Background and aim. Everolimus (EVR) use in liver transplantation (OLT) has been prescribed with calcineurin inhibitors (CNIs), steroids, and monoclonal antibodies. The aim of our study was to evaluate the safety, feasibility, and impact on renal function of EVR ab initio, in combination with enteric-coated mycophenolate sodium (EC-MPS) without the use of induction treatment, steroids, or CNIs.

Patients and methods. We retrospective analyzed nine consecutive patients who underwent OLT at our institution. The initial dose of EVR (1.5 mg/d) was adjusted to achieve trough levels of 8 to 12 ng/mL. EC-MPS introduced at 1080 mg/d was maintained at the same dose over time.

Results. At a mean follow-up of 21.48 (standard deviation [SD] 1.4) months from OLT, 7/9 recipients were alive with stable graft function. The 2-year patient and graft survivals were 77%. One recipient died due to cerebral hemorrhage and one, lung failure. No clinical evidence of an acute rejection episode was observed. Mean estimated glomerular filtration rate value, according to the Modification of Diet in Renal Disease formula increased from 59.5 (SD 9.89) mL/min/1.73 m$^2$ at OLT to 100.2 (SD 47.5) mL/min/1.73 m$^2$ ($P = .03$) after 12 months and 98.71 (SD 33.74) mL/min/1.73 m$^2$ ($P = .03$) after 24 months’ follow-up.

Conclusion. A double immunosuppression therapy with EVR and EC-MPS ab initio seemed to be efficacious and safe, representing a valid alternative to CNIs to prevent renal failure after OLT.

IMMUNOSUPPRESSIVE THERAPY based on calcineurin inhibitors (CNIs) in liver transplantation (OLT) reduces the risk of rejection but is associated with side effects including renal failure, neurotoxicity, cardiovascular complications, hypertension, and diabetes mellitus. Chronic renal dysfunction (CRD) among OLT recipients shows a cumulative incidence of 20% within 3 years and a fourfold increased risk of death. CRD among OLT recipients has multifactorial origins: female sex, renal disease pretransplantation, primary graft dysfunction, perioperative acute renal damage, recipient age, hepatitis C, hypertension, diabetes mellitus. The prescription of CNIs seems to be the only modifiable risk factor.

CNIs lead to acute nephropathy by renal arteriolar vasoconstriction and tubular vacuolization; pathological findings can be reversed by CNI withdrawal. In contrast, the chronic CNI-induced nephrotoxicity, which is characterized by arteriolopathy and tubulointerstitial fibrosis, develops irreversible structural damage. CNI therapy is also responsible for new-onset diabetes and hypertension, important risk factors for CRD.

The current clinical challenge is therefore to develop regimens that maintain high rates of efficacy while minimizing side effects. Everolimus (EVR; Certican; Novartis, Basel, Switzerland), a proliferation signal inhibitor (PSI), seems to not increase the risk of renal dysfunction while maintaining excellent efficacy as an immunosuppressant. A few studies have shown the safety and efficacy of PSI in midterm results; however, there was no renal functional
improvement after PSI introduction, possibly due to the onset of irreversible renal “stigmata” from chronic CNI use. Thus, it seems reasonable to introduce EVR earlier with CNI avoidance immediately after OLT to minimize the risk of renal dysfunction particularly among patients at high risk of renal failure. Based on the dogma of “earlier is better,” the aim of our study was to evaluate the safety, feasibility, and renal functional impact of EVR ab initio, as the main drug in OLT, in combination with enteric-coated mycophenolate sodium (EC-MPS) without the use of induction antibody treatment, steroids and CNIs.

PATIENTS AND METHODS
We retrospectively analyzed nine patients who consecutively underwent OLT between September 2009 and February 2010. The regimen consisted of EVR and EC-MPS ab initio. No patients received an induction therapy (basiliximab or daclizumab) and/or steroids. The initial EVR dose was 1.5 mg/d, seeking to achieve a C0 level of 8 to 12 ng/mL within 7 postoperative days (POD). The EVR dose was then modified to achieve an EVR target trough level of 6 to 10 ng/mL. EC-MPS was introduced and maintained at 1080 mg per day. EC-MPS and/or EVR administration was modified in the presence of side effects and/or the need for more intense immunosuppression.

The primary endpoint of the study was to evaluate the safety and efficacy of a CNI-free regimen without induction or steroid in de novo OLT recipients. The second aim was to assess renal function expressed as estimated glomerular filtration rate (eGFR) using the Modification of Diet in Renal Disease (MDRD) formula in OLT recipients who received EVR and EC-MPS ab initio.

Clinical and laboratory data were prospectively recorded at baseline as well as at months 3, 12, and 24 after OLT. The collected data included hematology and biochemistry tests and EVR trough levels.

EVR was assayed on aTDXFLx analyzer (Abbott Diagnostic, Ill, USA) using a Certican homogeneous fluorescence polarization immunoassay system.

Data were retrieved from a prospective database (Microsoft Access 2.0, Microsoft Corporation, USA). Categorical metrics were analysed using Fisher exact test for simple cross tables; continuous data included hematology and biochemistry tests and EVR trough levels.

RESULTS
Demographics Characteristics of the Study Population
Nine consecutive patients (eight male, one female) who underwent OLT at our institution between September 2009 and February 2010 received EVR and EC-MPS ab initio. Their indications for OLT are reported in Table 1. At the time of OLT the mean age was 48.6 ± 5.7 years and mean Model for End-stage Liver Disease (MELD) score was 22 (SD 6.4). Three recipients (33.3%) were hospitalized before OLT. The mean eGFR value according to MDRD was 59.5 (SD 9.89) mL/min/1.73 m² and 2 patients (22.2%) required dialysis for hepatorenal syndrome. None of the recipients had a significant additional medical history such as leukopenia, diabetes or dyslipidemia. All OLT were performed on the 9th table.

Table 1. Demographic Characteristics of the Study Population
<table>
<thead>
<tr>
<th>Patient</th>
<th>Gender/Age</th>
<th>Primary Diagnosis</th>
<th>Donor Gender/age</th>
<th>MELD pre-OLT</th>
<th>Follow-up</th>
<th>eGFR pre-OLT</th>
<th>eGFR post-OLT</th>
<th>Lower limb and edema</th>
<th>Leukopenia, wound dehiscence, incisional hernia</th>
<th>Pneumonia, wound dehiscence, incisional hernia</th>
<th>Graft Complication</th>
<th>Cause of Death</th>
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<td>F/66</td>
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<td>36</td>
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<td>F/18</td>
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<td>F/59</td>
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<td>Dialysis</td>
<td>Dialysis</td>
<td>Dialysis</td>
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</tr>
</tbody>
</table>

GFR, glomerular filtration rate; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GPT, gamma-glutamyl-transferase; HCV, hepatitis C virus; EVR, everolimus; EC-MPS, enteric-coated mycophenolate sodium; HBV, hepatitis B virus; MELD, model for End-stage Liver Disease; MDRD, Modification of Diet in Renal Disease formula.
using the venovenous bypass and all of them, a whole organ
graft, except one who received a right split liver from a
compatible group donor. The mean donor age was 46.4 (SD
19.8) years and the mean cold and warm ischemic times
were 402.4 ± 58.1 and 40.8 ± 7.7 minutes respectively.

Safety and Tolerability
After a mean of 21.4 (SD 1.4) months from OLT, 7/9
recipients (77.7%) were alive with stable graft function.
One recipient died due to a cerebral hemorrhage and one,
to lung failure after 4 and 35 days, respectively; both of
them experienced pre-OLT dialysis due to renal failure.

Regimen and Graft Function
Within the 6 months, the mean EVR trough levels were
between 8 and 12 ng/mL initially trending to < 10 ng/mL.
No patients required EVR therapy discontinuation during
the follow-up. EC-MPS was maintained at 1080 mg per day.
Neither graft lost nor clinical evidence of an acute rejection
episode or, liver dysfunction was observed at any of the
considered times. At 24 months’ follow-up, alanine amino-
transferase and gamma-glutamyl-transferase were 61 (SD
66.46) UI/L and 100 (SD 21.9) UI/L respectively.

Renal Function
At OLT, two patients presented severe renal impairment
requiring replacement therapy, both continued dialysis af-
after OLT. In the early posttransplant period one patient with
severe hepatorenal syndrome before OLT (eGFR = 36
mL/min/1.73 m²) developed acute renal dysfunction (eGFR =
19.41 mL/min/1.73 m²) that resolved after 30 days (eGFR of
68 mL/min/1.73 m²) without requiring any dialysis. At 22
months’ follow-up the patient was in good clinical condition
with stable renal function (eGFR = 84 mL/min/1.73 m²).
The overall mean eGFR value increased from 59.5 (SD
9.89) mL/min/1.73 m² at OLT to 113.63 (SD 63.5) mL/min/
1.73 m² (P = .04) at 3 months, remaining stable during the
follow-up: eGFR = 100.2 ± 47.5 mL/min/1.73 m² (P = NS)
at 12 months and 98.71 ± 33.74 mL/min/1.73 m² (P = NS)
at 24 months.

Adverse Events
In the early posttransplant period, one patient developed a
inferior vena cava thrombosis, that resolved with anticoag-
ulant therapy. One patient required EC-MPS withdrawal
after 9 months for severe leukopenia (white blood cell
count < 2000/µL) but remained under EVR monotherapy
with stable graft function.

Two patients developed pneumonia immediately after
OLT that resolved with antibiotic therapy; there was no
other infection including that due to cytomegalovirus. One
patient presented severe lower limb and eyelid edema, but
no evidence of mucositis/oral ulcer or dermatitis. Two
recipients (22.2%) experienced a surgical wound dehis-
cence, developing an incisional hernia. No gastrointestinal
side effects such as nausea, diarrhea, or abdominal pain
were observed. Dyslipidemia requiring medical therapy was
reported in three cases.

DISCUSSION
PSI acts at a later stage in the cell cycle blocking the
proliferation signal provided by growth factors, thereby
preventing cells from entering the S phase. The antiprolif-
erative effects of PSI are not limited to the immune system
but also to nonhematopoietic elements including vascular
smooth muscle cells. The safety and efficacy of EVR have
been demonstrated in renal 11 and heart 12 transplant recip-
ients. It is currently used in these patients to reduce CNI
toxicity and/or prevent CRD. There are few data concern-
ning the use of EVR in OLT recipients. Levy et al reported
no significant changes in laboratory parameters, infection
rates and an acceptable safety and toxicity profile of EVR in
combination with cyclosporin. 13,14 Preliminary studies of
PSI for a maintenance regimen in OLT recipients have
mainly focused on sirolimus (SRL) to treat patients with
CNI-related CRD. 15,16

These experiences have demonstrated that CNI minimi-
ization associated with SRL or conversion from a CNI to
SRL-based regimen was feasible. This strategy was associ-
ated with 5% to 15% risk of acute rejection with a variable
degree of improvement in renal function according to the
baseline creatinine clearance; 17–20 CNI-related renal dis-
case, and time from OLT. 21 Nevertheless, more recent data
have suggested the efficacy of SRL-based regimens on
long-term renal function post OLT. 22–25

CNI produces acute nephropathy characterized by vaso-
constriction of renal arterioles, as manifested clinically by
reduced glomerular filtration, hyperkalemia, hypertension,
increased sodium reabsorption, and oliguria. 6 The CNI-
induced chronic renal damage is characterized by the
development of arteriolopathy and tubulointerstitial fibro-
sis, which is irreversible and may lead to end-stage nephrop-
athy. 6 The toxicity may be reversed when CNI therapy is
reduced or withdrawn. Therefore, benefit from PSI therapy
may be expected only for early CNI minimization/with-
drawal or avoidance ab initio, namely while pathological
changes are still reversible. In OLT, most series have
reported EVR used as maintenance therapy seeking to
improve renal function but not CRD prevention. 5,7,12,26

In this scenario, the purpose of our study was to evaluate
whether a CNI-free regimen using EVR and EC-MPS as
the main drugs ab initio without induction or steroids was
safe and effective and ensured preservation of renal func-
tion among OLT recipients. Our data showed PSI use ab
initio to be safe in terms of patients and graft survival and
acute cellular rejection rate. Furthermore no patients with
baseline normal renal function developed post-OLT renal
dysfunction that required kidney replacement therapy. Only
one patient with hepatorenal syndrome before OLT devel-
oped severe renal impairment immediately there after, which
never required dialysis and recovered within 30 POD.
These results suggested that EVR and EC-MPS ab initio may be used to prevent renal dysfunction especially among patients who showed renal impairment before surgery. As reported by Masetti and Sanchez Fructuoso, hematologic side effects of the antimetabolite in association of PSI were rare. In our cohort only one patient who developed leukopenia after 9 months completely recovered after EC-MPS withdrawal, suggesting the hematotoxicity was rare and easily managed. A few patients experienced other side effects, including dyslipidemia and wound healing, completely recovered after dose adjustment and conservative management.

In conclusion, this series explores EVR associated with EC-MPS without CNI, induction therapy, or steroid in de novo OLT recipients. Our data suggested that this feasible and safe regimen was a valid alternative for patients who require a CNI-free protocol due to renal dysfunction before transplantation.

REFERENCES