2019 Summer Intern Proposal

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P-glycoprotein (P-gp) is a drug transporter recommended by regulatory agencies for clinical evaluation as its inhibition can cause clinically important drug-drug interactions (DDIs). P-gp inhibition has become part of routine transporter DDI investigations in drug development. Traditionally this DDI was assessed in vitro and clinically using digoxin as the probe drug. However, recent studies have indicated that digoxin is not selective for P-gp in vitro and in vivo, and exhibits low pharmacokinetic (PK) sensitivity to P-gp inhibition. As such, it is not an ideal probe substrate for studying P-gp inhibition.

Recently, dabigatran etexilate (DE), the prodrug of dabigatran, has been recommended by the EMA and FDA as a clinical probe for studying intestinal P-gp inhibition. Our internal clinical microdose studies have suggested that a microdose of DE could be a more sensitive and selective P-gp probe than digoxin as well as more sensitive than a therapeutic dose of DE to study intestinal P-gp inhibition. However, compared to digoxin, in vitro and clinical data using DE as a P-gp probe are still limited and additional in vitro and clinical studies with DE will greatly advance our understanding of its suitability and limitations as a P-gp probe.

DE is not stable in cell-based assays due to endogenous CES-catalyzed hydrolysis. Kishimoto et al and our internal studies indicate that DE is stable if dosed in the basal, but not the apical compartment of transwells in Caco-2 and P-gp-transfected MDCKII and LLC-PK1 cells, where DE has direct access to CES1. In 2017, the T & IVT group explored in vitro P-gp inhibition assay using DE as a probe by determining monolayer flux only in the basal-to-apical direction or as bi-directional transport in cells pretreated with esterase inhibitors. However, this method has not been further validated with known P-gp inhibitors and the in vitro to in vivo correlation (IVIVC) has not been evaluated. Given the recommendations from regulatory agencies, further evaluation of this assay is critically needed to validate the assay conditions, as well as exploring and understanding its translational impact. In addition, these studies will directly support our ongoing network initiative of developing an internal DE/dabigatran PBPK model for predicting P-gp related DDIs.

To that end, we would like to work with an intern over the summer to validate in vitro P-gp inhibition assay using DE as a probe and assess in vitro to clinical translation. The objectives of this work include:

- Transporter selectivity: confirming whether DE and parent dabigatran are substrates of other major drug transporters
- Refine the previously established DE in vitro P-gp inhibition protocol and assay conditions
- Determine IC50 for 8-10 P-gp inhibitors, evaluate IVIVC and compare with IC50s reported in the literature
- Explore the feasibility of determining the Km value of DE in P-gp transfected cells
In addition to P-gp, hepatic and renal uptake transporters also play a critical role in drug disposition and serve as important sites for potential DDIs. Recently, there is an increase interest in exploring the feasibility of endogenous biomarkers to assess DDI liability. Several endogenous biomarkers have been identified as substrates of hepatic and renal transporters; changes in the plasma or urine level of these biomarkers may implicate DDI at the transporter levels in early clinical phase. However, limited in vitro data are available to support endogenous biomarker as appropriate probe substrate for transporter inhibition and whether the impact is similar to those using clinical relevant drugs.

To this end, if time permitted, the summer intern will also have the opportunities to involve in some pilot studies and contribute to evaluate the inhibitory effect of several inhibitors on selected endogenous biomarkers. This work will include:

- Uptake of selected biomarkers in transporter expressing cell lines
- Establish the inhibition assay condition for biomarkers and determine IC50 of several known transporter inhibitors on biomarker uptake
- Comparison of IC50 of these inhibitors using biomarkers and clinical drugs as probe substrates

By the end of the internship, the student should be able to

- Develop basic sample and liquid handling skills
- Conduct transport studies in various transporter transfected cells
- Prepare samples for bioanalysis
- Analyze data to determine in vitro kinetic parameters
- Develop an understanding of the rapidly evolving field of drug transporters in drug discovery and development.

References:

3. Chu et al., Clinical pharmacology and therapeutics 2018 Accepted.
6. Chu et al., J Pharm Sci. 2017 Sep;106(9):2357-2367
7. Mariappan et al., Curr Drug Metab. 2017 Oct 16;18(8):757-768

Internship Timeframe: Mid May/Early June – Mid-August, 2019 (approx. 9-12 weeks)

Contact: Please send your CV to Xiaoyan Chu (xiaoyan_chu@merck.com)