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Hepatocellular carcinoma: Advances in diagnostic imaging

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Summary **Thanks to the growing knowledge on biological behaviors of hepatocellular carcinomas (HCC), as well as continuous improvement in imaging techniques and experienced interpretation of imaging features of the nodules in cirrhotic liver, the detection and characterization of HCC has improved in the past decade. A number of practice guidelines for imaging diagnosis have been developed to reduce interpretation variability and standardize management of HCC, and they are constantly updated with advances in imaging techniques and evidence based data from clinical series. In this article, we strive to review the imaging techniques and the characteristic features of hepatocellular carcinoma associated with cirrhotic liver, with emphasis on the diagnostic value of advanced magnetic resonance imaging (MRI) techniques and utilization of hepatocyte-specific MRI contrast agents. We also briefly describe the concept of liver imaging reporting and data systems and discuss the consensus and controversy of major practice guidelines.**

Keywords: Ultrasonography, tomography, X-Ray computed, magnetic resonance imaging, cirrhosis, diagnostic imaging, contrast media

1. Introduction

The common risk factors for hepatocellular carcinoma (HCC) are hepatitis B virus (HBV) or hepatitis C virus (HCV) infection and alcoholic liver disease. Less common causes include nonalcoholic fatty liver, hereditary hemochromatosis, autoimmune hepatitis, alpha-1 antitrypsin deficiency and Wilson disease, some porphyria and schistosomiasis (*1*). These risk factors can lead to remodel of texture with fibrotic progression of hepatic parenchyma. In patients with cirrhosis, the 5 year cumulative occurrence of HCC is between 5-30% depending on the causes of the disease, and associated cirrhosis is seen in 80-90% of patients with HCC (*2,3*). Due to the growing population of obesity and other metabolic syndromes, there is an increasing incidence of HCC due to non-alcoholic fatty infiltration liver disease; the incidence of HCC continues to grow in spite of the hepatitis B and C viruses' infection being prevented by the development of vaccines and anti-viral therapies (*4,5*). The fact that the classic imaging features could yield a definite diagnosis and the probability of needle track seeding are limiting the necessity of liver biopsy (*6*). Therefore, HCC is the unique malignancy to be diagnosed by diagnostic imaging, exempting the necessity of a needle biopsy (*7*).

Since imaging plays a decisive role in the diagnosis of HCC, it is critical that imaging examination might be performed according to generalized protocols (including the types of equipment, scanning parameters, administration of contrast agents and timing of acquisition) and the imaging findings might be interpreted and reported following a standardized terminology and categorization.

2. Imaging modalities of HCC

2.1. *Ultrasounography (US)*

US is a non-invasive examination and has no ionic radiation on the human body. It remains inexpensive as well, which is recommended as the first choice for the screening and surveillance of HCC by the guidelines of almost all international societies (*8*). Patients who have risk factors for developing HCC should undergo US surveillance every 3 to 6 months (*9*). However, the sensitivity varies from 58% to 70% and is even poor for small HCC less than 1 cm (*8-10*). Classic findings of

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HCC include hypoechoic nodules or mixed echogenic nodules due to tumor necrosis or fatty metamorphosis or a surrounding thin hypoechoic band indicating a capsule which is characteristic for HCC. Colored doppler flow imaging may show hypervascularity and tumor vascular shunting (*11*). Contrast enhanced ultrasound (CEUS) with microbubble agents could reflect the real time dynamics of blood supply of the lesion, which is helpful in both detection and characterization of HCCs (*12,13*).

2.2. *Multi-phasic enhanced computed tomography*

Multi-phasic enhanced computed tomography (CT) is the most common choice for the diagnosis of HCC. In the past decade, technical advances in CT scanners have yielded considerably faster acquisition time and a dramatically dropped radiation dose. There are technical requirements on the equipment and scanning parameters: at least 8 rows multi detector CT for fast acquisition, scanning with thin collimation not over 5mm, adequate amount of contrast medium used and a bolus injection rate over 3 mL/s (*14*). Accurate timing is critical, at least three phases should be acquired after administration of iodinated contrast agents, namely hepatic arterial phase, portal venous phase and delayed phase (*15*). Precontrast CT is suggested to provide a baseline to demonstrate the level of enhancement, and it may provide information on existence of fat content, iron, calcification, hemorrhage, and iodized oil after transarterial chemoembolization (TACE) treatment (*16*). The arterial phase is a time range with the hepatic artery fully enhanced while hepatic veins are not enhanced yet, it could be divided into early and subsequently late hepatic arterial phase (*17*). Late hepatic arterial phase is strongly recommended, because the hyperenhancement in HCC is more predominant in the late than the early arterial phase, and a majority of HCCs may show hyperenhancement only in the late hepatic arterial phase (*18,19*). Portal venous phase is acquired in which the images have the following characteristics: Portal veins and hepatic parenchyma are maximally enhanced, and hepatic veins are enhanced by antegrade flow as well (*20*). Delayed phase should be acquired at least 3 minutes after the initial of injection when liver parenchyma is less enhanced than in portal venous phase (*21*). The advantage of CT also affords the ability to perform three-dimensional reconstructions that may help with preoperative planning which is superior to MRI. Due to possible complications such as radiation, contrast media leaking, allergic reaction and contrast induced nephropathy, CT is not a choice of repeated surveillance (*22*).

2.3. *Magnetic resonance imaging (MRI)*

MRI is superior in both detection and characterization of HCC and is continuing to improve its performance and capability. The sensitivity and the specificity of MRI are reported at 91% and 95% as compared to 81% and 93% with MDCT (*23*). The standardized imaging protocol includes T2-weighted sequences to reveal the lesion in high resolution anatomic details, pre-contrast and multi-phasic enhanced 3D T1-weighted gradient echo sequences, and chemical shift in/opposed phase imaging which is sensitive to lipid content (*23,24*). The protocol of contrast examination is similar to contrast CT, and both early and late hepatic arterial phase might be acquired without fear of ionic radiation (*25*). The functional imaging is an added advantage of MRI. Among functional imaging techniques, diffusion weighted imaging (DWI) is the most promising method, it is based on differences of Brownian motion (diffusion) of water molecules within tissues in vivo. For tissues with increased cellularity and destroyed cell integrity such as malignancy, the diffusion of water molecules is restricted, which shows altered signal intensity and parametric changes on DWI (*26*). DWI is useful for detecting small HCC and differentiating compared to benign entities, however, it is not as robust and stable in image quality as T1WI and T2WI sequences and the positive predicting value and negative predicting value are controversial (Figure 1) (*27,28*). Currently, DWI is suggested but not required in most of the institutes.

The contrast medium commonly used for MRI is non-specific gadolinium-based contrast agents, however, hepatocyte specific contrast agents are promising in both detection and characterization of HCC (*29*). Among of several commercially available contrast agents, gadoxetate dimeglumine is a newer agent which enables both dynamic contrast and hepatocyte specific imaging with one administration (*30*). Approximately half of the agent is taken up by hepatocytes and excreted into the bile in about 20 min after routine contrast imaging, which is called hepatobiliary phase (*30*). Typically, HCCs appear hypointense in hepatobiliary phase because of lack of normal hepatocytes, which is a main feature for differentiating HCC from both regenerative nodules and dysplastic nodules which appear isointense

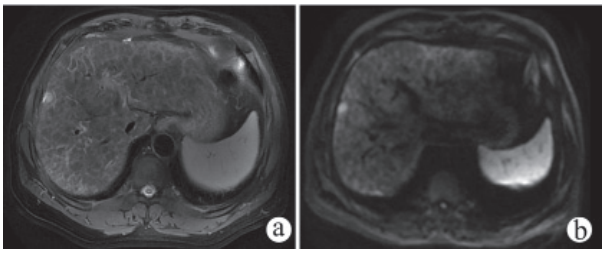

Figure 1. MRI feature of a small HCC associated with liver cirrhosis. (a) A moderate hyperintense nodule was revealed on fast suppressed T2WI sequence in the right margin, many small hypointense nodules could be seen in the background parenchyma suggesting the existence of cirrhosis. **(b)** The small HCC demonstrate remarkably hyperintense on diffusion weighted images, suggesting restriction of water molecule movement in the tumor.

(Figure 2) (*31,32*). However, about 10% of HCCs appear hyperintense compared to background parenchyma in hepatobiliary phase, because of overexpression of organic anion transporter peptide (OATP) proteins that are responsible for the transportation and uptake of the agent (*33*). Gadoxetate dimeglumine has proved its value in distinguishing small HCCs. The major limitation of the agent is lack of pure delayed phase, because the early uptake of the agent in delayed phase might superimpose true delayed enhancement, as a consequence, it might obscure the capsule which is diagnostic for HCC, the accumulation of the agent in the delayed phase might likewise mimic a tumor which is characteristic of delayed enhancement such as cholangiocarcinoma (*34*). Until now, in North America and European countries, gadoxetate dimeglumine is not widely used as compared to its use in East Asia (*35,36*).

3. Characteristics features of HCC

Cirrhotic nodules include regenerative nodules (RN), low-grade dysplastic nodules (LGDN), high-grade dysplastic nodules (HGDN), and HCCs (*37*). While the imaging modalities have greatly evolved and the detection rate of liver nodules has increased in the past decade, characterization of atypical hyperplastic/dysplastic nodules with small HCCs are still challenging (*38*). The following features are characteristic for HCC, the combination of these features could yield a definite diagnosis in most cases.

3.1. *Cirrhotic liver background*

In developing countries, many cirrhotic patients are unaware of their diseased condition until developing advanced HCC or liver decompensation or gastrointestinal bleeding, so the radiologist also need to determine the associated cirrhosis in the absence of clinical data (*39*). Therefore, the judgment of liver cirrhosis might also take part in the differential diagnosis of hepatic nodules. The presence of nodular liver contour, atrophy of right lobe and medial segment of left lobe, enlarged caudate lobe and lateral segment of left lobe, widened fissures, heterogeneity of parenchyma with fibrotic and fatty changes, varices, ascites and splenomegaly are indicative of cirrhotic liver (Figure 3a) (*40*). Because many benign entities such as cysts and hemangiomas may present with atypical appearance in cirrhotic liver background, the judgment of cirrhosis helps to distinguishing HCC and benign nodules (*41*).

3.2. *Hypoattenuation and moderate T1hypointense/T2 hyperintensity*

The classical imaging characteristics of HCC are hypoattenuation on precontrast CT and hypointense on T1-weighted images and moderately hyperintense on T2-weighted images (*42*). Low T1 and high T2 intensity represents increased water proton density in the tissue, which is caused by cytotoxic edema, tumor necrosis and hypervascularity (Figure 1a) (*42*). HCC with lower T1 signal and moderate higher signal is often recognized as poorly differentiated (*43*). High T1 intense represents the accumulation of starch, protein, or glycoprotein that is common in RNs and DNs, some of the high differentiation HCC can also have similar high intensity, and HCC with higher T1 intense suggests being well differentiated in classification of

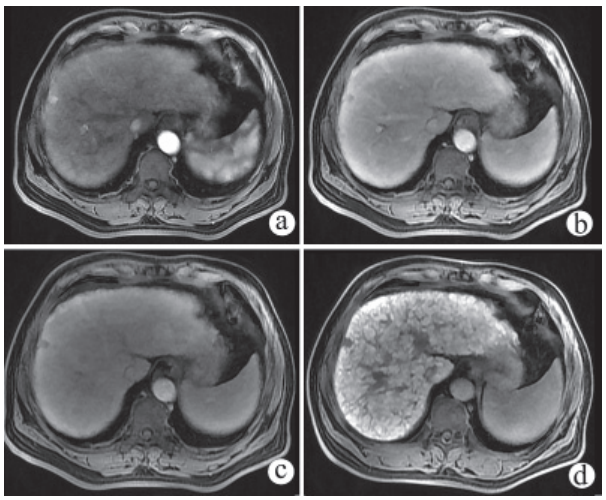

Figure 2. Gadoxetate dimeglumine enhanced T1 weighted MR imaging of a small HCC (the same case as in Figure 1). (a) The nodule shows remarkable hyperenhancement in arterial phase. **(b)** Rapid washout was observed in portal venous phase. **(c)** The nodule showed low intensity in delayed phase. **(d)** There was no uptake in the nodule in hepatobiliary phase.

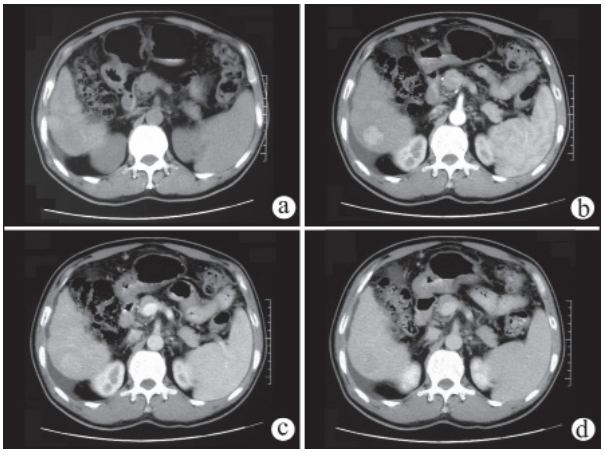

Figure 3. Multiphasic enhanced CT of a HCC associated with cirrhosis. (a) Precontrast CT showed a hypoattenuation nodule; while the nodular contour, parenchymal heterogeneity in attenuation, ascites and splenomegaly were indicative of cirrhosis. **(b)** In arterial phase, the nodule showed unequivocal hyperenhancement which had much higher enhancement than the adjacent background and precontrast baseline. **(c)** In portal venous phase, the nodule showed less enhancement but still higher than the background liver. **(d)** In delayed phase, the nodule demonstrated unequivocal washout which showed lower attenuation than the adjacent parenchyma.

which the prognosis is relatively good (*44*).

3.3. *Arterial hyperenhancement and washout appearance*

Arterial hyperenhancement is defined as more enhancement than liver parenchyma and higher attenuation/intensity in whole or in part of the lesion in the hepatic arterial phase compared to background liver. Washout is defined as an attenuation/intensity in whole or in part less than the earlier phase during the portal venous or delayed phase following the presence of arterial phase enhancement (*45*). If the lesion is surrounded by dense fibrosis then enhancement of the lesion should be compared to the comprehensive parenchyma. In some instances, delayed phase may be superior to portal venous phase for depicting washout appearance (Figures 3b-3d). Some HCC may show washout appearance only in the delayed phase (*20*). Neither arterial hyperenhancement or washout is characteristic of HCC, however, when combined together, the features are specific for HCC (*46,47*). A large nodule over 1.5-2 cm which appears to have hyperenhancement in the arterial phase and washout in the portal venous or delayed phase could be a diagnosis of HCC near 100% (*48*).

3.4. *Fibrous capsule or pseudocapsule*

The fibrous capsule of HCC consists of a dense fibrous tissue in the inner layer and a peripheral rim of sinusoids and small bile duct, while the pseudocapsule is made up of the dilated blood sinus and fibrous tissue around the tumor (*49,50*). Both fibrous capsule and pseudocapsule appear as slightly low signal on T1 and slightly high signal on T2 (Figure 4), and show a discrete ring of hyperenhancement along margin of HCC in the portal venous phase or delayed phase, the enhancement usually increases from portal venous phase to delayed phases. Compared to the ring along the margin of regenerative nodules in surrounding liver, capsule appearance is thicker and more conspicuous (*50*). The capsule appearance is characteristic of HCCs, regardless of whether it is tumor capsule or pseudocapsule, and it is also reported to be capable of predicting HCC progression, while HCC with complete capsule lesions has lower recurrence rate after treatment than that of incomplete capsular counterparts, suggesting that the fibrous capsule may be able to prevent the spread of HCC (*51,52*).

3.5. *Intratumoral lipid contents*

Lipid content is often seen in HCCs of 1.5-3 cm in size, and occasionally seen in larger tumors (*53*). On CT examination, a mass may be demonstrated as having intratumoral fat if its attenuation is below 40 Hounsfield units (HU) (Figure 5a). Loss of signal intensity on the opposed-phase T1-weighted images is more sensitive to fat content than CT (Figure 5b) (*54*). HCC with lipid content often shows slow progression and relatively better prognosis (*55*). HCCs with intratumoral lipid content need to be differentiated from angioleiomyolipoma or liposarcoma which is rarely seen in cirrhotic liver.

3.6. *Mosaic architecture*

Mosaic architecture is used to describe appearance consisting of randomly distributed nodules with different appearances in attenuation/intensity and enhancement pattern; it also refers to lesions with internal enhancing septations (*56*). "Nodule-in-nodule" is a subtype of mosaic architecture, which is defined as the presence of a small nodule within a larger nodule or mass, the latter are often DN, especially for HGDN, and it reflects the growth pattern of HCC (Figure 6) (*57*). The internal nodule differs in enhancement or other

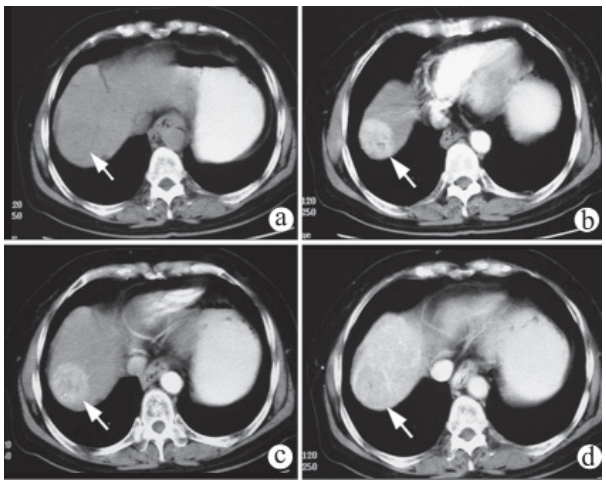

Figure 4. Capsule appearance of HCC (Arrow). (a) Precontrast CT showed an equivocal hypoattenuation nodule. **(b)** In early arterial phase, the nodule showed remarkable and heterogeneous hyperenhancement. **(c)** In late arterial phase, the nodule showed less enhancement but still higher than the background liver. **(d)** In portal venous phase, the nodule demonstrated unequivocal washout and a hyperattenuation ring was seen along margin of HCC namely capsule appearance.

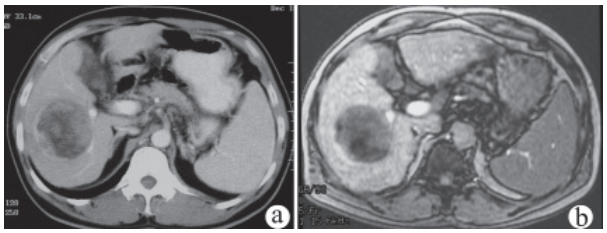

Figure 5. Intratumoral lipid contents in a masslike HCC. (a) Heterogeneous hypo-attenuation area (CT attenuation ranged from 25-38) in the mass on portal venous phase was indicative of intratumoral lipid. **(b)** Opposed phase imaging demonstrated obvious signal loss in the mass, which was specific for lipid content.

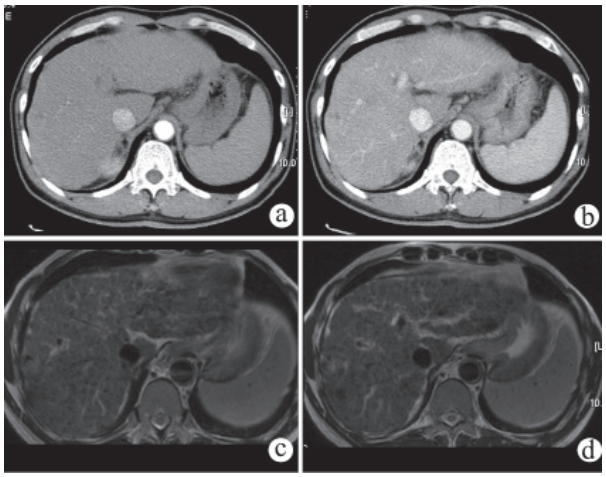

Figure 6. Nodule in nodule appearance in a cirrhotic liver. A heterogeneous enhanced nodule in arterial phase **(a)** and portal venous phase **(b).** CT could be seen adjacent to the right margin of the liver. **(c** and **d)** On T2WI sequence, the majority of nodules showed iso- and hypo- intense, there was a smaller nodule with moderately high T2 intense in the center of bigger nodule, histopathological findings had proved it was a small HCC in a high grade dysplastic nodule (HGDN).

features from the larger nodule. Mosaic architecture is a characteristic feature of HCCs (*56*).

3.7. *Hemorrhage*

Hemorrhage refers to presence of intra-tumoral or peritumoral blood products in absence of biopsy, trauma or local-regional treatment, it is an ancillary feature favoring HCC (*58*). On precontrast CT, hemorrhage could manifest as a heterogeneous hyper-attenuation area, but MRI is more sensitive and specific for detection of blood products than CT (Figure 7). On MRI, blood products usually manifest as areas of heterogeneous high T1 signal intensity and low T2 signal intensity due to T2* shortening (*59*).

3.8. *Tumoral thrombus*

Tumoral thrombus is defined when definite enhanced soft tissue is seen in the lumen of portal or hepatic vein. Vein occlusion with arterial phase hyperenhancement and washout within the lumen, lumen expanding, ill-defined walls and arterioles within lumen of vein are suggestive of tumor thrombus (Figure 8) (*60*). Comparatively, non-tumoral thrombus does not enhance and usually does not expand lumen to the same degree as tumor in vein (*61*). Tumor thrombus is a diagnostic feature of HCCs (*61,62*).

4. Imaging-based guidelines of HCC

Currently, there are at least 18 practice guidelines for the diagnosis and management of HCC since 2001. They are: Barcelona (BCLC) staging system; guideline 2010 from American Association for the Study of Liver Disease (AASLD); guideline from European

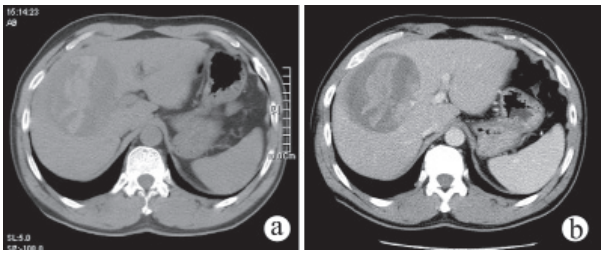

Figure 7. Intratumoral hemorrhage in a poorly differentiated HCC. 48-year-old man with sudden abdominal pain and history of hepatitis B virus infection. US revealed a mixed hyperechogenity mass in right lobe. **(a)** Precontrast CT showed a heterogeneous mass with irregular hyperattenuation in the central area. **(b)** Contrast CT demonstrated no enhancement in either hyperattenuation area or the majority of hypoattenueation area which were proved to be blood products in different stages, a small portion of soft tissue with moderate enhancement could be seen in the left margin on portal venous phase, which proved to be poorly differentiated HCC with intratumoral hemorrhage.

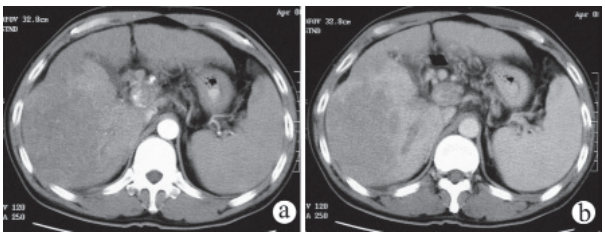

Figure 8. Tumoral thrombus in portal vein from a masslike HCC. (a) Hyperenhancement and arterials could be seen in the expanded but occluded portal vein lumen in arterial phase. **(b)** Rapid washout in vein could be seen in portal venous phase, no antegrade blood flow could be found in branches of the portal vein. A definite diagnosis of HCC with portal vein invasion could be made for the irregular mass in right lobe.

Association for the Study of Liver Disease (EASLD) updated in 2012; guideline from Asian Pacific Association for the Study of Liver Disease (APASL) in 2010, Japan Society of Hepatology (JSH) guideline 2014 and guideline from Korean Liver Cancer Study Group (KLCSG) in 2014 (*9,62-66*). These guidelines were developed to standardize the diagnosis of HCC mainly from the scope of clinical management. In 2011, Liver Imaging Reporting and Data System (LI-RADS) was proposed by American College of Radiology from a committee of radiologists, physicians, surgeons, pathologists and interventional radiologists (*67*).

LI-RADS is a system with a view of diagnostic imaging to provide standardized terminology and criteria for interpreting and reporting findings of CT and MRI in patients with cirrhosis or risk factors for HCC, which will help referring physicians to understand radiologic reports. It has been updated in 2013 and 2014 based on feedbacks from practice (*67*). The lexicon term "Observation" is used in the categorization instead of lesion or nodule, because observation might either be a hispathologically true lesion, perfusion alteration or artifacts. The features of arterial hyperenhancement and washout with size combination, capsule appearance

and interval growth are ancillary findings. LI-RADS categorizes radiological findings into five categorizations ranging from definitely benign to definitely HCC (Table 1) (*67*). LI-RADS is applied only for patients with cirrhosis or at high risk of HCC. Although it has more in common with AASLD compared to other guidelines, LI-RADS classified the "Indeterminate" category into probably benign, intermediate probability for HCC and probably HCC (LR 2, 3 and 4) to facilitate categorizing and reporting, especially for small nodules between 10 mm and 20 mm (*9,67*).

Because of the etiology, the incidence rate as well as treatment policies are different among international societies, and there is lack of consensus in the imaging techniques, diagnostic criteria, staging and treatment of HCCs. Some of the guidelines aim to enable ultimate specificity while others try to achieve higher sensitivity. The diagnostic strategies are different among LI-RADS and other clinical practice guidelines on several aspects: the application of CEUS in detection and characterizing of HCC, the application of specific imaging techniques of CT and MRI, the role of hepatocyte specific contrast agents, the diagnostic criteria of atypical HCC such as hypovascular HCC, diagnosis and management toward very small HCC, and the differential diagnostic spectrum of malignances other than HCC (*68-70*). Table 2 summarizes the controversies of the diagnostic strategy among major practice guidelines toward HCC.

In summary, with the growing knowledge of behavior of HCC, and the continuous improvement in

Table 1. Categories of LI-RADS v2014

LI-RADS	Category	Concept and definition
LR-1	Definitely benign	*Concept*: 100% certainty observation is benign. *Definition*: Observation with imaging features diagnostic of a benign entity, or definite disappearance at follow up in absence of treatment.
LR-2	Probably benign	*Concept*: High probability observation is benign. *Definition*: Observation with imaging features suggestive but not diagnostic of a benign entity.
LR-3	Intermediate probability for HCC	*Concept*: Both HCC and benign entity have moderate probability. *Definition*: Observation that does not meet criteria for other LI-RADS categories.
LR-4	Probably HCC	*Concept*: High probability observation is HCC but there is not 100% certainty. *Definition*: Observation with imaging features suggestive but not diagnostic of HCC.
LR-5*	Definitely HCC	*Concept*: 100% certainty observation is HCC. *Definition*: Observation with imaging features diagnostic of HCC or proven to be HCC at histology.
LR-5V	Definitely HCC with tumor in vein	*Concept*: 100% certainty that observation is HCC invading vein. *Definition*: Observation with imaging features diagnostic of HCC invading vein.
LR-5T	Treated observation	*Concept:* A loco-regionally treated HCC. *Definition*: LR5A or 5B observation or biopsy-proven HCC lesion that has undergone loco-regional treatment.
LR-M	Other malinancy	*Concept*: High probability that observation is a malignancy other than HCC. *Definition*: Observation with features suggestive of non-HCC malignancy.

*LR-5g, if there is ≥ 50% diameter increase in ≤ 6 months. *LR-5us, if there is both "washout" and visibility as discrete nodules at antecedent surveillance ultrasound. Modified from the original table from American College of Radiology. Liver Imaging Reporting and Data System. *http://www.acr.org/Quality-Safety/Resources/LIRADS. html*

Table 2. Major difference on the diagnosis of HCC among six major practice guidelines

Items	AASLD 2010	EASLD 2012	APASL 2010	JSH 2014	KLCSG 2014	LI-RADS 2014
Contrast enhanced ultrasonography	N	N*	Y	Y	Y	N
Angiographic assisted CTA/CTAP	N	N	N	Y	N	N
Inclusion of small HCC < 1.0 cm	N	N	Y	Y	Y	N
Diffusion weighted Imaging	N	N	N	N	N	Y
Hepatocyte specific contrast imaging	N	N	Y	Y	Y	Y
Criteria for hypovascular HCC	N	N	Y	Y	N	Y
Consideration of other malignances	N	N	N	N	N	Y

Y: agreed, included or recommended; N: disagreed, excluded or declined. * EASLD considers contrast enhanced ultrasonography to be used with caution.

imaging techniques and evidence-based interpretation in cirrhotic liver, the detection and characterization of HCC has improved in the past decade. Besides dynamic enhanced US/CT/MRI, hepatocyte-specific imaging and DWI are showing their potential for diagnosis of early HCCs. A number of practice guidelines for the imaging diagnosis have been developed to reduce interpretation variability and to help standardize management of HCC, and they are constantly updated with advances in imaging techniques and better understanding of features from clinical data.

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Review

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Recent advances in surgical treatment of hepatocellular carcinoma

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Summary **Hepatocellular carcinoma (HCC) is a common and lethal malignancy worldwide that arises within the context of a host of diseases. Surgery is the primary option for tumor treatment and is thus the most effective therapy to allow the best overall survival and recurrence-free survival for patients. One aim of this paper is to present and discuss recent advances in the surgical treatment of HCC such as associating liver partition and portal vein ligation for staged hepatectomy (ALPPS), laparoscopic hepatectomy, and robotic liver resection, and another aim of this paper is to highlight current issues in the surgical treatment of HCC such as extended indications.**

Keywords: Hepatocellular carcinoma, liver resection, laparoscopic hepatectomy, robotic, ALPPS, extended indications

1. Introduction

Hepatocellular carcinoma (HCC) is the sixth most common cancer and the second most common cause of cancer death; HCC is also the most common primary malignancy of the liver (*1*). The incidence of HCC is highest in East and Southeast Asia because of the prevalence of chronic hepatitis B in these regions. Standard potentially curative treatments for this cancer are either resection or transplantation, although radiofrequency ablation is considered a curative therapy in some cases (*2*). Better assessment of liver function, understanding of the segmental liver anatomy with more accurate imaging studies, and advances in surgical techniques are key factors that have led to a mortality rate of < 1% with an expected 5-year survival rate of 70% (*3-6*). One aim of the current paper is to present and discuss recent advances in the surgical treatment of HCC, and another aim of this paper is to highlight current issues in the surgical treatment of HCC.

2. Anatomical *vs.* non-anatomical resection

Hepatic resection and radiofrequency ablation are potentially curative treatments for HCC (*7*). Even after resection with curative intent, however, HCC has a high rate of recurrence, ranging from about 50% during the first 3 years after surgery to more than 70% during the first 5 years (*8-10*). The high incidence of HCC recurrence may be explained by the high incidence of both intrahepatic metastases and the multicentric occurrence of de novo HCC (*8*). In the past, portal vein dissemination was considered to be the main route for intrahepatic metastases, which led to the notion that anatomical resection, the site of which is based on where the blood flow to a tumor drains into the portal vein, might prevent the development of intrahepatic metastases of HCC (*11,12*). Data from a nationwide study in Japan (*13*) that included 5,781 patients with single HCCs revealed that anatomical subsegmentectomy (AS) was preferred over non-anatomical minor hepatectomy (MH) AS, especially when the size of the HCC ranged from 2 to 5 cm. Overall disease-free survival (DFS) was significantly better after an AS (p = 0.0089). If the HCC is smaller in size, one could reasonably deduce that there would be no statistical difference in the DFS after AS or MH since the risk of dissemination is presumably negligible, which means that both techniques had efficacy equivalent to that of local ablative therapy. If the HCC is larger, most patients will already have macroscopic vascular invasion or satellite nodules that will result in a high incidence of recurrence (*14*). This means a more advanced stage of HCC and evidence

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of the oncological behavior of the HCC, potentially offsetting the effects of the technique used. MH is comparable to AS. Therefore, AS is a strong prognostic factor, especially for HCCs measuring from 2 to 5 cm in size. In the cited study, there were no significant differences when the patients who underwent AS or MH were further stratified depending on the degree of liver damage (which is similar to the Child-Pugh score). This was true even in patients with HCCs of 2 to 5 cm in diameter. Therefore, AS is recommended particularly when the HCC ranges from 2 to 5 cm in diameter. However, MH is an option for treatment of a single HCC if AS cannot be safely performed.

Ishii *et al*. (*15*) analyzed 268 consecutive patients with HCC, including 110 patients who underwent anatomic liver resection (AR) and 158 who underwent non-anatomic liver resection (NAR). Forty-four patients from each group were selected and matched using logistic multivariate analysis followed by propensity score analysis. AR conveyed a survival advantage over NAR in specific subpopulations of patients with an HCC less than 5 cm in diameter, a single tumor, and good liver function.

Kamiyama *et al*. (*16*) analyzed 322 consecutive patients with HCC who met the Milan criteria and who underwent curative resection (R0). Patients were classified into two groups: Group A (patients with a single HCC having a diameter of 5 cm or less) and Group B (patients with multiple tumors, no more than three tumor nodules, each with a diameter of 3 cm or less). Kamiyama *et al*. found that anatomical resection improved surgical outcomes for patients with HCC that met the Milan criteria.

Yamamoto *et al*. (*17*) analyzed 174 patients with a single HCC 2-5 cm in diameter and without macroscopic vascular invasion. Studies investigating the survival benefits of AR compared to NAR have yielded results that are not completely consistent. Yamamoto *et al*. recommended that patients with an ICGR 15 < 20% and poor liver function undergo NAR rather than AR for the treatment of a solitary HCC 2-5 cm in diameter.

Marubashi *et al*. (*18*) investigated the pattern of HCC recurrence to evaluate whether non-anatomical resection, which is based on where the blood supply to a tumor drains, and anatomical resection, which restricts resection to the corresponding site where the tumor's blood supply drains into the portal vein, was more beneficial. Local dissemination as a pattern of HCC recurrence was observed in only 6 (1.4%) of the 424 patients included in their analysis. In the remaining patients, HCC recurrence was considered to be result of either systemic dissemination or de novo development of HCC. This "local dissemination" is a rather rare pattern of HCC recurrence, indicating that anatomical and non-anatomical resection are equally curative and that the selected technique did not influence patient outcomes. In other words, recurrence as a result of local dissemination can be ignored with both anatomical and non-anatomical resection. HCC recurring after curative resection is mostly caused by systemic dissemination of circulating tumor cells or de novo development of HCC.

In accordance with recent concepts and based on the evidence of HCC recurrence in patients with HCC who have undergone hepatic resection and liver transplantation, intrahepatic metastasis occurs because of the blood flow to a tumor or an aggregation of tumor cells in the remaining liver (*8,14,19*). Some studies (*20-22*) have demonstrated the superiority of anatomical resection over non-anatomical resection for treatment of HCC. Others (*23-25*) have questioned the validity of this suggestion, as they found no differences in HCC recurrence or overall survival rates for patients undergoing either form of resection after resection with curative intent. However, most reported studies had limited statistical power, and no case-matched or randomized clinical trials have compared the outcomes of anatomical and non-anatomical resection for treatment of HCC.

Clear evidence of the superiority of one technique over the other is not available since some studies have attributed a survival benefit to AR (*26,27*) while others have not (*28,29*). Two recent meta-analyses of observational studies have also reported conflicting results (*30,31*). Importantly, underlying cirrhosis was significantly more common in patients who underwent NAR and who also displayed greater hepatic dysfunction compared to patients who underwent AR. A meta-regression approach recently found that these aspects significantly affect the results of meta-analyses; that is, patient survival and DFS after AR seem to be superior to those after NAR because patients undergoing NAR have worse liver function reserve, which significantly affects prognosis (*32*).

Moreover, a recent meta-regression analysis was performed after adjusting for several key covariates, but results precluded the ready comparison of available studies on AR and NAR (*33*).

Thus, large randomized controlled trials are needed to define the best form of resection for patients with HCC developing from a cirrhotic liver (*32*).

3. Associating liver partition and PVL for staged hepatectomy (ALPPS)

Over the past few decades, advances in surgery, anesthesia, radiology, and oncology have resulted in an extension of the criteria for resectability of liver neoplasms (*34,35*), but a small volume of the future liver remnant (FLR) has been the Achilles heel limiting major hepatectomy (*36,37*). In general, most patients with HCC have an underlying liver disease, such as cirrhosis, that requires an FLR of at least 40% (*38*). Thus, surgeons

face the challenge of choosing either resection of the hepatic tumor with a potential risk of postoperative liver failure (PHLF) or giving the patient palliative treatment, such as transcatheter arterial chemoembolization or local ablative therapy, to avoid PHLF if the volume of the FLR is on the borderline (*39,40*). In recent years, some strategies, such as portal vein ligation (PVL), portal vein embolization (PVE), and two-stage liver resection have been developed to induce hypertrophy of the FLR prior to hepatectomy in primarily non-resectable liver tumors (*41*). Makuuchi *et al*. (*42*) first introduced the concept of PVE into clinical practice in the 1980s. In 2008, a meta-analysis of 37 studies conducted from 1990 to 2005 and involving 1088 patients revealed that 29 days, on average, elapsed from PVE to surgery, with an 8% to 27% increase in the FLR; in 14% of patients, resection was precluded after PVE due to disease progression or insufficient hypertrophy of the FLR (*43*). A systematic review and meta-analysis (*44*) compared PVL and PVE to assess the percentage increase in the FLR, morbidity, mortality, and tumor progression. All of the analyzed studies were retrospective. The 7 studies involved 218 patients, of whom 89 underwent PVL and of whom 129 underwent PVE. This meta-analysis comparing periprocedural outcomes of PVL and PVE revealed that the mean percentage increase in the FLR was 39% with PVE and 27% with PVL, but the difference in the percent increase was not significant. In addition, the two techniques resulted in similar morbidity and mortality rates after liver resection, a similar time to hepatectomy, and a similar time to disease progression. The two-stage hepatectomy was pioneered by surgeons at the Hôpital Paul Brousse in the 2000s. The procedure was designed when removal of all malignant lesions in the liver was not possible using a single procedure (*45*). The first hepatectomy was intended to keep the final FLR clear of all malignant lesions. During the wait prior to the second surgery, hypertrophy of the FLR was induced to make the second hepatectomy feasible and potentially curative (*46*). However, the major reason for failure of the two-stage hepatectomy was tumor progression while waiting for hypertrophy of the FLR or an insufficient increase in volume after portal vein occlusion (*47,48*). In a recent review analyzing short- and long-term outcomes of a large series of two-stage hepatectomies, morbidity was reported to range between 20 and 60% after the second surgery and the drop-out rate was reported to range between 8 and 31% (*49*).

ALPPS is a new 2-stage surgical strategy to increase the size of FLR, but the procedure was invented by chance. Professor Hans Schlitt from Regensburg, Germany first performed ALPPS in 2007. He planned to perform an extended right hepatectomy in a patient with hilar cholangiocarcinoma. During surgery, he realized that the FLR was too small to sustain the patient's life postoperatively. Hence, he made a good but unusual surgical decision to perform only a selective left hepaticojejunostomy for palliation. For optimal positioning of the hepaticojejunostomy, he divided the liver parenchyma along the falciform ligament, thereby completely devascularizing segment 4. He also ligated the right portal vein in order to induce hypertrophy of the left lateral section of the liver. Out of curiosity, he performed a computed tomography scan on day 8 postoperatively. To his surprise, the left lateral section had massively grown in size. He successfully removed the diseased liver with a second surgery. This novel approach was formally reported as a series of 3 cases in a presentation by Baumgart *et al*. (*50*), from Mainz, Germany, during the Ninth E-AHPBA Meeting in Cape Town, South Africa in April 2011. In 2012, de Santibanes and Clavien (*51*) proposed the acronym "ALPPS" for this novel technique. PVL and PVE are traditional approaches to induce hypertrophy of the FLR prior to hepatectomy in primarily nonresectable liver tumors. However, these approaches fail in about 14% of patients. Adequate hypertrophy of the FLR using PVL or PVE generally takes more than four weeks. ALPPS can induce rapid growth of the FLR, which is greater than that of reported with portal vein embolization or occlusion alone. Recent studies have noted the marked hypertrophy of the FLR, which enlarges by 40-80% within 6-9 days. Faster hepatocyte regeneration has resulted in a lower drop-out rate for the two-stage procedure. This waiting time can be critical, especially for patients with marginally resectable tumors or oncologically aggressive tumors (*52*).

The indications for ALPPS include an FLR of less than 30% in patients with a normal liver or an FLR of less than 40% in patients with a diseased liver caused by cholestasis, macrosteatosis, fibrosis, or pathologic changes associated with chemotherapy. Indications include marginally resectable or locally advanced unresectable liver tumors of any origin with an insufficient FLR either in terms of volume or quality. The pathologies that ALPPS is commonly used to treat include colorectal liver metastases, hilar cholangiocarcinoma, and HCC. Contraindications for ALPPS include unresectable liver metastases in the FLR, unresectable extrahepatic metastases, severe portal hypertension, high anesthetic risks, and a poor condition prior to major surgery (*53*).

In the beginning, ALPPS was mostly used to treat metastatic liver diseases. A liver with an underlying disease is known to have a lower capacity for regeneration and hypertrophy. In fact, a cirrhotic liver is less capable for hypertrophy after PVE than is a healthy liver. Vennarecci *et al*. (*54*) found that *i*) the ALLPS procedure is technically feasible and safe even when performing a major liver resection to treat HCC in a cirrhotic liver, that *ii*) the procedure is able to induce a significant increase in the volume of the FLR in a short period of time, allowing completion of the second stage of ALPPS, and that *iii*) the volume of

the FLR continues to increase even in a cirrhotic liver after the hepatectomy. Oncological results and short and long-term survival times of patients with a large HCC and major vascular invasion treated with ALPPS are not yet available. Now that the initial experimental phase has passed, prospective controlled studies with large samples are needed to properly evaluate ALPSS. An FLR to BWR ratio of at least 0.5% in a normal liver and a ratio of 0.8% in a cirrhotic liver should be achieved in order to avoid the considerable risk of hepatic decompensation and postoperative mortality.

ALPPS was reported to have high rates of operative morbidity, mortality, and bile leakage. Morbidity after ALPPS is reported to be 16%-64%, and mortality is reported to be 12-23%. The main forms of morbidity included bile leakage and sepsis, and the main cause of mortality was hepatic insufficiency. Total laparoscopic ALPPS has been reported (*55,56*) to cause fewer adhesions during the second stage of surgery. The long-term oncological outcomes following ALPPS are not yet available, but the 5-year overall survival rate after a standard two-stage hepatectomy is reported to be 51%. However, more studies are needed to evaluate the role of ALPPS in patients with hepatic fibrosis.

ALPPS has emerged as a new strategy to increase the resectability of hepatic malignancies. Due to the high morbidity and mortality rates of ALPPS, surgical candidates should be carefully selected. Moreover, there is very limited evidence of the technical feasibility, safety, and oncological outcomes of this procedure, so these aspects need to be evaluated further in large-scale studies.

4. Indications for and the current role of laparoscopy

Although laparoscopic surgery has been widely used in the field of abdominal surgery, such as colon and gastric surgery, use of laparoscopic liver resection is still limited to specialized facilities. Laparoscopic liver resection requires sufficient experience in both laparoscopic and open surgery. In addition, it requires advanced laparoscopic surgical techniques associated with parenchymal dissection and hemostasis. Laparoscopic liver resection has been used to treat various liver diseases as experience with laparoscopic surgery has increased and laparoscopic instruments have been developed (*57*).

Laparoscopic resection to treat HCC was first performed in 1992 (*58*). Initially, laparoscopic liver resection was only used to treat lesions located in the left lateral or peripheral segments. Due to the difficulty of controlling bleeding and visualization of the surgical field, lesions in the deep or posterior sections of the liver (segments I, VII, and VIII and the superior part of IV) were previously considered to be unsuitable for laparoscopic liver resection (*59,60*). A group of experts met in Louisville, Kentucky, United States in 2008 and determined that the best indications for laparoscopy were solitary lesions, less than 5 cm in diameter, located in the anterior segments at a distance from the line of transection, the hepatic hilum, and the vena cava (*61*). Since that time, surgical indications have continued to evolve: tumor size alone is no longer a contraindication for laparoscopic surgery (*62*) and experienced facilities use laparoscopic resection to treat tumors in the posterior segments or center of the liver (*63,64*).

Obtaining a safe margin for the inferior portion of a tumor is difficult when the tumor is located in the posterior or superior part of the liver. However, recent studies have noted the feasibility and safety of laparoscopic liver resection of lesions in those locations (*65,66*). Since Azagra *et al*. (*67*) performed the first anatomical resection, laparoscopic left lateral sectionectomy has become the standard treatment at some facilities (*68*). According to one study (*57*), laparoscopic left lateral sectionectomy was the most common form of anatomical liver resection. In the future, though, laparoscopic left lateral sectionectomy may become a routine procedure at most facilities. However, laparoscopic major hepatectomy is not used worldwide because of the complexity of the procedure and the fear of causing uncontrollable bleeding. This procedure should be performed by an experienced surgeon according to an expert consensus on laparoscopic liver surgery (*69*).

Laparoscopic resection is associated with significantly less intraoperative blood loss and less need for transfused blood, which can partly be explained by the hemostatic effect of pneumoperitoneum (*70*) and the magnified vision afforded by laparoscopy (*70,71*). In addition, transfusion rates have been identified as an independent prognostic factor for DFS in HCC (*72,73*), and blood loss was found to be independently associated with recurrence and decreased survival rates after resection of HCC (*74*). Laparoscopy should reduce the need for a transfusion and thus improve the prognosis for patients undergoing a resection to treat HCC (*75*).

A recent meta-analysis by Yin *et al*. (*76*) found that the postoperative morbidity rate after laparoscopic resection of HCC decreased significantly in comparison to open surgery. A meta-analysis indicated that laparoscopic resection consistently yields favorable results in terms of the duration of hospitalization (*77-79*), consequently reducing the overall morbidity rate and incidence of intractable ascites. The main concern about using laparoscopy to treat malignancies is the risk of inadequate tumor resection. In a number of studies (*76-79*) comparing laparoscopic and open liver resection for treatment of HCC, there was no significant difference in recurrence-free or overall survival, suggesting that laparoscopic surgery does not compromise oncological principles.

Nonetheless, some studies have criticized laparoscopic resection because of its low level of

reproducibility and because it is limited to a few specialized facilities. After a learning curve of 60 procedures (*80*), there is significant improvement in terms of operating time, conversion rate, blood loss, morbidity, and duration of hospitalization. This suggests that laparoscopic resection is reproducible at facilities regularly performing liver surgery but that it requires specific training in advanced laparoscopy.

Laparoscopic liver resection is a new strategy for liver surgery that is needed to treat liver tumors.

5. Robotic liver resection

Hepatobiliary (HB) surgery is a challenging surgical subspecialty that requires highly specialized training and an adequate level of experience in order to be performed safely. Although the technical feasibility of a laparoscopic approach has been extensively demonstrated, its use has not extended to the broader community of surgeons performing HB surgery. This is due in large part to the limitations of the procedure, since it can be safely performed by a few highly experienced surgeons. Recent development of robotic platforms has provided a tool that can overcome many of the limitations of conventional laparoscopic HB surgery. Augmented dexterity enabled by endowristed movements, software filtration of the surgeon's movements, and high-definition three-dimensional vision provided by the stereoscopic camera combine to allow steady and careful dissection of the structures of the liver hilum as well as prompt and precise endosuturing in cases of intraoperative bleeding. These advantages have allowed many facilities to expand the indications for minimally invasive HB surgery, with encouraging initial results (*81*). In 2003, Giulianotti *et al*. (*82*) reported the first use of a robotic liver resection. The indications for robotic hepatectomy are similar to those for laparoscopic hepatectomy. Both benign and malignant tumors can be resected robotically. Laparoscopic hepatectomy for lesions in the superoposterior segments, such as segments VII and VIII, is particularly challenging due to their positions and the curved transection lines. As a result, lesions in these segments may be more commonly resected *via* a right hepatectomy, sacrificing a substantial volume of the normal liver (*83*). Robotic hepatectomy helps overcome this problem and some authors have reported success with this approach (*84*). Thus the greatest theoretical advantage of robotic hepatectomy may lie in sectoral, segmental, or subsegmental resection of lesions in difficult-to-reach positions. As a result, patients may be spared the large incisions and extensive mobilization required in an open approach.

The most prolific use of robotic hepatectomy to date was reported by Giulianotti *et al*. (*85*), who described procedures performed by a single surgeon in 70 cases (60% malignant, 40% benign). Twenty-seven patients underwent a major hepatectomy; of those, 20 underwent right hepatectomy, 5 underwent left hepatectomy, and 2 underwent right trisectionectomy. Few studies have compared robotic to laparoscopic liver resection. Berber *et al*. found that the operating time, blood loss, and resection margins differed (*86*). Ji *et al*. found that robotic resection may involve a longer operating time than laparoscopic or open resection but comparable blood loss and complications (*87*). Lai *et al*. found a similar association in patients undergoing minor hepatectomy (< 3 segments) alone (*88*). The largest matched comparison of laparoscopic and robotic hepatectomy was reported by Tsung *et al*. and the University of Pittsburgh group (*89*). In this retrospective study, 57 patients undergoing robotic hepatectomy were matched with 114 patients undergoing laparoscopic hepatectomy based on underlying liver disease, the extent of resection, the diagnosis, ASA class, age, BMI, and gender. They found that the operating time was significantly longer for both major and minor hepatectomies that were performed robotically. There were no significant differences in complication rates, length of stay, mortality, and negative margin rates. A systematic review (*90*) identified 232 unique patients. Overall, the outcomes reported were similar to those seen in large laparoscopic series, but there was no clear difference in the outcomes of a robotic or laparoscopic approach. The key issue is to define the best indications for robotic liver resection. Despite the subjective advantages of a robotic system (maneuverability, ergonomics, and 3D vision), identifying its objective advantages is difficult in light of the current literature. Some authors have found that robotic technology provides an advantage mainly in two key steps during hepatectomy: dissection of the hilum and the hepatocaval dissection in the event of a right hepatectomy (*88*). In addition, the microsuturing capacity of a robotic system allows reconstruction of the biliary anatomy (*91*).

Overall, robotic technology has developed and it has expanded the indications for minimally invasive surgery. This technology might facilitate the treatment of large lesions and lesions that are posteriorly located.

The crucial point for a new technology is to offer advantages without sacrificing safety. With minimally invasive surgery, surgical stress is reduced, whether it is by a robotic or a laparoscopic approach. Like laparoscopy (*92,93*), a robotic approach yields results comparable to those of open surgery (*94*), primarily by minimizing blood loss and reducing the risk of a required transfusion (in the patients described). However, oncological outcomes rather than feasibility and peri-operative safety are the key to determining whether an approach is effective at treating malignancies; the same was true for laparoscopy several years ago (*95*). Even though only a limited number of patients were selected, the available data do not appear to indicate that oncological principles have been sacrificed. The reported outcomes compare favorably to those of a laparoscopic or open approach

(*92,96*) as indicated by a low rate of recurrence. A point worth noting is that the robotic approach has not been followed for a long enough period of time, so that approach needs to be examined in comparison to other approaches. Nevertheless, the robotic approach provides results, at 2 years for treatment of HCC (*88*) and at 3 years for treatment of colorectal metastasis (*97*), that are similar to those reported in systematic reviews of laparoscopic approaches (*96*). The increasing interest in robotic technology should help encourage randomized studies with larger samples and a longer follow-up. With quality evidence, the robotic approach might become the minimally invasive treatment of choice in advanced and complex cases.

One of the major disadvantages of robotic surgery is the high cost. In a systematic review of the literature in English, Turchetti *et al*. analyzed 11 studies that compared the cost of robotic surgery to that of a laparoscopic approach in various abdominal surgeries. The cost of the robotic approach was generally higher due to the longer operating time (and especially the set-up time) and instruments required, although the costs of hospitalization were similar (*98*). The purchase and maintenance costs are significant, particularly for lower-volume facilities, but many studies have not included these costs.

Robotic liver resection is safe and feasible when performed by an experienced surgeon. The procedure requires an expert patient-side surgeon with advanced laparoscopic skills. Wristed instruments are useful in a variety of maneuvers, such as looping Glissonian pedicles (especially on the left side of the liver) and in suturing bleeding points. The learning curve for robotic resection may be shorter than that of conventional laparoscopic liver surgery because the three dimensional imaging camera, wristed instruments, and better ergonomics will help experienced laparoscopic surgeons to quickly familiarize themselves with the robotic procedure (*99*). Despite the limited number of cases reported in the literature (*85,87,88*), the use of a robot to perform a minor or major liver resection appears to be a safe and feasible alternative to the open and laparoscopic approaches, resulting in lower postoperative morbidity and adequate oncological outcomes for primary and metastatic diseases. Most of the studies of robotic liver resection have focused on short-term perioperative outcomes (*100*). Long-term oncologic results and cost-effectiveness must be evaluated before the advantages and disadvantages of robotic liver resection can be conclusively determined. Robotic HB surgery has been rapidly increasing over the past few years. A prospective comparative study should be conducted to verify the advantages of robotic liver resection for the management of HCC. The development of new technologies and robotics will certainly expand the use of laparoscopy in the multimodal management of hepatocarcinoma (*101*).

6. Should indications for surgery be expanded beyond the BCLC criteria?

Curative treatments, including liver transplantation, surgical resection, and percutaneous ablation, are able to achieve a long-term survival rate of more than 50% at 5 years; however, only a small group of patients with early-stage HCC are eligible for these therapies (*102-107*). Most patients have advanced HCC when they are diagnosed. Thus, several HCC staging systems based on the tumor burden and liver function have been proposed over the past decades to guide therapeutic decisions (*108-113*). The Barcelona Clinic Liver Cancer (BCLC) staging system is accepted worldwide for clinical practice. The BCLC classification divides patients with HCC into 5 stages (0, A, B, C, and D) depending on tumor status-related variables (size, number, vascular invasion, N1, and M1), liver function (Child-Pugh grade), and health status (ECOG). The BCLC classification divides patients with HCC into stages depending on prognostic variables and allocates therapies depending on treatment-related status (*114*). For example, BCLC stage B is defined as an intermediate stage. Chemoembolization is recommended as the standard treatment of intermediate-stage (BCLC stage B) HCC (*114*).

According to the BCLC classification, liver resection should be performed only in patients with a small single HCC nodule without signs of portal hypertension or hyperbilirubinemia. Based on the BCLC classification, patients with multiple HCCs, a large HCC, or HCC with macrovascular invasion should undergo palliative treatment with unsatisfactory long-term results even if the lesion is resectable (*115-117*). However, recent studies have reported that surgical resection can lead to good short- and long-term survival rates for these patients (*118-121*).

Therefore, this classification has been criticized because it excludes many patients who could benefit from curative resection (*122-125*). Use of liver resection in cases of multiple HCCs is still controversial (*126,127*). According to the BCLC classification, all patients with multiple HCCs should be scheduled for percutaneous ablation or TACE if liver transplantation is contraindicated (*125*). Poon *et al*. (*128*) reported a 5-year survival rate of 60% after liver resection in patients with fewer than 3 HCC nodules ≤ 3 cm. Ruzzenente *et al*. (*121*) conducted a study of 464 patients with HCC from a multi-institutional database and they found that patients with fewer than 3 nodules who underwent liver resection had a higher survival rate than those who were treated with local ablative therapies (including percutaneous ablation and TACE), as indicated by a median survival time of 58 months versus 20 months with local ablative therapies ($p < 0.01$). These findings were verified by a subsequent randomized controlled trial. The authors reported that

patients with multifocal HCC meeting the Milan criteria had a 5-year survival rate of 69% after liver resection and 45% after radiofrequency ablation ($p = 0.042$) (*129*). In selected patients with multinodular BCLC B (more than 3 nodules) HCC and preserved liver function, liver resection yielded better long-term results than TACE with a 5-year survival rate of 36-37% compared to 11-14% with TACE (*130*). Although further studies need to verify these results, liver resection seems to offer satisfactory long-term results for patients with multiple HCCs. Macrovascular invasion (MVI) is one of the strongest predictors of survival in patients with HCC (*131,132*). The median survival time for patients with untreated HCC and PVTT is 2.7 months while that for patients with untreated HCC and MVI is 5 months (*133,134*). After sorafenib treatment, these patients are reported to have a survival time of 6 months (*135*). In recent series of surgical cases including MVI by HCC, the postoperative mortality rate ranged from 3.4% to 7.7% and the morbidity rate ranged from 30.8% to 37.1% (*120,136,137*). In a multicenter study of 102 patients with MVI by HCC who underwent surgical resection, Pawlik *et al*. reported that patients had a 5-year survival rate of 10% (*120*). Better survival rates are reported for selected patients with PVTT, with a 5-year survival rate ranging from 11% to 42% according to the literature (*137-140*). According to the Hong Kong Consensus Recommendations on the Management of Hepatocellular Carcinoma, resection may be considered in some patients with BCLC grade B or C liver disease, which the Consensus Recommendations classify as HKLC stage II. According to the Consensus Recommendations, resection of isolated extrahepatic metastasis after hepatic resection is justified in selected patients with HKLC stage II HCC (*141*). No meta-analyses have been performed, so the available evidence is limited.

Recent improvements in surgical techniques and perioperative care have enhanced the feasibility and safety of liver resection with satisfactory long-term results in selected patients with early HCC and PH and with intermediate-advanced HCC. Based on the BCLC algorithm, the EASL/AASLD guidelines currently exclude many patients from curative treatment even though they may benefit from liver resection. No other HCC classification has been recognized worldwide. Based on data in the literature, the treatment strategy should be tailored to the individual patient. Thus, the BCLC algorithm should be revised and clinical guidelines that possibly include new molecular classifications should be introduced (*142*).

7. Conclusion

The incidence of HCC has been increasing worldwide. HCC remains an aggressive malignancy that is one of the more common causes of cancer-related death. Surgery is the primary option for tumor treatment and is thus the most effective therapy to allow the best overall survival and recurrence-free survival for patients. Surgery will gain further importance since there are emerging insights into the indications for liver resection to treat HCC indicating that these indications can be expanded to tumor stages outside of previous recommendations. This malignancy can be cured in appropriately selected patients through use of advanced surgical techniques. Newer imaging modalities continue to advance, surgical approaches are being devised, and patient selection is improving, so there are grounds for believing that the outcomes of HCC treatment will improve.

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Review

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Liver transplantation for hepatocellular carcinoma

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Summary **Marked improvement in the prognosis for patients with liver cancer who undergo liver transplantation has been achieved as a result of advances in liver transplantation techniques. Given the current shortage of organs in China, a favorable long-term survival rate might be achieved with rigorous selection of suitable patients and therefore benefit society the most. Further study of the mechanism of cancer recurrence following liver transplantation, continuing to optimize pretreatment strategies prior to liver transplantation, and paying closer attention to the prevention and treatment of cancer recurrence following liver transplantation are important steps to improve the long-term clinical benefit of liver transplantation for patients with hepatocellular carcinoma. Perfecting the techniques of liver transplantation using a marginal donor liver is the main way to solve the current problem of an organ shortage for patients with liver cancer.**

Keywords: Hepatocellular carcinoma (HCC), liver transplantation, organ shortage

1. Introduction

There is a higher prevalence of hepatocellular carcinoma (HCC) in China, which accounts for 50% of the world's cases of liver cancer. HCC is the second most common cause of cancer mortality among all malignant tumors (*1*). HCC is accompanied by cirrhosis and has multiple foci, so fewer than 30% of patients with HCC can undergo surgical resection and the 5-year recurrence rate after surgery is 70% (*2*). Liver transplantation (LT) is surgery to remove a diseased liver and completely eliminates the cause of cancer recurrence, so LT is an effective way to cure liver cancer.

Data from the China Liver Transplant Registry (CLTR) shows that HCC currently accounts for about 50% of all liver transplants each year and that 50% of patients with HCC have advanced liver cancer falling outside the Milan criteria. Rational use of liver transplants cannot be achieved for high recurrence rate after transplant. As the number of patients waiting for LT increases, the problem of a shortage of organs is worsening. There is debate over whether to expand the use of donor resources by using marginal donor livers for LT. This paper discusses eligibility criteria for LT to treat HCC, perioperative prevention of the recurrence of HCC, and expanding the pool of donors for LT to treat HCC.

2. Eligibility criteria for LT

In clinical practice, factors for cancer recurrence after LT are key aspects of the eligibility criteria for LT. Therefore, the criteria for liver transplant recipients have been revised as LT techniques have been developed. Mazzaferro *et al*. proposed the earliest criteria for LT to treat HCC known as the Milan criteria. The 4-year overall survival rate was 85% and the disease-free survival rate after LT was 92% for patients with HCC who were selected in accordance with the Milan criteria (*3*). The Milan criteria were the first criteria for LT and were widely used by most transplant centers. However, the strict limitations of the Milan criteria meant that many patients with HCC falling outside the Milan criteria despite a lack of major vascular invasion or lymph node metastasis were not eligible to undergo LT. The Milan criteria attach greater importance to the size and number of tumors without considering the biological characteristics of HCC. Many transplant centers began to explore broader criteria for LT to treat HCC, leading to development of the Pittsburgh modified TNM criteria, the University of California San Francisco (UCSF)

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criteria, the new Kyoto criteria, the Canadian criterion of total tumor volume, and the up-to-seven criteria.

In 2001, Yao *et al*. at UCSF devised the UCSF criteria. Compared to the Milan criteria, the UCSF criteria reduced the rate of recipient loss and expanded the indications of LT for HCC without significantly increasing the rate of HCC recurrence (*4*). The UCSF criteria have gradually been adopted as the criteria for LT by many transplant centers because they have better reference values compared to the Milan criteria. Data on 2,610 liver transplants for primary HCC at two centers (Tianjin First Central Hospital and Beijing Armed Police Hospital) from January 1999 to December 2011 revealed that the 5-year cumulative survival rate was 77.1% for patients meeting the Milan criteria and 68.9% for those meeting the UCSF criteria (*5*). There was no significant difference in the cumulative survival rate of the two groups. Patients with HCC meeting the UCSF criteria but falling outside the Milan criteria accounted for 25.4% of all recipients, and those patients had a 5-year cumulative survival rate of 58.1%. This figure was greater than 50% but significantly lower than the survival rate for patients meeting the Milan criteria. Therefore, more than 50% of patients falling outside the Milan criteria and the UCSF criteria can undergo LT, representing a significant increase in potential liver transplant recipients (data showed that the number of recipients could be increased by 50% without significantly reducing the long-term survival rate).

Similar to the Milan criteria, the UCSF criteria mainly focus on preoperative imaging studies that may not coincide with actual pathology results. There are limitations on the eligibility criteria for LT to treat HCC depending on the number and size of tumors (*6,7*). One clinical pathological study at the authors' hospital found that the stage of HCC could not be determined accurately in 27% of patients prior to surgery even when sophisticated imaging studies were performed. In addition, these aforementioned criteria do not reflect the history of liver disease, prognostic factors for liver cancer, and other biological characteristics that often lead to marked discrepancies in prognosis. Over the past few years, the "Hangzhou criteria" and the "Fudan criteria" have been proposed in China (*8*). These criteria use the serum levels of alpha-fetoprotein and pathology according to a liver biopsy to evaluate tumors, but these criteria do not reflect the true histological grade due to the heterogeneity of liver cancer. Moreover, a liver biopsy may increase the risk of cancer spreading, so this approach is used by few transplant centers (*9*).

About 50% of Chinese patients with liver cancer have advanced cancer, so expanding eligibility criteria would benefit these patients, but this also means a higher rate of recurrence. Satisfactory survival rates and a quality of life like that with a normal liver transplant could not be achieved for patients with HCC falling outside the Milan criteria, and LT was only considered to be a palliative treatment. In light of the shortage of donor organs, more rigorous selection criteria are needed in order for donor resources to best benefit society.

3. Perioperative treatment

3.1. *Preoperative treatment*

Because of the shortage of organs in China, patients must wait significantly longer for LT. If patients do not receive interventional treatment while they are waiting for a donor, patients with a small tumor, much less those with a larger tumor, may cease to be eligible for surgery. This means that preoperative adjuvant treatment is absolutely necessary. Common treatments include transcatheter arterial chemoembolization (TACE), systemic chemotherapy (UFTM), percutaneous ethanol injection (PEIT), and radio-frequency catheter ablation (RFCA). The most prevalent of these treatments is TACE. A randomized controlled trial (RCT) and meta-analysis (*10*) found that patients with unresectable liver cancer who underwent TACE had a significantly improved 2-year survival rate compared to patients not undergoing that treatment (31-63% *vs*. 27%). However, whether TACE can improve the prognosis of LT is still being debated. In a control study by Decaens *et al*. (*11*), 100 patients with liver cancer underwent TACE prior to surgery and 100 patients underwent LT alone. Preoperative TACE had no effect on the 5-year survival rate (59.4% for TACE *vs*. 59.3% for non-TACE, $p = 0.7$). Treatment in the form of TACE, UFTM, or RFCA to down-stage a tumor prior to LT resulted in no significant difference in the 5-year survival rate for patients undergoing that treatment compared to patients not undergoing that treatment (unpublished data). However, patients who received that treatment can wait substantially longer. Thus, TACE, UFTM, or RFCA is recommended to delay the progression of cancer in light of the shortage of livers.

3.2. *Effects of postoperative therapies on cancer recurrence*

There is no consensus on whether patients undergoing LT for HCC should be treated with chemotherapy or not. Soderdahl *et al*. (*12*) found that epirubicin was ineffective at preventing cancer recurrence after LT. A study by Bernal *et al*. (*13*) also found that chemotherapy with cisplatin and doxorubicin was ineffective. However, a study did report that 25 patients who received chemotherapy combining 5-FU, cisplatin, and doxorubicin had a better 3-year survival rate compared to previous patients (*13*). Immunosuppressors are an important treatment after organ transplantation, so choosing the right immunosuppressors is vital to the prognosis after LT to treat HCC. Rapamycin is a novel macrolide immunosuppressor that is more frequently used in clinical settings as a basic immunosuppressor

because of its dual role of immunosuppression and anti-tumor action. Sorafenib, a new molecularly targeted drug, has an effect on advanced HCC according to a large RCT and its effect on treating the postoperative recurrence of cancer has been noted in studies. Studies on the combined use of rapamycin and sorafenib to prevent cancer recurrence after LT are underway, and initial results have been favorable.

4. Expanding the pool of donors for LT to treat liver cancer

4.1. *Hepatectomy or liver resection and transplantation for liver cancer*

Liver resection and transplantation refers to LT to treat the intrahepatic recurrence of cancer (single lesions smaller than 5 cm, and fewer than 3 lesions smaller than 3 cm) or liver failure following a previous hepatectomy to treat resectable primary HCC (single lesions smaller than 5 cm, and fewer than 3 lesions smaller than 3 cm) along with complimentary liver function. A hepatectomy prior to LT was previously assumed to potentially cure some patients with liver cancer, thus allowing other needier patients to receive donor livers. The progression of liver cirrhosis and not the recurrence of cancer is what leads to LT for certain patients with HCC following a hepatectomy. Forty to 80% of patients with cancer recurrence after hepatectomy can undergo LT (*14*). Thus, LT is considered to be a stopgap measure for patients with cancer recurrence after a hepatectomy. A study has reported that a hepatectomy prior to LT might increase the surgical mortality and the rate of cancer recurrence postoperatively, thus decreasing the survival rate of patients. A study found that the outcomes of LT were not satisfactory if cancer recurred soon after a hepatectomy (*15*). Surgical techniques have improved and data from the CLTR indicated that liver resection and transplantation has a 1-, 3-, and 5- year-survival rate of 73%, 51.77%, and 45.84%, respectively, while LT alone has a 1-, 3-, and 5-year-survival rate of 74.49%, 55.10%, and 48.81%, respectively (*16,17*). As these figures indicate, there was no significant difference in the survival rate as a result of liver resection and transplantation and LT alone. A hepatectomy might control the progression of cancer and allow recipients to wait longer, so it could increase the chances for other patients to receive a donor liver to some extent. A hepatectomy could also rule out patients who are likely to have cancer recur and enhance recipient selection. Accordingly, a hepatectomy prior to LT warrants consideration.

4.2. *Utilization of livers from hepatitis B-positive donors*

China has a massive population with hepatitis B, so most patients with liver cancer are also infected with the hepatitis B virus. Conversely, some donors are unable to donate merely because they are hepatitis B carriers. Therefore, use of livers from hepatitis B-positive donors might possibly relieve the shortage of donors. Livers from hepatitis B-positive donors consist of livers from anti-HBc-positive and HBsAg-positive donors. Livers from anti-HBc-positive donors have been widely utilized in LT thus far, but there is still disagreement about the use of livers from HBsAg-positive donors.

Early on, the use of livers from HBsAg-positive donors was precluded because they led to transplant failure. With the improvement in and maturity of prophylaxis against hepatitis B after transplantation, livers from HBsAg-positive donors have gradually been used by various transplant centers. Studies have found that suitable anti-viral therapy provides satisfactory effectiveness when using livers from hepatitis B-positive donors for LT. Loggi *et al*. (*18*) reported 10 liver transplants using livers from hepatitis B-positive donors and they noted no complications related to hepatitis B after transplantation. The current authors studied 39 liver transplants using livers from hepatitis B-positive donors at this Hospital. Most recipients had liver cirrhosis associated with hepatitis B along with primary liver cancer (with a TNM stage of T4N0M0). The selection criteria for donor livers were a good shape and appearance as well as normal function. In the 39 transplants studied, the 1-, 3-, and 5-year-survival rate was 65%, 38%, and 24%, respectively. All the recipients received adefovir along with entecavir, and no transplant failures were caused by the recurrence of hepatitis B. Thus, the use of potent anti-HBV therapy after transplantation should allow livers from hepatitis B-positive donors with no other risk factors to be used in LT for hepatitis B-positive patients with progressive liver cancer. The risk of using these types of donor livers should be fully explained to patients and their families, and informed consent must be obtained before transplantation.

4.3. *Use of other types of marginal donor livers*

Other types of marginal donor livers consisting of livers from deceased donors, livers from elderly donors, steatotic donor livers, ABO-incompatible donor livers, and donor livers with a long cold ischemia time could be utilized in suitable patients with liver cancer. This would therefore increase the sources of donor livers and shorten the waiting time for transplant patients.

5. Conclusion

Marked improvement in the prognosis for patients with liver cancer who undergo LT has been achieved as a result of advances in LT techniques. Given the current shortage of organs in China, a favorable long-term survival rate might be achieved with rigorous

selection of suitable patients and therefore benefit society the most. Further study of the mechanism of cancer recurrence following LT, continuing to optimize pretreatment strategies prior to LT, and paying closer attention to the prevention and treatment of cancer recurrence following LT are important steps to improve the long-term clinical benefit of LT for patients with HCC. Perfecting the techniques of LT using a marginal donor liver is the main way to solve the current problem of an organ shortage for patients with liver cancer.

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Review

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Extra vascular interventional treatment of liver cancer, present and future

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Summary **Interventional therapy for liver cancer is a new type of treatment, and its progress has been influenced by the development of the large scale imaging instrument and various therapeutic apparatus. This article, from these two aspects, discusses the status and progress of interventional treatment of liver cancer.**

Keywords: Functional magnetic resonance imaging, contrast-enhanced computed tomography, contrast-enhanced ultrasound, image fusion technology, radiofrequency tumor ablation, microwave ablation, cryoablation, percutaneous ethanol ablation, laser ablation, Nano-knife treatment

1. Introduction

The current treatment of liver cancer includes surgical resection, liver transplantation, interventional therapy, radiation therapy, and chemotherapy. Interventional treatment includes both extravascular and endovascular treatment. For liver cancer, the extravascular interventional treatment is addressed as precision to inactivate in-situ treatment of a tumor using energy generated by high-tech physics (*e.g.* radio frequency, microwave, argon, helium freezing, laser, high intensity focused ultrasound, Nano-knife, photodynamic *etc*.) and chemicals (*e.g*. ethanol, acetic acid, dilute hydrochloric acid, *etc*.). Advanced imaging equipment and technical guidance of ultrasound are used, digital subtraction angigraphy (DSA), computed tomography (CT), and magnetic resonance imaging (MRI), have advantages of positioning accuracy, less trauma, bearable pain and curative treatment. Nowadays, it can be a trend to build a hybrid high-tech operating room, equipped with ultrasound, DSA, CT, MRI and other medical imaging equipment in large hospitals at home and abroad. Radiologists, interventional radiologists, and surgeons break discipline restrictions, and work in close coordination and cooperation with each other, to achieve minimal trauma for patients, truly people-centered in accordance with evidence-based medical principles.

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2. Imaging and extra vascular interventional treatment of liver cancer

As far as increasing requirements in people's quality of life as well as the great development of medical imaging technology, digital technology, computer technology, biotechnology, molecular biology and cell immunity, therapeutic strategies are undergoing profound changes. How to use minimally invasive or non-invasive methods to inactivate and kill tumors in-situ while maximizing protection of the surrounding normal tissue, has become a hot spot and most urgent pursuit for tumor therapy physicians. Non-vascular interventional treatment, as the representatives of radiofrequency tumor ablation, microwave ablation, cryoablation, ethanol ablation, laser ablation, Nano-knife treatment, guided by MR, CT, ultrasound and other imaging examinations, has become an important part of clinical treatment. Imaging technology used in tumor treatment plays an important role in two aspects, one to locate the lesion, early diagnosis and postoperative follow-up, on the other hand, to guide treatment instrument arriving at target areas. Two imaging diagnoses plus elevated serological index can be diagnosed as liver cancer, which can be proof for extra vascular interventional treatment. Imaging detection of liver lesions is particularly important.

2.1. *Functional magnetic resonance imaging (fMRI)*

fMRI is a physiological function of the organizational structure, based on the image displayed in its state

imaging technology. It appears to provide a minimally invasive way for liver cancer preoperative diagnosis and postoperative evaluation. fMRI includes a variety of methods, T1WI diffusion tensor and T2WI combine diffusion weighted imaging (DWI), perfusion weighted imaging (PWI), magnetic resonance spectroscopy (MRS), diffusion tensor imaging (DTI) and blood oxygen level dependent functional magnetic resonance imaging (BOLD-fMRI), and contrast enhanced MRI after injection of the new hepatocyte-specific MRI contrast agent, Cypriot disodium (gadolinium ethoxybenzyl diethylenetdamine pentaacetic acid, Gd-EOB.DTPA). No other investigations can exceed MRI in the diagnostic value of small hepatocellular carcinoma (*1,2*). The use of all kinds of contrast agents should however be used with caution in patients with renal failure given the risk of nephrogenic systemic fibrosis which is a rare disorder associated with fibrosis of the skin, joints, eyes, as well as internal viscera (*3*). The other disadvantage to MRI is the relatively long time it takes to complete the study which may be a challenge for critically ill transplant candidates who need more detailed imaging before listing for transplant.

2.2. *CT and contrast-enhanced CT*

Contrast-enhanced CT has already become the most common and important method used in cancer diagnosis, but it is still highly restricted to smaller diameter tumor detection proportions. Multi-detector computed tomography (CT) scanning remains a very useful tool in the diagnosis of HCC. Advances over the last 10 years have seen CT scanners become considerably faster while attempting to limit the radiation dose. The sensitivity of multidetector CT (MDCT) is reported at 81% as compared to 91% with MRI in a meta-analysis of 15 comparative studies between MRI and MDCT. The specificity of MDCT was 93% compared to 95% in the MRI group. CT scans do afford the ability to perform three-dimensional reconstructions that may help with operative planning which is an advantage over MRI (*4*). Although a rare event, this mode of imaging does however place patients at risk for contrast induced nephropathy (*5*).

Although not included in standard diagnostic guidelines, modern advances show that perfusion CT scanning may offer more information regarding liver hemodynamics and blood flow directed toward tumors in the liver (*6*). This may become more useful as transarterial chemoembolization (TACE) is an evolving therapy for HCC. It also may aid in treatment monitoring. Current perfusion CT does, however, deliver a higher radiation dose as well as lower resolution (*7*).

2.3. *Ultrasound and contrast enhanced ultrasound (CEUS)*

CEUS is one of the representatives of these new technologies, and the use of contrast agents significantly improves the resolution of ultrasound diagnostic sensitivity and specificity. It has been widely used in clinical diagnosis of liver diseases, and in particular has an irreplaceable role in the diagnosis of liver tumors. Real-time CEUS dynamically reveals hepatic tumor hemodynamics by enhanced mode and enhanced phase. First, CEUS can early detect nodules in a liver cirrhosis background, and make a differential diagnosis; its diagnostic accuracy is at a high rate compared with enhanced CT and MRI. Ryu *et al*. (*8*) reported an analysis of 48 patients and a total of 50 liver tumors and found the use of a suitable acoustic window; CEUS had a similar diagnostic value compared with CT/MRI examination. Zhu *et al*. (*9*) studied 45 patients who had lesions in liver with cirrhosis that underwent both contrast-enhanced MRI examination and CEUS examination before surgery, and found that the diagnostic accuracy was 77.3% and 62.7%, respectively. Zheng *et al*. considered when evaluating response to therapy after HCC, although compared with the contrast-enhanced CT/MRI examination, CEUS showed great superiority. At the same time CEUS can make up CT/MRI deficiencies, such as CEUS offers real-time dynamic imaging, on the very early or very late phase to observe a lesion which may not appear on CT, and MRI image enhancement mode is extremely useful. Second, unique structure of micro bubble intravascular contrast agents favor depiction of the hemodynamic characteristics of HCC in CEUS examination, and will not cause the phenomenon of false-enhancement in delay phase in enhanced CT or MRI due to contrast agent leaks to tumor gaps. Third, CEUS allows multiple injections of contrast agent, for the quick clearance of contrast agent from blood, so as to observe enhanced mode repeatedly. Last, as a safe agent excluded by respiration, patients with heart and kidney failure can also tolerate the treatment.

However, limitations as well exist in CEUS examination, in such situations as physical obesity, gas interference in intestine, deep location in ultrasonic far field, or close to the top of the diaphragm or corners, the lesions can not be clearly displayed in two-dimensional ultrasound, let alone in CEUS. Time for each angiography contrast agent through the lesion is shorter, only no more than two lesions can be checked, so it can not observe the whole liver each time. For AFP increased patients with multiple small intra-hepatic nodules or abnormal situations, only conventional ultrasound examination repeatedly performed is needed (*11-14*).

Application of CEUS in clinic is later than enhanced CT and MRI, and there is examination with many similarities among them, but the enhanced mode is not the same. Ultrasound guided extra vascular interventional treatment of liver cancer is widely used compared to MRI and CT for its convenience, because it is less time-consuming, and gives a real-time and non-invasive examination.

2.4. *CEUS combined with CT and MRI*

At present, it has been reported that a new imaging method, navigated by image fusion technology by CT/MRI-ultrasound, pinpoint the location of the original tumor. This method is able to integrate the advantages of CT/MRI, three-dimensional or three-dimensional CEUS imaging and other kinds of ultrasound, make a good collection of CT/MRI static volumetric imaging and real-time ultrasound imaging technology, the CT/MRI good spatial resolution and real-time ultrasound good operation, simplicity complementary. Truly perfect "eye" (CT/MRI) and "hand" (ultrasound operation) combination. It will be the size of the tumor before surgery, location fusion superimposed and displayed on the ablation lesions, based on the joint use of three-dimensional ultrasound contrast can be more comprehensive and an objective assessment of ablation forecast and secure borders through images, CT/MRI an ultrasound navigation image fusion image is expected to become liver cancer diagnosis, guide treatment and postoperative evaluation of the most accurate method, and the accuracy of vascular interventional treatment of hepatocellular carcinoma has important clinical significance (*15-20*).

2.5. *Tumor ablation and image guidance*

Tumor ablation can be performed by percutaneous, laparoscopic surgery and surgery. Percutaneous tumor ablation guided by ultrasound, CT, MRI and other imaging methods has an advantage of less trauma, shorter hospitalization time, is less costly with faster recovery and retains the body's normal tumor tissues and organ function to the maximum, compared to laparoscopic and open surgical approach, but it cannot completely replace the other two ways in some instances. For example, for tumors near the bowel and diaphragm, the laparoscopic approach is a priority for bowel and diaphragm protection. When the main tumor is excised, other satellite lesions can be ablated at the same time.

Ultrasound, CT, MRI and other imaging that are used to guide tumor ablation also have their own advantages. As well as making good use of these examinations can help disease diagnosis and treatment better. Real-time ultrasound imaging, as a multi-angle detection, is safe and the cheapest imaging examination, is the priority method for guided ablation; contrast enhanced ultrasound helps to confirm the size and shape of the tumor, which can define the scope of tumor invasion, atypical small HCC detection and satellite lesions, as well as provide a more reliable reference for tumor ablation. Contrast-enhanced ultrasound can be performed for follow-up in case of local tumor progression or new lesions early. However, ultrasound has poor spatial resolution for deep lesions, especially in obesity. Sonic energy is badly absorbed or reflected by lungs, bones, intestine, ribs and so on, which make tumor there unrecognized. Besides, the former larger ablation lesions can produce steam after ablation; these mimic bubble artifacts and will obviously interfere with the subsequent puncturing and treatment.

CT with high spatial resolution and intensity resolution can be widely used in whole body. Nowadays, as well as three-dimensional reconstruction technology employed, CT can more clearly show size, accurate location, quantity and relationship with organs nearby, which can provide reasonable proposal for ablation. CT examination also can evaluate the efficacy after ablation and follow up. The biggest disadvantage is radiation restriction.

MRI image examination is a very suitable guidance and monitoring equipment for ablation. It has the following advantages: first, it has good resolution of tissue and anatomical structures that can highly proceed other examination, so it can clearly show the tumor and relationship with adjacent structures. Its fast imaging technique makes it efficient for real-time monitoring the whole procedure; second, its unique black blood or white blood technique provides a method to recognize blood vessels without a contrast agent administrated, which greatly reduces unnecessary iatrogenic injury; third, with multiple parameter imaging techniques, some blurred lesion on CT can be displayed clearly; fourth, with multi-planar imaging capability, we can select the plane and show puncture path of the lobe; fifth, with no ionizing radiation, MRI can be the best image examination helping interventional treatment; and last, it is the only imaging technology with real-time temperature monitoring, being highly sensitive to the temperature and amount of water molecule, which is important to control the scope and efficiency of ablation. The ultimate aim of minimally invasive tumor ablation is to achieve inactivation of tumor in situ and keep live function at a maximum, MRI can clearly distinguish the damaged area and normal tissue ablation without enhancement, an animal experiment showed that the actual ablation scope measured in pathology after ablation is close to that evaluated by MRI during ablation, no more than 2 mm. MRI has a more sensitive and accurate evaluation efficacy than any other image examination. It is the best option to determine whether the tumor has complete ablation. However, MRI and ablation relative equipment is expensive; patients with pacemakers and metal implants should not be guided by MRI treatment; non-magnetic compatibility rescue and monitoring equipment cannot enter the MRI operating room and so on, which greatly restrains its application. Expected because of development of industries, MRI guidance will become popular in clinical settings.

Currently, the preoperative ultrasound CT/MRI image fusion is used to locate the tumor ablation, and navigation and real-time evaluation of the efficacy

has gained great attention. Real-time ultrasound, CT or MRI fusion imaging can better guide and monitor ablation, equipped with a virtual navigation system not only helps to determine the scope of tumor invasion, develop and simulate puncture route, but also to predict ablation volume.

3. Ablation and treatment evaluation

Italian scholars Rossi *et al*. raised the possibility of percutaneous radiofrequency ablation of liver tumors in 1990 (*21*), and first published this in 1993 (*22*). In recent years, under the guidance of imaging technology, ablation plays an important role in the treatment of liver cancer. Among them, radiofrequency ablation (RFA) and microwave ablation (MWA) are better represented. Due to less invasion, and ease of operation, they can effectively inactivate tumor by coagulation. This brings a breakthrough for tumor treatment (*23,24*).

3.1. *Radiofrequency ablation (RFA)*

The principle of radiofrequency ablation is tissue coagulation caused by electromagnetic waves (usually 375-500 kHz). Electromagnetic waves in the needle electrode produce an alternating magnetic field, alternating magnetic field excited alternating current and collision, friction heat, heat deposition exceeds the tolerable level of tumor and causes necrosis; the small blood vessels around the tumor are occluded due to heat damage and thereby block tumor blood supply, in addition to the thermal effect of RFA it can enhance immune function, inhibit residual tumor cell growth and enhance tumor sensitivity to radiotherapy and chemotherapy. A cohort study (*25*), success rate of complete ablation in lesions less than 2 cm is over 90%, with local recurrence rate of less than 1%. A recent comparison of percutaneous RFA and liver resection of small liver cancer meta-analysis shows that, overall survival rates were similar in patients with small HCC by either percutaneous RFA or surgical resection, for compliance with standards of Milan and suitable for surgery or percutaneous RFA. The later would have little invasion, low incidence of complications and so on. Patients unwilling to accept surgery can be recommended to select percutaneous RFA (*26*).

Compared with microwave ablation, for a diameter > 3 cm tumor, the rate of local recurrence and complications, like biloma, is high. It is believed that with accumulated experience, medical technology update, and equipment advances, RFA treatment of liver cancer will be used wider and wider (*27*).

3.2. *Microwave ablation (MWA)*

MWA uses frequency > 900 MHz (usually 900-2,500 MHz) electromagnetic waves, the microwave heating effect causes biological tissue tumor tissue degeneration and coagulation necrosis. Not only heating by "ionic heating" like RFA, but also by "dipole heating", and the later works as well. Two electromagnetic wave frequency ranges, 915 MHz and 2,450 MHz are applied in clinics. The later is more commonly used. Theoretically, under the same energy output, ablation with 915 MHz can penetrate deeper, gaining a broader ablation range. In addition to possessing all the advantages of RFA MWA still has its some advantage: no limit to current poor conduction, fast temperature rise , a small subsidence effect caused by organized carbonization, a larger ablated range for single-needle, shorter ablation time, less pain and no need of grounding negative plates. In addition, compared to a simple "ionic heating", MWA mainly produces heat by water-based "dipole heat", and therefore it is suitable for the treatment of cystic tumors. The RFA effective tissue heating zone is limited to a few millimeters from the needle tip center, and the rest of the ablation zone is by thermal conduction, while microwaves have good transmission characteristics, and it can heat effectively all tissue as set up by the antenna (*28,30*).

Another scholar advised improving the efficacy of radiofrequency ablation and reduce medical risks by adjusting the output power, the application of digital technology and combined intravenous anesthesia reconstruction techniques.

3.3. *Cryoablation*

Cryotherapy is based on the argon helium frozen ablation technique, argon rapid expands quickly so that tissue temperature drops below zero rapidly. Cold causes cell necrosis by formation of ice crystals, then helium makes a rapid heating release causing ice hockey swell thawing, rupture, and further damage to tissue cell structure collapse, ultimately leading to cell necrosis. Permopongkosol S *et al*. (*31*) reported that cryoablation greatly reduced pain during treatment, unaffected by vasoactive effect "thermal sinking". Cryoablation is superior to RFA for a tumor near large vessels. However, there are many complications: including freezing without concerning the edges, when there is a hockey burst, tumor cells may break into the surrounding tissue, inadequate refrigeration may cause bleeding after treatment or even "freeze shock (cryoshock)", which is the main reason for death. Although most scholars agree that cryoablation efficiency can be equal to RFA treatment of liver cancer. Since local recurrence rate is lower when comparing RFA to cryoablation, there is a tendency to choose RFA as treatment for cancer, which needs further randomized verified trials.

3.4. *Percutaneous ethanol infusion (PEI)*

PEI is another commonly used interventional

extravascular treatment. Coagulating inactivation produced by alcohol induces cell dehydration, protein denaturation, chemical embolization of tumor blood vessels and other small pathways and leads to cell coagulation necrosis. Lencioni RA *et al*. reported that PEI was more effective than RFA for an enveloped tumor or that with adjacent large blood vessels, or vital organs (*32*). However, PEI treatment has a high lesion recurrence rate, for tumors less than 3 cm recurrence rate was 33%, lesion when larger than 3 cm recurrence rate of over 43%. This may be due because alcohol cannot evenly distribute throughout the tumor, especially for lesions including septum. This kind of treatment is just like "irrigation", and does not designate a valid "security border". It had little effect on tumors with satellite nodules (*33*).

On the contrary, RFA can outline a "security border" and have lower local recurrence rate. Randomized clinical trials (*32,34*) confirmed that RFA helped keep local treatment area stable for a clear "security border".

3.5. *Laser ablation (LA)*

LA uses thin, flexible optical fibers (diameter 300-600 μm) or optical fiber specially designed water-cooled in center to insert inside tumor under image guidance, tumor generates heat through absorption of laser and produces thermal effects, pressure effects, photochemical effects and electromagnetic effects, thus achieving the purpose of killing tumor by degeneration, coagulation, and vaporization (*35*).

Laser has characteristics of deep penetration, easily absorped by water, output power is adjustable, flexible operation, uniform energy distribution, and it is better for tumor treatment. A large randomized multicenter study has not yet been developed, there is a lack of long-term follow-up studies after treatment, and currently there is no treatment-related international consensus standards and guidelines. Laser ablation effects in combination with other treatments need further study (*36*).

3.6. *High intensity focused ultrasound (HIFU)*

The sonic frequency HIFU used is significantly higher than the applied ultrasonic diagnostic ultrasound (frequency 0.8-3.2 MHz, time-averaged intensity of the focus area is 100-10,000 W/cm^2, peak voltage and peak sparse concentrated pressure do not wind, 30 MPa and 10 MPa). The principle of HIFU is to gather low energy density beam convergence to target the body, sound propagation in body, and to transfer orderly ultrasonic vibrational energy into disorderly molecules energy, local tumor temperature soars to 65 (0.1 to 0.5 seconds -100°C), and causes tumor tissue coagulation necrosis, and achieves the purpose of non-invasive tumor inactivation without damage to the upper tumor tissue and adjacent normal tissue. HIFU can involve three-dimensional tumor structure as a scanning motion, this administrates tumor treatment with different shapes and size. As a kind of ultrasound, HIFU also has acoustic shadow, reflection, and refraction. HIFU has little effect on deep lesions, intestines or ribs nearby and may damage normal tissue due to refraction (*37*).

3.7. *Irreversible electroporation (IRE)*

Electroporation is a kind of physical phenomena with nanoscale pores in the cell membrane. Nanoscale pores are produced by potential instability due to a high-voltage field effect in the form of microsecond and millisecond pulsars in the phospholipid bilayer membrane (*38*). According to pulse amplitude and time applied to the cell membrane, nonporous membrane can be divided into temporary or permanent, to reverse electroporation (RE) and in reverse electroporation. In RE conditions, cells can be fully restored and survive, and IRE leads to cell death. IRE has a special pattern of non-thermal ablation of cells, without affecting the collagen support structure that allows regeneration of healthy tissue ablated in the tissue area, there is no scarring and other important characteristics, which has caused great attention (*39*) in the clinical treatment of cancer. This kind of reverse electroporation is also called "Nano knife".

IRE technology has ability to inactivate selected tumor only, there is no thermal conductivity effect, no sharp edges around the ablation zone, no neighboring tissue impairment like arteries, veins, peripheral nerves, urethra or intrahepatic bile duct, *etc*. IRE technology still has many shortcomings, such as electrical pulse induced arrhythmias and strong muscle contractions (and thus should be under general anesthesia treatment), the electrode needle placement has pneumothorax and bleeding risk, and this needs to be further studied and solved. A large standard randomized multi-center clinical study and long-term follow-up study needs be performed.

4. Conclusion

Extra vascular interventional treatment of liver cancer is safe, minimally invasive, repeatable and effective, *etc*. It is widely used in the treatment of solid tumors in the liver, plays an important role in tumor resolution and is minimally invasive. Selecting the appropriate therapeutic indication to give a comprehensive treatment is key to reduce complications and lower recurrence rate, which is a major challenge of extravascular interventional treatment of liver cancer in the future.

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Advances in endovascular therapy to treat primary hepatocellular carcinoma

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Summary **Transcatheter arterial chemoembolization (TACE) is a minimally invasive procedure to restrict a tumor's blood supply, and TACE has an established role in cancer therapy. An embolic material in the form of microspheres (such as drug-eluting beads) and transarterial radioembolization is effective at treating hepatocellular carcinoma (HCC). Endovascular therapy offers promise for the treatment of tumor thrombi in the portal vein. Many researchers are anticipating an era of TACE with microspheres. This review aims to provide an overview of advances in endovascular therapy to treat primary HCC.**

Keywords: Endovascular therapy, primary hepatocellular carcinoma, transcatheter arterial chemoembolization, microsphere, drug-eluting beads, transarterial radioembolization

1. Introduction

Transcatheter arterial chemoembolization (also called transarterial chemoembolization, or TACE) is a minimally invasive procedure to restrict a tumor's blood supply. Small embolic particles coated with chemotherapeutic agents are injected selectively into an artery directly supplying a tumor (*1*). Most investigative efforts are now focused on local control, with transarterial embolization (TAE) and TACE playing an established role in therapy. TACE is used as an effective means of palliation for unresectable tumors (*2-4*). TACE was first successfully performed for liver tumors by Doyon *et al*. in 1974 (*5,6*). Over the past few years, biological materials have consistently advanced and endovascular treatment of primary hepatocellular carcinoma (HCC) has improved with advances in medical science and technology. An embolic material in the form of microspheres (such as drug-eluting beads) and transarterial radioembolization is effective at treating HCC. Endovascular therapy offers promise for the treatment of tumor thrombi in the portal vein. Many researchers are anticipating an era of TACE with microspheres instead of conventional TACE involving lipiodol mixed with chemotherapeutic agents in combination with gelfoam. This review aims to provide an overview of advances in endovascular therapy to treat primary HCC.

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2. Drug-eluting beads (DEBs)

DEBs are microspheres copolymerized from polyvinyl alcohol and the monomer 2-acrylamido-2-methylpropane sulfonate (AMPS). This new system of drug delivery overcomes the drawbacks of a conventional system since anti-tumor drugs adsorb to the spheres. DEBs are widely used in the West to deliver drugs. The main DEBs on the market were DC Beads and Hepasphere microspheres. The former consists of a biocompatible polymer such as polyvinyl alcohol hydrogel while the latter consists of a super-absorbent polymer. DC Beads (the brand name in Europe) were approved by the FDA under the name LC Beads (*7,8*). Hepasphere microspheres were approved by the European Union in 2004 and by the FDA in 2006.

2.1. *Chemo-drugs and loading doses*

Doxorubicin and irinotecan were approved for elution by DEBs. Doxorubicin-eluting beads can release doxorubicin for 14 days or longer after they are

injected. Theoretically, the maximum loading dose of doxorubicin can reach 45 mg. In fact, the recommended safe dose is from 25 mg to 37.5 mg per ml to assure optimum elution. The dose of doxorubicin ranges from 100 mg to 150 mg depending on the patient's bilirubin levels. Another way to determine the dose is based on tumor size. For a tumor smaller than 5 cm, a dose of 75 mg is recommended, otherwise, a dose of 150 mg is suggested (*9*). A point worth noting is that all DEBs are made of a non-biodegradable material that absorbs chemotherapy drugs. In other words, beads degrade in a controlled manner to release drugs into a tumor. This is why DEBs are referred to as sustained-release drug-loaded beads. The ideal drug-loaded beads would consist of a biodegradable material and allow independent control of drug release.

2.2. *The diameter of DEBs*

Hepasphere microspheres come in sizes (dry) of 50-100 μm, 100-150 μm, and 150-200 μm. After hydration and loading, sphere sizes are 200-400 μm, 400-600 μm, and 600-800 μm. DC Beads come in sizes of 70-100 μm, 100-300 μm, 300-500 μm, and 500-700 μm. A size of 100-300 μm is recommended for optimum embolization in a clinical setting (*3*). DC Beads (M1) in a new size of 70-150 μm have appeared in Europe. New evidence suggests that small DC Beads provide a better objective response, downstage the tumor, and produce less tumor necrosis than beads 300-500 μm in size (*9*). DEBs 40 μm in size (Tandem; CeloNova BioSciences, Newnan, GA) have been used in clinical practice (*11*). However, beads 300-500 μm in size are common in clinical research.

2.3. *Clinical efficacy of drug-loaded microspheres*

Numerous studies have examined the use of TACE with drug-loaded microspheres in comparison to conventional TACE with iodized oil as a drug carrier, but they have failed to reach a uniform conclusion. A multi-center phase II prospective randomized controlled study has confirmed that doxorubicin-loaded microspheres were more efficient and caused less tumor necrosis than conventional TACE. Prajapati *et al*. (*12*) used the RECIST, WHO, EASL, and mRECIST guidelines to assess the efficacy of drug-eluting microspheres for the treatment of HCC, and they found that the WHO and RECIST1.1 guidelines had no obvious correlation with survival but that the EASL and mRECIST guidelines could indicate patient prognosis. Of the latter 2 guidelines, mRECIST was more effective. This finding indicates that TACE with drug-loaded microspheres needs to be evaluated in a substantially different manner from conventional TACE with iodized oil as a drug carrier.

PRECISION V, a phase IV trial of 212 patients with HCC (*12*), has indicated that the use of microspheres results in a higher rate of tumor necrosis at 6 months but no significant difference in the overall survival rate (51.6% vs. 43.5%). Research has shown that drug-loaded microspheres are effective in the short term, they are better tolerated, and they significantly decrease the incidence of severe hepatotoxicity events. TACE with these microspheres can partially replace conventional TACE with iodized oil as a drug carrier.

In a multi-center study by Malagari *et al*. (*7*) with a follow-up of 5 years, 41% of 173 patients had Barcelona Clinic Liver Cancer (BCLC) stage B unresectable HCC, and the 5-year survival rate was 22.5%. Patients with Child-Pugh grade A liver disease had a survival rate of 29.4% while patients with Child-Pugh grade B liver disease had a survival rate of 12.8%, and the median survival time was 43.8 months. Huang *et al*. (*15*) performed a meta-analysis comparing TACE with drug-loaded microspheres to conventional TACE. Their analysis included 7 clinical studies and 700 patients and they found that TACE with microspheres resulted in a significantly higher tumor response rate compared to conventional TACE (OR = 1.92, 95% CI (1.34, 2.77), $p = 0.0004$) and a lower risk (0.15 (0.07, 0.24) ($p = 0.0003$). The 1-year and 2-year survival rates increased significantly, but the 6-month and 3-year survival rates were 0.72 (0.46, 1.14) ($p = 0.16$) and 0.77 (0.55, 1.06) ($p = 0.11$), so there has no significant difference in survival rates.

Ferrer Puchol *et al*. (*16*) used the RECIST criteria to compare clinical outcomes of TACE with DEBs to conventional TACE. In their study, group A served as the control group ($n = 25$) and group B underwent TACE with DEBs ($n = 47$). The RECIST criteria were used to determine patient prognosis. A CR was achieved in 5.6% of patients in group A and 13.9% of patients in group B, and group A had a mean overall survival time of 686.24 days while group B had a mean overall survival time of 765.32 days. There were no significant differences in the rate at which a CR was achieved or in the mean overall survival time. Kalva *et al*. (*17*) noted that drug-loaded microspheres can prolong overall survival especially for patients with advanced liver cancer and that overall survival was correlated with the number of times DEB-TACE was undergone.

Some studies have found that drug-loaded microspheres have no obvious advantages compared to iodized oil. Scartozzi *et al*. (*18*) studied TACE with drug-loaded microspheres and TACE with iodized oil as a drug carrier in 150 patients with HCC. Patients who underwent TACE with drug-loaded microspheres had a median survival time of 46 months while patients who underwent TACE with iodized oil as a drug carrier had a median survival time of 19 months. The difference in median survival time was statistically significant. The time to progression was 30 months for patients who underwent TACE with drug-loaded microspheres

and 16 months for patients who underwent TACE with iodized oil as a drug carrier, indicating that drug-loaded microspheres are less effective than iodized oil.

Han *et al*. (*19*) performed a meta-analysis of literature from 1979 and 2013 on drug-eluting microspheres. They analyzed included 5 reports, 3 multi-center studies, and 2 case-control studies, and they found that drug-eluting microspheres had no advantages in terms of the rate of disease control and treatment-related complications. In a statistical analysis of numerous clinical studies, Tsuji *et al*. (*20*) found that TACE with drug-loaded microspheres had efficacy on par with that of conventional TACE. Kloeckner *et al*. (*21*) noted no significant difference between conventional TACE and DEB-TACE in terms of overall survival but they noted that TACE with microspheres required significantly less time than conventional TACE. This means that drug-loaded microspheres are crucial to the treatment of advanced liver cancer.

2.4. *Complications of drug-loaded microspheres*

According to clinical reports on embolization with drug-loaded microspheres (mainly DC Beads), the incidence of complications ranges from 4.2 to 11.4%. Complications mainly include pleural effusion, gastric ulcers, esophageal variceal bleeding, liver failure, cholangitis, and abscess formation (*22,23*). Aminotransferase levels also rise but they are generally believed to return to normal after a few days. The small diameter of the microspheres significantly increased the incidence of adverse reactions to drug-loaded microspheres, which were mainly high levels of alanine transferase and alkaline phosphatase. A point worth noting is that existing clinical studies of DC Beads loaded with doxorubicin have not found those beads to be associated with symptoms of doxorubicin-related systemic toxicity.

2.5. *Trends in research and development of drug-loaded microspheres*

Drug-loaded microspheres have become a focus of clinical research into TACE. Whether drug-loaded microspheres are used in combination with radiotherapy or liver transplantation, they have become the gold standard for TACE (*22*). A study by Xing *et al*. (*24*) found that drug-loaded microspheres can sustain quality of life for patients with advanced liver cancer while conventional TACE decreases their quality of life. From a health economics perspective, Vadot *et al*. (*26*) noted that TACE with drug-loaded microspheres cannot improve overall survival but that it can reduce drug toxicity and adverse reactions to TACE during hospitalization and ultimately reduce medical expenses. Thus, Vadot *et al*. consider TACE with drug-loaded microspheres to have benefits in terms of medical economics. These studies indicate that drug-loaded microspheres are in fact on par with or better than conventional TACE in clinical settings.

Recently, some researchers have begun to develop drug-loaded microspheres that are visually apparent in imaging studies (*27,28*). A contrast agent is added to beads with a porous structure or bonds in bead materials are chemically modified. During embolization, the beads can be observed in real-time, allowing the distribution of drug-loaded microspheres to be adjusted. These new materials may usher in a new generation of embolization agents.

2.6. *Existing problems and prospects for the future*

Almost all of the clinical studies that have compared drug-loaded microspheres with iodized oil as a drug carrier have found that drug-loaded microspheres resulted in a higher rate of tumor necrosis and fewer adverse reactions in the short term. However, there is still a lack of evidence regarding the efficacy of those microspheres over the long term. Conventional TACE is still the treatment of choice in treatment guidelines for HCC. A point worth noting is that almost all of the trials on drug-loaded microspheres thus far were not balanced and had too small a sample. Randomized, controlled prospective multicenter clinical studies are needed.

The appearance of drug-loaded microspheres has changed the nature and form of TACE (*7,9*). However, there is disparity in the development and use of those microspheres due to social and economic factors in various countries. The use of DEBs in Europe and the United States differs substantially from that in developing countries. In 2014, only 31% of Asian experts on the EPOIHCC expert committee regularly performed TACE with DEBs (*29*). This suggests that experts need to focus on the characteristics of the beads and procedure as well as conditions in different countries and use of the procedure in combination with other treatments. In other words, TACE with microspheres need to be studied clinically in light of conditions in China and the efficacy of that treatment in treating HCC needs to be compared to conventional TACE with iodized oil as a drug carrier.

Multicenter, randomized, controlled clinical trials with a large sample need to be conducted in order to further evaluate the advantages of TACE with drug-loaded microspheres in comparison to conventional TACE. This is essentially the consensus view of all experts in interventional oncology.

3. Transarterial radioembolization (TARE)

In 1962, Kim, Lafave, and MacLean successfully treated a tumor by local and transarterial injection of colloidal yttrium 90 (Y-90), marking the start of local irradiation to treat tumors (*30,31*). However, limitations

in materials science meant that, the only radioactive microspheres available were made of a colloid or resin since the microspheres could easily enter the blood. Radiation can cause myelosuppression and systemic radiation can cause severe reactions such as pulmonary fibrosis, limiting the development of local radiation to treat tumors.

In 1992, Gray *et al*. (*32*) reported using Y-90 to treat liver cancer. Yan *et al*. reported details on the experimental and clinical use of Y-90 glass microspheres to treat HCC (*33*), creating a new field involving radioactive microspheres. As materials science developed, the clinical use of stable radioactive microspheres has become a focus of attention in the endovascular treatment of liver cancer over the last 10 years.

3.1. *The principles and features of treatment with radioactive microspheres*

Y-90 is a pure beta-ray emitter with a half-life of 64.2 h (2.67 days); its beta particles have a maximum energy of 2.27 MeV (average: 0.937 MeV), a maximum range of 11 mm in soft tissue, and penetrate an average of 2.5 mm (*34,35*). Because of their structure and diameter, radioactive microspheres are primarily used to provide treatment through radiation rather than embolization. This differs from conventional embolization, which uses iodized oil and gelatin sponge particles.

Two types of nuclide microspheres have been approved for use. The first type is the Y-90 glass microsphere produced by the Canadian company Nordion. Marketed under the trade name TheraSphere, these microspheres contain Y-90 and have a diameter from 20 to 300 μm. TheraSphere appeared on the market in 1999 and its use in the palliative treatment of unresectable HCC was approved by the FDA.

The second type of nuclide microsphere is the Y-90 resin microsphere produced by the Australian company Sirtex Medical. Marketed under the trade name Sir-Spheres, these microspheres are coated with a Y-90 resin and have a diameter from 20 to 60 μm. Sir-Spheres appeared in 2002 for use in combination with chemotherapy to treat metastases of colorectal cancer liver. According to existing data, 4 million TheraSphere microspheres are used to deliver a radiation dose of 2,500 bq. Forty million Sir-Spheres microspheres are used to deliver a radiation dose of 50 bq. Since more Sir-Spheres microspheres are administered per dose, they can target a large or extensive lesion, but their administration requires more careful control.

3.2. *Evaluation of the curative effect of TARE and the rounds of treatment required*

Like TACE, the RECIST criteria are being used to evaluate the efficacy of radioactive microspheres in treating HCC, and TARE is reported to have an efficacy of 25-60%. When the EASL guidelines are used, TARE is reported to have an efficacy of 80% (*36,37*). Recent studies have indicated that the mRECIST criteria may be more objective.

Although a change in lesion size may be evident 1 month after TARE, most experts tend to evaluate the efficacy of TARE based on lesion size after 3-4 months and then decide whether a second round of TARE is needed (*35*).

3.3. *Clinical efficacy of TARE*

The characteristics of radioactive microspheres are responsible for the obvious differences between TARE and TACE. A tumor takes time to shrink after radiotherapy, so the maximum tumor shrinkage is generally observed after 3 to 6 months, with a mean time of 6.6 months. Thus, there are differences in the efficacy of treatment with radioactive microspheres. The shrinkage of a tumor is associated with the dose of Y-90, and this also causes differences in efficacy. The absorption of radiation depends on the rays emitted, the mechanics of hepatic arterial blood flow, tumor vascular density, and other factors (*36*).

3.3.1. *TARE as a treatment to downstage early HCC or as a bridging therapy prior to liver transplantation*

Due to the limited source of livers, the effective control of HCC prior to liver transplantation is a key factor affecting the prognosis for the patients with early HCC who are eligible for liver transplantation. Lewandowski *et al*. (*37*) retrospective analyzed 43 patients who underwent TARE and 43 patients who underwent TACE before liver transplantation. HCC was downstaged in 58% of the patients who underwent TARE, and patients had a median survival time of 42 months. These outcomes were markedly better than those for patients who underwent TACE (HCC was downstaged in 31% of patients, and patients had a median survival time of 42 months). Similar studies have found that using Y-90 microspheres can extend the time patients can await liver transplantation compared to patients who do not receive bridging therapy. There is no significant difference in the survival rate of the two groups of patients after liver transplantation.

3.3.2. *TARE as a treatment for unresectable advanced HCC*

Numerous studies have found that interventional therapy plays an important role in the treatment of advanced HCC, and it is the most effective treatment besides surgery. Such therapy can effectively reduce the tumor load, control or decrease the incidence of complications, prolong survival, and improve quality of life. TARE is gradually being used as an emerging

interventional treatment in advanced liver cancer. Research suggests that TARE with Y-90 microspheres can treat advanced liver cancer. Morosi *et al*. (*38*) reported the results of a phase II clinical study involving TARE with Y-90 microspheres. They found that patients had a median survival time of 15 months and a median time to progression of 11 months. A study by Hilgard *et al*. (*39*) found that patients with BCLC stage B HCC who underwent TARE with Y-90 microspheres had a median survival time of 16.4 months. In a prospective study, Salem *et al*. (*40*) analyzed the use of TARE with Y-90 microspheres to treat patients with BCLC stage B liver cancer, and they noted that patients had a median survival time of 17.2 months.

3.3.3. *TARE as a rescue treatment for recurrence after liver resection*

Recurrence after radical resection of liver cancer is one of the important factors affecting the prognosis of liver cancer. Related studies have found that the rate of recurrence within five years is 50-80%. Lau *et al*. (*41*) used Y-90 microspheres to treat 51 patients who were ineligible for resection of HCC and 20 patients in whom HCC recurred after resection. They compared the two groups in terms of the curative effect of treatment and prognosis, and they found that both treatments had a similar curative effect and that none of the patients had serious adverse reactions. These results suggest that TARE can be used as a rescue treatment for recurrent live cancer.

3.3.4. *TARE as a treatment for HCC and portal vein tumor thrombosis*

Literature since 2014 has focused mostly on portal vein tumor thrombosis (PVTT) in patients with HCC, so experts in interventional radiology are eagerly anticipating the use of TARE to treat PVTT (*42-44*). A study of the use of TARE to treat PVTT found that TARE can extend the overall survival time of patients with HCC and PVTT to 10-10.4 months (*40*). Patients with grade A liver function and a tumor thrombus in a branch of the portal vein who underwent TARE had an overall survival time of 16.6 months, but patients with grade B liver function and a tumor thrombus in a branch of the portal vein who underwent TARE had an overall survival of just 4.5 months (*41,42*).

3.4. *Adverse reactions to TARE*

The adverse reactions to radioembolization are relatively mild and include fatigue, mild abdominal pain or discomfort, cachexia, elevated bilirubin, and similar flu-like symptoms, which some experts have termed post-radioembolization syndrome (PRS) (*27,45*). PRS has an incidence of 12% to 54% and resolves spontaneously within ten hours. TARE combines embolization with radiation therapy, so adverse reactions to the treatment are mild. In Europe and the United States, TARE does not require hospitalization but only 1 day of observation. Due to the abnormal distribution of radioactive microspheres, adverse reactions often manifest as radiation injuries in the form of liver damage, pneumonia, and biliary complications. Although these adverse reactions are rare, they may be serious and even require surgical intervention. Lambert *et al*. (*46*) investigated the urinary excretion of Y-90 following treatment. They used a gamma counter to estimate urinary excretion of Y-90 in urine collected for 12 h after injection. Only 0.0025% of the administered Y-90 was excreted in the urine within the first 12 h following injection of TheraSpheres. Four of the patients in that study experienced clinically severe adverse events. One patient developed grade 4 hyperbilirubinemia and ascites and received a liver transplant. Another patient died 58 days after treatment due to spontaneous bacterial peritonitis and subsequent liver failure. Two patients presented with a subacute GI bleeding. Strigari (*47*) reported toxicity related to treatment of HCC with Y-90 SIR spheres. With a median liver dose of 36 Gy (range, 6-78 Gy), liver toxicity that was ≥ grade 2 (G2) was observed in 32% of patients (23/73), liver toxicity that was ≥ grade 3 (G3) was observed in 21% (15/73), and liver toxicity that was ≥ grade 4 (G4) was observed in 11% (8/73). This suggests that TARE still has certain risks. Preoperative assessment needs to be enhanced and modalities involving a multi-disciplinary team (MDT) need to be explored to ensure the safety of treatment.

3.5. *Clinical studies of radioactive microspheres and TARE*

P-32 and Y-90 microspheres are commonly used to perform local radiation and embolization. Radioactive microspheres containing ^{32}p are currently used in China. An emitter of β-rays, ^{32}p has a half-life of 14.28 ± 0.02 days. β particles penetrate an average of 3.2 mm and a maximum of 8 mm, though these figures vary depending on the tissue. The latest nucleotides to be studied are ^{166}Ho and ^{188}Re, both of which emit γ rays. Both have therapeutic value in nuclear imaging to facilitate follow-up after treatment. In the future, these nucleotides may display practical value in clinical settings.

TARE is the latest technique for endovascular treatment of liver cancer. TARE is often combined with drug therapy or other treatments.

PREMIERE (NCT00956930), a large randomized study, is currently underway in the United States (*48*). This study is comparing the value of radioactive microspheres to that of RFA, TACE, or a combination therapy to treat unresectable HCC. The SIRveNIB trial

(NCT01135056) in the Asian Pacific region is directly comparing radioactive microspheres and sorafenib. The SORAMIC trial (NCT01126645) in Europe is evaluating radioactive microspheres in combination with sorafenib and sorafenib alone for treatment of advanced HCC, but the results have yet to be published.

3.6. *Problems with TARE and areas for research*

Overall, studies of radioactive microspheres for treatment of HCC have been retrospective and non-randomized, providing evidence that is grade II-2 or II-3. No studies have provided quality evidence as to whether TARE or TACE is better. In a retrospective study with a large sample, 104 patients with HCC underwent TACE with radioactive microspheres and 100 underwent TACE alone. Patients with Child-Pugh A grade A liver disease who underwent TACE with radioactive microspheres had a median survival time of 22.1 months while patients who underwent TACE alone had a median survival time of 15.6 months (p = 0.24). Patients with Child-Pugh grade B liver disease who underwent TACE with radioactive microspheres had a median survival time of 13.5 months while patients who underwent TACE alone had a median survival time of 12.8 months (p = 0.64). Thus, TARE is comparable to TACE.

This is actually a disadvantage of evaluating TARE. Since there is a lack of quality evidence, TARE does not appear in the guidelines of the American Society of Clinical Oncology (ASCO). However, the European Society of Medical Oncology and the National Comprehensive Cancer Network (NCCN) recommend TARE as complementary treatment for liver metastasis in patients with HCC. Thus, randomized, controlled, multi-center studies need to be performed to study TARE further.

Currently, only two companies offer radioactive microspheres that are approved for clinical use. The cost of treatment per patient is about 50,000 US dollars, or about 300,000 RMB. This imposes a heavy burden on the patient or insurance company in developed European countries despite the fact that there medical insurance systems are better. Therefore, how to benefit more patients in Asian countries such as China, how to optimize treatment, its indications, local production of radioactive microspheres, and the health economics of those treatments all need to be studied further.

4. Interventional therapy for hepatic cancer and PVTT

PVTT results in a poor prognosis for patients with HCC and often indicates advanced liver disease with portal hypertension, acute upper digestive tract bleeding, refractory ascites, and even liver failure. The median survival time without any intervention is about 2-4 months since PVTT can lead to the wide dissemination of tumors throughout the liver and cause a marked deterioration in hepatic function (*49*). Based on the anatomical features of the portal vein in the liver and the way in which a tumor thrombus develops in HCC, PVTT can be classified into four types: Type I, with a tumor thrombus located in or above the segmental branches (secondary branches) of the portal vein; Type II, with a tumor thrombus in the right or left branch of the portal vein (primary branches); Type III, with a thrombus in the main trunk of the portal vein; and Type IV, with a tumor thrombus in the superior mesenteric vein or inferior vena cava. The classification system helps to evaluate the progression of disease, to guide therapy selection, and to improve the survival rate of patients with HCC and PVTT (*50*).

TACE has been the preferred palliative treatment for patients with HCC and type I-II PVTT (*51*), though other treatments (*52-54*) include transhepatic portal vein chemoembolization (PVCE), percutaneous ethanol injection (PEI), microwave coagulation therapy (MCT), radiofrequency ablation (RFA), and 1aser ablation (LA). In addition, radioactive seeds (iodine-125) can be directly implanted into a localized tumor thrombus to improve the local control rate (*55,56*) .

For patients with HCC and type III-IV PVTT, a portal vein stent (PVS) should be placed across stenosis caused by a tumor thrombus in order to reduce portal vein pressure, to alleviate esophageal varices and ascites, to improve portal vein blood supply to normal liver tissue, to prevent liver failure and hepatic encephalopathy, and to reduce the risk of upper gastrointestinal bleeding. The duration of stent placement depends on the control of tumor, and other treatments to eliminate the tumor, such as radiotherapy, brachytherapy, and TACE, should be considered. In a study of 27 patients with HCC and PVTT who underwent PVS and TACE, the median duration of stent patency was 6 months and the survival rates at 3, 6, and 12 months were 51.85%, 29.63%, and 18.52%, respectively (*57*). Recently, Luo *et al*. (*58*) reported on 32 patients with HCC and PVTT who were treated with a stent. 125I seeds were placed in the obstructed main portal vein and patients then underwent TACE. The 90-day, 180-day, and 360-day cumulative survival rates were 96.4%, 67.4%, and 39.3%, respectively, and the cumulative stent patency rates were 96.7%, 83.4%, and 83.4%, respectively.

Thus far, primary HCC and PVTT has been a challenging condition to treat with a poor prognosis. Combinations of multiple interventional techniques, such as RFA + TACE + PVS and TACE + PVS + 125I seeds are being explored, but the long-term efficacy of these combination needs to be studied further. Moreover, the combination of interventional therapy with other treatments such as radiotherapy, molecularly targeted therapy (such as sorafenib), immunotherapy, and other organic combinations also warrant further study (*59*).

5. Interventional treatment of HCC and portal hypertension

About 80% of patients with hepatocellular carcinoma have a history of liver cirrhosis along with portal hypertension. Of these, about 15% to 28% die due to bleeding from esophageal or gastric varices, accounting for the second cause of mortality in HCC. Interventional treatment of portal hypertension seeks to relieve portal pressure and reduce the rate of bleeding. Common treatments are described below.

5.1. *Interventional embolization of varices*

Esophageal and gastric varices are embolized in different ways in order to prevent or stop bleeding. Percutaneous transhepatic variceal embolization (PTVE) achieves the embolization of gastroesophageal varices via percutaneous transhepatic puncture of the intrahepatic branch of the portal vein. PTVE stops active bleeding with an efficacy of 82.2% to 100%, and a better level of liver function results in greater efficacy (*60*). Since PTVE cannot reduce portal pressure, it only reduces the mortality rate of patients with bleeding and it cannot guarantee long-term efficacy. For patients with PVTT or tumor at the puncture site, percutaneous transsplenic variceal embolization (PTSVE) represents a treatment alternative. This procedure is relatively difficult has more complications because of the fragility of the spleen. At present, PTSVE is the only alternative to PTVE. Balloon-occluded retrograde transvenous obliteration (BRTO) seeks to achieve embolization of gastric varices through the left renal vein (or a left gastric vein-inferior vena cava shunt). This procedure can be used in patients with gastric varices and refractory hepatic encephalopathy in conjunction with a left gastric vein-left renal vein shunt or a left gastric vein-inferior vena cava shunt (*61*).

5.2. *Interventional creation of a shunt*

A shunt is created between the portal vein and the inferior vena cava in order to decrease pressure in the portal vein. Transjugular intrahepatic portosystemic shunt (TIPS) has emerged over the past 20 years as an effective and minimally invasive way to treat portal hypertension and its associated complications. There is a dearth of literature on the use of percutaneous portosystemic shunting in patients with hepatic malignancies. Generally, a patient undergoes TACE to shrink the tumor and a shunt is placed such that it traverses the malignancy. According to the MD Anderson Cancer Center TIPS did not increase the risk of bleeding or tumor metastasis even though the shunt traversed the malignancy. However, TIPS had a high incidence of early stenosis or occlusion of the stent that may be due to damage from tumor tissue. A covered stent designed specifically for TIPS would reduce the rate of stenosis, extend long-term patency, and reduce the risk of tumor seeding within the liver, especially when the shunt traverses the malignancy, but the long-term efficacy of this treatment needs to be evaluated further. There is some dispute about whether patients with PVTT should be eligible for TIPS (*62,63*). A direct intrahepatic portacaval shunt (DIPS) is a modified form of TIPS that seeks to create an intrahepatic shunt between the inferior vena cava behind the liver and the portal vein. This technique was initially conceived to increase the duration of shunt patency and to extend the spectrum of patients with portal hypertension who would be eligible for endovascular portocaval shunting. DIPS is a reasonable choice for patients with hepatic veins that are not suitable for TIPS or patients with an occluded shunt after TIPS (*64*).

5.3 *Partial splenic arterial embolization*

Portal hypertension in cirrhosis commonly leads to splenomegaly and is frequently associated with decreased hematologic indices, including thrombocytopenia and anemia. Partial splenic arterial embolization (PSE) is an effective procedure that increases circulating platelet and leukocyte levels and that alleviates hepatic encephalopathy. Some authors set their initial target at embolization of 50-70% of the splenic blood volume. Others, however, embrace a more conservative approach and will target 30-40% of the spleen with the expectations of repeating the embolization with a higher target area (up to 70%) if clinical symptoms do not respond to initial treatment (*65*). However, patients with HCC may have a different degree of symptom improvement after PSE from non-cancer patients since patients with HCC have diminished liver function. The specific causes of and factors influencing these differences need to be studied further.

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Review

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Systemic therapies for hepatocellular carcinoma

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Summary **Hepatocellular carcinoma (HCC) is a common cancer with high incidence and mortality worldwide. The main treatments for HCC include radical hepatectomy, liver transplant, locoregional therapies, and systemic therapies. Systemic treatments include targeted agent treatment, chemotherapies, antiviral therapies, and nutritional treatments. According to the results of SHARP and ORIENTAL study, sorafenib became the standard first-line therapy since 2008 because of nearly three months of survival improvement in patients with advanced HCC. Subsequent studies on targeted agents found that neither sunitinib nor brivanib were superior to sorafenib as first-line therapy. After progression or intolerance of sorafenib, brivanib did not improve the overall survival (OS) compared with placebo as second-line therapy. Randomized controlled EACH study and retrospective AGEO study for systemic chemotherapy showed that oxaliplatin-based or gemcitabine-based regimen was effective for advanced HCC patients. Randomized controlled trial for adjuvant chemotherapy in China showed that capecitabine could reduce the risk of recurrence and improve postoperative survival of HCC. Comparing sorafenib with other treatments, several retrospective studies found that other treatments were not inferior to sorafenib in terms of OS. In the systemic treatment of HCC, antiviral treatment can decrease the recurrence of HBV-related HCC postoperation and prolong the survival of patients. Based on the etiology, symptoms, complications, and treatment-related side effects, nutritional treatment is also very important for HCC patients. Systemic chemotherapy, newer targeted agents, and immune therapy are the new directions in future research.**

Keywords: Hepatocellular carcinoma, systemic therapy, targeted agent, antiviral therapy, nutritional therapy

1. Introduction

Hepatocellular carcinoma (HCC) is common cancer with high incidence and high mortality worldwide, especially in less developed regions. GLOBOCAN showed that the estimated incidence of liver cancer (including cancers from intrahepatic bile ducts) in both sexes was 782,451 and the estimated mortality was 745,533 in 2012 (*http://globocan.iarc.fr/Pages/fact_sheets_cancer.aspx*). Liver cancer is the fifth most common cancer in men and ninth in women. Although it is the seventh most common solid tumor in terms of incidence, liver cancer is the second leading cause of cancer-related death. The main risk factors of HCC include hepatitis virus infection, alcoholic cirrhosis, and non-alcoholicsteatohepatitis (NASH). Without obvious symptoms in its early stage, most of the HCCs are advanced diseases without the opportunity of radical operations upon diagnosis. A percentage of the patients with advanced HCC present abnormal liver functions. With the development of cancer progression, aggravation of liver dysfunction makes systemic drug therapy unavailable. All these factors result in worse prognosis of advanced HCC.

Radical resection or liver transplantation is an important treatment for patients with resectable and transplantable HCC. Meanwhile, locoregional therapy, such as ablation, arterially directed therapies, and

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external-beam radiation therapy, as well as systemic therapy, are available for cases with unresectable HCC or those who are not transplant candidates. Systemic therapy includes targeted agent therapy, chemotherapy, antiviral treatment and nutritional support treatment, and so on. According to the results of Sorafenib Hepatocellular Carcinoma Assessment Randomized Protocol (SHARP), the targeted agent sorafenib has become the standard systemic therapy drug for patients with inoperable HCC (*1*). Systemic chemotherapy has also been considered as palliative treatment for advanced HCC, especially with extrahepatic spread. The response rates of traditional cytotoxic chemotherapy agents, such as adriamycin, fluorouracil, cisplatin, and mitomycin, are less than 10%. The EACH (*2*) and AGEO (*3*) study have shown the effectiveness of oxaliplatin-based or gemcitabine-based regimen in advanced HCC. Randomized controlled studies have also been carried out for adjuvant chemotherapy after radical resection and liver transplantation. Systemic nutrition is also one of the most important palliative treatments for advanced HCC. To date, more studies have focused on systemic therapies for HCC. Thus, the systemic treatments for HCC are reviewed in this study.

2. Targeted agents

In 2008, the SHARP study demonstrated an overall survival (OS) improvement of nearly three months for sorafenib compared with the best supportive care in patients with advanced HCC (*1*). Thereafter, studies on targeted agents for HCC treatments have increased. The ORIENTAL study in Asia-Pacific also obtained similar OS improvement (*4*). To explore more targeted agents for advanced HCC, sunitinib and brivanib have been investigated and compared with sorafenib as first-line therapy in phase III trials. Results showed that sunitinib and brivanib were not superior in terms of OS. Thus, the European Society for Medical Oncology (ESMO) 2012 and the latest National Comprehensive Cancer Network (NCCN) guideline recommended sorafenib as the standard first-line therapy for advanced HCC with liver function of Child-Pugh A (CPA) (*5,6*). The phase III clinical trials on targeted agents are summarized in Table 1.

2.1. *Sorafenib*

Sorafenib is a small molecule tyrosine kinase inhibitor of multitargets, such as VEGFR-1, VEGFR -2, VEGFR -3, PDGFR-β, Raf, RET, and FLT-3 (*7*). Thus, it has the double antitumor effect of antiproliferation and antiangiogenesis. First, sorafenib can inhibit the growth of cancer cells through the RAF/MEK/ERK pathway (*8*). Second, it can inhibit the angiogenesis of the tumor, which leads indirect antitumor effect (*9*). The 2010 ESMO clinical practice guidelines recommended

Table 1. Summary of phase III clinical trials in targeted agents in HCC

Year	Author (*Ref.*)	Phase	Total No. cases	Treatments	ORR	DCR	Median TTP (months)	Median PFS (months)	Median OS (months)
2008	Llovet (*1*)	III	602	Sorafenib *vs.* placebo	2.0% *vs.* 1%	43.0% *vs.* 32%	5.5 *vs.* 2.8	-	10.7 *vs.* 7.9
2009	Cheng (*4*)	III	271	Sorafenib *vs.* placebo	3.3% *vs.* 1.3%	35.3% *vs.* 15.8%	2.8 *vs.* 1.4	-	6.5 *vs.* 4.2
2013	Cheng (*24*)	III	1074	Sunitinib *vs.* sorafenib	-	-	4.1 *vs.* 3.8	3.6 *vs.* 3.0	7.9 *vs.* 10.2
2013	Johnson (*25*)	III	1155	Brivanib *vs.* sorafenib	12% *vs.* 9%	66% *vs.* 65%	4.2 *vs.* 4.1	-	9.5 *vs.* 9.9
2013	Llovet (*26*)	III, second-line	395	Brivanib + BSC *vs.* placebo + BSC	10% *vs.* 2%	61% *vs.* 40%	4.2 *vs.* 2.7	-	9.4 *vs.* 8.2
2014	Kudo (*27*)	III, adjuvant therapy after TACE	502	Brivanib + TACE *vs.* placebo+TACE	48% *vs.* 42%	79% *vs.* 79%	8.4 *vs.* 4.9	-	26.4 *vs.* 26.1

TACE: transarterial chemoembolization; BSC: best supportive care.

sorafenib as the standard first-line therapy option for advanced HCC in grade IA (*10*). Other studies on second-line and adjuvant therapy with sorafenib have also been reported.

2.1.1. *Sorafenib in treatment of advanced HCC*

The first phase III, randomized, placebo-controlled trial is the SHARP study, which involved 602 patients with advanced HCC or progression after surgical or locoregional therapies. All eligible patients were randomly assigned in a 1:1 ratio to receive either 400 mg of sorafenib twice a day or a placebo. The primary endpoints are OS and the time to symptomatic progression. The results showed that the OS of the sorafenib and placebo groups was 10.7 and 7.9 months, respectively ($p < 0.001$). Although the difference in time to symptomatic progression was not statistically significant (4.1 months *vs*. 4.9 months, $p = 0.77$), the time to radiologic progression was obviously longer in the sorafenib group, with 5.5 months (2.8 months in the placebo group; $p < 0.001$). The disease control rate (DCR) was significantly higher in the sorafenib group (43% *vs*. 32%, $p = 0.002$). The common adverse events (AEs) include diarrhea, weight loss, hand-foot skin reaction, and hypophosphatemia. This experiment is the first trial with great significance in proving that systemic therapy is effective in advanced HCC.

The second trial is the ORIENTAL study carried out in 23 sites of the Asia-Pacific region where chronic hepatitis B infection and virus-related HCC was prevailing. The design of the ORIENTAL study was similar to that of the SHARP study, except for the 2:1 ratio. A total of 226 patients were randomized in the study. The results showed that sorafenib treatment could also prolong the OS and time to progression (TTP) of patients in the Asia-Pacific region. The OS of the sorafenib and placebo groups were 6.5 and 4.2 months ($p = 0.014$), respectively. The TTP in the sorafenib and placebo groups were 2.8 and 1.4 months ($p = 0.0005$), respectively. In 2012, a subset analysis of the ORIENTAL study suggested that sorafenib was effective for patients from the Asia-Pacific region with advanced HCC, irrespective of the baseline status (*11*). Comparing the ORIENTAL study with the SHARP study, the OS of the patients significantly varied. The OS of the patients in the Asia-Pacific region was much worse than that in the SHARP study. This difference may be attributed to the following reasons: The patients in the Asia-Pacific region have more Eastern Cooperative Oncology Group Performance Status (ECOG PS) of 2, Barcelona Clinic Liver Cancer (BCLC) stage C, hepatitis B virus infection, tumor burden, and lung metastasis.

Sorafenib is also effective as a second-line therapy. A retrospective study in Korea showed the DCR was 58.3% in the second-line therapy after failure of the

Table 2. Response assessment by modified RECIST (mRECIST) in ESMO 2012 (*5*)

Target lesions	
Complete response (CR)	Disappearance of any intratumoral arterial enhancement in all target lesions
Partial response (PR)	At least a 30% decrease in the sum of the diameters of viable (enhancement in the arterial phase) target lesions, taking the baseline sum of the diameters of target lesions as reference
Stable disease (SD)	Any cases that do not qualify for either PR or PD
Progressive disease (PD)	An increase in at least 20% in the sum of the diameters of viable (enhancement in the arterial phase) target lesions recorded since treatment started
Non-target lesions	
Complete response (CR)	Disappearance of any intratumoral arterial enhancement in all non-target lesions
Stable disease (SD) or incomplete response (IR)	Persistence of intratumoral arterial enhancement in one or more non-target lesions
Progressive disease (PD)	Appearance of one or more new lesions and/ or unequivocal progression of existing non-target lesions
Additional recommendations	
New lesion	A new lesion can be classified as HCC if its longest diameter is at least 1 cm and the enhancement pattern is typical for HCC. A lesion with atypical radiological pattern can be diagnosed as HCC by evidence of at least 1 cm interval growth.
Pleural effusion or ascites	Cytopathological confirmation of the neoplastic nature of any effusion that appears or worsens during treatment is required to declare PD.
Lymph nodes in the porta hepatis	Lymph nodes detected at the porta hepatis can be considered malignant if the lymph-node short axis is at least 2 cm
Portal vein thrombosis	Malignant portal vein thrombosis should be considered as a non-measurable lesion and thus included in the non-target lesion group

first-line chemotherapy with fluorouracil plus cisplatin (*12*). The OS and progression-free survival (PFS) was 7.1 and 2.3 months, respectively. The effectiveness of sorafenib in second-line therapy was not inferior to that of the first-line therapy. Second-line therapy with sorafenib after the systemic chemotherapy did not augment the incidence of AEs. Phase III randomized clinic trials are still needed to confirm the results of this retrospective study involving 24 patients.

2.1.2. *Sorafenib in adjuvant treatment*

In 2014, the American Society of Clinical Oncology (ASCO) presented the results of sorafenib as adjuvant treatment after resection or ablation. Sorafenib as Adjuvant Treatment in the Prevention of Recurrence of Hepatocellular Carcinoma (STORM) is a phase III randomized, double-blind, placebo-controlled trial with 1114 patients. The primary endpoint is the recurrence-free survival (RFS) by independent review. The secondary endpoints included time to recurrence (TTR) and OS. However, the trial did not meet the primary endpoint of the study. No differences in RFS and TTR were observed between the sorafenib and placebo groups, with an RFS of 33.4 and 33.8 months and a TTR of 38.6 and 35.8 months, respectively. The OS was not yet reached (*13*). The results of the STORM study did not meet the primary endpoint either.

2.1.3. *Other questions about sorafenib usage*

The first question is about the safety and effectiveness of sorafenib in advanced patients with worse liver function. The SHARP and ORIENTAL phase III trials did not answer these questions because all patients involved had CPA liver function. All the current data are from retrospective studies with liver function of CPB, data about sorafenib in patients with CPC are limited. A retrospective study observed the effectiveness and safety of sorafenib in 41 advanced HCC with CPA (n = 25) and B (n = 16) liver functions (*14*). The results showed that toxicities led to treatment interruption in 7 patients with CPA and 3 with patients with CPB, as well as dose reduction in 10 patients with CPA and 6 patients with CPB. The incidence of toxicities was not higher in patients with CPB compared with that in patients with CPA. In terms of survival, TTP and OS were better in patients with CPA than those with CPB. TTP was 4 and 2 months (p = 0.0045), while OS was 8.4 and 3.2 months (p = 0.0007) in patients with CPA and CPB, respectively. Another retrospective study by Chiu *et al*. explored the efficacy, tolerability, and survival benefits of sorafenib in 64 patients with CPB liver function (*15*). The patients with CPB were divided into CPB7 (with a CPB score of 7) and CPB8-9 (with a CPB score of 8 and 9) subgroups and compared with those with CPA. The clinical benefit rate and PFS were similar in CPA, CPB7, and CPB8-9. However, the OS of patients with CPB8-9 was much worse because of advanced diseases. The incidence of grade 3/4 hand-foot syndrome, diarrhea, rash, leukopenia, thrombocytopenia, and anemia was similar. However, patients with CPB experienced more anemia, gastrointestinal bleeding, and hepatic encephalopathy partially because more patients had higher total bilirubin and alanine aminotransferase in the CPB subgroup. The third and largest study of the Global Investigation of therapeutic Decisions in hepatocellular carcinoma and its treatment with sorafenib (GIDEON) provided more data about the safety of sorafenib in HCC patients with CPB liver function (*16,17,18*). The GIDEON study is a global, non-interventional, prospective surveillance study with two interim analysis and one final analysis in 2012 and 2013 when approximately 500, 1,500, and 3,200 treated patients were followed up for ≥ 4 months. A total of 3,202 patients were evaluable for safety. In the second interim analysis with 1,571 patients, 61% of the patients had CPA status and 23% had CPB (*17*). In the final analysis, 61.5% had CPA status and 20.8% had CPB (*18*). The GIDEON study showed that the incidence rates of AEs were comparable between the Child-Pugh subgroups at 60% to 70%. Drug related serious AEs were more common in 14.1% of CPB than 8.8% of CPA patients. The Child-Pugh status did not affect the starting dose of sorafenib, and the average of daily dose of sorafenib in patients with CPB was not less than that with CPA. Survival analysis showed that the median OS was longer in patients with CPA at 13.6 months than those with CPB at 5.2 months. In patients with CPB, the median OS was 6.2, 4.8, and 3.7 months in patients with CPB7, CPB8, and CPB9 (*18*). Based on these data, the latest NCCN guideline of 2015 suggested sorafenib should be used with caution for HCC patients with CPB liver function.

The second question is about the safety and effectiveness of sorafenib in older HCC patients. In a retrospective study by Wong *et al*., the patients were divided into older (age ≥ 70 years, n = 35) and younger (age < 70 years, n = 172) groups. The PFS, OS, and Grade 3/4 AEs were similar in the older and younger groups. The median PFS was 2.99 months in the older group, while 3.09 months in younger group (p = 0.275), and the OS was 5.32 months versus 5.16 months (p = 0.310). Grade 3/4 AEs were observed in 68.6% of the older group and 62.7% of the younger group (p = 0.560). However, neutropenia, malaise, and mucositis were more frequent in the older cohort (*19*). The use of sorafenib in older patients was not mentioned in the NCCN or ESMO guidelines, caution should be included when sorafenib is used in older advanced HCC patients.

The third question is how to measure the tumor response of the targeted agents. The response evaluation of targeted therapy in advanced HCC is controversial. The Response Evaluation Criteria in Solid Tumors

(RECIST) is used to measure tumor response based on tumor size changes of target lesions and nontarget lesions. RECIST is an important and valuable method to evaluate the antitumor activity of cytotoxic drugs. In the SHARP and ORIENTAL studies, the evaluation method both applied the RECIST standard. Given that the targeted agents are often used solely in HCC with slow action, RECIST assessment is limited in response evaluation of targeted therapies. In 2010, the modified RECIST assessment (mRECIST) was proposed for response assessment of targeted agents and mentioned in detail in the 2012 ESMO guideline (Figure 1) (*5,20*). The mRECIST assessment is still not used as the standard evaluation method for targeted agents. Further studies are still needed to confirm the accuracy of this method. In some studies, symptoms from targeted agent treatment were reported to be related to antitumor response, such as diarrhea (*21*), hypertension (*14*), early skin toxicity (*22*), and early decrease in AFP (*23*). Given that the symptoms in some extent are subjective, they were not be used as routine assessment of antitumor response.

2.2. *Sunitinib*

Sunitinib is also an oral multitargeted tyrosine kinase inhibitor and effective in HCC. In 2013, an open-label, phase III trial comparing sunitinib and sorafenib was carried out by Cheng *et al*., in Taipei (*24*). A total of 1,074 patients were randomized for the study (530 patients in the sunitinib and 544 patients in the sorafenib groups). The median OS was 7.9 and 10.2 months in the sunitinib and sorafenib groups (p = 0.0014), respectively. The median PFS and TTP were not significantly different in the two groups. In terms of safety, more sAEs were observed in the sunitinib group, especially thrombocytopenia (29.7%) and neutropenia (25.7%). Meanwhile, more hand-foot syndrome (21.2%) was observed in the sorafenib group. The subgroup analysis showed that the median OS was similar in hepatitis B-infected patients in the two groups, but shorter in hepatitis C-infected patients with sunitinib (9.2 *vs*. 17.6 months; p = 0.9835). Sunitinib is significantly inferior to sorafenib in terms of OS. Therefore, sorafenib is still the standard systemic therapy for advanced or inoperable HCC patients.

2.3. *Brivanib*

Brivanib is a selective dual inhibitor of vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF) signaling. It is the third targeted agent that has been proven to be effective in advanced HCC. Most frequent grade 3/4 AEs are hyponatremia, AST elevation, fatigue, hand-foot-skin reaction, and hypertension. Several phase III trials have investigated brivanib for first-line, second-line, and adjuvant therapies for advanced HCC. However, the results showed that brivanib totally failed in advanced HCC. In the first-line therapy, brivanib exhibited similar survival and DCR with sorafenib (*25*). In the second-line therapy, the combination of brivanib with the best supportive care (BSC) was superior to BSC in terms of OS (*26*). As an adjuvant therapy, brivanib did not improve the OS after transarterial chemoembolization (TACE) (*27*).

In first-line therapy of the BRISK-FL study, advanced HCC patients were randomly assigned (1:1) to the sorafenib group (400 mg twice daily, n = 578) and brivanib group (800 mg once daily, n = 577) (*25*). Tumor response was assessed with the mRECIST standard. Results showed that the primary end point of OS noninferiority for brivanib was not met. The median OS was 9.9 and 9.5 months in the sorafenib and brivanib groups (p = 0.3116), respectively. The secondary end points of TTP, ORR, and DCR were also similar between two groups.

In the second-line therapy of the BRISK-PS study, brivanib was used after progression or intolerance to sorafenib in patients with advanced HCC (*26*). A total of 395 patients were randomly assigned (2:1) to brivanib (800 mg orally once daily) or placebo groups. Although TTP and ORR were better in brivanib, the OS was not significantly different between brivanib plus BSC and placebo plus BSC. TTP was 4.2 months in the brivanib group and 2.7 months in the placebo group (p = 0.001). ORR was 10% and 2% in the brivanib and the placebo groups by mRECIST standard. The median OS was 9.4 and 8.2 months in the brivanib and placebo groups (p = 0.3307), respectively. Therefore, patients with advanced HCC after progression or intolerance to sorafenib did not seem to benefit from brivanib in terms of OS.

Adjuvant therapy with brivanib after TACE did not prolong the survival time of the patients in a multinational, randomized, double-blind, placebo-controlled, phase III study. Patients with TACE-eligible HCC were assigned (1:1) to receive either brivanib (800 mg) or placebo orally every day after the first TACE. A total of 870 patients were planned to be randomized. However, the therapy was terminated after randomization of 502 patients (brivanib n = 249; placebo n = 253) when BRISK-FL and BRISK-PS studies failed to meet the OS objectives. The median OS was 19.1 months with brivanib versus 26.1 months with placebo (p = 0.5280). The most frequent grade 3-4 AEs included hyponatremia (18% with brivanib *vs*. 5% with placebo) and hypertension (13% *vs*. 3%). Thus, brivanib did not improve the OS of HCC as adjuvant therapy after TACE (*27*).

3. Chemotherapy drugs

Studies about traditional chemotherapy agents in

advanced HCC, especially after progression or failure of locoregional therapy, are limited because the OS time was short, with low ORR and obvious side effects. Newer chemotherapy agents, such as oxaliplatin, gemcitabine, irinotecan, taxus, and orally administered fluorouracil are widely used in digestive tract cancers to prolong the survival of patients. Oxaliplatin is one of the third generation platinum drugs with higher efficiency and good tolerance. It is also effective in advanced HCC in some phase II studies, and increasingly used in advanced HCC. Capecitabine and S-1 are oral anticancer drugs that are as effective as venous fluoropyrimidine in gastric and colorectal cancers. Gemcitabine is a standard chemotherapy drug for inoperable pancreatic cancer. Meanwhile, liver is tissue homologous with the gallbladder and pancreas. Thus, systemic chemotherapy in advanced or inoperable HCC has drawn lessons from the chemotherapy of other digestive tract cancers. Single agents are often used in patients with high PS score or worse tolerance. Combination of two or more drugs is used in patients with better conditions. Oxaliplatin-based or gemcitabine-based chemotherapy regimens are currently used in advanced HCC.

3.1. *Single-drug regimen*

Single-agent chemotherapy is frequently used in patients postoperation or those with high PS. At present, the investigated newer drugs include gemcitabine, oxaliplatin, capecitabine, and so on. Capecitabine is an orally administered anticancer drug that can be easily accepted by patients. Capecitabine is used in gastric, colorectal, and breast cancers, and has been proven effective by phase III trials. At present, capecitabine is used in advanced HCC, as well as adjuvant therapy postoperation. A retrospective study conducted by Patt *et al*. investigated the anticancer effect of capcitabine on 63 liver patients with 37 HCC, 18 cholangiocarcinoma, and 8 gallbladder cancer (*28*). The ORR of capecitabine in the HCC group was 1%, and one patient obtained radiological complete response; the OS was 10.1 months. The main side effects include hand-foot syndrome with 37% and grade 3 thrombocytopenia with 8%.

A randomized, controlled trial conducted by Xia *et al*. provided evidence on capecitabine in adjuvant chemotherapy after HCC operation (*29*). In two years, 60 postoperative HCC patients were randomized into the capecitabine group (n = 30) or control group (n = 30). The recurrence rate was lower in the capecitabine group (53.3% *vs*. 76.7%). The median TTR in capecitabine was twice that of the control group (40.0 months *vs*. 20.0 months, p = 0.046). The 5-year OS rate was also higher in the capecitabine group (62.5% *vs*. 39.8%, p = 0.216). Adverse reactions, such as nausea, vomiting, diarrhea, and decreased white blood cell and/or platelet counts, were all tolerable. Postoperative adjuvant chemotherapy with capecitabine can reduce the risk of recurrence and tends to improve postoperative survival of HCC.

3.2. *Two-drug regimen*

Combination of two drugs is an often used regimen in chemotherapy. Platinum plus fluoropyrimidine is one of the most frequently used combination regimen. A phase III trial, named EACH study, with systemic chemotherapy was sponsored by Chinese researchers in 2007 (*2*). This study is a multicenter, open-label, randomized trial comparing FOLFOX4 (infusional fluorouracil, leucovorin, and oxaliplatin, n = 184) and doxorubicin (n = 187). A total of 371 patients with advanced or metastatic HCC were included in the study. The primary end point was OS, and response rate was assessed by RECIST. The results showed that FOLFOX4 was superior in terms of PFS, ORR, and DCR. The median PFS was 2.93 months for FOLFOX4 and 1.77 months for doxorubicin (p = 0 .001; HR = 0.62). The ORR was higher in patients with FOLFOX4 than that with doxorubicin (8.15% *vs*. 2.67%, p = 0.02). The DCR was 52.17% and 31.55% (p < 0.001), respectively. Final analysis after 266 events showed that FOLFOX4 had a trend to improve the OS of advanced HCC compared with adriamycin. The median OS of patients with FOLFOX4 or adriamycin were 6.40 and 4.97 months (HR = 0.80; p = 0.07), respectively. Additional analysis was carried out after 305 events had occurred, approximately 7 months after the final analysis. The survival benefit was maintained for FOLFOX, and the median OS was 6.47 months for FOLFOX4 and 4.90 months for DOX (p = 0.04; HR = 0.79). According the results of the EACH study, oxaliplatin-based regimen was approved by the State Food & Drug Administration (SFDA) to be used in locally advanced or metastatic HCC ineligible for curative resection or local treatment. Several factors affect the final results, making it a negative study in terms of OS. The high proportion of hepatitis B virus (HBV) infection (approximately 90%) and Barcelona Clinic Liver Cancer (BCLC) stage C disease (approximately 80%) may result in worse tolerance of the patients. Subsequent therapies, including sorafenib or others, were not mentioned. Given that the EACH study started before the publication of the SHARP study, ADM was chosen as control. The evidence for ADM benefit in advanced HCC was marginal based on the two studies that showed ADM was superior to no antitumor therapy (*30*) or nolatrexed (*31*). An imbalance was noted between the two groups, such as more cycles of prior transarterial chemoembolization (3.46 *vs*. 2.77 cycles) and greater proportion with prior systemic therapy (30% *vs*. 21%). The lack of blinding and imbalance also resulted in more patients withdrawing

after randomization, but before treatment (13patients *vs*. 1 patient) (*32*). As a result, reaching significance in unplanned analysis did not make the EACH study positive. Given that EACH study is a negative study in terms of OS, it was not been recommended as category I evidence in the ESMO and NCCN guidelines.

Gemcitabine with oxaliplatin is another frequently used two-drug combination regimen. A retrospective AGEO study reported in 2011 ASCO meeting shed more light on the systemic treatment of advanced HCC (*3*). In 10 years, the trial involved 204 patients, wherein 38.2% had extra-hepatic metastasis. For liver function assessment, 51.0% of the patients had CPA, 20.6% had CPB, and 4.4% had CPC. The analysis of effectiveness showed that the ORR was 22% and the DCR was 66%. The survival analysis proved that the PFS, TTP, and OS were 4.5, 8, and 11 months, comparable with those of sorafenib. More importantly, the patients with an objective response obtained more than twice of OS than those without an objective response (19.9 months *vs*. 8.5 months). About 8.5% of the patients were eligible for curative-intent therapies. In terms of safety, in a total of 1522 cycles of chemotherapy, grade 3/4 toxicity occurred in 90 patients (44.1%) and 32 patients (16%) discontinued the treatment because of limiting toxicities or patient refusal. The main severe toxicities include thrombocytopenia, 24%; neutropenia, 18.1%; diarrhea, 13.7%, and neurotoxicity, 11.7%.

Based on the results of the EACH and AGEO studies, oxaliplatin- or gemcitabine-based chemotherapy is effective and tolerant in patients with advanced or metastatic HCC. Some phase II trials also investigated the effectiveness of oxaliplatin plus capcitabine (XELOX), cisplatin plus capcitabine, or gemcitabine plus cisplatin (GP). Phase III trials to compare FOLFOX or GEMOX in advanced HCC have not been conducted. Given the limited data, no obvious recommendations for systemic chemotherapy were given in the 2012 ESMO or 2015 ASCO guidelines.

3.3. *Comparison of sorafenib and other treatments*

Sorafenib is a standard therapy for advanced inoperable HCC, but it is expensive, especially for developing countries. To find inexpensive treatments that are not inferior in efficiency, sorafenib was compared with other treatments. Several studies showed that the OS of patients was similar between sorafenib and other treatments. Kim *et al*. investigated sorafenib (n = 123) versus other treatments (TACE, radiotherapy and chemotherapy, n = 253) (*33*), and found no obvious difference in the OS of sorafenib (8.4 months) and the other treatments (8.2 months) (p = 0.601). Prognostic factors include high alpha-fetoprotein, massive/infiltrative intrahepatic tumors, macrovascular invasion, extrahepatic spread, and higher tumor-node-metastasis stage. According to these factors, a subgroup analysis found that patients with extrahepatic spread and massive/infiltrative tumors treated with sorafenib had longer survival time. Meanwhile, other treatments were superior to sorafenib without these prognostic factors. A retrospective study by Pinter *et al*. obtained similar results. The OS was similar in patients with sorafenib (7.4 months, n = 63) and TACE (9.2 months, n = 34) (*34*). In 2011, a single center retrospective study by Lee *et al*. compared the effect of sorafenib (n = 44) and traditional chemotherapy (n = 129) in patients with inoperable HCC (*35*). The OS of patients with sorafenib and chemotherapy were 23 and 43.6 weeks (p = 0.105) and the median PFS was 11.1 and 12.4 weeks (p = 0.496), respectively. The ORR was 2.3% and 6.2% and DCR was 52.3% and 43.4%, respectively. In terms of side effects, grade 3/4 neutropenia and skin toxicity are more common in the chemotherapy and sorafenib groups, respectively. No randomized clinical trials for comparing the targeted agents with other treatments have been conducted. According to the results of the retrospective studies, chemotherapy and other treatments are at least not inferior to sorafenib. Thus, identifying which one could benefit more from targeted agents or other treatments is difficult.

4. Anti-virus therapy

HBV infection is associated with the incidence of HCC and has unfavorable influence on anticancer therapies of HCC (*36,37*). During the course of chemotherapy and other immunosuppressive treatment, HBV will be reactivated in HCC patients with chronic virus carriers. Thus, anti-viral therapy is very important, especially in patients with HCC. Anti-viral therapy can reduce the risk of developing HCC, as well as decrease the risk of HBV reactivation, reduce the recurrence, and improve OS and DFS of HCC patients.

First, antiviral therapy can reduce the risk of developing HCC. Retrospective analysis showed that HBV-infection resulted in 17-fold higher risk of HCC through a follow up time of 8.0 years (*38*). A US study involving 2,671 adult participants with chronic HBV infection (49% Asian) showed that antiviral therapy for chronic HBV can reduce the risk of HCC (*39*). With a median follow up of 5.2 years, 3% developed HCC: 20 among the 820 patients had a history of antiviral therapy and 47 among the 1,851 patients did not undergo antiviral treatment. In propensity-adjusted Cox regression, patients with antiviral therapy had lower risk of HCC (HR = 0.39; $p < 0.001$). When viral loads > 20,000 IU/mL, patents with antiviral treatment had a significantly lower risk of HCC than that without antiviral treatment.

Second, antiviral therapy can reduce the risk of recurrence and improve the survival of HCC patients postoperation, or treatment with sorafenib. Retrospective analysis showed that antiviral therapy improved the DFS

and OS of HBV-related HCC patients after hepatectomy (*40*). In 2015, a Japanese study reported similar results in 162 HBV-related HCC patients (*41*). Several meta-analysis showed that antiviral therapy was associated with reduced risk of recurrence, as well as significant reductions in liver-related overall mortality (*42,43*). In 2014, a meta-analysis including 20 studies with a total of 8,204 participants showed that nucleoside analogs (NAs) antiviral therapy improved the prognosis of HBV-related HCC postoperation. The analysis also found that high viral load was significantly related to the risk of recurrence (RR = 1.85; $p < 0.001$) and poorer OS (RR = 1.47; $p < 0.001$) of HBV-related HCC postoperation. NA antiviral therapy significantly decreased the risk of HCC recurrence (RR = 0.69; $p < 0.001$) and improved both DFS (RR = 0.70; $p < 0.001$) and OS (RR = 0.46; $p < 0.001$) (*44*). In 2015, a randomized controlled trial on antiviral therapy showed that adefovir (10 mg/d) antiviral therapy improved the long-term survival after hepatic resection in patients with HBV-related HCC. The RFS and OS of the antiviral group were significantly better than those of the control group ($p = 0.026$, $p = 0.001$). In the Cox analysis, the antiviral therapy was an independent protective factor of late tumor recurrence (HR = 0.348; $p = 0.002$) (*45*). When combined with sorafenib, antiviral treatment also improved the prognosis of HBV-related HCC patients. A retrospective from China also showed that the antiviral therapy with NAs improved the OS of HBV-related HCC patients treated with sorafenib, especially with higher HBV-DNA level. The OS was 17.47 months and 13.10 months in patients with NA treatment and without antiviral treatment (HR = 0.67; $p = 0.03$) (*46*).

Third, antiviral therapy can reduce the risk of reactivation and liver failure. A retrospective study involving 590 HCC patients who were HBV surface antigen-positive and accepted either surgical resection or TACE showed that the HBV-reactivation rate in TACE treatment was 1.5% with antiviral therapy and 17.5% without anti-HBV therapy. The rate of deterioration of liver function was much lower in the anti-HBV therapy (1.5% *vs*. 8.1%) (*47*). In 2014, a prospective-retrospective study of 404 HBV-related HCC patients with hepatectomy showed that antiviral therapy improved the survival and liver function reserved at the time of recurrence. With a mean follow-up time of 52.4 months, patients in the antiviral group had higher 5-year OS rate (66.7% *vs*. 56.0%, $p = 0.001$). Meanwhile, the 5-year DFS was significant different in the two groups (44.7% *vs*. 38.1%, $p = 0.166$). With disease recurrence, the patients who received antiviral therapy had better liver function reserve, and more patients can receive curative treatment (38.5% *vs*. 24.3%, $p = 0.041$) (*48*).

In 2015, the American Gastroenterological Association (AGA) presented a guideline on the prevention and treatment of HBV reactivation during immunosuppressive drug therapy. Antiviral prophylaxis in hepatitis B surface antigen (HBsAg)-positive or antibody to hepatitis B core antigen (anti-HBc)-positive patients was associated with a reduction of 87% relative risk of reactivation, as well as 84% relative risk of HBV-related hepatitis flared. The HBV reactivation was obviously associated with the types of immunosuppressive drugs, such as B cell-depleting agents, anthracycline derivatives, tumor necrosis factor alpha inhibitors, cytokine or integrin inhibitors, tyrosine kinase inhibitors, and traditional immunosuppressive agents. However, newer anticancer agents have not been mentioned in the guideline. According to the estimated reactivation with available evidence, the drugs are divided into high-, moderate- and low-risk groups. HBV screening (HBsAg and anti-HBc, followed by a sensitive HBV DNA test if positive) is recommended for patients with moderate- or high- risk, who will undergo immunosuppressive drug therapy (Strong recommendation; Moderate-quality evidence). In patients with high risk, such as HBsAg-positive/anti-HBc-positive patients treated with anthracycline derivatives, AGA recommended antiviral treatment for at least 6 months after discontinuation of immunosuppressive therapy (Strong recommendation, Moderate-quality evidence). In patients with moderate risk, such as HBsAg-positive/anti-HBc-positive or HBsAg-negative/anti-HBc-positive patients treated with tyrosine kinase inhibitors, AGA suggested antiviral prophylaxis over monitoring for patients (Weak recommendation; Moderate-quality evidence). In patients with low-risk, AGA did not suggest routine administration of antiviral prophylaxis for patients undergoing immunosuppressive drug therapy (Weak recommendation; Moderate-quality evidence) (*49*).

5. Nutritional therapy

Liver is an important organ for digestion and related to nutrition metabolism absorption and detoxification. Liver cancer affects the nutrition of the patients, especially with other liver illness. The effect of liver cancer on the nutrition of the patients can be divided into etiology, symptoms, complications, and treatments. First, the main etiology of HCC includes viral hepatitis, heavy alcohol intake, nonalcoholic steatohepatitis (NASH), and aflatoxins intake. In hepatitis and NASH, the structures and functions of the liver change, which in turn change the metabolism of foods and energies. Thus, the incidence of malnutrition is high. Second, nontypical symptoms in patients with HCC have unfavorable effect on digestion. Nausea, vomiting, dyspepsia, abdominal distension, and loss of appetite aggravate malnutrition of advanced HCC patients. Third, complications of hypoalbuminemia, portal hypertension, ascites, gastrointestinal hemorrhage, hepatic encephalopathy, and electrolyte disorder in advanced disease also affect the nutrition of advanced

HCC patients. Fourth, anticancer treatment of operation, TACE, targeted agents, and chemotherapy prolong the survival of HCC patients, as well as lead to several side effects. Reduced remnant liver volume, diarrhea of targeted agents, and digestive tract reaction of systemic chemotherapy all result in negative effect to the nutrition of HCC patients. As a result, nutritional therapy is also very important in advanced HCC patients, as well as in postoperation patients.

Based on nutrition screening and assessment, nutrition therapy is administered according to the individual situations of the patients. No guidelines on nutritional treatments of primary HCC have been reported. However, several guidelines have been provided as references: ESPEN guidelines on enteral nutrition: hepatology (*50*), surgery (*51*), and non-surgical oncology (*52*). Detailed recommendations for energy, lipid, and special substance have been provided in the guideline (*52*). Diet and nutrition directions are also provided by the experts (*53*).

6. Conclusion

With the development of systemic therapies in HCC, prognosis in HCC patients has been improved. Given the inadequacy of evidence, more phase III randomized clinical trials are needed to support the utility of systemic chemotherapy. Owing to the development of newer chemotherapy agents and immune therapy, systemic chemotherapy or targeted agents and immune therapy are the future therapeutic directions. China has high HCC prevalence, especially HBV-related advanced HCC. Thus, multicenter, randomized, and controlled clinical trials must be conducted. The EACH study and the capecitabine adjuvant therapy in Shanghai were a good start. Immune regulator thymalfasin had been proven effective by several pilot studies as an adjuvant therapy. A large-scale, multicenter, randomized, controlled study has been planned in China to investigate the effect of thymalfasin (1.6 mg twice a week for 12 months) on the 2-year RFS rate and tumor immune microenvironment (ClinialTrials.gov Identifier: NCT02281266). Results of the proposed study are worth expecting.

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Review

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Immunotherapy for hepatocellular carcinoma

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Summary **Hepatocellular carcinoma (HCC) is the most common type of hepatic malignancies, with poor prognosis. Treatment for HCC are limited, especially for patients with advanced disease who are not eligible for curative hepatectomy or hepatic transplantation. Mechanisms of immune response during tumor development have been investigated for decades. The efficacy and safety of immunotherapy have also been tested in clinical treatment of malignancies. Here we reviewed the immunotherapy strategies for HCC, as well as the particularity of liver immune system and the immune tolerance of HCC. Vaccines, adaptive therapy, immune checkpoint blockades and cytokines are included. We hope this review will give us an integral concept on HCC immunotherapy and help the readers to understand the mechanism of immune tolerance in liver cancer.**

Keywords: Hepatocellular carcinoma, immune tolerance, immunotherapy

1. Introduction

Primary liver cancer is the second leading cause of cancer related death worldwide, with an increasing incidence rate. Asia and Africa have the highest incidence rates of liver cancer all over the world, and China accounts for more than 50% of the whole burden (*1*). Hepatocellular carcinoma (HCC) is the most common type of hepatic malignancies, accounting for approximately 85% of primary liver cancer (*2*). Hepatitis B virus (HBV) or hepatitis C virus (HCV) infection is considered as the main risk factor for HCC, which induces a chronic inflammation microenvironment within infected liver (*3*). Other cause factors, including aflatoxin contact, alcohol consumption, obesity, tobacco abusing, *et al*., are also involved in the carcinogenesis and progression of HCC (*4*).

Although public health measures such as HBV vaccine immunization and health education have resulted in a decrease of HCC incidence (*3*), patients' median survival is approximately 6 to 20 months after establishing of diagnosis. Early detection of the disease makes better outcome for HCC patients. Partial hepatectomy is considered as an optimal treatment for patients with adequate liver function and no evidence of portal hypertension or vascular invasion. For other patients with earlier stage HCC but unfavorable liver function, liver transplantation is also a curative procedure (*5*). However, majority of HCC patients developed advanced-stage disease at first diagnosis. Transcatheter arterial chemoembolization (TACE) and chemotherapy are main options for patients with more advanced disease as palliative procedure, but the efficiency is undesirable (*6*). Additionally, sorafenib is the only multi-kinase inhibitor approved by Food and Drug Administration (FDA) for HCC treatment. In a phase III clinical trial, HCC patients receiving sorafenib had a better overall survival (OS) than patients receiving placebo (10.7 months *vs*. 7.9 months) (*7*).

Different from other organs, liver is considered as a lymphoid organ and chronic inflammation in HBV or HCV infected liver would also promote tumor development. Since new therapy strategy is urgent, immunotherapy has been paid more attention in recent years. We will discuss the complicated immune microenvironment within liver and focus on the current immunotherapy strategies for HCC. We hope this review would give a new horizon on HCC immunotherapy.

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2. The liver as a lymphoid organ

The liver has unique vasculature and distinctive dual blood supply, with large blood flow volume (1.5 L per minute). It receives blood from both the systemic circulation (25%, transmitting oxygen *via* hepatic artery) and the portal vein (75%, draining venous blood from the digestive tract, the pancreas and the spleen) (*8*). Thin wall capillaries formed by fenestrated, basement membrane absent liver sinusoidal endothelial cells (LSECs) separate the bloodstream from the hepatocytes and create a space so called hepatic sinusoid (*9*). Mixed blood from portal vein and hepatic artery imported into the hepatic sinusoid. Under physiological conditions, the liver undertakes multiple tasks, including metabolism, detoxification and immune reaction, within hepatic sinusoid. Myriad antigens and dietary component carried by the venous blood from gastrointestinal tract enter the sinusoid *via* blood vessels of the portal triad. The incomplete sinusoidal wall and low velocity of blood flow facilitate the material exchange and immune reaction.

Classic immune organs, such as spleen, lymph nodes and thymus, are well known since their anatomy and histology have been found to be related to immune function. However, organ like liver whose parenchymal cells may not carry the first physiological task as immunoreactivity still performs potential immunological functions. Hepatocytes are the parenchymal cells of liver cells accounting for 80% of total cells, and the remaining 20% are non-parenchymal cells, including LSECs, hepatic stellate cells (HSCs), Kupffer cells (KCs), dendritic cells (DCs) and lymphocytes. These cells have different functions and differential sources, along with hepatocytes together to regulate local and systemic immune function.

LSECs accounting for 50% of hepatic non-parenchymal cells and constitutively express scavenger receptor and mannose receptor that are responsible for recognition and elimination of pathogens, as well as major histocompatibility complex (MHC) I and MHC II and costimulatory molecule (*e.g.* CD80 and CD86). Pattern recognition receptors (PRRs) such as Toll-like receptors (TLRs) also express on LSECs (*10,11*). Furthermore, LSECs are considered as professional antigen presenting cells (APCs) and responsible for the defense against foreign antigens from dietary (*12*). However, it maintains a capability to induce immune tolerance. Antigen presentation through MHC molecules to T cells results in an upregulation of specific molecules, including the B7 family member programmed death ligand 1 (PD-L1) (*13*). Inhibitory signaling is transmitted by the formation of PD-L1 and its receptor programmed cell death protein 1 (PD-1), inducing T cell tolerance. Another tolerance-inducing mechanism is induction of rapid tolerization of antigen-specific CD8+ T cell. LSECs antigen cross-presentation to CD8+ T cell will induce rapid proliferation but no effector cytokine production (such as interferon-γ, IFN-γ, or interleukin-2, IL-2), which means a cellular cytotoxicity reduction (*14*). Suppressive cytokine interleukin-10 (IL-10) secreted by liver-resident macrophage KCs also can lead to an induction of LSECs antigen presentation capacity (*15*). Since liver is continuously exposed to massive molecule derived from food and commensal gut flora, hepatic immune tolerance is essential to maintain the immune homeostasis within the whole body.

HSCs are cells with an astral phenotype, located between hepatocytes and LESCs. These HSCs comprise 5-8% of total liver cells (*16*). Under normal conditions, HSCs serve as a storage place for vitamin A and lipid. HSCs act as immune cells by expressing antigen presenting associated molecules, similar to LSECs and KCs, including MHC I, MHC II, CD80 and CD86 (*17,18*). In another study, HSCs failed to perform as APCs since expression of key molecules required for antigen presentation were not observed (*19*). HSCs may participate in immune regulation by other manners. Under chronic inflammatory environment, HSCs differentiate to a more active phenotype, which is myofibroblasts and will promote formation of cirrhosis. Activated HSCs express the immunological modulator PD-L1 and can inhibit T cell responses by inducing T cell apoptosis (*20*).

KCs are special macrophages located within liver and the second majority of hepatic non-parenchymal cells (35%). KCs adhere to LSECs and directly capture pathogens from blood stream. To accomplish its mission as macrophage, KCs express immune receptors such as TLRs, scavenger receptors, complement receptors and so on, Activation of these receptors will activate KCs, which stimulates cytokines production, allowing KCs to function as immune sentinel (*21*). Studies have demonstrated that absence of KCs leaded to severe bacterial infection and even host death, indicating that KCs are essential for immunologic defense (*22*). KCs can eliminate pathogens by recruiting neutrophils, which indicates its capability of pathogen clearance and immune cell recruitment. Molecules associated with antigen presentation also express on KCs, such as MHC I, MHC II, as well as costimulatory molecules. Since the particularity of hepatic physiology by the myriad antigens it will encounter, KCs induce immune tolerance under physiological conditions (*23*). Continuous exposure to lipopolysaccharide can inhibit KCs to activate lymphocytes, which also stimulates KCs to release IL-10 (*23,24*). Prostaglandin E2 produced by KCs abrogates activation of antigen-specific CD4+ T cells (*25*). KCs interacting with regulatory T cells (Tregs), increase IL-10 production by Tregs, promote induction of systemic tolerance (*26*).

DCs locate in the portal triad in a high number, surrounding the central vein (*27*). According to their

different surface markers, they can be divided into five subpopulation, with the two main subpopulation myeloid and lymphoid DCs (*28,29*). Liver DCs internalize antigens and present them to regional lymph node to accomplish their tasks as APCs, but unlike DCs from other tissues, liver DCs appear to be poor activator of T cells response (*29,30*). Studies have demonstrated that cytokine milieu within liver (high IL-10 and low IL-12) contribute to the 'immature' status of DCs (*30*). Furthermore, interaction with LSECs and hepatocytes reduces the capacity of DCs to activate T cells, induced by high production of IL-10 by DCs (*31*). DC-derived IL-10 also promotes a shift from Th1-type responses to Th2-type responses, further suppressing cellular immunity and promoting the development of Tregs (*32*).

Stationary hepatic lymphocytes include significant numbers of natural killer (NK), T cells, B cells, and natural killer T (NKT) cells. They together play important roles in detection, elimination and response to potential pathogens. Among these cells, NK cells comprise the majority of total liver-resident lymphocytes (20-30%), while the percentage of NK cells is less than 5% seen peripheral blood. Enriched NK cells perform duties as a critical sentinel by surveillance for infection, killing of infected hepatocytes, or even for malignant transformation cells (*33*). Activated NK cells release cytotoxic granules containing perforin and granzyme in a cell-directed manner, which will kill target cells. NK cells produce a large amount of cytokines (such as IFN-γ) after being stimulated, which also enhance immune response (*34*). The conventional T cells express CD4 or CD8 molecule, along with a diverse type of T cell receptors (TCR) consisted by α and β chain. In the liver, the number of CD8+ T cells is one to two times the number of CD4+ T-cells, while the ratio is reversed in peripheral blood (*35*). The percentage of γδ T cells in the liver lymphocyesis approximately 20%, which is much higher than it in the blood (*36*). However, the role γδ T cells may play in maintaining liver immune homeostasis still remains unknown γδ TCR can bind to ligands in both an MHC-dependent and MHC-independent fashion (*37*). γδ T cells in the liver take part not only in bacterial infection, but also in tumor immunity. The protective role was performed by Vγ4 γδ T cells by IFN-γ and perforin production after activation, while Vγ1 γδ T cells, another principle subpopulation of γδ T cells, play a regulatory role in tumor immunity by IL-4 production (*38,39*). Some T cells do not express CD4 or CD8. These cells are known as "double negative" T cells and found in the liver, expressing αβ or γδ TCR, which may participate in liver autoimmunity (*40,41*). NKT cells are a particular group of T lymphocytes that express both NK and T cell surface markers. They are also enriched and important immunological component in liver. NKT cells express restricted TCR repertoire and recognize lipid presented by CD1 molecule (*42*). Cytokine production of NKT cells is fast and efficient, ensuring NKT cells to complete its task. NKT cells participate in immune procedure in liver injury, inflammation, fibrosis, and regeneration (*43*). IFN-γ and IL-4 are the main cytokine that produced by NKT cell, which involve in regulating innate and adaptive immunity (*42*). NKT cells also have the capacity to patrol the hepatic vasculature and search for pathogens (*44*).

Hepatic immunity is considered to be immunological tolerance rather than immunity. Since liver is continually exposed to abundant antigens and microbes contained in dietary, to maintain the immune homeostasis, complicated immunological tolerogenic activity is required in hepatic environment to not response to harmless molecules. This can not only reduce the rejection rate of allogeneic liver graft, but also weaken the immunosurveillance, which is detrimental in the case of HCC progression.

3. Immune escape mechanism of HCC

HCC has a unique self-protection mechanism to escape from the host's immunosurveillance. Secretion of immunosuppressive cytokines, abnormal expression of antigens and changes in the local immune microenvironment facilitate the HCC cells to avoid from immune attack (*45*). Evidence has also demonstrated that immunosuppressive factors expressed by tumor cells that inhibit APC or T cell function, which suppress the antigen presentation and immune response, facilitate the immune escaping of tumor cells.

Transforming growth factor-beta (TGF-β) is well known as a typical immunosuppressive factor. It has dual function: one is to inhibit tumor proliferation and initiate tumor cell differentiation and apoptosis in the early stage of tumorogenesis, the other is its immune suppressive potential in advanced stage disease. Moreover, TGF-β also has capability of angiogenesis promotion and epithelial-mesenchymal transition (EMT) induction (*46-48*), which facilitates tumor invasion and metastasis. TGF-β1 is a subtype of TGF-β, and a principal isoform in humans, which is considered as a biomarker for the occurrence and development of tumor. TGF-β1 is also a polypeptide cytokine abundant in the liver, with high biological activity. The expression of TGF-β1 are abnormally elevated in liver cancer (*49*), which mainly involves the inhibition of innate immune and stimulation of Tregs generation to destroy the anti-tumor immune response, resulting in progression of malignancies (*50*).

Another immunosuppressive cytokine is IL-10, which belongs to Th2-type cytokines, produced by monocyte-derived macrophages, Tregs and tumor cells. IL-10 plays a variety of ways in immunosuppression, promote tumor cell escape from immunosurveillance. It can activate the naive CD4+ T cells, and inhibit Th1 cells secretion, thereby affecting the maturation and function of Tregs. It

also reduces the expression of MHC II molecule, as well as CD80/86 or other costimulatory molecules on APCs, and decrease the ability of antigen-presenting. IL-10 also indirectly induces cytotoxic T cells (CTL) into anergy state (*51*).

Tumor antigens refers to new antigens occurred in tumor development or antigens abnormally expressed by tumor cells, which can induce anti-tumor immune response. If the difference between antigens expressed by tumor cells and normal proteins is small, or the antigens have low antigenicity, sufficient immune response will not be induced to remove the tumor cells. Alpha fetal protein (AFP) is an antigen associated with HCC, which synthesized by fetal liver and down-regulated for expression after birth. Malignant transformation will activate the expression of associated genes and the synthesis of the protein is restarted, so AFP is often overexpressed in HCC tumor cells. But due to the immune tolerance the system has established in fetal stage, only high level of AFP cannot induce sufficient immune response to kill tumor cells (*52*).

4. Immunotherapeutic strategies for HCC

As mentioned above, the instinct of hepatic immune system and the immune tolerance induced by HCC tumor cells result in disease progression rather than anti-tumor immunity. The targets involved in this procedure provide us an entry point for study of HCC immunotherapy.

Although AFP protein is considered as a tumor associated antigen (TAA) with low immunogenicity and well tolerated by the host immune system, it is the first target investigated in HCC vaccine therapy. Multiple strategies were used to overcome the limitation of AFP to generate sufficient immune response. In the first AFP vaccine clinical trial, 6 HLA-A*0201 HCC patients with elevated serum AFP were immunized with intradermal vaccinations of four AFP peptides (*53*). These peptides were derived from human AFP with HLA-A*0201-restriction and previously found to stimulate specific T cell responses in cultured peripheral blood lymphocytes (*54*). The result showed all of the patients (6/6) generated T cell responses to most or all of the peptides (*53*). In a subsequent phase I/II trial, AFP peptide-pulsed DCs was administrated and transient T cell response was detected in 6/10 HCC patients (*55*). Another TAA used in HCC vaccine study is glypican-3 (GPC3), which is overexpressed in more than 80% of HCC. HLA-A24-restricted $GPC3_{298-306}$ and HLA-A02-restricted $GPC3_{144-152}$ peptides were proven to induce specific CD8+ CTLs in HLA-A02 and HLA-A24 restricted HCC patients, respectively (*56*). Based on these encouraging results, a phase I clinical trial used these two peptides was performed. After GPC3 peptide vaccine administration, GPC3-specific CTL response was able to detected in 30 patients out of 33 patients. Overall survival was positively associated with GPC3-specific CTL response (*57*). Cell-free vaccines based on AFP and GPC3 DNA vaccines were both tested and showed anti-tumor effect and survival improvement in preclinical research (*58,59*). Elevated expression of telomerase was found in HCC, which makes telomerase a possible target for vaccine treatment. In a phase II study of GV1001, low-dose cyclophosphamide and GM-CSF were used, but did not lead to any responses. Additionally, decreasing in the number of CD4+CD25+Foxp3+ Tregs was observed in this trial (*60*). DC vaccines was found to induce antigen-specific CTLs (*61*), activate NK cells and inhibit Tregs in HCC patients (*62*). DCs fused with allogeneic hepatocellular carcinoma cell line HepG2 activated CD4+ and CD8+ T cells, and CTLs induced by the fusion cells were able to kill autologous HCC (*63*). An encouraging outcome was observed in a phase II clinical trial based on DC vaccine. DCs pulsed with autologous tumor lysates were administered. Among 31 treated patients, 4 patients (12.9%) exhibited partial response, 17 patients (54.8%) had stable disease. The overall 1-year survival rate of all 31 patients was 40.1% (*64*). In another phase II clinical trial, DCs pulsed with lysates of HepG2 cell line containing multiple antigens. 25 patients received at least 3 doses. The radiologically determined disease control rate was 28%. However, the survival was not favorable, with median survival of only 168 days (*65*). New vaccine treatment strategies were under investigation. Fusion antigen also performed better immunogenicity. A combination of full-length HBV core protein and melanoma antigen gene-A induced full development of antitumor response against the epitopes (*66*). Moreover, fusion antigen base on heat shock protein 65 containing different epitopes that involve initiating mechanisms in the immune response also acquired anti-tumor response in HCC bearing BALB/c mouse model (*67*). A highly immunogenic AFP created by computer-guided methodical epitope-optimization showed sufficient anti-tumor effects in mouse HCC model by activating CD8+ T cells (*68*). A phase II, open-label, randomized study on JX-594 for advanced HCC showed desirable result (*69*). JX-594 is an artificial genetic recombination vaccinia virus vaccine (*70-72*). JX-594 is designed to induce virus replication-dependent oncolysis and tumor-specific immunity (*73-75*). Low- or high-dose JX-594 was injected into liver tumors for two different groups of advanced HCC patients. JX-594 replication and granulocyte-macrophage colony-stimulating factor (GM-CSF) expression resulted in oncolysis and induction of anti-tumor immunity. Both doses showed tumor shrink in injected and distant non-injected tumors, with mild side effect such as influenza-like symptoms. Median survival was 14.1 months compared to 6.7 months on the high and low dose, respectively (*69*). In addition, a phase IIb trial on JX-594 is now recruiting advanced

HCC patients who failed sorafenib to detect therapy associated OS and recurrence-free survival (RFS) (NCT01387555).

Adoptive cell transfer (ACT) indicates autologous immune cells transfusion, which are extracted from patient's tumor or peripheral blood, then activated and expanded *in vitro*. This strategy is now promising and well developed in the treatment of solid tumors. ACT has showed considerable anti-tumor effects on HCC in several clinical trials.Cytokine-induced killer (CIK) cells are *in vitro* activated autologous and allogeneic T cells, which have acquired non-specific anti-tumor cytotoxicity and CD56 overexpression, and representing a cell population with double T and NK phenotype (*76*). Positive results were reported in studies on CIK adjuvant immunotherapy. A retrospectively study indicated that CIK cell treatment declined recurrence and metastasis in HCC patients after TACE and radiofrequency ablation (RFA) (*77*). In a randomized, controlled trial, postoperative CIK cell therapy was found to reduce the recurrence and metastasis of HCC. However, there was no improvement on OS (*78*). 150 patients who had undergone curative resection of HCC were enrolled in a randomized clinical trial. Among these patients, 76 patients accepted adoptive immunotherapy, and the remaining 74 patients underwent no adjuvant treatment. The median follow-up was 4.4 years. The trial showed that adoptive immunotherapy declined the frequency of tumor recurrence by 18%, with a better recurrence-free survival and disease-specific survival. No difference was observed in OS between treated and untreated groups (*79*). Several other studies demonstrated the same results (*80-82*). Combination with DC vaccine is another considerable strategy. After curative resection, HCC patients were treated with an autologous tumor lysate-pulsed DC vaccine combined activated T cell transfer combination. It was reported that HCC patients benefit from combination therapy. The median RFS and OS were 24.5 months and 97.7 months in the patients receiving combination therapy and 12.6 months and 41.0 months in the group receiving surgery alone (*83*). Other approaches such as NK cells or Chimeric antigen receptor-T cells (CAR-T) is also considered as a potential treatment for solid tumor. NK cells were found involved in the anti-tumor effect in HCC xenograft mouse models (*84,85*). Although CAR-T therapy has been evaluated in the treatment of hematological malignancies such as lymphoid leukemia (*86,87*) and acute myeloid leukemia (*88*), there is rare evidence for the application of CAR-T in HCC immunotherapy. The safety and efficiency of ACT should be considered and tested by further studies. The combination of immunotherapy also provides approach for in the development of new adaptive immune therapies.

With the deepening of the research, inhibitors targeted immune checkpoints promote the development of solid tumor immune therapy. Co-inhibitory signals transduced by PD-1 or CTLA-4 turn down the T-cell activation induced by antigen presentation. Blockage of such signals will gain an increasing in anti-tumor response. Among many investigated immune checkpoints, PD-1, PD-L1 and CTLA-4 molecules have been identified and antibodies against these targets were used in clinical. Ipilimumab (anti-CTLA-4), pembrolizumab and nivolumab (anti-PD-1) have been approved by the FDA for the treatment of melanoma. Tremelimumab is a monoclonal antibody that blocks CTLA-4. A phase II, non-controlled, multicenter clinical trial enrolled 21 patients with HCC and chronic HCV infection. Each patient received 15 mg/kg tremelimumab every 90 days until tumor progression or severe toxicity. Partial response rate was 17.6%, and disease control rate was 76.4%, with median OS of 8.2 months. A good safety profile was recorded. 45% of patients suffered above grade 3 transaminase toxicity after the first tremelimumab dose, which was not observed in the following doses. In most of the patients, tremelimumab induced a progressive decrease in viral load (*89*). Another phase I/II clinical trial is now under way to test the tremelimumab in combination with local therapies such as TACE or RFA (NCT01853618). Anti-PD-1 and anti-PD-L1 antibodies interfere with the signal transduction by the binding of PD-1 and PD-L1, which inhibits T cell activation and cytokine release (*90*). Among PD-1/PD-L1 targeted treatments, nivolumab is fully human IgG4 monoclonal antibody targeting PD-1 receptor. An active phase I dose escalation clinical trial is now recruiting. Safety and preliminary activity of nivolumab on patients with HCC with or without HBV or HCV infection will be detected in this trial (NCT01658878). A new PD-1 blockade pidilizumab (CT-011) was evaluated in a phase I clinical trial (NCT00966251), which unfortunately terminated because of slow accrual without reporting any results. In addition to PD-1 and CTLA-4, other potential checkpoints, like VISTA, OX40, TIM-3, LAG-3 and BTLA were under investigation (*91*). Preclinical studies have indicated anti-tumor activity of LAG3, TIM-3 and NK-inhibitory receptors, although efficacy and safety in HCC patients has not yet been reported (*92,93*). Studies on immune modulatory molecules such as CD244 (2B4), CD137 (4-1BB), and OX-40 are in progress (*94,95*). Immune checkpoint blockade therapy is considered to be a strategy with a bright future. Notably, CLTA-4 immune checkpoint involves in inhibition of antigen presenting procedure carried by DCs, which decreases CD4+ T cell activation to a specific antigen and increases the IL-10 production by DCs (*96*). Thus will strongly downregulate the antigen presenting capability (**97**). We suggest that combination of vaccine and immune checkpoint inhibitor will enhance TAA-specific immune activation.

Cytokine therapy showed mediate response for

treatment of HCC. Interferon (IFN) is used in the treatment of HCC infection and also shows anti-tumor activity. Several randomized clinical trials on IFN have been completed with mixed results. Although HCC patients may benefit from IFN, more attention should be paid on its side effect. Intratumoral application based on adenovirus-based approach may overcome these limitation (*98*). Chemokines are considered to regulate immune cell function by interacting with the receptors on the membrane. Tumor infiltrating immune cells, including T cells, NK cells and NKT cells, showed enhanced expression of certain receptors (***99***). Preclinical studies indicate that overexpression of certain chemokine genes, such as CXCL10 and CCL5 in HCC tissue predicted a better prognosis, which is correlated with CTL and NK cells (*100*). As we have discussed above, TGF-β is an immunosuppressor in HCC progression. There is a new cytokine targeting therapeutic approach, a novel small molecule inhibitor of TGF-b receptor I, LY2157299, is underinvestigation for HCC treatment. 109 HCC patients were enrolled in a phase II clinical trial. Median OS was 36 weeks. Median OS were 93.1 weeks and 29.6 weeks in AFP responders (> 20% decline from baseline) and non AFP responders, respectively. The trial is still active to further investigate the combination with sorafenib (NCT01246986).

5. Conclusion

Preclinical researches and clinical trials offer many opportunities for the development of HCC treatment. Immune therapeutic strategies such as vaccines, immune checkpoint blockade and ACT, have been proved safe and effective. Clinical application of immune checkpoint blockade provides a new version in malignancy immune therapy, which is also important in HCC. Combination of immune checkpoint blockade such as PD-1/CTLA-4 antibody and other immunotherapy approaches will be a trend and acquire excellent clinical benefits. More translational studies and randomized, controlled trials are needed to promote the development of HCC immunotherapy.

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Review

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Stereotactic body radiation therapy: A novel treatment modality for inoperable hepatocellular carcinoma

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Summary **Hepatocellular carcinoma (HCC) is the most common malignancy in the world and the most common cause of cancer-related death. Surgical resection is the standard of care for solitary liver-confined HCC and provides the best long-term survival, however, most HCCs are diagnosed at an intermediate to advanced stage, and few meaningful therapeutic options are available at this point. Stereotactic body radiation therapy (SBRT) is a type of external beam radiation therapy (EBRT) that delivers radiotherapy (RT) accurately and precisely to the tumor, more so than conventionally fractionated RT. Several series report high rates of local control and low incidence of complications in SBRT for inoperable HCC. Herein, we discuss the emerging role of SBRT as well as current indications, implementation, efficacy and toxicities after SBRT. It was noted that SBRT was a safe and effective therapeutic option for HCC lesions unsuitable for standard locoregional therapies, with acceptable local control rates and low treatment-related toxicity. The significant correlation between local control (LC) and higher doses and between LC and overall survival (OS) supports the clinical value of SBRT in these patients.**

Keywords: Stereotactic body radiation therapy, inoperable hepatocellular carcinoma, local control, overall survival, toxicities

1. Introduction

Hepatocellular carcinoma (HCC) is the most common malignancy in the world and the most common cause of cancer-related death. In the USA, the incidence is low compared with that in Eastern Asia because the majority of cases occur due to the increasing prevalence of viral infection (*1,2*). Surgical resection is the standard of care for solitary liver-confined HCC and provides the best long-term survival, as it treats both cancer and the underlying cirrhosis (*3*). However, most HCCs are diagnosed at an intermediate to advanced stage, and few meaningful therapeutic options are available at this point such as transcatheter arterial chemoembolization (*4-6*). Even though progress has been achieved for HCC diagnosis and treatment, the overall 5-year survival rate for all patients with HCC has remained steady at 3% to 5% (*7*). Therefore, it is important to depend upon a palliative treatment option for patients with inoperable HCC. In addition, radiation-induced liver disease (RILD) was the main issue that limited use of RT for HCC treatment, until technological advances provided improvements on the application of radiation therapy (*8*).

Stereotactic body radiation therapy (SBRT) is a type of external beam radiation therapy (EBRT) that delivers radiotherapy (RT) accurately and precisely to the tumor, more so than conventionally fractionated RT. It can be delivered either using a traditional linear accelerator or using a robotic arm (*i.e.* CyberKnife). Currently, the role of SBRT is not defined in the American Association for the Study of Liver Diseases (AASLD), the European Association for the Study of the Liver and the European Organization for Research and Treatment of Cancer (EASL-EORTC), and National Comprehensive Cancer Network (NCCN) guidelines for HCC treatment, while

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several series report high rates of local control and low incidence of complications in SBRT for inoperable HCC (*9-33*). Herein, we discuss the emerging role of SBRT as well as current indications, implementation, outcomes and toxicities after SBRT.

2. The indications of SBRT for inoperable HCC

Although the indications of SBRT for inoperable HCC have evolved, the role of SBRT in inoperable HCC is less clear. Currently, certain requirements and restrictions for patients with inoperable HCC who receive SBRT are as follows: (*i*) the number of tumor lesions (typically ≤ 3); (*ii*) the tumor size (the longest individual tumor diameter was less than 6 cm); (*iii*) no extrahepatic metastases, and (*iv*) Child-Pugh score A or B, *etc*. In addition, a number of other requirements and restrictions to assess the patient situation including a Karnofsky performance score ≥ 70; patient's life expectancy was more than 3 months; serum liver enzymes concentration was twice less than the upper limit of the normal range (*34-36*). Therefore, careful patient selection is required and SBRT should be considered only after thorough discussion within a multi-disciplinary team, with all legitimate treatment options also considered.

3. The implementation of SBRT for inoperable HCC

SBRT needs the image-guided radiation treatment planning system to ensure accurate implementation of radiation, and it can be delivered either using a traditional linear accelerator or using a robotic arm (*i.e*. Cyber-Knife). Except for computed tomography (CT), the role of magnetic resonance imaging (MRI) and positron emission tomography/computed tomography (PET/CT) images in SBRT were paid more and more attention because they can clearly display the actual tumor boundaries, and distinguish between edema, tumor and normal liver tissue, as well as help radiation oncologist to seek tumor target. The implementation of SBRT for inoperable HCC included the tumor target confirmation, the prescription dose and fractionation, normal tissues constraints confirmation, and quality control of SBRT.

3.1. *The tumor target confirmation of SBRT*

The gross target volume (GTV) of inoperable HCC was defined by most radiation oncologists as the visible gross tumor from imaging such as CT, MRI, PET-CT, or the combination. The planning target volume (PTV) was defined as the GTV with some margins in the x, y, and z-axis direction. Theoretically, the PTV was affected by tumor size, location of tumor lesion, the respiratory motion and setup errors, *etc*. The PTV was also amended according to adjacent organs at risk (*e.g*. duodenum, stomach, and small intestine bowels, *etc*.). In addition, the rapid fall off radiation dose outside the GTV has met the requirements of CTV because of the high SBRT fractionated dose, so the vast majority of studies have adopted that GTV with a margin to generated PTV.

3.2. *The prescribed dose and fractionation of SBRT*

There are wide variations in dose prescription and fractionation across published series even with the limited number working on the same protocol. The dose is prescribed in nearly all cases to the 80% isodose line covering the PTV (*34-36*). The dose per fraction and total dose were determined using the dose-volume histogram and organs at risks (OARs) specific report. The normal liver was defined as the volume of liver not included in the PTV (total liver volume minus PTV) and the dose constraints protocol for normal liver and to the OARs should respect the described constraints. In addition, overwhelming evidence confirmed that the prescribed dose and fractionation were specified according to tumor size, location of tumor lesion, the therapeutic purpose, and patient status, *etc*.

3.3. *The normal tissue constraints of SBRT*

Tolerance of the liver to SBRT derived from experimental models using conventional fractionation schemes and the linear-quadratic model has been well documented. The major dose-limiting concern in the use of SBRT for liver tumors is the risk of radiation-induced liver disease (RILD). The risk is generally proportional to the mean dose of radiation delivered to normal liver tissue because the liver obeys the parallel architecture model of radiobiology.

Although the liver dose limits currently vary, it was agreed that the need to ensure a certain volume of normal liver from the high doses of radiation. Each regimen provided a constraint to roughly one third of normal liver tissue and across all studies, threshold doses ranged from 7 to 21 Gray. Among them, it was generally acknowledged that a critical volume constraint of 700 mL of normal liver should not receive more than 15 Gray in 3 fractions, assuming that the liver volume was at least 2,000 cm^3 (*29,34*). The dose-volume planning objectives for other OARs were defined as follows: stomach, small intestine, maximal dose ≤ 21 Gray in 3 fractions; bilateral kidney, mean dose ≤ 21 Gray in 3 fractions; and spinal cord, ≤ 21 Gray in 3 fractions (*21,27*).

3.4. *The quality control of SBRT*

Considering that high doses are delivered in a few numbers of fractions, the movements of the liver during the treatment have to be taken into consideration

(*32,35*). The need for accurate repositioning from simulation to treatment and rigorous compensation for organ motion require control devices such as abdominal compression or breath-hold maneuvers to maintain the tumor in a reproducible stage of the respiratory cycle. The radiation ray periodically switched when patients received respiration and breathing control devices, and it was noted that it can effectively reduce the normal tissue radiation dose around the tumor. In addition, daily image guidance using on-board cone-beam computed-tomography (CBCT) imaging is mandatory to delocalize the target before each treatment delivery (*37*). Advanced techniques allow controlling the positioning of the fiducial markers during the irradiation with on-line verification of the positioning of the target.

4. The efficacy of SBRT for inoperable HCC

The treatment efficacy of inoperable HCC is undoubtedly the focus of radiation oncologists and clinical researchers. Currently, SBRT is an effective modality with good LC and acceptable toxicity for inoperable HCC. Further studies in more favorable patients and a longer follow-up period should further elucidate the dose-response relationship, the potential late toxicity profile, and the chances of long-term survival after SBRT. The updated results from the most important series are reported in Table 1.

4.1. *Local control rate*

At present, most studies show 1-year and 2-year LC rates of inoperable HCC treated with SBRT was about 72-89.8% and 64% in the best cases, respectively. In general, fixed doses of 40-60 Gray/3-5 fractions are employed for relatively small tumors with a median diameter of approximately 3 cm. In contrast, modified doses are employed for relatively larger targets according to normal liver tolerance depending on tumor size and normal liver volume (Table 1). Current evidence shows that many important factors affecting LC rate include total dose and per fractionation, BED, and tumor size, *etc*.

Several studies demonstrated that a dose-response relationship seems to be associated with local control. In the setting of HCC, Andolino *et al*. (*22*) compared their results (with a median total dose between 40 and 44 Gray) with those reported by Tse *et al*. (median dose 36 Gray) (*38*). The former reported a local control rate of 90% at 2 years, while the latter reported a local control of 65% at 1 year. The most likely explanation could be a higher median dose per fraction and a lower median tumor volume. Similarly, a Korean series of 108 patients suffering from inoperable HCC treated with an escalated dose from 33 Gray in 3 fractions to 60 Gray in 3 fractions demonstrated the role of the dose in a multivariate analysis for LC rate (*15*). Based on a tumor control probability model, the dose of 54.8 Gray is associated with 90% probability of local control at 2 years. However, in a study of 185 patients with HCC (median diameter, 27 mm) treated with SBRT of 35 Gray or 40 Gray in 5 fractions, both local control (91% and 89%, respectively; $p = 0.99$) rates were equivalent between the two dose groups (*11*). The reason for these discrepancies may in part be attributed to the histoloGray, patient selection, and other treatments used. In any case, the vast majority of studies have shown that a higher total dose of SBRT should be set if patients' general condition permitted and the surrounding normal tissues could be tolerated.

Scorsetti *et al*. (*9*) also demonstrated that a dose-response relationship between BED and LC in inoperable HCC and a higher more intense BED and dose contribute to higher LC. They conducted prospective clinical trial in 43 inoperable HCC patients with treatment pattern of 48-75 Gray/3f and 36-60 Gray/6f and the results showed actuarial LC rate at 6, 12, 24 months with BED > 100 Gray were much higher than that with BED < 100 Gray. So they preliminarily thought there would be a certain relationship between BED and LC rate. Though there is no approved definite total dose and fractionation pattern, most researchers thought that SBRT could cure tumors with BED > 100 Gray.

In addition, Mendez *et al*. found (*29*) that using doses ranging from 25 Gray (tumor size at least 4 cm) to 37.5 Gray (tumor larger than 4 cm) in 3 fractions, the 1-year and 2-year local control were 94% and 82%, respectively. Concurrent with the above study, Scorsetti *et al*. (*9*) conducted prospective clinical trials in 43 inoperable HCC patients with a treatment pattern of 48-75 Gray/3f and 36-60Gray/6f and the results showed actuarial LC rate with GTV < 5 cm was much higher than that with GTV ≥ 5 cm. So these preliminary outcomes demonstrated that there would be a certain relationship between tumor size and prescription dose/fractionation, thus affecting the LC rate. Other factors may also affect treatment outcomes including primary tumor histological type, progression free survival, and number of lesions. For example, our previous polled analyses showed that SBRT combined with TACE significantly improved local control rate (*39*).

4.2. *Overall survival*

There existed apparent differences in overall survival of inoperable HCC patients for influencing factors such as dose and fractionation pattern. Sanuki *et al*. (*34*). summarized that currently for inoperable HCC patients 1-year OS and 2-year OS were 21-69% and 30-38% after SBRT, respectively.

Bujold *et al*. (*13*) conducted phase I and II combined clinical trials in 102 inoperable HCC patients with a treatment pattern of 24-54 Gray/6f and the results showed the median follow-up time and the median

Table 1. Stereotactic body radiation therapy for inoperable HCC (sample ≥ 38)

Ref.	Type	Patient/ lesion number	Child-Pugh A/B/C number	Median volume (mL)	Median size (cm)	Median dose/fraction, Gy	Median follow-up (mo)	LC	OS	Toxicity ≥ G3 (%)
Bujold *et al*. (*13*)	Pro LA	102/-	A and B; 35/ C; 67	117	7.2	24-54/6	31.0	87% (1-yr)	Median 17.0 months	26.5
Kang *et al*. (*19*)	Pro CK	47/56	41/6/0	29	-	57/3	17	94.6% (2 yr)	68.7% (2 yr)	10.7
Scorsetti *et al*. (*9*)	Pro LA	43/63	A/B; all patients	-	4.8	48-75/3 36-60/6	8.0	85.5% (1 yr) 64.4% (2 yr)	77.9% (1 yr); 45.3% (2 yr)	16
Seo *et al*. (*23*)	Pro CK	38/-	34/4/0	40.5	-	33-57/3-4	15	78.5% (1 yr) 66.4% (2 yr)	68.4% (1 yr) 61.4% (2 yr) 42.1% (3 yr)	2.7
Sanuki *et al*. (*11*)	Retro LA	185/185	158/27/0	8	-	CPA:40/5 CPB:35/5	24	99% (1 yr) 93% (2 yr) 91% (3 yr)	95% (1 yr) 83% (2 yr) 70% (3 yr)	13.0
Jang *et al*. (*15*)	Retro LA	82/95	74/8/0	-	3	51/3	30	87% (2 yr) 82% (5 yr)	63% (2 yr) 39% (5 yr)	7
Yamashita *et al*. (*10*)	Retro LA	79/-	67/9/1/2	-	-	48/4	21.0	-	52.9% (2 yr)	4.6
Huertsa *et al*. (*33*)	Retro CK	77/97	35/6/36	11.7	2.4	45/3	12.0	99% (2 yr)	81.8% (1 yr); 56.6% (2 yr)	5.2
Andolino *et al*. (*22*)	Retro LA	60/71	36/24/0	29	3.2	CPA:44/3 CPB:40/5	27	90% (2 yr)	67% (2 yr)	0
Kwon *et al*. (*24*)	Retro CK	42/-	38/4/0	15.4 cc	-	30-39/3	28.7	72% (1 yr) 67.5% (3 yr)	92.9% (1 yr); 58.6% (3 yr)	2.3
Xi *et al*. (*14*)	Retro LA	41/-	-	65	-	36/6	10.0	-	50.3% (1 yr)	2.4

Abbreviations: Retro, Retrospective; Pro, Prospective; CK, CyberKnife; LA, linear accelerator; Gy, Gray; LC, Local control; OS, Overall survival; Yr, Year.

survival time were 31.0 and 17.0 months, respectively. Similar to above results, Andolino *et al*. (*22*) conducted a prospective phase I/II clinical trial study with total dose range from 24-48 Gray in 6-16 fractions showing that 2-year OS rates were 67%. Until now, the best LC rate provided by a large sample study, and 3-year LC rate was up to 91% when the patients received SBRT of 35 Gray/5 fractions (*11*).

Similarly, a Scorsetti *et al*. (*9*) study also demonstrated that there was a significant correlation between OS and BED as well as tumor size, with a median OS of 27 months in patients treated with BED > 100 Gray versus 8.1 months in those patients treated with BED < 100 Gray ($p < 0.05$). In addition, OS decreased significantly in the subgroup of patients with cumulative GTV > 5 cm (1-year OS rate of 48%), while patients with GTV < 5cm presented a 1-year OS rate of 85% ($p = 0.046$). Furthermore, several studies were consistent with the results of Scorsetti *et al*., which showed that tumor size was an independent prognostic factor for OS of patients (*15,18*). These results support the use of ablative dose in the treatment of inoperable HCC, not only to increase the local response, but also to improve the prognosis of these patient populations, even if there is no candidate for effective alternative care.

A number of studies have demonstrated that the primary histological type, progression free survival, number of lesions, tumor size and systematic treatment except for total radiation dose and fractionation pattern also affected the OS of patients with inoperable HCC receiving SBRT. Therefore, we expect to have more meticulous and comprehensive studies to further understand and correctly evaluate the curative effect of SBRT for inoperable HCC patients.

4.3. *SBRT for inoperable BCLC-C stage HCC*

There are recently published reports of various treatment modalities for BCLC-C stage. The median survival time of BCLC-C stage was 2-28 months. One-year and 3-year OS rates were 6-70% and 1-41%, respectively (*40-45*). Although the best treatment outcome was associated with surgery, however, surgery is indicated in highly selected patients among the BCLC-C stage.

Culleton *et al*. (*46*) conducted pooled analysis of prospective (14/29, 48.28%) and retrospective (15/29, 51.72%) clinical study in 29 patients, and most of them were BCLC-C stage inoperable HCC (CP class B; 28 and CP class C; 1). The median dose was 30 Gray in 6 fractions, and the median OS and the 1-year survival rate were 7.9 months and 32.3%, respectively. There was no significant difference in OS between prospective and retrospective groups of patients ($p = 0.308$). Though Bae *et al*. (*47*) treated 35 inoperable BCLC-C stage HCC patients (CP class A; 32 and CP class B; 3) with a totally different fractionation pattern (30-60 Gray/3-5fractions), they obtained better OS rates with 52% for 1-year and 21% for 2-years, respectively. The reason they analyzed was that patients with CP class A were the best candidates and at least SBRT dose of BED > 80Gray was required for BCLC-C stage. It is clear that SBRT would be considered a treatment option for BCLC-C stage, especially in Asian countries. We suggest that CP class A is the best candidate for SBRT in patients with BCLC-C stage. In addition, SABR dose of at least BED > 80 Gray would be required to achieve a considerable treatment outcome.

4.4. *SBRT successful bridge to transplantation for unresctable HCC*

Importantly and interestingly, there is always a waiting period between listing and transplantation, and this varies among institutions. Because of prolonged wait times on transplantation lists, the incidence of disease progression while listed for organ transplantation ranges from 10% to 23%. Many therapies have been used as a "bridge" to transplantation, and SBRT has also been evaluated as a means to bridge to transplantation. As a bridging therapy, SBRT has been reported to be feasible and well tolerated (*48-50*). Therefore, future studies should focus not only on maximizing efficacy, but also on determining how SBRT should be used in the context of other previously established therapies.

4.5. *SBRT combination with TACE for inoperable HCC*

Numerous clinical studies of TACE plus SBRT for patients with inoperable HCC have emerged recently. Among these trials, two strategies of combining SRT with TACE have been studied. The most common approaches included the use of SBRT follow by TACE procedures and TACE procedure follow by SBRT. The first involves using RT to treat portal vein and inferior vena cava tumor thrombus to complement TACE. The rationale for this approach is that TACE is less effective in patients with portal vein tumor thrombus, and RT may make TACE more effective if portal vein disease can be eradicated. A second approach is to deliver RT as a "consolidation" planned procedure to target residual hepatic tumor after TACE. The rational for this approach is that RT targets cancer cells at the tumors periphery that may remain viable through blood supply from collateral circulation or recanalization of the embolized artery (*51*). The third approach, tumor shrinkage after TACE allows the use of smaller irradiation fields, which permits higher tumor doses and improves normal liver tolerance (*52*). Furthermore, the TACE anticancer drugs retained in the tumor may have a radiosensitizing effect (*53,54*). Hence, we asserted that the combination of TACE with RT may remedy the limitation of each alone and have synergistic effects.

Although considerable evidence indicates that TACE plus SBRT is highly beneficial for treating

patients with UHCC. It is still unclear whether the existing evidence is scientifically rigorous enough to recommend its routine use to palliative treatment of UHCC. Hence, the methodological quality of clinical trials with TACE plus SBRT for inoperable HCC needs improvement in accordance with the Consolidated Standards of Reporting Trials statement (CONSORT). In particular, rigorously designed, multi-center, large, randomized, double-blind, controlled trials are required.

5. The toxicities of SBRT for inoperable HCC

The SBRT for inoperable HCC patients was considered to potentially cause risk of RILD. Therefore, how to avoid and predict the occurrence of RILD has became another key of inoperable HCC using SBRT. Some reviews summarized many studies showing normal liver dose was the important factor to predict the occurrence of RILD (*22-24*). When enough normal liver could avoid irradiation, the highest prescription dose of liver lesion was even up, and in those circumstances the non-irradiated normal liver tissue could maintain function. It was noted that average liver irradiation dose and normal liver volume after SBRT had a close relationship with adverse events, so those limits should be paid attention to when formulating a radiotherapy plan, especially for patients who had small normal liver volume (< 1000 mL) before SBRT.

The toxicities were mild (CTCAE Grade 1-2), with most patients experiencing constitutional symptoms, elevated liver enzyme, and leucopenia, *etc*. These symptoms were transient and resolved with conservative management. It has been reported that adverse events were relatively rarely observed in surrounding liver tissue, particularly in gastrointestinal tissue, but patients had the lesion in close proximity to the gstrointestinal tract and relatively high doses were delivered to the gastrointestinal tract who may experience Grade 3 and 4 gastrointestinal toxicity (*15*). For example, Tse *et al*. (*38*) reported several Grade 3 and 4 gastrointestinal complications after escalating the SBRT dose for inoperable HCC. Among these patients with gastrointestinal complication, one patient appearing with duodenal ulcer at the distal stomach and proximal duodenum received 20 Gray/4 fraction irradiation. Therefore, dose-volume constraints for OARs around the liver are strict especially in stomach and duodenum. Currently, the case of biliary stricture after SBRT has not been reported, nonetheless considering the hypofractionated dose compared to conventional radiotherapy are more likely to lead to biliary fibrosis narrow complications. Hence, radiation oncologists should place more emphasis on developing the treatment plan when GTV is close to the bile duct.

Above evidence suggests that we should pay close attention to the irradiation sensitive OARs near the target area in the implementation of SBRT. Meanwhile, longer follow-up is needed to assess the late adverse events of varieties of SBRT doses and fractionated regimens, to provide reliable evidence for improving efficacy and decreasing normal tissue adverse events.

6. Conclusion

The role of SBRT for inoperable HCC has evolved over the years. The technological advances that provided the means to deliver a tumoradical dose to liver lesions while sparing the surrounding normal parenchyma have given new insight into the treatment options for inoperable HCC. The published results of SBRT for inoperable HCC are encouraging; however, the optimal dose, target, and fractionated regimen now remain inconclusive. Combined with the above evidence, the higher dose rate was associated with better OS and LC rate, we recommend the prescription BED dose at least > 100 Gray.

Fortunately, clinical investigators should pay more attention to how to accurately target the tumor lesion and real time monitor the tumor movement, and thus maximize protection of the surrounding normal tissue except for prescribing sufficient doses into tumor lesions. With the extended follow-up time, a considerable number of patients with out-field failure after SBRT, therefore, the multimodality therapy of SBRT, chemotherapy, and targeted therapy may be the future of treatment strategies for these patients. In conclusion, we have successfully moved from the role of SBRT for inoperable HCC to a new era of radiotherapy given as an effective treatment for patients not suitable for other therapeutic approaches. Currently, two Korean Phase II prospective studies have been opened for evaluating SBRT for inoperable HCC (ClinicalTrials.gov IDs: NCT01165346 and NCT01910909, respectively) to determine the optimal fractionation modality.

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