

# Human ABC transporter ABCG2/BCRP expression in chemoresistance: basic and clinical perspectives for molecular cancer therapeutics

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**Abstract:** Adenine triphosphate (ATP)-binding cassette (ABC) transporter proteins, such as ABCB1/P-glycoprotein (P-gp) and ABCG2/breast cancer resistance protein (BCRP), transport various structurally unrelated compounds out of cells. ABCG2/BCRP is referred to as a “half-type” ABC transporter, functioning as a homodimer, and transports anticancer agents such as irinotecan, 7-ethyl-10-hydroxycamptothecin (SN-38), gefitinib, imatinib, methotrexate, and mitoxantrone from cells. The expression of ABCG2/BCRP can confer a multidrug-resistant phenotype on cancer cells and affect drug absorption, distribution, metabolism, and excretion in normal tissues, thus modulating the in vivo efficacy of chemotherapeutic agents. Clarification of the substrate preferences and structural relationships of ABCG2/BCRP is essential for our understanding of the molecular mechanisms underlying its effects in vivo during chemotherapy. Its single-nucleotide polymorphisms are also involved in determining the efficacy of chemotherapeutics, and those that reduce the functional activity of ABCG2/BCRP might be associated with unexpected adverse effects from normal doses of anticancer drugs that are ABCG2/BCRP substrates. Importantly, many recently developed molecular-targeted cancer drugs, such as the tyrosine kinase inhibitors, imatinib mesylate, gefitinib, and others, can also interact with ABCG2/BCRP. Both functional single-nucleotide polymorphisms and inhibitory agents of ABCG2/BCRP modulate the in vivo pharmacokinetics and pharmacodynamics of these molecular cancer treatments, so the pharmacogenetics of ABCG2/BCRP is an important consideration in the application of molecular-targeted chemotherapies.

**Keywords:** kinase inhibitor, SNP, single-nucleotide polymorphisms, molecular target

## Introduction

Various mechanisms are involved in the multidrug resistance of cancer cells, including reduced drug uptake, the efflux of intracellular drugs, the activation of deoxyribonucleic acid (DNA) repair pathways, and the induction of the antiapoptotic machinery.<sup>1</sup> The adenine triphosphate (ATP)-binding cassette (ABC) transporter proteins, particularly P-glycoprotein (P-gp) (P-gp/MDR1 [multidrug resistance 1]/ABCB1), multidrug resistance protein 1 (MRP1) (MRP1/ABCC1), and breast cancer resistance protein (BCRP) (BCRP/MXR [mitoxantrone resistance]/ABCP/ABCG2), function as key molecules in the multidrug-resistant phenotype of cancer cells.<sup>2-4</sup> They mediate the ATP-dependent unidirectional efflux of various compounds, both endogenous and exogenous, from cells.<sup>2-5</sup>

ABCG2/BCRP, a “half transporter” member of the ABCG subfamily,<sup>6</sup> has been found in cancer-drug-resistant human cancer cell lines isolated by in vitro selection.<sup>7-9</sup> The overexpression of ABCG2/BCRP confers resistance to various chemotherapeutic

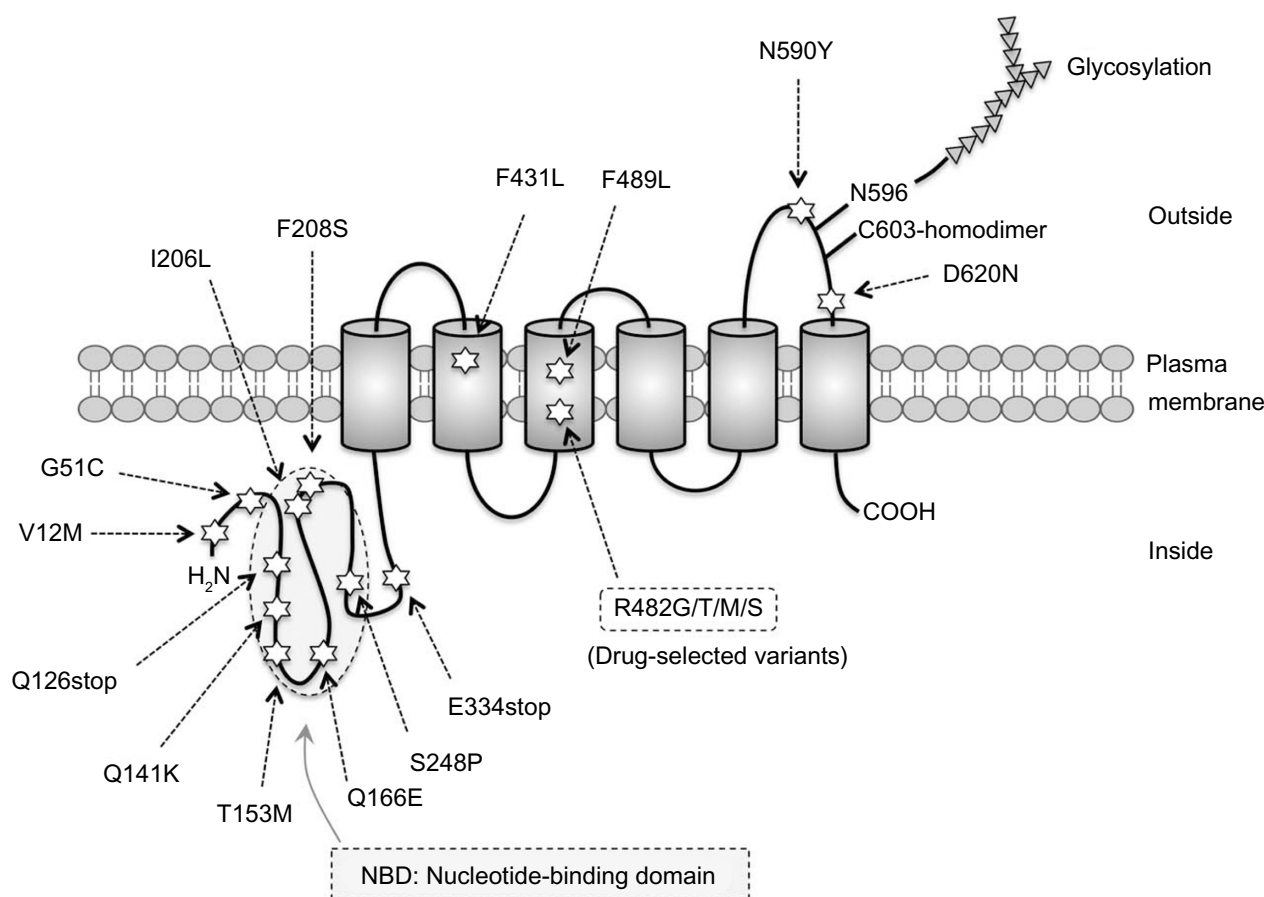
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drugs, such as the topoisomerase I inhibitor topotecan and the antifolate agent methotrexate,<sup>9,10</sup> and ABCG2/BCRP is probably associated with clinical drug resistance, including that in patients with acute myelogenous leukemia or acute lymphocytic leukemia.<sup>11–17</sup> Because ABCB1/P-gp is quite an important factor in the drug resistance observed in clinical leukemia, a large-scale analysis that assesses both ABCG2/BCRP and ABCB1/P-gp is essential to properly delineating the contribution of ABCG2/BCRP to drug resistance in cancer patients.

## ABCG2/BCRP structure and activity

ABCG2/BCRP is a 655 amino acid, 72 kDa protein with a single ABC signature domain within the nucleotide-binding domain and six transmembrane domains, as shown in Figure 1. Structural and functional studies of ABCG2/BCRP have provided valuable insight into the molecular mechanisms of ABCG2/BCRP-mediated transport. The cloning of ABCG2/BCRP cDNAs (complementary DNAs) from

drug-selected clone cells and normal tissues has revealed functional variations in the amino acid substitutions in the ABCG2/BCRP protein and altered substrate preferences. ABCG2/BCRP proteins in drug-selected cells, such as the S1-M1-80 and MCF7/AdVp3000 cell lines, are mutant forms, and unique mutations in ABCG2/BCRP have been identified at amino acid position 482.<sup>18</sup> MCF7/AdVp3000 and S1-M1-80 cells express R482T and R482G variants of ABCG2/BCRP, respectively, and are highly resistant to both mitoxantrone and doxorubicin. Anthracycline resistance and a rhodamine efflux capacity are also unique phenotypes of these two ABCG2/BCRP-overexpressing cell lines.<sup>18,19</sup> The substitution of Arg at position 482 in ABCG2/BCRP with Gly or Thr confers additional efflux activity for rhodamine 123, doxorubicin, and other anthracyclines, which are usually not good substrates of wild-type ABCG2/BCRP.<sup>18,20</sup> In contrast, the ABCG2/BCRP variants R482G and R482T lose their methotrexate-transporting activity but, at the same time, confer increased mitoxantrone resistance.<sup>19,21–23</sup> The COOH terminus of the transmembrane 3 region, in close



**Figure 1** Schematic diagram of ABCG2/BCRP protein and its amino acid variations. Amino acid variations by single-nucleotide polymorphisms are marked with stars. Transmembrane domains are shown as cylinders.

**Abbreviations:** ABC, adenine-triphosphate-binding cassette; BCRP, breast cancer resistance protein.

proximity to position 482, is involved in the substrate-binding pocket of ABCG2/BCRP, and the Arg residue at position 482 affects drug-ABCG2/BCRP interactions.<sup>23–25</sup> Moreover, 13 variant ABCG2/BCRP proteins with substitutions at R482 (R482N, C, M, S, T, V, A, G, E, W, D, Q, and H, but not Y or K) confer strong resistance to doxorubicin and mitoxantrone in PA317 cells, as in S1-M1-80 and MCF7/AdVp3000 cells.<sup>26</sup> Thus, structural variations in ABCG2/BCRP affect its drug efflux activity. In addition, mutations in ABCG2/BCRP at N557 and H630 severely affect this resistant phenotype. Cells expressing either N557D or H630E mutant ABCG2/BCRP displayed lower resistance to SN-38 (7-ethyl-10-hydroxycamptothecin), although the mitoxantrone resistance of these cells was similar to that of cells expressing wild-type ABCG2/BCRP.<sup>26</sup> Structural studies using three-dimensional homology modeling of ABCG2/BCRP have also indicated that the transmembrane domains of ABCG2/BCRP function as drug-recognition interfaces<sup>24,25,27</sup> and suggest that R482 is located in the central cavity of the protein, with its side chain pointing toward the drug translocation pathway.<sup>28</sup> Therefore, the drug efflux activity of ABCG2/BCRP is influenced by various mutations, which will necessarily affect the clinical efficacy of ABCG2/BCRP-transportable anticancer drugs.

ABCG2/BCRP functions as a homodimer.<sup>29,30</sup> A disulfide bridge at C603 is involved in homodimer formation,<sup>31</sup> and crystallization studies suggest a tetrameric complex composed of four ABCG2/BCRP homodimers.<sup>32</sup> ABC transporter research has also demonstrated that the activity of these proteins is affected by both posttranslational regulation<sup>33,34</sup> and genetic polymorphisms.<sup>35–37</sup> ABCG2/BCRP is targeted to the plasma membrane via the endoplasmic reticulum–Golgi pathway, and during its passage, undergoes N-linked glycosylation at N596<sup>38,39</sup> and the formation of an intramolecular disulfide bond between C592 and C608, which affects its stability.<sup>40</sup> Oligomeric glycosylated ABCG2/BCRP is degraded in the lysosome, whereas underglycosylated, misfolded ABCG2/BCRP lacking the intramolecular disulfide bond is targeted to the proteasomal system for degradation.<sup>39</sup>

## Single-nucleotide polymorphisms (SNPs) of ABCG2/BCRP

Primary structural variations of ABCG2/BCRP are associated with its drug-transporter function, as described above. Numerous germ-line mutations in the *ABCG2/BCRP* gene have been found in ethnically diverse populations,<sup>36,41–43</sup> as shown in Figure 1. Therefore, SNPs in the *ABCG2/BCRP*

gene would influence the pharmacological effects of ABCG2/BCRP differently in different patients.

## 421C>A (Q141K) ABCG2/BCRP SNP

Japanese population and human cancer cell lines have been shown to have three variant *ABCG2/BCRP* cDNAs with the substitutions: 34G>A (V12M), 421C>A (Q141K), and a nucleotide 944–949 deletion, removing A315 and T316 ( $\Delta$ 315–316).<sup>44</sup> The 34G>A and 421C>A variants are SNPs. The frequency of the 421C>A SNP in a normal Japanese population demonstrated that 57 of 124 samples had the A421 allele, and nine of these were homozygous for this polymorphism.<sup>36,45</sup> These data suggest that some Japanese individuals probably express low levels of ABCG2/BCRP. The 421C>A SNP appears to be very common in Asian populations, with reported allelic frequencies between 27% and 34%,<sup>35,44,46</sup> whereas this SNP is rare in sub-Saharan African and African-American populations, with frequencies of <5%.<sup>47</sup> Its frequency in Caucasian populations is approximately 10%.<sup>48</sup> The physiological significance of the 421C>A *ABCG2/BCRP* SNP has been analyzed in relation to the pharmacokinetics of diflomotecan, a new camptothecin derivative anticancer agent, during a Phase I study.<sup>49</sup> This study showed that five patients who were heterozygous for the A421 allele had much higher plasma levels of diflomotecan after its intravenous administration than 15 homozygous wild-type individuals (mean values of 138 ng h/mL/mg versus 46.1 ng h/mL/mg, respectively). Consistent with this, 421C>A *ABCG2/BCRP*-transfected murine fibroblast PA317 (PA/Q141K) cells expressed less exogenous ABCG2/BCRP protein levels than wild-type *ABCG2/BCRP*-transfected cells.<sup>44</sup> Drug accumulation was higher in PA/Q141K cells than in other *ABCG2/BCRP* transfectants, suggesting that the SNP 421C>A (Q141K) reduces ABCG2/BCRP function. These observations from laboratory and clinical studies suggest that the levels and functions of ABCG2/BCRP expressed from the 421C>A *ABCG2/BCRP* allele are reduced compared with those of the wild-type protein. The Q141K mutation is located within the functionally important ATP-binding region between the Walker A and B motifs of ABCG2/BCRP and thus is likely to affect the ATPase activity of the protein.<sup>35,50</sup>

In terms of the genetic polymorphisms of ABCG2/BCRP and the expression of the protein, other non-synonymous SNPs, F208S and S441N, have been shown to affect ABCG2/BCRP protein levels at the plasma membrane, indicating that these non-synonymous SNPs reduce the stability

of ABCG2/BCRP by enhancing its ubiquitin-mediated proteasomal proteolysis, resulting in reduced ABCG2/BCRP function.<sup>51</sup>

### 376C>T (Q126stop) ABCG2/BCRP SNP

Another SNP within the *ABCG2/BCRP* gene, 376C>T, is present at low frequencies in healthy Japanese samples as a heterozygosity (reported frequencies of 3/124 and 2/120 in two studies).<sup>44,47</sup> The frequency of the T376 allele of *ABCG2/BCRP* is low and is not observed in Caucasian or African-American groups. A combination of the 376C>T and 421C>A SNPs would be expected to occur in a considerable proportion of the Japanese population. Because these SNPs would have negative effects on ABCG2/BCRP activity, the combined 376C>T 421T/C>A variant is expected to show severely reduced ABCG2/BCRP activity.

### Additional ABCG2/BCRP SNPs

Other *ABCG2/BCRP* SNPs, such as 34G>A, 151G>T, 376C>T, 421C>A, 458C>T, 496C>G, 616A>C, 623T>C, 742T>C, 1000G>T, 1291T>C, 1465T>C, 1768A>T, and 1858G>A, cause amino acid substitutions. Synonymous mutations, such as 114T>C, 369C>T, 474C>T, 564A>G, 1098G>A, and 1425A>G, have been identified in the coding region of *ABCG2/BCRP*. The highest allele frequency for the 34G>A SNP is observed in Mexican-Indians, and there are significant differences in the frequencies of this SNP in Caucasian, Japanese, and Swedish populations.<sup>52,53</sup> Transfection studies of *ABCG2/BCRP* (V12M) encoding the 34G>A SNP showed that the expression levels and drug-resistance of this variant are similar to those of wild-type ABCG2/BCRP, suggesting that V12M does not affect ABCG2/BCRP protein activity.<sup>44</sup> However, a recent report suggested a possible association between this polymorphism and the alternative splicing of ABCG2/BCRP mRNA (messenger ribonucleic acid), particularly the liver-specific splicing of polymorphic exon 2 in these transcripts.<sup>54</sup> ABCG2/BCRP mRNA expression was significantly lower in Hispanic livers with the 34G>A variant genotype, and individuals with the 34G>A allele displayed reduced ABCG2/BCRP expression in their liver cells. Although these observations suggest that chemotherapy regimens containing ABCG2/BCRP-substrate anticancer drugs may have increased efficacy in these patients because the expression of ABCG2/BCRP is reduced, another study has shown that Chinese acute myelogenous leukemia patients with the wild-type 34GG genotype had longer disease-free survival and longer overall survival than those with the 34GA/AA genotypes.<sup>55</sup> The reason for the unpredictable

effects of the 32G>A SNP on ABCG2/BCRP is unknown. The polymorphic and differential expression of another splicing variant of ABCG2/BCRP mRNA, involving exon 1b in the liver, also seems to be associated with lower ABCG2/BCRP expression.<sup>56</sup> Approximately 90% of patients with the 34G>A SNP of *ABCG2/BCRP* show exon 2 skipping in the liver, and the lower level of ABCG2/BCRP mRNA in the liver may be associated with this SNP in the Hispanic population.<sup>54</sup>

## ABCG2/BCRP expression and physiological functions

ABCG2/BCRP is widely expressed in the placenta, blood-brain barrier, gastrointestinal tract, liver, kidney, testis, and lactating breast. ABCG2/BCRP localizes apically in the epithelia of the small intestine and colon,<sup>57</sup> on the luminal surfaces of the endothelial cells of the human brain,<sup>58</sup> and on the luminal surfaces of the kidney tubules.<sup>59</sup> Experimental inhibition of ABCG2/BCRP activity affected drug distribution in various in vivo experiments.<sup>60,61</sup> These observations suggest a possible role for ABCG2/BCRP in controlling the absorption/distribution of its substrate compounds. Various cancers also contain subpopulations of stem cells that are characterized by the expression of ABCG2/BCRP and other ABC transporters.<sup>62</sup> These transporters have been suggested to play an important role in the multidrug resistance of these cancer stem-like cells during chemotherapy.<sup>63</sup> Indeed, the experimental inhibition of ABCG2/BCRP has been shown to suppress the proliferation of side population cells in cancer cell lines.<sup>64</sup>

ABCG2/BCRP extrudes various types of compounds among its functional substrates, including sulfated hormone metabolites, the chlorophyll metabolite pheophorbide A, fluorescent dyes such as Hoechst 33342 and BODIPY (boron-dipyrromethene)-prazosin, cimetidine, various flavonoids, and some antibiotics.<sup>30,65</sup> Porphyrin/heme was the first-identified endogenous ABCG2/BCRP substrate,<sup>66</sup> and ABCG2/BCRP regulates heme homeostasis under hypoxic conditions.<sup>67</sup> ABCG2/BCRP also transports endogenous folates, such as the mono-, di-, and triglutamates of folic acid, and participates in the energy-dependent efflux of certain folates and antifolates.<sup>22</sup> Recently, genome-wide screening for the genetic determinants of gout found that an SNP of ABCG2/BCRP (Q141K) is associated with high uric acid levels, and demonstrated that uric acid is a natural substrate of ABCG2/BCRP.<sup>68,69</sup>

ABCG2/BCRP also appears to play a protective role against xenobiotics and their metabolites.<sup>30,70</sup> The typical ABCG2/BCRP substrates, irinotecan and SN-38, are detoxified by

glucuronidation with uridine-diphosphate–glucuronyltransferase, and ABCG2/BCRP can extrude SN-38–glucuronide.<sup>71</sup> Interestingly, ABCG2/BCRP can transport another of the glucuronide conjugates, 17- $\beta$ -estradiol 17-( $\beta$ -D-glucuronide), and the sulfated conjugates estrone-3-sulfate and dehydroepiandrosterone are also substrates of ABCG2/BCRP.<sup>22,70</sup> The apical localization of ABCG2/BCRP in the intestinal epithelium and the bile canalicular membrane also suggests the intestinal absorption and hepatobiliary excretion of ABCG2/BCRP substrates.<sup>57,72–74</sup> ABCG2/BCRP may also play a protective role by transporting dietary carcinogens. A study by van Herwaarden et al<sup>72</sup> showed that BCRP1 effectively restricts the exposure of mice to the ingested food carcinogen 2-amino-1-methyl-6-phenylimidazo(4,5-b)pyridine by reducing its uptake from the gut lumen and by mediating its hepatobiliary and intestinal elimination. Similarly, ABCG2/BCRP limits the intestinal uptake of the carcinogens 2-amino-3-methylimidazo(4,5-f)quinoline, 3-amino-1,4-dimethyl-5H-pyrido(4,3-b)indole, and aflatoxin B1 in a mouse model.<sup>75</sup> Overall, ABCG2/BCRP may restrict the bioavailability of orally administered ABCG2/BCRP-substrate anticancer agents, such as topotecan (and its metabolite SN-38), irinotecan, camptothecin derivatives, methotrexate, flavopiridol, and others.<sup>65</sup> Therefore, the functional activity of ABCG2/BCRP is an important consideration in ABCG2/BCRP-transportable drug absorption, distribution, metabolism, and excretion in patients.

## Pharmacological interaction of ABCG2/BCRP with molecular-targeted drugs

### ABCG2/BCRP and anticancer kinase inhibitors

A growing number of small-molecule inhibitors of oncogenic kinases have come into clinical use and have shown great potential as anticancer drugs.<sup>76,77</sup> Imatinib mesylate, which targets BCR-ABL (B-cell receptor-Abelson), was the first approved protein kinase inhibitor.<sup>78</sup> Imatinib is very effective against chronic myeloid leukemia and other cancers associated with the deregulation of these kinase pathways. Resistance to this drug is typically conferred by mutations in the target kinase within the region of the drug–kinase interaction,<sup>79–82</sup> but another mechanism leading to imatinib resistance correlates with ABCB1/P-gp expression.<sup>83,84</sup> As shown in Table 1, a number of recent studies have indicated possible interactions between many kinase inhibitors and ABC transporters, including ABCB1/P-gp and ABCG2/BCRP.<sup>85–90</sup> ABCG2/BCRP has

a potent ability to interact with numerous clinically important kinase inhibitors, including imatinib,<sup>87–89,91–99</sup> nilotinib,<sup>95</sup> dasatinib,<sup>96</sup> lapatinib,<sup>97</sup> gefitinib,<sup>42,98–105</sup> canertinib,<sup>106</sup> erlotinib,<sup>107–109</sup> sorafenib,<sup>110</sup> pazopanib,<sup>111</sup> vandetanib,<sup>112</sup> vemurafenib,<sup>113</sup> axitinib,<sup>114</sup> and ponatinib.<sup>115</sup> In contrast, bosutinib is unlikely to be a substrate of ABCG2/BCRP but probably inhibits it.<sup>116</sup> A recently developed anaplastic lymphoma kinase inhibitor, crizotinib, is also unlikely to interact with ABCG2/BCRP, although it is a good substrate of ABCB1/P-gp.<sup>117</sup>

A variety of kinase-inhibiting compounds appear to interact with ABC transporters.<sup>85,118–124</sup> These protein kinase inhibitors are designed to compete with ATP in the kinase domain and thus show competitive suppressive effects.<sup>125</sup> Therefore, most ABCG2/BCRP-interactive kinase inhibitors used at higher concentration were initially suspected to block the ATPase activity of this protein. However, Saito et al<sup>126</sup> demonstrated that gefitinib binds to ATP-bound ABCG2/BCRP, indicating that the as yet undetermined gefitinib-binding site in ABCG2/BCRP is not in the ATP-binding domain. Using photoaffinity labeling technique with <sup>125</sup>I-labeled iodoarylazidoprazosin, a typical substrate of ABCB1/P-gp and ABCG2/BCRP, Brendel et al<sup>95</sup> demonstrated that imatinib and nilotinib bind to ABCG2/BCRP at the substrate-interaction site, whereas Shi et al<sup>107</sup> showed that erlotinib does not compete with iodoarylazidoprazosin at the substrate-binding sites on ABCG2/BCRP or ABCB1/P-gp. Some interesting studies have proposed the presence of multiple drug-binding sites in this ABC transporter,<sup>127,128</sup> suggesting that kinase inhibitors may target these substrate-binding pockets. Further studies are required to properly clarify the modes of interaction between kinase inhibitors and ABCG2/BCRP.

## Pharmacological interactions affected by ABCG2/BCRP polymorphisms

Studies of the pharmacological interactions between ABCG2/BCRP and molecular-targeted kinase inhibitors have revealed that clinically used kinase inhibitors are in vivo substrates and/or inhibitors of this ABC transporter. Gefitinib is an orally administered, active, selective epidermal growth factor receptor tyrosine kinase inhibitor (TKI) used to treat patients with advanced non-small-cell lung cancer (NSCLC),<sup>129,130</sup> and ABCG2/BCRP is expressed in intestinal epithelial cells and at the blood–brain and blood–cerebrospinal barriers, where it restricts the penetration of the brain by xenobiotics.<sup>65,131</sup> Stewart et al<sup>98</sup> showed that the oral bioavailability of irinotecan, a good substrate of ABCG2/BCRP, is affected by the oral administration of gefitinib. Zhuang et al<sup>132</sup> have also shown that the oral

**Table 1** Interactions between tyrosine kinase inhibitors and ABCG2/BCRP

Drug	Target	ABCG2/BCRP	ABCB1/P-gp	Study
Imatinib	BCR-ABL	+	+	Ozvegy-Laczka et al, <sup>87</sup> Houghton et al, <sup>88</sup> Breedveld et al, <sup>89</sup> Burger et al, <sup>91</sup> Thomas et al, <sup>92</sup> Jordanides et al, <sup>93</sup> and Liu et al <sup>94</sup>
	c-Kit			
	PDGFR			
Nilotinib	BCR-ABL	+	+	Brendel et al <sup>95</sup> and Dohse et al <sup>142</sup>
	c-Kit			
	PDGFR			
Dasatinib	BCR-ABL	+	+	Hiwase et al <sup>96</sup> and Dohse et al <sup>142</sup>
	SRC			
Lapatinib	HER2	+	+	Polli et al <sup>97</sup> and Dai et al <sup>143</sup>
	EGFR			
Ponatinib	BCR-ABL with mutations, including T315I	+	+	Sen et al <sup>115</sup>
Bosutinib	BCR-ABL mutations, except V299L and T315I	±	±	Hegedus et al <sup>116</sup>
Gefitinib	EGFR	+	+	Sugimoto et al, <sup>42</sup> Stewart et al, <sup>98</sup> Yanase et al, <sup>99</sup> Carter et al, <sup>100</sup> Elkind et al, <sup>101</sup> Nakamura et al, <sup>102</sup> Cusatis et al, <sup>103</sup> Leggas et al, <sup>104</sup> and Katayama et al <sup>105</sup>
Erlotinib	EGFR	+	+	Shi et al, <sup>107</sup> Rudin et al, <sup>108</sup> and Kawahara et al <sup>109</sup>
Canertinib	EGFR	+	+	Erlichman et al <sup>106</sup> and Minocha et al <sup>111</sup>
	HER2			
	ERBB4			
Sunitinib	VEGFR-1, -2, and -3	+	+	Shukla et al <sup>144</sup>
	PDGFR- $\alpha/\beta$			
	c-Kit			
	FLT3			
	RET			
Pazopanib	VEGFR-1, -2, and -3	+	+	Minocha et al <sup>111</sup>
	PDGFR- $\alpha/\beta$			
	c-Kit			
Vandetanib	EGFR	+	+	Hegedus et al, <sup>112</sup> Zheng et al, <sup>145</sup> and Jovelet et al <sup>146</sup>
	VEGFR-2			
	RET			
Axitinib	VEGFR-1, -2, and -3	+	+	Poller et al <sup>114</sup> and Reyner et al <sup>147</sup>
	PDGFR			
	c-Kit			
Sorafenib	C-RAF	+	+	Hu et al <sup>110</sup> and Lagas et al <sup>148</sup>
	B-RAF			
	VEGFR-2			
	PDGFR- $\beta$			
Vemurafenib	B-RAF <sup>V600E</sup>	+	+	Mittapalli et al, <sup>113</sup> Durmus et al, <sup>149</sup> and Wu et al <sup>150</sup>
	C-RAF			
	ACK1			
	KHSI			
	SRMS			
Crizotinib	ALK	-	+	Tang et al <sup>117</sup>
	ROSI			
	c-MET			

**Abbreviations:** ABC, adenine-triphosphate-binding cassette; ABL, Abelson murine leukemia viral oncogene homolog 1; ACK1, activated CDC42 kinase 1; ALK, anaplastic lymphoma kinase; BCR, B-cell receptor; BCRP, breast cancer resistance protein; EGFR, epidermal growth factor receptor; ERBB4, erythroblastic leukemia viral oncogene homolog-4; FLT3, Fms-like tyrosine kinase 3; HER2, human epidermal growth factor receptor 2; PDGFR, platelet-derived growth factor receptor; P-gp, P-glycoprotein; VEGFR, vascular endothelial growth factor receptor; ROSI, reactive oxygen species I.

administration of gefitinib increases the penetration of topotecan across the brain extracellular fluid but, conversely, reduces its penetration of the ventricular cerebrospinal fluid.

Importantly, recent studies have tentatively implicated some ABCG2/BCRP polymorphisms in the pharmacokinetics

of molecular-targeted drugs,<sup>103</sup> as shown in Table 2. A common functional SNP in the ABCG2/BCRP gene, 421C>A, was shown to be associated with diarrhea in 124 patients treated with oral gefitinib (250 mg once daily); 44% of the patients with a heterozygous 421C>A allele developed diarrhea,

**Table 2** Single-nucleotide polymorphisms of the *ABCG2/BCRP* gene

Variation	Amino acid change	Location	Effect on drug action	Study
-15622C>T		Gene promoter	Increased risk of gefitinib-dependent, moderate-to-severe diarrhea. Increased pharmacokinetic parameters of erlotinib. Increased toxicity of sunitinib.	Rudin et al, <sup>108</sup> Lemos et al, <sup>133</sup> and van Erp et al <sup>136</sup>
-1379A>G				
Δ-654/-651				
-286G>C				
-476T>C				
Δ-235A				
-113A>G				
-29A>G				
34G>A	V12M	N-terminal		
114T>C	No change	N-terminal		
151G>T	G51C	N-terminal		
369C>T	No change	NBD		
376C>T	Q126stop	NBD		
421C>A	Q141K	NBD	Increased bioavailability of topotecan and lactone form of 9-aminocamptotecin. Increased plasma concentration of diflomotecan. Increased risk of gefitinib-induced diarrhea.	Sparreboom et al, <sup>49</sup> Cusatis et al, <sup>103</sup> Sparreboom et al, <sup>151</sup> and Zamboni et al <sup>152</sup>
458C>T	T153M	NBD		
474C>T	No change	NBD		
496C>G	Q166E	NBD		
564A>G	No change	NBD		
616A>C	I206L	NBD		
623T>C	F208S	NBD		
742T>C	S248P	Linker		
1000G>T	E334stop	Linker		
1098G>A	No change	Linker		
1143C>T	-	Intron	Increased risk of gefitinib-dependent, moderate-to-severe diarrhea. Increased pharmacokinetic parameters of erlotinib. Increased toxicity of sunitinib.	Rudin et al, <sup>108</sup> Lemos et al, <sup>133</sup> van Erp et al <sup>136</sup>
1291T>C	F431L	TMD	Insensitive to sunitinib-mediated inhibition.	Kawahara et al <sup>109</sup>
1425A>G	No change	TMD		
1465T>C	F489L	TMD		
1768A>T	N590Y	TMD		
1858G>A	D620N	TMD		
2237G>T	-			
2393G>T	-			
Mutations in selected cell lines		TMD	Increased rhodamine 123 efflux, resistant to mitoxantrone/anthracyclin, and decrease of methotrexate transport.	Honjo et al, <sup>18</sup> Allen et al, <sup>20</sup> Volk and Schneider, <sup>153</sup> and Wang et al <sup>154</sup>
R482T in MCF7 AdVp3000				
R482G in SI-MI-80				
R482M in MT4/Dox500				
R482S in 88.6/D800-B				

**Abbreviations:** ABC, adenine-triphosphate-binding cassette; BCRP, breast cancer resistance protein; NBD, nucleotide-binding domain; TMD, transmembrane domain.

whereas 12% of the other patients were homozygous for the wild-type allele. Other polymorphisms at the *ABCG2/BCRP* locus appear to affect gefitinib-induced diarrhea. A significant number of patients carrying the *ABCG2* (-15622C/T) polymorphism and the *ABCG2* (1143C/T, -15622C/T) haplotype developed gefitinib-dependent moderate-to-severe

diarrhea.<sup>133</sup> These studies suggest that patients with reduced *ABCG2/BCRP* activity arising from a genetic variation might be at increased risk of gefitinib-induced diarrhea, and these genetic markers should be considered in the optimization of NSCLC treatments with gefitinib. However, no clear association was reported between the 421C>A SNP of *ABCG2*/

*BCRP* and a susceptibility to gefitinib-induced adverse effects in a Japanese population.<sup>134</sup> The skin toxicity of gefitinib is also reported to be unrelated to this SNP.<sup>103</sup> More large-scale analyses are required to resolve these discrepancies.

Like gefitinib, erlotinib has been shown to interact with *ABCG2/BCRP*.<sup>107,135</sup> Two polymorphic loci identified in the *ABCG2/BCRP* promoter and intron,  $-15622C/T$  and  $1143C/T$ , which reduce the protein's expression, were reported to be associated with modulation of pharmacokinetic parameters for erlotinib.<sup>108</sup> The area under the curve and maximum observed concentration ( $C_{max}$ ) were higher for the *ABCG2*  $1143 C/T$  or  $T/T$  (lower expression) genotype, and  $C_{max}$  was higher in patients with the  $-15622 C/T$  or  $T/T$  (lower expression) genotype than in those with the  $C/C$  genotype. These observations indicate that *ABCG2/BCRP* recognizes erlotinib as a substrate in vivo.

Sunitinib, an oral multitargeted TKI for vascular endothelial growth factor receptors 1, 2, and 3, platelet-derived growth factor receptor  $\alpha$  and  $\beta$ , c-Kit, Fms-like tyrosine kinase 3 receptor, and the receptor encoded by the *RET* proto-oncogene, is used as a first-line treatment for metastatic renal cell carcinoma and imatinib-resistant metastatic gastrointestinal stromal tumors. The haplotype ( $-15622C/T$ ,  $1143C/T$ ) at the *ABCG2* locus that has been associated with gefitinib-associated adverse effects and increased erlotinib exposure, is also related to the development of increased sunitinib toxicity. The prevalence of toxicity higher than grade 2 increased when one or two copies of  $TT$  were present in the *ABCG2* ( $-15622C/T$ ,  $1143C/T$ ) haplotype.<sup>136</sup> Another SNP in *ABCG2/BCRP* has also been shown to be associated with adverse effects similar to those attributed to sunitinib-related toxicity. Among 12 different genetic polymorphisms examined, the *ABCG2/BCRP*  $421AA$  genotype correlated with the development of grade 3 or grade 4 thrombocytopenia and neutropenia in Korean patients suffering from metastatic renal cell carcinoma, and may be predominantly associated with the risk of sunitinib-related toxicity in those patients.<sup>137</sup> Intriguingly, the  $421C>A$  SNP of *ABCG2/BCRP* may have another effect on tumor lysis syndrome with hyperuricemia during TKI-based molecular-targeted therapy. Several studies have reported renal failure and tumor lysis syndrome during TKI-based molecular-targeted therapies, including with sorafenib for hepatocellular carcinoma, with imatinib for chronic myelogenous leukemia, and with flavopiridol for chronic lymphocytic leukemia.<sup>138–141</sup> Because the  $Q141K$  variant of *ABCG2/BCRP* corresponding to SNP  $421C>A$  reduces uric acid transport<sup>68</sup> and those TKIs are substrates/inhibitors of *ABCG2/BCRP*, TKI therapy in patients with

the SNP  $421C>A$  ( $Q141K$ ) may be at higher risk of tumor lysis syndrome.<sup>5</sup>

A defect in the pharmacological interaction has been suggested between sunitinib and another *ABCG2/BCRP* germ-line mutant allele,  $1291T>C$ .<sup>109</sup> Remarkably, sunitinib reversed wild-type *ABCG2/BCRP*-mediated drug resistance and competitively inhibited *ABCG2/BCRP*-mediated estrone 3-sulfate transport and the binding of  $^{125}I$ -iodoarylazidoprazosin to *ABCG2/BCRP*. The  $F431L$  variant of *ABCG2/BCRP*, which is expressed from a germ-line mutant allele  $1291T>C$ , was insensitive to sunitinib-mediated inhibition. Thus, residue  $F431$  of *ABCG2/BCRP* may have impact on the pharmacological interaction with sunitinib.

## Perspectives

Molecular analyses of the functional interactions between novel molecular targeted drugs and the ABC transporter *ABCG2/BCRP* have demonstrable utility as indicators of the clinical efficacy of these anticancer agents in individual patients. Such pharmacological interactions might be influenced by personal genotypes, and the increased risk of adverse effects from putative *ABCG2/BCRP* substrates should be evaluated when considering combinations of protein kinase inhibitors, even at their clinically used doses.

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## Disclosure

The authors have no conflicts of interest to disclose.

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