Clinical Pattern of Tolvaptan-Associated Liver Injury in Trial Participants With Autosomal Dominant Polycystic Kidney Disease (ADPKD): An Analysis of Pivotal Clinical Trials

David H. Alpers, MD, James H. Lewis, MD, Christine M. Hunt, MD, MPH, James W. Freston, MD, PhD, Vicente E. Torres, MD, PhD, Hui Li, MS, Wenchyi Wang, PhD, Molly E. Hoke, PhD, Sharin E. Roth, MS, Lucas Westcott-Baker, MS, Alvin Estilo, MD

PII: S0272-6386(22)00921-0

DOI: https://doi.org/10.1053/j.ajkd.2022.08.012

Reference: YAJKD 57781

To appear in: *American Journal of Kidney Diseases*

Received Date: 2 March 2022

Accepted Date: 6 August 2022

Please cite this article as: Alpers DH, Lewis JH, Hunt CM, Freston JW, Torres VE, Li H, Wang W, Hoke ME, Roth SE, Westcott-Baker L, Estilo A, Clinical Pattern of Tolvaptan-Associated Liver Injury in Trial Participants With Autosomal Dominant Polycystic Kidney Disease (ADPKD): An Analysis of Pivotal Clinical Trials, *American Journal of Kidney Diseases* (2022), doi: https://doi.org/10.1053/j.ajkd.2022.08.012.

Clinical Pattern of Tolvaptan-Associated Liver Injury in Trial Participants With Autosomal Dominant Polycystic Kidney Disease (ADPKD): An Analysis of Pivotal Clinical Trials

David H. Alpers, MD[1]; James H. Lewis, MD[2]; Christine M. Hunt, MD, MPH[3,4]; James W. Freston, MD, PhD[5]; Vicente E. Torres, MD, PhD[6]; Hui Li, MS[7]; Wenchyi Wang, PhD[7]; Molly E. Hoke, PhD[8]; Sharin E. Roth, MS[7]; Lucas Westcott-Baker, MS[7]; Alvin Estilo, MD[7]

[1]Washington University School of Medicine, St. Louis, MO; [2]Georgetown University School of Medicine, Washington, DC; [3]Duke University Medical Center, Durham, NC; [4]Durham Veterans Affairs Health Care System, Durham, NC; [5]University of Connecticut Health Center, Farmington, CT; [6]Mayo Clinic, Rochester, MN; [7]Otsuka Pharmaceutical Development & Commercialization, Rockville, MD; [8]Otsuka Pharmaceutical Development & Commercialization, Rockville, MD (formerly)

Corresponding Author
David Alpers, MD
Emeritus William B. Kountz Professor of Medicine, Division of Gastroenterology, John T. Milliken Department of Medicine, Washington University School of Medicine in St. Louis; Geriatrics, 660 S. Euclid Avenue, 8031-14-003, St. Louis, MO 63110
dalpers@wustl.edu

ABSTRACT

Rationale & Objective

Tolvaptan is associated with risk of drug-induced liver injury (DILI) when used to treat autosomal dominant polycystic kidney disease (ADPKD). After this risk was described based on the clinical trials TEMPO 3:4 and TEMPO 4:4, additional data from the REPRISE Study and a long-term extension of TEMPO 4:4, REPRISE, and other tolvaptan trials in ADPKD have become available. To further characterize the hepatic safety profile of tolvaptan, an analysis of the expanded dataset was conducted.

Study Design

Analysis of safety data from prospective clinical trials of tolvaptan.

Setting & Participants

Multicenter clinical trials including >2900 tolvaptan-treated subjects, >2300 with ≥18 months of drug exposure.

Intervention(s)

Tolvaptan administered twice daily in split-dose regimens.

Outcomes

Frequency of liver enzyme elevations detected by regular laboratory monitoring.

Results

In the placebo-controlled REPRISE trial, more tolvaptan- than placebo-treated participants (38/681 [5.6%] vs 8/685 [1.2%]) experienced alanine aminotransferase (ALT) elevations >3x upper limit of normal (ULN), similar to TEMPO 3:4 (40/957 [4.4%] vs 5/484 [1.0%]). No participant in REPRISE or the long-term extension experienced concurrent ALT >3x ULN and total bilirubin >2x ULN (Hy's Law laboratory criteria). Based on the expanded dataset, liver enzyme elevations most often occurred within 18 months after tolvaptan initiation and were less frequent thereafter. Elevations returned to normal or near normal after treatment interruption or discontinuation. Thirty-eight patients were rechallenged with tolvaptan after the initial DILI episode, with return of liver enzyme elevations in 30; 1 additional participant adapted after the initial episode, with resolution of the enzyme elevations despite continuation of tolvaptan.

Limitations

Retrospective analysis.

Conclusions

The absence of Hy's Law cases in REPRISE and the long-term extension trial support monthly liver enzyme monitoring during the first 18 months of tolvaptan exposure and every 3 months thereafter to detect and manage enzyme elevations as is recommended on the drug label.

Funding

Otsuka Pharmaceutical Development & Commercialization Inc (Rockville, MD).

Trial Registration

ClinicalTrials.gov: TEMPO 3:4 (NCT00428948); TEMPO 4:4 (NCT01214421); REPRISE (NCT02160145); long-term extension (NCT02251275).

Keywords: autosomal dominant polycystic kidney disease (ADPKD), tolvaptan, drug-induced liver injury (DILI), clinical trial, liver safety, Hy's Law, alanine aminotransferase

Plain Language Summary

In early clinical trials of tolvaptan (TEMPO 3:4 and TEMPO 4:4), liver enzyme elevations in tolvaptan-treated participants indicated risk for drug-induced liver injury (DILI). We evaluated data from two subsequent, large-scale clinical studies (REPRISE and a long-term extension of all 3 trials) conducted after monthly liver enzyme testing was required for patients enrolled in tolvaptan trials. No additional liver enzyme elevations meeting criteria for greatest risk (i.e., "Hy's Law" cases) were reported, and the less severe elevations that did occur were seen mainly during the first 18 months of treatment. These results support the conclusion that monthly liver enzyme testing of tolvaptan-treated patients during the first 18 months of therapy enabled timely detection and intervention before severe DILI could occur.

Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is the most common inherited kidney disorder and the fourth leading cause of kidney failure worldwide.[1] This condition is characterized by the development of slow-growing, fluid-filled cysts in the kidneys. Liver cysts appear in about 80% of patients by age 30 and less commonly, cysts on other organs may develop.[1] Tolvaptan, a selective arginine vasopressin receptor type 2 antagonist, has been shown to reduce the rate of growth in kidney volume and to slow decline in kidney function in subjects with ADPKD who are at risk of rapid disease progression, based on two pivotal, phase 3 trials (TEMPO 3:4 [NCT00428948] and REPRISE [NCT02160145]).[2,3] TEMPO 3:4 demonstrated significant slowing of renal function decline with tolvaptan versus placebo over 3 years in subjects with predominantly early-stage chronic kidney disease (CKD) at baseline (83% in CKD stages G1 and G2),[4] and REPRISE subsequently showed a similar result over 1 year of treatment in subjects with later-stage CKD (95% in CKD stages G3 and G4).[3]

The potential for drug-induced liver injury (DILI) is a major concern in the pharmaceutical development process. Drugs may cause liver injury in a predictable, dose-dependent manner in both preclinical models and in humans; such toxicity is termed "intrinsic DILI," with acetaminophen as the most common and well-known causative agent.[5] Complicating liver safety evaluation, rare, severe, and unpredictable DILI events known as "idiosyncratic DILI" may occur without a clear relationship to dose and only after weeks to months of treatment before detection. Several possible mechanisms are involved with idiosyncratic DILI, including induced stress placed on hepatocytes that results in neo-antigen generation, triggering an attack on hepatocytes by the adaptive immune system; disruption of hepatocyte transporters; impairment of the bile salt excretory pump; and damage to mitochondria.[5] In some cases, genetic predisposition to liver injury can be found, the result of various metabolic polymorphisms.[5] Idiosyncratic DILI can take the clinical, biochemical, and histological forms of all acute and chronic forms of liver disease, making its diagnosis challenging in the absence of a specific DILI biomarker.[6] While the incidence of idiosyncratic DILI is considered quite rare (on the order of 3 to 20 cases per 100,000

persons),[6,7] the most common causes of idiosyncratic DILI are due to well-established hepatotoxins, including antimicrobials, anticonvulsants, and various dietary and weight-loss supplements as well as herbal compounds; more than 650 drugs have the potential to cause liver injury.[8]

Idiosyncratic DILI events may not be seen during clinical trials due to their rarity or because many trials have limited numbers of patients. As a result, laboratory monitoring of liver tests to identify signs of liver injury is nearly universally performed to assess the hepatic safety risk.[9] Aminotransferase elevations >3 times the upper limit of normal (ULN) or elevations in serum alkaline phosphatase are potential early indicators of DILI.[10] The clinical observations of Dr. Hyman Zimmerman, starting in the 1960s, that drug-induced hepatocellular jaundice was associated with a poor prognosis, with a mortality rate (or need for liver transplant) due to acute liver failure in 10% or more affected patients, led the FDA to introduce stopping rules to reduce the risk of severe hepatotoxicity in clinical trials.[11] The FDA coined the term "Hy's Law," in which a drug needs to be stopped immediately whenever alanine aminotransferase (ALT) or aspartate aminotransferase (AST) rise above 3x ULN with total bilirubin exceeding 2x ULN. If these biochemical criteria are met, a causality assessment to exclude other possible causes of the liver injury must be conducted in order for Hy's Law to be invoked. Even a single verified Hy's Law case in a clinical trial can have severe regulatory consequences, including non-approval or removal of a drug from the market, based on a risk-benefit assessment.[12]

In the tolvaptan clinical trial program for ADPKD, three subjects met the criteria for Hy's Law: two from TEMPO 3:4 and one from the open-label extension TEMPO 4:4 (NCT01214421).[13] There was also a higher proportion of subjects with ALT >3x ULN in the tolvaptan arm (4.4%) relative to placebo (1.0%) in TEMPO 3:4.[14] Accordingly, upon unblinding of TEMPO 3:4, the frequency of liver chemistry monitoring was increased. In TEMPO 3:4, monitoring was performed every 4 months; in TEMPO 4:4, it started at every 6 months, but was changed to every 3 months and finally to once monthly. An independent, blinded, expert Hepatic Adjudication Committee (HAC) re-examined subject-level data from the TEMPO trials, as well as from non-ADPKD subjects who had received tolvaptan in clinical trials for other indications. A

signature pattern of susceptibility was identified in which the onset of hepatocellular injury was generally between 3 and 18 months of starting tolvaptan treatment, with injury gradually resolving over 1–4 months following drug cessation.[14] It should be noted that with rare exception, patients with ADPKD, including those with polycystic disease of the liver (seen in up to 94% of patients), have normal liver biochemical tests, including ALT, AST, alkaline phosphatase, and bilirubin.[15] As a result, ADPKD, even with polycystic disease of the liver, is not felt to be a likely cause of any liver abnormalities that develop.

Monthly liver chemistry testing was implemented in REPRISE, and no Hy's Law cases were reported.[3] Similarly, for subjects entering a long-term, open label extension trial (NCT02251275) from TEMPO 4:4, REPRISE, or other tolvaptan trials in ADPKD, testing was monthly until 18 months of tolvaptan exposure, then every 3 months.[16] The US label for JYNARQUE (tolvaptan) requires blood testing for ALT, AST, and bilirubin prior to drug initiation, at 2 and 4 weeks after initiation, monthly for 18 months, and once every 3 months thereafter.[17]

Drug-induced liver injury is a diagnosis of exclusion, and positive rechallenge data are one of the most confirmatory pieces of evidence. When rechallenge data are available, it is an important variable in causality assessment and adjudication.[18] Gathering data on negative rechallenge is not as useful for causality assessment, but is helpful in determining if a drug can safely be readministered, especially when the benefit outweighs the risk, such as with the treatment of drug-resistant tuberculosis with isoniazid.[19] However, rechallenging can potentially be dangerous and lead to severe liver injury and death and should only be performed in the absence of prior hypersensitivity or severe liver injury, and with patient consent, frequent liver enzyme testing, and the close follow-up of an experienced physician.

Since publication of the results from the HAC analysis,[14] additional safety data have become available from the REPRISE trial and the long-term, open-label extension trial. To further characterize the hepatic safety profile of tolvaptan in ADPKD, the HAC here presents an updated analysis based on the expanded dataset.

Methods

Analysis population

The safety databases reviewed were generated in clinical trials that examined the efficacy and safety of tolvaptan in ADPKD. The trials included TEMPO 3:4, TEMPO 4:4, REPRISE, and the long-term, open-label extension. Study design, enrollment, and tolvaptan exposure are discussed in detail in **Item S1**.

Adjudication of hepatic safety signals

The HAC comprised 4 expert hepatologists (DHA, JHL, JWF, CMH) who examined data from TEMPO 3:4, TEMPO 4:4, REPRISE, and the long-term extension in subjects with aminotransferases >3x ULN using the 5-point US DILI Network (DILIN) classification.[20] Per the adjudication charter, adjudication criteria included adverse events meeting any of the 5 hepatic standardized Medical Dictionary for Regulatory Activities queries or any of the following liver-related investigations: ALT >3x ULN and total bilirubin >2x ULN, AST >3x ULN and total bilirubin >2x ULN, and either ALT or AST >5x ULN (lowered to >3x ULN as a more stringent and conservative approach to understand tolvaptan DILI). A total bilirubin level >2x ULN was originally included in the adjudication criteria but later dropped, as the hepatology experts agreed that a patient with an isolated elevated serum total bilirubin in the absence of the other selection criteria was not a DILI concern.[14]

For causality assessment, the HAC utilized ‘‘expert opinion’’ [21,22] rather than a structured scoring instrument (e.g., Roussel Uclaf Causality Assessment Method).[23] The committee assessed causality of all adjudicated events based on co-morbid conditions, concomitant medication use, onset, offset, and dose relationship. Events of interest were allocated into the following 5 causality groups as defined by the DILIN, [20,21] based on the likelihood that the injury was caused by the drug: “definite” (>95%), “highly likely” (75%-95%), “probable” (50%-74%), “possible” (25%-49%), and “unlikely” (<25%).[14]

Evaluation of Drug-Induced Serious Hepatotoxicity (eDISH) assessments

Potential hepatocellular injury was visualized using the eDISH approach, which has been described previously.[14,24,25] In this graphical methodology, the log of the peak serum ALT concentration is plotted for each subject along the x-axis and the log of peak serum total bilirubin concentration is plotted along the y-axis. Each peak represents the maximal value of ALT or total bilirubin during an event and each may occur on different days during the event. Four quadrants on the eDISH plot are defined by lines at ALT 3x ULN and total bilirubin 2x ULN. The upper-right quadrant is the Hy's Law quadrant of potentially severe liver injury, although subjects may also appear there due to cholestatic liver injury. To separate out these latter confounders, US FDA guidance defines a subject in the upper-right quadrant as having severe DILI when the serum alkaline phosphatase is <2x ULN and all other possible explanations of the injury (e.g., viral hepatitis, alcohol hepatitis) have been ruled out.[11] An excess of subjects in the lower-right quadrant (i.e., the "Temple's Corollary Quadrant"[24]) for a trial drug relative to placebo also indicates a drug that may be capable of causing liver injury, even when examination of the Hy's Law quadrant is unrevealing.[11] This reflects the fact that ALT is a more sensitive indicator of hepatocellular injury than total bilirubin and that increases in ALT may occur before or without accompanying rises in total bilirubin.[26]

Rechallenge assignments

Rechallenge criteria were included in the trial protocols for REPRISE and the long-term extension for subjects who interrupted trial drug due to abnormal aminotransferase or bilirubin levels. The criteria specified that liver aminotransferase or bilirubin levels reaching or exceeding 2x ULN that had an uncertain or rapidly increasing trajectory should prompt at least temporary study drug interruption. The study drug should not be resumed until monitoring indicated that the abnormalities had resolved, were stable or were not rapidly increasing, and then only with an increased frequency of monitoring. Subjects would not typically be allowed to resume treatment with study drug if: 1) aminotransferase levels rose above 8x ULN; 2) aminotransferase levels were >5x ULN for more than 2 weeks, or; 3) there were concurrent elevations of aminotransferase >3x ULN and total bilirubin >2x ULN. Subjects with these levels of abnormality, however, could be

re-challenged if the abnormalities were adjudicated as having a <50% likelihood of being related to study drug (per DILIN probability criteria) by the independent HAC, and the investigator and medical monitor agreed to an intensive monitoring plan to mitigate risk. The subject must also have been willing to comply with these monitoring measures, be informed of the potential risks, and consent to study drug re-challenge.

There are no universally agreed threshold values to define a positive drug rechallenge, with suggested thresholds ranging from ALT 2-5x ULN with drug rechallenge.[23,27-29] In the clinical trials of tolvaptan in subjects with ADPKD, in some cases, the original ALT elevation did not reach >3x ULN and was still determined to be DILI. Therefore, in this analysis, a DILI case was deemed "positive rechallenge" when there was a doubling from baseline in ALT following tolvaptan rechallenge at any dose. A positive rechallenge was followed by a recovery to normal or near normal ALT levels while either remaining on or taken off tolvaptan. A negative rechallenge is commonly defined by ALT elevations observed temporally related to the suspect drug, followed by rechallenge with ALT levels that are unchanged or <3x ULN.[29] However, similar to what was found in DILI "positive rechallenge" with tolvaptan, a DILI was deemed "negative rechallenge" if the ALT was less than double from baseline following rechallenge. If discontinuation of tolvaptan never occurred, regardless of whether the dose remained the same or was reduced, and the ALT levels stabilized or returned to normal or near normal levels, then the DILI was deemed "adaptation." If the subject was dechallenged and not rechallenged, or if there were not sufficient data to definitively determine "positive rechallenge" or "negative rechallenge", then the subject was excluded from the rechallenge analysis.

Statistical analyses

For this assessment of safety data, summary statistics are presented. All comparisons were based on empirical results without hypothesis testing.

Compliance with ethical standards

All ADPKD clinical trials were supported by Otsuka Pharmaceutical Development & Commercialization Inc (Rockville, MD). All trials were conducted in compliance with the protocol the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use Good Clinical Practice guideline, the ethical principles originating in the Declaration of Helskinki, and all other applicable regional regulatory requirements. Each trial site was approved by its local institutional review board or ethics committee according to regional requirements. Written informed consent was obtained from all subjects prior to initiation of any procedure being performed.

Results

Subjects

Tolvaptan exposure in the 4 ADPKD trials was extensive (>2300 subjects had at least 18 months of exposure) (**Figure 1**). Nearly 800 subjects, namely, those entering the long-term extension from TEMPO 4:4, had at least 5 years of exposure, and 86 subjects had >10 years of exposure.

Hepatic events

As previously reported, more tolvaptan- than placebo-treated subjects with at least one post-baseline assessment of hepatic injury exhibited ALT >3x ULN in TEMPO 3:4 (40/957 [4.4%] vs 5/484 [1.0%], respectively).[14] Similar to TEMPO 3:4, more subjects treated with tolvaptan experienced ALT elevations >3x ULN compared to placebo subjects in REPRISE (38/681 [5.6%] vs 8/685 [1.2%]).[3] In all cases, the elevated liver enzyme levels returned to normal or near normal after the interruption or discontinuation of treatment.[3,14] Subjects with ALT >3x ULN and total bilirubin >2x ULN are depicted in eDISH plots in **Figure 2**. No subject in REPRISE or the long-term extension experienced a concurrent elevation of ALT >3x ULN and of total bilirubin >2x ULN (Hy's Law laboratory criteria).

A total of 125 events in as many tolvaptan- or placebo-treated subjects were identified that met the trigger criteria for adjudication in REPRISE (72 events) and the long-term

extension (53 events) (**Table 1**). Using data that were blinded to treatment, in REPRISE, 15 events were adjudicated as "probable," and 39 as "possible," and in the long-term extension, 2 were rated as "probable," and 24 as "possible." No events in REPRISE or the long-term extension were adjudicated as "definite" or "highly likely." **Figures S1-S42** illustrate liver enzyme levels over time in subjects with events adjudicated as having a "probable" or greater relationship to tolvaptan.

In REPRISE, ALT or AST elevations started to occur approximately 2 to 3 months after the initiation of tolvaptan and continued to be reported during the 12-month trial period.[3] The temporal pattern in REPRISE and the long-term extension (**Figure 3**) was consistent with the window of susceptibility observed in TEMPO 3:4, namely, elevations in the liver enzyme levels occurred within 18 months after the initiation of tolvaptan and were less frequent thereafter. No event observed after 18 months of tolvaptan treatment was adjudicated as having more than a "possible" relationship with tolvaptan, suggesting that monthly liver chemistry tests for the first 18 months of treatment and then every 3 months were sufficient. In **Figure 3B**, subjects entering the long-term extension from the REPRISE placebo arm were newly exposed to long-term tolvaptan after a year of receiving placebo, accounting for the higher rate of elevations. This rate was comparable to the REPRISE tolvaptan arm in **Figure 3A**, but not quite as high, and both curves plateaued at about the same time (350+ days of exposure).

In each of the trials, baseline characteristics were generally similar between tolvaptan-treated subjects who experienced hepatic events that were adjudicated as "probable," "highly likely," or "definite" and subjects who did not (**Table 2**). Subjects with events adjudicated as "probable" or higher had lower eGFR.

In subjects who provided DNA samples, no correlations between *PKD1* or *PKD2* genotype and HAC adjudication result were evident (**Table 3**). However, all subjects who were adjudicated as "probable" or higher also had a *PKD1* variant, which is associated with more rapidly progressive disease.

Thirty-nine patients were rechallenged with tolvaptan (n=38) or adapted while remaining on tolvaptan (n=1) after the initial DILI episode (**Table 4**). Following ALT recovery from the initial DILI, most of the 38 rechallenged patients (n=27; 71.1%) were rechallenged with a reduced tolvaptan dose. Among all rechallenged patients, most (n=30; 78.9%) experienced a positive rechallenge, with 23 (60.5%) exhibiting ALT recovery off tolvaptan and 7 (18.4%) experiencing ALT recovery despite continued tolvaptan administration after positive rechallenge. Eight of 38 rechallenged patients (21.1%) displayed a negative rechallenge, and an additional patient was not rechallenged but exhibited adaptation while remaining on tolvaptan. No events of liver failure were observed. Liver enzyme versus time plots shown in **Figures S43A-S43C** are representative examples of positive rechallenge when either the original ALT peak or the rechallenge ALT peak was ≤3x ULN but at least double of baseline.

The 8 patients deemed negative rechallenge all had a peak ALT ≤20x ULN, and half were rechallenged at a reduced dose (**Table 4**). **Figure S43D** depicts liver enzyme levels over time in a patient with negative rechallenge, showing a rapid rise in ALT, which fell back to baseline after dechallenge. Upon rechallenge, ALT levels remained normal to near normal for the remainder of the trial.

In another clinical pattern of tolvaptan DILI, adaptation (**Table 4**), the initial DILI episode typically results in dose reduction of 45 or 60 mg total daily dose (TDD) but can also be observed with maintaining the original dose of 120 mg TDD. One patient had 3 ALT peaks: after the first, the dose was reduced to 90 mg TDD, after the second, tolvaptan was discontinued, and after the third peak, the patient remained on tolvaptan at a TDD of 60 mg and was deemed as adaptation based on the third ALT peak (**Figure S43E**).

Discussion

Similar to TEMPO 3:4, more subjects treated with tolvaptan in REPRISE experienced ALT elevations >3x ULN compared to placebo subjects (5.6% vs 1.2%). Although comparisons to placebo are limited to the randomized trials TEMPO 3:4 and REPRISE, it is notable that no additional Hy's Law cases beyond those that occurred in the TEMPO program were

reported in either REPRISE or the long-term extension, even though the REPRISE population had more advanced ADPKD than the TEMPO 3:4 population. These findings suggest that increasing liver chemistry monitoring to monthly helped to identify hepatic enzyme elevations early and prevented severe liver injury with more rapid interruption or discontinuation of treatment. In most liver injury, ALT is more liver-specific and exhibits higher activity than AST.[30]

In REPRISE and the long-term extension, tolvaptan was found to be generally safe and well tolerated when administered twice daily in a split dose (i.e., 45/15, 60/30, 90/30 mg). Tolvaptan exposure in this population was extensive, with 1571 (87.3%) subjects having >18 months of tolvaptan treatment. Events of liver injury were reversible in REPRISE and the long-term extension, consistent with earlier experience in the TEMPO trials.

The temporal pattern of ALT elevations >3x ULN was consistent across TEMPO 3:4 and REPRISE; in both trials, the elevations occurred between 60 days and 240 days after the initiation of tolvaptan and became less frequent thereafter.[3] Additionally, as the signature of the drug has become clearer over time, a pattern has emerged whereby aminotransferases may continue to rise for up to several weeks after stopping the drug (as seen in many of the cases in **Figures S1-S42**), before returning to normal or near normal, a pattern usually indicative of an adaptive immune response.[5]

The mechanisms of DILI have been illustrated more fully in recent years.[9,31] Proposed tolvaptan-specific DILI mechanisms are described. Metabolized extensively by cytochrome P450 3A,[32] tolvaptan and its metabolites are largely eliminated through liver metabolism and fecal excretion.[33,34] Systemic tolvaptan exposure increases in patients with reduced creatinine clearance (<30 ml/min) compared to those with more preserved renal function,[35] an increase that may be associated with tolvaptan-related liver injury in susceptible patients.[36] Tolvaptan main metabolites include an oxybutyric acid metabolite (DM-4103), whose half-life in healthy subjects is >180 hours, and a hydroxybutyric acid metabolite (DM-4107).[33,34] Following one 60-mg dose of ^{14}C-tolvaptan, plasma concentrations of DM-4103 were detectable for more than 450 hours.[34,36] The long half-life

of DM-4103 may explain the observation that liver enzymes can continue to rise and stay elevated for days or weeks after stopping tolvaptan before returning to normal or near normal. This underscores the need to stop tolvaptan when liver injury is detected, as most drug-induced liver injury improves with prompt drug cessation.[9]

Tolvaptan's DM-4103 metabolite inhibits multiple human hepatic proteins involved in bile acid transport, which may negatively impact bile acid homeostasis. Compared with tolvaptan and its DM-4107 metabolite, the DM-4103 metabolite is a more potent inhibitor of the bile salt export pump (BSEP), with ~7.5- fold and ~29-fold more inhibitory potency as measured by IC_{50} than tolvaptan and DM-4107, respectively. Inhibition of BSEP by DM-4103 is best described as competitive inhibition, while tolvaptan appears to be a non-competitive inhibitor. Regarding the potential of causing an interaction with other BSEP inhibitors based on the maximal concentration observed at steady state (Cmax) versus the inhibitory potential (IC_{50}), DM-4103 was determined to be a potential inhibitor, whereas tolvaptan and DM-4107 were not of concern.[36]

Quantitative hepatic exposures of tolvaptan and its two metabolites *in vitro* were used in DILIsym™ pharmacokinetic modeling to simulate tolvaptan liver injury *in vivo*.[37] These analyses revealed that exposure to tolvaptan and the DM-4103 metabolite, combined with the inhibition of BSEP and mitochondrial respiration, could account for tolvaptan-initiated DILI.[37] DM-4107 did not affect bile acid transporters or mitochondrial function. As mitochondria provide the hepatocellular energy required by bile acid transporters for bile acid efflux, drugs impairing both bile acid transport and mitochondrial function are associated with more severe DILI, compared to those that exert only one mechanism of injury.[38] Bile acid accumulation and immune-mediated mechanisms of injury *in vivo* were not evaluated.

FDA research reports that oral medications of high lipophilicity (logP $\geq$3), daily doses of $\geq$100 mg, or which form reactive metabolites are associated with an increased risk of DILI.[39,40] Tolvaptan has high lipophilicity (logP of 4.31),[41] a total maximum daily dose of 120 mg, and no known reactive metabolites. Highly lipophilic drugs are more likely to

inhibit BSEP and mitochondrial function,[42] as noted for tolvaptan in DILIsym analyses.[37] Animal, *in vitro*, and DILIsym modeling have implicated multidrug resistance protein (MRP)2 dysfunction in polycystic kidney disease,[43] and reduced biliary efflux of DM-4103 in the susceptibility to tolvaptan-associated hepatocellular injury.[44] There is no known association between tolvaptan exposure and liver enzyme levels.[14]

With no alternative therapy licensed for the treatment of ADPKD and prior events of serious liver injury, tolvaptan rechallenge following DILI was performed infrequently, and occasionally resulted in adaptation to liver injury, although no known factors predict adaptation. When rechallenge was pursued, the tolvaptan dose was lowered in most cases (27/38, 71%), sometimes by half the initial dose (e.g., 60 mg from an initial 120 mg or a lesser amount if the original dose was 90 mg or less) (**Table 4**, **Figure S43**). The willingness to continue with tolvaptan treatment following a DILI event depends upon many factors, including the severity of the DILI episodes, the presence or absence of confounding factors (e.g., concomitant medications), and shared decision-making of the patient and/or physician to continue with therapy. For a critical medicine, suspect drug rechallenge after a DILI event may be considered when the patient is likely to derive objective benefit which exceeds the safety risk. Rechallenge should only be considered if: 1) no safer alternatives therapies are available; 2) the (potentially lower) tolvaptan dose will likely provide objective benefit; 3) the patient understands the benefits and risks, has not exhibited severe, symptomatic liver injury or hypersensitivity (fever, rash, eosinophilia), will report hepatitis symptoms (nausea, anorexia, fatigue, abdominal pain), and adhere to follow-up.[29] As seen in the presented cases, tolvaptan rechallenge was associated with reasonable safety when accompanied by more frequent enzyme testing and clinical follow-up after an informed decision in the absence of prior hypersensitivity or severe injury. Per FDA guidance, rechallenge should in general be performed only in patients who experienced mild aminotransferase elevations, and be avoided in those who experienced elevations >5x ULN.[11] The US label for tolvaptan specifies that the drug may be re-initiated with increased frequency of monitoring as long as ALT and AST remain <3x ULN. However, per US label, tolvaptan should not be restarted in patients who experience signs or symptoms consistent with hepatic injury or whose ALT or AST ever exceeds 3x ULN during

treatment with tolvaptan, unless there is another explanation for liver injury and the injury has resolved.[17]

The pattern of positive drug rechallenge was the most commonly observed, i.e., the initial DILI episode resulted in tolvaptan cessation, and a rechallenge with a lower tolvaptan dose was followed by a rapid elevation of ALT, and a later fall to normal or near normal levels with dechallenge. While recovery from the initial DILI episode could take 2-3 or more months, the tolvaptan rechallenge usually resolved within a month.

Importantly, a negative dechallenge does not exonerate the drug, as adaptation may have been responsible. While this pattern is usually referred to as a negative rechallenge, it may also represent adaptation or may be due to an alternative cause of the original liver injury.

Hepatic adaptation following an episode of DILI is a well-described phenomenon seen in ~20% of patients, although the mechanisms of adaptation are unknown.[45] Normalization of ALT values may be related to a dose effect or adaptation to drug injury or an alternative cause of the initial injury (e.g., biliary event) which is no longer present. While no dose-response relationship was evident in an earlier evaluation of tolvaptan-induced DILI cases,[14] lowering the tolvaptan dose led to enzyme normalization in 10 out of 27 DILI cases (**Table 4**). Therefore, a dose-relationship at the population level is still uncertain.

Predictive functional or genetic markers of tolvaptan-induced liver injury are needed. Research is ongoing to elucidate the mechanism of tolvaptan-induced liver injury and to identify safety biomarker(s) that can be used in the management of tolvaptan treatment. The availability of archived, broadly consented biospecimens has been instrumental in these research efforts, highlighting the importance of biobanking in clinical trials. [46]

A strength of this analysis is its inclusion of over 2000 subjects with ADPKD who received tolvaptan for 18 months or longer and were followed with more frequent liver chemistry monitoring over time to detect potential liver injury. An additional strength is the inclusion of ancillary studies describing the dual inhibition of bile acid efflux and mitochondria as a mechanism of tolvaptan-and its long-lived DM-4103 metabolite-related liver injury. This

analysis is limited by its retrospective methodology and the lack of mechanistic models that represent immune-mediated injury. Insufficient information to adjudicate causality precluded the evaluation of some liver injury events. In other cases, the available information was sufficient to adjudicate, but the level of proof required for a determination of a "probable" or greater relationship to drug (typically a positive re-challenge or data ruling out other causes of liver disease) was unavailable, and so an adjudication of "possible" DILI was made, as is standard for the expert opinion process. Additionally, DNA samples were available for only a subset of the trial subjects, restricting the dataset for genetic analysis.

Liver safety results from REPRISE and the long-term extension are consistent with those of TEMPO 3:4. Reversible elevations in ALT/AST developed with a latency period usually between 3 to 18 months. The results support the conclusion that monthly hepatic monitoring during the first 18 months of tolvaptan exposure and every 3 months thereafter, as required by the prescribing information in countries where tolvaptan has been approved to treat ADPKD, enables the effective early detection of aminotransferase elevations that could result in prompt action to cease tolvaptan therapy.

Supplementary Material

Item S1: Description of clinical trials included in the analysis

Table S1: Clinical trial enrollment of subjects prior to entry into TEMPO 4:4 and the long-term extension

Figures S1-S42: Liver enzyme levels over time in subjects with events adjudicated as having a "probable" or greater relationship to tolvaptan

Figure S43A-S43E: Liver chemistries over time plots of patients with ADPKD who were rechallenged after an initial ALT elevation due to tolvaptan-induced liver injury

Article Information

Authors' Contributions

Data acquisition: MEH, SER, AE

Data analysis/interpretation: DHA, JHL, CMH, JWF, VET, HL, WW, MEH, SER, LWB, AE

Statistical analysis: HL, WW

Each author contributed important intellectual content during manuscript drafting or revision and agrees to be personally accountable for the individual's own contributions and to ensure that questions pertaining to the accuracy or integrity of any portion of the work, even one in which the author was not directly involved, are appropriately investigated and resolved, including with documentation in the literature if appropriate.

Support

The trials analyzed here and retrospective safety analysis were supported by Otsuka Pharmaceutical Development & Commercialization (Rockville, MD, USA). The sponsor participated in the design of the study; collection, analysis, and interpretation of data; writing the report; and the decision to submit the report for publication. Alice Walton, PhD, and Andrew Horgan, PhD, of BioScience Communications, Inc (New York, NY, USA), assisted in drafting the manuscript, activity that was also funded by Otsuka. Each author participated in decisions on which data and outcomes were to be reported, and in the interpretation of the data and the conclusions to be drawn.

Financial Disclosure

David Alpers is a paid consultant to Otsuka and a member of the Hepatic Adjudication Committee (HAC) for tolvaptan, which is sponsored by Otsuka. Dr. Alpers chairs the HAC for the phase 3 ALERT trial for lixivaptan, which is sponsored by Palladio Biosciences.

James H. Lewis is a paid consultant to Otsuka and a member of the HAC for tolvaptan, which is sponsored by Otsuka. Dr. Lewis is a member of the HAC for the phase 3 ALERT trial for lixivaptan, which is sponsored by Palladio Biosciences.

Christine M. Hunt is a paid consultant to Otsuka and a member of the HAC for tolvaptan, which is sponsored by Otsuka. Dr. Hunt is a member of the HAC for the phase 3 ALERT trial for lixivaptan, which is sponsored by Palladio Biosciences, and reports consulting for Galmed and Akebia Therapeutics Inc.

James W. Freston is a member of the HAC, which is sponsored by Otsuka. Dr. Freston is a member of the HAC for the phase 3 ALERT trial for lixivaptan, which is sponsored by Palladio Biosciences.

Vicente E. Torres reports grants from Otsuka Pharmaceutical, Palladio Biosciences, Sanofi Genzyme, Blueprint Medicines, and Mironid, outside the submitted work.

Wenchyi Wang, Hui Li, Sharin E. Roth, Lucas Westcott-Baker, and Alvin Estilo are employees of Otsuka. Molly E. Hoke is a former employee of Otsuka.

Acknowledgements

The authors would like to thank Peter C. Harris, PhD, of Mayo Clinic (Rochester, MN) for generating the *PKD1/PKD2* genotyping data.

Data Sharing

To submit inquiries related to Otsuka clinical research, or to request access to individual participant data (IPD) associated with any Otsuka clinical trial, please visit https://clinical-trials.otsuka.com/. For all approved IPD access requests, Otsuka will share anonymized IPD on a remotely accessible data sharing platform.

Peer Review: Received March 2, 2022. Evaluated by 2 external peer reviewers and a methods reviewer, with direct editorial input from an Associate Editor and the Editor-in-Chief. Accepted in revised form August 6, 2022.

References

1. Chebib FT, Torres VE. Autosomal dominant polycystic kidney disease: core curriculum 2016. *Am J Kidney Dis*. 2016;67(5):792–810. doi: 10.1053/j.ajkd.2015.07.037
2. Torres VE, Chapman AB, Devuyst O, et. al.; on behalf of the TEMPO 3:4 Trial Investigators. Tolvaptan in patients with autosomal dominant polycystic kidney disease. *N Engl J Med.* 2012;367(25):2407-2418. doi: 10.1056/NEJMoa1205511
3. Torres VE, Chapman AB, Devuyst O, et al.; on behalf of the REPRISE Trial Investigators. Tolvaptan in later-stage autosomal dominant polycystic kidney disease. *N Engl J Med.* 2017;377(20):1930-1942. doi: 10.1056/NEJMoa1710030

4. Torres VE, Higashihara E, Devuyst O, et al; TEMPO 3:4 Trial Investigators. Effect of tolvaptan in autosomal dominant polycystic kidney disease by CKD stage: results from the TEMPO 3:4 trial. *Clin J Am Soc Nephrol*. 2016;11(5):803-811. doi: 10.2215/CJN.06300615
5. Mosedale M, Watkins PB. Drug-induced liver injury: advances in mechanistic understanding that will inform risk management. *Clin Pharmacol Ther*. 2017;101(4):469-480. doi: 10.1002/cpt.564
6. Leise MD, Poterucha JJ, Talwalkar JA. Drug-induced liver injury. *Mayo Clin Proc*. 2014;89(1):95-106. doi: 10.1016/j.mayocp.2013.09.016
7. Bell LN, Chalasani N. Epidemiology of idiosyncratic drug-induced liver injury. *Semin Liver Dis*. 2009;29(4):337-47. doi: 10.1055/s-0029-1240002
8. LiverTox: clinical and research information on drug-induced liver injury. Bethesda, MD: National Institute of Diabetes and Digestive and Kidney Diseases; 2022. https:// https://www.ncbi.nlm.nih.gov/books/NBK547852/. Accessed 18 April 2022.
9. European Association for the Study of the Liver. EASL Clinical Practice Guidelines: Drug-induced liver injury. *J Hepatol*. 2019;70(6):1222-1261. doi: 10.1016/j.jhep.2019.02.014
10. Moylan CA, Suzuki A, Papay JI, Yuen NA, Ames M, Hunt CM. A pre-marketing ALT signal predicts post-marketing liver safety. *Regul Toxicol Pharmacol*. 2012;63(3):433-439. doi: 10.1016/j.yrtph.2012.05.016
11. United States Food and Drug Administration. Guidance for industry, drug-induced liver injury: premarketing clinical evaluation. 2009. https://www.fda.gov/media/116737/download. Accessed 14 November 2021.
12. Senior JR. How can 'Hy's law' help the clinician? *Pharmacoepidemiol Drug Saf*. 2006;15(4):235-239. doi: 10.1002/pds.1210
13. Torres VE, Chapman AB, Devuyst O, et al.; on behalf of the TEMPO 4:4 Trial Investigators. Multicenter, open-label, extension trial to evaluate the long-term efficacy and safety of early versus delayed treatment with tolvaptan in autosomal dominant polycystic kidney disease: the TEMPO 4:4 Trial. *Nephrol Dial Transplant*. 2018;33(3):477-489. doi: 10.1093/ndt/gfx043
14. Watkins PB, Lewis JH, Kaplowitz NE, et al. Clinical pattern of tolvaptan-associated liver injury in subjects with autosomal dominant polycystic kidney disease: analysis of clinical trials database. *Drug Saf.* 2015;38(11):1103-1113. doi: 10.1007/s40264-015-0327-3
15. Zhang ZY, Wang ZM, Huang Y. Polycystic liver disease: Classification, diagnosis, treatment process, and clinical management. *World J Hepatol*. 2020;12(3):72-83. doi: 10.4254/wjh.v12.i3.72

16. Torres VE, Chapman AB, Devuyst O, et al. Multicenter study of long-term safety of tolvaptan in later-stage autosomal dominant polycystic kidney disease. *Clin J Am Soc Nephrol*. 2020;16(1):48-58. doi: 10.2215/CJN.10250620
17. JYNARQUE [US package insert]. Rockville, MD: Otsuka America Pharmaceutical, Inc.; 2020. https://www.otsuka-us.com/sites/g/files/qhldwo4671/files/media/static/JYNARQUE-PI.pdf. Accessed 14 November 2021.
18. Senior JR. Can rechallenge be done safely after mild or moderate drug-induced liver injury? *Hepatology*. 2016;63(3):691-693. doi: 10.1002/hep.28353
19. Mitchell JR, Long MW, Thorgeirsson UP, Jollow DJ. Acetylation rates and monthly liver function tests during one year of isoniazid preventive therapy. *Chest*. 1975;68(2):181-190. doi: 10.1378/chest.68.2.181
20. Fontana RJ, Watkins PB, Bonkovsky HL, et al. Drug-Induced Liver Injury Network (DILIN) prospective study: rationale, design and conduct. *Drug Saf.* 2009;32(1):55-68. doi: 10.2165/00002018-200932010-00005
21. Rockey DC, Seeff LB, Rochon J, et al. Causality assessment in drug-induced liver injury using a structured expert opinion process: comparison to the Roussel-Uclaf causality assessment method. *Hepatology*. 2010;51(6):2117-2126. doi: 10.1002/hep.23577
22. Regev A, Seeff L, Merz M, et al. Causality assessment for suspected DILI during clinical phases of drug development. *Drug Saf*. 2014;37(Suppl 1):S47–56. doi: 10.1007/s40264-014-0185-4
23. Danan G, Benichou C. Causality assessment of adverse reactions to drugs--I. A novel method based on the conclusions of international consensus meetings: application to drug-induced liver injuries. *J Clin Epidemiol*. 1993;46(11):1323-1330. doi: 10.1016/0895-4356(93)90101-6
24. Watkins PB, Desai M, Berkowitz SD, et al. Evaluation of drug-induced serious hepatotoxicity (eDISH): application of this data organization approach to phase III clinical trials of rivaroxaban after total hip or knee replacement surgery. *Drug Saf*. 2011;34(3):243–252. doi: 10.2165/11586600-000000000-00000
25. Senior JR. Evolution of the Food and Drug Administration approach to liver safety assessment for new drugs: current status and challenges. *Drug Saf*. 2014;37(Suppl 1):S9-17. doi: 10.1007/s40264-014-0182-7
26. Senior JR. Monitoring for hepatotoxicity: what is the predictive value of liver ‘‘function’’ tests? *Clin Pharmacol Ther.* 2009;85(3):331–334. doi: 10.1038/clpt.2008.262

27. Fontana RJ, Seeff LB, Andrade RJ, et al. Standardization of nomenclature and causality assessment in drug-induced liver injury: summary of a clinical research workshop. *Hepatology*. 2010;52(2):730-742. doi: 10.1002/hep.23696
28. Aithal GP, Watkins PB, Andrade RJ, et al. Case definition and phenotype standardization in drug-induced liver injury. *Clin Pharmacol Ther*. 2011;89(6):806-815. doi: 10.1038/clpt.2011.58
29. Hunt CM, Papay JI, Stanulovic V, Regev A. Drug rechallenge following drug-induced liver injury. *Hepatology*. 2017;66(2):646-654. doi: 10.1002/hep.29152
30. Dufour DR, Lott JA, Nolte FS, Gretch DR, Koff RS, Seeff LB. Diagnosis and monitoring of hepatic injury. I. Performance characteristics of laboratory tests. *Clin Chem*. 2000;46(12):2027-2049.
31. Bellos I. Safety profile of tolvaptan in the treatment of autosomal dominant polycystic kidney disease. *Ther Clin Risk Manag*. 2021;17:649-656. doi: 10.2147/TCRM.S286952
32. Shoaf SE, Bricmont P, Mallikaarjun S. Effects of CYP3A4 inhibition and induction on the pharmacokinetics and pharmacodynamics of tolvaptan, a non-peptide AVP antagonist in healthy subjects. *Br J Clin Pharmacol*. 2012;73:579-587. doi: 10.1111/j.1365-2125.2011.04114.x
33. Sorbera LA, Castaner J, Bayes M, Silvestre J. Tolvaptan: treatment for heart failure vasopressin V2 antagonist. *Drugs Future*. 2002;27:350–357.
34. Tammara BK, Sekar KS, Brumer SL. The disposition of a single dose of 14C OPC-41061 in healthy male volunteers. 1999 American Association of Pharmaceutical Scientists Annual Meeting and Exposition. Abstract 2025. New Orleans, LA.
35. Shoaf SE, Bricmont P, Mallikaarjun S. Pharmacokinetics and pharmacodynamics of oral tolvaptan in patients with varying degrees of renal function. *Kidney Int*. 2014;85:953-961. doi: 10.1038/ki.2013.350
36. Slizgi JR, Lu Y, Brouwer KR, et al. Inhibition of human hepatic bile acid transporters by tolvaptan and metabolites: contributing factors to drug-induced liver injury? *Toxicol Sci*. 2016;149(1):237–250. doi: 10.1093/toxsci/kfv231
37. Woodhead JL, Brock WJ, Roth SE, et al. Application of a mechanistic model to evaluate putative mechanisms of tolvaptan drug-induced liver injury and identify patient susceptibility factors. *Toxicol Sci*. 2017;155(1):61-74. doi: 10.1093/toxsci/kfw193
38. Aleo MD, Luo Y, Swiss R, Bonin PD, Potter DM, Will Y. Human drug-induced liver injury severity is highly associated with dual inhibition of liver mitochondrial function and bile salt export pump. *Hepatology*. 2014;60(3):1015-1022. doi: 10.1002/hep.27206

39. Chen M, Borlak J, Tong W. High lipophilicity and high daily dose of oral medications are associated with significant risk for drug-induced liver injury. *Hepatology* 2013;58:388–396. doi: 10.1002/hep.26208
40. McEuen K, Borlak J, Tong W, Chen M. Associations of drug lipophilicity and extent of metabolism with drug-induced liver injury. *Int J Mol Sci*. 2017;18(7):1335. doi: 10.3390/ijms18071335
41. Tolvaptan. DrugCentral 2021. https://drugcentral.org/drugcard/4110. Accessed 14 November 2021.
42. Chen M, Borlak J, Tong W. A model to predict severity of drug-induced liver injury in humans. *Hepatology*. 2016;64(3):931-940. doi: 10.1002/hep.28678
43. Bezençon J, Beaudoin JJ, Ito K, et al. Altered expression and function of hepatic transporters in a rodent model of polycystic kidney disease. *Drug Metab Dispos*. 2019;47:899-906. doi: 10.1124/dmd.119.086785
44. Beaudoin JJ, Brock WJ, Watkins PB, Brouwer KLR. Quantitative systems toxicology modeling predicts that reduced biliary efflux contributes to tolvaptan hepatotoxicity. *Clin Pharmacol Ther*. 2021;109(2):433-442. doi: 10.1002/cpt.2007
45. Dara L, Liu ZX, Kaplowitz N. Mechanisms of adaptation and progression in idiosyncratic drug induced liver injury, clinical implications. *Liver Int*. 2016;36(2):158-165. doi: 10.1111/liv.12988
46. Roth SE, Avigan MI, Bourdet D, et al. Next-generation DILI biomarkers: prioritization of biomarkers for qualification and best practices for biospecimen collection in drug development. *Clin Pharmacol Ther*. 2020;107(2):333-346. doi: 10.1002/cpt.1571

Table 1. Adjudication results of hepatic events, includes tolvaptan- and placebo-treated subjects

	Clinical Trial	TEMPO 3:4	TEMPO 4:4	REPRISE	Long-term extension	Total
Tolvaptan and Placebo Combined	**# Subjects Adjudicated**	46	39	72	53	210
	Definite (>95%)	0	0	0	0	0
	Highly Likely (75-95%)	1	3	0	0	4
	Probable (50-74%)	17	6	15*	2	40
	Possible (25-49%)	11	11	39	24	85
	Unlikely (<25%)	17	19	18	23	77
	Insufficient Data	0	0	0	4	4
Tolvaptan	**# Subjects Adjudicated**	35	39	62	53	189
	Definite (>95%)	0	0	0	0	0
	Highly Likely (75-95%)	1	3	0	0	4
	Probable (50-74%)	16	6	15	2	39
	Possible (25-49%)	9	11	33	24	77
	Unlikely (<25%)	9	19	14	23	65
	Insufficient Data	0	0	0	4	4
Placebo	**# Subjects Adjudicated**	11	0	10	0	21
	Definite (>95%)	0	0	0	0	0
	Highly Likely (75-95%)	0	0	0	0	0
	Probable (50-74%)	1	0	0	0	1
	Possible (25-49%)	2	0	6	0	8
	Unlikely (<25%)	8	0	4	0	12
	Insufficient Data	0	0	0	0	0

*Includes 11 events previously reported[3] and 4 additional events.

Table 2. Baseline characteristics of tolvaptan-treated subjects with and without hepatic event causality adjudicated as "probable" or higher (probable+)*

Parameter	TEMPO 3:4 Probable+	TEMPO 3:4 Other	TEMPO 4:4 Probable+	TEMPO 4:4 Other	REPRISE Probable+	REPRISE Other	Long-term Extension Probable+	Long-term Extension Other
Number of subjects	17**	944	9	1074	15	668	2	1801
Age (yrs)								
Mean (SD)	41.1 (6.1)	38.5 (7.1)	42.3 (3.3)	41.7 (7.8)	46.3 (8.8)	47.3 (8.2)	54.5 (12.0)	47.4 (8.1)
Sex								
Male	5 (29.4)	490 (51.9)	3 (33.3)	564 (52.5)	7 (46.7)	340 (50.9)	1 (50.0)	928 (51.5)
Female	12 (70.6)	454 (48.1)	6 (66.7)	510 (47.5)	8 (53.3)	328 (49.1)	1 (50.0)	873 (48.5)
Height (cm)								
Mean (SD)	168.5 (13.0)	173.6 (10.3)	176.6 (7.0)	174.6 (10.6)	174.5 (9.8)	173.7 (10.4)	170.5 (3.5)	173.8 (10.6)
Weight (kg)								
Mean (SD)	73.6 (19.7)	79.6 (18.2)	81.7 (14.1)	82.1 (18.3)	81.7 (15.5)	84.7 (19.9)	71.1 (7.0)	84.0 (19.4)
Race/Ethnicity								
White	12 (70.6)	798 (84.5)	9 (100.0)	1024 (95.3)	13 (86.7)	613 (91.8)	2 (100.0)	1687 (93.7)
Black	0	16 (1.7)	0	16 (1.5)	2 (13.3)	23 (3.4)	0	48 (2.7)
Hispanic***	0	13 (1.4)	0	1 (0.1)	1 (6.7)	43 (6.4)	1 (50.0)	101 (5.6)
Asian	5 (29.4)	116 (12.3)	0	10 (0.9)	0	22 (3.3)	0	42 (2.3)
Other	0	1 (0.1)	0	23 (2.1)	0	10 (1.5)	0	24 (1.3)
eGFR (ml/min/1.73 m^2)								
Mean (SD)	77.2 (21.5)	81.4 (21.0)	52.4 (24.6)	70.0 (25.2)	39.3 (11.6)	40.7 (10.9)	36.7 (7.9)	47.1 (20.7)

*"Probable," "highly likely," or "definite"; **1 subject in TEMPO 3:4 who was adjudicated as "probable" or higher was in the placebo group, hence 18 subjects are shown as "probable" or higher in Table 2 and 17 are shown here; ***As Hispanic ethnicity did not exclude race categories, percentages for race/ethnicity may add up to >100%.
eGFR, glomerular filtration rate estimated using the Chronic Kidney Disease Epidemiology Collaboration equation.

Table 3. *PKD1* and *PKD2* genotype for adjudicated tolvaptan-treated subjects with DNA samples

TEMPO 3:4 and TEMPO 4:4								
	Adjudication Categorization							
	Definite (n=0)	**Highly Likely (n=3)**	**Probable (n=7)**	**Possible (n=7)**	**Unlikely (n=11)**	**Insufficient Data (n=0)**	**Total (n=28)**	**All Tolvaptan Subjects With Mutation (N=982)***
PKD1 Truncating		2 (66.7%)	6 (85.7%)	4 (57.1%)	8 (72.7%)		20 (71.4%)	586 (59.7%)
PKD1 Non-truncating		1 (33.3%)	1 (14.3%)	2 (28.6%)	2 (18.2%)		6 (21.4%)	263 (26.8%)
PKD2 Truncating								87 (8.9%)
PKD2 Non-truncating				1 (14.3%)	1 (9.1%)		2 (7.1%)	19 (1.9%)
No Mutation Detected								27 (2.7%)
REPRISE and Long-Term Extension								
	Adjudication Categorization							
	Definite (n=0)	**Highly Likely (n=0)**	**Probable (n=16)**	**Possible (n=47)**	**Unlikely (n=25)**	**Insufficient Data (n=2)**	**Total (n=90)**	**All Tolvaptan Subjects With Mutation (N=1127)**
PKD1 Truncating			10 (62.5%)	21 (44.7%)	15 (60.0%)	1 (50.0%)	47 (52.2%)	634 (56.3%)
PKD1 Non-truncating			6 (37.5%)	20 (42.6%)	7 (28.0%)		33 (36.7%)	302 (26.8%)
PKD2 Truncating				5 (10.6%)	3 (12.0%)	1 (50.0%)	9 (10.0%)	125 (11.1%)
PKD2 Non-truncating								12 (1.1%)
HNF1B Truncating								1 (0.1%)
No Mutation Detected				1 (2.1%)			1 (1.1%)	53 (4.7%)

*Total excludes 4 subjects who withdrew consent and 2 for whom genotype could not be determined.

Subjects with >1 event are included in the table once under the highest adjudicated causality (i.e., “probable” > “possible” > “unlikely” > “insufficient data”). As all subjects in REPRISE underwent a tolvaptan run-in period, the table includes subjects who were randomized to placebo and had an event during the tolvaptan run-in, or who were not randomized due to an event during tolvaptan run-in.

Table 4. Comparison of baseline characteristics and first elevated ALT events between patients with negative rechallenge, positive rechallenge, or adaptation

Characteristic	Positive rechallenge[a] with recovery off drug n=23 (59.0%)	Positive rechallenge[a] with recovery on drug n=7 (17.9%)	Negative rechallenge[b] n=8 (20.5%)	Adaptation[c] n=1 (2.6%)
Age, years				
Mean (SD)	48 (8)	47 (6)	49 (11)	37 (-)
Median (min–max)	48 (33–63)	46 (39–57)	53 (33–63)	37 (37–37)
Female, n (%)	14 (60.9)	3 (42.9)	3 (37.5)	0
Race, n (%)				
Asian	1 (4.3)	0	0	0
White	22 (95.7)	7 (100.0)	8 (100.0)	1 (100.0)
Peak category of the first elevated ALT events, n (%)				
ALT ≤3x ULN	7 (30.4)	4 (57.1)	2 (25.0)	0
ALT >3–5x ULN	8 (34.8)	2 (28.6)	3 (37.5)	0
ALT >5–8x ULN	4 (17.4)	1 (14.3)	1 (12.5)	1 (100.0)
ALT >8–20x ULN	3 (13.0)	0	2 (25.0)	0
ALT >20x ULN	1 (4.3)	0	0	0
Time to onset of the first elevated ALT events, days				
Median (min–max)	214.0 (63–1391)	239.0 (118–307)	611.0 (185–1901)	8.0 (8–8)
Time from onset to recovery of the first elevated ALT events, days				
Median (min–max)	91.0 (17–482)	36.0 (30–112)	60.5 (15–110)	117.0 (117–117)
Rechallenged with reduced dose, n (%)				
Yes	17 (73.9)	6 (85.7)	4 (50.0)	n/a
No	6 (26.1)	1 (14.3)	4 (50.0)	n/a
Time to recurrence of elevated ALT following rechallenge, days				
Median (5th–95th percentile)	55.0 (14–114)	89.0 (27–147)	n/a	n/a

[a]Doubling in ALT following tolvaptan rechallenge; [b]ALT less than doubled following tolvaptan rechallenge; [c]If discontinuation of tolvaptan never occurred, regardless of whether the dose remained the same or was reduced, and ALT returned to normal or near normal levels.
ALT, alanine aminotransferase; SD, standard deviation; ULN, upper limit of normal.

Figure Titles and Legends

Figure 1. Duration of exposure to tolvaptan in the four phase 3 ADPKD Trials (TEMPO 3:4, TEMPO 4:4, REPRISE, long-term extension)

Figure 2. Evaluation of drug-induced serious hepatotoxicity (e-DISH) plots for the 4 pivotal ADPKD trials.

Peak ALT (x-axis) versus peak total bilirubin (y-axis). Vertical lines correspond to ALT >3x ULN. Horizontal lines correspond to total bilirubin >2x ULN. Subjects in the lower-left quadrant are relatively normal and subjects meeting Hy's Law laboratory criteria for potentially severe liver injury (ALT >3x ULN and total bilirubin >2x ULN with serum alkaline phosphatase <2x ULN) are shown in the upper-right quadrant. Panels B and D categorize subjects in the TEMPO 4:4 extension and the long-term extension by their trial prior to entry; TEMPO 4:4 subjects entered from TEMPO 3:4, TEMPO 2:4, NOCTURNE, Trial 156-06-260, Trial NCT01336972, and Trial NCT01210560, and long-term extension subjects entered from TEMPO 3:4, NOCTURNE, TEMPO 4:4, and REPRISE (see **Table S1**).

ADPKD, autosomal dominant polycystic kidney disease; ALT, alanine aminotransferase; PBO, placebo; TBili, total bilirubin; TOL, tolvaptan; ULN, upper limit of normal.

Panel A is reproduced with permission from Watkins PB, Lewis JH, Kaplowitz NE, et al. *Drug Saf.* 2015;38:1103-1113.

Figure 3. Kaplan-Meier curves of time to first elevation in ALT >3x ULN in REPRISE (A) and the long-term extension (B)

Arrow = For each subject, time to first elevation to >3x ULN adjudicated as having a "probable" relationship to tolvaptan. Panel A includes 11 events previously reported[3] and 2 additional events. The hepatic adjudication committee deemed two additional hepatic events (one subject at Day 382; the other at Day 387) with ALT elevations of <3x ULN (data not shown) to be probably related to tolvaptan. Panel B categorizes subjects in the long-term extension by their trial prior to entry; subjects in the Other Trial group had received tolvaptan previously in TEMPO 3:4, NOCTURNE, TEMPO 4:4, and/or REPRISE (see **Table S1**). The hepatic adjudication committee deemed one additional hepatic event in a subject who was on tolvaptan for 158 days and developed ALT elevations of <3x ULN (data not shown) to be probably related to tolvaptan in the long-term extension.

ALT, alanine aminotransferase; ULN, upper limit of normal.