

The role of mTOR inhibitors in the prevention of organ rejection in adult liver transplant patients: a focus on everolimus

Teresa Casanovas

Liver Transplant Unit, Bellvitge
University Hospital, Barcelona, Spain

Abstract: Liver transplantation remains the therapy of choice for patients with end-stage liver disease and in selected cases of hepatocellular carcinoma. While short-term allograft survival has improved significantly in recent years, there has been little improvement in long-term survival after liver transplantation. A growing body of evidence on factors influencing the long-term outcomes and the safety profiles of existing immunosuppressive agents after liver transplant points to a need to continue searching for alternative strategies. The calcineurin inhibitors (CNIs) (cyclosporine and tacrolimus) currently represent the backbone of most immunosuppressor regimens. They have had a revolutionary effect on the overall success of transplantation, as is reflected in greatly reduced rates of acute rejection. However, the CNIs have significant toxicities that produce renal dysfunction, cardiovascular disease, and other unwanted effects, such as malignancies. The recognition of these risk factors has sparked interest in regimens that limit exposure to CNIs. Nowadays, the use of immunosuppressive drugs with different mechanisms of action, which allow for a reduction or avoidance of CNIs, is common. Everolimus, which belongs to the mammalian target-of-rapamycin inhibitor family and is best known for its use in kidney and heart transplantation, has recently been approved for liver transplantation. This overview discusses the emerging evidence on the role of everolimus in the prevention of rejection after liver transplantation, in de novo transplants, conversion regimens, or as a rescue therapy. In addition, some of the most relevant and current clinical problems related to everolimus in this field are discussed.

Keywords: everolimus, mTOR inhibitors, tacrolimus, liver transplant, cyclosporine, renal impairment

Introduction

Although it has been recognized that the liver is an immunologically privileged organ and that liver transplanted patients receive a less intense immunosuppressor treatment, compared against other transplants, immunosuppressive drugs continue to be necessary to control the allogeneic response.¹ While short-term survival after liver transplantation has notably improved in recent years, there remains a significant need to improve long-term survival.

Immunosuppressor treatments have specific and known toxicities that can be modified using different strategies.² Although the calcineurin inhibitors (CNIs), cyclosporine (CsA) and tacrolimus (Tac), have been recognized as one of the contributing causes of chronic kidney disease, they are presently the basic immunosuppressor drugs. Undesirable effects, such as hyperglycemia, hypertension, and increased incidence of de novo malignancy after transplant have been associated with these treatments.^{3,4}

Correspondence: Teresa Casanovas
Liver Transplant Unit (Hepatitis
Program), Bellvitge University Hospital,
C/Feixa Llarga s/n 08907, Hospitalet
de Llobregat, Barcelona, Spain
Tel +34 9326 07940
Email teresacasanovas@bellvitgehospital.cat

Ojo et al, in a population-based cohort study, estimated that after 5 years, 18.1% of liver transplant recipients suffered from chronic renal failure and were at higher risk of death after transplant (relative risk: 4.55).⁵ Data were gathered prior to the introduction of the model for end-stage liver disease (MELD) score. This is a scoring system which assesses the severity of chronic liver disease, and which is used to prioritize patients on the waiting list. The MELD score has been applied since 2002 in the USA, with the aim of “transplanting the sickest first”.

The MELD score, which has been demonstrated to be a useful tool in predicting chronic liver disease outcome,⁶ combines prothrombin time (international normalized ratio), bilirubin, and creatinine in a single formula. The serum creatinine concentration has the greatest weight in the formula, which is why patients nowadays frequently arrive for liver transplantation with severe kidney dysfunction.^{7,8}

The consequences of renal dysfunction in the early postoperative phase are very serious and influence poor outcomes and progressive renal failure, with increased risk of death. Causes of progressive kidney disease in a liver transplant setting are multifactorial, but CNI treatment has been recognized as a principal culprit. Current strategies for immunosuppression in liver transplant recipients consider risk factors such as renal failure, hepatitis C, cardiovascular complications, and malignancies.⁹

As well as CNIs, other therapeutic options are being studied. New therapies and revived strategies include the administration of monoclonal antibodies to lymphocyte T-cells (as inductive therapy), antimetabolites, mammalian target-of-rapamycin inhibitors (mTORi) and the delay and/or minimization of CNIs.

Kawahara et al¹⁰ have drawn attention to the need for new immunosuppressive drugs with new properties, for liver transplant, in order to achieve unmet objectives. These drugs should not have the toxicities associated with CNIs and should not have an adverse impact on hepatitis C virus (HCV) recurrence. These agents should also be able to minimize the risk of hepatocellular carcinoma (HCC) recurrence. Everolimus, with its specific therapeutic effects, is believed to have some of these qualities. However, the key to solving these open issues probably lies in a repertoire of drugs and combinations with different profiles, administered at different times after transplant.

Early evidence suggests that the mTORi, everolimus and sirolimus, may offer effective immunosuppressive activity, together with less nephrotoxicity, and may cover unmet

needs in long-term therapeutic management of the liver transplanted patient.^{11,12}

Data relating to the administration of mTORi come mostly from kidney transplant patients, whereas experience of mTORi after liver transplant is rather limited.¹² Initial studies, which were published years ago, were inconclusive and raised concerns about their toxicities. Recently, new data have been reported on the practical aspects of everolimus administration, contributing to evolving issues, such as mechanism of action, clinical utility, drug monitoring, and side effects.

This review summarizes emerging evidence for the use of mTORi-based immunosuppression, and is focused on the effectiveness of everolimus, after liver transplantation, to maximize graft and patient survival, while minimizing the risks of adverse events and avoiding known risks associated with CNIs.

Methods

A PubMed search was conducted using the keywords “mTOR”, “everolimus”, and “liver transplant”, limiting articles to those published in English or Spanish within the past 15 years. A search of the archives of internationally-recognized journals on transplantation was also carried out. In addition, in order to gain insight into new developments, the ClinicalTrials.gov archive was examined, as well as relevant information presented at recent liver transplant meetings. After reviewing the literature, we selected relevant publications, focusing on the role of everolimus in liver transplant. As regards this drug, it is also essential to extrapolate information provided from other solid organ transplantations.

Aims

This article aims to be a systematic review, providing answers to key questions and summarizing the most prominent clinical studies on the administration of everolimus after liver transplant. An emphasis will be put on new developments with everolimus in rejection prevention, efficacy in avoiding renal dysfunction, safety (depending on when after transplant everolimus is administered), role in *de novo* or conversion protocols, and use as a rescue therapy.

Results

It was not until 2012 that a definitive trial on everolimus was published, by De Simone et al.¹³ The study, conducted in an early posttransplant setting, represents a milestone in the development of everolimus. This report clearly defines

that everolimus has to be started during the acute phase (in de novo liver transplant recipients) – a clinical situation that has to be differentiated from the maintenance phase. Now, it is widely accepted that Week 4 is the best time to introduce everolimus, in order to avoid wound complications, although it is believed that further studies could determine an earlier start time. In conversion protocols, everolimus represents a switch from the previous immunosuppressor drug.

Immunosuppressor drugs: current standard of care after liver transplant

After transplantation, and in order to avoid rejection, clinicians employ combined bitherapy or triple therapy, administering CNI as a cornerstone therapy.

In recent years, important research has been done into the study of new drugs and drug combinations in sequential treatments. Different strategies can be used to reduce drug dosages, taking into account their synergistic immunosuppressive actions. Since no consensual protocols exist, the timing, dosing, and choice of immunosuppressive agents differ widely between centers.

Induction therapy

Induction therapy is the prophylactic administration of perioperative antibodies in addition to baseline immunosuppression. The aim of these drugs is to induce hyporesponsiveness in the recipient toward the transplanted organ, in order to prevent early rejection, thereby delaying administration of the CNI or even allowing for its avoidance.¹⁴

The administration of induction agents after liver transplant has increased in recent years, especially in patients more at risk of rejection, which is at its highest early posttransplant. The use of antibodies early after transplant achieves potent immunosuppression to prevent acute rejection, giving the clinician the opportunity to optimize baseline immunosuppressive management and to delay the use of nephrotoxic agents (CNIs) while the liver graft and kidney reach a baseline function. Basiliximab (Simulect®, Novartis, Basel, Switzerland) is a monoclonal antibody that specifically binds and blocks the interleukin-2 receptor alpha chain on activated T-cells.¹⁴

Basiliximab is well-tolerated and administered in two doses: within 6 hours after reperfusion, and on Day 4 post-transplant. Usually, during these first few days, the recipient could begin with a low dose of Tac, with or without corticosteroids and/or mycophenolate mofetil (MMF).¹⁵

Alloreactivity tends to decline during the maintenance period. In cases of acute rejection, higher immunosuppression is required.⁹

Maintenance immunosuppressive regimens

The role of CNIs (CsA and Tac) has been crucial until now. Both drugs have comparable immunosuppressive effects, but accumulated experience has favored combining immunosuppressor drugs.

A few months after the transplant, the administration of two drugs is usual (a CNI combined with MMF), to maintain an immunosuppressive antirejection state. The major advantage in using MMF is the lack of renal toxicity. Since the late 1990s, patients with preexisting renal disease have been receiving MMF in conjunction with a low dose of CNIs, as part of a renal-sparing protocol.¹⁶

Natural course in patients receiving CNIs

The long-term administration of CNIs adversely affects renal function and can also worsen other clinical baseline conditions, such as neurotoxicity, glucose metabolism, hypertension, obesity, metabolic syndrome, and hepatitis C, which have also been involved in renal dysfunction.¹⁷ In an attempt to avoid the adverse effects linked to CNIs, mTORi has been proposed as an alternative to regimens based on them.¹⁸ In addition, organ transplant recipients are at higher risk than the general population of developing de novo malignancies; mTORi may play a role in the prevention and treatment of cancer in liver transplant receptors.¹⁹

Mammalian target-of-rapamycin inhibitors

The mTORi, sirolimus (Rapamune®, Pfizer Inc., New York, NY, USA) and everolimus (Certican®, Novartis), are potent immunosuppressors and proliferation signal inhibitors, with several advantages over CNIs, especially due to their lack of nephrotoxicity. The transplant community has been working on these drugs for the last 15 years, although only everolimus has been developed and approved for administration after liver transplantation.²⁰

Sirolimus was the first mTORi to be developed and approved for kidney transplants. Its efficacy and safety was demonstrated – combined with MMF or azathioprine, in order to avoid CNIs.²¹ The administration of sirolimus was then extended to selected liver transplanted patients, and some series on clinical experiences administering sirolimus were published (see below). But, the protocols used were heterogeneous and their results were inconclusive.²²

Sirolimus and everolimus: mechanisms of action and interesting pharmacological facts

After their absorption, sirolimus and everolimus form a complex with the cellular FK binding protein complex (FKBP-12), downregulating p70S6 kinase activity and, subsequently, the translation of specific mRNAs, which results in halting the G1/S phase of cell cycle progression. Despite their similarities, these two molecules have important pharmacokinetic differences. Notably, the half-life of everolimus (28 hours) is considerably shorter than that of sirolimus (62 hours), and whereas everolimus reaches steady state in 4 days, sirolimus takes 6 days.^{23,24}

Extensive drug–drug interactions exist (when mTORi is coadministered with drugs metabolized by the cytochrome P450 [CYP] system). This is an essential consideration that has to be taken into account when an mTORi is prescribed in combination with a CNI,²⁵ because the mTORi works synergistically with the CNI (especially with CsA), and this allows minimization of CNI exposure.

Everolimus was developed in an attempt to improve the pharmacokinetic characteristics of sirolimus; in particular, to increase its oral bioavailability and facilitate its clinical administration (due to its shorter half-life).²⁶ Everolimus is very similar to sirolimus, except for a structural difference – a chain substitution at position 40 on the sirolimus (rapamycin) structure confers different pharmacokinetic characteristics.²⁷

Although everolimus has been investigated in clinical development programs for the prophylaxis of organ transplant rejection since 1996,²⁸ its approval by the United States Food and Drug Administration (FDA), as an antirejection drug in de novo heart, kidney, and liver transplantations, has only been obtained recently. Everolimus is also administered in oncological treatments, and is additionally used in drug-eluting stents, considering its antiproliferative properties (Table 1).

Everolimus pharmacokinetics

Everolimus is rapidly absorbed orally, with a median time to maximal plasma concentration of 1 hour, after a single dose in stable, adult, transplanted recipients.²⁹ It is metabolized by the liver and eliminated in the bile, after having systemic exposure.³⁰

Everolimus safety and pharmacokinetics were studied in patients with moderate hepatic impairment and in healthy volunteers. In patients with moderate hepatic impairment (Child-Pugh classification: B), who may show a twofold

Table 1 Potential clinical benefits and adverse effects of the mTORi drugs: sirolimus and everolimus

Beneficial effects	Adverse effects
Prevents rejection and chronic graft dysfunction	Dyslipidemia
Improvement or prevention of atheromatosis	Proteinuria
Less left ventricular hypertrophy	Diabetes
Improves blood pressure	Edemas
Less risk of recurrent tumor	Immunological protection
Less incidence of CMV	Pneumonitis
Useful as a rescue drug in case of toxicity	Anemia, leukopenia
Related to other immunosuppressors	Skin rash

Abbreviations: mTORi, mammalian target-of-rapamycin inhibitor; CMV, cytomegalovirus infection.

prolongation in elimination half-life, the dosage of everolimus should be reduced. It should not be recommended for patients with severe hepatic impairment, unless the benefits outweigh the risks.³¹ In the event of impaired creatinine clearance, dose adjustment is not needed.

Drug–drug interactions

CsA, Tac, and mTORi (sirolimus, everolimus) interact in particular ways when administered concomitantly with substances that inhibit or induce CYP 3A4 and P-glycoprotein. These interactions may lead to a modification of the levels of immunosuppressive drugs in the blood.^{32,33}

The coadministration of everolimus/tacrolimus appears to have minimal effect on trough levels of everolimus, compared against the observed influence of CsA. This circumstance, confirmed in several studies, is why Tac is currently the preferred standard of care in protocols which administer everolimus concomitantly.³⁴

Adverse effects associated with sirolimus and everolimus in liver transplant

Some adverse events and clinical complications, secondary to the administration of mTORi, have been observed and well-documented. Initial studies with sirolimus observed proteinuria, hypercholesterolemia and hypertriglyceridemia, bone marrow suppression, interstitial pneumonitis, peripheral edema, dermatological effects (acne, mouth ulcers), and delayed wound healing.^{35,36}

Interstitial pneumonitis is a side effect related to mTORi drugs, which resolves on withdrawal of the drug.³⁷ In most recent studies on everolimus, adverse events have been less frequent and less severe, probably because transplant centers are more aware that lower doses of everolimus are needed in liver transplants.³⁸

Conversion to sirolimus in liver transplantation

Sirolimus was the first mTORi used in a transplant setting, but it was not until its results in kidney transplant were published that some clinicians considered it as a therapeutical option in selected liver transplanted patients. Table 2 shows a summary of selected studies on conversion to sirolimus in liver transplantation.

Despite its associated toxicities, sirolimus offered a therapeutic option for patients with renal or neurological impairment after liver transplant. It was administered for many years as a rescue drug.³⁹

The first reported study illustrating the effectiveness of sirolimus monotherapy for maintenance of immunosuppression in liver transplantation was published in 1999 by Watson et al.⁴⁰ However, two subsequent, larger studies that examined sirolimus in de novo therapy, in combination with Tac and corticosteroids, were terminated early, due to excess of hepatic artery thrombosis. As a result, sirolimus was not developed for liver transplant at the time.

Chang et al⁴¹ reported their experience using sirolimus in 14 liver transplant recipients, for whom CNIs were contraindicated, due to renal insufficiency or acute mental status impairment. Some relevant outcomes should be noted. Sirolimus was first administered at loading doses of 5–10 mg/day, and afterwards at fixed doses of 1–4 mg/day, combined with MMF and corticosteroids. The follow-up was short: only 2–7 months. CNIs were initially withheld in 9 patients. The remaining 5 patients could not receive sirolimus, due to toxicity. It is interesting to note that serum trough levels of sirolimus did not correlate with the doses administered. According to the authors, sirolimus was a

therapeutic option after liver transplantation in patients with neurological or renal complications, and was considered to be an attractive alternative when CNIs are undesirable. However, they recommend a prospective, randomized study of a sirolimus-based CNI-avoiding regimen, comparing with standard therapy, to further evaluate the role of sirolimus in liver transplantation.

Kniepeiss et al⁴² presented a retrospective follow-up study of late conversion in 7 patients, due to renal or neurological impairment. They switched to sirolimus and MMF from CsA or Tac. Doses were administered depending on blood levels, and selected trough levels were 4–10 ng/mL. Patient and graft survival was 100%; no rejection episodes or infections were observed. Renal function and neurological complications improved in all cases. The side effects of hypertriglyceridemia, hypercholesterolemia, and exanthema, were important in 3 patients; in 2 of them, it was necessary to stop therapy.

Zimmerman et al⁴³ in 2008 published a comparative study, administering sirolimus in a group of patients, and observed a potential survival benefit.

Di Benedetto et al⁴⁴ administered sirolimus monotherapy to 26 patients who developed nephrotoxicity, owing to CNIs. The initial doses were 3–5 mg/day, subsequently adjusted to achieve trough levels of 8–10 ng/mL. After a follow-up of 27.5 months (range: 2–71 months), renal function (creatinine, urea, and estimated glomerular filtration rate [GFR]) significantly improved. The authors recommended that sirolimus be initiated when renal dysfunction is first noted. Otherwise, the complication would be irreversible.⁴⁵

Campsen et al⁴⁶ compared patients receiving sirolimus or CNIs during the first year after liver transplant.

Table 2 Summary of selected studies on conversion to sirolimus in maintenance period after liver transplantation

Year of publication Author	N	Study design	IS treatment	Results	Limitations
1999 Watson et al ⁴⁰	15	Observational	SRL + reduced CsA ± corticosteroids	Rejection more common on monotherapy	Patients in poor clinical condition
2000 Chang et al ⁴¹	12	Observational	SRL + MMF + corticosteroids	Improvement of liver and kidney function	Heterogeneous population
2005 Kniepeiss et al ⁴²	58	Comparative study	Study group: SRL + MMF ± Tac	Improvement of renal function in study group	Authors suggest use of SRL combined with Tac low dose
2008 Zimmerman et al ⁴³	97	Comparative study (52 control vs 45 SRL)		Potential survival benefit in patients with sirolimus	
2009 Di Benedetto et al ⁴⁴	26	Observational	Conversion from CNI to SRL	eGFR improved significantly	Few cases. Relatively high doses and blood levels
2011 Campsen et al ⁴⁶	688	Center database	Five groups, depending on IS treatment	No differences in survival. Study group 50% less rejection	

Abbreviations: IS, immunosuppression; CsA, cyclosporine; Tac, tacrolimus; MMF, mycophenolate mofetil; SRL, sirolimus; CNI, calcineurin inhibitor; eGFR, estimated glomerular filtration rate.

They retrospectively assessed the results of 688 transplants at their center, divided into four groups, depending on treatment at the time of discharge: one group receiving CNI + mycophenolate sodium (MPS), and three groups receiving CNI + MPS + sirolimus (which was added at different times). The objectives were to review mortality, graft loss, GFR, and acute rejection. There were no significant differences in mortality or graft loss, but patients who received sirolimus as the primary immunosuppression had 50% less rejection, compared against controls.

In summary, previous data showed that sirolimus could optimize outcomes, preventing rejection, progression of kidney dysfunction, and other complications. However, the studies had limitations. They were retrospective, with few cases, and with short follow-up. These preliminary studies did not generate scientific evidence.

Everolimus: developing program on kidney and heart transplant

Phase I trials in kidney transplant were conducted in 1999 and established the safety and tolerability of everolimus.⁴⁷ In addition, preliminary data demonstrated that fixed doses of everolimus (2 mg/day or 4 mg/day) led to a lower incidence of acute rejection.⁴⁸

In controlled clinical trials for de novo kidney transplant recipients, fixed doses of everolimus were used (1.5 mg/day or 3 mg/day), combined with different doses of CsA, and were compared with a control group who received a combination of CsA and MMF. Results evaluated after 12 months and 36 months showed that CsA dose requirements for patients receiving everolimus were lower than for the control group.⁴⁹

Conflicting information came from Phase II and Phase III clinical trials in de novo heart transplantation. In a controlled, international, double-blind trial, a 2-year analysis of 634 de novo heart transplant recipients assessed the safety and efficacy of everolimus, compared against azathioprine. The overall results showed that the incidence of allograft vasculopathy was lower in the group that received everolimus, but the control arm had better creatinine clearance. In the study group, the development of adverse events was higher. This, which seems contradictory, was due to the administration of fixed doses of everolimus and the undetected toxicity associated with CsA (which was unknown at the time).⁵⁰ Consequently, studies following aimed to minimize CsA exposure.⁵¹

In the pivotal Phase III studies on kidney transplant, a target trough level of more than 3 ng/mL was established

for patients receiving everolimus. It was demonstrated that the addition of induction therapy in the acute period enabled the reduction of biopsy proved acute rejection (BPAR). Also, comparing two target trough levels (3–8 ng/mL and 6–12 ng/mL), although everolimus was administered initially at fixed doses of 1.5 mg or 3 mg daily, the best option was for target levels to be 3–8 ng/mL. These results led to the approval of everolimus in 2010 by the FDA, in low-to-moderate risk kidney transplant patients, in combination with basiliximab and steroids, administering everolimus combined with CsA.^{52,53}

Everolimus: developing program in liver transplant: clinical trials

Patients are selected for everolimus therapy based on when and how their condition could benefit from it. Relevant studies are classified as follows:

1. De novo liver transplant recipients (Table 3).
2. Maintenance liver transplant patients (Table 4).
3. Everolimus as rescue therapy.

I. Everolimus in de novo liver transplant recipients

Initial experiences were published by Levy et al⁵³ in 2006. They published an international, randomized, placebo-controlled, Phase II trial in liver transplanted recipients, comparing four groups who were treated with different doses of everolimus, associated with CsA and corticosteroids. The first objective was to evaluate the safety and tolerability of everolimus. Secondary objectives were to investigate the efficacy of everolimus in avoiding rejection and graft failure, at 12 months and 36 months. Results showed that trough levels of less than 3 ng/mL were associated with higher rates of rejection. Graft losses were related to posttransplant complications, but not connected with study medication or hepatic artery thromboses. Wound complications in everolimus-treated patients were not observed, but the higher-dose group suffered an increased number of adverse events.⁵³

The small number of patients in each group (about 30), the elevated dropout rate (related to adverse events), and the use of CsA as the basic CNI make this study difficult to interpret. Subsequent studies have discussed these issues.

Fischer et al⁵⁴ (PROTECT study) evaluated the renal protective effects of everolimus, which was started 30 days after transplantation in 203 patients, who were divided into four groups. GFR, which was assessed 11 months after randomization, showed a significant improvement in the everolimus group. But, the primary hypothesis – that everolimus was superior to CNI – was not verified, because patients receiving

Table 3 Summary of everolimus studies on de novo liver transplanted patients

Year of publication Author	N	Study design	Immunosuppressive treatment	Results	Limitations
2006 Levy et al ⁵³	119	Randomized, controlled trial (4 groups)	Everolimus: three different doses (0.5 mg, 1 mg, and 2 mg) twice-daily, combined with CsA or placebo	Trend toward less acute rejection	Inconclusive results, due to few cases for group
2010 Masetti et al ⁵⁵	78	Randomized, controlled trial	CsA for 10 days, after CsA + everolimus, then continuation or everolimus monotherapy	Early withdrawal of CsA, followed by everolimus: better renal function	No statistically significant differences
2012 De Simone et al ¹³	719	Randomized, controlled trial to receive everolimus at Day 30	Three arms: Tac elimination (N=231), everolimus + reduced Tac (N=245), and Tac standard (N=243)	eGFR superior in everolimus + reduced Tac, 12 months. After liver transplant	Tac elimination group was suppressed, due to the risk of rejection
2013 Saliba et al ⁵⁶		Extension to 24 months of the previous study		Early introduction of everolimus provided renal benefit at 2 years	
2012 Fischer et al ⁵⁴	203	Randomized, controlled, multicenter trial CNI (N=102) versus everolimus (N=101)		Safe alternative that deserves further investigation	
2014 Sterneck et al ⁵⁷	203	Randomized, controlled, multicenter study	3-year results from the PROTECT study population of the previous study	Only patients with mild renal dysfunction were randomized	

Abbreviations: CsA, cyclosporine; Tac, tacrolimus; eGFR, estimated glomerular filtration rate; CNI, calcineurin inhibitor.

everolimus had more infections, toxicities, and more cases of discontinuation than in the CNI group.

Masetti et al⁵⁵ reported the results of a randomized trial, assessing whether the early withdrawal of CsA, followed by the initiation of everolimus monotherapy, in de novo liver transplantation patients would result in superior renal function, compared against a CsA-based immunosuppression protocol. Seventy-eight patients were randomized to receive everolimus (N=52) or CsA (N=26). All patients were treated with CsA for the first 10 days. They were then randomized to receive everolimus in combination with CsA up to Day 30, and were then either continued on everolimus monotherapy (everolimus group) or maintained on CsA (CsA group), with or without MMF, in case of chronic kidney disease. There was no statistically significant difference in patient survival between the two groups, but renal function, as measured by GFR at 12 months, was significantly better in the everolimus group (87±26 mL/min versus 59±12 mL/min;

$P<0.001$). The incidence of advanced chronic kidney disease was higher in the CsA group at 1 year (52.2% versus 15.4%; $P=0.005$). The results of this study indicate that early withdrawal of CsA, followed by everolimus monotherapy, in de novo recipients is associated with an improvement in renal function, with similar incidence of rejection and other complications.

Recently, a definitive trial on everolimus in early posttransplant liver recipients has been published by De Simone et al.¹³ This study has proven everolimus safety, tolerance, and effectivity, and has become the registration protocol.

Moreover, Saliba et al specifically reported kidney function outcomes after 24 months in controlled patients of the above-mentioned study.⁵⁶

In the registration protocol, 716 de novo liver transplanted patients were stratified by their hepatitis C status and renal function. The design of this prospective, open-label,

Table 4 Selected studies of conversion to everolimus during maintenance period

Year of publication Author	N	Study design	Immunosuppressive treatment	Results	Limitations
2009 De Simone et al ⁵⁹	145	Prospective, randomized, multicenter	Everolimus with CNI reduction or discontinuation	80% discontinued CNI. Better renal function	Lack of trials targeting earlier period
2011 Saliba et al ⁶⁰	240	Multicenter, retrospective. Assessment of everolimus in daily practice	Changing CNI to everolimus in routine clinical practice	60% of patients were free of CNI. Low risk of rejection	Retrospective
2013 Alegre et al ⁷²	57	Observational	Everolimus: 24 cases in monotherapy versus 33 cases in combination	Improvement of renal function. Usual adverse events	Prevention of HCC was not demonstrated

Abbreviations: CNI, calcineurin inhibitors; HCC, hepatocellular carcinoma.

registration trial reflects accumulated learning experience. The most important features of this protocol are: the delayed introduction of everolimus (Day 30 after transplant); the moderate trough levels of everolimus (3–8 ng/mL, in combination with Tac); reduced target levels (3–5 ng/mL), and the duration of the study (12 months).

Saliba et al⁵⁶ reported renal function results of the H2304 Study at 2 years. The study demonstrated that the change in GFR, from randomization to Month 24, was superior with everolimus combined with reduced Tac, compared against the Tac control. Study medication was discontinued due to adverse events in 28.6% of patients in the everolimus group and in 18.2% of Tac control patients. The authors concluded that early introduction of everolimus, with reduced exposure to Tac, provided a significant and clinically relevant benefit for renal function. Common everolimus-related adverse events were reported (Table 5).

2. Conversion studies in maintenance liver transplant patients

Data on the conversion of patients to everolimus after liver transplantation are sparse. It should be noted that there is some overlap between de novo transplanted recipients (everolimus in induction studies) and patients in the maintenance period, which (theoretically) starts after 6 months posttransplant. Concepts such as early or late conversion to everolimus may be misleading, given that late conversion

means everolimus administration starts during the maintenance phase, 6 months after liver transplant.

The efficacy and safety of immunosuppressive regimens containing an mTORi with Tac minimization therapy in solid organ transplant recipients were reviewed by Peddi et al.⁵⁸ The authors identified and evaluated twenty-one relevant studies of conversion to mTORi combined with Tac at low doses, focusing on toxicities related to immunosuppressive drugs. They selected studies comprising 2,201 kidney, 260 heart, 108 lung, and 757 liver transplanted patients who were treated with an mTORi plus Tac at low doses. In a subanalysis of twelve controlled clinical trials of the previous groups, lower rates of infection (BK virus, cytomegalovirus, or Epstein–Barr virus) or malignancy (0%–7%) were observed, but with a high proportion of adverse events. Although significant changes in patient survival or graft rejection rates were not achieved, the authors concluded that regimens including an mTORi and Tac at low doses preserved better renal function, compared against standard-dose Tac.

A large, multicenter study on everolimus conversion was published in 2009 by De Simone et al,⁵⁹ evaluating the efficacy and safety of everolimus in 145 liver transplant patients in the maintenance period while eliminating or reducing Tac. This conversion study was prospective, randomized, and multicenter. Patients started everolimus therapy with a CNI reduction or discontinuation (N=72) or continued receiving standard CNI (N=73). At Month 6, 80% of patients who had converted to everolimus discontinued the CNI. The primary study end-point was not achieved, because the mean change in creatinine clearance from baseline to Month 6 was similar between groups. In line with a protocol amendment, monitoring continued for 6 months. No significant differences were detected among patients who continued everolimus. Renal dysfunction was irreversible in the majority of cases. The high frequency of CNI dose reductions in controls (77% of the patients) and the relatively long mean time posttransplant (>3 years) are likely to have contributed to the small difference between groups. The authors therefore recommended further trials targeting earlier conversion, to confirm the efficacy and safety of everolimus for improving renal function in liver transplant.

In 2011, Saliba et al⁶⁰ published a multicenter, retrospective analysis of the use of everolimus in routine clinical practice in maintenance liver transplant recipients. In this study, a survey was conducted to analyze current indications for everolimus conversion and the regimens employed. Exposure levels were examined, as well as the impact on efficacy and safety, in 240 maintenance liver transplant patients (Table 5).

Table 5 Adverse everolimus-related events and infections of clinical interest observed in patients of a French retrospective multicenter study (N=245)

Adverse event	N (%)
Any adverse event	203 (84.6%)
Gastrointestinal disorders	55 (22.9%)
Skin rash	45 (18.8%)
Edema	39 (16.3%)
Hypertriglyceridemia	35 (14.6%)
Stomatitis/mouth ulcerations	34 (14.2%)
Hypercholesterolemia	32 (13.3%)
Anemia	31 (12.9%)
Bacterial infection	30 (12.5%)
Leukopenia	22 (7.1%)
Pneumonia	17 (6.3%)
Thrombocytopenia	15 (5.8%)
Viral infection	14 (5.4%)
Proteinuria	13 (3.8%)
Acne	9 (2.9%)
Biopsy-proven acute rejection	4 (1.6%)

Note: Adapted with permission from Saliba F, Dharancy S, Lorho R, et al. Conversion to everolimus in maintenance liver transplant patients: a multicenter, retrospective analysis. *Liver Transpl*. 2011;17(8):905–913. Copyright 2013 The American Society of Transplantation and the American Society of Transplant Surgeons.⁶⁰

The mean time between transplantation and the introduction of everolimus was 4.9 ± 5.2 years. The mean everolimus trough levels were 7 ± 4 ng/mL at Month 1, and 8 ± 4 ng/mL at Month 12. After 12 months, 61.6% of the patients were no longer receiving CNI therapy. The mean GFR was 64 ± 30 mL/min at baseline, and 68 ± 32 mL/min at Month 12 ($P=0.007$). Four patients (1.6%) developed mild or moderate BPAR. In summary, this retrospective analysis demonstrated that $>60\%$ of the patients were kept free of CNIs, the risk of acute rejection was low, and the safety profile was acceptable.

Conversion protocols to everolimus after liver transplantation have focused on practical issues. What are the most appropriate and effective strategies for introducing everolimus for conversion, or in de novo liver transplantation? According to cases published so far, in current practice, CNIs are stopped or significantly reduced when everolimus is started. In our chronic patients, we usually overlap the CNI when starting everolimus at a very low dose (0.25 mg per 12 hours), to achieve trough levels of 3–5 ng/mL, and adjust both drugs over a period of 2 weeks while we check the tolerance. However, conversion can be performed abruptly, in cases of conversion for cancer or toxicity.

3. Everolimus as a rescue therapy

Some of the properties of mTORi are linked to the avoidance of specific disorders commonly observed after transplants, which is why the pros and cons of this family of drugs have to be taken into account.

Everolimus has been administered as a rescue medication when the clinical conditions of patients are poor. So, their baseline status could imply heterogeneous results.

Some typical clinical situations where everolimus could be beneficial are discussed below.

Everolimus may be chosen to manage malignant diseases after transplantation. In post kidney transplants, both mTORi have been associated with a significant decrease of malignancies. Specific clinical guidelines recommend them for renal transplant recipients who had a pretransplant malignancy or who have developed de novo cancer after transplant.

There is a lack of clinical, randomized, controlled trials to have examined the anticancer effects of mTORi in liver transplant recipients, in particular those related to HCC, but some studies are in progress.

Some research about renal-sparing strategies in liver transplant recipients that has been published recently suffers from poor methodology or a short duration of follow-up (usually 6–12 months). The results have failed to show

conclusive outcomes. Until recent years, the results of everolimus trials have been hampered by exclusions, due to clinical conditions and the heterogeneity of participants. Thus, their conclusions should be considered with caution, because the results did not address long-term benefits and outcomes.

Initially, renal dysfunction (as previously mentioned) was the main cause for adding everolimus.^{61,62} But, the use of everolimus may be indicated in other acute or chronic clinical situations. Neurotoxicity related to CNI administration is not a common problem, but the clinical presentation can be very serious, varying from headaches and tremors to agitation, confusion, hallucinations, and overt psychosis, which is why everolimus is a favorable option.⁶³

In posttransplant lymphoproliferative disorder, everolimus was added as a therapeutic option when planning specific treatment, surgical resection, or rituximab and/or chemotherapy when applicable.⁶⁴

In autoimmune diseases, including de novo autoimmune hepatitis, adding everolimus may allow for the withdrawal of corticosteroids. This entity requires an aggressive immunosuppressive regimen, the administration of corticosteroids being the standard of care.⁶⁵ Treatment of de novo autoimmune hepatitis, appearing during HCV therapy with interferon, results in a therapeutic dilemma for the liver transplanted patient. Autoimmune hepatitis concomitant with active HCV hepatitis would require corticosteroids, which are involved in reactivation of HCV hepatitis, which may lead to severe and progressive liver disease. In this scenario, everolimus may have a role in the minimization and withdrawal of corticosteroids.^{66,67}

Everolimus has been associated with a lower incidence of cytomegalovirus infection, compared with azathioprine and MMF, which may positively impact long-term outcomes.⁶⁸

Recent developments

Hepatitis C cirrhosis is the most common indication for liver transplantation. While the effect of immunosuppression on its recurrence is controversial, it has been shown that boluses to treat rejection episodes increase hepatitis C viral load and accelerate fibrosis and progression to cirrhosis. Usually, if acute rejection is mild, the dose of current immunosuppression is increased, instead of using steroid boluses. The role of everolimus has not been evaluated specifically.¹⁰

Prospective data evaluating fibrosis progression in HCV after liver transplant in patients receiving an mTORi versus CNI are lacking. Villamil et al⁶⁹ are in the process

Table 6 A selection of everolimus registered trials in acute or maintenance period after adult cadaveric donor liver transplantation

Title	Study phase NCT identifier	Studied condition	Immunosuppressor treatment	Objectives Primary end point
Preservation of renal function in liver transplant recipients with certican therapy	Phase III NCT00378014	Acute and maintenance period after liver transplant	Everolimus + basiliximab	Superiority of everolimus-based regimen in renal function, compared against continuation of CNl-based treatment, at 11 months post randomization
Extension study to evaluate the long-term efficacy and safety of everolimus in liver transplant recipients	Phase III NCT01150097	Maintenance period after liver transplant	Three arms: Tac Tac + everolimus Everolimus	Renal function by eGFR. Efficacy failure as treated biopsy proven acute rejection, graft loss or death. Rate of progression of HCV-related allograft fibrosis.
The impact of everolimus-based immunosuppression in the evolution of hepatitis C fibrosis after liver transplantation	Phase III NCT01707849	Hepatitis C recurrence after liver transplant	Everolimus arm MMF arm	Time frame: 36 months and 48 months posttransplant To evaluate the safety and efficacy of two steroid-free immunosuppressor regimens to reduce HCV recurrence associated with fibrosis progression ($F \geq 2$), at 1 year posttransplant
Everolimus after liver transplant	Phase II NCT01998789	Renal function outcome after liver transplant	Everolimus Tac	Change in eGFR Time frame: baseline and 12 months posttransplant
Efficacy of everolimus in combination with Tac in liver transplant recipients	Phase III NCT01551212	Renal function outcome after liver transplant	Everolimus as add-on: Tac group	eGFR (MDRD-4 formula) at Month 12 in de novo liver transplant Timeframe: 12 months after randomization
Efficacy of everolimus as inhibitor of fibrosis progression in liver transplant patients with recurrence of hepatitis C	Phase II NCT00382738	Recurrent hepatitis C	CsA/Tac (usual treatment) vs everolimus	Change from baseline in Fibrosis Staging Score (by the Ishak-Knodell score) – between baseline and 24 months posttransplant
Efficacy and safety of concentration-controlled everolimus to eliminate or to reduce Tac, compared to Tac in de novo liver transplant recipients	Phase III NCT00622869	Liver transplantation outcome	Two arms: experimental everolimus + reduced Tac versus reduced Tac + everolimus + corticosteroids	Incidence rate of composite efficacy failure – from randomization to Month 12

Abbreviations: NCT, national clinical trials registry; CNl, calcineurin inhibitor; Tac, tacrolimus; eGFR, estimated glomerular filtration rate; HCV, hepatitis C virus; MMF, mycophenolate mofetil; MDRD-4, modification of diet in renal disease 4; CsA, cyclosporine.

of publishing a randomized, multicenter, open-label study that evaluates fibrosis progression in 43 maintenance liver transplant patients with recurrent HCV infection, receiving everolimus or CNI-based immunosuppression. They reported 12-month findings, as follows. Fibrosis scores at baseline were 2.6 ± 0.9 with everolimus (N=14) versus 1.9 ± 1.1 with CNI (N=18) ($P=0.043$); at Month 12, fibrosis scores were 1.9 ± 1.2 vs 2.2 ± 1.3 , respectively. Interestingly, fibrosis scores decreased with everolimus but increased with CNI by 0.2 ± 1.2 ($P=0.046$). No acute rejection or graft losses occurred. At Month 12, GFR was similar – and preserved – in both groups. Adverse events led to everolimus discontinuation in 5 patients (22.7%). Although it had few patients, this study suggests that conversion from CNI to everolimus reduces progression of liver fibrosis, preserves renal function, and prevents rejection in liver transplant recipients with recurrent HCV. However, the study is associated with drug-related adverse events. These preliminary findings (with a short follow-up) deserve further examination in a larger trial.

Learning curve

Increasingly, as experience with liver transplantation grows, programs are shifting their therapeutic approaches toward minimizing exposure to CNIs. Everolimus has undergone drug development in other solid organ transplants, clinical and study trials, but finally, its role has been demonstrated in liver transplants.¹³ It has to be recognized that everolimus came to the liver transplant setting after its full development in other transplant settings.^{38,52} Everolimus is a critical-dose compound that requires therapeutic drug monitoring, because of the direct relationship between trough concentration and efficacy (versus toxicity). Everolimus introduces marked improvements, owing to its modest nephrotoxicity and possible vasoprotective and putative antineoplastic effects (as opposed to the adverse actions of CNIs).⁷⁶ At present, it is widely accepted that immunosuppression treatment should be less intense in liver transplant.⁷¹

Mechanistic causes of adverse events associated with mTORi and clinical strategies for their management have been studied.

Current status and future challenges

In these times of extreme organ shortage, two aspects related to liver allocation have to be considered. On one hand is disease severity, reflected by the MELD score; patients with more severe disease receive transplant earlier. But, on the other hand, the recipient's survival after transplantation may be hampered by renal dysfunction. For the first time, everoli-

mus has a role that has been adequately studied. We will soon have a drug that may prevent some of the potential complications after liver transplant – observed in the short and/or long term – which may determine prognosis and survival. Some refinements have been made recently, considering combining everolimus with low doses of Tac, starting after 30 days post-transplant, avoiding such risks as thrombotic events, wound healing disturbances, and other complications described in the use of sirolimus, which are not observed in more recent reports. Some clinical trials are in progress (Table 6).

At present, the majority of liver transplant recipients are surviving for decades, but complications such as renal insufficiency, malignancy, and metabolic syndrome have become a major burden on long-term outcomes. Hence, the main objective is to reduce the incidence of avoidable posttransplant complications.

Disclosure

The author reports no conflicts of interest in this work.

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