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

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

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 antimitabolite e.g., mycophenolate or azathioprine, which may be a more favorable oncological strategy (29). However, the influence of antimitabolite 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 antimitabolite 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
Additionally, the rate of HCC recurrence 1 year after transplant recipients with hepatitis C (67). 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 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

Conflicts of Interest: The authors have no conflicts of interest to declare.

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