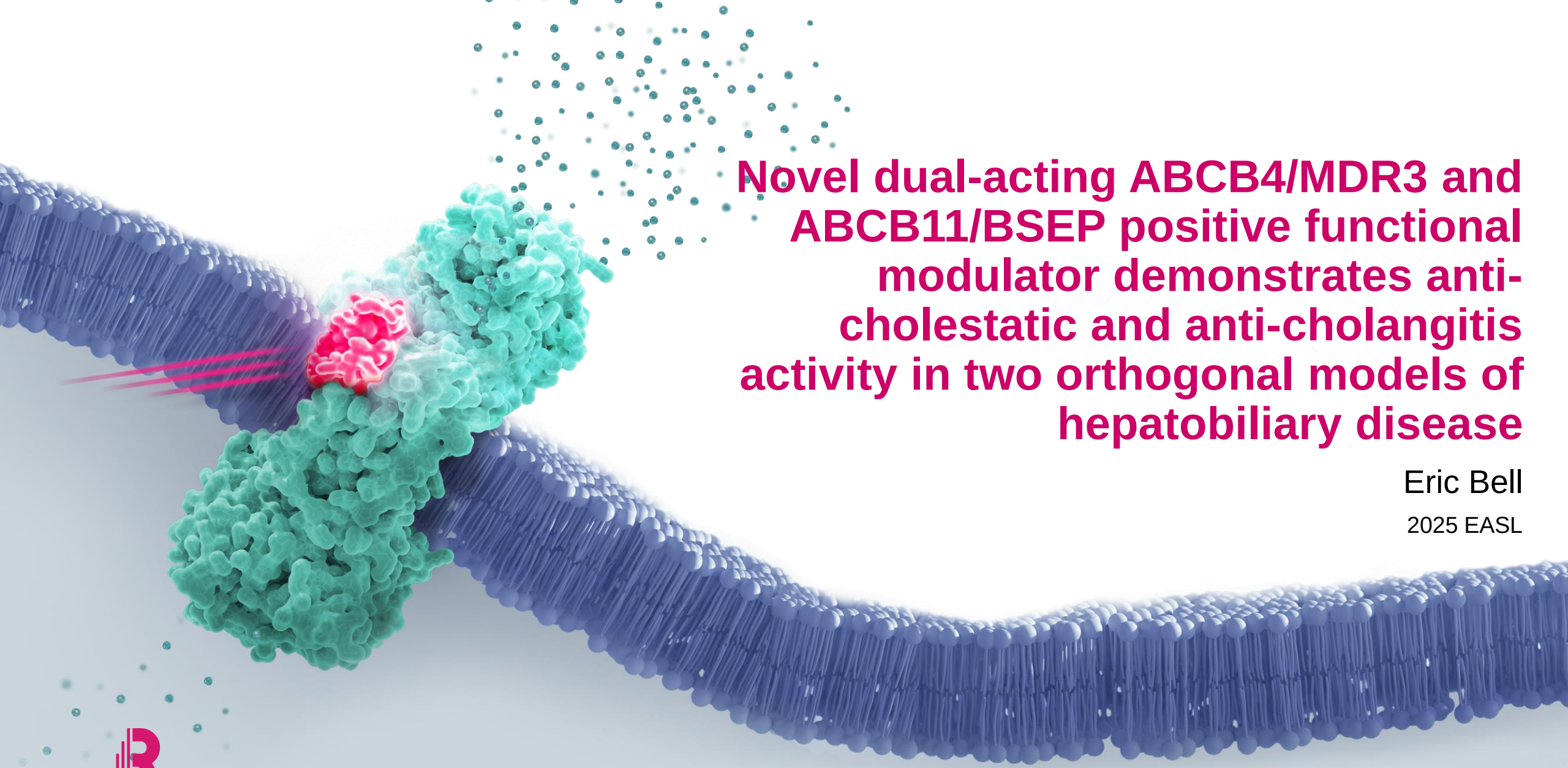


Novel dual-acting ABCB4/MDR3 and ABCB11/BSEP positive functional modulator demonstrates anti-cholestatic and anti-cholangitis activity in two orthogonal models of hepatobiliary disease

Eric Bell

2025 EASL

RECTIFY PHARMA

Disclosures slide

- E. Bell is an employee and holds stock of Rectify Pharmaceuticals

Executive summary

- ABCB4/BSEP Positive Functional Modulator (PFM) is a **new therapeutic mechanism of action** for hepatobiliary diseases

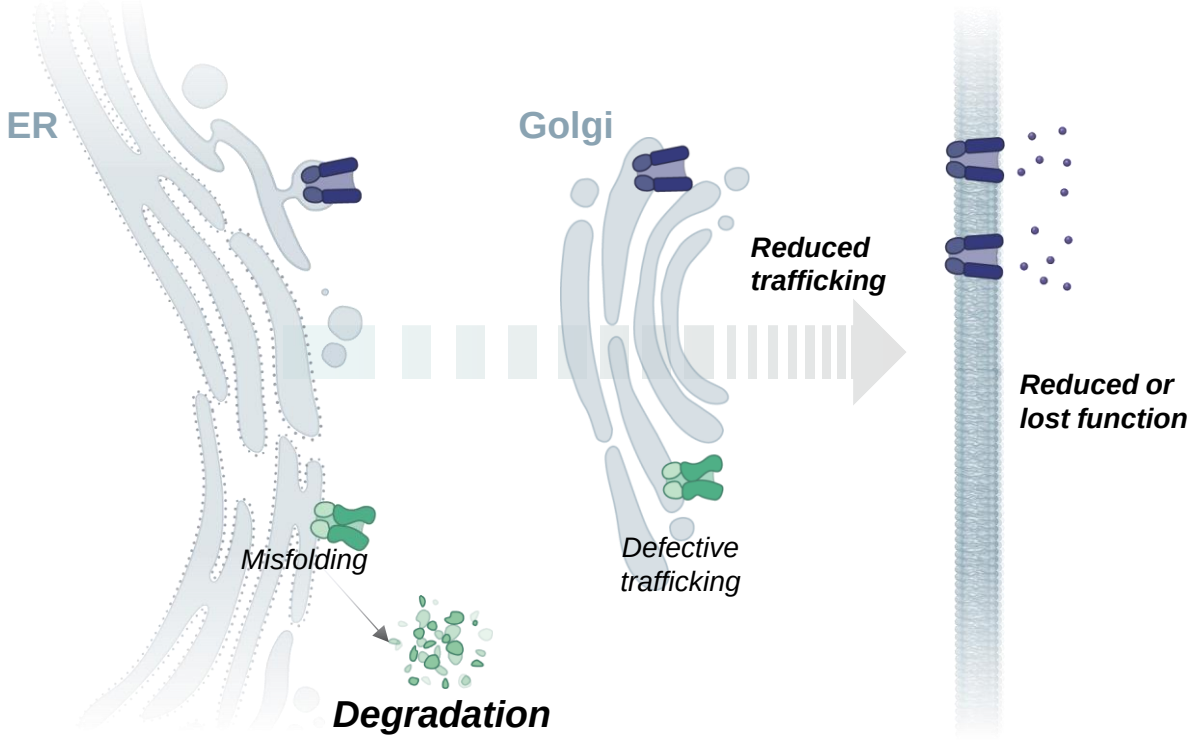
- The **dual ABCB4/BSEP PFM directly binds target transporters and increases efflux** of phospholipid (ABCB4) and bile acids (BSEP)

- Increased ABCB4/BSEP activity **improves downstream disease endpoints of ductular reaction, inflammation and fibrosis** in a mouse model of biliary disease **and demonstrates anti-cholestatic activity** in a genetic mouse model of PFIC2

- **RTY-694** is a **clinical candidate** for the treatment of **Primary Sclerosing Cholangitis (PSC)**

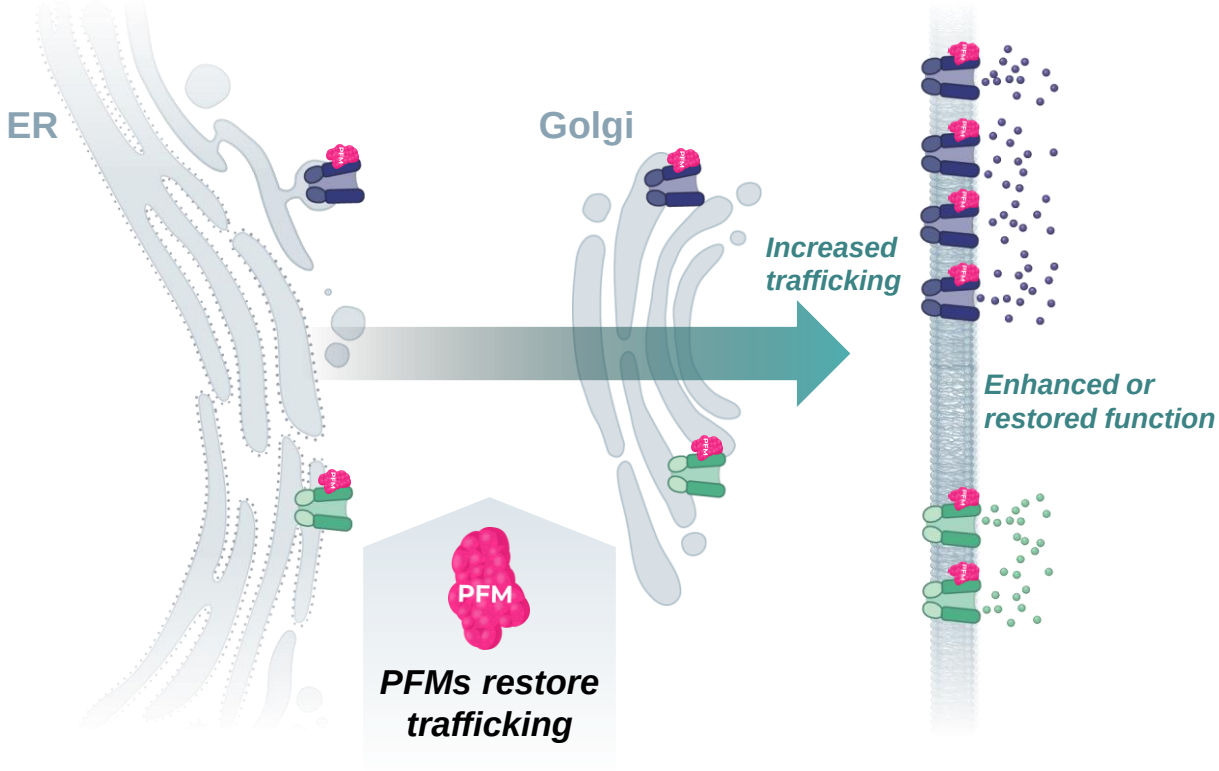
PFMs rectify protein dysfunction

DYSFUNCTIONAL



Both WT and mutant proteins are targeted for degradation by the cellular quality control machinery

PFMs RECTIFY FUNCTION



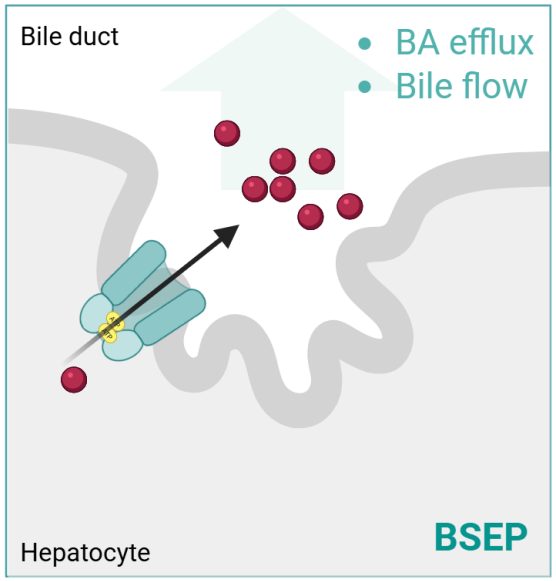
PFMs directly and specifically bind WT and mutant proteins to enhance or restore function

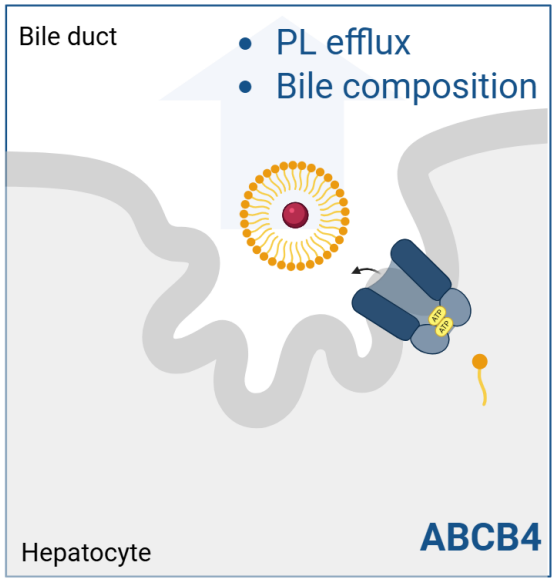

Wildtype protein

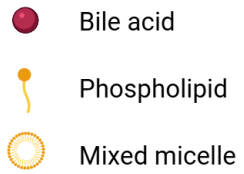

Mutant protein

ABCB4 and BSEP function are required for hepatobiliary homeostasis

ABCB4 and BSEP Biology



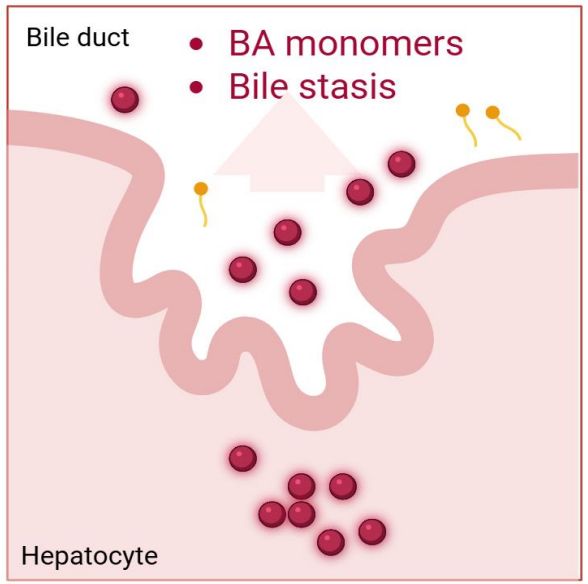




BSEP mutations → PFIC2
Bile stasis

ABCB4 mutations → PFIC3
BA monomers (Toxic Bile)

Hepatobiliary Disease



BSEP → BA efflux → bile flow

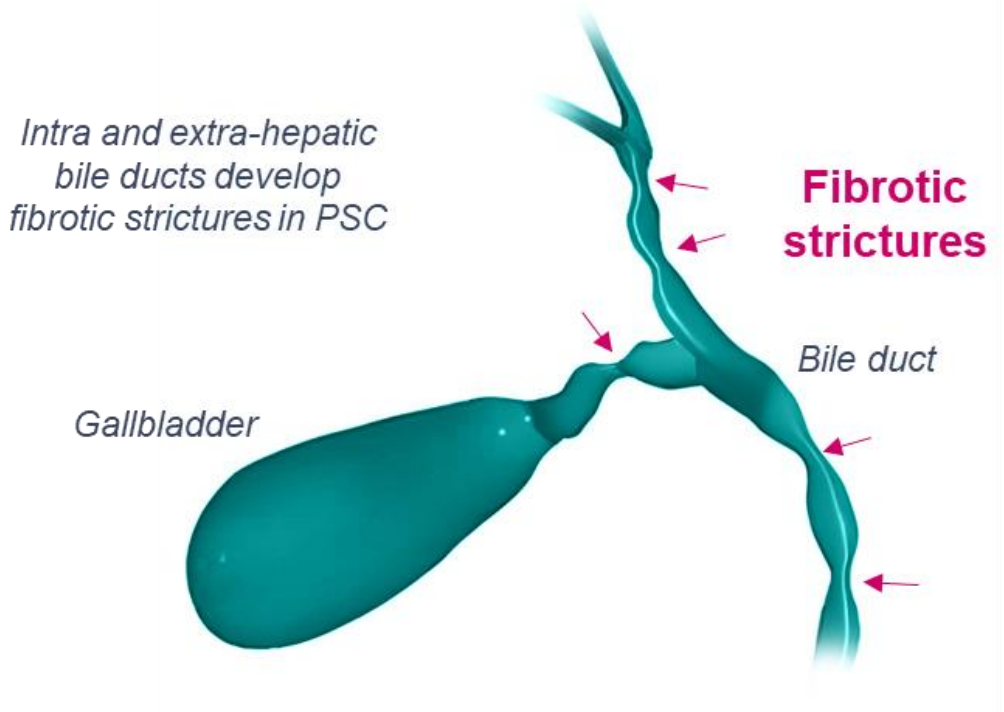
ABCB4 → PL efflux → bile composition

Functional deficits are implicated in complex hepatobiliary diseases

BA = bile acids; PL = phospholipid; MOA = mechanism of action; PFIC2 = Progressive familial intrahepatic cholestasis type 2; PFIC3 = Progressive familial intrahepatic cholestasis type 3

Targeting toxic bile in Primary Sclerosing Cholangitis (PSC)

Primary Sclerosing Cholangitis



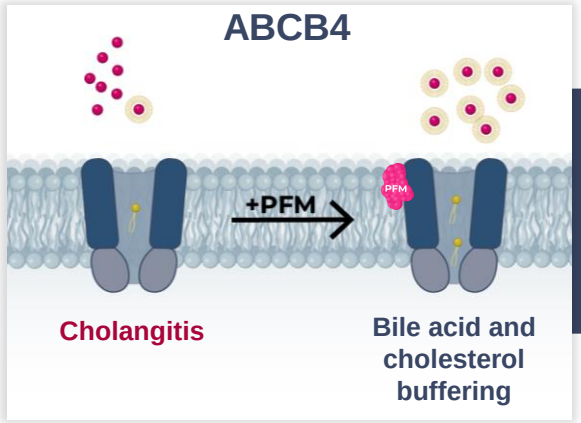
Bile Duct Injury

Increased biliary PL and BA efflux is a new MOA to address unmet need in PSC

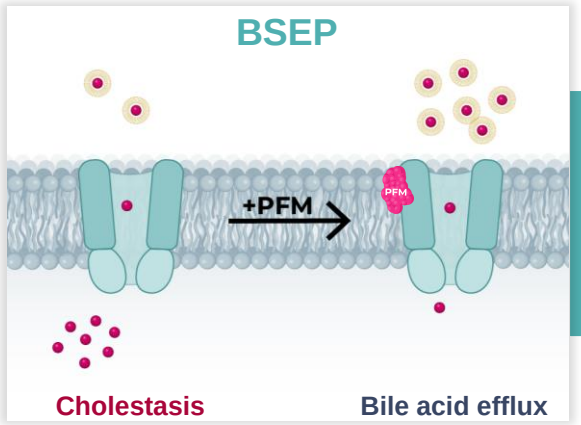
BA = bile acids; PL = phospholipid

ABCB4/BSEP PFM improves bile composition and enhances bile flow, a novel MOA to treat PSC and multiple hepatobiliary diseases

BSEP/ABCB4 dual-targeted PFM



Enhanced phospholipid efflux → Improved bile composition → Neutralization of bile acids and free cholesterol



Enhanced bile acid efflux → Improved bile flow → Reduced bile stasis

- Reduced cholestasis & cholangitis
- Improved biliary & liver health
- Reduced symptom burden
- Altered disease progression

Novel mechanism of action that is differentiated from PPARs, THRβ, and IBAT

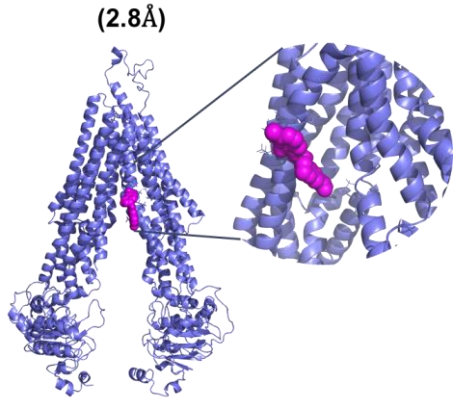
ABCB4/BSEP PFM binds transporter to increase function

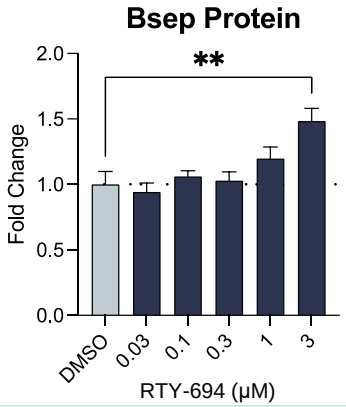
Directly Binds Target

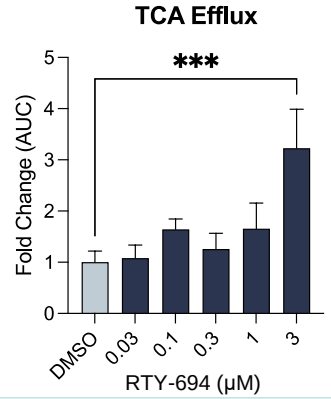
Increased Transporter

Increased Activity

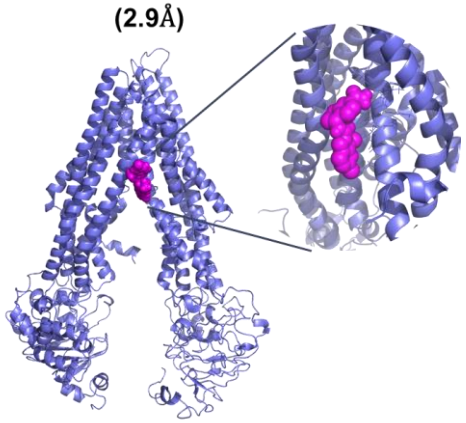
BSEP

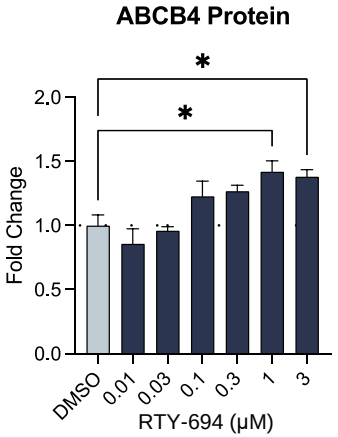


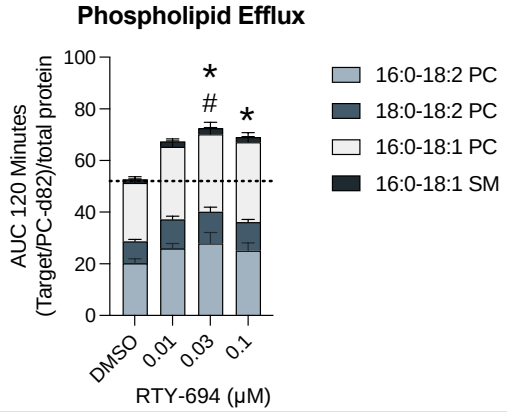




ABCB4







- **PFM** binds to both BSEP and ABCB4 in the transmembrane domain
- **PFM** increase protein levels of both BSEP and ABCB4 *in vitro*
- **PFM** increased transporter protein translates into increased substrate efflux

PFM = positive functional modulator; TCA = taurocholic acid; PC = phosphatidyl choline; SM = sphingomyelin

ABCB4/BSEP PFM evaluated in translational model of PFIC2

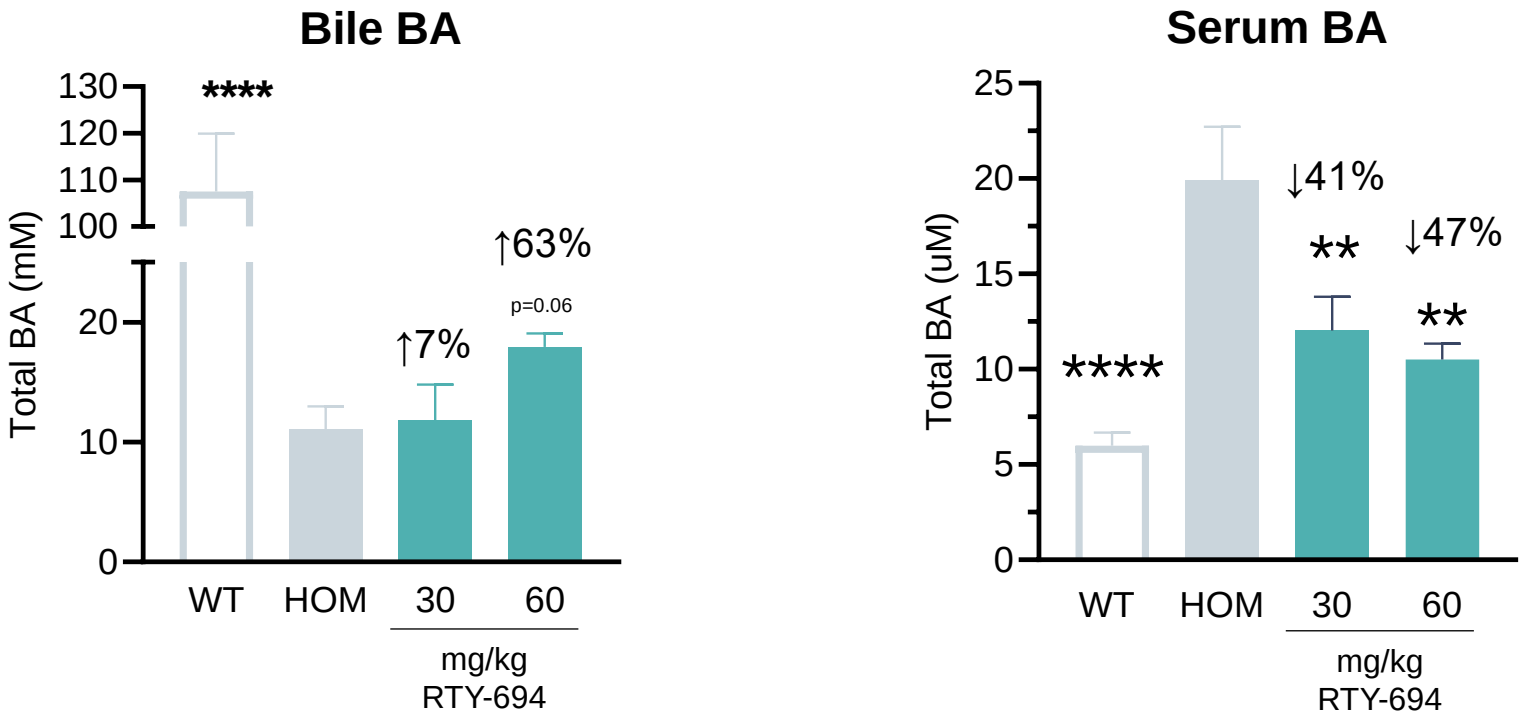
	PFIC2 mouse model
Model Mechanism	▪ $BSEP^{E297G}$ / $BSEP^{E297G}$
Phenotype	▪ Dysregulated enterohepatic bile acid distribution ▪ Elevated biochemical markers of liver injury
B4/BSEP PFM pharmacology	▪ Therapeutic dosing ▪ Increased biliary BA content consistent with disease modification ▪ Reduction in liver function tests and liver weight

Predictive genetic mouse model phenocopies PFIC2

PFM = positive functional modulator; BA = bile acids; PFIC2 = Progressive familial intrahepatic cholestasis type 2

Therapeutic dosing of ABCB4/BSEP PFM demonstrates disease modifying activity in a genetic PFIC2 mouse model

Target engagement and disease modification

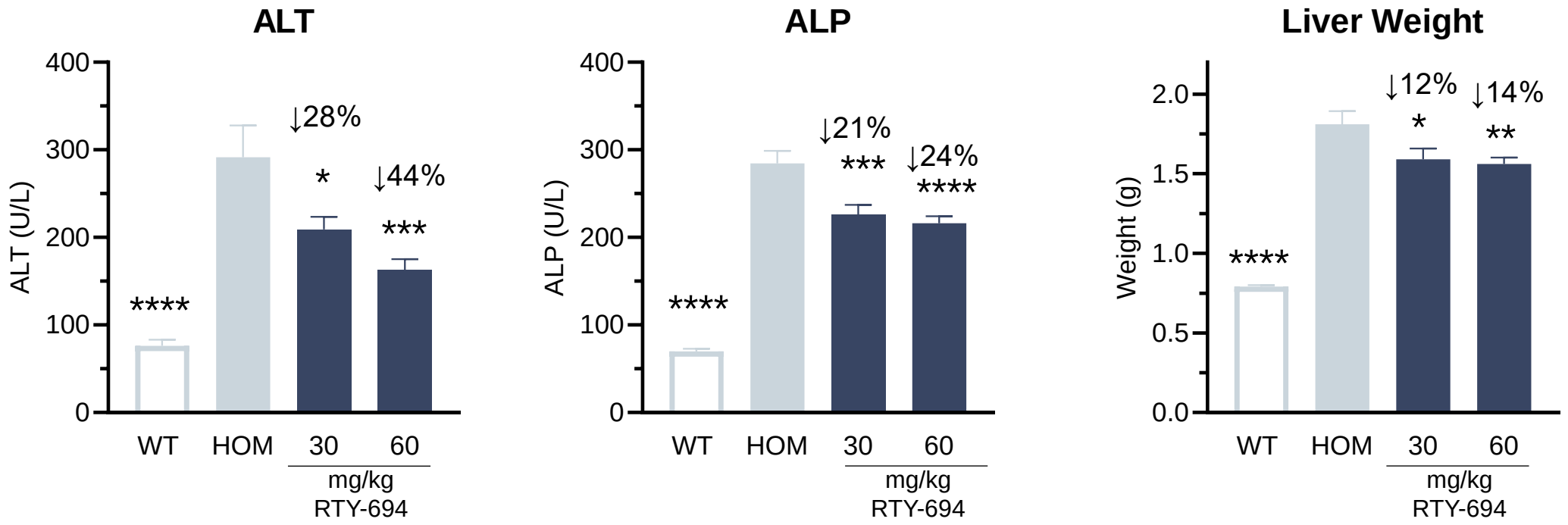


- Two weeks of therapeutic dosing is sufficient to reduce serum bile acids, consistent with disease modification

Statistics based on comparison to HOM; BA = bile acids; WT = wild type; HOM = homozygous *Bsep*^{E297G}

Therapeutic dosing of ABCB4/BSEP PFM demonstrates disease modifying activity in a genetic PFIC2 mouse model

Disease pathology

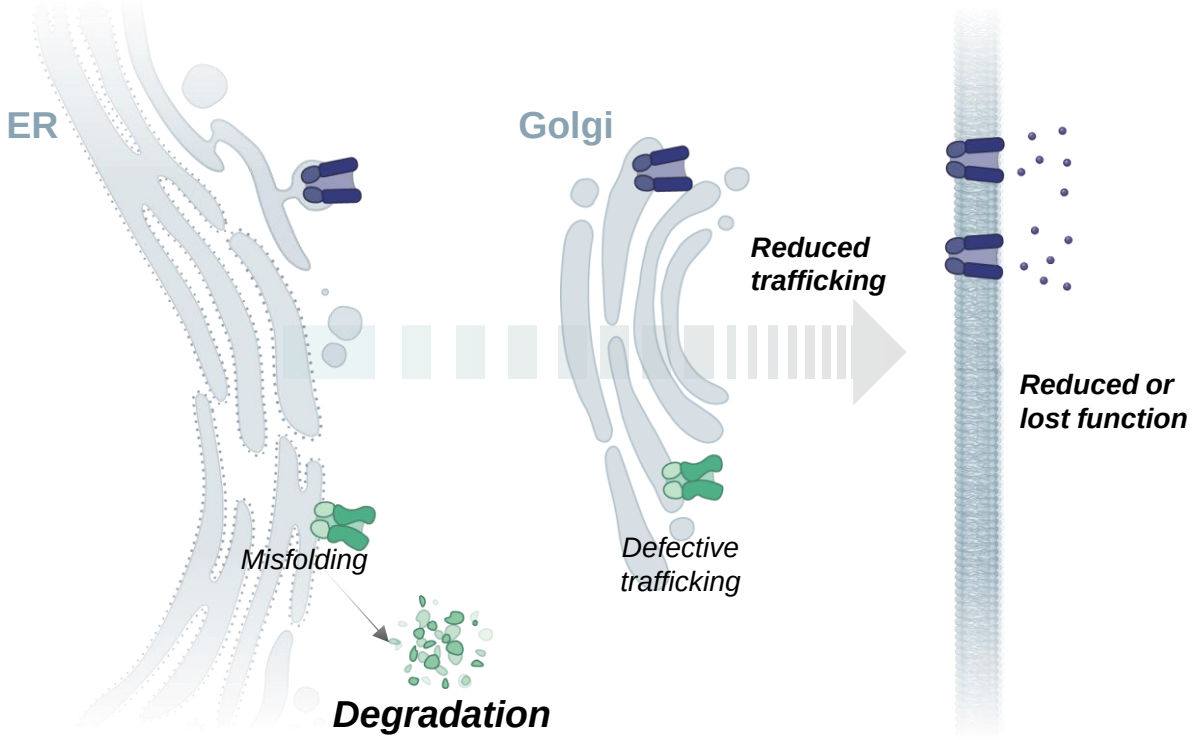


- Two weeks of dosing improved liver health with reduction in ALT, ALP, and liver weight

Statistics based on comparison to HOM; ALT = Alanine aminotransferase; ALP = alkaline phosphatase; WT = wild type; HOM = homozygous *Bsep*^{E297G}

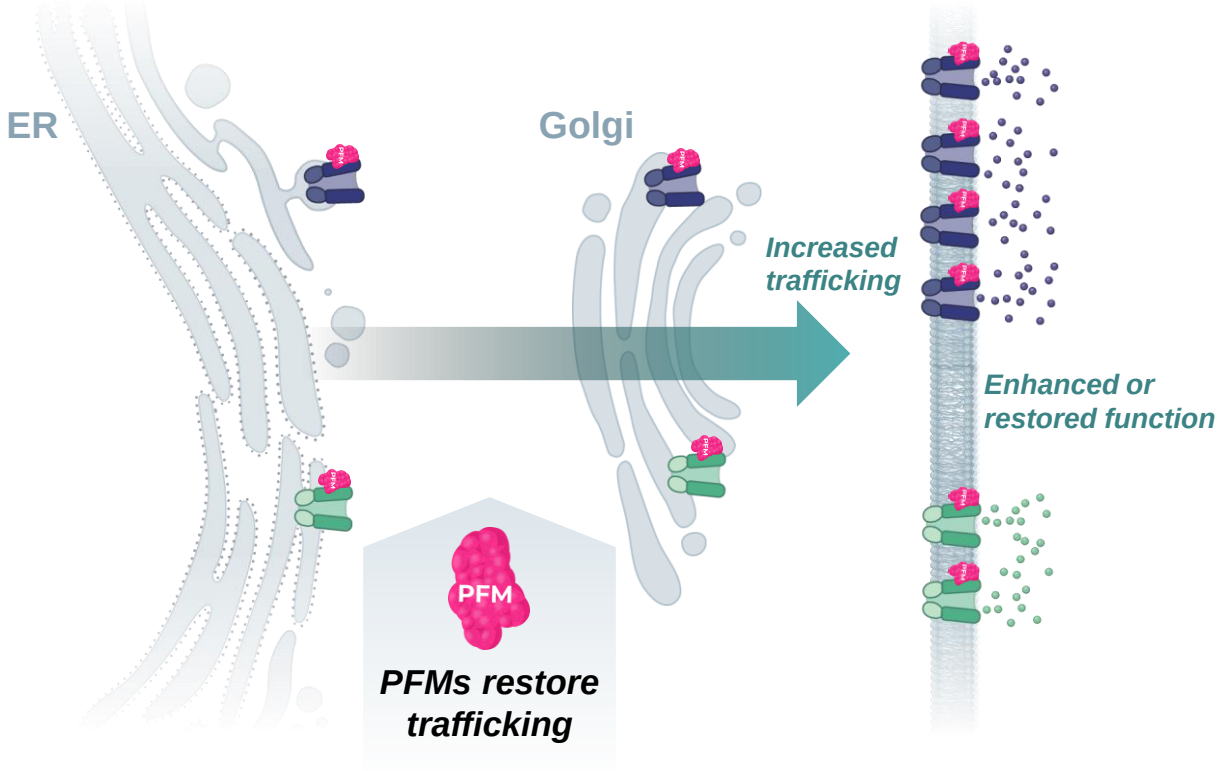
PFMs rectify protein dysfunction

DYSFUNCTIONAL



Both WT and mutant proteins are targeted for degradation by the cellular quality control machinery

PFMs RECTIFY FUNCTION



PFMs directly and specifically bind WT and mutant proteins to enhance or restore function


Wildtype protein


Mutant protein

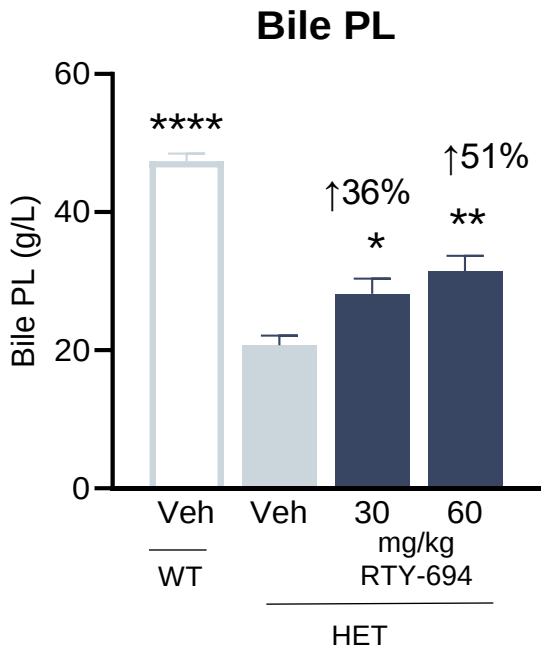
ABCB4/BSEP PFM evaluated in a novel phenotypic model for multiple biliary diseases

	Biliary disease mouse model
Model Mechanism	▪ ABCB4 heterozygous mice supplemented with lithogenic diet
Phenotype	▪ Primary toxic bile induced cholangiocyte injury ▪ Elevated biochemical markers of liver injury ▪ Presents preferentially in ABCB4 heterozygous vs WT mice
B4/BSEP PFM pharmacology	▪ Prophylactic dosing ▪ Enhances wild type ABCB4/BSEP ▪ Reduction of cholestasis, inflammation, and fibrotic markers

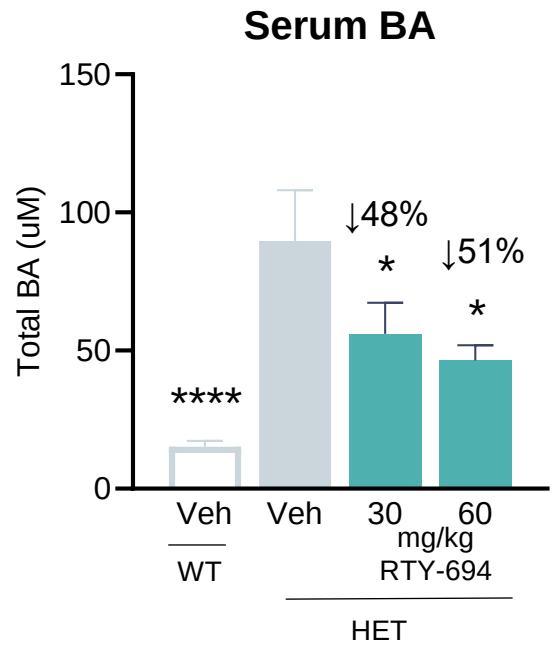
Novel mouse model of biliary diseases with phospholipid deficiency (e.g., PSC)

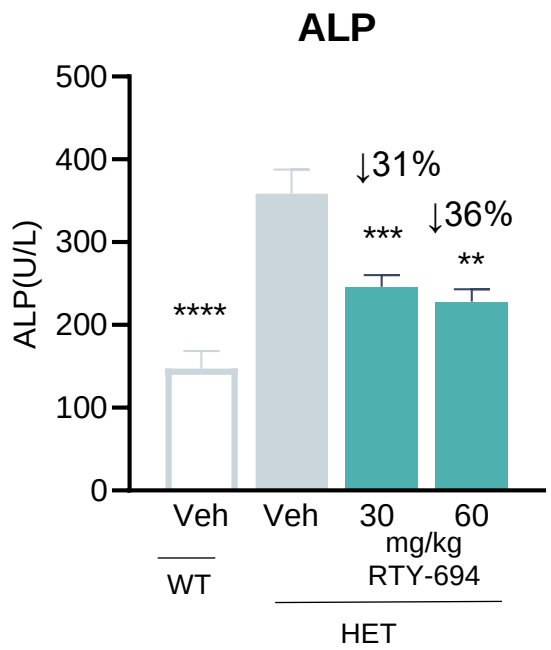
ABCB4/BSEP PFM changes bile composition and has anti-cholestatic activity in a mouse model of biliary disease

Bile composition (ABCB4)



Anti-cholestatic activity (BSEP)





Improved bile composition

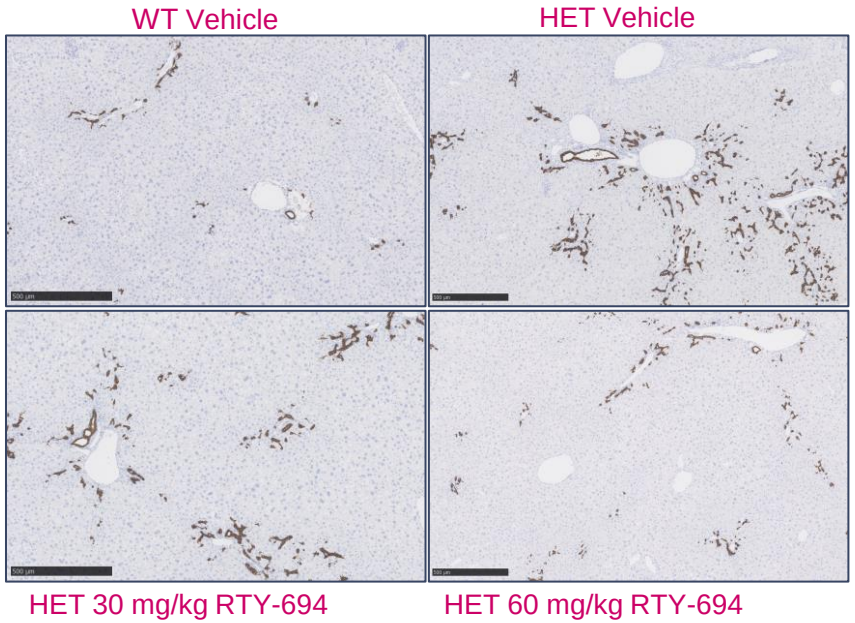
Anti-cholestatic activity

- Increased biliary PL indicates target engagement of ABCB4
- Reduced serum total BA and ALP indicate anti-cholestatic activity of BSEP

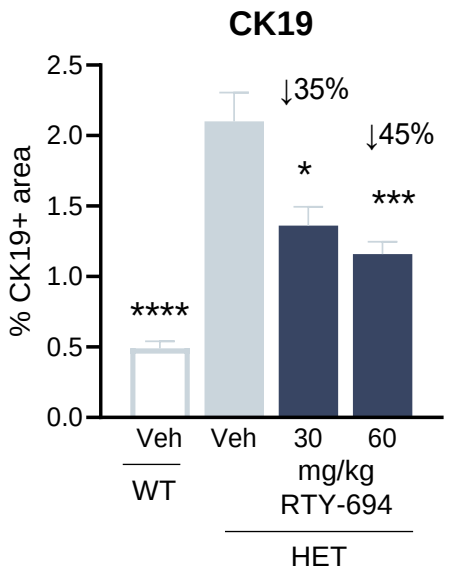
Statistics based on comparison to HET veh; PL = phospholipid; BA = bile acids; ALP = alkaline phosphatase; WT = wild type; HET = heterozygous

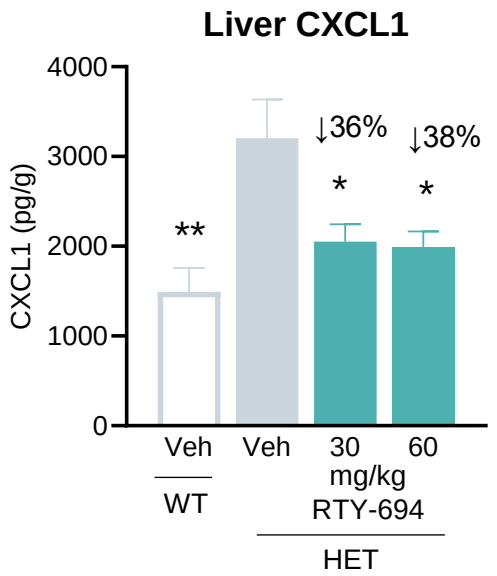
ABCB4/BSEP PFM reduces bile duct injury and inflammation in a mouse model of biliary disease

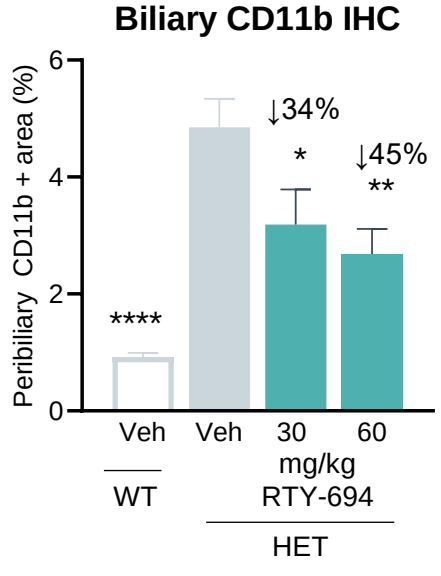
Ductular Reaction (CK19)



Cholangitis







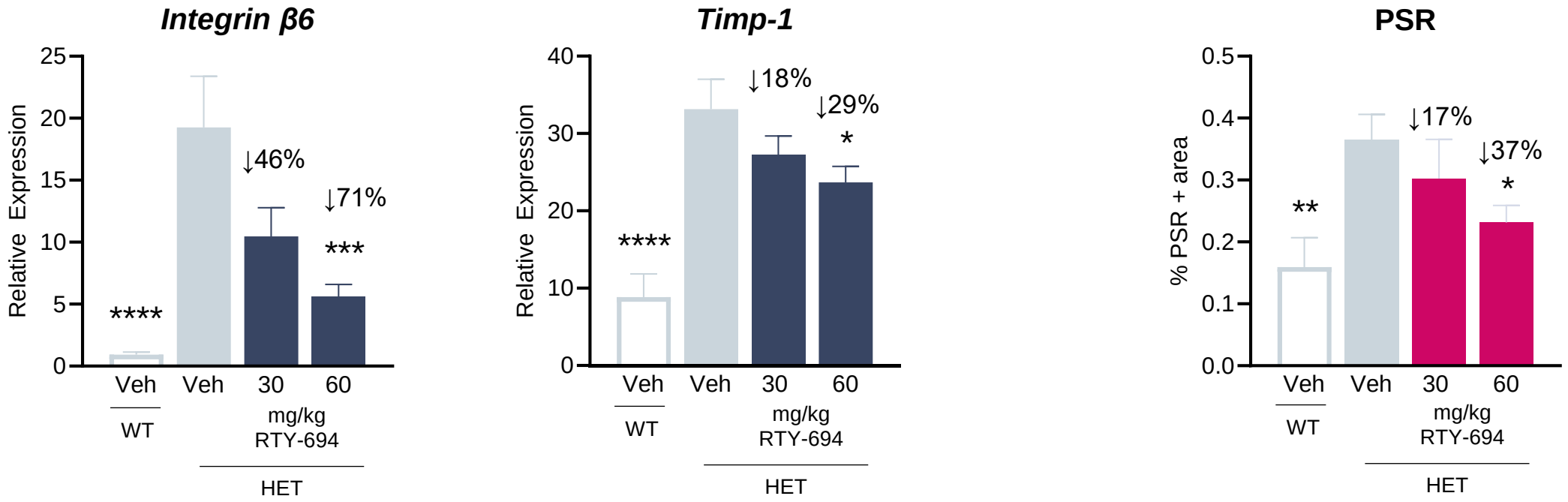
- Efficacy across multiple relevant endpoints provides confidence that ABCB4/BSEP PFM addresses bile duct injury

Statistics based on comparison to HET veh; CK19= cytokeratin 19; CXCL1 = C-X-C motif chemokine ligand 1; CD11b = cluster of differentiation 11b; WT = wild type; HET = heterozygous

ABCB4/BSEP PFM reduces fibrotic markers in a mouse model of biliary disease

Fibrosis modulators

Fibrosis histology



- Efficacy across fibrotic modulators and collagen deposition provides confidence that ABCB4/BSEP PFM can impact downstream pathophysiology in biologically rationalized indications

Statistics based on comparison to HET veh; Itgb6 = integrin beta 6; Timp-1 = tissue inhibitor of metalloproteinases 1; PSR = Picro Sirius Red ; WT = wild type; HET = heterozygous

Summary

- ABCB4/BSEP PFM is a **new therapeutic mechanism of action** for hepatobiliary diseases
- The **dual ABCB4/BSEP PFM directly binds target transporters and increases efflux** of phospholipid (ABCB4) and bile acids (BSEP)
- Increased ABCB4/BSEP activity **improves downstream disease endpoints of ductular reaction, inflammation and fibrosis** in a mouse model of biliary disease **and demonstrates anti-cholestatic activity** in a genetic mouse model of PFIC2
- **RTY-694** is a **clinical candidate** for the treatment of **Primary Sclerosing Cholangitis (PSC)**

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