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Drug resistance in the mouse cancer clinic

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ABSTRACT

Drug resistance is one of the most pressing problems in treating cancer patients today. Local and regional disease can usually be adequately treated, but patients eventually die from distant metastases that have become resistant to all available chemotherapy. Although work on cultured tumor cell lines has yielded a lot of information on potential drug resistance mechanisms, it has proven difficult to translate these results to clinical drug resistance in patients. The controversy regarding the contribution of ABC transporters to drug resistance in patients is one example. The study of genetically engineered mouse models (GEMMs), which closely resemble cancer in human patients, can help to bridge this gap. In models for BRCA1- or BRCA2-associated breast cancer, we observed a substantial synergy between the defect in homology-directed DNA repair and sensitivity to DNA-targeting drugs. Nevertheless, tumors are not easily eradicated and eventually drug resistance develops. In this review we will discuss the use of the new generation mouse models to address major clinical problems, such as mechanisms of drug resistance, predicting chemotherapy response or characterizing the nature of residual tumor cells that escape eradication. Moreover, we will address the contribution of ABC transporters to drug resistance in our model.

1. Introduction

Over the past 4 decades we have gained detailed knowledge of the role of ATP-binding cassette (ABC) transporters in cellular processes. In particular the contribution of ABC transporters to drug resistance has been the subject of many investigations. For prokaryotic or eukaryotic cells that are confronted with xenobiotics, drug extrusion is one of the most effective mechanisms to escape death. A recent example comes from a high-throughput chemical screening and genome-wide association analysis in the malaria parasite *Plasmodium falciparum* (Yuan et al., 2011). Mutations in only 3 genes appear to explain resistance to nearly all useful compounds, including several agents that are also used to treat cancer. Two of these genes mediate drug efflux, one encodes the ABC transporter PfMDR1.

The contribution of mammalian ABC transporters to anti-cancer drug resistance was initially studied in cell lines (Szakacs et al., 2006). Upregulation of transporters correlated with acquired resistance, and the role of transporters in causing resistance was directly proven by overexpression of specific transporter genes in transfected cells. ABCB1/P-glycoprotein (P-gp) serves as the prime example. It was the first MDR protein identified in a drug-selected cell line (Juliano and Ling, 1976), and since its discovery a plethora of investigators found ABCB1 to be overexpressed in their favorite drug-resistant cell line. Genetic ablation of the *Abcb1a* and *Abcb1b* genes that encode ABCB1 in mice (Schinkel et al., 1997) greatly increased the vulnerability of animals to many compounds, supporting the idea that ABCB1 protects both normal and neoplastic cell types from xenotoxins. These include various key anti-cancer drugs used every day in clinical oncology, such as taxanes, anthracyclines and vinca alkaloids. ABCB1 also has an impact on novel drugs such as protein kinase inhibitors (Hegedus et al., 2009) or new drug combinations, as recently observed for lenalidomide plus temsirolimus in patients with relapsing multiple myeloma (Hofmeister et al., 2011). Several other ABC transporters have been associated with anti-cancer drug resistance, in particular ABCG2 (Robey et al., 2010; Szakacs et al., 2006). Together, ABC transporters cover many classical and novel anti-cancer drugs and one would therefore expect these versatile drug eliminators to contribute to multidrug resistance in cancer patients.

Unfortunately, these preclinical studies on ABC transporters have not yet resulted in much benefit to medical oncologists and their patients. Several ABCB1 inhibitors that completely reversed resistance *in vitro* have yielded only marginal benefits to patients (Robey et al., 2010). Even the new generation ABCB1 inhibitor zosuquidar, which is more potent and specific than previous inhibitors, failed to improve the efficacy of standard chemotherapy for patients suffering from acute myeloid leukemia or breast

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cancer (Cripe et al., 2010; Ruff et al., 2009). In fact, some clinical oncologists have completely abandoned the concept of concurrent administration of an ABCB1 inhibitor with chemotherapy (Libby and Hromas, 2010). There are several factors that may explain these frustrating results: tumors of patients do not express the target; tools to detect the expression of ABC transporters are not reliable; redundancy of ABC transporters; the protection of normal tissues by ABC transporters requires dose reduction of the cytotoxic agent, if the transporter is inhibited; use of chemotherapy cocktails that contain drugs that are only poor substrates for the targeted transporter; inclusion of tumors for which ATP-dependent drug efflux is not a relevant mechanism of resistance; or a combination of some of these. The sobering conclusion is that we still do not know what drug transporters contribute to resistance in patients notwithstanding many years of intensive preclinical research. This is part of a broader frustration: we still do not know how tumors in patients become resistant to most classical cytotoxic chemotherapeutic agents.

2. The toolbox to study drug resistance in mouse models

Apparently, there is a disconnect between the knowledge gained from drug resistant cell lines studied in the test tube and the application of this knowledge to tumors in patients. The study of drug resistance in mouse models that mimic human cancer could bridge this gap. In mouse models various tools to detect ABC transporters in drug resistant tumors can be tested and optimized. Moreover, treatment schedules and the efficacy of inhibitors are more easily investigated in mice than in humans. Human clinical trials are time- and cost-intensive, and there are never enough patients to test all treatment options. Mice can also be genetically modified, an option not available in human patients. Genetic elimination of ABC transporter function, for instance, can provide unambiguous evidence that the transporter causes resistance. A disadvantage of using mouse models to study ABC transporters is that there may be species-specific properties in the regulation of gene expression or substrate handling.

Regarding the choice of animal model to study drug resistance, many options are available. Their advantages and disadvantages have been reviewed previously (Michalak and Jonkers, 2011; Politi and Pao, 2011; Rottenberg and Jonkers, 2008; Sharpless and DePinho, 2006), and the NCI has set up a useful homepage of available models (http://emice.nci.nih.gov). Classically, investigators have inserted human tumor pieces (xenografts) or human cancer cell lines into immunodeficient mice, but this provides a poor model for studying drug resistance. Such cell lines represent selected subpopulations of human cancers that have usually adapted to cell culture conditions for decades. Despite their advantages regarding experimental reproducibility, the resulting tumors are often a suboptimal surrogate of the original tumor they are derived from. They also rarely become resistant to drugs, presumably because even immunocompromized mice can still use their innate immune system to finish off the cancer cells that survive drug treatment.

Nevertheless, sophisticated xenografts can be useful in specific circumstances. Wu et al. (2009) used primary human breast epithelial organoids in which p53 knockdown was introduced together with HER2 or KRAS overexpression. These cells were grafted into 'humanized' mammary fat pads and eventually gave rise to preneoplastic lesions and carcinomas that resembled those found in patients. Moreover, the so-called "tumorgraft" models have regained popularity in recent years; an approach that was already established in the 1970s (Garber, 2009). Basically, fresh fragments of human tumors are propagated in immunodeficient animals, thereby preserving tumor heterogeneity and avoiding artificial *in vitro* selection and adaptation. One downside of this approach is that for some cancer types, *e.g.*, breast cancer, only a small fraction of individual cancers will eventually be useful for serial grafting (Marangoni et al., 2007). Those that do take also require several months to be established. We still do not understand the factors involved in this *in vivo* selection. However, those tumorgraft lines that do grow appear to resemble the original tumor well in morphology, genomic profile and drug response, as shown for breast, pancreatic and childhood cancers (Garrido-Laguna et al., 2011; Hidalgo et al., 2011; Houghton et al., 2007; Marangoni et al., 2007). The pediatric preclinical testing program is an instructive example of how tumorgraft models can be exploited to provide a useful complement to clinical trials with novel anti-cancer agents (http://pptp.nchresearch.org).

To study ABCB1-mediated drug resistance xenograft models are still frequently used. Patel and Tannock (2009) investigated ABCB1-overexpressing MCF-7 cells, in addition to a mouse mammary sarcoma cell line. Whereas tumor cells close to blood vessels did show an increased uptake of doxorubicin in combination with the ABCB1 inhibitors valspodar (PSC-833) and verapamil, an opposite effect was observed in distal tumor cells. The authors suggest that this unexpected (and unexplained) result may contribute to the poor clinical success of these inhibitors. Another example is the recent study by Emmink et al. (2011) in which spheres of colorectal tumor specimens were xenografted to investigate the role of ABCB1 to protect tumor-initiating cells from irinotecan. It was found that the ABCB1 inhibitor valspodar (PSC-833) increased the anti-tumor efficacy of irinotecan, but unexpectedly by inhibiting ABCB1 in more differentiated tumor cells, not the tumor-initiating ones.

For most drugs tested, xenografts in mice respond better to drug than tumors in patients. Differences in the setup of clinical trials and mouse experiments may explain part of this meagre congruence (Kelland, 2004; Kerbel, 2003). In most clinical trials patients have advanced, high-volume, metastatic disease, and they have already been treated with standard chemotherapy. In many animal studies a single drug-naïve microscopic or low-volume metastatic tumor is treated only a few days (or hours) after grafting. Another complication is that most anti-cancer drugs increase the immunogenicity of tumor cells in mice (Zitvogel et al., 2008). Once the xenograft has been debulked by drug, residual activity of the immune system of nude mice, *e.g.*, NK-T cells, may be sufficient to kill off the remaining foreign tumor cells.

For the study of drug resistance mechanisms xenograft models have one obvious disadvantage: once genes have been identified that might confer resistance, they cannot be modified genetically without prior tumor dissociation, *in vitro* culturing and selection. Here, genetically engineered mouse models (GEMMs) have an edge. GEMMs traditionally employ the introduction of germline modifications that result in the targeted expression or inhibition of selected genes in specific organs, as summarized elsewhere (Politi and Pao, 2011; Walrath et al., 2010). In particular the development of mouse strains with multiple gene replacements, guided by our knowledge of human cancer genetics, resulted in conditional GEMMs which mimic the stochastic tumorigenesis seen in patients (Jonkers and Berns, 2002). Tumors that arise in these models often resemble their human counterparts in their histopathological and genetic features. Moreover, the development of inducible models, in which genes within selected cells are switched on or off using a chemical or viral trigger, has further improved GEMMs. The generation and characterization of GEMMs requires years, however. The increasing influx of new drug target genes/networks derived from high-throughput genomic or proteomic analyses has therefore increased the need for non-germline methods to introduce genetic modifications more efficiently (Heyer et al., 2010). These include transplantation models in which genetically modified somatic stem

or progenitor cells are implanted into the corresponding adult tissue of a recipient mouse (Evers et al., 2010; Vafaizadeh et al., 2010; Zender et al., 2008). In case the isolation of stem or progenitor cells is a limiting factor, chimeric mice may be generated by modifying mouse embryonic stem cells (ESCs) (Watters et al., 2009; Zhou et al., 2010). Another promising approach is the possibility of generating chimeric mice using ES cells from already existing GEMMs (Huijbers et al., 2011). Potent and reversible inhibition of a gene of interest can be achieved in the mouse ESCs by the introduction of shRNA constructs (Premsrirut et al., 2011; Seibler et al., 2007). Hence, GEMMs offer a number of versatile tools that can be exploited to study drug responses (Kim and Sharpless, in press) and tackle anti-cancer drug resistance.

Thus far drug resistance to classical or targeted anti-cancer drugs has mainly been studied in GEMMs for lymphoma, lung, pancreatic and breast cancer (Olive et al., 2009; Pajic et al., 2009, 2010; Politi and Pao, 2011; Rottenberg et al., 2008; Rottenberg and Jonkers, 2008; Singh et al., 2010; Watters et al., 2009; Zander et al., 2010; Zhou et al., 2010; Zuber et al., 2009). In collaboration with the group of Jos Jonkers we have employed GEMMs for breast cancer to elucidate mechanisms underlying drug resistance. In these models conditional deletions of the *Brca1*, *Brca2*, or *E-cadherin* genes together with *p53*, have been introduced using the Cre/loxP system (Derksen et al., 2006, 2011; Jonkers et al., 2001; Liu et al., 2007). The stochastically developing mammary carcinomas that arise in these mice after several months mimic many aspects of the human disease. In particular, in the models for BRCA1/2-associated breast cancer the deficiency in proper DNA damage repair by homologous recombination (HR) is reflected by a high frequency of DNA copy number alterations that is comparable to the corresponding alterations in human tumors (Holstege et al., 2010a,b). A disadvantage of the BRCA1/2 models is the absence of macroscopically visible metastases. Nevertheless, the response to drug of distant metastases can be studied in the other models for invasive lobular breast cancer, in which the loss of E-cadherin drives metastasis formation. An advantage of these models is that tumors derived from the same original tumor can be orthotopically transplanted into fully immunocompetent, syngeneic mice (Rottenberg et al., 2007). Importantly, the grafted tumors preserve the morphology, the genomic fingerprint, and the response to anti-cancer agents of the original tumor. The orthotopic transplantation of tumor pieces or dissociated tumor cells, without any intermediate *in vitro* culturing step, is helpful in assessing how transplanted tumors originating from the same primary tumor respond to different chemotherapeutics.

3. Upfront lack of drug sensitivity

We have treated animals carrying *Brca1*$^{-/-}$,*p53*$^{-/-}$; *Abcb1a/b*$^{-/-}$,*Brca1*$^{-/-}$,*p53*$^{-/-}$; *Abcg2*$^{-/-}$,*Brca1*$^{-/-}$,*p53*$^{-/-}$; *Brca2*$^{-/-}$,*p53*$^{-/-}$; *E-cadherin*$^{-/-}$,*p53*$^{-/-}$; *Abcb1a/b*$^{-/-}$,*p53*$^{-/-}$ or *p53*$^{-/-}$ mammary tumors with the maximum tolerable dose (MTD) of the topoisomerase II inhibitor doxorubicin, the topoisomerase I inhibitor topotecan, the taxane docetaxel or the DNA crosslinking agent cisplatin, anti-cancer drugs that are regularly used in the clinic. We found that *Brca1*$^{-/-}$,*p53*$^{-/-}$ and *Brca2*$^{-/-}$,*p53*$^{-/-}$ tumors are highly sensitive to the DNA damage inflicted by doxorubicin, topotecan, or cisplatin ((Rottenberg et al., 2007; Zander et al., 2010) and unpublished). Examples of responses to the MTD of doxorubicin or cisplatin are shown in Fig. 1. This sensitivity is not unexpected given the function of BRCA1 or BRCA2 in homology-directed DNA repair. Many tumors also responded to the microtubule-targeting docetaxel (Rottenberg et al., 2007). In contrast, *E-cadherin*$^{-/-}$,*p53*$^{-/-}$ or *p53*$^{-/-}$ mammary tumors only showed a modest response, even tumors that grow as rapidly as the BRCA1/2-deficient tumors (unpublished observation). Hence, fast proliferation is not sufficient to make tumor cells susceptible to chemotherapy.

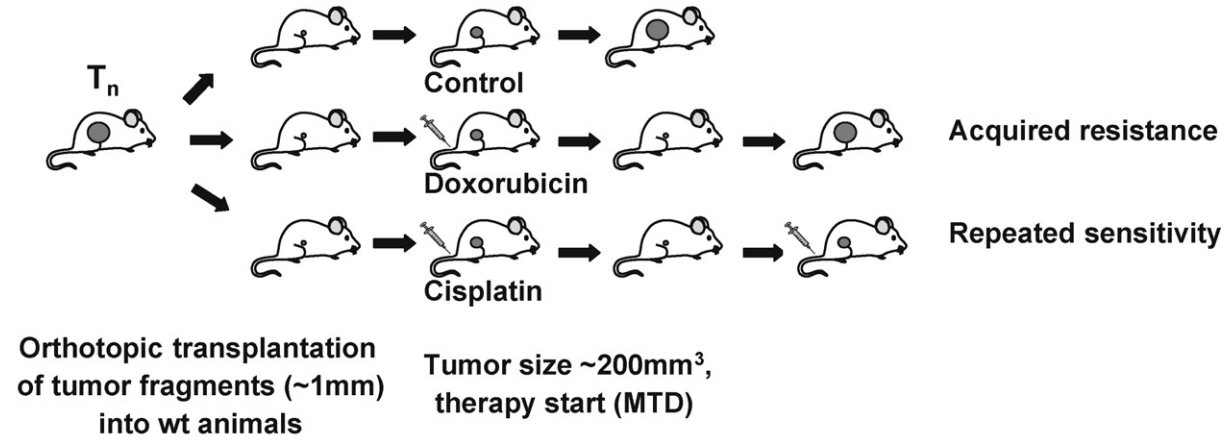
In contrast, defects in DNA repair have an enormous impact. This is illustrated by the success of the poly(ADP-ribose)-polymerase (PARP) inhibitor olaparib (AZD2281). PARP inhibition prevents repair of single strand DNA breaks, which are eventually converted into double strand breaks during DNA replication. Whereas homologous recombination-proficient cells are capable of repairing these breaks in an error-free manner, PARP inhibition induces synthetic lethality in BRCA1- or BRCA2-deficient cells (Bryant et al., 2005; Farmer et al., 2005). In line with this hypothesis, we observed in our BRCA models a high olaparib sensitivity whereas *E-cadherin*$^{-/-}$,*p53*$^{-/-}$ or *p53*$^{-/-}$ mammary tumors did not respond (Evers et al., 2008; Rottenberg et al., 2008). This olaparib sensitivity was also observed in other BRCA1/2 mouse models (Hay et al., 2009; Kortmann et al., 2011) and in human patients (Audeh et al., 2010; Fong et al., 2009, 2010; Tutt et al., 2010).

These results show that tumors do respond to chemotherapy if the tumor contains a well-defined tumor-specific target (*i.e.* a defect in DNA repair), if the drug specifically exploits this target, and if the drug reaches the target at sufficient concentrations. So why is there so much primary chemotherapy resistance in patients? The obvious answer is that we are still fairly ignorant. For most of the classical cytotoxic drugs, we still do not know exactly why they (sometimes) kill tumor cells more effectively than normal cells and why this killing does not occur or not sufficiently occur in real life.

This is where GEMMs can help to collect essential information. Obviously, the drug needs to reach its target to act, and this does not always happen, as shown for gemcitabine by Olive et al. (2009) using a GEMM for pancreatic cancer. In that GEMM drug delivery can be achieved by inhibiting the hedgehog signaling pathway. Even then the responses of the KRAS- and p53-mutated tumors are not impressive. A KRAS-driven GEMM for lung adenocarcinomas also barely responds to cisplatin (Oliver et al., 2010). Only by targeting translesion synthesis-mediated DNA repair, tumors were sensitized (Doles et al., 2010). These findings underline the urgent need to find new therapeutic approaches for tumors that are driven by oncogenic KRAS.

Based on the preclinical work, the identification of patients that carry tumors with a dysfunctional DNA damage response, such as HR deficiency (HRD), seems logical. At present, it is still unclear to what extend human tumors contain other HR deficiencies than *BRCA1/2* mutations. A recent integration of genomic and epigenomic data suggested that about half of the high-grade serous ovarian carcinomas are defective in HR (Cancer Genome Atlas Research Network, 2011). In addition, Rad51D was just identified as another HR pathway protein mutated in some ovarian cancers (Loveday et al., 2011). Features of BRCA dysfunction ("BRCAness") including mutations, promoter methylation, reduced RNA expression, typical histopathological features, or characteristic DNA copy number alterations are also frequent in the group of patients with hormone receptor- and HER2-negative ("triple-negative") breast cancer (Da Silva and Lakhani, 2010; Lips et al., 2011; Turner et al., 2004). In estrogen receptor-positive tumors, a BRCA2-like comparative genomic hybridization pattern and amplification of the *EMSY* gene, which encodes a BRCA2-inhibiting protein, were commonly found (Lips et al., 2011). In line with the preclinical data, recent studies have shown that tumors displaying features of HRD showed increased chemotherapy sensitivity (Graeser et al., 2010; Kriege et al., 2009; Lips et al., 2011; Silver et al., 2010; Vollebergh et al., 2011).

Nevertheless, these clinical trials also illustrate that the presence of a BRCA1/2 mutation is no guarantee for therapy success. Several mutation carriers did not benefit from olaparib or neoadjuvant chemotherapy. Moreover, BRCA1/2-associated cancers are



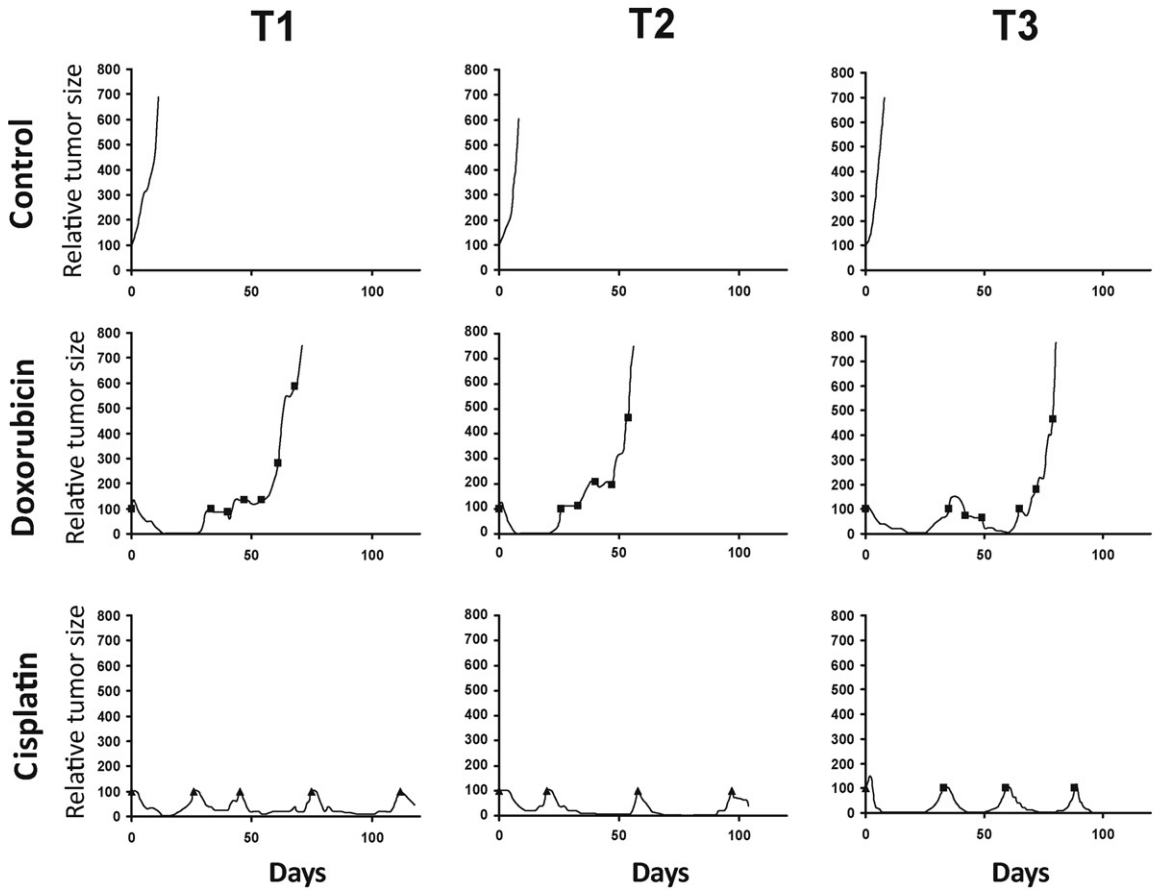


Fig. 1. Responses of BRCA1;p53-deficient tumors to the maximum tolerable dose of doxorubicin or cisplatin. Animals with 3 individual orthotopically transplanted BRCA1;p53-deficient mammary tumors (T1, T2 and T3; volume ~200 mm^3) were left untreated (control) or treated with 5 mg doxorubicin per kg i.v., or 6 mg cisplatin per kg i.v. When tumors relapsed or showed progressive growth (tumor size ≥50%) after a recovery time of 7 days, treatment was resumed as indicated by the filled squares (doxorubicin) and triangles (cisplatin).

considered highly aggressive, and one would not expect a poor prognosis of a highly drug sensitive tumor. How can this paradox be resolved? One explanation is re-activation of BRCA function. BRCA1/2 function can be restored *via* genetic reversion of a small mutation (Edwards et al., 2008; Sakai et al., 2008; Swisher et al., 2008), but we still do not know how frequent this is in patients. If *BRCA1/2* are silenced *via* promoter methylation, the genes may be re-activated by demethylation. Ovarian cancer patients with BRCA1/2 mutations live longer than patients with BRCA1/2 promoter methylation (65). Apparently, cells that lack promoter methylation may be selected out more easily during chemotherapy than drug resistant cells with a mutation in the *BRCA1/2* gene.

A second explanation for the poor survival of patients with BRCA-altered tumors comes from GEMMs generated in the Jonkers lab in which *BRCA1* founder mutations were introduced in mice (Drost et al., 2011). Although the C61G mutation, which affects the BRCA1 RING domain, promotes mammary tumor formation just as effectively as a large intragenic *Brca1* deletion, tumors with the C61G mutation respond poorly to cisplatin or olaparib treatment.

This suggests that hypomorphic activity of the BRCA1-C61G protein thwarts drug sensitivity.

A third explanation also comes from experiments on GEMMs. Screens in BRCA1-deficent mouse ES cells identified loss of 53BP1 as a mechanism to partially re-activate HR in cells without functional BRCA1 (Bouwman et al., 2010; Bunting et al., 2010). In BRCA1;p53-deficient mammary tumors we found that 53BP1 loss causes olaparib or topotecan resistance (Jaspers, Jonkers and Rottenberg, unpublished observation). Since 53BP1 is also often absent in triple-negative breast cancer (Bouwman et al., 2010), this may be another mechanism that corrupts the success of anti-cancer drugs.

As fourth explanation we found increased drug efflux. In 5 out of 22 BRCA1/p53-deficient mouse mammary tumors that did not respond to the MTD of docetaxel or doxorubicin, an increased expression of the mouse *Abcb1a/b* genes was observed (Rottenberg and Borst, unpublished observation). This is also the cause of resistance to doxorubicin that arises during long-term treatment, as illustrated in Fig. 1. Tumor-specific deletion of the *Abcb1a/b* genes resulted in docetaxel hypersensitivity. In contrast, ablation of *Abcb1a/b* did not sensitize p53-deficient tumors to docetaxel. This strongly suggests that loss of BRCA1 function is the critical factor that contributes to docetaxel sensitivity.

A fifth explanation is epithelial-mesenchymal transition (EMT) (Singh and Settleman, 2010). Only a small fraction of breast cancers in patients show a metaplastic phenotype, and these tumors are indeed usually drug resistant (Weigelt et al., 2009). In our BRCA1/2-deficient mouse mammary tumors we also found a small fraction of EMT tumors, and these tumors poorly responded to several anti-cancer drugs (unpublished observation). As expected, the EMT phenotype correlated with a drastic change in the gene expression profile. This also includes increased *Abcb1a/b* gene expression. We are currently investigating whether this is causal by itself, as suggested by Li et al. (2009).

4. Predicting resistance

The examples presented in the preceding section illustrate only some complications that can hamper the success of chemotherapy of tumors that one may classify as being drug sensitive. Several additional mechanisms can be envisioned, including lack of drug import, drug metabolism or drug target alterations. These examples also demonstrate why it is so difficult to predict chemotherapy response (Borst and Wessels, 2010). Small mutations of *BRCA1* or *53BP1* genes, that may be sufficient for restoration of DNA repair, will not be easily picked up. In addition, a moderate three-to five-fold increase of *Abcb1* gene expression, which is enough to cause complete doxorubicin or olaparib resistance in our model (Pajic et al., 2009; Rottenberg et al., 2008), may not be easily detected using ordered arrays.

Another complication is the presence of multiple mechanisms of drug resistance. Only if a mechanism occurs in the majority of resistant tumors, conventional analysis tools of large data sets will find a significant difference. This is shown in Fig. 2 for some *Brca1*$^{-/-}$;*p53*$^{-/-}$ mouse mammary tumors. 20 individual tumors that were sampled before treatment were analyzed by gene expression profiling (Rottenberg and Borst, unpublished observation). In subsequent experiments we found that 10 tumors (here named tumor 1–10) were sensitive to docetaxel, whereas 10 other tumors (tumor 11–20) were not. Supervized significance analysis of microarrays (SAM) based on the therapy outcome did not yield a single gene that correlated with therapy response. In contrast, when matched samples of 5 *Brca1*$^{-/-}$;*p53*$^{-/-}$ tumors (T21-25) before docetaxel treatment and of the docetaxel resistant tumor were analyzed, *Abcb1b* was identified using the same analysis (Fig. 2B). Quantitative analysis of *Abcb1b* gene expression

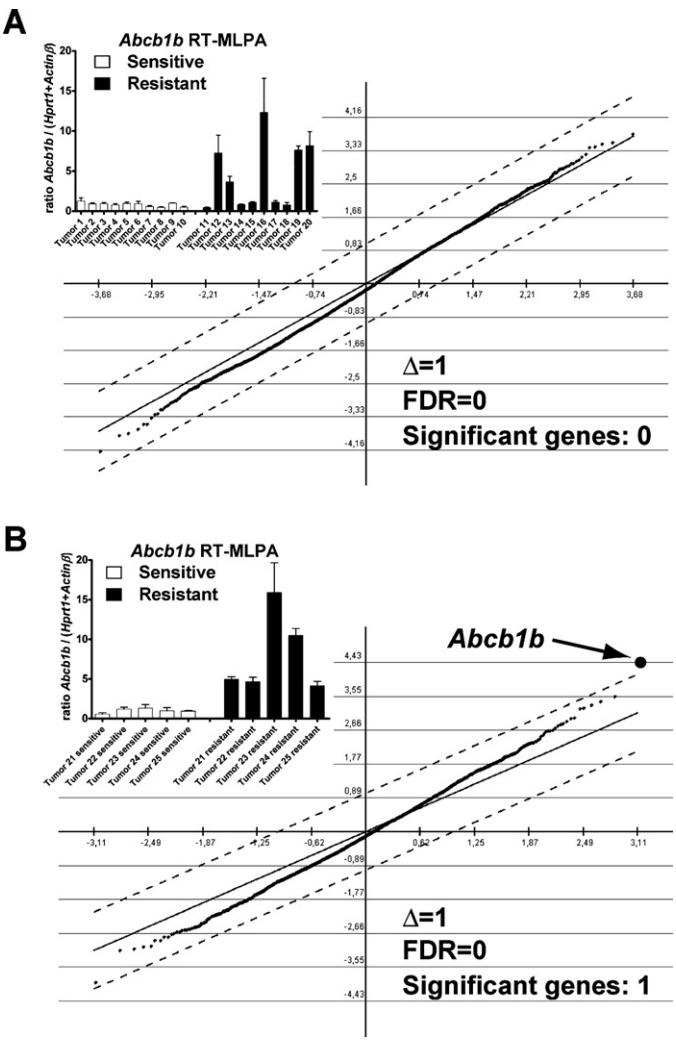


Fig. 2. Gene expression profiling of docetaxel-sensitive *versus* resistant *Brca1*$^{-/-}$;*p53*$^{-/-}$ mouse mammary tumors. (A) 20 drug-naïve tumors were analyzed for *Abcb1b* gene expression by quantitative reverse transcriptase-multiplex ligation-dependent probe amplification (RT-MLPA, top left insert). 10 of these tumors were docetaxel-sensitive (white bars, average of 3 experiments + SD) whereas 10 responded only poorly to docetaxel (black bars, average of 3 experiments + SD). 5 of the 10 resistant tumors contain *Abcb1b* RNA levels known to be high enough to make the tumors resistant to drug. The same samples were also analyzed by genome-wide expression profiling using 39K mouse exonic evidence based oligonucleotide (MEEBO) arrays and the 2 groups were subsequently compared by significance analysis of microarrays (SAM, *x* axis = expected values; *y* axis = observed values). (B) RNA of 5 docetaxel-sensitive *Brca1*$^{-/-}$;*p53*$^{-/-}$ mouse mammary tumors and RNA isolated from the matching tumor which eventually acquired docetaxel resistance with increased *Abcb1b* RNA levels were analyzed as described under (A).

showed for tumors 1–20 increased transcripts for 5 of the 10 poor responders, whereas all 5 tumors which acquired docetaxel resistance had increased *Abcb1b* RNA levels. Hence, even if a mechanism can explain drug resistance in half of the samples, it may not be detected by classical gene expression profiling.

The BRCA1 deficiency illustrates how a specific defect in DNA repair can sensitize tumors to chemotherapy, but the examples provided also show how easily such defects are sometimes reversed. A functional assay by which DNA repair can be measured in patients would therefore be useful. Such tests may be applied and optimized in GEMMs under controlled conditions. One example is the measurement of cell-free tumor DNA in the blood of patients (Leary et al., 2010). Next generation sequencing provides the possibility to identify tumor-specific DNA mutations in tumor samples. This

knowledge allows the design of a simple PCR strategy to detect DNA in the serum of patients that is leaking from the tumor. An increase in cell-free tumor DNA should reflect the death of many tumors cells, *e.g.*, due to successful chemo- or radiotherapy. This approach may be helpful in monitoring the success of therapy early on. It could also be used as a quick functional readout to determine whether a tumor has a defect in HR, using a test dose of olaparib, which has only few side effects.

5. Drug tolerance impedes tumor eradication

Another set-back for chemotherapy is the frequent lack of tumor eradication of drug-sensitive tumors. Our experiments with *Brca1*$^{-/-}$;*p53*$^{-/-}$ GEMM tumors using cisplatin have shed new light on this complication (Borst et al., 2008; Pajic et al., 2010; Rottenberg et al., 2007). Due to the large intragenic deletion, BRCA1 function cannot be restored in our model tumor (Liu et al., 2007). Moreover, cisplatin is not a substrate of P-gp, our mouse tumors usually express 53BP1, and tumors grow rapidly. This looks like the perfect scenario to achieve tumor eradication. Despite the high cisplatin sensitivity of this model, however, tumors are usually not eradicated, even not by dose-dense treatment (Pajic et al., 2010). Intriguingly, relapsing tumors are not resistant to cisplatin. Instead, tumors that grow back from remnants always respond again to cisplatin and the time until relapse is not shorter after repeated treatments (Fig. 1). Hence, the residual tumor cells that eventually repopulate the tumor are not selected for stable drug resistance. We investigated whether the remnant cells may represent tumor-initiating cells (TICs) that have increased biochemical defense mechanism (Pajic et al., 2010). In the cisplatin-surviving tumor remnants the TICs were not enriched (Pajic et al., 2010). This argues against the hypothesis that TICs have special biochemical defense mechanisms that would make them less vulnerable to drugs. Instead, our results are reminiscent of the drug-tolerant cells recently observed *in vitro* (Sharma et al., 2010). We are currently investigating whether cell cycle arrest of cells with tumor-initiating capability, is underlying such drug tolerance.

Is there a role for ABC transporters in drug tolerance? When we treated *Abcg2*$^{-/-}$,*Brca1*$^{-/-}$,*p53*$^{-/-}$ mammary tumors with topotecan (Zander et al., 2010), or *Abcb1a/b*$^{-/-}$,*Brca1*$^{-/-}$,*p53*$^{-/-}$ with docetaxel, olaparib or doxorubicin (Rottenberg, Jaspers, Zander, Jonkers and Borst, unpublished observation), we always found a significant increase in the time until tumors relapsed in comparison to *Brca1*$^{-/-}$,*p53*$^{-/-}$ tumors. Nevertheless, tumor eradication was usually not achieved. This suggests that in our model drug tolerance of drug-naïve tumors does not depend on these ABC transporters. Still, their presence helps tumors to re-grow more rapidly.

6. ABC transporters frequently cause acquired drug resistance to docetaxel, doxorubicin, topotecan, or olaparib in *Brca1*$^{-/-}$,*p53*$^{-/-}$ mouse mammary tumors

In contrast to our finding that relapsing tumors do not develop cisplatin resistance, *Brca1*$^{-/-}$,*p53*$^{-/-}$ tumors always acquire resistance to docetaxel, doxorubicin, topotecan or olaparib (Rottenberg et al., 2007, 2008; Zander et al., 2010). This is exemplified in Fig. 1 for doxorubicin. As underlying mechanism we frequently found an increased expression of the *Abcb1* or *Abcg2* genes. Inhibition of ABCB1 using tariquidar successfully reversed drug resistance (Pajic et al., 2009; Rottenberg et al., 2008). The relevance of ABCB1- or ABCG2-mediated drug efflux for acquired drug resistance was also confirmed in ABC transporter-deficient tumors. *Abcg2*$^{-/-}$,*Brca1*$^{-/-}$,*p53*$^{-/-}$ tumors showed a delay in acquiring topotecan resistance (Zander et al., 2010) and we observed the same delay for *Abcb1*$^{-/-}$,*Brca1*$^{-/-}$,*p53*$^{-/-}$ tumors treated with olaparib (Jaspers, Jonkers and Rottenberg, unpublished observation). Even more strikingly, *Abcb1*$^{-/-}$,*Brca1*$^{-/-}$,*p53*$^{-/-}$ tumors largely failed to acquire resistance to the MTD of docetaxel or doxorubicin (Rottenberg, Guyader, Zander and Borst, unpublished observation). Only by lowering the dose to 50%, resistance eventually occurred (Guyader, Zander, Borst and Rottenberg, unpublished observation). Together these data unambiguously show that in our model increased expression of ABC transporters is a relevant mechanism of acquired drug resistance.

Intriguingly, we have thus far no evidence that other ABC transporters than ABCB1 or ABCG2 contribute to resistance. Based on previous studies we expected to find topotecan or docetaxel resistant tumors in which *Mrp4* or *Mrp7* gene expression would be increased (Kruh et al., 2007; Leggas et al., 2004). However, in none of the resistant tumors studied thus far, the expression of these transporter genes was altered.

The precise mechanisms of increased *Abcb1* or *Abcg2* gene expression are still unclear. In human tumor samples and cell lines three types of activation of the human *ABCB1* gene have been reported: DNA amplification, DNA rearrangements linking the *ABCB1* gene to a strong promoter (Mickley et al., 1997, 1998) and activation of a distal promoter, 100 kb upstream of the *ABCB1* gene (Chen et al., 2005; Scotto, 2003). However, in our analysis of the 5′-ends of the *Abcb1a/b* or *Abcg2* mRNAs we did not find either DNA amplification, DNA rearrangements, or alternative promoter use (Pajic et al., 2009). Since the basal level of ABCB1 in rodent tissues is higher than in most human tissues, transcriptional activation of rodent ABCB1 may occur more readily. Consequently, the use of a mouse model probably results in an overestimation of the importance of ABC transporter-mediated drug efflux as a mechanism of drug resistance in human tumors. However, the *Abcb1*$^{-/-}$,*Brca1*$^{-/-}$,*p53*$^{-/-}$ and *Abcg2*$^{-/-}$,*Brca1*$^{-/-}$,*p53*$^{-/-}$ tumors also provide useful tools to detect ABCB1- or ABCG2-independent drug resistance mechanisms in mice. We have already found that loss of 53BP1 or reduced topoisomerase I expression explains some of the ABC transporter-independent olaparib or topotecan resistance (Zander et al., 2010). As a tool to identify the remaining mechanisms of resistance, we have also derived cell lines from several models on which we are performing functional screens using shRNAs or insertional mutagenesis. In tumors which have developed resistance, we can then validate which of the hits identified *in vitro* occur *in vivo*.

7. Is there a future for ABC transporters in human oncology?

Based on the results obtained thus far in breast cancer GEMMs, we infer that ABCB1 and ABCG2 may play a role in drug resistance of human tumors that cannot handle the damage by efficient DNA repair. If genetic re-arrangements are required to induce human ABCB1 or ABCG2, the frequency ABC transporter-mediated drug resistance might well be lower than in mouse tumors in which transcriptional activation may be available more readily. To provide further support for the relevance of ABC transporters in human tumors, the study of drug responses of xenografts with defined deficiencies in the HR pathway may be informative. Moreover, it would be helpful to investigate tumor samples of patients with known DNA repair deficiencies that were treated with a drug that is a substrate for ABCB1 or ABCG2. Ideally, tumor samples of both the drug-sensitive tumor before treatment and the drug-refractory tumor after treatment should be analyzed. Functional *in vivo* imaging using (99m)Tc-sestamibi in combination with tariquidar may also be helpful in identifying those patients that may benefit from the application of an ABCB1 inhibitor in combination with chemotherapy (Kelly et al., 2011). Given all the evidence that

drug efflux is such an efficient resistance mechanism for a wide range of prokaryotic and eukaryotic cells, human cancer cells would be really stupid if they forgot about it.

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References

Audeh, M.W., Carmichael, J., Penson, R.T., Friedlander, M., Powell, B., Bell-McGuinn, K.M., Scott, C., Weitzel, J.N., Oaknin, A., Loman, N., Lu, K., Schmutzler, R.K., Matulonis, U., Wickens, M., Tutt, A., 2010. Oral poly(ADP-ribose) polymerase inhibitor olaparib in patients with BRCA1 or BRCA2 mutations and recurrent ovarian cancer: a proof-of-concept trial. Lancet 376, 245–251.

Borst, P., Rottenberg, S., Jonkers, J., 2008. How do real tumors become resistant to cisplatin? Cell Cycle 7, 1353–1359.

Borst, P., Wessels, L., 2010. Do predictive signatures really predict response to cancer chemotherapy? Cell Cycle 9, 4836–4840.

Bouwman, P., Aly, A., Escandell, J.M., Pieterse, M., Bartkova, J., van der Gulden, H., Hiddingh, S., Thanasoula, M., Kulkarni, A., Yang, Q., Haffty, B.G., Tommiska, J., Blomqvist, C., Drapkin, R., Adams, D.J., Nevanlinna, H., Bartek, J., Tarsounas, M., Ganesan, S., Jonkers, J., 2010. 53BP1 loss rescues BRCA1 deficiency and is associated with triple-negative and BRCA-mutated breast cancers. Nat. Struct. Mol. Biol. 17 (6), 688–695.

Bryant, H.E., Schultz, N., Thomas, H.D., Parker, K.M., Flower, D., Lopez, E., Kyle, S., Meuth, M., Curtin, N.J., Helleday, T., 2005. Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. Nature 434, 913–917.

Bunting, S.F., Callen, E., Wong, N., Chen, H.T., Polato, F., Gunn, A., Bothmer, A., Feldhahn, N., Fernandez-Capetillo, O., Cao, L., Xu, X., Deng, C.X., Finkel, T., Nussenzweig, M., Stark, J.M., Nussenzweig, A., 2010. 53BP1 inhibits homologous recombination in Brca1-deficient cells by blocking resection of DNA breaks. Cell 141, 243–254.

Cancer Genome Atlas Research Network, 2011. Integrated genomic analyses of ovarian carcinoma. Nature 474, 609–615.

Chen, K.G., Wang, Y.C., Schaner, M.E., Francisco, B., Duran, G.E., Juric, D., Huff, L.M., Padilla-Nash, H., Ried, T., Fojo, T., Sikic, B.I., 2005. Genetic and epigenetic modeling of the origins of multidrug-resistant cells in a human sarcoma cell line. Cancer Res. 65, 9388–9397.

Cripe, L.D., Uno, H., Paietta, E.M., Litzow, M.R., Ketterling, R.P., Bennett, J.M., Rowe, J.M., Lazarus, H.M., Luger, S., Tallman, M.S., 2010. Zosuquidar, a novel modulator of P-glycoprotein, does not improve the outcome of older patients with newly diagnosed acute myeloid leukemia: a randomized, placebo-controlled trial of the Eastern Cooperative Oncology Group 3999. Blood 116, 4077–4085.

Da Silva, L., Lakhani, S.R., 2010. Pathology of hereditary breast cancer. Mod. Pathol. 23 (Suppl. 2), S46–S51.

Derksen, P.W., Braumuller, T.M., Van der Burg, E., Hornsveld, M., Mesman, E., Wesseling, J., Krimpenfort, P., Jonkers, J., 2011. Mammary-specific inactivation of E-cadherin and p53 impairs functional gland development and leads to pleomorphic invasive lobular carcinoma in mice. Dis. Model. Mech. 4, 347–358.

Derksen, P.W., Liu, X., Saridin, F., van der Gulden, H., Zevenhoven, J., Evers, B., van Beijnum, J.R., Griffioen, A.W., Vink, J., Krimpenfort, P., Peterse, J.L., Cardiff, R.D., Berns, A., Jonkers, J., 2006. Somatic inactivation of E-cadherin and p53 in mice leads to metastatic lobular mammary carcinoma through induction of anoikis resistance and angiogenesis. Cancer Cell 10, 437–449.

Doles, J., Oliver, T.G., Cameron, E.R., Hsu, G., Jacks, T., Walker, G.C., Hemann, M.T., 2010. Suppression of Rev3, the catalytic subunit of Pol{zeta}, sensitizes drug-resistant lung tumors to chemotherapy. Proc. Natl. Acad. Sci. U.S.A. 107, 20786–20791.

Drost, R., Bouwman, P., Rottenberg, S., Boon, U., Schut, E., Klarenbeek, S., Klijn, C., Van der Heijden, I., van der Gulden, H., Wientjens, E., Pieterse, M., Catteau, A., Green, P., Solomon, E., Morris, J.R., Jonkers, J., 2011. BRCA1 RING function is essential for tumor suppression but dispensable for therapy resistance. Cancer Cell 20, 797–809.

Edwards, S.L., Brough, R., Lord, C.J., Natrajan, R., Vatcheva, R., Levine, D.A., Boyd, J., Reis-Filho, J.S., Ashworth, A., 2008. Resistance to therapy caused by intragenic deletion in BRCA2. Nature 451, 1111–1115.

Emmink, B.L., van Houdt, W.J., Vries, R.G., Hoogwater, F.J., Govaert, K.M., Verheem, A., Nijkamp, M.W., Steller, E.J., Jimenez, C.R., Clevers, H., Borel Rinkes, I.H., Kranenburg, O., 2011. Differentiated human colorectal cancer cells protect tumor-initiating cells from irinotecan. Gastroenterology 141, 269–278.

Evers, B., Drost, R., Schut, E., de Bruin, M., Van der Burg, E., Derksen, P.W., Holstege, H., Liu, X., Van Drunen, E., Beverloo, H.B., Smith, G.C., Martin, N.M., Lau, A., O'Connor, M.J., Jonkers, J., 2008. Selective inhibition of BRCA2-deficient mammary tumor cell growth by AZD2281 and cisplatin. Clin. Cancer Res. 14, 3916–3925.

Evers, B., Speksnijder, E.N., Schut, E., Ciampricotti, M., Smalley, M.J., Derksen, P.W., Jonkers, J., de Visser, K.E., 2010. A tissue reconstitution model to study cancer cell-intrinsic and -extrinsic factors in mammary tumourigenesis. J. Pathol. 220, 34–44.

Farmer, H., McCabe, N., Lord, C.J., Tutt, A.N., Johnson, D.A., Richardson, T.B., Santarosa, M., Dillon, K.J., Hickson, I., Knights, C., Martin, N.M., Jackson, S.P., Smith, G.C., Ashworth, A., 2005. Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. Nature 434, 917–921.

Fong, P.C., Boss, D.S., Yap, T.A., Tutt, A., Wu, P., Mergui-Roelvink, M., Mortimer, P., Swaisland, H., Lau, A., O'Connor, M.J., Ashworth, A., Carmichael, J., Kaye, S.B., Schellens, J.H., de Bono, J.S., 2009. Inhibition of poly(ADP-ribose) polymerase in tumors from BRCA mutation carriers. N. Engl. J. Med. 361, 123–134.

Fong, P.C., Yap, T.A., Boss, D.S., Carden, C.P., Mergui-Roelvink, M., Gourley, C., De Greve, J., Lubinski, J., Shanley, S., Messiou, C., A'Hern, R., Tutt, A., Ashworth, A., Stone, J., Carmichael, J., Schellens, J.H., de Bono, J.S., Kaye, S.B., 2010. Poly(ADP)-ribose polymerase inhibition: frequent durable responses in BRCA carrier ovarian cancer correlating with platinum-free interval. J. Clin. Oncol. 28, 2512–2519.

Garber, K., 2009. From human to mouse and back: 'tumorgraft' models surge in popularity. J. Natl. Cancer Inst. 101, 6–8.

Garrido-Laguna, I., Uson, M., Rajeshkumar, N.V., Tan, A.C., De Oliveira, E., Karikari, C., Villaroel, M.C., Solomon, A., Taylor, G., Sharma, R., Hruban, R.H., Maitra, A., Laheru, D., Rubio-Viqueira, B., Jimeno, A., Hidalgo, M., 2011. Tumor engraftment in nude mice and enrichment in stroma-related gene pathways predicts poor survival and resistance to gemcitabine in patients with pancreatic cancer. Clin. Cancer Res. 17, 5793–5800.

Graeser, M., McCarthy, A., Lord, C.J., Savage, K., Hills, M., Salter, J., Orr, N., Parton, M., Smith, I.E., Reis-Filho, J.S., Dowsett, M., Ashworth, A., Turner, N.C., 2010. A marker of homologous recombination predicts pathologic complete response to neoadjuvant chemotherapy in primary breast cancer. Clin. Cancer Res. 16, 6159–6168.

Hay, T., Matthews, J.R., Pietzka, L., Lau, A., Cranston, A., Nygren, A.O., Douglas-Jones, A., Smith, G.C., Martin, N.M., O'Connor, M., Clarke, A.R., 2009. Poly(ADP-ribose) polymerase-1 inhibitor treatment regresses autochthonous Brca2/p53-mutant mammary tumors in vivo and delays tumor relapse in combination with carboplatin. Cancer Res. 69, 3850–3855.

Hegedus, C., Ozvegy-Laczka, C., Szakacs, G., Sarkadi, B., 2009. Interaction of ABC multidrug transporters with anticancer protein kinase inhibitors: substrates and/or inhibitors? Curr. Cancer Drug Targets 9, 252–272.

Heyer, J., Kwong, L.N., Lowe, S.W., Chin, L., 2010. Non-germline genetically engineered mouse models for translational cancer research. Nat. Rev. Cancer 10, 470–480.

Hidalgo, M., Bruckheimer, E., Rajeshkumar, N.V., Garrido-Laguna, I., De Oliveira, E., Rubio-Viqueira, B., Strawn, S., Wick, M.J., Martell, J., Sidransky, D., 2011. A pilot clinical study of treatment guided by personalized tumorgrafts in patients with advanced cancer. Mol. Cancer Ther. 10, 1311–1316.

Hofmeister, C.C., Yang, X., Pichiorri, F., Chen, P., Rozewski, D.M., Johnson, A.J., Lee, S., Liu, Z., Garr, C.L., Hade, E.M., Ji, J., Schaaf, L.J., Benson Jr., D.M., Kraut, E.H., Hicks, W.J., Chan, K.K., Chen, C.S., Farag, S.S., Grever, M.R., Byrd, J.C., Phelps, M.A., 2011. Phase I trial of lenalidomide and CCI-779 in patients with relapsed multiple myeloma: evidence for lenalidomide-CCI-779 interaction via P-glycoprotein. J. Clin. Oncol. 29, 3427–3434.

Holstege, H., Horlings, H.M., Velds, A., Langerod, A., Borresen-Dale, A.L., van de Vijver, M.J., Nederlof, P.M., Jonkers, J., 2010a. BRCA1-mutated and basal-like breast cancers have similar aCGH profiles and a high incidence of protein truncating TP53 mutations. BMC Cancer 10, 654.

Holstege, H., van Beers, E., Velds, A., Liu, X., Joosse, S.A., Klarenbeek, S., Schut, E., Kerkhoven, R., Klijn, C.N., Wessels, L.F., Nederlof, P.M., Jonkers, J., 2010b. Cross-species comparison of aCGH data from mouse and human BRCA1- and BRCA2-mutated breast cancers. BMC Cancer 10, 455.

Houghton, P.J., Morton, C.L., Tucker, C., Payne, D., Favours, E., Cole, C., Gorlick, R., Kolb, E.A., Zhang, W., Lock, R., Carol, H., Tajbakhsh, M., Reynolds, C.P., Maris, J.M., Courtright, J., Keir, S.T., Friedman, H.S., Stopford, C., Zeidner, J., Wu, J., Liu, T., Billups, C.A., Khan, J., Ansher, S., Zhang, J., Smith, M.A., 2007. The pediatric preclinical testing program: description of models and early testing results. Pediatr. Blood Cancer 49, 928–940.

Huijbers, I.J., Krimpenfort, P., Berns, A., Jonkers, J., 2011. Rapid validation of cancer genes in chimeras derived from established genetically engineered mouse models. BioEssays 33, 701–710.

Jonkers, J., Berns, A., 2002. Conditional mouse models of sporadic cancer. Nat. Rev. Cancer 2, 251–265.

Jonkers, J., Meuwissen, R., van der Gulden, H., Peterse, H., Van der Valk, M., Berns, A., 2001. Synergistic tumor suppressor activity of BRCA2 and p53 in a conditional mouse model for breast cancer. Nat. Genet. 29, 418–425.

Juliano, R.L., Ling, V., 1976. A surface glycoprotein modulating drug permeability in Chinese hamster ovary cell mutants. Biochim. Biophys. Acta 455, 152–162.

Kelland, L.R., 2004. Of mice and men: values and liabilities of the athymic nude mouse model in anticancer drug development. Eur. J. Cancer 40, 827–836.

Kelly, R.J., Draper, D., Chen, C.C., Robey, R.W., Figg, W.D., Piekarz, R.L., Chen, X., Gardner, E.R., Balis, F.M., Venkatesan, A.M., Steinberg, S.M., Fojo, T., Bates, S.E., 2011. A pharmacodynamic study of docetaxel in combination with the P-glycoprotein antagonist tariquidar (XR9576) in patients with lung, ovarian, and cervical cancer. Clin. Cancer Res. 17, 569–580.

Kerbel, R.S., 2003. Human tumor xenografts as predictive preclinical models for anticancer drug activity in humans: better than commonly perceived-but they can be improved. Cancer Biol. Ther. 2, S134–S139.

Kim, W.Y., Sharpless, N.E., in press. Drug efficacy testing in mice. Curr. Top. Microbiol. Immunol.

Kortmann, U., McAlpine, J.N., Xue, H., Guan, J., Ha, G., Tully, S., Shafait, S., Lau, A., Cranston, A.N., O'Connor, M.J., Huntsman, D.G., Wang, Y., Gilks, C.B., 2011. Tumor growth inhibition by olaparib in BRCA2 germline-mutated patient-derived ovarian cancer tissue xenografts. Clin. Cancer Res. 17, 783–791.

Kriege, M., Seynaeve, C., Meijers-Heijboer, H., Collee, J.M., Menke-Pluymers, M.B., Bartels, C.C., Tilanus-Linthorst, M.M., Blom, J., Huijskens, E., Jager, A., van den Ouweland, A., van Geel, B., Hooning, M.J., Brekelmans, C.T., Klijn, J.G., 2009. Sensitivity to first-line chemotherapy for metastatic breast cancer in BRCA1 and BRCA2 mutation carriers. J. Clin. Oncol. 27, 3764–3771.

Kruh, G.D., Guo, Y., Hopper-Borge, E., Belinsky, M.G., Chen, Z.S., 2007. ABCC10, ABCC11, and ABCC12. Pflugers Arch. 453, 675–684.

Leary, R.J., Kinde, I., Diehl, F., Schmidt, K., Clouser, C., Duncan, C., Antipova, A., Lee, C., McKernan, K., De La Vega, F.M., Kinzler, K.W., Vogelstein, B., Diaz Jr., L.A., Velculescu, V.E., 2010. Development of personalized tumor biomarkers using massively parallel sequencing. Sci. Transl. Med. 2, 20ra14.

Leggas, M., Adachi, M., Scheffer, G.L., Sun, D., Wielinga, P., Du, G., Mercer, K.E., Zhuang, Y., Panetta, J.C., Johnston, B., Scheper, R.J., Stewart, C.F., Schuetz, J.D., 2004. Mrp4 confers resistance to topotecan and protects the brain from chemotherapy. Mol. Cell. Biol. 24, 7612–7621.

Li, Q.Q., Xu, J.D., Wang, W.J., Cao, X.X., Chen, Q., Tang, F., Chen, Z.Q., Liu, X.P., Xu, Z.D., 2009. Twist1-mediated adriamycin-induced epithelial-mesenchymal transition relates to multidrug resistance and invasive potential in breast cancer cells. Clin. Cancer Res. 15, 2657–2665.

Libby, E., Hromas, R., 2010. Dismounting the MDR horse. Blood 116, 4037–4038.

Lips, E.H., Mulder, L., Hannemann, J., Laddach, N., Vrancken Peeters, M.T., van de Vijver, M.J., Wesseling, J., Nederlof, P.M., Rodenhuis, S., 2011. Indicators of homologous recombination deficiency in breast cancer and association with response to neoadjuvant chemotherapy. Ann. Oncol. 22, 870–876.

Liu, X., Holstege, H., van der Gulden, H., Treur-Mulder, M., Zevenhoven, J., Velds, A., Kerkhoven, R.M., Van Vliet, M.H., Wessels, L.F.A., Peterse, J.L., Berns, A., Jonkers, J., 2007. Somatic loss of BRCA1 and p53 in mice induces mammary tumors with pathologic and molecular features of human BRCA1-mutated basal-like breast cancer. Proc. Natl. Acad. Sci. U.S.A. 104, 12111–12116.

Loveday, C., Turnbull, C., Ramsay, E., Hughes, D., Ruark, E., Frankum, J.R., Bowden, G., Kalmyrzaev, B., Warren-Perry, M., Snape, K., Adlard, J.W., Barwell, J., Berg, J., Brady, A.F., Brewer, C., Brice, G., Chapman, C., Cook, J., Davidson, R., Donaldson, A., Douglas, F., Greenhalgh, L., Henderson, A., Izatt, L., Kumar, A., Lalloo, F., Miedzybrodzka, Z., Morrison, P.J., Paterson, J., Porteous, M., Rogers, M.T., Shanley, S., Walker, L., Eccles, D., Evans, D.G., Renwick, A., Seal, S., Lord, C.J., Ashworth, A., Reis-Filho, J.S., Antoniou, A.C., Rahman, N., 2011. Germline mutations in RAD51D confer susceptibility to ovarian cancer. Nat. Genet. 43, 879–882.

Marangoni, E., Vincent-Salomon, A., Auger, N., Degeorges, A., Assayag, F., de Cremoux, P., de Plater, L., Guyader, C., De Pinieux, G., Judde, J.G., Rebucci, M., Tran-Perennou, C., Sastre-Garau, X., Sigal-Zafrani, B., Delattre, O., Dieras, V., Poupon, M.F., 2007. A new model of patient tumor-derived breast cancer xenografts for preclinical assays. Clin. Cancer Res. 13, 3989–3998.

Michalak, E.M., Jonkers, J., 2011. Studying therapy response and resistance in mouse models for BRCA1-deficient breast cancer. J. Mammary Gland Biol. Neoplasia 16, 41–50.

Mickley, L.A., Lee, J.-S., Weng, Z., Zhan, Z., Alvarez, M., Wilson, W., Bates, S.E., Fojo, T., 1998. Genetic polymorphism in *MDR-1*: a tool for examining allelic expression in normal cells, unselected and drug-selected cell lines, and human tumors. Blood 91, 1749–1756.

Mickley, L.A., Spengler, B.A., Knutsen, T.A., Biedler, J.L., Fojo, A.T., 1997. Gene rearrangement: a novel mechanism for MDR-1 gene activation. J. Clin. Invest. 99, 1947–1957.

Olive, K., Jacobetz, M., Davidson, C., Gopinathan, A., McIntyre, D., Honess, D., Madhu, B., Goldgraben, M., Caldwell, M., Allard, D., Frese, K., Denicola, G., Feig, C., Combs, C., Winter, S., Ireland-Zecchini, H., Reichelt, S., Howat, W., Chang, A., Dhara, M., Wang, L., Rückert, F., Grützmann, R., Pilarsky, C., Izeradjene, K., Hingorani, S., Huang, P., Davies, S., Plunkett, W., Egorin, M., Hruban, R., Whitebread, N., McGovern, K., Adams, J., Iacobuzio-Donahue, C., Griffiths, J., Tuveson, D., 2009. Inhibition of Hedgehog signaling enhances delivery of chemotherapy in a mouse model of pancreatic cancer. Science 324, 1457–1461.

Oliver, T.G., Mercer, K.L., Sayles, L.C., Burke, J.R., Mendus, D., Lovejoy, K.S., Cheng, M.H., Subramanian, A., Mu, D., Powers, S., Crowley, D., Bronson, R.T., Whittaker, C.A., Bhutkar, A., Lippard, S.J., Golub, T., Thomale, J., Jacks, T., Sweet-Cordero, E.A., 2010. Chronic cisplatin treatment promotes enhanced damage repair and tumor progression in a mouse model of lung cancer. Genes Dev. 24, 837–852.

Pajic, M., Iyer, J.K., Kersbergen, A., Van der Burg, E., Nygren, A.O., Jonkers, J., Borst, P., Rottenberg, S., 2009. Moderate increase in Mdr1a/1b expression causes in vivo resistance to doxorubicin in a mouse model for hereditary breast cancer. Cancer Res. 69, 6396–6404.

Pajic, M., Kersbergen, A., van Diepen, F., Pfauth, A., Jonkers, J., Borst, P., Rottenberg, S., 2010. Tumor-initiating cells are not enriched in cisplatin-surviving BRCA1;p53-deficient mammary tumor cells in vivo. Cell Cycle 9, 3780–3791.

Patel, K.J., Tannock, I.F., 2009. The influence of P-glycoprotein expression and its inhibitors on the distribution of doxorubicin in breast tumors. BMC Cancer 9, 356.

Politi, K., Pao, W., 2011. How genetically engineered mouse tumor models provide insights into human cancers. J. Clin. Oncol. 29, 2273–2281.

Premsrirut, P.K., Dow, L.E., Kim, S.Y., Camiolo, M., Malone, C.D., Miething, C., Scuoppo, C., Zuber, J., Dickins, R.A., Kogan, S.C., Shroyer, K.R., Sordella, R., Hannon, G.J., Lowe, S.W., 2011. A rapid and scalable system for studying gene function in mice using conditional RNA interference. Cell 145, 145–158.

Robey, R.W., Massey, P.R., Amiri-Kordestani, L., Bates, S.E., 2010. ABC transporters: unvalidated therapeutic targets in cancer and the CNS. Anticancer Agents Med. Chem. 10, 625–633.

Rottenberg, S., Jaspers, J.E., Kersbergen, A., Van der Burg, E., Nygren, A.O., Zander, S.A., Derksen, P.W., de Bruin, M., Zevenhoven, J., Lau, A., Boulter, R., Cranston, A., O'Connor, M.J., Martin, N.M., Borst, P., Jonkers, J., 2008. High sensitivity of BRCA1-deficient mammary tumors to the PARP inhibitor AZD2281 alone and in combination with platinum drugs. Proc. Natl. Acad. Sci. U.S.A. 105, 17079–17084.

Rottenberg, S., Jonkers, J., 2008. Modeling therapy resistance in genetically engineered mouse cancer models. Drug Resist. Updat. 11, 51–60.

Rottenberg, S., Nygren, A.O.H., Pajic, M., Van Leeuwen, F.W.B., Van der Heijden, I., Van de Wetering, K., Liu, X., de Visser, K.E., Gilhuijs, K.G., Van Tellingen, O., Schouten, J.P., Jonkers, J., Borst, P., 2007. Selective induction of chemotherapy resistance of mammary tumors in a conditional mouse model for hereditary breast cancer. Proc. Natl. Acad. Sci. U.S.A. 104, 12117–12122.

Ruff, P., Vorobiof, D.A., Jordaan, J.P., Demetriou, G.S., Moodley, S.D., Nosworthy, A.L., Werner, I.D., Raats, J., Burgess, L.J., 2009. A randomized, placebo-controlled, double-blind phase 2 study of docetaxel compared to docetaxel plus zosuquidar (LY335979) in women with metastatic or locally recurrent breast cancer who have received one prior chemotherapy regimen. Cancer Chemother. Pharmacol. 64, 763–768.

Sakai, W., Swisher, E.M., Karlan, B.Y., Agarwal, M.K., Higgins, J., Friedman, C., Villegas, E., Jacquemont, C., Farrugia, D.J., Couch, F.J., Urban, N., Taniguchi, T., 2008. Secondary mutations as a mechanism of cisplatin resistance in BRCA2-mutated cancers. Nature 451, 1116–1120.

Schinkel, A.H., Mayer, U., Wagenaar, E., Mol, C.A.A.M., Van Deemter, L., Smit, J.J.M., Van der Valk, M.A., Voordouw, A.C., Spits, H., Van Tellingen, O., Zijlmans, J.M.J.M., Fibbe, W.E., Borst, P., 1997. Normal viability and altered pharmacokinetics in mice lacking mdr1-type (drug-transporting) P-glycoproteins. Proc. Natl. Acad. Sci. U.S.A. 94, 4028–4033.

Scotto, K.W., 2003. Transcriptional regulation of ABC drug transporters. Oncogene 22, 7496–7511.

Seibler, J., Kleinridders, A., Kuter-Luks, B., Niehaves, S., Bruning, J.C., Schwenk, F., 2007. Reversible gene knockdown in mice using a tight, inducible shRNA expression system. Nucleic Acids Res. 35, e54.

Sharma, S.V., Lee, D.Y., Li, B., Quinlan, M.P., Takahashi, F., Maheswaran, S., McDermott, U., Azizian, N., Zou, L., Fischbach, M.A., Wong, K.K., Brandstetter, K., Wittner, B., Ramaswamy, S., Classon, M., Settleman, J., 2010. A chromatin-mediated reversible drug-tolerant state in cancer cell subpopulations. Cell 141, 69–80.

Sharpless, N.E., DePinho, R.A., 2006. The mighty mouse: genetically engineered mouse models in cancer drug development. Nat. Rev. Drug Discov. 5, 741–754.

Silver, D.P., Richardson, A.L., Eklund, A.C., Wang, Z.C., Szallasi, Z., Li, Q., Juul, N., Leong, C.O., Calogrias, D., Buraimoh, A., Fatima, A., Gelman, R.S., Ryan, P.D., Tung, N.M., De Nicolo, A., Ganesan, S., Miron, A., Colin, C., Sgroi, D.C., Ellisen, L.W., Winer, E.P., Garber, J.E., 2010. Efficacy of neoadjuvant Cisplatin in triple-negative breast cancer. J. Clin. Oncol. 28, 1145–1153.

Singh, A., Settleman, J., 2010. EMT, cancer stem cells and drug resistance: an emerging axis of evil in the war on cancer. Oncogene 29, 4741–4751.

Singh, M., Lima, A., Molina, R., Hamilton, P., Clermont, A.C., Devasthali, V., Thompson, J.D., Cheng, J.H., Bou Reslan, H., Ho, C.C., Cao, T.C., Lee, C.V., Nannini, M.A., Fuh, G., Carano, R.A., Koeppen, H., Yu, R.X., Forrest, W.F., Plowman, G.D., Johnson, L., 2010. Assessing therapeutic responses in Kras mutant cancers using genetically engineered mouse models. Nat. Biotechnol. 28, 585–593.

Swisher, E.M., Sakai, W., Karlan, B.Y., Wurz, K., Urban, N., Taniguchi, T., 2008. Secondary BRCA1 mutations in BRCA1-mutated ovarian carcinomas with platinum resistance. Cancer Res. 68, 2581–2586.

Szakacs, G., Paterson, J.K., Ludwig, J.A., Booth-Genthe, C., Gottesman, M.M., 2006. Targeting multidrug resistance in cancer. Nat. Rev. Drug Discov. 5, 219–234.

Turner, N., Tutt, A., Ashworth, A., 2004. Hallmarks of 'BRCAness' in sporadic cancers. Nat. Rev. Cancer 4, 814–819.

Tutt, A., Robson, M., Garber, J.E., Domchek, S.M., Audeh, M.W., Weitzel, J.N., Friedlander, M., Arun, B., Loman, N., Schmutzler, R.K., Wardley, A., Mitchell, G., Earl, H., Wickens, M., Carmichael, J., 2010. Oral poly(ADP-ribose) polymerase inhibitor olaparib in patients with BRCA1 or BRCA2 mutations and advanced breast cancer: a proof-of-concept trial. Lancet 376, 235–244.

Vafaizadeh, V., Klemmt, P., Brendel, C., Weber, K., Doebele, C., Britt, K., Grez, M., Fehse, B., Desrivieres, S., Groner, B., 2010. Mammary epithelial reconstitution with gene-modified stem cells assigns roles to Stat5 in luminal alveolar cell fate decisions, differentiation, involution, and mammary tumor formation. Stem Cells 28, 928–938.

Vollebergh, M.A., Lips, E.H., Nederlof, P.M., Wessels, L.F., Schmidt, M.K., van Beers, E.H., Cornelissen, S., Holtkamp, M., Froklage, F.E., de Vries, E.G., Schrama, J.G., Wesseling, J., van de Vijver, M.J., Van Tinteren, H., de Bruin, M., Hauptmann, M., Rodenhuis, S., Linn, S.C., 2011. An aCGH classifier derived from BRCA1-mutated breast cancer and benefit of high-dose platinum-based chemotherapy in HER2-negative breast cancer patients. Ann. Oncol. 22, 1561–1570.

Walrath, J.C., Hawes, J.J., Van Dyke, T., Reilly, K.M., 2010. Genetically engineered mouse models in cancer research. Adv. Cancer Res. 106, 113–164.

Watters, J.W., Cheng, C., Majumder, P.K., Wang, R., Yalavarthi, S., Meeske, C., Kong, L., Sun, W., Lin, J., Heyer, J., Ware, C., Winter, C., Reilly, J.F., Demuth, T., Clark, S., Chiu, M.I., Robinson, M.O., Kohl, N., Kannan, K., 2009. De novo discovery of a gamma-secretase inhibitor response signature using a novel in vivo breast tumor model. Cancer Res. 69, 8949–8957.

Weigelt, B., Kreike, B., Reis-Filho, J.S., 2009. Metaplastic breast carcinomas are basal-like breast cancers: a genomic profiling analysis. Breast Cancer Res. Treat. 117, 273–280.

Wu, M., Jung, L., Cooper, A.B., Fleet, C., Chen, L., Breault, L., Clark, K., Cai, Z., Vincent, S., Bottega, S., Shen, Q., Richardson, A., Bosenburg, M., Naber, S.P., DePinho, R.A., Kuperwasser, C., Robinson, M.O., 2009. Dissecting genetic requirements of human breast tumorigenesis in a tissue transgenic model of human breast cancer in mice. Proc. Natl. Acad. Sci. U.S.A. 106, 7022–7027.

Yuan, J., Cheng, K.C., Johnson, R.L., Huang, R., Pattaradilokrat, S., Liu, A., Guha, R., Fidock, D.A., Inglese, J., Wellems, T.E., Austin, C.P., Su, X.Z., 2011. Chemical genomic profiling for antimalarial therapies, response signatures, and molecular targets. Science 333, 724–729.

Zander, S.A., Kersbergen, A., Van der Burg, E., de Water, N., Van Tellingen, O., Gunnarsdottir, S., Jaspers, J.E., Pajic, M., Nygren, A.O., Jonkers, J., Borst, P., Rottenberg, S., 2010. Sensitivity and acquired resistance of BRCA1;p53-deficient mouse mammary tumors to the topoisomerase I inhibitor topotecan. Cancer Res. 70, 1700–1710.

Zender, L., Xue, W., Zuber, J., Semighini, C.P., Krasnitz, A., Ma, B., Zender, P., Kubicka, S., Luk, J.M., Schirmacher, P., McCombie, W.R., Wigler, M., Hicks, J., Hannon, G.J., Powers, S., Lowe, S.W., 2008. An oncogenomics-based in vivo RNAi screen identifies tumor suppressors in liver cancer. Cell 135, 852–864.

Zhou, Y., Rideout III, W.M., Zi, T., Bressel, A., Reddypalli, S., Rancourt, R., Woo, J.K., Horner, J.W., Chin, L., Chiu, M.I., Bosenberg, M., Jacks, T., Clark, S.C., DePinho, R.A., Robinson, M.O., Heyer, J., 2010. Chimeric mouse tumor models reveal differences in pathway activation between ERBB family- and KRAS-dependent lung adenocarcinomas. Nat. Biotechnol. 28, 71–78.

Zitvogel, L., Apetoh, L., Ghiringhelli, F., Kroemer, G., 2008. Immunological aspects of cancer chemotherapy. Nat. Rev. Immunol. 8, 59–73.

Zuber, J., Radtke, I., Pardee, T.S., Zhao, Z., Rappaport, A.R., Luo, W., McCurrach, M.E., Yang, M.M., Dolan, M.E., Kogan, S.C., Downing, J.R., Lowe, S.W., 2009. Mouse models of human AML accurately predict chemotherapy response. Genes Dev. 23, 877–889.