Review

Breast Cancer Resistance Protein (ABCG2) in Clinical Pharmacokinetics and Drug Interactions: Practical Recommendations for Clinical Victim and Perpetrator Drug-Drug Interaction Study Design

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Running Title: BCRP clinical DDI study design recommendations (46/60 characters)

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Review Format

Abstract: 247/250 words

Body Text: (review: no word limit)

References: (review: no reference limit)

Number of Tables: 7

Number of Figures: 1

Abbreviations: AUC, area under the concentration-time curve; BCRP (ABCG2), Breast Cancer Resistance Protein; DDI, drug-drug interaction; EMA, European Medicines Agency; FDA, Food and Drug Administration; IR, immediate release; ITC, International Transporter Consortium; MRP, multidrug resistance associated protein; NAT, *N*-acetyltransferase; OATP, organic anion transporting polypeptide; P-gp, P-glycoprotein; SNP, single nucleotide polymorphism; TKIs, tyrosine kinase inhibitors; PPIs, proton pump inhibitors;

ABSTRACT (247/250 words)

BCRP limits intestinal absorption of low-permeability substrate drugs and mediates biliary excretion of drugs and metabolites. Based on clinical evidence of BCRP-mediated drug-drug interactions (DDIs) and the c.421C>A functional polymorphism affecting drug efficacy and safety, both the FDA and EMA recommend preclinical evaluation, and when appropriate, clinical assessment of BCRP-mediated DDIs. Although many BCRP substrates and inhibitors have been identified in vitro, clinical translation has been confounded by overlap with other transporters and metabolic enzymes. Regulatory recommendations for BCRP-mediated clinical DDI studies are challenging, as consensus is lacking on the choice of the most robust and specific human BCRP substrates and inhibitors and optimal study design. This review proposes a path forward based on a comprehensive analysis of available data. Oral sulfasalazine (1000 mg, IR tablet) is the best available clinical substrate for intestinal BCRP, oral rosuvastatin (20 mg) for both intestinal and hepatic BCRP, and intravenous rosuvastatin (4 mg) for hepatic BCRP. Oral curcumin (2000 mg) and lapatinib (250 mg) are the best available clinical BCRP inhibitors. To interrogate the worst-case clinical BCRP DDI scenario, study subjects harboring the BCRP c.421C/C reference genotype are recommended. In addition, if sulfasalazine is selected as the substrate, subjects having the rapid acetylator phenotype are recommended. In the case of rosuvastatin, subjects with the OATP1B1 c.521T/T genotype are recommended, together with monitoring of rosuvastatin cholesterol lowering effect at baseline and DDI phase. A proof-of-concept clinical study is being planned by a collaborative consortium to evaluate the proposed BCRP DDI study design.

Introduction

Breast cancer resistance protein (BCRP, ABCG2) is an ATP-binding cassette efflux transporter comprised of a homodimer of two half subunits (Giacomini et al., 2010). BCRP is expressed in the small intestine, liver, blood-brain barrier, testis, placenta and mammary glands (Endres et al., 2006) and limits systemic and organ exposure of relevant substrates. BCRP transports endogenous and exogenous substrates with diverse physicochemical properties, which generally include a positive and/or negative charge, but otherwise span a large range of molecular weight, lipophilicity, and permeability (Mao and Unadkat, 2005; Ni et al., 2010; Robey et al., 2011). However, the impact of BCRP on pharmacokinetics is the most pronounced for compounds with low passive permeability (Poirier et al., 2014).

From a drug disposition perspective, BCRP functions primarily as an apical efflux pump in enterocytes, attenuating intestinal absorption of low-permeability substrate drugs, and as a canalicular efflux pump, transporting substrates from hepatocytes into bile. Unlike intestinal BCRP, which is rate-determining in the absorption of low-permeability substrate drugs, in the liver, uptake is generally rate-determining in hepatic clearance of drugs that predominantly rely on carrier-facilitated transport into the liver prior to excretion into bile via BCRP. However, when a basolateral efflux mechanism is operational (e.g. MRP4), BCRP biliary excretion can become rate-determining in systemic drug clearance when it is impaired to a sufficiently large extent (Pfeifer et al., 2013a; Pfeifer et al., 2013b). Such shifting of the rate-determining step in hepatobiliary clearance is a kinetically complex phenomenon and is best described with both systemic pharmacokinetic data, as well as a measure of hepatic drug exposure, which in humans can be assessed directly (e.g. imaging of a metabolically-stable drug) or indirectly using a hepatic pharmacodynamic biomarker (e.g. cholesterol for statins).

Pharmacogenetic studies identified a c.421C>A (p.Q141, rs2231142) single nucleotide polymorphism (SNP) of BCRP that results in reduced activity (Giacomini et al., 2013). In vitro studies demonstrated that this BCRP variant exhibits maintained mRNA expression, but 50-70% reduced protein expression and function due to enhanced susceptibility to proteasomal degradation (Kondo et al., 2004; Furukawa et al., 2009). Several marketed drugs including sulfasalazine, rosuvastatin, and atorvastatin have increased systemic exposure due to impaired BCRP activity in carriers of this functional polymorphism (Giacomini et al., 2013). For example, rosuvastatin demonstrated 2.4-fold higher exposure in homozygous BCRP c.421A/A individuals compared to homozygous c.421C/C individuals (Keskitalo et al., 2009b). In addition to changes in the pharmacokinetic profile, clinical studies involving BCRP c.421A/A homozygous carriers demonstrated an increased incidence of gefitinib side effects (Cusatis et al., 2006).

BCRP has been demonstrated to be an important mediator of drug-drug interactions (DDIs) in humans (Giacomini et al., 2010). For example, the herbal product curcumin increased sulfasalazine oral exposure 3.2-fold. This clinical DDI has been attributed to BCRP inhibition, because it could be replicated in wild-type, but not Bcrp-knockout mice, clearly proving Bcrp to be the DDI mechanism (Shukla et al., 2009; Kusuhara et al., 2012).

The clinical evidence of BCRP inhibition as a mechanism of DDIs with potential safety and efficacy consequences prompted the International Transporter Consortium (ITC) to highlight BCRP as an important transporter to evaluate during drug development (Giacomini et al., 2010). Subsequently, the FDA and EMA recommended that all investigational drugs should be evaluated as substrates (victims) or inhibitors (perpetrators) of BCRP, and when warranted, that victim and perpetrator DDI potential should be characterized in humans (FDA, 2012; EMA

2012). The exception to the BCRP substrate evaluation is when the drug candidate meets

biopharmaceutical classification system 1 criteria, where intestinal solubility and permeability

are too high for oral absorption to be attenuated by BCRP efflux (FDA, 2012; EMA 2012).

These regulatory recommendations are reasonable and justified by overall clinical evidence

supporting BCRP as a contributor to, although typically not the sole site of, victim and

perpetrator DDIs.

Many drugs from various therapeutic areas have been identified as substrates or inhibitors

of BCRP in vitro, yet clinical DDIs attributed directly and specifically to BCRP are limited due

to overlap with other transport, as well as metabolic, pathways (Poirier et al., 2014). For

example, the 8-fold increase in atorvastatin exposure during tipranavir and ritonavir co-

administration was attributed mechanistically to inhibition of BCRP, OATP1B1 and CYP3A4

(Pham et al., 2009); however, quantification of the relative contribution of each of these

pathways to the overall DDI is challenging. Since well-characterized clinical BCRP substrate

probes and inhibitors are lacking, DDIs attributed solely to BCRP are rare. As a consequence, a

consensus is lacking on how to optimally evaluate clinical BCRP victim and perpetrator DDI

potential.

Recognizing the challenges faced by the pharmaceutical industry regarding the clinical

evaluation of BCRP-mediated drug disposition and DDIs, the intent of this review is 1) to

identify the best available clinical probes and inhibitors that can be used to understand the

contribution of BCRP to intestinal, hepatic, or overall systemic drug disposition in humans, and

2) to propose a clinical study design to test the validity of the selected substrate/inhibitor pairs to

evaluate BCRP-mediated DDIs, as well as delineate organ-specific BCRP contributions in

humans.

Clinical Relevance of BCRP Polymorphisms

Impaired BCRP activity is associated with a non-synonymous SNP, c.421C>A (Furukawa et al., 2009). Clinically, this polymorphism is linked to increased exposure to rosuvastatin, sunitinib, an active metabolite of leflunomide, sulfasalazine, flavopiridol, diflomotecan and gefitinib (Giacomini et al., 2013). For example, homozygous carriers of the variant allele (c.421A/A) showed up to a 3.5-fold increase in sulfasalazine oral exposure compared to homozygous carriers of the reference allele (c.421C/C) (Yamasaki et al., 2008; Kusuhara et al., 2012). Clinical examples of elevated statin exposure in homozygous carriers of the c.421C>A SNP range from 1.7-fold increase for both atorvastatin and fluvastatin to 2.4-fold increase for rosuvastatin (Keskitalo et al., 2009a; Keskitalo et al., 2009b).

In general, the effect of the BCRP c.421C>A variant on the pharmacokinetics of most BCRP substrates has been relatively modest; however, a major limitation of many of these studies is the low number of subjects homozygous for this SNP due to low population frequency of the recessive allele. For example, intravenous diflomotecan exhibited 3-fold higher intravenous exposure in c.421C/A (n=5) versus c.421C/C carriers (n=15) but no change in AUC following oral drug administration (Sparreboom et al., 2004), while rosuvastatin exposure was 1.8-fold higher in a combined cohort of c.421C/A and A/A (n=5 C/A and n=2 A/A) versus c.421C/C carriers (n=7) (Zhang et al., 2006) (Table 1). In contrast, no effect of the c.421C/A SNP was observed on the oral pharmacokinetics of the following BCRP substrates: nitrofurantoin, lamivudine, pravastatin, pitavastatin, and intravenous irinotecan (Ferrante et al., 1991; de Jong et al., 2004; Han et al., 2007; Ieiri et al., 2007; Jada et al., 2007; Adkison et al., 2008; Keskitalo et al., 2009a; Zhou et al., 2013). Lack of effect of BCRP c.421C>A on pravastatin exposure (Keskitalo 2009a) stands in contrast to rosuvastatin (which has comparable

low passive permeability to pravastatin) and supports the hypothesis that P-gp and MRP2 play more prominent roles than BCRP in pravastatin absorption.

The above examples highlight the critical importance of thoughtful design of BCRP pharmacogenetic studies when intended to be used in lieu of traditional DDI inhibitor studies to delineate the victim DDI potential of a BCRP substrate drug. As demonstrated by the above examples, the number of study subjects in typical DDI studies is not high enough to randomly enroll enough carriers of the BCRP c.421A/A and c.421C/A genotypes, making a post-hoc comparison of BCRP genotype effects impossible. As such, when BCRP pharmacogenetic studies are intended to be used as a substitute for a DDI study with a BCRP inhibitor, subjects must be genotyped during enrollment, and the study should be powered with an equal and adequate number of subjects in each of the three groups (C/C, C/A, A/A). The two sulfasalazine BCRP pharmacogenetic studies by Yamasaki et al. and Adkison et al. provide good examples of how to proactively investigate victim DDI potential of a BCRP substrate drug in a properly designed study (Yamasaki et al., 2008; Adkison et al., 2010) (Table 2).

Recently, several null allelic mutations in the BCRP gene were reported to correlate with the Junior(a-) blood group antigen (Saison et al., 2012; Zelinski et al., 2012). The Junior(a-) phenotype has been associated with clinically relevant adverse events, including transfusion reactions and fetal anemia. This phenotype has been detected at a low frequency in individuals of northern European and Romani ancestry and in Bedouin Arabs, but at a relatively higher frequency in Asians, particularly Japanese (0.02-1.7%) (Zelinski et al., 2012; Tanaka et al., 2014). All of these mutations result in non-functional protein, thereby identifying individuals with the Junior(a-) blood group as a population lacking BCRP activity.

As BCRP deficiency has recently been elucidated as the mechanism underlying the Junior(a-) phenotype, these populations have not yet been used to study BCRP-mediated drug disposition in humans. Initial investigations into the safety and efficacy implications of BCRP deficiency demonstrated conflicting results with respect to elevated blood concentrations of uric acid (no change and hyperuricemia) (Matsuo et al., 2009; Saison et al., 2012); however, uric acid disposition is complex and involves several transporters besides BCRP, thus these conflicting findings are not entirely surprising (Reginato et al., 2012).

Theoretically, the Junior(a-) population may provide an opportunity to tease out the contribution of BCRP by comparing the effect to healthy controls; however, such a clinical study paradigm remains to be established. In practice, these studies would have to be conducted in Japan, where the prevalence of the Junior(a-) phenotype is sufficiently high and the phenotype and study subject recruitment is enabled by tracking of this phenotype by blood banks.

Potential Clinical BCRP Probe Substrates

Based on studies with BCRP-transfected polarized cell lines and membrane vesicles

generated from BCRP over-expressing cells, many marketed drugs and dietary/natural

constituents have been identified as substrates of BCRP. A list of 81 substrates was evaluated

based on reported efflux ratios >2 in cell monolayers and/or reported K_m values <10 μM (Table

3). These substrates have a broad range of physicochemical properties, with passive

permeability ranging from low to high (0.07-21.8 x 10^{-6} cm/s). Many BCRP substrates are

extensively metabolized, whereas others are excreted unchanged. The contribution of other

transporters to the disposition of these substrates is common (Poirier et al., 2014). In contrast to

the numerous BCRP substrates identified in vitro, the number of potential clinical substrates is

limited. Theoretically, the ideal clinical probe substrate should be selective for BCRP with

minimal contribution from other transporters and metabolizing enzymes in order to minimize the

complexity in data interpretation and enable extrapolation to other BCRP substrate drugs.

Analogous to digoxin safety studies for P-glycoprotein inhibitor drugs, if the candidate BCRP

probe substrate has a narrow therapeutic window and/or identified safety concerns, then the DDI

will have direct clinical relevance by informing dose adjustment for the substrate drug. An

example of the latter is the case of statins where increased exposure can result in myopathy

(Egan and Colman, 2011). Several potential clinical BCRP substrates for consideration are

described below.

Sulfasalazine. Sulfasalazine, an anti-inflammatory and immunomodulatory drug used to

treat ulcerative colitis, rheumatoid arthritis, and Crohn's disease (Azulfidine Prescribing

Information, 2011), has been proposed as a clinical probe for intestinal BCRP . Sulfasalazine is

restricted largely to the gastrointestinal tract due to low permeability, low solubility, and efficient

intestinal BCRP efflux. As such, oral bioavailability at therapeutic doses is low (<10%) and

highly variable. The low fraction of a sulfasalazine oral dose that is absorbed into the systemic circulation is mostly excreted unchanged in urine (via glomerular filtration) and bile (Azulfidine Prescribing Information, 2011; Giacomini et al., 2010).

Sulfasalazine is metabolized extensively by bacteria in the distal small intestine and colon to sulfapyridine, which is relatively well absorbed and cleared predominantly by *N*-acetyltransferase (NAT) 2. Inhibition of intestinal BCRP reduces sulfapyridine formation by increasing sulfasalazine absorption, which in turn decreases sulfasalazine exposure to intestinal bacteria. As such, the metabolite-to-parent AUC ratio is a more sensitive indicator of intestinal BCRP modulation in humans than parent sulfasalazine AUC, but only in intermediate and rapid acetylator phenotypes (Yamasaki et al., 2008). Specifically in NAT2 rapid acetylators, the range of BCRP clinical effect is ~10-fold as measured by the decrease in sulfapyridine-to-sulfasalazine AUC ratio, while the clinical magnitude of parent sulfasalazine AUC increase is ≤3.5-fold (Yamasaki et al., 2008).

A wealth of preclinical data support sulfasalazine as an essentially specific probe for intestinal BCRP, although a potentially small contribution from P-gp and MRP2 has been proposed (Zaher et al., 2006; Dahan and Amidon, 2009; Zamek-Gliszczynski et al., 2012). Overall, Bcrp-knockout rodent data support BCRP limiting sulfasalazine oral bioavailability by at least an order of magnitude, while MRP2 and perhaps P-gp attenuate intestinal absorption by a lesser extent (≤2 to 3-fold). However, the fact that sulfasalazine intestinal absorption is not strictly limited by BCRP cannot be ignored in the interpretation of sulfasalazine interaction data, especially considering possible overlap between BCRP and P-gp, as well as BCRP and MRP2, inhibitors.

The possibility of uptake transporter(s) contributing to sulfasalazine absorption has been proposed, and intestinal OATP2B1 has been shown to transport sulfasalazine in vitro (Kusuhara et al., 2012; Tomaru et al., 2013). Intestinal uptake could be an important consideration concerning interpretation of DDI data should a decrease in sulfasalazine exposure be observed. Furthermore, saturation of uptake transporter(s) may underlie pharmacokinetic differences between sub-therapeutic and therapeutic doses, which cannot be explained by drug solubility alone (Kusuhara et al., 2012).

Topotecan. Topotecan is a topoisomerase inhibitor for second-line treatment of patients with metastatic ovarian carcinoma and small cell lung cancer, available in both intravenous and oral formulations (HYCAMTIN[®] Prescribing Information (06/18/2014;

http://www.accessdata.fda.gov/drugsatfda_docs/label/2014/200199s003lbl.pdf and

http://www.accessdata.fda.gov/drugsatfda_docs/label/2014/020981s006lbl.pdf). Although oral absorption is limited by intestinal BCRP (Jonker et al., 2000; Kruijtzer et al., 2002), topotecan is a substrate for other efflux transporters as well, including P-gp and MRP2 (Lin et al., 2011). Nonetheless, in P-gp-knockout mice, the nonspecific BCRP and P-gp inhibitor, elacridar, increased topotecan oral exposure 6-fold, thus demonstrating a major role of Bcrp in limiting topotecan oral absorption (Jonker et al., 2000). Co-administration of elacridar with topotecan to cancer patients increased plasma exposure 2- to 3-fold (Kruijtzer et al., 2002), which is the upper limit of the possible increase in humans, where baseline bioavailability is 40% (HYCAMTIN[®] Prescribing Information (06/18/2014;

http://www.accessdata.fda.gov/drugsatfda_docs/label/2014/020981s006lbl.pdf). The effect of BCRP c.421C>A SNP on the pharmacokinetics of topotecan showed 1.3-fold higher oral bioavailability in c.421C/A versus c.421C/C individuals, a modest effect that would be expected

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to increase in patients who are c.421A/A homozygous but were not included in this study (Sparreboom et al., 2005). Although topotecan has some attractive characteristics as a BCRP probe substrate, due to its safety profile as a cytotoxic agent, topotecan is impractical for routine use in healthy volunteer BCRP DDI studies.

Statins. The physicochemical properties of statins, such as atorvastatin, cerivastatin, fluvastatin, pitavastatin, rosuvastatin, and pravastatin, are similar with respect to molecular weight, pKa, and anionic charge, but differ in terms of lipophilicity. Consequently, the involvement of a variety of metabolizing enzymes and drug transporters in statin disposition differs based on a statin's lipophilic versus hydrophilic character (Elsby et al., 2012; Shitara et al., 2013). Likewise, passive permeability varies with lipophilicity, and on one extreme is low for pravastatin and rosuvastatin, while on the other is high for cerivastatin and fluvastatin (Jones et al., 2012; Menochet et al., 2012; Li et al., 2014). With respect to clearance pathways, cerivastatin, fluvastatin, simvastatin lactone, and atorvastatin are extensively metabolized, whereas pravastatin, rosuvastatin, and pitavastatin undergo minimal metabolism and are cleared mainly through biliary and urinary excretion (Elsby et al., 2012). All statins to a varying degree are substrates for hepatic uptake transporters and some are BCRP substrates (Elsby et al. 2012).

Studies in Bcrp-knockout mice have demonstrated an important role for Bcrp in the hepatobiliary disposition of pitavastatin and rosuvastatin (Hirano et al, 2005; Kitamura et al., 2008). Following intravenous infusion to Bcrp-knockout mice, the biliary excretion rate of both drugs was reduced by approximately an order of magnitude relative to wild-type mice. In contrast, the systemic clearance of pitavastatin was not affected by Bcrp knockout, whereas that of rosuvastatin was reduced by approximately 2-fold. Likewise clinically, the BCRP c.421 C>A polymorphism had a negligible effect on pitavastatin pharmacokinetics (Ieiri et al., 2007).

Pitavastatin is a sensitive clinical probe for OATP1B1 DDI study (Prueksaritanont et al., 2014), but it is not recommended as a clinical probe in BCRP DDI study because of a minimal role of this transporter in its absorption, disposition and clearance. Although the most pronounced effects on C_{max} is reported for fluvastatin and rosuvastatin, the high permeability of fluvastatin combined with less compelling BCRP pharmacogenetic effects and DDI potential relative to rosuvastatin preclude consideration of this statin as a sensitive probe for intestinal BCRP-mediated DDI studies (Hirano et al., 2005; Ieiri et al., 2007; Elsby et al., 2012).

Rosuvastatin has limited ability to permeate cellular membranes via passive diffusion due to low lipophilicity ($LogD_{7.4}$ = -0.33) (Kitamura et al., 2008; Menochet et al., 2012). Intestinal absorption is ≤3.2-fold limited by BCRP in both preclinical species and humans (Karibe et al., 2014; Prueksaritanont et al., 2014). Rosuvastatin is primarily cleared by the liver (72%) and to a lesser extent by the kidneys (28%) (Elsby et al., 2012). Several hepatic uptake transporters (OATP1B1, OATP1B3, and sodium-taurocholate co-transporting polypeptide) contribute to its distribution in the liver, while BCRP and MRP2 secrete rosuvastatin into the bile, such that it can undergo extensive enterohepatic recirculation (Ho et al., 2006; Kitamura et al., 2008; van de Steeg et al., 2013). In addition to glomerular filtration, the tubular secretion of rosuvastatin is mediated by organic anion transporter 3 uptake and efflux by MRP2.

Based on pharmacogenetic assessment of all the statins, the disposition of rosuvastatin is affected primarily by BCRP polymorphisms in vivo, as summarized in Table 1. Because the BCRP c.421 C>A variant does not represent a complete knockout of BCRP activity, it is likely that more profound changes may be observed if rosuvastatin was used in DDI studies with a potent BCRP inhibitor or in the Junior(a-) population, which lacks BCRP altogether. Moreover, reduced biliary transport of rosuvastatin via BCRP (due to either inhibition or a polymorphism)

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may lead to more pronounced changes in hepatic concentrations compared to systemic

concentrations. Taken together, the evidence for BCRP as a major contributor to rosuvastatin

disposition relative to the other statins is the strongest. However, the use of rosuvastatin as a

BCRP DDI probe requires an appropriate clinical study design and careful consideration of

perpetrator properties, as discussed below.

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Clinical Evidence-Based Recommendations for BCRP Substrate Selection

Intestinal Probe Substrate. Four clinical studies have employed oral sulfasalazine as an intestinal BCRP probe to investigate the pharmacokinetic effects of BCRP inhibitors and the c.421C>A SNP in humans (Urquhart et al., 2008; Yamasaki et al., 2008; Adkison et al., 2010; Kusuhara et al., 2012) (Table 2 and Table 4). Although the number of human DDI studies is limited, important lessons can be drawn for future study design, specifically with respect to subject population, dose, formulation, and study endpoints. These considerations are discussed in detail below.

Population variability in sulfasalazine exposure can be large, as high as 81-fold (Adkison et al., 2010) (Table 4). As such, it is critical that clinical sulfasalazine interaction studies use a crossover design. To maximize the observed human intestinal BCRP interaction effects, study subjects with high BCRP and NAT2 activities should be enrolled, i.e., BCRP c.421C/C homozygous carriers and rapid NAT2 acetylators (Yamasaki et al., 2008). Rapid NAT2 acetylators provide the greatest sensitivity (~10-fold) for determination of clinical DDIs in terms of the sulfapyridine/sulfasalazine AUC ratio, followed by intermediate acetylators (~3-4-fold) versus slow acetylators, who show no apparent change in the ratio (Yamasaki et al., 2008). Notably, an unexpected increase in the sulfapyridine/sulfasalazine AUC ratio, or a decrease not commensurate with the increase in oral sulfasalazine exposure, may be indicative of NAT2 inhibition, which should be further investigated. In summary, intestinal BCRP DDI studies with sulfasalazine should enroll BCRP c.421C/C homozygous, rapid acetylator subjects and measure both parent and sulfapyridine oral exposure. As discussed in the preceding section, the sulfapyridine/sulfasalazine AUC ratio decrease appears to be a more sensitive measure of intestinal BCRP inhibition than parent drug exposure increase, and it is recommended that the DDI be reported using both endpoints.

The choice of sulfasalazine dose and formulation used for intestinal BCRP interaction studies also merits careful consideration (Table 2). BCRP pharmacogenetic and inhibition effects on oral sulfasalazine exposure were readily apparent and statistically significant following oral administration of a 2000 mg dose as immediate release (IR) tablets (Yamasaki et al., 2008; Kusuhara et al., 2012). In contrast, following oral administration of 500 mg sulfasalazine enteric coated tablets, systemic pharmacokinetics were not altered by BCRP pharmacogenetics or by oral co-administration of the BCRP inhibitor, pantoprazole (Adkison et al., 2010). The exact mechanistic reasons for why sulfasalazine administered as enteric coated tablets is not sensitive to BCRP modulation remain unknown. One plausible explanation is that this type of formulation releases drug further down the GI tract, where relative BCRP expression is up to 2-fold lower (Englund et al., 2006; Enokizono et al., 2007b; MacLean et al., 2008) and much less concentrated per unit of surface area (Pang, 2003), such that sulfasalazine is released past BCRP in the upper small intestine (Adkison et al., 2010). Although the extended release formulation appears to be the most likely culprit for the lack of BCRP impairment effect in the Adkison et al. (2010) study, because three independent clinical studies using sulfasalazine immediate release tablets or suspension showed consistent pharmacokinetic changes (Urquhart et al., 2008; Yamasaki et al., 2008; Kusuhara et al., 2012), further work is needed to provide an unequivocal mechanistic explanation for the negative findings of Adkison et al. (2010). Other formulations have been evaluated, including an oral suspension prepared from crushed IR tablets. At a therapeutic dose of 1000 mg, oral exposure was 1.6-fold higher following administration of the suspension versus IR tablet but was not statistically significant (Urquhart et al., 2008; Kusuhara et al., 2012). However, following oral administration of a 0.1 mg suspension (microdose) versus a 2000 mg IR tablet, the dose-normalized exposure was an order of

magnitude higher for the microdose suspension compared to the IR tablet, an effect possibly

attributed primarily to potential saturation of the speculated intestinal absorptive transporter(s) at

the therapeutic dose and secondarily to solubility effects (Kusuhara et al., 2012). Since the

apparent fraction absorbed is greater for the microdose suspension, the magnitude of the

intestinal BCRP DDI is not as large as that observed for the therapeutic IR tablet (AUC ratio of

2.0 vs. 3.2) (Kusuhara et al., 2012).

Total daily doses of up to 2000 mg appear to be reasonably well tolerated in most

subjects; however, gastric side effects are common, with about one-third of subjects experiencing

these side effects to some degree and increasing with dose level (Azulfidine Prescribing

Information, 2011; Kusuhara et al., 2012). Serious side effects such as Stevens-Johnson

syndrome can occur and have been observed following a single dose. To avoid unnecessary

adverse event complications, DDI studies involving sulfasalazine should exclude subjects with

known "sulfa" allergy, viral infections, a weakened immune system, a history or family history

of Stevens-Johnson syndrome, or with the HLA-B *1502 allele. Based on currently available

clinical experience, 1000 mg administered orally as IR tablets is the most optimal and practical

sulfasalazine dosing regimen for intestinal BCRP DDI studies. Considering the maximal (<4-

fold) clinical increase in sulfasalazine exposure due to BCRP inhibition, clinical exposure

following administration of the 1000 mg dose would not exceed the exposure observed following

administration of the highest approved dose level of 4000 mg/day. Finally, while the subject

genotyping recommendations for robust response with sulfasalazine as a marker of intestinal

BCRP activity (BCRP c.421 C/C; NAT2 rapid acetylator phenotype, non-carriers of HLA-B

*1502 allele) might seem onerous, these are all routine clinical genotyping tests, which add

insignificant cost to the overall study and do not present a serious enrollment hurdle, because the predominant genotype is recommended for all three genes.

Systemic Probe Substrate.   Oral rosuvastatin represents a sensitive probe to assess the magnitude of inhibition of both intestinal and hepatic BCRP. Allowing for assessment of the worst-case DDI scenario, a rosuvastatin BCRP DDI study should enroll subjects homozygous for the BCRP reference allele c.421 C/C (most prevalent in non-Asian ethnicities) and exclude any carriers of OATP1B1 c.521T/C or C/C. Consideration of CYP2C9 polymorphism (rosuvastatin undergoes minimal metabolism via this enzyme), is not of relevance because CYP2C9*1/*3 or *3/*3 was reported to have no effect on rosuvastatin (or its metabolite) PK or PD (Table 1). Alternatively, a clinical study should consider the genotype of the subjects enrolled and investigate the impact of multiple genetic covariates (e.g., co-existence of OATP1B1 and BCRP polymorphisms in the same subject) together with ethnicity data on the BCRP DDI sensitivity. An adequately powered study in terms of sample size would allow for identification of multiple high-risk combination patterns that could cause an increase in rosuvastatin exposure in conjunction with reduced BCRP activity, as recently reported for simvastatin (Tsamandouras et al., 2014).

Considering the complexity of rosuvastatin disposition and potential for enterohepatic recycling, it is evident that interpretation of increased rosuvastatin exposure in the DDI study following oral administration may be confounded by multiple factors. A key factor is the inability to differentiate between the contribution of intestinal and hepatic BCRP to the overall magnitude of a rosuvastatin-based interaction. Perpetrators that are potent BCRP as well as OATP inhibitors represent an additional issue of concern in that either or both can result in elevated blood levels of rosuvastatin. BCRP DDI study design involving oral and intravenous

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rosuvastatin formulations in the same subjects would allow delineation of the contribution of intestinal and hepatic BCRP. Based on the modeling and simulation work for compounds with comparable disposition to rosuvastatin (Watanabe et al., 2009; Chu et al., 2013b), the expectation is that the inhibition of hepatic BCRP may not cause significant changes in the plasma exposure, whereas drug accumulation in the hepatocytes and increase in liver exposure would occur. To account for this, cholesterol lowering effect of rosuvastatin will also be monitored in the control and inhibitor phase of the clinical studies, as a direct reflection of any changes in rosuvastatin liver exposure.

Rosuvastatin is commonly administered at an oral dose of 5–40 mg/day, with the majority of the clinical DDIs or pharmacogenetic studies conducted using a 10 or 20 mg dose (Simonson et al., 2004; Keskitalo et al., 2009b; Allred et al., 2011). Although a rosuvastatin intravenous formulation is not available currently, clinical data after infusion of 8 mg rosuvastatin have been reported as safe and well tolerated (Martin et al., 2003).

Rosuvastatin administered orally at a dose of 20 mg represents a good clinical probe for both hepatic and intestinal BCRP function (Table 1). The selected oral dose should be sufficient to capture BCRP DDI, while minimizing any potential safety concerns associated with increased exposure at the upper limit of known clinical DDIs. Selection of a 20 mg oral rosuvastatin dose for the clinical DDI study can theoretically be paired with a 4 mg intravenous dose, reflecting the reported 20% oral bioavailability (Martin et al., 2003). In the case of intravenous rosuvastatin, inhibition of hepatic BCRP would interrupt enterohepatic recycling; monitoring of the changes in rosuvastatin pharmacodynamic effect relative to the control data would allow assessment of its contribution. Rosuvastatin DDI studies should be conducted in a cross-over design, ideally in subjects with the highest BCRP and OATP activity, and report rosuvastatin pharmacokinetics

and cholesterol lowering effect at baseline and in the presence of a perpetrator. Comparison of

the increase in rosuvastatin exposure after intravenous and oral administration and any changes

in the PD effect will enable differentiation of the magnitude of hepatic and intestinal BCRP

interaction.

Potential Clinical BCRP Inhibitors

Identifying a drug or diet-derived/natural product that can be used as a selective BCRP

inhibitor in a clinical DDI study is challenging, as currently available compounds are not

selective for BCRP and inhibit other transporters and drug metabolizing enzymes (Leggas et al.,

2006; Lainey et al., 2012; Fujita et al., 2013; Jamei et al., 2014; Poirier et al., 2014; Shukla et al.,

2014). Upon careful consideration of inhibition data generated from various in vitro and in vivo

studies, there is a high likelihood that any known BCRP inhibitor will inhibit other membrane

transporters or metabolic enzymes. Given these limitations, the most potent and selective BCRP

inhibitors were identified that complement the proposed BCRP substrates for their potential use

in clinical DDI studies. Such inhibitors were selected through a comprehensive literature search

using the University of Washington Drug Interaction Database

(http://www.druginteractioninfo.org) and the University of California, San Francisco-FDA

TransPortal (http://bts.ucsf.edu/fdatransportal).

Identifying Potential BCRP Inhibitors. Known BCRP inhibitors with IC_{50} (or K_i)

values <10 µM were identified that could potentially be used in a clinical DDI study (Table 5).

Compounds for which the IC_{50} could not be obtained from the literature or considered

impractical for use in a clinical study (e.g., cyclosporine) were not considered further. C_{max}

values for the identified BCRP inhibitors were used to determine $[I_1]/IC_{50}$ values, which were

then used to predict the potency of systemic BCRP inhibition (FDA, EMA 2012). Similarly,

maximum intestinal concentrations, estimated as the highest approved dose/250 mL, were used

to calculate $[I_2]/IC_{50}$ values, which were used to predict the potency of intestinal BCRP

inhibition (Supplemental Table 1) (FDA, EMA 2012). If only a K_i was available, the IC_{50} was

assumed to be equivalent to $2xK_i$ when substrate concentration is equal to the K_m (Zhang et al.,

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2008). Drugs for which literature data supported inhibition of BCRP in vivo were included in the final assessment of potential inhibitors of systemic and intestinal BCRP. To undergo further consideration, a compound had to meet our criteria set at $[I_1]/IC_{50}>1$ or $[I_2]/IC_{50}>20$ for a high likelihood of clinical inhibition, which had to be supported by literature evidence that the compound either inhibits or is a BCRP substrate in vivo (i.e. a competitive inhibitor), BCRP inhibitors were rank ordered from highest to lowest $[I_1]/IC_{50}$ (Table 6) and $[I_2]/IC_{50}$ (Table 7).

In addition to a rank ordering of systemic and intestinal BCRP inhibition potential, a detailed assessment of the inhibition potential of other transporters and drug metabolizing enzymes was considered (Tables 6 and 7). The comprehensiveness of these tables reflects the difficulty encountered when selecting a viable substrate-inhibitor pair, as multiple inhibitory interactions occurring simultaneously will confound data interpretation concerning specific BCRP involvement in substrate pharmacokinetics. Such potential nonselective interactions were carefully considered, after which most identified BCRP inhibitors were eliminated. The final selected BCRP inhibitors have inherent pros and cons, which are detailed below.

Sulfasalazine. Sulfasalazine is a BCRP substrate with limited absorption due to efficient intestinal BCRP efflux into the lumen, as well as low permeability and low solubility (Azulfidine Prescribing Information, 2011). Being a substrate, sulfasalazine may act as a competitive inhibitor of BCRP, with reported IC_{50} values as low as 0.46 µM (Elsby et al., 2011). The C_{max} range for a single 3 to 4 gram dose of sulfasalazine is 38-79 µM (Goodman et al., 2001), resulting in a maximum $[I_1]/IC_{50}$ value of 170 (Table 6), and an $[I_2]/IC_{50}$ value of 87,000 (Table 7). As such, sulfasalazine could be used as a BCRP inhibitor in a clinical study when paired with a BCRP substrate such as rosuvastatin. However, sulfasalazine also inhibits OATP-mediated uptake, in particular OATP1B1 [$IC_{50} = 0.56$ µM (Karlgren et al., 2012b)]. As OATP1B1 is one

of the primary transporters responsible for taking up rosuvastatin into the liver (Simonson et al., 2004; Ho et al., 2006; Pasanen et al., 2007), and the inhibitory potency towards OATP1B1 is similar to that of BCRP, OATP1B1 becomes a major confounding factor. Therefore, sulfasalazine was excluded from consideration for use as a clinical BCRP inhibitor.

Elacridar. Elacridar was originally designed and developed as a reversal agent of P-glycoprotein and BCRP to overcome multidrug resistance. Although, elacridar increases the accumulation of several anticancer agents in preclinical tumor models (Hyafil et al., 1993; Agarwal et al., 2012), it is not a systemically-potent efflux inhibitor at chronically-tolerated doses in humans (Kalvass et al., 2013). However in the intestine, elacridar has been shown to increase topotecan bioavailability via inhibition of BCRP (Kruijtzer et al., 2002; Kuppens et al., 2007). These observations are consistent with elacridar ranking low in terms of systemic BCRP inhibition ($[I_1]/IC_{50} = 1.1$, Table 6), but high with respect to intestinal BCRP inhibition ($[I_2]/IC_{50} = 9,200$, Table 7). The use of elacridar as a clinical intestinal BCRP inhibitor is precluded by the fact that this compound is not currently approved by the FDA or EMA for use in humans. Furthermore, elacridar is an even more potent inhibitor of P-gp than BCRP ($[I_2]/IC_{50} = 180,000$) and would not be suitable for elucidating the intestinal BCRP contribution to limiting the absorption of dual substrates.

Proton Pump Inhibitors. Multiple proton pump inhibitors (PPIs) inhibit BCRP in vitro; however, pantoprazole and rabeprazole are the only two that meet the aforementioned criteria for consideration as clinical inhibitors (Tables 6 and 7); however, clinical and/or preclinical proof-of-concept studies have not been reported. Despite clear advantages to using an inhibitor with an acceptable safety profile such as pantoprazole (Remes-Troche et al., 2014) or rabeprazole (Zouboulis-Vafiadis et al., 2014), these drugs are not as potent BCRP inhibitors as the tyrosine

kinase inhibitors with respect to the $[I_2]/IC_{50}$ values (Table 7) where preclinical proof of concept

has been demonstrated (Zaher et al., 2006). Similarly, pantoprazole marginally meets the pre-

defined criterion for systemic BCRP inhibition, with an $[I_1]/IC_{50}$ value of 1.2 (Table 6). As such,

although using a PPI may minimize the safety risks, other drugs are expected to offer a superior

in vivo inhibition profile, rendering data interpretation more straightforward. Finally, any

observed DDIs with PPIs are fundamentally confounded by increased gastrointestinal pH (Ware

et al., 2013), and a separate intestinal pH control arm must be incorporated into the clinical study

with a PPI, which is not a BCRP inhibitor to control for this effect (Adkison et al., 2010).

Natural Products. Several diet-derived/natural product constituents such as curcumin,

daidzein, genistein, chrysin, 7,8-benzoflavone and glycyrrhetic acid have been shown to inhibit

human BCRP in vitro (Zhang et al., 2005a; Yoshida et al., 2008; Merino et al., 2010; Tamaki et

al., 2010). In addition, a mixture of the soy isoflavones genistein and daidzein was shown to

inhibit Bcrp in wild-type but not Bcrp-knockout mice, supporting specificity for Bcrp inhibition

(Merino et al., 2010). Of these constituents, only curcumin has been evaluated clinically as a

BCRP inhibitor. Curcumin is the principal curcuminoid in turmeric, a commonly used spice

derived from the rhizomes of *Curcuma longa*, a member of the ginger family. Curcuminoids are

polyphenols that render turmeric yellow in color (Sharma et al., 2005). Curcumin is a potent

inhibitor of BCRP, with a K_i of 0.7 μM determined with membrane vesicles over-expressing

human BCRP and sulfasalazine as the probe substrate (Kusuhara et al., 2012). Based on the

$[I_2]/IC_{50}$, curcumin is predicted to inhibit intestinal BCRP in vivo. Several clinical studies

involving oral curcumin have been conducted, with doses ranging from 2000 to 8000 mg (Cheng

et al., 2001; Kusuhara et al., 2012). Curcumin was well tolerated, with no signs of toxicity

observed during a phase I study in which the subjects were administered oral doses of 8000

mg/day for 3 months (Cheng et al., 2001). At 2000 mg/day, curcumin increased sulfasalazine

exposure 3.2-fold, which was attributed to inhibition of intestinal BCRP (Kusuhara et al., 2012).

Because curcumin is considered a food or dietary supplement, and thus not subject to strict

manufacturing controls as drugs, the purity and reproducibility with respect to constituent

composition, stability, and dissolution characteristics is not assured. Accordingly, a natural

products chemistry laboratory should be consulted for sourcing and analyzing the test product.

Tyrosine kinase inhibitors. Tyrosine kinase inhibitors (TKIs) are a relatively new

generation of anti-cancer drugs that are generally well tolerated. Several TKIs (erlotinib,

gefitinib, lapatinib, nilotinib, and sunitinib) are potent BCRP inhibitors with acceptable safety

margins to be considered for use in healthy volunteers. One clear advantage to using a TKI as a

clinical BCRP inhibitor is that, with the exception of sunitinib, TKIs are predicted to inhibit

intestinal as well as systemic BCRP. Based on the rank ordering of inhibitory potency for TKIs,

lapatinib is the most potent when considering both intestinal and systemic inhibition of BCRP.

Although in vitro data suggest that lapatinib may inhibit P-gp, OATP1B1, and CYP2C8

concomitantly, these interactions are based on a high therapeutic dose of 1250 mg. However, at

a sub-therapeutic dose of 250 mg, based on $[I_1]/IC_{50}$ and $[I_2]/IC_{50}$ values (22 and 40,000,

respectively), lapatinib is predicted to effectively inhibit BCRP (Table 6 and 7). Although

lapatinib carries a black box warning for hepatotoxicity rarely observed during chronic use in

cancer patients (TYKERB® product label, 10/18/2013,

http://www.accessdata.fda.gov/drugsatfda_docs/label/2013/022059s016s017lbl.pdf), it is well

tolerated acutely, as doses up to 7000 mg/day have shown no dose limiting toxicities (Chien et

al., 2014). At clinical doses, the diarrhea associated with patient treatment would be the only

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potential concern; however, this adverse effect typically resolves within 24-48 hours of treatment

cessation (MacFarlane and Gelmon, 2011; Chien et al., 2014).

Clinical Evidence-Based Recommendations for BCRP Inhibitor Selection.

Considering the totality of available information, two compounds emerge as potential clinical BCRP inhibitors for use in DDI studies. To discern the effect of intestinal-specific BCRP inhibition, a single 2000 mg dose of curcumin is proposed to extensively inhibit intestinal, but not systemic, BCRP (Tables 6-7). Due to poor absorption, curcumin is not detectable in plasma at this proposed dose (Garcea et al., 2005; Kusuhara et al., 2012), rendering the compound a practical choice for intestinal-specific BCRP inhibition. The second proposed BCRP inhibitor is lapatinib. Compared to a food product such as curcumin, the benefit of using a marketed drug to assess DDIs is that, among other things, a natural products chemistry laboratory need not be consulted. The recommended sub-therapeutic oral dose of 250 mg should have minimal adverse effects and possibly attenuate the impact of inhibition of other transporters and metabolic enzymes (Castellino et al., 2012). At this dose, lapatinib is predicted to inhibit both intestinal and systemic BCRP based on $[I_1]/IC_{50}$ and $[I_2]/IC_{50}$ values (Tables 6-7). One potential concern is that lapatinib may also inhibit OATP1B1, as rosuvastatin uptake is dependent on this transporter (the $[I_1]/IC_{50}$ is 0.29, which is marginally above the cut off value of 0.1). However, lapatinib is highly protein bound (>99%) so circulating levels of free drug available to inhibit OATP1B1 systemically will be significantly less than the total C_{max} reported in Table 6. Taking into account the concentration of lapatinib in the intestine and in the systemic circulation, lapatinib is likely to inhibit BCRP but not OATP1B1.

Recommendations for Clinical BCRP Drug Interaction Study Design

This comprehensive review of potential clinical BCRP probe substrates and inhibitors identified two substrates and two inhibitors to assess BCRP-mediated DDIs in humans. The two recommended probe substrates are sulfasalazine (1000 mg, IR, oral) and rosuvastatin (20 mg oral and 4 mg, intravenous), and the two inhibitors are curcumin (2000 mg, oral) and lapatinib (250 mg, oral). The combination of oral sulfasalazine with either inhibitor provides a substrate-inhibitor pair to assess the extent of a potential intestinal BCRP DDI. The dynamic range of sulfasalazine will provide a measure of the highest extent of an interaction due to inhibitor co-administration. Further, the combination of oral and intravenous rosuvastatin with both inhibitors will enable deconvolution of intestinal versus systemic BCRP contribution to rosuvastatin pharmacokinetics. Based on these aims, a clinical study design is proposed to validate these approaches (Figure 1).

Sulfasalazine Intestinal BCRP DDI. Oral sulfasalazine (1000 mg, IR, single dose) is recommended to evaluate intestinal BCRP-mediated DDIs. The study will be an open-label, 3-period cross-over study involving 10 subjects genotyped for the BCRP reference allele and phenotyped for rapid NAT2 acetylator status prior to enrollment. Sulfasalazine will be administered alone and in combination with oral curcumin (2000 mg, single dose) or lapatinib (250 mg, single dose) (Figure 1). Subjects will undergo three treatments with at least a 1-week washout between treatments as follows: sulfasalazine alone, sulfasalazine plus curcumin, and sulfasalazine plus lapatinib. Pharmacokinetics of both sulfasalazine and sulfapyridine will be determined, with the DDI reported as both an increase in sulfasalazine exposure and decrease in the sulfapyridine/sulfasalazine AUC ratio.

Rosuvastatin Intestinal and Hepatic BCRP DDI. Oral rosuvastatin (20 mg, single dose) is recommended to evaluate intestinal and hepatic BCRP DDIs, whereas intravenous rosuvastatin (4 mg, single dose) is recommended to evaluate hepatic BCRP DDIs. Each rosuvastatin administration route will include an open-label, 3-period cross-over study involving subjects genotyped for the BCRP and OATP1B1 reference alleles prior to enrollment. Rosuvastatin will be administered alone and with oral curcumin (2000 mg, single dose) or lapatinib (250 mg, single dose) (Figure 1). Treatments include intravenous rosuvastatin alone, intravenous rosuvastatin plus oral curcumin, intravenous rosuvastatin plus oral lapatinib, oral rosuvastatin alone, oral rosuvastatin plus oral curcumin, and oral rosuvastatin plus oral lapatinib. Inclusion of cholesterol monitoring will provide insight towards hepatic accumulation of rosuvastatin due to inhibition of hepatic BCRP. Changes in rosuvastatin exposure relative to the control will be the DDI study endpoint.

Conclusions

The regulatory recommendation to evaluate the contribution of BCRP to drug disposition and DDIs in clinical studies has posed challenges across the pharmaceutical industry. These challenges primarily reflect the lack of selective and specific human BCRP substrates and inhibitors, as well as a lack of established and accepted optimal clinical study designs. As described throughout this review, BCRP substrates and inhibitors suitable for human use were identified through a comprehensive analysis of available pharmacogenetics and DDI data. Two substrates were selected to probe intestinal and/or hepatic BCRP-mediated DDIs. Oral sulfasalazine (1000 mg, IR tablets) was deemed the best available probe for intestinal BCRP, oral rosuvastatin (20 mg) as the best available probe for both intestinal and hepatic BCRP, and intravenous rosuvastatin (4 mg) as the best available probe for hepatic BCRP. Oral curcumin (2000 mg) and oral lapatinib (sub-efficacious 250 mg dose) were deemed the best available BCRP inhibitors. Optimal study designs that would elicit the worst-case clinical BCRP DDI scenarios are proposed (Figure 1). The pharmacokinetic data obtained from the proposed 9-arm clinical study should provide the pharmaceutical industry and regulatory agencies insight into the extent of BCRP-mediated clinical DDIs, as well as validate clinical study designs that enable optimal assessment of intestinal and combined intestinal and hepatic BCRP-mediated DDIs.

Author Contributions

Participated in research design: Lee, O'Connor, Galetin, Cook, Ellens, Feng, Taub, Paine, Polli,

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Contributed new reagents: NA

Conducted experiments: NA

Performed data analysis: O'Connor, Ragueneau-Majlessi, Ritchie

Wrote or contributed to the writing of the manuscript: Lee, O'Connor, Ritchie, Galetin, Cook,

Feng, Ellens, Ragueneau-Majlessi, Taub, Paine, Polli, Ware, and Zamek-Gliszczynski

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Table 1. Summary of rosuvastatin BCRP pharmacogenomic studies and associated effect on its pharmacokinetics

Dose*	Subject Race & Sex	BCRP c.421C>A Genotype	OATP1B1 Phenotype	Effect of c.421C>A SNP	Reference
20 mg SD	Asian Chinese male	7- CC, 5 –CA, 2-AA	All subjects had OATP1B1 521TT and CYP2C9*1/*1 genotypes ((wild type)	1.78-fold AUC increase in 421CA/AA genotype (combined)[a]	Zhang 2006
20 mg SD	Caucasian (50% female)	16-CC 12-CA, 4-AA	Individuals with the OATP1B1 521CC genotype not included[b]	1.2-fold AUC increase in 421CA (NS); 2.44- and 2.3-fold AUC and Cmax increase, respectively in 421AA[a]	Keskitalo 2009b
5, 10 and 20 mg SD (1 week washout)	Asian Chinese	15-CC, 15-CA, 6-AA	No significant effect of CYP2C9*3 and OATP1B1 c.521 T > C	1.6-fold AUC increase in 421 AA[c]	Zhou 2013
10 mg/day (min 4 weeks)	Asian Chinese (52% female)	129-CC, 108- CA, 39-AA	205- OATP1B1 521TT and 62/5- TC/CC. Rosuvastatin plasma concentrations affected by both BCRP 421C>A and OATPB1 521T>C [e]	2.2-fold increase in mean plasma concentrations in 421AA[a,d] BCRP 421C>A (but not OATP1B1521 T>C) strongly associated with the LDL-C response to rosuvastatin	Lee 2013

*The route of administration was oral in all cases
[a] Denotes significant change, $p<0.05$
[b] Subjects had ABCB1 1199GG reference genotype
[c] Dose-normalized changes (based on 5 mg)
[d] 1.99-fold increase in mean plasma concentration of N-desmethyl rosuvastatin
[e] No effect of CYP2C9*1/*3 or *3/*3 on rosuvastatin PK or PD

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Table 2. Summary of clinical studies using sulfasalazine as the intestinal BCRP probe.

Dose / Formulation*	Subject Race & Sex	BCRP c.421C>A Genotype	NAT2 Phenotype	Sulfasalazine AUC Variability	Effect of c.421C>A SNP or BCRP inhibitor on PK	Reference
2000 mg IR tablet	Asian men	12-CC, 16-CA, 9-AA	13-RA, 16-IA, 8-SA	1.9-fold (95% CI, genotype controlled)	1.9-fold AUC increase in 421CA, 3.5-fold AUC increase in 421AA; clear gene-dose effect	Yamasaki et al., 2008
1000 mg suspension (also tested IR tablet)	Caucasian men and women	9-CC, 5-CA, 0-AA	Not genotyped, sulfapyridine PK not studied	20.5-fold (max/min; genotype controlled)	2.4-fold AUC increase in 421CA genotype	Urquhart et al., 2008
500 mg enteric-coated tablet	Asian men	12-CC, 12-CA, 12-AA	4-RA, 20-IA, 12-SA	21- to 81-fold (max/min; genotype controlled)	None (trend for 2-fold AUC increase in AA genotype, NS); no clear gene-dose effect; no significant effect of BCRP inhibitor (40 mg pantoprazole)	Adkison et al., 2010
2000 mg / 100 µg IR tablet / suspension	Asian men	8-CC	Not genotyped, sulfapyridine PK not studied	AUC CV = 24-40%	Curcumin (2 g*) increased AUC 3.2-fold (therapeutic dose) and 2-fold (microdose)	Kushuhara et al., 2012

*The route of administration was oral in all cases
RA – rapid acetylator; IA – intermediate acetylator, SA – slow acetylator

Table 3. Compounds considered as in vivo human BCRP substrates based on available in vitro data.

Object	Object Therapeutic Class	Experimental System	Efflux Ratio [a]	K_m (µM)
(–)–pantoprazole	Proton Pump Inhibitors	MDCK–transfected cells		0.59 [1]
(+)–pantoprazole	Proton Pump Inhibitors	MDCK–transfected cells		5.32 [1]
2'–hydroxyflavone–O–glucuronide	Food Products	HeLa–transfected cells		5.4 [2]
3,2'–dihydroxyflavone–O–glucuronide	Food Products	HeLa–transfected cells		35.9 [2]
3,3'–dihydroxyflavone–O–glucuronide	Food Products	HeLa–transfected cells		45.2 [2]
3,4'–dihydroxyflavone–O–glucuronide	Food Products	HeLa–transfected cells		35.9 [2]
3,5–dihydroxyflavone–O–glucuronide	Food Products	HeLa–transfected cells		12.8 [2]
3,5–di–O–caffeoylquinic acid	Food Products	Caco–2 cells	5.1 [3]	
3,6–dihydroxyflavone–O–glucuronide	Food Products	HeLa–transfected cells		31.9 [2]
3,7–dihydroxyflavone–O–glucuronide	Food Products	HeLa–transfected cells		3.5 [2]
3'–hydroxyflavone–O–glucuronide	Food Products	HeLa–transfected cells		5.6 [2]
3–hydroxyflavone–O–glucuronide	Food Products	HeLa–transfected cells		9.7 [2]
4–hydroxyflavone–O–glucuronide	Food Products	HeLa–transfected cells		17.5 [2]
5–hydroxyflavone–O–glucuronide	Food Products	HeLa–transfected cells		48.5 [2]
6–hydroxyflavone–O–glucuronide	Food Products	HeLa–transfected cells		26.8 [2]
7–hydroxyflavone–O–glucuronide	Food Products	HeLa–transfected cells		9 [2]

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aconitine	Herbal Medications	MDCK–transfected cells	19.8 [4]	
apixaban	Anticoagulants and Antiplatelets	MDCK–transfected cells	12 [5]	
axitinib	Kinase Inhibitors	MDCK–transfected cells	3.4 [6]	
barasertib hydroxy–QPA	Kinase Inhibitors	MDCK–transfected cells	2.31 [7]	
boceprevir	Antivirals	MDCK–transfected cells	2 [8]	
bupropion	Dopamine–Norepinephrine Reuptake Inhibitors	Membrane vesicles		3 [9]
canertinib	Kinase Inhibitors	MDCK–transfected cells	2.8 [10]	
chlorothiazide	Diuretics	MDCK–transfected cells	36 [11]	
cimetidine	H–2 Receptor Antagonists	MDCK–transfected cells	3.4 [12]	
ciprofloxacin	Antibiotics	MDCK–transfected cells	2.9 [13]	
dantrolene	Muscle Relaxants	Caco–2 cells	5 [14]	
daunorubicin	Anthracyclines	Caco–2 cells	4.9 [15]	
dimenhydrinate	H–1 Receptor Antagonists	Caco–2 cells	9.8 [16]	
dipyridamole	Anticoagulants and Antiplatelets	MDCK–transfected cells	2.7 [17]	
dolutegravir	HIV–Integrase Strand Transfer Inhibitors	MDCK–transfected cells	3.1 [18]	
edaravone sulfate	Neuroprotectors	Membrane vesicles		16.5 [19]
erythromycin	Antibiotics	Caco–2 cells	8.1 [15]	
estradiol–17–β–glucuronide	Estrogens	Membrane vesicles		44.2 [20]
estrone–3–sulfate	Estrogens	Caco–2 cells	48.5 [21]	6.8 [22,b]
ethinyl estradiol sulfate	Estrogens	Membrane vesicles		2.9 [23]
etoposide	Topoisomerase Inhibitors	Caco–2 cells	12.7 [15]	
flavopiridol (alvocidib)	Kinase Inhibitors	MDCK–transfected cells	6 [24]	

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fluvastatin	HMG CoA Reductase Inhibitors (Statins)	Caco–2 cells	4.9 [15]	
furosemide	Diuretics	Caco–2 cells	17.5 [15]	
gemifloxacin	Antibiotics	MDCK–transfected cells		145.69 [25]
genistein	Food Products	LLC–PK1–transfected cells	2 [26]	
glyburide (glibenclamide)	Sulfonylureas	MDCK–transfected cells	9.3 [27]	13.07 [28,c]
grepafloxacin	Antibiotics	MDCK–transfected cells	9.14 [29]	
hematoporphyrin	Photosensitizing agent	Membrane vesicles		17.8 [30]
hypaconitine	Herbal Medications	MDCK–transfected cells	7.6 [4]	
imatinib	Kinase Inhibitors	MDCK–transfected cells	2.4 [24]	
irinotecan	Topoisomerase Inhibitors	Caco–2 cells	3.7 [15]	
–SN–38	Topoisomerase Inhibitors	MDCK–transfected cells	8.4 [10]	4 [31,b]
–SN–38 glucuronide	Topoisomerase Inhibitors	Membrane vesicles		26 [31]
lamivudine	Nucleoside Reverse Transcriptase Inhibitors (NRTIs)	MDCK–transfected cells	3 [32]	216.5 [32]
lapatinib	Kinase Inhibitors	MDCK–transfected cells	2.6 [33]	
mesaconitine	Herbal Medications	MDCK–transfected cells	11.1 [4]	
methotrexate	Antimetabolites	MDCK–transfected cells	6.78 [34]	681 [35,b]
mitoxantrone	Other Antineoplastics	MDCK–transfected cells	6.1 [10]	
nitrofurantoin	Anti–Infective Agents	Caco–2 cells	24.1 [15]	69.4 [36]
norfloxacin	Antibiotics	Caco–2 cells	3 [15]	
olomoucine II	Kinase Inhibitors	MDCK–transfected cells	2.27 [37]	
pemetrexed	Antimetabolites	Membrane vesicles		390 [38]

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pitavastatin	HMG CoA Reductase Inhibitors (Statins)	Membrane vesicles		5.73 [39]
prazosin	Alpha/Beta Adrenergic Antagonists	MDCK–transfected cells	11.1 [40]	
rivaroxaban	Anticoagulants and Antiplatelets	Caco–2 cells	2.82 [41]	
rosuvastatin	HMG CoA Reductase Inhibitors (Statins)	Caco–2 cells	83.2 [40]	2.02 [42,b]
sildenafil	Erectile Dysfunction Treatments	MDCK–transfected cells	3.59 [43]	22.4 [43]
sorafenib	Kinase Inhibitors	MDCK–transfected cells	2.7 [44]	
sulfasalazine	NSAIDS	Caco–2 cells	87 [45]	0.7 [46,b]
sunitinib	Kinase Inhibitors	MDCK–transfected cells	1.9 [47,d]	
–N–desethyl sunitinib (SU12662)	Kinase Inhibitors	MDCK–transfected cells	13.5 [48]	
tandutinib	Kinase Inhibitors	Caco–2 cells	5 [49]	4 [49]
temocapril	Angiotensin Converting Enzyme (ACE) Inhibitors	Caco–2 cells	5.56 [50]	
teniposide	Topoisomerase Inhibitors	Caco–2 cells	10.8 [15]	
teriflunomide	Other Immunomodulators	Caco–2 cells	9.37 [51]	
topotecan	Topoisomerase Inhibitors	Caco–2 cells	6.9 [15]	213.28 [52,e]
troglitazone sulfate	Thiazolidinediones	MDCK–transfected cells	6.42 [53]	
ulifloxacin	Antibiotics	MDCK–transfected cells	2.95 [29]	
vardenafil	Erectile Dysfunction Treatments	MDCK–transfected cells	4.12 [43]	14.3 [43]

Metabolites are listed below the respective parent compound. K_m and efflux ratio values are presented as reported in the respective references.

[a] compounds with efflux ratios >2 were selected for further consideration

[b] kinetic experiments were performed in membrane vesicles

[c] kinetic experiments were performed in HEK–transfected cells

[d] included in the list because metabolite has an efflux ratio >2

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[e] kinetic experiments were performed in MDCK–transfected cells

[1] (Wang et al., 2012); [2] (Wei et al., 2013); [3] (Farrell et al., 2012); [4] (Ye et al., 2013); [5] (Zhang et al., 2013); [6] FDA (2012) Drug approval package: INLYTA® (axitinib) [FDA application no, (NDA) 202324].; [7] (Marchetti et al., 2013); [8] (Chu et al., 2013a); [9] (Hemauer et al., 2010); [10] (Xiao et al., 2006); [11] (Beery et al., 2012); [12] (Pavek et al., 2005); [13] (Merino et al., 2006); [14] (Xiao et al., 2012); [15] (Lin et al., 2011); [16] (Crowe and Wright, 2012); [17] (Zhang et al., 2005b); [18] (Reese et al., 2013); [19] (Mizuno et al., 2007); [20] (Chen et al., 2003); [21] (Li et al., 2011a); [22] (Imai et al., 2003); [23] (Han et al., 2010); [24] (Zhou et al., 2009); [25] (Jin et al., 2013); [26] (Imai et al., 2004); [27] (Varma et al., 2014); [28] (Pollex et al., 2010); [29] (Ando et al., 2007); [30] (Tamura et al., 2006); [31] (Nakatomi et al., 2001); [32] (Kim et al., 2007); [33] (Polli et al., 2008); [34] (Xia et al., 2007); [35] (Volk and Schneider, 2003); [36] (Wright et al., 2011); [37] (Hofman et al., 2013); [38] (Li et al., 2011b); [39] (Hirano et al., 2005); [40] (Li et al., 2012); [41] (Gong et al., 2013); [42] (Kitamura et al., 2008); [43] (Choi and Song, 2012); [44] (Lagas et al., 2010); [45] (Wang et al., 2008); [46] (Jani et al., 2009); [47] (Tang et al., 2012a); [48] (Tang et al., 2012b); [49] (Yang et al., 2010); [50] (Ohura et al., 2011); [51] FDA (2012) Drug approval package: AUBAGIO® (teriflunomide) [FDA application no, (NDA) 202992].; [52] (Li et al., 2008); [53] (Enokizono et al., 2007a)

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Table 4. *In vivo* drug interaction studies involving rosuvastatin and sulfasalazine indicating at least partial contribution by BCRP.

Object	Object Dose*	Precipitant	Precipitant Therapeutic Class	Precipitant Dose*	% Change in AUC[a]
sulfasalazine	2 g SD	curcumin	Food Products	2 g SD	220 [1]
	500 mg SD	pantoprazole	Proton Pump Inhibitors	40 mg SD	83 [2,b]
	100 µg SD	curcumin	Food Products	2 g SD	83 [1]
rosuvastatin	10 mg QD x 10D	cyclosporine	Immunosuppressants	75 to 200 mg BID (stable dose for at least 2 months)	610 [3,c]
	10 mg SD	atazanavir + ritonavir	Protease Inhibitors	300 mg atazanavir + 100 mg ritonavir QD x 6D	210 [4]
	20 mg QD x 7D	lopinavir + ritonavir	Protease Inhibitors	400 mg lopinavir + 100 mg ritonavir BID x 7D	110 [5]
	10 mg SD	eltrombopag	Platelet Stimulating Agents	75 mg QD x 5D	55 [6]
	10 mg SD	elvitegravir + cobicistat	AIDS Treatments	150 mg elvitegravir + 150 mg cobicistat QD x 10D	40 [7]
	10 mg SD	tipranavir + ritonavir	Protease Inhibitors	500 mg tipranavir + 200 mg ritonavir BID x 11D	37 [8]

*The route of administration was oral in all cases

[a] - Values were extracted from the DIDB and calculated as the change in mean AUC values.

[b] - AUC change observed with 421A/C genotype and was not statistically different from control

[c] - change in AUC was calculated using healthy historical controls

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[1] (Kusuhara et al., 2012); [2] (Adkison et al., 2010); [3] (Simonson et al., 2004); [4] (Busti et al., 2008); [5] (Kiser et al., 2008); [6] (Allred et al., 2011); [7] (Custodio et al., 2014); [8] (Pham et al., 2009)

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Table 5. Compounds considered as in vivo BCRP inhibitors, based on in vitro IC_{50} or K_i values < 10 μM

Precipitant	Therapeutic Class	K_i (μM)	IC_{50} (μM)
afatinib	Kinase Inhibitors	–	0.75 [1]
aripiprazole	Antipsychotics	–	3.5 [2]
axitinib	Kinase Inhibitors	–	4.4 [3]
curcumin	Food Products	–	1.6 [4]
cyclosporine	Immunosuppressants	6.7 [5]	13.4 [a]
erlotinib	Kinase Inhibitors	0.15 [6]	0.13 [6]
elacridar	drug distribution modulator	–	0.31 [7]
fluvastatin	HMG CoA Reductase Inhibitors	5.43 [8]	10.86 [a]
fumitremorgin C (FTC)	None	0.55 [6]	0.25 [6]
gefitinib	Kinase Inhibitors	–	1.01 [9]
ivermectin	Antiparasitics	1.4 [10]	2.8 [a]
ko143	Transporter Modulators	–	0.013 [5]
lapatinib	Kinase Inhibitors	–	0.025 [11]
nilotinib	Kinase Inhibitors	0.69 [12]	1.38 [a]
novobiocin	Antibiotics		0.063 [13]
pantoprazole	Proton Pump Inhibitors	–	5.5 [14]
pitavastatin	HMG CoA Reductase Inhibitors	2.92 [8]	5.84 [a]
ponatinib	Kinase Inhibitors	–	0.013 [15]
quercetin	Food Products	–	0.6 [16]
quizartinib (AC220)	Kinase Inhibitors	–	0.5 [17]
rabeprazole	Proton Pump Inhibitors	–	8.5 [14]
regorafenib	Kinase Inhibitors	–	0.0447 [18]
rilpivirine	NonNucleoside Reverse Transcriptase Inhibitors	–	1.5 [19]
sulfasalazine	NSAIDS	–	0.46 [13]
sunitinib	Kinase Inhibitors	0.32 [20]	0.64 [a]
tacrolimus	Immunosuppressants	–	6 [16]
teriflunomide	Other Immunomodulators	–	0.146 [21]

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trametinib	Kinase Inhibitors	–	1.1 [22]
trifluoperazine	Antipsychotics	–	7.56 [23]
vismodegib	Cancer Treatments	–	2.4 [24]

IC_{50} and K_i values are presented as reported in the respective references.

[a] IC_{50} value was calculated as $2 \times K_i$, assuming linearity

[1] FDA (2013) Drug approval package: GILOTRIF® (afatinib dimaleate) [FDA application no. (NDA) 201292]; [2] (Nagasaka et al., 2012); [3] (Reyner et al., 2013); [4] (Kusuhara et al., 2012); [5] (Xia et al., 2007); [6] (Noguchi et al., 2009); [7] (Si et al., 2013); [8] (Hirano et al., 2005); [9] (Yanase et al., 2004); [10] (Jani et al., 2011); [11] (Polli et al., 2008); [12] (Tiwari et al., 2009); [13] (Elsby et al., 2011); [14] (Suzuki et al., 2009); [15] FDA (2012) Drug approval package: ICLUSIG® (ponatinib hydrochloride) [FDA application no. (NDA) 203469]; [16] (Saito et al., 2006); [17] (Bhullar et al., 2013); [18] FDA (2012) Drug approval package: STIVARGA® (regorafenib) [FDA application no. (NDA) 203085]; [19] (Weiss and Haefeli, 2013); [20] (Kawahara et al., 2010); [21] FDA (2012) Drug approval package: AUBAGIO® (teriflunomide) [FDA application no. (NDA) 202992]; [22] FDA (2013) Drug approval package: MEKINIST® (trametinib dimethyl sulfoxide) [FDA application no. (NDA) 204114]; [23] (Pan et al., 2013); [24] FDA (2012) Drug approval package: ERIVEDGE® (vismodegib) [FDA application no. (NDA) 203388]

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Table 6. In vitro and in vivo data and considerations for potential systemic BCRP inhibitors.

Precipitant	I_1 (µM, C_{max})	Plasma Binding (%)	Dose	BCRP parameters				Other possible systemic interactions			
				K_i (µM)	IC_{50} (µM)	$[I_1]/IC_{50}$	In vivo PK–based DDI data	Transporter / Enzyme	IC_{50} (µM)	$[I_1]/IC_{50}$	In vivo PK–based DDI data
regorafenib	8.1 [1]	99.5 [1]	160 mg (SS) [1]	–	0.0447 TOP [2]	180	No published inhibition data [3]	P–gp	0.8 [2]	10	
								CYP3A4	22.2 [a,2]	0.36	
								CYP2C8	1.2 [a,2]	6.8	
								CYP2C9	9.4 [a,2]	0.86	
								CYP2B6	10.4 [a,2]	0.78	
								CYP2C19	32.8 [a,2]	0.25	
								UGT1A1	6 [2]	1.4	SN–38 glucuronide AUC +44% (when co–administered with irinotecan) [2]
								UGT1A9	4.2 [2]	1.9	
sulfasalazine	79 [4]	>99.7 [4]	3–4 g SD [4]	–	0.46 E3S [5]	170	No effect with methotrexate [6], however in vivo substrate [7]	OATP1B1	0.56 [8]	140	
								OATP1B3	12 [8]	6.6	
								OATP2B1	3 [8]	26	

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Drug								Target			
lapatinib	4.2 [b,9]	>99 [9]	1250 mg QD (SS) [9]	–	0.025 CIM [10]	170 [c]	Decreased topotecan clearance, −18% (renal −22%) [11], SN–38 +45% AUC [12]	P–gp	1.5 [13]	2.8	Digoxin AUC +2.8–fold [9]
								OATP1B1	4 [10]	1.0	SN–38 AUC +45% [12]
								CYP3A	39 [14]	0.11	Midazolam AUC +45% (oral) +22% (IV) [9]
								CYP2D6	13.8 [14]	0.30	
								CYP2C8	1.2 [a,14]	3.5	Paclitaxel AUC +23% [8]
								CYP2C9	11.8 [14]	0.36	
								CYP2C19	27.2 [14]	0.15	
erlotinib	6.06 [15]	90 [15]	150 mg QD x 21D [15]	0.15 E3S [16]	0.13 E3S [16]	47	No effect on topotecan [17]	P–gp	2 [16]	3.0	
								OATP1B1	21 [8]	0.29	
								OATP1B3	44 [8]	0.14	
								OATP2B1	0.55 [8]	11	
								CYP3A4	2.56 [a,18]	2.4	2–fold increase in exposure of OSI–930 [23], everolimus AUC +21% [24], case report of toxicity with simvastatin [25]
								CYP3A5	80 [a,19]	0.08	2–fold increase in exposure of OSI–930 [23], everolimus AUC +21% [24], case report of toxicity with simvastatin [25]

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Drug								Enzyme/Transporter	Value	Ratio	Comment
								CYP2D6	37% [20]	–	
								CYP2C8	8.04 [a,18]	0.75	No effect on paclitaxel [26]
								CYP2C9	49% [20]	–	Case report of increased INR with warfarin [27]
								CYP1A2	30% [20]	–	
								CYP2C19	42.9 [21]	0.14	
								UGT1A1	1.28 [a,22]	4.7	No effect on irinotecan/SN–38 [28]
								P–gp	1.7 [29]	2.5	
								OCT1	2.92 [31]	0.47	
								OCT2	>30 [31]		
								OCT3	0.345 [31]	4.0	
nilotinib	4.3 [29]	98 [29]	400 mg BID x 15D [29]	0.69 MTX [30]	1.38	3.1	No inhibition data	CYP3A4	0.4 [32]	11	Midazolam AUC +30% [29]
								CYP2C8	0.4 [21]	11	
								CYP2C9	0.264 [a,29]	16	No effect on S–warfarin [34]
								CYP2D6	2.92 [a,29]	1.5	
								CYP2C19	7.64 [a,29]	0.56	

								UGT1A1	0.28 [a,33]	15	
								UGT1A7	7.8 [a,33]	0.55	
								P–gp	17.9 [39]	0.36	No effect on digoxin [44,45]
								OATP1B1	54% [40]	–	
pantoprazole	6.5 [35]	98 [35]	40 mg SD [35]	–	5.5 MTX [36]	1.2	Sulfasalazine AUC +83.3% (not significant) with 421A/C genotype [37], Case report of toxicity with methotrexate [38]	CYP3A4	1 [41]	6.5	No effect on carbamazepine [46], nifedipine [47,48], tacrolimus [49]
								CYP2D6	15 [41]	0.43	No effect on metoprolol [50]
								CYP2C9	13.0 [a,42]	0.50	No effect on S–warfarin [51], diclofenac [52,53]
								CYP2C19	4.1 [43]	0.46	No effect on phenytoin [54,55], diazepam [56]
								CYP2B6	200 [41]	0.03	
								P–gp	0.0156 [60]	21	Doxorubicin AUC +46% [57], topotecan (IV) AUC +143% [59], paclitaxel AUC increase [63]
elacridar	0.327 [57]	Not available	400 mg BID x 3D [57]	–	0.31 MIT [58]	1.1	Topotecan (IV) AUC +143% [59]	OATP1B1	<<20 [61]	–	
								OATP1B3	>20 [8]	–	
								OATP2B1	<20 [8]	–	
								CYP3A4	4.9 [62]	0.07	

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								CYP			
								CYP2C9	19.1 [62]	0.02	
								CYP1A2	16 [62]	0.02	
								CYP2C19	2.05 [62]	0.16	
								CYP3A4	9.6 [a,18]	0.08	No effect on docetaxel [66], everolimus [67]
gefitinib	0.8 [64]	90 [64]	225 mg QD x 14D [64]	–	1.01 E3S [65]	0.79	No inhibition data	CYP2D6	13.8 [21]	0.06	Metoprolol AUC +49.5% [68]
								CYP2C8	17.4 [a,18]	0.05	
								CYP2C19	36 [62]	0.02	

IC$_{50}$ and K$_i$ values are presented as reported in the respective references, along with the substrate used: CIM – cimetidine, E3S – estrone-3-sulfate, MTX – methotrexate, MIT – mitoxantrone, TOP – topotecan.

[a] – IC$_{50}$ was calculated as 2 x K$_i$, assuming linearity

[b] – C$_{max}$ for proposed 250 mg dose = 0.54 μM (Bence et al., 2005)

[c] – [I$_1$]/IC$_{50}$ value for proposed 250 mg dose = 22

[1] STIVARGA® product label (05/29/2013, http://www.accessdata.fda.gov/drugsatfda_docs/label/2013/203085s001lbl.pdf); [2] FDA (2012) Drug approval package: STIVARGA® (regorafenib) [FDA application no, (NDA) 203085]; [3] ongoing clinical trial to investigate effect as BCRP inhibitor: http://clinicaltrials.gov/show/NCT02106845; [4] (Goodman et al., 2001); [5] (Elsby et al., 2011); [6] (Haagsma et al., 1996); [7] (Kusuhara et al., 2012); [8] (Karlgren et al., 2012b); [9] TYKERB® product label (10/18/2013, http://www.accessdata.fda.gov/drugsatfda_docs/label/2013/022059s016s017lbl.pdf); [10] (Polli et al., 2008); [11] (Molina et al., 2008); [12] (Midgley et al., 2007); [13] (Sugimoto et al., 2011); [14] FDA (2012) Drug approval package: TYKERB® (lapatinib ditosylate) [FDA application no, (NDA) 022059]; [15] (Yamamoto et al., 2008); [16] (Noguchi et al., 2009); [17] (Stewart et al., 2014); [18] (Wang et al., 2014); [19] (Li et al., 2010); [20] (Dong et al., 2011); [21] (Kim et al., 2013); [22] (Liu et al., 2010); [23] (Macpherson et al., 2013); [24] (Bullock et al., 2011); [25] (Veeraputhiran and Sundermeyer, 2008); [26] (Tran et al., 2011); [27] (Thomas et al., 2010); [28] (Messersmith et al., 2004); [29] FDA (2012) Drug approval package: TASIGNA® (nilotinib hydrochloride monohydrate) [FDA application no, (NDA) 022068]; [30] (Tiwari et al., 2009); [31] (Minematsu and Giacomini, 2011); [32] (Kenny et al., 2012); [33] (Ai et al., 2014); [34] (Yin et al., 2011); [35] PROTONIX® product label (12/10/2013, http://www.accessdata.fda.gov/drugsatfda_docs/label/2013/020987s048,022020s010lbl.pdf); [36] (Suzuki et al., 2009); [37] (Adkison et al., 2010); [38] (Ranchon et al., 2011); [39] (Pauli-Magnus et al., 2001); [40] (Oostendorp et al., 2009); [41] (Moody et al., 2013); [42] (Li et al., 2004); [43] (Zvyaga et al., 2012); [44] (Hartmann et al., 1995); [45] (Hartmann et al., 1996); [46] (Huber et al., 1998); [47] (Bliesath et al., 1996b); [48] (Bliesath et al., 1996a); [49] (Lorf et al., 2000); [50] (Koch et al., 1996); [51] (Duursema et al., 1995); [52] (Bliesath et al., 1996d); [53] (Bliesath et al., 1996c); [54] (Middle et al., 1995); [55] (Middle et al., 1996); [56] (Gugler et al., 1996); [57] (Planting et al., 2005); [58] (Ahmed-Belkacem et al., 2005); [59] (Kruijtzer et al., 2002); [60] (Dickens et al., 2013); [61] (Karlgren et al., 2012a); [62] (Englund et al., 2014); [63] (Malingre et al., 2001); [64] (Goodman et al., 2005); [65] (Yanase et al., 2004); [66] (Wilding et al., 2006); [67] (Milton et al., 2007); [68] (Swaisland et al., 2005)

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Table 7. In vitro and in vivo data and considerations for potential intestinal BCRP inhibitors.

Precipitant	I_2 (μM)	Dose	BCRP parameters				Other possible intestinal Interactions			
			K_i (μM)	IC_{50} (μM)	I_2/IC_{50}	In vivo PK–based DDI data	Transporter/ Enzyme	IC_{50} (μM)	I_2/IC_{50}	In vivo PK–based DDI data
lapatinib [a]	5300 [b]	1250 mg QD (SS) [1]	–	0.025 CIM [2]	210000 [c]	Decreased topotecan clearance, -18% (renal -22%) [2], SN–38 +45% AUC [4]	P–gp	1.5 [5]	3500	Digoxin AUC +2.8–fold [1]
							CYP3A	39 [6]	140	Midazolam AUC +45% (oral) +22% (IV) [1]
sulfasalazine [a]	40000	3–4 g SD [7]	–	0.46 E3S [8]	87000	No effect with methotrexate [9], however in vivo substrate [10]	OATP2B1	3 [11]	13000	
regorafenib [a]	1300	160 mg (SS) [12]	–	0.0447 TOP [13]	28000	No inhibition data [14]	P–gp	0.8 [13]	1600	SN–38 glucuronide AUC +44% (when co–administered with irinotecan) [13]
							CYP3A4	22.2 [d,13]	58	
							UGT1A1	6 [13]	210	
curcumin [e]	22000	2 g SD [10]	0.7 SSZ [10]	1.6 SSZ [10]	14000	Sulfasalazine exposure increased significantly [10]	P–gp	~20 [15]	1100	Talinolol AUC +33–54% [17, 18]
							CYP3A4	13.9 [16]	1600	
erlotinib [a]	1500	150 mg QD x 21D [19]	0.15 [20]	0.13 E3S [20]	12000	No effect on topotecan [21]	P–gp	2 [20]	760	
							OATP2B1	0.55 [11]	2800	
							CYP3A4	2.56 [d,22]	600	2–fold increase in exposure of OSI–930 [25], everolimus AUC +21% [26], case

Drug		Dose				Inhibition	Transporter/Enzyme			Comment
										report of toxicity with simvastatin [27]
							CYP3A5	80 [c,23]	19	2–fold increase in exposure of OSI–930 [25], everolimus AUC +21% [26], case report of toxicity with simvastatin [27]
							UGT1A1	1.28 [d,24]	1200	No effect on irinotecan/SN–38 [28]
elacridar [a]	2800	400 mg BID x 3D [29]	–	0.31 MIT [30]	9200	Topotecan AUC +143% [31]	P–gp	0.0156 [32]	180000	Doxorubicin AUC +46% [29], topotecan AUC +143% [31], paclitaxel AUC increase [34]
							OATP2B1	<20 [11]	–	
							CYP3A4	4.9 [33]	580	
nilotinib [a]	2800	400 mg BID x 15D [35]	0.69 MTX [36]	1.38	2000	No inhibition data	P–gp	1.7 [35]	1700	Midazolam AUC +30% [35]
							CYP3A4	0.4 [37]	7100	
							UGT1A1	0.28 [d,38]	10000	
gefitinib [a]	2000	225 mg QD x 14D [39]	–	1.01 E3S [40]	2000	No inhibition data	CYP3A4	9.6 [d,22]	210	No effect on docetaxel [41], everolimus [42]
sunitinib	500	50 mg QD x 28D	0.32 E3S [44]	0.64	780	No inhibition data, in vivo substrate [45]	P–gp	15.2 [d,44]	33	Docetaxel AUC +44.7% [47], Case report of toxicity with colchicine [48]

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	[43]					CYP3A4	19.0 [d,46]	26	Docetaxel AUC +44.7% [47]
pantoprazole [a]	370	40 mg SD [49]	–	5.5 MTX [50]	67	Sulfasalazine AUC +83.3% with 421A/C genotype [51], Case report of toxicity with methotrexate [52]	P–gp	17.9 [53]	21	No effect on digoxin [55,56]
							CYP3A4	1 [54]	370	No effect on carbamazepine [57], nifedipine [58,59], tacrolimus [60]
rabeprazole	440	40 mg AD x 8D [61]	–	8.5 MTX [50]	52	Delayed methotrexate elimination [50]	CYP3A4	4.9 [54]	91	No effect on tacrolimus [62]

IC_{50} and K_i values are presented as reported in the respective references, along with the substrate used: CIM – cimetidine, E3S – estrone-3-sulfate, MTX – methotrexate, MIT – mitoxantrone, SSZ – sulfasalazine, TOP – topotecan.

[a] – Also present in $[I_1]/IC_{50}$ list

[b] – I_2 value for proposed 250 mg dose = 1000 μM

[c] – $[I_2]/IC_{50}$ value for proposed 250 mg dose = 40,000

[d] – IC_{50} value was calculated as 2 x K_i, assuming linearity

[e] – Curcumin also caused a small, but significant, decrease in the clearance of the CYP1A2 substrate, caffeine (–29%) (Juan et al., 2007)

[1] TYKERB® product label (10/18/2013, http://www.accessdata.fda.gov/drugsatfda_docs/label/2013/022059s016s017lbl.pdf); [2] (Polli et al., 2008); [3] (Molina et al., 2008); [4] (Midgley et al., 2007); [5] (Sugimoto et al., 2011); [6] FDA (2012) Drug approval package: TYKERB® (lapatinib ditosylate) [FDA application no, (NDA) 022059]; [7] (Goodman et al., 2001); [8] (Elsby et al., 2011); [9] (Haagsma et al., 1996); [10] (Kusuhara et al., 2012); [11] (Karlgren et al., 2012b); [12] STIVARGA® product label (05/29/2013, http://www.accessdata.fda.gov/drugsatfda_docs/label/2013/203085s001lbl.pdf); [13] FDA (2012) Drug approval package: STIVARGA® (regorafenib) [FDA application no, (NDA) 203085]; [14] ongoing clinical trial to investigate effect as BCRP inhibitor: http://clinicaltrials.gov/show/NCT02106845; [15] (Si et al., 2013); [16] (Appiah-Opong et al., 2007); [17] (Juan et al., 2007); [18] (He et al., 2012); [19] (Yamamoto et al., 2008); [20] (Noguchi et al., 2009); [21] (Stewart et al., 2014); [22] (Wang et al., 2014); [23] (Li et al., 2010); [24] (Liu et al., 2010); [25] (Macpherson et al., 2013); [26] (Bullock

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et al., 2011); [27] (Veeraputhiran and Sundermeyer, 2008); [28] (Messersmith et al., 2004); [29] (Planting et al., 2005); [30] (Ahmed-Belkacem et al., 2005); [31] (Kruijtzer et al. 2002); [32] (Dickens et al., 2013); [33] (Englund et al., 2014); [34] (Malingre et al., 2001); [35] FDA (2012) Drug approval package: TASIGNA® (nilotinib hydrochloride monohydrate) [FDA application no, (NDA) 022068]; [36] (Tiwari et al., 2009); [37] (Kenny et al., 2012); [38] (Ai et al., 2014); [39] (Goodman et al., 2005); [40] (Yanase et al., 2004); [41] (Wilding et al., 2006); [42] (Milton et al., 2007); [43] SUTENT® product label (08/30/2013, http://www.accessdata.fda.gov/drugsatfda_docs/label/2013/021938s024s025lbl.pdf); [44] (Kawahara et al., 2010); [45] (Oberoi et al., 2013); [46] FDA (2006) Drug approval package: SUTENT® (sunitinib malate) [FDA application no, (NDA) 021938].; [47] (Robert et al., 2010); [48] (Abodunde et al., 2013); [49] PROTONIX® product label (12/10/2013, http://www.accessdata.fda.gov/drugsatfda_docs/label/2013/020987s048,022020s010lbl.pdf); [50] (Suzuki et al., 2009); [51] (Adkison et al. 2010); [52] (Ranchon et al., 2011); [53] (Pauli-Magnus et al., 2001); [54] (Moody et al., 2013); [55] (Hartmann et al., 1995); [56] (Hartmann et al., 1996); [57] (Huber et al., 1998); [58] (Bliesath et al., 1996b); [59] (Bliesath et al., 1996a); [60] (Lorf et al., 2000); [61] ACIPHEX® product label (04/19/2013, http://www.accessdata.fda.gov/drugsatfda_docs/label/2013/020973s032lbl.pdf); [62] (Itagaki et al., 2004)

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Figure 1