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Outcomes of immunosuppression minimization and withdrawal early after liver transplantation

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The Immune Tolerance Network ITN030ST A-WISH assessed immunosuppression withdrawal in liver transplant recipients with hepatitis C or nonimmune nonviral liver disease. Of 275 recipients enrolled before transplantation, 95 were randomly assigned 4:1 to withdrawal (n = 77) or maintenance (n = 18) 1- to 2-years posttransplant. Randomization eligibility criteria included stable immunosuppression monotherapy; adequate liver and kidney function; ≤Stage 2 Ishak fibrosis; and absence of rejection on biopsy. Immunosuppression withdrawal followed an 8-step reduction algorithm with ≥8 weeks per level. Fifty-two of 77 subjects (67.5%) reduced to ≤50% of baseline dose, and 10 of 77 (13.0%) discontinued all immunosuppression for ≥1 year. Acute rejection and/or abnormal liver tests were treated with increased immunosuppression; 5 of 32 rejection episodes required a methylprednisolone bolus. The composite end point (death or graft loss; grade 4 secondary malignancy or opportunistic infection; Ishak stage ≥3; or >25% decrease in glomerular filtration rate within 24 months of randomization) occurred in 12 of 66 (18%) and 4 of 13 (31%) subjects in the withdrawal and maintenance groups. Early immunosuppression minimization is feasible in selected liver recipients, while complete withdrawal is successful in only a small proportion. The composite end point comparison was inconclusive for noninferiority of the withdrawal to the maintenance group.

KEYWORDS
clinical research/practice, clinical trial, immunosuppression/immune modulation, immunosuppressive regimens - minimization/withdrawal, infection and infectious agents - viral: hepatitis C, liver transplantation/hepatology, tolerance

1 | INTRODUCTION

Standard practice patterns for liver transplant recipients include multiple immunosuppressive drugs aimed at predetermined trough levels, adjusted to time after transplantation. Excellent graft and patient survival rates support this approach. However, there are significant short- and long-term risks associated with immunosuppression, such as infections, malignancies, cardiovascular disease, metabolic disorders, and renal and other complications.[1,2]

Single-center reports have demonstrated that many recipients can tolerate reduced doses of immunosuppression, suggesting that liver transplant recipients are a diverse cohort for whom immunosuppression may be personalized.[3-5] Prospective clinical trials report that >40% of highly selected liver transplant recipients can

Abbreviations: GFR, glomerular filtration rate; HCV, hepatitis C virus; NINV, nonimmune nonviral.

withstand complete withdrawal of immunosuppression when done at a mean of 10.2 years after transplantation in adult, and 8.5 years in pediatric patients.[6,7]

The multiple systemic complications that are the direct outcomes of standard immunosuppressive regimens continue to justify research into the potential elimination of multiple drug use and dose minimization. Other options under investigation include substitution of current standard immunosuppression with drugs that are less toxic, aiming to reduce side effects; however, these drugs may be associated with a different range of toxicities.[8-10]

The Immune Tolerance Network ITN030ST A-WISH trial (NCT00135694) was a prospective randomized study designed to assess the safety of immunosuppression withdrawal in liver transplant recipients with hepatitis C or nonimmune nonviral causes of liver failure initiated in the first 1-2 years posttransplantation.

2 | PATIENTS AND METHODS

Subjects were enrolled at 7 liver transplantation centers in the United States from November 2005 to April 2011. Entry eligibility criteria included the following: liver failure due to infection with the hepatitis C virus (HCV), demonstrated by viral genomes in blood; or to nonimmune, nonviral (NINV) causes.

Exclusion criteria included the following: previous, multiorgan, or split liver, other than right trisegment, transplant; living or HCV-infected donor, or donation after cardiac death; liver failure due to autoimmune disease; hepatitis B infection; stage III or higher hepatocellular cancer detected in the explanted liver; and clinically significant renal, cardiovascular, or cerebrovascular disease. Subjects with stage III or higher cancer in the liver explant were replaced.

All subjects provided written informed consent prior to transplantation, and again at the point of assessment for randomization eligibility. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the institutional review boards of all participating centers.

2.1 | Study design

Following transplantation, subjects received immunosuppression with corticosteroids and a calcineurin inhibitor and/or antimetabolite. Corticosteroids were planned to be tapered within 3 months. At 6 months after transplant, tacrolimus dosing was adjusted to maintain trough blood levels of 5-10 ng/mL. Between 1 and 2 years after transplantation, once eligibility criteria were met and at the discretion of the investigator and after review by the study chair and NIH medical monitor, eligible subjects could be randomly assigned in a 4:1 ratio to immunosuppression withdrawal or to immunosuppression maintenance. Randomization was stratified by HCV or NINV stratum. Those assigned to immunosuppression maintenance continued study visits for 2 years. Those assigned to immunosuppression withdrawal underwent a planned taper consisting of eight 8-week withdrawal steps. The initial taper dose was defined as the daily dose at the time of random assignment but with an adjustment to once-a-day administration. These subjects continued visits for another 2 years at the conclusion of their tapering.

Eligibility for randomization included the following: immunosuppression monotherapy with a calcineurin inhibitor or antimetabolite for at least 3 months, Stage 2 (of 6) or less Ishak fibrosis, no post-transplant interferon, adequate hepatic and renal function, no biopsy-proven rejection within the prior 3 months by local pathology review, and absence of Banff moderate or severe acute rejection or chronic rejection by central review of a biopsy obtained within 4 weeks.[11] Adequate hepatic function was defined for participants with hepatitis C infection as total bilirubin of <3 mg/dL and for participants with non-immune nonviral causes of liver failure as total bilirubin, alanine aminotransferase (ALT), and alkaline phosphatase all ≤2 times the upper limit of normal.

Those assigned to withdrawal underwent a scheduled taper planned to last ≈1 year with doses modified in 8-week steps. The daily baseline immunosuppression dose was initially administered as a single morning dose, then reduced to 75%, and then to 50% of the baseline dose. This dose was subsequently reduced to every other day, biweekly, weekly, and every-other-week administration, and finally discontinued.

2.2 | Protocol-specified biopsies

Protocol biopsies were planned for the day of transplant, at eligibility for randomization evaluation (12-24 months posttransplant), and at 24 and 36 months posttransplant. Additional protocol biopsies were planned for HCV subjects at 6 and 12 months posttransplant.

2.3 | Allograft dysfunction, resolution, and biopsy

A liver biopsy was planned when allograft dysfunction was detected. For HCV subjects, allograft dysfunction was defined as an elevation in aspartate aminotransferase (AST) or ALT >3 times the upper limit of normal, except during withdrawal when it was defined as >2 times the most recent value before a change in immunosuppression. For NINV subjects, allograft dysfunction was defined as an elevation in aspartate aminotransferase or alanine aminotransferase >2 times the upper limit of normal. A biopsy was also performed when clinically indicated at the investigator's discretion. Liver tests (alkaline phosphatase, ALT, AST, γ-glutamyl transferase [GGT], or total bilirubin) were considered resolved when all liver function tests (LFTs) were <150% from the higher of the value at randomization or the upper limit of normal.

2.4 | Definition and treatment of rejection

Rejection was diagnosed according to Banff criteria.[12] Treatment was based on the local site pathologist's finding. In order to ensure uniformity and comparability with other studies, the study analysis was based only on the findings of the central pathologist.

2.5 | Definition of operational tolerance

Subjects were considered operationally tolerant if they remained off immunosuppressive medications for at least 1 year and did not have clinical evidence of acute or chronic rejection as determined by liver tests.

2.6 | Objectives

The A-WISH study was designed to determine the outcomes of immunosuppression minimization and withdrawal starting within 2 years after liver transplantation.

2.7 | Study end points

The primary end point was a composite defined as the occurrence of death or graft loss, grade 4 secondary malignancy, grade 4 opportunistic infection, stage 3 or higher fibrosis, or decrease in renal function. Grades for malignancy and opportunistic infection were taken from Common Terminology Criteria for Adverse Events Version 3.0. The end point was assessed as the occurrence at any time up to 24 months after random assignment for all components except for renal function, which was assessed using the assessment closest to 24 months up to 36 months post–random assignment. Subjects without a renal assessment in this range were considered unevaluable for the primary end point. A decrease in renal function was defined as a 25% decrease in glomerular filtration rate (GFR) if GFR at randomization was between 30 and 90 mL/min per 1.73^2 and a 25% decrease and a GFR <90 mL/min per 1.73^2 for subjects with a GFR >90 mL/min per 1.73^2 at randomization. The Modification of Diet in Renal Disease formula was used to calculate GFR.[13] Secondary end points were eligibility for random assignment, immunosuppression withdrawal completion, immunosuppression-free duration, hepatitis C viral load, fibrosis, and graft loss or death.

2.8 | Sample size

The planned sample size was based on assessment of the primary end point after random assignment in the combined HCV and NINV strata and was intended to test whether the withdrawal arm was noninferior to the maintenance arm with respect to immunosuppression-related complications.

We intended to enroll enough individuals prior to transplantation so that enough patients would be available for the primary comparison after accounting for the proportion eligible for random assignment.

The original sample size was 275 subjects with the assumption that 75% of those would be eligible for random assignment, allowing 200 available subjects for the primary comparison. This would have allowed an assessment of noninferiority with a 5% margin, a 97.5% 1-sided confidence interval, and 80% power with a 10% dropout rate.

However, we observed early in enrollment that only 37% of enrolled subjects were in fact eligible for random assignment. We therefore re-estimated the power for the primary comparison. We assumed 104 individuals would be available for random assignment. This allowed an assessment of noninferiority with a 10% margin, a 95% 1-sided confidence interval, and 80% power.

2.9 | Randomization implementation

Subjects were randomly assigned using a random assignment website hosted by the Data Coordinating Center, RhoFed. Of the 275 enrolled transplant recipients, 95 were eligible and were randomly assigned 4:1 to immunosuppression withdrawal (n = 77) or maintenance (n = 18) using an allocation sequence developed by the Data Coordinating Center.

2.10 | Statistical analysis

Categorical variables were compared using a Fisher's exact test and continuous variables were compared between groups using a *t*-test or Wilcoxon test, depending on normality, with a 2-tailed 0.05 alpha level. Mixed model analyses were used to test for differences among the treatment groups for longitudinal data. Analyses were conducted using SAS (SAS Institute Inc., Cary, NC), SAS Institute Inc.100 SAS Campus DriveCary, NC 27513-2414, USA version 9.3 or above.

3 | RESULTS

3.1 | Study subjects

Between November 2005 and April 2011, 286 consented participants underwent transplantation at 7 clinical sites (Figure 1). Of these, 11 had stage III hepatocellular carcinoma in the explanted liver and were excluded without further follow-up, to achieve the target study accrual of 275. The last follow-up was in September 2015. Baseline characteristics are in Table S1.

3.2 | Eligibility for random assignment

Of the 275 subjects who continued in the study, 95 subjects (95/275) were randomly assigned to immunosuppression withdrawal (30 HCV and 47 NINV) or maintenance (7 HCV and 11 NINV). One hundred eighty (93 HCV, 87 NINV) were determined to be ineligible for random assignment (Figure 1). The most common reasons for study termination prior to random assignment were voluntary withdrawal in 41 (22.8%) subjects; followed by complications related to hepatitis C (such as treatment with interferon, fibrosis above stage 2, recurrent or severe hepatitis C, or fibrosing cholestatic hepatitis) in 39 (21.7%); protocol deviations in 19 (10.6%); adverse events in 17 (9.4%); and death in 14 (7.8%). There were no differences in baseline characteristics at time of transplant between those randomly assigned to the withdrawal or maintenance groups (Table 1).

Ninety-one of 95 subjects were on tacrolimus monotherapy at the time of random assignment. Of the 4 subjects who were not on tacrolimus, 2 (1 maintenance, 1 withdrawal) were on cyclosporine monotherapy; 1 maintenance subject was on mycophenolic acid monotherapy; and 1 withdrawal subject was on mycophenolate mofetil monotherapy.

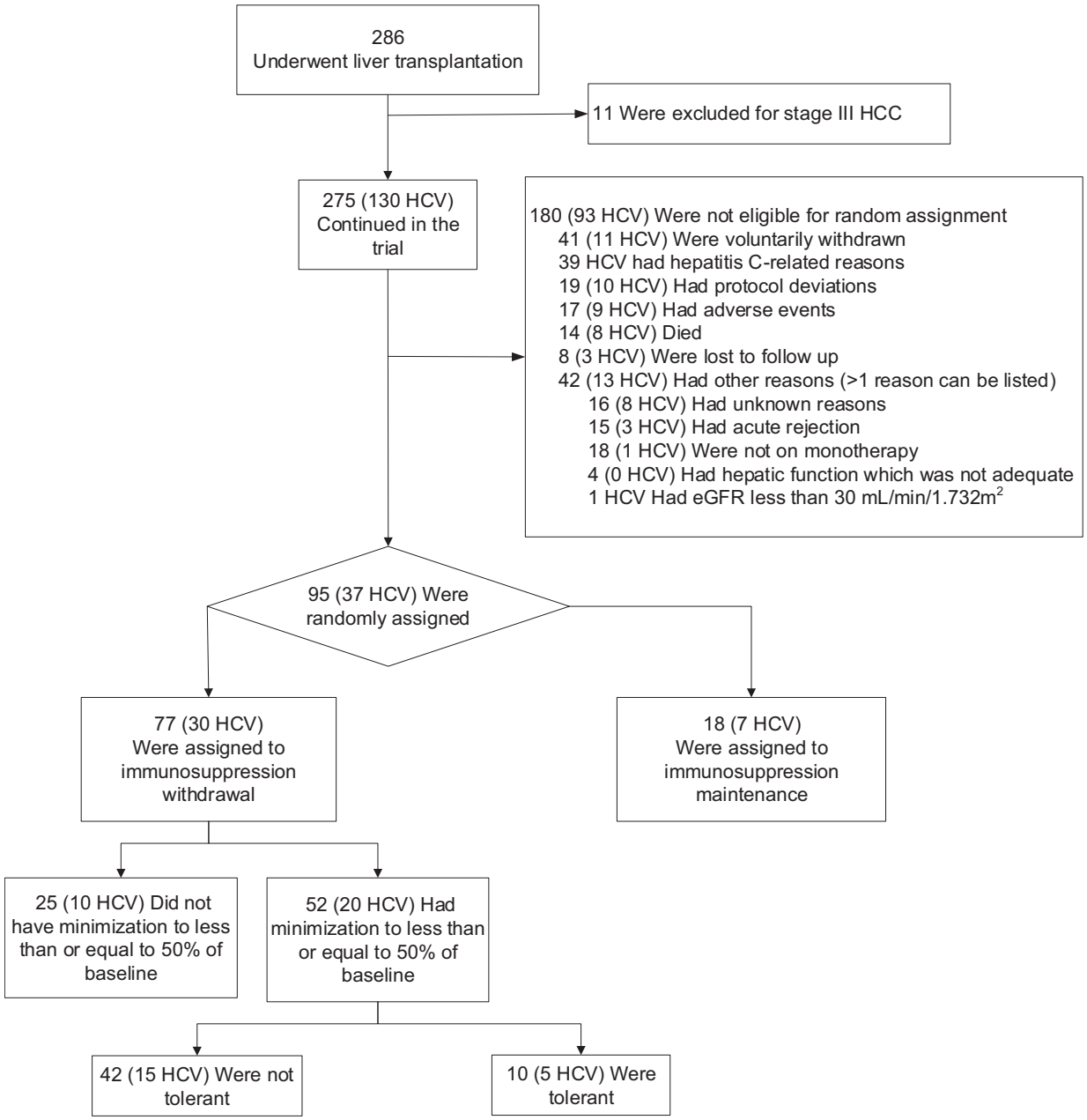

FIGURE 1 Disposition of enrolled subjects. All subjects who were assessed for eligibility for random assignment, as well as those who were replaced, are included. eGFR, estimated glomerular filtration rate; HCC, hepatocellular carcinoma; HCV, hepatitis C virus

There were also no differences either in liver tests (ALT, AST, alkaline phosphatase, direct bilirubin, GGT) or in immunosuppression trough levels at the time of randomization between those randomly assigned to the withdrawal or maintenance groups (Table 2). Furthermore, there were no differences in tacrolimus trough levels among sites at the time of random assignment.

A review of the randomization eligibility biopsies of the 95 subjects who were randomly assigned shows that 6 subjects (1 NINV subject in the maintenance group and 2 HCV and 3 NINV subjects in the withdrawal group) had findings that were indeterminate/borderline for acute rejection (Table S2). The incidence of this and other findings was similar between the maintenance and withdrawal groups. Although allowed by protocol, there were no patients with mild rejection in the randomized cohort.

3.3 | Immunosuppression outcomes

3.3.1 | Withdrawal outcomes

Most of the 77 subjects assigned to immunosuppression withdrawal achieved substantial reduction in immunosuppression dose while maintaining stable allograft function without evidence of clinically suspected rejection (Figure 2). Seventy-one (92.2%) tolerated once-a-day dosing and 52 (67.5%) tolerated a reduction to ≤50% of baseline dose.

TABLE 1 Demographics and baseline characteristics of randomized subjects at transplant

Characteristics	Total randomized (N = 95)	Maintenance (N = 18)	Withdrawal (N = 77)	*P* value
Age (y)	54.9 (9.59)	57.4 (7.70)	54.3 (9.92)	.21
Sex (male) — n (%)	76 (80.0)	13 (72.2)	63 (81.8)	.35
Race — n (%)				.31
White	84 (88.4)	17 (94.4)	67 (87.0)	
Black	7 (7.4)	0	7 (9.1)	
Asian	2 (2.1)	1 (5.6)	1 (1.3)	
Other	2 (2.1)	0	2 (2.6)	
Donor age (y)	44.7 (17.39)	40.7 (19.79)	45.6 (16.79)	.29
Age matched[a] (yes) — n (%)	61 (64.2)	10 (55.6)	51 (66.2)	.42
Race matched (yes) — n (%)	59 (62.1)	11 (61.1)	48 (62.3)	1.00
Sex matched (yes) — n (%)	57 (60.0)	8 (44.4)	49 (63.6)	.18
BMI (kg/m^2)	30.7 (5.42)	30.6 (5.08)	30.8 (5.52)	.89
Creatinine (mg/dL)	1.2 (0.65)	1.1 (0.68)	1.3 (0.65)	.45
eGFR (mL/min per 1.73^2)	76.9 (39.47)	85.3 (46.30)	75.0 (37.78)	.32
Total bilirubin (mg/dL)	7.3 (9.70)	6.4 (11.78)	7.5 (9.22)	.66
HCV viral load (log base 10)				
N	14	4	10	
Mean (standard deviation)	5.3 (1.18)	5.6 (0.32)	5.2 (1.39)	.49
Primary cause of liver disease — n (%)				
Chronic hepatocellular disease — Hepatitis C	37 (38.9)	7 (38.9)	30 (39.0)	1.00
Chronic hepatocellular disease — NINV[b]	58 (61.1)	11 (61.1)	47 (61.0)	
Alcoholic liver disease	26 (44.8)	4 (36.4)	22 (46.8)	
Cryptogenic cirrhosis	6 (10.3)	2 (18.2)	4 (8.5)	
Nonalcoholic steatohepatitis	19 (32.8)	5 (45.5)	14 (29.8)	
Metabolic diseases	3 (5.2)	0	3 (6.4)	
Hepatocellular carcinoma	3 (5.2)	0	3 (6.4)	
Other	1 (1.7)	0	1 (2.1)	

Values are expressed as mean (standard deviation), except otherwise noted.
BMI, body mass index; eGFR, estimated glomerular filtration rate; HCV, hepatitis C virus; NINV, nonimmune nonviral.
[a]Subjects are considered age matched if both the recipient and donor are >55 y of age or both are ≤55 y of age.
[b]NINV subjects can have more than 1 primary reason for liver failure and percents for subcategories are out of total NINV subjects.

3.3.2 | Operationally tolerant subjects

Ten subjects (13.0%) remained off all immunosuppression for at least 1 year with no clinical evidence of rejection and were termed operationally tolerant (Figure 1). We cannot exclude the possibility of subclinical rejection since biopsies were not available for all operationally tolerant subjects (Table 3). Immunosuppression was discontinued in these subjects at a median of 33 months (range 28-44 months) from transplantation and 15 months (range 12-24 months) from random assignment. Nine of these subjects remained off immunosuppression therapy for the 2 years of study follow-up. One subject remained off immunosuppression therapy for 14 months but was retransplanted due to recurrent hepatitis C.

Laboratory values at the time of randomization and at time of last report are shown in Figure S1. Laboratory values were available on average 696 days (range 283-790 days) following completion of immunosuppression withdrawal. No clinical parameters assessed at time of random assignment were found to be associated with operational tolerance (Figure S2).

The last available liver function laboratory tests postimmunosuppression withdrawal for the 10 tolerant subjects are shown in Table 3. For NINV subjects, ALT was normal or improved compared to baseline in all subjects; GGT was normal or improved except in subject 212; and alkaline phosphatase was improved or normal in all subjects. For HCV subjects, ALT was slightly elevated compared to baseline in 106 and 220; GGT was normal in all subjects with assessments; and alkaline phosphatase was normal in all subjects except 273.

TABLE 2 Characteristics of randomized subjects at randomization

Characteristics	Total randomized (N = 95)	Maintenance (N = 18)	Withdrawal (N = 77)	*P* value
ALT (U/L)	45.0 (37.93)	57.7 (64.46)	42.1 (28.35)	.33
AST (U/L)	38.8 (30.33)	50.7 (52.86)	36.1 (21.71)	.26
Alkaline phosphatase (U/L)	107.0 (41.62)	102.1 (36.03)	108.2 (42.95)	.58
Direct bilirubin (mg/dL)				.93
n	78	15	63	
Mean (standard deviation)	0.2 (0.08)	0.2 (0.10)	0.2 (0.08)	
GGT (U/L)				.28
n	68	13	55	
Mean (standard deviation)	75.9 (79.1)	97.5 (100.44)	70.8 (73.34)	
Tacrolimus trough levels (ng/mL)				
n	90	16	74	
Mean (standard deviation)	6.4 (2.35)	6.0 (2.36)	6.5 (2.35)	.42
Tacrolimus trough levels by site (ng/mL)				.53
Site 1				
n	3			
Mean (standard deviation)	7.4 (1.66)			
Site 2				
n	7			
Mean (standard deviation)	5.7 (1.09)			
Site 3				
n	22			
Mean (standard deviation)	5.8 (1.42)			
Site 4				
n	2			
Mean (standard deviation)	6.0 (2.26)			
Site 5				
n	6			
Mean (standard deviation)	6.9 (1.90)			
Site 6				
n	41			
Mean (standard deviation)	6.9 (2.99)			
Site 7				
n	9			
Mean (standard deviation)	5.6 (1.82)			

Site trough levels were compared using an analysis of variance test.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyl transferase.

Central biopsy findings at the time of random assignment and at time of last report are also shown in Table 3. Nine of the 10 operationally tolerant subjects had a biopsy an average of 212 days (range 14 to 406 days) following completion of withdrawal; however, 1 of these was read locally only with no central reading available. One HCV subject had a clinically indicated biopsy 396 days following completion of withdrawal with findings of recurrent HCV that ultimately resulted in graft loss. Postimmunosuppression withdrawal follow-up biopsies for the 8 subjects with a central reading available demonstrated stable findings in NINV subjects but some degree of histologic progression compared to time of randomization in HCV subjects, as follows:

1. Increased fibrosis of 1 stage in 3 HCV subjects and from stage 1 to stage 5 in 1 HCV subject,
2. Increased periportal/interface hepatitis in 3 HCV subjects and stable periportal/interface hepatitis in 2 HCV subjects, and
3. Increased inflammation in 4 HCV subjects.

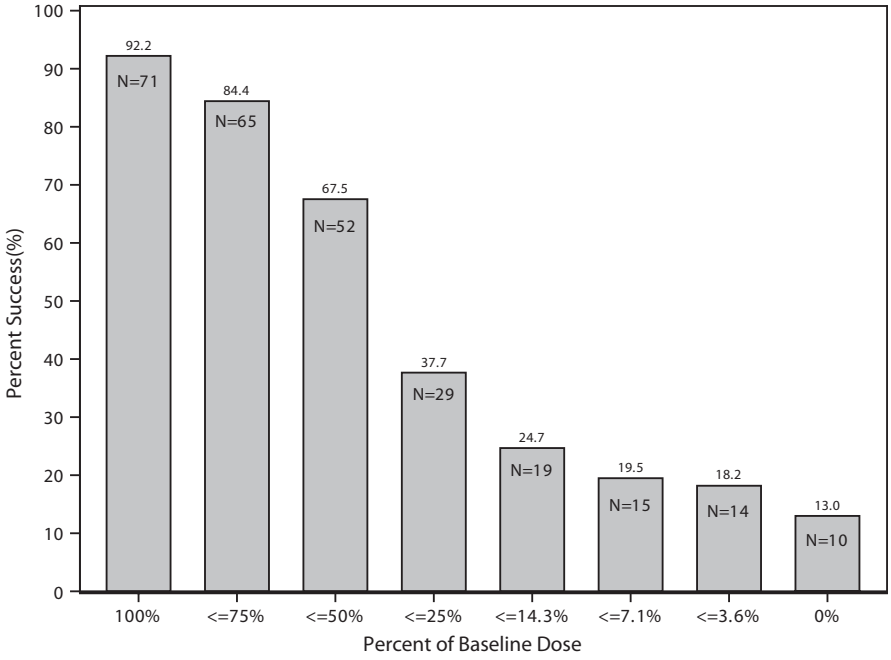

FIGURE 2 Percent of subjects who successfully completed each protocol-specified dose reduction. Subjects withdrew from immunosuppression at protocol-specified levels with the target dose indicated on the horizontal axis. Four subjects withdrew off all immunosuppression temporarily but restarted at a median time of 165 days later

3.3.3 | Nontolerant subjects

Of the 67 nontolerant subjects, 45 had a biopsy (41 for elevated LFTs and 4 for other reasons) at the time of failing withdrawal. Of these 45, 32 had a finding of rejection (18 mild, 2 mild-to-moderate, 9 moderate, 2 moderate-to-severe, and 1 severe) (Figure 3). Immunosuppression was increased for all subjects with rejection. Five of the rejection episodes also required treatment with at least 1 bolus of methylprednisolone 500 mg. No antibody treatment was administered. Twenty-nine rejection episodes were considered resolved, ie, with normal LFTs, at a median of 69 days (range 4-562 days) after failing withdrawal. Among the 13 subjects with elevated LFTs who had a biopsy where rejection was not diagnosed, immunosuppression was nonetheless increased as a conservative measure. Liver tests resolved in 11 of these subjects in a median of 148 days (range 26-903 days) after failing withdrawal.

Among the 22 subjects who did not have a biopsy at the time of failing withdrawal, 19 had elevated LFTs. Of these 19, 12 (63%) resolved in a median of 206 days (range 41-779 days). Subject-specific information for the nontolerant subjects is in Table S3.

Fifty-four nontolerant subjects were receiving the same or a lower amount of immunosuppression at study completion or termination compared to at the time of randomization (Figure 4). Dosing information for the 13 who were receiving more immunosuppression is shown in Table S4.

Recipients in whom liver enzymes did not return to normal limits (NINV n = 5, HCV n = 7) were not found to have chronic allograft injury and/or allograft failure for the postwithdrawal 2-year observation period.

3.3.4 | Maintenance outcomes

Fifteen of the 18 subjects randomly assigned to maintenance were on the same or a lower dose of immunosuppression at their final visit compared to randomization (Figure 4). Of the 3 on higher doses, 2 were no longer on monotherapy and 1 was on an increased total dose of a single agent at last follow-up (Table S4). Regimen changes were in response to rejection or elevated liver tests, or to maintain within-range trough levels. All maintenance subjects stayed on twice-a-day dosing. One subject had severe rejection after random assignment.

3.4 | Primary end point: clinical complications

A composite primary end point was used to assess whether immunosuppression withdrawal was at least not inferior to maintenance with respect to key posttransplant clinical complications in the 24 months after random assignment.

Such clinical complications were identified in 12 (18%, 90% confidence interval 10.4-26.0%) of the 66 evaluable subjects assigned to withdrawal and in 4 (31%, 90% confidence interval 9.7-51.8%) of the 13 evaluable subjects assigned to maintenance (Table 4 and Table S5). This gives a difference between withdrawal and maintenance of −13%, with a 90% confidence interval of -35% to 10%. This interval includes both zero and the specified noninferiority margin of 10%, and therefore renders the findings inconclusive for noninferiority.

3.5 | Rejection and adverse events

Transplant rejection was the most common adverse event in this trial reported after random assignment (Table 5 and Table S6) and was reported in 31 (40.3%) subjects in the withdrawal group and 1 (5.6%) subject in the maintenance group.

Other frequently occurring adverse events included liver function abnormalities in 19 (24.7%) subjects in the withdrawal group

TABLE 3 Laboratory and biopsy findings on tolerant subjects with central readings

Subject	Stratum	Completion of IS to last labs (d)	Lab findings eligibility/last available			Completion of IS withdrawal to biopsy (d)	Central biopsy findings eligibility/last available			
			ALT (U/L)	GGT (U/L)	Alk Phos (U/L)		Fibrosis	Periportal/ interface hepatitis	mHAI inflammation grade	Steatosis severity
106	HCV	790	58/71	47/32	84/55	156	1/2	1/2	3/5	Mild/moderate
141	HCV	772	53/52	60/23	96/65	202	0/1	0/2	1/5	Mild/mild
186	HCV	736	43/33	91/38	90/78	14	1/1	1/2	4/6	Mild/mild
220	HCV	740	85/101	97/47	108/97	29	1/2	2/2	6/5	None/none
273	HCV	283	93/68	329/–	125/168	396	1/5	2/2	7/9	Mild/none
084	NINV	735	51/46	26/22	80/87	240	0/0	0/0	0/0	Severe/severe
098	NINV	781	17/34	–/–	116/118	277[a]	0/–	0/–	0/–	None/–
159	NINV	747	11/19	23/23	160/52	260	0/0	0/0	0/0	Mild/mild
206	NINV	646	11/12	9/41	62/63	–	0/–	0/–	0/–	Mild/–
212	NINV	728	13/29	37/266	120/137	406	0/0	0/0	0/0	Severe/moderate

"–" Indicates not done.

Alk Phos, alkaline phosphatase; ALT, alanine transaminase; GGT, γ-glutamyl transpeptidase; HCV, hepatitis C virus; IS, immunosuppression; mHAI, modified hepatic activity index; NINV, nonimmune nonviral.

[a]Biopsy read locally only with no central read data available.

and 2 (11.1%) in the maintenance group and incisional hernia in 6 (7.8%) subjects in the withdrawal group and 4 (22.2%) subjects in the maintenance group. Neoplasms were reported in 16 subjects, 1 (5.6%) in the maintenance group, and 15 (19.5%) subjects in the withdrawal group. Six subjects had grade 4 secondary malignancies, adverse events that were considered life-threatening or disabling, that were counted as events for the primary end point. They were 1 lung neoplasm in the maintenance group, and 2 hepatic malignant recurrent neoplasms, 1 melanocytic nevus, 1 multiple myeloma, and 1 myelodysplastic syndrome in the withdrawal group. Ten subjects had less than grade 4 malignancies, which did not contribute to the primary end point. The most frequent were basal cell (3) or squamous cell (3) carcinomas. Serious adverse events were reported in 43 (55.8%) subjects in the withdrawal group and in 7 (38.9%) subjects in the maintenance group (Table S7).

3.6 | Biopsy features in follow-up

No differences between the maintenance and withdrawal groups in histological features in liver biopsies were observed in follow-up biopsies at a median of 583 days (range 140-1206 days) after random assignment for progression of at least 1 point for fibrosis (50% vs 31%), periportal/interface hepatitis (17% vs 27%), modified hepatic activity index inflammation (25% vs 31%), and steatosis (8% vs 24%); nor in the other histological features.

4 | DISCUSSION

This prospective study of 275 liver transplant recipients was designed to test early immunosuppression minimization and complete withdrawal in liver transplant recipients receiving standard immunosuppression drugs. The study end points were designed to determine whether an early decrease of immunosuppression drug exposure would reduce the incidence of immunosuppression-related complications and be associated with measurable clinical benefits.

A key aspect of the study design was enrollment of participants prior to transplantation. However, only 95 (35%) of the 275 enrolled met the eligibility criteria for random assignment within 2 years of transplantation. The 2 leading reasons for discontinuation prior to random assignment were voluntary withdrawal (41/180, 23%) or findings related to active HCV infection (39/180, 22%). In future studies the latter group would more likely be suitable for random assignment given current curative therapy for HCV.[14,15]

Among 77 subjects randomly assigned to withdrawal, 71 (92%) were able to tolerate once-a-day dosing. Among subjects who had further minimization, 52 (67.5%) were reduced to 50% or less of baseline monotherapy dose without any biochemical evidence of allograft dysfunction. The study also demonstrates that early attempts at complete immunosuppression withdrawal, starting in the second year after transplantation, can be tolerated in a

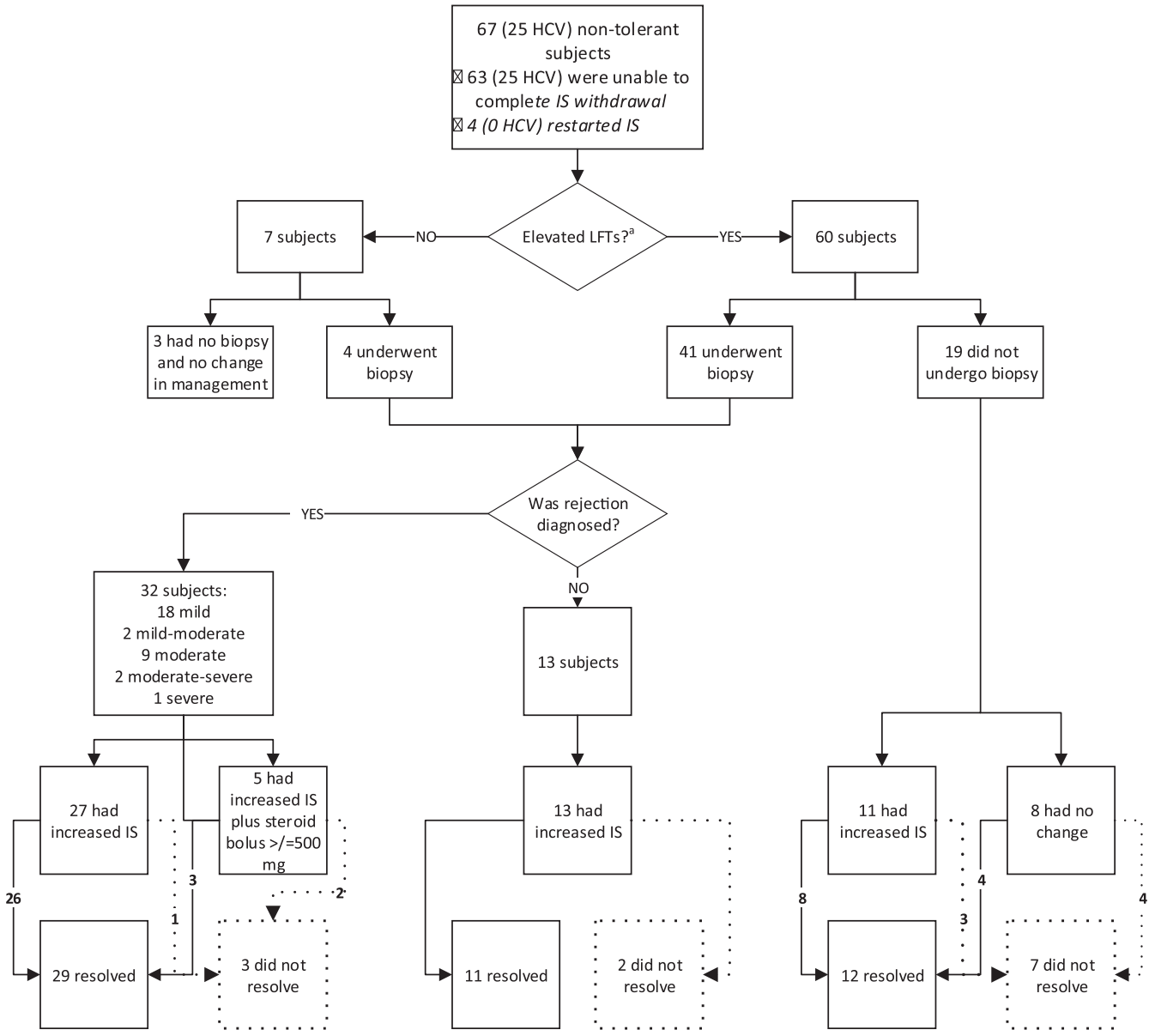

FIGURE 3 Disposition of subjects randomly assigned to immunosuppression withdrawal who were nontolerant. Those with or without elevated LFTs, and who did or did not undergo biopsy, are indicated. Those whose elevated LFTs did or did not resolve, and who had modification of immunosuppression, are also indicated. LFTs included γ-glutamyl transferase, alanine aminotransferase, aspartate aminotransferase, bilirubin, and alkaline phosphatase. [a]LFTs are considered elevated if any of the 5 tests were increased >150% from the higher of the value at random assignment or the upper limit of normal. LFTs function tests were considered resolved when all tests were <150% from the higher of the value at randomization or the upper limit of normal. HCV, hepatitis C virus; IS, immunosuppression; LFTs, liver function tests

limited number of recipients who have normal LFTs and no histologic findings of rejection in protocol biopsies. A limited number of the randomly assigned recipients (10/77, 13%) tolerated complete withdrawal at a mean of 2.8 years after transplantation. This is a unique finding since operational tolerance was achieved very early after transplantation using a standard immunosuppression strategy.

Previous studies have demonstrated operational tolerance in a larger proportion of study subjects; however, these studies enrolled stable recipients long after the transplant procedure. A European study enrolled 102 recipients, of whom 40% completed withdrawal at a mean of 10.9 years after transplantation, and a smaller US study in pediatric recipients of whom 12 of 19 recipients (63%) completed withdrawal at a mean of 8.3 years.[6,7]

The safety of clinically guided minimization and withdrawal must be measured against the ability to reverse graft injury. Our study and others demonstrate that with careful monitoring, clinical allograft dysfunction can be reversed with adjustments in immunosuppression management. Liver function at the end of the trial was similar between the withdrawal and maintenance groups, suggesting

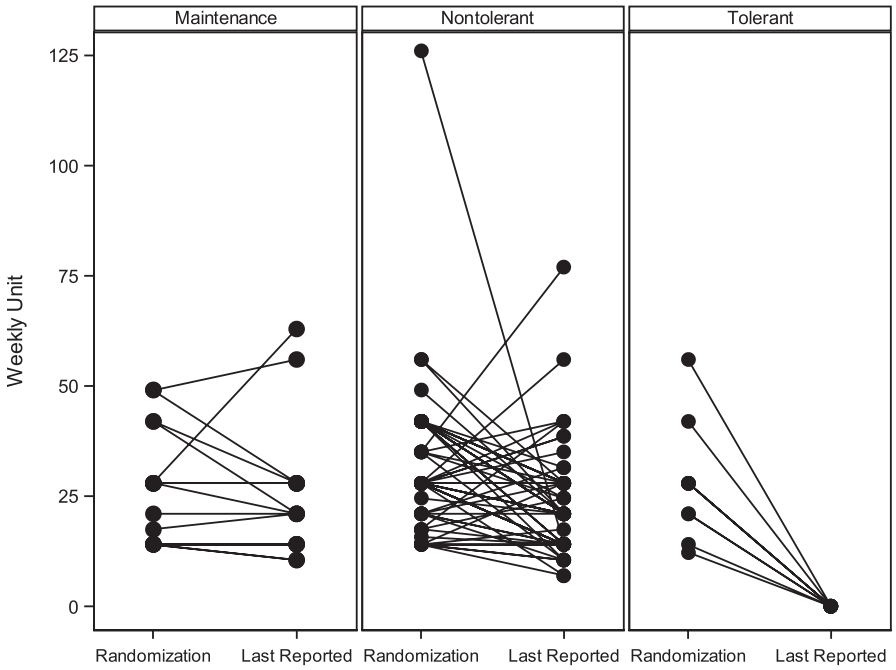

FIGURE 4 Subject dosing information at random assignment and last reported follow-up. The 18 subjects assigned to immunosuppression maintenance are depicted in the left panel. The 67 nontolerant subjects and the 10 tolerant subjects among those assigned to immunosuppression withdrawal are shown in the middle and right panels, respectively. Dosing units are as follows: 1 unit is equal to tacrolimus 1 mg, cyclosporine 100 mg, sirolimus 1 mg, mycophenolate mofetil 1000 mg, mycophenolic acid 720 mg, azathioprine 50 mg, or prednisone 5 mg. Any antibody use equaled 20 units. Unit scores are based on Vasudev et al[22]

TABLE 4 Primary end point of immunosuppression complications assessed 2 years after randomization

End point complication	Immunosuppression withdrawal (N = 77)	Immunosuppression maintenance (N = 18)	Difference[a]
Evaluable — n[b]	66	13	
One or more immunosuppression complications	12 (18%)	4 (31%)	−13% (−35%, 10%)
Death or graft loss	1 (2%)	0	
Grade 4 secondary malignancy	4 (6%)	1 (8%)	
Grade 4 opportunistic infection	0	0	
Stage 3 or higher fibrosis on Ishak scale	3 (5%)	2 (17%)	
GFR decrease[c]	6 (9%)	2 (17%)	

[a]Difference in percentage of subjects with 1 or more immunosuppression complication (withdrawal — maintenance) and corresponding 90% confidence interval. The confidence interval includes both zero and the noninferiority margin of 10%, so the results are inconclusive.
[b]The primary end point could not be assessed in those subjects who did not undergo complete assessment of outcome measures due to subject noncompliance or preference.
[c]GFR decrease was defined as a 25% decrease in GFR if GFR at randomization was between 30-90 mL/min per 1.73^2 and a 25% decrease and a GFR <90 mL/min per 1.73^2 for subjects with a GFR >90 mL/min per 1.73^2 at randomization. GFR was calculated using the Modification of Diet in Renal Disease formula.[5]

that there was no long-lasting injury related to attempts to minimize immunosuppression beyond monotherapy. The recipients in whom liver enzymes did not completely return to normal limits were not found to have chronic allograft injury and/or allograft failure during the 2-year postwithdrawal observation period.

Allograft dysfunction and clinically suspected rejection with or without biopsy-proven rejection was reversed by reinstitution of calcineurin inhibitors, with few subjects needing steroid therapy, and with no clinical evidence of long-lasting injury to the allograft.

It is likely that minimization or complete withdrawal of immunosuppression can minimize toxicities associated with prolonged exposure to high-dose medications, improve host immune surveillance, improve compliance with once-daily dosing, and reduce medication costs. A recent meta-analysis in 957 patients demonstrated that lower tacrolimus troughs early after transplant were associated with less renal impairment at 1 year without an increase in the rate of rejection.[16] Similarly the rate of recurrence of hepatocellular carcinoma was lower in those with less tacrolimus exposure.[17]

However, previous studies of immunosuppression withdrawal have failed to demonstrate such clinical benefit with respect to renal function, infection risk, secondary malignancies, or other complications related to immunosuppressive medications.[6,18-21] These studies were

TABLE 5 Adverse events postrandomization with an incidence >5%

	Immunosuppression maintenance (N = 18)	Immunosuppression withdrawal (N = 77)
Total number of adverse events	54	527
Number of subjects with at least 1 adverse event, n (%)	11 (61.1)	72 (93.5)
Infections and infestations, n (%)	5 (27.8)	38 (49.4)
Investigations, n (%)	3 (16.7)	37 (48.1)
Immune system disorders, n (%)	2 (11.1)	31 (40.3)
Gastrointestinal disorders, n (%)	3 (16.7)	26 (33.8)
Metabolism and nutrition disorders, n (%)	3 (16.7)	21 (27.3)
Injury, poisoning, and procedural complications, n (%)	4 (22.2)	19 (24.7)
Musculoskeletal and connective tissue disorders, n (%)	2 (11.1)	20 (26.0)
Nervous system disorders, n (%)	4 (22.2)	17 (22.1)
General disorders and administration site conditions, n (%)	1 (5.6)	16 (20.8)
Respiratory, thoracic, and mediastinal disorders, n (%)	2 (11.1)	15 (19.5)
Neoplasms benign, malignant, and unspecified (incl cysts and polyps) , n (%)	1 (5.6)	15 (19.5)
Skin and subcutaneous tissue disorders, n (%)	2 (11.1)	11 (14.3)
Hepatobiliary disorders, n (%)	3 (16.7)	8 (10.4)
Renal and urinary disorders, n (%)	0 (0)	10 (13.0)
Vascular disorders, n (%)	0 (0)	10 (13.0)
Psychiatric disorders, n (%)	2 (11.1)	6 (7.8)
Cardiac disorders, n (%)	2 (11.1)	4 (5.2)
Eye disorders, n (%)	0 (0)	5 (6.5)
Surgical and medical procedures, n (%)	2 (11.1)	3 (3.9)
Reproductive system and breast disorders, n (%)	1 (5.6)	3 (3.9)

The total number of adverse events counts all postrandomization adverse events for all randomized subjects. A subject is counted once if the subject reported 1 or more events, and percents are based on the number of subjects in the randomization group. Adverse events are coded according to Medical Dictionary for Regularly Activities V11.1.

done long after transplantation when drug-related systemic damage with limited reversibility had already been established. In the current study we observed a lower but not statistically significant incidence of a composite end point related to immunosuppression complications. However, the small number of subjects, and small number of events, and the relative short-term follow-up prevent us from making a conclusion about the impact of early immunosuppression withdrawal on such clinical complications.

5 | STUDY LIMITATIONS

Interpretation of the A-WISH trial outcomes is limited by several factors:

1. The trial design overestimated the proportion of participants who would be eligible for random assignment. The ability to detect differences between the withdrawal and maintenance group is therefore limited by the small number who were randomly assigned, 4:1, to immunosuppression withdrawal vs maintenance and by the fact that the maintenance participants were followed for only 2 years after random assignment. In contrast, the withdrawal participants were followed during the withdrawal attempts and then for a further 2 years.
2. The study population included hepatitis C participants with potentially active disease. This group is now less relevant in clinical practice given the current effective treatments for hepatitis C. Furthermore, conduct of the trial began in 2005 and continued to 2014, spanning changing patterns of practice with generally reduced immunosuppression.
3. The use of a composite end point to compare complication rates between groups does not allow for direct comparison of individual complications. In addition, we were not able to assess the primary end point in those participants who did not have complete outcome data (specifically, those who declined follow-up biopsies due to clinical stability).
4. The lack of mechanistic results further limits insight into the achievement of tolerance among liver transplant recipients.
5. The study design could have been improved by specifying the timing of protocol-mandated biopsies relative to time of completion of immunosuppression withdrawal rather than time from transplant.

6. The per-protocol definition of operational tolerance did not require a biopsy. Thus, some patients who were determined to be tolerant did not have protocol biopsies to confirm histological characteristics. In addition, in some cases, biopsies intended by the protocol were not obtained at the time of abnormal LFTs, due to patient noncompliance or preference.

6 | CONCLUSION

We demonstrated that clinically guided minimization can be performed in selected patients early after transplantation with manageable risk and acceptable safety. We also showed that such minimization within the first 2 years after transplantation only rarely results in complete immunosuppression withdrawal. In this short follow-up time there was no statistical difference in the primary end point outcome between the maintenance and withdrawal groups.

Thus, we conclude that broad-based immunosuppression withdrawal trials conducted early after transplant without specific selection are unlikely to be successful. However, if biomarkers can be defined to guide patient selection to enrich the small population of potentially tolerant individuals, this approach to early withdrawal could be revisited. In addition, we now recognize the challenges inherent in attempting to mandate complex patient withdrawal and assessment algorithms over many sites, especially in patients with very different time courses relative to key clinical milestones.

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DISCLOSURE

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

REFERENCES

1. Watt KD, Pedersen RA, Kremers WK, Heimbach JK, Charlton MR. Evolution of causes and risk factors for mortality post-liver transplant: results of the NIDDK long-term follow-up study. *Am J Transplant*. 2010;10(6):1420-1427.
2. Ojo AO, Held PJ, Port FK, et al. Chronic renal failure after transplantation of a nonrenal organ. *N Engl J Med*. 2003;349(10):931-940.
3. Lerut JP, Pinheiro RS, Lai Q, et al. Is minimal, [almost] steroid-free immunosuppression a safe approach in adult liver transplantation? Long-term outcome of a prospective, double blind, placebo-controlled, randomized, investigator-driven study. *Ann Surg*. 2014;260(5):886-891; discussion 891-2.
4. Lerut J, Mathys J, Verbaandert C, et al. Tacrolimus monotherapy in liver transplantation: one-year results of a prospective, randomized, double-blind, placebo-controlled study. *Ann Surg*. 2008;248(6):956-967.
5. Londoño MC, Rimola A, O'Grady J, Sanchez-Fueyo A. Immunosuppression minimization vs. complete drug withdrawal in liver transplantation. *J Hepatol*. 2013; 59(4):872-879.
6. Benítez C, Londoño MC, Miquel R, et al. Prospective multicenter clinical trial of immunosuppressive drug withdrawal in stable adult liver transplant recipients. *Hepatology*. 2013;58(5):1824-1835.
7. Feng S, Ekong UD, Lobritto SJ, et al. Complete immunosuppression withdrawal and subsequent allograft function among pediatric recipients of parental living donor liver transplants. *JAMA*. 2012;307(3):283-293.
8. De Simone P, Nevens F, De Carlis L, et al. Everolimus with reduced tacrolimus improves renal function in de novo liver transplant recipients: a randomized controlled trial. *Am J Transplant*. 2012;12(11):3008-3020.
9. Saliba F, Duvoux C, Gugenheim J, et al. Efficacy and safety of everolimus and mycophenolic acid with early tacrolimus withdrawal after liver transplantation: a multicenter randomized trial. *Am J Transplant*. 2017;17(7):1843-1852.
10. Fischer L, Saliba F, Kaiser GM, et al. Three-year outcomes in de novo liver transplant patients receiving everolimus with reduced tacrolimus: follow-up results from a randomized, Multicenter Study. *Transplantation*. 2015;99(7):1455-1462.
11. Demetris A, Adams D, Bellamy C, et al. Update of the International Banff Schema for Liver Allograft Rejection: working recommendations for the histopathologic staging and reporting of chronic rejection. An International Panel. *Hepatology*. 2000;31(3):792-799.
12. Banff Working Group, Demetris AJ. Liver biopsy interpretation for causes of late liver allograft dysfunction. *Hepatology*. 2006;44(2):489-501.
13. Levey AS, Coresh J, Balk E, et al. National kidney foundation practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Ann Intern Med*. 2003;139:137-147.
14. Azab M, Shah S, Liu X, et al. Extended DAA indications for hepatitis C patients awaiting liver transplantation and further statistical considerations. *J Hepatol*. 2017;68:626-627.
15. Ahmed A, Gonzalez SA, Cholankeril G, et al. Treatment of patients waitlisted for liver transplant with all-oral direct-acting antivirals is a cost-effective treatment strategy in the United States. *Hepatology*. 2017;66(1):46-56.
16. Rodriguez-Peralvarez M, Germani G, Darius T, Lerut J, Tsochatzis E, Burroughs AK. Tacrolimus trough levels, rejection and renal impairment in liver transplantation: a systematic review and meta-analysis. *Am J Transplant*. 2012;12(10):2797-2814.
17. Rodríguez-Perálvarez M, Germani G, Darius T, Lerut J, Tsochatzis E, Burroughs AK. Reduced exposure to calcineurin inhibitors early after liver transplantation prevents recurrence of hepatocellular carcinoma. *J Hepatol*. 2013;59(6):1193-1199.
18. Perito ER, Mohammad S, Rosenthal P, et al. Posttransplant metabolic syndrome in the withdrawal of immunosuppression in

Pediatric Liver Transplant Recipients (WISP-R) pilot trial. *Am J Transplant*. 2015;15(3):779-785.
19. Tryphonopoulos P, Ruiz P, Weppler D, et al. Long-term follow-up of 23 operational tolerant liver transplant recipients. *Transplantation*. 2010;90(12):1556-1561.
20. Mazariegos GV, Reyes J, Marino IR, et al. Weaning of immunosuppression in liver transplant recipients. *Transplantation*. 1997;63(2):243-249.
21. Takatsuki M, Soyama A, Torashima Y, et al. Weaning of immunosuppression in living donor liver transplant recipients. *Transplantation*. 2001;72(3):449-454.
22. Vasudev B, Hariharan S, Hussain SA, et al. BK virus nephritis: risk factors, timing, and outcome in renal transplant recipients. *Kidney Int*. 2005;68(4):1834-1839.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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