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Timing of sirolimus conversion influences recovery of renal function in liver transplant recipients

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Abstract: The long-term use of calcineurin inhibitors (CNI) leads to renal dysfunction in many liver transplant (LT) recipients. The purpose of this analysis is to evaluate renal function in patients converted from CNI to sirolimus (SRL). From May 2002–November 2006, 137 LT were performed in 125 patients, 72 of which were converted to SRL. Evaluation of SRL conversion was stratified by early conversion (< 90 d from LT) (EC) vs. late conversion (LC). Renal function was evaluated using the six-point modification of diet in renal disease formula (estimated glomerular filtration rate [eGFR]). Forty-two patients on SRL and 40 on CNI had at least three months of follow-up and are included in the eGFR evaluation. At all time points after conversion, the EC group demonstrated a significantly higher mean eGFR than those in the LC group. A significant improvement in eGFR was seen within the EC group when comparing eGFR at time of conversion to eGFR at three, six, nine, and 12 months after conversion and last follow-up. The only improvement in the LC group was from conversion to the three-month time point. We conclude that EC to SRL results in a profound improvement in eGFR that begins at three months and is sustained beyond one yr.

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The significant improvements in patient and graft survival over the past 10 yr in liver transplantation can largely be attributed to the introduction of calcineurin inhibitors (CNI). However, the clinical use of CNI is often limited by their nephrotoxic effects. The use of CNI based immunosuppression is the leading cause of post-transplant renal dysfunction in non-renal transplant recipients (1) and chronic renal failure (CRF) develops in up to 18% of liver transplant recipients at five yr post-transplant (1). The development of CRF in the liver transplant population has been shown to adversely impact both patient and graft survival (1, 2). Development of effective immunosuppressive strategies that avoid or minimize the use of CNI is imperative to overcome this problem.

Sirolimus (SRL) (Rapamune; Wyeth Inc., Philadelphia, PA, USA) is an alternative immunosuppressive agent that may be used in place of CNI. SRL is FDA approved for the prevention of rejection in renal transplant recipients (3). Despite its limited FDA indication, SRL is often used off label in non-renal transplant recipients. SRL has been shown to be both safe and efficacious when used as a primary immunosuppressive agent in a CNI free regimen in kidney transplant recipients. Flechner et al. demonstrated that use of a SRL based regimen resulted in longer death censored graft survival, higher glomerular filtration rate (GFR) and fewer graft losses from chronic allograft nephropathy in the kidney transplant population (4). Numerous single center reports have

shown the utility of using SRL as an alternative option to CNI in non-renal transplant recipients. There are conflicting data as to whether or not SRL conversion improves renal function in the liver transplant population (5–12).

The purpose of our retrospective analysis was to determine if there is an improvement in renal function after conversion from a CNI to an SRL based regimen. Additionally, we sought to evaluate the influence of timing of SRL conversion on the response to therapy.

Methods

We retrospectively collected data on all liver transplant recipients from May 2002 to November 2006. Our immunosuppression protocol in May 2002 included induction with basiliximab, microemulsion cyclosporine (target trough: 0–6 months, 200–250; 6–12 months, 150–200; and 12 months and beyond 100–150), mycophenolate mofetil 1000 mg BID dose adjusted for WBC or gastrointestinal (GI) intolerance and a prednisone tapered to 5 mg by six months. In January 2004, we modified our cyclosporine protocol and began monitoring C2 levels, (target levels of 1000 ng/dL for the first six months, 800 ng/dL for 6–12 months and 600 ng/dL). Further modification occurred in May 2004 with tacrolimus replacing cyclosporine (target trough levels for the first six months were 8–10 ng/dL, for months 6–12 was 6–8 ng/dL and after month 12 was 6 ng/dL). Basiliximab induction was removed from the standard protocol in July 2005.

Liver transplant recipients with renal dysfunction (defined as an increase in serum creatinine [Scr] of $>30\%$ from baseline or a GFR of ≤ 55 mL/min but >30 mL/min) or an underlying diagnosis of hepatocellular carcinoma (HCC) are converted to SRL as part of our standard protocol if they are a minimum of six wk post-transplant and have a completely healed surgical incision. Other indications for conversion included CNI toxicity (neurotoxicity or GI intolerance). Doppler ultrasound demonstrating patent hepatic vasculature was required prior to SRL initiation. Laboratory parameters such as WBC count ($>3000/mm^3$), platelet count ($>75\,000/mm^3$), fasting triglycerides (<350 mg/dL), and fasting cholesterol (<300 mg/dL) were confirmed to be within acceptable limits prior to conversion. When SRL was initiated the CNI dose was decreased by 50% and continued until the SRL level was >6 mg/dL. No other alterations in immunosuppression therapy were made upon addition of SRL. Target SRL levels for the first three months post-conversion were 8–10 ng/dL, 6–8 ng/dL for months 3–6 and after month 12 it was 5–6 ng/dL.

Change in renal function was assessed by evaluating Scr and by utilizing the six-point modification of diet in renal disease (MDRD) study equation estimated glomerular filtration rate (eGFR) (13). Renal function was evaluated at the time of transplant, time of conversion to SRL, every three months thereafter and at the date of last follow-up for patients converted to SRL. Patients remaining on CNI had their renal function evaluated at the time of transplant and at the time of last follow-up (last clinic visit date with laboratory value assessment). Length of follow-up for those converted to SRL was defined as time from initiation of SRL therapy to the date of last available laboratory testing while on SRL. Additionally, we evaluated whether timing of conversion to SRL impacted recovery of renal function. Patients converted to SRL within three months post-transplant were evaluated in the early conversion (EC) group whereas those converted to SRL after three months were considered to be in the late conversion (LC) group. All patients converted to SRL were included in the rejection and adverse event analysis where as only those with a minimum of three months of follow-up were included in the renal function analysis.

Logistic regression was performed to identify factors that would predict stabilization in renal function over time. The logistic regression was performed in a forward stepwise fashion with stabilization of CKD class as the dependent variable. Variables in the univariate analysis with $p < 0.10$ were included in the analysis. In order to evaluate this in all patients we evaluated the change in renal function (eGFR) from the time of transplant to last follow-up. Chronic kidney disease (CKD) scores were assigned to eGFR measurements at all time points (14). Patients were defined as having stable renal function (dependent variable) if they remained in the same CKD class or had an improvement in CKD class (i.e., CKD class 2 [eGFR 60–89 mL/min] to CKD class 1 [eGFR 90 mL/min or greater]) from the time of transplant to the time of last follow-up. Variables entered into the model included age >50, MELD score at time of transplant (<10, 11–15, 16–20, 21–25 or 26+), cause of liver disease (hepatitis C virus cirrhosis [HCV], hepatitis B virus cirrhosis [HBV], alcohol, HCC), presence of hypertension or diabetes post-transplant and the use of SRL.

Categorical variables were compared using the chi-square or Fisher's exact test where indicated. Continuous variables were compared by ANOVA

or unpaired *t*-tests as appropriate. We performed all statistical analysis using SAS (SAS Institute, Cary NC, USA). Data are presented as mean ± SD unless otherwise noted. This study was approved by the investigational review board.

Results

One hundred and thirty-seven liver transplants were performed in 125 patients from May 2002 to November 2006. Seventy-two patients (57.6%) were converted to SRL therapy. Fifty-three patients (74%) were converted to SRL therapy because of renal insufficiency while on a CNI, eight (11%) were converted due to CNI induced neurotoxicity, six (8%) were converted for a diagnosis of HCC, three (4%) were converted due to rejection and two (3%) were converted due to GI intolerance. Demographic features of the patients converted to SRL as well as those remaining on CNI are presented in Table 1.

Renal function

Forty-three of the 72 transplants converted to SRL were treated with SRL for at least three months and are included in the primary analysis of renal function. Forty liver transplant recipients who remained on CNI based immunosuppression for a minimum of three months were also included in the analysis of renal function. Fifty-four transplants were excluded from the primary analysis of renal function due to the fact that they did not receive a minimum of three months of SRL or CNI therapy (n = 36), were a repeat transplants (n = 9), were a combined kidney/liver transplant recipients (n = 6) or were maintained on a combination of SRL with a CNI long-term (n = 3). The flow diagram in Fig. 1 describes the distribution of patients in the EC, LC and CNI groups as well the various reasons why patients were excluded from the renal function analysis.

Change in renal function from the time of transplant (CNI group) or time of conversion (SRL conversion group) to the last follow-up visit was evaluated both by change in mean Scr and eGFR over time. Renal function as assessed by Scr at the time of transplant was found not to differ between the EC, LC and CNI groups (p = 0.48). When renal function was evaluated by eGFR, there was no difference across the three groups (p = 0.09); however, a significant difference in eGFR at the time of transplant was observed between those on CNI therapy and those in the LC group (p = 0.03). A significant deterioration was seen in the renal function of both the EC and LC groups from the time of transplant to SRL conversion. At the time of conversion there was no difference in renal function as assessed by both Scr and eGFR between the EC and LC groups (p = 0.08).

At all time points after conversion to SRL, the EC group demonstrated a significantly higher mean eGFR and a significantly lower Scr than the LC group (Fig. 2, Table 2). Patients in the EC group demonstrated a marked and significant improvement in renal function as measured by change in mean eGFR from the time of conversion to the time of last follow-up (p = 0.002). Those in the LC group did not demonstrate the same improvement from conversion to last follow-up (p = 0.27). A significant improvement in mean eGFR was seen within the EC group when comparing eGFR at time of conversion to eGFR

Table 1. Demographics

	Early conversion % (n = 47)	Late conversion % (n = 25)	CNI based immunosuppression % (n = 65)	p-Value
Age (yr)	53 ± 7	53 ± 11	51 ± 11	ns
Gender (male)	76 (35)	76 (19)	71 (46)	ns
Lab MELD at the time of LT	22 ± 10	23 ± 11	21 ± 10	ns
Cause of liver disease				
Alcohol	34 (16)	12 (3)	15 (10)	<0.05[a]
Hepatitis C	45 (21)	56 (14)	47 (31)	ns
Hepatitis B	6 (3)	4 (2)	6 (4)	ns
Cholestatic	4 (2)	0	6 (4)	ns
Others	10 (5)	24 (6)	25 (16)	ns
Mean time from LT to conversion (d)	50 ± 20	309 ± 292	n/a	<0.001
Mean duration of F/U (d)	422 ± 328[c]	486 ± 285[c]	616 ± 459[d]	<0.05[b]

CNI, calcineurin inhibitors; ns, not significant; n/a, not applicable; LT, liver transplant.
[a]Early vs. CNI and early vs. late.
[b]Early vs. CNI.
[c]From conversion.
[d]From transplantation.

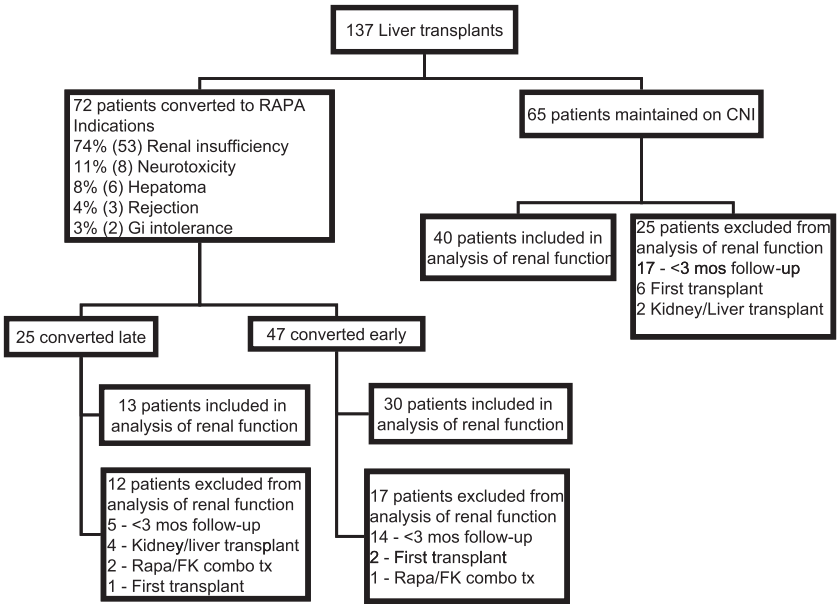

Fig. 1. Study population.

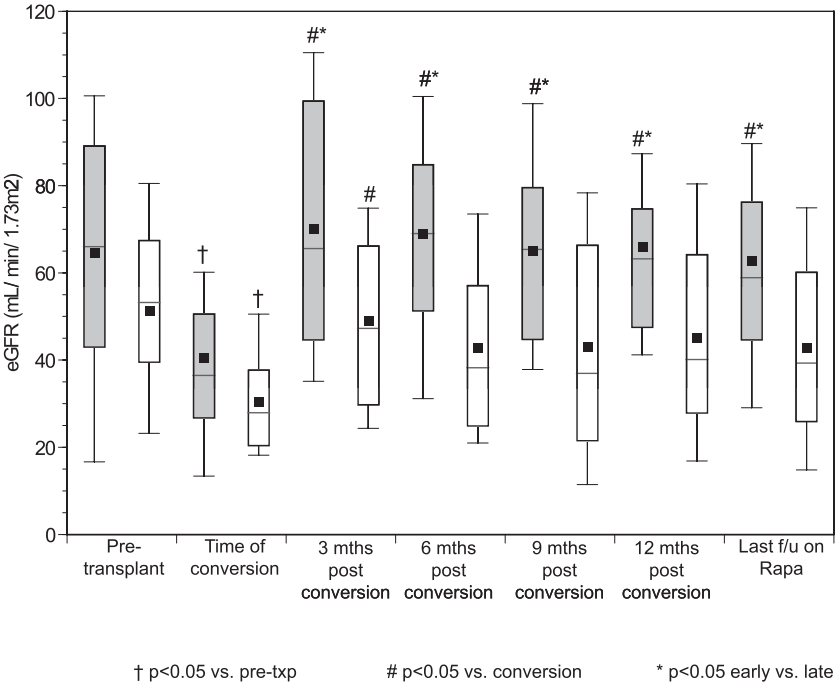

Fig. 2. Comparison of renal function between early and late conversion groups. Early (grey) vs. Late (white). Data are depicted in box and whisker plots. The central box represents the values from the lower to upper quartile (25–75 percentile). The middle line represents the median. The horizontal line extends from the minimum to the maximum.

at three ($p = 0.0001$), six ($p = 0.0001$), nine ($p = 0.007$) and 12 months ($p = 0.008$). The LC group demonstrated only an early and non-sustained improvement in eGFR from conversion to the three month post-conversion time point ($p = 0.02$). The LC group failed to show improvements in mean eGFR at all other time points post-conversion. When eGFR was compared between EC and LC groups at three, six, nine, 12 months and last follow-up, the EC group had a significantly higher eGFR at all time points ($p < 0.05$). The EC group demonstrated improved and sustained renal function over time whereas both the LC and CNI groups exhibited a progressive and significant deterioration in renal function from the time of transplant to the time of last follow-up (Fig. 3). Fourteen patients included in the analysis of renal function had to be converted back to CNI. Nine patients were in the EC group and five were in the LC group. At the time of conversion back to CNI the Scr in the EC group was 0.99 ± 0.6 mg/dL and the eGFR was 85.4 ± 40.5 mL/min/1.73 m^2. Patients were on SRL for a mean of 296 ± 321 d. At the time of conversion back to CNI, the Scr in

Table 2. Change in eGFR over time

	eGFR pre-transplant (mL/min/1.73 m^2)	eGFR at conversion (mL/min/1.73 m^2)	eGFR three months post-conversion (mL/min/1.73 m^2)	eGFR six months post-conversion (mL/min/1.73 m^2)	eGFR nine months post-conversion (mL/min/1.73 m^2)	eGFR 12 months post-conversion (mL/min/1.73 m^2)	eGFR at last follow-up (mL/min/1.73 m^2)
Early conversion	64.8 ± 33.5	40.7 ± 24.7	70.4 ± 30.5*†	69.4 ± 25.5*†	65.2 ± 25.4*†	66.2 ± 24.3*†	62.9 ± 30.3*†
Late conversion	51.4 ± 21.0	30.6 ± 12.6	49.1 ± 20.1†	43.0 ± 20.8	43.1 ± 26.4	45.3 ± 24.3	42.9 ± 22.1‡
CNI maintenance	73.4 ± 34.24	n.a	n.a	n.a	n.a	n.a	53.3 ± 19.3‡
Hepatitis C positive population							
Early conversion	72.7 ± 28.3	51.8 ± 31.2	84.4 ± 25.3*	81.9 ± 15.4*	78.4 ± 14.9*	77.6 ± 28.7*	82.6 ± 34.0*
Late conversion	50.8 ± 17.1	32.1 ± 12.6	49.7 ± 19.0	45.1 ± 18.5	46.7 ± 25.5	48.4 ± 19.9	41.3 ± 19.4
Hepatitis C negative population							
Early conversion	59.5 ± 36.4	33.4 ± 16.2	62.4 ± 33.7	66.3 ± 39.9	56.8 ± 27.6	56.2 ± 15.0	49.8 ± 18.9
Late conversion	52.5 ± 28.4	28.3 ± 13.6	48.3 ± 24.2	37.8 ± 29.2	34.7 ± 32.2	38.0 ± 36.8	45.5 ± 28.1

Values presented as mean ± SD.
CNI, calcineurin inhibitors.
*$p < 0.05$ when comparing early vs. late.
†$p < 0.05$ when compating time-point to conversion.
‡$p < 0.05$ when comparing pre-transplant to last follow-up.

the LC group was 2.1 ± 1.4 mg/dL and the eGFR was 34.7 ± 23.1 mL/min/1.73 m^2. Patients were on SRL for a mean of 237 ± 152 d. After a mean of 1074 ± 203 d on CNI therapy there was a significant deterioration in renal function in the EC group; Scr increased to 1.2 ± 0.5 mg/dL ($p = 0.05$) and eGFR declined to 65.2 ± 27.2 mL/min/1.73 m^2 ($p = 0.03$). Though there was a significant deterioration in renal function when placed back on CNI therapy no patient developed stage 4 or 5 CKD. After a mean of 1465 ± 209 d on CNI therapy renal function continued to decline in the LC group; Scr increased to 2.5 ± 3.4 mg/dL and the eGFR improved to 54.8 ± 24.3 mL/min/1.73 m^2. Of note, the two patients who had stage 5 CKD at the time of conversion back to CNI had normal eGFR at the date of last follow-up. One of these patients had a renal transplant and the other patient expired with a low Scr that did not truly reflect renal function. One patient who had stage 4 CKD at the time of conversion back to CNI progressed on to hemodialysis.

In a multivariate logistic regression, SRL conversion conferred an increased odds ratio of 20.2 (95%CI, 3.5–115.7) vs. those maintained on CNI to remain in the same CKD class or improve in class over time ($p = 0.0007$).

A subgroup analysis was performed to evaluate the effects of SRL conversion on renal function in the HCV positive population. Interestingly, when the study population was grouped according to HCV serostatus, only the HCV positive subjects demonstrated a benefit from early SRL conversion. The eGFR was significantly higher in the EC HCV positive patients when compared to LC HCV positive at three, six, nine, 12 months and at the time of last follow-up. No difference was seen in eGFR when comparing the EC to LC HCV negative patients. Fig. 4A and B as well as Table 2 illustrate the changes in renal function over time in the HCV subpopulation.

Rejection

Rejection was analyzed regardless of whether or not patients completed three months of therapy on SRL; therefore, all 137 transplanted subjects are included in this analysis. Patients who were converted to SRL and rejected while on a CNI prior to conversion to SRL are evaluated in the CNI group ($n = 9$). Those who rejected while on a CNI and also rejected when converted to SRL are evaluated in both groups ($n = 3$). For the purpose of this analysis, rejection is defined as any histologic evidence of rejection on biopsy regardless of how it is treated. Rejection data are presented in

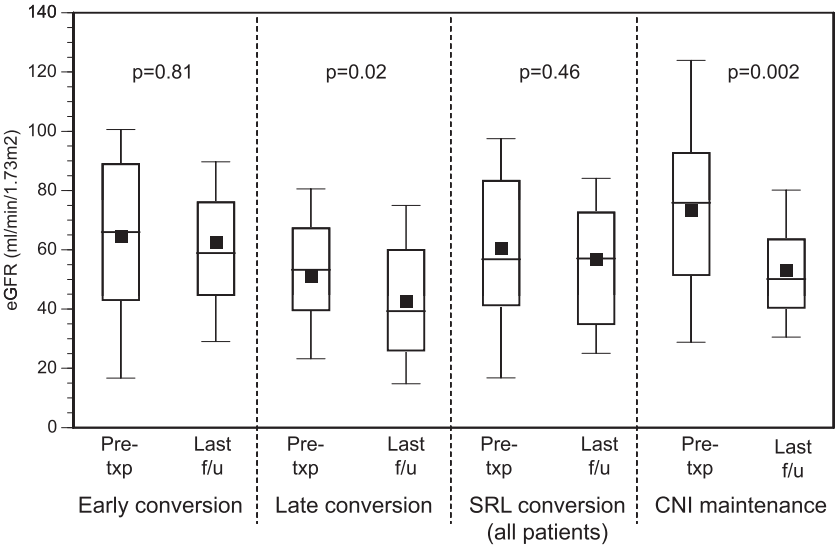

Fig. 3. Comparison of renal function at transplant and at last follow-up. Data is depicted in box and whisker plots. The central box represents the values from the lower to upper quartile (25–75 percentile). The middle line represents the median. The horizontal line extends from the minimum to the maximum.

Table 3. A similar proportion of rejections occurred in the EC (35%) and LC (38%) SRL groups. All four of the moderate rejections occurred in the EC group. Time between conversion and first rejection episode did not differ between the EC and LC groups (102.6 ± 101.5 d vs. 99 ± 52.9 d, p = 0.91). Four rejection episodes occurred within the first month of conversion to SRL, three of the four were in the EC group. Two of the three rejections occurred while SRL was still overlapped with CNI.

Safety

Of the 72 patients converted to SRL 46 patients (55%) discontinued therapy. The various reasons for discontinuation of SRL are listed in Table 4. The two most common reasons for discontinuation were rejection (32.5% of discontinuations) and anticipation of hernia repair (20% of discontinuations). The most commonly reported adverse events included anemia requiring treatment with erythropoietin (44%), the need for lipid lowering therapy (31%), hypertriglyceridemia (200–500 mg/dL) (22%), leukopenia requiring granulocyte-colony stimulating factor (20%), the occurrence of oral ulcers (15%) and incisional hernias (14%). The mean time to conversion in patients developing hernias on SRL was 57 ± 53 d. The incidence of anemia and leukopenia requiring treatment while on concomitant interferon and ribavirin is not reported in the analysis. All adverse events occurred at a similar rate between the early and late conversion groups (data not shown). When comparing adverse events in the SRL conversion group to those who remained on CNI there is a significantly higher incidence of anemia requiring the use of erythropoietin, hypertriglyceridemia (> 500 mg/dL) and oral ulcers in the SRL conversion group. There were significantly more patients who experienced delayed wound healing and portal vein thrombosis in the CNI maintenance group. Adverse event data are presented in Table 5.

Discussion

The widespread use of calcineurin inhibitors has largely contributed to the increase in success of liver transplantation. With our patients living longer it is essential to focus therapeutic management towards minimizing the long-term morbidity and mortality seen with long-term use of immunosuppression. The development of chronic renal dysfunction after liver transplant is a significant contributor to the morbidity and mortality in the liver transplant population. Numerous factors have been shown to contribute including pre-transplant hepatorenal syndrome, intrinsic renal disease, drug toxicity as well as other co-morbidities such as diabetes, hypertension and hepatitis C (15). CNI nephrotoxicity can either be reversible or permanent. Early CNI withdrawal increases the likelihood of affecting the reversible component of CNI toxicity (16, 17). CNI cause reversible renal vasoconstriction and a reduction in GFR, which lead to tubulo-intersititial fibrosis, a potentially irreversible change (18). At five yr after liver transplant, 80% of recipients may have some evidence of mild to moderate renal impairment (Scr > 1.4 mg/dL) (19) and up to 15% may progress on to CRF (1). The use of CNI sparing protocols which utilize agents such as SRL and mycophenolate mofetil offer some hope in addressing long-term CNI nephrotoxicity.

There are now many published studies reporting single center experiences with converting liver

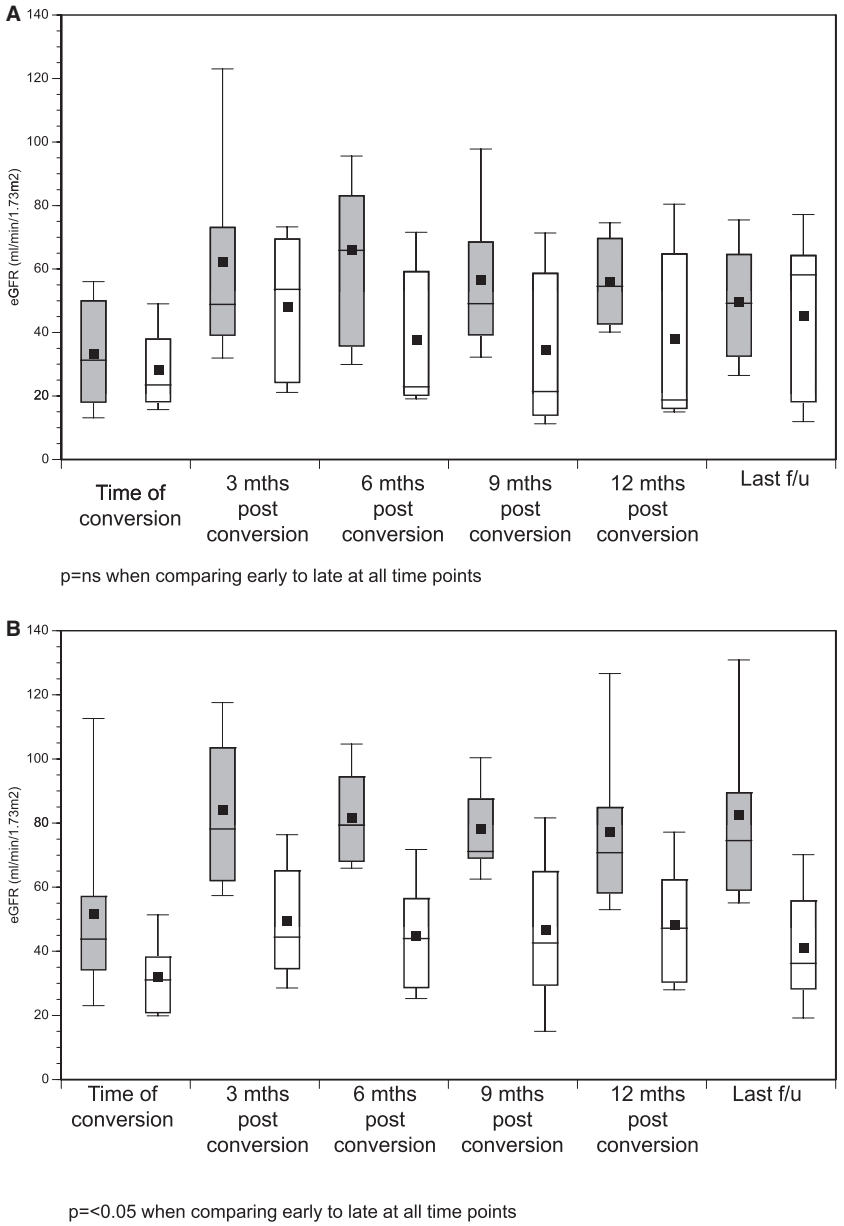

Fig. 4. (A) Estimated glomerular filtration rate (eGFR) in HCV – population converted to sirolimus. Early (grey) vs. late (white). (B) eGFR in HCV + population converted to sirolimus. Early (grey) vs. Late (white). Data are depicted in box and whisker plots. The central box represents the values from the lower to upper quartile (25–75 percentile). The middle line represents the median. The horizontal line extends from the minimum to the maximum.

transplant patients from a CNI based regimen to an SRL based regimen with a goal to recover renal function (5–7, 9, 11, 12, 16, 17, 20, 21). The majority of liver transplant recipients in these reports had CNI withdrawal after the first year post-transplant. Aside from our experience there is only one other small report of EC from CNI to SRL for known renal dysfunction or neurotoxicity secondary to CNI (22). Vivareli et al. (22) reported a case series of six patients who were converted to SRL for CNI neurotoxicity or nephrotoxicity within the first month post-transplant. Renal function was found to improve in two of the three patients converted for renal insufficiency. Three of the six patients developed acute cellular rejection and responded to pulse steroids.

Our study is unique in that it is the first reported experience evaluating the effect of timing of SRL conversion with extensive follow-up of over one yr. We acknowledge that the design of our study was not optimal to compare the difference in renal function seen in those who remained on CNI to those who were converted to SRL. The comparison of renal function at the time of transplant to that of the last follow-up visit in all patients demonstrated a key point about the timing of conversion. Only those converted to SRL within three months of transplant had stabilization in renal function at the time of last follow-up. Both those converted to SRL after three months and the patients remaining on CNI exhibited a significant deterioration in mean eGFR over this time period. Additionally,

Table 3. Rejection

Rejection	Sirolimus conversion (n = 63)	CNI maintanance (n = 78)	Cyclosporine (n = 43)	Tacrolimus (n = 35)
Mild	30% (19)	36% (28)	47% (20)	23% (8)
Moderate	6% (4)	6% (5)	12% (5)	0
Severe	0	1% (1)	0	2% (1)
Total	36% (23)	43% (34)	58% (25)*	26% (9)
Mean time to rejection from transplant (d)	193 ± 132	93 ± 110	95 ± 120	88 ± 77
Mean time to rejection from conversion (d)	101 ± 82	n.a	n.a	n.a

CNI, calcineurin inhibitors; n.a, not applicable.
Three patients experienced a rejection while on CNI and again rejected when converted to sirolimus; these patients are counted in both groups. Nine patients rejected on CNI prior to being converted to Rapa; these patients are included in the CNI group.
*p = 0.01 cyclosporine vs. tacrolimus.

Table 4. Reason for discontinuation of sirolimus

Reason	No. patients n (%)
Rejection	13 (32.5)
Anticipation of hernia repair	8 (20)
Pulmonary toxicity	5 (12.5)
Poor wound healing	2 (5)
Inability to achieve therapeutic levels	1 (2.5)
Viral syndrome	1 (2.5)
Proteinurea	1 (2.5)
Need for interacting medication	1 (2.5)
Hypertriglyceridemia	1 (2.5)
Hepatic artery stenosis	1 (2.5)
Facial rash	1 (2.5)
Depression	1 (2.5)
Pancytopenia	1 (2.5)
Anemia	1 (2.5)
Angioedema	1 (2.5)
Avacular necrosis	1 (2.5)
Total	40 (55)

Table 5. Adverse events

Event	Sirolimus conversion n (%) (n = 72)	CNI maintenance n (%) (n = 65)	p-Value
Anemia requiring use of erythropoientin	32 (44)	7 (11)	<0.001
Rejection	23 (36)	34 (43)	0.135
Use of lipid lowering agents	22 (31)	11 (17)	0.060
Hypertriglyceridemia (>200)	16 (22)	10 (15)	0.310
Leukopenia requiring use of filgrastim	15 (20)	8 (12)	0.184
Oral ulcers	11 (15)	2 (3)	0.019
Incisional hernia	10 (14)	10 (15)	0.860
Hypertriglyceridemia (>500)	9 (13)	0 (0)	0.003
CMV disease	9 (13)	4 (6)	0.250
Pulmonary toxicity (pneumonia)	8 (11)	3 (5)	0.193
Facial rash	6 (8)	3 (5)	0.498
Hepatic artery thrombosis	5 (7)	7 (11)	0.549
Poor wound healing	4 (6)	13 (20)	0.017
Deep vein thrombosis	3 (4)	2 (3)	1.000
Avascular necrosis	2 (3)	0 (0)	0.498
Angioedemia (facial/neck/ periobital swelling)	2 (3)	1 (1)	1.000
Portal vein thrombosis	0 (0)	5 (8)	0.020

CNI, calcineurin inhibitors.
Denominator of 63 used to calculate rejection for sirolimus group, denominator of 78 and 67 used to calculate rejection and hernia rate respectively in the CNI group. This accounts for those who rejected or developed hernia's prior to conversion. p = ns for all comparisons of events between early and late conversion groups.

mean eGFR was significantly higher in patients who were converted early vs. those who were converted late at all time points after conversion. When comparing eGFR after conversion to the baseline eGFR value at conversion, the LC group demonstrated a significant improvement only at three months whereas a significant improvement was seen at all time points post-conversion for the EC group. Two additional studies have shown that the duration of time between transplant and conversion to SRL may impact the likelihood of recovering of renal function (16, 17). Though the patients converted to SRL in these two studies were converted much later than our population, renal function was shown to be more likely to improve in those converted at 60 month vs. those converted at 112 months in one study and at 91 ± 46 months vs. 147 ± 43 months in the other. In both this study and other recent reports, there is a substantial impact of EC on the improvement in eGFR observed. Three recent studies that followed patients for over one yr all found a 6–9 mL/min improvement in renal function at 12 months or later after conversion to SRL (5, 12, 21). We saw a difference in mean eGFR in the EC group of 26 mL/min and of 15 mL/min in the LC group at 12 months following conversion.

Another striking finding of our analysis was seen in the subset analysis of our HCV population. We found that only the HCV positive subjects demonstrated a benefit from early SRL conversion. The HCV negative patients did not show a significant benefit to early SRL conversion at any time point after conversion. There are data supporting the fact that HCV may be an independent risk factor for the development of renal insufficiency in liver and in other non-renal transplant recipients (1, 23). The addition of CNI may be compounding the underlying renal dysfunction that may already be present in these patients. Additionally, there are reports of SRL use leading

to spontaneous clearance of HCV in LT recipients as well as reversal of liver fibrosis in various animal models (24–26). These data are intriguing but must be confirmed in a prospective study with a larger sample size.

The rejection rate following SRL conversion in our study was over 30%. The time to rejection from transplant was significantly longer in the SRL conversion group compared with those who remained on CNI. This may be reflective of the fact that nine patients who were in the SRL conversion group but rejected on CNI prior to conversion are counted in the CNI group. The difference may also reflect the increase in risk of rejection seen when altering immunosuppression within the first year post-transplant. Our rejection rate is slightly higher than the than other series of SRL conversion. The reason for this difference may be the fact that our rejection rate includes all rejections (including mild) as well as the fact that most other studies did not initiate SRL conversion until six months or later. The studies often had mean or median times of conversion often over three yr. These studies have rejection rates of <10 % after conversion (5, 12, 21). When converting to SRL within the first year of transplant it is important to ensure adequate immunosuppression with adjunctive immunosuppressive agents. Overlapping therapy with CNI until two therapeutic SRL levels are achieved may minimize rejection caused by early under-immunosuppression.

In our experience more than 50% of patients discontinued SRL therapy. We feel that this high discontinuation rate is reflective of the retrospective nature of this study and illustrates the variability seen in clinical practice when patients are not enrolled in a prospective clinical trial. The two primary indications for discontinuation were rejection and anticipation of hernia repair. Seven of the eight patients who discontinued SRL for hernia repair were in the EC group. This may raise concern with the appropriateness of EC and the adverse effects it may have on healing of sub-facsial layers. The difference in the rate of incisional hernias between the EC and LC groups did not show a significant difference. Small numbers and a lack of power may be the reason why there is not a difference. The adverse events seen more often in the SRL group were those that are often reported at a high incidence with SRL therapy. These events included anemia, hypertriglyceridemia and oral ulcers. An important question than still needs to be addressed is the cost effectiveness of SRL in the LT population. Studies have showed that SRL is more cost effective than both tacrolimus and cyclosporine in the renal transplant population (27, 28). It is unknown how much of a cost impact the management of adverse events may have on therapy. Treatment of anemia and leukopenia with growth factors present a long-term cost that must be evaluated. Additionally, the cost of inpatient admissions for the management adverse events such as incisional hernia repairs, pulmonary toxicity and hepatic artery stenosis/thrombosis should be evaluated.

Although this study demonstrated that EC to SRL preserves renal function, it is important to acknowledge its limitations. The retrospective nature of this study made it difficult to compare the difference in renal function seen over time in patients remaining on CNI to those converted to SRL. It was also difficult to adequately judge the incidence of adverse events and the actions taken in response to them. A prospective randomized trial would address these issues more clearly. Other limitations include the lack of measurement of proteinuria and the use of the six-point MDRD equation to calculate renal function instead of the gold standard inulin clearance. Measurement of proteinurea was added to our protocol; however, there were not enough data available to summarize for the manuscript.

Conversion to SRL therapy within three months of LT maximizes the improvement in renal function seen after withdrawal of CNI therapy. This improvement in renal function with SRL was not accompanied by a significant increase in the risk of rejection compared to remaining on CNI therapy. The risk of rejection was clearly higher in patients who we converted early and were not on optimal concomitant immunosuppression. When converting to SRL therapy it is imperative to try to maintain adequate mycophenolate and CNI exposure until SRL levels are therapeutic. Despite the significant improvement in renal function numerous adverse effects occurred, requiring discontinuation of therapy in over 60% of patients. Prior to converting to SRL therapy it is imperative to individualize therapy and weigh the risks and benefits that may occur with that specific patient.

References

1. Ojo AO, Held PJ, Port FK et al. Chronic renal failure after transplantation of a nonrenal organ. N Engl J Med 2003: 349: 931.
2. Gonwa TA, Mai ML, Melton LB et al. End-stage renal disease (ESRD) after orthotopic liver transplantation (OLTX) using calcineurin-based immunotherapy: risk of development and treatment. Transplantation 2001: 72: 1934.

3. Rapamune. [package insert]. Philadelphia (PA): Wyeth Pharmaceuticals; 2009.
4. Flechner SM, Goldfarb D, Solez K et al. Kidney transplantation with sirolimus and mycophenolate mofetil-based immunosuppression: 5-year results of a randomized prospective trial compared to calcineurin inhibitor drugs. Transplantation 2007: 83: 883.
5. Shenoy S, Hardinger KL, Crippin J et al. Sirolimus conversion in liver transplant recipients with renal dysfunction: a prospective, randomized, single-center trial. Transplantation 2007: 83: 1389.
6. Campbell MS, Rai J, Kozin E et al. Effects of sirolimus vs. calcineurin inhibitors on renal dysfunction after orthotopic liver transplantation. Clin Transplant 2007: 21: 377.
7. Morard I, Dumortier J, Spahr L et al. Conversion to sirolimus-based immunosuppression in maintenance liver transplantation patients. Liver Transpl 2007: 13: 658.
8. Maheshwari A, Torbenson MS, Thuluvath PJ. Sirolimus monotherapy versus sirolimus in combination with steroids and/or MMF for immunosuppression after liver transplantation. Dig Dis Sci 2006: 51: 1677.
9. Montalbano M, Neff GW, Slapak-Green G, Berney T, Meyer D. Sirolimus therapy in orthotopic liver transplant (OLT) recipients with acute renal insufficiency. Dig Dis Sci 2004: 49: 1986.
10. Nair S, Eason J, Loss G. Sirolimus monotherapy in nephrotoxicity due to calcineurin inhibitors in liver transplant recipients. Liver Transpl 2003: 9: 126.
11. Heffron TG, Smallwood GA, Davis L, Martinez E, Stieber AC. Sirolimus-based immunosuppressive [correction of immunosuppresive] protocol for calcineurin sparing in liver transplantation. Transplant Proc 2002: 34: 1522.
12. Fairbanks KD, Eustace JA, Fine D, Thuluvath PJ. Renal function improves in liver transplant recipients when switched from a calcineurin inhibitor to sirolimus. Liver Transpl 2003: 9: 1079.
13. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. Ann Intern Med 1999: 130: 461.
14. Foundation NK. K/DOQI Clinical practice guidelines for chronic kidney disease: evaluation, classification and stratification. Am J Kidney Dis 2002: 39(Suppl. 1): S1.
15. Miller BW. Chronic kidney disease in solid-organ transplantation. Adv Chronic Kidney Dis 2006: 13: 29.
16. Sindhi R, Seward J, Mazariegos G et al. Replacing calcineurin inhibitors with mTOR inhibitors in children. Pediatr Transplant 2005: 9: 391.
17. Cotterell AH, Fisher RA, King AL et al. Calcineurin inhibitor-induced chronic nephrotoxicity in liver transplant patients is reversible using rapamycin as the primary immunosuppressive agent. Clin Transplant 2002: 16 (Suppl. 7): 49.
18. Olyaei AJ, de Mattos AM, Bennett WM. Nephrotoxicity of immunosuppressive drugs: new insight and preventive strategies. Curr Opin Crit Care 2001: 7: 384.
19. Fisher NC, Nightingale PG, Gunson BK, Lipkin GW, Neuberger JM. Chronic renal failure following liver transplantation: a retrospective analysis. Transplantation 1998: 66: 59.
20. Neff GW, Montalbano M, Slapak-Green G et al. Sirolimus therapy in orthotopic liver transplant recipients with calcineurin inhibitor related chronic renal insufficiency. Transplant Proc 2003: 35: 3029.
21. Watson CJ, Gimson AE, Alexander GJ et al. A randomized controlled trial of late conversion from calcineurin inhibitor (CNI)-based to sirolimus-based immunosuppression in liver transplant recipients with impaired renal function. Liver Transpl 2007: 13: 1694.
22. Vivarelli M, Vetrone G, Zanello M et al. Sirolimus as the main immunosuppressant in the early postoperative period following liver transplantation: a report of six cases and review of the literature. Transpl Int 2006: 19: 1022.
23. Asfandiyar S, Abouljoud M, Kim D et al. Influence of hepatitis C on renal function after liver transplantation. Transplant Proc 2006: 38: 3643.
24. Samonakis DN, Cholongitas E, Triantos CK et al. Sustained, spontaneous disappearance of serum HCV-RNA under immunosuppression after liver transplantation for HCV cirrhosis. J Hepatol 2005: 43: 1091.
25. Zhu J, Wu J, Frizell E et al. Rapamycin inhibits hepatic stellate cell proliferation in vitro and limits fibrogenesis in an in vivo model of liver fibrosis. Gastroenterology 1999: 117: 1198.
26. Biecker E, De Gottardi A, Neef M et al. Long-term treatment of bile duct-ligated rats with rapamycin (sirolimus) significantly attenuates liver fibrosis: analysis of the underlying mechanisms. J Pharmacol Exp Ther 2005: 313: 952.
27. McEwan P, Baboolal K, Conway P, Currie CJ. Evaluation of the cost-effectiveness of sirolimus versus cyclosporin for immunosuppression after renal transplantation in the United Kingdom. Clin Ther 2005: 27: 1834.
28. McEwan P, Dixon S, Baboolal K, Conway P, Currie CJ. Evaluation of the cost effectiveness of sirolimus versus tacrolimus for immunosuppression following renal transplantation in the UK. Pharmacoeconomics 2006: 24: 67.