Published in final edited form as:

Drug Resist Updat. 2012 ; 15(0): 50–61. doi:10.1016/j.drup.2012.02.002.

Targeting MDR in Breast and Lung Cancer: Discriminating its Potential Importance from the Failure of Drug Resistance Reversal Studies

Laleh Amiri-Kordestani^a, Agnes Basseville^a, Karen Kurdziel^b, Antonio Tito Fojo^a, and Susan E. Bates^{a,*}

^aMedical Oncology Branch, Center for Cancer Research, National Cancer Institute, NIH, Bethesda, MD 20892

^bMolecular Imaging Program, Center for Cancer Research, National Cancer Institute, NIH, Bethesda, MD 20892

Abstract

This special issue of Drug Resistance Updates is dedicated to multidrug resistance protein 1 (MDR-1), 35 years after its discovery. While enormous progress has been made and our understanding of drug resistance has become more sophisticated and nuanced, after 35 years the role of MDR-1 in clinical oncology remains a work in progress. Despite clear *in vitro* evidence that P-glycoprotein (Pgp), encoded by MDR-1, is able to dramatically reduce drug concentrations in cultured cells, and that drug accumulation can be increased by small molecule inhibitors, clinical trials testing this paradigm have mostly failed. Some have argued that it is no longer worthy of study. However, repeated analyses have demonstrated MDR-1 expression in a tumor is a poor prognostic indicator leading some to conclude MDR-1 is a marker of a more aggressive phenotype, rather than a mechanism of drug resistance. In this review we will re-evaluate the MDR-1 story in light of our new understanding of molecular targeted therapy, using breast and lung cancer as examples. In the end we will reconcile the data available and the knowledge gained in support of a thesis that we understand far more than we realize, and that we can use this knowledge to improve future therapies.

Keywords

MDR-1/Pgp; 99mTc-sestamibi; 18F fluoropaclitaxel; breast cancer; lung cancer; drug penetration

Introduction

The last decade in clinical oncology has been noteworthy for advancing our understanding of the molecular foundations thought responsible for the origin and maintenance of the malignant phenotype. While we still do not understand for every tumor the critical pathways involved, we increasingly understand that both breast and lung cancer, two major solid tumors of adults, represent a collection of molecular subtypes beyond those previously recognized, and no longer assessable as single diseases (Hayes et al., 2006; Perou et al.,

*Corresponding author at: Medical Oncology Branch, Center for Cancer Research, National Cancer Institute, NIH, Building 10, Room 12N226, Bethesda, MD 20892, sebates@helix.nih.gov.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

2000). Along with this recognition has come an effort to develop therapies directed toward specific molecular aberrations for distinct tumor subtypes. We thus have erlotinib and gefitinib for lung cancers harboring epidermal growth factor receptor (EGFR) mutations and crizotinib for tumors with echinoderm microtubule associated protein like 4-anaplastic lymphoma kinase (EML4-ALK) rearrangements. Breast cancers that express hormone receptors and human epidermal growth factor receptor 2 (HER2) have a different spectrum of agents under development; and there are also agents that may be active in breast cancers that harbor BRCA mutations. And while “cytotoxic agents” remain a cornerstone of many therapies, even for patients with defined mutations, the advent of new, “targeted therapies” has added to the never-answered question of the etiology of drug resistance.

1. Background

Adenosine triphosphate (ATP) binding cassette transporters (ABC) were so named because a conserved ATP binding domain provides the energy required for a conformational change that effectively transfers substrates across the cell membrane to the cell exterior. This energy-consuming process is capable of transferring drug against very steep concentration gradients (Dean et al., 2001). The first discovered human ABC transporter was P-glycoprotein (Pgp), encoded by the MDR1 gene; in all 48 ABC transporters have been identified in humans (Szakacs et al., 2006). These are classified in 7 subfamilies, based on sequencing of the highly conserved ATP-binding domains. Over time, increasing evidence of the involvement of ABC transporters in normal physiology and disease has been gathered, including an ABCG2 variant with impaired uric acid efflux and a role in gout (Woodward et al., 2009), and a recent report that ABCC9 influences sleep requirement (Allebrandt et al., 2011). Many of the ABC transporters have dedicated physiological functions, but the role of several seems to be normal tissue protection – achieved by its expression in the GI tract, kidney, liver, pancreas, and the endothelium of vessels of the brain and testes. Amongst these, the ABC transporters thought most likely to mediate chemotherapy drug resistance are ABCB1 (Pgp/MDR1), ABCC1 (multidrug resistance-associated protein-1 (MRP1)) and ABCG2 (breast cancer resistance protein (BCRP)) (Gottesman et al., 2002; Robey et al., 2007).

Given that classical chemotherapeutics are numbered among the substrates for these transporters, including but not limited to doxorubicin, epirubicin, etoposide, paclitaxel, and docetaxel, the potential for a link between drug efflux and drug resistance in the clinic was readily apparent. The discovery by Tsuruo and colleagues (Tsuruo et al., 1981) that verapamil could inhibit the function of Pgp and reverse drug resistance led to clinical trials attempting to reverse drug resistance, beginning with inhibitors already “on the shelf” – verapamil, amiodarone, and cyclosporine – followed by agents such as valspodar and VX710, and finally more potent and specific agents such as tariquidar and zosuquidar. Tariquidar and CBT-1, an orally bioavailable inhibitor, are still in clinical development. The importance of ABC transporters in drug resistance was asked in clinical studies with inhibitors, but due to several considerations including potency of the agents and the design of the trials the question was left unanswered.

2. Clinical trials with inhibitors of Pgp

Tables 1 and 2 outline clinical trials in breast cancer and are consistent with what was observed in lung cancer and other tumor types, including acute myelogenous leukemia (Shaffer, et al., 2012). Many phase I studies tested the safety, tolerability, pharmacokinetic and pharmacodynamics of MDR inhibitors in combination with other agents (selected studies targeting breast cancer populations are shown in Table 1). In early studies the safety of verapamil and r-verapamil in combination with adriamycin and vincristine (Ries and

Dicato, 1991; Wilson et al., 1995) and with paclitaxel (Berg et al., 1995; Tolcher et al., 1996) were confirmed. Hematologic toxicity often required a reduction in the dose of the chemotherapeutic agent.

The efficacy of combining an MDR inhibitor with different chemotherapy agents in metastatic breast cancer was studied in several phase II and phase III trials (Table 2). Two randomized Phase II clinical trials compared epirubicin to epirubicin with either verapamil (Mross et al., 1993a) or quinidine (Wishart et al., 1994). Neither of these studies could show a significant improvement in the response rate (Table 2). While in similar patients with metastatic breast cancer, phase III trials gave dissimilar results. The first trial randomized 99 patients to receive vindesine 3 mg/m² on days 1 and 10 with continuous infusion 5-FU on days 1 through 10 of each 28 day cycle with or without oral verapamil 240 mg/day. Patients treated with verapamil had a longer overall survival 323 vs 209 days ($p = 0.036$) and a higher response rate, 27% vs 11% ($p = 0.04$) (Belpomme et al., 2000). However, verapamil is not a potent Pgp inhibitor, and a second study with a higher potency agent failed to demonstrate a similar improvement. Saeki et al conducted a phase III randomized double blind control study of six cycles of CAF (100 mg cyclophosphamide administered orally on days 1 through 14, together with 25 mg/m² adriamycin and 500 mg/m² fluorouracil administered intravenously on days 1 and 8) with or without 900 mg dofequidar administered orally on days 1 - 14 in 221 patients with metastatic breast cancer. Although both the overall response rates of 42.6% and 53.1% ($P = 0.077$) and median progression-free survivals of 241 days and 366 days ($P = 0.145$) for CAF alone and CAF plus dofequidar, respectively, suggested a benefit from adding dofequidar, the results did not reach statistical significance (Saeki et al., 2007).

Multiple explanations including trial design can be offered for the failure of these trials to convincingly show clinical benefit. One is that many of the studies used inhibitors that were low in potency. Additionally, toxicities of the earlier agents such as calcium channel blockers (verapamil and other analogues) and amiodarone, prevented dose escalation and yielded an ineffective dose, almost certainly true of the verapamil studies (Mross et al., 1993a; Wishart et al., 1994). Later agents such as valspodar required reduction in the dose of the anticancer agent due to CYP3A4-related pharmacokinetic interactions, a strategy that undermined any potential value of the combination, as sub-therapeutic peak concentrations likely resulted (Bates et al., 2004). It is also likely that, if drug transporters are important resistance mechanism in breast cancer, they are important in only a subset of tumors. The inhibitors were quickly developed and reached the clinic before there was understanding that clinical trials need to enrich for the patient population under study. The development of trastuzumab in breast cancer was illustrative. In an unselected population, a low response rate of 26% (5% in FISH- vs. 41% FISH+) was noted, and only in HER2+ tumors did clinical benefit clearly emerge (Vogel et al., 2001). Similarly, in an unselected population of patients with lung cancer, the EGFR inhibitor, erlotinib, had a response rate of 8.9% and only in tumors harboring a mutant EGFR did clinical benefit emerge (Shepherd et al., 2005). These examples show that the failure to select patients for enrollment alone could have doomed the strategy to failure. However, enrichment of a patient population for the trial of an inhibitor of drug efflux would have required a reliable assay that indicated MDR as a dominant mechanism of resistance, and for solid tumors this was never developed.

A further confounding problem with design is that while some were randomized studies (Mross et al., 1993a; Wishart et al., 1994), a larger number used a run-in phase or cross-over design (Bates et al., 1995) in which patients who initially received the chemotherapy agents alone were then treated with an MDR inhibitor, in some studies only after clear progression on chemotherapy had been documented (Warner et al., 1998). If a drug transporter were important, one could envision these designs leading to greater resistance mediated by both

the transporter as well as other mechanism that could emerge. Development of additional mechanisms of tolerance could also undermine as targeting a single mechanism resulted in less efficacy (Germano and O'Driscoll, 2009).

Not encountered in the breast cancer trials was a problem noted in trials in leukemia and in lung cancer – increased toxicity in the experimental arm. The observation that leukemias often express high levels of Pgp, and that expression correlates with poor survival, together with the continued poor outcome of patients with acute myeloid leukemia, prompted the conduct of a large number of randomized studies with Pgp inhibitors (Shaffer et al., 2012). Notably, higher rates of mortality and adverse events were observed in the experimental arms of several studies. A similar finding was observed with the Pgp inhibitor, tariquidar, in two large randomized Phase III trials in patients with non-small cell lung cancer. Both trials closed early for adverse events and mortality rates that would have made it impossible to demonstrate any survival benefit from the combination. While not proven, inhibition of Pgp in bone marrow stem cells or early progenitor cells and in drug metabolizing organs such as the liver are viewed as the most likely explanations for the increased toxicity.

Other variables that may have contributed to the failure of clinical trials may have been the inclusion of patients with polymorphic variants of transporters that we now understand can impair function. These polymorphisms may make bone marrow stem cells or early progenitors more susceptible to Pgp inhibition (Cascorbi, 2006). Interestingly, a synonymous polymorphism in *ABCB1* -- 3435C>T SNP, together with two others, 2677G>T/A, and/or 1236C>T, comprise a haplotype that in general has been associated with impaired protein function. The mutation at the 3435 C>T SNP site may cause ribosome stalling and different speeds of protein translation, impacting protein folding (Fung and Gottesman, 2009). Other transporters potentially involved in drug resistance are also subject to polymorphic variation. For example, a variant ABCG2, C421A, replaces a glutamine with a lysine at amino acid residue 141 and is associated with impaired protein trafficking so that the protein is degraded rather than trafficked to the cell surface (Furukawa et al., 2009; Morisaki et al., 2005). Variants encoding stop codons have also been described (Saison et al., 2012). Such polymorphisms, unknown during early clinical trials, could confound results by including some patients whose tumors will not develop significant drug transporter-mediated resistance but whose bone marrow might be more sensitive to chemotherapy substrates when combined with a transport inhibitor. Although a hypothesis, it is possible that selection of patients could have benefitted in two directions – identifying patients whose tumors had high expression of Pgp, who may have benefited from addition of an inhibitor and those whose tumors had low expression and were not likely to benefit but instead had greater toxicity.

These comments make clear that the trials were conducted too early, with insufficient understanding. Despite multiple trials, few actually confirmed expression of Pgp in tumor tissue, none required expression for enrollment and none demonstrated inhibition of drug efflux and increased drug accumulation in tumors with addition of the Pgp inhibitor. No trials demonstrated that the Pgp inhibitor was able to penetrate tumor tissue. No trials evaluated genotype to determine the impact of polymorphic variants. Despite the lack of such pharmacodynamic data, the clinical results were considered by many to be conclusive and interest in ABC transporters as a mechanism of drug resistance faded.

3. Beyond Pgp inhibitors: ABC transporter expression and correlation with clinical outcomes

Despite the largely negative results in clinical trials summarized above, expression studies have repeatedly shown correlations with clinical outcome. In leukemia three decades of data

support an adverse outcome for patients whose leukemias express high levels of Pgp. In breast and lung cancer, the data are also fairly compelling. A 2005 meta-analysis of Pgp expression in breast cancer concluded that a significant number of breast cancer samples demonstrate Pgp expression, that expression is increased after chemotherapy, and that expression correlates with a worse response to treatment (Clarke et al., 2005). Even in the last decade, as interest in trials has waned, studies in breast cancer examining expression of the three ABC transporters most often linked to drug resistance have again reported that expression is often, although not always associated with adverse outcome, as shown in Table 3A. Similarly, ABC transporter expression in lung cancer has been associated with poor outcome (Stewart, 2010). The most recent decade of studies shown in Table 3B confirms that association. The question is whether expression is related to decreased drug accumulation or is a marker for another feature of poor outcome, such as invasiveness (Colone et al., 2008; Mignogna et al., 2006).

While overexpression of Pgp and other ABC transporters in tumor tissue has been associated with outcome, the mechanism underlying that overexpression has not been fully explored. Apart from a few specific examples, such as gene rearrangement and capture of the MDR1 gene by a constitutively active promoter (Mickley et al., 1997), overexpression is related to the state of differentiation or as a response to drug exposure - both a consequence of epigenetic regulation. We observed that MDR1 induction is one of the most consistent changes in gene expression that occurs following the histone acetylation that results from addition of histone deacetylase inhibitors (Bates et al., 2010). MDR1 transcription is also upregulated by the histone methyltransferase mixed lineage leukemia 1 protein (MLL 1), through the activating methylation at lysine 4 of histone H3 (Huo et al., 2010). Mutated, rearranged or duplicated, MLL is leukemogenic and may concurrently upregulate MDR1. It is likely that a parallel mechanism exists in solid tumors. Other epigenetic changes associated with increased gene expression, such as MDR1 promoter CpG hypomethylation, have been observed following chemotherapy (Baker et al., 2005). Finally, chemotherapeutic agents activate the pregnane X receptor, a master transcription factor for drug metabolizing enzymes, and a mediator of MDR1 transcription. Induction of MDR1 by this mechanism has been observed in breast and prostate cancer cells (Chen et al., 2009; Chen et al., 2007). Together, these studies show that MDR1, and by extension other ABC transporters, are regulated genetically and epigenetically, and suggest that overexpression may occur both during oncogenesis and in response to the administration of chemotherapeutic agents.

4. The complexity of drug accumulation

A less well studied explanation for the failure of clinical trials to confirm the MDR hypothesis is that drug transporters may simply be one of several factors affecting drug accumulation in cells with additional factors affecting drug penetration into tumors. Indeed, although numerous animal models have been evaluated, the mechanisms underlying drug penetration into tumors have not been well studied in patients. Three early studies that measured doxorubicin levels in breast cancer reported a disturbingly broad range. Cummings et al reported a mean doxorubicin concentration of 819 ± 482 ng/g at 30 minutes, while Stallard noted a 7-fold range from 220 – 1590 ng/g at one hour, and Rossi a 16-fold range from 1.86 to 30 ug/g at 24 hours (Cummings and McArdle, 1986; Rossi et al., 1987; Stallard et al., 1990). In a subsequent study, Lankelma and colleagues reported steep doxorubicin gradients just microns away from a tumor's vasculature and showed these gradients varied among patients (Lankelma et al., 1999). A more recent evaluation of paclitaxel in cervical and ovarian cancers demonstrated median concentrations of 324 and 305 ng/g, respectively; with concentrations (ng/g) at the 25th and 90th percentile concentrations of 160 and 736.8 for cervical cancer and 185 and 862 for ovarian cancer [(Koshiba et al., 2009), and Hisato Koshiba, personal communication]. Given the steep

dose-response curves for most cancer chemotherapies, these data strongly suggest adequate drug concentrations may not be achieved in some tumors and mandate a better understanding of factors responsible for drug penetration and persistence in tumor tissue. Yet we still do not have a means of assessing chemotherapy gradients, nor altering them in patients. It is thought that multiple factors, among them pH, interstitial pressure, and hypoxia, influence these gradients and that these factors are linked at least in part with the disordered vasculature that is the hallmark of cancer. Thus, drug transporter expression may influence drug accumulation in cells and possibly in tissues, but likely is only one of multiple factors regulating penetration into tumor tissue.

5. Imaging drug uptake and accumulation in tissues and in tumors

A first assumption in cancer therapy is that a drug always reaches its target. We infer this when we see clinical responses, and then assume other mechanisms account for treatment failure. But in fact we know very little about the extent of variation in drug penetration and to what extent that can account for treatment failure. An evolving strategy for evaluating drug uptake in tumors is radiolabeled imaging of either anticancer drugs or surrogates. There are considerable data regarding ^{99m}Tc -sestamibi, a radionuclide imaging agent approved by the FDA for cardiac imaging and for its ability to detect breast cancers. ^{99m}Tc -sestamibi has been shown to be promising in the detection of microcalcifications (Fondrinier et al., 2004), occult breast carcinomas (Coover et al., 2004), breast cancer (Sampalis et al., 2003), as well as in staging, axillary lymph node evaluation (Myslivecek et al., 2004; Ozulker et al., 2010; Zhou et al., 2009) and sentinel node mapping (Arcan et al., 2005; Sadeghi et al., 2010). Although ^{99m}Tc -sestamibi is approved by the FDA for breast imaging, current evidence does not support its use for breast cancer screening [NCCN guidelines v2 2011], in part due to the difficulty of detecting lesions smaller than 10 mm.

Multiple small studies have evaluated imaging with ^{99m}Tc -sestamibi, or a similar agent tetrafosmin, as a surrogate for chemotherapy in patients with lung cancer, finding that imaging uptake was highly associated with paclitaxel-based chemotherapy response (Ceriani et al., 1997; Fuster et al., 2003; Komori et al., 2000; Mohan and Miles, 2009; Nishiyama et al., 2000; Shih et al., 2003; Yuksel et al., 2002). A meta-analysis concluded the test had predictive value for this purpose (Mohan and Miles, 2009). Given that sestamibi and tetrafosmin are substrates for both Pgp and MRP1-mediated efflux, co-expression in lung cancer could reduce accumulation. Similarly in breast cancer, sestamibi washout has been correlated with response to neoadjuvant chemotherapy (Alonso et al., 2002; Ciarmiello et al., 1998; Mankoff et al., 1999; Sciuto et al., 2002), and linked to Pgp expression (Sun et al., 2000).

The functional activity of the Pgp transporter in a tumor can be measured with ^{99m}Tc -sestamibi scans, if coupled with administration of a MDR efflux inhibitor. This has been evaluated in solid tumors (Abraham et al., 2009; Agrawal et al., 2003; Bates et al., 2004) including breast cancer (Pusztai et al., 2005; Sun et al., 2000). We observed marked heterogeneity of uptake in sestamibi in lung cancer, with minimal change following tariquidar (Kelly et al., 2010), presumably due to non-Pgp factors, such as hypoxia, limiting uptake.

Despite potential utility, the limitations of single photon imaging with ^{99m}Tc -sestamibi have kept it from entering clinical practice. The optimal imaging parameters (i.e. imaging times, retention index calculation methods) have not been established, and the overall predictive value of these studies has not been validated to warrant change in clinical therapy. Single photon imaging is limited by overall low count rate requiring relatively long imaging times.

Furthermore, routine dynamic imaging is performed by planar imaging; resulting in only relative count data (i.e. not truly quantitative).

In contrast, positron emission tomography (PET) imaging is quantitative, provides improved spatial resolution (~5 mm), and has high-count rate sensitivity. Carbon-11 (^{11}C), a PET radionuclide, has been used to label several Pgp substrates including verapamil (Hendrikse et al., 2001), colchicine (Levchenko et al., 2000), daunorubicin (Elsinga et al., 1996), loperamide and [N-methyl]N-desmethyl-loperamide (Lazarova et al., 2008). Radiolabeled therapy agents have also been explored including [^{11}C]paclitaxel (Ravert et al., 2002), [^{111}In]paclitaxel (Li et al., 1997), and [^{11}C]docetaxel (van Tilburg et al., 2004). However, the 20.4-minute physical half-life of [^{11}C] and corresponding need for on-site synthesis limits the clinical utility of [^{11}C]-tracers.

Paclitaxel, a chemotherapeutic agent and a Pgp substrate, has been labeled with [^{18}F], a positron emitter with a longer half-life which should allow imaging of slowly changing physiological phenomena, and like $^{99\text{m}}\text{Tc}$ -sestamibi, offers the possibility of evaluating drug uptake as well as Pgp function. Paclitaxel is not a substrate for multidrug resistant protein (MRP) (Huang et al., 1997), is a neutral compound, and does not require an electropotential gradient for intracellular retention. The overall ^{18}F fluoropaclitaxel (FPAC) kinetics are dependent on microtubule specific binding, non-specific FPAC binding and Pgp-related efflux.

Preclinical animal FPAC imaging data showed a similar biodistribution between unlabeled fluoropaclitaxel and paclitaxel (Gangloff et al., 2005; Jagoda et al., 2002; Kiesewetter and Eckelman, 2001; Kurdziel et al., 2007; Schinkel, 1998). In a human breast cancer xenograft model, FPAC was shown to predict chemotherapeutic response (Hsueh et al., 2006). FPAC PET was used *in vivo* to image and quantify Pgp inhibition following the intravenous administration of tariquidar (Kurdziel et al., 2011). To date, three normal volunteers and three breast cancer patients have been imaged with FPAC. While the overall tumor uptake was small, there was low background activity in the chest, breast, brain, head and neck, making even small differences in FPAC accumulation apparent (Figure 1).

6. Drug uptake and accumulation in sanctuary sites - the CNS as a paradigm

Another aspect of drug resistance is the existence of sanctuary sites such as the central nervous system (CNS), an example of an environment protected from both the toxic and beneficial effects of chemotherapeutics (Lin et al., 2004; Steeg et al., 2011). For patients whose tumors express HER2, the increase in survival that has resulted from the use of HER2 targeting agents has been complicated by the emergence of CNS metastases (Dawood et al., 2009; Eichler et al., 2008; Kirsch et al., 2005; Ricciardi and de Marinis, 2010), (Brufsky et al., 2011). This is accompanied by morbidity associated with the CNS disease itself (Witgert and Meyers, 2011), as well as the adverse effects of treatment, which centers on surgery and radiation therapy (Platta et al., 2010). Often occurring in patients whose systemic disease is well-controlled, it is assumed that the metastases arise from dormant tumor cells that have crossed the blood brain barrier (BBB) and have failed to be eliminated or controlled by chemotherapeutics that do not have access to the CNS (Eichler et al., 2011). The BBB separates circulating blood from the extracellular fluid of CNS. It consists of tight junctions around capillaries, perivascular astrocytes, as well as a number of transporters including ABCB1 and ABCG2 (Abbott et al., 2006) (Deeken and Loscher, 2007).

The contribution of transporters to the BBB has been evaluated in mouse models in which ABCB1 and ABCG2 have been deleted. Early experiments with mice lacking only the

ABCB1 orthologues showed minimal impact on brain uptake, suggesting the tight junctions or other facets were more important. Subsequent studies in mice lacking both ABCB1 and ABCG2 revealed a dramatic impact on brain uptake with the obvious caveat that we cannot be certain this is predictive of their role in the human BBB. Figure 2 compiles several reports on the effect of the double knockout on uptake of anticancer drugs in the CNS (de Vries et al., 2007; Kodaira et al., 2010; Lagas et al., 2010; Poller et al., 2011; Polli et al., 2009; Tang et al., 2012). It is noteworthy that most of the agents included are targeted agents. Uptake of drugs known to be substrates for drug transporters including lapatinib, topotecan, mitoxantrone, sunitinib, sorafenib, and axitinib showed minimal alteration when either the ABCB1 or ABCG2 gene had been deleted, but 10 – 25-fold increases in brain concentrations when both genes were deleted. While providing proof of concept that ABC transporters could limit drug uptake, these studies also demonstrated the protection conferred by redundancy.

Although there is evidence the BBB is only partially intact in metastatic tumors (Taskar et al., 2011), studies in patients with CNS metastases suggest brain concentrations are limiting. For example, response rates to lapatinib of only 2.6 and 6% were observed in CNS metastases due to breast cancer (Lin et al., 2008; Lin et al., 2009). Although in a small series of patients with lung cancers bearing EGFR mutations, erlotinib given on a weekly “high dose” schedule induced a better response rate at 67% (Grommes et al., 2011). Together these studies suggest prevention or treatment of metastatic disease in the CNS may be one potentially important area for the study of efflux inhibitors or novel drugs developed to avoid drug transporters.

7. Perspective

Unless there is an unaccountable level of publication bias influencing the findings discussed above, the fundamental conclusion seems to be that expression of Pgp or other MDR transporters and associated drug efflux in a tumor is bad, but that so far Pgp has not been successfully translated to a therapeutic target. Unfortunately, too many have taken this to mean that Pgp is not important clinically, a conclusion not supported by the wealth of data both in pre-clinical models and most importantly in clinical studies. Many analogies come to mind, all of which illustrate the folly of reaching such a conclusion. The taxanes and the vinca alkaloids for example, specifically target tubulin and the microtubules, but are effective in only a fraction of patients. Notably, they are inactive in colon cancer. To conclude from this that microtubules are not essential for colon cancer would be at best misguided and certainly incorrect. Similarly, the recent near universal disappointment with agents targeting mitosis including inhibitors of the aurora kinases, mitotic spindle protein and polo-like kinase would never be interpreted as evidence that mitosis is not important in cancer. So too can the only conclusion drawn from the data with Pgp be that we have been, to date, unable to inhibit its function effectively in tumors or that its inhibition alone has not been sufficient – not that Pgp is not important.

This distinction is important as one considers the way forward in this field of research. Because studies correlating expression with poor outcomes have usually examined cancers treated with drugs we consider Pgp substrates, one can conclude that the poorer outcomes of those patients whose tumors express Pgp can be in part explained by less effective chemotherapy. While we cannot exclude that Pgp might be a marker or surrogate of other more important resistance mechanisms or of “bad tumor biology”, it is also possible, indeed most likely, that Pgp by conferring resistance is in part responsible for the poorer outcomes. Together, these data can be taken as compelling evidence to develop agents for cancer that are not substrates for Pgp or other MDR transporters. Additional benefits from developing agents that are not substrates for multidrug transporters would include enhanced oral

bioavailability and increased permeability into the brain and other sanctuary sites where penetration is prevented by ABC transporters. The latter has been seen as a limitation of traditional cytotoxic agents such as the taxanes and other microtubule targeting agents. Novel analogs now in development such as GRN1005 (<http://clinicaltrials.gov/ct2/show/NCT01480583>), a paclitaxel drug conjugate, offer the hope of overcoming this obstacle. Similarly the tyrosine kinase inhibitors (TKIs) have proven to be substrates for transporters as a class and clinical data suggest brain penetration is limited (Brozik et al., 2011). A TKI that is not a substrate for ABC transporters could be a useful addition to the armamentarium.

As for continuing to evaluate Pgp or other MDR transporter as a therapeutic target, any such trials should enroll patients only when transporter expression is documented and blood is stored for genotyping. An effort should be directed toward developing a real test for transporter expression. At this time a specific recommendation for a particular antibody-based assay for Pgp cannot be made – one of the most commonly used antibodies was shown a decade ago to have cross-reactivity with c-erbB2 (Liu et al., 1997). Measuring expression by PCR has been problematic – the greater sensitivity of that assay renders most tumors positive for expression; a cut-off of significance has not been determined in any tumor type, and RNA expression would not solve the potential role of protein variants in altering function. These difficulties reinforce the point that a major effort should be made to develop imaging agents that would allow assessment of drug uptake, and to understand rate-limiting factors of uptake.

We will probably come to understand drug transporter expression as part and parcel of the malignant phenotype, sometimes less important than other features and sometimes a dominant mechanism of resistance. In the end, personalized medicine will be about making this distinction, and 35 years on, we might be able to convincingly determine the role of drug uptake in clinical drug resistance.

References

- Abbott NJ, Ronnback L, Hansson E. Astrocyte-endothelial interactions at the blood-brain barrier. *Nat Rev Neurosci.* 2006; 7:41–53. [PubMed: 16371949]
- Abraham J, Edgerly M, Wilson R, Chen C, Rutt A, Bakke S, et al. A phase I study of the P-glycoprotein antagonist tariquidar in combination with vinorelbine. *Clin Cancer Res.* 2009; 15:3574–3582. [PubMed: 19417029]
- Agrawal M, Abraham J, Balis FM, Edgerly M, Stein WD, Bates S, et al. Increased 99mTc-sestamibi accumulation in normal liver and drug-resistant tumors after the administration of the glycoprotein inhibitor, XR9576. *Clin Cancer Res.* 2003; 9:650–656. [PubMed: 12576431]
- Allebrandt KV, Amin N, Muller-Myhsok B, Esko T, Teder-Laving M, Azevedo RV, et al. A K(ATP) channel gene effect on sleep duration: from genome-wide association studies to function in *Drosophila*. *Mol Psychiatry.* 2011 in press.
- Alonso O, Delgado L, Nunez M, Vargas C, Lopera J, Andruskevicius P, et al. Predictive value of (99m)Tc sestamibi scintigraphy in the evaluation of doxorubicin based chemotherapy response in patients with advanced breast cancer. *Nucl Med Commun.* 2002; 23:765–771. [PubMed: 12124482]
- Arcan P, Ibis E, Aras G, Cam R, Kucuk NO. Identification of sentinel lymph node in stage I-II breast cancer with lymphoscintigraphy and surgical gamma probe: comparison of Tc-99m MIBI and Tc-99m sulfur colloid. *Clin Nucl Med.* 2005; 30:317–321. [PubMed: 15827399]
- Atalay C, Deliloglu Gurhan I, Irkkan C, Gunduz U. Multidrug resistance in locally advanced breast cancer. *Tumour Biol.* 2006; 27:309–318. [PubMed: 17033200]
- Atalay C, Demirkazik A, Gunduz U. Role of ABCB1 and ABCC1 gene induction on survival in locally advanced breast cancer. *J Chemother.* 2008; 20:734–739. [PubMed: 19129072]
- Baker EK, Johnstone RW, Zalcborg JR, El-Osta A. Epigenetic changes to the MDR1 locus in response to chemotherapeutic drugs. *Oncogene.* 2005; 24:8061–8075. [PubMed: 16091741]

- Bates SE, Bakke S, Kang M, Robey RW, Zhai S, Thambi P, et al. A phase I/II study of infusional vinblastine with the P-glycoprotein antagonist valspodar (PSC 833) in renal cell carcinoma. *Clin Cancer Res.* 2004; 10:4724–4733. [PubMed: 15269145]
- Bates SE, Meadows B, Goldspiel BR, Denicoff A, Le TB, Tucker E, et al. A pilot study of amiodarone with infusional doxorubicin or vinblastine in refractory breast cancer. *Cancer Chemother Pharmacol.* 1995; 35:457–463. [PubMed: 7882454]
- Bates SE, Zhan Z, Steadman K, Obrzut T, Luchenko V, Frye R, et al. Laboratory correlates for a phase II trial of romidepsin in cutaneous and peripheral T-cell lymphoma. *Br J Haematol.* 2010; 148:256–267. [PubMed: 19874311]
- Belpomme D, Gauthier S, Pujade-Lauraine E, Facchini T, Goudier MJ, Krakowski I, et al. Verapamil increases the survival of patients with anthracycline-resistant metastatic breast carcinoma. *Ann Oncol.* 2000; 11:1471–1476. [PubMed: 11142488]
- Berg SL, Tolcher A, O'Shaughnessy JA, Denicoff AM, Noone M, Ognibene FP, et al. Effect of R-verapamil on the pharmacokinetics of paclitaxel in women with breast cancer. *J Clin Oncol.* 1995; 13:2039–2042. [PubMed: 7636546]
- Brozik A, Hegedus C, Erdei Z, Hegedus T, Ozvegy-Laczka C, Szakacs G, et al. Tyrosine kinase inhibitors as modulators of ATP binding cassette multidrug transporters: substrates, chemosensitizers or inducers of acquired multidrug resistance? *Expert Opin Drug Metab Toxicol.* 2011; 7:623–642. [PubMed: 21410427]
- Brufsky AM, Mayer M, Rugo HS, Kaufman PA, Tan-Chiu E, Tripathy D, et al. Central nervous system metastases in patients with HER2-positive metastatic breast cancer: incidence, treatment, and survival in patients from registHER. *Clin Cancer Res.* 2011; 17:4834–4843. [PubMed: 21768129]
- Budd GT, Bukowski RM, Lichtin A, Bauer L, Van Kirk P, Ganapathi R. Phase II trial of doxorubicin and trifluoperazine in metastatic breast cancer. *Invest New Drugs.* 1993; 11:75–79. [PubMed: 8349440]
- Burger H, Foekens JA, Look MP, Meijer-van Gelder ME, Klijn JG, Wiemer EA, et al. RNA expression of breast cancer resistance protein, lung resistance-related protein, multidrug resistance-associated proteins 1 and 2, and multidrug resistance gene 1 in breast cancer: correlation with chemotherapeutic response. *Clin Cancer Res.* 2003; 9:827–836. [PubMed: 12576456]
- Carlson RW, O'Neill AM, Goldstein LJ, Sikic BI, Abramson N, Stewart JA, et al. A pilot phase II trial of valspodar modulation of multidrug resistance to paclitaxel in the treatment of metastatic carcinoma of the breast (E1195): a trial of the Eastern Cooperative Oncology Group. *Cancer Invest.* 2006; 24:677–681. [PubMed: 17118777]
- Cascorbi I. Role of pharmacogenetics of ATP-binding cassette transporters in the pharmacokinetics of drugs. *Pharmacol Ther.* 2006; 112:457–473. [PubMed: 16766035]
- Ceriani L, Giovannella L, Bandera M, Beghe B, Ortelli M, Roncari G. Semi-quantitative assessment of ⁹⁹Tcm-sestamibi uptake in lung cancer: relationship with clinical response to chemotherapy. *Nucl Med Commun.* 1997; 18:1087–1097. [PubMed: 9423210]
- Chen Y, Tang Y, Chen S, Nie D. Regulation of drug resistance by human pregnane X receptor in breast cancer. *Cancer Biol Ther.* 2009; 8:1265–1272. [PubMed: 19746521]
- Chen Y, Tang Y, Wang MT, Zeng S, Nie D. Human pregnane X receptor and resistance to chemotherapy in prostate cancer. *Cancer Res.* 2007; 67:10361–10367. [PubMed: 17974979]
- Chen YJ, Huang WC, Wei YL, Hsu SC, Yuan P, Lin HY, et al. Elevated BCRP/ABCG2 expression confers acquired resistance to gefitinib in wild-type EGFR-expressing cells. *PLoS One.* 2011; 6:e21428. [PubMed: 21731744]
- Chintamani Singh JP, Mittal MK, Saxena S, Bansal A, Bhatia A, et al. Role of p-glycoprotein expression in predicting response to neoadjuvant chemotherapy in breast cancer--a prospective clinical study. *World J Surg Oncol.* 2005; 3:61. [PubMed: 16164742]
- Ciarmiello A, Del Vecchio S, Silvestro P, Potena MI, Carriero MV, Thomas R, et al. Tumor clearance of technetium ^{99m}-sestamibi as a predictor of response to neoadjuvant chemotherapy for locally advanced breast cancer. *J Clin Oncol.* 1998; 16:1677–1683. [PubMed: 9586878]
- Clarke R, Leonessa F, Trock B. Multidrug resistance/P-glycoprotein and breast cancer: review and meta-analysis. *Semin Oncol.* 2005; 32:S9–15. [PubMed: 16360717]

- Colone M, Calcabrini A, Toccaceli L, Bozzuto G, Stringaro A, Gentile M, et al. The multidrug transporter P-glycoprotein: a mediator of melanoma invasion? *J Invest Dermatol.* 2008; 128:957–971. [PubMed: 17943188]
- Coover LR, Caravaglia G, Kuhn P. Scintimammography with dedicated breast camera detects and localizes occult carcinoma. *J Nucl Med.* 2004; 45:553–558. [PubMed: 15073249]
- Cummings J, McArdle CS. Studies on the in vivo disposition of adriamycin in human tumours which exhibit different responses to the drug. *Br J Cancer.* 1986; 53:835–838. [PubMed: 3718837]
- Dawood S, Broglio K, Esteva FJ, Yang W, Kau SW, Islam R, et al. Survival among women with triple receptor-negative breast cancer and brain metastases. *Ann Oncol.* 2009; 20:621–627. [PubMed: 19150943]
- de Vries NA, Zhao J, Kroon E, Buckle T, Beijnen JH, van Tellingen O. P-glycoprotein and breast cancer resistance protein: two dominant transporters working together in limiting the brain penetration of topotecan. *Clin Cancer Res.* 2007; 13:6440–6449. [PubMed: 17975156]
- Dean M, Rzhetsky A, Allikmets R. The human ATP-binding cassette (ABC) transporter superfamily. *Genome Res.* 2001; 11:1156–1166. [PubMed: 11435397]
- Deeken JF, Loscher W. The blood-brain barrier and cancer: transporters, treatment, and Trojan horses. *Clin Cancer Res.* 2007; 13:1663–1674. [PubMed: 17363519]
- Eichler AF, Chung E, Kodack DP, Loeffler JS, Fukumura D, Jain RK. The biology of brain metastases-translation to new therapies. *Nat Rev Clin Oncol.* 2011; 8:344–356. [PubMed: 21487419]
- Eichler AF, Kuter I, Ryan P, Schapira L, Younger J, Henson JW. Survival in patients with brain metastases from breast cancer: the importance of HER-2 status. *Cancer.* 2008; 112:2359–2367. [PubMed: 18361426]
- Elsinga PH, Franssen EIJ, Hendrikse NH, Fluks L, Weemaes AMA, vanderGraaf WTA, et al. Carbon-11-labeled daunorubicin and verapamil for probing P- glycoprotein in tumors with PET. *J Nucl Med.* 1996; 37:1571–1575. [PubMed: 8790221]
- Faneyte IF, Kristel PM, Maliepaard M, Scheffer GL, Scheper RJ, Schellens JH, et al. Expression of the breast cancer resistance protein in breast cancer. *Clin Cancer Res.* 2002; 8:1068–1074. [PubMed: 11948115]
- Filipits M, Pohl G, Rudas M, Dietze O, Lax S, Grill R, et al. Clinical role of multidrug resistance protein 1 expression in chemotherapy resistance in early-stage breast cancer: the Austrian Breast and Colorectal Cancer Study Group. *J Clin Oncol.* 2005; 23:1161–1168. [PubMed: 15718312]
- Fondrinier E, Muratet JP, Anglade E, Fauvet R, Berger V, Lorimier G, et al. Clinical experience with 99mTc-MIBI scintimammography in patients with breast microcalcifications. *Breast.* 2004; 13:316–320. [PubMed: 15325666]
- Fracasso PM, Blum KA, Ma MK, Tan BR, Wright LP, Goodner SA, et al. Phase I study of pegylated liposomal doxorubicin and the multidrug-resistance modulator, valspodar. *Br J Cancer.* 2005; 93:46–53. [PubMed: 15942626]
- Fung KL, Gottesman MM. A synonymous polymorphism in a common MDR1 (ABCB1) haplotype shapes protein function. *Biochim Biophys Acta.* 2009; 1794:860–871. [PubMed: 19285158]
- Furukawa T, Wakabayashi K, Tamura A, Nakagawa H, Morishima Y, Osawa Y, et al. Major SNP (Q141K) variant of human ABC transporter ABCG2 undergoes lysosomal and proteasomal degradations. *Pharm Res.* 2009; 26:469–479. [PubMed: 18958403]
- Fuster D, Vinolas N, Mallafre C, Pavia J, Martin F, Pons F. Tetrofosmin as predictors of tumour response. *Q J Nucl Med.* 2003; 47:58–62. [PubMed: 12714956]
- Gangloff A, Hsueh WA, Kesner AL, Kiesewetter DO, Pio BS, Pegram MD, et al. Estimation of paclitaxel biodistribution and uptake in human-derived xenografts in vivo with (18)F-fluoropaclitaxel. *J Nucl Med.* 2005; 46:1866–1871. [PubMed: 16269601]
- Germano S, O'Driscoll L. Breast cancer: understanding sensitivity and resistance to chemotherapy and targeted therapies to aid in personalised medicine. *Curr Cancer Drug Targets.* 2009; 9:398–418. [PubMed: 19442059]
- Gottesman MM, Fojo T, Bates SE. Multidrug resistance in cancer: role of ATP-dependent transporters. *Nat Rev Cancer.* 2002; 2:48–58. [PubMed: 11902585]

- Grommes C, Oxnard GR, Kris MG, Miller VA, Pao W, Holodny AI, et al. “Pulsatile” high-dose weekly erlotinib for CNS metastases from EGFR mutant non-small cell lung cancer. *Neuro Oncol*. 2011; 13:1364–1369. [PubMed: 21865399]
- Hayes DN, Monti S, Parmigiani G, Gilks CB, Naoki K, Bhattacharjee A, et al. Gene expression profiling reveals reproducible human lung adenocarcinoma subtypes in multiple independent patient cohorts. *J Clin Oncol*. 2006; 24:5079–5090. [PubMed: 17075127]
- Hendrikse NH, de Vries EGE, Franssen EJF, Vaalburg W, van der Graaf WTA. In vivo measurement of [C-11]verapamil kinetics in human tissues. *Eur J Clin Pharmacol*. 2001; 56:827–829. [PubMed: 11294373]
- Hsia TC, Lin CC, Wang JJ, Ho ST, Kao A. Relationship between chemotherapy response of small cell lung cancer and P-glycoprotein or multidrug resistance-related protein expression. *Lung*. 2002; 180:173–179. [PubMed: 12177731]
- Hsueh W, Kesner A, Gangloff A, Pegram M, Beryt M, Czernin J, et al. Predicting Chemotherapy Response to Paclitaxel with 18F-Fluoropaclitaxel and PET. *J Nucl Med*. 2006; 47:1995–1999. [PubMed: 17138742]
- Huang Y, Ibrado AM, Reed JC, Bullock G, Ray S, Tang C, et al. Co-expression of several molecular mechanisms of multidrug resistance and their significance for paclitaxel cytotoxicity in human AML HL-60 cells. *Leukemia*. 1997; 11:253–257. [PubMed: 9009089]
- Huo H, Magro PG, Pietsch EC, Patel BB, Scotto KW. Histone methyltransferase MLL1 regulates MDR1 transcription and chemoresistance. *Cancer Res*. 2010; 70:8726–8735. [PubMed: 20861184]
- Jagoda E, Kiesewetter D, Shimoji K, Ravasi L, Eckelman W. Comparison of the Biodistribution of Fluorinated and Tritiated paclitaxel (PAC) in *mdr1a(-)/1b(-)* knockout vs wildtype mice. *J Nucl Med*. 2002; 43:277P.
- Kelly RJ, Robey R, Draper D, Chen C, Venkatesan AM, Figg WD, et al. A pharmacodynamic study of docetaxel in combination with the p-glycoprotein antagonist, tariquidar (XR9576) in patients with lung, ovarian, and cervical cancer. *Proc Amer Assoc Can Res*. 2010; 51
- Kiesewetter D, Eckelman W. Radiochemical Synthesis of [18F]fluoropaclitaxel ([18F]FPAC). *J Labelled Cpd Radiopharm S*. 2001:903–S905.
- Kim YH, Ishii G, Goto K, Ota S, Kubota K, Murata Y, et al. Expression of breast cancer resistance protein is associated with a poor clinical outcome in patients with small-cell lung cancer. *Lung Cancer*. 2009; 65:105–111. [PubMed: 19036469]
- Kirsch DG, Ledezma CJ, Mathews CS, Bhan AK, Ancukiewicz M, Hochberg FH, et al. Survival after brain metastases from breast cancer in the trastuzumab era. *J Clin Oncol*. 2005; 23:2114–2116. author reply 2116–2117. [PubMed: 15774813]
- Kodaira H, Kusuhara H, Ushiki J, Fuse E, Sugiyama Y. Kinetic analysis of the cooperation of P-glycoprotein (P-gp/Abcb1) and breast cancer resistance protein (Bcrp/Abcg2) in limiting the brain and testis penetration of erlotinib, flavopiridol, and mitoxantrone. *J Pharmacol Exp Ther*. 2010; 333:788–796. [PubMed: 20304939]
- Komori T, Narabayashi I, Matsui R, Sueyoshi K, Aratani T, Utsunomiya K. Technetium-99m MIBI single photon emission computed tomography as an indicator of prognosis for patients with lung cancer-preliminary report. *Ann Nucl Med*. 2000; 14:415–420. [PubMed: 11210093]
- Koshiba H, Hosokawa K, Mori T, Kubo A, Watanabe A, Honjo H. Intravenous paclitaxel is specifically retained in human gynecologic carcinoma tissues in vivo. *Int J Gynecol Cancer*. 2009; 19:484–488. [PubMed: 19509540]
- Kurdziel K, Kalen J, Hirsch J, Wilson J, Agarwal R, Barrett D, et al. Imaging multidrug resistance with 4-[18F]fluoropaclitaxel. *Nucl Med Biol*. 2007; 34:823–831. [PubMed: 17921033]
- Kurdziel KA, Kalen JD, Hirsch JI, Wilson JD, Bear HD, Logan J, et al. Human dosimetry and preliminary tumor distribution of 18F-fluoropaclitaxel in healthy volunteers and newly diagnosed breast cancer patients using PET/CT. *J Nucl Med*. 2011; 52:1339–1345. [PubMed: 21849404]
- Lagas JS, van Waterschoot RA, Sparidans RW, Wagenaar E, Beijnen JH, Schinkel AH. Breast cancer resistance protein and P-glycoprotein limit sorafenib brain accumulation. *Mol Cancer Ther*. 2010; 9:319–326. [PubMed: 20103600]
- Lankelma J, Dekker H, Luque FR, Luykx S, Hoekman K, van der Valk P, et al. Doxorubicin gradients in human breast cancer. *Clin Cancer Res*. 1999; 5:1703–1707. [PubMed: 10430072]

- Larkin A, O'Driscoll L, Kennedy S, Purcell R, Moran E, Crown J, et al. Investigation of MRP-1 protein and MDR-1 P-glycoprotein expression in invasive breast cancer: a prognostic study. *Int J Cancer*. 2004; 112:286–294. [PubMed: 15352042]
- Lazarova N, Zoghbi SS, Hong J, Seneca N, Tuan E, Gladding RL, et al. Synthesis and evaluation of [N-methyl-11C]N-desmethyl-loperamide as a new and improved PET radiotracer for imaging P-gp function. *J Med Chem*. 2008; 51:6034–6043. [PubMed: 18783208]
- Lehnert M, Mross K, Schueller J, Thuerlimann B, Kroeger N, Kupper H. Phase II trial of dexverapamil and epirubicin in patients with non-responsive metastatic breast cancer. *Br J Cancer*. 1998; 77:1155–1163. [PubMed: 9569055]
- Lemos C, Giovannetti E, Zucali PA, Assaraf YG, Scheffer GL, van der Straaten T, et al. Impact of ABCG2 polymorphisms on the clinical outcome and toxicity of gefitinib in non-small-cell lung cancer patients. *Pharmacogenomics*. 2011; 12:159–170. [PubMed: 21332310]
- Levchenko A, Mehta BM, Lee JB, Humm JL, Augensen F, Squire O, et al. Evaluation of C-11-colchicine for PET imaging of multiple drug resistance. *J Nucl Med*. 2000; 41:493–501. [PubMed: 10716325]
- Li C, Yu DF, Inoue T, Yang DJ, Tansey W, Liu CW, et al. Synthesis, biodistribution and imaging properties of indium-111-DTPA-paclitaxel in mice bearing mammary tumors. *J Nucl Med*. 1997; 38:1042–1047. [PubMed: 9225788]
- Li J, Li ZN, Du YJ, Li XQ, Bao QL, Chen P. Expression of MRP1, BCRP, LRP, and ERCC1 in advanced non-small-cell lung cancer: correlation with response to chemotherapy and survival. *Clin Lung Cancer*. 2009a; 10:414–421. [PubMed: 19900859]
- Li J, Li ZN, Yu LC, Bao QL, Wu JR, Shi SB, et al. Association of expression of MRP1, BCRP, LRP and ERCC1 with outcome of patients with locally advanced non-small cell lung cancer who received neoadjuvant chemotherapy. *Lung Cancer*. 2010; 69:116–122. [PubMed: 19875192]
- Li XQ, Li J, Shi SB, Chen P, Yu LC, Bao QL. Expression of MRP1, BCRP, LRP and ERCC1 as prognostic factors in non-small cell lung cancer patients receiving postoperative cisplatin-based chemotherapy. *Int J Biol Markers*. 2009b; 24:230–237. [PubMed: 20082278]
- Lin NU, Bellon JR, Winer EP. CNS metastases in breast cancer. *J Clin Oncol*. 2004; 22:3608–3617. [PubMed: 15337811]
- Lin NU, Carey LA, Liu MC, Younger J, Come SE, Ewend M, et al. Phase II trial of lapatinib for brain metastases in patients with human epidermal growth factor receptor 2-positive breast cancer. *J Clin Oncol*. 2008; 26:1993–1999. [PubMed: 18421051]
- Lin NU, Dieras V, Paul D, Loissignol D, Christodoulou C, Stemmler HJ, et al. Multicenter phase II study of lapatinib in patients with brain metastases from HER2-positive breast cancer. *Clin Cancer Res*. 2009; 15:1452–1459. [PubMed: 19228746]
- Liu B, Sun D, Xia W, Hung MC, Yu D. Cross-reactivity of C219 anti-p170(mdr-1) antibody with p185(c-erbB2) in breast cancer cells: cautions on evaluating p170(mdr-1). *J Natl Cancer Inst*. 1997; 89:1524–1529. [PubMed: 9337349]
- Lopez M, Vici P, Di Lauro L, Paoletti G, Gionfra T, Conti F, et al. Inpatient comparison of single-agent epirubicin with or without lonidamine in metastatic breast cancer. *Eur J Cancer*. 1995; 31A:1611–1614. [PubMed: 7488410]
- Mankoff DA, Dunnwald LK, Gralow JR, Ellis GK, Drucker MJ, Livingston RB. Monitoring the response of patients with locally advanced breast carcinoma to neoadjuvant chemotherapy using [technetium 99m]-sestamibi scintimammography. *Cancer*. 1999; 85:2410–2423. [PubMed: 10357412]
- Mickley LA, Spengler BA, Knutsen TA, Biedler JL, Fojo T. Gene rearrangement: a novel mechanism for MDR-1 gene activation. *J Clin Invest*. 1997; 99:1947–1957. [PubMed: 9109439]
- Mignogna C, Staibano S, Altieri V, De Rosa G, Pannone G, Santoro A, et al. Prognostic significance of multidrug-resistance protein (MDR-1) in renal clear cell carcinomas: a five year follow-up analysis. *BMC Cancer*. 2006; 6:293. [PubMed: 17177989]
- Mohan HK, Miles KA. Cost-effectiveness of 99mTc-sestamibi in predicting response to chemotherapy in patients with lung cancer: systematic review and meta-analysis. *J Nucl Med*. 2009; 50:376–381. [PubMed: 19223414]

- Morisaki K, Robey RW, Ozvegy-Laczka C, Honjo Y, Polgar O, Steadman K, et al. Single nucleotide polymorphisms modify the transporter activity of ABCG2. *Cancer Chemother Pharmacol*. 2005; 56:161–172. [PubMed: 15838659]
- Moureau-Zabotto L, Ricci S, Lefranc JP, Coulet F, Genestie C, Antoine M, et al. Prognostic impact of multidrug resistance gene expression on the management of breast cancer in the context of adjuvant therapy based on a series of 171 patients. *Br J Cancer*. 2006; 94:473–480. [PubMed: 16434992]
- Mross K, Bohn C, Edler L, Jonat W, Queisser W, Heidemann E, et al. Randomized phase II study of single-agent epirubicin +/- verapamil in patients with advanced metastatic breast cancer. An AIO clinical trial Arbeitsgemeinschaft Internistische Onkologie of the German Cancer Society. *Ann Oncol*. 1993a; 4:45–50. [PubMed: 8435362]
- Mross K, Hamm K, Hossfeld DK. Effects of verapamil on the pharmacokinetics and metabolism of epirubicin. *Cancer Chemother Pharmacol*. 1993b; 31:369–375. [PubMed: 8431970]
- Murren JR, Durivage HJ, Buzaid AC, Reiss M, Flynn SD, Carter D, et al. Trifluoperazine as a modulator of multidrug resistance in refractory breast cancer. *Cancer Chemother Pharmacol*. 1996; 38:65–70. [PubMed: 8603454]
- Myslivecek M, Koranda P, Kaminek M, Husak V, Hartlova M, Duskova M, et al. Technetium-99m-MIBI scintimammography by planar and SPECT imaging in the diagnosis of breast carcinoma and axillary lymph node involvement. *Nucl Med Rev Cent East Eur*. 2004; 7:151–155. [PubMed: 15968602]
- Nishiyama Y, Yamamoto Y, Satoh K, Ohkawa M, Kameyama K, Hayashi E, et al. Comparative study of Tc-99m MIBI and TI-201 SPECT in predicting chemotherapeutic response in non-small-cell lung cancer. *Clin Nucl Med*. 2000; 25:364–369. [PubMed: 10795697]
- O'Connor R, O'Leary M, Ballot J, Collins CD, Kinsella P, Mager DE, et al. A phase I clinical and pharmacokinetic study of the multi-drug resistance protein-1 (MRP-1) inhibitor sulindac, in combination with epirubicin in patients with advanced cancer. *Cancer Chemother Pharmacol*. 2007; 59:79–87. [PubMed: 16642371]
- Ota S, Ishii G, Goto K, Kubota K, Kim YH, Kojika M, et al. Immunohistochemical expression of BCRP and ERCC1 in biopsy specimen predicts survival in advanced non-small-cell lung cancer treated with cisplatin-based chemotherapy. *Lung Cancer*. 2009; 64:98–104. [PubMed: 18823676]
- Ozulker T, Ozulker F, Ozpacaci T, Bender O, Degirmenci H. The efficacy of (99m)Tc-MIBI scintimammography in the evaluation of breast lesions and axillary involvement: a comparison with X-rays mammography, ultrasonography and magnetic resonance imaging. *Hell J Nucl Med*. 2010; 13:144–149. [PubMed: 20808988]
- Paredes Lario A, Blanco Garcia C, Echenique Elizondo M, Lobo C. [Expression of proteins associated with multidrug resistance and resistance to chemotherapy in lung cancer]. *Arch Bronconeumol*. 2007; 43:479–484. [PubMed: 17919413]
- Park S, Shimizu C, Shimoyama T, Takeda M, Ando M, Kohno T, et al. Gene expression profiling of ATP-binding cassette (ABC) transporters as a predictor of the pathologic response to neoadjuvant chemotherapy in breast cancer patients. *Breast Cancer Res Treat*. 2006; 99:9–17. [PubMed: 16752223]
- Perou CM, Sorlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, et al. Molecular portraits of human breast tumours. *Nature*. 2000; 406:747–752. [PubMed: 10963602]
- Platta CS, Khuntia D, Mehta MP, Suh JH. Current treatment strategies for brain metastasis and complications from therapeutic techniques: a review of current literature. *Am J Clin Oncol*. 2010; 33:398–407. [PubMed: 19675447]
- Poller B, Iusuf D, Sparidans RW, Wagenaar E, Beijnen JH, Schinkel AH. Differential impact of P-glycoprotein (ABCB1) and breast cancer resistance protein (ABCG2) on axitinib brain accumulation and oral plasma pharmacokinetics. *Drug Metab Dispos*. 2011; 39:729–735. [PubMed: 21282407]
- Polli JW, Olson KL, Chism JP, John-Williams LS, Yeager RL, Woodard SM, et al. An unexpected synergist role of P-glycoprotein and breast cancer resistance protein on the central nervous system penetration of the tyrosine kinase inhibitor lapatinib (N-{3-chloro-4-[(3-fluorobenzyl)oxy]phenyl}-6-[5-({[2-(methylsulfonyl)ethyl]amino}methyl)-2-furyl]-4-quinazolinamine; GW572016). *Drug Metab Dispos*. 2009; 37:439–442. [PubMed: 19056914]

- Pusztai L, Wagner P, Ibrahim N, Rivera E, Theriault R, Booser D, et al. Phase II study of tariquidar, a selective P-glycoprotein inhibitor, in patients with chemotherapy-resistant, advanced breast carcinoma. *Cancer*. 2005; 104:682–691. [PubMed: 15986399]
- Ravert H, Klecker R, Collins J, Mathews W, Pomper M, Wahl R, et al. Radiosynthesis of [C-11]paclitaxel. *J Labelled Cpd Radiopharm*. 2002; 45:471–477.
- Ricciardi S, de Marinis F. Multimodality management of non-small cell lung cancer patients with brain metastases. *Curr Opin Oncol*. 2010; 22:86–93. [PubMed: 20009927]
- Ries F, Dicato M. Treatment of advanced and refractory breast cancer with doxorubicin, vincristine and continuous infusion of verapamil. A phase I-II clinical trial. *Med Oncol Tumor Pharmacother*. 1991; 8:39–43. [PubMed: 2041381]
- Rijavec M, Silar M, Triller N, Kern I, Cegovnik U, Kosnik M, et al. Expressions of topoisomerase IIalpha and BCRP in metastatic cells are associated with overall survival in small cell lung cancer patients. *Pathol Oncol Res*. 2011; 17:691–696. [PubMed: 21455636]
- Robey RW, Polgar O, Deeken J, To KW, Bates SE. ABCG2: determining its relevance in clinical drug resistance. *Cancer Metastasis Rev*. 2007; 26:39–57. [PubMed: 17323127]
- Rossi C, Gasparini G, Canobbio L, Galligioni E, Volpe R, Candiani E, et al. Doxorubicin distribution in human breast cancer. *Cancer Treat Rep*. 1987; 71:1221–1226. [PubMed: 3690533]
- Rudas M, Filipits M, Taucher S, Stranzl T, Steger GG, Jakesz R, et al. Expression of MRP1, LRP and Pgp in breast carcinoma patients treated with preoperative chemotherapy. *Breast Cancer Res Treat*. 2003; 81:149–157. [PubMed: 14572157]
- Rybarova S, Hodorova I, Hajdukova M, Schmidtova K, Mojzis J, Kajo K, et al. Expression of MDR proteins in breast cancer and its correlation with some clinical and pathological parameters. *Neoplasma*. 2006; 53:128–135. [PubMed: 16575468]
- Sadeghi R, Zakavi SR, Forghani MN, Aryana K, Kakhki VR, Ayati NK, et al. The efficacy of Tc-99m sestamibi for sentinel node mapping in breast carcinomas: comparison with Tc-99m antimony sulphide colloid. *Nucl Med Rev Cent East Eur*. 2010; 13:1–4. [PubMed: 21154308]
- Saeki T, Nomizu T, Toi M, Ito Y, Noguchi S, Kobayashi T, et al. Dofequidar fumarate (MS-209) in combination with cyclophosphamide, doxorubicin, and fluorouracil for patients with advanced or recurrent breast cancer. *J Clin Oncol*. 2007; 25:411–417. [PubMed: 17179098]
- Saison C, Helias V, Ballif BA, Peyrard T, Puy H, Miyazaki T, et al. Null alleles of ABCG2 encoding the breast cancer resistance protein define the new blood group system Junior. *Nat Genet*. 2012; 44:174–177. [PubMed: 22246505]
- Sampalis FS, Denis R, Picard D, Fleischer D, Martin G, Nassif E, et al. International prospective evaluation of scintimammography with (99m)technetium sestamibi. *Am J Surg*. 2003; 185:544–549. [PubMed: 12781883]
- Schinkel AH. Pharmacological insights from P-glycoprotein knockout mice. *Int J Clin Pharmacol Ther*. 1998; 36:9–13. [PubMed: 9476142]
- Sciuto R, Pasqualoni R, Bergomi S, Petrilli G, Vici P, Belli F, et al. Prognostic value of (99m)Tc-sestamibi washout in predicting response of locally advanced breast cancer to neoadjuvant chemotherapy. *J Nucl Med*. 2002; 43:745–751. [PubMed: 12050317]
- Shaffer BC, Gillet JP, Patel C, Baer MR, Bates SE, Gottesman MM. Drug resistance: Still a daunting challenge to the successful treatment of AML. *Drug Resist Updates*. 2012 in press.
- Shepherd FA, Rodrigues Pereira J, Ciuleanu T, Tan EH, Hirsh V, Thongprasert S, et al. Erlotinib in previously treated non-small-cell lung cancer. *N Engl J Med*. 2005; 353:123–132. [PubMed: 16014882]
- Shih CM, Shiau YC, Wang JJ, Ho ST, Kao A. Using technetium-99m tetrofosmin chest imaging to predict taxol-based chemotherapy response in non-small cell lung cancer but not related to lung resistance protein expression. *Lung*. 2003; 181:103–111. [PubMed: 12953149]
- Stallard S, Morrison JG, George WD, Kaye SB. Distribution of doxorubicin to normal breast and tumour tissue in patients undergoing mastectomy. *Cancer Chemother Pharmacol*. 1990; 25:286–290. [PubMed: 2295114]
- Steeg PS, Camphausen KA, Smith QR. Brain metastases as preventive and therapeutic targets. *Nat Rev Cancer*. 2011; 11:352–363. [PubMed: 21472002]

- Stewart DJ. Tumor and host factors that may limit efficacy of chemotherapy in non-small cell and small cell lung cancer. *Crit Rev Oncol Hematol*. 2010; 75:173–234. [PubMed: 20047843]
- Sun SS, Hsieh JF, Tsai SC, Ho YJ, Lee JK, Kao CH. Expression of mediated P-glycoprotein multidrug resistance related to Tc-99m MIBI scintimammography results. *Cancer Lett*. 2000; 153:95–100. [PubMed: 10779636]
- Surowiak P, Materna V, Matkowski R, Szczuraszek K, Kornafel J, Wojnar A, et al. Relationship between the expression of cyclooxygenase 2 and MDR1/P-glycoprotein in invasive breast cancers and their prognostic significance. *Breast Cancer Res*. 2005; 7:R862–870. [PubMed: 16168133]
- Szakacs G, Paterson JK, Ludwig JA, Booth-Genthe C, Gottesman MM. Targeting multidrug resistance in cancer. *Nat Rev Drug Discov*. 2006; 5:219–234. [PubMed: 16518375]
- Tang SC, Lagas JS, Lankheet NA, Poller B, Hillebrand MJ, Rosing H, et al. Brain accumulation of sunitinib is restricted by P-glycoprotein (ABCB1) and breast cancer resistance protein (ABCG2) and can be enhanced by oral elacridar and sunitinib coadministration. *Int J Cancer*. 2012; 130:223–233. [PubMed: 21351087]
- Taskar KS, Rudraraju V, Mittapalli RK, Samala R, Thorsheim HR, Lockman J, et al. Lapatinib distribution in HER2 overexpressing experimental brain metastases of breast cancer. *Pharm Res*. 2011 in press.
- Tolcher AW, Cowan KH, Solomon D, Ognibene F, Goldspiel B, Chang R, et al. Phase I crossover study of paclitaxel with r-verapamil in patients with metastatic breast cancer. *J Clin Oncol*. 1996; 14:1173–1184. [PubMed: 8648372]
- Toppmeyer D, Seidman AD, Pollak M, Russell C, Tkaczuk K, Verma S, et al. Safety and efficacy of the multidrug resistance inhibitor Incel (biricodar; VX-710) in combination with paclitaxel for advanced breast cancer refractory to paclitaxel. *Clin Cancer Res*. 2002; 8:670–678. [PubMed: 11895894]
- Triller N, Korosec P, Kern I, Kosnik M, Debeljak A. Multidrug resistance in small cell lung cancer: expression of P-glycoprotein, multidrug resistance protein 1 and lung resistance protein in chemo-naïve patients and in relapsed disease. *Lung Cancer*. 2006; 54:235–240. [PubMed: 16934363]
- Trump DL, Smith DC, Ellis PG, Rogers MP, Schold SC, Winer EP, et al. High-dose oral tamoxifen, a potential multidrug-resistance-reversal agent: phase I trial in combination with vinblastine. *J Natl Cancer Inst*. 1992; 84:1811–1816. [PubMed: 1359155]
- Tsuruo T, Iida H, Tsukagoshi S, Sakurai Y. Overcoming of vincristine resistance in P388 leukemia in vivo and in vitro through enhanced cytotoxicity of vincristine and vinblastine by verapamil. *Cancer Res*. 1981; 41:1967–1972. [PubMed: 7214365]
- Ushijima R, Takayama K, Izumi M, Harada T, Horiuchi Y, Uchino J, et al. Immunohistochemical expression of MRP2 and clinical resistance to platinum-based chemotherapy in small cell lung cancer. *Anticancer Res*. 2007; 27:4351–4358. [PubMed: 18214043]
- Usuda J, Tsunoda Y, Ichinose S, Ishizumi T, Ohtani K, Maehara S, et al. Breast cancer resistant protein (BCRP) is a molecular determinant of the outcome of photodynamic therapy (PDT) for centrally located early lung cancer. *Lung Cancer*. 2010; 67:198–204. [PubMed: 19477032]
- Vaclavikova R, Nordgard SH, Alnaes GI, Hubackova M, Kubala E, Kodet R, et al. Single nucleotide polymorphisms in the multidrug resistance gene 1 (ABCB1): effects on its expression and clinicopathological characteristics in breast cancer patients. *Pharmacogenet Genomics*. 2008; 18:263–273. [PubMed: 18300948]
- van Kalken CK, van der Hoeven JJ, de Jong J, Giaccone G, Schuurhuis GJ, Maessen PA, et al. Bepridil in combination with anthracyclines to reverse anthracycline resistance in cancer patients. *Eur J Cancer*. 1991; 27:739–744. [PubMed: 1829915]
- van Tilburg EW, Franssen EJJ, van der Hoeven JJM, van der Meij M, Elshove D, Lammertsma AA, et al. Radiosynthesis of [C-11]docetaxel. *J Label Compd Radiopharm*. 2004; 47:763–777.
- Vogel CL, Cobleigh MA, Tripathy D, Gutheil JC, Harris LN, Fehrenbacher L, et al. First-line Herceptin monotherapy in metastatic breast cancer. *Oncology*. 2001; 61(2):37–42. [PubMed: 11694786]

- Warner E, Hedley D, Andrulis I, Myers R, Trudeau M, Warr D, et al. Phase II study of dexverapamil plus anthracycline in patients with metastatic breast cancer who have progressed on the same anthracycline regimen. *Clin Cancer Res.* 1998; 4:1451–1457. [PubMed: 9626462]
- Wilson WH, Bates SE, Fojo A, Bryant G, Zhan Z, Regis J, et al. Controlled trial of dexverapamil, a modulator of multidrug resistance, in lymphomas refractory to EPOCH chemotherapy. *J Clin Oncol.* 1995; 13:1995–2004. [PubMed: 7636540]
- Wishart GC, Bissett D, Paul J, Jodrell D, Harnett A, Habeshaw T, et al. Quinidine as a resistance modulator of epirubicin in advanced breast cancer: mature results of a placebo-controlled randomized trial. *J Clin Oncol.* 1994; 12:1771–1777. [PubMed: 8083699]
- Witgert ME, Meyers CA. Neurocognitive and quality of life measures in patients with metastatic brain disease. *Neurosurg Clin N Am.* 2011; 22:79–85. vii. [PubMed: 21109152]
- Woodward OM, Kottgen A, Coresh J, Boerwinkle E, Guggino WB, Kottgen M. Identification of a urate transporter, ABCG2, with a common functional polymorphism causing gout. *Proc Natl Acad Sci U S A.* 2009; 106:10338–10342. [PubMed: 19506252]
- Xiang L, Su P, Xia S, Liu Z, Wang Y, Gao P, et al. ABCG2 is associated with HER-2 expression, lymph node metastasis and clinical stage in breast invasive ductal carcinoma. *Diagn Pathol.* 2011; 6:90. [PubMed: 21943250]
- Yeh JJ, Hsu NY, Hsu WH, Tsai CH, Lin CC, Liang JA. Comparison of chemotherapy response with P-glycoprotein, multidrug resistance-related protein-1, and lung resistance-related protein expression in untreated small cell lung cancer. *Lung.* 2005; 183:177–183. [PubMed: 16078039]
- Yeh JJ, Hsu WH, Wang JJ, Ho ST, Kao A. Predicting chemotherapy response to paclitaxel-based therapy in advanced non-small-cell lung cancer with P-glycoprotein expression. *Respiration.* 2003; 70:32–35. [PubMed: 12584388]
- Yoh K, Ishii G, Yokose T, Minegishi Y, Tsuta K, Goto K, et al. Breast cancer resistance protein impacts clinical outcome in platinum-based chemotherapy for advanced non-small cell lung cancer. *Clin Cancer Res.* 2004; 10:1691–1697. [PubMed: 15014021]
- Yuksel M, Cermik TF, Doganay L, Karlikaya C, Cakir E, Salan A, et al. 99mTc-MIBI SPET in non-small cell lung cancer in relationship with Pgp and prognosis. *Eur J Nucl Med Mol Imaging.* 2002; 29:876–881. [PubMed: 12111127]
- Zhou M, Johnson N, Gruner S, Ecklund GW, Meunier P, Bryn S, et al. Clinical utility of breast-specific gamma imaging for evaluating disease extent in the newly diagnosed breast cancer patient. *Am J Surg.* 2009; 197:159–163. [PubMed: 19185109]

Figure 1.

FPAC imaging of a patient with breast cancer. Fused PET/CT (top row, axial and coronal views) and PET (bottom row, axial and coronal views) of a female patient with breast cancer 80 minutes after the injection of 6.1mCi ^{18}F fluoropaclitaxel (FPAC). Right breast tumor (arrow) showing increased FPAC uptake (SUV of 1.3). Note absence of uptake in the brain due to the blood-brain-barrier (BBB) (a portion of the pituitary gland outside the BBB seen (solid arrow head)). Physiologic uptake in the heart, liver, bowel and bone marrow are also seen, as is residual tracer in the vasculature of the injected arm (brackets). Due to the extensive hepatic clearance and subsequent excretion into bowel, the diagnostic value of FPAC PET in the abdomen and pelvis is limited. A pilot study of FPAC PET in patients with a renal, adrenal, lung and breast cancer patients is ongoing (<http://clinicaltrials.gov/ct2/show/NCT01086696>).

Figure 2.

The impact of ABC transporters on CNS uptake in murine knockout studies. Data from 6 separate studies were compiled to generate the bar graphs shown (de Vries et al., 2007; Kodaira et al., 2010; Lagas et al., 2010; Poller et al., 2011; Polli et al., 2009; Tang et al., 2012). All of the studies employed mice bearing knockout of ABCB1a/b, ABCG2, or both ABCB1a/b and ABCG2 mice. Brain concentrations were reported as relative to wild type and were measured at different timepoints – lapatinib, 24 h; topotecan, 24 h; mitoxantrone, 2 h; axitinib, 1 h; sorafenib, 6 h; and sunitinib, 6 h. Mitoxantrone data were expressed as C_{brain}/C_{plasma} and estimated from Figure 4 in the reference (Kodaira et al., 2010), while topotecan data were reported as area under the concentration curve (AUC) (de Vries et al., 2007).

Table 1

Phase I studies in breast cancer

Modulator	Agent	Cancer type	N	Result/Toxicity	Reference
HD Tamoxifen	Vinblastine	Epithelial tumors	53	DLT: neurotoxicity	(Trump et al., 1992)
Verapamil	Epirubicin	Breast cancer	10	+ Significant interaction	(Mross et al., 1993b)
R-verapamil	Paclitaxel	Breast cancer	6		(Berg et al., 1995)
R-verapamil	Paclitaxel	Breast cancer	34	Paclitaxel alone: 6/34 18% PR	(Tolcher et al., 1996)
			29	Crossover: 0/29 PR Hematologic toxicity from paclitaxel + r-verapamil	
Valspodar	PEG-LD	Advanced cancer	14	1 PR in breast and ovarian carcinoma.No DLTs.	(Fracasso et al., 2005)
Sulindac	Epirubicin	Advanced cancer	17	2/15 PR (malignant melanoma and breast cancer)DLT 800 mg: 1 renal impairment, 1 fatal haemoptysis in lung cancer	(O'Connor et al., 2007)

HD: high dose

DLT: dose limiting toxicity

PR: partial response

N: number of patients

Table 2
Phase II studies in breast cancer

Inhibitor	Agent	N	Result	Reference
Verapamil	Adriamycin + Vincristine	16	3 PR (21%). Median OS 6 m	(Ries and Dicato, 1991)
Bepiridil	Anthracycline	14	3 SD	(van Kalken et al., 1991)
Trifluoperazine	Doxorubicin	20	9 PR (45%). Median DOR 17 wks	(Budd et al., 1993)
Verapamil (+/-)	Epirubicin	26	- EPI + verapamil: CR (4%), 7 PR (29%)	(Mross et al., 1993a)
		25	- EPI alone: 8 PR (28%)	
Quinidine	Epirubicin	106	- EPI + placebo: 6% CR, 38% PR; OS: 59 wk	(Wishart et al., 1994)
		107	- EPI + quinidine: 4% CR, 39% PR; OS: 47 wk	
Amiodarone	Doxorubicin or vinblastine	33	9/33 PR	(Bates et al., 1995)
Lonidamine	Epirubicin	45	EPI alone: 6 CR, 14 PR; DOR 6.5 mo	(Lopez et al., 1995)
		25	Crossover: EPI + lonidamine: 5 PR; DOR 7 mo	
Trifluoperazine	Vinblastine	16	1 PR (6%). Lasted 16 wks	(Murren et al., 1996)
Dexverapamil	Epirubicin	25	4 PR (17%). Lasted 3, 8, 11, 11+ m	(Lehnert et al., 1998)
Dexverapamil	Anthracycline	20	2 PR (10%). Lasted for 6 m	(Warner et al., 1998)
Biricodar	Paclitaxel	35	4 PR (11.4%). DOR 5.5 mo	(Toppmeyer et al., 2002)
Tariquidar	Doxorubicin or taxane-containing regimens	17	1 PR	(Pusztai et al., 2005)
Valspodar	Paclitaxel	34	2/34 CR 6%; 5/34 PR 15 %	(Carlson et al., 2006)

CR: complete response

PR: partial response

SD: stable disease

OS: overall survival

EPI: epirubicin

LND: lonidamine

N: number of patients

DOR: Duration of response

Table 3
Expression studies for MDR-1/Pgp, ABCB1/MRP, and ABCG2/BCRP: Correlation with clinical outcome

n	* treatment	**	MDR1 / Pgp / ABCB1	Correlation with outcome	MRP1 / ABCB1	***	Correlation with outcome	****	BCRP / ABCG2	***	Correlation with outcome	****	Reference
TABLE 3A: Breast cancer													
196													
25	A or E		Pgp induction	shorter PFS (p=, 0004) shorter OS (p=, 0025)	MRP1 induction		NS for PFS NS for OS		high ABCG2		lymph node metastasis (p=, 049), stage (p=, 015) HER2 expression (p=, 001)		(Xiang et al., 2011)
90			mRNA	NS for clinical or pathological characteristics									(Vaclavikova et al., 2008)
25	FAC or FEC		Pgp induction Pgp positive	poorer RR (p<, 001) poorer RR (p<, 001)	MRP1 induction MRP1 positive		NS for RR NS for RR						(Atalay et al., 2006)
171	FEC +/- radiotherapy +/- hormonal therapy		mRNA	NS for RFS NS for OS	mRNA		NS for RFS NS for OS						(Moureaud-Zabotto et al., 2006)
21	FEC + paclitaxel		mRNA	NS for RR	mRNA		NS for RR		mRNA		NS for RR		(Park et al., 2006)
87			Pgp	neg correlation with histological grade	MRP1		NS for histological grading status						(Rybarova et al., 2006)
50	FAC		Pgp positive	poorer RR (p<, 05)									(Chintamani et al., 2005)
516	CMF tamoxifen+goserelin				MRP1 positive MRP1 positive		shorter RFS (p=, 002) shorter OS (p=, 02) NS for OS and RFS						(Filipits et al., 2005)
104	CMF or tamoxifen +/- radiotherapy +/- hormonal therapy		high Pgp	higher grade (p<, 001) shorter OS (p<, 001)									(Surowiak et al., 2005)

n	* treatment	**	MDR1 / Pgp / ABCB1 ***	Correlation with outcome ****	MRP1 / ABCC1 ***	Correlation with outcome ****	BCRP / ABCG2 ***	Correlation with outcome ****	Reference
TABLE 3A: Breast cancer									
shorter PFS (p<.0001)									
177	CMF	high Pgp	NS for RFS NS for OS	high MRP1	shorter RFS (p=.0181) shorter OS (p=.0171)	NS for OS NS for RFS	high mRNA	NS for OS NS for PFS	(Larkin et al., 2004)
59	CMF+ FAC-FEC	high mRNA	shorter OS (p=.05) shorter PFS (p<.001)	high mRNA	NS for OS NS for RFS	NS for OS NS for RFS	high mRNA	NS for OS NS for PFS	(Burger et al., 2003)
	FAC-FEC	high mRNA	shorter OS (p<.001) shorter PFS (p=.007)	high mRNA	shorter OS (p=.056) shorter PFS (p=.04)	shorter OS (p=.056) shorter PFS (p=.04)	high mRNA	NS for OS NS for PFS	
	CMF	high mRNA	NS for OS or PFS	high mRNA	NS for OS or PFS	NS for OS or PFS			
80	CMF, anthracycline-based or taxane-based chemotherapy	Pgp pre-vs post-chemo Pgp positive pre-chemo	higher expression after treatment (p<.001) positive lymph nodes (p=.008)	MRP1 pre-vs post-chemo MRP1 positive pre-chemo	higher expression after treatment (p<.001) distant metastases and shorter PFS (p=.02)	higher expression after treatment (p<.001) distant metastases and shorter PFS (p=.02)			(Rudas et al., 2003)
52	anthracycline						mRNA/ ABCG2	NS for PFS or RR no anthracycline-induced expression	(Faneyte et al., 2002)

n	* treatment	**	MDR1 / Pgp / ABCB1 ***	Correlation with outcome ****	MRP1 / ABCC1 ***	Correlation with outcome ****	BCRP / ABCG2 ***	Correlation with outcome ****	Reference
TABLE 3B: Lung Cancer									
49	NSCLC	gefitinib					ABCG2 positive	shorter PFS (p=.026) shorter OS (p=.005)	(Chen et al., 2011)
94	NSCLC	gefitinib					ABCG2	NS for RR, time to progression and OS	(Lemos et al., 2011)
21	both	cisplatin-etoposide and/or CAVE	mRNA	NS for OS NS for RR	mRNA	NS for OS NS for RR	high mRNA	shorter OS (p=.034) NS for RR	(Rijavec et al., 2011)
46	NSCLC	cisplatin-based chemotherapy			high mRNA	poorer RR (p=.032), shorter PFS (p=.043)	mRNA	NS for RR NS for PFS	(Li et al., 2010)

TABLE 3B: Lung Cancer

n *	treatment **	MDR1 / Pgp / ABCB1 ***	Correlation with outcome ****	MRP1 / ABCC1 ***	Correlation with outcome ****	BCRP / ABCG2 ***	Correlation with outcome ****	Reference
TABLE 3B: Lung Cancer								
81	CLELC PDT				shorter OS (p=.019)		NS for OS	
						high ABCG2	poorer RR (P = .04) for lesion >1cm	(Usuda et al., 2010)
130	SCLC platinum-based chemotherapy	Pgp positive	NS for RR NS for PFS	MRP1 positive	NS for RR NS for PFS	ABCG2 positive	poorer RR (p=.026) shorter PFS (p=.0103)	(Kim et al., 2009)
60	NSCLC cisplatin-based chemotherapy			high mRNA	shorter PFS (p=.034) shorter OS (p=.021)	mRNA	NS for PFS NS for OS	(Li et al., 2009b)
66	NSCLC cisplatin-based chemotherapy			high mRNA	poorer RR (p=.046) shorter PFS (p=.012) shorter OS (p=.017)	mRNA	NS for RR NS for PFS NS for OS	(Li et al., 2009a)
156	NSCLC platinum-based chemotherapy					high ABCG2	shorter OS (p=.02) NS for RR or PFS	(Ota et al., 2009)
101	both not specified	Pgp positive	poorer RR (p<.05)					(Paredes Lario et al., 2007)
61	SCLC platinum-based/CAV	Pgp positive	poorer RR (p=.03)	MRP1 positive	NS for RR			(Ushijima et al., 2007)
17	SCLC cisplatin-etoposide	high Pgp	poorer RR (p<.0001)	high MRP1	poorer RR (p=.0002)			(Triller et al., 2006)
40	SCLC cisplatin-etoposide	Pgp positive	poorer RR (p<.01)	MRP1 positive	poorer RR (p<.01)			(Yeh et al., 2005)
72	NSCLC platinum-based chemotherapy	Pgp	NS for RR NS for OS NS for PFS	MRP1	NS for RR NS for OS NS for PFS	ABCG2 positive	poorer RR (p=.08) shorter PFS (p=.0003) shorter OS (p=.004)	(Yoh et al., 2004)
50	NSCLC paclitaxel	Pgp positive	poorer RR (p<.05)					(Yeh et al., 2003)
50	SCLC cisplatin-etoposide	high Pgp + high MRP1	poorer RR (p<.05)	high MRP1 + high Pgp	poorer RR (p<.05)			(Hsia et al., 2002)

* n, number of patients; "SCLC", "NSCLC" or "both" refer to study on small cell lung cancer, non-small cell lung cancer, or both cancers, respectively; CLELC: centrally located early lung cancer.

A: adriamycin (doxorubicin), C: cyclophosphamide, E: epirubicin, F: fluorouracil, M: methotrexate, V: vincristine; PDT: photodynamic therapy

Pgp, MRP1 and ABCG2 refer to the protein product. "High protein" and "protein positive" indicate that a threshold was set for high/low or for positivity/negativity, respectively

OS, overall survival; PFS, progression-free survival; RR, response rate; NS, no significant correlation