Maralixibat in progressive familial intrahepatic cholestasis (MARCH-PFIC): a multicentre, randomised, double-blind, placebo-controlled, phase 3 trial

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Summary

Background Progressive familial intrahepatic cholestasis (PFIC) is a group of autosomal recessive disorders, the most prevalent being BSEP deficiency, resulting in disrupted bile formation, cholestasis, and pruritus. Building on a previous phase 2 study, we aimed to evaluate the efficacy and safety of maralixibat—an ileal bile acid transporter inhibitor—in participants with all types of PFIC.

Methods MARCH-PFIC was a multicentre, randomised, double-blind, placebo-controlled, phase 3 study conducted in 29 community and hospital centres across 16 countries in Europe, the Americas, and Asia. We recruited participants aged 1–17 years with PFIC with persistent pruritus (>6 months; average of ≥1·5 on morning Itch-Reported Outcome [Observer; ItchRO(Obs)] during the last 4 weeks of screening) and biochemical abnormalities or pathological evidence of progressive liver disease, or both. We defined three analysis cohorts. The BSEP (or primary) cohort included only those with biallelic, non-truncated BSEP deficiency without low or fluctuating serum bile acids or previous biliary surgery. The all-PFIC cohort combined the BSEP cohort with participants with biallelic FIC1, MDR3, TJP2, or MYO5B deficiencies without previous surgery but regardless of bile acids. The full cohort had no exclusions. Participants were randomly assigned (1:1) to receive oral maralixibat (starting dose 142·5 µg/kg, then escalated to 570 µg/kg) or placebo twice daily for 26 weeks. The primary endpoint was the mean change in average morning ItchRO(Obs) severity score between baseline and weeks 15–26 in the BSEP cohort. The key secondary efficacy endpoint was the mean change in total serum bile acids between baseline and the average of weeks 18, 22, and 26 in the BSEP cohort. Efficacy analyses were done in the intention-to-treat population (all those randomly assigned) and safety analyses were done in all participants who received at least one dose of study drug. This completed trial is registered with ClinicalTrials.gov, NCT03905330, and EudraCT, 2019-001211-22.

Findings Between July 9, 2019, and March 4, 2022, 125 patients were screened, of whom 93 were randomly assigned to maralixibat (n=47; 14 in the BSEP cohort and 33 in the all-PFIC cohort) or placebo (n=46; 17 in the BSEP cohort and 31 in the all-PFIC cohort), received at least one dose of study drug, and were included in the intention-to-treat and safety populations. The median age was 3·0 years (IQR 2·0–7·0) and 51 (55%) of 93 participants were female and 42 (45%) were male. In the BSEP cohort, least-squares mean change from baseline in morning ItchRO(Obs) was –1·7 (95% CI –2·3 to –1·2) with maralixibat versus –0·6 (–1·1 to –0·1) with placebo, with a significant between-group difference of –1·1 (95% CI –1·8 to –0·3; p=0·0063). Least-squares mean change from baseline in total serum bile acids was –176 µmol/L (95% CI –257 to –94) for maralixibat versus 11 µmol/L (–58 to 80) for placebo, also representing a significant difference of –187 µmol/L (95% CI –293 to –80; p=0·0013). The most common adverse event was diarrhoea (27 [57%] of 47 patients on maralixibat *vs* nine [20%] of 46 patients on placebo; all mild or moderate and mostly transient). There were five (11%) participants with serious treatment-emergent adverse events in the maralixibat group versus three (7%) in the placebo group. No treatment-related deaths occurred.

Interpretation Maralixibat improved pruritus and predictors of native liver survival in PFIC (eg, serum bile acids). Maralixibat represents a non-surgical, pharmacological option to interrupt the enterohepatic circulation and improve the standard of care in patients with PFIC.

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Research in context

Evidence before this study

Progressive familial intrahepatic cholestasis (PFIC) is a group of rare autosomal recessive liver diseases characterised by canalicular bile transport defects, resulting in disrupted bile formation, progressive cholestasis with elevation of serum bile acids, and pruritus. In children, PFIC represents 10–15% of indications for liver transplantation, either due to severe pruritus or end-stage liver disease. In bile salt export pump (BSEP) and familial intrahepatic cholestasis-associated protein 1 (FIC1) deficiencies, there is a clear relationship between reductions in serum bile acid and native liver survival after surgical biliary diversion. More recently, pharmacological agents have been developed to interrupt the enterohepatic circulation via ileal bile acid transporter inhibition in patients with genetic cholestasis. We conducted a PubMed search for research articles published from database inception to Oct 25, 2023, with no language restrictions, using the terms "progressive familial intrahepatic cholestasis" AND "clinical trial". We identified published clinical trial data on five other agents from 10 trials being investigated for efficacy in pruritus and fat-soluble vitamin deficiency in PFIC. Most studies had no comparator or had poor follow-up. Two ileal bile acid transporter inhibitors have been evaluated to treat PFIC. Odevixibat reduced pruritus and serum bile acids versus placebo in a placebo-controlled study in patients with BSEP and FIC1 deficiencies. Maralixibat reduced pruritus and serum bile acids and led to 5-year transplantation-free survival in children with BSEP deficiency who had clinically relevant and statistically significant serum bile acid reductions in a long-term, open-label study.

Added value of this study

To our knowledge, MARCH-PFIC is the first placebo-controlled, randomised clinical study evaluating the efficacy and safety of an ileal bile acid transporter inhibitor in patients with PFIC types beyond BSEP and FIC1 deficiencies. The study assessed the efficacy and safety of oral maralixibat (maximum dose 570 µg/kg twice daily) versus placebo during 26 weeks. MARCH-PFIC met its primary and secondary efficacy endpoints of changes in morning pruritus severity and bile acids in patients with biallelic, non-truncated BSEP deficiency, as well as these endpoints in patients with FIC1, MDR3, TJP2, or MYO5B deficiencies. Improvements in bilirubin concentrations and growth with maralixibat were in keeping with the potential benefits associated with ileal bile acid transporter inhibition beyond pruritus relief. We observed improved efficacy in serum bile acids than that described in the previous phase 2 study that used a dose of 266 µg/kg maralixibat once daily. Maralixibat was generally well tolerated throughout the treatment period.

Implications of all the available evidence

MARCH-PFIC presents the first data showing the therapeutic benefit of an ileal bile acid transporter inhibitor across PFIC types. Maralixibat improved pruritus and predictors of native liver survival in PFIC (serum bile acids and total bilirubin). Maralixibat represents a non-surgical, pharmacological option to interrupt the enterohepatic circulation and improve the standard of care in patients with PFIC.

Introduction

Progressive familial intrahepatic cholestasis (PFIC) is a group of rare autosomal recessive liver diseases characterised by canalicular bile transport defects, resulting in disrupted bile formation, progressive cholestasis with elevation of serum bile acids, and pruritus.[1] In children, PFIC represents 10–15% of indications for liver transplantation, either due to intractable pruritus or end-stage liver disease.[2–4] The most prevalent PFIC types are bile salt export pump (BSEP) deficiency, also known as PFIC2 (50–60%), multidrug resistance protein 3 (MDR3; phosphatidylcholine translocator ABCB4) deficiency, also known as PFIC3 (30–40%), and familial intrahepatic cholestasis-associated protein 1 (FIC1; phospholipid-transporting ATPase IC) deficiency, also known as PFIC1 (10–20%).[5] Other types include tight junction protein 2 (TJP2; tight junction protein ZO-2) deficiency, also known as PFIC4, and unconventional myosin-VB (MYO5B) deficiency, also known as PFIC6.[6–8] In addition, a minority of patients with a PFIC phenotype elude genetic diagnosis with current methods.[9]

Severe pruritus is a common feature of PFIC, greatly affecting quality of life and leading to liver transplantation when symptoms are refractory.[5,10–13] The accumulation of bile acids in PFIC is an important mediator of pruritus and driver of liver disease progression. Interruption of enterohepatic bile acid recirculation has the potential to alleviate pruritus and prevent liver damage.[1] Surgical biliary diversion has been performed to interrupt the enterohepatic circulation of bile acids in children with pruritus who do not respond to clinical management.[6] In a large retrospective analysis in patients with BSEP deficiency, reduction of serum bile acid concentrations by at least 75% or to less than 102 µmol/L after surgical biliary diversion was associated with long-term native liver survival.[14]

More recently, pharmacological agents have been developed to interrupt the enterohepatic circulation via ileal bile acid transporter (ileal sodium/bile acid cotransporter) inhibition in patients with genetic cholestasis, including PFIC.[1,15] The efficacy and safety of ileal bile acid transporter inhibitors, however, remain to be established in the broader PFIC group beyond BSEP and FIC1 deficiencies. Maralixibat is a minimally absorbed ileal bile acid transporter inhibitor approved for the treatment of cholestatic pruritus in patients with Alagille syndrome aged 3 months or older in the USA and

2 months or older in the EU. In a phase 2 open-label study, maralixibat 266 µg/kg per day improved pruritus and led to 5-year transplantation-free survival in children with BSEP deficiency who had clinically relevant and statistically significant reductions in serum bile acid levels after drug administration.[1] Here, we aimed to evaluate the efficacy and safety of a higher dose of maralixibat in a phase 3, placebo-controlled study—the largest to date, to our knowledge—in which patients with all PFIC types were eligible to participate.

Methods

Study design and participants

MARCH-PFIC was a 26-week, multicentre, randomised, double-blind, parallel-group, placebo-controlled, phase 3 study of maralixibat in children with PFIC. The study was performed across 29 community and hospital centres in Europe, North America, South America, and west and southeast Asia (Lebanon, Brazil, Mexico, Colombia, Poland, Italy, the USA, Argentina, Austria, Belgium, Canada, France, Germany, Singapore, Türkiye, and the UK). Participants who completed the 26-week placebo-controlled study were eligible to enrol in an open-label, long-term extension study (MRX-503).

The trial adhered to the Declaration of Helsinki and the International Conference on Harmonisation guidelines for Good Clinical Practice. The protocol, informed consent form, and other relevant study documents were submitted to an institutional review board/institutional ethics committee (IRB/IEC) by the investigator and reviewed and approved by the IRB/IEC before study initiation (see appendix p 1 for additional details on IRB centres). Any amendments to the protocol required IRB/IEC approval before the implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study participants. In addition, the IRB/IEC approved all advertising used to recruit participants for the study. Notification of serious adverse drug reactions was provided to the IRB/IEC according to local regulations and guidelines. Caregivers and patients (when applicable) provided written informed consent and assent before the study. The protocol can be found online.

Participants aged 12 months or older and younger than 18 years with a clinical diagnosis of PFIC with persistent (>6 months) pruritus and biochemical abnormalities, pathological evidence of progressive liver disease, or both were included. All participants had an average morning pruritus severity score by Itch-Reported Outcome (Observer; ItchRO[Obs]) of 1·5 or higher during the last 4 consecutive weeks of screening. The study was open to all patients with a clinical diagnosis of PFIC regardless of documentation of pathogenic variants or previous biliary surgery.

Participants were not eligible if they had a history of other diseases associated with pruritus (added at the beginning of recruitment with a protocol amendment on Nov 22, 2019; no participants with a history of other diseases associated with pruritus were enrolled), decompensated cirrhosis, possible malignant liver mass on imaging, or an alanine aminotransferase (ALT) or total serum bilirubin greater than 15 times the upper limit of normal (ULN). Medications to treat cholestasis or pruritus were allowed during the study if participants had been on stable dosing for 30 days before screening, and dosing was expected to remain stable during the study. Complete eligibility criteria are provided in the appendix (pp 2–3).

Randomisation and masking

After confirmation of study eligibility, participants were randomly assigned (1:1) to receive maralixibat or placebo in the primary cohort (participants with biallelic, non-truncated BSEP deficiency without low or fluctuating serum bile acid concentrations or previous biliary surgery) or in the supplemental cohort (other PFIC types, regardless of serum bile acids or previous surgery). In the supplemental cohort, participants were stratified by FIC1, MDR3, or other PFIC. Participants with BSEP deficiency with complete absence of BSEP function (ie, truncated BSEP deficiency) or non-biallelic disease, previous biliary surgery, or with low (<3×ULN) or fluctuating serum bile acid concentrations (intermittent cholestasis) were stratified into the other PFIC category (participants with another known mutation [not FIC1 or MDR3] or an unknown mutation). Randomisation was performed via a computer-generated list by use of a stratified block design with a block size of 4. The randomisation sequence was prepared by an unmasked statistician not associated with the conduct of the clinical trial. Participants were assigned to treatment groups by use of an interactive response technology system. All participants, investigators, and study personnel involved in the conduct of the study, including data management, were masked to treatment assignment. All packaged study medication components, including the dosing dispensers, were identical to maintain blinding.

Procedures

Study medication was provided as an oral solution. The placebo contained all components of the active drug except the active drug substance. Study products were dosed by bodyweight on a twice-daily regimen. Participants who met eligibility criteria after completion of all screening visit (visit 0) assessments entered the 26-week, double-blind treatment period. This period did not commence until all screening assessments required to confirm eligibility for randomisation had been completed and the PFIC genotype results had been confirmed by sponsor or designee. Standard-of-care genotyping results, performed according to the clinical practice of each study site, were reviewed and documented by the sponsor for confirmation of PFIC type and for determination of cohort assignment. The

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See **Online** for appendix

For the **protocol** see https://clinicaltrials.gov/study/NCT03905330#more-information

treatment period included a dose escalation period lasting 4–6 weeks and a stable dosing period lasting 20–22 weeks, with a clinic visit every other week in the first 6 weeks and every 4 weeks thereafter, totalling nine (appendix p 6). In the dose escalation period, doses of maralixibat free base were increased weekly (starting dose of 142·5 µg/kg twice daily) to a maximum dose of 570 µg/kg twice daily or to a maximum tolerated dose. Dose reductions were permitted for safety and tolerability reasons down to a minimum dose of 142·5 µg/kg twice daily. Patients who could not tolerate that dose were discontinued from the study.

The ItchRO(Obs) diaries, intended for completion by caregivers, have been validated in paediatric cholestasis to evaluate pruritus.[6] In MARCH-PFIC, pruritus was primarily assessed using the ItchRO(Obs) scale administered as a twice-daily electronic diary (morning and evening). The ItchRO(Obs) severity score is based on a discrete 0–4 scale, for which 0 represents no itch, 1 represents mild, 2 represents moderate, 3 represents severe, and 4 represents very severe itch.[6] A reduction of 1 point or more in the ItchRO(Obs) severity score is considered to be a clinically meaningful improvement.[16] The Exploratory Diary Questionnaire (Observer; EDQ[Obs]) was also administered twice daily (morning and evening) and is a 1–5 scale (1=never or no itch to 5=almost always or very severe itch) that includes a question focused on sleep disturbances related to pruritus (ie, "Because of itch, my child had trouble staying asleep"). The Clinician Scratch Scale provided the clinician's assessment of pruritus severity (scratching), as observed at the clinic visit.[16] The Clinician Scratch Scale is scored on a 0–4 scale, for which 0 represents none and 4 represents cutaneous mutilations, haemorrhage, and scarring (worst scratching). The ItchRO(Obs), EDQ(Obs), and Clinician Scratch Scale were completed from the start of screening to week 26. Additional information on the scales appears in the appendix (pp 3–4).

Blood samples were collected at every clinic visit. Laboratory measurements included complete blood count, coagulation, chemistry, lipid panel, and total serum bile acid concentration, which were measured at every clinical visit apart from visit 3, and fat-soluble vitamins, which were measured at every clinic visit apart from visit 2 and 3. Weight and height were measured every 2 weeks during the first 10 weeks of the trial, and every 4 weeks for the remainder of the trial. Abdominal ultrasound was performed at the screening visit and at week 26 (end of treatment; visit 9) to determine the presence of any liver mass. As the trial enrolled children, biological sex data were provided by caregivers using the options of "female" or "male".

Safety and adverse events were monitored throughout the study and during 7 days of follow-up for those who did not enrol in the extension study. The severity of adverse events was determined as follows: mild (an adverse events that is usually transient and might require only minimal treatment or therapeutic intervention; the event does not generally interfere with usual activities of daily living); moderate (an adverse event that is usually alleviated with specific therapeutic intervention; the event interferes with usual activities of daily living, causing discomfort, but poses no clinically significant or permanent risk of harm to the participant); and severe (an adverse event that interrupts usual activities of daily living, considerably affects clinical status, or might require intensive therapeutic intervention). Descriptions of the adverse events of clinical interest can be found in the appendix (appendix p 15).

We defined three cohorts for analysis in our study. The BSEP cohort included only those who had biallelic variations in *ABCB11*, coding for BSEP, with potential residual BSEP function (non-truncated BSEP deficiency), without low (<3×ULN) or fluctuating serum bile acid concentrations (the same as the primary cohort used for randomisation). The all-PFIC cohort included patients in the BSEP cohort combined with participants (regardless of bile acid concentrations) with biallelic FIC1, MDR3, TJP2, or MYO5B deficiencies. The BSEP and all-PFIC cohorts excluded any participants with biliary surgery at baseline and genotype variations affecting only one allele (heterozygous). The full cohort had no exclusions and included all enrolled participants. The analysis populations and their definitions are depicted in the appendix (p 7).

Outcomes

The primary efficacy endpoint was the mean change in the average morning ItchRO(Obs) severity score between baseline and the last 12 weeks of treatment (weeks 15–26) in the BSEP cohort, as reported by caregivers. The key secondary efficacy endpoint was the mean change in total serum bile acid concentration between baseline and the average of weeks 18, 22, and 26 in the BSEP cohort. Additional secondary efficacy endpoints were the primary and key secondary endpoints analysed in the all-PFIC cohort and the proportions of participants with improvements in pruritus and serum bile acids (pruritus and serum bile acid responders, respectively) in both the BSEP and all-PFIC cohorts. Pruritus response was defined as having an average morning ItchRO(Obs) severity score reduction from baseline to the last 12 weeks of treatment (weeks 15–26) of at least 1 point or an average severity score of 1·0 or less over the same time period. A serum bile acid response was defined as having an average (at weeks 18, 22, and 26) serum bile acid concentration of less than 102 µmol/L (applied only if baseline serum bile acid was ≥102 µmol/L) or an average decrease of 75% or more from baseline.[14]

Exploratory efficacy endpoints included mean change from baseline in additional parameters of pruritus (highest daily, evening, and morning frequency ItchRO[Obs] and Clinician Scratch Scale scores),

EDQ(Obs) sleep disturbance score, liver chemistry, and growth (height and weight Z scores). Safety endpoints comprised the incidence of adverse events, adverse events by severity, and adverse events by relationship to study drug.

Statistical analysis

The sample size was selected to provide a power of at least 80% for the primary analysis. A between-treatment group (placebo to active) difference in least-squares mean change from baseline of 0·663 and a pooled SD of 0·563 were assumed. The estimations of difference in least-squares mean change from baseline, SD, and effect size for the sample size justification were based on ItchRO(Obs) data from maralixibat phase 2 studies. Participants with BSEP deficiency given maralixibat from study LUM001-501 (NCT02057718) were compared with participants with Alagille syndrome given placebo from studies LUM001-301 (NCT02057692) and LUM001-302 (NCT01903460) as no placebo data are available from study LUM001-501.[1,16,17] This calculation required 26 participants based on a two-sided, two-sample *t* test at the 0·05 significance level. Assuming a 10% dropout rate, approximately 30 participants (15 participants per group) were required in the BSEP cohort for the primary analysis.

All efficacy analyses were conducted in the intention-to-treat population, defined as all randomly assigned participants, separately for the BSEP and all-PFIC cohorts. Safety was evaluated in all three analysis cohorts and conducted in the safety population, defined as all participants who received at least one dose of study drug.

Baseline ItchRO(Obs) and EDQ(Obs) average morning, evening, and highest scores were constructed as the average of four weekly (7-day) average scores in the period consisting of the 28 days immediately before the date of first dose of study drug. The numerator and denominators used in the derivation of scores were based on the number of non-missing scores. For liver biochemistry laboratory parameters (ie, ALT, aspartate aminotransferase, alkaline phosphatase, total bilirubin, direct bilirubin, gamma-glutamyl transferase, and albumin), baseline was defined as the average of data collected between screening and visit 1 (day 0), or the last non-missing measurement before receiving the first dose of study drug if only one value was available. For all other parameters, baseline values were defined as data measured or collected at the baseline visit, visit 1 (day 0), before the first dose of study drug was administered. If data were not measured or collected at the baseline visit, then the last non-missing measurement before receiving the first dose of study drug was used as the baseline value.

A hierarchical testing procedure was used for the primary and secondary efficacy endpoints (see appendix pp 4–5 for order of testing). A restricted maximum likelihood-based longitudinal mixed-effects model for repeated measures in the intention-to-treat population of the BSEP cohort was used as the primary analysis method. For the all-PFIC cohort analyses, PFIC types (ie, FIC1, non-truncated BSEP, MDR3, and the combination of TJP2 and MYO5B) were also included in the mixed-effects model for repeated measures as covariates. The repeated measures included post-baseline time periods during the dose escalation period (ie, weeks 1–6) and stable dosing period (ie, weeks 7–10, 11–14, 15–18, 19–22, and 23–26), with change from baseline in the 6-week or 4-week average morning ItchRO(Obs) severity score as the dependent variable. The model included the fixed, categorical effects of treatment group, time period, and treatment-group-by-time-period interaction, as well as the continuous, fixed covariates of baseline 4-week average morning ItchRO(Obs) severity score and the baseline-score-by-time-period interaction. An unstructured variance and covariance matrix was used to model the variances and covariances for the six timepoints included in the model. Unstructured variance and covariance does not impose any restrictions on the pattern of the matrix elements. The primary efficacy analysis compared maralixibat and placebo using the difference in least-squares mean change from baseline between treatment groups across the last 12 weeks of the study (ie, weeks 15–18, 19–22, and 23–26 combined). The analytical solution of the overall treatment effect obtained from the mixed-effects model for repeated measures is an equally weighted average of the three individual visit-specific estimates over the time period of interest (ie, the last 12 weeks of the study). Significance tests were based on least-squares means, with a two-sided significance level (two-sided 95% CIs). The underlying assumptions of the mixed-effects model for repeated measures of normality and homogeneity of variance of residuals were assessed and confirmed graphically.

Analyses similar to those described for the primary efficacy endpoint were performed for each of the change from baseline secondary efficacy endpoints. For serum bile acids, seven post-baseline visits were used (weeks 2, 6, 10, 14, 18, 22, and 26). Similar to the primary endpoint, the average of the last three visits (weeks 18, 22, and 26) was used in the analysis.

Analyses with longitudinal models included all available data from participants with non-missing change from baseline data. The only exclusion was for participants without baseline data (and thus no change from baseline could be calculated) or no post-baseline data.

Multiple imputation was used as a sensitivity analysis for missing data. Missing values were imputed by use of a regression-based multiple imputation model. For participants with complete data up to a particular timepoint (eg, time period average score for the primary endpoint and analysis visit for the key secondary endpoint), a multiple regression model was fit that included the outcome at that timepoint as the dependent

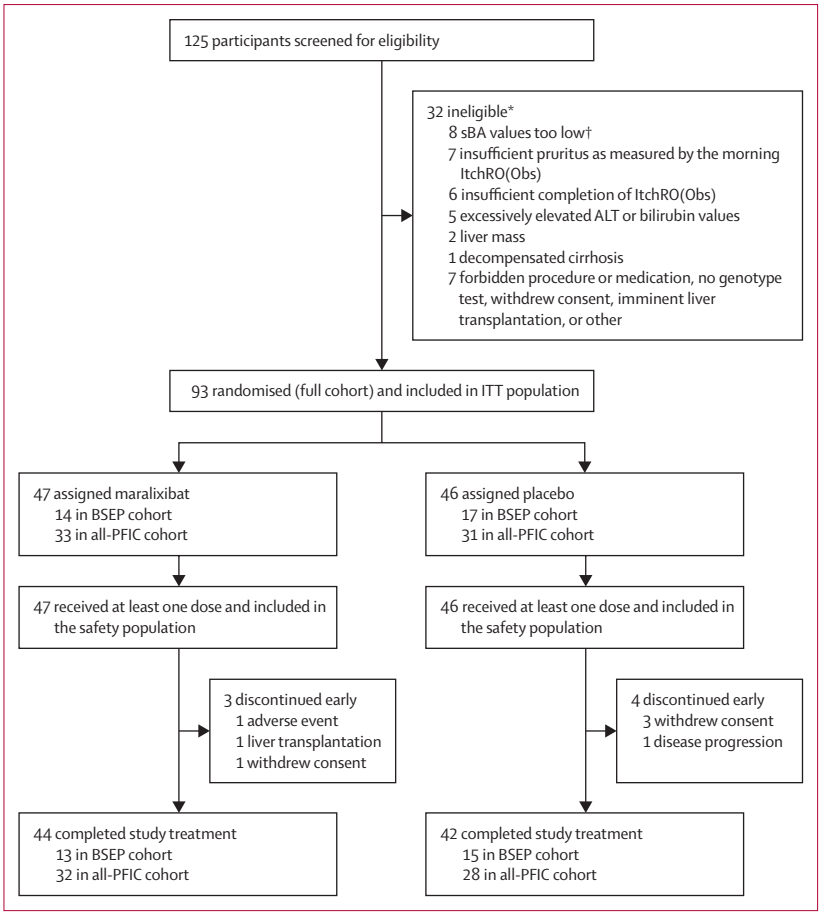

Figure 1: **Trial profile**
The BSEP (or primary) cohort includes only those with biallelic, non-truncated BSEP deficiency without low or fluctuating serum bile acids or previous biliary surgery. The all-PFIC cohort combines the BSEP cohort with participants with biallelic FIC1, MDR3, TJP2, or MYO5B deficiencies without previous surgery but regardless of bile acids. BSEP=bile salt export pump. ALT=alanine aminotransferase. ItchRO(Obs)=Itch-Reported Outcome (Observer). ITT=intention-to-treat. PFIC=progressive familial intrahepatic cholestasis. sBA=serum bile acid. *Participants might have more than one reason for ineligibility. †Participants were excluded for having a sBA concentration of less than three times the upper limit of normal in all cohorts until a protocol amendment restricted this exclusion criterion to the primary cohort only, after which these participants were randomly assigned in the supplemental cohort.

variable and observed data (eg, outcomes at previous timepoints, treatment, and baseline) as independent variables. Separate models were similarly constructed for each timepoint. Using these regression models, a missing value for a participant at a particular timepoint was imputed as a draw from the predictive distribution given the outcomes at previous timepoints (some possibly imputed), treatment group, and so on. This process was repeated 20 times, and the mixed-effects model for repeated measures analyses were performed separately for each of the completed analysis datasets; the results were combined into one multiple imputation inference.

For responder endpoints, the number and proportion of participants who were considered responders were summarised by treatment group for each analysis visit or time period, as appropriate. Barnard's exact unconditional test was used to calculate the p value for the difference between treatment groups. For responder endpoints, participants without data were excluded. Exact CIs were obtained using the method of Chan and Zhang. Additional information can be found in the appendix (pp 4–5). For safety, qualitative variables were summarised using counts and percentages and quantitative variables were summarised using descriptive statistics (eg, mean [SD]).

Post-hoc sensitivity analyses were performed in additional populations to further evaluate the efficacy findings: the population of patients with FIC1, MDR3, TJP2, or MYO5B deficiencies; the population of patients with no pathogenic variants identified; and the population of patients with truncated BSEP deficiency. Of note, the FIC1, MDR3, TJP2, and MYO5B subgroup was composed of the all-PFIC cohort excluding the BSEP cohort to examine whether inclusion of the BSEP cohort in the all-PFIC cohort affected the results. Post-hoc analyses testing normalisation of total and direct bilirubin were performed to evaluate findings in the all-PFIC cohort.

Statistical analyses were done using SAS, version 9.4. An independent data monitoring committee oversaw the safety of study participants. This study is registered with ClinicalTrials.gov, NCT03905330, and EudraCT, 2019-001211-22.

Role of the funding source

This study was funded by Mirum Pharmaceuticals, which was involved in study design, data collection, data analysis, and data interpretation. Mirum Pharmaceuticals provided support for writing this report.

Results

125 children with PFIC were screened for the trial between July 9, 2019, and March 4, 2022 (figure 1). The last participant completed the study on Sept 1, 2022. 93 participants were randomly assigned to maralixibat (n=47) or placebo (n=46) and were included in the intention-to-treat population (figure 1). 32 participants were ineligible at screening. The top four reasons for exclusion from the trial were serum bile acid concentrations too low (n=8), insufficient pruritus (n=7), insufficient completion of ItchRO (Obs; n=6), and excessively elevated ALT or bilirubin (n=5). 86 (92%) of 93 participants completed the 26-week treatment period, with seven participants discontinuing from the study across all groups (withdrawal of consent [n=4]; adverse event [diarrhoea; n=1]; liver transplantation [n=1]; and disease progression [n=1]). All randomly assigned participants also received at least one dose of study drug and were included in the safety population.

Baseline demographic and clinical characteristics are depicted in table 1. One participant had intermittent cholestasis (fluctuating serum bile acid concentrations) before study enrolment (BSEP deficiency). This participant was included only in the full cohort. At baseline, in the

	BSEP cohort		All-PFIC cohort		Full cohort	
	Maralixibat (n=14)	Placebo (n=17)	Maralixibat (n=33)	Placebo (n=31)	Maralixibat (n=47)	Placebo (n=46)
Age, years	6·3 (5·2); 4·0 (3·0 to 11·0)	4·2 (3·6); 3·0 (1·0 to 7·0)	4·9 (4·1); 3·0 (2·0 to 7·0)	4·4 (3·6); 3·0 (1·0 to 7·0)	4·8 (4·2); 3·0 (2·0 to 7·0)	4·7 (3·6); 3·5 (1·0 to 7·0)
Sex						
Male	7 (50%)	6 (35%)	17 (52%)	13 (42%)	20 (43%)	22 (48%)
Female	7 (50%)	11 (65%)	16 (48%)	18 (58%)	27 (57%)	24 (52%)
Race						
White	9 (64%)	9 (53%)	24 (73%)	19 (61%)	36 (77%)	34 (74%)
American Indian or Alaska Native	3 (21%)	3 (18%)	3 (9%)	4 (13%)	3 (6%)	4 (9%)
Black	1 (7%)	2 (12%)	1 (3%)	2 (6%)	1 (2%)	2 (4%)
Asian	0	0	3 (9%)	0	3 (6%)	0
>1 race (mixed)	1 (7%)	2 (12%)	2 (6%)	4 (13%)	3 (6%)	4 (9%)
Not reported	0	1 (6%)	0	2 (6%)	1 (2%)	2 (4%)
PFIC type						
Non-truncated BSEP	14 (100%)	17 (100%)	14 (42%)	17 (55%)	18 (38%)	18 (39%)
Truncated BSEP	..	..	..	..	5 (11%)	4 (9%)
FIC1	..	..	7 (21%)	6 (19%)	9 (19%)	8 (17%)
MDR3	..	..	4 (12%)	5 (16%)	4 (9%)	5 (11%)
TJP2	..	..	6 (18%)	1 (3%)	6 (13%)	2 (4%)
MYO5B	..	..	2 (6%)	2 (6%)	2 (4%)	2 (4%)
Heterozygous variant	..	..	..	..	0	2 (4%)
No pathogenic variant	..	..	..	..	3 (6%)	5 (11%)
Surgery*	..	..	..	..	5 (11%)	3 (7%)
Pruritus						
ItchRO(Obs)†	2·9 (0·9); 3·0 (1·8 to 3·6)	2·6 (0·9); 2·2 (1·8 to 3·1)	2·9 (0·9); 2·9 (2·3 to 3·6)	2·7 (0·9); 2·6 (1·9 to 3·7)	2·8 (0·8); 2·9 (2·1 to 3·6)	2·9 (0·8); 3·0 (2·0 to 3·7)
Clinician Scratch Scale	2·8 (0·8); 3 (2 to 3)	2·5 (0·9); 3 (2 to 3)	2·8 (0·7); 3 (2 to 3)	2·6 (1·0); 3 (2 to 3)	2·7 (0·9); 3 (2 to 3)	2·7 (1·0); 3 (2 to 3)
Sleep disturbance	n=9	n=15	n=27	n=26	n=39	n=38
EDQ(Obs)	3·7 (1·0); 3·5 (3·1 to 4·5)	3·6 (1·1); 3·7 (2·8 to 4·5)	3·7 (0·8); 3·7 (3·1 to 4·5)	3·7 (1·0); 3·7 (3·0 to 4·5)	3·6 (0·8); 3·6 (3·0 to 4·4)	3·8 (0·9); 3·7 (3·2 to 4·5)
Total serum bile acid	n=12	n=17	n=33	n=31	n=45	n=46
Total serum bile acid, µmol/L	312 (158); 352 (219 to 423)	312 (152); 313 (218 to 442)	254 (140); 246 (162 to 363)	272 (147); 262 (184 to 348)	263 (151); 255 (162 to 389)	243 (153); 226 (155 to 336)
Alkaline phosphatase, U/L	536 (321); 414 (377 to 632)	443 (156); 423 (336 to 516)	630 (286); 517 (423 to 754)	518 (290); 471 (339 to 596)	595 (292); 498 (389 to 733)	513 (255); 471 (339 to 604)
Gamma-glutamyl transferase, U/L	18 (14); 15 (9 to 28)	22 (17); 19 (9 to 27)	65 (140); 22 (14 to 31)	70 (119); 22 (10 to 51)	52 (119); 21 (11 to 31)	60 (107); 20 (11 to 34)
Aspartate aminotransferase, U/L	104 (74); 83 (54 to 110)	158 (126); 104 (77 to 246)	97 (55); 83 (60 to 110)	130 (101); 86 (74 to 157)	120 (100); 87 (60 to 124)	126 (96); 90 (62 to 152)
Alanine aminotransferase, U/L	98 (80); 83 (50 to 87)	155 (128); 119 (66 to 199)	88 (62); 73 (50 to 102)	127 (104); 92 (58 to 149)	108 (90); 81 (50 to 129)	121 (99); 85 (58 to 142)
Total bilirubin, mg/dL	3·48 (3·90); 1·73 (0·60 to 5·50)	2·71 (2·96); 1·85 (0·80 to 3·85)	4·12 (3·80); 2·80 (1·35 to 5·50)	4·04 (4·46); 2·55 (0·80 to 5·50)	4·10 (3·74); 2·80 (1·33 to 5·30)	3·80 (4·64); 2·38 (0·80 to 3·95)
Direct bilirubin, mg/dL	2·42 (2·92); 1·08 (0·35 to 3·20)	1·92 (2·14); 1·30 (0·45 to 2·95)	2·98 (2·82); 2·05 (0·90 to 4·00)	2·93 (3·35); 1·85 (0·45 to 4·30)	2·99 (2·80); 2·05 (0·90 to 3·95)	2·77 (3·52); 1·70 (0·55 to 2·97)
International normalised ratio	1·38 (0·83); 1·05 (0·95 to 1·25)	1·17 (0·37); 1·08 (0·96 to 1·20)	1·44 (1·50); 1·04 (0·99 to 1·15)	1·14 (0·30); 1·07 (0·96 to 1·20)	1·36 (1·26); 1·05 (0·99 to 1·22)	1·12 (0·25); 1·07 (0·99 to 1·18)
Albumin, g/dL	4·56 (0·16); 4·55 (4·45 to 4·70)	4·88 (0·35); 4·95 (4·70 to 5·03)	4·57 (0·22); 4·60 (4·45 to 4·75)	4·72 (0·34); 4·70 (4·45 to 4·95)	4·67 (0·29); 4·65 (4·45 to 4·85)	4·69 (0·37); 4·70 (4·40 to 4·90)
Platelets	n=10	n=15	n=26	n=25	n=38	n=37
Platelets, $\times 10^3$/µL	350 (219); 286 (246 to 464)	397 (171); 381 (245 to 558)	370 (199); 333 (257 to 452)	372 (222); 339 (215 to 544)	364 (185); 362 (257 to 453)	371 (234); 327 (215 to 522)
Height Z score	−1·96 (1·39); −1·80 (−2·58 to −1·06)	−2·19 (1·41); −2·34 (−3·39 to −1·81)	−2·08 (1·29); −1·96 (−2·83 to −1·19)	−2·06 (1·48); −2·34 (−3·37 to −0·96)	−2·01 (1·33); −1·96 (−2·83 to −1·06)	−1·91 (1·32); −1·97 (−2·93 to −0·96)

(Table 1 continues on next page)

	BSEP cohort		All-PFIC cohort		Full cohort	
	Maralixibat (n=14)	Placebo (n=17)	Maralixibat (n=33)	Placebo (n=31)	Maralixibat (n=47)	Placebo (n=46)
(Continued from previous page)						
Weight Z score	−1·53 (1·38); −1·43 (−1·75 to −1·17)	−1·24 (1·50); −1·09 (−2·08 to −0·65)	−1·75 (1·29); −1·46 (−2·45 to −0·95)	−1·28 (1·33); −1·09 (−2·26 to −0·33)	−1·56 (1·36); −1·40 (−2·41 to −0·91)	−1·22 (1·22); −1·14 (−2·10 to −0·33)
Ursodeoxycholic acid use	11 (79%)	17 (100%)	27 (82%)	30 (97%)	39 (83%)	39 (85%)
Rifampicin use	6 (43%)	9 (53%)	18 (55%)	15 (48%)	26 (55%)	23 (50%)

Data are n (%) or mean (SD); median (IQR). The BSEP (or primary) cohort includes only those with biallelic, non-truncated BSEP deficiency without low or fluctuating serum bile acids or previous biliary surgery. The all-PFIC cohort combines the BSEP cohort with participants with biallelic FIC1, MDR3, TJP2, or MYO5B deficiencies without previous surgery but regardless of bile acids. BSEP=bile salt export pump. EDQ(Obs)=Exploratory Diary Questionnaire (Observer). FIC1=familial intrahepatic cholestasis-associated protein 1. ItchRO(Obs)=Itch-Reported Outcome (Observer). MDR3=multidrug resistance protein 3. MYO5B=unconventional myosin-VB. PFIC=progressive familial intrahepatic cholestasis. TJP2=tight junction protein 2. *Surgery participants had the following PFIC types: non-truncated BSEP (maralixibat: three; placebo: zero), FIC1 (maralixibat: two; placebo: two), and TJP2 (maralixibat: zero; placebo: one). †4-week morning average severity score.

Table 1: **Baseline characteristics**

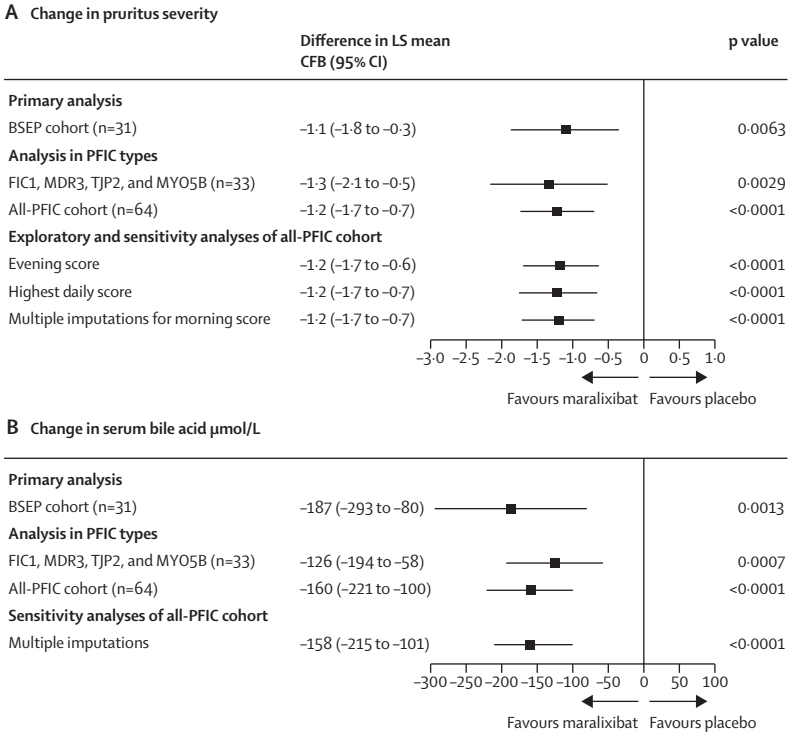

Figure 2: **Efficacy in the intention-to-treat population**
(A) Change in pruritus severity, comprising the primary endpoint (morning ItchRo[Obs] in the BSEP cohort), a secondary endpoint (morning ItchRo[Obs] in the all-PFIC cohort), exploratory endpoints in the all-PFIC cohort (evening and highest daily ItchRo[Obs]), and additional post-hoc analyses in participants with FIC1, MDR3, TJP2, and MYO5B deficiencies and in the all-PFIC cohort after multiple imputations. (B) Change in serum bile acids for the BSEP and all-PFIC cohorts (secondary endpoints), in participants with FIC1, MDR3, TJP2, and MYO5B deficiencies (post-hoc analysis), and in the all-PFIC cohort after multiple imputations (post-hoc analysis). BSEP=bile salt export pump. CFB=change from baseline. FIC1=familial intrahepatic cholestasis-associated protein 1. ItchRo(Obs)=Itch-Reported Outcome (Observer). LS=least-squares. MDR3=multidrug resistance protein 3. MYO5B=unconventional myosin-VB. PFIC=progressive familial intrahepatic cholestasis. TJP2=tight junction protein 2.

full cohort, the median age was 3·0 years (IQR 2·0–7·0) and 51 (55%) of 93 participants were female and 42 (45%) were male. Most participants were receiving ursodeoxycholic acid (78 [84%] of 93) or rifampicin (49 [53%]). In all cohorts, serum bile acid concentrations and pruritus severity were similar between the maralixibat and placebo groups.

In the BSEP cohort, treatment with maralixibat led to significantly greater reductions in least-squares mean change from baseline in the morning ItchRO(Obs) score versus placebo (–1·7 [95% CI –2·3 to –1·2]; p<0·0001 *vs* –0·6 [–1·1 to –0·1]; p=0·017), with a difference in least-squares mean change from baseline between groups of –1·1 (95% CI –1·8 to –0·3; p=0·0063; figure 2A; appendix pp 13–14). Results were consistent for the highest daily and evening ItchRO(Obs) scores (appendix pp 13–14). Significantly greater reductions from baseline in total serum bile acids were also noted with maralixibat than with placebo, with a least-squares mean change from baseline of –176 µmol/L (95% CI –257 to –94; p=0·0001) for maralixibat versus an increase of 11 µmol/L (–58 to 80; p=0·74) for placebo, representing a significant difference of –187 µmol/L (95% CI –293 to –80; p=0·0013; figure 2B).

Similar findings were observed in the all-PFIC cohort. The difference in least-squares mean change from baseline in morning ItchRO(Obs) score in the maralixibat versus placebo groups was –1·2 (95% CI –1·7 to –0·7; p<0·0001), with a least-squares mean reduction of –1·8 (95% CI –2·2 to –1·4; p<0·0001) in the maralixibat group versus –0·6 (–1·0 to –0·2; p=0·0026) with placebo (appendix p 14). Results were consistent for the evening and highest daily pruritus severity scores (figure 2A). In the all-PFIC cohort, there was a significant difference in least-squares mean change from baseline in serum bile acid concentrations between groups (–160 µmol/L [–221 to –100]; p<0·0001; figure 2B), with a least-squares mean reduction of –157 µmol/L (–200 to –115; p<0·0001) for maralixibat versus an increase of 3 µmol/L (–42 to 48; p=0·90) for placebo. Post hoc, similar reductions in serum bile acid concentrations and pruritus severity were observed in the subgroup composed of participants with FIC1, MDR3, TJP2, and MYO5B deficiencies (figure 2A, B; appendix p 8) and in participants with no pathogenic variants identified (appendix pp 9–10). In participants with truncated BSEP deficiency assigned maralixibat, reductions from baseline to week 26 in serum bile acid were seen in individual participants post hoc, with limited improvements in pruritus (appendix pp 9–10).

Sensitivity analyses using multiple imputation for the change from baseline for morning ItchRO(Obs) score and serum bile acid concentration were nearly identical to the observed results (appendix p 14).

In the BSEP and all-PFIC cohorts, improvements in pruritus severity and reductions in serum bile acid concentrations in maralixibat-treated participants were observed by week 2 and maintained during the study (figures 3A, B). Greater pruritus and serum bile acid response rates in the maralixibat group versus placebo group were observed in both cohorts, although the difference in pruritus responders in the BSEP cohort was not statistically significant, meaning, per the hierarchical testing procedure, reported p values are nominal (figures 3C, D). There were significant reductions in ItchRO(Obs) morning frequency score, Clinician Scratch Scale, and EDQ(Obs) sleep disturbance scores in participants given maralixibat versus placebo in the BSEP and all-PFIC cohorts (appendix p 11).

Treatment with maralixibat led to reductions in total and direct bilirubin in the all-PFIC cohort, with a difference of total bilirubin in least-squares mean change from baseline versus placebo of –2·0 mg/dL (95% CI –4·0 to –0·03; p=0·047; appendix pp 12–13), but there was no significant difference in the BSEP cohort. In a post-hoc analysis (all-PFIC cohort) in 43 participants with elevated total bilirubin at baseline (>1·2 mg/dL), ten (40%; BSEP n=4, FIC1 n=3, MDR3 n=1, TJP2 n=1, and MYO5B n=1) of 25 participants (BSEP n=9, FIC1 n=7, MDR3 n=4, TJP2 n=4, and MYO5B n=1) on maralixibat had normalisation of total bilirubin (averaged over weeks 18, 22, and 26) versus zero of 18 participants in the placebo group (difference 40·0 percentage points [95% CI 16·9 to 61·3]; p=0·0024; appendix p 12). Maralixibat also normalised direct bilirubin in more patients on maralixibat than on placebo (appendix p 12). There was no difference in change in ALT between groups (appendix p 14). Additional comparisons for liver chemistry and select laboratory measurements of interest are shown in the appendix (p 14).

In the all-PFIC cohort, there was a significant difference in least-squares mean change from baseline in weight Z score for maralixibat versus placebo (0·23 [95% CI 0·01–0·44]; p=0·039; appendix p 13), but the difference was not significant in the BSEP cohort (0·11 (–0·16 to 0·39; p=0·41). The difference in the least-squares mean change from baseline in height Z score for maralixibat versus placebo was 0·21 (–0·04 to 0·45;

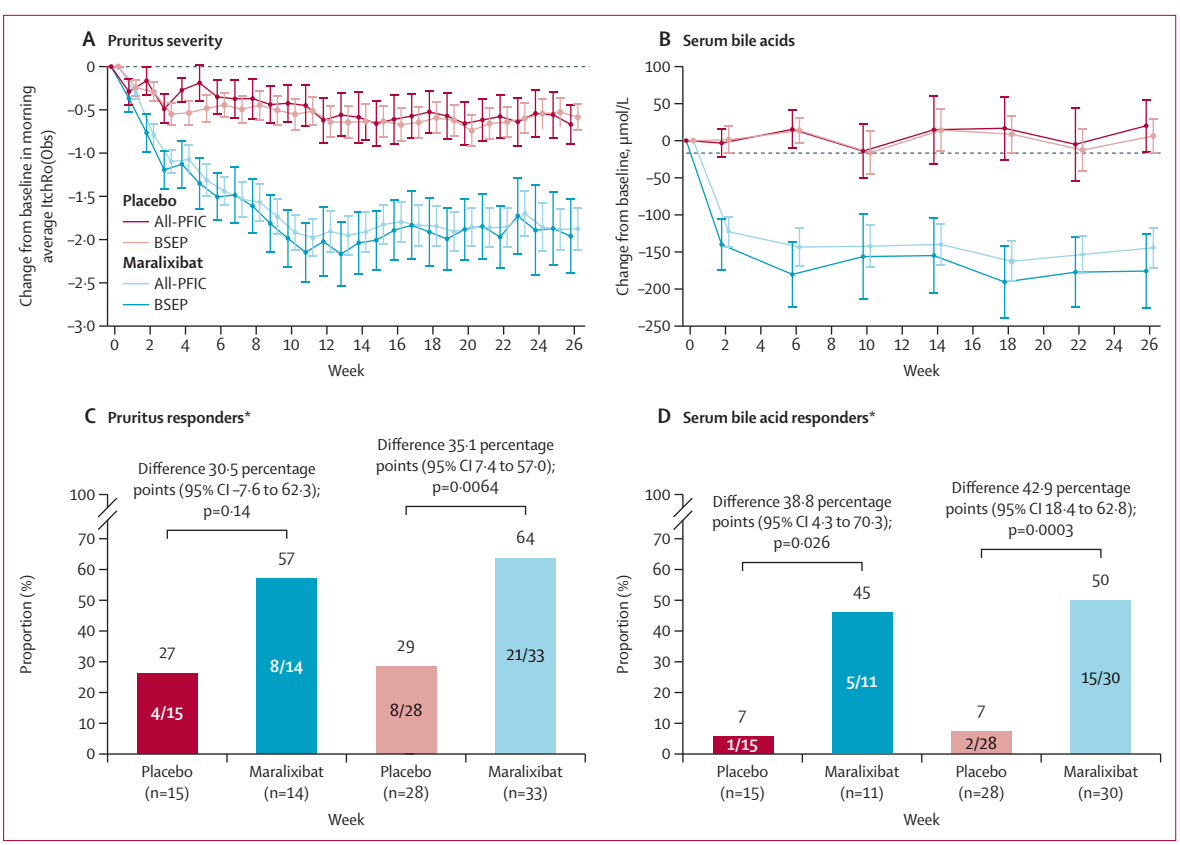

Figure 3: **Pruritus severity, serum bile acids, and responders by week 26**
Changes in pruritus severity (A) and serum bile acid concentrations (B) over time (baseline to week 26). Pruritus (C) and serum bile acid (D) responders. The p values reported for the responders are nominal p values. BSEP=bile salt export pump. ItchRo(Obs)=Itch-Reported Outcome (Observer). PFIC=progressive familial intrahepatic cholestasis. *Only observed cases were classified.

	Maralixibat (n=47)	Placebo (n=46)
Any TEAE	47 (100%)	43 (93%)
Mild	44 (94%)	41 (89%)
Moderate	20 (43%)	25 (54%)
Severe	3 (6%)	3 (7%)
Serious TEAE	5 (11%)	3 (7%)
TEAEs leading to discontinuation	1 (2%)	0
Liver-related TEAEs*	13 (28%)	11 (24%)
TEAEs occurring in ≥5% of patients in either group†	47 (100%)	43 (93%)
Diarrhoea	27 (57%)	9 (20%)
Pyrexia	17 (36%)	13 (28%)
Abdominal pain‡	12 (26%)	6 (13%)
Rhinorrhoea	8 (17%)	5 (11%)
Vitamin D deficiency§	8 (17%)	7 (15%)
Cough	7 (15%)	5 (11%)
Hyperbilirubinaemia¶	7 (15%)	9 (20%)
Vitamin E deficiency‖	7 (15%)	7 (15%)
Alanine aminotransferase increased	6 (13%)	3 (7%)
Influenza	6 (13%)	2 (4%)
Nasopharyngitis	5 (11%)	2 (4%)
Non-specific viral upper respiratory tract infection**	5 (11%)	9 (20%)
Pruritus	5 (11%)	8 (17%)
Constipation	4 (9%)	2 (4%)
Coronavirus infection	3 (6%)	4 (9%)
Gastroenteritis	3 (6%)	2 (4%)
Haematochezia	3 (6%)	1 (2%)
Headache	3 (6%)	0
Urinary tract infection	3 (6%)	1 (2%)
Vomiting	3 (6%)	5 (11%)
Vitamin K deficiency††	2 (4%)	5 (11%)
Drug-related TEAEs†	18 (38%)	2 (4%)
Diarrhoea	13 (28%)	1 (2%)
Abdominal pain	3 (6%)	0
Blood bilirubin increased	3 (6%)	0

Data are n (%). TEAE=treatment-emergent adverse event. *Liver-related TEAEs include preferred terms of blood bilirubin increased (maralixibat: 7; placebo: 4), hyperbilirubinaemia (maralixibat: 0; placebo: 5), hepatic enzyme increased (maralixibat: 1; placebo: 0), aminotransferases increased (maralixibat: 1; placebo: 0), alanine aminotransferase increased (maralixibat: 6; placebo: 3), and aspartate aminotransferase increased (maralixibat: 2; placebo: 1). Participants were counted once among all possible terms reported. †Preferred term. ‡Includes preferred terms of abdominal pain and abdominal pain upper. §Includes preferred terms of vitamin D decreased, vitamin D deficiency, and blood 25-hydroxycholecalciferol decreased. ¶Includes preferred terms of blood bilirubin increased and hyperbilirubinaemia. ‖Includes preferred terms of vitamin E decreased and vitamin E deficiency. **Includes preferred terms of upper respiratory tract infection, viral upper respiratory tract infection, and respiratory tract infection viral. ††Includes preferred terms of international normalised ratio increased, prothrombin time abnormal, activated partial thromboplastin time prolonged, prothrombin time prolonged, and vitamin K deficiency.

Table 2: **Safety findings from baseline to week 26 (TEAEs) in the full cohort (safety population)**

p=0·09) in the all-PFIC cohort and 0·22 (–0·14 to 0·59; p=0·22) in the BSEP cohort (appendix p 13).

The proportion of participants with severe treatment-emergent adverse events was similar between treatment groups (table 2). Serious treatment-emergent adverse events were reported in five (11%) of 47 participants receiving maralixibat and in three (7%) of 46 participants receiving placebo. Serious treatment-emergent adverse events (grouped by patient) in the maralixibat group were idiopathic pneumonia syndrome (n=1); blood bilirubin increased and cholelithiasis (n=1); pneumonia, urinary tract infection, and constipation (n=1); cholestasis and cholangitis (n=1); and urinary tract infection (n=1). Serious treatment-emergent adverse events (grouped by patient) in the placebo group were coagulopathy, vitamin K deficiency, and seizure (n=1); accidental exposure to product (n=1); and viral gastroenteritis (n=1). None were deemed treatment-related (except one event of mild increased bilirubin in the maralixibat group) and all resolved without any dose modifications. Only one participant had an adverse event that led to permanent discontinuation of study drug (non-serious, mild diarrhoea in the maralixibat group). No participant had a treatment-emergent adverse event categorised as severe and related to the study drug or had an event resulting in death. Five participants had an adverse event that resulted in dose reduction (four in the maralixibat group [one with diarrhoea, frequent bowel movements, abdominal pain, and haematochezia; one with increased aminotransferase; one with diarrhoea; and one with diarrhoea and abdominal pain]; one in the placebo group [one with increased ALT and AST]). Diarrhoea events occurred in a greater proportion of maralixibat-treated participants than placebo-treated participants (table 2; appendix pp 14–15). All diarrhoea events were considered mild or moderate in severity, with no serious diarrhoea events being reported. Most diarrhoea events were transient in nature (median duration of 5·5 days [IQR 3·0–13·0]) and resolved while treatment was ongoing. The two participants on maralixibat with MYO5B deficiency both reported mild diarrhoea. There were no clinically significant differences between groups in adverse events associated with fat-soluble vitamin deficiencies. Descriptions of the adverse events of clinical interest can be found in the appendix (p 15). One patient receiving placebo (MDR3 deficiency) had a hepatic mass at study day 183 (lymph node in the hepatic hilum). The participant continued under observation and completed the study. For safety in the BSEP and all-PFIC cohorts seperately, see appendix pp 14–15.

Discussion

In MARCH-PFIC, maralixibat at a maximum dose of 570 µg/kg twice daily significantly improved cholestatic pruritus and reduced serum bile acid concentrations versus placebo in children with PFIC in both the BSEP and all-PFIC cohorts. We observed improved

efficacy in both pruritus and bile acids with the higher maralixibat dose of 570 µg/kg twice daily than that observed with the 266 µg/kg once-daily dose used in the previous phase 2 study,[1] as well as serum bile acid reductions and pruritus improvements in patients with BSEP deficiency and also in patients with deficiencies other than BSEP. In MARCH-PFIC, reductions in pruritus severity and serum bile acid concentrations were clinically meaningful, occurred rapidly, and were sustained until week 26. Overall, maralixibat was well tolerated, with no unexpected treatment-emergent adverse events observed. Diarrhoea events were more frequently observed in the maralixibat group than in the placebo group; these events were mild or moderate in severity and mostly transient (no severe or serious diarrhoea events were reported).

To our knowledge, MARCH-PFIC is the first placebo-controlled, randomised study that included participants with a broad range of PFIC types and characteristics, including multiple PFIC-related genotypes beyond BSEP and FIC1 deficiencies, participants who had surgery for cholestasis, and those with no disease-causing variants observed in their genotyping tests. Most of these disease types are studied here for the first time.

Improvements in pruritus severity and reductions in serum bile acid concentrations with maralixibat treatment were observed across PFIC types. The robustness of these results was confirmed by greater reductions in pruritus and serum bile acids with maralixibat versus placebo in the group composed of patients with FIC1, MDR3, TJP2, and MYO5B deficiencies, as well as by reductions seen in patients without a known variant. In patients with truncated BSEP deficiency, reductions in serum bile acids were observed in some patients, which is an unexpected finding given the predicted loss of BSEP function associated with this genotype. Due to the small numbers of participants with truncated BSEP deficiency enrolled, a conclusion cannot be drawn, but our results could suggest the presence of residual enterohepatic circulation of bile acids in the absence of predicted BSEP function in some patients with truncated BSEP deficiency.

Improvements in pruritus severity in all studied groups in favour of maralixibat were robust, with similar results in the morning, evening, and highest daily scores, as well as in the post-hoc analysis after multiple imputation. These improvements were clinically and statistically significant in the BSEP and all-PFIC cohorts. In the all-PFIC cohort, 64% of participants assigned maralixibat had a response in pruritus versus 29% assigned placebo. Significant improvements in pruritus morning frequency and sleep disturbance with maralixibat indicate the encompassing impact of maralixibat on the burden of pruritus.

A serum bile acid concentration of less than 102 µmol/L or a decrease of 75% or more after surgical biliary diversion have been associated with longer native liver survival in patients with BSEP deficiency.[14] Another serum bile acid reduction threshold associated with native liver survival has been described for FIC1 deficiency (<65 µmol/L), but no cutoff exists for other PFIC types.[18] Therefore, the well established serum bile acid reduction threshold for BSEP deficiency constituted the serum bile acid response definition for all PFIC types in our study. In the all-PFIC cohort, 50% of participants receiving maralixibat versus 7% of participants receiving placebo reached the serum bile acid response definition. The serum bile acid response in the BSEP cohort was higher than the 36% described in the previous phase 2 study,[1] indicating improved efficacy with a higher daily dose of maralixibat.

Improvements in bilirubin concentrations and growth with maralixibat versus placebo were consistent with the potential benefits associated with ileal bile acid transporter inhibition beyond pruritus relief. Reductions in bilirubin concentrations after surgical biliary diversion have been shown to predict native liver survival in patients with BSEP deficiency.[14,19] Our findings suggest that improvements in liver health could occur after pharmacological interruption of the enterohepatic circulation with maralixibat without the clinical burden associated with surgery.

Maralixibat was generally safe and well tolerated. As expected for a minimally absorbed locally active drug, the most common treatment-emergent adverse events were gastrointestinal, with diarrhoea being the most common. Most diarrhoea events were transient in nature and all were considered mild or moderate in severity. The increase in bile acid load arriving at the colon after successful interruption of the enterohepatic circulation is likely to be responsible for the safety profile. The fast remission of diarrhoea after a median duration of 5·5 days of treatment could be explained by the initial elimination of previously synthesised bile acids followed by a new steady state in which colonic bile acids only reflect newly synthesised bile acids. Importantly, unlike with bile acid-binding resins, fat-soluble vitamin deficiencies did not worsen during 26 weeks of maralixibat treatment.

The strengths of this study include an adequate duration of the placebo-controlled period (26 weeks) and the inclusion of participants with different PFIC types, including groups studied, to our knowledge, for the first time in a placebo-controlled manner (participants with MDR3, TJP2, MYO5B, and truncated BSEP deficiencies, and participants with a PFIC phenotype but no variants observed in the genotype test). As a limitation, due to the lower numbers of participants with PFIC types that were not BSEP or FIC1 deficiencies, powered analyses for each PFIC type were not planned as they were not deemed feasible. Differences across specific PFIC types within the groupings might have introduced heterogeneity. The robustness of the results across all studied groups, however, indicate a true drug effect across the PFIC types studied.

In conclusion, maralixibat 570 µg/kg administered twice daily represents a new non-surgical, pharmacological option to interrupt the enterohepatic circulation and reduce bile acids in patients with PFIC, showing efficacy beyond BSEP and FIC1 deficiencies. Maralixibat improved cholestatic pruritus and markers of sleep disturbances and has the potential to improve the standard of care in patients with PFIC, regardless of genotype.

Contributors

All authors contributed to data collection, data analysis, data interpretation, and the writing and review of the manuscript. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication. AGM, TN, AL, LL, DBM, WG, PV, and RJT have accessed and verified the data.

Declaration of interests

AGM is a consultant and has a sponsored research agreement with Mirum Pharmaceuticals. JCE has received support to his institution from Mirum Pharmaceuticals. PC is a consultant for Ipsen-Albireo. FO is a speaker for Alexion Pharmaceuticals and Valentech Pharma. AAA is a consultant and adviser for Mirum Pharmaceuticals and Ipsen-Albireo. ES is the founder and chairperson of Cellaion; an investigator for Mirum Pharmaceuticals, Albireo, and Intercept; and an adviser for Mirum Pharmaceuticals and Albireo. UB is a consultant for and has received research funding from Mirum Pharmaceuticals, Ipsen-Albireo, Nestle, and Vivet Pharmaceuticals; and has served as a speaker for Mirum Pharmaceuticals and Ipsen-Alibreo. LD is a consultant and adviser for Mirum Pharmaceuticals, Albireo, Selecta, Vivet Pharmaceuticals, Tome, Spark, Genespire, and Alexion. NK is a consultant for Mirum Pharmaceuticals. C-HL has received a research grant from Mirum Pharmaceuticals. SG is an adviser for and has received research support to her institution from Mirum Pharmaceuticals. NM is an investigator for Mirum Pharmaceuticals. SPH is a hepatic safety adjudication committee member at Ipsen-Albireo and has received a research grant from Mirum Pharmaceuticals. TJ is a shareholder and has intellectual property rights in Mirum Pharmaceuticals, and is an employee of and shareholder in Galapagos. VFH is a consultant for and has received research support to her institution from Mirum Pharmaceuticals. RPG-P has received a research grant from Mirum Pharmaceuticals, and is an adviser, teacher, and educator for Mirum Pharmaceuticals and Albireo. UE is a steering committee member for Mirum Pharmaceuticals. JH has received a research grant from Mirum Pharmaceuticals. NO has received research support to her institution from Mirum Pharmaceuticals, Albireo, and Travere. RJT is a consultant, adviser, and speaker for and has received research support to his institution from Mirum Pharmaceuticals. TN, AL, LL, DBM, WG, and PV are employees of and shareholders in Mirum Pharmaceuticals. AMou, GP, AMos, RHS, DD, NLab, CA, FKC, W-DH, and NLav declare no competing interests.

Data sharing

Beginning 6 months and ending 5 years after publication, de-identified participant data for data meta-analysis might be made available to investigators whose proposed use of the data has been approved by a review committee, including the primary authors and the study funder. The study protocol will also be available via weblink. Proposals should be directed to medinfo@mirumpharma.com. Before being granted access, data requesters will be required to sign a data access agreement.

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