Efficacy and Safety of Immunosuppression Withdrawal in Pediatric Liver Transplant Recipients: Moving Toward Personalized Management

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BACKGROUND AND AIMS: Tolerance is transplantation's holy grail, as it denotes allograft health without immunosuppression and its toxicities. Our aim was to determine, among stable long-term pediatric liver transplant recipients, the efficacy and safety of immunosuppression withdrawal to identify operational tolerance.

APPROACH AND RESULTS: We conducted a multicenter, single-arm trial of immunosuppression withdrawal over 36-48 weeks. Liver tests were monitored biweekly (year 1), monthly (year 2), and bimonthly (years 3-4). For-cause biopsies were done at investigators' discretion but mandated when alanine aminotransferase or gamma glutamyltransferase exceeded 100 U/L. All subjects underwent final liver biopsy at trial end. The primary efficacy endpoint was operational tolerance, defined by strict biochemical and histological criteria 1 year after stopping immunosuppression. Among 88 subjects (median age 11 years; 39 boys; 57 deceased donor grafts), 33 (37.5%; 95% confidence interval [CI] 27.4%, 48.5%) were operationally tolerant, 16 were nontolerant by histology (met biochemical but failed histological criteria), and 39 were nontolerant by rejection. Rejection, predicted by subtle liver inflammation in trial entry biopsies, typically (n = 32) occurred at ≤32% of the trial-entry immunosuppression dose and was treated with corticosteroids (n = 32) and/or tacrolimus (n = 38) with resolution (liver tests within 1.5 times the baseline) for all but 1 subject. No death, graft loss, or chronic, severe, or refractory rejection occurred. Neither fibrosis stage nor the expression level of a rejection gene set increased over 4 years for either tolerant or nontolerant subjects.

CONCLUSIONS: Immunosuppression withdrawal showed that 37.5% of selected pediatric liver-transplant recipients were operationally tolerant. Allograft histology did not deteriorate for either tolerant or nontolerant subjects. The timing and reversibility of failed withdrawal justifies future trials exploring the efficacy, safety, and potential benefits of immunosuppression minimization. (HEPATOLOGY 2021;73:1985-2004).

For children with liver transplants, the primary barriers to optimal allograft and patient health are chronic allo-immune graft injury[(1-8)] and

the cumulative toxicities of immunosuppression.[9-12] The perception that the liver is "tolerogenic" has spawned intense interest in mitigating the cumulative toxicities of immunosuppression by reducing dosage or discontinuing immunosuppression. Multiple single-center reports have described recipients who, off immunosuppression, have apparently maintained normal allograft function (summarized in Feng and Bucuvalas[13] and Levitsky and Feng[14]). However, studies showing a high prevalence of graft injury in patients with normal liver tests on standard-of-care immunosuppression[1-8] and those refuting the benign nature of rejection[15-18] have raised concerns regarding the wisdom of immunosuppression withdrawal (ISW). These concerns have been partly assuaged by two adult and one pediatric multicenter ISW trials.[19-21] With respect to efficacy, only the adult trials estimated the prevalence of operational tolerance. With respect to safety, clinical and histological follow-up of operationally tolerant subjects was limited to 1 and 3 years in the two adult trials and extended to 5 years in the pilot pediatric trial. Neither allograft inflammation nor fibrosis was reported to increase. However, no trials afforded histologic follow-up to those who failed ISW. It remains unknown whether rejection that occurred during ISW resulted in histological sequelae. Thus, the safety of attempted ISW has not been fully elucidated.

We report on a multicenter trial conducted at 12 North American transplant centers to determine the efficacy and safety of ISW in children with stable, long-term liver transplants. Our primary objective was to ascertain the prevalence of operational tolerance with a sufficiently narrow confidence interval to guide

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clinicians and patients. A critical secondary objective was the safety of attempted ISW. Immunosuppression Withdrawal for Stable Pediatric Liver Transplant Recipients (iWITH) mandated that all subjects who initiated ISW, regardless of outcome, undergo the same duration of follow-up with liver tests, donor-specific antibody (DSA) testing, and liver biopsy. Finally, as an exploratory objective, we aimed to identify predictors of operational tolerance. Elucidating conditions that permit immunosuppression dose reduction and delineate appropriate monitoring thereafter, inclusive of histological evaluation, may free children with liver transplants from the current impossible dichotomy of "too little" or "too much" immunosuppression.

Patients and Methods

TRIAL DESIGN AND SUBJECTS

iWITH (NCT01638559) was a prospective, single-arm, multicenter trial to determine the efficacy and safety of ISW in pediatric liver transplant recipients. Each participant and his or her guardian provided informed assent and consent. The trial protocol and statistical analysis plan are submitted as Supporting Information.

INCLUSION/EXCLUSION CRITERIA

Inclusion and exclusion criteria are detailed in the trial protocol (Supporting Information). The key criteria were:

Inclusion

- Liver transplant recipient at ≤6 years of age;
- ≥4 years after transplant and <18 years at enrollment;
- Alanine aminotransferase (ALT) and gamma-glutamyltransferase (GGT) consistently <50 U/L;
- No acute or chronic rejection within 2 years;
- On calcineurin inhibitor monotherapy for the preceding year without ≥50% dose increase during the preceding 6 months; and
- Eligible screening biopsy per central pathology (Supporting Table S1).[22]

Exclusion

- Transplant secondary to autoimmune etiologies, hepatitis B virus, or hepatitis C virus;

- Recipient of second organ transplant before, simultaneous to, or after liver transplant; and
- Calculated glomerular filtration rate (GFR; modified Schwartz formula) <60 mL/min/1.73 m^2.

No subjects participated in a previous ISW trial.

TRIAL PROCEDURES AND ENDPOINTS

Immunosuppression was reduced in seven steps of 4-week or 6-week duration. The total daily dose was reduced by 25% (steps 1 and 2), followed by the number of dosing days per week (steps 3 through 7) (Supporting Fig. S1). Step progression could be paused for ≤4 weeks; each subject was limited to three pauses, so ISW ranged from 36 to 48 weeks. Liver tests were checked every 2 weeks during ISW and for an additional 12 weeks thereafter. For-cause liver biopsies were performed at the investigators' discretion but mandated if ALT or GGT exceeded 100 U/L. Local pathology assessment guided clinical decision making, including immunosuppression management; central pathology assessment was used for data analysis.

Subjects completing ISW with stable liver tests and without rejection were evaluated 1 year after the last dose for operational tolerance using the primary endpoint criteria of ALT and GGT < 50 U/L and liver biopsy with no more than minimal change compared with the eligibility biopsy[22] (Supporting Table S1). All subjects were required to undergo biopsy at trial end. The primary safety endpoint was absence of histologically severe rejection, refractory rejection (rejection requiring treatment with a lymphocyte depleting drug), chronic rejection, allograft loss, or death. Secondary safety endpoints included histological grade, clinical severity, and time to resolution of acute rejection, overall immunosuppression exposure, and fibrosis progression.

SAFETY MONITORING

Site investigators reported adverse events through 30 days after trial completion. Investigators graded severity according to the Common Terminology Criteria for Adverse Events (version 4.03) and assessed seriousness and relatedness to trial procedures (phlebotomy, biopsy, and ISW). Severe adverse events were reviewed by a National Institute of Allergy and

Infectious Diseases (NIAID) medical monitor who determined final severity and attribution assessments.

MECHANISTIC STUDIES

Detailed methods for mechanistic studies described subsequently are provided in the Supporting Information. Human leukocyte antigen and eplet mismatch were determined for donor-recipient pairs. Serum specimens were tested for autoantibodies, quantitative immunoglobulin G, and class II DSA.[3]

Formalin-fixed liver biopsies were scored for inflammation and fibrosis. Batched slide sets underwent multiplex immunohistochemistry for (1) leukocytes (CD45+), antigen-presenting cells (MHCII+), and endothelial cells (CD34+); (2) T cells (CD3+) and recent infiltrating monocytes/macrophages (MAC387+); and (3) T-cell subsets (CD4+, CD8+, T-box protein expressed in T cells+ and forkhead box protein P3+). Fully automated tissue-tethered cytometry was performed using image analysis software and applied using automated batch processing without human intervention.

We conducted tissue transcriptional profiling of 222 cryopreserved liver-tissue samples using Affymetrix U219 microarrays and validated the results using the NanoString nCounter platform. To assess the probability of T cell–mediated rejection (TCMR) based on changes in microarray gene expression, we analyzed the transcript levels of a previously published 12-gene TCMR signature.[23] To assess overrepresentation of biological pathways in the microarray data set, we used gene-set enrichment analysis (GSEA) and three publicly available gene sets. Finally, real-time PCR experiments assessed the transcript levels of a five-gene predictor of ISW success for adult liver transplant recipients.[24]

STATISTICAL ANALYSES

The sample size was based on an estimated operational tolerance rate of 35% and a specified 95% confidence interval (CI) half-width of 10%. Categorical and continuous variables were compared using Fisher exact and two-sample Student t tests. Logistic regression analyses were used to identify associations between operational tolerance and clinical, serological, histological, immunohistochemical, and transcriptional parameters. Statistical analyses were performed using SAS, version 9.4 (SAS Institute, Cary, NC).

All data and analyses are available on the Immune Tolerance Network TrialShare analysis portal (https://www.itntrialshare.org/iWITH_primary.url).

TRIAL OVERSIGHT

The first two authors designed the trial in collaboration with NIAID, National Institute of Diabetes and Digestive and Kidney Diseases, Immune Tolerance Network, and iWITH investigators. The trial was conducted in accordance with the Declaration of Helsinki. Adverse events were reviewed by the NIAID medical monitor and data and safety monitoring board Clinical data and mechanistic data were submitted to and analyzed by a central data coordinating center and by the Immune Tolerance Network. The manuscript was drafted by the first author and edited and reviewed by all. All authors confirm that the trial was conducted according to the protocol and that the data and analyses presented are accurate and complete.

Results

PATIENT COHORT

Among the 2,909 children with liver transplants followed at 12 centers (Fig. 1), 1,731 were excluded for transplant within <4 years ago (n = 963), at age ≥7 years (n = 507), a second liver or other transplant (n = 186), or transplant for viral or auto-immune disease (n = 75). After additional exclusions (n = 823), 355 patients remained. Among 276 approached, 161 patients and guardians provided assent and informed consent between August 2012 and June 2014. Final assessment excluded 73 subjects, 69 secondary to histopathology and 4 secondary to abnormal liver tests. Key histological features of the 88 eligible subjects are shown in Supporting Fig. S2.

PRIMARY ENDPOINT

Among the 88 subjects who initiated ISW, 33 subjects (37.5%; 95% CI: 27.4%-48.5%) were operationally tolerant, meeting biochemical and histological criteria (Fig. 2A). The remaining 55 were nontolerant (Fig. 2B). Table 1 lists the comparisons of operationally tolerant and nontolerant subjects. All subjects were assessed for the primary endpoint; 3 did not complete the trial.

2,909 Pediatric liver transplant recipients followed at 12 centers, born after 1/3/1996

1,731 Patients excluded
   963 Transplanted after 1/2/2012 (< 4 years post-transplant)
   507 Transplanted when they were ≥ 7 years of age
   186 Undergone repeat liver transplantation or had second organ transplant
   75 Transplanted for hepatitis C, hepatitis B, primary sclerosing cholangitis or
         autoimmune hepatitis

1,178 Potentially eligible patients

594 Patients medically excluded
  180 Baseline ALT OR GGT > 50 U/L
  31 Rejection within 2 years of enrollment
  213 Not on calcineurin inhibitor monotherapy within 1 year of enrollment
  49 ≥ 50% increase in calcineurin inhibitor within 6 months of enrollment
  13 Evidence of hepatitis C, hepatitis B, or human immunodeficiency virus infection
  16 Glomerular filtration rate < 60 mL/min/1.73m^2
  84 Medical condition not suitable for study
  1 Received an investigational drug given within 4 weeks of enrollment
  5 Received live or attenuated vaccine within 8 weeks of enrollment

584 Met medical inclusion/exclusion

229 Excluded
  227 Unsuitable logistical factors (distance, financial, non-adherence)
  2 Unwilling to use accepted mode of contraception

355 Met all clinical inclusion/exclusion

79 Patients not approached (but eligible)

276 Approached for consent

115 Refused consent

161 Consented

73 ineligible
  69 Secondary to biopsy findings
  4 Secondary to liver test abnormalities

88 Eligible; initiated ISW

88 Evaluated for primary endpoint

3 Terminated trial participation early
  1 Lost to follow-up
  1 Withdrew assent/consent
  1 Declined final biopsy

85 Completed trial participation

FIG. 1. iWITH enrollment diagram. Beginning with 1,178 potentially eligible recipients, 1,090 patients were sequentially excluded, resulting in 88 subjects who were fully eligible and initiated ISW.
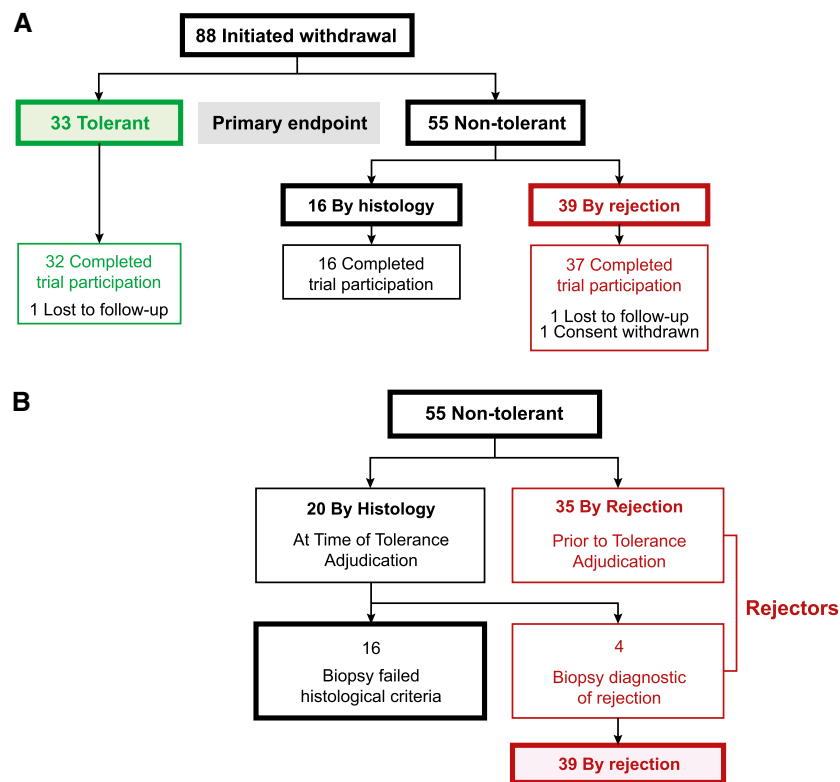
FIG. 2. iWITH primary endpoint: the outcome of immunosuppression withdrawal. Eighty-eight subjects initiated immunosuppression withdrawal in seven steps according to a protocol-specified algorithm provided in Supporting Fig. S2. (A) Thirty-three subjects met the biochemical and histological criteria for operational tolerance. Fifty-five subjects were nontolerant: 39 failed secondary to rejection, and 16 failed secondary to histological findings, even though they met biochemical criteria. Three subjects did not complete trial participation, secondary to being lost to follow-up (operationally tolerant subject, after tolerance adjudication), withdrawal of consent, and refusal to travel for the end-of-trial (year 4) biopsy (both subjects who rejected). (B) Among the 55 nontolerant subjects, 35 rejected before tolerance adjudication. The remaining 20 subjects were determined to be nontolerant based on adjudication biopsy findings: 16 subjects failed to meet the histologic criteria of operational tolerance (Supporting Table S1), and 4 subjects met the biopsy criteria for rejection despite stable relative to baseline serum ALT and GGT levels. We classified 39 subjects as nontolerant by rejection: 35 before the tolerance adjudication biopsy and 4 at the time of the adjudication biopsy.

OPERATIONALLY TOLERANT SUBJECTS

For the 33 operationally tolerant subjects, Fig. 3A shows the baseline, peak, and final ALT and GGT over 4 years; 4% of ALT and 2% of GGT values exceeded 50 U/L (Supporting Fig. S3A). Serial DSA testing demonstrated that 12 (36%) never had, 15 (45%) had at trial entry, and 5 (15%) developed class II DSA (Supporting Fig. S4A). Biopsies at trial start and end (4-year separation) were compared for inflammation (Supporting Fig. S5A) and fibrosis (Fig. 3B). Liver allograft fibrosis scores (LAFSc)[25] were unchanged or minimally changed (−1 ≤ Δ ≤ 1) for 25 of 32, improved for 5 of 32 (2 with −3, and 3 with −2 change), and worsened for 2 of 32 (1 each with +2 and +3 change).

Consistent with histological observations, TCMR probability scores were unchanged, comparing trial end to start biopsies (4-year separation; Fig. 3C). Consistency was confirmed at the gene pathway level by whole-transcriptome pairwise comparisons of the three longitudinal, protocol-directed biopsies. Pro-inflammatory pathway enrichment scores did not increase after immunosuppression discontinuation (Supporting Tables S5 and S6).

NONTOLERANT SUBJECTS BY REJECTION

Among the 55 nontolerant subjects, 39 were nontolerant by rejection (Fig. 2B) and 16 were nontolerant by histology. There were 37 biopsy-proven and 2

TABLE 1. Characteristics of 88 Subjects Undergoing Immunosuppression Withdrawal by Tolerance Status

Characteristic			Tolerant (n = 33)	Nontolerant (n = 55)
Donor	Age (years)		15 (2-27)	10 (3-31)
	Male gender		18 (55)	26 (47)
	Race	White	22 (67)	39 (71)
		Black	5 (15)	5 (9.1)
		Other	6 (18)	11 (20)
	Deceased		22 (67)	35 (64)
Recipient	Age at transplant (years)		1 (1-2)	1 (1-3)
	Male gender		13 (39)	26 (47)
	Race	White	30 (91)	46 (84)
		Black	1 (3.0)	3 (6)
		Other	2 (6)	6 (11)
	Transplant indication	Acute liver failure	2 (6)	5 (9)
		Biliary atresia	20 (61)	31 (56)
		Tumor	2 (6)	3 (6)
		Metabolic liver disease	2 (6)	7 (13)
		Other	7 (21)	9 (16)
Transplant	Whole graft		15 (46)	26 (47)
	Previous rejection episodes	0	24 (73)	32 (58)
		1	7 (21)	12 (22)
		2 or more	2 (6)	11 (20)
	Time since last rejection (years)	8 (7-9)	6 (4-8)	8 (24)
	Received induction immunosuppression			
At trial entry*	Tacrolimus		29 (88)	52 (95)
	Tacrolimus dose (n = 81; mg/kg/day)		0.04 (0.02-0.05)	0.05 (0.04-0.07)
	Age (years)		11 (7-13)	11 (8-13)
	Time since transplant (years)		9 (6-10)	8 (6-11)
	ALT (U/L)		26 (21-30)	23 (19-30)
	GGT (U/L)		14 (12-19)	15 (12-19)
	Anti-nuclear antibody (n = 77)	Positive (≥1:40)	4 (13)	12 (26)
	Anti-smooth muscle antibody (n = 77)	Positive (1:80)	3 (10)	0
	Quantitative immunoglobulin G (n = 73; mg/dL)		629 (562-822)	701 (616-801)
	Eplet mismatch (n = 86)	Total (DR + DQ)	27 (16-45)	28 (21-40)
		DQ only	10 (5-15)	10 (6-16)
	α-Class II DSA (n = 87)	Positive	15 (47)	29 (53)
		Maximum MFI > 20,000	4 (13)	5 (9)
		MFI sum > 20,000	6 (19)	8 (15)

Note: Continuous variables are summarized using median and IQR. Categorical variables are summarized by counts and percentages.
*Seven subjects were on cyclosporine at trial entry.

clinical rejection episodes, defined per protocol (Fig. 4A). Rejection was diagnosed during ISW (n = 33), after ISW but before tolerance adjudication (n = 2), or at the time of tolerance adjudication (n = 4). Most episodes (33 of 39; 85%) occurred at ≤32% of the entry immunosuppression dose. Histological severity[26] of the 37 biopsy-proven episodes was predominantly indeterminate (n = 16) or mild (n = 17). Median (interquartile range [IQR]) peak ALT was 136 (101-205) U/L and peak GGT was 63 (42-104) U/L (Fig. 4B). During the 4-year trial, 12% of ALT and 9% of GGT values exceeded 50 U/L (Supporting Fig. S3B).

Rejection was treated with corticosteroids in 32 of 39 subjects, with a median (IQR) total dose of 34.7 (16.4-50.7) mg/kg over 63 (42-121) days. Nearly all subjects (38 of 39) were also treated with re-initiation or

A

	Baseline	Peak	Final
ALT (U/L)	26 (21-30)	54 (44-75)	30 (19-35)
GGT (U/L)	14 (12-19)	34 (19-45)	15 (13-24)

B
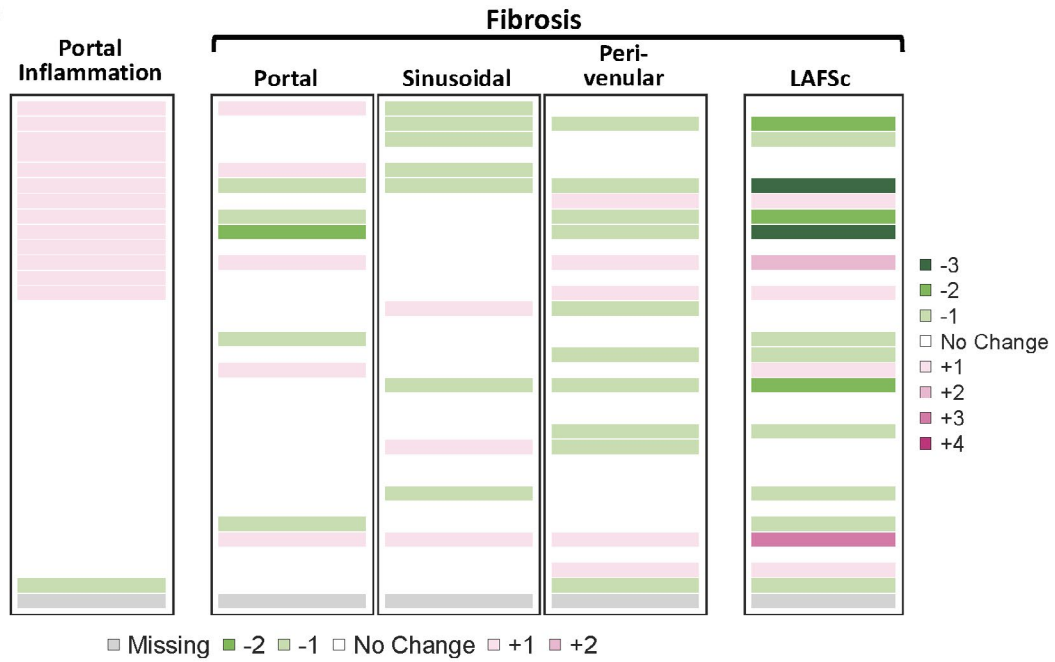
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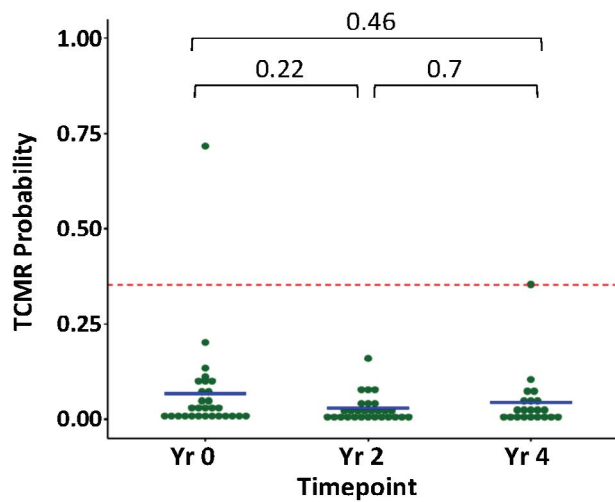
FIG. 3. Data regarding the 33 tolerant subjects. (A) Trial entry, peak, and end-of-trial ALT and GGT values (mean [IQR]) for operationally tolerant subjects; ALT and GGT levels over time are shown in Supporting Fig. S3A. (B) Change in key features of the final (year 4) compared with the baseline (year 0) biopsy for operationally tolerant subjects; changes in additional biopsy features are presented in Supporting Fig. S5A. Each row represents a single subject. In both figures, subjects are presented in the same order, sorted by change in portal inflammation and then subject identification number. To calculate change over time, absolute scores at year 0 were subtracted from scores at year 4 for the following parameters: portal inflammation, portal, sinusoidal, and perivenular fibrosis and the LAFSc. All score scales ranged from 0 to 3 except the LAFSc scale, which ranged from 0 to 9.[25] Pink indicates progression and green indicates regression; increasing intensity of either pink or green indicates larger magnitude of change. Gray indicates missing data. One operationally tolerant subject was lost to follow-up after 3 years of trial participation. (C) The TCMR probability is plotted for protocol-driven liver biopsies collected at three timepoints (year 0: trial entry; year 2: tolerance adjudication; and year 4: end of trial). The transcriptional probability of rejection was calculated based on the expression levels of genes in the TCMR signature. The dotted line corresponds to the optimal probability threshold to identify biopsies diagnostic of acute rejection. *P* values correspond to an unpaired Mann-Whitney U test.

increased tacrolimus dosing. In 5 subjects, azathioprine or mycophenolate was added. No rejection episodes were steroid-refractory. For 4 subjects with stable ALT and GGT but whose tolerance adjudication biopsy showed rejection, biochemical resolution could not be assessed. For the remaining 35, biochemical resolution, defined per protocol as ALT and GGT < 1.5-times the baseline, occurred for 34 in a median (IQR) of 13 (7.1-19.1) weeks (Fig. 4C); 1 subject did not resolve. All 35 subjects achieved ALT and GGT < 50 U/L in a median (IQR) of 5.0 (3.3-12.3) weeks.

At trial end, 36 of 39 subjects who rejected were on monotherapy. For those on tacrolimus (n = 35), the end-of-trial compared with entry tacrolimus dose was lower, the same, and higher for 46%, 26%, and 29%, respectively. Over 4 years, 50% of subjects received less total tacrolimus exposure than if they had been maintained on their entry dose (Fig. 4D).

Serial DSA testing demonstrated that 6 (15%) never had, 21 (54%) had pre-existing, and 12 (31%) developed class II DSA (Supporting Fig. S4B). Biopsies at trial start and end (4-year separation) were compared for inflammation (Supporting Fig. S5B) and fibrosis (Fig. 4E). LAFSc[25] was unchanged or minimally changed (–1 ≤ Δ ≤ 1) for 17 of 37, improved for 14 of 37 (5 with –3, and 9 with –2 change) and worsened for 6 of 37 (4 with +2, and 2 with +3 change).

Longitudinal transcriptional profiling revealed a significant increase in rejection-related transcripts at rejection diagnosis (Fig. 4F), with most (85%; 23 of 27) of the tested samples classified as rejection by the TCMR score. Following treatment, TCMR transcriptional changes resolved. Resolution was confirmed at the functional pathway level using GSEA, which showed no differences in either rejection or inflammatory-related transcriptional pathways between trial start and end (4-year separation) biopsies (Supporting Tables S5 and S6).

NONTOLERANT SUBJECTS BY HISTOLOGY

Among the 55 nontolerant subjects, 16 subjects met biochemical but not histological criteria for operational tolerance (Supporting Table S1 and Fig. 2B). The primary criterion for nontolerant designation was interface hepatitis (n = 15); bile duct damage and isolated arteriopathy without other chronic rejection features developed in 1 subject each (Fig. 5A). Baseline, peak, and final ALT and GGT are shown in Fig. 5B; 2% of ALT and 3% of GGT values collected during the 4-year trial exceeded 50 U/L (Supporting Fig. S3C).

At the discretion of the subjects' physicians, 8 reinitiated and 8 remained off immunosuppression (Supporting Information). The latter underwent an additional biopsy 6 to 20 months after tolerance adjudication, prompting immunosuppression re-initiation in 2 subjects (carets in Fig. 5A,D). The other 6 subjects remained off immunosuppression through trial end. Over 4 years, 12 of 13 received less tacrolimus than if they were maintained on their entry dose (Fig. 5C).

Serial DSA testing showed that, of the 16 nontolerant by biopsy subjects, 2 never had, 8 had pre-existing, and 6 developed DSA (Supporting Fig. S4C). Biopsies at trial start and end (4-year separation) were compared for inflammation (Supporting Fig. S5C) and fibrosis (Fig. 5D). LAFSc[25] was unchanged or minimally changed (–1 ≤ Δ ≤ 1) for 10 of 15, improved for 3 of 15 (1 with –3, and 2 with –2 change) and worsened for 2 of 15 (1 each, +2 and +4 change). The single subject whose fibrosis score increased by +4 was diagnosed with and required treatment for biliary stricture.

Comparison of biopsies performed at trial entry to tolerance adjudication (2-year separation) showed increased expression of TCMR genes (Fig. 5E and Supporting Fig. S6), which did not reach statistical

significance. Only 5 of 14 tested adjudication biopsies exhibited a TCMR probability above the suggested rejection threshold (Fig. 5E). Furthermore, whole-genome pair-wise analysis of tolerance adjudication compared with trial entry biopsies identified 149 and 107 overexpressed and underexpressed (false
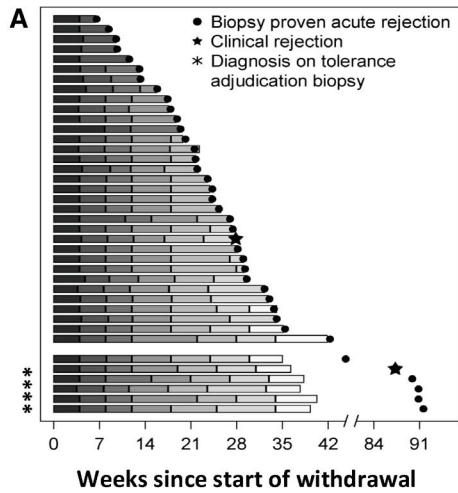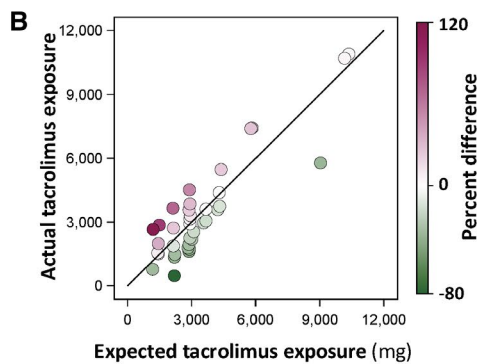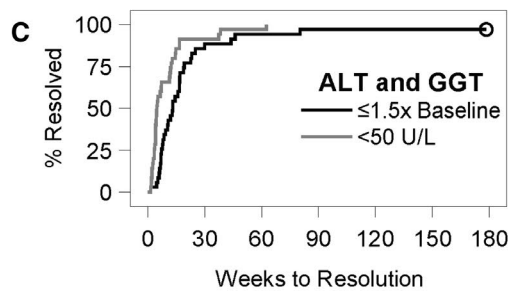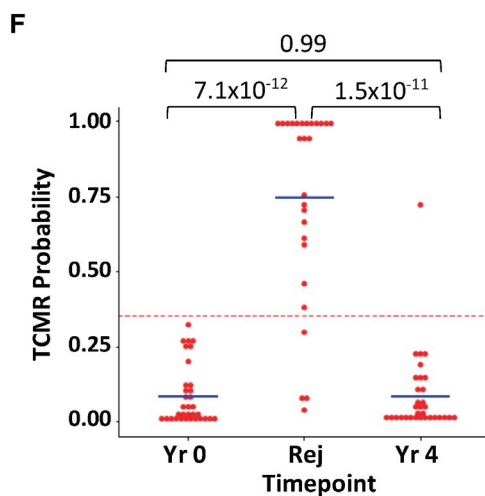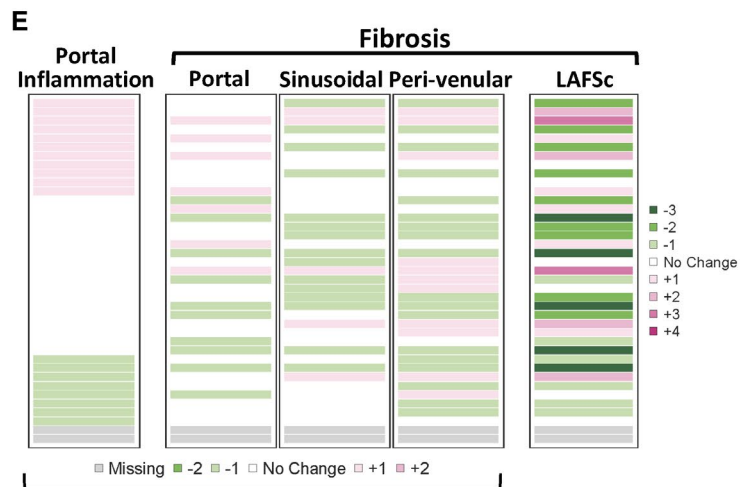
FIG. 4. Data regarding 39 nontolerant by rejection subjects. (A) Timing of rejection episodes. The time of rejection for each subject diagnosed with rejection is represented by a bar. Bar segments represent ISW steps (Supporting Fig. S2); segment length represents step duration. Time of rejection diagnosis is marked by a circle for biopsy-proven acute rejection (n = 37), based on central pathology assessment according to Banff criteria[22] or by a star for clinical rejection (n = 2), defined by the trial protocol (Supporting Information) as elevated liver tests treated with increased or re-initiation of immunosuppression but without biopsy confirmation. Rejection occurred during withdrawal for 33 subjects and after stopping immunosuppression for 6 subjects. Of these 6, 4 subjects who met the biopsy criteria for rejection despite stable relative to baseline liver tests were diagnosed with biopsy-proven acute rejection based on the tolerance adjudication biopsy and are noted with an asterisk. (B) Trial entry, peak, and end-of-trial ALT and GGT values (mean [IQR]) for nontolerant by rejection subjects; ALT and GGT levels over time are shown in Supporting Fig. S3B. (C) Time to resolution of rejection for those with elevated liver tests are shown (n = 35); 4 subjects with stable relative to baseline ALT and GGT values but biopsy-proven acute rejection at the tolerance adjudication biopsy are excluded. Two definitions for resolution are presented: (1) ALT and GGT values ≤ 1.5 times the baseline, as defined in the trial protocol (black) (one unresolved episode is censored [O] at the end of the trial); and (2) ALT and GGT values <50 units per milliliter (gray). (D) Immunosuppression exposure over the 4-year trial is shown for those who were nontolerant by rejection and on tacrolimus (n = 38); 1 subject who converted to azathioprine monotherapy was excluded. Expected exposure (x-axis) was calculated assuming that the subject was maintained on the dose at trial entry and plotted against actual exposure (y-axis). Pink circles (n = 19) identify subjects with higher actual than expected exposure, while green circles (n = 19) identify subjects with lower actual than expected exposures. Color intensity increases with larger differences between actual and expected exposures. (E) Change in key features of the final (year 4) compared with the baseline (year 0) biopsy for nontolerant by rejection subjects; changes in additional biopsy features are presented in Supporting Fig. S5B. Each row represents a single subject. In both figures, subjects are presented in the same order, sorted by change in portal inflammation and then subject identification number. To calculate change over time, absolute scores at year 0 were subtracted from scores at year 4 for the following parameters: portal inflammation, portal, sinusoidal, and perivenular fibrosis and the LAFSc. All score scales ranged from 0 to 3 except the LAFSc scale, which ranged from 0 to 9.[25] Pink indicates progression and green indicates regression; increasing intensity of either pink or green indicates larger magnitude of change. Gray indicates missing data. Two subjects did not complete trial participation; 1 withdrew assent/consent after 3 years; and the other refused to travel for the end-of-trial biopsy. (F) The TCMR probability is plotted for liver biopsies collected at three timepoints (yr 0: trial entry; rej: time of rejection diagnosis; yr 4: end-of-trial). The transcriptional probability of rejection was calculated based on the expression levels of genes in the TCMR signature. The dotted line corresponds to the optimal probability threshold to identify biopsies that are diagnostic of acute rejection. P values correspond to an unpaired Mann-Whitney U test.

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discovery rate <0.05) genes. However, none are known to be involved in allograft rejection (https://www.itntrialshare.org/iWITH_primary.url). Similarly, GSEA did not reveal enrichment in pro-inflammatory pathways (Supporting Tables S5 and S6).

Comparison of tolerance adjudication to end-of-trial biopsies (2-year separation) showed similar TCMR signature gene-expression levels (Fig. 5E), without much change for either subjects who were kept off of immunosuppression (Supporting Fig. S6A) or subjects who re-initiated immunosuppression (Supporting Fig. S6B). Comparison of trial end to start biopsies (4-year separation) demonstrated no change in either the TCMR score (Fig. 5E) or in rejection-associated molecular pathways (Supporting Tables S5 and S6).

SAFETY OF ISW

No death, graft loss, or chronic, refractory, or severe rejection occurred. Among 1,023 nonrejection adverse events, 47 were possibly or definitely related trial procedures: phlebotomy (n = 4), ISW (n = 21), or liver biopsy (n = 22; 4 serious: 1 episode each of cholangitis, bile leak, abdominal pain, and skin infection) (Supporting Tables S2 and S3). Operationally tolerant and nontolerant subjects did not differ in the frequency of serious adverse events. Immunosuppression escalation to treat rejection did not increase infectious events (data not shown). Tolerant and nontolerant subjects did not differ in calculated GFR at either trial start or end. Moreover, they did not differ in the 4-year change in calculated GFR (Supporting Table S4). Longitudinal biopsies over 4 years from subjects with *de novo* class II DSA or those with DSA at trial entry did not exhibit increased fibrosis change compared with those who remained DSA-free (Supporting Fig. S7).

HISTOLOGICAL AND IMMUNOHISTOCHEMICAL BUT NOT TRANSCRIPTIONAL PARAMETERS OF THE ENTRY BIOPSY PREDICTED ISW OUTCOME

At trial entry, no clinical or serological variables predicted operational tolerance, including age at or

time after transplant, living versus deceased donor, allele or eplet mismatch, and class II DSA status (Table 2). However, prospectively scored histological and immunohistochemical features of the eligibility biopsy were associated with operational tolerance. Operationally tolerant subjects more frequently had no portal inflammation and decreased leukocytes (CD45+), antigen-presenting cells (MHCII+),
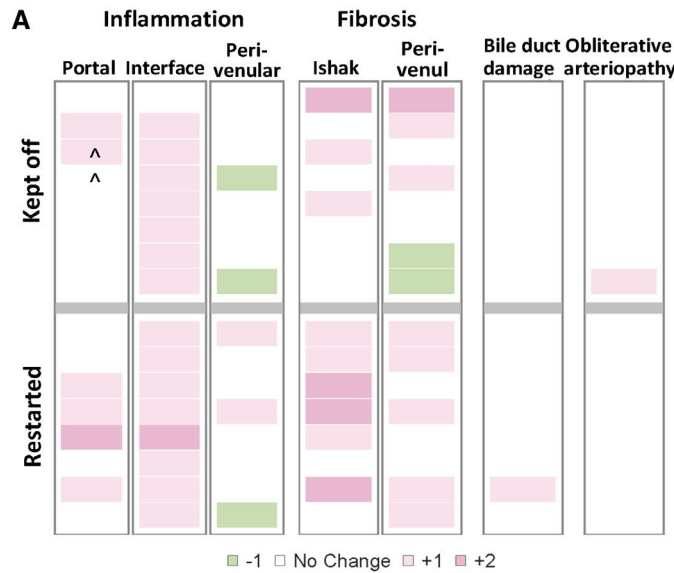
FIG. 5. Data regarding 16 non-tolerant by histology subjects. (A) Changes in the specific histological features used to adjudicate operational tolerance (Supporting Table S1); each row represents a single subject. The upper eight rows represent subjects who were kept off immunosuppression; the lower eight rows represent subjects who were restarted on immunosuppression as a result of the tolerance adjudication biopsy. Two subjects, identified by carets, were re-initiated on 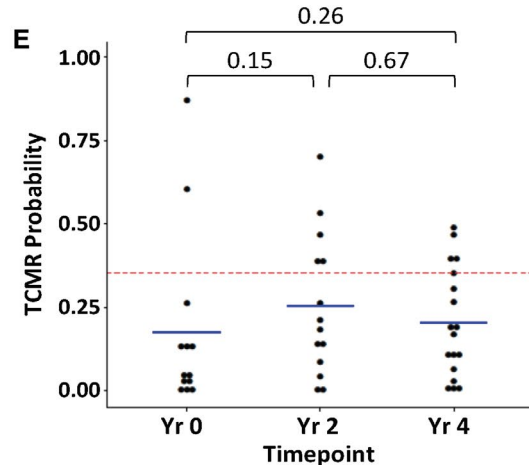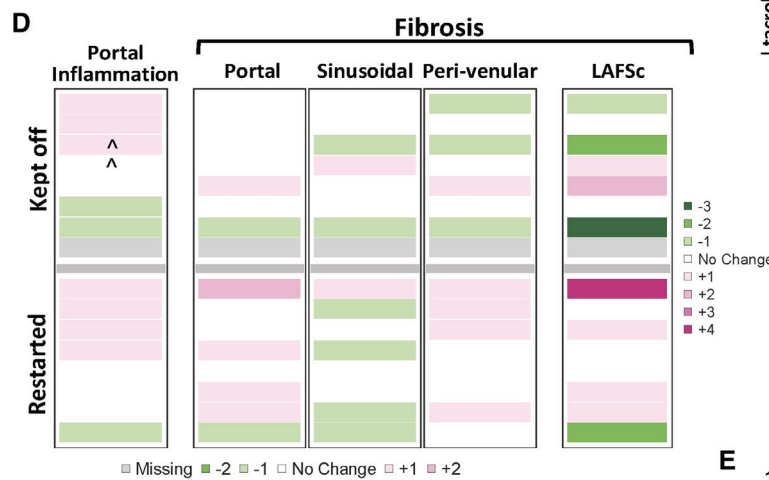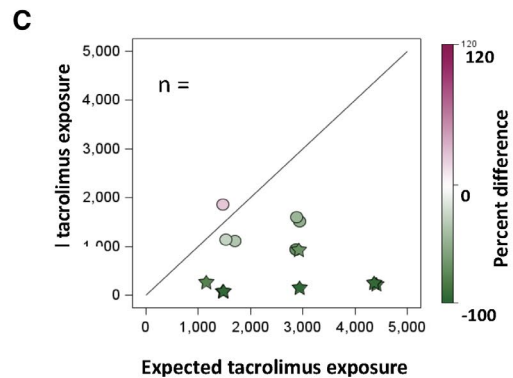immunosuppression before the end of the trial. To calculate change over time, absolute scores at year 0 were subtracted from those at year 2 for the following: three parameters of inflammation (portal, interface, and perivenular), two parameters of fibrosis (Ishak and perivenular), bile duct damage, and isolated arteriopathy. All score scales ranged from 0 to 3 except the Ishak fibrosis stage, which ranged from 0 to 6.[22] Pink indicates progression and green indicates regression; increasing intensity indicates larger magnitude of change. Gray indicates missing data. All except 1 subject failed the primary endpoint due to new onset necro-inflammatory-type interface activity with or without other disqualifying features, such as increase in fibrosis stage of 2 or new onset isolated arteriopathy. (B) Trial entry, peak, and end-of-trial ALT and GGT values (mean [IQR]) for nontolerant by histology subjects; ALT and GGT levels over time are shown in Supporting Fig. S3B. (C) Immunosuppression exposure over the 4-year trial for those who were nontolerant by histology and on tacrolimus (n = 13); 3 subjects on cyclosporine were excluded. Expected exposure (x-axis) was calculated assuming that the subject was maintained on the dose at trial entry and plotted against actual exposure (y-axis). The pink circle identifies the single subject with higher actual than expected exposure. The remaining subjects (n = 12) with lower actual than expected exposures are identified by green symbols: Green circles (n = 5) identify subjects who resumed tacrolimus, and green stars (n = 7) identify subjects who remained off immunosuppression after the tolerance adjudication biopsy (year 2). Color intensity increases with larger differences between actual and expected exposures. (D) Change in key features of the final (year 4) compared with the baseline (year 0) biopsy for nontolerant by histology subjects; changes in additional biopsy features are presented in Supporting Fig. S5C. Each row represents a single subject. The upper eight rows represent subjects who were kept off immunosuppression, whereas the lower eight rows represent subjects who were restarted on immunosuppression as a result of the biopsy. In both figures, subjects in the groups of eight are presented in the same order, sorted by change in portal inflammation and then subject identification number. Two subjects, identified by carets, were re-initiated on immunosuppression before the end of the trial (Supporting Information). To calculate change over time, absolute scores at year 0 were subtracted from scores at year 4 for the following parameters: portal inflammation, portal, sinusoidal, and perivenular fibrosis and the LAFSc. All score scales ranged from 0 to 3 except the LAFSc scale, which ranged from 0 to 9.[25] Pink indicates progression and green indicates regression; increasing intensity indicates larger magnitude of change. Gray indicates missing data. One subject did not undergo the end-of-trial biopsy secondary to a for cause biopsy within the preceding 6 months. (E) The TCMR probability for protocol-driven liver biopsies collected at three timepoints (yr 0: trial entry; yr 2: tolerance adjudication; yr 4: end of trial). The transcriptional probability of rejection was calculated based on the expression levels of genes in the TCMR signature. The dotted line corresponds to the optimal probability threshold to identify biopsies that are diagnostic of acute rejection. *P* values correspond to an unpaired Mann-Whitney U test.

leukocyte/antigen-presenting cell pairs, infiltrating monocytes/macrophages (MAC387+), and effector T cells (CD8+) (Table 2). Plotting three immunohistochemical parameters shows clustering of operationally tolerant subjects (Fig. 6A). As a predictor of operational tolerance, immunohistochemical clustering offered sensitivity and specificity of 94% and 66%, respectively, whereas portal inflammation offered sensitivity and specificity of 70% and 55%, respectively. Differential performance is illustrated when eligibility biopsies with similar portal (and lobular) inflammation grades but different immunohistochemical inflammatory loads are juxtaposed (Fig. 6B).

At trial entry, whole-genome microarray tissue transcriptional profiling did not reveal differences between tolerant and nontolerant subjects: no genes showed significant differential expression at a false discovery rate of <10% (https://www.itntrialshare.org/iWITH_primary.url). Similarly, the expression level of neither the TCMR gene set nor a five-gene tolerance classifier, derived from a European adult ISW trial,[24] predicted operational tolerance (Table 2).

DEVELOPMENT OF *DE NOVO* DSA WAS ASSOCIATED WITH ISW FAILURE

In addition to trial-entry DSA status, we analyzed DSA development during ISW (year 0 to 1). Among subjects who never exhibited DSA, 64% (14 of 22) were operationally tolerant (Fig. 6C), compared with 34% (15 of 44) for those with DSA at entry and 15% (3 of 20) for those who developed DSA. Compared with subjects who never exhibited DSA, subjects with DSA at entry and those who developed DSA were less likely to be operationally tolerant (30% [95% CI 10%-86%] and 10% [95% CI 2%-45%], respectively; Table 2).

Discussion

iWITH has shown that, among selected children with liver transplants, 37.5% were operationally tolerant. The 10% CI width on either side of this point estimate is sufficiently narrow to guide clinicians and

TABLE 2. Univariable Models of Factors Potentially Associated With Operational Tolerance*

Factor		Reference Group	OR	95% CI
Demographical and clinical	Age at transplant (per year)		0.92	0.70-1.20
	Age at trial entry (per year)		0.98	0.86-1.11
	Time since transplant (per year)		1.00	0.87-1.13
	Living donor	Deceased donor	0.88	0.35-2.17
	Whole liver	Partial liver	0.93	0.39-2.21
	Induction at time of transplant	None	1.84	0.62-5.50
	History of rejection	None	0.52	0.21-1.33
	Alanine aminotransferase (per year)		1.04	0.98-1.10
	Gamma glutamyl transferase (per year)		0.98	0.93-1.05
Serological	Class II eplet mismatch (per unit increment)		0.99	0.97-1.02
	Class II DSA present at trial entry	No Class II DSA at trial entry	0.61	0.25-1.48
	Class II present at baseline (n = 44)	No Class II DSA during year 1	**0.30**	**0.10-0.86**
	Class II DSA develops *de novo* (n = 20)	(n = 22)	**0.10**	**0.02-0.45**
Histological	Mild portal inflammation	None	**0.36**	**0.14-0.90**
	Mild lobular inflammation	None	0.29	0.06-1.42
	Mild perivenular inflammation[†]	None	NA	NA
	Ishak fibrosis stage (per unit increment)		1.45	0.69-3.06
	Portal fibrosis (per unit increment)		1.37	0.57-3.25
	Sinusoidal fibrosis (per unit increment)		0.63	0.27-1.47
	Perivenular fibrosis (per unit increment)		0.54	0.21-1.39
	Liver allograft fibrosis score (per unit increment)		0.87	0.59-1.29
Immunohisto-chemical	CD45+ cells (per unit increment; n = 70)	Portal	**0.87**	**0.79-0.96**
		Lobular	**0.96**	**0.94-0.99**
		Total	**0.97**	**0.95-0.99**
	MHC II+ cells (per unit increment; n = 70)	Portal	0.99	0.97-1.01
		Lobular	**0.98**	**0.97-1.00**
		Total	0.99	0.98-1.00
	CD45+/MHC II+ pairs (per unit increment; n = 70)	Portal	**0.78**	**0.64-0.94**
		Lobular	**0.80**	**0.70-0.92**
		Total	**0.82**	**0.74-0.92**
	MAC 387+ cells (per unit increment; n = 68)		**0.91**	**0.85-0.97**
	CD4+ cells (per unit increment; n = 72)		1.00	1.00-1.00
	CD8+ cells (per unit increment; n = 72)		0.99	0.97-1.00
Transcriptional	TCMR probability score[26] (per unit increment; n = 75)		0.10	0.002-5.23
	5-Gene tolerance biomarker[25] (per unit increment; n = 83)		0.96	0.19-4.74

Abbreviations: NA, not available; OR, odds ratio.
*Significant associations are identified in bold. Numbers are provided when data is not available for all 88 subjects.
†All subjects with mild peri-venular inflammation were in the non-tolerant group.

patients. ISW failure typically occurred after substantial dose reduction, suggesting that modest immunosuppression dose reduction might have been possible and safe for nontolerant subjects. Rejection episodes were, with few exceptions, histologically mild, biochemically reversed by treatment, and, most importantly, not associated with histological sequelae over 4 years.

The threat of life-long immunosuppression is particularly potent for children for whom liver transplantation is expected to secure decades of healthy and productive life.[10,27] Two-thirds of late mortality is directly attributed to immunosuppression complications such as infection or malignancy.[27,28] A population-based study from four Nordic countries reported that children with liver transplants experience nearly 10-fold higher

standardized incidence ratio for all cancers.[9] The cumulative incidence of cancer rises steeply in young adulthood, increasing from 2% to 6% and 22% at 10, 20, and 25 years, respectively, after transplant. The dramatic change in slope confirms suspicions that morbidity and mortality imposed on children by cumulative immunosuppression exposure manifests *decades* after transplant, a timeline well beyond that of a clinical trial. iWITH collected and analyzed longitudinal data relevant to the toxicities of immunosuppression. Successful ISW did not yield perceptible benefit; failed ISW did not result in perceptible harm.
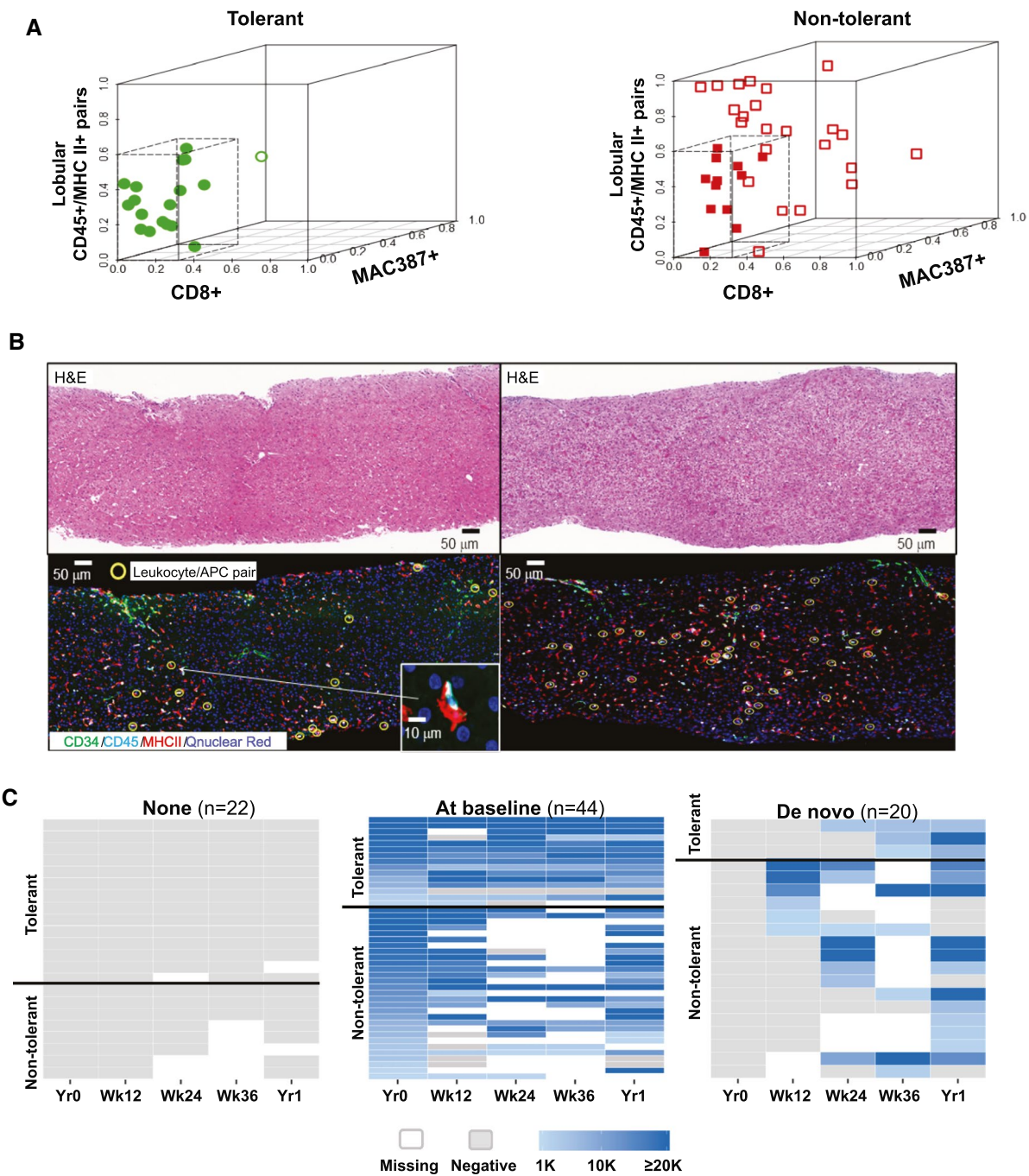
FIG. 6. Factors associated with operational tolerance. (A) Multiplex immunohistochemistry parameters of the eligibility biopsy separate tolerant from nontolerant subjects. Shown is a 3D scatter plot of tolerant (green circles; n = 18) and nontolerant (red squares; n = 35) subjects according to the number of CD8+ cells per square millimeter (T effector cells; x-axis), lobular CD45+/MHCII+ pairs per square millimeter (leukocyte/antigen-presenting cell pairs; y-axis), and MAC387+ cells per square millimeter (infiltrating macrophages; z-axis) in the eligibility biopsy. The inner cube identifies thresholds that, simultaneously, maximize the number of tolerant subjects (17 of 18; 94%) and minimize the number of nontolerant subjects (12 of 35; 34%). Subjects within the inner cube are closed symbols; those outside are open symbols. Plots only show subjects for whom values of all three parameters were available. (B) Eligibility biopsies with comparable portal and lobular inflammation grade but different immunohistochemical inflammatory loads. Hematoxylin and eosin sections are shown in the top row, while corresponding immunostained sections (green, CD34; teal, CD45; and red, MHCII) are shown in the bottom row. The left column is the eligibility biopsy from an operationally tolerant subject, while the right column is a nontolerant subject. Using a scale from 0 to 3, both biopsies were graded as 0 for both portal and lobular inflammation. Algorithmically detected pairings of leukocytes (CD45+) and antigen-presenting cells (MHCII+), shown in high magnification in the inset, are highlighted in yellow circles in the immunostained sections. The number of pairings was $7.6/mm^2$ for the tolerant (lower left) and $15.3/mm^2$ for the nontolerant (lower right) subject. (C) Class II DSA presence during ISW (year 0 to 1) is shown in three heatmaps. A minimum mean fluorescence intensity (MFI) threshold of 1,000 was used to identify a positive class II DSA. Heatmaps show the maximum MFI for class II DSA with a range from 1,000 to 20,000; white indicates missing data. Immunosuppression was reduced stepwise according to a protocol-specified algorithm (Supporting Fig. S2). Class II DSA was determined at baseline (year 0), weeks 12, 24, 36, and year 1 as long as subjects continued to withdraw immunosuppression. After diagnosis of rejection, subjects were not tested for class II DSA until the year 1 visit. Hence, nontolerant subjects have a high frequency of missing data, particularly at the week 24 and 36 timepoints. Subjects are divided into those who did not show any DSA during year 1 (n = 22), those who have detectible DSA at trial entry (n = 44), and those who develop DSA as immunosuppression is reduced (n = 20); 2 subjects with missing data at trial entry were excluded. Subjects within each group were ordered first by tolerance status, then timepoint, and finally MFI. Univariable logistic regression models were used to explore class II DSA status during ISW for association with operational tolerance. The corresponding odds ratios and 95% CIs are shown in Table 2. Abbreviations: APC, antigen-presenting cell; H&E, hematoxylin and eosin.

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iWITH targeted the prevalence of operational tolerance as the primary endpoint and was powered to provide a prevalence estimate that is useful to clinicians and patients. iWITH uniquely used an active and rigorous definition of operational tolerance, requiring no or minimal change in ALT, GGT, and liver biopsy 1 year after stopping immunosuppression, compared with trial entry 2 years earlier. Any subject failing to meet the criteria for operational tolerance was deemed nontolerant. The validity of histological assessment to adjudicate operational tolerance was corroborated by tissue transcriptional data; biopsies that met operational tolerance criteria had low TCMR probability scores, consistent with immunologic quiescence. In contrast to iWITH's strict criteria, most previous single-center studies considered subjects to be operationally tolerant if liver tests did not escalate and rejection had not occurred. An American multicenter adult trial similarly adjudicated operational tolerance according to clinical and biochemical criteria.[21] The European multicenter adult trial included histological assessment[19] but criteria were lax, stipulating absence of rejection rather than a comparison with pre-withdrawal biopsies. The high prevalence of silent allograft injury with histopathological findings nondiagnostic of acute rejection but reflective of an allo-immune response[3,29] indicates that operational tolerance must be actively determined, with inclusion of rigorous histological assessment.

Our approach to adjudicate operational tolerance led to the identification of a phenotype not previously reported. Twenty of 88 subjects were "biochemically tolerant" but "histologically nontolerant," a group almost certainly characterized by previous trials as "tolerant." Compared with trial-entry biopsies, tolerance adjudication biopsies showed distinctly but modestly increased TCMR gene expression, supporting our hypothesis that the histopathological features driving the nontolerant designation reflected mild T cell–mediated graft injury.[3,23,29] The transcriptional probability of rejection for the nontolerant by biopsy subjects strongly mirrored the levels observed for a previously described cluster of iWITH eligibility biopsies[3] that was deemed ineligible for ISW.

Although iWITH's primary objective was efficacy, many of iWITH's secondary objectives related to safety. Histologically severe, steroid-refractory, or chronic rejection along with graft loss or patient death did not occur. However, iWITH's design enabled us to address several subtle metrics of safety. First, iWITH mandated histological follow-up of those who rejected. Previous ISW trials followed those who

rejected with laboratory assessments alone, inadequate in the context of subclinical graft injury and fibrosis progression. Standard histological assessment confirmed that, at trial end, liver allografts of those who rejected did not exhibit changes over time in either inflammatory or fibrosis parameters. This interpretation is strengthened by two considerations: (1) Subjects entered iWITH with nearly pristine biopsies such that, for many histological parameters, only deterioration was possible; and (2) tissue transcriptional profiles at trial start and end were comparable. As such, we suggest that rejection precipitated by monitored ISW, promptly diagnosed and treated, did not compromise midterm allograft health. Although nearly all subjects who rejected were exposed to corticosteroids, and approximately half were exposed to additional tacrolimus, we did not identify deterioration of either renal or infectious parameters during the 4-year trial.

In addition to assessing those who rejected, iWITH rigorously assessed allograft health of the other two cohorts: operationally tolerant and nontolerant by biopsy. Reassuringly, inflammation and fibrosis parameters did not change over the 4-year trial. Histological stability was confirmed by tissue transcriptional stability in TCMR and immune activation genes.

A final critical metric of safety that we assessed was the impact of pre-existing and *de novo* DSA. The literature is replete with evidence that lowering and/or discontinuing immunosuppression after solid organ and cellular transplants engenders a humoral alloimmune response.[20,30-32] Although *de novo* DSA is associated with injury and diminished graft survival for non-liver allografts,[33-35] a negative effect on liver allograft health is less well-established.[36-41] The relative resistance and resiliency of the liver allograft to antibody-mediated injury almost certainly reflects unique, liver-specific, innate, and adaptive mechanisms that attenuate potential immunologic insults.[42] Our findings that a substantial number of subjects with class II DSA had healthy allografts at trial entry, which remained healthy after attempted ISW—regardless of outcome, provides reassurance that pre-existing or *de novo* DSA does not portend inevitable or aggressive structural deterioration.[20,38] The histological stability almost certainly reflects the robust allograft health of this cohort at trial entry. iWITH excluded patients with any significant necro-inflammatory activity

and/or fibrosis, as these damaged allografts have upregulated expression of microvascular, endothelial class II antigens, which likely increases their vulnerability to class II DSA.[37,43]

iWITH specifically aimed to identify predictors of operational tolerance. No clinical, biochemical, or serological factors at trial entry, including time after transplant, living or deceased donor, or class II DSA status, was associated with ISW outcome. Tissue transcriptional profiling and, specifically, the biomarker associated with successful ISW in 2 adult ISW trials,[24,44] did not predict iWITH outcomes, possibly suggesting different mechanisms of operational tolerance in children and adults. However, differences in trial design, inclusion/exclusion criteria, and cohort demographics preclude robust conclusions. Currently, a European multicenter trial (NCT02498977), specifically designed to prospectively assess the biomarker's diagnostic accuracy, is ongoing. Although perhaps counterintuitive, iWITH, along with other trials, have consistently shown that DSA presence, in and of itself, should not preclude ISW and does not contradict operational tolerance.[20,21,38] However, iWITH has uniquely shown that the development of DSA during ISW was associated with nontolerance. A trial of ISW involving adults early after transplant reported that *de novo* DSA predicted acute rejection.[32] These findings raise the question as to why, when immunosuppression is reduced, some subjects develop DSA while others do not. Understanding the mechanisms that determine whether DSA will emerge is necessary to inform rational approaches to therapy. We are currently using longitudinal biospecimens collected during iWITH to address this critical issue.

Although clinical, biochemical, and serological metrics did not predict operational tolerance, several histological and immunohistochemical parameters of the trial entry biopsy did. The negative effect of prospectively scored portal inflammation emerged, despite excluding subjects whose grafts showed more than minimal or focal mild abnormalities. In a pilot trial, we similarly observed an inverse association between portal inflammation and operational tolerance.[20] An adult center has also reported that increased numbers of CD8+ cells correlated with failed ISW.[45] These observations recapitulate animal data in which pre-existing

inflammation promotes liver damage by facilitating effector T-cell maturation.[46,47] Although some investigators have speculated that allograft infiltrates may be regulatory in nature, facilitating tolerance,[48,49] our data indicate otherwise. ISW appeared to activate even the few scattered inflammatory cells and precipitate rejection. Multiplex immunohistochemistry analyses of allograft biopsies yielded quantitative, objective, and relational data and suggested thresholds of inflammatory cells prohibitive of successful ISW.

Although iWITH was rigorously executed, with crisply defined entry criteria, a detailed ISW algorithm, strict guidelines mandating for-cause biopsies, and comprehensive data and specimen collection, we acknowledge limitations. First, the strict eligibility criteria and moderate sample size limits generalizability to the overall pediatric liver transplant population with respect to immunosuppression reduction. Second, the nonrandomized design and, as a result, the lack of a control group, rendered it impossible to assess whether ISW yielded benefit. The one-arm design reflects regulatory and equipoise considerations that govern clinical trial participation for children. Finally, the 4-year follow-up, typical for a clinical trial, may be insufficient to support definitive long-term conclusions regarding the efficacy, safety, or durability of operational tolerance.

In conclusion, iWITH has shown that more than one-third of selected pediatric liver-transplant recipients, clinically stable on a single immunosuppression drug with normal allograft histology, are operationally tolerant. Just as importantly, we have shown that ISW, successful or unsuccessful, was safe according to clinical, biochemical, histological, and transcriptional assessment over 4 years. Because withdrawal failure almost always occurred at one-third or less of the baseline dose, some subjects may have been receiving more pharmacologic immunosuppression than necessary. Although DSA emerging as immunosuppression is reduced portends nontolerance, it may not compromise a robustly healthy allograft. Intermittent histological evaluation is, however, critical. The development of interface activity and/or other histopathology, even with stable relative to baseline liver tests, should prompt consideration of stopping further dose reduction and perhaps even dose escalation. As such, optimal long-term immunosuppression management requires intermittent histological assessment. Longitudinal decision making embodies the enormous challenge facing physicians caring for children with liver transplants with expectations of robust graft function and overall health for many decades. The insights gained from iWITH, the culmination of effort exerted over nearly a decade, should spur future interventional trials and mechanistic investigations that address the steep challenge of improving lifelong graft and patient outcomes.

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