A Positive Functional Modulator of ABCC6 Decreases Vascular Calcification and Improves Kidney Function in a Rat Adenine Diet Model

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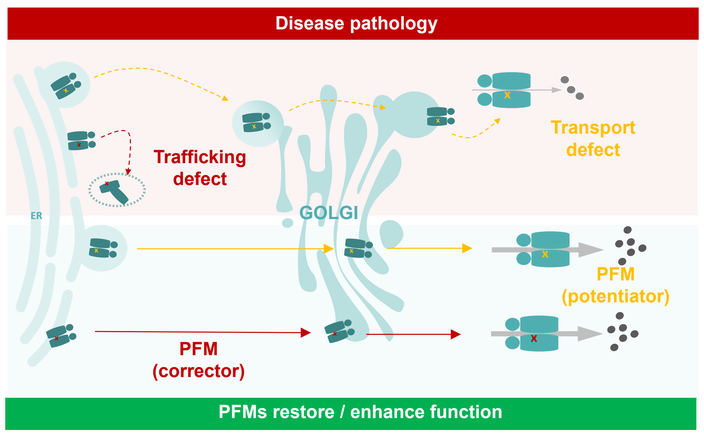
Background

- ATP-binding cassette (ABC) transporters are a large, phylogenetically conserved family with broad physiological and pathological relevance
- CFTR is currently the only ABC transporter targeted for activation by approved drugs, which address underlying genetic defects driving cystic fibrosis (CF) to reestablish chloride transport by correcting or potentiating transporter function
- Rectify is leveraging understanding across the ABC superfamily to pursue Positive Functional Modulators (PFMs) of ABC transporters to treat a broad range of human diseases

Positive Functional Modulators (PFMs) to unlock the pharmacotherapeutic potential of the ABC proteome

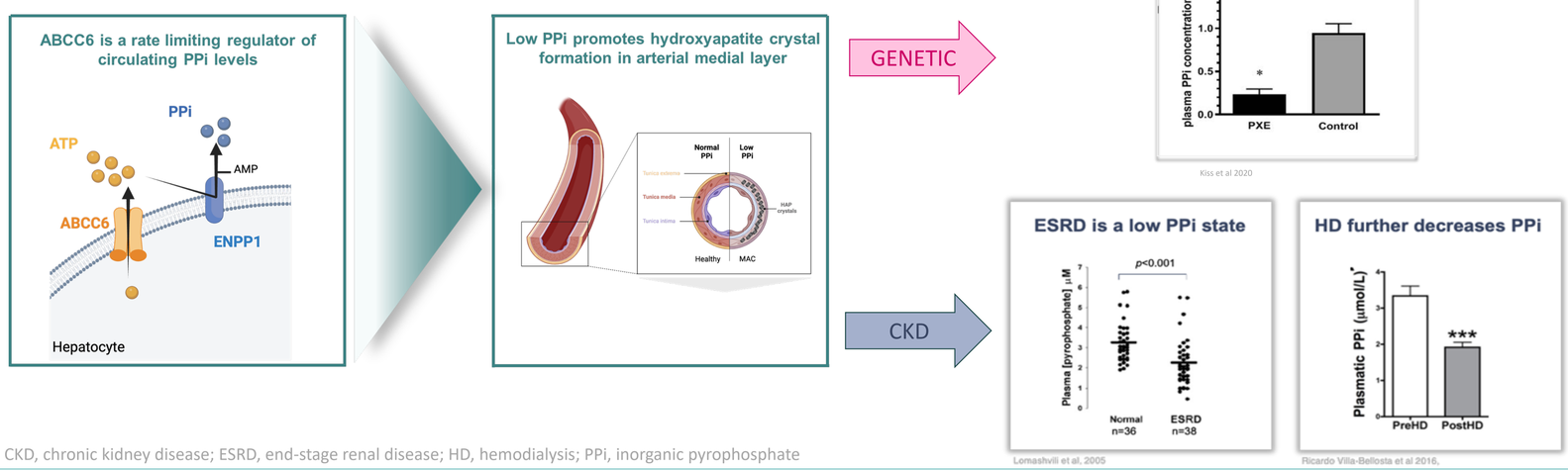
- ABC transporters are folded in the ER and trafficked to lipid membranes where they transport diverse substrates
- Genetic mutations can cause:
  - **Protein trafficking defects**
  - **Substrate transport defects**
- ABC transporter proteins are associated with multiple human diseases:
  - Etiological causes of **rare monogenic diseases**
  - Predisposition to symptoms & severity of **complex disease**
  - Mechanistic association to pathways implicated **in common disease**

ABC transporter dysfunction causes monogenic disease and is relevant to many common diseases



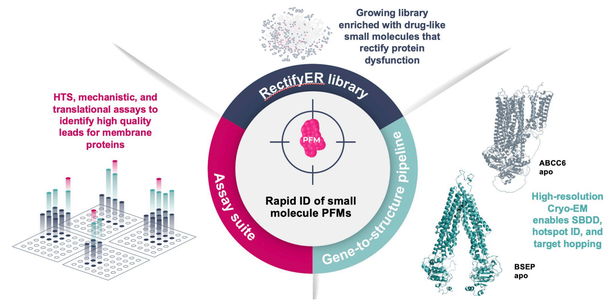
- **Positive Functional Modulators (PFMs):**
  - Small molecule compounds that rescue mutant ABC transporter function
  - **PFM correctors** can rescue mutant protein **trafficking** to reestablish membrane localization
  - **PFM potentiators** can rescue mutant protein functional activity to reestablish substrate **transport**
  - PFMs also have potential to **enhance WT ABC transporter biology**

ABCC6 is a critical determinant of circulating PPi levels



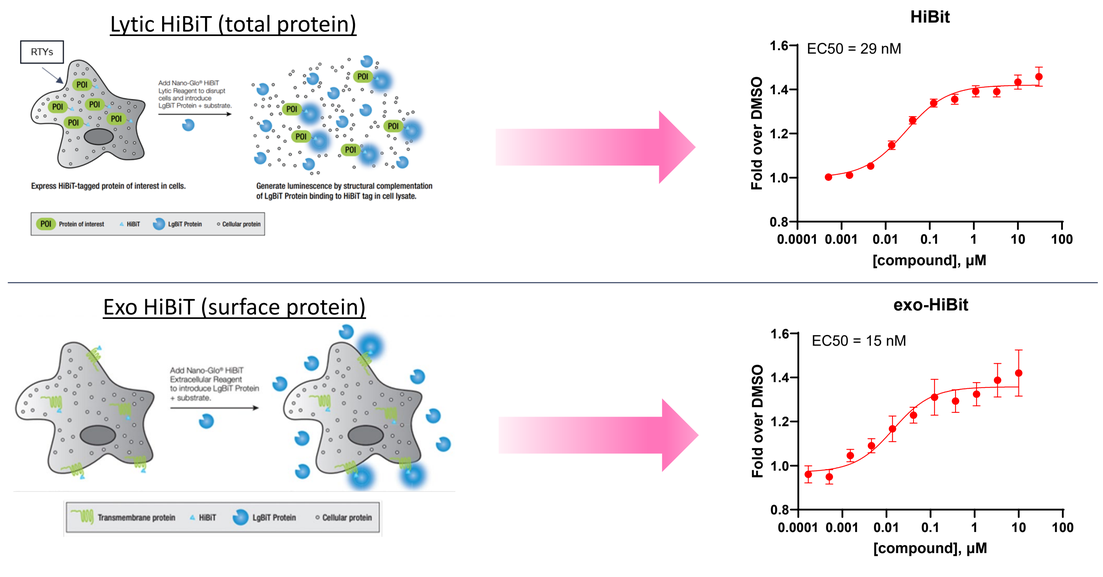
CKD, chronic kidney disease; ESRD, end-stage renal disease; HD, hemodialysis; PPi, inorganic pyrophosphate

RectifyER library accelerates PFM discovery



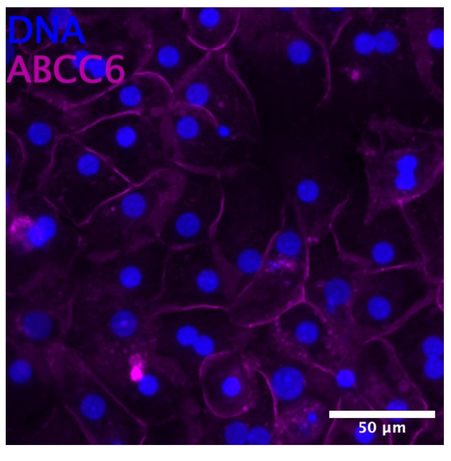
- Proprietary library is a rich collection of lead-like compounds enabling pan-ABC transporter drug discovery
- Screening diversity libraries allows enrichment of library through elaboration of new scaffold hits
- Deep structural and computational mining generates novel and diverse privileged scaffolds

HiBiT assays used to identify PFMs that increase ABCC6 protein

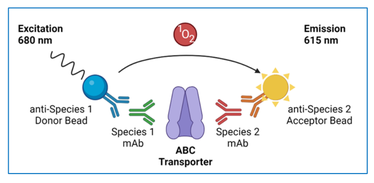


Rectify screen using HiBiT split luciferase technology identifies hits that increase ABCC6 protein expression in 2 different formats: Lytic (quantifying total protein) or Exo (quantifying surface protein only)

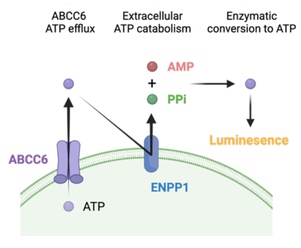
RTY-822 increases ABCC6 protein and function *in vitro* in PHH

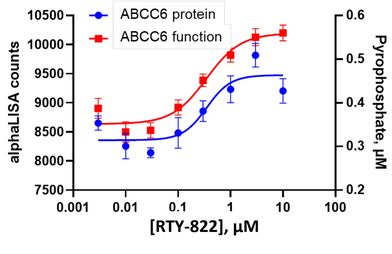


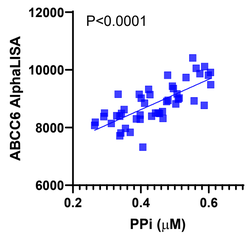
ABCC6 AlphaLISA assay concept



PPi assay concept







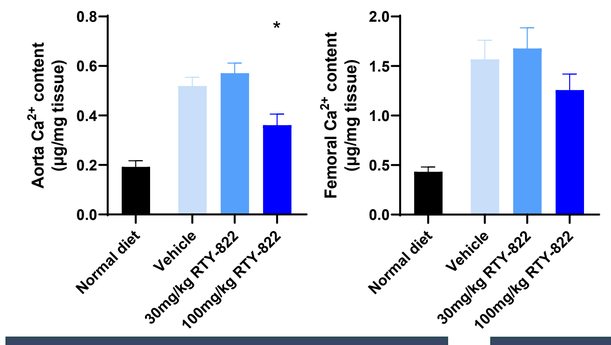
Compound testing in primary human hepatocytes (PHH) demonstrated correlated increases in ABCC6 protein (EC_{50} 211nM) and function (EC_{50} 271nM) by RTY-822. ABCC6 mRNA levels were unchanged in these cells.

RTY-822 significantly decreased vascular calcification & improved kidney function *in vivo*

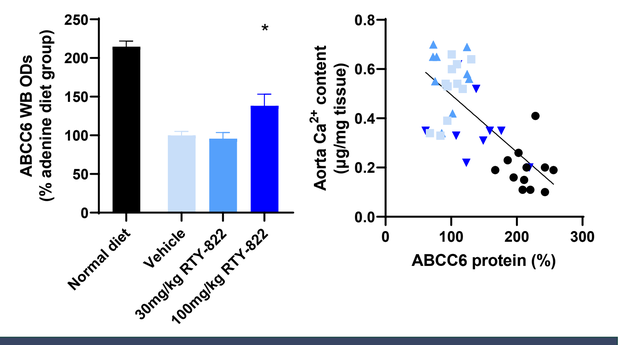
- Rats were on 0.75% adenine diet for the 1st week and then 0.5% adenine diet for the remainder of the study.
- Diet composition was modified to ↑Ca, ↑P, and ↓Mg compared to standard diet.
- Rats were dosed with 100ng/kg calcitriol SC M, W, F each week.
- RTY-822 was dosed PO QD at 30 and 100mg/kg.

Stats: One-way ANOVA with Dunnett's post-hoc test, n=9-12 rats/group. * p<0.05; ** p<0.01; *** p<0.001

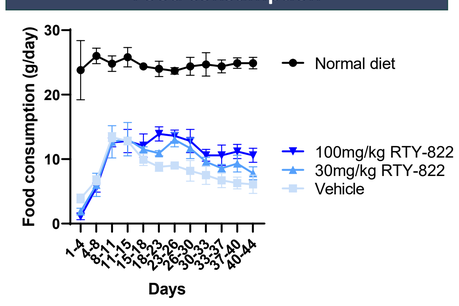
100mg/kg RTY-822 reduced vascular calcification



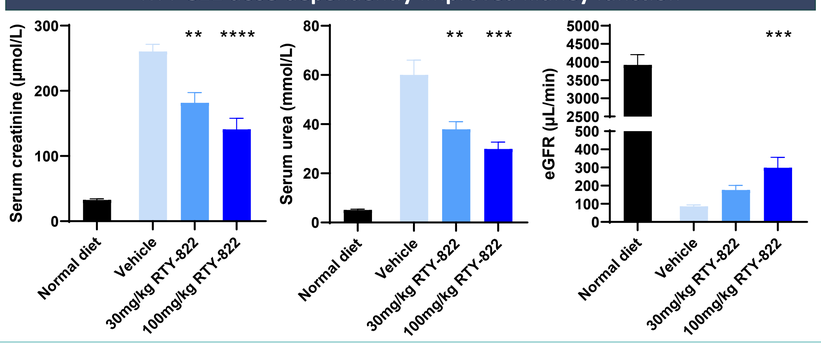
100mg/kg RTY-822 increased liver ABCC6 protein



Food consumption



RTY-822 dose-dependently improved kidney function



Conclusions

Positive Functional Modulation demonstrated for ABC Transporters

- Compounds identified in screening the proprietary RectifyER library increased ABCC6 protein expression.
- Lead compound RTY-822 increased ABCC6 protein and function in primary human hepatocytes, a translational cell system.
- RTY-822 decreased vascular calcification *in vivo* in a rat CKD model. The amount of vascular calcification in the aorta was significantly correlated with liver ABCC6 protein levels.
- RTY-822 also dose-dependently improved kidney function.
- These results validate the Rectify approach to identify Positive Functional Modulators of ABC Transporters, enabling drug discovery across a range of targets and therapeutic areas.