Strategies to improve outcome of patients with hepatocellular carcinoma receiving a liver transplantation

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However, tumour recurrence rates are as high as 20%, and once the recurrence is established the therapeutic options are scarce and with little impact on prognosis. Strategies to minimize tumour recurrence and thus to improve outcome may be classified into 3 groups: (1) An adequate selection of candidates for liver transplantation by using the Milan criteria; (2) An optimized management within waiting list including prioritization of patients at high risk of tumour progression, and the implementation of bridging therapies, particularly when the expected length within the waiting list is longer than 6 mo; and (3) Tailored immunosuppression comprising reduced exposure to calcineurin inhibitors, particularly early after liver transplantation, and the addition of mammalian target of rapamycin inhibitors. In the present manuscript the available scientific evidence supporting these strategies is comprehensively reviewed, and future directions are provided for novel research approaches, which may contribute to the final target: to cure more patients with hepatocellular carcinoma and with an improved long term outcome.

Key words: Hepatocellular carcinoma; Recurrence; Bridging therapy; Milan criteria; Immunosuppression; Liver transplantation

Core tip: Liver transplantation is the only therapeutic option which allows to treat both, the hepatocellular carcinoma and the underlying liver disease. However, tumour recurrence rates are 15%-20% with a very poor prognosis. Strategies to minimize tumour recurrence and thus to improve outcome are focused in a careful selection of candidates for liver transplantation, an optimized management within waiting list and a tailored immunosuppression. The available scientific evidence supporting these strategies is reviewed, and future directions are provided for novel research approaches, which may contribute to the final target: to cure more patients with hepatocellular carcinoma and with an improved long term outcome.
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Guerrero-Misas M, Rodríguez-Perálvarez M, De la Mata M. Strategies to improve outcome of patients with hepatocellular carcinoma receiving a liver transplantation. World J Hepatol 2015; 7(4): 649-661 Available from: URL: http://www.wjgnet.com/1948-5182/full/v7/i4/649.htm DOI: http://dx.doi.org/10.4254/wjh.v7.i4.649

INTRODUCTION

Hepatocellular carcinoma (HCC) is the most frequent primary liver cancer, which accounts for 70%-80% of the hepatic malignancies[11]. It generally appears on a cirrhotic liver, and thus the common causes of cirrhosis are also involved in the oncogenesis of HCC, being particularly relevant hepatitis B virus (HBV) and HCV chronic viral infections[2-4]. It has been estimated that HBV is responsible for 50%-80% of HCC cases, whereas 10%-20% of cases are related to HCV[3].

HCC represents the fifth most common malignancy (554000 cases worldwide) and the second leading cause of cancer-related mortality, (746000 deaths annually)[5]. HCC rates are 2-4 fold increased in male population. The incidence is higher in developing countries, although its incidence in developed countries is increasing, mainly due to HCV infection[6]. Among developed countries the highest incidence rates are found in North America (9.3 cases/100000) and southern Europe (9.5 cases/100000)[5]. However this picture may change in the next decades given the exponential raising of infant obesity in United States and Europe which may trigger the number of HCCs attributable to non-alcoholic fatty liver disease[7-9].

Liver Transplantation (LT) is the only therapeutic option able to treat both the HCC and the underlying liver disease, and it is currently considered the standard of care for patients with small unresectable HCC. The proportion of patients diagnosed at early stage, who potentially would benefit from LT, is expected to increase due to the screening programs with ultrasound implemented for patients with chronic liver disease[10].

Compared to liver resection and locoregional ablative therapies, LT offers an improved long term survival (70% at 5 years). However the tumour recurrence rates after LT are 15%-20%, and the therapeutic options are very limited in this situation[11].

Current strategies to minimize HCC recurrence after LT are grouped in: (1) Adequate selection of candidates for LT; (2) Waiting list management and bridging locoregional therapies; and (3) Tailored immunosuppression. In the present manuscript the scientific evidence supporting these strategies is comprehensively reviewed, and future directions are drawn in order to improve long term outcome of LT patients with HCC.

SELECTION OF HCC PATIENTS FOR LT

The initial experiences of LT to treat HCC were disappointing since the tumour recurrence was frequent, and survival was shorter when compared with other LT aetiologies (5-year survival rates 18%-40%)[12]. Many institutions included the HCC as a formal contraindication for LT until the early 90’s, when some studies showed that patients with small HCC undergoing LT had reduced tumour recurrence rates when compared to liver resection. Clinicians soon became aware of the improved outcomes when LT was restricted to patients with a limited tumour burden. Indeed in a cohort of 221 consecutive LT patients [38 patients with small HCC (< 5 cm) and 136 with cirrhosis without HCC], the survival rates were similar irrespective of whether HCC was present or not (63% vs 68% at 5 years respectively; P = 0.84). In 1996 Mazzaferro et al[13] prospectively analyzed a cohort of 48 patients with small unresectable HCC undergoing LT. The authors considered LT when there was a single nodule ≤ 5 cm or up to 3 nodules, ≤ 3 cm each, in the absence of macrovascular invasion or extrahepatic metastases. The 4-year survival rate was 75%, with a recurrence-free survival rate of 83%. These premises, currently known as Milan criteria, found a wide acceptance and most LT institutions implemented them as the standard of care to select HCC patients for LT[14]. However according to the Milan criteria, only 6% of patients with HCC would be eligible for LT[14], and therefore these criteria may be considered too restrictive. A significant proportion of patients above Milan criteria could still benefit from LT without increasing HCC recurrence rates[15].

There have been many attempts to expand Milan criteria, being the most frequently used summarized in Table 1. The expansion of the Milan criteria may be performed by increasing the diameter and/or the number of nodules allowed, and in some cases with additional criteria such as histological tumour differentiation, serum PIVKA-Ⅱ or α-fetoprotein.

One of the most popular criteria is the so called “up-to-seven”, according to which patients without macrovascular invasion could be candidates for LT as long as the sum of the number of nodules and the diameter of the largest nodule is ≤ 7. These criteria were derived from a retrospective multicenter study with 1556 patients with HCC, from which 1070 had a HCC beyond Milan criteria. Patients outside Milan criteria but within the up-to-seven criteria had an overall 5 year-survival rate of 71.2%, similar to those found in patients within Milan criteria (73.3%). HCC recurrence rates were also similar provided that microvascular invasion was absent[16]. However this study had important limitations. The tumour was evaluated in the explanted liver rather than by using radiological techniques, and a certain grade of disagreement between both approaches is expected in clinical practice. Most importantly, the favorable outcome showed by patients within the up-to-seven criteria was only present after excluding patients
with microvascular invasion, which cannot be assessed pre-LT\textsuperscript{[27]}. Thus the up-to-seven criteria cannot be considered a safe approach to expand Milan criteria.

The UCSF criteria were described in 2001 by Yao et al\textsuperscript{[28]}, who prospectively analyzed the outcome of 70 patients with HCC undergoing LT in a single institution. Patients with a solitary tumor \( \leq 6.5 \text{ cm} \), or up to 3 nodules, being the largest lesion \( \leq 4.5 \text{ cm} \) and the total tumor diameter \( \leq 8 \text{ cm} \), had 1-year survival rates of 90% (vs 50% for patients exceeding these criteria; \( P = 0.0005 \)). Although the tumour assessment was performed in the explanted liver, the UCSF criteria have been subsequently validated in independent retrospective studies, some of them based on pre-LT imaging techniques\textsuperscript{[19-22]}.

The criteria from the University of Navarra include a single tumor \( \leq 6 \text{ cm} \) or 2-3 nodules up to 5 cm, without macrovascular invasion or extra-hepatic disease\textsuperscript{[29]}. The original study included 160 LT patients from a single institution (47 patients had HCC, and 12 were above Milan criteria). There were no differences between patients within Milan criteria and above Milan criteria but within the Navarra criteria. The limitations of this proposal are the limited number of patients included, the retrospective design and the lack of external validation.

In the Kyoto criteria up to 10 nodules, less than 5 cm each, were permitted if serum PIVKA-II was < 400 mU/mL\textsuperscript{[24]}. These factors were obtained by a multivariate approach in a retrospective study with 125 LT with HCC (70 patients met the Milan criteria, and 55 patients did not). Patients above Milan criteria but within the Kyoto criteria had similar 5-year tumour recurrence rates when compared with patients within the Milan criteria (7.3% vs 9.7%; \( P = 0.87 \)). When serum PIVKA-II, which is a tumour marker related to an aggressive histological behavior of HCC, was included in the selection criteria the patients within Kyoto criteria showed reduced 5-year HCC recurrence rates (4.9%) when compared to patients above Kyoto criteria (60.5%) (\( P = 0.0001 \)). Similarly, 5-year survival rates significantly differed between these groups (86.7% vs 34.4%, respectively; \( P = 0.0001 \)). Similar results were described in independent cohorts\textsuperscript{[25,26]}.

In the Toronto criteria there were no limits in terms of diameter of the main nodule or the number of nodules, provided that extrahepatic metastasis, macrovascular invasion and poor histological differentiation were ruled out, and there was a preserved performance status\textsuperscript{[27]}. There were similar 5-year survival rates and 5-year disease-free survival rates between the patients within the Milan criteria and patients beyond Milan criteria but within Toronto criteria. Interestingly, a histological parameter was implemented in the Toronto criteria, as it is histological differentiation according to the Edmonson scale\textsuperscript{[28]}. Far from being a limitation, the addition of histological differentiation may add relevant information\textsuperscript{[29,30]} and it can be assessed, although with some limitations, in a regular liver biopsy performed before the LT. However most of the patients with HCC are diagnosed by imaging techniques and the liver biopsy would not be performed otherwise. The risk of needle track seedi

### Table 1 Summary of the most relevant criteria used to select candidates with hepatocellular carcinoma for liver transplantation

| Ref. | Name of criteria | No. of nodules | Diameter | Tumour different | PIVKA-II | Prospective validation | \( n \) | Outcomes within Milan criteria | Outcomes within proposed criteria |
|------|------------------|----------------|----------|------------------|----------|------------------------|------|-----------------------------|----------------------------------|
| Mazzaferro et al\textsuperscript{[31]} | Milan | 1 or \( \leq 3 \) | \( \leq 5 \text{ cm} \) or \( \leq 3 \text{ cm} \) each | - | - | + | 48 | 4-yr survival rate 75% | 5-yr survival rate 73.3% | 5-yr survival rate 76% |
| Mazzaferro et al\textsuperscript{[31]} | Up-to-seven | TTN + DLN \( \leq 7 \) | - | - | - | 1556 | 5-yr survival rate 73.3% | 5-yr survival rate 71.2% | > 0.05 |
| Yao et al\textsuperscript{[32]} | UCSF criteria | 1 or \( \leq 3 \) | \( \leq 6.5 \text{ cm} \) or \( \leq 4.5 \text{ cm} \) the largest one but with a TTD \( \leq 8 \text{ cm} \) | - | - | - | 70 | 5-yr survival rate 72.4% | 5-yr survival rate 75.2% | 0.87 |
| Herrero et al\textsuperscript{[33]} | Universidad de Navarra criteria | 1 or 2-3 | \( \leq 6 \text{ cm} \) or \( \leq 5 \text{ cm} \) | - | - | - | 47 | Not reported | 5-yr recurrence free survival rate 79% | - |
| Lee et al\textsuperscript{[34]} | Asan criteria | \( \leq 6 \) | \( \leq 5 \text{ cm} \) | - | - | - | 221 | 5-yr survival rate 76% | 5-yr survival rate 81.6% | 0.34 |
| DuBay et al\textsuperscript{[35]} | Toronto criteria | No | No | + | - | - | 294 | 5-yr overall survival rate 72% | 5-yr overall survival rate 70% | 0.63 |
| Ito et al\textsuperscript{[36]} | Kyoto criteria | \( \leq 10 \) | \( \leq 5 \text{ cm} \) | - | + | - | 125 | Not reported | 5-yr disease free survival rate 79% | 5-yr disease free survival rate 66% | 0.25 |

TTN: Total tumor nodules; DLN: Diameter of the largest nodule; TTD: Total tumor diameter; NA: Not applicable.
after the liver biopsy\(^{31}\) casts doubts on recommending this procedure to all patients with HCC before LT. Unfortunately the Toronto criteria have not been validated in independent cohorts.

In the Asan criteria \(\leq 6\) tumors with a maximum tumor diameter of \(\leq 5\) cm, and without macrovascular invasion or extra-hepatic involvement were considered\(^{32}\). The original study analyzed the outcome of 221 patients with HCC undergoing LT in a single institution. The 5-year survival rates within Milan criteria were 76%, similar to those found in patients above Milan criteria and within Asan criteria (76.3%) \((P = 0.334)\).

Hitherto these attempts to expand Milan criteria had a little impact in clinical practice because of inherent methodological limitations. Further studies are needed to identify the best approach to expand Milan criteria safely. Although there is a general agreement to exclude HCC patients with macrovascular invasion or extrahepatic spreading, the intrahepatic tumour burden allowed is a matter of debate, as they are the additional parameters to be included. The ideal criteria to select candidates with HCC for LT should be based on solid data, and future studies addressing this issue should fulfill the following premises: (1) Enough sample size and statistical power; (2) Criteria based on objective parameters with prognostic capability, easily measured before LT; (3) Cut-off points derived from robust statistical methods; (4) Similar overall and disease free survival rates as Milan criteria; and (5) External validation in a prospective multicentric cohort.

On the other hand the expansion of the Milan criteria should be tempered. A liberal policy would not only impair outcomes, but would also limit the access to LT of patients with other liver diseases, particularly in areas with increased incidence rates of HCC. Thus any attempt to expand Milan criteria need to provide similar long term outcome when compared to other aetiologies for LT, and specific studies will be needed in each area considering the HCC prevalence, the number of donors available, and the impact of this strategy on the waiting list.

The idea of expanding Milan criteria by using only the size of the tumour and the number of nodules is too simplistic. Each series may show a different threshold for the maximum tumour diameter or for the number of nodules permitted, but the results may not be reproducible in other countries, or even in a different institution within the same country. The reason may be that the biological tumour behavior is widely variable between patients with similar HCC burden. There are many surrogate markers related to an aggressive tumour behavior in HCC, which can be categorized into histological and serum markers.

Microvascular invasion (mVI) occurs when the tumour phenotype is sufficiently evolved to degrade extracellular matrix which surround vascular structures, and invades the vascular lumen. HCC cells are then free to metastasize either in a different location of the liver (multinodular disease), or in other organs (extrahepatic spreading). Thus mVI is a critical hallmark in HCC progression, and the strongest prognostic factor as demonstrated in a metaanalysis of observational studies \((RR = 3.41\) for tumour recurrence and \(RR = 2.41\) for mortality at 3 years\(^{17}\). However the diagnosis of mVI has proven to be difficult even for experienced pathologists with the whole HCC specimen\(^{33}\). The mVI assessment in a regular liver biopsy has not been validated, but implementing this information to the selection of HCC patients for LT would allow to expand safely the Milan criteria\(^{30,34}\). Hitherto there have been many attempts to identify surrogate biomarkers of mVI, including serum markers \([i.e.,\alpha -\text{fetoprotein (AFP), PIVKA-} II,\text{ neutrophil to lymphocyte ratio}]^{35-38}\), histological markers \([i.e.,\text{ poor differentiation and extranodular growth}]^{39-41}\), and imaging techniques \([i.e.,\text{ presence of capsule in an magnetic resonance imaging, smooth margin in computed tomography scan or positive positron emission tomography}]^{42-44}\), but none of them are reliable enough to impact on the decision-making process. Further studies are needed either to identify new not invasive biomarkers of mVI, or to validate its diagnosis in a regular liver biopsy. There are other histological features related to poor prognosis in HCC patients such as capsular invasion, lymphatic permeation, presence of satellite nodules and tumour differentiation, being the later the only one able to be detected in a liver biopsy before LT. Many studies have shown that patients with poorly differentiated tumours have increased risk of recurrence and reduced survival rates\(^{39-41}\).

Among serum markers, AFP is the most widely used. Monitoring AFP levels was used in the past as a screening to detect early HCC in patients with chronic liver disease, but in the most recent guidelines the only recognized screening technique was liver ultrasound\(^{40}\). AFP was abandoned because of its suboptimal sensitivity. In patients with HCC candidates to LT there is controversy about what is the best threshold to exclude a patient from the waiting list\(^{45,46}\). In addition AFP serum levels may be modified within waiting list by the use of locoregional therapies such as transarterial chemoembolization (TACE)\(^{47}\). Serum PIVKA-II, also known as Des-gamma-carboxyprothrombin, is preferred to AFP in some LT institutions, particularly in eastern countries, because of an increased accuracy reported in some studies\(^{48}\). However AFP appears more sensitive than PIVKA-II for early HCC\(^{49}\). Increased serum PIVKA-II concentrations are found in patients with more advanced HCC, and in patients with mVI\(^{50,52}\). The combination of PIVKA-II and AFP provided increased accuracy than any of them alone\(^{53}\). Other biomarkers related to systemic inflammation such as neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio and inflammation-based index have been associated with poor survival in HCC\(^{54,55}\), but their role in predicting HCC recurrence after LT is controversial\(^{20,56,57}\), probably because these parameters are highly influenced by
many environmental factors different from tumour progression.

The combination of demographic features, the underlying liver disease, tumour burden, histological characteristics and serum biomarkers by using novel multivariate approaches allowing to manage an increased amount of information such as machine learning classifiers or artificial neural networks, which have already proven their utility in other LT scenarios[68], might be the key for a safe expansion of the Milan criteria.

## WAITING LIST MANAGEMENT AND BRIDGING THERAPY

The shortage of donors is universal and causes an imbalance between candidates for LT and number of organs available. The patients included in the waiting list should face a risk of drop-out, either because of death or due to a significant worsening of the underlying liver disease. In patients with HCC the drop-out is usually related to tumour progression. The waiting list management should be programmed carefully, and adapted to each clinical scenario in order to guarantee an equal access to LT for patients with different aetiologies of liver disease.

From 2002 the Model for end stage liver disease (MELD) was widely accepted as the standard of care to predict short term survival within waiting list, and has been adopted to prioritize the sickest patients for LT. However the MELD score does not reflect the risk of HCC progression. The proposal to palliate this problem consisted in adding extra-MELD points on an empirical basis according to the time within the waiting list. First experiences resulted in an increased benefit to patients with HCC, with more patients transplanted, decreased waiting list mortality and drop-out rates[59,60]. Indeed this was a very positive picture for HCC patients but it was linked to an imbalance in the access to LT between HCC patients and patients with other liver diseases[59-63].

A moderate delay within the waiting list would allow for a better selection of HCC candidates for LT according to some authors. The patients with the most aggressive tumours would experience an early tumour progression and they would not be transplanted. Indeed a recent analysis of a nationwide American database showed that a longer waiting time for LT resulted in improved survival rates after LT for HCC patients, while the disparities in the access to LT among different aetiologies were reduced[64]. The optimal balance between the length within waiting list and the outcome after LT for HCC patients has not been established yet. The current allocation policy for patients with HCC is to add extra-MELD points only when there is a significant risk of drop-out (i.e., T2 HCC stage). This may palliate the problem but it is far from solving it. A recent study used Monte-Carlo simulations and multiple logistic regression to calculate a corrected MELD score for HCC patients. The so called HCC-MELD formula included also AFP and provided the same priority for HCC patients as the equivalent of conventional MELD score did for other LT aetiologies[65]. The main limitations were the lack of consideration of tumour volume at listing and tumour progression within the waiting list. In addition changes in AFP after bridging therapies may decrease the HCC-MELD score in patients with positive response to therapy, and these patients have shown particularly reduced tumour recurrence rates.

Neoadjuvant locoregional therapies are recommended when the expected time to LT is longer than 6 mo in order to prevent drop-out and increase long term survival, while minimizing the risk of tumour recurrence after LT[73,66-72]. However many LT institutions treat most of the patients within the waiting list, since the actual time to LT may be unpredictable, and this strategy has demonstrated a favourable cost-effect balance[73]. The radiological response to bridging therapy may also help to assess the HCC biological behavior[74-76] and to prioritize HCC patients for LT[77-81]. Patients with tumour growth beyond Milan criteria after locoregional therapies should not undergo LT.

There are different modalities of locoregional therapies to be used as bridging for LT. The most frequently applied are liver resection (LR), TACE and radiofrequency ablation (RFA). None of these therapies have shown to be superior to the others and the selection should be tailored according to the BCLC schema[72]. LR can be used as a first line-bridging therapy procedure to LT in experienced units, with a morbidity of 39%, and an early mortality rate of 3%[82]. The main advantage of LR is that the whole HCC specimen will be available for histological examination. The information coming from the histology is very valuable as noted above, and may serve to identify predictors of poor outcome. In the presence of these factors the tumour recurrence is almost universal and many LT teams include high-risk patients within waiting list for LT immediately after LR. Other authors would consider LT only in patients with tumour recurrence after LR (salvage LT), but this strategy may result in worse survival rates and increased recurrence rates, unless a careful selection of cases is carried out[83-87].

TACE is the most frequently used locoregional bridging therapy for LT. It has been hypothesized an increased risk of arterial and biliary complications after LT in patients with a previous TACE due to an endothelial damage, but this was not confirmed in a recent study with 456 HCC transplanted patients[88]. The use of TACE with drug eluting beads has improved the performance of the technique with complete tumour necrosis rates as high as 76.2%, and with a better safety profile[87,89]. In spite of this, RFA is preferred for single tumours less than 5 cm[80]. The available studies comparing RFA vs TACE suggested that complete response is more frequent with RFA, while drop-out rates are diminished[80,90]. In addition, RFA is a safer procedure with reduced rates of adverse events (4.6%)[91,92]. However the new protocols of TACE with drug eluting...
beads have not been tested against RFA in a randomized fashion. The heterogeneity in reporting outcome and in the inclusion criteria among the available studies make it impossible to perform pooled data analysis, and no recommendation can be made of which is the optimal bridging protocol in HCC patients candidates for LT. Other locoregional therapies have been evaluated with promising results (i.e., percutaneous ethanol injection, percutaneous laser ablation, microwave ablation, and radioembolization)\cite{172,29,103}, but further studies are needed to confirm their utility, and to describe which patients may benefit the most of these novel approaches.

Sorafenib is an oral multi-tyrosine kinase inhibitor with antiangiogenic properties which has shown to prolong survival in patients with advanced HCC\cite{94}. The role of sorafenib in the LT setting has been nicely reviewed by Castelli et al\cite{85}. Theoretically, sorafenib would be used as an adjuvant therapy to locoregional ablation to reduce tumour recurrence after LT, and this approach is thought to be cost-effective for T2 HCC patients\cite{96}. However the antiangiogenic effects of sorafenib could be deleterious in the perioperative period, and important safety concerns were arisen in the available series including biliary complications and hepatic artery thrombosis\cite{97}. The combination of radioembolization and sorafenib as bridging for LT was poorly tolerated in a pilot prospective study with 23 patients, and the risk of biliary complications after LT was enhanced\cite{98,99}. The combination of sorafenib with locoregional therapies as bridging for LT should not be recommended.

### STRATEGIES TO IMPROVE OUTCOME AFTER LIVER TRANSPLANTATION

Despite a careful selection of candidates for LT by the Milan criteria, and an optimization of bridging locoregional therapies within the waiting list, HCC recurrence rates are 15%-20%\cite{100}. In addition pre-LT imaging techniques may lead to misdiagnosis either by not detecting HCC nodules (incidental HCC), or by inducing a wrong staging, which usually means patients transplanted above Milan criteria and increased tumour recurrence rates\cite{106}. Even in some patients fulfilling Milan criteria the histological evaluation shows features of poor prognosis such as mVI, poor differentiation or capsular invasion. In these situations the implementation of post-LT strategies to minimize HCC recurrence may be the only option to improve outcome\cite{101}.

The whole concept of tumour recurrence requires that a remnant of circulating HCC cells should be left behind after the LT, and remained unnoticed by the immune system. The use of immunosuppressive drugs after LT is needed to prevent the consequences of acute cellular rejection, including chronic rejection and graft loss\cite{102}. In normal conditions the immune system is able to detect tumour cells and to destroy them\cite{103}. However the use of high doses of immunosuppressants may abolish the immune surveillance in the early post-LT period\cite{104-106}, as happens in other immunosuppressive conditions such as HIV chronic infection\cite{109}.

In LT patients with HCC the relationship between immunosuppression and tumour recurrence is poorly understood, but it is attracting more attention in the recent years. However the variability in the immunosuppression protocols among different institutions make it difficult to design studies addressing this issue with reduced risk of bias\cite{106}. The current evidence is mainly based in observational studies, most of them retrospective and with a limited sample size, and thus their results should be taken with caution. Among immunosuppressive drugs used in LT patients, only calcineurin inhibitors and mammalian target of rapamycin (mTOR) inhibitors have shown to influence HCC recurrence, increasing and decreasing the risk respectively. Azathioprine is preferred in some centres for patients transplanted with HCV claiming for amelioration of viral recurrence and prevention of graft loss\cite{110}. Long term use of azathioprine increases the risk of non-melanoma skin cancer\cite{111,112} and lymphoma, the later when high doses are used especially in elderly patients\cite{112}, but there is no proved role on HCC. On the other hand mycophenolate seems to be protective against malignancy in the transplant population\cite{113,114} but again there is no evidence supporting any significant effect on HCC. With regard to induction agents, the anti-interleukin-2 receptor basiliximab does not increase the risk of cancer, but anti-thymocyte globulins have been associated with an increased risk of lymphoma\cite{115}.

The use of calcineurin inhibitors, which are the mainstay of immunosuppression protocols after LT, is able to activate proto-oncogenes and pathways of cancer in a dose-dependent fashion such as transforming growth factor beta, thus promoting tumour proliferation, resistance to apoptosis and metastasis\cite{116,117}. In a retrospective study with 70 LT patients receiving cyclosporine, the drug exposure calculated with the trapezoidal rule was increased in patients with HCC recurrence (trough concentrations 278.3 ng/mL vs 169.9 ng/mL; \(P < 0.001\))\cite{118}. However there were only 7 patients with HCC recurrence in this cohort, and it was not possible to control for confounding factors. In another study from the same group, 139 LT patients with HCC were analyzed, being 60 patients under tacrolimus and 79 patients under cyclosporine\cite{119}. The rates of HCC recurrence were increased in patients with higher exposure to calcineurin inhibitors defined as tacrolimus > 10 ng/mL or cyclosporine > 220 ng/mL (RR = 4.01; \(P = 0.014\)). However the wide interval of recruitment with patients transplanted before and after the implementation of the Milan criteria, the heterogeneous length of drug exposure considered for each patient, and the lack of control for concomitant immunosuppression weakened the
conclusions. Another study with 219 LT patients from two European institutions evaluated the exposure to calcineurin inhibitors within the first month after LT with respect to HCC recurrence\[120\]. After controlling for possible confounding factors such as tumour features and concomitant immunosuppression, the increased exposure to calcineurin inhibitors within the first month after LT (tacrolimus > 10 ng/mL or cyclosporine > 300 ng/mL) was an independent predictor of HCC recurrence (RR = 2.82; \( P = 0.005 \)), either if the patient was within or above Milan criteria. The exposure to calcineurin inhibitors after the first month post-LT was similar in patients with and without tumour recurrence, highlighting the early post-LT period as one in which the minimization of calcineurin inhibitors should be encouraged.

The mTOR is a serine/threonine kinase involved in cellular growth, proliferation, metabolism and angiogenesis. The mTOR pathway is up-regulated in approximately half of patients with HCC\[121\]. The mTOR inhibitors sirolimus and everolimus have shown anti-cancer properties in animal models including HCC\[121-123\]. The mTOR inhibitors are able to prevent acute cellular rejection after LT, and allow for reducing the exposure to calcineurin inhibitors, and thus acting as renal sparing agents\[124\]. Regarding prevention of HCC recurrence there are five retrospective studies with sirolimus\[125-129\], whose results have been recently summarized in two meta-analyses\[130,131\] with the same conclusion: sirolimus may protect against HCC recurrence after LT, and patients treated with sirolimus showed improved overall survival rates. However the level of evidence is poor. These studies are heterogeneous, retrospective, and with an increased risk of reporting bias. There are no randomized controlled trials evaluating the role of mTOR inhibitors in preventing HCC recurrence. The SILVER study is a multicentre randomized controlled trial which preliminary results are expected to be available in 2016, and may shed some light in the actual role of sirolimus in LT patients with HCC\[132\]. The major concern with sirolimus relies in its safety profile. A large phase II randomized trial (\( n = 222 \)) evaluated de novo sirolimus and reduced tacrolimus after liver transplantation compared with a control arm composed by conventional tacrolimus\[133\]. The study had to be prematurely stopped due to an imbalance of adverse outcomes between groups. Patients under sirolimus experienced increased rates of graft failure (26.4% vs 12.5%) and mortality (20% vs 8% at 24 mo; \( P = 0.010 \)), and a trend towards more hepatic artery thrombosis (8.3% vs 2.7%; \( P = 0.065 \)). In addition the analysis of the Scientific Registry of Transplant Recipients (SRTR) database (\( n = 26414 \)) showed an increased risk of all-cause mortality in patients with hepatitis C treated with sirolimus (HR = 1.29; 95%CI: 1.08-1.55)\[134\]. Everolimus has a selective effect on mTOR complex 1, and it has been proposed to be more potent than sirolimus\[135\], and with an improved metabolic profile\[136\]. Unfortunately the evidence linking everolimus and HCC recurrence after LT is lacking. In the current scenario the systematic use of mTOR inhibitors after LT to prevent HCC recurrence may not be justified, but selected patients either with features of poor prognosis in the explanted liver (i.e., above Milan criteria, poor tumour differentiation and/or microvascular invasion), or with up-regulated mTOR pathway may benefit of combining early tacrolimus minimization and de novo everolimus. Future randomized controlled trials should evaluate the convenience, efficacy and safety of this approach.

Sorafenib have shown to delay HCC recurrence and metastasis after LT in a rat model\[137\]. In a prospective not randomized pilot study, 7 patients with HCC above Milan criteria were treated with sorafenib after LT, and compared with 12 matched historical controls in whom sorafenib had not been used\[138\]. Sorafenib was well tolerated with no severe adverse effects and there was a trend to less HCC recurrence in the group treated with sorafenib (29% vs 75%; \( P = 0.07 \)). The combination of sorafenib and mTOR inhibitors should be avoided because of increased risk of severe adverse events\[95\]. At any rate these are very early experiences and no further recommendations should be derived until larger randomized controlled trials are performed.

**CONCLUSION**

The efforts to improve outcome of patients with HCC undergoing LT should be driven to prevent tumour recurrence by combining the following approaches: (1) Adequate selection of candidates for LT by using Milan criteria. A moderate expansion of the Milan criteria may be possible without a significant increase in HCC recurrence rates, but this expansion should be based in objective criteria strongly associated with the biological tumour behavior; (2) Optimization in waiting-list management. Bridging locoregional therapies should be used whenever possible to prevent drop-out and to minimize HCC recurrence after LT, particularly when the expected time to LT is longer than 6 mo. The best protocol to be used remains as a matter of debate; and (3) Tailored immunosuppression protocols: Currently, early minimization of calcineurin inhibitors combined with an mTOR inhibitor may be the most rationale schema, but specific randomized controlled trials are needed for a general recommendation. Taken as a whole the scientific evidence regarding strategies to prevent HCC recurrence after LT needs to be strengthened. Research projects addressing this issue face important caveats such as the increased sample size needed, prolonged length of recruitment and follow up of patients, and increased costs. Further studies are needed to identify non-invasive biomarkers of HCC with prognostic capability, to establish the optimal management within waiting list, and to develop new immunosuppressive drugs with antiproliferative properties, able to prevent tumour recurrence in high-
risk patients.

ACKNOWLEDGMENTS

We would like to thank Encarna Díaz Silleró and Maribel Gómez Núñez for their continuous effort in improving medical care, and to make possible the clinical research within our department.

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