Limiting Hepatitis C Virus Progression in Liver Transplant Recipients Using Sirolimus-Based Immunosuppression

G. J. McKenna a, *, J. F. Trotter b, E. Klintmalm a, N. Onaca a, R. Ruiz a, L. W. Jennings a, M. Neer b, J. G. O’Leary b, G. L. Davis b, M. F. Levy a, R. M. Goldstein a and G. B. Klintmalm a

Departments of aSurgery and bMedicine, Baylor Transplant Institute, Baylor University Medical Center, Dallas, TX
*Corresponding author: Greg J. McKenna, greg.mckenna@baylorhealth.edu

Hepatitis C virus (HCV) causes progressive liver fibrosis in liver transplant recipients and is the principal cause of long-term allograft failure. The antifibrotic effects of sirolimus are seen in animal models but have not been described in liver transplant recipients. We reviewed 1274 liver recipients from 2002 to 2010 and identified a cohort of HCV recipients exposed to sirolimus as primary immunosuppression (SRL Cohort) and an HCV Control Group of recipients who had never received sirolimus. Yearly protocol biopsies were done recording fibrosis stage (METAVIR score) with biopsy compliance of >80% at both year one and two. In an intent-to-treat analysis, the SRL Cohort had significantly less advanced fibrosis (stage ≥2) compared to the HCV Control Group at year one (15.3% vs. 36.2%, p < 0.0001) and year two (30.1% vs. 50.5%, p = 0.001). Because sirolimus is sometimes discontinued for side effects, the SRL Cohort was subgroup stratified for sirolimus duration, showing progressively less fibrosis with longer sirolimus duration. Multivariate analysis demonstrated sirolimus as an independent predictor of minimal fibrosis at year one, and year two. This is the first study among liver transplant recipients with recurrent HCV to describe the positive impact of sirolimus in respect of reduced fibrosis extent and rate of progression.

Key words: Fibrosis, hepatitis C virus, liver transplantation, mTOR inhibitor/inhibition, sirolimus

Introduction

Recurrent hepatitis C virus (HCV) disease in the liver allograft is the primary cause of long-term graft failure in transplant recipients, and any successful strategy that minimizes recurrence, or its effects, would have a major impact on liver transplantation. Recurrent HCV is marked by progressive liver fibrosis caused by deposition of collagen in the liver allograft. Thus, an approach that affects collagen deposition may be a valuable strategy to alter the natural progression of HCV recurrence. In vitro studies have established the mammalian target of rapamycin (mTOR) as a mediator of collagen production by hepatic stellate cells thereby making mTOR a potential target for antifibrotic therapy. As the immunosuppressive effects of sirolimus also occur through inhibition of mTOR, sirolimus should by extension inhibit collagen production from hepatic stellate cells, and therefore make sirolimus a tool for limiting the effects of HCV recurrence.

Animal models of liver fibrosis have demonstrated that sirolimus can attenuate progression to advanced liver fibrosis. An animal model of cirrhosis created via bile duct ligation has shown that mTOR inhibition by sirolimus leads to prolonged survival. Despite these animal models that highlight the antifibrotic effects of sirolimus, its impact on fibrosis and cirrhosis in humans has never been definitively evaluated.

De novo sirolimus use in liver transplantation carries a black box warning from the FDA. Despite this black box warning, sirolimus has been used off-label for liver transplant recipients in several centers, and reports describe up to 11% of all recipients nationwide using sirolimus sometime during the first year of transplant. Our center has been using sirolimus by protocol since 1998 and as a result we have accumulated a large experience of de novo sirolimus use in liver transplant recipients. In addition, our center employs annual protocolized liver biopsies for all recipients. Using our large sirolimus experience, combined with...
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protocolized liver biopsy data we define how sirolimus impacts liver fibrosis progression due to recurrent HCV.

Materials and Methods

Study inclusion/exclusion criteria

Institutional review board approval from Baylor University Medical Center was obtained. This is an 8-year single center retrospective intention-to-treat cohort study from a prospectively obtained database. We reviewed all of the liver transplant recipients from March 2002 to March 2010 (including retransplants and multivisceral transplants) and identified a cohort group of HCV patients who received de novo sirolimus as part of their initial immunosuppression regimen (SRL Cohort).

Before receiving sirolimus at our center, all liver transplant recipients sign an informed consent. Complications specific to sirolimus, as well as the details in the FDA black box warning for sirolimus are explained to the patient with this consent.

Inclusion criteria:

1. Sirolimus initiated within 7 days posttransplant. (See “Indications for Sirolimus Use”)
2. Positive HCV RNA PCR.

Exclusion criteria:

1. Age <18 years.

A control group (HCV Control Group) consisted of all of the remaining HCV transplant recipients transplanted from March 2002–March 2010 to whom sirolimus was never given at any point posttransplant (either de novo or as a conversion).

Study design

The objective was to define the impact of sirolimus on both HCV fibrosis progression and the incidence of advanced fibrosis (biopsy stage >2) comparing HCV fibrosis on first and second year protocol biopsies between the SRL Cohort and HCV Control Group. The SRL Cohort was subsequently stratified by duration of sirolimus exposure over the 2-year study period with a subgroup analysis. The independent predictors of minimal HCV (biopsy stage 0–1) were identified with a stepwise linear regression multivariate analysis using the following variables thought to potentially impact fibrosis: recipient age, sex, MELD score, cold ischemia time (CIT), warm ischemia time (WIT), donor age, race, donor/recipient racial mismatch, pretransplant diabetes, interferon, genotypes 1, hepatocellular carcinoma (HCC), chemotherapy, cyclosporine, tacrolimus, mycophenolate, steroids, antibody therapy, acute rejection (ACR), steroid-resistant rejection, cytomegalovirus (CMV) infection and sirolimus, including subgroups sirolimus >90, >300 and >700 days. Additionally, a separate multivariate analysis was done using the duration of sirolimus as a continuous variable.

Indication for sirolimus use

The main indications for de novo sirolimus at our center were (i) HCC, (ii) posttransplant renal dysfunction (oliguria and Cr >3.0 persisting within 7 days posttransplant) and (iii) neurotoxicity where calcineurin inhibitor use was relatively contraindicated. The most common reasons for discontinuing sirolimus were pending surgical procedures, wound healing issues, rejection, infection, mouth ulcers and cytopenias.

Immunosuppression protocol

In the SRL Cohort, the sirolimus dose was 2 mg daily without a loading dose beginning on the first postoperative day. A second agent, namely tacrolimus, cyclosporine or mycophenolic acid (MMF) was used in addition to sirolimus. The initial serum concentration of tacrolimus was targeted at 10–15 ng/mL for the first 6 weeks and titrated to 7–10 ng/mL. Cyclosporin was initially targeted to 300–400 ng/mL for the first 6 weeks before titrating to 150–200 ng/mL. The initial MMF dose was 2000 mg/day in divided doses. In the HCV Control Group, the immunosuppression was tacrolimus or cyclosporine (titrated to a similar level as in the SRL Cohort) with a second agent of MMF. From March 2002 to June 2005 in both the SRL Cohort and the HCV Control Group, a standard prednisolone taper was used. This was tapered to 5 mg daily by one year and discontinued by postoperative day 420. From June 2005 to March 2010, a steroid-free protocol was used with dacluzimab 2mg/kg induction on days 1, 3 and 8.

Indication for interferon therapy

Recipients were considered for interferon therapy if they remain HCV RNA positive and they have a biopsy demonstrating stage ≥2 fibrosis, and they do not have any contraindications to therapy.

Follow-up and liver biopsies

Upon discharge from hospital, all recipients were followed in clinic for 3 months posttransplant. Yearly protocol liver biopsies were done at year one and two as part of our routine follow-up with quantitative HCV RNA PCR performed in conjunction with each biopsy. In addition, liver biopsies were performed in response to elevated liver enzymes on a nonprotocol basis and all episodes of allograft rejection were biopsy diagnosed. Biopsies were reviewed by one of our two liver pathologists using the METAVIR system to score for grade of inflammation and stage of fibrosis.

Statistical methods

The Wilcoxon two-sample test was used to compare continuous variables. Categorical variables were compared using the two-sided Fisher’s exact test for 2 × 2 tables and the likelihood ratio chi-square test for larger tables. Survival curves were estimated using the Kaplan–Meier method and compared using the Log Rank test. A multivariate analysis was done applying a stepwise multiple logistic regression obtaining odds ratio estimates with 95% Wald Confidence limits. Variables identified by univariate analysis to have a p < 0.10 were included in the stepwise model. Statistical significance was defined as p < 0.05. Groups of variables are expressed as mean values. All analyses were performed using SAS 9.1.3 (SAS Institute, Cary, NC).

Results

Demographics and complications

Of 1274 deceased donor liver recipients from March 2002 to March 2010, 540 were HCV patients. There were 258 HCV patients who received sirolimus, of whom 85 were later conversions to sirolimus occurring >7 days posttransplant, leaving 173 de novo sirolimus HCV patients representing the SRL Cohort. The HCV Control Group was made up of the remaining 282 HCV patients from the same period who never received sirolimus posttransplant at any point.

Patient demographics are outlined in Table 1. The SRL Cohort had a higher mean recipient age (53.7 years vs. 50.4 years, p = 0.0001), and a lower mean calculated MELD score (13.6 vs. 18.1, p < 0.0001). The SRL Cohort has significantly more HCC (84.9% vs. 17.0%, p < 0.0001) as expected given our described immunosuppression...
The mean pretransplant HCV RNA was significantly greater in the SRL Cohort (1.46 × 10^6 vs. 0.2 × 10^6, p = 0.0003) but was not different at either the first or second year posttransplant (Table 3). There was no difference in the number of patients with HCV genotype 1. Interferon therapy was used significantly more in the HCV Control Group compared to the SRL Cohort (24.7% vs. 8.4%, p < 0.0001).

### Sirolimus duration

The mean duration of sirolimus over the 2-year study period was 328.5 days and the median duration was 242 days.

### Biopsy compliance

Protocol biopsies were performed in 93.1 and 82.7% of the SRL Cohort surviving to the first and second year follow-up respectively. This was statistically similar to the biopsy compliance in the HCV Control Group, where biopsies were performed in 90.4 and 83.6% surviving to the first and second year follow-up.

### Protocol biopsy analysis of HCV fibrosis progression

In an intent-to-treat analysis, the SRL Cohort had significantly reduced mean fibrosis stage at both 1 year posttransplant (0.62 vs. 1.24, p = 0.0001) and at 2 years posttransplant (1.15 vs. 1.74, p = 0.0003) compared to the HCV Control Group (Figure 2). There was also a significantly lower incidence of advanced fibrosis (stage ≥2) in the SRL Cohort at 1 year posttransplant (15.3% vs. 36.2%, p = 0.0001) and 2 years posttransplant (30.1% vs. 50.5%, p = 0.0005) compared to the HCV Control Group (Figures 3 and 4).

In a subgroup analysis that factored the impact of the duration of sirolimus therapy on fibrosis, the SRL Cohort was stratified into groups <90 days, >90 days and >300 days of sirolimus at 1 year posttransplant and <90, >90 days, >300 days and >700 days of sirolimus at year two. In the <90 day SRL Cohort subgroup there was no difference in advanced fibrosis compared to the HCV Control Group at both 1 year (25.4% vs. 36.2%, p = NS) and 2 years (41.3% vs 50.5%, p = NS). However, after 1 year, the HCV Control Group had 36.2% advanced fibrosis compared to 8.8% of the >90 day SRL Cohort subgroup (p < 0.0001) and 4.3% of the >300 day SRL Cohort subgroup (p < 0.0001) (Figure 3). After 2 years, the HCV Control Group had 50.5% advanced fibrosis compared to 22.4% of the >90 day SRL Cohort subgroup (p = 0.0002), 17.0% of the >300 day

Limiting HCV with Sirolimus

Table 1: Comparison of patient demographics of recipient, donor organ criteria between SRL Cohort and HCV Control

<table>
<thead>
<tr>
<th></th>
<th>SRL cohort (n = 173)</th>
<th>HCV control (n = 282)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recipient age (mean)</td>
<td>53.7 y</td>
<td>50.4 y</td>
<td>0.0001</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>76.7%</td>
<td>75.8%</td>
<td>NS</td>
</tr>
<tr>
<td>Black</td>
<td>8.7%</td>
<td>8.5%</td>
<td>–</td>
</tr>
<tr>
<td>Hispanic</td>
<td>20.9%</td>
<td>16.0%</td>
<td>–</td>
</tr>
<tr>
<td>Other</td>
<td>4.7%</td>
<td>1.5%</td>
<td>–</td>
</tr>
<tr>
<td>Racial mismatch (donor/recipient)</td>
<td>56.0%</td>
<td>49.6%</td>
<td>NS</td>
</tr>
<tr>
<td>Pretransplant diabetes</td>
<td>14.0%</td>
<td>15.4%</td>
<td>NS</td>
</tr>
<tr>
<td>HCC</td>
<td>84.9%</td>
<td>17.0%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Donor age (mean)</td>
<td>41.5 y</td>
<td>41.6 y</td>
<td>NS</td>
</tr>
<tr>
<td>CIT (mean)</td>
<td>8.03</td>
<td>8.14</td>
<td>NS</td>
</tr>
<tr>
<td>Calculated MELD (mean)</td>
<td>13.6</td>
<td>18.1</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

NS = not significant.

Table 2: Incidence of complications acute cellular rejection (ACR), steroid resistant rejection (SRR), cytomegalovirus infection (CMV) and hepatic artery thrombosis (HAT) between SRL Cohort and HCV Control

<table>
<thead>
<tr>
<th></th>
<th>SRL cohort (n = 173)</th>
<th>HCV control (n = 282)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACR incidence</td>
<td>38.3%</td>
<td>41.8%</td>
<td>NS</td>
</tr>
<tr>
<td>SRR incidence</td>
<td>5.2%</td>
<td>7.5%</td>
<td>NS</td>
</tr>
<tr>
<td>CMV incidence</td>
<td>13.3%</td>
<td>20.2%</td>
<td>0.07</td>
</tr>
<tr>
<td>HAT incidence</td>
<td>1.2%</td>
<td>5.6%</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Table 3: HCV relevant parameters including HCV RNA levels at pretransplant, and years 1 and 2 posttransplant. HCV genotype and the use of interferon therapy

<table>
<thead>
<tr>
<th></th>
<th>SRL cohort (n = 173)</th>
<th>HCV control (n = 282)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCV RNA (mean)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pretransplant</td>
<td>1.46 × 10^6</td>
<td>0.2 × 10^6</td>
<td>0.003</td>
</tr>
<tr>
<td>1 year</td>
<td>9.16 × 10^6</td>
<td>6.90 × 10^6</td>
<td>NS</td>
</tr>
<tr>
<td>2 years</td>
<td>6.12 × 10^6</td>
<td>4.83 × 10^6</td>
<td>NS</td>
</tr>
<tr>
<td>HCV Genotype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genotype 1</td>
<td>78.9%</td>
<td>81.8%</td>
<td>NS</td>
</tr>
<tr>
<td>Genotype 2–4</td>
<td>21.1%</td>
<td>18.2%</td>
<td>–</td>
</tr>
<tr>
<td>Post-OLT interferon therapy</td>
<td>8.4%</td>
<td>24.7%</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

NS = not significant.

(Figure 1). The most common reason for discontinuation of sirolimus was pending surgery (18.5%), wound healing issues (5.8%), wound infection (4.6%), neutropenia (4.0%), mouth ulcers (4.0%), rejection (3.5%) and pneumonia (3.5%). Sirolimus was restarted in 33.5% of the SRL Cohort where it had been discontinued.
SRL Cohort subgroup (p < 0.0001) and 0% of the >700 day SRL Cohort subgroup (p < 0.0001) (Figure 4).

Because the significantly greater percentage of HCC patients in the SRL Cohort represented a major bias, an additional subgroup analysis was done comparing the 51 HCC patients in the HCV Control Group who never received any sirolimus (HCC/HCV Subgroup) to both the entire HCV Control Group and the SRL Cohort Subgroup. At both year one and year two, there was no difference in advanced fibrosis between the HCC/HCV Subgroup and the entire HCV Control Group (47.6% vs. 36.2%, p = NS at year one and 57.6% vs. 50.5%, p = NS at year two). Comparing the HCC/HCV Subgroup to the SRL Cohort, there was significantly less advanced fibrosis in the SRL Cohort at both 1 year (15.3% vs. 47.6%, p = 0.0001) and 2 years posttransplant (30.1% vs. 57.6%, p = 0.007).

**Multivariate analysis**

We identified factors that might impact HCV fibrosis progression, including recipient factors (age, race, sex, HCC, diabetes, racial mismatch, genotype 1, calculated MELD score and warm ischemia time), donor factors (donor age and cold ischemia time), immunosuppression type (mycophenolate, steroids, calcineurin inhibitor and antibody), rejection, infection and chemotherapy. A multivariate analysis applying a stepwise linear regression was performed using these factors, as well as using the various sirolimus subgroups as variables (Table 4). We set the linear regression as the inverse, so it defined the likelihood of these variables to predict minimal HCV fibrosis (stage <2). At year one posttransplant, sirolimus of >300 days duration was the strongest independent predictor of minimal HCV fibrosis (odds ratio 8.91, CI 2.56–30.97) followed by an absence of steroid resistant rejection, sirolimus of >90 days duration, CIT < 7 h and donor age <50. Posttransplant interferon therapy was a strong independent predictor of advanced fibrosis (stage ≥2) (odds ratio 0.15, CI 0.07–0.32). At year two, sirolimus of >300 days duration was the strongest predictor of minimal fibrosis (odds ratio of 18.46, CI 4.30–79.20) followed by non–African American race, while posttransplant interferon therapy was an independent predictor of advanced fibrosis, followed by CMV infection.

An additional multivariate analysis performed with sirolimus duration as a continuous variable shows sirolimus to be a significant predictor of fibrosis <2 at any time period, with an exponentially increasing odds ratio that increases as sirolimus duration increases (Figure 5).

**Survival**

The 1 year actual patient survival between the SRL Cohort and HCV Control Group (92.5% vs. 87.9%, p = 0.15) was statistically similar. A Kaplan–Meier analysis showed that similar patient and graft survival extended up to 5 years posttransplant (Figure 6).

**Discussion**

Sirolimus is an immunosuppressant from the class termed mTOR inhibitors, which inhibits the mTOR signal transduction pathway and prevents IL2 stimulation of T-lymphocytes. In regards to immunosuppression, mTOR
inhibition prevents translation of cell cycle regulating proteins such as cyclin E and cyclin A-dependent kinase (4) via regulation of the p70–S6 kinase gene. This prevents the cell cycle transition from G1 to S phase, limiting cellular proliferation and activation of lymphocytes.

In addition to its regulation of the cell cycle, mTOR affects several different cellular processes that impact fibrogenesis. This allows mTOR inhibitors several mechanisms for limiting development of liver fibrosis from the hepatic stellate cells: (i) decreasing type 1 collagen mRNA synthesis through a pathway independent of p70S6 kinase (5); (ii) decreasing fibroblast proliferation by inhibiting expression of profibrotic growth factors MGP-1, PDGF and TGF-β (6); (iii) interfering with fibroblast attachment by impacting the α-1β-3 integrin pathway and (4) decreasing expression of plasminogen activator inhibitor-1, a key promoter of fibrosis (7–9). In addition, mTOR may also mediate inhibition of the JAK-STAT pathway by SOCS3 (suppressor of cytokine 3) increasing the production of interferon stimulating genes and accordingly suppressing HCV replication, thereby impacting HCV fibrogenesis (10–12).

Building on in vitro data regarding the impact of sirolimus on fibrogenesis are in vivo animal models that describe its impact on liver fibrosis. Two different animal models—long-term CCl4 treatment in rats, as well as bile duct ligation rat models—have demonstrated that sirolimus limits disease progression to advanced liver fibrosis (2), and will prolong survival in a setting of cirrhosis (3). In vivo sirolimus treatment halves the mRNA expression of TGF-β and PDGF, which in vitro studies show to be a major stimulator of fibrosis pathways.

Recurrent HCV is characterized by progressive fibrosis of the liver allograft and is universal in liver recipients who are HCV positive at the time of transplant (13). Most patients develop histologic features of acute HCV 4–12 weeks post-transplant which parallel an increase in HCV RNA (14,15). In liver transplant recipients, the natural history of chronic
HCV and progression of fibrosis is accelerated due to the effects of immunosuppression, with 10–30% of recipients progressing to cirrhosis by 5 years and more than 40% by 10 years (16).

Our center uses annualized protocol biopsies in HCV patients to define and track HCV disease progression. There was a very high biopsy compliance rate among our subjects, exceeding 80% in both the SRL Cohort and HCV Control Group for both the 1 and 2 year biopsy. The use of protocol biopsies, with a high compliance rate is what allows us to definitively describe sirolimus’ impact on HCV recurrence.

The natural history of progression of HCV fibrosis in post-transplant recipients has been described by several groups that perform protocol biopsies. Some have described a linear fibrosis progression rate of +0.8 fibrosis stage units per year (17). Other groups describe a nonlinear fibrosis progression, with mean fibrosis scores of 1.2 at year one and 1.7 at year two posttransplant (18). Data from these groups correspond closely to the HCV fibrosis progression in our HCV Control Group, which had a mean fibrosis score of 1.24 at year one and 1.74 at year two posttransplant. Thus, our HCV Control Group experienced a typical fibrosis progression when compared to those described by historical reports (Figure 2).

Evaluating on an intent-to-treat basis—involving all de novo sirolimus HCV patients including those in whom sirolimus was discontinued—the SRL Cohort had significantly less fibrosis compared to the HCV Control Group for both the mean fibrosis score and the incidence of advanced fibrosis. This significant reduction in fibrosis with sirolimus was seen at both 1 and 2 years posttransplant.

One of the realities of using sirolimus is that many patients have it discontinued at some point for a variety of reasons. At our center, almost half of the SRL Cohort had sirolimus stopped by 8 months with the main reasons for discontinuation including pending surgery, wound healing issues, mouth ulcers, rejection and cytopenias. To address the impact of variable sirolimus duration on our results, a subgroup analysis of the SRL Cohort was done stratifying for the duration of sirolimus. This demonstrated that duration of sirolimus is the key to the outcomes. As the duration of sirolimus increases the incidence of advanced fibrosis significantly decreases, so that patients with >300 days of sirolimus have one quarter of the incidence of advanced fibrosis compared to nonsirolimus patients at year one, and >700 days of sirolimus have no patients with advanced fibrosis at year two compared to more than half of the nonsirolimus patients. To further underline the impact of sirolimus duration, those patients receiving <90 days of sirolimus had no difference in advanced fibrosis at years one and two compared to nonsirolimus patients.

Fibrosis progression is abrogated with continual sirolimus therapy, as the difference in mean fibrosis in the >300 days SRL Cohort at 1 year and >700 days SRL Cohort at 2 years was only +0.06 compared to a progression of +0.50 in the HCV Control Group between years one and two. Thus, while on sirolimus therapy, the level of fibrosis in the liver stays essentially unchanged. Additionally, by the end of year two, 20.4% of the HCV Control Group had progressed to cirrhosis (biopsy stage 4) compared to none of patients receiving >700 days sirolimus.

A stepwise linear regression multivariate analysis was performed to define the independent predictors of HCV fibrosis, considering variables thought to impact HCV fibrosis as well as the duration of sirolimus therapy. The strongest independent predictor of minimal fibrosis (stage <2) on biopsy at the first year was a sirolimus duration of >300 days, followed by an absence of steroid resistant rejection, a sirolimus duration of >90 days, CIT < 7 h and donor age <50 years, while posttransplant interferon therapy was a strong independent predictor of advanced fibrosis. At year two, the strongest independent predictor of minimal fibrosis was >300 days of sirolimus, followed by non–African American with posttransplant interferon being a strong predictor of advanced fibrosis, followed by CMV infection. The multivariate analysis reiterates the importance that longer duration of sirolimus has upon limiting fibrosis in the allograft. Examining the sirolimus duration instead as a continuous variable within in the multivariate analysis showed
that sirolimus was an independent predictor of minimal fibrosis at each time point, with the odds ratio exponentially increasing with sirolimus duration.

The impact of sirolimus on HCV progression does not seem to be explained by favorable HCV elements in the SRL Cohort. Viral load was either similar or significantly higher in the SRL Cohort at all time points, there was no difference in the incidence of genotype 1, and there was significantly less interferon use in the SRL Cohort compared to the HCV Control Group. It is expected that interferon therapy would be a predictor of advanced fibrosis in the multivariate analysis since biopsy stage $\geq 2$ is our indication to initiate therapy, and the higher interferon therapy in the HCV Control Group reflects the higher incidence of advanced fibrosis occurring in that group.

One possible confounding factor for the difference in fibrosis between the two groups might be related to the difference in HCC incidence. As HCC patients get an upgrade to their MELD score, they often have a lower calculated MELD score compared to their assigned MELD score, and the higher HCC incidence explains the lower MELD score in the SRL Cohort since our center’s immunosuppression protocol uses sirolimus for HCC patients. To control for this potential confounding factor, the subgroup analysis of HCC patients in the HCV Control Group who never received sirolimus (HCC/HCV Control Subgroup) demonstrated no difference in fibrosis compared to the entire HCV Control Group yet was significantly greater than in those patients on sirolimus therapy. This subgroup analysis removes HCC as a confounding factor for explaining sirolimus’ impact on fibrosis.

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**Figure 5:** Graphical result of the multivariate analysis for biopsy fibrosis stage $<$2 at year two, with sirolimus duration as a continuous variable. The odds ratio is plotted against specific sirolimus durations. The table shows the odds ratio along with the Wald 95% confidence intervals from the multivariate analysis.

**Figure 6:** A Kaplan–Meier survival analysis of patient survival and graft survival comparing the SRL Cohort to the HCV Control Group.

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Another explanation for the reduction in HCV fibrosis with sirolimus use might be that sirolimus provides an overall lower level of immunosuppression. However, there was no statistical difference in either the incidence of acute cellular rejection or steroid resistant rejection between the SRL Cohort and the HCV Control Group. If sirolimus generated a lower overall immunosuppression, one would have expected a higher rate of rejection in the SRL Cohort.

A third explanation for the differences in outcomes between the SRL Cohort and the HCV Control Group is that those patients with longer sirolimus duration (i.e. > 700 days) may reflect a more stable group of patients. Patients with rejection or posttransplant surgical procedures often get their sirolimus doses held. A prolonged sirolimus course may just be a surrogate marker of a patient with a stable posttransplant course and therefore portend a better outcome. We are unable to exclude this explanation as a possible reason for the difference. However, as a counter to this, the HCV Control Group is healthier compared to the SRL Cohort with respect to renal function. Our centers protocol is to convert patients with posttransplant renal insufficiency to sirolimus and because the patients in the HCV Control Group have never received sirolimus, this HCV Control group contains a fewer patients with renal insufficiency.

Resistance to exploring the use of sirolimus *de novo* posttransplant in HCV patients is a result of the FDA black box warning against sirolimus describing increased HAT incidence and increased graft loss and mortality. Contrary to the black box warning, in our study the SRL Cohort had a significantly lower incidence of HAT compared to controls, and a higher graft and patient survival although this was not statistically significant (Figure 5). These data reassure that sirolimus can be used safely in liver transplant patients, and that the black box warning should not limit clinicians from exploiting the antifibrotic effects of sirolimus effects to limit HCV fibrosis progression.

Conclusions

In summary, these data suggest that sirolimus administration is associated with reduced fibrosis from HCV as seen by protocol biopsies, and this is the first study to demonstrate sirolimus’ potential benefit on limiting HCV recurrence. The effects of sirolimus appear related to the duration of sirolimus, with a greater reduction in fibrosis occurring with increased duration of sirolimus therapy. Maintaining patients on long-term sirolimus may represent a way to contain the effects of HCV recurrence and limit disease progression. Because this was not a randomized trial, there is significant consideration that the two populations of recipients may be sufficiently different to explain these findings, however the data are bolstered by the use of protocol biopsies with a very high compliance rate. These data warrant further consideration in a controlled prospective trial to delineate sirolimus’ impact on HCV recurrence.

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Disclosure

The authors of this manuscript have no conflict of interest to disclose as described by the *American Journal of Transplantation*.

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