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REVIEW ARTICLE

# Management strategies for hepatocellular carcinoma: old certainties and new realities

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**Abstract** Hepatocellular carcinoma (HCC) is a highly prevalent disease ranking among the ten most common cancers worldwide with increasing trend of incidence in most developed countries. The great healthcare costs and economic burden of HCC dictate proper preventive interventions as well as surveillance and screening programs to decrease disease incidence and allow early diagnosis. HCC treatment outcomes are affected by several variables, including liver function, patient's performance status, and tumor stage. In line with the Barcelona Clinic Liver Cancer (BCLC) staging curative treatments, such as surgery or radio-frequency ablation, are indicated in early-stage HCC (BCLC-A), and the noncurative treatments are indicated in intermediate and advanced stages of HCC (BCLC-B, C). Transarterial chemoembolization (TACE) represents the treatment of choice for intermediate-stage HCC with Child–Pugh A cirrhosis, and the long-term survival after liver transplantation is inferior to that of early-stage HCCs. In advanced-stage HCC or when complete necrosis is not achieved or early recurrence after TACE develops, individualized treatments such as systemic treatment or combined radiation therapy are indicated. The increasing knowledge of the genomic landscape of HCC and the development of molecular-targeted therapies is heading toward expanding the armamentarium for HCC management.

**Keywords** Hepatocellular · Cancer · Therapy

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## Introduction

Hepatocellular carcinoma (HCC) represents the major histological subtype among primary liver cancers, accounting for 70–85 % of the total burden worldwide. The incidence is characterized by gender-related differences, and rates are more than twice as high in males as in females. HCC in men is the fifth most frequently diagnosed cancer worldwide but the second most frequent cause of cancer death, whereas in women is the seventh most commonly diagnosed cancer and the sixth leading cause of cancer death [1–3]. There are also differences in geographical distribution. The highest liver cancer rates are found in East and Southeast Asia and in Middle and Western Africa, whereas rates are low in South-central and Western Asia, as well as Northern and Eastern Europe [1, 2]. Chronic hepatitis B virus (HBV) infection determines the high HCC rates in parts of Asia and sub-Saharan Africa. In particular, HBV infection accounts for about 60 % of the total liver cancer in developing

countries and for about 23 % of cancer in developed countries, while hepatitis C virus (HCV) infection accounts for about 33 % of the total liver cancer in developing countries and 20 % in developed countries [1, 2, 4, 5]. The role of aflatoxin B1 exposure in determining HCC burden in parts of Africa and Asia is indefinite. Alcohol-related cirrhosis and probably obesity-associated nonalcoholic fatty liver disease cause the majority of liver cancers in the USA and numerous other low-risk Western countries [6–9]. The incidence of HCC increases progressively with age, but in countries with a high incidence the average age at diagnosis is in the third decade of life, while in other regions has moved in two or three decades later [1]. The different distribution of risk factors and the different pathogenetic mechanisms underlay the variability of HCC incidence and clinical presentation. In this regard, it is noted a significant difference in the stage at diagnosis between countries with low and high incidence. In the former, in fact, HCC is still frequently identified in the asymptomatic phase because of routine instrumental checks underwent by patients at risk; in the latter, instead, the diagnosis is often delayed for the absence of regular checks and for earliness of hazardous conditions, resulting in detection of infiltrating or advanced-stage HCC [2].

## Prevention

The best prevention strategy for HCC is to avoid the development of liver diseases and their progression to cirrhosis. The main cause of HCC in the world is represented by HBV infection; therefore, vaccination may be an effective method of prevention [6]. Encouraging results have been achieved in some countries with a high incidence, where the vaccination of children has led to a drastic decrease of HCC with a very positive result in terms of cost/benefit ratio [10]. In other geographic areas where the incidence of HCC is related to alcohol abuse and HCV infection, the preventive measure is aimed at the early identification of those at risk and the prevention and treatment of viral infection and alcohol abuse. The appropriate preventive measures should therefore be adapted to different types of patients. For example, primary prevention of HCC in cirrhotic patients is indicated in the Western world. The use of screening for liver disease and the search for noninvasive methods validated for detecting liver cirrhosis, a disease that continues to be under-diagnosed, are therefore important. Still debated is the role of any preventive drug therapies in order to reduce hepatocarcinogenesis in subjects with chronic active hepatitis [11].

## Surveillance

The clinical and instrumental monitoring procedures have the dual purpose of reducing disease-specific mortality and increasing survival through early detection of the disease in question in order to give the patient the best therapeutic strategy. HCC has all the features that justify the commitment to a program of surveillance, and therefore, the screening of HCC has become a shared aspect of the management of patients with advanced-stage liver disease. Screening refers to the execution of a test that permits the diagnosis of a disease to a stage at which therapeutic intervention can significantly increase the survival of the patient. Surveillance consists of the application of this test repeated over time. In 2001, a group of experts (EASL—European Association for the Study of the Liver) met and established guidelines for surveillance of HCC in cirrhotic patients. The most commonly used screening tests for HCC are the dosage of serum α-fetoprotein (AFP) [12] and ultrasound examination [13]. The benefit of surveillance in terms of reduced mortality was evidenced by a single randomized study from China comparing surveillance and nonsurveillance in HBV patients using serum AFP and abdominal ultrasound at 6-month intervals [14]. Besides, a survival advantage for patients under HCC screening was revealed by cohort studies; this evidence was corroborated, and cost-effectiveness was shown by retrospective studies [15–18]. AFP was the most extensively used marker in the early studies on the surveillance of HCC, but the sensitivity of α-fetoprotein for screening of HCC varies widely, and if the threshold level of AFP for the diagnosis of HCC increases for example from 20 to 100 mg/L, the test sensitivity decreases from 39 to 13 %, while the specificity increases [19]. AFP in any case is not specific for HCC, and values can also increase to the peak activity of hepatitis. Out of 44 HBV-positive patients with elevated AFP levels found during a surveillance program for HCC, only 6 were actually diagnosed with HCC. Further investigations have shown that 28/44 (41 %) patients had an exacerbation of the underlying hepatic disease or changes in the state of HBV replication [20]. Individuals with viral infection without HCC may show elevated levels of AFP with transient, persistent, or intermittent pattern. These elevations are to be attributed to an exacerbation of hepatitis, more certainly when accompanied by a concomitant increase in the levels of transaminases, indicative of active infection or seroconversion. It becomes difficult to make a differential diagnosis between viral infection and HCC when the increase of AFP does not correlate with an increase in transaminases. However, the levels of AFP, in the presence of hepatocellular carcinoma, tend to increase the range and exceed that observed in the absence of tumor.

There are no guidelines indicating when an increase in the level of AFP in the presence of a normal ultrasound picture needs further investigation for a diagnosis of exclusion of HCC. In a direct test of AFP in diagnosing HCC in HBV-positive and HBV-negative patients, the specificity was only 50 % in HBV-positive patients compared to HBV-negative patients [21]. The characteristics of AFP assay as a screening test render a sensitivity of 39–64 %, a specificity from 76 to 91 %, and a positive predictive value from 9 to 32 % [22, 23]. Serum AFP has overall reduced sensitivity and specificity and thus should not be used as a screening tool unless ultrasonography (or other imaging modality) is unavailable. Regarding ultrasound imaging, the characteristics of the study method as a screening test for HCC were defined both in healthy carriers (noncirrhotic) of hepatitis B surface antigen and in patients with cirrhosis. Diagnostic medical sonography showed a sensitivity of 71 and 78 %, respectively, a specificity of 93 %, and the positive predictive value was 14 and 73 % [22, 23]. Various clinical studies have been conducted in order to identify the target population for surveillance. Male sex, age over 55 years, positivity for HCV, and the alteration of liver function tests were identified as risk factors for the development of HCC in a study conducted on 463 subjects with cirrhosis monitored for an average of 38 months [24]. In several studies, the intervals of monitoring range from 3 months to 1 year, but, on the basis of the data of tumor growth, an interval of 6 months seems appropriate. In fact, the time it takes to become an instrumentally detectable neoplastic lesion (at least 2 cm in diameter) is between 4 and 12 months [25], with a median tumor doubling time of 117 days [26]. The most recent EASL guidelines propose the combined use of ultrasound and AFP assay to be performed every 6–12 months in patients with cirrhosis [27, 28]. Applying these surveillance programs, diagnosis at an early stage was possible in a proportion of 40 % of HCC patients in a Spanish center highly specialized in the management and treatment of HCC [28]. In the presence of a nodule detected by ultrasound imaging, subsequent steps depend strictly on the size of the nodule, according to the following algorithm: (i) nodules <1 cm: pathological studies have shown that over half of the suspicious nodules with a diameter <1 cm are not really HCC. Furthermore, the identification of small hypervascular spots by magnetic resonance imaging (MRI) or computed tomography (CT) scan may not be indicative of neoplastic nodule. However, these small nodules can become malignant over time and increase in size. Therefore, the sub-centimeter nodules must be followed by ultrasound imaging at intervals of 3–4 months, for at least 2 years; if in that period of time they show no dimensional changes, the patient may be followed through a regular monitoring program; (ii) nodules 1–2 cm: the EASL guidelines [29] recommended the use of biopsy for these nodules, given the high likelihood of being cancerous nodules. However, for small nodules, biopsy may be difficult to perform and not diagnostic, with values of false negatives that fluctuate between 30 and 40 % [29]. Therefore, the current recommendations include the use of two imaging methods that assess the vascularization of the nodule. In the presence of a typical vascular pattern (arterial hypervascularity with portal venous phase washout), the nodule is interpreted as HCC. The biopsy should instead be performed in the presence of an atypical vascular pattern (e.g., a nodule not uptaking contrast medium in the arterial phase) or in the event of a discrepancy between the two imaging modalities. If the biopsy is negative, the patient should continue to perform a close instrumental follow-up with the use of contrast medium (CT or MRI); (iii) nodule >2 cm: In this case, a single imaging modality (CT or MRI) is sufficient to provide a proper diagnosis in the presence of the typical pattern of contrast effect. If, however, this pattern is not found and the nodule is atypical, with AFP values <200 ng/mL, it is necessary to perform a biopsy, as already proposed for the nodules between 1 and 2 cm. In conclusion, it can be said that, while the dosage of AFP was abandoned as a screening method and only maintains a role in identifying patients at high risk and in activating a noninvasive diagnostic program when it is markedly elevated, among the imaging methods, diagnostic medical sonography remains the only one that can be used for screening. In fact, despite the known limitations related to operator dependence and the possible difficulties of interpretation related to patient characteristics (obesity) or tumor (isoechogenicity), the location of the lesion (subdiaphragmatic area), and the appearance of the remaining parenchyma (inhomogeneous pattern in steatosis or macronodular liver cirrhosis), ultrasound is able to recognize HCC before the critical diameter of 3 cm in more than 85 % of the cases [28].

## Diagnosis

HCC is usually diagnosed by gastroenterologists or hepatologists during checks of the underlying liver disease. The diagnosis is made considering the clinical presentation, the imaging features, the AFP dosage, and possibly a liver biopsy [28]. Often, the cancer is at an advanced stage when the patient reaches the observation of the clinician due to several factors: the possible rapid tumor growth, the need for the tumor to reach a noticeable size to be symptomatic, the considerable reserve capacity of the liver that makes liver failure occur only when most of the parenchyma is replaced, the rare and late metastatic spread, and the presence of an underlying disease that can mask for a long time the symptoms referable to the tumor [4]. The clinical

onset of HCC is insidious, and disease progresses silently in the early stages, making timely diagnosis difficult. The earliest symptoms are as follows: dull, deep, and worsening pain in the right upper quadrant and epigastrium, feeling of abdominal distension, and other nonspecific symptoms such as fever, malaise, anorexia, postprandial feeling of fullness, and weight loss [4]. Other symptoms of lower frequency are to be referred to the peculiar localization of the tumor: jaundice, if the tumor compresses the primary bile ducts; severe abdominal pain, if there is distension of the capsule or capsular rupture into the peritoneum [4]. The presence of a chronic hepatic disease involves a whole set of symptoms referable to secondary liver failure. However, HCC is frequently diagnosed at an asymptomatic stage during a preoperative evaluation (e.g., in anticipation of a liver transplant) or during the screening program in patients with cirrhosis. For the detection of HCC, in addition to the clinical characterization, is fundamental an accurate imaging study, which must include the use of equipment of high diagnostic profile, with acquisition dynamics after intravenous administration of contrast medium, obtaining scans in the arterial, portal, and late phases [4]. The diagnosis of HCC is based on fundamentally, early identification of impregnation contrast enhancement in the arterial phase with subsequent disposal in the portal and late phases both for ultrasound examination, CT and MRI. In fact, while the normal liver parenchyma is vascularized predominantly (75 %) from the portal blood and the remainder (25 %) from the hepatic artery, the HCC has a predominantly arterial vasculature. The identification of the typical contrastographic pattern allows making the diagnosis of HCC with a positive predictive value of approximately 90–100 %, which was comparable for the three imaging modalities [30–32]. At ultrasound imaging, the echogenicity of the lesion varies depending on the size; in fact, while the nodules smaller than 3 cm are usually hypoechoic, homogenous and with well-defined margins, lesions >3 cm appear most often inhomogeneous, for the alternation of areas of necrosis, hemorrhage, and interstitial fibrosis. When visible, the capsule appears as a hypoechoic rhyme [33]. Similarly, at MRI, visualization of HCC is influenced by many factors (composition of the lesion, size, vascularity, state of the perilesional parenchyma), which tend to vary from subject to subject: Typically, HCC is hypointense on T1 and hyperintense on T2; however, the presence of glycogen, clear cell, copper or fatty degeneration, is the basis of the considerable signal variability of HCC [34]. The significant technological development in recent years, with the validation of contrast-enhanced ultrasound (CEUS) imaging, the spread of multidetector CT and the development of liver-specific contrast agents and high spatial and temporal resolution sequences in MRI, has greatly increased the sensitivity and specificity of imaging methods, making the diagnostic angiography examination obsolete and the use of biopsy increasingly rare. Anyway, if the radiological imaging features on the dynamic studies do not coincide, an image-guided core biopsy can be considered. Consequently, while in 2000 the histological examination was recommended for the diagnosis of HCC, the 2005 guidelines recommend biopsy only in cases of lesions with contrast-atypical pattern, therefore lacking the typical impregnation in the arterial phase and washout in the portal phase [27]. These nodules may in fact be an expression of what has recently been described as very early HCC in which only a few foci of neoplastic degeneration are histologically recognized and early treatment could significantly increase patient's survival. An interesting study evaluated the diagnostic accuracy of CT and MRI of cirrhotic livers with lesions suspicious for HCC, subsequently subjected to removal and transplantation, documenting a higher diagnostic accuracy of MRI over CT in the identification of HCC nodules sized between 1 and 2 cm [35].

## Staging

The purpose of the staging of neoplastic diseases is to cluster patients into distinct groups so as to identify the most appropriate therapeutic strategy, define an estimate of prognosis, and provide a valuable tool for evaluating the results of treatment. Unlike most other cancers, for which the staging systems are well codified and universally accepted, in HCC, the proposed systems are not shared by all. One of the reasons that render difficult the staging of HCC is related to the fact that in almost all cases, this cancer occurs in patients with liver cirrhosis. Therefore, the staging must take into account the severity of the underlying disease, which most often impacts prognosis. According to EASL Guidelines [29], an appropriate staging of HCC should take into account four main aspects: tumor characteristics, liver function, general condition of the patient, and therapeutic perspectives for each individual. An evaluation of the underlying hepatic function, using the Child–Pugh scoring system (bilirubin, albumin, prothrombin time, ascites, and encephalopathy) and the MELD score (creatinine, International Normalized Ratio, bilirubin), should represent the preliminary phase in assessing HCC patients and allows patient stratification and prognostic estimation based on the degree of hepatic function impairment [6]. The Child–Pugh classification is the most widely used classification for the evaluation of hepatic impairment alone, but the main flaw is represented by the lack of predictive ability, related to the fact that it does not take into account the clinical and biochemical variability of the disease. One of the staging systems for HCC is the

TNM system, now in its sixth revision, which has two main limitations: It does not include liver function tests and requires invasive procedures. The first system of classification of HCC was proposed by Okuda in 1985, and it included variables assessing liver function in association with tumor size. In this system, however, important parameters such as the unifocality or multifocality of the tumor, vascular invasion, and the presence of extrahepatic localizations were not included, and it not adequately distinguished HCC in the initial stage from the advanced one. The staging system of Cancer of the Liver Italian Program (CLIP) was created using a database of 435 patients with the aim of overcoming the TNM and Okuda classifications. Compared to the latter, CLIP staging system showed greater accuracy and greater ability to discriminate homogeneous groups [36]. Currently, the most widely used staging is the Barcelona Clinic Liver Cancer (BCLC), the only one that takes into account the aforementioned four aspects (tumor characteristics, liver function, general condition of the patient, and therapeutic perspectives for each case) [37], which, although has room for improvement, is a valuable tool to include the patient in most appropriate therapeutic option. This classification uses variables related to tumor stage (single or multiple nodules, size, portal invasion, positive lymph node, presence of metastasis), hepatic function (Child–Pug score), and disease-related symptoms (performance status) and outlines five stages (stage 0, A, B, C, and D), which are flanked by different therapeutic protocols [27]. It is particularly effective in the selection of patients with HCC at an early stage (stage A) who undergo curative treatment, such as surgical resection, liver transplantation, or local ablative therapies. This staging system although widely accepted and used in clinical practice as a guideline both for the treatment of HCC and for scientific and clinical purposes for stratification of HCC patients in prospective clinical trials has limitations in defining optimally the various possible clinical situations and the appropriate treatment, does not clearly indicate whether patients in Child–Pugh class C with small HCC are candidates for transplantation or not [38], and does not differentiate in a comprehensive manner class B patients by those of class C.

## The genomic landscape of hepatocarcinogenesis

The genomic background of HCC has not been entirely discovered, and knowledge of genetic aberrations involved in hepatocarcinogenesis allows finding new strategies for HCC-targeted therapy. Genetic drivers frequently altered take part in different signaling pathways comprising telomere maintenance, cell cycle regulators, chromatin remodeling, Wnt/β-catenin, RAS/RAF/MAPK kinase, and AKT/mTOR pathway. HCC onset and progression are related to molecular signatures from cirrhotic livers and single nucleotide polymorphism, and personalized clinical decision making to improve survival of HCC patients needs a translational approach, particularly in patients with refractory advanced/metastatic HCC referred for experimental therapies. HCC is a complex disease hallmarked by genomic diversity and classification of high-risk populations with a poor prognosis related to early tumor recurrence due to metastasis and late tumor recurrence due to de novo carcinogenesis after curative treatment would be valuable to design chemopreventive trials or to target cancer genes by biotherapies and tumor-specific suicide gene therapy [39–43]. Molecular profiling of HCC permits the progress of novel approaches to disease diagnosis, prognosis prediction, and treatment management as a paradigm of stratified medicine adapted to tumor biology through integration of clinical staging systems and molecular-based information to support clinical decision making. For up-to-date and comprehensive description of the molecular mechanisms involved in hepatocellular carcinogenesis, refer to some recent articles [44–52].

## Natural history and prognosis

Until recently, the prognosis of HCC was poor. Many patients died within a year of diagnosis regardless of treatment strategy. In developed countries, this result has changed due to early diagnosis possible today in approximately 30–40 % of patients who may therefore benefit from curative treatments. Therefore, on the basis of the BCLC classification, we can distinguish 5 stages: (i) very early stage (stage 0); (ii) early stage (stage A); (iii) intermediate stage (stage B); (iv) advanced stage (stage C); and (v) end-stage (stage D).

### HCC in very early and early stage (BCLC stage 0 and A)

The natural history of HCC at an early stage is unknown because nearly all subjects are treated in this phase [53]. Up to 10 years ago, the best survival was 65 % at 3 years for class Child–Pugh A patients with a single tumor [54]. At present, a 5-year survival of 50–70 % is reported [55]. The prognosis in early stage is linked to the state of the tumor, the liver function, and the treatment used. The status of the tumor is defined by the size of the nodule and the multicentricity. Liver function is particularly relevant to the choice of the most appropriate treatment. Among patients in Child–Pugh class A undergoing resection, positive predictors of survival were bilirubin concentration <17.1 μmol/L, absence of portal hypertension (hepatic

venous pressure gradient <10 mmHg), and indocyanine green (ICG) retention rate at 15 min <20 % [56]. The definition of early HCC has changed over time. Until a few years ago was described as an early tumor smaller than 5 cm in diameter; subsequently, also multiple nodules were considered early HCC, in the maximum number of 3, each with a diameter of <3 cm. However, clinical and pathological data dispute this definition, as the response to treatment and survival in this group of patients is variable, and the term early HCC actually covers different stages with a heterogeneous biological behavior. For example, the rates of complete response after percutaneous treatments vary according to the size of the nodule (90–100 % in the tumors of 2 cm, 50 % in the tumors of 5 cm) [57]. The same thing happens for the survival rates. We must also consider that the possibility to identify small tumors has led to the definition of very early HCC, which from a pathological point of view refers to carcinoma in situ, that is, to a well-differentiated HCC that contains bile ducts and portal vessels of nodular form and, as by definition, which has not yet invaded any structure, and which usually has a diameter <2 cm. You should be aware that the invasion by the tumor is a phenomenon independent of the size of the nodule. So you may incur in tumors smaller than 2 cm, which behave as carcinoma in situ or already scattered. Some authors have differentiated "indistinct nodular"-type HCC (average size of 1–2 cm), with no local invasion, the "distinct nodular" type (average size of 1–6 cm), with local invasiveness. In the indistinct nodular type, local metastases were observed around the nodule in 10 % of cases, and microscopic portal invasion in 25 %. Although both types have been identified at the ultrasound examination, the indistinct type appears hypovascular on CT, while the second type appears hypervascular. This correlation between radiological and pathological anatomies agrees with the finding that early cancers have portal vasculature without the typical hypervascular pattern visible in the dynamic study. The very early identification of HCC (very early stage) is extremely important because these patients, if properly treated, have a especially favorable prognosis, with 5-year survival of 89–93 % after surgical resection [58–60] and 71 % after percutaneous treatment [59], compared with 71 and 54 %, respectively, in patients with conventional early HCC (early stage) smaller than 2 cm [55]. Moreover, very early HCC does not tend to recur after curative treatment of HCC differently from the traditional type smaller than 2 cm (8 versus 74 % at 3 years) [60]. Clearly, these tumors should be distinguished from nodules of high-grade dysplasia, for which there is no general agreement on histological criteria to be applied. The neoplastic invasion of the portal vessels within the tumor might help identify the malignant nature of the very early cases, but in the future molecular analysis could become the optimal tool. In the scientific literature, however, are not reported randomized trials comparing surgical resection, transplantation, and thermal radio-frequency ablation (RFA) or percutaneous alcohol injection in early HCC; recent study projects designed to compare resection and RF ablation failed in Italy and Japan. Therefore, there is not a firm and established scientific eviFrom this experience, howeverdence on what is the best radical treatment for a patient suffering from early HCC with preserved liver function. However, numerous not randomized cohort studies have shown that resection and transplantation reach excellent results of survival (60–70 % at 5 years) in well-selected patients and are therefore regarded as first-choice treatment of early HCC [61, 62].

### Intermediate- and advanced-stage HCC (BCLC stage B and C)

In the majority of patients, HCC is diagnosed in intermediate and advanced stages, thus precluding the use of radical therapies. The natural course and prognostic factors that define these stages are now quite well known in comparison with past decades when the patients had a prognosis of approximately 1 year after the diagnosis of HCC [63]. Survival rates at 1 and 2 years in 25 randomized controlled trials in which patients received no treatment were, respectively, 10–72 % and 8–50 % [61]. The wide variability emphasizes the heterogeneity of the population with HCC not candidate for surgery and the need to break it down into subcategories. For this purpose, Llovet et al. enrolled a cohort of 102 patients from two untreated control groups, recruited among patients of randomized controlled trials. The survival at 1, 2, and 3 years was 54, 40, and 28 %, respectively. The independent prognostic factors were the presence of cancer-related symptoms (performance status 1–2) and the presence of vascular invasion or extrahepatic dissemination. The survival among patients in the intermediate stage (asymptomatic, noninvasive pattern) at 1, 2, and 3 years was 80, 65, and 50 %, while among those with advanced disease (symptomatic, with invasive pattern or both), the survival was 29, 16, and 8 %. The concentration of AFP and alkaline phosphatase, the Child–Pugh class, and the presence of ascites could further clarify the prognosis [62].

### HCC in end-stage (BCLC stage D)

A high percentage of patients is still identified with symptoms related to cancer in terminal phase, especially in Asian and African countries where monitoring programs are not possible for logistical and economic reasons, and their prognosis is poor, with a life expectancy of <3 months.

## Treatment options

Currently, the choice of the therapeutic strategy to be adopted in patient with HCC is mainly based on the EASL guidelines [27] and the BCLC staging [37]. According to this algorithm, patients with early-stage HCC may benefit from curative treatments, comprising hepatic resection, liver transplantation, percutaneous radio-frequency ablation (RFA), or percutaneous ethanol injection (PEI) [61, 64, 65]. In case of HCC in the intermediate and advanced stages, patients may be amenable to palliative treatments, in order to increase survival, and/or could be included in prospective studies, especially if randomized controlled trials, in order to be able to identify therapies more appropriate to achieve an increase in survival.

### HCC in very early or early stage: healing treatments

It is estimated that at the time of diagnosis, approximately 30–40 % of patients with HCC in the Western world can benefit from curative treatment [61]; in Japan, this percentage reaches 60–90 %, thanks to the spread of surveillance programs and a wide application of the treatments [55]. Since, for ethical reasons, it is not possible to carry out a study that compares the main curative treatments, no solid evidence establishes which is the optimal treatment for patients with early-stage disease. Non-randomized cohort studies report 5-year survival rates of 60–70 %, in carefully selected patients, with results essentially matched for hepatic resection, orthotopic liver transplantation, and percutaneous ablative treatments (thermal ablation RF, PEI, laser therapy) [64, 65]. The selection of the patient remains the critical factor for obtaining an appropriate clinical outcome in the long term. According to the data available today can in fact benefit from curative treatment patients with single nodule <5 cm or with <3 nodules each <3 cm [66]. These criteria are, however, currently under discussion.

#### *Surgical resection*

Hepatic resection is the treatment of choice in noncirrhotic patients with HCC, accounting for about 5 % of patients with HCC in the Western world and 40 % in Asia (Bruix, 2002). Hepatic resection offers excellent results in cirrhotic patients with well-preserved liver function and with single nodule. The classification of Child–Pugh class A is not sufficient alone to identify patients who are candidates for resection and must be supported by additional indicators. Very restrictive selection criteria are in fact required to avoid complications related to treatment, especially related to liver failure. The minimal critical size of remnant liver after resection is 25 % in presence of normal liver and 50 % in patients with cirrhosis [67]. Japanese researchers have used the ICG retention rate at 15 min to identify the best candidates, while in Europe the values of portal pressure and bilirubin are used [68, 69]. It was therefore identified the so-called Child–Pugh hyperclass A: patients with asymptomatic HCC, normal bilirubin, and no signs of portal hypertension. According to these criteria, approximately 5–10 % of patients with HCC may benefit from hepatic resection, with an operative mortality of <1–3 %, less need for blood transfusions in 10 % of cases, and 70 % survival rates at 5 years. The main complication in the follow-up after resection is tumor recurrence, which occurs in about 70 % of cases at 5 years. Recognized predictors of recurrence following resection are the presence of microvascular invasion, poor histologic differentiation, and multiplicity of tumors with presence of satellite lesions predict the occurrence of relapses. Some strategies were evaluated for the prevention of post-surgical recurrence: Adoptive immunotherapy, chemoembolization, or adjuvant chemotherapy has not demonstrated any benefit, while the internal irradiation with 131 I-labeled lipiodol and interferon therapy have shown promising results [70–73].

#### *Orthotopic liver transplantation*

HCC is the only solid tumor in which the graft has an important role and has in fact completely changed the therapeutic strategy of HCC. It has the advantage, compared to surgery, to remove the cirrhotic liver with the tumor itself, thus eliminating the underlying disease. Unfortunately, this theoretical superiority is faced with the scarcity of donors and the consequent need to carefully select patients for transplantation. In addition, in the patient on the waiting list, the cancer may progress and preclude the intervention (dropout). The broad selection criteria applied two decades ago led to poor results, because of the frequent recurrences (32–54 %) with 5-year survival <40 % [74]. From this experience, however, were derived the data useful for identifying the best selection criteria in order to transplant only patients who can really benefit from it, with a significant increase in survival [75]. These criteria, although still under discussion, are represented by the presence of a single nodule <5 cm or <3 tumor nodules with a diameter <3 cm each (the so-called Milan criteria) [66]. Their adoption is associated with a 5-year survival of 70 % with recurrence rates of <15 %. Some studies based on the examination of the explanted liver have suggested that these criteria could be expanded, but this decision should be based on a sound analysis of imaging data at the time of the therapeutic indication rather than pathological anatomy data that become available only when the operation is finished. However, many authors have proposed to

expand the criteria for patients with HCC with the possibility therefore of increasing the survival, including among the possible new selection criteria the presence of a single tumor <7 cm or 3 nodules below 5 cm, 5 nodules <3 cm, and finally evidence of tumor regression after locoregional treatment [76]. The most important problem in patients with HCC candidates for transplant is time on the waiting list, which often exceed 12 months, given the scarcity of organs, resulting in dropout from the list caused by tumor progression in approximately 20–50 % cases. The problem of dropouts was also addressed by the use of adjuvant treatments such as intra-arterial chemoembolization in patients waiting for transplantation to prevent tumor progression. However, the real effectiveness of adjuvant therapies in pretransplant is still the subject of debate [77, 78]. The living donor transplantation is emerging as an alternative to cadaveric transplantation, to increase the availability of organs. However, enthusiasm for living donor transplantation is mitigated by the need for highly specialized surgical team, given the considerable complexity of the intervention, with rates of perioperative morbidity of 20–40 %; also, the mortality of the donors is 0.3–0.5 % [79].

*Radiofrequency thermoablation/percutaneous ethanol injection*

The treatment of HCC by percutaneous ablative techniques has been an important progress made by the interventional radiology and changed the therapeutic approach to patients not candidates for transplantation. The percutaneous alcoholization (percutaneous ethanol injection, PEI) was the first technique used: It consists of the intratumoral injection of ethyl alcohol, producing coagulative necrosis, as a consequence of cellular dehydration, denaturation of protein, and chemical occlusion of tumor small vasculature. It is generally performed under ultrasound guidance, which allows for real-time assessment of the diffusion of alcohol into the tumor while avoiding an excessive spreading outside of the margins of the lesion. The therapeutic protocol involves running 4–6 sessions of alcoholization with a frequency of once or twice a week. The number of sessions, as well as the amount of ethanol injection, varies in relation to the size of the lesion, the type of perfusion, and the tolerability of the patient. Typically, PEI is a well-tolerated treatment, easy to perform, inexpensive, with few side effects, and with a high antitumor efficacy, in particular in single and small size nodules [80], with survival rates of 47–79 % at 3–5 years for tumors <5 cm in diameter [81]. The major limitation of PEI is represented by the high rate of local recurrence, which can reach 33 % in lesions smaller than 3 cm and 43 % in lesions larger than 3 cm [82–85]. This is attributable to the uneven diffusion of ethanol within the lesion, resulting in inadequate positioning of the needle and/or the presence of intratumoral septa, as well as to the limited effects on extracapsular spread of neoplastic cells. Furthermore, PEI is not able to create an ablation margin of safety in the liver parenchyma surrounding the lesion, where satellite nodules are most frequently located. Currently, PEI is therefore indicated in patients with HCC at an early stage, when neither transplantation nor surgical resection is a feasible option. Alternative methods of tumor ablation were developed, with the use of microwave, laser, or radio frequency (RF), with the aim to increase the antitumor efficacy of percutaneous treatments, in terms of tumor necrosis. RF ablation is the method currently most used in the treatment of hepatocellular carcinoma, with promising results [86]. The purpose of RF ablation is to cause thermal tissue damage through deposition of electromagnetic energy. The cells subjected to thermal damage undergo coagulation necrosis during the days following treatment. To achieve effective destruction of the lesion, the entire tumor volume must be subjected to cytotoxic temperatures including a surrounding area of apparently healthy tissue of 0.5–1 cm, to eliminate any microscopic foci of disease and compensate for the uncertainty that often exists as to the actual location of the tumor margins. In the course of the first experiences with RF, the major limitation was represented by the small volume of ablation induced by conventional electrodes. These devices were capable of producing ablation zones of cylindrical shape not exceeding 1.6 cm [87]. Multiple sessions were then required to treat even small lesions. Subsequently, numerous strategies have been developed to increase the ablation volume obtainable with RF, for example, by optimizing the production of heat and minimizing the dispersion within the area to be treated [88] or through the introduction of new needle-electrodes with more tips. RF can achieve response rates similar or even superior to PEI in fewer sessions and seems more effective than PEI in tumors larger than 3 cm [86, 89]. In a randomized study of 104 patients with HCC at an early stage, it was found that RF ablation appears to be associated with a disease-free survival rate higher than PEI [86]. However, even with this treatment, tumor recurrence is high, similar to that obtainable after surgical resection (50 % at 3 years and >70 % at 5 years).

## HCC in intermediate–advanced stage: precision TACE and TACE

Surveillance programs have increased the percentage of patients who are diagnosed with HCC at an early stage, although in half of the patients the disease continues to be diagnosed at an intermediate–advanced stage, no longer indicating curative treatments. Nowadays, the optimal

treatment of HCC in the intermediate–advanced phase represents the subject of extensive debate, within a scenario represented by multiple systemic or locoregional therapeutic alternatives, which have the purpose of inducing tumor necrosis, thus slowing the progression of disease and possibly improving survival.

*TACE (transcatheter arterial chemoembolization)*

In this context, selective embolization with (TACE) or without (TAE) intra-arterial (hepatic artery) injection of chemotherapeutic drugs represents a possible treatment strategy, with demonstrated possibility of obtaining an objective tumor response of 16–60 % resulting in an increase in survival of 10–50 % [90, 91]. TACE, described for the first time by Kato et al. in 1981, consists in conveying a mixture composed of one or more cytotoxic drugs and an oily medium (usually Lipiodol) through an intra-arterial catheter in more or less extensive areas of hepatic parenchyma with HCC followed by mechanical occlusion (embolization) of the afferent vessels. The rationale of TACE method is based on the peculiar characteristic of the HCC nodule to be preferentially perfused by branches of the hepatic artery, unlike the nontumor liver parenchyma that receives more than 70 % of blood perfusion from the portal vascular branches. It is therefore possible to selectively attack the tumor nodule through the arterial approach sparing the remaining liver parenchyma and thus reducing local and systemic side effects. The block of the arterial circulation through embolization favors the stagnation of chemotherapy in neoplastic nodules, slowing down the washout, and simultaneously causes sudden intralesional ischemia, which favors the necrosis and at the same time increases the cytotoxic effect of the anticancer agent for hypoxic effect. Necrosis induced by TACE therefore derives from the synergistic effect of high concentrations of drug within the diseased tissue and ischemia, which works inducing damage to tumor tissue and allowing the prolonged action of the drug in the target lesions. Recent research has shown that many cytotoxic drugs are actively expelled from the tumor cell with the aid of a specific ATP-dependent glycoprotein, produced by a multidrug resistance (MDR) gene, linked to drug resistance of tumor strains: Probably, ischemia induced by TACE also acts on this specific mechanism. The oily medium (Lipiodol) used in cytotoxic mixture is taken up preferentially into tumor cells where it concentrates from 3 to 5 times higher than in nontumor parenchyma, remaining in the tumor up to a few weeks or months, mainly due to a slow elimination for the absence at that level of the lymphatic vessels and reticuloendothelial cells. In this way, it is possible to achieve in the tumor tissue drug concentrations 10–100 times higher compared to systemic chemotherapy. Some authors still consider Lipiodol an embolizing material; however, there is no evidence of its ability of embolizing and can only be considered a carrier for transporting the chemotherapy into the tumor. Studies comparing the effectiveness of TACE and RFA in patients with small single-nodule HCC have evidenced that TACE is an effective treatment permitting to attain long-term survival rates comparable to those with RFA and may be a feasible choice when RFA is not feasible [92, 93].

*Chemotherapeutic agents used for TACE*

Several chemotherapeutic agents used for TACE are reported in the scientific literature, and the chemotherapy regimen used may comprise a single chemotherapeutic drug, two drugs, or three drugs. The most widely used agent in monotherapy is doxorubicin (epirubicin), followed by cisplatin, mitoxantrone, and mitomycin C. Two different types of double therapy are used, doxorubicin plus mitomycin C and doxorubicin plus cisplatin and a triple therapy (doxorubicin plus cisplatin plus mitomycin C). At present, there is no scientific evidence of the superiority of any chemotherapy agent used in monotherapy or in combination with the TACE [94].

*Radioembolization*

A different form of hepatic arterial therapy, applicable also in patients with portal vein thrombosis [95], is represented by transarterial radioembolization (TARE) with intra-arterial injection of yttrium-90 microspheres (Y-90) existing as glass (TheraSpheres; Theragenics Corp., Ottawa, Canada) or resin (Sirtex; Sirtex Medical, Wilmington, MA, USA), distributed to single or multiple segments through selective arterial cannulation, and preferably retained in the tumor capillary bed as a result of their small size (20–60 μm) [96]. The microspheres induce tumor necrosis by microscopic embolization, occluding the tumor capillary bed, and by radiation, transporting up to 150 Gy of β radiation to the neoplastic tissue with a half-life of 62 h and action radius of up to 1 cm, and fairly sparing the contiguous nontumor tissue [97]. Pre-treatment procedures comprising an angiogram and a macroaggregated albumin scan are necessary for patient selection, to perform prophylactic embolization if variant anatomy is identified to avoid nontarget delivery of Y-90 and to confirm that hepatic artery-to-lung shunting is <16 % to prevent lung injury [98]. Gastrointestinal ulcerations, pancreatitis, pneumonitis, and cholecystitis represent possible complications caused by nontarget delivery of Y-90 [99]. Controlled data comparing TARE with TACE and evaluating impact on survival in intermediate-stage HCC patients evidenced that both treatments resulted in similar survival probabilities

despite more advanced disease in the TARE Y-90 group, which was better tolerated and associated with less hospitalization and treatment sessions [100]. Interestingly, in cirrhotic patients with intermediate–advanced or not-otherwise-treatable HCC, sorafenib and TARE have been shown to provide similar survivals, and down-staging allowing liver transplantation only occurred after TARE [101].

*HCC in advanced stage (N1, M1, portal invasion, PST1-2): Sorafenib and beyond*

Not all of the advanced-stage HCC can be subjected to TACE. HCC at an advanced stage with extrahepatic metastases (M1) and/or lymph node metastases (N1) and/or portal invasion established by imaging techniques and/or hepatic impairment (performance status 1–2), which make up about 40–70 % of the total population of HCC patients, according to the guidelines of the BCLC, should be treated with systemic chemotherapy. Until 2005, no chemotherapy was effective in the treatment of advanced-stage HCC with TACE contraindication: various systemic therapies, including chemotherapy (doxorubicin, epirubicin, cisplatin, etc.), hormonal compounds (anti-estrogens, anti-androgens, octreotide), immunotherapy (interferon), and other drugs such as nolatrexed or seocalcitol—an antiproliferative molecule similar to vitamin D—have yielded negative results [102]. However, in recent years, cancer research had provided encouraging results with molecular therapy. Among these, Sorafenib (Nexavar), a multikinase inhibitory molecule that blocks the signal transmitted by various growth factors (Raf-1, B-Raf, VEGFR2, PDGFR, c-Kit), showed antiproliferative and antiangiogenic capacity toward HCC in vitro and in animal models, leading in 2006 to the first phase II clinical study that showed a potential clinical benefit in advanced-stage HCC [103]. Then in 2008 came the excellent results of the SHARP trial, a phase III clinical trial conducted on 602 patients with advanced HCC, who was arrested for the obvious superiority in terms of survival of the sorafenib group ($n = 299$) compared to placebo ($n = 303$). The study showed a sorafenib/placebo hazard ratio of death of 0.69, expression of a 31 % reduction in the risk of death with a median survival of 10.6 months in the sorafenib group compared with 7.9 months in the placebo group [104]. Similar and encouraging results in terms of survival have come from another phase III randomized clinical trial of sorafenib versus placebo, conducted in the Asia–Pacific region on 226 patients with HCC at an advanced unresectable or metastatic stage [105]. To date, therefore, Sorafenib ranks as the undisputed drug of choice in the treatment of HCC at an advanced stage that may not be treated with TACE and showing HCC progression after locoregional therapies. Besides, retrospective and randomized studies have suggested that the combined use of Sorafenib, TACE, and RFA may be useful in patients with unresectable HCC [106, 107]. In particular, in a retrospective study, a therapeutic approach with curative intent for all detectable lesions in patients with BCLC stage 0-B1 HCC comprising of Sorafenib combined with RFA was evaluated for efficacy [108]. The study involved 128 HCC patients: 64 patients were treated with Sorafenib plus RFA and 64 patients were treated with RFA alone; the primary end-point of the study was the incidence of post-RFA HCC recurrence, whereas secondary end-points were overall survival (OS) and treatment toxicity. Combined therapy with Sorafenib and RFA was associated with a lower incidence of post-RFA recurrence and better OS than RFA alone, whereas no statically significant difference was evidenced in morbidity and mortality and the two groups had similar Dindo–Clavien class complications [108]. Besides, the final results of the START trial, a phase II, investigator-initiated, prospective single-arm multinational study that evaluated sorafenib in combination with doxorubicin-based transarterial TACE in patients with intermediate-stage, unresectable HCC, evidenced that TACE/sorafenib cycles repeated every 6–8 weeks were well tolerated, and 52.6 % of patients achieved complete response in target lesions; 16.8 % achieved partial response, and 5.8 % had progression of disease as their best response, assessed by modified response evaluation criteria in solid tumors (RECIST). Median progression-free survival and time to progression were 384 and 415 days, respectively, and the estimated 3-year overall survival was 86.1 % [109].

*HCC in terminal phase: symptomatic treatment*

HCC patients in terminal phase have no benefit from the aforementioned treatments, and there is only indication for palliative/support therapy and psychosocial intervention in order to relief symptoms, to provide to patients a final less painful disease, and to improve health-related quality of residual life [110].

## Conclusion

HCC represents an important cause of cancer-related mortality worldwide and imposes a huge social and economic burden on healthcare systems. HCC treatment outcomes are influenced by a number of variables, comprising liver function, patient's performance status, and tumor stage. In recent times, improved surveillance programs have allowed detection of the disease at earlier stage and increased therapeutic options have advanced patients'

survival. Curative therapies, such as liver transplantation, surgical resection, or ablative therapies; locoregional therapies, such as radio-frequency ablation, percutaneous ethanol injection, and transcatheter arterial chemoembolization; molecular-targeted agents such as sorafenib, gene therapy, and immunotherapy represent the treatment strategies exploitable depending on tumor size, number, and location, as well as anatomical and functional parameters. The progressive unveiling of genomic changes underlying hepatocarcinogenesis would allow better stratification of risk of developing HCC and more precise selection of patients for targeted therapy according to individual molecular signatures.

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## References

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin. 2011;61:69–90.
2. Forner A, Llovet JM, Bruix J. Hepatocellular carcinoma. Lancet. 2012;379:1245–55.
3. International Agency for Research on Cancer. Cancer incidence in five continents, Vol. X (electronic versions) Lyon 2014. http://ci5.iarc.fr/. Last accessed on 16 Feb 2015.
4. Bruix J, Gores GJ, Mazzaferro V. Hepatocellular carcinoma: clinical frontiers and perspectives. Gut. 2014;63(5):844–55.
5. Wang H, Wang AH, Gressner OA, et al. Association between HBV Pre-S mutations and the intracellular HBV DNAs in HBsAg-positive hepatocellular carcinoma in China. Clin Exp Med. 2014. [Epub ahead of print].
6. Cabrera R, Nelson DR. Review article: the management of hepatocellular carcinoma. Aliment Pharmacol Ther. 2010;31(4):461–76.
7. Calle EE, Rodriguez C, Walker-Thurmond K, Thun MJ. Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. N Engl J Med. 2003;348:1625–38.
8. El-Serag HB, Tran T, Everhart JE. Diabetes increases the risk of chronic liver disease and hepatocellular carcinoma. Gastroenterology. 2004;126:460–8.
9. Veldt BJ, Chen W, Heathcote EJ, et al. Increased risk of hepatocellular carcinoma among patients with hepatitis C cirrhosis and diabetes mellitus. Hepatology. 2008;47:1856–62.
10. Chang MH, Chen CJ, Lai MS, et al. Universal hepatitis B vaccination in Taiwan and the incidence of hepatocellular carcinoma in children. N Engl J Med. 1997;336:1855–9.
11. Nishiguchi S, Shiomi S, Nakatami S, et al. Prevention of hepatocellular carcinoma in patients ith chronic active hepatitis C and cirrhosis. Lancet. 2001;357:196–7.
12. Biselli M, Conti F, Gramenzi A, et al. A new approach to the use of α-fetoprotein as surveillance test for hepatocellular carcinoma in patients with cirrhosis. Br J Cancer. 2015;112(1):69–76.
13. Collier J, Sherman M. Screening for Hepatocellular carcinoma. Hepatology. 1998;27:273–8.
14. Zhang BH, Yang BH, Tang ZY. Randomized controlled trial of screening for hepatocellular carcinoma. J Cancer Res Clin Oncol. 2004;130:417–22.
15. Oka H, Kurioka N, Kim K, et al. Prospective study of early detection of hepatocellular carcinoma in patients with cirrhosis. Hepatology. 1990;12:680–7.
16. McMahon BJ, Bulkow L, Harpster A, et al. Screening for hepatocellular carcinoma in Alaska natives infected with chronic hepatitis B: a 16-year population-based study. Hepatology. 2000;32:842–6.
17. Wong LL, Limm WM, Severino R, Wong LM. Improved survival with screening for hepatocellular carcinoma. Liver Transpl. 2000;6:320–5.
18. Bolondi L, Sofia S, Siringo S, et al. Surveillance program of cirrhotic patients for early diagnosis and treatment of hepatocellular carcinoma: a cost-effectiveness analysis. Gut. 2001;48:251–9.
19. Oka H, Tamori A, Kuroki T, Kabayashi K, Yamamoto S. Prospective study of alphafetoprotein in cirrhotic patients monitored for development of hepatocellular carcinoma. Hepatology. 1994;19:61–6.
20. Lok ASF, Lai C-L. Alpha-fetoprotein monitoring in Chinese patients with chronic hepatitis B virus infection: role in the early detection of hepatocellular carcinoma. Hepatology. 1989;9:110–5.
21. Lee HS, Chung YH, Kim CY. Specificities of serum alpha-fetoprotein in HBsAg+ and ABsAg- patients in the diagnosis of hepatocellular carcinoma. Hepatology. 1991;14:68–72.
22. Sherman M, Peltekian KM, Lee C. Screening for hepatocellular carcinoma in chronic carriers of hepatitis B virus: incidence and prevalence of hepatocellular carcinoma in a North American urban population. Hepatology. 1995;22:432–8.
23. Pateron D, Ganne N, Trinchet JC, et al. Prospective study of screening for hepatocellular carcinoma in Caucasian patients with cirrhosis. J Hepatol. 1994;20:65–71.
24. Velazquez RF, Rodriguez M, Navascues CA, et al. Prospective analysis of risk factors for hepatocellular carcinoma in patients with liver cirrhosis. Hepatology. 2003;37:520–7.
25. Llovet JM, Burroughs A, Bruix J. Hepatocellular carcinoma. Lancet. 2003;362:1907–17.
26. Sheu JC, Sung JL, Chen DS, et al. Growth rates of asymptomatic hepatocellular carcinoma and its clinical implications. Gastroenterology. 1985;89:259–66.
27. Bruix J, Sherman M. Practice guidelines committee, American Association for the Study of Liver Disease. Management of hepatocellular carcinoma. Hepatology. 2005;42:1208–36.
28. Llovet JM, Fuster J, Bruix J. The Barcelona approach: diagnosis, staging, and treatment of hepatocellular carcinoma. Liver Transpl. 2004;10(2 Suppl 1):S115–20.
29. Bruix J, Sherman M, Llovet JM, et al. Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. European Association for the Study of the Liver. J Hepatol. 2001;35(3):421–30.
30. Valls C, Cos M, Figueras J, et al. Pretransplantation diagnosis and staging of hepatocellular carcinoma in patients with cirrhosis: value of dual-phase helical CT. AJR Am Roentgenol. 2004;182(4):1011–7.
31. Marrero JA, Hussain HK, Nghiem HV, et al. Improving the prediction of hepatocellular carcinoma in cirrhotic patients with

an arterially enhancing liver mass. Liver Transpl. 2005;11(3):281–9.
32. Bolondi L, Gaiani S, Celli N, et al. Characterization of small nodules in cirrhosis by assessment of vascularity: the problem of hypovascular hepatocellular carcinoma. Hepatology. 2005;42(1):27–34.
33. Choi BI, Kim CW, Han MC, et al. Sonographic characteristics of small hepatocellular carcinoma. Gastrointest Radiol. 1989;14:255–61.
34. Marti-Bonmati L. MR imaging characteristics of hepatic tumors. Eur Radiol. 1997;7:249–58.
35. Burrel M, Llovet JM, Ayuso C, et al. MRI angiography is superior to helical CT for detection of HCC prior to liver transplantation: an explant correlation. Hepatology. 2003;38(4):1034–42.
36. Levy I, Sherman M, Liver Cancer Study Group of the University of Toronto. Staging of hepatocellular carcinoma: assessment of the CLIP, Okuda and Child-Pugh staging systems in a cohort of 257 patients in Toronto. Gut. 2002;50(6):881–5.
37. Llovet JM, Bruix J. Prognosis of hepatocellular carcinoma: the BCLC staging classification. Semin Liver Dis. 1999;19:329–38.
38. Befeler AS, Di Bisceglie AM. Hepatocellular carcinoma: diagnosis and treatment. Gastroenterology. 2002;122:1609–19.
39. Kakinoki K, Nakamoto Y, Kagaya T, et al. Prevention of intrahepatic metastasis of liver cancer by suicide gene therapy and chemokine ligand 2/monocyte chemoattractant protein-1 delivery in mice. J Gene Med. 2010;12(12):1002–13.
40. Niess H, Bao Q, Conrad C, et al. Selective targeting of genetically engineered mesenchymal stem cells to tumor stroma microenvironments using tissue-specific suicide gene expression suppresses growth of hepatocellular carcinoma. Ann Surg. 2011;254(5):767–74.
41. Wang G, Dong X, Tian W, et al. Evaluation of miR-122-regulated suicide gene therapy for hepatocellular carcinoma in an orthotopic mouse model. Chin J Cancer Res. 2013;25(6):646–55. doi:10.3978/j.issn.1000-9604.2013.11.07.
42. Kawashita Y, Deb NJ, Garg MK, et al. An autologous in situ tumor vaccination approach for hepatocellular carcinoma. 2. Tumor-specific immunity and cure after radio-inducible suicide gene therapy and systemic CD40-ligand and Flt3-ligand gene therapy in an orthotopic tumor model. Radiat Res. 2014;182(2):201–10.
43. Lai YH, Lin CC, Chen SH, Tai CK. Tumor-specific suicide gene therapy for hepatocellular carcinoma by transcriptionally targeted retroviral replicating vectors. Gene Ther. 2015;22(2):155–62. doi:10.1038/gt.2014.98 **Epub 2014 Oct 30**.
44. Fang S, Huang SF, Cao J, Wen YA, Zhang LP, Ren GS. Silencing of PCDH10 in hepatocellular carcinoma via de novo DNA methylation independent of HBV infection or HBX expression. Clin Exp Med. 2013;13(2):127–34.
45. Rappa F, Greco A, Podrini C, et al. Immunopositivity for histone macroH2A1 isoforms marks steatosis-associated hepatocellular carcinoma. PLoS ONE. 2013;8(1):e54458.
46. Pinyol R, Nault JC, Quetglas IM, Zucman-Rossi J, Llovet JM. Molecular profiling of liver tumors: classification and clinical translation for decision making. Semin Liver Dis. 2014;34(4):363–75.
47. Quetglas IM, Moeini A, Pinyol R, Llovet JM. Integration of genomic information in the clinical management of HCC. Best Pract Res Clin Gastroenterol. 2014;28(5):831–42.
48. Janku F, Kaseb AO, Tsimberidou AM, Wolff RA, Kurzrock R. Identification of novel therapeutic targets in the PI3K/AKT/mTOR pathway in hepatocellular carcinoma using targeted next generation sequencing. Oncotarget. 2014;5(10):3012–22.
49. Neumann O, Kesselmeier M, Geffers R, et al. Methylome analysis and integrative profiling of human HCCs identify novel protumorigenic factors. Hepatology. 2012;56(5):1817–27.
50. Wang J, Liu G, Li Q, Wang F, et al. Mucin1 promotes the migration and invasion of hepatocellular carcinoma cells via JNK-mediated phosphorylation of Smad2 at the C-terminal and linker regions. Oncotarget. 2015 [**Epub ahead of print**].
51. McKee C, Soeda J, Sigala B, et al. Amphiregulin activates human hepatic stellate cells and is upregulated in non alcoholic steatohepatitis. Sci Rep. 2015;5:8812. doi:10.1038/srep08812.
52. Sha L, Dong L, Lv L, Bai L, Ji X. HOXB9 promotes epithelial-to-mesenchymal transition via transforming growth factor-β1 pathway in hepatocellular carcinoma cells. Clin Exp Med. 2015;15(1):55–64.
53. Bruix J, Llovet JM. Prognostic assessment and evaluation of the benefits of treatment. J Clin Gastroenterol. 2002;35(Suppl. 2):S138–42.
54. Barbara L, Benzi G, Gaiani S, et al. Natural history of small untreated hepatocellular carcinoma in cirrhosis: a multivariate analysis of prognostic factors of tumor growth rate and patient survival. Hepatology. 1992;16:132–7.
55. Arii S, Yamaoka Y, Futagawa S, et al. Results of surgical and nonsurgical treatment for small-sized hepatocellular carcinoma: a retrospective and nationwide survey in Japan. Hepatology. 2000;32:1224–9.
56. The Liver Cancer Study Group of Japan. Predictive factors for long term prognosis after partial hepatectomy for patients with hepatocarcinoma in Japan. Cancer. 1994;74:2272–80.
57. Livraghi T, Bolondi L, Buscarini L, et al. No treatment, resection and ethanol injection in hepatocellular carcinoma: a retrospective analysis of survival in 391 patients with cirrhosis. Italian Cooperative HCC Study Group. J Hepatol. 1995;22:522–6.
58. Kojiro M. Pathology of early hepatocellular carcinoma: progression from early to advanced. Hepatogastroenterology. 1998;45:1203–5.
59. Sakamoto M, Hirohashi S. Natural history and prognosis of adenomatous hyperplasia and early hepatocellular carcinoma: multiinstitutional analysis of 53 nodules followed up for more than 6 months and 141 patients with single early hepatocellular carcinoma treated by surgical resection or percutaneous ethanol injection. Jpn J Clin Oncol. 1998;28:604–8.
60. Takayama T, Makuuchi S, Hirohashi S, et al. Early hepatocellular carcinoma as an entity with high rate of surgical cure. Hepatology. 1998;28:1241–6.
61. Bruix J, Llovet JM. Prognostic prediction and treatment strategy in hepatocellular carcinoma. Hepatology. 2002;35:519–24.
62. Llovet JM, Burroughs A, Bruix J. Hepatocellular carcinoma. Lancet. 2003;362(9399):1907–17.
63. Forner A, Gilabert M, Bruix J, Raoul JL. Treatment of intermediate-stage hepatocellular carcinoma. Nat Rev Clin Oncol. 2014;11(9):525–35.
64. Wayne JD, Lawers GY, Ikai I, et al. Preoperative predictors of survival after resection of small hepatocellular carcinomas. Ann Surg. 2002;235:722–31.
65. Yao FY, Ferrell L, Bass NM, et al. Liver transplantation for hepatocellular carcinoma: expansion of tumor size limits does not adversely impact survival. Hepatology. 2001;33:1394–403.
66. Mazzaferro V, Regalia E, Doci R, et al. Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. N Engl J Med. 1996;334:693–9.
67. Breitenstein S, Apestegui C, Petrowsky H, Clavien PA. ''State of the art'' in liver resection and living donor liver transplantation: a worldwide survey of 100 liver centers. World J Surg. 2009;33:797–803.

68. Ohwada S, Kawate S, Hamada K, et al. Perioperative real-time monitoring of indocyanine green clearance by pulse spectrophotometry predicts remnant liver functional reserve in resection of hepatocellular carcinoma. Br J Surg. 2006;93: 339–46.
69. Bruix J, Castells A, Bossch J, et al. Surgical resection of hepatocellular carcinoma in cirrhotic patients: prognostic value of preoperative portal pressure. Gastroenterology. 1996;11:1018–22.
70. Shwartz JD, Schwartz M, Mandeli J, Sung M. Neoadjuvant and adjuvant therapy for resectable hepatocellular carcinoma: review of the randomised clinical trials. Lancet Oncol. 2002;3: 593–603.
71. Lau WJ, Leung TW, Ho SK, et al. Adjuvant intra-arterial iodine-131-labelled lipiodol for resectable hepatocellular carcinoma: a prospective diagnosis with MR imaging and explantation correlation. Radiology. 2001;219:445–54.
72. Takayama T, Sekine T, Makuuchi M, et al. Adoptive immunotherapy to lower postsurgical recurrence rates of hepatocellular carcinoma: a randomised trial. Lancet. 2000;356 (9232):802–7.
73. Kawata A, Une Y, Hosokawa M, et al. Adjuvant chemoimmunotherapy for hepatocellular carcinoma patients. Adriamycin, interleukin-activated killer cells versus adriamycin alone. Am J Clin Oncol. 1995;18(3):257–62.
74. Ringe B, Pichlmayr R, Wittekind C, Tusch G. Surgical treatment of hepatocellular carcinoma in Caucasian patients with cirrhosis. J Hepatol. 1994;20:65-71
75. Bhoori S, Mazzaferro V. Current challenges in liver transplantation for hepatocellular carcinoma. Best Pract Res Clin Gastroenterol. 2014;28(5):867–79.
76. Otto G, Herber S, Heise M, et al. Response to transarterial chemoembolization as a biological selection criterion for liver transplantation in hepatocellular carcinoma. Liver Transplant. 2006;12:1260–7.
77. Bismuth H, Majno PE, Adam R. Liver transplantation for hepatocellular carcinoma. Semin Liver Dis. 1999;19:311–22.
78. Lesurtel M, Müllhaupt B, Pestalozzi BC, Pfammatter T, Clavien PA. Transarterial chemoembolization as a bridge to liver transplantation for hepatocellular carcinoma: an evidence-based analysis. Am J Transpl. 2006;6(11):2644–50.
79. Trotter J, Washs M, Everson G, Kam I. Adult-to-adult transplantation of the right hepatic lobe from living donor. N Engl J Med. 2002;14:1074–82.
80. Bartolozzi C, Lencioni R. Ethanol injection for the treatment of hepatic tumors. Eur Radiol. 1996;6:682–96.
81. Livraghi T, Giorgio A, Marin G, et al. Hepatocellular carcinoma and cirrhosis in 746 patients: long-term results of percutaneous ethanol injection. Radiology. 2005;197:101–8.
82. Pompili M, Rapaccini GL, Covino M, et al. Prognostic factors for survival in patients with compensated cirrhosis and small hepatocellular carcinoma after percutaneous ethanol injection therapy. Cancer. 2001;92:126–35.
83. Teratani T, Ishikawa T, Shiratori Y, et al. Hepatocellular carcinoma in elderly patients: beneficial therapeutic efficacy using percutaneous ethanol injection therapy. Cancer. 2002;95: 816–23.
84. Kan KN, Yatsuhashi H, Yamasaki K, et al. Prospective analysis of risk factors for early intrahepatic recurrence of hepatocellular carcinoma following ethanol injection. J Hepatol. 2000;32: 269–78.
85. Koda M, Murawaki Y, Mitsuda A, et al. Predictive factors for intrahepatic recurrence after percutaneous injection therapy for small hepatocellular carcinoma. Cancer. 2000;88:529–37.
86. Lencioni R, Allgaier HP, Cioni D, et al. Small hepatocellular carcinoma in cirrhosis: randomized comparison of radiofrequency thermal ablation versus percutaneous ethanol injection. Radiology. 2003;228:235–40.
87. Goldberg SN, Gazelle GS, Halpern EF, et al. Radiofrequency tissue ablation: importance of local temperature along the electrode tip exposure in determining lesion size and shape. Acad Radiol. 1996;3:212–8.
88. Goldberg SN, Gazelle GS, Mueller PR. Thermal ablation therapy for focal malignancies: a unified approach to underlying principles, techniques, and diagnostic imaging guidance. AJR Am J Roentgenol. 2000;174:323–31.
89. McGahan JP, Dodd GD III. Radiofrequency ablation of the liver: current status. AJR Am J Roentgenol. 2000;176:3–16.
90. Llovet JM, Real MI, Montana X, et al. Arterial embolization or chemoembolization versus symptomatic treatment in patients with unresectable hepatocellular carcinoma: a randomised controlled trial. Lancet. 2002;359:1734–9.
91. Llovet JM, Bruix J. Systematic review of randomized trials for unresectable hepatocellular carcinoma: chemioembolization improves survival. Hepatology. 2003;37(2):429–42.
92. Kim JW, Kim JH, Sung KB, et al. Transarterial chemoembolization versus radiofrequency ablation for the treatment of single hepatocellular carcinoma 2 cm or smaller. Am J Gastroenterol. 2014;109(8):1234–40.
93. Yang HJ, Lee JH, Lee DH, et al. Small single-nodule hepatocellular carcinoma: comparison of transarterial chemoembolization, radiofrequency ablation, and hepatic resection by using inverse probability weighting. Radiology. 2014;271(3): 909–18.
94. Marelli L, Stigliano R, Triantos C, et al. Transarterial therapy for hepatocellular carcinoma: which technique is more effective? A systematic review of cohort and randomized studies. Cardiovasc Intervent Radiol. 2007;30(1):6–25.
95. Lau WY, Sangro B, Chen PJ, et al. Treatment for hepatocellular carcinoma with portal vein tumor thrombosis: the emerging role for radioembolization using yttrium-90. Oncology. 2013;84(5): 311–8.
96. Salem R, Mazzaferro V, Sangro B. Yttrium 90 radioembolization for the treatment of hepatocellular carcinoma: biological lessons, current challenges, and clinical perspectives. Hepatology. 2013;58(6):2188–97.
97. Kulik LM, Atassi B, van Holsbeek L, et al. Yttrium-90 microspheres (TheraSphere) treatment of unresectable hepatocellular hepatocellular carcinoma: downstaging to resection, RFA and bridge to transplantation. J Surg Oncol. 2006;94: 572–86.
98. Carr BI. Hepatic arterial 90yttrium glass microspheres (Therasphere) for unresectable hepatocellular carcinoma: interim safety and survival data on 65 patients. Liver Transpl. 2004;10:S107–10.
99. Salem R, Lewandowski R, Roberts C, et al. Use of yttrium-90 glass microspheres (TheraSphere) for the treatment of unresectable hepatocellular carcinoma in patients with portal vein thrombosis. J Vasc Interv Radiol. 2004;15:335–45.
100. Fouly AE, Ertle J, Dorry AE, et al. In intermediate stage hepatocellular carcinoma: radioembolization with Yttrium 90 or Chemoembolization? Liver Int. 2015;35(2):627–35.
101. Gramenzi A, Golfieri R, Mosconi C, et al. BLOG (Bologna Liver Oncology Group). Yttrium-90 radioembolization versus sorafenib for intermediate-locally advanced hepatocellular carcinoma: a cohort study with propensity score analysis. Liver Int. 2015;35(3):1036–47.
102. Lopez PM, Villanueva A, Llovet JM. Systematic review: evidence-based management of hepatocellular carcinoma–an updated analysis of randomized controlled trials. Aliment Pharmacol Ther. 2006;23(11):1535–47.

103. Abou-Alfa GK, Schwartz L, Ricci S, et al. Phase II study of sorafenib in patients with advanced hepatocellular carcinoma. J Clin Oncol. 2006;24(26):4293–300.
104. Llovet JM, Ricci S, Mazzaferro V, et al. Sorafenib in advanced hepatocellular carcinoma. N Engl J Med. 2008;359(4):378–90.
105. Cheng AL, Kang YK, Chen Z, 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. 2009;10(1):25–34.
106. Li Y, Zheng YB, Zhao W, et al. Sorafenib in combination with transarterial chemoembolization and radiofrequency ablation in the treatment for unresectable hepatocellular carcinoma. Med Oncol. 2013;30:730.
107. Zhao Y, Wang WJ, Guan S, et al. Sorafenib combined with transarterial chemoembolization for the treatment of advanced hepatocellular carcinoma: a large-scale multicenter study of 222 patients. Ann Oncol. 2013;24:1786–92.
108. Feng X, Xu R, Du X, et al. Combination therapy with sorafenib and radiofrequency ablation for BCLC stage 0-B1 hepatocellular carcinoma: a multicenter retrospective cohort study. Am J Gastroenterol. 2014;109(12):1891–9.
109. Chao Y, Chung YH, Han G, et al. The combination of transcatheter arterial chemoembolization and sorafenib is well tolerated and effective in Asian patients with hepatocellular carcinoma: final results of the START trial. Int J Cancer. 2015;136(6):1458–67.
110. Palmieri VO, Santovito D, Margari F, et al. Psychopathological profile and health-related quality of life (HRQOL) in patients with hepatocellular carcinoma (HCC) and cirrhosis. Clin Exp Med. 2015;15(1):65–72.