

Clinical Pattern of Tolvaptan-Associated Liver Injury in Subjects with Autosomal Dominant Polycystic Kidney Disease: Analysis of Clinical Trials Database

Paul B. Watkins¹ · James H. Lewis² · Neil Kaplowitz³ · David H. Alpers⁴ ·
Jaime D. Blais⁵ · Dan M. Smotzer⁵ · Holly Krasa⁵ · John Ouyang⁵ ·
Vicente E. Torres⁶ · Frank S. Czerwiec⁵ · Christopher A. Zimmer⁵

Published online: 19 July 2015

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Abstract

Introduction Subjects with autosomal dominant polycystic kidney disease (ADPKD) who were taking tolvaptan experienced aminotransferase elevations more frequently than those on placebo in the TEMPO 3:4 (Tolvaptan Efficacy and Safety in Management of Autosomal Dominant Polycystic Kidney Disease and its Outcomes) clinical trial.

Methods An independent, blinded, expert Hepatic Adjudication Committee re-examined data from TEMPO 3:4 and its open-label extension TEMPO 4:4, as well as from long-term (>14 months) non-ADPKD tolvaptan trials, using the 5-point Drug-Induced Liver Injury Network classification.

Results In TEMPO 3:4, 1445 subjects were randomized 2:1 (tolvaptan vs. placebo) and 1441 had post-baseline assessments of hepatic injury. Sixteen patients on tolvaptan

and one on placebo had significant aminotransferase elevations judged to be at least probably related to study drug. No association with dose or systemic exposure was found. Two of 957 subjects taking tolvaptan (0.2 %) and zero of 484 taking placebo met the definition of a Hy's Law case. One additional Hy's Law case was identified in a TEMPO 4:4 subject who had received placebo in the lead study. The onset of a hepatocellular injury occurred between 3 and 18 months after starting tolvaptan, with gradual resolution over the subsequent 1–4 months. None of the events were associated with liver failure or chronic liver injury/dysfunction. No imbalance in hepatic events was observed between tolvaptan and placebo in lower-dose clinical trials of patients with hyponatremia, heart failure, or cirrhosis. **Conclusions** Although hepatocellular injury following long-term tolvaptan treatment in ADPKD subjects was infrequent and reversible, the potential for serious irreversible injury exists. Regular monitoring of transaminase levels is warranted in this patient population.

✉ Christopher A. Zimmer
Christopher.Zimmer@otsuka-us.com

¹ Hamner-UNC Institute for Drug Safety Sciences, University of North Carolina-Chapel Hill, Research Triangle Park, Chapel Hill, NC, USA

² Georgetown University School of Medicine, Washington, DC, USA

³ Keck School of Medicine, University of Southern California, Los Angeles, CA, USA

⁴ Washington University School of Medicine, St Louis, MO, USA

⁵ Global Clinical Development, Otsuka Pharmaceutical Development & Commercialization, Inc., 2440 Research Blvd, Rockville, MD 20850, USA

⁶ Nephrology, Mayo Clinic College of Medicine, Rochester, MN, USA

Key Points

In patients with autosomal dominant polycystic kidney disease (ADPKD), long-term treatment with tolvaptan can rarely cause severe and potentially life-threatening liver injury.

This injury is typically hepatocellular, occurs between 3 and 18 months after starting tolvaptan, and resolves within 4 months after stopping the drug.

A risk of similar liver injury was not detected following exposure to tolvaptan in non-ADPKD patients.

1 Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is an inherited disorder characterized by the appearance and slow growth of fluid-filled cysts primarily in the kidneys, but also in the liver and other organs [1, 2]. Over time, the expanding cysts physically displace and obstruct renal tubules, blood vessels and lymphatics, as well as promote apoptosis, atrophy and fibrosis of the renal parenchyma, leading to progressive renal failure [3]. ADPKD was responsible for 2.6 % of patients on dialysis and 9.9 % of patients receiving renal transplants in the USA in 2012 [4].

Studies of animal models implicate the antidiuretic hormone arginine vasopressin and its secondary messenger 3',5'-cyclic adenosine monophosphate (cAMP) as promoters of kidney-cyst cell proliferation and luminal fluid secretion [5, 6]. In early animal models the suppression of vasopressin release by vasopressin V₂-receptor inhibition slowed disease progression [7, 8]. Because tolvaptan is a V₂ receptor antagonist, its use in treating ADPKD was investigated. In the pivotal TEMPO 3:4 (Tolvaptan Efficacy and Safety in Management of Autosomal Dominant Polycystic Kidney Disease and its Outcomes) trial (ClinicalTrials.gov identifier: NCT00428948 [24]), long-term treatment with tolvaptan was associated with favorable outcomes in subjects with early ADPKD. These favorable outcomes included lower rate of growth in total kidney volume (2.8 vs. 5.5 %; $p < 0.001$) and slower decline in kidney function [reciprocal of the serum creatinine level, -2.61 vs. -3.81 (mg per mL)⁻¹ per year; $p < 0.001$] [9].

TEMPO 3:4 and its extension trial TEMPO 4:4 (ClinicalTrials.gov identifier: NCT01214421 [25]) were continuously monitored by an Independent Data Monitoring Committee (IDMC) that assessed overall safety, including hepatic function and injury. During the course of continued monitoring of the TEMPO trials, the IDMC recommended increasing the frequency of liver monitoring in the TEMPO 4:4 extension study, from every 6 months to every 3 months. Upon unblinding of the TEMPO 3:4 database, an imbalance in hepatic injury, defined as alanine aminotransferase (ALT) >3 times the upper limit of normal (ULN), was observed for subjects receiving tolvaptan (4.4 %) relative to placebo (1.0 %). To assess the risk of hepatotoxicity, an independent, blinded, expert Hepatic Adjudication Committee (HAC) re-examined subject-level data from all ADPKD clinical trials, as well as data from non-ADPKD subjects who had received long-term tolvaptan therapy in other clinical trials, including patients with hyponatremia [syndrome of inappropriate anti-diuretic hormone release (SIADH)], heart failure, and cirrhosis. The results of this analysis are presented here.

2 Methods

2.1 Subjects

The safety databases reviewed here were generated in clinical trials that examined the efficacy and safety of tolvaptan in the treatment of ADPKD. The vast majority of included subjects were from the pivotal, randomized, placebo-controlled TEMPO 3:4 trial (NCT00428948 [24]) [9] and its open-label extension TEMPO 4:4 (NCT01214421 [25]). To be eligible for inclusion in TEMPO 3:4, all subjects had to have an image-confirmed diagnosis of ADPKD, total kidney volume ≥ 750 mL and an estimated creatinine clearance rate ≥ 60 mL/min. 1445 subjects were enrolled (tolvaptan, 961; placebo, 484) and 1441 had at least one post-baseline assessment of hepatic injury. Tolvaptan was administered twice daily, starting at a morning/afternoon dose of 45/15 mg and titrated weekly to 60/30 mg and 90/30 mg based on tolerability; subjects could down-titrate at any time to as low as the starting dose (45/15 mg). Subjects were treated for 36 months or until early discontinuation. In TEMPO 4:4, 871 subjects from the lead TEMPO 3:4 trials (tolvaptan, 557; placebo, 314), received open-label tolvaptan at their highest tolerated dose for a minimum of 24 additional months. The study drug was discontinued when pre-determined criteria were met. Re-challenge was conducted on an individual basis.

To provide further context for this analysis, safety databases from the non-ADPKD clinical trials evaluating the efficacy and safety of tolvaptan in subjects with heart failure or hyponatremia (etiologies included SIADH, heart failure, and cirrhosis) were also examined. In total, 6155 subjects were enrolled (tolvaptan, 3403; placebo, 2752) across all non-ADPKD trials, and 4664 subjects (tolvaptan, 2414; placebo, 2250) were enrolled in long-term placebo-controlled trials. All trials were sponsored by Otsuka Pharmaceuticals.

2.2 Evaluation of Drug-Induced Serious Hepatotoxicity (eDISH) Assessments

The Evaluation of Drug-Induced Serious Hepatotoxicity (eDISH) approach was used to efficiently visualize potential hepatocellular injury in the large subject populations examined in this study and to supply supportive data for the adjudication process. This is a graphical methodology in which the log of the peak serum ALT concentration is plotted for each subject along the x-axis and the log of peak total serum total bilirubin (BT) concentration is plotted along the y-axis [10]. Four quadrants on the eDISH plot are defined by lines at $3 \times$ ULN for ALT and $2 \times$ ULN for BT. The upper-right quadrant is the Hy's Law quadrant,

although patients 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 drug-induced liver injury (DILI) when the serum alkaline phosphatase is $<2 \times \text{ULN}$ [11].

An excess of subjects in the lower-right quadrant for a study 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 BT and that increases in ALT may occur before or without accompanying rises in serum bilirubin [12]. Subjects discovered to have elevated serum ALT may be discontinued from treatment before the injury progresses to the point of bilirubin elevations. Finally, an excess of patients in the upper-left quadrant for a study drug relative to placebo is generally observed in drugs associated with cholestatic liver reactions, or patients with Gilbert's syndrome or other causes of isolated hyperbilirubinemia.

2.3 Adjudication of Hepatic Safety Signals

The HAC consisted of four expert hepatologists (PBW, JHL, NK, and DHA) who reviewed safety data from ADPKD and non-ADPKD (hyponatremia, heart failure, and cirrhosis) tolvaptan trials. Five general categories of adverse events were identified for further adjudication:

1. *Category 1* A non-serious treatment-emergent event leading to discontinuation of treatment or any serious treatment-emergent adverse event matching a lower level term in any one of the following hepatic standard MedDRA^{®1} queries (SMQs):
 - (a) cholestasis and jaundice of hepatic origin,
 - (b) hepatic failure, fibrosis, and cirrhosis and other liver damage-related conditions,
 - (c) non-infectious hepatitis,
 - (d) liver-related investigations, signs, and symptoms,
 - (e) liver-related coagulation and bleeding disturbances.
2. *Category 2* ALT $>3 \times \text{ULN}$ and BT $>2 \times \text{ULN}$.
3. *Category 3* aspartate aminotransferase (AST) $>3 \times \text{ULN}$ and BT $>2 \times \text{ULN}$.
4. *Category 4* ALT or AST $>5 \times \text{ULN}$.
5. *Category 5* BT $>2 \times \text{ULN}$.

¹ MedDRA[®] (The Medical Dictionary for Regulatory Activities) terminology is the international medical terminology developed under the auspices of the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). MedDRA[®] trademark is owned by the International Federation of Pharmaceutical Manufacturers and Associations (IFPMA) on behalf of ICH.

In cases where ULN could not be obtained, 40 IU/L and 1 mg/dL (17 $\mu\text{M/L}$) were used for ALT and BT, respectively. Laboratory evaluations were completed during the TEMPO 3:4 trial at baseline, 3 weeks, 4 months, and every 4 months thereafter.

For causality assessment, the four hepatologists comprising the HAC agreed to utilize "expert opinion" rather than a structured scoring instrument [e.g., Roussel Uclaf Causality Assessment Method (RUCAM)] [13]. 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 five causality groups, as defined by the Drug-Induced Liver Injury Network [14, 15]: "definite", i.e., evidence that the drug is causing the injury is beyond reasonable doubt; "highly likely", i.e., evidence that the drug is causing the injury is clear and convincing but not definite; "probable", i.e., the preponderance of evidence supports the link between the drug and liver injury; "possible", i.e., the evidence for the drug causing the injury is equivocal but present; and "unlikely", i.e., evidence is available that an etiological factor other than the drug caused the injury. Cases judged "not classifiable" in regard to the role of the study drug occurred when the data were insufficient to render an opinion. Such situations arose when other confounding factors were present (e.g., diseases or other medications) but details could not be provided for the intensity/dose and timing of the underlying conditions (e.g., congestive heart failure, ischemia, multiple drug administration, underlying liver disease). Insufficient data were infrequently encountered [0/98 (0 %) ADPKD cases and 5/52 (10 %) non-ADPKD cases], but when it was the consensus opinion, it was always in cases with significant underlying diseases other than ADPKD.

The four hepatologists on the committee performed their assessments independently and without knowledge of treatment assignment. If committee members did not arrive at an identical independent causality score for a particular case, the case was discussed further in conference and a final causality determination was achieved by consensus, although majority rule was used in rare instances where consensus could not be achieved.

Because TEMPO 4:4 was ongoing at the time the adjudication was initiated, the committee performed two rounds of adjudication, one with a cut-off date of 31 March 2012 to provide initial feedback and guide future treatment regimens, and a later one with a cut-off date of 31 March 2015. The selection criteria for adjudicated cases were identical during the two periods, with the exception that Category 5 (BT $>2 \times \text{ULN}$) was not employed between October 2012 and February 2014. This change was prompted by discussions among the hepatology experts, who agreed that a patient with an isolated elevated serum

bilirubin in the absence of the other selection criteria was not a safety concern.

3 Results

3.1 Subjects

The duration of exposure to tolvaptan in all of the ADPKD studies is shown in Table 1. Up until the first cut-off date (31 March 2012), 1581 subjects had received tolvaptan therapy, of whom 838 (53.0 %) and 814 (51.5 %) had received the drug for 18 and 24 months, respectively. By the 31 March 2015 data capture, 1636 ADPKD subjects had received tolvaptan, of whom 1330 (81.3 %) and 1266 (77.4 %) had been treated for 18 and 24 months, respectively. Clinical demographics for subjects adjudicated as probable or higher were comparable with the total population from the pivotal TEMPO 3:4 study [mean (standard deviation) age: 42 (5) vs. 39 (7) years; *n* (%) male: 12 (40 %) vs. 746 (52 %)].

3.2 Hepatic Events in the TEMPO 3:4 Study

The pivotal TEMPO 3:4 trial included 957 ADPKD subjects who received tolvaptan and 484 who received placebo. An imbalance between tolvaptan- and placebo-treated subjects with ALT $>3 \times$ ULN and BT $<2 \times$ ULN was also observed in TEMPO 3:4 [40/957 (4.4 %) vs. 5/484 (1.0%), respectively] (Fig. 1a, lower-right quadrant). No imbalance in ALT $>3 \times$ ULN between treatment groups was evident at baseline [3/946 (0.32 %) vs. 1/479 (0.21 %), respectively]. Using adjudication criteria, 35 tolvaptan- and 11 placebo-treated subjects were investigated by the independent HAC (Table 2). The likelihood that an event was caused by study medication was assessed as probable or higher in 17 of these subjects, of whom 16 had received tolvaptan and one had received placebo. The presence of possible confounding diagnoses, including risk factors for viral hepatitis, autoimmune hepatitis, fatty liver

disease, alcohol consumption, and concomitant use of medications with potential for idiosyncratic transaminase elevations, may have exacerbated the tolvaptan-related hepatotoxicity in some subjects.

Two of the probable events in the tolvaptan group [2/957 (0.2 %)] and zero in the placebo group [0/484 (0 %)] met Hy's Law laboratory criteria (ALT $>3 \times$ ULN and BT $>2 \times$ ULN) (Fig. 1a, upper-right quadrant).

The two Hy's Law cases had received the highest dose of tolvaptan administered in the study (120 mg/day split into morning/evening doses of 90/30 mg). In both cases ALT and BT returned to $<3 \times$ ULN and $<2 \times$ ULN, respectively, following withdrawal from tolvaptan (Fig. 2). Although both Hy's Law cases occurred at the highest administered dose, subjects adjudicated as probable or higher were generally distributed across all doses and exposures, with no significant difference in the area under the concentration–time curve ($p = 0.7543$), suggesting no clear dose dependence (Fig. 3). This finding is supported by a Fisher exact test that failed to identify a statistical relationship between dose and hepatotoxicity ($p = 0.3464$). While no association with dose of exposure was found, additional research has been initiated to further investigate the role of dose and exposure on the risk of hepatic injury.

3.3 Open-Label Extension Study

Up until the cut-off date of the first adjudication period, nine of 846 (1.11 %) subjects receiving open-label tolvaptan met hepatic adjudication criteria. One subject met Hy's Law laboratory criteria (Table 2) and eight subjects (0.95 %) experienced ALT/AST elevations $>3 \times$ ULN, with BT $<2 \times$ ULN. Among these nine subjects with ALT $>3 \times$ ULN, the liver signal was attributed to tolvaptan (causality assessed as probable or higher) for the Hy's Law subject and four of eight subjects with ALT elevations; all had received placebo during TEMPO 3:4 prior to crossing over to tolvaptan in TEMPO 4:4.

During the second adjudication period, 31 additional cases from the extension study were referred to the HAC (Table 2). Of these, four were adjudicated as highly likely ($n = 1$) or probable ($n = 3$) for tolvaptan causality. Two of the previous four subjects had received tolvaptan in the pivotal study and two had received placebo. No new Hy's Law cases were identified in the second reporting period.

3.4 Hepatic Events in the Non-Autosomal Dominant Polycystic Kidney Disease Trials

In total, 2414 subjects received tolvaptan and 2250 subjects received placebo in the long-term non-ADPKD trials (hyponatremia, heart failure, and cirrhosis). Of these, 589

Table 1 Extent of exposure to tolvaptan: all autosomal dominant polycystic kidney disease studies

Total tolvaptan exposure time	October 2012 cut-off [<i>n</i> (%)]	February 2014 cut-off [<i>n</i> (%)]
≥ 1 day	1581 (100)	1588 (100)
≥ 6 months	1017 (64.3)	1509 (95.0)
≥ 12 months	886 (56.0)	1368 (86.1)
≥ 18 months	838 (53.0)	1275 (80.3)
≥ 24 months	814 (51.5)	1195 (75.3)
≥ 36 months	661 (41.8)	962 (60.6)

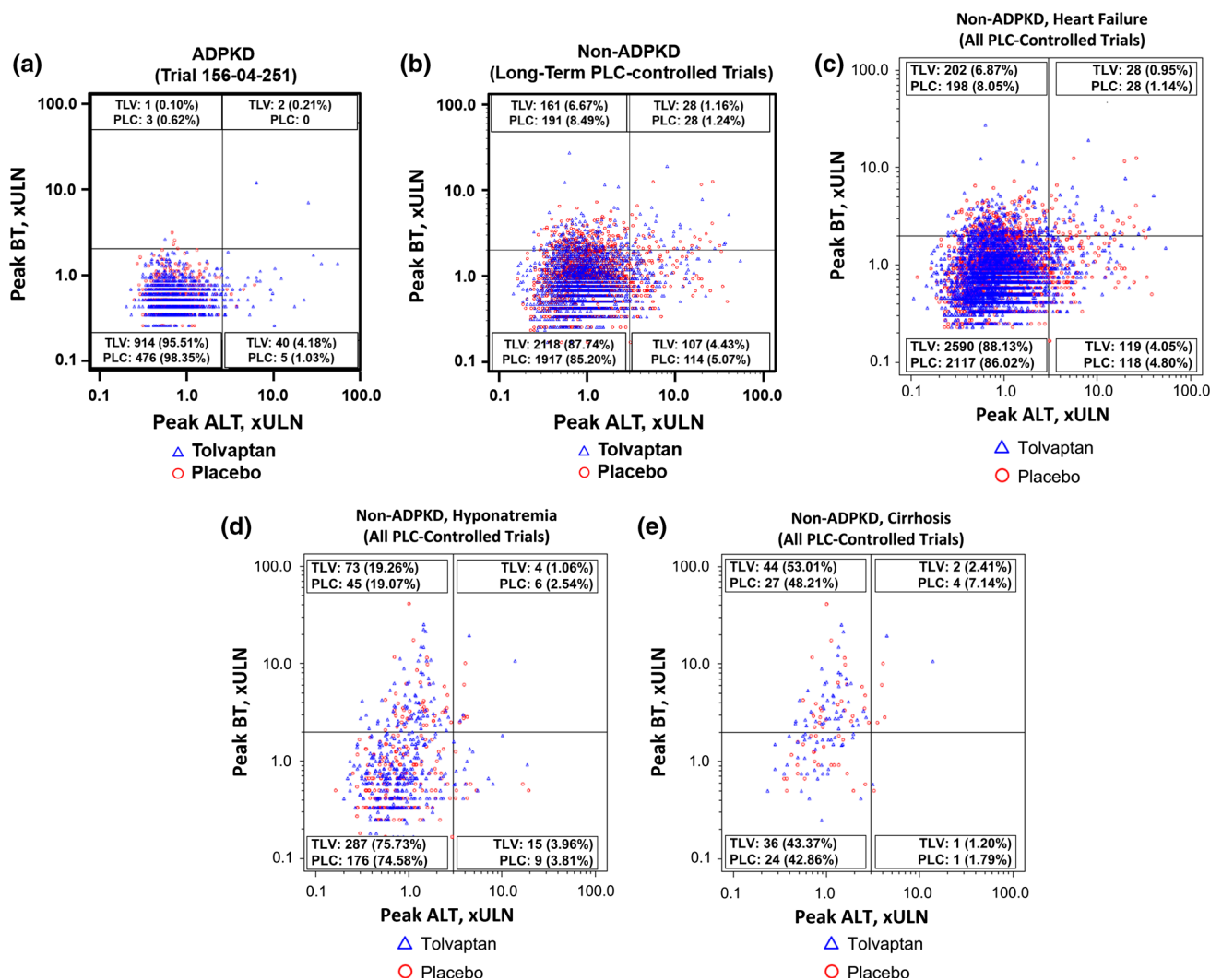


Fig. 1 Evaluation of drug-induced serious hepatotoxicity (e-DISH) plots for **a** ADPKD (TEMPO 3:4); **b** non-ADPKD subjects; and **(c–e)** non-ADPKD subjects by etiology. Peak ALT (*x*-axis) versus peak total bilirubin (*y*-axis). Vertical lines correspond to $3 \times \text{ULN}$ for ALT. Horizontal lines correspond to $2 \times \text{ULN}$ for BT. Subjects in the

lower-left quadrant are relatively normal and subjects meeting Hy's laboratory criteria are shown in the upper-right quadrant. ADPKD autosomal dominant polycystic kidney disease, ALT alanine aminotransferase, BT total bilirubin, PLC placebo, TLV tolvaptan, ULN upper limit of normal

were enrolled in clinical studies that exposed subjects to tolvaptan for at least 14 months, the majority of whom had heart failure (553). No evident imbalance between tolvaptan- and placebo-treated subjects was observed in the upper- or lower-right quadrants of eDISH plots (Fig. 1b–e). Given the similar number of subjects in both groups that experienced elevations in ALT $>3 \times \text{ULN}$, the HAC decided to adjudicate only subjects with higher degrees of hepatic dysfunction as reflected by ALT $>5 \times \text{ULN}$ and concomitant bilirubin $>3 \times \text{ULN}$. This amounted to 28 cases across all three subject populations. Only one of the 28 cases was judged to be possibly due to study drug, whereas the remaining 27 cases were considered to be unlikely. After unblinding the treatment

assignment, the single possible case was found to have received placebo.

3.5 Identifying a Signature Pattern

In TEMPO 3:4, the 16 cases with ALT elevations $>3 \times \text{ULN}$ that were attributable to tolvaptan (probable or highly likely) were detected between 3 and 18 months after initiation (Fig. 4). For the two Hy's Law cases, ALT elevations $>3 \times \text{ULN}$ first occurred between 5 and 9 months post initiation of tolvaptan. All 35 cases adjudicated in the tolvaptan group returned to $\leq 3 \times \text{ULN}$. The majority of subjects who had discontinued tolvaptan (14/35) returned to $\leq 3 \times \text{ULN}$ within 40 days, whereas the majority of subjects

Table 2 Adjudication results for subjects meeting criteria for adjudication based on Drug-Induced Liver Injury Network criteria

Clinical trial	DILIN criteria				
	Definite	Highly likely	Probable	Possible	Unlikely
First adjudication period (cut-off: 31 March 2012)					
TEMPO 3:4					
Tolvaptan	0	1	15	10	9
Placebo	0	0	1	2	8
TEMPO 4:4					
Placebo → tolvaptan	0	2	3	0	2
Tolvaptan → tolvaptan	0	0	0	1	1
Other ADPKD studies					
Tolvaptan	0	0	2	1	2
Second adjudication period (cut-off: 31 March 2015)					
TEMPO 4:4 extension ^a					
Placebo → tolvaptan	0	1	1	3	6
Tolvaptan → tolvaptan	0	0	2 ^b	7	10
Other ADPKD studies					
Tolvaptan	0	0	2	2	2
Placebo	0	0	0	1	0

ADPKD autosomal dominant polycystic kidney disease, DILIN Drug-Induced Liver Injury Network

^a In addition, there was one subject (tolvaptan → tolvaptan) that had insufficient data for adjudication

^b Both subjects received a modified release formulation of tolvaptan for 8 weeks prior to enrolling in TEMPO 4:4

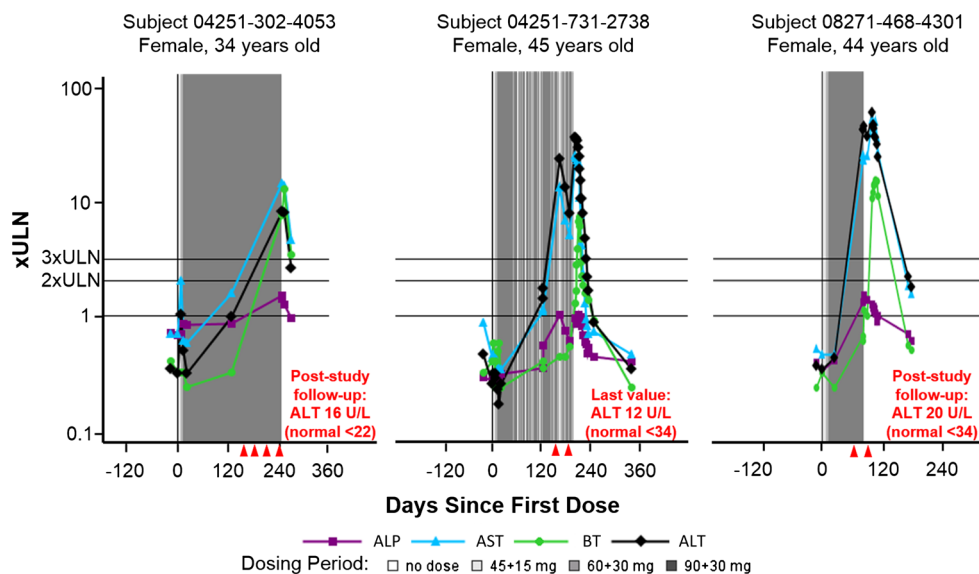


Fig. 2 Patterns of hepatic transaminase/total bilirubin elevations in the three Hy's Law cases in TEMPO 3:4 and its open-label extension study TEMPO 4:4. Cases A and B are from TEMPO 3:4, and case C was from TEMPO 4:4 (a prior placebo subject from TEMPO 3:4). Additional information on each case study is presented in Sect. 3.

who had continued therapy (21/35) returned to $\leq 3 \times \text{ULN}$ within 120 days (data not shown). There was no correlation between the height of the peak serum ALT and duration of the ALT elevation $>3 \times \text{ULN}$ (data not shown).

Gray shading in the background represents periods of dosing; white lines correspond to dosing interruptions. ALP alkaline phosphatase, ALT alanine aminotransferase, AST aspartate aminotransferase, BT total bilirubin, ULN upper limit of normal

The timing of the onset of the liver injury was not always known; the first blood sample obtained by the central laboratory was at the end of the 3-week titration period. Per protocol, the next assessment of liver

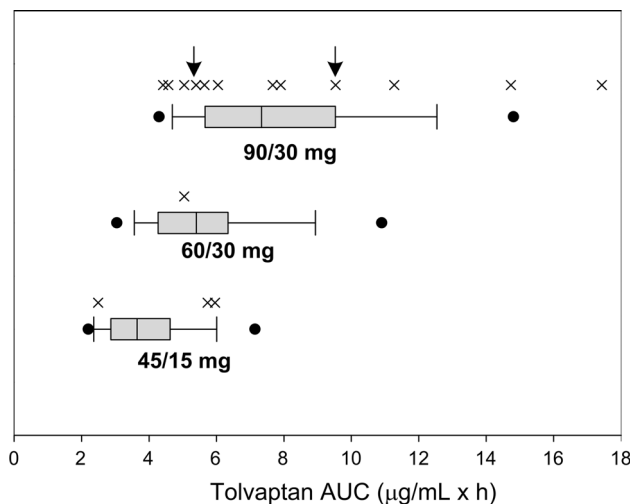


Fig. 3 Estimated tolvaptan exposure in TEMPO 3:4 for subjects adjudicated as probable or higher. Exposures at each of the three doses for all subjects with measurable tolvaptan concentrations are represented by *box and whisker plots* which summarize daily steady-state exposure, AUC, predicted using individual empirical Bayesian estimates from the population pharmacokinetic model at the individual modal dose. Tolvaptan exposures at the dose taken at the time of the onset of the first hepatic event, for each of the subjects adjudicated as probable or higher is represented as an “x”. Hy’s Law cases are shown as *arrows*. *Whiskers* represent the 10th and 90th percentiles, *black dots* represent the 5th and 95th percentiles. *AUC* area under the plasma concentration–time curve

chemistries was not until the 4-month visit (Fig. 4), but local laboratory values were sometimes available prior to this date. Examples of liver enzyme patterns from representative cases are shown in Fig. 5. Nearly half of the patients (10/21) were able to continue therapy with ALT levels that remained $<3 \times \text{ULN}$ after recovery, whereas the remaining patients (11/21) exhibited rapid transaminase elevations following re-challenge with therapy that led to discontinuation, but subsequently returned to normal over 1–4 months. No patient who was re-challenged in this manner met criteria for acute liver failure.

Based on the characteristics of the positively adjudicated cases, the HAC suggested a potential signature pattern for the observed events defined as development of acute hepatocellular injury with onset between 3 and 18 months after starting tolvaptan therapy.

3.6 Hy’s Law Case Studies

3.6.1 Case 1

Eight months after initiating tolvaptan treatment in TEMPO 3:4, a 34-year-old woman presented with jaundice and increased BT, ALT, and AST (Fig. 2a). She had consumed a single 8 g dose of Augmentin[®] between 2 and 3 months before liver injury was detected.

Augmentin[®] is a relatively frequent cause of liver injury and characteristically presents as a mixed hepatocellular/cholestatic reaction, often associated with evidence of immunoallergy (e.g., rash, fever, eosinophilia) [16]. Hepatocellular injury is a less common presentation, but is more frequently observed in patients under 45 years. In the HAC’s experience, there have been no reports of Augmentin[®] causing clinically important liver injury after a single dose (albeit an overdose in this case). Also, the latency to presentation was long (typically 1–2 months after the start of treatment). It was noted that the timing of the event was consistent with the tolvaptan signature presentation; however, resolution following tolvaptan discontinuation was more rapid than is characteristic. The event was adjudicated as probably related to tolvaptan by blinded consensus.

3.6.2 Case 2

A 45-year-old woman presented with complaints of nausea and stomach discomfort and elevations in ALT and AST liver enzymes 4 months after initiating tolvaptan treatment in TEMPO 3:4. Transaminase elevations were resolving on continued treatment when she experienced worsening nausea and a more severe injury at around 7 months, resulting in hospitalization (Fig. 2b). She was treated with prednisone during the resolution of the second peak, raising the possibility of autoimmune hepatitis. She experienced bleeding into her liver cysts, which suggested the presence of coagulopathy. It is unclear if she remains on immunosuppression. The event was adjudicated as probably related to tolvaptan by blinded consensus.

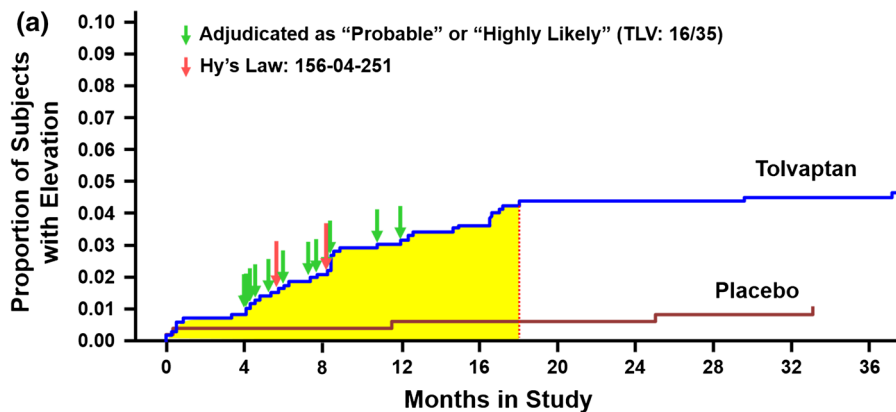
3.6.3 Case 3

A 44-year-old woman received placebo in TEMPO 3:4 prior to the open-label study (Fig. 2c). She developed nausea, abdominal pain, and jaundice and was hospitalized with hepatocellular injury 3 months after initiating tolvaptan treatment. Liver biopsy (performed by the investigator at the request of a consulting hepatologist 30 days after the event) showed “cytolytic hepatitis” with evidence of centrilobular necrosis, suggestive of DILI. The pattern of liver injury fit the clinical signature, and the event was adjudicated as highly likely related to tolvaptan by blinded consensus.

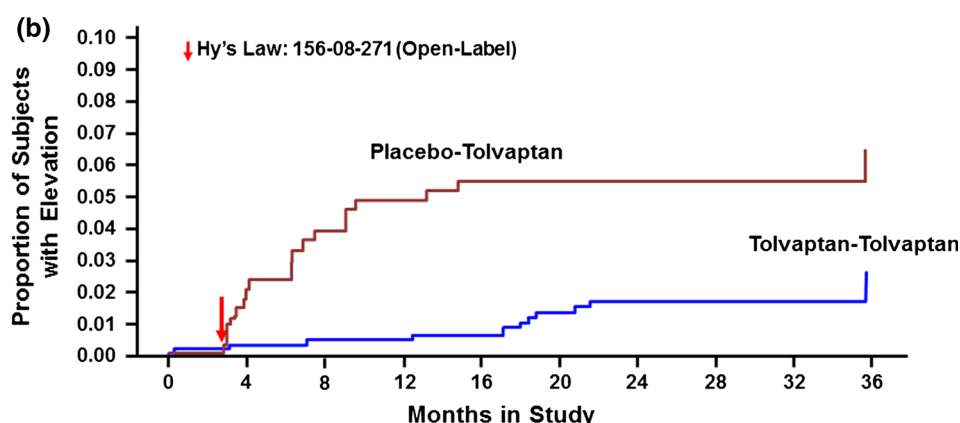
3.6.4 Summary of Cases

In all three Hy’s Law cases, ALT and BT returned to normal with no chronic liver injury reported following permanent discontinuation of tolvaptan.

Fig. 4 Time to first elevation of alanine aminotransferase to $>3 \times$ upper limit of normal in adjudicated subjects. **a** TEMPO 3:4: the apparent window of susceptibility is shown as shaded in yellow. All of the tolvaptan cases adjudicated as probable or higher are shown as green arrows and the two Hy's Law cases are shown as red arrows. **b** TEMPO 4:4: the Hy's Law case is shown as a red arrow. TLV tolvaptan



Days in Study	0	100	200	300	400	500	600	700	800	900	1000	1100
Tolvaptan N=	961	884	836	812	796	774	765	751	740	734	726	268
Placebo N=	483	476	468	459	452	445	442	433	425	422	415	147



Days in Study	0	100	200	300	400	500	600	700	800	900	1000	1100
Prior Placebo N=	364	340	326	318	306	297	295	293	283	272	255	212
Prior Tolvaptan N=	639	634	628	620	613	610	603	592	578	551	525	541

4 Discussion

No cases of acute liver failure have been reported in clinical trials of tolvaptan in ADPKD, and all subjects experiencing hepatic injury have recovered. Nonetheless, tolvaptan treatment was associated with elevations in serum aminotransferases exceeding $3 \times$ ULN in subjects with ADPKD. This finding alone is not considered to be a reliable liver safety signal [11], but three tolvaptan-treated subjects in the TEMPO 3:4 clinical trial and its open-label extension were confirmed to have met criteria for Hy's Law (ALT $>3 \times$ ULN and BT $>2 \times$ ULN). Based on FDA guidance [11], the identification of Hy's Law cases indicates that tolvaptan has the potential to cause hepatic injury capable of progressing to liver failure in patients with ADPKD. The apparent rarity of hepatic injury observed with tolvaptan has all the characteristics of an idiosyncratic reaction, suggesting the vast majority of patients should be able to receive long-term treatment without risk of liver injury.

A signature presentation of liver injury was discerned from the reviewed data. Tolvaptan-associated hepatocellular injury had an onset between 3 and 18 months, with little evidence of similar events before or after this period of apparent susceptibility. The injury typically progressed by biochemical criteria for a median of 28 [interquartile range (IQR) 15–50] days after discontinuation of treatment, and resolved slowly over a median of 46 (IQR 32.5–70) days.

Liver biopsies were obtained in only four of 25 (16 %) subjects adjudicated as probable or higher and, as is typical with idiosyncratic DILI, no pathomonomic features were evident [17].

A number of patients were re-challenged with tolvaptan after elevated ALT values subsided. Approximately half of these subjects were able to tolerate the drug when it was re-introduced, suggesting that a form of adaptation or drug tolerance occurs. However, the other half experienced rapid ALT elevations upon re-exposure (positive re-challenge) and tolvaptan treatment had to be permanently withdrawn. The more rapid recurrence of injury upon re-

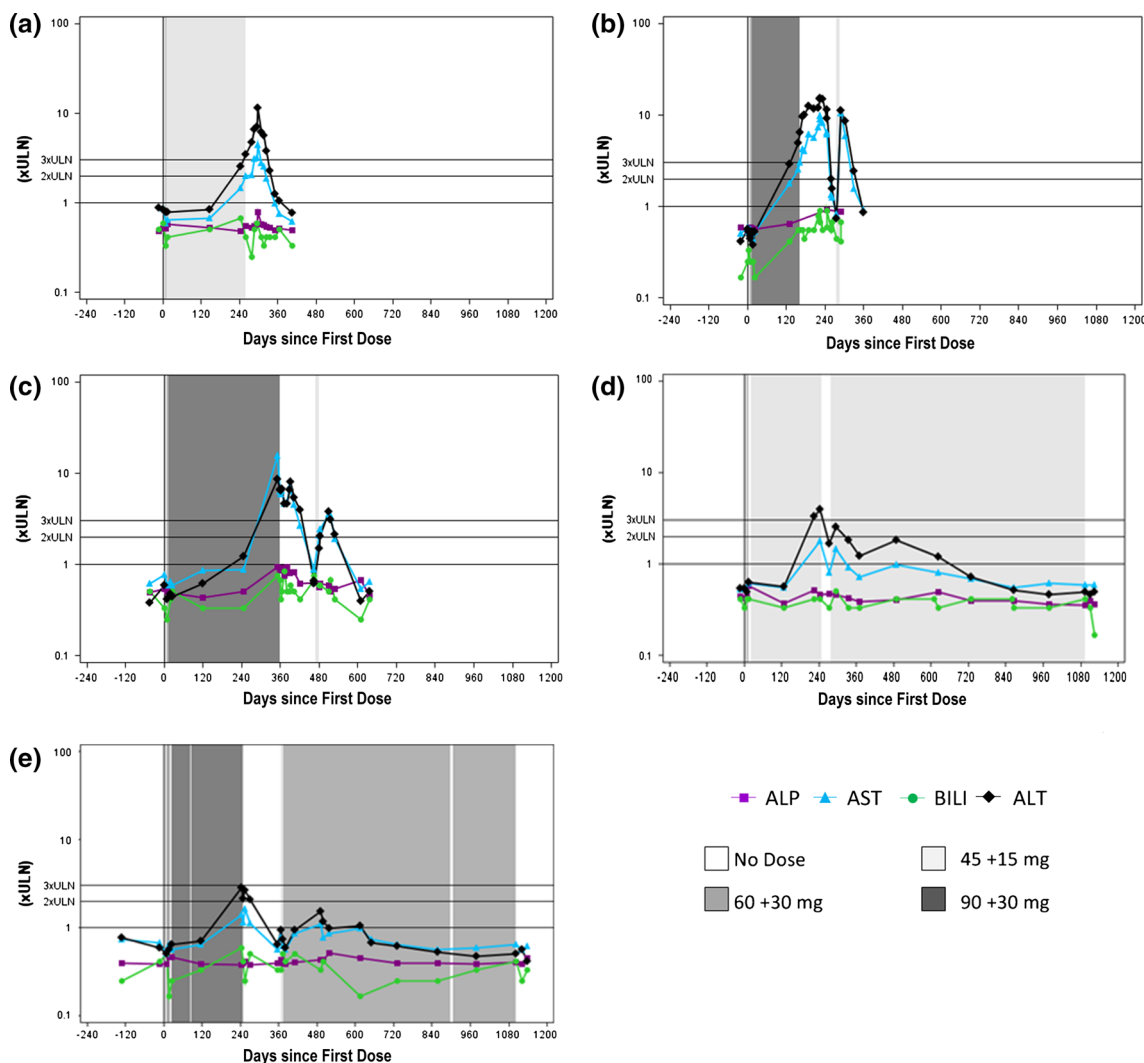


Fig. 5 Patterns of hepatic transaminases/total bilirubin elevations in cases representative of the signature profile. Case A represents a subject whose ALT continued to rise transiently post-tolvaptan discontinuation prior to a return to $<1 \times \text{ULN}$ (a). Cases B and C experienced similar ALT elevations to case A; however, both observed an immediate elevation in ALT upon re-challenge with tolvaptan (b and c). Both subjects subsequently returned to

$<1 \times \text{ULN}$ upon discontinuation. Cases D and E are representative examples of subjects whose ALT normalized while on tolvaptan (d and e). Gray shading in the background represents periods of dosing, darker shades of gray represent higher doses of tolvaptan; white lines correspond to dosing interruptions. ALP alkaline phosphatase, ALT alanine aminotransferase, AST aspartate aminotransferase, BILI bilirubin, ULN upper limit of normal

exposure may indicate an adaptive immune mechanism and studies are underway to search for possible human leukocyte antigen (HLA) risk alleles as have been demonstrated for delayed DILI caused by other agents, including ximelagatran, lumiracoxib, and lapatinib [18–20].

Following a recommendation from the TEMPO Steering Committee to increase the frequency of monitoring to monthly, no additional Hy’s Law cases have been identified to date ($n = 1275$ subjects exposed for ≥ 18 months) (Table 1), further lowering the incidence of potential liver failure to approximately 1:4000. It should be noted that since liver chemistry monitoring was relatively infrequent

in TEMPO 3:4 and its open-label extension, more frequent monitoring is expected to further lower the risk of liver failure. In some cases, liver injury did progress for weeks after stopping drug treatment, followed by slow resolution, and it seems unlikely that the risk of serious liver injury could be eliminated solely by more frequent monitoring. While no additional Hy’s cases have been identified since implementation of a comprehensive monitoring program consisting of monthly liver enzyme testing went into effect, the number of additional patients treated does not provide sufficient power to eliminate the possibility of another Hy’s case from occurring.

Of note, there was no evidence of a similar liver safety signal or signature presentation observed among subjects with cirrhosis, congestive heart failure, or hyponatremia exposed to tolvaptan in non-ADPKD clinical trials. This was a surprising finding, as the expectation might be that patients with liver disease or severe congestive heart failure would be more susceptible to DILI. The explanation is unlikely due to sample size, since the number of subjects treated for at least 14 months in the non-ADPKD populations ($n = 589$) was not grossly different from the corresponding number of ADPKD subjects ($n \sim 860$). Although the dose of tolvaptan received by the non-ADPKD population (single daily dose up to 60 mg/day) was lower than that received by most subjects in the ADPKD trials (split daily dose up to 120 mg/day), the overall exposure for most chronic heart failure subjects was similar to subjects receiving a split dose of 45/15 mg/day in the ADPKD population (data on file). It is possible that patients with ADPKD may be more susceptible to tolvaptan-associated liver injury than other patient populations due to an unknown feature of disease pathology. In this regard, it should be noted that the most common extra-renal manifestation of ADPKD is polycystic liver disease, which is characterized by the presence of multiple hepatic cysts originating from biliary ducts and peribiliary glands [21]. In the CRISP (Consortium for Radiologic Imaging Studies of Polycystic Kidney Disease) study, hepatic cysts were observed in 58 % of 15- to 24-year-olds, 85 % of 25- to 34-year-olds, and 94 % of 35- to 46-year-olds with ADPKD [22]. While imaging to document the presence of hepatic cysts was not included in the study protocols, more than 60 % of subjects in TEMPO 3:4 had liver cysts as determined by medical history or a retrospective review of their study MRIs. While it was not possible to correlate the presence of hepatic cysts with susceptibility to tolvaptan-associated liver injury in this study, 76 % of subjects adjudicated as probable or higher in TEMPO 3:4 and TEMPO 4:4 demonstrated some degree of hepatic cyst burden. It should also be noted that 18 of 30 (60 %) of subjects adjudicated as probable or higher and all three Hy's law cases occurred in women, but it remains unknown whether this reflects true sex-specific differences.

Several risk-management strategies seem plausible for mitigating the potential for irreversible liver damage in the setting of long-term tolvaptan treatment for ADPKD, including more frequent liver testing at monthly intervals through the window of observed susceptibility. In addition, a personalized medicine approach might be feasible. Targeted genetic studies are currently underway to examine the potential role of the *HLA* genotype, as are studies to determine if subjects with *PKD1* or *PKD2* have similar risks for hepatocellular injury [23]. Alternatively, the observed liver injury may have a metabolic or mechanistic

etiology. To address this possibility, drug-metabolizing enzymes, drug transporters, stress response proteins, mitochondrial markers, extracellular vesicle trafficking, and biliary disposition are all being examined. Finally, large-scale screening methodologies are being employed to detect other potential biomarkers that might be used to identify the rare ADPKD patient susceptible to tolvaptan-mediated liver injury.

5 Study Limitations

This was a retrospective analysis. The interval between protocol-driven assessments of liver chemistries changed after an interim analysis by the Data and Safety Monitoring Board but remained relatively long in these studies. This limited the ability to time the onset of injury and it is also possible that transient but treatment-emergent liver events were not captured. Finally, subjects were not randomized to the doses they were receiving at the time of liver events as all would have escalated within 3 weeks to the highest dose (120 mg/day) had they not experienced titration-limiting symptoms or events. This confounds the dose:event analysis.

6 Conclusions

In the pivotal TEMPO 3:4 clinical trial and its open-label extension, an imbalance in the number of subjects experiencing serum ALT elevations exceeding $3 \times$ ULN was observed. The risk of liver failure in ADPKD patients receiving long-term tolvaptan therapy was estimated to be approximately 1:4000, with the latency of onset occurring primarily between 3 and 18 months of receiving therapy. Among the positively adjudicated cases, there were no reports of liver failure and all subjects experiencing hepatic injury recovered. Of note, the liver safety risk for tolvaptan-treated subjects in the ADPKD population was not evident in the tolvaptan-treated subjects in the non-ADPKD population, possibly due to enhanced susceptibility in the ADPKD population.

To further reduce the risk of liver injury in patients receiving long-term tolvaptan, we recommend frequent monitoring of liver function tests.

Acknowledgments The authors thank David Norris, PhD (Ecosse Medical Communications, LLC), for editorial assistance provided during the preparation of this manuscript.

Compliance with Ethical Standards

All ADPKD and non-ADPKD clinical trials were supported by Otsuka Pharmaceutical Development & Commercialization Inc. All

trials were conducted in compliance with the protocol the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) Good Clinical Practice (GCP) guideline and all other applicable regional regulatory requirements. Each trial site was approved by their 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.

Each author contributed important intellectual content during manuscript drafting or revision and accepts accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved.

Funding The TEMPO 3:4 study and editorial assistance was supported by Otsuka Pharmaceutical Development & Commercialization. All members of the HAC are paid consultants to Otsuka but were not reimbursed for any time spent in the preparation of this manuscript.

Conflicts of Interest Jaime D. Blais, Dan M. Smotzer, Frank S. Czerwiec, John Ouyang, Holly Krasa, and Christopher A. Zimmer are employees of Otsuka Pharmaceuticals and Otsuka Pharmaceutical Development & Commercialization.

Paul B. Watkins, James H. Lewis, Neil Kaplowitz, David H. Alpers, and Vicente E. Torres are consultants to Otsuka Pharmaceutical Development & Commercialization and numerous other pharmaceutical companies.

The authors have no other potential conflicts of interest relevant to the content of this article.

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References

1. Grantham JJ. Clinical practice. Autosomal dominant polycystic kidney disease. *N Engl J Med.* 2008;359(14):1477–85.
2. Torres VE, Harris PC, Pirson Y. Autosomal dominant polycystic kidney disease. *Lancet.* 2007;369(9569):1287–301.
3. Grantham JJ, Mulamalla S, Swenson-Fields KI. Why kidneys fail in autosomal dominant polycystic kidney disease. *Nat Rev Nephrol.* 2011;7(10):556–66.
4. United States Renal Data System. 2014 annual data report: an overview of the epidemiology of kidney disease in the United States. Bethesda: National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases; 2014.
5. Yamaguchi T, Nagao S, Wallace DP, Belibi FA, Cowley BD, Pelling JC, et al. Cyclic AMP activates B-Raf and ERK in cyst epithelial cells from autosomal-dominant polycystic kidneys. *Kidney Int.* 2003;63(6):1983–94.
6. Hanaoka K, Guggino WB. cAMP regulates cell proliferation and cyst formation in autosomal polycystic kidney disease cells. *J Am Soc Nephrol.* 2000;11(7):1179–87.
7. Gattone VH 2nd, Wang X, Harris PC, Torres VE. Inhibition of renal cystic disease development and progression by a vasopressin V2 receptor antagonist. *Nat Med.* 2003;9(10):1323–6.
8. Torres VE, Wang X, Qian Q, Somlo S, Harris PC, Gattone VH 2nd. Effective treatment of an orthologous model of autosomal dominant polycystic kidney disease. *Nat Med.* 2004;10(4):363–4.
9. Torres VE, Chapman AB, Devuyst O, Gansevoort RT, Grantham JJ, Higashihara E, et al. Tolvaptan in patients with autosomal dominant polycystic kidney disease. *N Engl J Med.* 2012;367(25):2407–18.
10. Watkins PB, Desai M, Berkowitz SD, Peters G, Horsmans Y, Larrey D, 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):243–52.
11. United States Food and Drug Administration. Guidance for industry, drug-induced liver injury: premarketing clinical evaluation. 2014. <http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM174090.pdf>. Accessed 11 Feb 2015.
12. Senior JR. Monitoring for hepatotoxicity: what is the predictive value of liver “function” tests? *Clin Pharmacol Ther.* 2009;85(3):331–4.
13. Regev A, Seeff L, Merz M, Ormarsdottir S, Aithal GP, Gallivan J, et al. Causality assessment for suspected DILI during clinical phases of drug development. *Drug Saf.* 2014;37(Suppl 1):S47–56.
14. Rockey DC, Seeff LB, Rochon J, Freston J, Chalasani N, Bonacini M, 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):2117–26.
15. Fontana RJ, Watkins PB, Bonkovsky HL, Chalasani N, Davern T, Serrano J, et al. Drug-Induced Liver Injury Network (DILIN) prospective study: rationale, design and conduct. *Drug Saf.* 2009;32(1):55–68.
16. Augmentin [package insert]. Research Triangle Park, NC: GlaxoSmithKline; 2006.
17. Teschke R, Frenzel C. Drug induced liver injury: do we still need a routine liver biopsy for diagnosis today? *Ann Hepatol.* 2013–2014;13(1):121–6.
18. Spraggs CF, Budde LR, Briley LP, Bing N, Cox CJ, King KS, et al. HLA-DQA1*02:01 is a major risk factor for lapatinib-induced hepatotoxicity in women with advanced breast cancer. *J Clin Oncol.* 2011;29(6):667–73.
19. Singer JB, Lewitzky S, Leroy E, Yang F, Zhao X, Klickstein L, et al. A genome-wide study identifies HLA alleles associated with lumiracoxib-related liver injury. *Nat Genet.* 2010;42(8):711–4.
20. Kindmark A, Jawaid A, Harbron CG, Barratt BJ, Bengtsson OF, Andersson TB, et al. Genome-wide pharmacogenetic investigation of a hepatic adverse event without clinical signs of immunopathology suggests an underlying immune pathogenesis. *Pharmacogenomics J.* 2008;8(3):186–95.
21. Hogan MC, Abebe K, Torres VE, Chapman AB, Bae KT, Tao C, et al. Liver involvement in early autosomal-dominant polycystic kidney disease. *Clin Gastroenterol Hepatol.* 2015;13(1):155–64.
22. Bae KT, Zhu F, Chapman AB, Torres VE, Grantham JJ, Guay-Woodford LM, et al. Magnetic resonance imaging evaluation of hepatic cysts in early autosomal-dominant polycystic kidney disease: the Consortium for Radiologic Imaging Studies of Polycystic Kidney Disease cohort. *Clin J Am Soc Nephrol.* 2006;1(1):64–9.
23. Hughes J, Ward CJ, Peral B, Aspinwall R, Clark K, San Millan JL, et al. The polycystic kidney disease 1 (PKD1) gene encodes a novel protein with multiple cell recognition domains. *Nat Genet.* 1995;10(2):151–60.
24. Otsuka Pharmaceutical Development & Commercialization, Inc. Tolvaptan phase 3 efficacy and safety study in ADPKD (TEMPO3/4) [ClinicalTrials.gov identifier NCT00428948]. US National Institutes of Health, ClinicalTrials.gov. <http://www.clinicaltrials.gov>. Accessed 8 Jul 2015.
25. Otsuka Pharmaceutical Development & Commercialization, Inc. Open-label tolvaptan study in subjects with ADPKD (TEMPO 4/4) [ClinicalTrials.gov identifier NCT01214421]. US National Institutes of Health, ClinicalTrials.gov. <http://www.clinicaltrials.gov>. Accessed 8 Jul 2015.