



Liver transplantation: immunosuppression and oncology

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Purpose of review

Long-term survival of liver transplant recipients is threatened by increased rates of de-novo malignancy and recurrence of hepatocellular carcinoma (HCC), both events tightly related to immunosuppression.

Recent findings

There is accumulating evidence linking increased exposure to immunosuppressants and carcinogenesis, particularly concerning calcineurin inhibitors (CNIs), azathioprine and antilymphocyte agents. A recent study including 219 HCC transplanted patients showed that HCC recurrence rates were halved if a minimization of CNIs was applied within the first month after liver transplant. With mammalian target of rapamycin (mTOR) inhibitors as approved immunosuppressants for liver transplant patients, pooled data from several retrospective studies have suggested their possible benefit for reducing HCC recurrence.

Summary

Randomized controlled trials with sufficiently long follow-up are needed to evaluate the influence of different immunosuppression protocols in preventing malignancy after LT. Currently, early minimization of CNIs with or without mTOR inhibitors or mycophenolate seems a rational strategy for patients with risk factors for de-novo malignancy or recurrence of HCC after liver transplant. A deeper understanding of the immunological pathways of rejection and cancer would allow for designing more specific and safer drugs, and thus to prevent cancer after liver transplant.

Keywords

cancer, hepatocellular carcinoma, immunosuppression, liver transplantation, malignancy

INTRODUCTION

The improvement in surgical techniques and medical care has prolonged survival after liver transplantation, leading to a parallel increase of long-term complications such as de-novo malignancy, which is becoming a major source of morbidity and mortality [1[•]]. Several population-based studies worldwide have reported a two- to three-fold increased cancer rates in liver transplant patients, when compared with age and sex-matched populations [2–12]. Moreover, in patients transplanted with hepatocellular carcinoma (HCC), tumour recurrence affects 15–20% of patients despite a careful selection of candidates based on the Milan criteria [13], and therapeutic options are very limited in this situation. In a recent analysis of 93 634 patients from the European Liver Transplant Registry (1968–2009), 21% of deaths occurred because of de-novo tumours or recurrence of HCC, demonstrating the critical importance of these complications in the current liver transplantation scenario [14].

The link between immunosuppression and oncogenesis is well established, as the integrity of the immune system is one of the defenses against cancer [15]. In the initial stages of carcinogenesis, several components of the immune system are able to locate and destroy cancer cells, delay tumour progression and prevent vascular invasion and metastasis. The immune system also allows for

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KEY POINTS

- De-novo malignancy after liver transplantation has become a major source of morbidity and mortality.
- The immunosuppression plays a central role in the increased risk of cancer after liver transplantation, including recurrence of hepatocellular carcinoma.
- CNIs, azathioprine and antithymocyte globulins have a pro-oncogenic effect, whereas mTOR inhibitors (everolimus and sirolimus) and mycophenolate seem to be protective against cancer.
- An early minimization of CNIs should be universally applied after liver transplantation and for selected patients with increased risk of rejection, the addition of either mTOR inhibitors or mycophenolate would be the most rational approach to prevent malignancy.

control of viral infections related to cancer. Animal models with defective function of natural killer cells and/or T cells (CD8⁺ cytotoxic or CD4⁺ T helper) have increased risk and aggressiveness of tumours, suggesting a cumulative cancer promoting effect, when both the innate and the adaptive immune pathways are impaired [16]. Conversely, cancer cells from highly aggressive tumours are able to paralyze infiltrating immune cells by secreting immunosuppressive molecules such as transforming growth factor (TGF)- β and CCL21 [17,18]. Indeed the types of cancer with the highest standardized incidence ratio after liver transplantation are related to infections (Kaposi sarcoma, nasopharyngeal carcinoma, cervical and vulvar cancer) [19], have an origin in the immune system (lymphoproliferative disorders particularly Burkitt lymphoma [20]), or are located in exposed areas (skin cancer, head and neck cancer) (Table 1) [2,3,5–8,10–12]. Thus, the increased risk of overall malignancy after liver transplantation is partly related to these otherwise less frequent tumours, leading to a specific ‘cancer pattern’ related to immunosuppression. It is not surprising that this ‘cancer pattern’ is reproduced in AIDS wherein effective antiretroviral therapies have prolonged survival [21], and establish a chronic immunosuppressive status [22,23]. New therapies that enhance the immune system are becoming a reality in the management of several types of cancer.

However, there are few studies evaluating immunosuppression protocols to prevent or reduce malignancy after liver transplantation, and they have a poor level of evidence (Fig. 1). There are no randomized controlled trials powered to detect differences in de-novo tumours or recurrence of HCC, mainly because of the heterogeneity in the biology of different types of cancer, and the

prolonged follow-up required. The available evidence comes from observational studies, and thus results should be interpreted with caution because of the great variability in clinical practice between liver transplant institutions, which increases the risk of bias when analyzing multicentre pooled data. Despite this situation, the general behaviour among clinicians is to minimize the exposure to immunosuppressants as much as possible after liver transplantation. With these background caveats, we present a comprehensive review of the available evidence about the relationship between the immunosuppressive drugs most frequently used in liver transplant patients and the risk of malignancy. We conclude with some recommendations to prevent de-novo malignancy and recurrence of HCC after liver transplantation.

CALCINEURIN INHIBITORS

Calcineurin inhibitors (CNIs) are the mainstay of immunosuppression after liver transplantation. Tacrolimus is the most frequently used because of reduced acute rejection rates and better graft and patient survival, compared with cyclosporine [24]. In-vitro studies and animal models have shown that both tacrolimus and cyclosporine are able to activate proto-oncogenes and pathways of cancer such as TGF- β in a dose-dependent fashion, thus promoting tumour proliferation, resistance to apoptosis and metastasis [25–27].

The risk of malignancy with tacrolimus or cyclosporine should be similar in clinical practice. To our knowledge, only one single-centre study has described an increased risk of malignancy in liver transplant patients treated with cyclosporine compared with tacrolimus [28]. However, the authors admit that a different monitoring was used for cyclosporine, and lower rejection rates were detected in this group, suggesting higher immunosuppressive potency with cyclosporine than with tacrolimus in this series. The only randomized controlled trial aiming to detect differences in de-novo malignancy compared two regimens with cyclosporine in kidney transplant recipients: conventional trough levels (i.e. 150–250 ng/ml) vs. reduced trough levels (i.e. 75–125 ng/ml). The group with conventional exposure had increased rates of de-novo tumours (32 vs. 19%; $P=0.03$) [29]. Thus, the risk of malignancy related to CNI in clinical practice may come from the dosage rather than the type of CNI used.

Several retrospective studies have shown a dose-dependent relationship between CNI and recurrence of HCC after liver transplantation [30,31,32^{***}]. The over-exposure to CNI may be particularly relevant

Table 1. Studies reporting types of cancer and their standardized incidence ratio after liver transplantation published in the last decade. Only selective data on liver transplant recipients are shown. The marked standardized incidence ratio values (*) indicate statistical significance at $P < 0.05$

Authors	Year	Country	Type	Period	n	Overall	Lymphoma	Skin	Head-neck	Renal	Others
Krynitz <i>et al.</i> [11]	2013	Sweden	National Survey	1970–2008	10476	3.4*	9.6*	1.6*	4.6*	1.9	Colon: 2.2; Breast: 1; Prostate: 0.5; Lung: 1.8.
Chairath <i>et al.</i> [10]	2013	United States	Single centre	1997–2004	534	3.1*	7.1*	–	–	–	–
Schrem <i>et al.</i> [6]	2013	Germany	Single centre	1983–2010	2000	1.94*	10.9*	–	Oral: 1.7; Larynx: 2.3.	2.6*	Colorectal: 1.41*; Breast: 0.83; Vulvar: 23.8*; Prostate: 0.62; Lung: 1.85*.
Engels <i>et al.</i> [8]	2011	United States	National survey	1987–2008	37888	–	Non-Hodgkin: 7.77*	–	–	1.8*	Lung: 1.95*; Liver: 43.8*.
Baccarani <i>et al.</i> [3]	2010	Italy	Two centres	1991–2005	417	2.6*	13.8*	–	7*	–	Colon: 1.4; Lung: 1.6; Breast: 0.6.
Jiang <i>et al.</i> [5]	2008	Canada	National survey	1983–1998	2034	2.5*	Non-Hodgkin: 20.8*	–	2.5	3.1	Colorectal: 2.6*; Breast: 0.6; Prostate: 1.
Aberg <i>et al.</i> [2]	2008	Finland	National survey	1982–2005	540	2.59*	Non-Hodgkin: 13.9*; Hodgkin: 14.7	38.5*	Lip: 21.3; Mouth: 14.8.	4.17	Colorectal: 1.59*; Breast: 0.26; Prostate: 1.24; Stomach: 4.97.
Collet <i>et al.</i> [7]	2010	United Kingdom	National survey	1980–2007	6846	2.2*	Non-Hodgkin: 13.3*; Hodgkin: 8.9*.	6.6*	Lip: 20*; Oral: 10*.	1.8	Colorectal: 2.3*; Breast: 0.8; Lung: 1.6*.
Oo <i>et al.</i> [12]	2005	United Kingdom	Single centre	1982–2004	1778	2.07*	10.3*	5.8*	–	–	Colon: 4.9*; Breast: 0.97*; Lung: 1.96*.

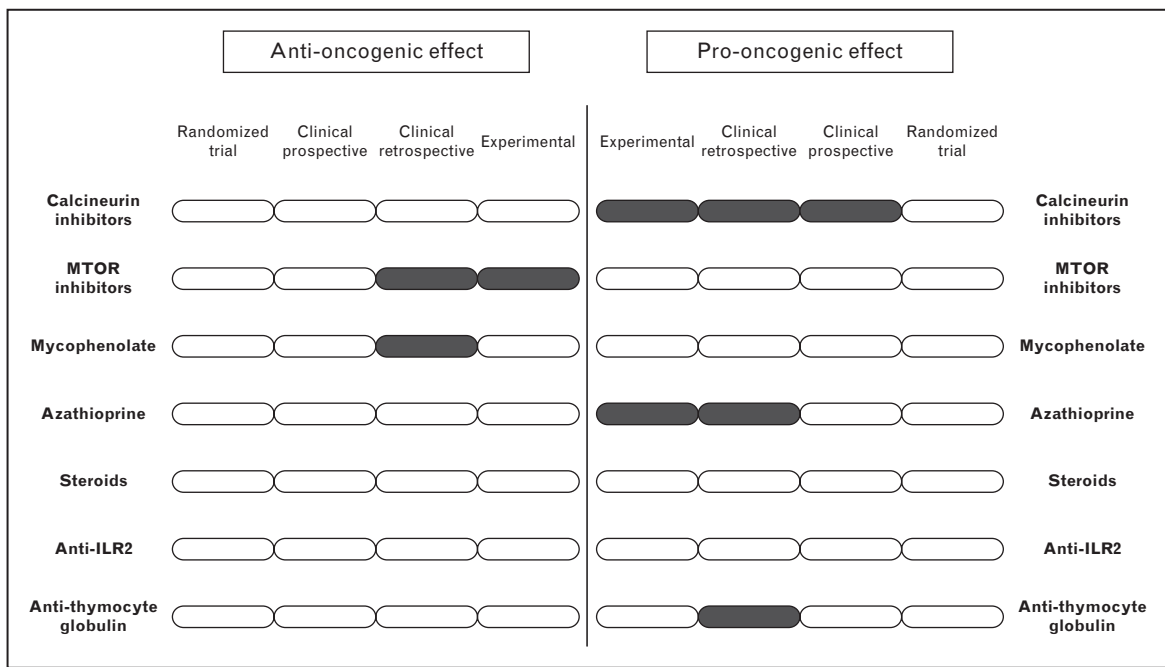


FIGURE 1. Summary of the level of evidence regarding the influence of the most frequently used immunosuppressive drugs after liver transplantation and the risk of malignancy (including lymphoproliferative disorders, any type of solid malignancy and recurrence of hepatocellular carcinoma). The figure points out the scarce number of studies available and the reduced level of evidence.

early after liver transplantation when the immune system should be able to detect and destroy remaining HCC cells in the bloodstream. Indeed, an observational study with 219 patients transplanted with HCC showed that those patients having mean trough concentrations more than 10 ng/ml for tacrolimus or more than 300 ng/ml for cyclosporine within the first 30 days after liver transplantation had nearly a three-fold increased risk of HCC recurrence, after controlling for possible confounding factors [32²²]. Moreover, tacrolimus trough concentrations 7–10 ng/ml within the first 30 days after liver transplantation result in similar rejection rates [33²¹], halved renal impairment [33²¹] and longer graft survival [34], when compared with trough concentrations more than 10 ng/ml, which is the ‘conventional’ exposure in randomized trials [35]. Therefore, tacrolimus trough concentrations 7–10 ng/ml should be the standard of care within the first month after liver transplantation, and future randomized trials should implement reduced tacrolimus trough concentrations in their design.

MTOR INHIBITORS

The mammalian target of rapamycin (mTOR) is a serine/threonine kinase involved in cellular growth, proliferation, metabolism and angiogenesis, and it comprises two signalling pathways: mTOR complex 1, responsible for cellular growth and proliferation

in response to immune regulatory signals, and mTOR complex 2 involved in aspects of cell metabolism [36²³]. There are two mTOR inhibitors available for liver transplant patients. Sirolimus is a non-selective inhibitor of mTOR complexes 1 and 2, and everolimus has a restricted effect on mTOR complex 1. Both drugs have shown immunosuppressive and anticancer properties in animal models including HCC [37–40].

In liver transplantation, the mTOR inhibitors are used mainly as renal sparing agents, owing to a potent immunosuppressive effect, which allows reduction of CNI exposure, or even for a conversion from a CNI based to a mTOR inhibitor-based regimen [41,42]. There are no published randomized controlled trials evaluating the effect of mTOR inhibitors in preventing de-novo malignancy or recurrence of HCC after LT. The available evidence is based on clinical reports and retrospective studies, thus making it difficult to extract solid conclusions. Despite this, many transplant centres frequently add or convert to a mTOR inhibitor when there are risk factors for malignancy after liver transplantation, or even when a tumour has been diagnosed. There are several reports of improved outcome of lymphoproliferative disorders and Kaposi sarcoma after switching to a mTOR inhibitor [43].

Regarding prevention of HCC recurrence, there are five retrospective studies evaluating the role of sirolimus-based immunosuppression after liver

transplantation [44–48], the results of which have been summarized in two meta-analyses [49,50]. The conclusions from both are similar suggesting a protective effect of sirolimus against HCC recurrence with an odds ratio (OR) ranging from 0.30 [95% confidence interval (CI) 0.16–0.55 to 0.42 (95% CI 0.21–0.83), and also a benefit in 5-year overall survival with OR from 0.35 (95% CI 0.20–0.61) to 0.40 (95% CI 0.28–0.58). However an analysis of 26 414 patients from the Scientific Registry of Transplant Recipients Database (USA) showed an increased risk of all-cause mortality in patients with hepatitis C treated with sirolimus (hazard ratio = 1.29; 95% CI 1.08–1.55) [51]. The limitations of these studies are evident: observational and retrospective design, some based on registries originally designed for a different purpose, and a high risk of reporting bias. The ongoing SiLVER study, which is a multicentre randomized controlled trial evaluating the role of sirolimus in HCC transplanted patients, may determine the true effect of mTOR inhibitors in preventing tumour recurrence, but the results will not be available before 2016 [52].

With respect to everolimus, there are no clinical studies suggesting a significant protective effect against HCC recurrence or de-novo malignancy after liver transplantation. As everolimus has a selective inhibition on mTOR complex 1, a more specific antitumour effect would be expected [37], and an improved metabolic profile has been hypothesized [53]. However, the activation of feedback signals makes prediction of the consequences difficult in clinical practice. The selective blocking of mTOR complex 1 with everolimus on the severity of hepatitis C recurrence and graft loss also deserves further investigation.

ANTIMETABOLITES

The antimetabolites interfere with de novo synthesis of nucleotides, and herein have a potent cytostatic effect on lymphocytes, which is more pronounced than in other cell types. Mycophenolate is the most widely used after liver transplantation, mainly in combination with CNI as a renal sparing agent [54,55]. To date there is no evidence suggesting a link between the use of mycophenolate and de novo malignancy after liver transplantation, even in the long term. A large observational study included 6751 renal transplant patients who received mycophenolate from the United Network for Organ Sharing (UNOS) and the collaborative transplant study registries. There was no significant increase in either lymphoma or any malignancy when compared with a matched cohort without mycophenolate. In the

subgroup from the collaborative transplant study, a longer time to malignancy was found for mycophenolate-treated patients (log rank 0.026) [56]. In 3895 heart transplant patients, the use of mycophenolate had a protective effect against de-novo malignancy (relative risk = 0.75; 95% CI 0.56–0.95) [57]. The studies evaluating the role of immunosuppression on HCC recurrence showed no influence of mycophenolate [31,32].

Azathioprine used as 1 mg/kg/day is preferred in some institutions for patients transplanted with hepatitis C because of a slower fibrosis progression and reduced risk of decompensation of severe recurrent disease, as recently shown in a randomized controlled trial with a median follow-up of 8 years and with protocol biopsies [58]. The prolonged use of azathioprine has been associated with an increased risk of nonmelanoma skin cancer, which may be explained by a dual mechanism, including an increased DNA damage powered by the interaction between ultraviolet-A radiation and 6-thioguanine, and an impaired DNA repair system [59,60]. The actual implication of nonmelanoma skin cancer, which usually is diagnosed at early stages, on prognosis is a matter of debate [61,62]. In inflammatory bowel disease patients treated with azathioprine, there is a four- to five-fold increased risk of lymphoproliferative disorders, especially in elderly patients, but still lymphoma is a very uncommon malignancy [63]. However, these data are hardly transferred to the liver transplant population, as doses of azathioprine are at least twice in patients with inflammatory bowel disease. The risk of Burkitt lymphoma is increased in liver transplantation patients compared with renal recipients according to a large registry from the USA, and azathioprine appeared as an independent risk factor in this cohort [20]. In liver transplantation patients, Benlloch *et al.* [64] retrospectively analyzed a single-institution experience (n = 772) on the development of non-skin malignancy and the risk factors involved. They found azathioprine as an independent predictor of any tumour development after liver transplantation (OR = 3.8; 95% CI 1.7–8.6; $P = 0.004$).

OTHER IMMUNOSUPPRESSIVES

Corticosteroids form part of most immunosuppression protocols within the first months after liver transplantation. The heterogeneity in dosage and tapering schedule between institutions makes it difficult to obtain a reliable analysis of pooled data [65], and there is no evidence suggesting that the use of corticosteroids influences de novo malignancy after liver transplantation. In a single registry-based

study, the use of corticosteroids was protective against Burkitt lymphoma [20].

The interleukin-2 receptor blockers (basiliximab and daclizumab – the latter has been withdrawn from the market) are frequently used immediately after liver transplantation as induction immunosuppression agents in order to delay the introduction of CNI as part of a renal-sparing strategy [66]. A large observational study with 929 renal transplant patients was unable to find any relationship between the use of anti-interleukin-2 receptors and de-novo malignancy. However, induction with anti-thymocyte globulins, which are polyclonal antibodies directed against multiple T- and B-cell antigens responsible for a depletion of peripheral lymphocytes, is associated with an increased risk for lymphoma after renal transplantation (0.67 vs. 0.43% with no induction; $P=0.002$), whereas basiliximab was not (0.38 vs. 0.43% with no induction; $P=0.33$) [67]. However, studies in liver transplant population are lacking.

FUTURE PERSPECTIVES

The reduced clinical significance of acute cellular rejection after liver transplantation, which progresses to chronic rejection and graft loss in less than 5% of patients, together with the increasing risk of renal impairment, infections and malignancy, demonstrates that liver transplant patients have been, and are still, over-immunosuppressed in many transplant centres [33,68]. There are still recent randomized trials using quadruple immunosuppression as a standard of care for liver transplantation patients [69], leading to unnecessarily reduced rejection rates, a more severe recurrence of hepatitis C, more frequent infections and new-onset diabetes [70]. A certain grade of acute rejection early after liver transplantation provides a benefit in terms of long-term survival [34,71], and it has been hypothesized that a complete suppression of acute rejection may prevent operational tolerance, and therefore it is neither necessary nor appropriate [71,72].

The current immunosuppression protocols in liver transplantation interfere with the immune system at many different levels simultaneously, impairing both the innate and the adaptive immune responses. A better understanding of the mechanisms underlying rejection is needed to develop more specific drugs, able to target selective rejection effectors, while keeping the ability to fight cancer cells, infections and to develop tolerance. In the future, a tailored 'cocktail' of monoclonal antibodies against specific cytokines, receptors and growth factors depending on individual patient's

immunological features will form the standard of care to prevent chronic rejection and graft loss. The therapeutic paradigm will change from immunosuppression to immunomodulation [73]. With the available immunosuppressants, the best approach to prevent malignancy after liver transplantation nowadays would be to decrease the number and the dosage of the immunosuppressive drugs to the minimum, starting as soon as possible after liver transplantation. A certain grade of immune response is desirable to prevent cancer, as it is to facilitate long-term graft tolerance. A single episode of acute rejection early after liver transplantation is easily managed and derived mortality is zero, whereas a single cancer forms a life-threatening condition with very limited therapeutic options.

CONCLUSION

The studies linking immunosuppression and cancer after liver transplantation have serious biases, which weaken the evidence: heterogeneity of immunosuppression protocols between centres, variable biologic tumour behaviour and inadequate follow-up. Future studies if these biases are removed will have a problem of increased follow-up costs. However, the evidence taken as a whole suggests that the immunosuppression regimen plays a central role in de-novo malignancy and in the recurrence of HCC after liver transplantation. The minimization of CNI should be universally applied, beginning as soon as possible after liver transplantation. In selected patients requiring a more potent immunosuppression, the addition of either mTOR inhibitors or mycophenolate would be the most rational approach. Prospective studies and randomized controlled trials with a large number of patients and sufficiently long follow-up are urgently needed to support the recommendations given, and to explore the best 'anti-tumour' immunosuppression regimen. The next steps should be to identify the critical nodes in the pathophysiology of rejection, and to develop more specific drugs with a better safety profile including reduced pro-oncogenic effects.

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Conflicts of interest

The authors of the present manuscript do not have any conflict of interest to disclose.

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