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EXPERT REVIEW

ABC Transporters in Multi-Drug Resistance and ADME-Tox of Small Molecule Tyrosine Kinase Inhibitors

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ABSTRACT The past decade has seen tremendous efforts in the research and development of new chemotherapeutic drugs using target-based approaches. These efforts have led to the discovery of small molecule tyrosine kinase inhibitors (TKIs). Following the initial approval of imatinib by the US FDA in 2001, more than 15 TKIs targeting different tyrosine kinases have been approved, and numerous others are in various phases of clinical evaluation. Unlike conventional chemotherapy that can cause non-discriminating damage to both normal and cancerous cells, TKIs attack cancer-specific targets and therefore have a more favorable safety profile. However, although TKIs have had outstanding success in cancer therapy, there has been increasing evidence of resistance to TKIs. The enhanced efflux of TKIs by ATP-binding cassette (ABC) transporters over-expressed in cancer cells has been found to be one such important resistance mechanism. Another major drawback of TKI therapies that has been increasingly recognized is the extensive inter-individual pharmacokinetic variability, in which ABC transporters seem to play a major role as well. This review covers recent findings on the interactions of small molecule TKIs with ABC transporters. The effects of ABC transporters on anticancer efficacy and the absorption, distribution, metabolism, excretion, and toxicity (ADME-Tox) of the small molecule TKIs are summarized in detail. Since TKIs have been found to not only serve as substrates of ABC transporters, but also as modulators of these proteins via inhibition or induction, their influence upon ABC transporters and potential role on TKI-drug interactions are discussed as well.

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ABBREVIATIONS

ABC	ATP-binding cassette
ADME-Tox	Absorption, distribution, metabolism, excretion, and toxicity
ALL	Acute lymphoblastic leukemia
BCRP	Breast cancer resistance protein
CML	Chronic myelogeous leukemia
EGFR	Epidermal growth factor receptor
MDR	Multi-drug resistance
MRP1	Multi-drug resistance protein 1
PDGFR	Platelet-derived growth factor receptor
P-gp	P-glycoprotein
SAR	Structure activity relationship
TKIs	Tyrosine kinase inhibitors
VEGFR	Vascular endothelial growth factor receptor

INTRODUCTION

For several decades, conventional cytotoxic agents have represented the cornerstone of anticancer chemotherapy. Most of these cytotoxic agents exhibit their antitumor effect through an interaction with DNA or its precursors, inhibiting the synthesis of new genetic material, thereby killing the rapidly dividing tumor cells. As these compounds cannot inherently differentiate tumor cells from normal cells, they also harm normal cells that divide rapidly, such as cells in bone marrow, digestive tract, and hair follicles, resulting in the most common side effects associated with chemotherapy: myelosuppression, mucositis, and alopecia, respectively [1–4]. As a result, although cytotoxic agents have a wide spectrum of antitumor activities, their clinical use is often hampered by the

occurrence of severe toxicities such as those mentioned above. Therefore, there is a great unmet need in developing anticancer drugs with increased selectivity and lower side effect burden. In the last 15 years, tremendous progress has been made in human genome mapping, structural biology, and in molecular and cell biology technologies, which correspondingly advances our understanding of the genetics, genomics, biochemistry, and pharmacology of human cancers. The rapidly expanding knowledge of cancer has driven the current cancer chemotherapy development to target-based approaches rather than conventional approaches. Among the various targets identified in cancer, protein tyrosine kinases have been exploited the most [5–7], and are found to play a central role in regulating multiple cellular processes that contribute to tumor development and progression, including cell growth, differentiation, migration, angiogenesis, and apoptosis [5, 8, 9]. An abnormality in tyrosine kinase structure or function, such as overexpression of a tyrosine kinase or mutations that stabilize the active kinase conformation, could lead to cell-autonomous signaling, resulting in malignant transformation and unregulated cell growth [10–12]. It has been reported that more than 80% of the oncogenes and proto-oncogenes involved in human cancer are derived from protein tyrosine kinases [13]. To date, 90 unique tyrosine kinase genes have been identified in the human genome [14]. Considering the critical role of tyrosine kinases in the development and progression of many types of cancer, the inhibition of the catalytic activity of tyrosine kinases represents a very promising strategy to control the deregulated activity of tyrosine kinases.

In the past decade, substantial efforts have been made in the development of new chemotherapeutic drugs using target-based approaches and has led to the discovery of small molecule tyrosine kinase inhibitors (TKIs), an entirely new class of more target-specific anticancer drugs [6, 15]. Imatinib was the first molecularly-targeted TKI that received FDA approval in 2001. It targets the BCR-ABL tyrosine kinase, a constitutively active tyrosine kinase that drives hyperproliferation of stem and progenitor cells and the consequent panmyelosis associated with chronic myelogenous leukemia (CML) [16, 17]. Imatinib has been demonstrated to be a highly effective treatment for the chronic phase of CML, with high rates of complete remission observed [18–20]. Indeed, the clinical success of imatinib revolutionized CML therapy, and imatinib is currently viewed as the first-line therapy for CML. Following imatinib, a variety of TKIs targeting different tyrosine kinases, such as epidermal growth factor receptor (EGFR), vascular endothelial growth factor receptor (VEGFR), and platelet-derived growth factor receptor (PDGFR) have been developed, and many studies are now underway world-wide to investigate their clinical efficacy [21–24]. Presently, more than 15 TKIs have received FDA approval, with numerous more in various phases of clinical evaluation. Unlike conventional chemotherapy that can cause non-discriminating damage to both normal and cancer cells, TKIs attack cancer-specific targets and therefore have a more favorable safety profile. Because of their low toxicity profile, TKIs can be taken orally on a daily basis. However, although TKIs have had outstanding success in treating selected types of cancer, unfortunately, there is accumulating and documented evidence of acquired resistance to TKIs [25, 26]. Cellular resistance to TKIs can arise by many mechanisms, including unveiling point mutations within the kinase domain, modifications of signaling pathway, and target gene amplification or overexpression [27]. In addition, enhanced efflux of TKIs by an over-expression of ATP-binding cassette (ABC) transporters in cancer cells has recently been found to be an important resistance mechanism (Fig. 1) [28–30]. Besides incomplete response due to acquired resistance, a further limitation of TKI therapies has been the recognition that extensive inter-individual pharmacokinetic variability exists [31–33], in which ABC transporters appear to play prominent roles as well (Fig. 1) [29, 34].

In the present review we cover recent findings on the interactions of small molecule TKIs with ABC transporters. The influence of ABC transporters on anticancer efficacy and the absorption, distribution, metabolism, excretion, and toxicity (ADME-Tox) of the small molecule TKIs are summarized. As TKIs have been found not only to be transported by ABC transporters but also to serve as modulators of these proteins as inhibitors or inducers, their modulatory effect on ABC transporters and subsequent TKI-drug interactions are discussed as well. The information presented here may provide guidance in minimizing TKI resistance, optimizing TKI dose regimen, as well as recognizing and avoiding unwanted TKI-drug interactions.

TKIS AND CANCER CHEMOTHERAPY

Tysosine Kinases and Their Role in Cancer

Tyrosine kinases are classified as receptor tyrosine kinases and non-receptor tyrosine kinases, with the former translating extracellular signals into active intracellular cues and the latter transducing signals within the cells. Of the 90 tyrosine kinase genes identified in the human genome to date, 58 encode receptor tyrosine kinase proteins [35]. Receptor tyrosine kinases have 20 subfamilies, including the well-known EGFR, VEGFR, PDGFR, insulin-like growth factor receptor (IGFR), nerve growth factor receptor (NGFR), fibroblast growth factor receptor (FGFR), and others [36]. All receptor tyrosine kinases have a similar molecular structure, which includes an extracellular ligand-binding domain, a single transmembrane-spanning region, and an intracellular tyrosine kinase domain [37]. Upon ligand binding, receptor tyrosine kinases undergo a dimerization process or a conformational change, resulting in auto-phosphorylation of the tyrosine kinase domains and

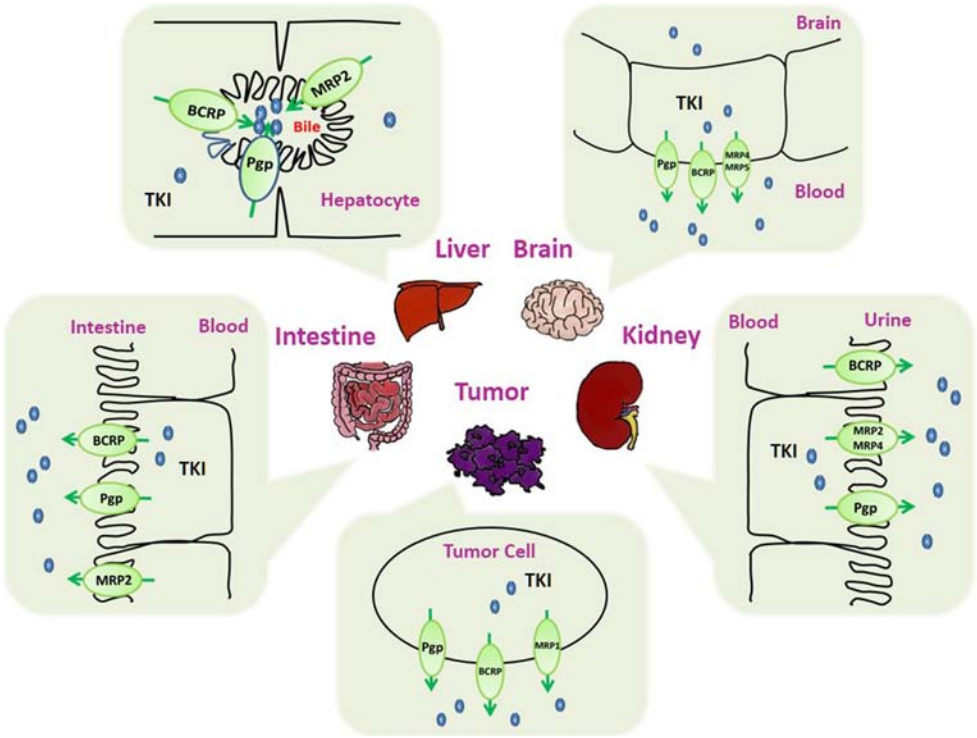


Fig. 1 Schematic representation of potential interactions between TKIs and ABC transporters. The efflux transporters, including P-gp, BCRP and MRPs, are localized in the apical side of the polarized cells, such as the luminal side of the intestinal epithelial cells, bile canalicular membrane of hepatocytes, the proximal renal tubules and the luminal side of the brain endothelial cells. The efflux transporters pump TKI molecules back to the luminal side of the polarized cells mentioned above, leading to decreased intestinal absorption, increased biliary excretion, increased urinary excretion, and deceased brain penetration of TKIs. In addition to normal tissues, these efflux transporters are also expressed in tumor cells and can pump the TKI molecules out of the tumor, resulting in decreased intracellular TKI concentrations, which subsequently leads to TKI resistance. TKI, tyrosine kinase inhibitor; P-gp, P-glycoprotein; BCRP, Breast Cancer Resistance Protein; MRP, Multi-drug Resistance Protein.

increased kinase activity. The activated receptor tyrosine kinases will further initiate a cascade of intracellular signaling pathways. Ultimately, the complex signaling network triggered by receptor tyrosine kinases leads to altered DNA synthesis and cell division as well as a variety of biological processes, including cell growth, migration, differentiation and death [38]. Constitutive activation of receptor tyrosine kinases can lead to malignant transformations via a variety of mechanisms including the overexpression of wild-type receptors, mutations in the kinase gene that cause ligand-independent constitutive receptor activation, and trans-activation through receptor dimerization [39–42].

Unlike receptor tyrosine kinases, non-receptor tyrosine kinases lack transmembrane domains and are found in the cytoplasm, nucleus, and the inner surface of the plasma membrane. At present, 32 non-receptor tyrosine kinases are recognized, which fall into ten subfamilies based on kinase domain sequences [38]. Examples of non-receptor tyrosine kinases include Src, Abl, and Janus kinases. The non-receptor tyrosine kinases are integral components of the signaling cascades triggered by receptor tyrosine kinases and by other cell surface receptors. Dysregulation of non-receptor tyrosine kinase activity has been revealed in the pathogenesis of many human cancers [13]. Similar to receptor tyrosine kinases, the involvement of non-receptor tyrosine kinases in cancer can also occur through various mechanisms such as mutation, overexpression, and translocation.

Molecular Targeted Small Molecule TKIs

Increased knowledge and recognition of the critical role of tyrosine kinases in cancer development, its activation mechanisms, and the signaling pathways controlled by tyrosine kinases provided the impetus for the development of novel and target-specific classes of anticancer drugs, among which small molecule TKIs represents an extremely promising and rapidly expanding group. Small molecule TKIs in most cases compete with ATP binding to the intracellular catalytic domain of tyrosine kinases. This subsequently inhibit protein autophosphorylation that involves in the post-receptor signal cascade, resulting in antiproliferative and antiangiogenic effect [42]. In addition to small molecule TKIs, there are a few other promising approaches to inhibit the activation of tyrosine kinases, such as targeted monoclonal antibodies (mAbs) which are directed against the extracellular domain of receptor

tyrosine kinases [43], and antisence oligonucleotides that block receptor translation [44].

TKIs Targeting Epidermal Growth Factor Receptor Family

EGFR belongs to the ErbB family of receptor tyrosine kinases. EGFR, also known as ErbB1 or HER1, has been reported to be essential for the growth and differentiation of epithelial cells [45]. Among the various receptor tyrosine kinases implicated in human cancers, the deregulation of EGFR system seems to be the most prevalent. EGFR has been found to be commonly overexpressed in a variety of solid tumors, including non-small cell lung cancer (NSCLC), colorectal adenocarcinoma, glioblastoma, squamous cell carcinoma of the head and neck, ovarian, breast and prostate cancer [46–49]. In addition to EGFR, HER2, also known as ErbB2, is an important oncogenic kinase in the ErbB family. HER2 is a possible heterodimerization partner of EGFR and it has been found to be up-regulated in many types of human cancer, especially in human breast cancer where HER2 gene amplification (2 to 20-fold) has been identified with a frequency of 30% [40, 50]. The formation of EGFR/HER2 heterodimer is the most active form of EGFR. Considering the wide-spread contribution of the EGFR family in tumorgenesis, these receptors represent one of the most promising targets for anticancer therapy. A large number of small molecule TKIs targeting EGFR have been developed and several of them have already received FDA approval.

Gefitinib. A prominent representative of anti-EGFR TKIs is gefitinib, which received an accelerated FDA approval in 2003 for the treatment of patients with NSCLC [51]. Gefitinib is a selective EGFR (ErbB1) tyrosine kinase inhibitor and it has 200-fold greater affinity for ErbB1 (IC50 of 20–80 nM) compared to ErbB2 [52]. Early results with gefitinib in lung cancer are promising, but further trials that recruited primarily non-Asian NSCLC patients could not demonstrate a survival advantage [24]. Retrospective analysis of multiple studies revealed that patients who were Asian, nonsmokers, or women had significantly higher response rates [53, 54]. Tumors from these patients frequently have characteristic activating mutations in EGFR. The standard dose of gefitinib is 250 mg daily. Gefitinib has a favorable safety profile and the most frequent adverse effects were mild acneiform skin rash and diarrhea.

Erlotinib. Erlotinib is also a selective and potent inhibitor of the EGFR tyrosine kinase. Erlotinib competitively inhibits ATP binding at the active site of the EGFR kinase domain, with an IC_{50} of 2 nM. Erlotinib was approved by the FDA in 2004 for the second-line treatment of patients with locally advanced or metastatic NSCLC. FDA approval was based on the improvement demonstrated in overall survival in a large placebo-controlled trial conducted in advanced stage III or IV NSCLC patients [55]. Results from two phase III studies showed no clinical benefit from the combination of erlotinib with conventional chemotherapy over chemotherapy alone [56]. In addition to NSCLC, it was subsequently approved for use in combination with gemcitabine as a first-line treatment for patients with locally advanced, unresectable, or metastatic pancreatic cancer [23]. Similar to gefitinib, the most frequent adverse effects of erlotinib were also acneiform skin rash and diarrhea [57].

Lapatinib. Lapatinib is a reversible and specific EGFR tyrosine kinase inhibitor which has been shown to have activity against both EGFR (ErbB1) and HER2 (ErbB2), with IC_{50} values of approximately 10 nM [58, 59]. Due to its nonselective inhibition of EGFR, lapatinib has a broader spectrum of antitumor activity and improved efficacy. Evidence of clinical efficacy, including notable tumor responses and improved survival, has been reported in several clinical trials in HER2 positive breast cancers for lapatinib used either alone or in combination with other anticancer drugs [22]. Lapatinib was approved by the FDA in 2007 for the treatment of HER2 positive breast cancer in combination with capecitabine. The most frequent adverse effects of lapatinib were diarrhea, rash, nausea and fatigue.

Afatinib. Afatinib is an irreversible inhibitor against ErbB receptor family members, EGFR/ErbB1 (IC_{50}=0.5 nM), HER2/ErbB2 (14 nM), HER4/ErbB4 (1 nM), and the oncogenic mutants $EGFR^{L858R}$ (0.4 nM) [60]. Afatinib received FDA approval in 2013 as a first-line treatment for metastatic NSCLC in patients whose tumors have EGFR exon 19 deletions or exon 21 (L8558R) substitution mutations. The approval of afatinib was based on a head-to-head phase III trial of afatinib or cisplatin plus pemetrexed in which patients with metastatic NSCLC were stratified according to EGFR mutation status. A significant prolongation of median progression-free survival was found among patients with EGFR mutations in the afatinib treatment arm as compared to the chemotherapy arm [61]. Common side effects of afatinib include diarrhea, rash, blisters or other skin lesions.

Canertinib (CI-1033). Canertinib is a novel, nonselective, irreversible EGFR inhibitor. As it is active against all four ErbB members, canertinib is expected to have a greater efficacy and broader spectrum of antitumor activity than the other presently available anti-EGFR TKIs. It may also have the advantage of prolonged clinical effects with less frequent dosing requirement. Canertinib has demonstrated activity against a variety of human breast tumors in both *in vitro* and *in vivo* in xenograft tumor models [62]. Clinical trials of canertinib conducted in patients with breast cancers and NSCLC are currently ongoing [35].

TKIs Targeting Receptor Tyrosine Kinase Families Involved in Angiogenesis

Angiogenesis, a process through which new blood vessels form from pre-existing vessels, is an essential property of cancers. It has been well documented that cancer cells secrete angiogenic factors which can induce the formation of new blood vessels which assure the flow of nutrients to the tumor cells. These angiogenic factors include VEGF, PDGF, fibroblast growth factor (FGF), and transforming growth factor (TGF) which are found to be overexpressed in many tumor types [35]. The most studied angiogenic factor is VEGF, which is secreted by almost all solid tumors. VEGF initiates endothelial cell proliferation when binding to members of the VEGF receptor family. This receptor family includes a group with intracellular tyrosine kinase domains containing VEGFR1 (FLT1), VEGFR 2 (KDR), and VEGFR3 (FLT4) [63]. Elevated activities of the VEGF-VEGFR ligand-receptor system correlate with increased tumor vascularization and metastasis, as well as decreased survival [63]. In addition to VEGFR, PDGFR also plays an important role in promoting angiogenesis. Two distinct PDGFR, PDGFRa and PDGFRb, have been identified. Both receptor types have been found to be overexpressed in many solid tumors, including dermatofibrosarcoma, glioblastoma, NSCLC, and others [64]. The establishment of the role of receptor tyrosine kinases in promoting angiogenesis has provided a new avenue of development for cancer therapeutics. Many small molecule TKIs targeting VEGFR, PDGFR, and other tyrosine kinases with similar functions have been developed in the past 10 years.

Sorafenib. Sorafenib is a novel tyrosine kinase inhibitor that inhibits both tumor cell proliferation and angiogenesis. It targets multiple tyrosine kinases, including VEGFR1, VEGFR2, VEGFR3, PDGFR-b, c-KIT, FLT-3 and b-RAF. Sorafenib was the first multikinase inhibitor receiving FDA approval (2005) for the treatment of patients with advanced renal cell carcinoma and was later granted an additional indication for the treatment of patients with unresectable hepatocellular carcinoma [21, 65, 66]. Overall sorafenib has a favorable safety profile. Sorafenib administration has been associated with some adverse cardiovascular effects including hypertension and arterial thromboembolic events which have also been documented other anti-angiogenic medications.

Sunitinib. Similar to the aforementioned sorafenib, sunitinib is also a multikinase inhibitor and inhibits the cellular signaling of multiple targets including VEGFR1, VEGFR2, PDGFRa, PDGFRb, FLT3, c-KIT, and RET. Sunitinib was approved by the FDA in 2006 as a first-line treatment of advanced renal cell carcinoma and in imatinib-resistant gastrointestinal stromal tumor [67]. Sunitinib displays an intrinsically high degree of brain penetration among TKIs, and its effect in recurrent glioblastoma multiforme is currently under investigation. The common side effects of sunitinib include fatigue, hypothyroidism, bone marrow suppression and diarrhea.

Axitinib. Axitinib is a newly developed and potent tyrosine kinase inhibitor which inhibits VEGFR-1, −2, and −3 at picomolar levels and PDGFRb at nanomolar levels [68]. Axitinib showed efficacy against many solid tumors in Phase II clinical trials and produced a significant increase in progression-free survival compared with sorafenib in a Phase III study conducted in patients with advanced renal cell carcinoma [69–71]. Axitinib received FDA approval in early 2012 for treatment of advanced renal cell carcinoma in patients who had failed one prior systemic therapy. Axitinib appears to be generally well tolerated. The dose-limiting toxicities of axitinib were elevated blood pressure, hemoptysis, and stomatitis.

Pazopanib, Vandertanib and Cediranib. Pazopanib is a second generation tyrosine kinase inhibitor targeting VEGFR-1, −2, and −3, PDGFRa, PDGFRb, and c-Kit. It was recently approved by the FDA for the treatment of metastatic renal cell carcinoma [72, 73]. Vandertanib is also a newly developed tyrosine kinase inhibitor which targets VEGFR and EGFR [74]. It recently received the FDA approval for the treatment of metastatic medullary thyroid carcinoma [75]. Finally, cediranib is a potent inhibitor of VEGFR-2 with IC_{50} less than 1 nM. It also has activity against VEGFR-1, VEGFR-3, PDGFR and c-Kit. Cediranib has been evaluated in several clinical trials for the treatment of various tumors, including recurrent GBM, NSCLC, and colorectal cancer [76–78].

TKIs Targeting Non-receptor Tyrosine Kinases

Non-receptor tyrosine kinases are confined to the cellular cytoplasm or nuclear compartment, and the dysregulation of their activity has been implicated in the pathogenesis of many human cancers. The most clinically relevant non-receptor tyrosine kinase is BCR-ABL, a protein derived from a translocation event of chromosomes 9 and 22, resulting in a fusion of the c-ABL and the breakpoint cluster region gene (BCR) [35]. This new fusion gene, BCR-ABL, encodes an unregulated, cytoplasm-targeted tyrosine kinase which allows the cells to proliferate without being regulated by cytokines and this permits the cells to become malignant. BCR-ABL oncogene is seen in 95% of patients with CML and 15 to 30% of adult patients with acute lymphoblastic leukemia (ALL) [79, 80]. The increased understanding of this pathogenetic defect at the molecular level led to the development of a number of small molecule TKIs targeting BCR-ABL tyrosine kinases.

Imatinib. Imatinib is a potent inhibitor of the BCR-ABL tyrosine kinase and specifically kills proliferating myeloid cell lines containing BCR-ABL with minimal harm to normal cells [81]. In addition to BCR-ABL, imatinib also inhibits c-Kit and PDGFR tyrosine kinases. In clinical trials, imatinib has been shown to be highly effective in CML. Imatinib induced major cytogenetic response in 80 to 90% of patients with previously untreated CML [82]. Imatinib is also effective in the treatment of BCR-ABL positive refractory adult ALL [19]. Imatinib was the first molecularly targeted TKI to receive FDA approval. It is approved for the treatment in BCR-ABL positive CML, and in the treatment of gastrointestinal stromal tumors that harbor c-Kit mutations, both of which were difficult to treat before the clinical introduction of imatinib [18, 83]. Imatinib is generally well tolerated, with neutropenia, thrombocytopenia, and anemia representing the most common adverse effects.

Dasatinib and Nilotinib. Although imatinib initially improved the outcome of CML dramatically, its beneficial effects were unfortunately limited by intrinsic or acquired drug resistance. The recognition of imatinib resistance led to the development of a second generation of BCR-ABL TKIs, such as dasatinib and nilotinib [84]. Dasatinib is a second generation tyrosine kinase inhibitor targeting BCR-ABL and was approved by the FDA in 2006 for the treatment of imatinib-resistant CML patients. In terms of BCR-ABL inhibition, dasatinib has an IC_{50} of less than 1 nM, which is significantly more potent than imatinib (IC_{50} = 100 nM). Similar to dasatinib, nilotinib is another second generation BCR-ABL inhibitor that was likewise approved by the FDA in 2007 for the treatment of imatinib-resistant or imatinib-intolerant disease. The IC_{50} of nilotinib on BCR-ABL inhibition is less than 20 nM. Nilotinib is also more potent than imatinib, and can overcome imatinib resistance in some instances [85].

Bosutinib, Ponatinib, and Danusertib. Bosutinib is a potent TKI against c-Src (IC_{50} = 1–2.4 nM) and Abl kinases (3.5 nM) [86]. Bosutinib was approved by the FDA in 2012 for treatment of patients with chronic, accelerated, or blast phase Philadelphia chromosome positive (Ph+) CML that have resistance to prior medications, such as imatinib, dasatinib, or nilotinib. Ponatinib is an effective oral TKI against ABL (0.37 nM), c-Src (5.4 nM), c-Kit (12.5 nM), VEGFR2 (1.5 nM), FGFR1 (2.2 nM), PDGFRα (1.1 nM), as well as several ABL kinase variants [87]. As a multi-targeted TKI effective against many BCR-ABL variants, ponatinib received FDA approval in 2012 for treatment of CML or Ph+ALL patients that have been resistant to prior TKI therapy. However, presently, ponatinib is temporarily suspended by the FDA due to findings that a high percentage of patients enrolled in ponatinib phase I and II trials experienced serious adverse vascular effects.

THE ABC TRANSPORTERS IN CANCER DRUG RESISTANCE AND DRUG DISPOSITION

The ABC transporter superfamily is one of the largest protein superfamilies known. The vast majority of its members are responsible for the transport of a diverse array of substrates, including lipids, amino acids, sugars, bile salts, peptides, steroids, endogenous metabolites, ions, drugs and other xenobiotics [86–92]. To date, at least 49 ABC transporters have been identified in humans. Based on phylogenetic analysis, they have been classified into seven subfamilies labeled A-G [93]. ABC transporters represent active transporters and they efflux their substrates against a concentration gradient using the energy of ATP hydrolysis. The functional unit of an ABC transporter contains two membrane spanning domains (MSDs) and two nucleotide binding domains (NBDs). The conformation changes within the MSDs to form a permeation pathway which are believed to be responsible for the transport of substrates. NBDs, where the energy of ATP is harvested, act as the energy supplier [94].

Multi-drug resistance (MDR), a phenomenon in which cancer cells become resistant to various structurally and functionally unrelated chemotherapeutic agents, is a major obstacle in the effective chemotherapy of human cancer patients. MDR can be intrinsic or acquired. In intrinsic resistance, tumors fail to respond to chemotherapy from the outset; whereas acquired resistance is the situation that tumors initially respond to chemotherapy but eventually become insensitive to treatment upon relapse [95]. Several molecular mechanisms have been reported to be associated with the development of MDR. These mechanisms include alterations in drug targets such as DNA topoisomerase II [96], increased repair of DNA damage [97], reduced apoptosis [98], increased drug metabolism, e.g. by glutathione (GSH) conjugation [99], down-regulation of the uptake system (especially for those water soluble anticancer drugs) [100], and enhanced efflux of drugs by ABC transporters [101]. Although various cellular mechanisms have been shown to underlie the development of MDR, enhanced anticancer drug efflux via over-expression of ABC transporters in cancer cells is the most commonly observed and best characterized mechanism impairing the effectiveness of chemotherapy.. Among the 49 ABC transporters identified, P-glycoprotein (P-gp, ABCB1), Multi-drug Resistance Protein 1 (MRP1, ABCC1) and Breast Cancer Resistance Protein (BCRP, ABCG2) account for the majority of the observed efflux transporter-mediated MDR in humans and rodents [102].

ABC Transporters and MDR of Conventional Cytotoxic Agents

P-gp and MRP1

The role of P-gp and MRP1 in MDR has been extensively studied since their initial discovery by Ling and co-workers

in 1976 and Cole and associates in 1992, respectively [103, 104]. Both transporters are known to transport numerous conventional anticancer drugs that are utilized in first line chemotherapy against different cancers, such as anthracyclines (doxorubin and daunorubicin), vinca alkaloids (vinblastine and vincristine), camptothecins (topotecan) and epipodophyllotoxinx (etoposide and teniposide) [90, 91, 105]. The expression of P-gp and MRP1 has been evaluated in a number of investigations and have been detected in almost every tumor type examined, including both solid tumors and hematological malignancies [106]. P-gp expression in clinical tumor samples has been characterized and was found both during diagnosis as well as relapse, indicating its important role in both intrinsic and acquired MDR [107, 108]. A number of clinical investigations have suggested that P-gp over-expression correlates well with poor response to chemotherapy [109, 110].

BCRP

Relative to P-gp and MRP1 which were discovered several decades ago, BCRP is represents a relatively new identified ABC transporter. BCRP has generated significant interest since it was first cloned from a doxorubicin selected human breast cancer cell line (MCF-7/AdrVP) in 1998 [111]. Although BCRP was initially termed Breast Cancer Resistance Protein, the expression of this protein is not limited to breast cancer cells. In a study conducted by Diestra *et al.* [112], among 150 human solid tumors comprising 21 different tumor types, the expression of BCRP was detected in all tumor types examined, with a high frequency in colorectal carcinoma, gastric carcinoma, hepatocellular carcinoma, bladder carcinoma, ovarian carcinoma, small cell lung cancer, and melanoma. Many structurally distinct conventional anticancer drugs, including mitoxantrone, topotecan, irinotecan, etoposide, and flavopiridol, are substrates of BCRP [91, 113, 114]. A few other anti-cancer agents, such as bisantrene and anthracyclines (doxorubicin and daunorubicin), although not transported by wild-type BCRP, are substrates of the BCRP mutant R482T [92]. The correlation of BCRP with MDR is supported by several clinical studies. For example, Candeil *et al.* [115] examined the expression of BCRP in tumor samples from 42 patients with colon cancer and observed higher BCRP mRNA levels in tumors in the irinotecan-treated group compared to the untreated group.

ABC Transporters and Drug Disposition

The efflux transporters that are present in cancer cells are also present in normal tissues. Due to their wide distribution in normal tissues and their broad substrate spectra, ABC transporters have been found to play an important role in the ADME-Tox of their substrates [89].

P-gp

In 1987, Thiebaut *et al.* investigated the expression and cellular localization of P-gp in normal human tissues using the monoclonal antibody MRK16 [116]. They found that P-gp was expressed exclusively in the apical membrane of enterocytes of the gastrointestinal tract, hepatocytes renal proximal tubules and capillary endothelial cells of the brain and testis. The apical orientation of P-gp in normal tissues indicated that physiologically, P-gp may serve as a defense mechanism: it pumps xenotoxins back into the gastrointestinal tract to decrease absorption and expels xenotoxins into bile and urine to increase their elimination. P-gp appears to also a similar function in the capillary endothelial cells comprising the blood–brain and blood-testis barrier where it is also highly expressed. P-gp.has a very broad substrate spectrum and a structurally diverse group of clinically important therapeutic agents are known to serve as substrates including anticancer drugs, HIV-protease inhibitors, H2-receptor antagonists, immunosuppressive agents, calcium-channel blockers, antibiotics and an array of neuropsychiatric medications (Doran *et al.* 2005 [119]) [90, 91, 117]. Although the substrates of P-gp are structurally dissimilar, most of them are amphipathic and lipid soluble compounds, with aromatic rings and a positive charge at physiological pH. The important role of P-gp in the disposition of its substrates has been verified by numerous investigations. For example, after intravenous and oral administration, the plasma AUC of paclitaxel was 2-fold and 6-fold, higher, respectively, in mdr1a (−/−) mice than mdr1a (+/+) mice [118].

Because of the importance of P-gp in drug disposition, the screening for P-gp transport has become routine in the pharmaceutical industry. Indeed, promising lead compounds can be found to be severely hampered in their ability to produce their intended pharmacological effects *in vivo* if they are good substrates for P-glycoprotein, particularly if the route of administration is intended to be oral or the ultimate target tissues is one highly expressing P-gp. Additionally, the potential for drug-drug interactions arises under the circumstances of a P-gp substrate being coadministered with another agent that can significantly inhibit P-gp activity [119].

MRP1

MRP1 is expressed in most tissues in the human body, with relative high levels detected in the lung, testis, peripheral blood mononuclear cells, choroid plexus and kidney [120]. Unlike P-gp, which is expressed in apical membranes, MRP1 expression in polarized cells is restricted to the basolateral side, and correspondingly its substrates are transported towards the basolateral side of epithelia [121]. Therefore, in contrast to P-gp,

physiologically, MRP1 may serve as a cellular defense mechanism rather than organismal defense. Although MRP1 also has the capacity to mediate the transport of a wide array of compounds and P-gp and MRP1 share some common substrates, generally MRP1 can transport anionic compounds while P-gp substrates tend to be neutral or positively charged drugs at physiologic pH. The compounds which have been identified to be MRP1 substrates include anticancer drugs, various glutathione conjugates, glucuronide or sulfate conjugates, HIV protease inhibitors, fluorescent compounds such as calcein, and antibiotics [91, 122].

BCRP

Similar to P-gp and MRP1, BCRP is also expressed in a number of normal tissues and not limited to cancer cells. The highest expression of BCRP has been observed in human placental tissue followed by prostate, small intestine, brain, colon, liver and ovary [111]. In contrast to P-gp, there is little expression of BCRP mRNA in the human kidney. On the other hand, murine Bcrp1 is abundantly expressed in both mouse and rat kidney [123]. In terms of cellular localization, similar to P-gp, BCRP is expressed in the apical side of epithelial and endothelial membranes which are important for drug absorption, distribution and elimination, suggesting that BCRP also functions as a protective efflux pump and has the potential to limit bioavailability and increase the biliary and urinary excretion of xenobiotics that are BCRP substrates. BCRP has a very broad substrate spectrum which encompasses both positively and negatively charged compounds, including anticancer agents, antibiotics, HMG-CoA reductase inhibitors, antiviral drugs, porphyrins, chemical toxins, carcinogens, fluorescent dyes, endogenous compounds, and several glucuronide and sulfate conjugates, have been found to be transported by BCRP [92, 124–126]. BCRP is also found to play an important role in drug disposition. For example, with the co-administration of 5,7-dimethoxyflavone, a potent BCRP inhibitor, the concentration of mitoxantrone in mouse liver and kidney increased more than 90% and 60%, respectively, compared with the mitoxantrone alone group [127].

INTERACTION OF ABC TRANSPORTERS WITH SMALL MOLECULE TKIS

Role of ABC Transporters in TKI Resistance in Cancer

In the past decade, a significant number of small molecule TKIs have been approved for cancer treatment and these targeted agents have produced impressive clinical benefits in many type of tumors. However, although TKIs have increased selectivity and fewer side effects relative to conventional cytotoxic agents, much like these conventional anticancer drugs, the development of acquired TKI resistance TKIs after an initially favorable response has been frequently observed. This acquired resistance represents a major obstacle for future development of successful tyrosine kinase targeted anticancer therapy. Among several reported cellular mechanisms, enhanced cellular efflux TKIs of TKI due to overexpression of ABC transporters in cancer cells represents an important mechanism associated with observed TKI resistance (Fig. 1).

Bcr-Abl TKIs

The first report of efflux transporter-mediated TKI resistance was described in 2000 by Mahon and coworkers [128]. They determined that BCR-ABL positive cells with MDR1 overexpression were resistant to imatinib. Similar phenomenon of P-gp-mediated resistance to imatinib was also reported by different research groups using several other cell lines [129–131]. Illmer and colleagues showed that the intracellular levels of imatinib were decreased in P-gp-positive leukemic cells and CysA, a well-known P-gp inhibitor, could restore imatinib cytotoxicity in these cells [130]. Research conducted by Widmer and associates showed that imatinib intracellular concentration increased by 4- to 9-fold in P-gp positive K562 cells when the expression of ABCB1 was down regulated by RNAi [132]. In addition to P-gp, imatinib was also found to be transported by BCRP. It has been reported by several research groups that BCRP protected CML-derived Bcr-Abl K562 leukemia cells from the cytotoxic effect of imatinib by decreasing its intracellular concentration [29]. Further investigations on the interaction of ABC transporters with imatinib indicated that imatinib interacts with P-gp and BCRP only at low micromolar concentrations within a narrow concentration range and exhibits inhibitory effect at high concentrations [133–135]. In addition to P-gp and BCRP, the interaction between MRP1 and imatinib has also been evaluated and imatinib was found to be a poor substrate of MRP1 [136].

Similar to imatinib, the second generation BCR-ABL TKIs nilotinib and dasatinib have also been found to be substrates of both P-gp and BCRP [137, 138]. It has been reported that P-gp and BCRP-overexpressing K562 cells are resistant to nilotinib and dasatinib, indicating that these two drugs are transported by Pgp and BCRP, and that these two transporters could convey inherent resistance to nilotinib and dasatinib [137]. For nilotinib, it seems BCRP plays a larger role than P-gp in nilotinib resistance. For example, Hegedus *et al.* reported that compared to parental K562 cells, BCRP expressing K562 cells were 8.8-fold more resistant to nilotinib, whereas Pgp-expressing K562 cells only produced a small (albeit significant) resistance to nilotinib [137]. In

contrast to imatinib, nilotinib and dasatinib, bosutinib, a newly developed BCR-ABL inhibitor, was not a substrate of either Pgp or BCRP, indicating that the presence of these transporters will not confer resistance to bosutinib [137]. *In vitro* investigations into the mechanism of resistance of danusertib, another novel and potent BCR-ABL inhibitor, revealed that danusertib is a BCRP substrate, and BCRP overexpression was identified and validated as the predominant mechanism of acquired danusertib resistance in BCR-ABL positive cells [139].

EGFR Family TKIs

Compared with BCR-ABL TKIs, the information related to the role of ABC transporters in the cancer resistance of EGFR TKIs is less abundant, and the evaluations were mainly focused on BCRP. It has been reported that the resistance to gefitinib was consistently observed when BCRP was transduced to several different cancer cell lines, including human epidermal carcinoma A431 cells, human NCSLC PC-9 cells, human colon cancer Caco-2 cells, and adenocarcinoma WiDr cells [135, 140, 141]. Usuda *et al.* reported that BCRP expression can be detected in the wtEGFR-expressing patient with acquired gefitinib resistance [30]. These studies suggested that BCRP-mediated gefitinib efflux may account for the poorer clinical outcomes of gefitinib, and BCRP may be used as at least one predictor of clinical response to gefitinib. In addition to gefitinib, the role of the ABC transporter in the resistance of several other EGFR TKIs has also been reported. Canertinib, a nonselective and irreversible EGFR tyrosine kinase inhibitor, was found to be transported by BCRP [142]. In addition, the role of the ABC transporter in the resistance to erlotinib was also investigated and the result revealed that BCRP does not seem to be the major determinant of erlotinib resistance.

VEGFR TKIs and Other Emerging New TKIs

The information of ABC transporter-mediated resistance of TKIs targeting VEGFR, PDGFR, and other receptors are not available at this point. However, since most of these TKIs have been found to be substrates of ABC transporters, efflux transporter-mediated acquired resistance may be anticipated to occur after chronic administration of these TKIs as well.

Role of ABC Transporters in ADME-Tox of TKIs

Beyond resistance in cancer, another significant factor that may compromise the therapeutic efficacy of TKIs is the extensive inter-individual variability in pharmacokinetics. Large inter-individual variability in systemic exposure has been consistently observed in numerous TKIs, including imatinib, gefitinib, erlotinib, sorafenib, dasatinib, nilotinib, lapatinib, and sunitinib [33]. It has been reported that the steady-state trough concentrations of gefitinib varied 20-fold following the administration of a single 250 mg daily oral dose to 14 adult cancer patients [143]. The clearance of imatinib was found to vary 60-fold in patients with gastrointestinal stromal tumors [144]. As noted earlier, ABC transporters are expressed not only in cancer cells but also in a variety of normal tissues that are known to play critical roles in ADME-Tox of numerous xenobiotics, and are frequently found to be a major source of the inter-individual variability observed in the disposition of their substrates [89]. The role of ABC transporters in ADME-Tox of TKIs, especially with regard to their brain penetration, has been extensively investigated in the recent years, with detailed information summarized below.

Bcr-Abl TKIs

In vitro data have indicated that imatinib is a good substrate of both Pgp and BCRP [145]. Oostendorp *et al.* performed an animal study of imatinib and comprehensively evaluated the impact of P-gp and Bcrp1 on imatinib ADME in Pgp-knockout, Bcrp1-knockout, Pgp/Bcrp1-knockout, and wild-type mice [34]. Their results indicated that the bioavailability of imatinib was significantly increased when it was coadministered with elacridar (a dual Pgp and BCRP inhibitor), indicating that these two transporters play an important role in imatinib absorption. In the presence of elacridar, the plasma concentrations of imatinib were also greatly increased. Compared to wild-type mice, fecal excretion of imatinib was significantly diminished by 3.4-fold in P-gp knockout mice and 5.2-fold in P-gp/Bcrp1 knockout mice, but was not significantly different in BCRP knockout mice, suggesting that P-gp may play a larger role in the elimination of imatinib [34]. The role of P-gp and Bcrp1 in imatinib tissue distribution was evaluated in mice liver, kidneys and brain following an administration of 50 mg/kg dosage. The results showed that these two transporters have minimal role in imatinib liver and kidney distribution, but have a significant impact on imatinib brain penetration [34]. Interestingly, P-gp and BCRP seem to work synergistically in limiting imatinib brain penetration - imatinib brain levels increased only 2.3-fold in P-gp knockout mice and 1-fold in Bcrp1 knockout mice at 1 h, whereas in Pgp/Bcrp1 knockout mice imatinib brain level increased 12.6-fold compared to that of wild-type mice [34]. The observation that Pgp and BCRP appear to work in concert in limiting imatinib brain penetration has also been confirmed by several other research groups [145–147]. In addition to imatinib, the apparent synergistic roles of Pgp and BCRP in limiting drug delivery across the BBB was also observed for dasatinib [148–150]. For example, dasatinib brain concentrations did not change significantly in Bcrp1 knockout mice, increased 3.6-fold in Pgp knockout mice,

and increased 13.2-fold in Pgp/Bcrp1 knockout mice [150]. Although *in vitro* data showed that both Pgp and Bcrp1 efficiently transport imatinib and dasatinib, based on the *in vivo* results it appears that P-gp plays a more prominent role than BCRP in the brain penetration of imatinib and dasatinib. As it has been reported that the expression of Pgp on BBB is significantly higher than that of Bcrp1 [151], the difference in their expression may be the reason behind the subdued effect of Bcrp1-mediated efflux at BBB. Apart from imatinib and dasatinib, investigations into the role of ABC transporters in the transport of nilotinib and other newly developed BCR-ABL TKIs are mainly limited to *in vitro* studies. *In vitro* data indicated that nilotinib is a poor P-gp substrate and a good BCRP substrate.. Ponatinib is transported by both P-gp and BCRP, whereas bosutinib is not a substrate of either transporter [137, 152].

EGFR Family TKIs

Similar to BCR-ABL TKIs, most of the EGFR TKIs are also substrates of both P-gp and BCRP, and these two transporters have recently also been found to play an important role in the pharmacokinetics and tissue distribution of these TKIs, especially with regard to their brain penetration. Agarwal *et al.* reported that the transport of gefitinib across the BBB was significantly limited by P-gp and BCRP [153]. Interestingly, consistent with that observed for imatinib and dasatinib, the brain level of gefitinib was substantially increased (70-fold higher) in P-gp/Bcrp1 knockout mice compared to wild-type mice, whereas the increase in gefitinib brain concentration was only modest in P-gp knockout and Bcrp1 knockout mice, again suggesting a synergistic role of P-gp and Bcrp1 in gefitinib brain penetration [153]. This phenomenon was also observed in several other EGFR TKIs, including erlotinib and lapatinib, from several independent laboratories [154–157]. These findings are of clinical significance for therapy in brain cancers, where co-administration of a dual P-gp and BCRP inhibitor can increase brain delivery of TKIs and correspondingly enhance drug efficacy.

In addition, ABC transporters have also been found to be associated with the toxicity of TKIs. For example, Cusatis *et al.* reported that cancer patients with reduced BCRP activity resulting from a common genetic variant were at increased risk of gefitinib-induced diarrhea [158].

VEGFR TKIs and Other Emerging New TKIs

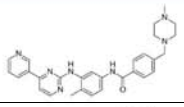
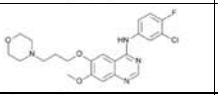
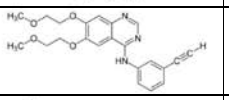
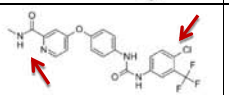
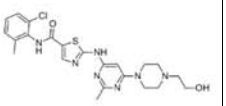
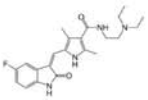
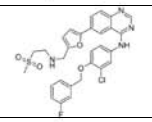
TKIs targeting VEGFR, PDGFR, and other receptors involved in angiogenesis represent a rapidly emerging class of TKIs. The role of P-gp and BCRP in the disposition of this novel class of TKIs, particularly regarding their brain penetration, has been investigated extensively. Consistent with the results obtained in BCR-ABL and EGFR TKIs, Pgp and BCRP work synergistically and play a crucial role in restricting the brain penetration of many TKIs targeting angiogenesis, including sorafenib [159, 160], sunitinib [160], axitinib [161], pazopanib [142], and vandetanib [162]. For example, relative to wild-type mice, the brain accumulation was increased 9.3- to 36-fold for sorafenib, 24-fold for sunitinib, 21-fold for axitinib in Pgp/Bcrp1 knockout mice [160, 161]. In addition, Elarida, a dual Pgp/Bcrp1 inhibitor, significantly enhanced the brain levels of pazopanib and vandertanib by 5-fold [162]. For the TKIs mentioned above, although Pgp and BCRP work in concert in restricting their brain access, for the majority of these agents, P-gp has a greater impact than Bcrp1 on brain penetration. Sorafenib is a notable exception however, in that the role of BCRP in active efflux at the BBB is greater than that of P-gp [163]. Unlike other VEGFR TKIs, for the agent cediranib, P-gp and Bcrp1 do not appear to exert a synergistic effect on it brain penetration, with only P-gp playing a role in the transport of cediranib across BBB [164].

Inhibitory Effect of TKIs on ABC Transporters – Potential Chemosensitizers?

While numerous TKIs have been reported to be substrates of ABC transporters, many of them have also been found to be inhibitors of these transporters at the same time [137, 138, 152, 165–169]. For example, imatinib, a substrate of P-gp and BCRP at low concentration, can efficiently inhibit the function of these transporters at high concentration and has been shown to sensitize resistant cells against co-administered conventional cytotoxic agents that are substrates of these transporters, including mitoxantrone, vincristine, topotecan and SN-38 [134, 170]. Similar to imatinib, both nilotinib and dasatinib have been shown to interact with P-gp and BCRP as substrates within narrow concentration ranges and as inhibitors at higher concentrations [137, 138]. For these BCR-ABL TKIs, their effect on ABC transporters are concentration-dependent, whether they behave as substrate or inhibitor will predominate in the *in vivo* system could depend on location: in the gastrointestinal tract where the concentrations are usually high, it is very likely that the role of inhibitor predominates; while in the tumor cells or in brain where the concentrations may be low, the substrate phenotype could prevail. Among these three BCR-ABL TKIs, Dohse *et al.* reported that nilotinib is a more potent P-gp and BCRP inhibitor in *ex vivo* and *in vitro* models than imatinib and dasatinib [138]. Unlike imatinib, nilotinib and dasatinib, which all have biphasic effects on ABC transporters, bosutinib, a newly developed BCR-ABL tyrosine kinase inhibitor currently in clinical trials, was recently found to be an inhibitor, but not a substrate of Pgp or BCRP [137].

Similar to BCR-ABL TKIs, many EGFR TKIs, including gefitinib, erlotinib, and lapatinib, which are all dual substrates

Table I Interaction of P-gp and BCRP with TKIs

TKI / Approval Year	Indications*	Target	P-gp**		BCRP**		Pgp/BCRP Synergy for TKI brain penetration	Structure	Ref.
			Substrate	Inhibitor	Substrate	Inhibitor			
Imatinib (Gleevec®) 2001	CML GIST ALL	BCR-ABL PDGFR	+++	+++	+++	+++	Yes		(31, 133, 138, 146, 184, 185)
Gefitinib (Iressa®) 2003	NSCLC	EGFR	+++	NA	+++	+++	Yes		(30, 141, 153, 171, 186)
Erlotinib (Tarceva®) 2004	NSCLC PC	EGFR	+++	+++	+++	+++	Yes		(142, 154, 155, 187)
Sorafenib (Nexavar®) 2005	HCC RCC	RAF VEGFR PDGFR	×	NA	+++	NA	Yes		(159, 160, 163)
Dasatinib (Sprycel®) 2006	CML ALL	BCR-ABL	+++	+	+++	++	Yes		(148-150, 160)
Sunitinib (Sutent®) 2006	PC RCC GIST	c-KIT; FLT-3 PDGFR VEGFR2 RET	+++	+++	+++	+++	Yes		(160, 168, 174, 188)
Lapatinib (Tykerb®) 2007	BC	EGFR HER2	+++	+++	+++	+++	Yes		(156, 167, 173)

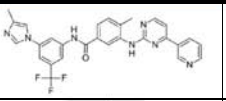
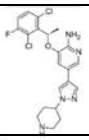
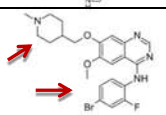
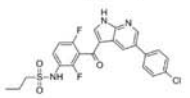
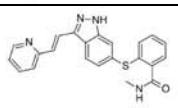
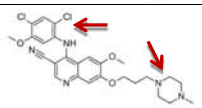
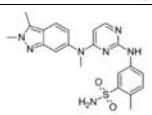
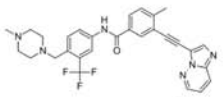
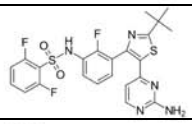
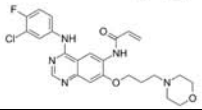
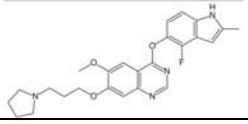
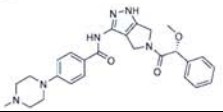
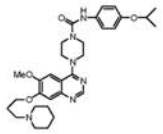
TKI / Approval Year	Indications*	Target	P-gp**		BCRP**		Pgp/BCRP Synergy for TKI brain penetration	Structure	Ref.
			Substrate	Inhibitor	Substrate	Inhibitor			
Nilotinib (Tasigna®) 2007	CML GIST	BCR-ABL c-Kit PDGFR	+	+++	+++	+++	NA		(137, 166, 180, 189)
Crizotinib (Xalkori®) 2011	NSCLC	ALK c-MET	+++	+++	×	×	Yes		(176, 190)
Vandetanib (Caprelsa®) 2011	ThyC NSCLC	VEGFR EGFR	×	NA	+++	+++	Yes		(162, 177)
Vemurafenib (Zelboraf®) 2011	Melanoma	B-Raf	+++	+	+++	+++	Yes		(175, 191, 192)
Axitinib (Inlyta®) 2012	RCC	VEGFR PDGFR c-KIT	+++	+++	+	+++	Yes		(161, 169)
Bosutinib (Bosulif®) 2012	CML	BCR-ABL	×	+++	×	+++	NA		(137)
Pazopanib (Votrient®) 2012	RCC	VEGFR PDGFR c-Kit	++	NA	+++	NA	Yes		(142)

Table I (continued)

TKI / Approval Year	Indications*	Target	P-gp**		BCRP**		Pgp/BCRP Synergy for TKI brain penetration	Structure	Ref.
			Substrate	Inhibitor	Substrate	Inhibitor			
Ponatinib (Iclusig®) 2012	CML ALL	BCR-ABL FLT3	+++	++	+++	+++	NA		(152)
Dabrafenib (Tafinlar®) 2013	Melanoma	BRAF	+++	NA	+++	NA	Yes		(193)
Canertinib (CI-1033 or PD183805)	NA	EGFR	NA	+++	+++	+++	NA		(142, 178)
Cediranib (Recentin®)	NA	VEGFR PDGFR, c-Kit	+++	+++	+++	×	No		(164, 179)
Danusertib (PHA-739358)	CML NSCLC	BCR-ABL Aurora Kinase	NA	NA	+++	NA	NA		(139)
Tandutinib (MLN518)	NA	FLT3, c-Kit PDGFR	+++	NA	+++	NA	Yes		Yang JJ et al DML2010

Regarding the inhibitor characterizations of TKIs, the evaluation is mainly based upon literature reported IC50 values. With regard to the substrate characterization, evaluations were based upon documented efflux ratios (*i.e.* B-to-A/A-to-B ratio obtained from bi-directional transport studies). For several TKIs, their IC50 values and/or efflux ratios have not been reported yet. In those cases the evaluation was made based upon the concentrations assessed in the available studies and the evaluation and conclusion(s) of the authors of these reports

**Abbreviations*; *RCC* (Renal cell carcinoma), *CML* (Chronic myelogenous leukemia), *ALL* (Acute lymphoblastic leukemia), *HCC* (Hepatocellular carcinoma), *PC* (Pancreatic carcinoma), *GIST* (Gastrointestinal stromal tumor), *NSCLC* (Non-small-cell lung carcinoma), *BC* (Breast carcinoma), *ThyC* (Thyroid carcinoma)

***Symbol*; +++ (Good substrate or inhibitor), ++ (Moderate substrate or inhibitor), + (Weak substrate or inhibitor), × (Not substrate or inhibitor)

of Pgp and BCRP, were also found to be inhibitors of these ABC transporters [135]. For example, gefitinib reversed SN-38 resistance in BCRP-transduced leukemia cells, but not in those parental cells [165]. Yanase *et al.* reported that gefitinib increased intracellular accumulation of topotecan in K562/BCRP cells, and P388/BCRP transplanted mice treated with the combination of irinotecan and gefitinib survived significantly longer than those treated with irinotecan alone or gefitinib alone [165]. In addition, gefitinib was also reported to significantly enhance the bioavailability of oral irinotecan by 4-fold in pediatric patients with refractory solid tumors [171]. As with gefitinib, erlotinib reversed resistance to vincristine and paclitaxel in P-gp-overexpressing cancer cells as well as resistance to mitoxantrone and SN-38 in BCRP-overexpressing cancer cells [172]. Similarly, lapatinib enhanced the cytotoxic effect of conventional chemotherapeutic drugs that are substrates of P-gp or BCRP in cancer cells expressing these transporters [167, 173]. In addition, the inhibitory effect of lapatinib was found to be more potent against BCRP than P-gp (IC_{50} values of 0.025 and 3.9 μM, respectively) [157].

Similar to the BCR-ABL and EGFR TKIs, the VEGFR tyrosine kinase inhibitor sunitinib has also been reported to be an inhibitor of both P-gp and BCRP, and at low micromolar concentrations it was able to reverse cancer drug resistance mediated by these transporters *in vitro* [168, 174]. The IC_{50} of sunitinib for inhibition of IAAP binding was 14.2 and 1.33 μM for P-gp and BCRP, respectively [168]. Similarly, axitinib produced inhibition of P-gp-mediated transport of digoxin and BCRP-mediated transport of topotecan with IC_{50} values of 3 μM and 4.4 μM, respectively [169].

In addition to the TKIs mentioned above, many other newly developed TKIs, including vemurafenib [175], crizotinib [176], vandetanib [177], canertinib [178], cediranib [179], and ponatinib [152], have also been reported to be not only substrates but also inhibitors of P-gp and/or BCRP

and can reverse transporter mediated drug resistance in various cancer cells *in vitro*.

The combination of small molecule TKIs with conventional chemotherapeutic drugs has been commonly employed in the clinic based on the rationale that they have different anticancer mechanisms of action. The findings that most TKIs have inhibitory effects on ABC transporters add more validity to the use of combination therapy since most conventional chemotherapeutic drugs are known to be ABC transporter substrates. When used in combination, TKIs not only exert anticancer effects but also act as chemosensitizers to reverse ABC transporter-mediated resistance against co-administered conventional anticancer drugs, thus improving overall treatment outcomes synergistically. The synergistic interaction between TKIs and conventional chemotherapeutic agents has been observed in numerous studies conducted either *in vitro* utilizing ABC transporter expressing cancer cell lines or *in vivo* in murine xenograft tumor models [29, 135, 173, 180, 181]. In addition to combination therapy of a TKI with a conventional cytotoxic drug, combined use of different TKIs has also been investigated by several research groups and may be regarded as an additional strategy to enhance anticancer effects [182–184]. For example, Hiwase *et al.* reported that nilotinib increased the intracellular concentration of dasatinib in CML cells through P-gp inhibition and the authors proposed that a combination of low dose dasatinib and nilotinib may provide an additive/synergistic anti-leukemic effect in P-gp-expressing leukemia cells that are refractory to tyrosine kinase therapy. Results from an investigation by Weisberg *et al.* demonstrated that imatinib increased the intracellular concentration of nilotinib through inhibition of ABC transporters, which may explain the observed synergy between these two TKIs. It should be noted that while combination therapy with TKIs and conventional agents (or among TKIs themselves) seems to synergize anticancer effects, improve tumor response and overall survival, these combinations may also hold the potential to increase the toxicity of administered conventional anticancer drugs due to their elevated systemic and tissue concentrations, particularly in those normal tissues expressing high levels of these ABC transporters.

Structure Activity Relationship of the Interaction Between TKIs and ABC Transporters

To our knowledge, there are no comprehensive research reports describing Structure Activity Relationships (SAR) between TKIs and ABC transporters. As most TKIs are found to be inhibitors and/or substrates of ABC transporters, it is difficult to establish the structure activity relationship. Based on our preliminary and somewhat cursory analysis, we identified some structural similarities of those TKIs that are not transported by P-gp (indicated by red arrows in Table 1). The backbone structures of these non-P-gp substrate TKIs are noted to be flanked by secondary amines with halogen substitutions on phenyl groups on one end of the molecule, and by nitrogen-containing heterocycles with N-methylation on the other. Therefore, TKIs with these common structural features may be less likely to be transported by P-gp and correspondingly, will have less MDR efflux potential. No structure similarity was observed for those TKIs that are inhibitors of ABC transporters. In order to produce a more robust prediction of the interaction between TKI and ABC transporters, further quantitative structure activity relationship (QSAR) analysis would be required. For TKIs that are efflux transporter inhibitors, their IC_{50} values can be used to quantitatively evaluate their inhibitory potency. For TKIs that are substrates of efflux transporters, their efflux ratio (*i.e.* B-to-A/A-to-B ratio obtained from those bi-directional transport studies) can be used to quantitatively evaluate their transport activity. It should be noted that for those TKIs that are inhibitors of ABC transporters, IC_{50} values have not been examined for many of them. Similarly, efflux ratio values are unavailable for many TKIs. Thus, to have a robust QSAR analysis, further experiments are needed to obtain IC_{50} and efflux ratio values that haven't been reported.

CONCLUSIONS

Small molecule TKIs represent a novel class of anticancer drugs that attack cancer-specific targets and have a generally more favorable safety and tolerability profiles relative to conventional cytotoxic agents. However, although TKIs have better selectivity and fewer side effects, they share several limitations with conventional chemotherapeutic drugs, including drug resistance and high inter-individual pharmacokinetic variability. ABC transporters, particularly P-gp and BCRP, have been found to play important roles in treatment resistance and inter-individual variability. For small molecule TKIs that are actively transported by ABC transporters, recent investigations suggest that the co-administration of non-toxic ABC transporter inhibitors may overcome TKI resistance and improve TKI bioavailability. In addition, ABC transporters appear to play a crucial role in the brain penetration of TKIs, and numerous studies have confirmed that P-gp and BCRP work in a synergistic fashion to restrict the brain penetration of TKIs. Additionally, the co-administration of a dual inhibitor (*i.e.* P-gp and BCRP) with a TKI may represent a promising new strategy in increasing brain delivery of TKIs and enhancing their efficacy in the treatment of brain tumors. As many TKIs interact with ABC transporters as both substrates and inhibitors, combination therapies of TKIs with conventional cytotoxic agents or other TKI's that are ABC transporter substrates may enhance their anticancer effect through ABC

transporter-mediated pharmacodynamic interactions in the cancer cells. A broadening in the understanding of the role(s) of ABC transporters in MDR and ADME-Tox of small molecule TKIs will ultimately prove helpful to the clinician tasked with TKI dose optimization balanced with patient safety. Finally, it is important to delineate interactions of ABC transporters with currently approved TKIs with regard to MDR efflux and ADME-Tox so as to provide guidance in the future design of TKI molecules which can overcome or circumvent these impediments to more successful chemotherapy.

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