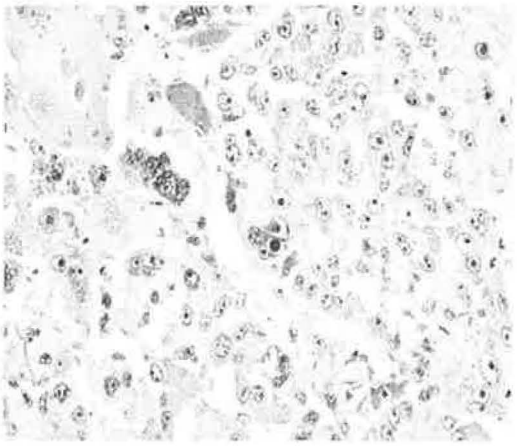
New Insights into Hepatocellular Carcinoma



Courtesy of Thomas Rogers, M.D.

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Dr. Brown has a particular interest in diseases involving inflammation in the liver and the gastrointestinal tract. She has an interest in the treatment of hepatitis B and C and in the treatment of the complications of cirrhosis, including hepatocellular carcinoma

CASE PRESENTATION

In 1996, an HBsAg+, HBeAg+ 24 year old African American male, who presented with decompensated liver disease, expired after a 28 day hospitalization. Five weeks prior to admission, the patient had increasing abdominal girth. Ultrasound demonstrated ascites, small nodular liver with inhomogeneous hepatic parenchyma. Despite antibiotics for spontaneous bacterial peritonitis, volume resuscitation and supportive care for gastroesophageal variceal bleed, the patient expired. Hepatocellular carcinoma was noted on autopsy.

Prior to this case, the number of hepatocellular carcinomas, related risk factors, initial presentation and alpha-feto protein (AFP) levels in patients seen in consult on the inpatient service at Parkland Memorial Hospital from May-June,1992 were reviewed. There was a 50-59 year old male predominance, viral and alcoholic chronic liver disease were important risk factors, decompensated liver disease, including ascites and gastrointestinal bleeding was a common presenting diagnosis, and AFP levels were markedly elevated in some of the patients (Table1)(personal communication, D Thiele). This case presentation and subsequent patient data provides a frame work for the beginning of this grand round, supplying demographics, risk factors, physical findings, and diagnostic testing, all of which continue to be relevant today.

Table 1 Patients with Hepatocellular Carcinoma Evaluated by the Liver Consult Service at PMH from May, 1992 thru July, 1992

Patient	Age	Sex	HBsAg	HCV	Lifestyle Risks	Presenting complaint	Mean Diameter	a-FP (ng/ml)
1	63	M	+	-	ETOH X 30 yr	Ascites	2.5 cm	11,143
2	50	M	-	+	IVDA X 25 yr	Variceal Bleeding	N.M.	77
3	84	M	-	-	None	Wt loss	15 cm	<3
4	57	F	-	+	Transfusion 22 yr PTA	Ascites	10 cm	390,000
5	52	M	-	-	ETOH X 35 yr	Pain	5 cm	<3
6	48	M	-	+	Penitentiary X 10 yr, prior ETOH	Variceal Bleeding	10 cm	3,264
7	52	M	-	-	ETOH X 25 yr	Ascites	7 cm	4,538
8	19	M	+	-	None	Pain	19 cm	4,824
9	55	F	-	+	Transfusion, 23 y PTA	Variceal Bleeding	3 cm (multiple)	9

However, there have been advances and changes in not only the clinical presentation of this disease but in our understanding of this disease. During grand rounds, the changing epidemiology, the increased understanding of the pathogenesis, the improved and future diagnostic test and therapeutic options for hepatocellular carcinoma will be discussed.

EPIDEMIOLOGY

The American Cancer Society estimates that, in 2005, there were over 667,000 new cases of hepatocellular cancer (HCC) worldwide. The 5-year survival rate of individuals with HCC in the United States was only 8.9%, marking this malignancy as the second most lethal cancer after pancreatic ductal adenocarcinoma (1, 2). The lethality of liver cancer stems in part from its resistance to existing anticancer agents, a lack of biomarkers that can detect surgically resectable incipient disease, and underlying liver disease that limits the use of chemotherapeutic drugs.

The incidence varies according to gender, geographical location, and exposure to chronic viral infection. World age-adjusted incidence is 14.67/ 100k for men and 4.92/ 100k for women. While the incidence also varies by geographical location, this variance is associated with prevalence of hepatitis B viral infection. In the 1980's, the epidemiological studies among Chinese male, government employees, demonstrated that chronic hepatitis B virus infection was associated with a 100 fold relative risk of HCC among the population (3). In 2005, the incidence is still higher in Africa and Asia with incidence ranging from <10/100K population in North America and rising to 50-150/ 100K in Africa and Asia (4) and corresponding to HBV prevalence, which ranges from 0.1-1 % in North America and Europe to up to 20 % in Southeast Asia and Africa. Asia continues to accounts for 70-80% for all HCC world-wide (5).

The relative risk for HCC is higher in patients with chronic liver diseases, including chronic hepatitis C and chronic alcoholic liver disease. A prospective population-based (12,008 men) study of the risk of HCC with hepatitis C demonstrated that being anti-HCV-positive conferred a 20-fold increased risk of HCC compared to anti-HCV-negative subjects (6). In patients with chronic alcoholic liver disease, the risk factor approximated by the hospitalization rate of HCC related to ETOH (7), is similar to the increased risk of HCC for HCV positive individuals.

Investigators have recently begun to examine the effect of the HCV epidemic and the HBV vaccine recommendations on changes in HCC incidence. Gender- and age-specific and standardized incidence rates of primary liver cancer in 23 populations from 21 registries were retrieved and were age-adjusted to the world standard population (8, 9). Among men, the incidence of primary liver cancer has increased in populations in Central Europe and North America, as well as in Oceania. The largest percentage increases were in New South Wales, Australia; France; Italy; and Alberta, Canada, with overall increases of 108%, 90%, 83%, and 70%, respectively. In comparison, the most striking decreases were seen in Asia, particularly among Chinese populations: -30% in Singapore, -18% in Shanghai, -27% in Sweden, and -23% in Zaragoza, Spain (8). Of interest, in an analysis of the U.S. Surveillance, Epidemiology, and End Results (SEER) registry, the incidence among U.S. white and African American population increased by 36 and 40% respectively (9). The increase in HCC appears to correlate with the aging of the population with chronic hepatitis C viral infection.

A progressive increase in HCC-related mortality over the past 3 decades has been observed in the United States. According to the U.S. vital statistics, the overall age-adjusted mortality rate for HCC (ICD-9 code 150.0) has risen significantly from 1.7 per 100,000 during 1981–1985 to 2.4 per 100,000 during 1991–1995 (8). Furthermore, the increase in mortality in African American males due to HCC continues to rise (9).

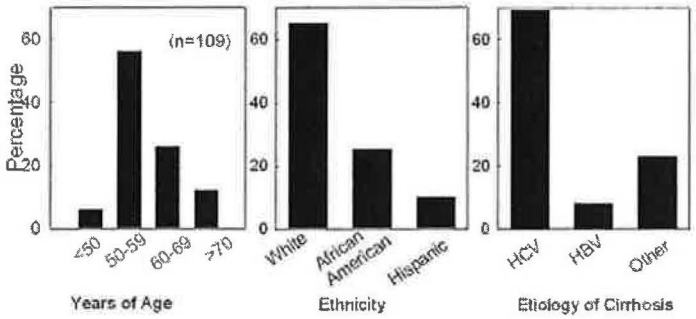
Dallas VA North Texas Health Care System

Veterans have a high risk of HCV and subsequently, a high risk for HCC. The prevalence of HCV infection in the veteran population has ranged from a reported 10% to as high as 20% among veterans that served in the Viet Nam war (10, 11). After 2-3 decades of hepatitis C virus (HCV) carriage, 20% of carriers will develop cirrhosis and its related complications, and about 1-2% of subjects may develop HCC (12-16). The risk of HCC in the veteran population may be as high as 4%. A retrospective chart review was undertaken at the Dallas VA North Texas Health Care System from 2002-2006 to examine the prevalence of HCC and the associated demographics, risk factors, and therapeutic opportunities.

Initially, HCC were identified by either a data base that was initiated and maintained by health care providers in the Dallas VA North Texas Health Care System liver clinics, with

accepted laboratory, pathological and image findings of HCC. Other HCC were identified by lists extracted from the electronic charts, with ICD 9 codes of 155.0 and 155.2 maintained by the Dallas VA North Texas Health Care System. The chart review included patient identifier, age, gender, ethnicity, presence of HCV, HBV, body mass index, survival, size and number of cancer, and type of treatment received.

The absolute documented number of HCC has increased from 12 to 48 from 2002 to 2006. In this VA population, the rate is 50/100K (veterans seen at the Dallas VA) or about 5 fold higher than the general population. The characteristics of patients with HCC reflected the veterans enrolled in health care for HCV treatment at the Dallas VA North Texas Health Care System (17). There were 99% male. The median age was 58 years old, with subjects ranging from 45 to 91 years of age. Of the patients identified, 66% were Caucasian, 25% were African American, and 10% were Hispanic. Etiology of cirrhosis included HCV (69%), chronic Hepatitis B (HBV), (8%) and other [non-alcoholic fatty liver disease (NAFLD) alcohol (ETOH)] (23%). Patient survival was defined as the time of diagnosis to death. Fifty-three percent of patients survived for less than 12 mos. while 47% survived for more than 12 mos.



HCC PATHOGENESIS

Though some of the major risk factors for HCC are known, the mechanisms by which these risk factors induce carcinogenesis have only partially been elucidated. The most prominent factors associated with HCC include chronic hepatitis B and C viral infection, chronic alcohol consumption, aflatoxin–B1-contaminated food and cirrhosis inducing conditions (18) Two billion people are infected with hepatitis B virus worldwide, with 320,000 deaths annually from HCC. Thirty-fifty percent of HBV infected patients die from HCC. In contrast, 170 million people are infected with HCV worldwide and only 2.5% die from HCC (19). Though the difference in the mortality is known, the reason behind the difference is unclear. Contrasting investigational studies support different pathways to carcinogenesis for each virus, respectively, which may in turn lead to the differences in the observed mortality.

HCV and HBV induced carcinogenesis

Common to all pathways to carcinogenesis are the characteristics of self sufficiency in growth signals, insensitivity to growth inhibitor signals, evasion of apoptosis, limitless replication potential, sustained angiogenesis and tissue invasion and metastasis (20). The predominant cellular event necessary for the development of primary liver cancer may be the unconstrained cell proliferation. Cell growth must be balanced with the apoptotic signals pathways. Disruption of the innate homeostatic mechanisms may be the critical second hit that allows unregulated proliferation (20). Important growth pathways are the tyrosine kinase pathways. Tyrosine kinases are cell membrane receptors that include several growth factors, ie.

hepatocyte growth factor and the vascular endothelial growth factor. As demonstrated on the slide, these growth factors drive cell proliferation by induction of transcription of genes (20). These processes may become unregulated and play a major role in the HCV induced carcinogenesis.

Hepatitis C virus is a non-cytopathic virus of the *flaviviridae* family. The HCV positive stranded RNA genome encodes nonstructural proteins (NS2, NS4A, NS5A and NS5B, which associate with the endoplasmic reticulum (ER) membrane to form the viral replicase and the viral envelope proteins (E1 and E2). HCV possess 3 important distinctions from HBV that are relevant to carcinogenesis. First, HCV show a higher propensity to yield chronic infection (60-80% of patients exposed to HCV) compared to 10% of patients exposed to HBV, resulting in long-standing chronic inflammation and cellular turnover. The second different is the greater propensity of HCV to promote cirrhosis, (5-10% of HCV after 10 years, which is 20 fold higher than HBV). Third, HCV is an RNA virus without a DNA intermediate form, suggesting that DNA integration inducing mutations in growth genes is not a critical pathway in HCV induced carcinogenesis (18).

HCV may promote HCC by causing a continuous cycle of hepatocyte death and subsequent regeneration, which in turn, provides a context for the accumulation and propagation of multiple mutations. Alternatively, the HCV proteins may interact with signaling pathways of cell proliferations such as a tyrosine kinase pathway or other proteins Finally, HCV core proteins may induce hepatic steatosis, inducing reactive oxygen species and the development of oxidative stress mediated mechanisms. Finally, tumor growth may be due to the inhibition of cell death (apoptosis) of the hepatocyte.

Like HCV, host immune response to HBV induces sustained cycles of necrosis-inflammation and regeneration (21, 22). However, HBV induced carcinogenesis likely follows additional alternative pathways. Two significant findings suggest that there are alternative pathways in patients with chronic hepatitis B. Importantly, 25-40% of the HCC in patients infected with chronic HBV occurs in a noncirrhotic liver. Furthermore, the impact of hepatitis B virus (HBV) infection on HCC development is reflected by the correlation between increased incidence of HCC in patients with increasing liver of HBV DNA in serum (23).

HBV is non-cytopathic, partially double stranded hepatotropic DNA virus classified as a member of the *hepadnaviridae* family. The HBV genome encodes several viral proteins essential to life cycle, including a reverse transcriptase/DNA polymerase (pol), the capsid protein known as the hepatitis B core antigen (HBcAg) and the L, M and S envelope proteins that associate with the endoplasmic reticulum (ER) membrane. HBV also encodes a number of proteins that may be associated with carcinogenesis, such as the protein x (HBX) (21).

Direct involvement of HBV in the transformation from non-malignant to malignant cells may occur. First, HBV genome integration has been associated with host DNA microdeletions (24) that can target cancer relevant genes including telomerase reverse transcriptase (TERT), platelet derived growth factor receptor β (PDGFRβ , PDGFβ) and mitogen activated protein kinase 1 (MAPK1), and cellular proliferation genes, including retinoic acid receptor B (RAR-B) gene, and human cyclin A gene. HBV genome encodes the HBX, which can alter the expression of growth factors like the insulin like growth factor II, the insulin receptor substrate I and transforming growth factors (TGFα and TGFβ) or growth control genes like tyrosine kinases (25-28). Furthermore, HBX can bind and inactivate the tumor suppressor p53 gene, which may compromise DNA damage check points (28, 29).

Recently, investigators have suggested that action of HBV on the transformation of a cell to a malignant cell is indirect. Investigators have demonstrated that HBV by itself is an inefficient carcinogen in mice containing the entire HBV genome. However, HBV can efficiently promote hepatocarcinogenesis initiated by a known carcinogen diethylnitrosamine (DEN). This effect of HBV does not involve chronic liver inflammation and is apparently due to enhanced hepatocellular apoptosis and compensatory regeneration following DEN treatment. With removal of the HBX protein, the mice continued to have high levels of tumor burden following DEN treatment, suggesting that HBX was not required (30, 31).

Carcinogenesis induced by other etiology

Alcohol is also an important HCC risk factor. Like HCV, there is a continuous cycle of hepatocyte death and subsequent regeneration which provides a context for the accumulation and propagation of multiple mutations. Furthermore, there is an increase in oxidative stress mechanisms (32). Unlike alcohol and hepatitis C, aflatoxin-B1 induced carcinogenesis seems to function as a mutagen and is associated with a specific p53 mutation and there is no connection between cirrhosis and the mutational actions of this toxin (18). Other etiological factors associated with HCC are outlined in Table 2 and the mechanisms continue to involve toxic metabolites in the liver with potential mutagenic properties, continuous hepatocellular injury, hepatocyte apoptosis, and increased oxidative stress.

Table 2 Other etiologies associated with HCC

Long term use of oral contraceptives in women
Metabolic disorders including hemochromatosis, porphyria cutanea tarda, alpha-1 antitrypsin deficiency, Hereditary tyrosinaemia
Diabetes mellitus
Non-alcoholic fatty liver disorders (NAFLD) and non-alcoholic steatohepatitis, Autoimmune hepatitis and Primary Biliary Cirrhosis

In summary, some of the common carcinogenic pathways may include inflammation and continuous rounds of necrosis and regeneration, especially in HCV, HBV and alcohol induced cirrhosis. A secondary pathway in HBV likely involves the integration of HBV DNA into the genome, causing mutations in cell cycle genes or in p53 and other cancer specific genes. A final pathway may include the indirect pathway whereby HBV increases the sensitivity to known carcinogens.

Multi-Step Histological Process and Genetic alteration

The neoplastic evolution of normal hepatocytes toward HCC likely proceeds through a multi-step histological process. Hyperplastic nodules of regenerating hepatocytes may represent a potential first step toward HCC. The next step may be a premalignant dysplastic nodule which has abnormal cytological features. The molecular analysis of human HCC has shown many genetic and epigenetic alterations that result in dysregulation of key ongcogenes and tumor suppressor genes (18).

The general developmental sequence of genomic changes during the process of carcinogenesis may drive hepatocytes to develop a malignant phenotype. Genomic alterations develop randomly beginning in preneoplastic lesions and their development escalates in dysplastic hepatocytes and HCC. Of importance, in the chronic hepatitis stage or the cirrhosis stage, there are no detected changes in the structures of genes or chromosomes. Elevated expression of transforming growth factor–α (TGF-α) and insulin-like growth factor-2 (IGF-2)

are detected in cirrhosis and chronic hepatitis but mutations are not observed. The upregulation of these factors results from actions of cytokines produced by chronic inflammatory cells and the regenerative response of the liver (33, 34). In contrast, a reduced expression of insulin-like growth factor binding protein-3 (IGFBP-3) has been correlated with tumor size, histological differentiation, capsular invasion and portal venous invasion, being associated with poor survival (34,35).

Genomic alteration, including both quantititave changes in gene expression occurring in the absence of structural abnormality and qualitative changes in gene expression resulting from aberations in gene structure of HCC, is only partially understood. Chromosome segregation defect, such as aneuploidy, a common cytogenetic feature of cancer cells has been noted in HCC. Significant effort has been directed toward charting the genomic events in HCC. Karyotypic analysis, chromosomal comparative genomic hybridization or CGH and loss of heterozygosity (LOH) mapping have identified recurrent regions of copy number change and allelic imbalances. LOH is frequent in chromosome 1, 4, 5, 6, 8, 9,13,14,16, and 17 (36). Of interest, specific candidate tumor suppressor genes are localized to a number of these chromosomes, p53 on 17p, IGF-IIR on 6q and breast cancer gene 2(BRCA2) and RARA-B on 13q (37, 38).

Mutations may proceed or associate with LOH. For example, the mutation arginine for serine substitution on codon 249 of the p53 gene had been noted on patients with HCC secondary to aflatoxin B1 exposure. Both RAR-B1 (15% of HCC) and β catenin mutations (18-41%) have been reported in HCC (36) More recently, investigators have demonstrated that oncogene-specific gene expression signatures at preneoplastic stage in mice define distinct mechanisms of hepatocarcinogenesis. Specific gene overexpression was associated with a strong alteration in lipid metabolism, while others with increase in liver mass or with an increased ATP synthesis (39).

Pathological Classification and Associated Genetic Differences

Recently, pathologists have been investigating the ability to discern prognosis by their microscopic appearances and their relation to genetic differences. HCC displays considerable diversity in their macroscopic appearances. The tumors can be single or multiple, with or without encapsulation, and often bulge beneath the hepatic capsule. They can range from <1 to >30 cm. in diameter (40, 41). The traditional classification is based on general tumor disposition and distinguishes three patterns: nodular, massive, and diffuse (42). The nodular pattern, the most common type, is typically seen in cases associated with cirrhosis and characterized by multiple nodules scattered across the liver. In the massive pattern, a large single mass occupies a substantial portion of a hepatic lobe; this is the standard variety of noncirrhotic livers. The rare diffuse pattern is distinguished by widespread infiltration. Like other cancer cells, HCC cells are distinguished from normal hepatocytes by the presence of cytologic atypia. This atypia is characterized by varying degrees of nuclear enlargement and hyperchromasia with clumping and irregularity of the chromatin and asymmetric nuclear contours; prominent nucleoli are a common but not invariable feature. Although the cells may be smaller or larger than their normal counterparts, the nuclear-cytoplasmic ratio is customarily increased. There is no discernable normal lobular architecture.

The spectrum of differentiation can be divided into various grades, and the four-tiered grading system proposed by Edmondson and Steiner is commonly cited (43). These histologic grades have been shown to correlate with the DNA content and cellular proliferation indices of the tumor (44, 45, 46). Most small carcinomas tend to be well-differentiated (grade I), but they are often not uniform in their differentiation. Larger tumors, on the other hand, exhibit more

homogenous differentiation, but the preponderance of cases are moderately to poorly-differentiated range (grades II and III) (47, 48).

Utilizing gene array technology to look at mutation rate of tumors, investigators have found that there seem to be two different classes of liver cancers: about half of liver cancers seem to be rapidly mutating, rapidly metastasizing, very aggressive and very poor prognosis lesions, while the others seem to have a more indolent course, a much different pattern of gene expression. A number of genetic changes are associated with the degree of differentiation. For example, P53 mutation, LOH and comparative genomic hybridization (CGH gains and losses) are associated with the degree of differentiation (36). Other investigators have demonstrated genetic alterations may help clarify the process of carcinogenesis in patients with Hepatitis B (with and without cirrhosis) versus patients with Hepatitis C as illustrated in table 3 (18).

Table 3 Genetic alterations in HCC

	Gain	Loss
Genomic alterations in >50% of studies	1a, 6p, 8q, 11q, 17q,	1p, 4q, 8p, 13q, 17p
Dysplastic lesions	17q	16q, 4q, 17p
Early stage HCC (small and well differentiated	8q24	6q
Late stage HCC (large, moderate/poorly differentiated	11q13, 8q, 20q	13q, 8p, 17p
HCC metastases	NR	8p11.2, 8p23.2, 17p13.1, 4q21, 4q32, 13q, 6q, 19p13.1
HBV (no cirrhosis)	8q,20q	4q
HCV versus HBV	10q	10q
HBV versus HCV	11q13	NR

Others have reported on specific molecular signature that discriminate dysplastic nodules from early HCC in patients with HCV cirrhosis. The investigators developed a practical real time PCR test that provided a 3-gene set signature with 94% accuracy to differentiate dysplastic nodules from early HCC (49).

Cell of Origin

Besides understanding the etiological specific induced carcinogenesis pathways, the subsequent genetic mutations and genomic changes, less is understood of the actual cell that undergoes the malignant transformation. The question of whether hepatocellular carcinoma arises from differentiation block of stem cells or de-differentiation of mature cells remains controversial. HCC may originate from transdifferentiation of bone marrow cells. Interestingly, there are four levels of cells in the hepatic stem cell linage, bone marrow cells, hepato-pancreas stem cells, oval cells and hepatocytes that may be involved in tumorigenesis. The differences in the histopathology may provide a clue that there are at least two cells that may be the cell of origin. Poorly differentiated HCC may originate from bone marrow stem cells and oval cells and well differentiated HCC may originate from mature hepatocytes. As illustrated, all four levels of the differentiation of the hepatocyte may be targets for carcinogenesis (50).

Summary

HCC is an heterogenous collection of molecular subtypes and specific etiologies may drive distinct genomic events. The cellular origin of HCC may provide clues to the pathogenesis. The role of oval cells, which give rise to both hepatocytes and bile duct epithelial cells, will be important to discern. There may be multiple cells of origin, reflecting the developmental plasticity of the hepatocyte lineage. In addition, understanding the host immune response and the role of the cirrhotic microenvironment and its constituents may aid in understanding HCC

development. Understanding mechanisms giving genomic instability might provide new approaches designed to detect or eliminate this process. For example, understanding telomere shortening processes, chromosome segregation defect mechanisms, or inactivation of DNA damage response molecules may provide further advances.

CLINCIAL CASES 2007 Case 2 - Mr J with HCV+, Child's A cirrhosis was noted to have a 2 cm liver mass in '04, with normal AFP, with well differentiated HCC on biopsy. He received transareterial chemoembolization (TACE) X 1 in '04, X 2 in '05 and X 3 in '06. He has recurrent HCC.

Case 3 - Mr. S with HBV+, Child's A cirrhosis was noted to have a 2 cm liver lesion in 2004, and received an orthotopic liver transplantation (OLTx) in November, 2004. Computed tomography image in 2006 had no evidence of recurrent HCC. On adefovir and lamivudine, no hepatitis on liver biopsy was noted.

Therapeutic options may now include TACE, radiofrequency ablation (RFA) and OLTx. At the Dallas VA North Texas Health Care Center, TACE has increased to 30 procedures being performed annually. A variety of therapies, including TACE, RFA, resection and orthotopic liver transplantation was received by 48% of the patients.

Presentation of patients in past range form obstructive jaundice, to decompensated liver disease, to paraneoplastic syndromes including hypercalcemia and polycythemia (51, 52). As in our case at the beginning of the grand rounds, the patient presented with ascites and esophageal variceal bleed. With the advent of better diagnostic tests, which include both imaging and screening techniques, patients are now presenting in asymptomatic stages.

DIAGNOSIS, IMAGING AND SCREENING

Regarding imaging, cancers in the liver are hypervascular and receive their blood supply from the hepatic artery, whereas the liver itself has a dual blood supply and two thirds of its blood comes from the portal vein. Injection of contrast intravenously arrives in the liver via the hepatic artery, which makes the HCC visibly lighter than the rest of the liver. HCC may be invisible in the absence of contrast, and invisible late in the study when contrast reaches the portal vein and fills out the liver; but in the arterial phase, cancers are visible. HCC may be demonstrated by MRI by infusing gadolinium or with a triple phase (non-contrast phase, arterial phase, and portal venous phase) CT by infusing standard IV contrast.

The guidelines from the AASLD recommend radiology, biopsy and AFP serology for the diagnosis of HCC. Some form of imaging is always required to determine the extent of the disease. The sequence of test used to diagnose HCC depends on the size of the lesion. Lesions >2 cm in diameter in the setting of cirrhosis is highly suspicious of HCC. If AFP is >200 ng/mL and the radiological appearance of the mass is suggestive of HCC (large and/or mutifocal disease with arterial hypervascularity), the likelihood that the lesion is HCC is high and biopsy is not essential (53, 54, 55). If the imaging appearances are atypical, the differential diagnosis is broader, a tumor biopsy should be considered. The EASL conference (56) recommended that the diagnosis of HCC can be made without biopsy in patients with cirrhosis who have a mass >2 cm that shows characteristic arterial vascularization that is seen on two imaging modalities, e.g., triphasic CT scan and MRI. If the vascular profile on dynamic imaging is not characteristic and the AFP is <200 ng/mL, a biopsy is recommended. Lesions between1-2 cm in a cirrhotic liver found during surveillance also have a high likelihood of being HCC. The EASL conference recommended that these lesions should be biopsied irrespective of their vascular profile (53-56). Patients with lesions which are 1-2 cm in diameter with a non-specific vascular profile who have

a negative biopsy should continue to undergo enhanced follow-up. Finally, lesions <1 cm in diameter on ultrasound should be followed up every few months (53).

Recently, investigators have reported that noninvasive assessment of reticuloendothelial (RE) function using super paramagnetic iron oxide (SPIO) enhanced magnetic resonance (MR) imaging allows differentiation of malignant versus benign focal hypervascular lesions in end stage liver disease. The investigators hypothesized that diminished RE function as assessed on SPIO MR may predict malignancy. The reference standard was liver explant histology (27 lesions), biopsy (6 lesions) and long term imaging follow up (47 lesions). RE function was reduced in 47 lesions, of which 39 were HCC and was not reduced in 33 lesions, of which 32 were benign. Thus, the reduced RE function as assessed on SPIO enhanced images (57).

As seen in the first presentation, the AFP is also used as a marker of HCC. AFP is a fetal-specific glycoprotein. During adulthood its synthesis is repressed. As a tumor marker for HCC in humans it has been used for decades, although its sensitivity and specificity varies from 40 to 65% and 76 to 96%, respectively (58-64). With increasing cutoff value its specificity rises but sensitivity falls. AFP values > 400 ng/mL are considered to be diagnostic for HCC (64). It is well known that patients with chronic viral hepatitis have elevated AFP levels even without detectable HCC. AFP levels seem to be more useful in detecting HCC in patients without viral hepatitis. For all patients at risk for HCC development, a progressively increasing AFP value has to be taken seriously and additional diagnostic tests are warranted.

Besides the total AFP, three different AFP variants differing in their sugar chains (AFP-L1, AFP-L2, AFP-L3) have been described. The variant AFP-L3 has a high binding affinity to the lectin lens culinaris agglutinin and seems to be more specific for HCC than total AFP (14, 15). AFP-L3 is found in approximately one third of patients with HCC when cutoff values of 10 to 15% are used. Its sensitivities and specificities range from 36 to 96% and 89 to 94% (65-72) respectively. Currently in Japan, the ratio of AFP-L3 to total AFP is calculated. As seen in the following table, the specificity of 10% is higher than the AFP level of 10ng/ml, suggesting that this test may be a better test to assess for HCC.

Table 4. Sensitivity, specificity and relative risk of AFP-L3% and AFP

	AFP-L3%	AFP
Cutoff value	10%	10ng/ml
Sensitivity	51.3%	79.5%
Specificity	92.3%	64.8%
Relative risk (95% confidence interval	7.0 (4.1-12)	5.6 (2.7-11.8

Regarding screening, the guidelines published by American Association for the Study of Liver Disease (AASLD) state that the following patients should be screened: Hepatitis B carriers starting at age 40 for men and 50 for women. Patients from Africa should be screened at age>20 years. All non hepatitis B cirrhotics should be screened. Both imaging and AFP, should be used for screening (53).

PROGNOSIS

The prognosis for most patients with HCC continues to be poor as indicated by the average survival rates of less than 1 year. However with the advent of screening, some tumors are noted early. The prognosis of HCC depends on the stage of the tumor, i.e. the extent of tumor, size of the lesion(s), the number of lesions, the presence in both lobes, invasion of the vessels and the presence of metastases. The aggressive tumor is often associated with very high AFP. In HCC prognosis, the severity of cirrhosis is another very important factor, as is the

functional status of the patient. Among patients without cirrhosis, the two-year survival rate is approximately 25%, but is under 5% in patients with cirrhosis (73-75).

Tumor staging systems include the tumor, nodes and metastases (TNM) staging system which accounts for the number of nodules, tumor size, presence of portal vein thrombosis and presence of metastases (75-76). The American Liver Transplant Society (ALTS) has modified the TNM classification to reflect the stages that are amenable to transplant (Milan critera). Stage 1 including solitary tumors less than 2 cm, Stage 2 including solitary tumors between 2 and 5 cm or 3 tumors≤ 3 cm, stage 3 which is 3 tumors irrespective of size and Stage 4a 4or more tumors, 4a2 which is large vessel invasion and 4b metastasis. A retrospective survival analysis of 850 patients with HCC was published in 1985 by Okuda et al. The Okuda scoring system included the tumor size, ascites, jaundice and serum albumin which indirectly assessed the functioning hepatic mass and degree of cirrhosis. His stages included Stage 1 (not advanced, tumor size - smaller than 50% with no ascites, albumin > 3 and bilirubin < 3), Stage II (moderately advanced, one or 2 signs of advanced disease) and Stage III (very advanced, 3 or 4 of the advanced signs were present (76).

The Barcelona Clinic Liver Cancer (BCLC) staging system is detailed below: Stage A included patients with asymptomatic early tumors suitable for radical therapies, Intermediate stage B, comprised patients with asymptomatic multi-nodular HCC, advanced Stage C which included patients with symptomatic tumors and/or invasion and D end stage disease patients(77).

Table 5 The BCLC Staging classification

Stage	Performance	Tumor Stage	Okuda Stage	Function
A1	0	Single	I	Nl Bilirubin
A2	0	Single	I	PH + nl bilirubin
A3	0	Single	I	PH and abnl bili
A4	0	3 tumors <3cm	I-II	Child-Pugh A-B
B (intermediate)	0	Multinodular	I-II	Child-Pugh A-B
C (advanced)	1-2	Invasion	I-II	Child Pugh A-B
D (end stage)	3-4	Any	III	Child-Pugh C

The Chinese University Prognostic Index (CUPI) staging system is the weight of the following factors, bilirubin, ascites, alkaline phosphatase, a fetoprotein and asymptomatic disease in addition to the TNM stage. Summation of the weights of TNM staging + asymptomatic disease on presentation + ascites + AFP + TB + ALP predicts the prognosis of the patient (low-risk group, CUPI score ≤1; intermediate risk group, CUPI score = 2-7; high-risk group, CUPI score ≥8) (78)

Table 6 Weight of the six prognostic factors in CUPI

TNM I and II	-3
TNM IIIa and IIIb	-1
IVa and IVb	0
Asymptomatic disease	-4
AFP >500 ng/ml	2
TB μmol/L <34	0
TB μmol/L 34-51	3
TB μmol/L l>52	4
ALP >200	3

The Cancer of the Liver Italian Program (CLIP) investigators determined another scoring system by assigning weights to the following variables with a minimum of 0 and maximum of 6 (79).

Table 7. Weight of the prognostic factors in CLIP

Child Pugh stage	
A	0
B	1
C	2
Tumor morphology	
Uninodular and extension <50%	0
Multinodular and extension<50%	1
Massive or extension >50%	2
AFP <400	0
AFP >400	1
Portal vein thrombosis	
No	0
Yes	1

Recently investigators assessed the value of the following staging systems in assessing the best predictors of prognosis: Okuda, TNM, CLIP, CUPI and BCLC. The BCLC had the best independent predictive power for survival when compared with the other prognostic systems (80). In all, these staging systems may be useful in determining the appropriate management for patients with HCC. Clearly patients with a higher tumor burden and reduced hepatic function have a less favorable outcome.

MANAGEMENT

Management of HCC may be categorized as surgical and nonsurgical therapeutic options. Both hepatic resection and orthotopic liver transplantation are considered surgical options in selected patients. Percutaneous therapies including percutaneous ethanol injection (PEI), RFA, TACE, and theraspheres are all considered options for patients that are not surgical candidates.

SURGICAL THERAPY

Surgical therapy for HCC should be considered in absence of extra hepatic disease. Hepatic resection is the treatment of choice for HCC in noncirrhotic patients (5% of cases in the West, 40% in Asia) (81-83). In 1986, Makuuchi et al reported an algorithm for hepatic resection for cirrhotics that was further completed by his group in 1995, (84) based on three variables: ascites, bilirubin, and indocyanine green retention rate at 15 minutes (ICG15). The BCLC group identified the absence of clinically relevant portal hypertension and normal bilirubin as the key variables to select the best candidates (85, 86) A hepatic venous pressure gradient > 10 mm Hg was shown to be a predictor of unresolved hepatic decompensation after surgery (86) Afterward, clinically relevant portal hypertension, defined as the presence of either a hepatic venous pressure gradient > 10 mm Hg, esophageal varices, or splenomegaly with platelet count <100,000/mm^3, was associated with overall survival. Subjects without relevant portal hypertension and normal bilirubin achieve 70% survival at 5 years, whereas survival is 50% in patients with portal hypertension and even lower with both adverse factors (85)

Absolute contraindication to resection includes the presence of extra hepatic disease, lack of sufficient hepatic functional reserve, multi-focal hepatic disease, tumors in locations not amenable to resection, and severe co-morbid disease. The markers of poor outcome after resection include multiple lesions, lesion size >5 cm, elevated AFP level vascular invasion, advanced tumor stages and lymph node metastases. Five year survival rate between cirrhotic with well preserved liver function and noncirrhotic after resection is similar (36-51 %).

Changing Role of Liver Transplantation for HCC

While surgical resection is an option for a small minority of patients, over the past 20 years, the role of another surgical procedure, liver transplantation for HCC has continued to

escalated dramatically. In 1987, a quote from the "future research direction" for HCC (87, 88) stated that the bleak prognosis of patients undergoing OLTx for cancer was related "mainly to the extent of the disease, may be too advanced by the time the patients received the transplant" (87) and to the lack of biologic markers for selection of a "less aggressive tumor variant." (88) This bleak prognosis improved in the early 1990's. Analysis of the previous experience suggested that patients with incidental asymptomatic tumors had the same outcome as patients with nonmalignant disease (103). Following this concept, some pioneering groups selecting "optimal candidates" reported 70% 5-year survival with a recurrence rate below 15% (85, 90, 91, 92). This was supported by Bismuth et al (93) and by Mazzaferro et al, (90) who established in a landmark manuscript which established the Milan criteria-patients with a single HCC $\leq$ 5 cm or up to three nodules $\leq$ 3 cm. The excellent results obtained reflected the selection of favorable tumors in an era of short waiting times of less than 6 months. Vascular invasion and poor tumor differentiation were the strongest predictors of recurrence (92, 94, 95).

At present, increasing number of candidates and the shortage of donors have led to extremely long waiting times, resulting in a decrease of survival (85, 96-99). The concern now is that with the disparity between supply and demand and the lack of centers removing patients for the list, the rates originally quoted in the asymptomatic tumor and the selected group in the early studies may not be as accurate Furthermore, the risk of recurrence because of micrometastases has become an important consideration. Early results in 2003 demonstrated that the staging of HCC prior to OLTx was not the same as after OTLx Freeman reviewed the UNOS data base, consisting of 796 transplants with 666 reports. Microvascular invasion was noted in 7% of cases; 46% had HCC lesions meeting Milan criteria. Explant staging varied from preoperative staging in 30% of the OLTx patients. Explant HCC demonstrated a pathologic stage 0 - 23%, Stage 1 - 8%, Stage 2 - 37% , Stage 3 10% , Stage 4a 8% and Stage 4b 125 (100). This disparity in the staging before and after OLTx likely accounts for the increase in recurrence of HCC.

NONSURGICAL THERAPY

Nonsurgical therapy for HCC is utilized in tumors considered too advanced for transplantation or surgical resection. Available strategies in tumors in intermediate to advanced stages of HCC include percutaneous ablation. Percutaneous ablation induces coagulative necrosis by cellular dehydration, by changes in temperature, protein denaturation and small vessel.

Percutaneous ethanol injection

One of the chemical approaches is percutaneous ethanol injection (PEI). Ethanol induces tumor necrosis by protein denaturation and thrombosis of small vessels. Ethanol ablation is usually performed under ultrasound guidance using a 21 gauge needle and 95% absolute ethanol which is injected into several sites inside and around the lesion. In PEI, the rate of complete necrosis correlates with tumor size. Specifically, there is a: 90-100% complete necrosis if the tumor < 2 cm, 70% for tumors between 2-3 cm, and 50% if the tumor is between 3-5 cm. A 5 year survival rate of 40-65% has been reported in Child A patients with single HCC's smaller than 3 cm (101). In the study by Livraghi et al, 5 year survival rates were 47, 29 and 0% in patients with Child Pugh class A, B and C, respectively (102). The rate of recurrence is 26-32% at 1 year, 51-81% at 2 year, 61-83% at 3year. The risk factors for recurrence include Childs Pugh score (CPS) and tumor size (>3 cm). Complications for PEI include death, post ablation syndrome and needle track seeding. The treatment related death < 1 %, needle track seeding < 0.6%. The therapy is not effective if the tumor>3 cm because it is unable to interrupt the intratumoral septa.

Transareterial embolization and chemoembolization

Another therapeutic option based on HCC blood supply is arterial embolization . As HCC grows the blood supply becomes progressively arterialized and becomes dependant on hepatic artery. Arterial obstruction/infusion was hypothesized to be an effective therapeutic option. These options include Transarterial embolization (TAE) Transarterial chemotherapy (TAC), Transarterial chemoembolization (TACE) and Transarterial radiation (Iodine mixed with lipoidol) and 90 Yttrium glass microspheres (theraspheres) (101-104). TAE is defined as the occlusion of arterial flow by either synthetic gelfoam cubes or natural particles (blood clots). TACE is defined as the same process preceded by the administration of chemotherapeutic agents such as doxorubicin/cisplatin/mitomycin C +/- lipiodol (vehicle) and then finally obstruction of hepatic artery with gelatin sponge, starch microspheres or metallic coils. TACE is judged to be successful if there is disappearance of arterial enhancement on imaging. Follow up imaging is performed 4-6 weeks after all areas have been treated and after initial response: follow up q 3-4 months with further TACE.

Selection of patients inclusion criteria are patients with HCC not suitable for surgical therapy. Exclusion criteria include poor synthetic function: Child's Puch Score C, GI bleeding, hepatic encephalopathy, refractory ascites, total biliburin>3 mg/L; Alb<2.8 g/dl, PT>4 sec over control, extrahepatic spread or vascular invasion, portosystemic shunt, hepatofugal flow, complete PV obstruction, renal failure, vascular contraindications for procedure or poor performance status.

One of the first randomized controlled trials assessed the efficacy of TACE in patients with unresectable HCC. In 1996, 40 out of 80 Asian patients with newly diagnosed unresectable HCC were assigned to either hepatic artery chemoembolization with a variable dose of emulsion of cisplatin and lipiodol and gelatin sponge patches or observation. Actuarial survival was significantly better in the chemoembolization group than in the control group. One, 2, and 3 year survival were 57%, 31% and 26%, respectively versus 32%, 11% and 3% in the control group. Complications included fever in 32%, abdominal pain in 26%, vomiting in 16% and ascites in 5% (103). Another trial of patients classified as Child-Pugh class A or B and Okuda stage I or II were randomized to receive either regularly repeated arterial embolization (gelatin sponge) or chemoembolization (gelatin sponge plus doxorubicin). Survival was compared between the groups and with patients that received a conservative treatment. Twenty-five of 37 patients assigned embolization, 21 of 40 assigned chemoembolization, and 25 of 35 assigned conservative treatment died. Survival probabilities at 1 and 2 years were 75% and 50% for embolization; 82% and 63% for chemoembolization, and 63% and 27% for control (104,105). This finding was upheld in a systematic review of randomized trials published from 1978 to 2002. Most of the studies include Childs Pugh A patients (70-100% of the reported cases), Okuda stage 1 (47-90% of the cases), mutilobular disease without vascular invasion (>95%)(106). TACE improves survival in well selected candidates in selected candidates, such as a CPS A with multinodular asymptomatic tumors without vascular invasion (53).

Minor TACE complications include post embolization syndrome (80-90%), elevation in AST/ALT while major complications (10%) include liver failure, ischemia, GI ulceration/perforation, hepatic abscesses, biliary strictures, pulmonary embolus and death as high as 4% in selected candidates but as high as 20% in CPS B-C (106).

In addition, there have been a number of studies that looked at TACE as an interventional bridge to liver transplantation. Conflicting reports have been published. Overall, the 1 year survival was 95% in selected patients with small tumors but decreased dramatically to 50% or

less with larger tumors (5-7 cm)(107). However in another case control study, 100 patients with TACE before LT and 100 patients without TACE and matched for tumor characteristics and time on waiting list, there was no difference in 5 year tumor recurrence: 59.4% with TACE and without TACE 59.3% (108).

Radiofrequency ablation

Another percutaneous therapy is radiofrequency ablation (RFA), where a 17-gauge, cooled-tip electrode is inserted under real-time ultrasound guidance into the tumor. An electrode with an exposed tip is connected to a 500-kHz RF generator which produces 200 watts. The equipment also allows the measurements of generator output, tissue impedance, and electrode tip temperature. A tip temperature of 10-20 °C is maintained by a peristaltic pump infusing chilled saline solution. Ablation is started at a low wattage. The power is increased in regular increments. The duration of a single ablation is 5-10 minutes. When the target nodule is > 2 cm in diameter, multiple ablations are performed. Total ablation time is always less than 60 minutes. In one study, investigators noted 40 major complications (4.0% per treatment, 1.9% per session) and 17 minor complications (1.7% per treatment, 0.82% per session) were observed,. There were no treatment-related deaths. Surgical intervention was required in one case each of bile peritonitis and duodenal perforation. The cumulative survival rates at 1 and 5 years were 94.7% and 54.3% for naive patients, whereas the cumulative survival rates were 91.8%, and 38.2% for nonnaive patients, respectively (109).

RFA is favored for tumors> 2 cm. In RFA, the necrosis rate is between 88-98% for tumors <3 cm and 80-90% for tumors between 3-5 cm. Recurrence rate: 20% at 1 year, 60% at 3 year and 66% at 4 years. Advanced liver dysfunction and bigger size of tumor (>3 cm) are predictive factors for recurrence. Complications include death: 0.1-0.5%, Minor: up to 36%: pain, post ablation syndrome (malaise, low grade fevers than can last up to 3 weeks) and Major: 0-12% and needle tract seeding: up to 0.5%. Major complications are categorized to the timing of the complication, immediate, <24 hours and periprocedural, <30 days. The immediate are intraperitoneal hemorrhage, hepatic infarction, pneumothorax, hemothorax, pleural effusion, bile peritonitis wjhile the reported Periprocedural complications are bronchobiliary fistula, Hepatic abscess, duodenal perforation, gastric perforation and colonic perforation. Follow up CT is recommended at 1 month after necrosis. The following is the table detailing survival in patients with varying size of tumors and CPS class. RFA has higher 5 year survival rates than PEI in all sizes of tumors and hepatic function. However, survival of patients with tumor > 5 cm is markedly lower than patients with tumor < 2 cm (102,109).

Table 8 Survival with RFA and PEI based on tumor size and hepatic function

Survival	1 year RFA	1 year PEI	5 year RFA	5 year PEI
CPS				
A	96%	98%	62%	47%
B-C	90%	81%	31%	1&
Tumor (cm)				
<2	93%	97%	83%	40%
2-5	93%	84%	45%	37%
>5	87%	85%	33%	30%

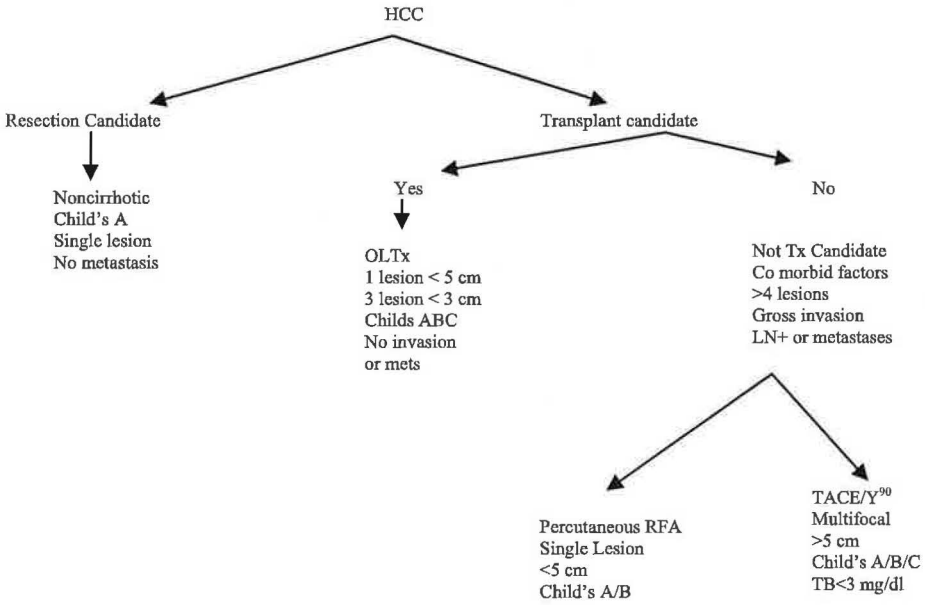
Internal Radiation technology

Microsphere and particle technology represent the next generation agents that have formed the basis of interventional oncology, an evolving subspecialty of interventional radiation(110-115). One of these yttrium-90 microspheres (^{90}Y) is rapidly being adopted in the

management of liver malignancies. Yttrium-90 microspheres are 20- to 40-μm particles that emit β-radiation The biodegradable glass microspheres that contain yttrium-90 are injected. Because the microspheres are delivered via the hepatic arterial route, the process can be considered as internal rather than external radiation. TheraSphere (glass microsphere; MDS Nordion, Kanata, ON, Canada) was approved in 1999 by the U. S. Food and Drug Administration (FDA) under a humanitarian device exemption for the treatment of unresectable HCC)(110-113,) SIR-Spheres (resin microsphere; Sirtex Medical, Lane Cove, Australia) were granted full premarketing approval in 2002 by the FDA for the treatment of colorectal metastases in conjunction with intrahepatic floxuridine (114-115). In one study, using 43 patients with the following criteria: HCC confined to the liver, Tbil<2 mg/dl, Cr <2.0 mg/dl, ANC >1500, Plt>60, survival ranged from 370 days to 633 days for Okuda II and I(110). Complications include abdominal pain, worsening ascites, acute cholecystitis, decrease in ANC, lymphocyte count(110). Other studies have had similar results (110-112).

With these new therapeutic options, treatment algorithms have been suggested by a number of investigational groups. In the following algorithm, the patient is first categorized to whether he is a surgical candidate. If he is a surgical candidate, the next step is to determine whether he is a resection or a transplant candidate. If the patient meets the Milan criteria then the patient is a transplant candidate. If the patient is not a transplant candidate than the patient is randomized to percutaneous RFA procedure if the HCC is a single lesion and less than 5 cm or randomized to TACE or Y^{90} procedure if the patient has a multifocal tumor or the tumor>5 cm and the bilrubin is less than 3 mg/dl. The criteria for each of the procedures continue to evolve with further information about these patients (53).

Treatment Algorithm



OTHER THERAPIES

Standard radiation to the liver tumors is ineffective because the liver does not tolerate radiation. However there are novel approaches that my limit liver injury, including proton beam radiotherapy, carbon ion radiotherapy and intensity modulated radiotherapy. All of these types of radiation are available only through clinical trials.

In addition, there are a multiple of new therapeutic studies being performed that are targeting growth factors, proteosome, and immunomodulators. These experimental protocols may in the future provide another therapeutic modality or a better understanding of this disease (116).

Table 9 Ongoing Clinical trials in the U.S.

Sorafenib	Multiple tyrosine kinase inhibitor	Phase II/III	
Nolatrexed	Thymidylate Synthase inhibitor	I	
Megestrol	Anti-estrogen	III	
DENSPM	Depletion of cellular pool of polyamines	1/II	
MB07133	Antiproliferative (precursor of AraC)	II/II	
PHY906	Potentiate anti-tumor effect of capecitabine	I/II	
Aresenic trioxide	Antineoplastic	II	
Bortezomib	Preoteosome inhibitor	II	
Thalidomide	Antiantiogenic	II	
Bevaacizumab	antiVEGF mab	II	
Erlotinib	EGFR tyrosine kinase inhibitor	II	
AGI-PEG20	Arginine depletion	II	
Thymasalfasin	Immunomodulator	II	
Ispinesib	Disrupts kinases	II	
Lapatinimb	Tyrosine kinase inhibitor	II	

SUMMARY

HCC has become a more frequent malignancy secondary to the aging population of the hepatitis C epidemic. A better understanding of the pathogenesis of this fatal malignancy has occurred with the availability of new genomic technology. Further delineation of molecular pathogenesis will provide information for targeted and gene therapy. In addition, the prognosis has improved because of the screening algorithms. Clearly, the staging systems are assisting with assessing the best therapeutic option for the patients but a unifying staging system will be advantageous. Surgical options and nonsurgical options are improving the survival of selected patients. Percutaneous ablation has improved the survival in patients with early stage HCC and preserved hepatic function. Importantly, the continuation of hepatitis B vaccination is imperative in this disease process along with the treatment and prevention of Hepatitis C.

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