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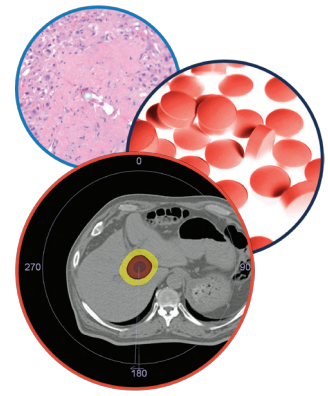

When to consider liver transplantation in hepatocellular carcinoma patients?

Hepatic Oncology

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Practice points

- Liver resection is an effective treatment option associated with a 5-year survival of 70%, yet, the recurrence rate is also high.
- Liver transplantation (LT) is regarded as the best treatment for cirrhotic patients with hepatocellular carcinoma (HCC).
- Tumor biology is not completely reflected by the tumor size and number as depicted by Milan Criteria, extended criteria allows larger HCC which have good tumor biology to be transplanted.
- New scoring systems and prognostic markers help in the process of patient selection, and post-transplant surveillance planning.
- Down-staging therapy serves to provide a last hope of LT for HCC beyond criteria; patients transplanted after the 6-month waiting period should have the similar post-transplant outcome when compared with those who are transplanted within criteria.
- Bridging therapy halts tumor progression and helps to reduce dropout rate of the wait-listed HCC patients.
- Development of nontransplantable HCC recurrence remains the Achilles heel of the salvage transplantation policy, close posthepatectomy surveillance is therefore warranted.
- There is no oncological difference between living donor liver transplantation and deceased donor liver transplantation as a treatment for HCC, while the former approach negates the concern of graft allocation.
- Post-transplant HCC recurrence is associated with dismal prognosis, though long-term survival is still possible for selected patients with graft hepatectomy and pulmonary metastatectomy.
- Apart from maintaining good long-term graft survival, modern immunosuppression is associated improved side-effect profile, and additional antitumor effects.

Orthotopic liver transplantation (LT) has been regarded as the best cure among the three curative treatment modalities. However, when to consider LT in hepatocellular carcinoma (HCC) patients remains a complicated clinical question. In this article, we will look into the recent updates in the context of LT for HCC, including the timing of orthotopic LT (primary or salvage LT), patient selection criteria, newer prognostic markers and scoring systems, down-staging and bridging therapy, salvage LT and treatment option of post-LT HCC recurrence. Evolution of immunosuppressive therapy and future development of the LT for HCC will also be discussed.

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Hepatocellular carcinoma (HCC) is the fifth most common cancer and third most common cancer-related mortality worldwide, leading to over one million deaths every year [1,2]. Majority of the HCC arise from a cirrhotic liver, and due to the presence of impaired liver function, curative therapies such as partial hepatectomy and tumor ablation are not suitable options. Orthotopic liver transplantation (OLT) was thought to be an ideal treatment for cirrhotic patients with HCC, since it removes the tumor with the largest possible margin and replaces it with a noncirrhotic liver. However, initial experience of OLT for HCC was disappointing. In the 1980s, HCC patients treated by OLT had high early recurrence rate [3] and poor long-term outcome of 5-year survival less than 50% [4,5]. The poor treatment outcome was attributed to suboptimal patient selection. Since the landmark paper published by Mazzaferro *et al.* in 1996, a clear cut patient selection criteria according to tumor number and size had been adopted by most transplant centers, and the long-term survival of over 80% was consistently achieved [6]. Nonetheless, the number of HCC patients who fulfill the stringent criteria and benefit from OLT remains a minority group. Researchers have been focusing on how to extend the benefit of OLT to more HCC patients without jeopardizing oncological outcomes. In this article, we will explore the world trend of HCC patient selection for OLT. Issue about primary liver transplantation (LT) versus salvage LT, extended criteria and various scoring system for patient selection, downstaging therapy, deceased and living donor liver transplantation (DDLT vs LDLT) for HCC will be covered.

Liver resection for HCC

Patients with preserved liver function and small HCC are often not necessary for LT. Such lesion can be treated by percutaneous radiofrequency ablation (RFA) [7] and liver resection [8] with 5-year survival of over 60 and 70%, respectively [7,8]. Unfortunately, vast majority of the HCC are not suitable for ablation due to a number of size and geographic limitations. Given the fact that most HCC are developed in the background of liver cirrhosis [9], posthepatectomy hepatic insufficency remains a concern particularly following major resection. On the other hand, around 70–80% of the cirrhotic patients are expected to recur after hepatectomy as a result intrahepatic tumor spread or *de novo* development of HCC [8,10–12]. This suboptimal oncological outcome urges clinician to rethink the best approach in the management of HCC.

LT for HCC

LT has been indicated for liver transplantation since its inception back in 1963 [13,14]. By removing HCC with greatest possible margin and replacing the cirrhotic liver with a normal healthy organ, a 5-year survival of as high as 90% has been reported in selected series [15], and was, thus, regarded as the best treatment for HCC. Unlike hepatectomy for HCC, transplantation is an ultramajor operative procedure which often challenges the physiological reserve of the patients. Cardiovascular complications during the course of LT is a major cause of non-graft-related mortality, making a thorough pre-transplant cardiac/anaesthetic assessment mandatory [16]. Patients with significant ischemic heart disease, impaired ejection fraction or portopulmonary hypertension are not suitable transplant candidates. Patient should also be aware of the life-time risk of immunosuppression and graft dysfunction which is unique in this treatment modality. Last but not the least, increase in the donor pool burden translates into a longer waiting time, around 20% of the wait-listed HCC patients would be delisted in the first year chiefly as a result of disease progression, deteriorated physical condition or death [17,18]. In order to mitigate the problem of long waiting time, strategies such as bridging therapy and salvage LT had been proposed.

Extended criteria of OLT for HCC patients

Introduction of Milan Criteria marked a new era in the development of LT for HCC as it gave a clear cut clinical guidance on which group of HCC patients would better benefit from LT [6]. However, it has been criticized of being too restrictive [19,20], leading to a low LT rate for HCC in some countries [21]. On the other hand, aggressive tumor pathology was identified in a fourth of the explants of the 'within Milan Criteria' patient which deemed LT inappropriate [22,23]. Based on the template provided by the Milan Criteria, a number of newer criteria had been developed (Table 1). Initial attempts were made over modification of size and number of the tumor [15,20,24–26]. Since the association between tumor size, tumor number and tumor biology were not absolute [27,28]; in addition, there is often discrepancy between radiological and

pathological tumor size and number, therefore, some centers incorporated biochemical markers such as protein-induced vitamin K antagonist (PIVKA)-II and alpha-fetal protein (AFP) in the selection criteria [29,30]. Recently, a tumor size/number-independent selection criteria had been proposed by the group from Toronto General Hospital [31,32]. In their center, patients with advanced HCC would be transplanted regardless of the tumor number and size, only patients presented with systemic cancer-related symptoms, percutaneous tumor biopsy shows poor tumor differentiation or AFP level over 500 ng/ml would be rejected. This policy resulted in a 5-year survival of 69% for patient who had HCC beyond Milan Criteria [32]. There is little doubt about the relationship between tumor pathology and aggressiveness of HCC. A recent systemic review found that microvascular invasion is associated with 3.4 times worse in 3-year disease-free survival after LT for HCC [33]. However, percutaneous tumor biopsy is not always possible especially for cirrhotic patients with ascites, thrombocytopenia and coagulopathy; tumor located at the dome of liver and caudate lobe are literally impossible to be biopsied; tumor seeding and rupture are practical concerns; It has been reported that due to the presence of intratumoral heterogeneity, analysis of microvascular invasion through tumor biopsy would be inadequate [34]. There were studies suggesting that positron emission tomography using tracers carbon-11 acetate and 18-fluoro-deoxy-glucose could predict vascular invasion [35,36] and post-transplant survival [37]. The role of positron emission tomography scan in patient selection and risk stratification is expected to be further elucidated in future studies.

Other prognostic markers & scoring system in the context of LT for HCC

Since it is well-known that tumor diameter and number are insufficient to reflect tumor biology [38], LT performed for HCC within Milan Criteria still carries a 15% recurrence rate [39,40]. It implies that there is still ample room to refine the current patient selection criteria, newer prognostic parameters had been investigated for better prognostication. Total tumor volume has been introduced as a superior substitute for size and number parameters [41,42]. It has been shown that total tumor volume of <65.5 cm^3 (equivalent to a solitary HCC of 5 cm) has a better prognostic accuracy than Milan Criteria [43]. In order to better predict the chance of HCC recurrence after LT, Agopian *et al.* introduced a normogram, which comprised of seven factors, namely nuclear grading, vascular invasion, pre-LT down-staging treatment, radiological maximum tumor diameter, AFP level, neutrophil/lymphocyte ratio and total cholesterol level. This model was shown to have higher predictive value than using Milan and UCSF criteria in terms of HCC recurrence [44]. Halazun *et al.* recently

Table 1. Comparison of different liver transplant criteria for hepatocellular carcinoma.

Criteria	Tumor number	Tumor size	Additional restriction	Overall 5-year survival (%)	Ref.
UCSF	Solitary 3 or less	<6.5 cm <4.5 cm Total <8 cm	–	75.2	[24]
University of Tokyo	5 or less	5 cm or less	–	75	[25]
Chang Guan University	1 3 or less	6.5 cm 4.5 cm	–	90	[15]
Asan	6 or less	5 cm or less	–	82	[26]
Up to 7	7 or less	7 or less	Numerical sum of tumor size and number must be less than 7	71.2	[20]
Kyoto University	10 or less	5 cm or less	PIVKA-II ≤400 mAU/ml	87	[29]
Kyushu University	No limit	5 cm or less	PIVKA-II <300 mAU/ml	83	
Hangzhou	No limit	Total size 8 cm or less	For total tumor larger than 8 cm, histological grade must be I or II and AFP must be 400 ng/l or below	72	[30]
Dubay	No limit	No limit	Only biopsy confirmed poorly differentiated HCC would be excluded	72	[31]
Extended Toronto Criteria	No limit	No limit	Presence of cancer-related systemic symptoms, poor differentiation or AFP >500 ng/ml	70	[32]

AFP: Alpha-fetal protein; HCC: Hepatocellular carcinoma.

published a novel scoring system, the MORAL score [45] composed of AFP, neutrophil/lymphocyte ratio and tumor size showed high correlation with post-LT HCC recurrence. Another score known as the RETREAT score had been developed and validated in a multicenter study involving over 1000 HCC patients, by using pre-LT AFP, presence of microvascular invasion and the sum of the largest diameter of viable tumor (cm) plus the number of viable tumors on explant; such score was able to stratify patients into different risk groups, patients who scored 0 and over 5 would have a predicted 5-year HCC recurrence rate of less than 3% and 75% respectively [46]. More scoring systems are expected to emerge in the future. Internal and external validations are necessary before it can be generalized to different populations.

Down-staging & bridging therapy in the context of LT

Down-staging therapy allows patients with HCC beyond criteria to become eligible for listing again. Tumor down-staging programs differ from center to center (Table 2). Some centers set limits to number and size of tumor [47,48] while others set no restriction for patient inclusion for down-staging therapy [49,50]. Some centers used only transarterial chemoembolization (TACE) as the down-staging treatment [47,50], while the other employed various approaches including TACE, transarterial radioembolization, percutaneous, laparoscopic or open tumor ablation and resection in different combination [47–49,51]. In these series, around 44–78% of the patients could reach the predefined endpoint after receiving down-staging therapy and became eligible for LT [47–50]. The reported long-term overall and disease-free survival ranged from 75 to 94% [47,49,51–52] and 50–92%, respectively [47–48,50–51]. Despite the presence of heterogeneity among these series, the oncological outcomes were comparable to HCC patients who were within criteria and, hence, making the down-staging policy justified [53,54]. In addition, transarterial radioembolization with the use of Yttrium-90 had been recently shown to be an effective down-staging and bridging approach even for HCC with macrovascular invasion [55,56].

The 'ablate and wait' period after down-staging therapy is an essential component of the whole policy; by providing this 'test of time' which usually last for 6 months [57,58], tumors with unfavorable biology would manifest themselves with early recurrence/progression, thus help screening out 'high-risk' poor transplant candidate [57,59]. Future studies using standardized inclusion/exclusion criteria, treatment end point and outcome measurement for down-staging therapy shall further clarify its role.

For patients who are within criteria and waitlisted, bridging therapy is given so as to halt tumor progression [60,61], ultimately to reduce dropout rate and improve survival [59,62–63]. TACE is a commonly used bridging therapy, which was shown to achieve complete tumor necrosis in 30–50% of the patients [64,65]. It has been suggested that higher tumor necrosis rate could be achieved by the use of doxorubicin eluting bead TACE (DEB-TACE) [66], because of the potentially more severe complications after TACE [67]; further study data are needed before it could replace conventional TACE. Percutaneous RFA is another popular bridging therapy for HCC patients on waiting list; its efficacy depends on the size of tumor [68,69], however, poor liver function and presence of ascites limit its use in cirrhotic patients. Emerging methods of bridging therapy includes transarterial radioembolization using Yttrium-90 and external radiotherapy [70,71]. Both of them were shown to have high tumor necrosis and safety profile making them ideal alternatives for HCC which respond poorly or contraindicated to conventional bridging treatments.

Primary & salvage LT

Since Majno *et al.* introduced the concept of salvage LT in 2000, which referred to the treatment algorithm of upfront hepatectomy for resectable HCC followed by LT when recurrence or decompensated cirrhosis arose [72], this policy gained world-wide popularity as supported by the good results from various single center and meta-analyses series [73,74]. Belghiti *et al.* [73] compared 70 patients who had undergone primary LT with 17 patients who had salvage LT for HCC, wherein it was found that the reoperation rate was higher in the salvage group but there was no difference in 5-year overall survival (OS) between the groups (61 vs 59%). On the contrary, Adam *et al.* [75] reported a lower transplantability after tumor recurrence, significantly higher operative mortality (28.6 vs 2.1%) and inferior 5-year OS (61 vs 50%) after comparing 195 primary transplant patients with 17 salvage transplant patients. Nonetheless, salvage LT is

currently a more popular approach since some of the patients might be cured by resection alone; primary LT for all HCC patients would unnecessarily put these patients at extra risk related to graft rejection and immunosuppression; moreover, transplanting resectable HCC increases the burden to the already tight donor pool, and the waitlist mortality of the non-HCC patients would be increased. Resection-first approach can actually serve as a selection tool, those who develop early nontransplantable HCC recurrence after resection are not ideal candidates for primary LT either. Through close postoperative surveillance, transplantable recurrence should still be identified. Although a more recent intention-to-treat comparison between primary LT and primary resection followed by salvage LT from Adam's group demonstrated superior survivals with primary LT patients who succeeded salvage LT after primary resection, actually had similar survival as compared with the primary LT group [76]. Low transplantability rate for recurrent HCC remains the Achilles heel in the 'resection-first, salvage if recur' policy. Since randomized controlled trial in this context is unlikely practical, these options should be opened to the patients for discussion especially when there is a keen living donor.

LDLT & DDLT for HCC

Organ shortage has been a common problem faced by the transplant community. It has been reported that the dropout rate for HCC patients on waiting list were 25 and 43% for first and second year, respectively [77]. Since the implementation of MELD exception scheme, the chance of HCC patients getting a liver graft has greatly increased by sixfold [78]. Different centers have their own MELD exception policy according to the spectrum of the waitlisted patients and donor pool situations, subjecting to interval audit and modification so as to ensure equitable organ allocation among HCC and non-HCC patients by considering their respective dropout rate and waitlist mortality. Recently, a new '6-month delay' granting in MELD exception policy was implemented in the States – patients with HCC who remain within UNOS stage II (i.e., solitary tumor <5 cm, more than 3 tumors and each <3 cm) for half a year would be granted a starting MELD of 28, followed by additional MELD bonus score every 2 months thereafter, eventually capped at 34 [79]. In the authors' center, the policy is similar except the starting point is set at 18 without ceiling MELD score [80]. Nonetheless, the 'two blades knife' of MELD exception policy improves the survival of HCC patients but inevitably compromises the transplantation rate of non-HCC patients, resorting to LDLT seems to be the only way to beat this 'zero-sum game'. In regions of ultralow deceased donor rate such as Korea, Japan and Hong Kong, LDLT has been a dominating majority over DDLT [81,82]. Living donor graft is a dedicated gift, usually from a loved one's selfless sacrifice negating the concern of resources utility [83,84]. The hope of LT rekindled especially for HCC patients who have low MELD score or even tumor staging beyond standard LT criteria. Our earlier series suggested a worse

Table 2. Illustration of down-staging programs and outcomes from different series.

Study (year)	Criteria for DS	Mode of DS	Criteria for LT	Successful DS (%)	Proceed LT (%)	OS	DFS	Ref.
Barakat *et al.* (2010)	No limit	Mixed	UNOS T2	56	44	75% (2 years)	–	[52]
Yao *et al.* (2008)	Solitary≤8cm, 2–3 tumors ≤5 cm, 4–5 tumors ≤3 cm, total ≤8 cm	Mixed	UNOS T2, tumor necrosis	70	57	92% (4 years)	92% (4 years)	[47]
Cillo *et al.* (2007)	No limit	Mixed	Not stated	Not stated	7	79% (5 years)	Not stated	[49]
Chapman *et al.* (2008)	No limit	TACE	ALTSG stage 2	24	Not stated	94% (5 years)	50% (5 years)	[51]
Ravaioli *et al.* (2008)	Solitary ≤6 cm, 2 tumors ≤5 cm, 3–5 tumors ≤4 cm, total ≤12 cm	Mixed	Milan	90	67	Not stated	71% (3 years)	[48]
Otto *et al.* (2006)	No limit	TACE	RECIST (partial response)	55	44	Not stated	75% (5 years)	[50]

ALTSG: American Liver Tumor Study Group; DFS: Disease-free survival; DS: Down-staging; LT: Liver transplantation; OS: Overall survival; RECIST: Response Evaluation Criteria in Solid Tumors; TACE: Transarterial chemoembolization; UNOS: United Network for Organ Sharing.

oncological outcome of HCC treated by LDLT when compared with DDLT [85]; similar findings were reported in a multicenter series from the USA [86]. One reason for the inferiority of LDLT might be related to patient selection of the studies. The other possible cause is the 'fast-tracking' effect in LDLT. Since the waiting time for LDLT is usually shorter than those in the DDLT waiting list, the lack of the 'test of time' made transplanting a poor risk HCC patient more likely, hence the worse treatment result. A number of subsequent series, including those from our center, did not find significant survival difference between LDLT and DDLT in the context of HCC treatment [87,88]. This change could be related to the 'fast-track' effect after implementation of MELD exception scheme. Nonetheless, before further evidence emerged, LDLT remains a very important treatment for patient with HCC.

Unlike the case of DDLT, more advanced HCC could still be considered for LT as long as the patient and donor understand and accept the chance and implication of post-LT HCC recurrence. Threshold of performing LDLT for beyond criteria HCC varies from center to center. Most experiences were reported by the Korean groups [89,90]. In a series by Lee *et al.* [89], 11 patients diagnosed to have advanced HCC with portal vein tumor thrombi, had undergone LDLT. The 5-year disease-free and OS of the patients in that series was 45.5 and 63.6%, respectively; hence, they concluded that, HCC with portal vein tumor thrombi is not a contraindication for LDLT provided that main portal vein is not involved and the AP (AFP*PIVKA-II) score is low. Albeit a much inferior long-term oncological outcome as compared with those transplanted within standard criteria, the 5-year survivals according to that series was still much better than those who received palliative or systemic treatment [91,92]. In the context of LDLT for advanced HCC, implication of inferior oncological outcomes such as early recurrence and disease dissemination, should be conveyed to patient and potential donor. Donor morbidity and mortality must be minimized so as to justify this high recurrence risk operation.

Treatment for post-transplant HCC recurrence

Despite compliance to Milan Criteria, HCC recurrence after LT is still seen in 10–25% of the patients [6,39,93–95]. Recurrence commonly occurs within 2 years after LT and is associated with poor survival [96]. Late recurrence (>2 years) and well differentiated HCC seems to have a better survival [94,95]. The median survival of patients who developed post-LT HCC recurrence, is around 8–12 months [54,94,97–98]. Patients who developed resectable (hepatic and pulmonary) recurrence still have the median survival of around 5 years [94,99–100]. For unresectable recurrence, RFA, transarterial therapy, stereotactic body radiation therapy, high-intensity focused ultrasound and sorafenib are potential treatment modalities [101]; some of them were shown to improve survival and slow down disease progression [102,103]. The importance of close surveillance to detect resectable recurrence cannot be overemphasized.

Modern antitumor immunosuppressive therapy

The decade-by-decade evolution of immunosuppressive agents has been one of the thrust of transplantation medicine. Before the era of cyclosporine in the 1970s, the 1-year graft survival was around 30%. Subsequent development of cyclosporine improved the 1-year survival to over 50%. In the 1990s, a new calcineurin inhibitor, tacrolimus had substituted cyclosporine as the chief immunosuppressive due to its higher potency and more favorable side-effect profile, as shown in multiple trials [104]. However, in the context of HCC, some studies suggested that calcineurin inhibitor may promote primary tumor growth and distant metastasis and hence compromising disease-free survival [23,105–107]. A newer agent known as sirolimus, which is a natural fermentation product of *Streptomyces hygroscopicus*, contains potent antifungal and immunosuppressive properties [108]. By binding to and inhibiting the mammalian target of rapamycin (mTOR) and leading to interruption of the IL-2 pathway, it exerts its immunosuppressive effect by arresting cell cycle of T-lymphocytes. The antitumor effect had been reported in a number of retrospective series [109,110]. Until recently, a randomized multicenter open-label Phase 3 trial comparing post-transplant HCC recurrence rate of over 260 patients who were assigned to mTOR-free immunosuppression and sirolimus base immunosuppression regimen found that sirolimus use was associated with lower 3-year recurrence in patients who fulfilled Milan Criteria [111]. Despite the fact that long-term benefit of sirolimus in post-transplant HCC

patients could not be demonstrated, this trial provided an initial high-level evidence to suggest substituting tacrolimus with sirolimus in HCC patient after LT.

Future perspective of HCC treatment in the context of LT

LT will continue to be an effective treatment for HCC, problem of graft shortage would be partially alleviated by LDLT, yet, donor safety and morbidity issues have to be well addressed – shifting of the use of right lobe to left lobe living donor graft and maturation of laparoscopic donor hepatectomy would be the world trend.

Prediction of post-LT HCC recurrence will continue to be the research interest in the coming future. Apart from various new prognostic scoring system and markers mentioned, advances in the molecular biotechnology allows stratifying recurrence risk by comparing the DNA content of the tumor cells and normal reference cells. Jonas *et al.* introduced the DNA index, and suggested that index of 1.5 or less is associated with good outcome regardless of the tumor number, size and degree of tumor differentiation [112]. 'Signature genes' for rapid tumor progression had also been identified [113] which might be extrapolated to aid selecting appropriate patient for LT in the future.

Conclusion

LT is the best treatment for patients with HCC. Careful patient selection is the key to success in LT for HCC. Good results from series using extended criteria suggested that there are other factors associated with aggressive tumor biology in addition to tumor size and number. Selected patients who have HCC beyond criteria would also benefit from LT after down-staging therapy. Use of bridging therapy improves the survival and transplantation rate of waitlisted HCC patients. Primary and salvage are both viable options as a treatment of HCC provided that the patient understands the risk and benefit of each of them. Future development of the field would focus on the ways to select HCC patients for LT so as to achieve the best organ utility and minimize post-LT HCC recurrence

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References

1 El-Serag HB. Hepatocellular carcinoma: an epidemiologic view. *J. Clin. Gastroenterol.* 35(5 Suppl. 2), S72–S78 (2002).

2 Rossi L, Zoratto F, Papa A *et al.* Current approach in the treatment of hepatocellular carcinoma. *World J. Gastrointest. Oncol.* 2(9), 348–359 (2010).

3 Yokoyama I, Todo S, Iwatsuki S, Starzl TE. Liver transplantation in the treatment of primary liver cancer. *Hepatogastroenterology* 37(2), 188–193 (1990).

4 Yokoyama I, Todo S, Iwatsuki S, Starzl TE *et al.* Hepatic resection versus transplantation for hepatocellular carcinoma. *Ann. Surg.* 214(3), 221–228; discussion 228–229 (1991).

5 O'Grady JG, Polson RJ, Rolles K, Calne RY, Williams R. Liver transplantation for malignant disease. Results in 93 consecutive patients. *Ann. Surg.* 207(4), 373–379 (1988).

6 Mazzaferro V, Regalia E, Doci R *et al.* Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. *N. Engl. J. Med* 334(11), 693–699 (1996).

7 Cho YK, Kim JK, Kim WT, Chung JW. Hepatic resection versus radiofrequency ablation for very early stage hepatocellular carcinoma: a Markov model analysis. *Hepatology (Baltimore, MD)* 51(4), 1284–1290 (2010).

8 Poon RT, Fan ST, Lo CM, Liu CL, Wong J. Long-term survival and pattern of recurrence after resection of small hepatocellular carcinoma in patients with preserved liver function: implications for a strategy of salvage transplantation. *Ann. Surg* 235(3), 373–382 (2002).

9 Fattovich G, Stroffolini T, Zagni I, Donato F. Hepatocellular carcinoma in cirrhosis: incidence and risk factors. *Gastroenterology* 127(5 Suppl. 1), S35–S50 (2004).

10 Regimbeau JM, Abdalla EK, Vauthey JN *et al.* Risk factors for early death due to recurrence after liver resection for hepatocellular carcinoma: results of a multicenter study. *J. Surg. Oncol.* 85(1), 36–41 (2004).

11 Imamura H, Matsuyama Y, Tanaka E *et al.* Risk factors contributing to early and late phase intrahepatic recurrence of hepatocellular carcinoma after hepatectomy. *J. Hepatol.* 38(2), 200–207 (2003).

12 Fong Y, Sun RL, Jarnagin W, Blumgart LH. An analysis of 412 cases of hepatocellular carcinoma at a western center. *Ann. Surg.* 229(6), 790–799; discussion 799–800 (1999).

13 Starzl TE. *The Puzzle People: Memoirs of a Transplant Surgeon*. University of Pittsburgh Press, PA, USA (1992).

14 Starzl TE, Marchioro TL, Rowlands DT Jr *et al.* Immunosuppression after experimental and clinical homotransplantation of the liver. *Ann. Surg.* 160, 411–439 (1964).

15 Concejero A, Chen CL, Wang CC *et al.* Living donor liver transplantation for hepatocellular carcinoma: a single-center

experience in Taiwan. *Transplantation* 85(3), 398–406 (2008).

16 Dec GW, Kondo N, Farrell ML, Dienstag J, Cosimi AB, Semigran MJ. Cardiovascular complications following liver transplantation. *Clin. Transplant.* 9(6), 463–471 (1995).

17 Salvalaggio PR, Felga G, Axelrod DA, Della Guardia B, Almeida MD, Rezende MB. List and liver transplant survival according to waiting time in patients with hepatocellular carcinoma. *Am. J. Transplant.* 15(3), 668–677 (2015).

18 Majno P, Lencioni R, Mornex F, Girard N, Poon RT, Cherqui D. Is the treatment of hepatocellular carcinoma on the waiting list necessary? *Liver Transp.* 17(Suppl. 2), S98–S108 (2011).

19 Toso C, Asthana S, Bigam DL, Shapiro AM, Kneteman NM. Reassessing selection criteria prior to liver transplantation for hepatocellular carcinoma utilizing the Scientific Registry of Transplant Recipients database. *Hepatology (Baltimore, MD)* 49(3), 832–838 (2009).

20 Mazzaferro V, Llovet JM, Miceli R *et al.* Predicting survival after liver transplantation in patients with hepatocellular carcinoma beyond the Milan criteria: a retrospective, exploratory analysis. *Lancet Oncol.* 10(1), 35–43 (2009).

21 Shirabe K, Taketomi A, Morita K *et al.* Comparative evaluation of expanded criteria for patients with hepatocellular carcinoma beyond the Milan criteria undergoing living-related donor liver transplantation. *Clin. Transplant.* 25(5), E491–E498 (2011).

22 Margarit C, Charco R, Hidalgo E, Allende H, Castells L, Bilbao I. Liver transplantation for malignant diseases: selection and pattern of recurrence. *World J. Surg.* 26(2), 257–263 (2002).

23 Vivarelli M, Bellusci R, Cucchetti A *et al.* Low recurrence rate of hepatocellular carcinoma after liver transplantation: better patient selection or lower immunosuppression? *Transplantation* 74(12), 1746–1751 (2002).

24 Yao FY, Ferrell L, Bass NM *et al.* Liver transplantation for hepatocellular carcinoma: expansion of the tumor size limits does not adversely impact survival. *Hepatology (Baltimore, MD)* 33(6), 1394–1403 (2001).

25 Sugawara Y, Tamura S, Makuuchi M. Living donor liver transplantation for hepatocellular carcinoma: Tokyo University series. *Dig. Dis. (Basel, Switzerland)* 25(4), 310–312 (2007).

26 Lee SG, Hwang S, Moon DB *et al.* Expanded indication criteria of living donor liver transplantation for hepatocellular carcinoma at one large-volume center. *Liver Transpl.* 14(7), 935–945 (2008).

27 Jonas S, Bechstein WO, Steinmuller T *et al.* Vascular invasion and histopathologic grading determine outcome after liver transplantation for hepatocellular carcinoma in cirrhosis. *Hepatology (Baltimore, Md.)* 33(5), 1080–1086 (2001).

28 Kirimlioglu H, Dvorchick I, Ruppert K *et al.* Hepatocellular carcinomas in native livers from patients treated with orthotopic liver transplantation: biologic and therapeutic implications. *Hepatology (Baltimore, MD)* 34(3), 502–510 (2001).

29 Takada Y, Uemoto S. Liver transplantation for hepatocellular carcinoma: the Kyoto experience. *J. Hepatobiliary Pancreat. Sci.* 17(5), 527–532 (2010).

30 Zheng SS, Xu X, Wu J *et al.* Liver transplantation for hepatocellular carcinoma: Hangzhou experiences. *Transplantation* 85(12), 1726–1732 (2008).

31 Dubay D, Sandroussi C, Sandhu L *et al.* Liver transplantation for advanced hepatocellular carcinoma using poor tumor differentiation on biopsy as an exclusion criterion. *Ann. Surg.* 253(1), 166–172 (2011).

32 Sapisochin G, Goldaracena N, Laurence JM *et al.* The extended Toronto criteria for liver transplantation in patients with hepatocellular carcinoma: a prospective validation study. *Hepatology (Baltimore, MD)* 64(6), 2077–2088 (2016).

33 Rodriguez-Peralvarez M, Luong TV, Andreana L, Meyer T, Dhillon AP, Burroughs AK. A systematic review of microvascular invasion in hepatocellular carcinoma: diagnostic and prognostic variability. *Ann. Surg. Oncol.* 20(1), 325–339 (2013).

34 Banerjee S, Wang DS, Kim HJ *et al.* A computed tomography radiogenomic biomarker predicts microvascular invasion and clinical outcomes in hepatocellular carcinoma. *Hepatology (Baltimore, Md.)* 62(3), 792–800 (2015).

35 Cheung TT, Chan SC, Ho CL *et al.* Can positron emission tomography with the dual tracers [11 C]acetate and [18 F] fludeoxyglucose predict microvascular invasion in hepatocellular carcinoma? *Liver Transpl.* 17(10), 1218–1225 (2011).

36 Kobayashi T, Aikata H, Honda F *et al.* Preoperative fluorine 18 fluorodeoxyglucose positron emission tomography/computed tomography for prediction of microvascular invasion in small hepatocellular carcinoma. *J. Comput. Assist. Tomogr.* 40(4), 524–530 (2016).

37 Lee SD, Kim SH, Kim SK, Kim YK, Park SJ. Clinical impact of 18F-fluorodeoxyglucose positron emission tomography/computed tomography in living donor liver transplantation for advanced hepatocellular carcinoma. *Transplantation* 99(10), 2142–2149 (2015).

38 Mehta N, Yao FY. Moving past "One size (and number) fits all" in the selection of candidates with hepatocellular carcinoma for liver transplantation. *Liver Transpl.* 19(10), 1055–1058 (2013).

39 Levi DM, Tzakis AG, Martin P *et al.* Liver transplantation for hepatocellular carcinoma in the model for end-stage liver disease era. *J. Am. Coll. Surg.* 210(5), 727–734, 735–726 (2010).

40 Pomfret EA, Washburn K, Wald C *et al.* Report of a national conference on liver allocation in patients with hepatocellular carcinoma in the United States. *Liver Transpl.* 16(3), 262–278 (2010).

41 Hsu CY, Huang YH, Hsia CY *et al.* A new prognostic model for hepatocellular carcinoma based on total tumor volume: the Taipei Integrated Scoring System. *J. Hepatol.* 53(1), 108–117 (2010).

42 Grat M, Kornasiewicz O, Holowko W *et al.* Evaluation of total tumor volume and pretransplantation alpha-fetoprotein level as selection criteria for liver transplantation in patients with hepatocellular cancer. *Transplant. Proc.* 45(5), 1899–1903 (2013).

43 Lee YH, Hsia CY, Hsu CY, Huang YH, Lin HC, Huo TI. Total tumor volume is a better marker of tumor burden in hepatocellular carcinoma defined by the Milan criteria. *World J. Surg.* 37(6), 1348–1355 (2013).

44 Agopian VG, Harlander-Locke M, Zarrinpar A *et al.* A novel prognostic nomogram accurately predicts hepatocellular carcinoma recurrence after liver transplantation: analysis of 865 consecutive liver transplant recipients. *J. Am. Coll. Surg.* 220(4), 416–427 (2015).

45 Halazun KJ, Najjar M, Abdelmessih RM *et al.* Recurrence after liver transplantation for hepatocellular carcinoma: a new MORAL to the story. *Ann. Surg.* 265(3), 557–564 (2017).

46 Mehta N, Heimbach J, Harnois DM *et al.* Validation of a risk estimation of tumor recurrence after transplant (RETREAT) score for hepatocellular carcinoma recurrence after liver transplant. *JAMA Oncol.* doi:10.1001/jamaoncol.2016.5116 (2016) (Epub ahead of print).

47 Yao FY, Kerlan RK Jr, Hirose R *et al.* Excellent outcome following down-staging of hepatocellular carcinoma prior to liver transplantation: an intention-to-treat analysis.

Hepatology (Baltimore, MD) 48(3), 819–827 (2008).

48 Ravaioli M, Grazi GL, Piscaglia F *et al.* Liver transplantation for hepatocellular carcinoma: results of down-staging in patients initially outside the Milan selection criteria. *Am. J. Transplant.* 8(12), 2547–2557 (2008).

49 Cillo U, Vitale A, Grigoletto F *et al.* Intention-to-treat analysis of liver transplantation in selected, aggressively treated HCC patients exceeding the Milan criteria. *Am. J Transplant.* 7(4), 972–981 (2007).

50 Otto G, Herber S, Heise M *et al.* Response to transarterial chemoembolization as a biological selection criterion for liver transplantation in hepatocellular carcinoma. *Liver Transpl.* 12(8), 1260–1267 (2006).

51 Chapman WC, Majella Doyle MB, Stuart JE *et al.* Outcomes of neoadjuvant transarterial chemoembolization to downstage hepatocellular carcinoma before liver transplantation. *Ann. Surg.* 248(4), 617–625 (2008).

52 Barakat O, Wood RP, Ozaki CF *et al.* Morphological features of advanced hepatocellular carcinoma as a predictor of downstaging and liver transplantation: an intention-to-treat analysis. *Liver Transpl.* 16(3), 289–299 (2010).

53 Volk ML, Vijan S, Marrero JA. A novel model measuring the harm of transplanting hepatocellular carcinoma exceeding Milan criteria. *Am. J. Transplant.* 8(4), 839–846 (2008).

54 Clavien PA, Lesurtel M, Bossuyt PM, Gores GJ, Langer B, Perrier A. Recommendations for liver transplantation for hepatocellular carcinoma: an international consensus conference report. *Lancet Oncol.* 13(1), e11–22 (2012).

55 Ettorre GM, Levi Sandri GB, Laurenzi A *et al.* Yttrium-90 radioembolization for hepatocellular carcinoma prior to liver transplantation. *World J. Surg.* 41(1), 241–249 (2017).

56 Levi Sandri GB, Ettorre GM, Colasanti M *et al.* Hepatocellular carcinoma with macrovascular invasion treated with yttrium-90 radioembolization prior to transplantation. *Hepatobiliary Surg. Nutr.* 6(1), 44–48 (2017).

57 Roberts JP, Venook A, Kerlan R, Yao F. Hepatocellular carcinoma: ablate and wait versus rapid transplantation. *Liver Transpl.* 16(8), 925–929 (2010).

58 Halazun KJ, Patzer RE, Rana AA *et al.* Standing the test of time: outcomes of a decade of prioritizing patients with hepatocellular carcinoma, results of the UNOS natural geographic experiment. *Hepatology (Baltimore, MD)* 60(6), 1957–1962 (2014).

59 Toso C, Mentha G, Kneteman NM, Majno P. The place of downstaging for hepatocellular carcinoma. *J. Hepatol.* 52(6), 930–936 (2010).

60 Cescon M, Cucchetti A, Ravaioli M, Pinna AD. Hepatocellular carcinoma locoregional therapies for patients in the waiting list. Impact on transplantability and recurrence rate. *J. Hepatol.* 58(3), 609–618 (2013).

61 Fujiki M, Aucejo F, Choi M, Kim R. Neo-adjuvant therapy for hepatocellular carcinoma before liver transplantation: where do we stand? *World J. Gastroenterol.* 20(18), 5308–5319 (2014).

62 Majno P, Giostra E, Mentha G. Management of hepatocellular carcinoma on the waiting list before liver transplantation: time for controlled trials? *LiverTtranspl.* 13(11 Suppl. 2), S27–S35 (2007).

63 Hayashi PH, Ludkowski M, Forman LM *et al.* Hepatic artery chemoembolization for hepatocellular carcinoma in patients listed for liver transplantation. *Am. J. Transplant.* 4(5), 782–787 (2004).

64 Alba E, Valls C, Dominguez J *et al.* Transcatheter arterial chemoembolization in patients with hepatocellular carcinoma on the waiting list for orthotopic liver transplantation. *AJR Am. J. Roentgenol.* 190(5), 1341–1348 (2008).

65 Tsochatzis E, Garcovich M, Marelli L *et al.* Transarterial embolization as neo-adjuvant therapy pretransplantation in patients with hepatocellular carcinoma. *Liver Int.* 33(6), 944–949 (2013).

66 Nicolini A, Martinetti L, Crespi S, Maggioni M, Sangiovanni A. Transarterial chemoembolization with epirubicin-eluting beads versus transarterial embolization before liver transplantation for hepatocellular carcinoma. *J. Vasc. Interv. Radiol.* 21(3), 327–332 (2010).

67 She WH, Chan AC, Cheung TT *et al.* Acute pancreatitis induced by transarterial chemoembolization: a single-center experience of over 1500 cases. *Hepatobiliary Pancreat. Dis. Int.* 15(1), 93–98 (2016).

68 Mazzaferro V, Battiston C, Perrone S *et al.* Radiofrequency ablation of small hepatocellular carcinoma in cirrhotic patients awaiting liver transplantation: a prospective study. *Ann. Surg.* 240(5), 900–909 (2004).

69 Rodriguez-Sanjuan JC, Gonzalez F, Juanco C *et al.* Radiological and pathological assessment of hepatocellular carcinoma response to radiofrequency. A study on removed liver after transplantation. *World J. Surg.* 32(7), 1489–1494 (2008).

70 Riaz A, Kulik L, Lewandowski RJ *et al.* Radiologic-pathologic correlation of hepatocellular carcinoma treated with internal radiation using yttrium-90 microspheres. *Hepatology (Baltimore, MD)* 49(4), 1185–1193 (2009).

71 Sandroussi C, Dawson LA, Lee M *et al.* Radiotherapy as a bridge to liver transplantation for hepatocellular carcinoma. *Transpl. Int.* 23(3), 299–306 (2010).

72 Majno PE, Sarasin FP, Mentha G, Hadengue A. Primary liver resection and salvage transplantation or primary liver transplantation in patients with single, small hepatocellular carcinoma and preserved liver function: an outcome-oriented decision analysis. *Hepatology (Baltimore, MD)* 31(4), 899–906 (2000).

73 Belghiti J, Cortes A, Abdalla EK *et al.* Resection prior to liver transplantation for hepatocellular carcinoma. *Ann. Surg.* 238(6), 885–892; discussion 892–883 (2003).

74 Cherqui D, Laurent A, Mocellin N *et al.* Liver resection for transplantable hepatocellular carcinoma: long-term survival and role of secondary liver transplantation. *Ann. Surg.* 250(5), 738–746 (2009).

75 Adam R, Azoulay D, Castaing D *et al.* Liver resection as a bridge to transplantation for hepatocellular carcinoma on cirrhosis: a reasonable strategy? *Ann. Surg.* 238(4), 508–518; discussion 518–509 (2003).

76 Bhangui P, Allard MA, Vibert E *et al.* Salvage versus primary liver transplantation for early hepatocellular carcinoma: do both strategies yield similar outcomes? *Ann. Surg.* 264(1), 155–163 (2016).

77 Dubay D, Sandroussi C, Sandhu L *et al.* Liver transplantation for advanced hepatocellular carcinoma using poor tumor differentiation on biopsy as an exclusion criterion. *Ann. Surg.* 253(1), 166–172 (2011).

78 Ioannou GN, Perkins JD, Carithers RL. Liver transplantation for hepatocellular carcinoma: impact of the MELD allocation system and predictors of survival. *Gastroenterology* 134(5), 1342–1351 (2008).

79 Elwir S, Lake J. Current status of liver allocation in the United States. *Gastroenterol. Hepatol.* 12(3), 166 (2016).

80 Chan SC, Sharr WW, Chok KS, Chan AC, Lo CM. Wait and transplant for stage 2

hepatocellular carcinoma with deceased-donor liver grafts. *Transplantation* 96(11), 995–999 (2013).

81 Lee SG. Living-donor liver transplantation in adults. *Br. Med. Bull.* ldq003 (2010).

82 De Villa V, Lo CM. Liver transplantation for hepatocellular carcinoma in Asia. *Oncologist* 12(11), 1321–1331 (2007).

83 Song GW, Lee SG. Living donor liver transplantation. *Curr. Opin. Organ Transplant.* 19(3), 217–222 (2014).

84 Akamatsu N, Sugawara Y, Kokudo N. Living donor liver transplantation for patients with hepatocellular carcinoma. *Liver Cancer* 3(2), 108–118 (2014).

85 Lo CM, Fan ST, Liu CL, Chan SC, Ng IO, Wong J. Living donor versus deceased donor liver transplantation for early irresectable hepatocellular carcinoma. *Br. J. Surg.* 94(1), 78–86 (2007).

86 Fisher RA, Kulik LM, Freise CE *et al.* Hepatocellular carcinoma recurrence and death following living and deceased donor liver transplantation. *Am. J. Transplant.* 7(6), 1601–1608 (2007).

87 Liang W, Wu L, Ling X *et al.* Living donor liver transplantation versus deceased donor liver transplantation for hepatocellular carcinoma: a meta-analysis. *Liver Transpl.* 18(10), 1226–1236 (2012).

88 Chan SC, Fan ST, Lo CM *et al.* A decade of right liver adult-to-adult living donor liver transplantation: the recipient mid-term outcomes. *Ann. Surg.* 248(3), 411–419 (2008).

89 Lee KW, Suh SW, Choi Y *et al.* Macrovascular invasion is not an absolute contraindication for living donor liver transplantation. *Liver Transpl.* 23(1), 19–27 (2016).

90 Lee HW, Suh KS. Liver transplantation for advanced hepatocellular carcinoma. *Clin. Mol. Hepatol.* 22(3), 309–318 (2016).

91 Nishikawa H, Kita R, Kimura T *et al.* Proposal of the performance status combined Japan Integrated Staging system in hepatocellular carcinoma complicated with cirrhosis. *Int. J. Oncol.* 46(6), 2371–2379 (2015).

92 Llovet JM, Ricci S, Mazzaferro V *et al.* Sorafenib in advanced hepatocellular carcinoma. *N. Engl. J. Med.* 359(4), 378–390 (2008).

93 Klintmalm GB. Liver transplantation for hepatocellular carcinoma: a registry report of the impact of tumor characteristics on outcome. *Ann. Surg.* 228(4), 479–490 (1998).

94 Roayaie S, Schwartz JD, Sung MW *et al.* Recurrence of hepatocellular carcinoma after liver transplant: patterns and prognosis. *Liver Transpl.* 10(4), 534–540 (2004).

95 Chok KS, Chan SC, Cheung TT, Chan AC, Fan ST, Lo CM. Late recurrence of hepatocellular carcinoma after liver transplantation. *World J. Surg.* 35(9), 2058–2062 (2011).

96 Rubin J, Ayoub N, Kaldas F, Saab S. Management of recurrent hepatocellular carcinoma in liver transplant recipients: a systematic review. *Exp. Clin. Transplant.* 10(6), 531–543 (2012).

97 Zimmerman MA, Ghobrial RM, Tong MJ *et al.* Recurrence of hepatocellular carcinoma following liver transplantation: a review of preoperative and postoperative prognostic indicators. *Arch. Surg.* 143(2), 182–188; discussion 188 (2008).

98 EASL Clinical Practice Guidelines: Liver transplantation. *J. Hepatol.* 64(2), 433–485 (2016).

99 Regalia E, Fassati LR, Valente U *et al.* Pattern and management of recurrent hepatocellular carcinoma after liver transplantation. *J. Hepatobiliary Pancreat. Surg.* 5(1), 29–34 (1998).

100 Viola C, Asselah T, Samuel D *et al.* Solitary pulmonary metastasis arising thirteen years after liver transplantation for HBV-related hepatocellular carcinoma. *World J. Gastroenterol* 12(30), 4911–4913 (2006).

101 Chok K. Management of recurrent hepatocellular carcinoma after liver transplant. *World J. Hepatol.* 7(8), 1142–1148 (2015).

102 Zhou B, Shan H, Zhu KS *et al.* Chemoembolization with lobaplatin mixed with iodized oil for unresectable recurrent hepatocellular carcinoma after orthotopic liver transplantation. *J. Vasc. Interv. Radiol.* 21(3), 333–338 (2010).

103 Ko HK, Ko GY, Yoon HK, Sung KB. Tumor response to transcatheter arterial chemoembolization in recurrent hepatocellular carcinoma after living donor liver transplantation. *Korean J. Radiol.* 8(4), 320–327 (2007).

104 McAlister VC, Haddad E, Renouf E, Malthaner RA, Kjaer MS, Gluud LL. Cyclosporin versus tacrolimus as primary immunosuppressant after liver transplantation: a meta-analysis. *Am. J. Transplant.* 6(7), 1578–1585 (2006).

105 Hojo M, Morimoto T, Maluccio M *et al.* Cyclosporine induces cancer progression by a cell-autonomous mechanism. *Nature* 397(6719), 530–534 (1999).

106 Vivarelli M, Cucchetti A, Piscaglia F *et al.* Analysis of risk factors for tumor recurrence after liver transplantation for hepatocellular carcinoma: key role of immunosuppression. *Liver Transpl.* 11(5), 497–503 (2005).

107 Guba M, Von Breitenbuch P, Steinbauer M *et al.* Rapamycin inhibits primary and metastatic tumor growth by antiangiogenesis: involvement of vascular endothelial growth factor. *Nat. Med.* 8(2), 128–135 (2002).

108 Neuhaus P, Klupp J, Langrehr JM. mTOR inhibitors: an overview. *Liver Transpl.* 7(6), 473–484 (2001).

109 Zimmerman MA, Trotter JF, Wachs M *et al.* Sirolimus-based immunosuppression following liver transplantation for hepatocellular carcinoma. *Liver Transpl.* 14(5), 633–638 (2008).

110 Toso C, Merani S, Bigam DL, Shapiro AM, Kneteman NM. Sirolimus-based immunosuppression is associated with increased survival after liver transplantation for hepatocellular carcinoma. *Hepatology (Baltimore, MD)* 51(4), 1237–1243 (2010).

111 Geissler EK, Schnitzbauer AA, Zulke C *et al.* Sirolimus use in liver transplant recipients with hepatocellular carcinoma: a randomized, multicenter, open-label Phase III trial. *Transplantation* 100(1), 116–125 (2016).

112 Jonas S, Al-Abadi H, Benckert C *et al.* Prognostic significance of the DNA-index in liver transplantation for hepatocellular carcinoma in cirrhosis. *Ann. Surg.* 250(6), 1008–1013 (2009).

113 Villa E, Critelli R, Lei B *et al.* Neoangiogenesis-related genes are hallmarks of fast-growing hepatocellular carcinomas and worst survival. results from a prospective study. *Gut* 65(5), 861–869 (2016).