocytes have a relatively high glucose-6-phosphatase activity, which allows dephosphorylation of FDG and subsequent leakage of the tracer from cells. Studies have shown a variable sensitivity of 50–55% HCC detection using this tracer alone [119]. This has led to the search for other PET tracers that may be used to screen for the presence of HCC. In a study using two agents, 11C-acetate and 18F-FDG, poorly differentiated HCCs were detected by 18F-FDG and the well-differentiated HCCs were detected by 11C-acetate, leading to a 100% sensitivity using both tracers [119].
Established & novel treatments for HCC
With numerous treatment options available, a treatment strategy based on available evidence is essential for the optimal management of HCC. In Europe, the most widely used strategy is the Barcelona Clinic Liver Cancer Treatment Schedule [120-122,75]. Small (<2 cm), early-stage tumors with background Child’s A cirrhosis and no portal hypertension are amenable to curative resection. Depending on the size of the tumor, resection can be considered. However, in the case of cirrhosis, the function of the remnant liver following resection has to be adequate to avoid liver failure. If up to three tumors less than 3 cm are present with background liver disease, then liver transplant is an option. Alternatively, if comorbidities are present that contraindicate transplantation, percutaneous ethanol injection or radiofrequency ablation remain an option. Multinodular, intermediate-stage tumors are amenable to chemoembolization. It is possible that advanced-stage tumors might receive some benefit from a trial of sorafenib, although hard evidence for this from large multicenter studies for tumors arising on the background of all underlying functional CTP grades of cirrhosis is currently lacking.

Liver resection
In noncirrhotic or Child’s-A cirrhosis patients, liver resection remains the curative treatment of choice. In Western countries, this accounts for 5% of patients, and in Asia, this is as high as 40%. With carefully selected candidates, 5-year survival can exceed 50% following HCC resection [75]. In addition to cirrhosis, the presence of portal hypertension (hepatic vein gradient > 10 mmHg) adversely affects the outcome after resection [123]. It is, therefore, recommended that patients undergo portal pressure measurements prior to resection. Unfortunately, the risk of recurrence after resection exceeds 70% at 5 years, with the most important factors being the presence of microvascular invasion and additional tumor sites other than the primary lesion [75].

Liver transplantation
After the seminal study of Mazzefero and colleagues, the Milan criteria for patients most likely to benefit from transplant were established. Patients with a single tumor of less than 3 cm or up to three tumors all of which were less than 3 cm displayed 5-year survival exceeding 70% after transplantation [124]. However, patients outside these criteria may also benefit from transplant. A recent retrospective analysis by the same group, found that a subsection of patients, outside the Milan criteria, characterized by the ‘rule of seven’ (largest tumor size [cm] + number of lesions ≤ 7) achieved 5-year survival rates of 71% [125].

Percutaneous ethanol injection & radiofrequency ablation
For ablative measures, the majority of evidence exists for the success of percutaneous ethanol injection or radiofrequency ablation for the treatment of patients with intermediate disease. Currently, there have been no randomized, controlled trials comparing ablative to resective measures. Percutaneous ethanol injection is an inexpensive technique, performed under ultrasound guidance, which can result in HCC necrosis of 100% in lesions smaller than 2 cm. This drops to 50% in large, 5-cm lesions. Child’s A patients with successful tumor necrosis can display 5-year survival rates of 50% [75].

Radiofrequency ablation is the application of heat energy via electrodes inserted into the tumor. It compares well to percutaneous ethanol injection, with fewer applications required to cause tumor necrosis in lesions smaller than 2 cm, and its efficacy in larger tumors is superior. Trials have also displayed better local disease control with radiofrequency ablation [75]. However, complications such as peritoneal bleeding and pleural effusions are more prevalent with this technique.

Transarterial embolization & chemoembolization
Established tumors are usually highly vascularized with a hepatic artery (HA) supply. This, therefore, makes them amenable to necrosis via arterial occlusion. Transarterial embolization and chemoembolization involves the selective angiographic catheterization of the hepatic artery with the introduction of chemotherapy (usually adriamycin or cisplatin), followed by embolization with gelfoam, alcohol or metallic coils. The procedure is the same for transarterial embolization, without the introduction of chemotherapy [75]. A 20–60% improvement in survival has been observed at 2 years, but this is highly dependent on the underlying liver disease and the tumor stage of the patient [126].

Figure 4. MRI-guided laser and focused ultrasound ablation. (A) MRI. (B) Focused ultrasound allows heat to be applied to a cancer without inserting needles. (C) The tumor can be seen before application of heat and (D) again after heat ablation.
**High-intensity focused ultrasound**

High-intensity focused ultrasound (HIFU) is a novel technique that is still in the early stages of use for HCC treatment (Figure 4). Ultrasound energy is generated outside the patient and directed through intervening tissue onto a focal point of tumor tissue. Cancerous tissue is destroyed by a circumferentially radiating heat wave. The advantages of this technique are that no instrument is required to be inserted into the patient, and there is no cumulative damage to intervening tissue, so it can be repeated if necessary. A trial comparing HIFU with no therapy showed improved short- and long-term outcomes for patients with HCC [127]. However, the ultrasound beam needs to be focused accurately, which requires a single point within the liver to be targeted. Respiratory movements of the liver require the procedure to be performed in ventilated patients, so tidal volume can be predicted and controlled [128]. Lesions that are immediately below the ribs are not amenable to HIFU as bone acts a deflector of ultrasound energy. Some studies have used HIFU with partial rib resection to good effect [129]. Further studies comparing HIFU to other modes of ablative therapy are required before this technique can be applied more widely. There are currently no studies comparing HIFU to other ablative techniques.

**MR-guided laser thermal ablation**

As with other forms of ablation, the delivery of thermal energy can be delivered to the center of an HCC using laser energy (Figure 4). This has been trialed using MR real-time guidance and has the advantage of accurately targeting lesions. Dick and colleagues performed MR-guided laser thermal ablation on 35 patients with HCC and found improved survival in treated versus untreated patients [130]. Laser thermal ablation, unlike radiofrequency ablation, which generates magnetic field interactions, is amenable to periprocedural MR monitoring and thermal mapping of tumor destruction. This is a novel technique that needs further evaluation. There are currently no studies comparing MRI laser thermal ablation to other ablative techniques.

**Pharmacological therapy**

Chemotherapeutic agents for HCC have been relatively limited in their role, given the slow-growing nature of the tumor and lack of insight into the molecular pathways that contribute to oncogenesis [131]. However, the recent availability of sorafenib, an oral multikinase inhibitor, has altered this perception.

**Sorafenib**

Sorafenib is an oral multikinase inhibitor that inhibits tumor cell proliferation and angiogenesis in a wide range of tumor models [132]. It acts by inhibiting VEGF receptor, PDGF receptor and Raf. These pathways have been implicated in the molecular pathogenesis of HCC. Llovet and colleagues published a landmark multicenter, randomized, controlled study in 2008 [132]. A total of 602 patients with advanced HCC (defined as those not eligible for or had disease progression after surgical or locoregional therapy) were randomized to oral sorafenib (299 patients) or placebo (303 patients). Median overall survival was 10.7 months in the sorafenib group and 7.9 months in the placebo group (hazard ratio in the sorafenib group: 0.69; 95% CI: 0.55–0.87; p < 0.001). Diarrhea, weight loss, hand–foot skin reaction and hypophosphatemia were more frequent in the sorafenib group. Other pathway-inhibiting molecules are also being investigated for treatment of HCC. Erlotinib, an inhibitor of EGF receptor/human EGF receptor-1 was used in a Phase II trial. The median survival after treatment was 10.7 months [133].

**Expert commentary**

While HBV remains the second most common known carcinogen after tobacco, the advent of universal vaccination at birth in East Asian countries is leading to a decline in HCC incidence and mortality, although neonatally acquired HBV transmission remains unstemmed in sub-Saharan Africa through lack of resources and infrastructure in most affected African countries. In Europe and North America, the HCV-related epidemic, coupled with the increasing prevalence of obesity, suggests that overall HCC incidence will continue to rise for the foreseeable future, although there have been decreases in women and young individuals.

Although alcohol-related liver disease cannot be singled out as causing particularly high rates of HCC morbidity or mortality, the increased prevalence of alcohol consumption will continue to complicate the course of other end-stage liver diseases, such as HCV-related cirrhosis and, as a result, increase carcinogenic potential. However, formalized surveillance programs for HCC detection should lead to earlier tumor detection in the at-risk populations. Extended Milan criteria for liver transplantation suggest that more patients may be eligible for this procedure, provided that organ-donation mechanisms are improved (along the lines of the system in place in Spain, where the highest organ donation rates exist per head of the population). The advent of multikinase inhibitor therapy offers some promise for inoperable and nonablatable tumors, where there was previously none.

**Five-year view**

Hepatocellular carcinoma is now recognized as an important global health issue and changes in the incidence, diagnosis and treatment are certain to occur over the next 5 years. The incidence is likely to remain high in the African subcontinent but, in Asia, where HBV vaccination regimes are now established and widely available, there will be a steady fall. In the developed world, there will be a steady rise in incidence, owing to the burden of HCV infection in the 1960s and 1970s. When this will plateau is difficult to predict, and it is likely that emerging risk factors, such as obesity-related liver disease, may significantly contribute to the burden of HCC.

The key to effective and potentially curative treatment is early diagnosis of the tumor. Effective screening of at-risk patients is dependent on the sensitivity and specificity of diagnostic tests. The widespread search for more effective biomarkers of HCC, using promising novel proteomic and metabolomic techniques, will possibly uncover potential candidates, superior to the current gold-standard of AFP and ultrasound.

Finally, there is likely to be a paradigm shift in the treatment of HCC. Liver transplantation and resection will remain the mainstay of curative treatment in those with curable disease, but there is likely
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Key issues

- Worldwide, hepatitis B virus is the major risk factor for hepatocellular carcinoma (HCC), owing to its high prevalence in the developing world. Hepatitis B virus vaccination programs have and will cause a sustained fall in new cases of HCC.
- In the developed world, HCC incidence has almost doubled over the last two decades. This is probably owing to chronic hepatitis C virus infection, contracted by intravenous drug use and unscreened blood product transfusion in previous decades.
- Although alcohol-related liver disease does not carry a particularly high burden of HCC incidence, chronic alcohol consumption complicates and exacerbates the course of other conditions, such as viral hepatitis, and can increase carcinogenic potential in this way.
- Owing to the increasing epidemic of obesity-related morbidity, obesity-related liver disease is likely to be a major cause of HCC in the coming decades.
- Current surveillance regimes of α-fetoprotein and ultrasound may be augmented by novel biomarkers of HCC discovered through new technologies, such as proteomics and metabolomics.
- Novel ablative treatments such as high-intensity focused ultrasound and MRI laser thermal ablation are likely to play a more prominent role in the treatment of HCCs not amenable to resection or transplantation.
- Pharmacological treatments for advanced HCC, such as sorafenib, are likely to play a more prominent role in its treatment.

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