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The controversial role of ABC transporters in clinical oncology

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Abstract

The phenomenon of multidrug resistance in cancer is often associated with the overexpression of the ABC (ATP-binding cassette) transporters Pgp (P-glycoprotein) (ABCB1), MRP1 (multidrug resistance-associated protein 1) (ABCC1) and ABCG2 [BCRP (breast cancer resistance protein)]. Since the discovery of Pgp over 35 years ago, studies have convincingly linked ABC transporter expression to poor outcome in several cancer types, leading to the development of transporter inhibitors. Three generations of inhibitors later, we are still no closer to validating the 'Pgp hypothesis', the idea that increased chemotherapy efficacy can be achieved by inhibition of transporter-mediated efflux. In this chapter, we highlight the difficulties and past failures encountered in the development of clinical inhibitors of ABC transporters. We discuss the challenges that remain in our effort to exploit decades of work on ABC transporters in oncology. In learning from past mistakes, it is hoped that ABC transporters can be developed as targets for clinical intervention.

Introduction

Despite recent developments in anticancer drug discovery, various obstacles hinder successful cancer treatment. One such complication is the phenomenon of multidrug resistance (MDR), which is similar to the well-studied occurrence of antibiotic resistance in micro-organisms. Cellular resistance can be linked to the original genetic make-up of cancer cells, but may also develop in response to exposure to anticancer agents during treatment.

One intensively studied mechanism of MDR relies on the efflux of cytotoxic drugs from cancer cells by ABC (ATP-binding cassette) transporters. ABC transporters are energy-dependent transporters that normally function in the detoxification and protection of normal cells from xenobiotics. The substrates of ABC transporters include a wide range of structurally unrelated compounds that include numerous anticancer drugs. Although there are a number of ABC transporters that have been identified as potential transporters of anticancer drugs, three have received the most attention in the laboratory and in clinical oncology: Pgp (P-glycoprotein) (ABCB1/MDR1), MRP1 (multidrug-resistance protein 1) (ABCC1) and ABCG2 [BCRP (breast cancer resistance protein)/MXR (mitoxantrone-resistance protein)]. A model of Pgp based on the elucidated crystal structure [1] is shown in

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Figure 1. Whereas these transporters have been shown to confer resistance in *in vitro* and *in vivo* model systems, proof that they are responsible for a significant fraction of drug resistance in clinical oncology is lacking. The prevailing strategy used over the last decade to evaluate the contribution of the transporters to clinical drug resistance has been to investigate the efficacy of anticancer therapy in combination with ABC transporter inhibitors. Three generations of ABC transporter inhibitors have been developed and tested for clinical application. Unfortunately, these clinical trials have had minimal success. It is important to examine and understand the failure of these trials if ABC transporters are to be developed as therapeutic targets in clinical oncology.

Localization and expression

ABC transporters are endogenously expressed in a wide range of human tissues. Both Pgp and ABCG2 appear to play a major role in cellular protection from cytotoxins and xenobiotics. Pgp and ABCG2 are highly expressed in pharmacological barriers, including the brain microvessel endothelium, syncytiotrophoblasts of the placental chorionic villus, interstitial cells of the testes and haemopoietic stem cells in the bone marrow [2,3]. In addition to these barrier sites, Pgp is expressed on the canalicular surface of hepatocytes in the liver, in the epithelial cells of the proximal convoluted tubule in the kidney, the apical surface of gastrointestinal epithelial cells, the cortex and medulla of the adrenal glands, myoepithelium and in cells of the immune system [4]. ABCG2 is localized in the hepatocytes of the liver, zona reticularis layer of the adrenal glands, alveolar pneumocytes of the lung, prostate epithelium, uterine endocervical cells, cortical tubules of the kidney, islet and acinar cells in the pancreas, epithelial cells of the gastrointestinal tract and ducts and lobules of the mammary glands [5–7]. MRP1 is known to transport metabolic by-products, including glucuronide, glutathione and sulfate conjugates [8]. Key localizations of MRP1 also suggest protection at blood-normal tissue barriers, including testicular tubules, the choroid plexus, where it contributes to the blood-CSF (cerebrospinal fluid) barrier, and in bone marrow precursor cells [9,10].

In addition to their role in normal physiology, ABC transporters are highly expressed in multiple tumour types. In breast cancer, sarcoma and certain leukaemias, increased expression of Pgp was observed in recurrent or relapsed disease compared with expression at diagnosis [11]. In AML (acute myelogenous leukaemia), approximately 50% of clinical samples show Pgp expression with increasing levels in recurrent leukaemia cells, and expression has been repeatedly linked with poor outcome [12–16]. Although still under debate, numerous studies have reported increased Pgp expression following chemotherapy in tumours of the breast, ovaries, bladder, CNS (central nervous system) and cervix [2]. Pgp expression in these cancers is generally correlated with poor clinical outcome [14,17–19].

Pgp is by far the best characterized among the three ABC transporters, whereas MRP1 and other members of the ABC family are less well studied. MRP1 has not been found to be a significant factor in drug resistance in AML [12,20]. Its prognostic value in CLL (chronic lymphocytic leukaemia), lung cancer and breast cancer remains indeterminate [21–24]. To date, few studies have shown expression changes in MRP1 following treatment or have correlated expression with clinical outcome.

Association of ABCG2 with clinical outcome has also been inconclusive. Data concerning the expression of ABCG2 in AML are inconsistent, with high levels of expression being reported in some studies and lower levels in others [25–28]. The association of ABCG2 with response in AML is also debated, although some studies conclude that higher ABCG2 expression is associated with poor response to chemotherapy [15,29–31]. Notably, co-expression of Pgp and ABCG2 were linked with a lower complete response rate, worse event-free survival and worse overall survival in three studies of patients with AML[14,20]. In a study that did associate Pgp with poor outcome in breast cancer, there was no significant impact of ABCG2 expression [17]. In lung cancer, analysis of biopsy specimens from NSCLC (non-small-cell lung carcinoma) treated with cisplatin-based chemotherapy found that ABCG2 expression was associated with shorter survival, although there was no impact on response rate [32]. The same group found that ABCG2 expression in SCLC impaired response and progression free survival during platinum-based treatment [33]. Both associations were found despite the fact that platinum is not a substrate for transport.

Data such as those obtained from the lung cancer studies raise the possibility that other explanations may exist for the poor clinical outcome linked with transporter overexpression. One hypothesis is that cancer stem cells, existing as a separate and identifiable compartment of the tumour, are responsible for drug resistance. The model predicts a small subpopulation of drug-resistant pluripotent cells that is long-lived, quiescent, evades initial treatment and leads to the relapse of a drug-resistant tumour [34]. In this model, ABCG2, and Pgp in some cases, serves only as a marker for the stem cell and is responsible for the Hoechst-dim ‘side population’ phenotype, serving as a drug-resistance mechanism only for those stem cells. The stem cells are then responsible for repopulating a tumour following therapy, and drug resistance is due to this repopulation. Increasing numbers of these cells in a tumour could be linked with a poor outcome unrelated to the ability to extrude chemotherapy. Although the stem cell model is as yet unproven, it has led to the documentation of functional ABCG2 in putative cancer stem cells.

Substrates and inhibitors of ABC transporters

ABC transporter substrates include a diverse array of compounds, many of them structurally unrelated. In general, Pgp transports large hydrophobic compounds, whereas MRP1 and ABCG2 transport both hydrophobic drugs and large anionic compounds [3]. This wide range of substrates originally led to speculation that the transporters could be responsible for significant MDR in cancer cells. The list of substrates is striking not only in the number of anticancer agents, but also in the number of non-oncologic compounds. This highlights the potential role of ABC transporters in protection against xenobiotics and in pharmacology. A comprehensive but not exhaustive list of ABC transporter substrates found among anticancer drugs is provided in Table 1. This chapter focuses on the three transporters most studied clinically: Pgp, MRP1 and ABCG2. It is entirely possible that other ABC transporters may be clinically relevant, but the association has yet to be discovered. Transporters in the ABCC subfamily, for example, generally efflux methotrexate and other anionic substrates such as SN-38 [2], but clinical information is lacking.

Attempts to overcome MDR by preventing anticancer drug efflux have led to the development of a number of ABC transporter inhibitors. It has not been difficult to identify inhibitors due to the broad range of compounds that interact with the transporters; a partial list is provided in Table 2.

Clinical trials

It was hypothesized that inhibition of ABC drug transporter activity during cancer therapy could sensitize drug-resistant tumours and/or improve the initial activity of anticancer agents. Laboratory models showed promise for the clinical application of Pgp inhibitors to circumvent drug resistance. Unfortunately, translation of this knowledge to clinical application was unexpectedly difficult. Oncology drugs are generally developed in three steps, termed phases, which delineate how far along the drugs are in clinical development. Earliest trials, Phase I studies, are dose-finding studies. The goal is to define a safe and potentially effective dose to take to the next phase. Typically, these trials are carried out in a standardized sequential dose escalation until a pre-defined level of toxicity is observed that suggests that further escalation would be harmful to patients. Phase II studies seek to find some sign of efficacy in a given patient population. Phase III studies then compare two treatments. In drug-resistance reversal trials, where a modulator of resistance is being tested, it is critical that the activity of the modulator be tested in a Phase III trial design. Given that modulators seldom have intrinsic activity, a modulator is studied in combination with an anticancer agent that has been proven safe in the Phase I or II setting and then is compared in the Phase III setting against the anticancer agent alone. As described below, many Phase II trials of modulators were considered promising and then the results not borne out in Phase III trials. A list of clinical trials examining ABC transporter inhibitors is provided in Table 3. These inhibitors have been classified into three generations: the first indicating compounds that were already U.S. FDA (Food and Drug Administration)-approved for other medical uses; the second comprising compounds developed specifically as Pgp inhibitors, some with notable pharmacokinetic interactions; and the third including compounds intentionally developed, but lacking major pharmacokinetic interactions [2].

First generation

The first-generation MDR inhibitors included verapamil, quinidine and cyclosporin A. Clinical trials generally found these drugs ineffective and/or toxic at doses required to inhibit ABC transporter function [35]. However, several promising trials generated optimism for the use of ABC transporter inhibitors in clinical oncology. For example, quinine was shown to increase complete remission and survival rates in Pgp-positive myelodysplastic syndromes treated with intensive chemotherapy [36]. Also, cyclosporin A combined with daunorubicin and cytarabine in patients with poor risk AML revealed a statistically significant improvement in overall survival during a 2-year follow-up from the Phase III clinical trials [37]. Addition of dexverapamil to EPOCH chemotherapy (a regimen comprising etoposide, doxorubicin, vincristine, cyclophosphamide and prednisone) in a cross-over design resulted in an increased response rate in lymphoma patients [38]. These findings motivated investigators to develop more potent inhibitors of drug efflux. Interestingly, limited confirmation of the cyclosporin A trial was reported in two sequential Phase II trials in

which the addition of cyclosporin A in the second trial again improved relapse-free survival [39].

Second generation

The second generation of inhibitors was developed to improve potency over the first-generation inhibitors. The most developed inhibitor was valspodar (PSC-833), a 10–20-fold more potent analogue of cyclosporine D [40,41]. Although valspodar fulfilled the requirement for a higher-affinity non-toxic Pgp inhibitor, the drug presented unanticipated pharmacokinetic interactions. Through concurrent inhibition of CYP3A4 (cytochrome P450 3A4), valspodar interfered with drug metabolism and elimination, thus increasing anticancer drug exposure [42,43]. The Pgp drug-binding site is similar to the drug-binding site on CYP3A4, and many anticancer agents are substrates for Pgp and also CYP3A4. Valspodar reduced CYP3A4-mediated intestinal or liver metabolism of the anticancer agents at the same time that it blocked Pgp-mediated efflux from cancer cells and normal tissues. This resulted in reduced drug metabolism that elevated drug exposure, and increased the severity and incidence of adverse effects associated with the anticancer therapy. These were often bone marrow toxicities, including neutropenia and thrombocytopenia that could be attributed to increased AUCs (areas under the concentration curves) for a chemotherapeutic agent. In order to accommodate this pharmacokinetic interaction in valspodar trials, anticancer drug doses were reduced by 25–50% [44,45]. However, because of interpatient variation in CYP3A4 metabolism, some patients were underdosed and others overdosed [46,47]. A Phase III CALGB (Cancer and Leukemia Group B) trial using valspodar in previously untreated AML patients over age 60 was closed early due to excessive mortality in the experimental arm during induction [45]. Despite this problematic result, a subset of patients with detectable leukaemic cell drug efflux had a statistically significant improvement in complete remission rates and a trend towards an improved disease-free survival [45]. Another interesting result was seen in a study with AML patients under the age of 60. The trial observed that the addition of valspodar to daunorubicin, etoposide and cytarabine showed an advantage in disease-free and overall survival in a subset of patients 45 years old or younger [48]. Unfortunately, the result has not been duplicated, and development of valspodar has since been discontinued.

Similar findings were obtained with VX-710 (Biricodar, Incel™), which has the ability to inhibit Pgp, MRP1 and ABCG2 [49]. Responses were observed in trials with sarcoma and ovarian cancer, but because of the non-randomized design, it was not possible to determine their significance [17,50,51]. Development of Biricodar also appears to have been discontinued.

Third generation

The third-generation compounds were better agents: potent, non-toxic and with minimal pharmacokinetic interaction. These new inhibitors include tariquidar (XR9576), zosuquidar (LY335979), laniquidar (R101933) and CBT-1.

Tariquidar, as well as the second-generation inhibitor elacridar, have the added benefit of extended Pgp and ABCG2 inhibition [52,53]. A Phase I study demonstrated that

administration of elacridar combined with oral topotecan resulted in complete apparent oral bioavailability of topotecan [54]. Flow cytometry studies using rhodamine 123, a fluorescent substrate of Pgp, showed that the Pgp and ABCG2 antagonist tariquidar is able to inhibit Pgp-mediated rhodamine efflux for up to 48 hours after a single dose [55]. Phase I studies of tariquidar in combination with vinorelbine, paclitaxel or doxorubicin showed no significant side effects or pharmacokinetic interactions [56]. However, two large Phase III trials with tariquidar closed early due to toxicity. Both trials combined tariquidar with first-line chemotherapy for patients with NSCLC [57,58].

Zosuquidar is one of the most potent Pgp inhibitors in development. It has been evaluated in patients with AML, where zosuquidar is able to completely inhibit Pgp function, and results are awaited in these trials [59]. A previous clinical study showed a 75% response rate among 16 patients receiving zosuquidar in combination with daunorubicin and cytarabine for AML [60]. A Phase I/II trial demonstrated that administration of zosuquidar with standard chemotherapy in patients with untreated non-Hodgkin's lymphoma had little effect on the pharmacokinetics of the anticancer drugs [61]. A similar Phase II trial tested the effects of docetaxel with zosuquidar administration in breast cancer patients. Although the trial found no significant difference in progression-free survival, overall survival or response rate, the treatment regimens were found to be safe [62].

Additional third-generation inhibitors include laniquidar and CBT-1. Laniquidar has shown promise as a potent orally active MDR inhibitor with no observed pharmacokinetic interactions. A Phase II study of laniquidar in combination with docetaxel or paclitaxel in refractory breast cancer has been conducted, but results have not yet been reported [63]. Pre-clinical studies examining CBT-1 have affirmed the drug's ability to inhibit Pgp function at low concentrations [64]. Phase I trials testing CBT-1 with paclitaxel or doxorubicin have been completed, and the agent is now in Phase II and III trials in patients with NSCLC [65]. The initial Phase I studies demonstrated that CBT-1 had no effect on the pharmacokinetics of doxorubicin or paclitaxel [66].

Inhibitor trials revisited

To date, clinical trials using ABC transporter inhibitors have not met the expectations of the scientific community. Whereas the negative results may be explained by several factors, such as the effect of the inhibitors on pharmacokinetics, it is also possible that the hypothesis that ABC transporter inhibition will increase drug accumulation in tumours and thereby efficacy is simply incorrect. It will be difficult to be certain which conclusion is correct without further clinical and translational work. Some of the flaws in earlier trials that support the first conclusion are as follows.

- The initial enthusiasm for the Pgp hypothesis led to a large number of trials and a rapid loss of optimism when these trials were unable to achieve the magnitude of benefit anticipated based on *in vitro* models. More realistic expectations may have allowed identification of a subset of patients with true benefit.
- Clinical trials were guided by highly drug-resistant intraperitoneal murine tumour models. A recent study using a hereditary breast cancer mouse model

showed that modest increases in Pgp were sufficient to cause resistance to doxorubicin [67], and to the PARP [poly(ADP-ribose) polymerase] inhibitor olaparib (AZD2281) [68]. Increased sensitivity to both drugs was shown with the addition of tariquidar. Better pre-clinical models could have aided in the selection of appropriate drug and inhibitor combinations.

- A clinically validated assay for Pgp or other ABC transporters has never been established. This meant that correlative studies detecting Pgp in tumour tissue were not conducted in a standardized fashion. Since no definitive guidelines for clinical analysis existed, clinical trials differed in assay methodology, which resulted in confusing data that could not be used to interpret clinical trial results, or compared across institutions.
- Patients were not selected based on tumour expression of Pgp. To conduct a trial with power to determine the true impact of ABC transporter inhibitors in MDR, it is crucial to select the subset of patients whose tumours express ABC transporters as a dominant mechanism of resistance. For example, a Phase III clinical trial used tariquidar in patients with NSCLC, despite a lack of evidence suggesting that NSCLC expresses Pgp to a significant extent [57]. Much like trastuzumab for HER2-overexpressing breast cancers, imatinib in CML (chronic myeloid leukaemia) and erlotinib for patients with lung cancers containing epidermal growth factor receptor mutations, it is not likely that a targeted therapy will succeed without presence of the target.

A corollary to the inadequate Pgp detection methods is that other transporters, both uptake and efflux, were not assessed. A diagnostic imaging test would allow identification of tumours in which Pgp was a dominant factor in drug accumulation [69]. In many tumours, ABC transporters other than Pgp are likely to be equally important in reducing drug accumulation. It is now understood that there is a large family of uptake transporters that also determine drug accumulation. The relative importance of uptake compared with efflux transporters is not known, but is likely to vary among tumours or even across degrees of differentiation. Only a tumour in which Pgp is a dominant mechanism of resistance would be expected to be sensitive to modulation.

The earlier trials did not confirm that the Pgp inhibitor under question was actually able to inhibit the ABC transporter *in vivo.* In time, *ex vivo* assays confirming Pgp inhibition by second and third-generation inhibitors in $CD56^+$ circulating mononuclear cells were developed [70]. The radionuclide imaging agent [^{99m}Tc]sestamibi was identified as a Pgp substrate and was shown in patients to increase in tissues and tumours known to express high levels of Pgp, when administered in the presence of a Pgp inhibitor [56,71].

Most of the trials were conducted with ‘home-run’ Phase II designs in patients whose tumours did not necessarily overexpress ABC transporter, and in which it was not known whether or not an ABC transporter was a dominant mechanism of resistance. The hope was that benefit would be substantial and obvious; thus this design did not allow determination of the benefit of adding an inhibitor to the treatment regimen. Randomized studies were

needed, but those that came later again failed to select patients where the dominant mechanism of resistance was transporter-mediated.

Thus the authors would conclude that the hypothesis that Pgp mediates drug resistance was never adequately tested in the clinic. The failure to document the expression of Pgp in tumours, that it conferred resistance and that the inhibitors reached the tumour to block efflux and increase drug accumulation all suggest that the negative results from the clinical trials were flawed. Indeed, pharmaceutical companies are sufficiently wary of drug transporter-mediated drug resistance that compounds are often optimized during development to make them poorer substrates for transport.

However, it is also possible that Pgp inhibitors cannot increase drug uptake in tumours. Like anticancer drugs, inhibitors access solid tumours via blood vessels and must penetrate tumour tissue to reach all cancer cells. A recent study in a xenograft model demonstrated that Pgp inhibitors increase uptake of doxorubicin only in cells close to blood vessels and have little effect on drug uptake at intermediate distances [72]. Pgp inhibition may not have a significant impact if there is impaired permeability and drug diffusion, particularly in solid tumours. Imaging studies to evaluate drug uptake in tumours are critically needed to answer this question.

A third possibility is that Pgp, or other ABC transporter inhibitors that reside in bone marrow cells, will not succeed in the clinic because of the lack of a therapeutic window. To the extent that transporters protect normal bone marrow cells, normal tissue sanctuaries, and are involved in drug excretion, effective inhibitors have the potential to block these vital roles and increase toxicity to patients. It has been felt that a therapeutic window would exist because transporter levels are lower in tumours than in normal cells. Further, Pgp-knockout models have generally shown only a modest impact on blood levels of cancer chemotherapeutics, presumably related to complex and redundant metabolic pathways. To date, clinical trials have suggested that a therapeutic window does exist. But this question will remain inconclusive as long as clinically effective ABC transporter inhibition has not been achieved.

Implications from pharmacology

ABC transporters have emerged as an important variable in pharmacology and drug distribution. A number of SNPs (single nucleotide polymorphisms) have been identified that may contribute to inter-individual variation in drug metabolism. It is possible that some of the increased toxicity observed in the earlier trials occurred in patients with polymorphic ABC transporters with altered folding and impaired function or lower expression levels, described for certain ABC transporter variants [73–78]. A patient whose genetics constrain expression or function of Pgp or ABCG2 would not be expected to benefit from the addition of a transport inhibitor since both normal tissues and tumours would be affected. The data showing that, in the trial with valspodar mentioned earlier, patients with efflux-positive leukaemia did not have high mortality and actually had some evidence of treatment benefit support this hypothesis [45]. Understanding the impact of inter-individual variation in pharmacogenomics could clarify patient selection in future clinical trials.

Non-traditional applications of ABC transporters as targets

As a component of the blood-brain barrier and the gastrointestinal epithelium, the clinical applications of ABC transporter inhibitors can be expanded to trials examining ways to increase CNS uptake of non-toxic drugs and improve oral drug bioavailability. This is of increased importance in diseases such as breast, lung and renal cancer, where newer targeted therapies are increasing systemic control, and more CNS metastases are emerging [79]. TKIs (tyrosine kinase inhibitors), such as erlotinib, lapatinib and sorafenib, are effective in the systemic control of these tumours, but are also substrates for the transporters [80–84]. Remarkably, murine studies show that, whereas knockout of either transporter alone has minimal impact, deletion of the orthologues for Pgp and ABCG2 results in a 22-fold increase in relative CNS uptake of dasatinib, a 40-fold enhancement of CNS uptake of lapatinib, an 8.5-fold increase for erlotinib and a 9-fold increase for sorafenib. The ability to increase CNS penetration of these agents could thus have a major impact on decreasing the occurrence of CNS disease in solid tumours sensitive to these agents. Studies to assess accumulation of radionuclide-imaging agents in the brain are in early stages and may help to identify agents that could increase the accumulation of TKIs for patients whose systemic disease has been controlled [85–88]. The mouse knockout models also show an impact of the transporters on pharmacokinetics, although to a much lesser extent. Nonetheless, the ability of transport inhibitors to increase oral bioavailability and to equalize blood levels among patients is an area for investigation that could lead to increased access to anticancer agents, particularly where infusional therapy is either too cumbersome or too costly to administer.

Future directions

The most important question is where the field should go from here, and whether this family of proteins warrants continued investigation as potential therapeutic targets in cancer. Since Pgp and ABCG2 expression continue to be linked to poor outcome, we argue that it would be a mistake to discontinue studying the role of ABC transporters in clinical oncology. Data from clinical trials being conducted with zosuquidar in AML and with CBT-1 in NSCLC are awaited. However, negative results from these trials are still subject to many of the same caveats as the older trials, if conducted without selection of patients whose tumours have been shown to have a Pgp-mediated reduction in drug accumulation.

Whereas the list of ABC transporter substrates has steadily expanded, investigation into the implications of these compounds being substrates has waned. For example, the drugs imatinib, nilotinib and dasatinib, which are used in the treatment of CML, are both Pgp and ABCG2 substrates [89,90]. Therefore drug transporters could contribute to drug resistance in CML [91]. Mutation of the target and altered uptake are also relevant mechanisms of resistance for CML, hence the need to study the individual contribution of the different mechanisms, determine which are dominant and develop strategies to overcome resistance. Whereas resistance that manifests clinically is a problem for a relatively small subset of patients with CML in chronic phase, it is an inevitable problem for patients who receive TKIs as therapy for solid tumours. In this setting, resistance is again certain to be multifactoral, and the contribution of ABC transporters is one among several mechanisms. The sanctuary site data in murine knockout models that demonstrate marked redundancy

between Pgp and ABCG2 in limiting the CNS uptake of dasatinib, lapatinib, sorafenib, erlotinib and sunitinib suggest that together these transporters could markedly limit the uptake of the TKIs in solid tumours. Approaches that successfully increase accumulation of the TKIs in the CNS could reignite the question of whether systemic ABC transporter inhibition in solid tumours could be successful.

ABC transporters have received considerable attention since being discovered in putative cancer stem cells. Whether one accepts the idea that cancer cells and progeny divide stochastically, i.e. at random, or the revisionist hypothesis that cancer stem cells represent a unique subpopulation of cancer cells that persist and repopulate a tumour following each therapy-mediated reduction in cell number, the molecular pathways that produce the ‘sternness’ phenotype can be targeted. Inhibitors of Notch, Wnt and Hedgehog pathways could easily be ABC transporter substrates. Since putative cancer stem cells identified to date have high levels of ABCG2, it will be important to know whether inhibitors of these stem cell pathways are substrates. If the role of ABCG2 is to protect stem cells, then the stem cell pathway inhibitors need to circumvent drug efflux.

With the discovery of Pgp, MRP1 and ABCG2, each ABC transporter in turn was scrutinized as the crucial new transporter in MDR. As a result, research and literature focused on that one transporter rather than forming a cohesive study on multiple ABC transporters responsible for MDR. In order to avoid this bias, and to clarify tumour tissue expression of transporters in drug resistance, it is critical that transporters not be studied as individual entities in clinical samples. To understand mechanistically why drug accumulation may be limited, unbiased assays of known uptake and efflux transporters should be conducted within a single study, as they may function as a synergistic unit to reduce drug accumulation. Fortunately, advanced array technologies offer this possibility.

When Pgp inhibitors were introduced as a means of circumventing MDR, expectations for the inhibitors were very high. Early clinical trials were unable to meet these expectations and resulted in disappointment for many in the scientific community. Since then, changes have occurred in the standards of anticancer drugs as well as our perception of cancer therapy. For instance, cancer is more often viewed as a chronic disease and incremental improvements more acceptable. This concept has brought many new agents to the anticancer stage, and it may be worth examining transporter inhibitors in this current context. Indeed, AZD2281, a PARP inhibitor found to be active in BRCA1 (breast cancer early-onset 1)-deficient breast cancer, is also a substrate for Pgp-mediated drug resistance. A genetically engineered mouse model for BRCA1-associated breast cancer shows marked improvement in response and duration of response with the addition of tariquidar to AZD2281 [68].

However, clinical trials should not be conducted again until patients can be selected who have tumours in which resistance is dominated by ABC transporters. We lack a validated assay for detecting any of the transporters, i.e. an assay with known sensitivity, specificity and reproducibility for detection of Pgp, MRP1, ABCG2 or any of the transporters, in the clinical setting. The specificity of antibody-based assays has been a major problem; RNA assays appear too sensitive. More important is the development of a functional assay; indeed, unrelated to ABC transporters, determination of actual drug accumulation in solid

tumours has been a neglected area of cancer research. Currently, there is no test by which a treating physician can determine whether an ineffective agent is reaching the tumour or its therapeutic target. Diagnostic imaging has the potential to determine whether Pgp or other transporters are functioning to reduce drug accumulation and whether inhibition can change drug uptake in solid tumours. A number of new PET (positron-emission tomography) imaging agents that could remedy this problem are under study [85–87,92,93]. Indeed, it can be easily argued that laboratory investigations aimed at identifying transporter inhibitors should not be conducted further until a clinical assay exists to define which tumours have transporter-dominated resistance mechanisms. It has been much easier to select new inhibitors *in vitro* than it has been to show that these agents increase drug accumulation *in vivo.* This latter question should be the focus of future research in this field.

Conclusion

> “I have made some progress. But why so late and with such difficulty?”
>
> —Paul Cezanne

Progress in science, and apparently in art, is often painstakingly slow. The effort to exploit ABC transporters to reverse drug resistance in clinical oncology has been characterized by missteps that ultimately impeded our ability to answer the question of their true role in cancer. Koch’s postulates regarding the causal role of bacteria in disease initially insisted that the micro-organism should not be found in healthy animals, but was later revised when asymptomatic carriers were discovered. In the case of ABC transporters, presence of a transporter does not define that transporter as the dominant cause of drug resistance. Thirty-five years after the discovery of Pgp, we still do not know whether or in which tumours the transporter reduces anticancer drug accumulation. This is a question that needs to be answered before the field can begin to move again. Emerging understanding of the redundancy of ABC transporters in limiting drug distribution to sanctuary sites such as the CNS has provided proof-of-concept for their ability to limit drug accumulation in solid tumours. Methods must be developed to determine whether the latter is true, and whether it matters, in patients with cancer.

Note added in proof (received 19 July 2011)

Results from a randomized Phase III trial in older patients diagnosed with AML treated with cytarabine and daunorubicin with zosuquidar or placebo were reported recently [101]. The trial found that addition of zosuquidar to the treatment regimen did not significantly affect the complete response rate, outcome or progression-free survival.

Summary

- ABC transporters are overexpressed in a variety of tumour types and are associated with poor outcome in some malignancies.
- The ‘Pgp hypothesis’ conveys the notion that overexpression of Pgp or other ABC transporters confers clinical drug resistance, which could be overcome through inhibition of drug efflux mediated by the ABC transporter.

- Efforts to circumvent drug resistance through ABC transporter inhibition largely failed, with Pgp inhibitors the most intensively studied. Problems encountered during early development suggest, from one perspective, that the Pgp hypothesis was never adequately tested. It is also possible that non-transporter mechanisms limiting drug delivery also limited delivery of the inhibitors, and that these non-transporter mechanisms are more crucial to adequate drug exposure.
- Pharmacogenomic variation in ABC transporter expression or function could have made some individuals markedly more sensitive to the inhibitors, with loss of a therapeutic window.
- Normal tissue expression may provide the most relevant strategy to exploit ABC transporters as therapeutic targets. Thus inhibition of drug efflux at the blood-brain barrier may allow increased CNS uptake and retention of anticancer agents. Inhibition of efflux in the gastrointestinal tract could allow improved oral absorption of anticancer agents.
- Many novel targeted agents are substrates for ABC transporters in *in vitro* assays. It is not known whether Pgp or other ABC transporters are relevant in clinical resistance to targeted agents such as lapatinib, sorafenib, dasatinib or imatinib. These compounds are important candidates for strategies aimed at increasing CNS uptake.
- Development of imaging agents is critical to determining whether Pgp or another ABC transporter is a dominant mechanism of drug resistance, whether there is tumour heterogeneity, and whether expression might be critical in a small subset of stem-cell-like cancer cells. Imaging anticancer agents or surrogates *in vivo* has the potential to tell us whether ABC transporters are rate-limiting for drug uptake, whether they are a dominant mechanism of drug resistance and whether they should re-emerge as therapeutic targets.

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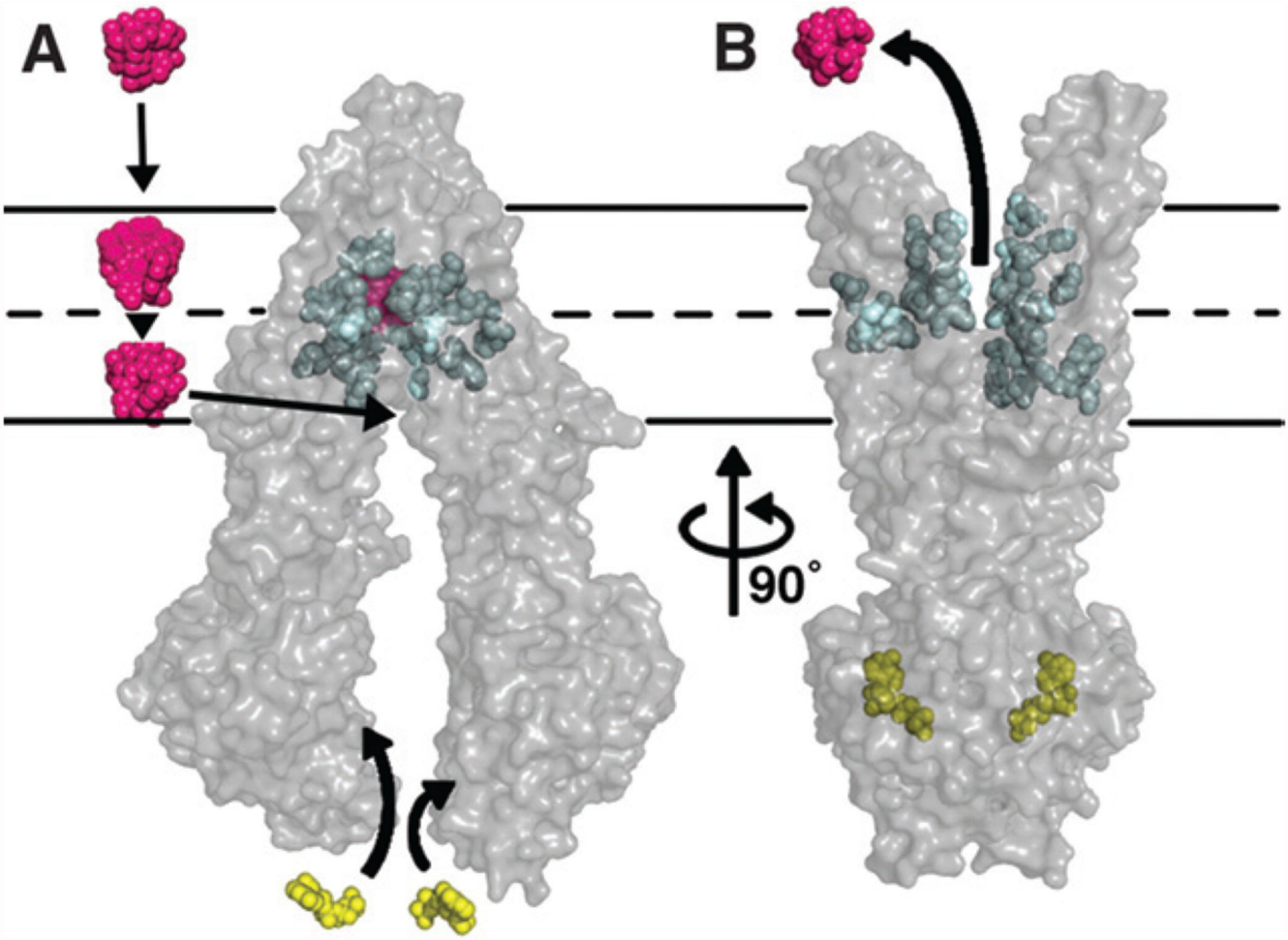

Figure 1. Substrate transport by Pgp

(**A**) The substrate, shown in magenta, enters the membrane and diffuses through a portal in the transporter. (**B**) Once in the drug-binding pocket, coloured blue, ATP, shown in yellow, binds to the nucleotide-binding domains and causes a conformational change, leaving the drug-binding pocket facing the extracellular space. ATP then binds again to reset the transporter to its original conformation shown in (**A**). From [1]: Aller, S., Yu, J., Ward, A., Weng, Y., Chittaboina, S., Zhuo, R., Harrell, P., Trinh, Y., Zhang, Q., Urbatsch, I. and Chang, G. (2009) Structure of P-glycoprotein reveals a molecular basis for poly-specific drug binding. Science **323**, 1718–1722. Reprinted with permission from AAAS.

Table 1.
Clinically relevant substrates of the MDR-related ABC transporters

Data compiled from [2,94].

Class	Substrate	ABCB1	ABCC1	ABCG2
Vinca alkaloids	Vinblastine	✓	✓	
	Vincristine	✓	✓	
	Vinorelbine	✓	✓	
Anthracyclines	Daunorubicin	✓	✓	✓
	Doxorubicin	✓	✓	✓
	Epirubicin	✓	✓	✓
Epipodophyllotoxins	Etoposide	✓	✓	✓
	Teniposide	✓		✓
Taxanes	Docetaxel	✓		
	Paclitaxel	✓		
Kinase inhibitors	Dasatinib	✓	✓	✓
	Erlotinib	✓		✓
	Gefitinib	✓		✓
	Imatinib (Gleevec)	✓	✓	✓
	Lapatinib	✓		✓
	Nilotinib	✓	✓	✓
	Sorafenib	✓		✓
	Sunitinib	✓		✓
	Vandetanib		✓	✓
Campthotecins	Irinotecan (CPT-11)	✓	✓	✓
	SN-38	✓	✓	✓
	Arsenite		✓	
	AZT			✓
	Bisantrene	✓		✓
	Cisplatin		✓	
	Colchicine	✓	✓	
	Digoxin	✓		
	Flavopiridol			✓
	Methotrexate	✓	✓	✓
	Mitoxantrone	✓		✓
	Saquinivir	✓		

Table 2.
Inhibitors of MDR-related ABC transporters

Data compiled from [2,94].

Substrate	ABCB1	ABCC1	ABCG2
Amiodarone	✓		
Cyclosporine A	✓	✓	✓
Nifedipine	✓		
Quinidine	✓	✓	
Quinine	✓	✓	
Verapamil	✓	✓	
Biricodar (VX-710)	✓	✓	✓
Dexniguldipine	✓		
Dofequidar (MS209)	✓	✓	
S9788	✓		
Valspodar (PSC-33)	✓		
CBT-I	✓	✓	
Elacridar (GF120918)	✓		✓
Laniquidar (Rl01 933)	✓		
LY475776	✓	✓	
ONT-093	✓		
Tariquidar (XR-9576)	✓		✓
Zosuquidar (LY335979)	✓		
Curcumin		✓	✓
Flavonoids		✓	
FTC			✓
Mitotane	✓		
MK57I		✓	

Table 3.

Phase III drug resistance inhibitor trials

ADE, cytarabine, daunorubicin and etoposide; MDS, myelodysplastic syndrome; OS, overall survival; PFS, progression-free survival; RAEB-t, refractory anaemia with excess blasts in transformation; VAD, vincristine, adriamycin and dexamethasone.

Generation	Modulator	Cancer type	Anticancer drug(s)	Outcome	Reference
First	Cyclosporin	Relapsed and refractory AML	ADE	No benefit	[95]
	Cyclosporin	Poor risk AML, RAEB-t	Daunorubicin, cytarabine	Improved OS in cyclosporin A group	[37]
	Cyclosporin	Myeloma	VAD	No benefit	[96]
Second	Valspodar (PSC-833)	AML	Daunorubicin, cytarabine	No benefit	[97]
	Valspodar (PSC-833)	Untreated AML	ADE	Terminated early due to toxicity	[45]
	Valspodar (PSC-833)	Untreated AML	ADE	No OS advantage age >45 years, OS benefit age <45 years	[48]
	Valspodar (PSC-833)	Ovarian	Carboplatin, paclitaxel	No benefit	[98]
	Valspodar (PSC-833)	Refractory AML, high-risk MDS	Mitoxantrone, etoposide, cytarabine	No benefit	[99]
	Valspodar (PSC-833)	NSCLC	Carboplatin, paclitaxel	Terminated early due to toxicity	Novartis
	Valspodar (PSC-833)	Multiple myeloma	VAD	No benefit; increased toxicity with valspodar	[44]
	VX-710 (Biricodar, Incel™)	Lung	Doxorubicin, vincristine	Unknown	http://clinicaltrials.gov
Third	CBT-1	NSLC	Paclitaxel, carboplatin	Ongoing	http://clinicaltrials.gov
	Dofequidar (MS-209)	Breast	Cyclophosphamide, doxorubicin, fluorouracil	Improved PFS in subset	[100]
	XR-9576 (Tariquidar)	NSCLC	Vinorelbine	Terminated early due to toxicity	http://clinicaltrials.gov
	XR-9576 (Tariquidar)	NSCLC	Paclitaxel, carboplatin	Terminated early due to toxicity	http://clinicaltrials.gov
	Zosuquidar (LY335979)	AML, MDS	Daunorubicin, cytarabine	Ongoing	http://clinicaltrials.gov