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Outcomes of immunosuppression minimization and withdrawal early after liver transplantation

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Abstract

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 post-transplant. 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 endpoint (death or graft loss; grade 4 secondary malignancy or opportunistic infection; Ishak stage ≥3; or > 25% decrease in glomerular filtration

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Supporting Information

Additional supplemental material may be found online in the Supporting Information section of this article.

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 endpoint comparison was inconclusive for non-inferiority of the withdrawal to the maintenance group.

Introduction

Standard practice patterns for liver transplant recipients include multiple immunosuppressive drugs aimed at predetermined trough levels, adjusted to time after transplantation. The 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 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 one to two years post-transplantation.

Patients and Methods

Subjects were enrolled at seven liver transplantation centers in the United States from November 2005 to April 2011. Entry eligibility criteria included: 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: 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.

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 to 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 two 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 two years at the conclusion of their tapering.

Eligibility for randomization included: 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 nonimmune nonviral causes of liver failure as total bilirubin, ALT, and alkaline phosphatase all ≤2 times the upper limit of normal.

Those assigned to withdrawal underwent a scheduled taper planned to last approximately 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.

Protocol-specified Biopsies

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

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 or alanine aminotransferase >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, GGT, or total bilirubin) were considered resolved when all LFTs were less than 150% from the higher of the value at randomization or the upper limit of normal

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.

Definition of Operational Tolerance

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

Objectives

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

Study Endpoints

The primary endpoint 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 endpoint 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 endpoint. A decrease in renal function was defined as a 25% decrease in glomerular filtration rate (GFR) if GFR at randomization was between 30-90 mL/min/1.73^2 and a 25% decrease and a GFR<90 mL/min/1.73^2 for subjects with a GFR >90 mL/min/1.73^2 at randomization. The Modification of Diet in Renal Disease formula was used to calculate GFR [13]. Secondary endpoints were: eligibility for random assignment, immunosuppression withdrawal completion, immunosuppression-free duration, hepatitis C viral load, fibrosis and graft loss or death.

Sample Size

The planned sample size was based on assessment of the primary endpoint after random assignment in the combined HCV and NINV strata and was intended to test whether the withdrawal arm was non-inferior 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 for the primary comparison. This would have allowed an assessment of non-inferiority with a 5% margin, a 97.5% one-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 non-inferiority with a 10% margin, a 95% one-sided confidence interval, and 80% power.

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.

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 two-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, version 9.3 or above.

Results

Study Subjects

Between November 2005 and April 2011, 286 consented participants underwent transplantation at seven clinical sites (Fig. 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 Supplementary Table S1.

Eligibility for Random Assignment

Of the 275 subjects who continued in the study, ninety-five 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 (Fig. 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

deviation 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 four subjects who were not on tacrolimus, two (one maintenance, one withdrawal) were on cyclosporine monotherapy; one maintenance subject was on mycophenolic acid monotherapy; and one withdrawal subject was on mycophenolate mofetil monotherapy.

There were also no differences in liver tests (ALT, AST, alkaline phosphatase, direct bilirubin, GGT) nor in immunosuppression trough levels at the time of randomization between those randomly assigned to the withdrawal or maintenance groups (Table 2). Further, 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 which were indeterminate/borderline for acute rejection. (Supplementary 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.

Immunosuppression Outcomes

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 (Fig. 2). Seventy-one (92.2%) tolerated once a day dosing and 52 (67.5%) tolerated a reduction to ≤ 50% of baseline dose.

Operationally Tolerant Subjects

Ten subjects (13.0%) remained off all immunosuppression for at least one year with no clinical evidence of rejection and were termed operationally tolerant (Fig. 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 Supplementary Fig. 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 (Supplementary Fig. S2).

The last available liver function labs post immunosuppression 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.

Central biopsy findings at the time of random assignment and at time of last report are also shown in Table 3. Nine of the ten operationally tolerant subjects had a biopsy an average 212 days (range 14 to 406 days) following completion of withdrawal; however one 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. Post immunosuppression 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:

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

Non-tolerant Subjects

Of the 67 non-tolerant subjects, 45 had a biopsy (41 for elevated liver function tests 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) (Fig. 3). Immunosuppression was increased for all subjects with rejection. Five of the rejection episodes also required treatment with at least one bolus of methylprednisolone 500 mg. No antibody treatment was administered. Twenty-nine rejection episodes were considered resolved, i.e. with normal liver function tests, at a median of 69 days (range 4 – 562 days) after failing withdrawal. Among the 13 subjects with elevated liver function tests who had a biopsy where rejection was not diagnosed, immunosuppression was nonetheless increased as 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 liver function tests. Of these 19, 12 (63%) resolved in a median of 206 days (range 41 – 779 days). Subject-specific information for the non-tolerant subjects is in Supplementary Table S3.

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

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

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 (Fig. 4). Of the three on higher doses, two were no longer on monotherapy and one was on an increased total dose of a single agent at last follow up (Supplementary 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.

Primary Endpoint: Clinical Complications

A composite primary endpoint was used to assess whether immunosuppression withdrawal was at least not inferior to maintenance with respect to key post-transplant 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 Supplementary 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 non-inferiority margin of 10%, and therefore renders the findings inconclusive for non-inferiority.

Rejection and Adverse Events

Transplant rejection was the most common adverse event in this trial reported after random assignment (Table 5 and Supplementary 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 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 counted as events for the primary endpoint. They were: 1 lung neoplasm in the maintenance group; and 2 hepatic malignant recurrent neoplasms, 1 melanocytic naevus, 1 multiple myeloma and 1 myelodysplastic syndrome in the withdrawal group. Ten subjects had less then grade 4 malignancies, which did not contribute to the primary endpoint. 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 7 (38.9%) subjects in the maintenance group (Supplementary Table S7).

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.

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 endpoints 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 enrolment of participants prior to transplantation. However, only 95 (35%) of the 275 enrolled met eligibility criteria for random assignment within two years of transplantation. The two 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 limited number of recipients who have normal liver function tests 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 paediatric recipients of whom 12/19 recipients (60%) 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 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 suffer from chronic allograft injury and/or allograft failure during the two-year post-withdrawal 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 one year without an increase in the rate of rejection[16]. Similarly the rate of recurrence of hepatocellular carcinoma were 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 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 endpoint 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.

Study Limitations

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

- 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.
- The study population included hepatitis C participants with potentially active disease which less relevant in clinical practice given current treatment advances. Further, conduct of the trial began in 2005 and continued to 2014 spanning changing patterns of practice with generally reduced immunosuppression.
- The use of a composite endpoint 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 endpoint in those participants who did not have complete outcome data. Specifically, those who declined follow-up biopsies due to clinical stability.

- The lack of mechanistic results further limits insight into the achievement of tolerance among liver transplant recipients.
- 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.
- 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 non-compliance or preference.

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 two years after transplantation only rarely results in complete immunosuppression withdrawal. In this short follow-up time there was no statistical difference in the primary endpoint outcome between the maintenance and withdrawal groups.

We conclude then 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

HCV	Hepatitis C virus

NINV	nonimmune nonviral
GFR	glomerular filtration rate

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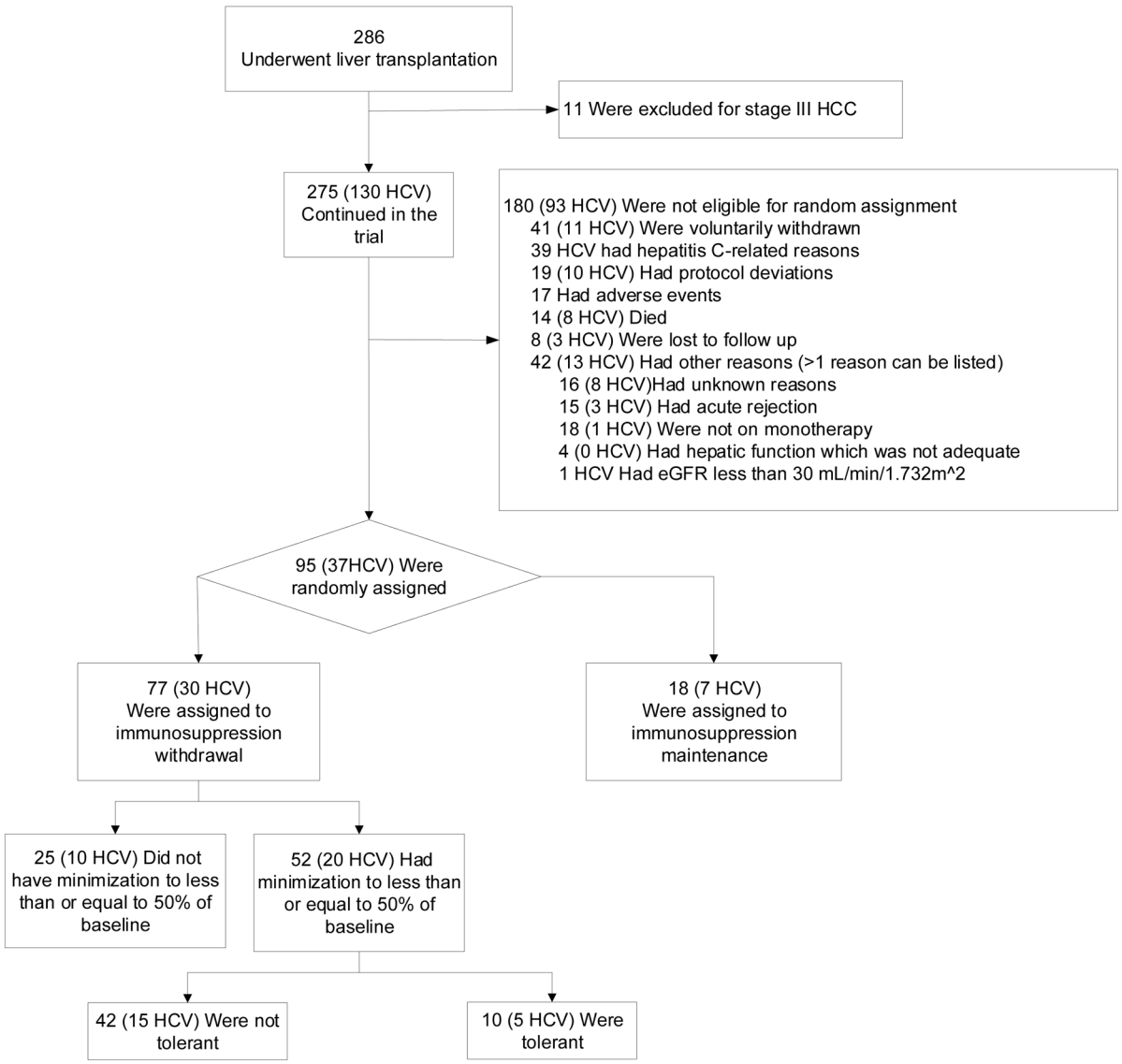

Figure 1:

Disposition of enrolled subjects. All subjects who were assessed for eligibility for random assignment, as well as those who were replaced, are included.

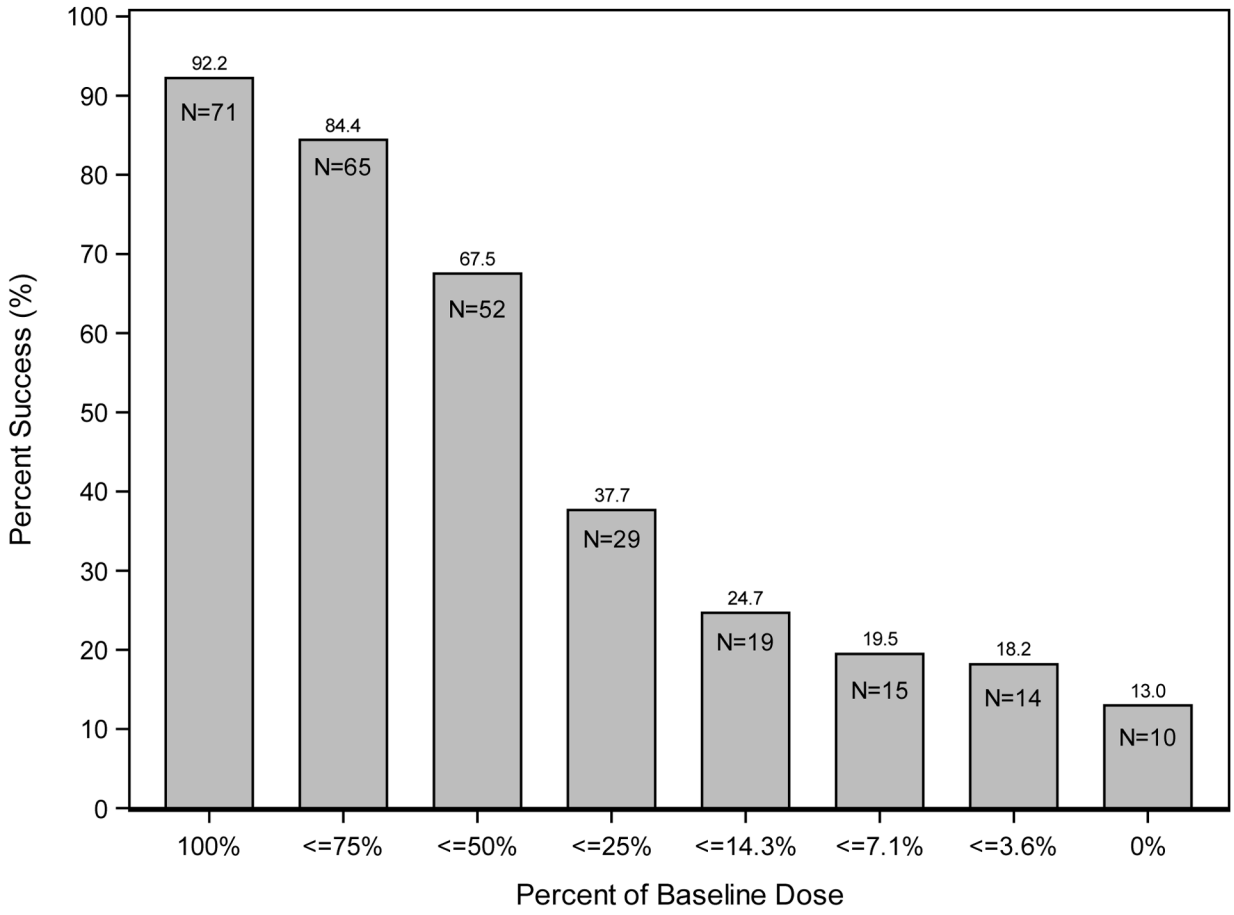

Figure 2:

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.

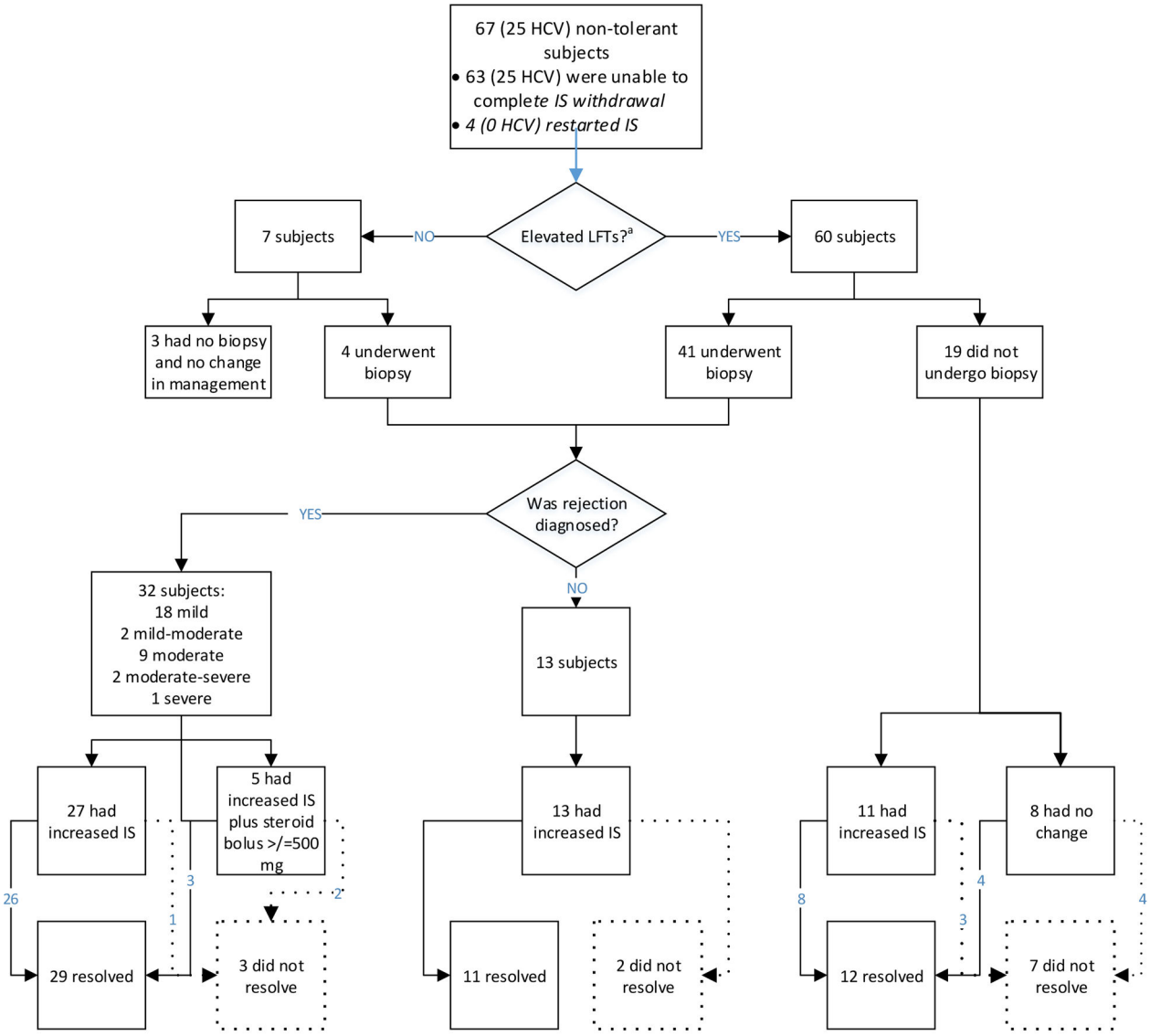

Figure 3:

Disposition of subjects randomly assigned to immunosuppression withdrawal who were non-tolerant. 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. Liver function tests included gamma-glutamyl transferase, ALT, aspartate aminotransferase, bilirubin, and alkaline phosphatase.

[a] Liver function tests are considered elevated if any of the five tests were increased more than 150% from the higher of the value at random assignment or the upper limit of normal. Liver function tests were considered resolved when all tests were less than 150% from the higher of the value at randomization or the upper limit of normal.

IS, immunosuppression; LFT, liver function tests

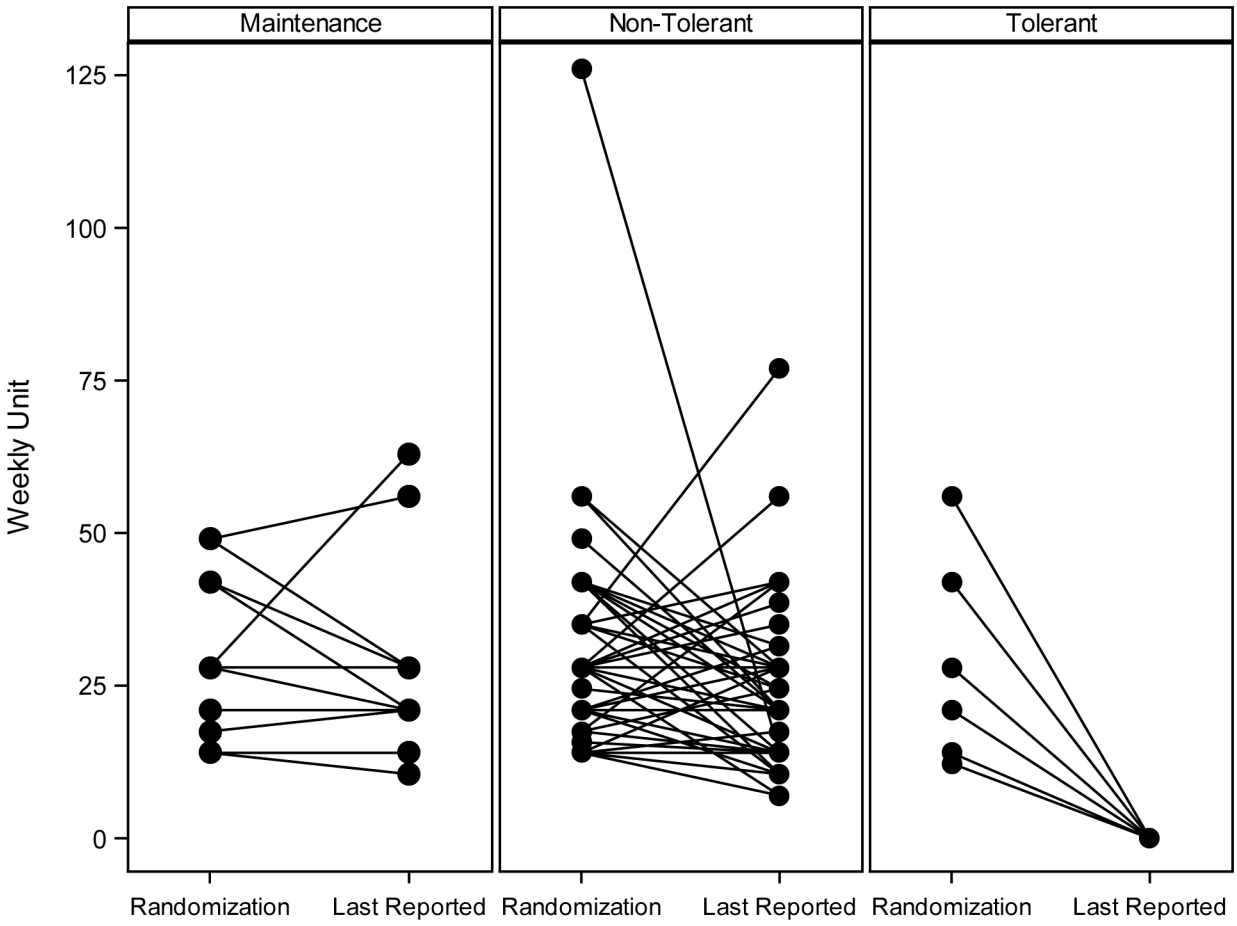

Figure 4:

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 non-tolerant 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 a 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 equalled 20 units. Unit scores based on Vasudev B, et al. [22].

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 (years)	54.9 (9.59)	57.4 (7.70)	54.3 (9.92)	0.21
Sex (Male) – n (%)	76 (80.0)	13 (72.2)	63 (81.8)	0.35
Race – n (%)				0.31
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 (years)	44.7 (17.39)	40.7 (19.79)	45.6 (16.79)	0.29
Age Matched[b] (Yes) – n (%)	61 (64.2)	10 (55.6)	51 (66.2)	0.42
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)	0.18
BMI (kg/m^2)	30.7 (5.42)	30.6 (5.08)	30.8 (5.52)	0.89
Creatinine (mg/dL)	1.2 (0.65)	1.1 (0.68)	1.3 (0.65)	0.45
eGFR ($mL/min/1.73^2$)	76.9 (39.47)	85.3 (46.30)	75.0 (37.78)	0.32
Total Bilirubin (mg/dL)	7.3 (9.70)	6.4 (11.78)	7.5 (9.22)	0.66
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)	0.49
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[c]	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.

[b] Subjects are considered age matched if both the recipient and donor are >55 years of age or both are <= 55 years of age.

[c] NINV subjects can have more than one primary reason for liver failure and percents for sub-categories are out of total NINV subjects.

Abbreviations: CI, confidence interval; eGFR, estimated glomerular filtration rate; BMI, body mass index

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)	0.33
AST (U/L)	38.8 (30.33)	50.7 (52.86)	36.1 (21.71)	0.26
Alkaline Phosphatase (U/L)	107.0 (41.62)	102.1 (36.03)	108.2 (42.95)	0.58
Direct Bilirubin (mg/dL)				0.93
n	78	15	63	
Mean (standard deviation)	0.2 (0.08)	0.2 (0.10)	0.2 (0.08)	
GGT (U/L)				0.28
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)	0.42
Tacrolimus Trough Levels by Site (ng/mL)				0.53
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)			

Values are expressed as mean (standard deviation). Site trough levels were compared using an ANOVA test.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase

Table 3:

Laboratory and Biopsy Findings on Tolerant Subjects with Central Readings

Subject	Stratum	Completion of IS to Last Labs (days)	Lab Findings Eligibility / Last Available			Completion of IS withdrawal to Biopsy (days)	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.

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

Abbreviations: IS, immunosuppression; mHAI, modified hepatic activity index; ALT, alanine transaminase; GGT, gamma-glutamyl transpeptidase; Alk Phos, alkaline phosphatase

Table 4:

Primary Endpoint of Immunosuppression Complications Assessed Two Years after Randomization

Endpoint 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 one or more immunosuppression complication (withdrawal – maintenance) and corresponding 90% confidence interval. The confidence interval includes both zero and the non-inferiority margin of 10%, so the results are inconclusive.

[b]The primary endpoint could not be assessed in those subjects who did not undergo complete assessment of outcome measures due to subject non-compliance or preference.

[c]GFR decrease was defined as a 25% decrease in GFR if GFR at randomization was between 30-90 mL/min/1.732 and a 25% decrease and a GFR<90 mL/min/1.732 for subjects with a GFR >90 mL/min/1.732 at randomization. GFR was calculated using the Modification of Diet in Renal Disease formula.[5]

Table 5:

Adverse Events Post-Randomization 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 One Adverse Event	11 (61.1)	72 (93.5)
Infections and infestations	5 (27.8)	38 (49.4)
Investigations	3 (16.7)	37 (48.1)
Immune system disorders	2 (11.1)	31 (40.3)
Gastrointestinal disorders	3 (16.7)	26 (33.8)
Metabolism and nutrition disorders	3 (16.7)	21 (27.3)
Injury, poisoning and procedural complications	4 (22.2)	19 (24.7)
Musculoskeletal and connective tissue disorders	2 (11.1)	20 (26.0)
Nervous system disorders	4 (22.2)	17 (22.1)
General disorders and administration site conditions	1 (5.6)	16 (20.8)
Respiratory, thoracic and mediastinal disorders	2 (11.1)	15 (19.5)
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	1 (5.6)	15 (19.5)
Skin and subcutaneous tissue disorders	2 (11.1)	11 (14.3)
Hepatobiliary disorders	3 (16.7)	8 (10.4)
Renal and urinary disorders	0 (0)	10 (13.0)
Vascular disorders	0 (0)	10 (13.0)
Psychiatric disorders	2 (11.1)	6 (7.8)
Cardiac disorders	2 (11.1)	4 (5.2)
Eye disorders	0 (0)	5 (6.5)
Surgical and medical procedures	2 (11.1)	3 (3.9)
Reproductive system and breast disorders	1 (5.6)	3 (3.9)

The total number of adverse events counts all post-randomization adverse events for all randomized subjects. A subject is counted once if the subject reported one 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.