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Placebo-Controlled Randomized Trial of an Intestinal Bile Salt Transport Inhibitor for Pruritus in Alagille Syndrome

Short Title: Maralixibat Therapy for Alagille Syndrome

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FOOTNOTES

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Abbreviations: AE, adverse event; ALGS, Alagille syndrome; ALP, alkaline phosphatase; ALT, alanine aminotransferase; ANCOVA, analysis of covariance; ASBT, apical sodium-dependent bile acid transporter; ASBTi, ASBT inhibitor; AST, aspartate aminotransferase; ChiLDReN, Childhood Liver Disease Research Network; CRADA, Cooperative Research and Development Agreement; CSS, Clinical Scratch Score; DB, direct bilirubin; DILI, drug-induced liver injury; GGT, gammaglutamyl transpeptidase; IE, ileal exclusion; INR, international normalized ratio; IQR, interquartile range; ItchRO, itch-reported outcome; LDL-C, low-density lipoprotein cholesterol; LOCF, Last Observation Carried Forward; MITT, modified intention-to-treat population; NIDDK, National Institute of Diabetes and Digestive and Kidney Diseases; Obs, observation; PBC, primary biliary cholangitis; PEBD, partial external bile diversion; pH, potential of hydrogen; PI, principal investigator;

Pt, patient; SAE, serious adverse event; SBA, serum bile acid; TB, total bilirubin; ULN, upper limit of normal

ABSTRACT

Medically refractory, severe, cholestasis-induced pruritus in Alagille syndrome may be improved by surgical interruption of the enterohepatic circulation. This multicenter trial tested the hypothesis that the intestinal bile acid transport inhibitor, maralixibat, would similarly reduce pruritus in Alagille syndrome (NCT02057692). Thirty-seven children with Alagille syndrome were randomly assigned to double-blinded administration of placebo, 70, 140, or 280 µg/kg/day of maralixibat for 13 weeks. Pruritus was assessed by caregiver (itch-reported outcome instrument [ItchRO^{TM}]) and clinician report (range 0-4 [severe]). Liver chemistries and serum bile acids were measured. The primary outcome was change from baseline to week 13 in ItchRO relative to placebo. In the *a priori* first analysis of the primary efficacy endpoint, the mean adjusted difference between participants receiving 140 or 280 µg/kg/d and placebo was -0.47 (95% confidence interval [CI]:-1.14, 0.20, p=0.16). Statistically significant decreases were observed with the 70 and 140 µg/kg/d doses (-0.89, 95%CI:-1.70,-0.08, p=0.032 and -0.91, 95%CI:-1.62,-0.19, p=0.014), but not 280 µg/kg/d (-0.04 95%CI:-0.94,0.86, p=0.44) or all doses combined (-0.61, 95%CI:-1.24,0.20, p=0.055). A 1-point reduction in pruritus was more common in maralixibat-versus placebo-treated participants (caregiver ItchRO: 65 vs. 25% p=0.06, clinician score: 76 vs. 25% p=0.01). Relative to placebo, there were no significant

changes in liver chemistries or bile acids. Adverse and serious adverse events were similar between maralixibat and placebo.

Conclusion: Although the pre-specified primary analyses of ItchRO were not all statistically significant, the data suggest that maralixibat was safe and may reduce pruritus in Alagille syndrome.

Clinical Trials: ClinicalTrials.gov (NCT02057692)

Alagille syndrome (ALGS) is a rare autosomal dominant disorder, classically manifested by cholestatic liver disease and variable involvement of the heart, eyes, face, skeleton, kidneys, and vasculature.(1) The liver disease of ALGS can present in infancy with marked cholestasis and fat malabsorption, with the subsequent development of intense pruritus, which may be debilitating, causing cutaneous mutilation and disruption of sleep and school activities. In cholestatic liver disease, pruritus appears to be associated with elevated total serum bile acids (SBAs), although the specifics of the relationship are not well-understood.

The management of pruritus in ALGS is challenging, and a variety of therapies are often used. These include antihistamines, rifampin, ursodeoxycholic acid, cholestyramine, naltrexone, and sertraline. Clinical experience suggests that these drugs have variable efficacy in reducing pruritus; however, no prospective clinical trial has quantified the effect of any of these therapies, either alone or in combination. Partial external bile diversion (PEBD) or ileal exclusion (IE), which interrupt the enterohepatic circulation, have had moderate success in reducing pruritus in ALGS patients; however, the procedures require surgery, and biliary diversion presents the long-term burden of caring for a stoma.(2, 3) Thus, refractory pruritus is, in some circumstances, an indication for liver transplantation in ALGS. Hence, there is an unmet need for the development and testing of improved medical therapies for ALGS-associated pruritus.

Maralixibat (SHP625, LUM001, Shire) is a potent inhibitor of the ileal bile acid transporter/apical sodium-dependent bile acid transporter (ASBT) (SLC10A2), that was initially developed as a cholesterol lowering agent. This transporter mediates the uptake of conjugated bile acids across the brush border membrane of the ileal enterocyte from where they are ultimately transported to the liver in the enterohepatic circulation. ASBT expression is under negative feedback regulation by luminal bile acids; thus, in the setting of cholestasis and reduced intraluminal bile acid concentrations, ASBT is maladaptively upregulated.(4, 5) Therefore, inhibiting the ileal reabsorption of bile acids may represent a useful strategy for reducing SBAs in cholestatic disease and potentially reducing pruritus.(6) Since PEBD and IE have been shown to reduce SBAs and improve pruritus and xanthomas in ALGS, pharmacological blockade of intestinal reuptake of bile acids with an ASBT inhibitor (ASBTi) may be a viable alternative to surgical intervention for pruritus in ALGS.(3, 7)

We conducted a randomized, double-blind, placebo-controlled, parallel group, multicenter trial of maralixibat in children with ALGS and pruritus. The study was designed to determine the effects of graduated doses of maralixibat for 13 weeks, compared with placebo, on pruritus, SBAs, liver enzymes, and other biochemical markers associated with cholestatic liver disease. A novel tool to assess pruritus, the itch-reported outcome (ItchRO) instrument, was administered via an electronic diary to capture twice-daily pruritus scores, the primary endpoint for this study.(8)

EXPERIMENTAL PROCEDURES

Study Population

This study enrolled children aged 1 through 18 years, who had cholestasis and pruritus caused by ALGS, which was diagnosed based upon study criteria (Supplemental Table 1) and confirmed by *JAGGED1* or *NOTCH2* genotyping. Eligibility (i.e., presence of significant pruritus) was determined using twice-daily caregiver-based assessment of pruritus via ItchRO(Obs^TM [observation of child

reported by parent/guardian/caregiver]).(8) ItchRO scores range from 0-4, with higher scores indicating increasing pruritus severity. The average daily score was derived from the highest score of the morning and evening observations, which reflects the worst pruritus of that day. Eligibility for this study required an average daily ItchRO(Obs) score of ≥ 2 for 2 consecutive weeks. Patients were excluded if they had chronic diarrhea requiring intervention, surgical interruption of the enterohepatic circulation, prior liver transplant, ALT >15 x upper limit of normal (ULN) or decompensated cirrhosis (full inclusion and exclusion criteria in Supplemental Table 2).

Written informed consent was obtained from caregivers, and assent was obtained when appropriate from the child according to local Institutional Review Board (IRB) rules. This study was approved by local IRBs and complied with the Declaration of Helsinki and Good Clinical Practice Guidelines. The study was registered at ClinicalTrials.gov (02057692). All authors had access to the study data and reviewed and approved the final manuscript. The study was developed with and conducted in collaboration with Lumena Pharmaceuticals, now part of the Shire Group of Companies, in the context of a Cooperative Research and Development Agreement (CRADA) with the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK). Lumena/Shire was not involved in the data analysis or the reporting and interpretation of the results, which was independently performed by the NIDDK-funded Childhood Liver Disease Research Network (ChiLDReN). As set out by the CRADA, Lumena/Shire was permitted to read and comment on the manuscript prior to submission.

Study Design

This was a double-blind, randomized, placebo-controlled phase 2b trial conducted at 12 NIDDK-funded ChiLDReN sites listed in Supplemental Table 3. Originally, participants were randomized to one of three treatment arms in a 2:1 randomization ratio between maralixibat and placebo (n=8 each in placebo, 70 µg/kg/day, or 140 µg/kg/day), with the primary comparison between the pooled

maralixibat groups and placebo. Approximately 1 year after the start of the study (after nine participants had begun investigational drug administration), an additional higher dose arm (280 μg/kg/day, n=8) was added to the study based upon preliminary results from a similar but smaller study conducted in the United Kingdom (IMAGO – design and preliminary results reported [NCT01903460]). To maintain the original design features of a 2:1 randomization ratio between placebo and active drug, four additional participants were randomized to placebo (n=12 total), and the primary comparison became the pooled two highest-tolerated active doses versus placebo. (A dose was considered "not tolerated" if >50% of participants in that dose cohort did not tolerate the treatment, as evidenced by dose reduction, suspension, or discontinuation due to gastrointestinal tolerability related to maralixibat.)

Randomization was performed by the Central Pharmacy using schedules (original and amended), which were prepared by a Clinical Research Organization, using permuted blocks of size 3 for the original design and size 9 for the amended study. The caregivers, participants, investigators, and the sponsor were unaware of treatment assignment until the last participants completed Week 13, at which time, the database was locked and the blind broken.

Study drug was administered once daily in the morning at least 30 minutes prior to breakfast. Dosing was escalated over 5 weeks to enhance tolerability of the study drug (Figure 1). The final dose of study drug was then maintained for 8 weeks. Study visits occurred at weeks 0, 2, 4, 8, and 13 and phone interviews at weeks 1, 3, and 6. Changes in the use of antipruritic medications during the study were not permitted.

The primary endpoint was the change in pruritus as measured by the ItchRO(Obs). The average daily ItchRO(Obs) score for 7 days pre-treatment was compared with the last 7 days of treatment (i.e., change from baseline to week

13 or end of treatment for those who discontinued early [designated Week 13]). Secondary endpoints included changes from baseline to Week 13 for SBAs, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gammaglutamyl transpeptidase (GGT), total bilirubin (TB), and direct bilirubin (DB).

Other efficacy endpoints included changes from baseline over time (weeks 2, 4, 8, and 13) for ItchRO(Obs), SBAs, ALT, ALP, GGT, TB, DB, total cholesterol, low-density lipoprotein cholesterol (LDL-C), and 7α-hydroxy-4-cholesten-3-one (C4; surrogate marker of bile acid biosynthesis). Changes from baseline in the Clinical Scratch Score (CSS; Supplemental Table 4), which has been used in pediatric studies and is based upon a scale developed by Whitington, were examined.[7, 9] Changes from baseline for ItchRO(Pt[TM] [patient]; Itch Report Outcome completed by participants if $\geq$9 years old or by caregivers with input from participants 5-8 years old) and xanthoma severity (Supplemental Table 5) were also assessed.[2] SBAs were quantified by stable-isotope dilution analysis using liquid chromatography-electrospray ionization-mass spectrometry (Division of Pathology and Laboratory Medicine, Cincinnati Children's Hospital Medical Center, Cincinnati, OH).[10]

Adverse events (AEs), serious adverse events (SAEs), treatment discontinuations due to AEs, and AEs of special interest (e.g., gastrointestinal symptoms, liver injury, fat-soluble vitamin level abnormalities, and growth retardation) were used in order to characterize the safety and tolerability of maralixibat.

Statistical Methods

Sample Size Determination. The planned sample size of 36 evaluable ALGS subjects was based on practical considerations, rather than a desired power for a pre-specified difference. With the proposed sample of 28 subjects for the primary efficacy analyses (16 maralixibat, from the two highest tolerated doses, and 12

placebo), there would be 80% power to detect an effect size of ≥ 1.12, using a two-sided Type I error of 5% and a two-sample t-test. Analyses were not adjusted for multiple comparisons, and nominal p-values are reported.

Analytic Methods. Efficacy analyses were performed using the modified intention-to-treat population (MITT), defined as all participants randomized, receiving at least one dose of study drug, and having at least one post-baseline efficacy assessment. Participants were analyzed by assigned treatment. Sensitivity analyses were performed using the Per Protocol population, defined as the MITT population who did not have a major protocol violation and the pure ITT population (all randomized and dosed participants), if it differed from the MITT (which it did not). For participants who prematurely discontinued from the study, a Last Observation Carried Forward (LOCF) approach, which only utilized values within 7 days of the last dose of study drug, was used to impute missing efficacy values. Safety, subject disposition, and baseline characteristics were analyzed using the safety population, defined as all randomized participants who received at least one dose of study drug. Participants were analyzed by treatment received. Statistical significance is defined as $p < 0.05$; no adjustments for multiplicity are applied in this phase 2b study.

For efficacy analyses, the first statistical test performed for each primary and secondary outcome measure was the comparison between the two highest-tolerated active dose groups combined (designated hereafter as maralixibat*) and placebo. In addition, all active doses combined (designated as maralixibat[a]), as well as each individual dose, were compared with placebo.

Treatment comparisons of the primary endpoint, and secondary and exploratory efficacy endpoints that are continuous, were made using an analysis of covariance (ANCOVA) model with treatment and baseline measures as covariates. Estimates of least squares mean changes and associated standard errors and 95% confidence intervals (CIs) are reported. Active treatment groups

(combined and individual) are tested against the placebo group, with adjusted mean treatment differences, standard errors, 95% CIs, and pairwise p-values reported. In addition, changes from baseline to each visit were summarized and tested. Analyses of discrete outcomes were analyzed using Fisher's exact or Cochran-Mantel-Haenszel test.

Exploratory responder analyses were defined *a priori* for ItchRO(Obs) (responder if change from baseline to Week 13 $\leq$ -1 or $\leq$ -2), CSS (responder if change from baseline to Week 13 $\leq$ -1), and clinician xanthoma scale (responder if change from baseline to Week 13 $\leq$ -1) analyzed using Fisher's exact test.

RESULTS

Participant Characteristics

Fifty-three participants were enrolled and assessed for eligibility, of whom 37 were randomized to investigational drug between November 24, 2014 and November 16, 2016 (Figure 2). Fifteen participants were ineligible due to failing the screening ItchRO(Obs) criteria. Twenty-five participants received maralixibat (8 - 70 µg/kg/d, 11 - 140 µg/kg/d, 6 - 280 µg/kg/d), while 12 received placebo. All but two participants completed the 13-week treatment period; one placebo participant was lost to follow-up on day 28, and one participant randomized to 70 µg/kg/d withdrew on day 1 with a rash and elevated liver biochemistries after receiving one dose (14 µg/kg). The mean age of participants was 6.8 years, and the majority (65%) was between 2 and 8 years old. All had a history of use of anti-pruritic medications, prescribed as per clinical practice prior to enrollment (anti-histamines 73%, ursodeoxycholic acid 84%, rifampin 68%). Laboratory parameters were characteristic of individuals with cholestasis and ALGS (mean SBAs 216.3 µM, ALT 158.7 IU/L, GGT 494.9 IU/L, total bilirubin 5.3 mg/dL, total cholesterol 405.7 mg/dL). Baseline characteristics were similar among the four groups (Supplemental Table 6).

Efficacy

In this phase 2b study, comparisons of maralixibat*, maralixibat[a] and each individual dose of maralixibat, relative to placebo, for the primary efficacy endpoint of change in pruritus, as measured by ItchRO(Obs) from baseline to Week 13, are shown in Table 1. In the first analysis of the primary efficacy endpoint, the mean adjusted difference between maralixibat* and placebo was -0.47 (95% CI: -1.14, 0.20, p=0.16). Relative to placebo, significant decreases were observed with the individual 70 and 140 µg/kg/d doses (-0.89, p=0.032 and -0.91, p=0.014, respectively). No change was observed in the 280 µg/kg/d group (-0.04, p=0.44). Change in maralixibat[a] relative to placebo was not statistically significant: -0.61 (p=0.055).

Compared with baseline, the placebo group had a significant decrease in ItchRO(Obs) at week 13 (-0.58, p=0.024). Individual responses over time to maralixibat or placebo are illustrated in Figure 3. The mean reduction from baseline in ItchRO(Obs) was similar between the groups in the first 2-4 weeks of the study during dose escalation, as all participants received the same doses of study drug. There was accentuation of the response in the 70 and 140 µg/kg/d group after 4 weeks, when participants were receiving their maximal dose of maralixibat (Figure 4).

Changes from baseline to Week 13 in SBAs and liver biochemistries were assessed as secondary endpoints (Table 2 and Supplemental Table 7). Individual responses over time for SBAs and C4 are in Supplemental Figure 1. TB and DB tended to diminish in participants receiving maralixibat, with significant decreases from baseline to Week 13 observed for maralixibat* and maralixibat[a] (Table 2 and Supplemental Table 7). Although there was no statistically significant change in TB or DB in the placebo group, when the changes in the maralixbat* and maralixibat[a] groups were compared with placebo, the changes were no longer statistically significant. For ALT, there were statistically insignificant increases during maralixibat treatment (Table 2). Pooled data over time for TB and ALT for maralixibat- and placebo-treated participants

are seen in Figure 5. No changes in GGT, alkaline phosphatase, or total cholesterol were observed (Supplemental Table 7). Individual responses over time for TB and ALT are in Supplemental Figure 1. LDL, but not total, cholesterol was significantly reduced relative to placebo with maralixibat* treatment (Supplemental Table 7). SBAs were variable and were not significantly reduced during maralixibat treatment (Table 2 and Figure 5). C4 tended to increase with therapy, although the changes were not significant, and the levels were highly variable (Table 2 and Figure 5). One participant receiving 70 µg/kg/d of maralixibat had extraordinarily high baseline SBAs (1014 µmol/L) and C4 (161.9 ng/mL). Pooled data for those participants receiving maralixibat revealed a reduction in SBAs in the first 4 weeks of the study with a potentially compensatory increase in C4 by Week 8 (Figure 5).

Additional responder analyses were conducted to further assess the impact of maralixibat on pruritus. Only five participants (four maralixibat and one placebo) had a decrease of at least two units in ItchRO(Obs), and none of the maralixibat groups yielded a significant difference relative to placebo (Supplemental Table 8). With a less stringent threshold of -1, overall response rates were higher and statistically significantly greater in maralixibat[a] (68% vs. 25%, p=0.03, Supplemental Table 8). ItchRO(Pt) could only be assessed in 23 of the study participants (Supplemental Table 9). The improvement in placebo group was nearly the same as maralixibat* and maralixibat[a] [-1.189 (0.3734), p=0.843, and -1.281 (0.2831), p=0.685, respectively]. Maralixibat had a significant impact on changes from baseline to Week 13 in CSS. Improvement was significantly greater in maraxibat*, maralixibat[a],140, and 280 µg groups relative to placebo (Supplemental Table 10). Using a pre-defined responder analysis at a cut-off of $\leq$-1 and a post-hoc cut-off of $\leq$-3, significant changes from baseline to Week 13 were observed (maralixibat* 76% vs. placebo 25%, p=0.01, and maralixibat* 35% vs. placebo 0%, p=0.028, respectively, Supplemental Table 10). Individual changes over time for CSS are seen in Figure 6. Changes in xanthomas with

maralixibat* treatment were not significant (31% vs. 9%, p=0.350; Supplemental Table 11).

Safety

No deaths occurred during the study. There was one SAE of vomiting leading to hospitalization in a participant receiving 70 μg/kg/d of maralixibat that was not felt to be related to study drug. Maralixibat was stopped in the first week of the study for one participant who developed a rash and elevated ALT, which the investigator considered unlikely to be related to the study drug. Treatment-emergent AEs were common and comparable in participants receiving maralixibat and placebo (Supplementary Table 12). Given the proposed mechanism of action of maralixibat, gastrointestinal AEs, including diarrhea and abdominal pain, were of special interest and found to occur at similar rates in maralixibat[a]- and placebo-treated participants (overall gastrointestinal 52% vs. 58%, diarrhea 32% vs. 50%, abdominal pain 16% vs. 17%, respectively). There was no clinically significant difference in change in weight from baseline to Week 13 in maralixibat versus placebo-treated participants (maralixibat[a] 0.73 ± 0.12 kg vs. 0.69 ± 0.17, mean $\pm$ SE, p=0.842).

Criteria for identification of drug-induced liver injury (DILI) or therapy-related hepatotoxicity in the setting of chronic cholestasis are not well-defined, either in the literature or at a regulatory level. For the purposes of this study, specific changes from baseline were developed *a priori* as potential safety signals requiring enhanced monitoring, and stopping rules were established (Supplemental Table 13). There were no participants in either the maralixibat- or placebo-treated groups who met these stopping criteria (data not shown). The criteria were also not met if baseline was defined as the average of screening and baseline laboratory values (data not shown).

Fat-soluble vitamin levels were also examined as a potential safety signal, given the profound cholestasis in these participants, the high dosing requirements of

fat-soluble vitamins as part of their routine care, and the potential for changes in intestinal luminal bile acid concentrations. Changes were characterized relative to baseline and as shifts from sufficient to insufficient or the converse, from insufficient to sufficient, as previously defined (Supplemental Table 14).(11) In general, the numbers of participants who changed their sufficiency status during the course of the study was low. In the maralixibat group, vitamin D levels changed in equal percentages to insufficient and sufficient during the study (13% for each). Vitamin A levels became insufficient in one maralixibat-treated participant, while vitamin E levels became sufficient in two maralixibat-treated participants. Excess levels of vitamin A (n=4) and vitamin D (n=1) were observed at week 13 of maralixibat therapy. In three maralixibat-treated participants, international normalized ratio (INR) increased as a potential marker of change in vitamin K sufficiency. None of these changes were observed in the placebo group.

DISCUSSION

Data from this randomized placebo-controlled trial in children with ALGS suggest that maralixibat is generally safe, well-tolerated, and may reduce pruritus. The first pre-defined analysis of the primary endpoint did not meet the pre-defined statistical level for efficacy, while other analyses of the primary endpoint did. The reason for the heterogeneous responses cannot be determined from these investigations, and further study to assess safety and efficacy of maralixibat as a treatment for cholestasis-associated pruritus in children with cholestasis is warranted.

The expected therapeutic benefit of maralixibat is based on the response of ALGS patients to surgical interruption of the enterohepatic circulation, primarily via PEBD or IE.(2, 3, 7) This response may not be as dramatic as has been observed in some individuals with PFIC. In general, responses tend to be favorable, with improvement in pruritus, xanthomas, and hypercholesterolemia.

There are less clear or minimal effects on SBAs, bilirubin, and ALT.(3) In most cases, surgery is performed for severe pruritus equivalent to CSS grade 4. Not all patients have complete resolution of their pruritus, but most have clinically-significant improvement, equivalent to a reduction in CSS of 2 or more. ItchRO was not available to assess response in any of these previously-published experiences. The favorable response to surgery has been generally documented over the first 12 to 24 months after surgery. None of the published studies of surgical intervention have examined results at 3 months after surgery, so direct comparison to this study is not possible. The relatively short time course of the current study may not have fully captured the potential efficacy of maralixibat. Ongoing, long-term follow-up studies (NCT02047318, NCT02117713) may shed light on this matter, although these studies are not placebo-controlled, with the exception of a 4-week randomized placebo-controlled withdrawal of maralixibat in ICONIC (NCT02160782).

The primary endpoint for this study was pruritus as assessed by ItchRO(Obs). This endpoint was chosen in recognition of the profound impact of pruritus on children with ALGS and their families. Pruritus is notoriously difficult to objectively assess in clinical settings, and this challenge is amplified in research trials. Instruments for assessing pruritus can broadly be categorized into patient-reported outcome tools and those that are independent of patient reporting, such as the CSS. An intermediate level of reporting is by parental/guardian observation of children, as was reported in this study. No single instrument, including actigraphy, has been fully validated to capture the multidimensional features of pruritus and its change over time, especially in children. To address this, a novel tool, ItchRO, was developed using rigorous tool development methodology to assess pruritus in children with cholestasis, specifically those with ALGS, for the purposes of this clinical trial.(8) Detailed analyses of ItchRO and the effect of maralixibat on quality of life, which is quite complex and beyond the scope of this report, will be the subject of a future report from this study.(12)

The placebo effect in this study was evident and highlights the critical need for double-blind placebo-controlled study design in clinical trials addressing pruritus. It is well-recognized that somatic symptoms, such as pain and fatigue, can be improved by placebo due to positive expectations, but the effect of placebo on itch in cholestasis has not been extensively studied. In the placebo-treated arm of a study of the effect of another ASBTi (GSK2330672) on pruritus in patients with primary biliary cholangitis (PBC), there was a 23% improvement in itch on a 10-point numerical rating scale.(13) A similar reduction of 0.6 in ItchRO was observed in the unpublished results of IMAGO (NCT01903460).

Pharmacologic inhibition of ASBT is an evolving potential approach to the treatment of constipation, cholestasis, diabetes, and fatty liver disease.(14) The findings of the current study should be considered in the context of recent related investigations of cholestatic liver disease. The only peer-reviewed published report of an ASBTi is in adults with PBC.(13) This was a 2-week double-blind, randomized, placebo-controlled, crossover phase 2a trial of 22 patients receiving GSK2330672 (NCT01899703). The primary endpoints of the study were safety and tolerability, which were deemed acceptable. Pruritus, quantified using three separate scores, was reduced by 30 to 57% with 2 weeks of therapy. Decreased SBAs and compensatory increases in C4 supported the expected biological effect on intestinal bile acid transport. The biochemical characteristics of the cholestasis in the adult PBC study participants at baseline were less severe than in the children with ALGS in the current study (e.g., mean TB: 12.2μM PBC and 90.6μM ALGS; SBAs: 48.6μM PBC and 216.3 μM ALGS). Additional studies of ASBTi in cholestasis have been preliminarily reported as abstracts or as registered trials. An open-label dose-ranging phase II study of A4250 (Albireo Pharma) in 19 children with a variety of cholestatic conditions demonstrated improvement in pruritus in 14 children as assessed by a visual itch score recorded by caretakers.(15) The small sample size (14 maralixibat and 6 placebo) in IMAGO, which was conducted in the United Kingdom, may have limited the power of the study to identify a potential effect (NCT01903460).

SBA levels are an attractive choice as an endpoint for the use of ASBTi in pediatric cholestasis. Levels represent a complex dynamic interplay of intestinal absorption and hepatic extraction, synthesis, and excretion. The experiences in this study of ALGS raise important concerns about this possible endpoint. First, there is significant variability in SBAs in children with ALGS, which necessitates a fairly large sample size to demonstrate a potential therapeutic effect. Ursodeoxycholic acid, which was used by 84% of the participants, is not actively transported by ASBT and, as such, its common use in ALGS may contribute significantly to SBAs and thus complicate the use of SBAs to define ASBTi effect. Fecal bile acid determination, which would be a direct assay of ASBTi activity, is cumbersome and was not employed in this study. Changes in C4 are used as an alternative surrogate marker of ASBTi activity, as diminished ileal absorption is predicted to lead to reduced fibroblast growth factor-19 mediated ileal signaling to the liver and de-repression of bile acid biosynthesis reflected by elevated C4. In this study, C4 levels were highly variable and may in part explain the lack of a statistically significant increase with maralixibat. The magnitude of changes in C4 suggest that a maximal ASBTi effect was not induced by the doses of drug used in this study. Alternatively, luminal bile acids in children with severe cholestasis with ALGS may be so low that an effect on C4 is difficult to demonstrate. LDL cholesterol levels were reduced, consistent with enhanced conversion of cholesterol to bile acids, as has been observed in surgical interruption of the enterohepatic circulation.(16) Total cholesterol levels, that are reflective of lipoprotein X accumulation, would not be expected to be impacted as quickly by ASBTi.

No significant safety issues were identified in this study of maralixibat. One of the potentially attractive features of ASBTi is that they can act at the luminal ileal brush border membrane without significant systemic absorption, thereby reducing potential risk of toxicity. The major potential predicted side effect of ASBTi relates to sequelae of bile acid malabsorption with related diarrhea and

abdominal pain. Approximately half of the children who received maralixibat had gastrointestinal symptoms, none of which were severe. The critical importance of a blinded placebo control is reconfirmed by the finding of similar gastrointestinal problems in the placebo-treated group. One of the complexities of clinical trials in cholestatic liver disease is the lack of understanding of approaches to monitoring for adverse effects on the liver itself.(17) DILI is a major concern in the development of new drugs, but is poorly characterized in chronic liver disease, especially cholestatic disease. Cholestatic features can be the most worrisome for serious drug-related injury. Given the exceptionally limited systemic absorption of maralixibat, DILI was of limited concern. Despite this, prospective methods for monitoring potential drug toxicity needed to be established. The parameters chosen in this study (Supplemental Table 13) did not reveal hepatotoxicity related to this treatment approach. These parameters might be considered for future studies in ALGS. The placebo treatment group, along with the screening and enrollment laboratory studies, provide additional novel insight into the natural variability of key liver parameters in ALGS that may guide future clinical studies of novel pharmacologic agents in pediatric cholestasis.

One of the major limitations of this study is the unexpected lack of response in the children receiving 280 μg/kg/d of maralixibat. It is unlikely that this is the result of an excessive dose of drug, especially in light of the relatively limited increase in C4. ALGS is a rare disorder, and the number of participants in the study was selected more on practical rather than power considerations. The randomization process in this small phase 2b study allocated only six participants to 280 μg/kg/d dosing, and this limited sample size may have skewed the findings. Both the placebo and 280 μg/kg/d groups were characterized by a preponderance of children younger than 5 years of age (Supplemental Table 6). ItchRO is a new tool, whose performance has had limited experience, and it is unclear if it performs equally at all ages. The positive CSS response in the 280 μg/kg/d group suggests that this could be an issue.

In conclusion, despite the inconsistent findings of the analyses of the primary endpoint of this study, the data in total suggest that maralixibat is generally safe and well-tolerated, and may reduce pruritus in ALGS. There is a clear unmet and significant need in the management of pruritus in ALGS and other cholestatic liver diseases in children. Continued investigation of maralixibat is warranted. These studies should continue to consider ongoing potential placebo effects and seek methods to determine dose responses on ileal ASBT activity and their relationship to biochemical and symptomatic effects.

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FIGURE LEGENDS

Figure 1. Study schema

Figure 2. CONSORT diagram

Figure 3. Spaghetti plots of changes in ItchRO(Obs) over time. Each line represents an individual participant. The participants are grouped by the target dose of study drug.

Figure 4. Pooled analysis of change from baseline of ItchRO(Obs). Mean change from baseline in ItchRO(Obs) is pooled amongst participants receiving the same dose of maralixibat. Dashed lines represent transition of some participants to a new dose of maralixibat.

Figure 5. Box and whisker plots of changes in measured laboratory parameters (A: Total bilirubin; B: Alanine aminotransferase; C: Serum bile acids; D: C4) over time. Maralixibat[a] is compared with placebo. Box and whiskers legend: Mean=o or + inside box, median=line inside box, box=interquartile range (25th to 75th percentiles), whiskers values within 1.50*interquartile range (IQR), and outliers as individual data points beyond the whiskers.

Figure 6. Spaghetti plots of changes in the clinician scratch scale (CSS) over time. Each line represents an individual participant. The participants are grouped by the target dose of study drug.

TABLES

Placebo-Controlled Randomized Trial of an Intestinal Bile Salt Transport Inhibitor

for Pruritus in Alagille Syndrome

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Table 1. Analysis of Primary Endpoint – Change from Baseline to Week 13 in ItchRO(Obs)

Outcome Measure Treatment Group Maralixibat	N	Baseline Mean (SE)	LS Means Change (SE)	Change from Baseline 95% CI	p-value	Treatment Comparison to Placebo Difference in LS Means (SE)	95% CI	p-value
ItchRO(Obs) Average Daily Score								
70 μg/kg/day	8	3.2 (0.23)	-1.5 (0.30)	(-2.1, -0.9)	<.001	-0.89 (0.40)	(-1.70, -0.08)	0.032
140 μg/kg/day	11	2.7 (0.16)	-1.5 (0.26)	(-2.0, -1.0)	<.001	-0.91 (0.35)	(-1.62, -0.19)	0.014
280 μg/kg/day	6	3.3 (0.24)	-0.6 (0.36)	(-1.3, 0.1)	0.093	-0.04 (0.44)	(-0.94, 0.86)	0.930
Maralixibat*	17	2.9 (0.15)	-1.1 (0.21)	(-1.5, -0.6)	<.001	-0.47 (0.33)	(-1.14, 0.20)	0.159
Maralixibat[a]	25	3.0 (0.13)	-1.2 (0.18)	(-1.6, -0.8)	<.001	-0.61 (0.31)	(-1.24, 0.01)	0.055
Placebo	12	2.8 (0.15)	-0.6 (0.25)	(-1.1, -0.1)	0.024			
LS = least-squares; SE = standard error; CI = confidence interval								

Table 2. Analysis of Secondary Efficacy Endpoints – Change from Baseline to Week 13

Outcome Measure Treatment Group Maralixibat	N	Baseline Mean (SE)	LS Means Change (SE) [1]	Change from Baseline 95% CI	p-value	Treatment Comparison to Placebo Difference in LS Means (SE)	95% CI	p-value

Outcome Measure Treatment Group Maralixibat	N	Baseline Mean (SE)	Change from Baseline LS Means Change (SE) [1]	95% CI	p-value	Treatment Comparison to Placebo Difference in LS Means (SE)	95% CI	p-value
Serum Bile Acid (μmol/L)								
70 μg/kg/day	7	392 (126.8)	-117 (46.2)	(-212, -23)	0.016	-107 (57.2)	(-224, 10)	0.071
140 μg/kg/day	11	151 (37.3)	-40 (34.9)	(-111, 31)	0.256	-30 (47.5)	(-127, 67)	0.534
280 μg/kg/day	6	188 (44.0)	-27 (46.3)	(-122, 67)	0.558	-17 (56.5)	(-132, 98)	0.766
Maralixibat*	17	164 (28.2)	-34 (29.2)	(-93, 26)	0.255	-23 (43.6)	(-112, 65)	0.594
Maralixibat[a]	24	231 (45.6)	-62 (23.9)	(-111, -13)	0.015	-51 (40.6)	(-134, 32)	0.216
Placebo	12	205 (46.9)	-10 (32.7)	(-77, 56)	0.751			
Total Bilirubin (mg/dL)								
70 μg/kg/day	7	7.96 (3.39)	-0.29 (0.38)	(-1.06, 0.48)	0.447	-0.39 (0.47)	(-1.35, 0.56)	0.407
140 μg/kg/day	11	3.36 (1.06)	-0.35 (0.30)	(-0.97, 0.26)	0.251	-0.46 (0.42)	(-1.31, 0.40)	0.284
280 μg/kg/day	6	4.22 (2.10)	-0.80 (0.40)	(-1.62, 0.02)	0.054	-0.91 (0.49)	(-1.92, 0.10)	0.076
Maralixibat*	17	3.66 (0.97)	-0.58 (0.25)	(-1.09, -0.06)	0.029	-0.68 (0.38)	(-1.47, 0.10)	0.086
Maralixibat[a]	24	4.92 (1.23)	-0.48 (0.21)	(-0.91, -0.06)	0.027	-0.59 (0.35)	(-1.31, 0.13)	0.107
Placebo	12	6.41 (1.95)	0.10 (0.28)	(-0.48, 0.68)	0.719			
Alanine Aminotransferase (ALT) (U/L)								
70 μg/kg/day	7	155 (33.7)	15 (17.9)	(-22, 51)	0.422	27 (22.8)	(-20, 73)	0.253
140 μg/kg/day	11	117 (17.4)	13 (14.9)	(-17, 44)	0.383	25 (21.0)	(-18, 68)	0.241
280 μg/kg/day	6	191 (42.4)	30 (19.6)	(-10, 70)	0.142	41 (23.7)	(-7, 90)	0.090

Outcome Measure Treatment Group Maralixibat	N	Baseline Mean (SE)	Change from Baseline			Treatment Comparison to Placebo		
			LS Means Change (SE) [1]	95% CI	p-value	Difference in LS Means (SE)	95% CI	p-value
Maralixibat*	17	143 (20.0)	21 (12.1)	(-3, 46)	0.086	33 (18.6)	(-5, 71)	0.082
Maralixibat[a]	24	146 (16.9)	19 (10.0)	(-1, 40)	0.066	31 (17.3)	(-4, 66)	0.082
Placebo	12	188 (26.9)	-12 (14.0)	(-40, 17)	0.400			
LS = least-squares; SE = standard error; CI = confidence interval								
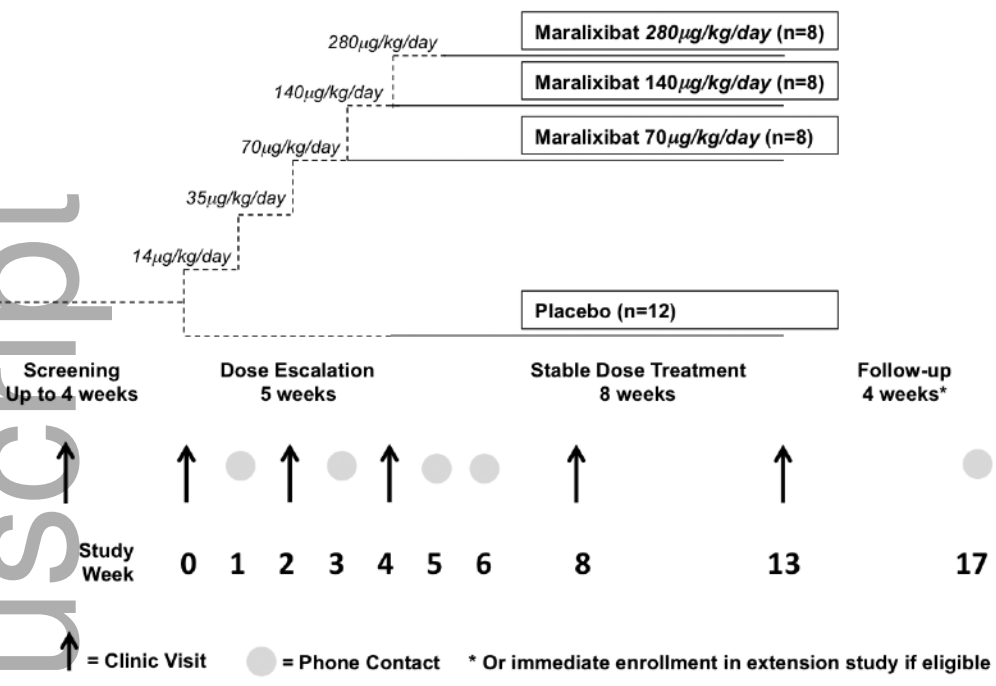
hep4_1244_f1.tif

hep4_1244_f2.tif

Maralixibat 70 μg/kg/day   Maralixibat 140 μg/kg/day

Maralixibat 280 μg/kg/day   Placebo

ItchRO (Obs) Average Daily Score

Baseline   Week 2   Week 4   Week 8   Week 13

hep4_1244_f3.tif

Dashed line (——) represents Maralixibat dose escalation from prior dose.
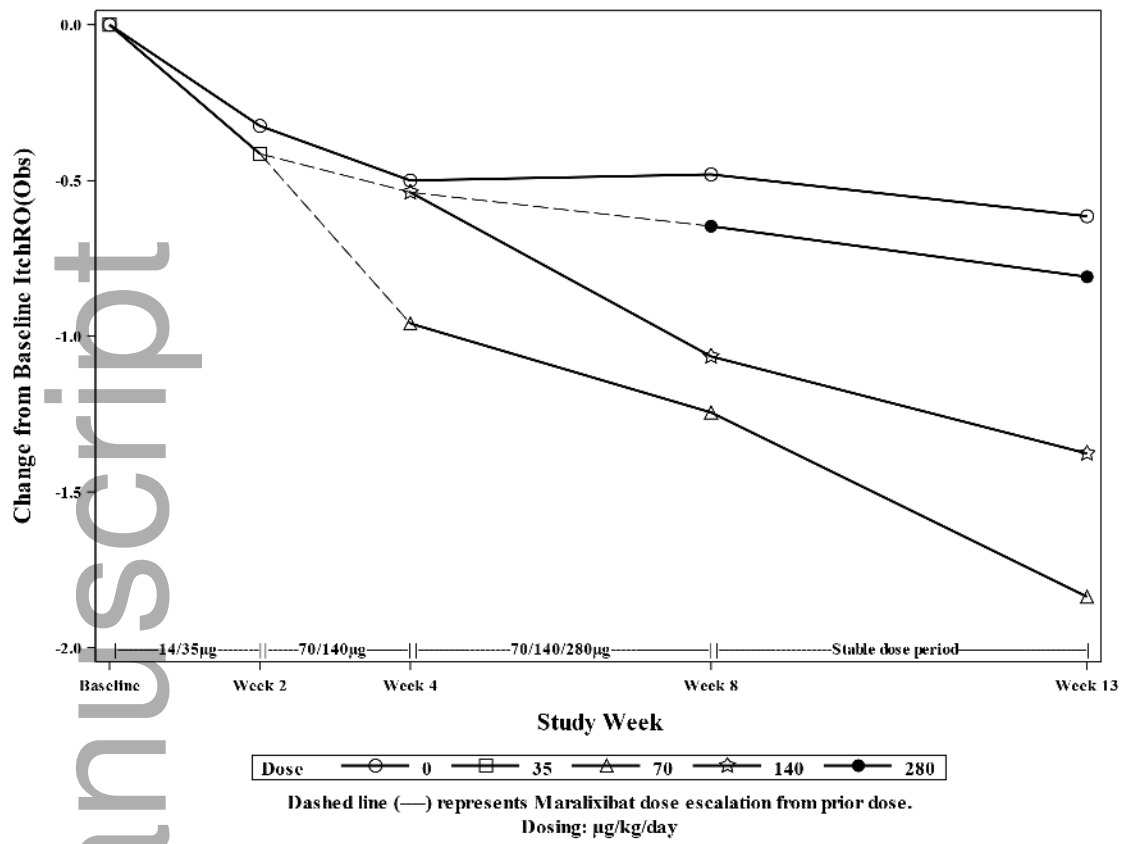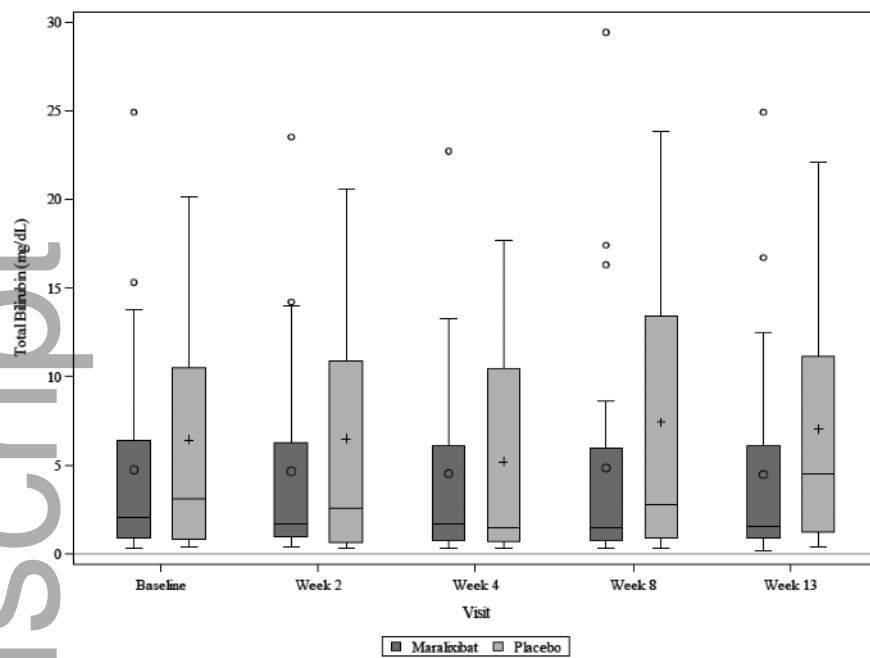Dosing: μg/kg/day
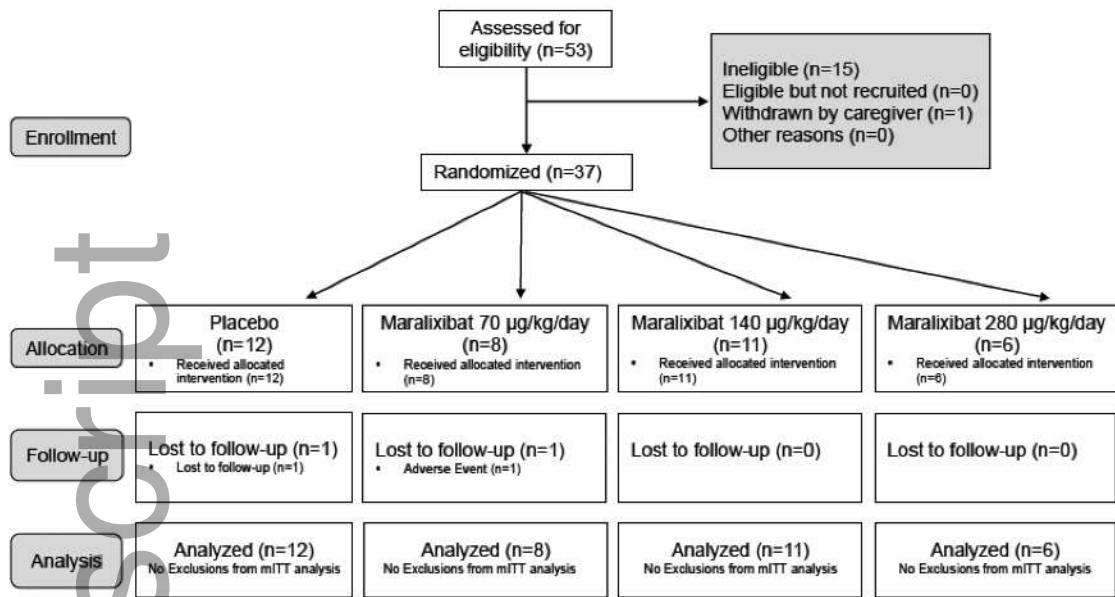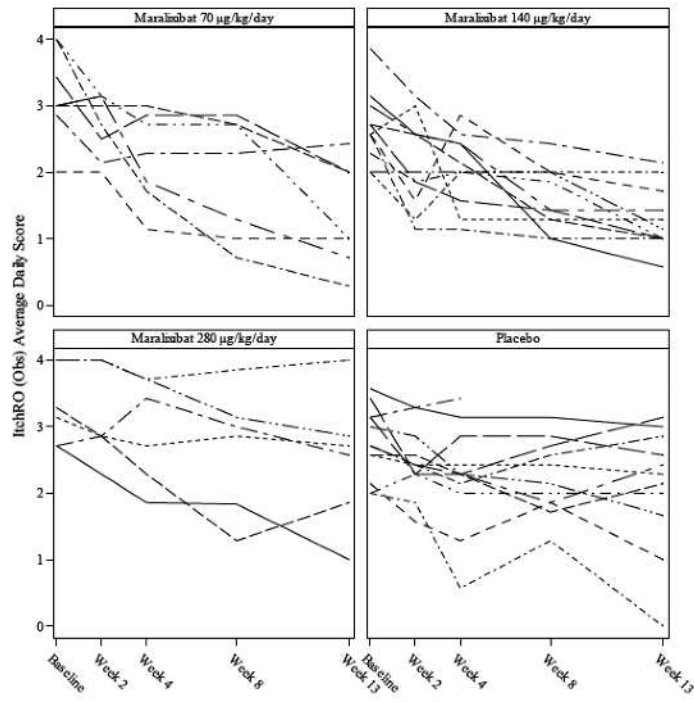
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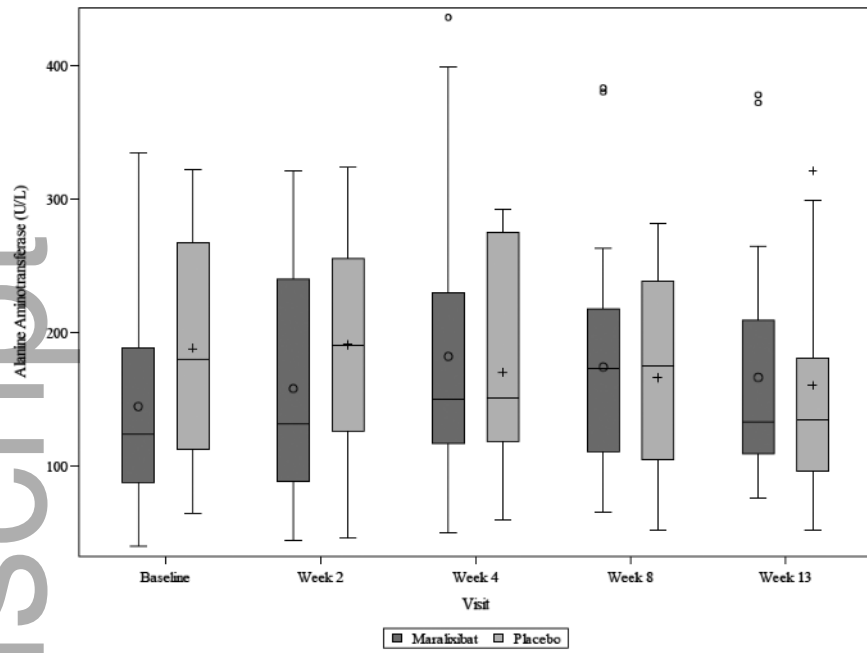
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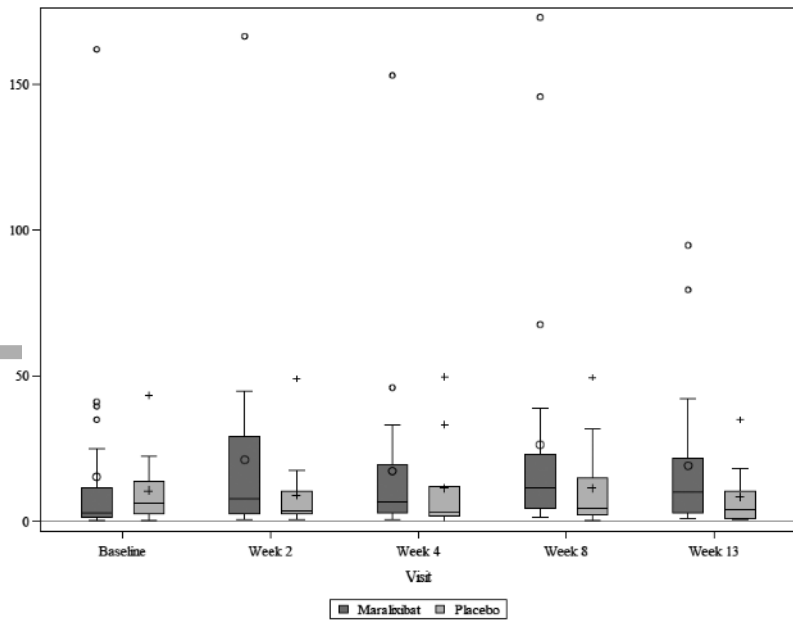
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