Review Article

Optimization of immunosuppressive medication upon liver transplantation against HCC recurrence

Shirin Elizabeth Khorsandi, Nigel Heaton

Institute of Liver Studies, King's Healthcare Partners at Denmark Hill, King's College Hospital NHSFT, London, SE5 9RS, UK

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Correspondence to: Professor Nigel Heaton. Institute of Liver Studies, King's College Hospital, London, SE5 9RS, UK. Email: nigel.heaton@nhs.net.

Abstract: The introduction of liver transplant listing criteria for hepatocellular cancer (HCC) has significantly improved oncological outcomes and survival. But despite this HCC recurrence is still problematic. There is emerging evidence that the choice of immunosuppression (IS) after transplant for HCC can influence oncological survival and HCC recurrence. The following is a short summary of what has been published on HCC recurrence with the different classes of immunosuppressive agents in present use, concluding with the possible rationalization of the use of these immunosuppressive agents in the post-transplant patient at high risk of recurrence.

Keywords: Liver transplant; immunosuppression (IS); hepatocellular cancer (HCC); chemotherapy

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Introduction

Globally, hepatocellular cancer (HCC) is the sixth most common cancer, the third commonest cause of cancer death and the commonest primary cancer of the liver (1). Liver transplantation is an attractive treatment option for the cirrhotic liver complicated by HCC. Treating both the hepatological and oncological aspects of this cancer, by providing a solution to impaired liver function and removing cirrhotic parenchyma with its inherent risk of hepatocarcinogenesis. However, liver transplant is not available in all countries and is restricted to patients with a low risk of HCC recurrence after transplant (2).

The main factors that determine HCC recurrence after liver transplant are related to the tumor burden and its underlying biology, as represented by number and size of tumor nodules, presence of vascular invasion, degree of differentiation and level of serum alpha-fetoprotein. The Milan Criteria (MC) are widely accepted for listing criteria for liver transplant and are associated with a recurrence rate of less than 10% at 5 years. However, attempts have been made to widen these criteria to include more patients and to be able to predict outcome after transplant. But widening of the listing criteria does lead to increased recurrence, typically within the first 2 years after liver transplant (3,4). The prognosis of recurrent HCC is poor, with a median survival of less than one year after diagnosis (5,6).

The introduction of the MC (solitary HCC <5 cm, or 3 nodules <3 cm) established the framework to select HCC patients on pre-transplant criteria that produces a 5-year survival of 70% and recurrence less than 10% (7,8). Being more restrictive on the selection of patients for transplant has improved outcome of liver transplant for HCC but recurrence is still problematic, and can affect up to 20% of recipients. Recurrent HCC can be divided in to early, within the first 2 years of transplant and is related to the primary tumor. While, late recurrent HCC, 2 years or more after transplant typically, represents *de novo* HCC on a background of graft cirrhosis. Presently, the treatment options, for recurrent HCC post-transplant are limited (3,9,10).

The factors that are established to be determinants of HCC recurrence are the surrogate markers of tumor biology

as represented by tumor volume, microvascular invasion, macrovascular invasion and differentiation (11). There is no convincing evidence that graft type has a significant influence on recurrence rates. Two recent meta-analysis comparing HCC recurrence rates between living donor liver transplant and deceased donor liver transplant came to opposite conclusions (12,13). Additionally, there is no evidence that a donor after cardiac death (DCD) compared to donor after brainstem death (DBD) liver has any influence on HCC recurrence rates post-transplant (14,15).

Nevertheless, there is emerging evidence that the choice of immunosuppression (IS) after transplant for HCC can influence oncological survival and HCC recurrence. IS is primarily used to reduce the risk of graft rejection, but these drugs also have a variety of direct and indirect oncogenic properties that may influence HCC recurrence (16-18). A competent immune system is recognized to be an important element in the body's early defense against cancer, by its ability to identify and destroy cancer cells. Thereby influencing local growth of cancer as well as the sequence of events involved in vascular invasion and metastasis (19).

From animal models, when natural killer cells and/or T cells (CD8+ cytotoxic or CD4+ T helper) are knocked out, cancers become more aggressive, highlighting the involvement of both a competent innate and adaptive immune system in cancer surveillance (20). Similarly, cancer cells can secrete immunosuppressive cytokines such as transforming growth factor beta 1 (TGF-β1) and chemokine (C-C motif) ligand 21 (CCL21) to prevent immune cell infiltration of the tumor (21,22).

In the clinical scenario, the innate immune system is able to recognize and destroy circulating cancer cells to reduce metastasis. But in the early post-transplant period, when IS levels are typically high, because of concerns regarding the occurrence of graft rejection, circulating HCC cells remain unchallenged by the immune system and this may contribute to HCC recurrence that is observed after transplant. Additionally, changes in the peritumoral lymphocyte subsets, with increased regulatory T cells over cytotoxic lymphocytes have also been associated with higher rates of HCC recurrence after transplant (23). What factors influence, whether recurrence occurs within the graft, or at extrahepatic sites such as lung, bone and nodes is unknown.

In the literature there is mounting evidence that the use of IS increases the risk of cancer in the transplant recipient either in the form of a *de novo* cancer or from recurrent HCC. The immune system has a critical role in preventing malignancy and metastasis. How the different classes of IS actually influence HCC recurrence after transplant is not fully understood (19). Additionally, there is a lack of good quality clinical studies on the effect of IS regimes on preventing or reducing HCC recurrence after transplant, mainly because of heterogeneity in IS protocols and HCC listing criteria between transplant centres. The following is a short summary of what has been published on HCC recurrence with the different classes of immunosuppressive agents in use (see *Table 1*). The final paragraph then summarizes the possible rationalization of the use of these immunosuppressive agents in the post-transplant patient at high risk of HCC recurrence.

IS classes and strategies

Steroids

Steroids modulate cellular and inflammatory responses by altering transcription of target genes in a cell type specific manner (37). The majority of IS protocols in the initial months after liver transplantation involve the use of steroids. Dosage and tapering schedules vary between transplant institutions and as a consequence makes it difficult to come to a clear conclusion of the importance of steroids in HCC recurrence (38). Nevertheless, there is some data that suggests the use of steroids might increase the recurrence in this setting (39). Based on this observation a randomized clinical trial was undertaken and demonstrated that withdrawal of steroids at 3 months was safe and significantly reduced HCC recurrence rates (24). This observation then has to be balanced against a subsequent retrospective study that reported no differences in HCC recurrence between early or late steroid withdrawal (25).

Calcineurin inhibitors (CNIs)

CNIs are the main immunosuppressant drug class used in liver transplantation. Cyclosporin, the first CNI used clinically in liver transplant has now been superseded by tacrolimus with its profile of improved graft and recipient survival, and lower rates of acute cellular rejection (40). The main mechanism of action for the CNIs is their binding of immunophilins to inhibit calcineurin phosphatase activity, which is part of the signaling cascade that up regulates the expression of interleukin 2 (IL-2), that in turn, stimulates the growth and differentiation of the T cell response. A number of *in vitro* and *in vivo* experiments have demonstrated that CNIs in addition to their immunoregulatory activity can also

Table 1 Summary of representative studies on immunosuppressive regimes and their effect on HCC recurrence after transplant

Reference	Immunosuppressive class	Immunosuppressive strategy	Study design	Patient No.	Within MC (%)	Recurrence free survival (%)			HCC recurrence reduction (P<0.05)
						1 yr	3 yr	5 yr	
Chen *et al*., 2007 (24)	Steroids	Steroid withdrawal at 3/12 *vs.* steroid maintenance	RCT; single centre	54	None	60.8	–	–	Yes
Kim *et al*., 2010 (25)		Steroid withdrawal at <5/12 *vs.* >5/12	Retrospective; observational; single centre	342	59	89.1	–	–	No
Vivarelli *et al*., 2002 (26)	CNIs	Low *vs.* high dose cyclosporin	Retrospective; observational; single centre	106	77	97.0	–	93.0	Yes
Vivarelli *et al*., 2005 (16)		Low *vs.* high dose cyclosporin	Retrospective; observational; single centre	70	81	–	–	–	Yes
Vivarelli *et al*., 2008 (27)		Low *vs.* high dose CNI (cyclosporin/tacrolimus)	Retrospective; observational; single centre	139	81	–	–	–	Yes
Decaens *et al*., 2006 (28)		Tacrolimus *vs.* cyclosporin	Retrospective; observational; multicentre	412	64	–	–	70.8	Yes
Rodríguez-Perálvarez *et al*., 2013 (29)		Low *vs.* high dose tacrolimus	Retrospective; observational; two centres	219	65	95.7	–	85.3	Yes
Vivarelli *et al*., 2010 (30)	mTOR inhibitors	Sirolimus added *vs.* sirolimus free IS regime	Retrospective; matched cohort; single centre	62	100	96.0	86.0	–	Yes
Chinnakotla *et al*., 2009 (31)		Sirolimus added *vs.* sirolimus free IS regime	Retrospective; case control; single centre	227	100	94.0	85.0	80.0	Yes
Zimmerman *et al*., 2008 (32)		Sirolimus added *vs.* sirolimus free IS regime	Retrospective; observational; single centre	97	Not stated	93.0	82.0	79.0	Yes
Zhou *et al*., 2008 (33)		Sirolimus added *vs.* sirolimus free IS regime	Retrospective; observational; single centre	73	None	90.7	–	80.6	Yes
Toso *et al*., 2010 (34)		Sirolimus added *vs.* sirolimus free IS regime	SRTR enquiry	2,491	Not stated	–	85.6	83.1	Yes
Geissler *et al*., 2016 (35)		Sirolimus added *vs.* sirolimus free IS regime	RCT; multicentre	525	Yes	85.2	72.3	68.4	No
Decaens *et al*., 2006 (28)	Induction IS	No ATG *vs.* ATG	Retrospective; observational; multicentre	412	64	83.0	–	58.8	Yes
Xing *et al*., 2013 (36)		Basiliximab *vs.* steroids	Retrospective; observational; single	178	36	93.0	–	88.0	Yes (if within MC)

The main points that have been included in the table, where data is available, is related to the main immunosuppressive regime studied, study design, number of patients included in study, percentage (%) of patients within Milan Criteria at time of transplant, % recurrence free survival at 1, 3 and 5 years, and whether the given immunosuppressive regime had a significant reduction on HCC recurrence after transplant. The studies summarized are highly heterogeneous in terms of patient numbers and whether the HCC was within Milan Criteria at time of transplant. Many of the studies are also retrospective and observational in design. Additionally, in some studies, the primary aim was not to determine whether HCC recurrence was related to a particular immunosuppressive regime. All of these factors make it hard to compare the studies. HCC, hepatocellular cancer; MC, Milan Criteria; RCT, randomized control trial; CNIs, calcineurin inhibitors; IS, immunosuppression; SRTR, scientific registry of transplant recipients; ATG, antithymocyte globulin.

switch on oncogenes to promote cancer cell proliferation, survival and metastasis (41,42).

A number of retrospective studies have demonstrated a dose related association between CNI IS and HCC recurrence after liver transplant (16,27,43). In the first year after liver transplant it has been shown that the dose of cyclosporin influences HCC recurrence (35). Additionally, when the use of cyclosporin has been compared to steroids and azathioprine, a significant association between recurrent HCC was found (35). However, oncologically there appears to be no significant clinical difference between cyclosporin and tacrolimus. One study comparing cyclosporin and tacrolimus described a better 5-year HCC disease free survival with cyclosporin (27), while another study reported poorer survival (36).

The clearest data regarding the effect of CNIs on HCC recurrence have been with tacrolimus, with higher tacrolimus trough concentrations being found in patients that experienced HCC recurrence (16,27). Suggesting that over IS, especially in the first few months after transplant, where the focus is on preventing acute cellular rejection is oncologically detrimental, as well as being harmful to renal function (44). More recent work from a single institute has corroborated these conclusions, by showing an increased risk of HCC recurrence when there is early exposure to high levels of CNIs. The first month after liver transplant appears to be critical, at a time when high trough levels of CNIs are typically encouraged (>10 ng/mL tacrolimus or >300 ng/mL cyclosporin) and at these levels, the risk of recurrent HCC has been found to be increased, by up to 3 times (29).

The threshold of tacrolimus trough concentration (10 ng/mL) has been found to increase the risk of HCC recurrence in two separate studies (27,29). Additionally, tacrolimus trough concentrations of 7–10 ng/mL in the first month after liver transplant produce similar rejection rates, halved the occurrence of renal impairment and were associated with longer graft survival (29,44) when compared to trough concentrations >10 ng/mL. A tacrolimus trough concentration of >10 ng/mL is often regarded as the standard/reference for many clinical trials on liver transplant IS being derived from the IS thresholds established for kidney transplant (44). Based on these findings and increasing experience in the use of tacrolimus in the liver transplant population has led to lower trough levels of tacrolimus being aimed for in the immediate post-transplant period in many liver transplant programmes throughout the world. Ideally, aiming for a tacrolimus trough level of 8 ng/mL for the first 3 months after transplant, then 5–8 ng/mL from then onwards.

CNI minimization can be either achieved by aiming for lower troughs. However, if there are concerns that there is a need for robust IS early post-transplant e.g., young recipient, autoimmune disease, or normal liver function tests at the time of transplant, then a CNI sparing regime can be adopted rather than aiming for a higher CNI trough. This would include the use of adding in an antimetabolite e.g., mycophenolate or azathioprine, which may be a more favorable oncological strategy (29). However, the influence of antimetabolite dose on HCC recurrence is not fully established (see later).

Acute cellular rejection after liver transplant that progresses to chronic rejection and subsequent graft loss occurs in less than 5%. The other risks of over IS include renal impairment, infection, new onset diabetes and malignancy (both de novo and recurrent cancer). Emphasizing that over IS is detrimental to the liver transplant recipient on a number of levels (29,44,45). Additionally, there is some evidence that early acute cellular rejection after liver transplantation may improve long-term survival (29,45) as it has been suggested that complete suppression of acute cellular rejection may prevent operational tolerance from developing (45,46). Operational tolerance is where stable normal graft function is achieved without the need for IS (47).

Overall, CNI minimization is to be encouraged, both because of the oncological and the additional benefits of reducing the metabolic, cardiovascular and renal complications associated with this immunosuppressive strategy. Building on from the benefits of CNI minimization a number of transplant centers are trying to identify recipients with a genetic/biomarker profile that will favor operational tolerance where CNIs can be stopped without the risk of rejection. For now no definitive recommendations on how and when to stop IS can be made for the HCC and non HCC transplant recipient (47).

Antimetabolites (mycophenolate and azathioprine)

The antimetabolites block nucleotide synthesis to inhibit the proliferation of T cells and B cells. At this point in time, mycophenolate mofetil is the most widely used antimetabolite in liver transplantation, typically, as part of a renal sparing IS regime in combination with a CNI (48,49). The alternative is azathioprine. Mycophenolate mofetil is hydrolyzed in the gut to mycophenolic acid, which then reversibly inhibits inosine-5'-monophosphate

dehydrogenase (IMPDH) to block guanine synthesis. The antiproliferative activity of mycophenolic acid led to it being initially developed as a chemotherapy agent (50) and more recently its antiangiogenic properties in cancer have been highlighted (51). However, little is known about the influence of mycophenolate on HCC recurrence after liver transplant. In observational studies that have assessed the effect of IS on HCC recurrence mycophenolate was not found to be clinically significant (30,52).

Azathioprine a prodrug for mercaptopurine that in turn inhibits an enzyme for DNA synthesis, used at a dose of 1 mg/kg/day is favored by some transplant institutes because of the clinical demonstration of a slower progression of fibrosis in hepatitis C and reduced risk of decompensation with recurrent disease (53). However, azathioprine has been found to be an independent predictor of any tumor development after liver transplant (54) and in its own right is classified as a carcinogen. The contribution that azathioprine makes to the patterns of HCC recurrence after transplant has yet to be established.

Mammalian target of rapamycin inhibitors (mTOR)

mTOR is a serine/threonine kinase and is a component of two signaling pathways. mTOR complex 1 that is triggered immunologically to influence cell growth and proliferation, and mTOR complex 2 that modulates cell metabolism (55). In 60% of patients with primary liver cancer, mTOR signaling has been demonstrated to be involved (56,57) making mTOR inhibitor based IS an attractive option in HCC patients after transplant (58).

The mTOR inhibitors available for use in liver transplant are sirolimus and everolimus. Sirolimus is a non-selective inhibitor of both mTOR complex 1 and 2, while everolimus targets mTOR complex 1. Both agents have been demonstrated to have anticancer properties (59-62). mTOR based IS is primarily used as part of a renal sparing strategy to allow either a CNI free or CNI reduced regime to be adopted (63,64).

There are a number of retrospective studies that have assessed the effect of sirolimus on HCC recurrence after liver transplant (30-34). On meta-analysis the conclusion was that sirolimus reduces HCC recurrence and improves oncological survival (65,66). However, these conclusions have to be balanced against the observation that the use of sirolimus resulted in an increased risk of death from all causes in transplant recipients with hepatitis C (67). Additionally, the rate of HCC recurrence 1 year after transplant with sirolimus in the meta-analysis were 8.6% and 13.6% (65,66) which is a higher recurrence rate when compared to that described in a CNI minimization study where the recurrence was 4.3% (29).

In order to establish the importance of the effect of sirolimus on HCC recurrence post liver transplant, Sirolimus in Liver Transplant Recipients with HCC study (SiLVER) was undertaken (68). SiLVER was a multicenter prospective randomized trial designed to compare recurrence free survival in sirolimus (mTOR inhibitor) containing versus mTOR inhibitor free immunosuppression in patients undergoing liver transplant for HCC (35). The study ran over 8 years, involved 45 transplant units and recruited 525 patients with a minimum follow-up of 5 years (35). Liver transplant recipients were randomized to mTOR free (n=264) or mTOR immunosuppression regimes (n=261), 19.2% of the mTOR immunosuppression regimes were monotherapy with sirolimus. In the study design, centre specific immunosuppression regimes were maintained, with steroids typically been withdrawn at 3 months. In the mTOR arm sirolimus was started at 1 month after transplant, because of concerns regarding its effect on surgical wound healing and hepatic artery thrombosis (69). Following the introduction of sirolimus, maintenance immunosuppression was then half dosed. The results were disappointing in that the study's overall conclusion was that sirolimus did not affect HCC recurrence free survival (35). This finding maybe related to the introduction of sirolimus being delayed by a month after transplant, and that micrometastatic disease that occurs at the time of transplant maybe the critical determinant of recurrent HCC which can be modulated by a given immunosuppression regime.

Regarding everolimus, there are some data to suggest that it can protect against HCC recurrence after transplant as well as to be of use in the management of patients with recurrent HCC after transplant (70,71). Data from phase 1 and 2 studies showed a stabilization of HCC progression with everolimus (72,73). As yet there are no clinical studies to establish its true role.

Induction immunosuppression

Induction immunosuppression is typically used to allow for early CNI minimization as part of a renal sparing strategy. The main induction immunosuppression agents available are divided into lymphocyte depleting and non-depleting. Antithymocyte globulin (ATG) is a lymphocyte

depleting agent that is a polyclonal antibody targeting a variety of T and B cell antigens. Basiliximab is a non-depleting lymphocyte agent in clinical use, that is a chimeric monoclonal antibody targeting the α chain (CD25) of IL-2 receptors of T cells.

There is little clinical data on the effect of induction immunosuppression on HCC recurrence patterns (74). A retrospective multicenter study showed the use of ATG was associated with a lower recurrence free survival (28). While another compared basiliximab to steroids led to better overall survival rates in recipients who were within MC (36). There is some research evidence on the use of anti-CD25 antibodies in cancer immunotherapy, but the doses of basiliximab used, were different from that used in transplant. Low concentrations of basiliximab (<0.06 μg/mL) were found to selectively inhibit $CD4^{+}CD25^{high}$ regulatory T (T-reg) cells allowing cancer cells to avoid immune elimination (75). In liver transplantation a higher dose of basiliximab, typically 20 mg on day 1 and 4 after transplant is used. The immediate serum concentration of basiliximab then ranges from 5–10 mg/L, with a half-life of 13.4 days (76). This regime produces a complete disappearance of all $CD25^{+}$ cells, including the tumor specific effector cytotoxic T cells that target tumor cells. As yet there is insufficient data on the importance of induction IS in HCC recipients and no clear cut recommendations can be made regarding their use in HCC outside specifically designed trials (77).

Immunosuppression and sorafenib

Sorafenib, is a small molecule oral multikinase inhibitor which has been demonstrated in randomized studies to increase the survival of patients with advanced HCC by 3 months (78,79). In the context of liver transplantation there are only retrospective cohort studies that have looked at the effect of sorafenib on recurrent HCC (77,80-82). How sorafenib is used and in combination with which immunosuppression regime is becoming established in the post-transplant patient with HCC recurrence (83). Typically the combination of sorafenib and mTOR inhibitors has been preferred, as mTOR inhibition has the strongest evidence and rationale for an anticancer effect. However, sorafenib is poorly tolerated in liver transplant patients, with or without mTOR immunosuppression, making it difficult to achieve a therapeutic dose of sorafenib (84-87). Nonetheless, there are a couple of case series that have described a survival benefit from using adjuvant sorafenib in post-transplant patients at high risk of recurrence (88-90). But at this moment in time it is unknown what the optimal dose of sorafenib is for preventing or treating recurrent HCC in transplant patients, as both the patient population and malignant state is different to that which the usage guide of sorafenib is based upon.

Rationalising IS

HCC is a heterogeneous cancer at a molecular and cellular level, with a variety of different etiologies, making it unlikely that one immunosuppressive regime will provide an optimal strategy to minimize HCC recurrence after transplant (58). Hyperactivation of mTOR signaling pathways occurs in 15–20% liver tumors (59,91) with mTOR activated HCC being associated with higher levels of alpha-fetoprotein and higher recurrence rates (59). Additionally, IMPDH enzyme activity has been demonstrated to have a cancer variation and could be a marker for mycophenolate immunosuppression being the optimal strategy to prevent HCC recurrence in a given HCC patient (92). Determining the molecular signature of HCC and identifying reliable biomarkers, will be of importance in the future to enable to rationalize and develop ideal immunosuppressive regimes for maintenance and for the prevention of HCC after transplant (47,58).

Conclusions

The introduction of liver transplant listing criteria for HCC has significantly improved oncological outcomes. But despite this HCC recurrence is problematic and more study into transplant cancer biology is needed to understand the basis of HCC recurrence, such as determining, if the main mechanism of recurrence is related to seeding at time of transplant or it is a pre-transplant event, in order to rationalize HCC prevention. Initial immunosuppression protocols may influence HCC recurrence after transplant and competency of the immune system is a component that is involved in preventing recurrent HCC. It should not be forgotten that recurrent HCC has high mortality and is difficult to treat, whereas, early acute cellular rejection is treatable and has a low morbidity and mortality.

With regards to the influence of immunosuppression, the evidence that is available demonstrates that the best approach to preventing HCC recurrence after liver transplant is to reduce the number and levels of immunosuppressant agents to a minimum, early after transplant. Presently, the optimal immunosuppressant regime for HCC recurrence appears to be early CNI

minimization and in recipients at high risk of rejection to consider the addition of mTOR inhibitor or mycophenolate. As ever, well designed, prospective and randomized studies are needed, with sufficient patient numbers and follow up, to help establish an oncologically considered immunosuppressive regime.

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Footnote

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References

1. Bosetti C, Turati F, La Vecchia C. Hepatocellular carcinoma epidemiology. Best Pract Res Clin Gastroenterol 2014;28:753-70.
2. Cucchetti A, Vitale A, Del Gaudio M, et al. Harm and benefits of primary liver resection and salvage transplantation for hepatocellular carcinoma. Am J Transplant 2010;10:619-27.
3. Welker MW, Bechstein WO, Zeuzem S, et al. Recurrent hepatocellular carcinoma after liver transplantation - an emerging clinical challenge. Transpl Int 2013;26:109-18.
4. Sotiropoulos GC, Molmenti EP, Lösch C, et al. Meta-analysis of tumor recurrence after liver transplantation for hepatocellular carcinoma based on 1,198 cases. Eur J Med Res 2007;12:527-34.
5. Kornberg A, Kupper B, Tannapfel A, et al. Long-term survival after recurrent hepatocellular carcinoma in liver transplant patients: clinical patterns and outcome variables. Eur J Surg Oncol 2010;36:275-80.
6. Hollebecque A, Decaens T, Boleslawski E, et al. Natural history and therapeutic management of recurrent hepatocellular carcinoma after liver transplantation. Gastroenterol Clin Biol 2009;33:361-9.
7. Mazzaferro V, Regalia E, Doci R, et al. Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. N Engl J Med 1996;334:693-9.
8. Mazzaferro V, Bhoori S, Sposito C, et al. Milan criteria in liver transplantation for hepatocellular carcinoma: an evidence-based analysis of 15 years of experience. Liver Transpl 2011;17:S44-57.
9. Bruix J, Sherman M. Management of hepatocellular carcinoma. Hepatology 2005;42:1208-36.
10. 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 2008;143:182-8.
11. Rodríguez-Perálvarez M, Luong TV, Andreana L, et al. A systematic review of microvascular invasion in hepatocellular carcinoma: diagnostic and prognostic variability. Ann Surg Oncol 2013;20:325-39.
12. Liang W, Wu L, Ling X, Schroder PM, et al. Living donor liver transplantation versus deceased donor liver transplantation for hepatocellular carcinoma: a meta-analysis. Liver Transpl 2012;18:1226-36.
13. Grant RC, Sandhu L, Dixon PR, et al. Living vs. deceased donor liver transplantation for hepatocellular carcinoma: a systematic review and meta-analysis. Clin Transplant 2013;27:140-7.
14. Croome KP, Lee DD, Burns JM, et al. The Use of Donation After Cardiac Death Allografts Does Not Increase Recurrence of Hepatocellular Carcinoma. Am J Transplant 2015;15:2704-11.
15. Khorsandi SE, Yip V, Cortes M, et al. Does Does donation after cardiac death utilization adversely effect hepatocellular cancer survival? Transplantation 2016. [Epub ahead of print].
16. 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 2005;11:497-503.
17. Hojo M, Morimoto T, Maluccio M, et al. Cyclosporine induces cancer progression by a cell-autonomous mechanism. Nature 1999;397:530-4.
18. Huynh H, Chow KH, Soo KC, et al. RAD001 (everolimus) inhibits tumour growth in xenograft models of human hepatocellular carcinoma. J Cell Mol Med 2009;13:1371-80.
19. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011;144:646-74.
20. Kim R, Emi M, Tanabe K. Cancer immunoediting from immune surveillance to immune escape. Immunology 2007;121:1-14.
21. Yang L, Pang Y, Moses HL. TGF-beta and immune cells: an important regulatory axis in the tumor microenvironment and progression. Trends Immunol 2010;31:220-7.
22. Shields JD, Kourtis IC, Tomei AA, et al. Induction of lymphoid like stroma and immune escape by tumors that

express the chemokine CCL21. Science 2010;328:749-52.
23. Cescon M, Bertuzzo VR, Ercolani G, et al. Liver transplantation for hepatocellular carcinoma: role of inflammatory and immunological state on recurrence and prognosis. World J Gastroenterol 2013;19:9174-82.
24. Chen ZS, He F, Zeng FJ, et al. Early steroid withdrawal after liver transplantation for hepatocellular carcinoma. World J Gastroenterol 2007;13:5273-6.
25. Kim JM, Joh JW, Kim SJ, et al. Steroid withdrawal in adult liver transplantation: occurrence at a single center. Transplant Proc 2010;42:4132-6.
26. Vivarelli M, Bellusci R, Cucchetti A, et al. Low recurrence rate of hepatocellular carcinoma after liver transplantation: better patient selection or lower immunosuppression? Transplantation 2002;74:1746-51.
27. Vivarelli M, Cucchetti A, La Barba G, et al. Liver transplantation for hepatocellular carcinoma under calcineurin inhibitors: reassessment of risk factors for tumor recurrence. Ann Surg 2008;248:857-62.
28. Decaens T, Roudot-Thoraval F, Bresson-Hadni S, et al. Role of immunosuppression and tumor differentiation in predicting recurrence after liver transplantation for hepatocellular carcinoma: a multicenter study of 412 patients. World J Gastroenterol 2006;12:7319-25.
29. Rodríguez-Perálvarez M, Tsochatzis E, Naveas MC, et al. Reduced exposure to calcineurin inhibitors early after liver transplantation prevents recurrence of hepatocellular carcinoma. J Hepatol 2013;59:1193-9.
30. Vivarelli M, Dazzi A, Zanello M, et al. Effect of different immunosuppressive schedules on recurrence-free survival after liver transplantation for hepatocellular carcinoma. Transplantation 2010;89:227-31.
31. Chinnakotla S, Davis GL, Vasani S, et al. Impact of sirolimus on the recurrence of hepatocellular carcinoma after liver transplantation. Liver Transpl 2009;15:1834-42.
32. Zimmerman MA, Trotter JF, Wachs M, et al. Sirolimus-based immunosuppression following liver transplantation for hepatocellular carcinoma. Liver Transpl 2008;14:633-8.
33. Zhou J, Wang Z, Wu ZQ, et al. Sirolimus-based immunosuppression therapy in liver transplantation for patients with hepatocellular carcinoma exceeding the Milan criteria. Transplant Proc 2008;40:3548-53.
34. Toso C, Merani S, Bigam DL, et al. Sirolimus-based immunosuppression is associated with increased survival after liver transplantation for hepatocellular carcinoma. Hepatology 2010;51:1237-43.
35. Geissler EK, Schnitzbauer AA, Zülke C, et al. Sirolimus Use in Liver Transplant Recipients With Hepatocellular Carcinoma: A Randomized, Multicenter, Open-Label Phase 3 Trial. Transplantation 2016;100:116-25.
36. Xing T, Huang L, Yu Z, et al. Comparison of steroid-free immunosuppression and standard immunosuppression for liver transplant patients with hepatocellular carcinoma. PLoS One 2013;8:e71251.
37. Schlossmacher G, Stevens A, White A. Glucocorticoid receptor-mediated apoptosis: mechanisms of resistance in cancer cells. J Endocrinol 2011;211:17-25.
38. Sgourakis G, Radtke A, Fouzas I, et al. Corticosteroid-free immunosuppression in liver transplantation: a meta-analysis and meta-regression of outcomes. Transpl Int 2009;22:892-905.
39. Mazzaferro V, Rondinara GF, Rossi G, et al. Milan multicenter experience in liver transplantation for hepatocellular carcinoma. Transplant Proc 1994;26:3557-60.
40. McAlister VC, Haddad E, Renouf E, et al. Cyclosporin versus tacrolimus as primary immunosuppressant after liver transplantation: a meta-analysis. Am J Transplant 2006;6:1578-85.
41. Maluccio M, Sharma V, Lagman M, et al. Tacrolimus enhances transforming growth factor-beta 1 expression and promotes tumor progression. Transplantation 2003;76:597-602.
42. Datta D, Contreras AG, Basu A, et al. Calcineurin inhibitors activate the proto- oncogene Ras and promote protumorigenic signals in renal cancer cells. Cancer Res 2009;69:8902-9.
43. Rodríguez-Perálvarez M, Germani G, Papastergiou V, et al. Early tacrolimus exposure after liver transplantation: relationship with moderate/severe acute rejection and long-term outcome. J Hepatol 2013;58:262-70.
44. Rodríguez-Perálvarez M, Germani G, Darius T, et al. Tacrolimus trough levels, rejection and renal impairment in liver transplantation: a systematic review and meta-analysis. Am J Transplant 2012;12:2797-814.
45. Wiesner RH, Fung JJ. Present state of immunosuppressive therapy in liver transplant recipients. Liver Transpl 2011;17:S1-9.
46. Starzl TE. Immunosuppressive therapy and tolerance of organ allografts. N Engl J Med 2008;358:407-11.
47. Martínez-Llordella M, Puig-Pey I, Orlando G, et al. Multiparameter immune profiling of operational tolerance in liver transplantation. Am J Transplant 2007;7:309-19.
48. Boudjema K, Camus C, Saliba F, et al. Reduced-dose tacrolimus with mycophenolate mofetil vs. standard-dose tacrolimus in liver transplantation: a randomized study.

Am J Transplant 2011;11:965-76.
49. Neuberger JM, Mamelok RD, Neuhaus P, et al. Delayed introduction of reduced-dose tacrolimus, and renal function in liver transplantation: the 'ReSpECT' study. Am J Transplant 2009;9:327-36.
50. Suzuki S, Kimura T, Ando K, et al. Antitumor activity of mycophenolic acid. J Antibiot (Tokyo) 1969;22:297-302.
51. Domhan S, Muschal S, Schwager C, et al. Molecular mechanisms of the antiangiogenic and antitumor effects of mycophenolic acid. Mol Cancer Ther 2008;7:1656-68.
52. Rodríguez-Perálvarez M, Manousou P, Lerut J, et al. How much immunosuppression is needed after liver transplantation? Clin Transplant 2014;28:6-7.
53. Manousou P, Cholongitas E, Samonakis D. Reduced fibrosis in recurrent HCV with tacrolimus, azathioprine and steroids versus tacrolimus: randomised trial long term outcomes. Gut 2014;63:1005-13.
54. Benlloch S, Berenguer M, Prieto M, et al. De novo internal neoplasms after liver transplantation: increased risk and aggressive behavior in recent years? Am J Transplant 2004;4:596-604.
55. Xu X, Ye L, Araki K, et al. mTOR, linking metabolism and immunity. Semin Immunol 2012;24:429-35.
56. Hui IC, Tung EK, Sze KM, et al. Rapamycin and CCI-779 inhibit the mammalian target of rapamycin signalling in hepatocellular carcinoma. Liver Int 2010;30:65-75.
57. Sieghart W, Fuereder T, Schmid K, et al. Mammalian target of rapamycin pathway activity in hepatocellular carcinomas of patients undergoing liver transplantation. Transplantation 2007;83:425-32.
58. Chen K, Man K, Metselaar HJ, et al. Rationale of personalized immunosuppressive medication for hepatocellular carcinoma patients after liver transplantation. Liver Transpl 2014;20:261-9.
59. Villanueva A, Chiang DY, Newell P, et al. Pivotal role of mTOR signaling in hepatocellular carcinoma. Gastroenterology 2008;135:1972-83.
60. Gaumann A, Schlitt HJ, Geissler EK. Immunosuppression and tumor development in organ transplant recipients: the emerging dualistic role of rapamycin. Transpl Int 2008;21:207-17.
61. Schumacher G, Oidtmann M, Rueggeberg A et al. Sirolimus inhibits growth of human hepatoma cells alone or combined with tacrolimus, while tacrolimus promotes cell growth. World J Gastroenterol 2005;11:1420-5.
62. Piguet AC, Semela D, Keogh A, et al. Inhibition of mTOR in combination with doxorubicin in an experimental model of hepatocellular carcinoma. J Hepatol 2008;49:78-87.
63. De Simone P, Nevens F, De Carlis L, et al. Everolimus with reduced tacrolimus improves renal function in de novo liver transplant recipients: a randomized controlled trial. Am J Transplant 2012;12:3008-20.
64. Watson CJ, Gimson AE, Alexander GJ, et al. A randomized controlled trial of late conversion from calcineurin inhibitor (CNI)-based to sirolimus-based immunosuppression in liver transplant recipients with impaired renal function. Liver Transpl 2007;13:1694-1702.
65. Menon KV, Hakeem AR, Heaton ND. Meta-analysis: recurrence and survival following the use of sirolimus in liver transplantation for hepatocellular carcinoma. Aliment Pharmacol Ther 2013;37:411-9.
66. Liang W, Wang D, Ling X, et al. Sirolimus-based immunosuppression in liver transplantation for hepatocellular carcinoma: a meta-analysis. Liver Transpl 2012;18:62-9.
67. Watt KD, Dierkhising R, Heimbach JK, et al. Impact of sirolimus and tacrolimus on mortality and graft loss in liver transplant recipients with or without hepatitis C virus: an analysis of the Scientific Registry of Transplant Recipients Database. Liver Transpl 2012;18:29-1036.
68. Schnitzbauer AA, Zuelke C, Graeb C, et al. A prospective randomised, open-labeled, trial comparing sirolimus-containing versus mTOR-inhibitor-free immunosuppression in patients undergoing liver transplantation for hepatocellular carcinoma. BMC Cancer 2010;10:190.
69. Massoud O, Wiesner RH. The use of sirolimus should be restricted in liver transplantation. J Hepatol 2012;56:288-90.
70. Saliba F, Dharancy S, Lorho R, et al. Conversion to everolimus in maintenance liver transplant patients: a multicenter, retrospective analysis. Liver Transpl 2011;17:905-13.
71. Valdivieso A, Bustamante J, Gastaca M, et al. Management of hepatocellular carcinoma recurrence after liver transplantation. Transplant Proc 2010;42:660-2.
72. Zhu AX, Abrams TA, Miksad R, et al. Phase 1/2 study of everolimus in advanced hepatocellular carcinoma. Cancer 2011;117:5094-102.
73. Shiah HS, Chen CY, Dai CY, et al. Randomised clinical trial: comparison of two everolimus dosing schedules in patients with advanced hepatocellular carcinoma. Aliment Pharmacol Ther 2013;37:62-73.
74. Turner AP, Knechtle SJ. Induction immunosuppression in liver transplantation: a review. Transpl Int 2013;26:673-83.

75. Okita R, Yamaguchi Y, Ohara M, et al. Targeting of CD4+CD25high cells while preserving CD4+CD25low cells with low-dose chimeric anti-CD25 antibody in adoptive immunotherapy of cancer. Int J Oncol 2009;34:563-72.
76. Onrust SV, Wiseman LR. Basiliximab. Drugs 1999;57:207-13; discussion 214.
77. Bhoori S, Toffanin S, Sposito C, et al. Personalized molecular targeted therapy in advanced, recurrent hepatocellular carcinoma after liver transplantation: a proof of principle. J Hepatol 2010;52:771-5.
78. Llovet JM, Ricci S, Mazzaferro V, et al. Sorafenib in advanced hepatocellular carcinoma. N Engl J Med 2008;359:378-90.
79. Cheng AL, Kang YK, Chen Z, et al. Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomised, double-blind, placebo-controlled trial. Lancet Oncol 2009;10:25-34.
80. Tan WF, Qiu ZQ, Yu Y, et al. Sorafenib extends the survival time of patients with multiple recurrences of hepatocellular carcinoma after liver transplantation. Acta Pharmacol Sin 2010;31:1643-8.
81. Weinmann A, Niederle IM, Koch S, et al. Sorafenib for recurrence of hepatocellular carcinoma after liver transplantation. Dig Liver Dis 2012;44:432-7.
82. Waidmann O, Hofmann WP, Zeuzem S, et al. mTOR inhibitors and sorafenib for recurrent hepatocellular carcinoma after orthotopic liver transplantation. J Hepatol 2011;54:396-8.
83. Toso C, Mentha G, Majno P. Integrating sorafenib into an algorithm for the management of post-transplant hepatocellular carcinoma recurrence. J Hepatol 2013;59:3-5.
84. Sposito C, Mariani L, Germini A, et al. Comparative efficacy of sorafenib versus best supportive care in recurrent hepatocellular carcinoma after liver transplantation: a case-control study. J Hepatol 2013;59:59-66.
85. Zavaglia C, Airoldi A, Mancuso A, et al. Adverse events affect sorafenib efficacy in patients with recurrent hepatocellular carcinoma after liver transplantation: experience at a single center and review of the literature. Eur J Gastroenterol Hepatol 2013;25:180-6.
86. Staufer K, Fischer L, Seegers B, et al. High toxicity of sorafenib for recurrent hepatocellular carcinoma after liver transplantation. Transpl Int 2012;25:1158-64.
87. Finn RS, Poon RT, Yau T, et al. Phase I study investigating everolimus com- bined with sorafenib in patients with advanced hepatocellular carcinoma. J Hepatol 2013;59:1271-7.
88. Shetty K, Dash C, Laurin J. Use of adjuvant sorafenib in liver transplant recipients with high-risk hepatocellular carcinoma. J Transplant 2014;2014:913634.
89. Teng CL, Hwang WL, Chen YJ, et al. Sorafenib for hepatocellular carcinoma patients beyond Milan criteria after orthotopic liver transplantation: a case control study. World J Surg Oncol 2012;10:41.
90. Saab S, McTigue M, Finn RS, et al. Sorafenib as adjuvant therapy for high-risk hepatocellular carcinoma in liver transplant recipients: feasibility and efficacy. Exp Clin Transplant 2010;8:307-13.
91. Bhat M, Sonenberg N, Gores GJ. The mTOR pathway in hepatic malignancies. Hepatology 2013;58:810-8.
92. Brouwer C, Vermunt-de Koning DG, Trueworthy RC, et al. Monitoring of inosine monophosphate dehydrogenase activity in mononuclear cells of children with acute lymphoblastic leukemia: enzymological and clinical aspects. Pediatr Blood Cancer 2006;46:434-8.

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