RTY-406 is a dual-acting ABCB4/MDR3 and ABCB11/BSEP positive functional modulator that demonstrates efficacy in a model of Primary Sclerosing Cholangitis

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Introduction

ATP binding cassette subfamily B members 4 (ABCB4/MDR3) and 11 (ABCB11/BSEP) facilitate the translocation of phospholipid (PL) and the efflux of bile acids (BA) from hepatocytes to the canalicular space, respectively[1]. Mutations in these transporters cause monogenic cholestatic diseases (PFIC3 [MDR3 deficiency] / PFIC2 [BSEP deficiency]) and are associated with adult-onset hepatobiliary diseases[2]. Primary Sclerosing Cholangitis (PSC) is a biliary disease that is in part driven by bile composition changes[3] that may benefit from increased ABCB4-mediated PL in bile and BSEP mediated BA efflux from hepatocytes. We have discovered RTY-406, a novel, potent, dual-acting ABCB4/BSEP positive functional modulator (PFM) that increases protein levels and functional output of ABCB4 and BSEP. In a toxic bile induced model of PSC[4], RTY-406 attenuated core disease pathologies of cholestasis, cholangitis, ductular reaction, and fibrosis.

Methods

Study design: Nine to ten-week-old female heterozygous (HET) FVB.129P2-Abcb4tm1Bor/J strain (002539) or wild type (WT) litter mate control mice were obtained from a breeding colony established at Jackson Laboratory. WT mice were divided into 2 groups with N=5 and HET mice were divided into 4 groups with N=15. One group of WT mice were fed control diet (CD) and the other was fed lithogenic diet (LD) composed of 15% fat, 1.25% cholesterol, and 0.5% cholic acid (Research Diets, D12336). All HET groups were fed LD. WT groups and one HET group were dosed with vehicle (0.5% methylcellulose with 0.1% Tween-80 in water), and the remaining 3 HET groups were dosed with RTY-406 at 15 mg/kg, 30 mg/kg, and 60 mg/kg P.O. B.I.D.. Two days after start of dosing the diet of appropriate groups was changed to LD. Terminal collection was performed at 6 weeks. Prior to bile collection, gallbladders were scored on a scale of 0-6 for cholesterol crystal formation using the following criteria: 0) gallbladder is filled with clear bile; 1) a few fine crystals are present; 2) around 10 fine crystals are present; 3) more than 10 fine crystals are present covering less than half of the gallbladder; 4) half of the gallbladder has crystals present; 5) leaflet or stratified crystals occupy over half of the gallbladder; 6) round gallstones. Liver tissue was split for formalin fixation and embedding or flash freezing. Flash frozen liver was sent to Pharmaron for analysis and embedded tissue was sent to DTR Labs for histology. The in-life portion of this study and serum analysis was performed at Physiogenex (Toulouse, France) in accordance with the Guide for the Care and Use of Laboratory Animals and French law.

Serum and bile: Total bile acids were measured by microplate enzyme cycling assay from Abcam (AB239702). Serum alkaline phosphatase (ALP) levels were determined using Horiba Pentra c400 clinical with the Pentra assay kit for ALP (Horiba, A11A01626) according to manufacturer's protocol. Biliary phospholipid concentrations were determined using the enzyme cycling assay LabAssay™ Phospholipid kit (FUJIFILM, 295-4401).

Liver TCA: Tissue was pulverized in precooled extraction buffer (1:1:1 mix of acetone:methanol:water with internal standard) with TissueLyser II. Samples were centrifuged and supernatant was injected into LC-MS/MS to determine TCA concentrations back calculated from calibration curve.

Gene expression: Frozen liver tissue was ground into powder using liquid N2 and then genomic DNA-free RNA was extracted using RNeasy Plus mini-Kit (Qiagen). Isolated RNA was converted to cDNA with High-Capacity RNA-to-cDNA Kit (Invitrogen). Using TaqMan® FAST Gene Expression Master Mix, 0.5µL of cDNA was analyzed using Thermo Fisher TaqMan® probe sets. Relative expression was calculated using the ΔΔCt method using the average of *Gapdh*, *Gusb*, and *Tbp* as housekeeping genes

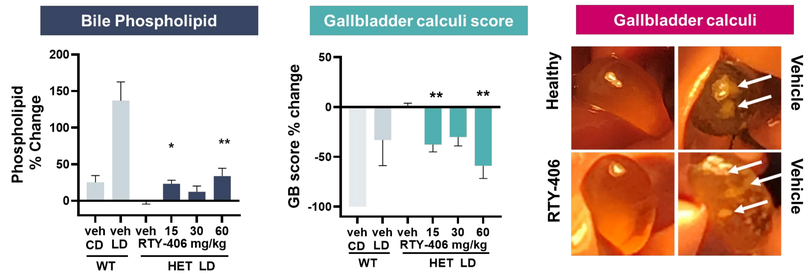
Cytokines: Liver tissues were homogenized in RIPA lysis buffer supplemented with protease and phosphatase inhibitors in TissueLyser II. 300µg of liver tissue were added to V-PLEX Proinflammatory panel 1 mouse (MSD K15048D) and V-PLEX Cytokine panel 1 mouse (MSD K15245D) and manufacturer instructions were followed.

Histology: Five slides were sectioned at 4 mm and stained histochemically for hematoxylin and eosin (H&E) or PSR, or by immunohistochemistry (IHC) for Ck19, Cd11b. Slides for Ck19 were collected serially to the slides stained for Cd11b and Vcam (slide order: Cd11b, Ck19, Vcam). All IHC staining was conducted on the Leica Bond Rx automated stainer and imaged using a Leica Aperio AT2 digital slide scanner. Whole slide images were imported into Visiopharm software for analysis. An initial deep learning algorithm (DLA) trained to detect tissue was used to establish the analyzable tissue area. Ck19 was used to define the peribiliary space that was overlayed with the aligned Cd11b images.

Statistical analysis: GraphPad Prism software was used to analyze data for statistical significance. Outliers were removed by ROUT method and then distribution was determined, followed by use of ANOVA or Kruskal-Wallis to determine significance.

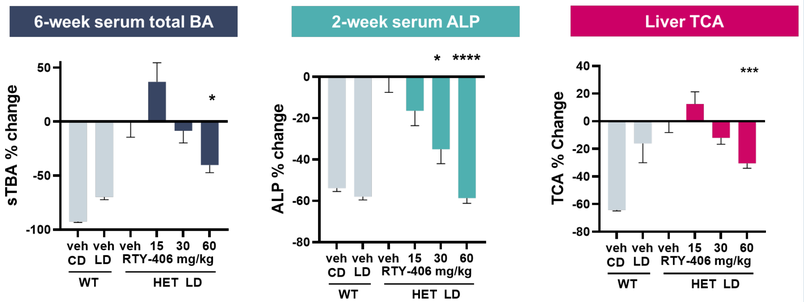
Results

Figure 1. RTY-406 increased biliary levels of PL, demonstrating Abcb4 target engagement, and results in reduced gallbladder crystal formation.



- RTY-406 increased biliary phospholipid
- RTY-406 reduced calculi (cholesterol crystal) formation in the gallbladder

Figure 2. RTY-406 reduced serum total BA, ALP, and liver TCA, demonstrating Bsep target engagement and reduction in cholestasis



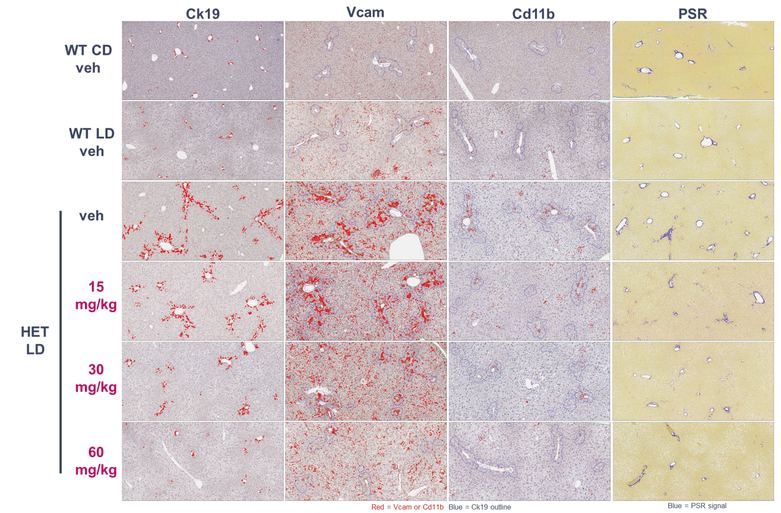
- RTY-406 reduced serum total BA and ALP after 6 weeks
- RTY-406 reduced liver TCA consistent with a reduction in cholestasis and Bsep target engagement

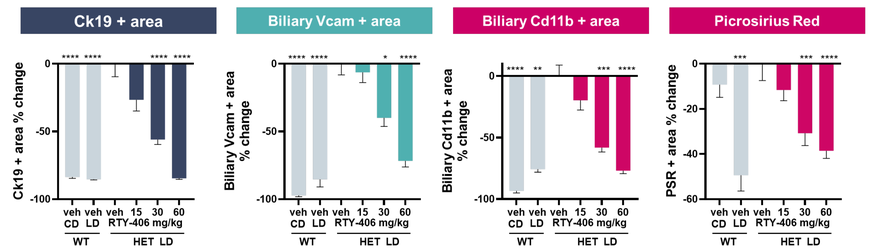
Table 1. RTY-406 reduced markers of ductular reaction, fibrosis and cholangitis

	mRNA % change							Protein % change	
RTY-406	*Ck19*	*Vcam*	*Itgb6*	*Spp1*	*Timp-1*	*Col1a1*	*Cola1a2*	Cxcl-1	Mcp-1
15 mg/kg	-9	-1	-7	-13	-6	0	0	-17	-23
30 mg/kg	-21	-14	-54	-44	-30	-10	-18	-37	-44
60 mg/kg	-76	-27	-95	-79	-25	-20	-37	-35	-57

- RTY-406 reduced mRNA for ductular reaction markers *Ck19* and *Vcam*
- Expression of fibrotic markers *Itgb6*, *Spp1*, *Timp-1*, and *Col1a1/2* are reduced by RTY-406
- RTY-406 reduced proinflammatory mediators of immune cell recruitment Mcp-1 and Cxcl-1

Figure 3. RTY-406 reduced ductular reaction, immune cell infiltration, and fibrosis.





- RTY-406 reduced Ck19 and biliary Vcam indicating reduced cholangiocyte proliferation and activation
- RTY-406 reduction in biliary Cd11b+ and PSR indicates improved cholangitis and fibrosis.

Conclusions

In a mouse model of toxic bile-induced PSC, twice daily dosing of RTY-406 reduced cholelithiasis and demonstrated an improvement in markers of cholestasis, cholangitis, ductular reaction, and fibrosis. Efficacy across multiple pathologically relevant endpoints provides high confidence that RTY-406 addresses core disease drivers and is a candidate drug for PSC.

References

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