

Complete Immunosuppression Withdrawal and Subsequent Allograft Function Among Pediatric Recipients of Parental Living Donor Liver Transplants

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SOLID ORGAN TRANSPLANTATION generally incurs a lifelong burden of immunosuppression with many accompanying toxic effects, including renal dysfunction, metabolic perturbation, opportunistic infection, and malignancy.¹⁻⁴ However, in liver transplantation, several single-center experiences suggest that a proportion of liver recipients can maintain normal allograft function without immunosuppression, termed operational tolerance.⁵⁻¹⁶ Based on these studies, the estimated frequency of operational tolerance has been proposed to be as high as 20%.¹⁷ Although withdrawal of immunosuppression therapy in liver allograft recipients can precipitate rejection, most episodes are reversible without long-term consequences, rendering this patient population ap-

See also Patient Page.

Context Although life-saving, liver transplantation burdens children with lifelong immunosuppression and substantial potential for morbidity and mortality.

Objective To establish the feasibility of immunosuppression withdrawal in pediatric living donor liver transplant recipients.

Design, Setting, and Patients Prospective, multicenter, open-label, single-group pilot trial conducted in 20 stable pediatric recipients (11 male; 55%) of parental living donor liver transplants for diseases other than viral hepatitis or an autoimmune disease who underwent immunosuppression withdrawal. Their median age was 6.9 months (interquartile range [IQR], 5.5-9.1 months) at transplant and 8 years 6 months (IQR, 6 years 5 months to 10 years 9 months) at study enrollment. Additional entry requirements included stable allograft function while taking a single immunosuppressive drug and no evidence of acute or chronic rejection or significant fibrosis on liver biopsy. Gradual immunosuppression withdrawal over a minimum of 36 weeks was instituted at 1 of 3 transplant centers between June 5, 2006, and November 18, 2009. Recipients were followed up for a median of 32.9 months (IQR, 1.0-49.9 months).

Main Outcome Measures The primary end point was the proportion of operationally tolerant patients, defined as patients who remained off immunosuppression therapy for at least 1 year with normal graft function. Secondary clinical end points included the durability of operational tolerance, and the incidence, timing, severity, and reversibility of rejection.

Results Of 20 pediatric patients, 12 (60%; 95% CI, 36.1%-80.9%) met the primary end point, maintaining normal allograft function for a median of 35.7 months (IQR, 28.1-39.7 months) after discontinuing immunosuppression therapy. Follow-up biopsies obtained more than 2 years after completing withdrawal showed no significant change compared with baseline biopsies. Eight patients did not meet the primary end point secondary to an exclusion criteria violation (n=1), acute rejection (n=2), or indeterminate rejection (n=5). Seven patients were treated with increased or reinitiation of immunosuppression therapy; all returned to baseline allograft function. Patients with operational tolerance compared with patients without operational tolerance initiated immunosuppression withdrawal later after transplantation (median of 100.6 months [IQR, 71.8-123.5] vs 73.0 months [IQR, 57.6-74.9], respectively; $P=.03$), had less portal inflammation (91.7% [95% CI, 61.5%-99.8%] vs 42.9% [95% CI, 9.9%-81.6%] with no inflammation; $P=.04$), and had lower total C4d scores on the screening liver biopsy (median of 6.1 [IQR, 5.1-9.3] vs 12.5 [IQR, 9.3-16.8]; $P=.03$).

Conclusion In this pilot study, 60% of pediatric recipients of parental living donor liver transplants remained off immunosuppression therapy for at least 1 year with normal graft function and stable allograft histology.

JAMA. 2012;307(3):283-293

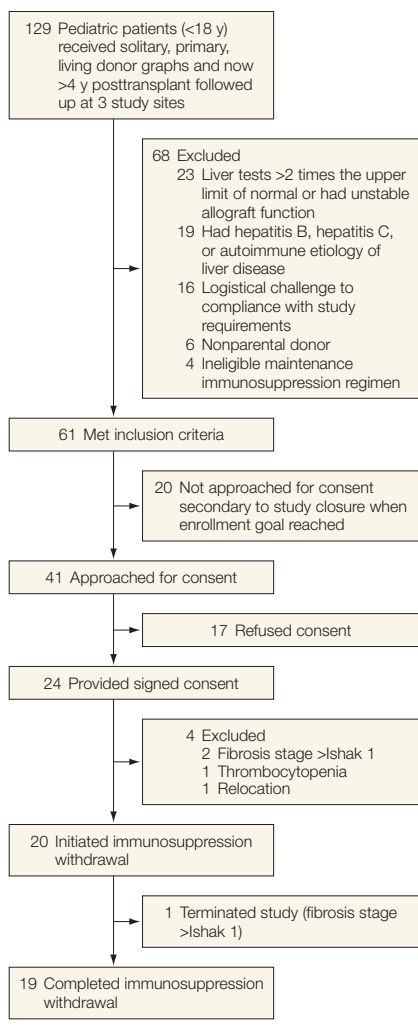
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Figure 1. Patient Flow Diagram



appropriate for drug minimization, discontinuation, or both.

To determine the feasibility of withdrawal of immunosuppression therapy in children, we conducted a prospective, multicenter, open-label, single-group pilot trial of withdrawal of immunosuppression therapy in a selected subgroup of pediatric liver transplant recipients. The study's primary end point was the proportion of operationally tolerant patients, defined as those remaining off immunosuppression therapy for 1 year with normal graft function. Secondary clinical end points included the durability of operational tolerance, and the incidence, timing, severity, and reversibility of rejection.

METHODS

The study was limited to pediatric (<18 years) recipients of parental living donor liver transplants 4 or more years prior to enrollment, who also had stable allograft function during the preceding 6 months while taking a single immunosuppressive drug. Eligible patients were required to undergo liver biopsy and permitted to undergo withdrawal of immunosuppression therapy if the biopsy did not show evidence of acute or chronic rejection or significant fibrosis (Ishak stage >1). Patients were excluded if they underwent transplantation for liver failure due to viral hepatitis or an autoimmune disease. Patients with serological evidence of autoimmunity defined as abnormal anti-nuclear, anti-smooth muscle, anti-mitochondrial, or anti-liver or anti-kidney microsomal antibody titers also were excluded. Similarly, patients with hepatitis B infection (defined by the presence of hepatitis B surface antigen or active treatment for hepatitis B) or those with hepatitis C infection (defined by the presence of antibody against hepatitis C) were not eligible for the study. Full study entry criteria appear in the eAppendix (at <http://www.jama.com>) and details regarding patient selection appear in FIGURE 1.

Age-appropriate written informed assent of all study patients and informed consent of all parents or legal guardians and all parental liver donors were obtained in person. The study was approved by the institutional review boards of all participating centers.

Immunosuppression Withdrawal

Patients underwent stepwise immunosuppression reduction over a minimum of 36 weeks at 1 of 3 transplant centers between June 5, 2006, and November 18, 2009 (FIGURE 2). Withdrawal was temporarily suspended for allograft dysfunction. Liver biopsy was required for any episode of allograft dysfunction unexplained by concurrent illness or other circumstance. Patients were considered to have failed immuno-

suppression withdrawal if the medication reduction was suspended for longer than 4 weeks or for any episode of rejection requiring treatment.

Assessments

During immunosuppression withdrawal and for 3 months after the last dose, patients underwent liver tests, which included levels of aspartate transaminase, alanine transaminase (ALT), alkaline phosphatase, total and direct bilirubin, and γ -glutamyl transpeptidase (GGT), every 2 weeks and at clinic visits every 3 months. Next, patients were transitioned to monthly liver tests and biannual clinic visits for 2 years followed by liver tests every 2 months and annual clinic visits for 2 additional years. Alloantibodies, autoantibodies, and quantitative immunoglobulin G (IgG) were monitored every 3 months during immunosuppression withdrawal and for 3 months after the last dose and every 6 months thereafter. Patients who did not complete immunosuppression withdrawal were managed according to the treatment center's standard of care and were followed up for 1 year.

Four protocol-specified liver biopsies were required at study entry, at 4 to 8 weeks, at 2 years, and at 4 years after the last immunosuppression dose after study entry. Patients who did not complete immunosuppression withdrawal were not required to undergo additional protocol biopsies. Patients were followed up for a median of 32.9 months (interquartile range [IQR], 1.0-49.9 months).

Allograft Dysfunction

Allograft dysfunction was defined as elevation of ALT or both alkaline phosphatase and GGT compared with values at baseline. Baseline values were calculated as the mean of 3 separate assessments using values obtained prior to screening, at screening, and at the liver biopsy visit. If values were normal or below normal at baseline, dysfunction was considered to have occurred when values reached twice the upper limit of normal. If above nor-

mal at baseline, dysfunction was considered to have occurred when values reached twice the baseline value. Resolution was defined as values less than or equal to the upper limit of normal if baseline tests were normal or below normal and less than or equal to $1.2 \times$ baseline if baseline tests were above normal.

Diagnosis and Treatment of Acute Rejection

Clinical diagnosis of acute rejection was based on the site pathologist’s assessment of the liver biopsy according to the Banff criteria.^{18,19} Treatment was given according to the discretion of the principal investigator and not specified by the trial protocol. The central pathologist’s blinded prospective readings were used for data analysis and reporting.

End Points

The primary end point was the proportion of patients who remained off immunosuppression therapy for at least 1 year and retained normal graft function. Secondary end points included rates and severity of acute rejection, graft loss, death, and adverse events.

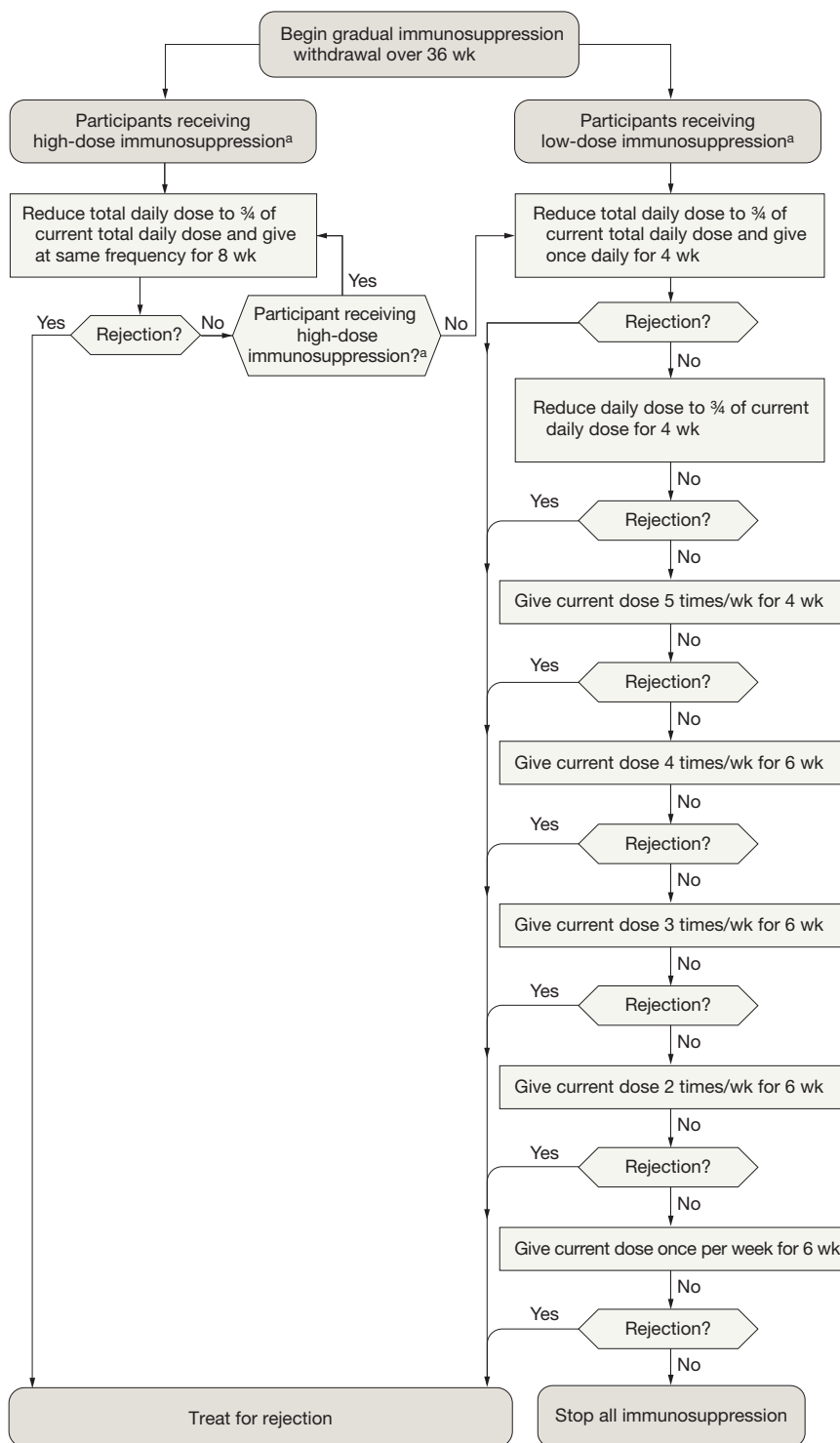
HLA Typing, Alloantibody Detection, and Flow Crossmatch

HLA typing was performed by automated DNA sequencing (University of California, San Francisco). HLA antibody screening and specificity determination were performed at Emory University using the FlowPRA Screening (One Lambda Inc) and LabScreen Single Antigen (One Lambda Inc) assays. Flow cytometry crossmatches were performed and reported as previously described.^{20,21}

Histology and Immunohistochemistry

High resolution $40 \times$ whole slide images of formalin-fixed, paraffin-embedded, and hematoxylin-eosin-stained $4\text{-}\mu\text{m}$ tissue sections were prospectively scored for 48 histopathologic criteria. C4d deposition was evaluated blindly on frozen preweaning bi-

Figure 2. Immunosuppression Withdrawal Algorithm



^aAll patients entered the study while taking either cyclosporine or tacrolimus monotherapy. For cyclosporine, high dose is defined as 3 mg/kg/day or greater; low dose is less than 3 mg/kg/day. For tacrolimus, high dose is defined as 0.08 mg/kg/day or greater; low dose is less than 0.08 mg/kg/day.

opsies using multiplex quantum dot immunostaining for C4d (rabbit polyclonal BI-RC4D, Alpco Diagnostics, 1:30) and CD31 (mouse monoclonal JC/70A, ThermoFisher, 1:25).²² Each vascular compartment and surrounding stroma (portal vein and capillary, portal stroma, hepatic artery, sinusoid, central vein and perivenular stroma) was separately scored (0=none; 1=minimal; 2=focal; 3=diffuse) and summed for a total C4d score.

Statistical Analysis

Individual baseline characteristics were analyzed to identify those that differed between tolerant and nontolerant patients. Categorical variables were compared between the 2 groups using the Fisher exact test and the *t* test for continuous variables with a 2-tailed α level of .05. The 1-sample 95% confidence intervals were constructed using an exact binomial distribution. Analyses were conducted using SAS statisti-

cal software version 9.1 (SAS Institute Inc). Data were analyzed up to July 31, 2010.

RESULTS

Demographics and Transplant History

Donor and recipient demographics showed a median recipient age of 6.9 months (IQR, 5.5-9.1 months) at transplantation and 8 years 6 months (IQR, 6 years 5 months to 10 years 9 months) at enrollment. Eleven patients (55%) were male and all were white (TABLE 1). Biliary atresia was the liver disease etiology for 16 patients (80%). The median donor age was 32.5 years (IQR, 30.8-37.3 years); 6 donors (30%) were fathers and all were white.

None of the patients received induction immunosuppression. After transplantation, all were discharged from the hospital while taking corticosteroids and calcineurin inhibitors (cyclosporine: n=17 [85%]; tacrolimus: n=3

[15%]). Fifteen patients (75%) also were discharged while taking a third immunosuppression drug (mycophenolate mofetil: n=11 [55%]; azathioprine: n=4 [20%]). Prior to enrollment, 9 patients (45%) had 1 episode, 2 patients (10%) had 2 episodes, and a single patient (5%) had 3 episodes of acute rejection. At study entry, 13 patients (65%) were taking tacrolimus and 7 patients (35%) were taking cyclosporine monotherapy.

Outcomes

Of the 20 patients, 12 (60%; 95% CI, 36.1%-80.9%) met the primary end point of remaining off immunosuppression therapy for at least 1 year with normal graft function and were termed *tolerant*. Of the 8 patients (40%) who did not meet the primary end point and who were termed *nontolerant*, 5 did not meet the primary end point during withdrawal of immunosuppression therapy and 3 did not meet the pri-

Table 1. Demographic Characteristics of Study Participants and Parental Living Liver Donors

Patient No.	Age		Period From Transplant to Study Entry	Sex	Liver Disease	Immunosuppression Therapy at Study Entry	
	At Transplant	At Study Entry				Calcineurin Inhibitor	Dose
1	3.8 mo	8 y 3 mo	7 y 11 mo	Male	Biliary atresia	Tacrolimus	1.00 mg twice daily
2	6.8 mo	9 y 2 mo	8 y 7 mo	Male	Biliary atresia	Tacrolimus	0.35 mg twice daily
3	6.6 mo	8 y 9 mo	8 y 2 mo	Female	Biliary atresia	Tacrolimus	0.25 mg twice daily
4	7.0 mo	12 y 2 mo	11 y 7 mo	Male	Biliary atresia	Tacrolimus	0.50 mg twice daily
5	4.6 mo	10 y 4 mo	10 y	Male	Biliary atresia	Cyclosporine	45 mg twice daily
6	9.1 mo	7 y	6 y 2 mo	Female	Biliary atresia	Tacrolimus	1.00 mg twice daily
7	4.8 mo	11 y 9 mo	11 y 4 mo	Female	Biliary atresia	Cyclosporine	38 mg twice daily
8	7 y 6 mo	15 y 3 mo	7 y 10 mo	Female	Ornithine transcarbamylase deficiency	Tacrolimus	4.50 mg twice daily
9	9.1 mo	5 y 3 mo	4 y 6 mo	Male	Biliary atresia	Tacrolimus	0.50 mg twice daily
10	5.3 mo	6 y 8 mo	6 y 3 mo	Female	Biliary atresia	Cyclosporine	25 mg twice daily
11	7.4 mo	5 y 6 mo	4 y 11 mo	Male	Biliary atresia	Cyclosporine	50 mg twice daily
12	6.7 mo	11 y 2 mo	10 y 7 mo	Female	Biliary atresia	Cyclosporine	50 mg/d
13	8.4 mo	13 y 5 mo	12 y 9 mo	Female	Biliary atresia	Tacrolimus	1.00 mg/d
14	3.8 mo	10 y 4 mo	10 y	Female	2 ₁ -Antitrypsin deficiency	Cyclosporine	35 mg/d
15	1 y 1 mo	6 y 3 mo	5 y 2 mo	Male	Biliary atresia	Tacrolimus	1.50 mg/d
16	2 y 5 mo	8 y 10 mo	6 y 5 mo	Male	Familial cholestasis or Byler disease	Tacrolimus	2.00 mg/d
17	1 y 5 mo	7 y 1 mo	5 y 7 mo	Male	Neonatal sclerosing cholangitis	Tacrolimus	1.00 mg/d
18	5.6 mo	6 y 7 mo	6 y 1 mo	Female	Biliary atresia	Tacrolimus	1.00 mg/d
19	5.6 mo	5 y 3 mo	4 y 10 mo	Male	Biliary atresia	Tacrolimus	0.50 mg twice daily
20	7.7 mo	5 y	4 y 5 mo	Male	Biliary atresia	Cyclosporine	37.50 mg twice daily

mary end point after immunosuppression withdrawal. The median time to not meeting the primary end point was 5.68 months (IQR, 3.15-9.91 months) after initiation of immunosuppression withdrawal. One patient was terminated from the study shortly after initiating immunosuppression withdrawal secondary to an exclusion criteria violation and was excluded from the subsequent analysis.

Tolerant Patients

The 12 tolerant patients were off immunosuppression therapy for a median of 35.7 months (IQR, 28.1-39.7 months) and had a median follow-up of 44.7 months (IQR, 36.4-47.8 months). Levels of ALT and GGT before, during, and after immunosuppression withdrawal appear in FIGURE 3. Six of the 12 patients exhibited a stable profile throughout the study (Figure 3A). Three patients had discrete spikes in ALT and GGT, consistent with the diagnosis of biliary obstruction made during the study (Figure 3B).

The remaining 3 patients exhibited predominantly GGT abnormalities (Figure 3C). For patient No. 9, an indication for liver biopsy was nondiagnostic and liver tests subsequently normalized. For patient No. 16, the first postwithdrawal protocol biopsy was performed 55 days after the last immunosuppression dose and it showed central mild perivenulitis without rejection. An indication biopsy was performed 37 days later and it showed decreased inflammation. A subsequent protocol biopsy was performed 697 days later when liver tests were back at baseline levels and it showed an absence of inflammation but mild perivenular fibrosis. Patient No. 17 had 2 indication biopsies on days 355 and 364 after the last immunosuppression dose that were read as indeterminate and mild acute rejection, respectively, by the central pathologist; however, the site pathologist did not read acute rejection for either biopsy so the patient remained off immunosuppression therapy. Portal vein stenosis was diagnosed and treated by dilatation and

stenting on day 606 and liver tests improved substantially. The follow-up biopsy was performed on day 969 (719 days after the last immunosuppression dose) and it showed indeterminate rejection with minimal inflammation but increased perivenular fibrosis per the central pathologist.

All 12 patients considered operationally tolerant had protocol biopsies at 4 to 8 weeks after the last dose of immunosuppression therapy; 11 of 12 patients had a second protocol biopsy 2 years after the last immunosuppression dose. Compared with baseline biopsies, follow-up biopsies did not exhibit increased inflammation (portal, lobular, or central venous; TABLE 2) or fibrosis (portal, Disse, or central venous; TABLE 3).

Nontolerant Patients

Seven of 19 evaluable patients did not meet the primary end point (TABLE 4) and had a median follow-up of 18.1 months (IQR, 14.8-21.7 months). Four patients developed allograft dysfunction 43 to 169 days (median, 121.5 days) after initiating immunosuppression withdrawal. Three patients were diagnosed by liver biopsy as having indeterminate or borderline acute rejection and 1 patient as having moderate acute rejection.

Three nontolerant patients did not meet the primary end point at 48, 51, or 69 days after discontinuation of immunosuppression (Table 4). For 2 patients (Nos. 18 and 19), protocol liver biopsy obtained 4 to 8 weeks after the last immunosuppression dose in the setting of normal ALT and GGT levels was read as mild acute rejection by the site pathologist. The third patient (No. 10) developed allograft dysfunction 69 days after the last immunosuppression dose. Local assessment of the liver biopsy was diagnosed as indeterminate acute rejection.

Treatment of allograft dysfunction or acute rejection was determined by the site principal investigator and not stipulated by the trial protocol. All 7 nontolerant patients were treated with increased calcineurin inhibitor dosing

(Table 4). Three patients also received a short course of corticosteroids. No antibody treatment was administered. Study participation for nontolerant patients ended 1 year after resolution of allograft dysfunction. At the end of follow-up, all 7 nontolerant patients had normal liver tests (eFigure at <http://www.jama.com>) while taking calcineurin inhibitor monotherapy. Three patients were receiving higher and 1 patient was receiving lower calcineurin inhibitor doses than at study entry, 2 patients were receiving the same dose as at study entry, and 1 patient had been switched from cyclosporine to tacrolimus (Table 4).

Adverse Events

No incidences of death, graft loss, or opportunistic infections were observed. Four children (3 tolerant; 1 nontolerant) were diagnosed with and treated for biliary obstruction during the trial. A complete listing of all adverse events (including serious adverse events) related to immunosuppression withdrawal appears in eTable 1. Of the 10 serious adverse events, 7 corresponded to not completing immunosuppression withdrawal. The 3 others, 1 episode each of cholangitis (patient 1), elevated liver enzyme levels (patient 9), and abdominal pain accompanied by fever and vomiting (patient 12) resolved with either no treatment or concomitant medications.

Donor-Specific Antibody, C4d Staining, Autoantibody, and Quantitative IgG Profiles

HLA antibody profiles were available for all 12 tolerant patients and for 6 of the 7 nontolerant patients (eTable 2). Four of the 12 tolerant patients had donor-specific antibodies at study entry. A new donor-specific antibody was detected in 7 of the 12 tolerant patients either during or after withdrawal, although it was transient in 3 patients (eTable 2). Five of the 6 nontolerant patients had detectible donor-specific antibodies at study entry; 2 had new donor-specific antibodies at the time of not completing

Figure 3. Alanine Transaminase (ALT) and γ -Glutamyl Transpeptidase (GGT) Profiles for 12 Tolerant Patients



immunosuppression withdrawal (eTable 3). Autoantibody and quantitative IgG profiles fluctuated but without correlation with significant clinical events such as allograft dysfunction.

Factors Associated With Operational Tolerance

Demographic, clinical, histological, and alloantibody characteristics prior to attempted withdrawal were analyzed for association with tolerance (TABLE 5). Tolerant compared with nontolerant

patients initiated immunosuppression withdrawal later after transplantation (median of 100.6 months [IQR, 71.8-123.5] vs 73.0 months [IQR, 57.6-74.9 months], respectively; $P=.03$), had less portal inflammation (91.7% [95% CI, 61.5%-99.8%] vs 42.9% [95% CI, 9.9%-81.6%] with no inflammation; $P=.04$), and had lower total C4d scores on the screening liver biopsies (median of 6.1 [IQR, 5.1-9.3] vs 12.5 [IQR, 9.3-16.8]; $P=.03$). HLA mismatch, sensitization status, and presence of donor-

specific antibodies were not associated with operational tolerance. The small number of patients rendered statistical assessment of correlations between C4d score and alloantibody or donor-specific antibody unfeasible.

COMMENT

We completed a multicenter pilot trial designed to assess the feasibility of immunosuppression withdrawal for pediatric recipients of parental living donor liver transplants with stable liver

Table 2. Portal, Lobular, and Central Venous Inflammation on Baseline and Follow-up Biopsies for Operationally Tolerant Participants

Patient No.	Baseline Biopsy			Follow-up Biopsy After Discontinuation of Immunosuppression Therapy					
	Portal	Lobular	Central	At 4-8 wk			At ≥2 y		
				Portal	Lobular	Central	Portal	Lobular	Central
1 ^a	None	None	None	Mild	None	None	None	None	None
2 ^a	None	None	None	Mild	None	None	None	None	None
3	None	Mild	None	Mild	None	None	None	None	None
4	None	None	None	None	None	None	None	None	None
5	None	None	None	None	None	None	None	None	None
7	None	None	None	Mild	None	None	NA ^b	NA ^b	NA ^b
9	None	None	None	None	None	None	None	None	None
11	None	None	None	None	None	None	None	None	None
12	Mild	None	None	Mild	None	None	Mild	None	None
14 ^a	None	None	None	None	None	None	Mild	None	None
16	None	None	None	Mild	Mild	None	None	None	None
17	None	None	None	None	None	None	Mild	Mild	Mild

Abbreviation: NA, not available.
^aDiagnosed with biliary obstruction during trial.
^bRefused protocol liver biopsy.

Table 3. Portal, Disse, and Central Venous Fibrosis on Baseline and Follow-up Biopsies for Operationally Tolerant Participants

Patient No.	Baseline Biopsy			Follow-up Biopsy After Discontinuation of Immunosuppression Therapy					
	Portal	Disse	Central	At 4-8 wk			At ≥2 y		
				Portal	Disse	Central	Portal	Disse	Central
1 ^a	Moderate	Mild	None	Moderate	None	Mild	Moderate	Mild	Mild
2 ^a	Mild	Mild	None	None	None	None	None	Mild	None
3	Mild	Mild	None	Mild	None	None	Mild	Mild	None
4	Mild	Mild	None	Mild	Mild	None	Mild	Mild	None
5	Mild	None	None	None	Mild	None	None	None	None
7	None	None	None	None	None	None	NA ^b	NA ^b	NA ^b
9	None	None	None	None	Mild	None	None	Mild	None
11	None	Mild	None	None	Mild	Mild	None	Mild	None
12	Mild	Mild	None	Mild	None	None	Mild	Mild	None
14 ^a	Mild	None	None	None	None	None	Moderate	Mild	None
16	Mild	None	None	Mild	None	None	None	None	Mild
17	None	Moderate	None	None	Moderate	Mild	Mild	Mild	Moderate

Abbreviation: NA, not available.
^aDiagnosed with biliary obstruction during trial.
^bRefused protocol liver biopsy.

tests. Of the 20 enrolled patients, 12 were operationally tolerant (60%), maintaining normal allograft function for more than 1 year after immunosuppression cessation. Follow-up biopsies from more than 2 years after immunosuppression discontinuation showed preservation of allograft his-

tology. All 7 nontolerant patients were managed with immunosuppression that promptly returned liver tests to their baseline levels. Of these 7, only 3 patients met Banff criteria for acute rejection. We did not observe severe or steroid refractory acute rejection, chronic rejection, graft loss, or death.

These outcomes demonstrate that immunosuppression withdrawal in this clinical trial setting appears to be feasible for both tolerant and nontolerant patients.

Our definition of operational tolerance is similar to that used in previous reports of immunosuppression with-

Table 4. Laboratory Data, Biopsy Results, and Immunosuppression Doses Associated With Discontinuation of the Immunosuppression Withdrawal Protocol for 7 Nontolerant Participants

Patient No.	Rejection Episode			Biopsy					Maximum Treatment CNI Dose (% of Dose at Entry) ^b	Day of Last Follow-up	End of Study CNI Dose (% of Dose at Study Entry) ^c
	Start; End Day ^a	CNI Dose (% of Dose at Entry)	Peak ALT; GGT	Local Read		Central Read					
				Day	Primary; Secondary Diagnosis	RAI	Primary; Secondary Diagnosis	RAI			
8	43; 162	Tacrolimus 3.50 mg in AM; 3.25 mg in PM (75%)	121; 244	59	Nonspecific change; Nonspecific inflammation	NA	Nonspecific inflammation; Acute rejection, indeterminate or borderline	2	Tacrolimus 4.50 mg twice daily (100%); No steroids	430	Tacrolimus 4.50 mg twice daily (100%)
15	88; 190	Tacrolimus 0.90 mg 5 times/wk (43%)	318; 98	108	Acute rejection, moderate	5	Acute rejection, moderate	5	Tacrolimus 4.50 mg/d (300%); Intravenous and oral steroids	472	Tacrolimus 2.00 mg/d (133%)
6	155; 515	Tacrolimus 1.10 mg 4 times/wk (31%)	562; 127	157	Nonspecific change; Nonspecific inflammation	NA	Acute rejection, indeterminate or borderline; Nodular regenerative hyperplasia	3	Tacrolimus 3.00 mg twice daily (300%); No steroids	661	Tacrolimus 3.00 mg twice daily (300%)
20	169; 222	Cyclosporine 32 mg/d (43%)	312; 197	184	Acute rejection, moderate	5	Acute rejection, indeterminate or borderline; Biliary obstruction or cholangitis	2	Tacrolimus 1.00 mg twice daily (NA ^d); Oral steroids	551	Tacrolimus 0.30 mg twice daily (NA ^d)
18	NA	None	NA	295	Acute rejection, moderate	4	Acute rejection, indeterminate or borderline; Nonspecific inflammation	1	Tacrolimus 2.00 mg twice daily (400%); Oral steroids	450	Tacrolimus 2.00 mg twice daily (400%)
19	NA	None	NA	298	Acute rejection	4	Acute rejection; Nodular regenerative hyperplasia	3	Tacrolimus 0.50 mg twice daily (100%); No steroids	645	Tacrolimus 0.50 mg twice daily (100%)
10	333; 402	None	134/25	358	Nonspecific change; Nonspecific inflammation	NA	Acute rejection, indeterminate or borderline; Drug exposure	2	Cyclosporine 25 mg twice daily (100%); No steroids	830	Cyclosporine 25 mg twice daily on Tue, Thu, and Sat; 25 mg/d on Mon, Wed, Fri, and Sun (71%)

Abbreviations: ALT, alanine transaminase; CNI, calcineurin inhibitor; GGT, γ -glutamyl transpeptidase; NA, data not applicable; RAI, rejection activity index.
^aThe days provided are based on liver test abnormalities and are relative to the first day of immunosuppression withdrawal. Patients 18 and 19 do not have start or end days because rejection was diagnosed during the protocol biopsy, which was required between 4 and 8 weeks after the last immunosuppression dose.
^bTreatment of acute rejection was left to the discretion of the site principal investigator and not specified by the trial protocol.
^cStudy participation ended 1 year after resolution of acute rejection.
^dPercentage of dose at study entry cannot be calculated because patient was switched from cyclosporine at study entry to tacrolimus monotherapy.

drawal. The definition is functional and based on clinical allograft status rather than on immunological assessment of donor-specific vs third-party alloreactivity. The 60% rate that we observed was higher than previously reported.⁵⁻¹⁶ Reports of adult transplantation have demonstrated tolerance rates ranging from 8% to 33% for liver allograft recipients.^{5,7,9-12,15,16} There are few reported pediatric trials of immunosuppression withdrawal. The University of Pittsburgh's tolerant cohort included a significant number of children, but success rates were not specified.^{9,11,23} A Kyoto University Hospital study reported that 88 of 581 pediatric living donor liver recipients (15%) were operationally tolerant.^{8,14} At Kyoto, immunosuppression was withdrawn more than 2 years after transplant for all children who had not experienced rejection in the preceding year. Among the tolerant Kyoto children, 38% underwent immunosuppression withdrawal after developing a contraindication to ongoing immunosuppression. Our study differed by requiring a longer time from transplant (4 years vs 2 years) and a liver biopsy prior to study entry, while excluding those with an autoimmune or viral disease. Despite differences in how immunosuppression was withdrawn, our experience, combined with that of Pittsburgh and Kyoto, suggests that operational tolerance occurs more frequently in children than in adults.

Our longitudinal data showing the emergence and disappearance of donor-specific antibodies in tolerant patients suggests that functionally defined operational tolerance may be a dynamic state. This is consistent with emerging data from studies in humans and in animals that indicate a requisite role for active regulation of antidonor responses to maintain tolerance rather than the complete absence of antidonor responses.²⁴ Importantly, our study offers protocol biopsies obtained 2 years after immunosuppression withdrawal. Central pathological assessment of these biopsies compared with baseline biopsies did not show increased inflammation or fi-

Table 5. Factors Associated With Operational Tolerance of Organ Recipient

	Median (IQR)		P Value ^a
	Nontolerant (n = 7)	Tolerant (n = 12)	
Recipient age, y			
At transplant	7.7 mo (5.6 mo-1 y 1 mo)	6.8 mo (4.7 mo-8.3 mo)	.40
At study entry	6 y 7 mo (5 y 3 mo-7 y)	9 y (7 y 7 mo-10 y 9 mo)	.08
Time from transplant to study entry, mo	73.0 (57.6-74.9)	100.6 (71.8-123.5)	.03
At study entry			
Alanine transaminase, U/L	30 (24-34)	31 (25-34)	.92
γ-Glutamyl transpeptidase, U/L	16 (14-68)	27 (18-43)	.58
C4d score at baseline biopsy	12.5 (9.3-16.8)	6.1 (5.1-9.3)	.03
Donor age at donation, y	32.5 (29.6-39.6)	33.2 (32.1-37.1)	.71
	No. (%) [95% CI]		
Male sex			
Recipient	3 (42.9) [9.9-81.6]	8 (66.7) [34.9-90.1]	.38
Donor	3 (42.9) [9.9-81.6]	3 (25.0) [5.5-57.2]	.62
Liver transplant indication			
Biliary atresia	6 (85.7) [42.1-99.6]	9 (75.0) [42.8-94.5]	.50
Other diseases	1 (14.3) [0.4-57.9]	3 (25.0) [5.5-57.2]	
History of rejection	4 (57.1) [18.4-90.1]	7 (58.3) [27.7-84.8]	>.99
Calcineurin inhibitor at study entry			
Cyclosporine	2 (28.6) [3.7-71.0]	5 (41.7) [15.2-72.3]	.66
Tacrolimus	5 (71.4) [29.0-96.3]	7 (58.3) [27.7-84.8]	
HLA antigen mismatch			
A + B + DR			
3	4 (57.1) [18.4-90.1]	4 (33.3) [9.9-65.1]	.38
<3	3 (42.9) [9.9-81.6]	8 (66.7) [34.9-90.1]	
Class I (A + B + C)			
3	4 (57.1) [18.4-90.1]	7 (58.3) [27.7-84.8]	>.99
<3	3 (42.9) [9.9-81.6]	5 (41.7) [15.2-72.3]	
Class II (DR + DQA + DQB)			
3	2 (28.6) [3.7-71.0]	3 (25.0) [5.5-57.2]	>.99
<3	5 (71.4) [29.0-96.3]	9 (75.0) [42.8-94.5]	
Sensitization at study entry (positive class I and/or II flow percentage reactive antibody)	5 (71.4) [29.0-96.3]	5 (41.7) [15.2-72.3]	.15
Donor-specific antibody at study entry	5 (71.4) [29.0-96.3]	4 (33.3) [9.9-65.1]	.13
At baseline biopsy			
Portal inflammation			
None	3 (42.9) [9.9-81.6]	11 (91.7) [61.5-99.8]	.04
Mild or moderate	4 (57.1) [18.4-90.1]	1 (8.3) [0.2-38.5]	
Rejection activity index			
0	5 (71.4) [29.0-96.3]	12 (100) [73.5-100]	.12
1	2 (28.6) [3.7-71.0]	0 [0-26.5]	
Hepatocyte apoptosis			
0	7 (100) [59.0-100]	11 (91.7) [61.5-99.8]	>.99
1	0 (0) [0-41.0]	1 (8.3) [0.2-38.5]	
Central fibrosis			
None	5 (71.4) [29.0-96.3]	12 (100) [73.5-100]	.12
Mild or moderate	2 (28.6) [3.7-71.0]	0 (0) [0-26.5]	
Interface activity			
None	5 (71.4) [29.0-96.3]	12 (100) [73.5-100]	.12
Mild or moderate	2 (28.6) [3.7-71.0]	0 (0) [0-26.5]	
Steatosis			
None	6 (85.7) [42.1-99.6]	5 (41.7) [15.2-72.3]	.15
Mild	1 (14.3) [0.4-57.9]	7 (58.3) [27.7-84.8]	

Abbreviation: IQR, interquartile range.

^aCategorical variables were compared between the 2 groups using the Fisher exact test and continuous variables were compared using an exact Wilcoxon 2-sample test with a 2-tailed α level of .05.

brosis. In contrast, the Kyoto group reported that grafts of tolerant compared with nontolerant children showed increased allograft fibrosis that regressed with reinstatement of immunosuppression therapy.⁸ However, our study cohort and the Kyoto cohort were not comparable because tolerant children received transplants at a younger age and immunosuppression therapy withdrawal occurred further out from the date of transplant. This issue can only be definitively resolved with well-designed controlled trials of adequate size and duration.

Although operational tolerance may be a dynamic condition, thus far it has been durable. Many of the 12 operationally tolerant children have weathered serious adverse events such as biliary obstruction or cholangitis, portal vein stenosis, and bacterial or viral infections associated with increased levels on liver tests. None required reinitiation of immunosuppression therapy. As a result, these 12 children were off all immunosuppression therapy for 36.0 to 56.7 months. For the 7 nontolerant patients, frequent biochemical and histological assessments ensured early diagnosis of allograft dysfunction, expeditious intervention, and prompt resolution. Perhaps the most notable and unexpected adverse event that occurred was the diagnosis of biliary obstruction in 4 children (3 tolerant and 1 nontolerant). Two patients experienced cholangitis immediately prior to and less than 3 months into immunosuppression withdrawal. A third patient was diagnosed with a diaphragmatic defect with herniation of the Roux en Y hepaticojejunostomy as the etiology of intermittent biliary obstruction. The final patient was not diagnosed until 22 months after immunosuppression discontinuation. The clinical details of these 4 cases argue strongly against a primary causative role for immunosuppression withdrawal in the development of biliary obstruction.

Our study, while limited in size, elucidated several factors that may be associated with operational tolerance.

Although these associations are preliminary, they deserve careful assessment in future, larger trials. Increased time interval between transplantation and immunosuppression withdrawal, consistent with the adult experience,²⁵ was the most important clinical factor associated with successful immunosuppression withdrawal. Histological factors such as portal inflammation, even in the absence of tissue damage, and increased total C4d score were associated with the absence of operational tolerance,²⁶ suggesting that active and inadequately regulated anti-donor cellular and humoral responses interfered with tolerance. The negative association of overall C4d staining intensity with operational tolerance is a novel observation. Baseline sensitization and donor-specific antibody status were not associated with tolerance, perhaps reflecting the small size of our study. The clinical impact of both humoral rejection and C4d staining are less well understood in liver transplantation than in other solid organ transplantation.²⁷ Because C4d staining has been explored almost exclusively in the setting of allograft dysfunction, its functional significance in long-term stable allografts such as those in our study is unknown.²⁸⁻³⁰ Future withdrawal trials should assess baseline and longitudinal patterns of donor-specific antibody and C4d staining to identify associations with tolerance, rejection, or both.

In conclusion, this pilot trial demonstrates that immunosuppression therapy can be completely withdrawn in 60% of highly selected pediatric recipients of parental living donor liver transplants with maintenance of normal allograft function and stable allograft histology. While there have been sporadic reports of immunosuppression discontinuation after liver transplantation over the past 2 decades, they have been limited to case series and isolated center experiences. Many featured atypical patients, such as those who had developed severe malignant, infectious, or metabolic complications that mandated immunosuppres-

sion cessation or those who were non-compliant with their prescribed medical regimen. While these reports have provided anecdotal evidence that liver allografts may continue to function in the absence of immunosuppression, they have not yielded generalizable knowledge. As a result, they neither changed perception nor practice.

Our preliminary study was based on a rigorous design, clearly defined entry criteria, a strict protocol for drug withdrawal, and histological follow-up. Our surprising finding that an unexpectedly high proportion of a well-defined pediatric cohort are operationally tolerant with stable allograft function and histology sets the agenda for larger studies with longer follow-up to define the frequency, assess the durability, and derive a predictive profile of operational tolerance for pediatric liver transplant recipients.

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Author Contributions: Dr Feng had full access to all of the data and takes responsibility for the integrity of the data integrity and the accuracy of the data analysis.

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Conflicts of Interest Disclosure: All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Dr Demetris reported that he has received fees as a consultant for pathology services related to clinical trials of immunosuppression drugs in adult de novo liver transplant recipients from Novartis/DCL Laboratory and Bristol-

Myers Squibb; and is a co-inventor of Omnyx Digital Pathology Solution, a joint venture between the University of Pittsburgh Medical Center and General Electric to create a complete digital pathology solution. Dr Turka reported that he owns stock in and has a family member employed by Novartis. No other disclosures were reported.

Funding/Support: The Immune Tolerance Network, an international clinical research consortium founded by the National Institutes of Health, funded the study. This study also was supported by grant UL1 RR024131 from the National Institutes of Health/National Center for Research Resources awarded to the University of California, San Francisco, Clinical & Translational Science Institute.

Role of the Sponsors: The sponsors had a role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, and approval of the manuscript.

Disclaimer: The contents of this article are solely the responsibility of the authors and do not necessarily represent the official views of the National Institutes of Health.

Online-Only Material: The eFigure and eTables 1-3 are available at <http://www.jama.com>.

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