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REVIEW – CANCER RESEARCH

Models used to screen for the treatment of multidrug resistant cancer facilitated by transporter-based efflux

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Abstract

Purpose Efflux transporters of the adenosine triphosphate-binding cassette (ABC)-superfamily play an important role in the development of multidrug resistance (multidrug resistant; MDR) in cancer. The overexpression of these transporters can directly contribute to the failure of chemotherapeutic drugs. Several in vitro and in vivo models exist to screen for the efficacy of chemotherapeutic drugs against MDR cancer, specifically facilitated by efflux transporters.

Results This article reviews a range of efflux transporter-based MDR models used to test the efficacy of compounds to overcome MDR in cancer. These models are classified as either in vitro or in vivo and are further categorised as the most basic, conventional models or more complex and advanced systems. Each model's origin, advantages and limitations, as well as specific efflux transporter-based MDR applications are discussed. Accordingly, future modifications to existing models or new research approaches are suggested to develop prototypes that closely resemble the true nature of multidrug resistant cancer in the human body.

Conclusions It is evident from this review that a combination of both in vitro and in vivo preclinical models can provide a better understanding of cancer itself, than using a single model only. However, there is still a clear lack of progression of these models from basic research to high-throughput clinical practice.

Keywords Cancer · Cell culture · Efflux transporter · Genetically engineered mouse model · Multidrug resistance · Preclinical screening model

Introduction

According to the International Agency for Research on Cancer, 18.1 million new cancer cases were registered around the world in 2018. The estimated number of deaths due to cancer in 2018 were approximately 9.6 million and this number is expected to increase to 29.5 million by 2040 (https://gco.iarc.fr/), mainly because of phenomena such as multidrug resistance (multidrug resistant; MDR). MDR is defined as the ability of a living cell to display resistance to multiple unrelated anticancer drugs that are structurally and/or functionally different (Krishna and Mayer 2000). To date, MDR has been shown to develop via cell death inhibition, drug inactivation, deoxyribonucleic acid (DNA) damage repair, drug target alteration, the epithelial–mesenchymal transition and epigenetic modifications, but the most widely studied MDR mechanism is that of drug efflux involving adenosine triphosphate (ATP)-binding cassette (ABC) membrane transporters (Saraswathy and Gong 2013; Wang et al.

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2017a). At least 49 structurally related transporters, known collectively as the ABC-superfamily, have been identified and categorised into seven subfamilies. Of these, 16 are primarily involved in human diseases (Tarling et al. 2013), of which P-glycoprotein (P-gp; MDR1/ABCB1), multidrug resistance-associated protein 1 (MRP1/ABCC1) and breast cancer resistance protein (BCRP1/ABCG2) are the major drug transporters widely implicated in drug resistant cancers (Goldstein et al. 1989; Luker et al. 2001). P-gp, MRP1 and BCRP1 are present in the epithelial lining of the lungs, testes, skin, kidneys, small intestines, and in heart and muscle tissues, acting as a barrier against many xenobiotics (Flens et al. 1996; Qian et al. 2013). Through staining, P-gp and BCRP1 have been observed predominantly in the apical side of certain epithelium, whereas MRP1 has a basolateral cell localisation (Szakacs et al. 2008). All three transporters have significantly overlapping resistance profiles (Szakacs et al. 2008), but MRP1 does not confer resistance to taxanes and some classes of tyrosine kinase inhibitors (Brooks et al. 2004; Sodani et al. 2012), while BCRP1 is not inhibited by arylmethyloxy-derivatives (Colabufo et al. 2008).

The more ABC transporters are expressed in the cancer tissue, the lower the chance of effective results with chemotherapy. For example, a recent study on children with acute myeloid leukaemia found a strong association between the relapse of patients and the number of overexpressed ABC transporters such as ABCA3, ABCB1, ABCC3 and ABCG2 in the cancer cells (Bartholomae et al. 2016). Furthermore, a compensatory effect between different ABC transporters has been proposed in the sense that the down-regulation of one transporter is interchanged by the up-regulation of another (Aberuyi et al. 2017; Efferth et al. 2006). This highlights the challenge of developing new anticancer drugs able to deal with complex interactions between different efflux transporters.

This review presents and discusses the different in vitro and in vivo models currently available to evaluate the treatment of efflux transporter-based MDR in cancer. In this review, "efflux transporter-based models" refer to models that can be used to investigate efflux transporter characteristics including (but not limited to) expression, activity and substrate affinity, as well as the response of these efflux pumps to anticancer treatments. The in vitro models that are discussed include both conventional cell culture-based models cultured as basic two-dimensional (2D) flat cultures and more complex cell-based models that make use of specialised matrices or objects to culture non-conventional or three-dimensional (3D) models. The in vivo models that are discussed include cell- and patient-derived xenografts, as well as genetically engineered mouse models (GEMMs). In addition to the discussions of these selected models, a comprehensive summary of both in vitro (Table 1) and in vivo models (Table 2) are given which have been used in previous studies to investigate the anticancer efficacy of compounds by influencing efflux transporter activity and/or expression. Lastly, a few suggestions are provided on future modifications that can be made to existing models, as well as newly developed approaches, that can accurately imitate the true drug response of tumours in the human body.

Multidrug resistant cancer treatment approaches

Common treatments for cancer include surgery, radiation therapy and chemotherapy, or combinations thereof (Yura 2017). Chemotherapy specifically is important in the treatment of metastatic and haematological malignancies (Pluchino et al. 2012). The phenomenon of MDR is, however, a major challenge causing failures in chemotherapy, but the mechanisms responsible for both intrinsic and acquired MDR also provide potential targets for new anticancer drugs to overcome MDR. Therefore, many innovative therapeutic strategies focus specifically on reversing, suppressing or evading MDR mechanisms in tumours (Krishna and Mayer 2000; Ozben 2006; Saraswathy and Gong 2013).

Inhibition of efflux transporters, specifically P-gp, by co-administration of transporter inhibitors has been explored as a treatment strategy to overcome MDR. Different P-gp inhibitors have been investigated (including first, second and third generation inhibitors) using in silico, in vitro and in vivo models (Saraswathy and Gong 2013; Srivalli and Lakshmi 2012; Wang et al. 2017a). However, many clinical trials that tested the efficacy of co-administration of P-gp inhibitors with anticancer drugs could not provide sufficient evidence that this strategy could overcome MDR in most cancers. Several reasons were given for this outcome such as associated toxicity, poor planning or lack of randomisation in clinical trial design and the fact that little effort was made to determine if the tumours in the subjects did actually express P-gp (Gottesman and Pastan 2015). Besides competitive and non-competitive inhibition of P-gp, modulation of P-gp expression has also been investigated as a strategy to overcome chemoresistance. Several signalling pathways that modulate P-gp expression in cancer cells have been targeted to regulate P-gp function and expression. However, P-gp modulators appeared to have selective efficacy depending on the specific anticancer drug that it was combined with (Callaghan et al. 2014). Despite the failure to produce significant improvements in the treatment of MDR cancer with co-administering efflux transporter inhibitors, research relevant to this approach remains ongoing. In a recent study, it was shown that combining an approved anticancer drug that suppresses efflux activity (e.g., regorafenib) with another anticancer drug (e.g., paclitaxel), resulted in increased

Table 1 In vitro efflux transporter-based models used for anticancer drug screening

Model	Drug target	Transporter studied	Outcome	References
2D flat cultures				
MCF-7 (MCF-7/ADR) cells (doxorubicin-resistant human breast cancer cells)	Resveratrol Doxorubicin	MDR1 (P-gp) MRP1/MRP2 BCRP	Doxorubicin-induced cell death is potentiated by resveratrol Enhanced intracellular doxorubicin accumulation is facilitated by resveratrol MDR1 and MRP1 mRNA-expression is down regulated Resveratrol acts as a P-gp inhibitor No effect was witnessed on MRP2 and BCRP	Kim et al. (2014)
LoVo and LoVoDX cells (colon cancer cells resistant to doxorubicin)	Melatonin Doxorubicin	P-gp	In some concentrations melatonin intensified the cytotoxicity effect of doxorubicin in the LoVoDX cells	Fic et al. (2017)
H460/MX20; HEK293/pcDNA3.1; HEK/R482T cells (large cell lung cancer cells; human embryonic kidney cells)	CCTA-1523 (ABCG2 inhibitor) Mitoxantrone Doxorubicin SN-38 Cisplatin	ABCG2	CCTA-1523 is a potent, selective & reversible modulator of ABCG2 CCTA-1523 enhances the cytotoxicity of mitoxantrone & SN-38	Patel et al. (2017)
SW620/Ad300 cells (human colon cancer cells)	Regorafenib (multikinase inhibitor) Paclitaxel Doxorubicin Vincristine Cisplatin	ABCB1	Regorafenib reversed the ABCB1-mediated MDR & increased the accumulation of [3H]-paclitaxel in ABCB1-overexpressing cells, by suppressing efflux activity of ABCB1, but not altering expression level & localization of ABCB1 Regorafenib & paclitaxel synergistically shrink resistant colorectal tumours. Regorafenib inhibits the efflux activity of ABCB1 transporter	Wang et al. (2017b)
K562 and K562/ADM cells [human chronic myeloid leukaemia (CML) cells]	Imatinib Etoposide	MRP1 MDR1/P-gp	Use of imatinib may be preferable over the use of etoposide in the treatment of CML	Husaini et al. (2017)
BxPC3, Cfpac-1, and HPAC cells (human pancreatic cancer cells)	CG200745 (HDAC inhibitor) Erlotinib Gemcitabine	MRP3 MRP4	A synergistic inhibitory & apoptotic effect with CG200745 was witnessed when combined with gemcitabine/erlotinib in pancreatic cancer cells Decreased expression of MRP mRNA	Lee et al. (2017)
KB_{v200} (drug resistant) and HEK293/ABCB1 stable transfected cells (human embryonic kidney cells)	Pristimerin	P-gp/ABCB1/MDR1	Pristimerin inhibited cell proliferation & induced apoptosis in both cell lines Decreased P-gp activity in a dose-dependent manner was independent of mRNA levels but primarily owing to its protein stability Disturbed the subcellular distribution of P-gp with decreased location in the plasma membrane	Yan et al. (2017)

Table 1 (continued)

Model	Drug target	Transporter studied	Outcome	References
Micelles developed				
MCF-7/ADR cells (human drug resistant breast cancer cells)	Doxorubicin Hyaluronic acid-g-poly(L-histidine) (HA-PHis) and d-α-Tocopheryl polyethylene glycol 2000 (TPGS2k) copolymers	P-gp	Incorporation of doxorubicin into a nanoparticle limited distribution (thus reducing cardio toxicity) The doxorubicin-loaded mixed micelles (HPHM/TPGS2k) caused increased MDR sensitisation due to reversal of transporter efflux Blank HPHM/TPGS2k inhibited P-gp activity by reducing mitochondrial membrane potential & depletion of ATP but without inhibition of P-gp expression	Qiu et al. (2014)
MCF-7 and MCF-7/ADR cells (human breast cancer cells and their multidrug-resistant phenotype)	Paclitaxel d-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) and the mPEG-SS-PTX conjugate	P-gp	TPGS is a P-gp inhibitor that can block the cancer cell action of pumping drugs out of cells Mixed micelles can effectively improve the accumulation of paclitaxel in multidrug resistant MCF-7 cells	Zhao et al. (2016)
SKOV-3TR and A2780-Adr cells (ovarian carcinoma cells)	Paclitaxel Tariquidar (P-gp inhibitor) Transferrin (Tf)-modified polyethylene glycol-phosphatidyl ethanolamine (PEG-PE)-based micellar delivery system	P-gp	Tariquidar increased the intracellular paclitaxel levels & its cytotoxicity Tf-targeting of these micellar nanoformulations can further enhance their efficacy by targeting deeper layers of tumours	Zou et al. (2017)
Nanoparticles developed				
MCF-7/T cells (Taxol-resistant breast cancer cells)	Selenium/ruthenium (nanoparticles)	P-gp	The delivery of siRNA by means of a Se/Ru- Metal–Organic Framework nanoparticles, decreased MDR genes in MCF-7/T	Chen et al. (2017b)
HCT 116 cells (human colon cancer cells)	Paclitaxel combined with rod-shaped gadolinium arsenite nanoparticles	P-gp	The hybrid paclitaxel-loaded gadolinium arsenite nanoparticle is used to decrease MDR of paclitaxel	Chen et al. (2017a)
MCF-7 and MCF-7/ADR cells (doxorubicin resistant breast cancer cells)	Doxorubicin in combination with siMDR1	P-gp	The liposome decreased doxorubicin resistance	Saw et al. (2017)
T24 and TCC-SUP cells (bladder cancer cells & acquired resistant cells towards gemcitabine and vinblastine)	Nanoparticle albumin-bound (nab) paclitaxel	ABCB1 (P-gp)	Hyperexpressive ABCB1 cell lines showed a comparable resistance pattern towards both paclitaxel & nab paclitaxel	Vallo et al. (2017)
MCF-7/ADR cells (doxorubicin resistant breast cancer cells)	Doxorubicin combined with microRNAs	P-gp	The higher miR-129-5p expression led to P-gp inhibition & decreased cellular efflux of doxorubicin	Yi et al. (2016)

Table 1 (continued)

Model	Drug target	Transporter studied	Outcome	References
Advanced complex cell cultures				
HEK cells cultured as scaffold-free, 3D spheroids formed in nonadhesive micromolds (human embryonic kidney cells)	Ko143 (inhibitor) Gefitinib Elacridar	ABCG2	This model gave a better idea of pharmacokinetic characteristics of transporter inhibitors Drug uptake and penetration was modelled more effectively Overall effectiveness of inhibitors was lower than in 2D models due to diffusion barriers in spheroids Elacridar showed effectiveness 5 h after removal, thus showing differentiation abilities of the model between static and dynamic inhibitors	Curran et al. (2015)
NHLF, SAEC and HMVEC-L co-cultured cells dispensed on poly-HEMA-coated plates and centrifuged to form 3D aggregates	Recombinant human Wnt5a Cisplatin LiCl (chemical activator of Wnt5a) IWR-1 (pathway inhibitor)	ABCB1 ABCG2	Transporter expression was induced in primary healthy lung tissue formulated as 3D aggregates, by adding precursors responsible for transporter expression Chemical manipulation of the Wnt5a pathway induced or reduced transporter expression Cisplatin presence induced MDR in other combinatory drugs through the Wnt5a modulation	Vesel et al. (2017)
MCF-7 and DOX resistant MCF-7/ADR cells entrapped in alginate-oligochitosan microcapsules to form 3D aggregates	Doxorubicin derivatives (Palm-N2H-DOX, N-Palm-DOX, DOX-5FU, DOX-TPP and DOX-AMG)	BCRP P-gp	Determining the most effective derivative (Palm-N2H-DOX) in the MDR 3D cancer model This model had increased resistance towards drugs when comparing flat culture models	Akasov et al. (2017)
Adriamycin-resistant cells, MCF-7R, and parental control cell line, MCF 7, seeded in silk-collagen scaffolds to form 3D aggregate models	Doxorubicin Carboplatin Paclitaxel	P-gp MRP2	3D aggregates had distinct cell proliferation, cellular aggregate formation and expression of drug resistance-related genes/proteins compared to that of 2D models The distinctive characteristics made the model more comparable to the in vivo environment Drug resistance of the 3D model increased due to alterations in the cell cycle distribution and increased presence of breast cancer stem cells	Ding et al. (2018)

Table 1 (continued)

Model	Drug target	Transporter studied	Outcome	References
HER2-positive breast cancer cell lines cultured as 3D models using the poly-HEMA method	Neratinib (HER2 targeted therapy) Docetaxel (chemotherapy)	HER2	This 3D model indicated a higher innate resistance due to alterations in cell survival precursors such as the receptor proteins, drug transporters and metabolic enzymes when compared to the 2D cells An overall increased expression of drug targets, cell survival proteins and drug transporters was also reported when compared to the 2D counterpart An overall increased cellular viability was reported when 3D cells were dosed with toxic concentrations in 2D	Breslin and O'Driscoll (2016)

concentrations of paclitaxel in the tumours and thereby provided a synergistic anti-tumour effect (Wang et al. 2017a).

Some of the other strategies that have been attempted to overcome MDR in chemotherapy, besides efflux inhibition, are briefly outlined here. Collateral sensitivity is the use of compounds that selectively kill MDR cells without killing the non-resistant parental cells from which they were derived. The genetic alterations accrued by cancer cells to obtain resistance against one compound may provide hypersensitivity towards another compound, which can be exploited. An important aspect of this strategy is that its efficacy is independent from other mechanisms of MDR in cancer cells (Pluchino et al. 2012). Another strategy that was proposed to overcome MDR is ribonucleic acid (RNA) interference (RNAi) therapy. In brief, this approach aims to silence or down-regulate the expression of specific genes by means of destructing mRNA molecules as triggered by other RNA molecules (Saraswathy and Gong 2013). Metronomic chemotherapy is the long-term administration of low, but active, concentrations of chemotherapeutic drugs on a frequent basis to target endothelial cells and thereby provide an anti-angiogenic effect (Hida et al. 2017). The combination of metronomic chemotherapy and immunotherapy exhibited promising results in both preclinical and clinical studies, but more research is necessary to be conclusive (Chen et al. 2017c). Microparticles have been identified as vectors to spread MDR to drug sensitive cancer cells by means of a non-genetic mechanism (Bebawy et al. 2009). Compounds that are capable of inhibiting the formation of microparticles are under investigation to potentially overcome MDR in cancer (Vysotski et al. 2016). Replication competent viruses have been used to kill cancer cells without harming normal cells. To improve the anticancer activities of oncolytic viruses, they can be engineered to express exogenous transgenes of tumour proteins (e.g., tumour protein p53) (Bressy et al. 2017). Several models are available to study the anti-tumour effect of potential compounds in an attempt to overcome MDR caused by efflux transporter up-regulation.

In vitro models for multidrug resistant cancer treatment screening

To screen for the effectiveness of anticancer drugs, it is essential to use a viable test system that truly represents the disease as it appears in human subjects. Systems used as preclinical screening models include, among others, in vitro approaches such as cultured unicellular or multicellular cell lines, as well as stem cells (Du et al. 2016; Shaheen et al. 2016; Vandenhaute et al. 2016). Cultured human cancer-derived cell lines form a strong pillar in anticancer drug screening and an exhaustive number of high-throughput

Table 2 In vivo efflux transporter-based models used for anticancer drug screening

Model	Drug target	Transporter studied	Outcome	References
Cell-derived xenografts				
Subcutaneous implantations				
ABCG2-HEK293 cells (human embryonic kidney cells)	MBLI-87 (ABCG2 inhibitor) Irinotecan	ABCG2/BCRP	Significant sensitisation to irinotecan Increased irinotecan effect MBLI-87 prevents drug efflux by ABCG2 inhibition	Arnaud et al. (2011)
ABCB1-KB-C2; ABCG2-H460/MX-20 & ABCC10-HEK/MRP7 cells (human non-small cell lung cancer cells, human embryonic kidney cells)	Nilotinib	ABCB1; ABCG2; ABCC10	Nilotinib potentiates the anticancer effect of paclitaxel & doxorubicin in MDR xenograft models	Tiwari et al. (2013)
SKOV-3 cells (human ovarian cancer cell line)	NSC23925 (P-gp inhibitor) Paclitaxel	P-gp/MDR1	Combination of paclitaxel & NSC23925 showed inhibition of tumour growth NSC23925 prevented development of paclitaxel resistance in vivo	Yang et al. (2015)
MCF-7/ADR cells (human breast cancer cells resistant to adriamycin)	Psi-Pgp-tGC nanoparticles Doxorubicin	P-gp	Psi-Pgp-tGC nanoparticles down-regulated P-gp expression Nanoparticles potentiated doxorubicin-mediated inhibition of tumour growth & showed lower tumour volume	Yhee et al. (2015)
KB_{V200} cells (human keratin-forming HeLa-derivative, ABCB1-overexpressing cells)	Trametinib Vincristine Doxorubicin	ABCB1	Trametinib in combination with vincristine inhibited the tumour growth of ABCB1-overexpressing xenografts Trametinib used in combination therapy has the potential to combat efflux pump related MDR	Qiu et al. (2015)
MDA-MB-231 and BT-474 cells transfected with shRNA targeting ABCC1 or ABCC3 (human breast cancer cells)	Doxorubicin Mitoxantrone 5-Fluorouracil	ABCC1 & ABCC3	Like ABCC1, ABCC3 is also overexpressed in primary breast cancers ABCC3 was responsible for drug resistance, whereas ABCC3 knock-down reversed resistance	Balaji et al. (2016)
C4-2B, TaxR and DU145-DTXR cells (docetaxel resistant human prostate cancer cells)	Bicalutamide Enzalutamide Docetaxel	ABCB1	Combination of bicalutamide & docetaxel overcomes resistance Bicalutamide and enzalutamide inhibit ABCB1 transporter activity	Zhu et al. (2015)

Table 2 (continued)

Model	Drug target	Transporter studied	Outcome	References
HCT-15 cells (human colorectal adenocarcinoma)	Fluoxetine (Prozac) Doxorubicin Bevacizumab (avastin)	P-gp; MRP & BCRP—tested, but not detected	Combination of doxorubicin & fluoxetine showed significant slow-down of tumour progression, comparable to aggressive treatment with bevacizumab Fluoxetine improved doxorubicin intracellular accumulation & uptake Ability of fluoxetine to modulate resistance in vivo	Argov et al. (2009)
JFCR013-2 cells (human non-small cell lung cancer cells resistant to ceritinib)	Ceritinib Crizotinib Alectinib	P-gp	P-gp overexpression is the major cause of ceritinib & crizotinib resistance in NSCLC cells Ceritinib & crizotinib are P-gp substrates	Katayama et al. (2016)
T24 cells (human bladder cancer cells)	Emodin Cisplatin	MRP1	By downregulating the MRP1 expression, emodin improves the chemosensitivity to cisplatin	Li et al. (2016)
H460 cells (human large cell lung carcinoma cells)	Sulindac Doxorubicin	MRP1	Sulindac reduced doxorubicin resistance by inhibiting MRP1 activity	O'Connor et al. (2004)
JC cells (mouse primary mammary gland adenocarcinoma cells)	Paclitaxel Poly(D,L-lactide-co-glycolide) nanoparticles	P-gp	Nanoparticles, containing both paclitaxel and P-gp targeted siRNA, showed effective MDR1 gene silencing & the improved accumulation of paclitaxel in drug-resistant cells	Patil et al. (2010)
Orthotopic implantations				
MCF-7/DOX cells (human breast cancer cells)	Nelfinavir (inhibitor) Doxorubicin	P-gp	Co-exposure to the anti-HIV-drug nelfinavir enhanced the antitumor efficacy of doxorubicin	Chakravarty et al. (2016)
MDA-MB-231 cells (human breast cancer cells)	Curcumin Paclitaxel Cisplatin Doxorubicin	ABCG2 ABCC1	Curcumin downregulated ABCG2 and ABCC1 expression Curcumin enhanced the sensitivity of the cancer to paclitaxel, cisplatin, doxorubicin & mitomycin C	Zhou et al. (2015)
Patient-derived xenografts				
Subcutaneous implantations				
Small cell lung cancer	Cyclophosphamide Cisplatin Doxorubicin Etoposide Verapamil (inhibitor)	P-gp/MDR1	The co-administration of verapamil enhanced the antitumor efficacy of CyCav treatment regimen	Arvelo et al. (1995)
Human colorectal tumours	PF-309 (inhibitor)	P-gp/ABCB1 gene	PF-309 efficacy is affected by the expression of P-gp in tumours	Bradshaw-Pierce et al. (2013)

Table 2 (continued)

Model	Drug target	Transporter studied	Outcome	References
Merkel cell carcinoma (MCC)	Carboplatin Etoposide	ABCB5	MCC cancers showing carboplatin and etoposide resistance, had high ABCB5 expression levels Tumour growth was reduced by the reversal of drug resistance, through the administration of ABCB5 blockade	Kleffel et al. (2016)
Non-small cell lung cancer	Etoposide Carboplatin Gemcitabine Paclitaxel Erlotinib	BCRP MDR1 MRP1	A low correlation between BCRP expression & PDX response to etoposide was found No other efflux transporter expression levels were correlated to any drug responses in vivo	Rolff et al. (2009)
Retinoblastoma (eye cancer)	Topotecan Melphalan	ABCB1 ABCC1 ABCG2	The single exposure to melphalan or topotecan did not influence the expression levels of the efflux transporters in the PDXs	Winter et al. (2016)
Genetically engineered mouse models				
Knock-in mice				
mdr1a.fLUC knock-in mice	Paclitaxel Docetaxel Pregnenolone-16α-carbonitrile (PCN)	MDR1	By inserting a firefly luciferase (fLUC) gene into the mdr1a locus of the murine host, luminescence intensities could be accurately detected mdr1a.fLUC expression was shown to change with paclitaxel, docetaxel or PCN treatments	Gu et al. (2009)
Knockout mice				
Mdr1a/1b$^{-/-}$ double knockout mice	BMS-275,183 (analogue of paclitaxel) Pantoprazole (proton pump inhibitor)	P-gp/ABCB1 MRP2/ABCC2 BCRP/ABCG2	By genetically deleting P-gp, it was shown that P-gp plays a role in the pharmacokinetics & brain distribution of BMS-275,183	Marchetti et al. (2014)
Mrp4$^{-/-}$ knockout mice	Topotecan	MRP4	The distribution of the anticancer drug, topotecan, is restricted by the Mrp4 transporter The overexpression of Mrp4 causes topotecan resistance	Leggas et al. (2004)
Mdr1a/b/Mrp2$^{-/-}$ triple knockout mice	Irinotecan Methotrexate Doxorubicin	P-gp/MDR1 MRP2	Methotrexate is a good substrate for Mrp2, whereas irinotecan is poorly effluxed	Vlaming et al. (2006)

Table 2 (continued)

Model	Drug target	Transporter studied	Outcome	References
Doxorubicin-sensitive & doxorubicin-resistant $Brca1^{-/-}$;$p53^{-/-}$ tumours in K14cre;$Brca1^{F/F}$;$p53^{F/F}$ mice	Doxorubicin Tariquidar	MDR1	Doxorubicin resistance is caused by increased expression of the Mdr1 gene encoding P-gp The third-generation P-gp inhibitor, tariquidar, can reverse this resistance	Pajic et al. (2009)
$Brca1^{-/-}$;$p53^{-/-}$ tumours in K14cre;$Brca1^{F/F}$;$p53^{F/F}$ mice	Doxorubicin AZD2281 (inhibitor) Tariquidar (inhibitor)	P-gp/Abcb1a and Abcb1b	The up-regulation of P-gp induced acquired resistance to AZD2281 The addition of the P-gp inhibitor, tariquidar, reversed this resistance	Rottenberg et al. (2008)
Abcg2-deleted alleles bred with K14cre;$Brca1^{F/F}$;$p53^{F/F}$ mice	Topotecan	BCRP/ABCG2	ABCG2 contributes to topotecan resistance in vivo	Zander et al. (2010)
Slco1a/1b$^{-/-}$;$1A2^{tg}$, Slco1a/1b$^{-/-}$;$1B1^{tg}$, Slco1a/1b$^{-/-}$;$1B3^{tg}$ mice	Paclitaxel Methotrexate	OATP1B1 OATP1B3 OATP1A2	Methotrexate is a substrate of all three human OATP1A/B transporters Paclitaxel is transported by OATP1B3 and OATP1A2, but not OATP1B1	Van de Steeg et al. (2013)

assays and types of cellular material are available for measuring anticancer effects (Barretina et al. 2012; Clarke and Holyoake 2017). Furthermore, it is relatively easy to perform genetic manipulation of these cells that can provide information on the genetic mutations occurring in cancer cells, thus correlating with tumour tissues (Ashraf et al. 2012; Clarke and Holyoake 2017; Wilding et al. 2010). Essentially, in vitro approaches refine preclinical results before advancing to animal and human trials; therefore, reducing ethical concerns, time and cost. However, in vitro cell culture-based models also have certain drawbacks as outlined in this review.

Cultured cell models can range from very simple systems to more intricate and complex models consisting of multiple cell types (i.e., co-cultures), additional matrices or mechanical support. For the purpose of this review, the in vitro cell-based models used to screen for the anticancer efficacy of efflux-targeting MDR treatments are divided into conventional (basic mammalian cell lines cultured as 2D or flat cultures) and complex (mammalian cell lines cultured with additional matrices, foreign objects or as 3D cultures) cell-based models.

Conventional cell-based models

In this review, "conventional models" include all adherent cell cultures which are grown in an artificial environment in a 2D state as a monolayer or flat culture, although some suspension cells can also be classified as conventional cell-based models. Conventional mammalian cell culture models have served as the primary support for the evaluation of tumour biology, drug screening and mechanistic studies (Barretina et al. 2012).

The introduction of cell cultures in 1950 provided an exciting new method for cancer drug screening in an in vitro environment (Hickman et al. 2014) and to date, it is still widely used to evaluate the activity of potential anticancer drugs. In the 1980′s, the United States National Cancer Institute (NCI) listed 60 human tumour cell lines that can be used for in vitro drug screening and discovery (Shoemaker 2006), which are available online on the NCI's website (https://dtp.cancer.gov/discovery_development/nci-60/). These cell lines encompass nine different tumour organs/systems including blood, prostate, colon, central nervous system, ovaries, kidney, skin, breast and lung. This NCI-60 cell panel is considered an important platform for high-throughput models for characterisation and a biologically based approach to investigate the treatment of malignancies (Zampella et al. 2016). The effect of the ABC transporter family on chemotherapeutics, and potential inhibitors of these transporters, has been studied using the conventional cell lines as listed by the NCI (see Table 1).

Non-cancerous human-derived cell lines: Given that normal kidney epithelia express drug efflux transporter proteins, such related cell lines can be used to investigate drug efflux. A human embryonic kidney (HEK293) cell line was used to modify an inverted membrane vesicle and estradiol-17-β-glucuronide was subsequently identified as a substrate for MRP2, P-gp and BCRP. This shared substrate was then used to determine the transport inhibition ability of nine inhibitors, showing different inhibitory properties towards the studied ABC transporters (Pedersen et al. 2017). Furthermore, an exosome-encapsulated paclitaxel delivery system was tested in P-gp expressing Madin-Darby canine kidney (MDCK) and RAW 264.7 macrophage cells, indicating a potential strategy for future chemotherapeutic treatment (Kim et al. 2014).

The simultaneous screening of compounds against chemosensitive, drug resistant and non-cancerous cell lines can help to evaluate the overall level of drug resistance, the mechanism responsible for resistance and the cytotoxicity of the compounds. The cytotoxic ability of fascaplysin (a natural product with anticancer activity) was screened between 134 and 1 740 nM against a panel of cell lines including non-small cell lung cancer (NSCLC), small cell lung cancer (SCLC), unrelated cell lines and the HEK293 cell line. The concluding remarks suggested the alteration of reactive oxygen species (ROS), topoisomerase I and poly-(adenosine diphosphate (ADP)-ribose) polymerase (PARP) activity through the influence of several cellular mechanisms (Hamilton 2014). More recently, it was shown that the efflux of doxorubicin and rhodamine 123, through ABCB1 and ABCG2 transporters, was inhibited by abemaciclib in hyper-expressing cell lines such as KB, MCF-7, the human colon carcinoma cell line S1 and HEK293 cells (Wu et al. 2017).

Drug induced resistant cell lines: Retrospectively, during the mid-1970′s the Chinese hamster ovary cell line was the first cell model to indicate a correlation between the drug-resistant phenotype and the drug efflux P-gp membrane transporter, which was indicated by a change in the drug permeation rate (Juliano and Ling 1976). Drug resistance was induced in the cultured cells through exposure to actinomycin D, which resulted in a 2500-fold increase in resistance compared to the parental cell line (Biedler and Riehm 1970). The initial technique used in developing the drug-resistant Chinese hamster cell model in the mid-1970′s is still used in modern cell culture laboratories today. It entails the continuous exposure of a parental cell line to an anticancer drug resulting in a drug-resistant daughter cell line. Furthermore, conventional efflux cell culture models and modified cell culture models are used together with suitable assays during drug screening to investigate MDR. Determination of P-gp protein levels can be done through a Western blot assay (Kim et al. 2016). Several other cell viability, proliferation and cytotoxicity assays [e.g., 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)], bidirectional transport studies and clonogenic assays are commonly used to compare the sensitivity or resistance of both the parental and daughter cell lines towards anticancer drugs (Corcoran et al. 2012; Hamilton 2014; Wong et al. 2006; Xue et al. 2016). Furthermore, the inhibition of ABC transporters and the extent of substrate efflux can be determined through fluorescent dye accumulation and vesicular transport assays (Ghavami et al. 2011; Whitt et al. 2016).

In a study conducted by Kim et al. (2014), a doxorubicin-resistant breast cancer cell line (MCF-7/ADR) was obtained by exposing the parental MCF-7 cell line to 0.3 μM doxorubicin (Adriamycin®) over an extended period of time. The combined effect of doxorubicin and resveratrol subsequently decreased the gene expression of MDR1 and MRP1 in this breast cancer model, which simultaneously increased the intracellular doxorubicin concentration. The same MDR model was also used to establish a novel micelle-based drug delivery system to increase the uptake of paclitaxel into the tumour tissue (Zhao et al. 2016). The MDR MCF-7/ADR cell line is one of the most widely used conventional models in cancer research, although its true origin is still questionable. Therefore, it should rather be used for hormone-responsive breast cancer studies due to its expression of oestrogen and progesterone receptors (Ke et al. 2011; Vickers et al. 1988).

One of the critical points of acquired drug resistance following drug exposure is the lack of standardisation of the methods and techniques used for these cell lines. As a consequence, results obtained from different laboratories are not always comparable and may be attributed to the type of chemotherapeutic drugs used or specific concentrations applied. On the other hand, drug induced resistant models can be informative when comparing the activity of a new drug in parental chemosensitive and resistant daughter lines. Kars et al. (2006) developed resistant sub-lines from the parental MCF-7 cell line (MCF-7/S) through stepwise selection in dose increments over 2 years. It was found that the cell lines MCF-7/120nMPaclitaxel, MCF-7/600nMDoxorubicin and MCF-7/80nMDocetaxel developed cross-resistance to the other anticancer drugs, to a higher extent, than the MCF-7/40nMVincristine cells. This showed that the parental MCF-7 cell line acquired cross-resistance to different anticancer drugs, at different levels, and each daughter cell line may have developed different drug resistance mechanisms. In agreement with the aforementioned study, at specific doses of chemotherapeutic drugs the MCF-7 cells have been shown to acquire drug resistance not only to the selected agent, but also cross-resistance to other agents of a similar drug class, and in some instances, even to drugs of other classes (Hembruff et al. 2008).

Transfected cell lines: Contributing further to the development of drug screening models is the transfection of a parental cell line with microRNA or small interfering RNA (siRNA). These models enable targeted alteration of the transporter expression of the parental line in the hope of modifying the extent of drug efflux, resulting in anticancer drug accumulation in the tumour and consequently increased therapeutic effects (Chen et al. 2015, Yhee et al. 2015). However, the shortcomings of using nucleic acid (RNA and DNA) interference therapies include ineffective tumour targeting, reduced RNA stability and short circulation time, which limit its clinical use (Yi et al. 2016). In a study conducted by Yang et al. (2017), a co-delivery system containing both siRNA (to silence MDR1 mRNA) and paclitaxel, showed increased cytotoxicity of paclitaxel in MCF-7 and MCF7/taxol cells. The ovarian carcinoma cell line (SKOV-3) was also used to evaluate the effectiveness of a nanoscale metal–organic framework co-delivery system of siRNA and cisplatin, to decrease the expression of MDR genes, and an increased chemotherapeutic efficacy was observed (He et al. 2014). More recently, a hollow carbon nanosphere particle was used to treat MCF-7 and MCF-7/ADR cells by delivering either cisplatin or doxorubicin, in combination with siRNAs targeting MDR1. The findings included a down-regulation of up to 96% of MDR1 protein expression in the MCF-7/ADR cells (Zhang et al. 2017). In a sub-clone of human colorectal adenocarcinoma cells (Caco-2; sub-clone C2BBe1), the zinc finger nuclease-mediated gene knockout approach was used to reduce the extent of transport facilitated by several ABC transporters. This subsequently produced a successful tool for explaining transport interactions (Sampson et al. 2015). Human cervix carcinoma (HeLa) cells have also been used for transcription activator-like effector nucleases (TALEN)-mediated *Nanog* gene knockout, resulting in weakened malignancy (Ding et al. 2014).

Although continuous 2D cell lines are easily maintained and the same cell line can be used for long-term experimental evaluations, these models do not fully correlate with in vivo conditions (Phelan and May 2016). The monolayer growth pattern of conventional cells is a problem, since few primary tumours can grow as a monolayer ex vivo (Naipal et al. 2016). Moreover, not only efflux transporters are responsible for drug resistance. In fact, other mechanisms such as cell-to-cell and cell-to-extracellular matrix interactions also alter drug penetration and metabolism (Hembruff et al. 2008). Conventional cell models lack the proper 3D architecture needed to produce an extracellular matrix (ECM) (Breslin and O'Driscoll 2013; Sarisozen et al. 2014; Wrzesinski et al. 2014). All these drawbacks add to the need for development of more advanced cell culture systems in an attempt to overcome these limitations.

Complex cell-based models

The lack of models that consider and include the organisational physiological structure of human tissues is deemed the major precursor of clinical drug failure during in vivo trials (Katt et al. 2016; Mak et al. 2014), while large scale screening of anticancer drug compounds in in vivo models (e.g., rodent models) is for the most part unpractical and subject to ethical scrutiny (Linzey 2013; Tannenbaum and Bennett 2015). Intrinsic limitations of conventional models cause isolation of certain characteristics, thus ignoring major pharmacokinetic parameters. This inability to accurately extrapolate to humans caused a major void in clinical trials and eventually stimulated the design of more complex systems resulting in advanced in vitro models (Griffith and Swartz 2006). These models are designed to better reproduce the primary elements of MDR, allowing better screening of drug compounds before they advance to the later stages of clinical trials. For the purpose of this review, complex cell-based models are defined as mammalian cells incorporated into advanced systems with more realistic intercellular communications and other features that better resemble those of the in vivo microenvironment. Table 1 provides some examples of complex cell-based models. For schematic illustrations of various complex cell-based models, please refer to the following reviews: Verjans et al. (2017), Katt et al. (2016) and Nath and Devi (2016).

Integrated discrete multiple organ culture (IdMOC) system

The integrated discrete multiple organ culture (IdMOC) system was designed to consist of individual wells, each containing their own cell culture and growth medium, embedded in a larger outer chamber (Li 2009). Once the cells in each well reached experimental viability, a universal medium containing the test compound is added to such an extent that it overflows into all the wells and fills up the outer chamber, thereby creating an interconnected system. This universal medium serves as a blood surrogate between the different cells in the system, containing metabolites and waste products produced by each organ-representative well, and thereby mimic the in vivo state by exposing the blood surrogate to every part of the system. This overcomes the problem of cell type isolation that is present in traditional 2D cell culture studies. The universal medium can be analysed to determine accumulative system metabolism, whereas each individual cell culture can be separately analysed for organ toxicity, bio-accumulation and cytotoxicity (Gayathri et al. 2018; Li et al. 2004, 2012; Li 2005). The IdMOC system was designed to incorporate primary hepatocytes, cell cultures representing several organs and a cancer cell line to allow more effective testing of potential anticancer drugs on multiple organ systems (Li 2008). This model currently

permits for the screening of organ selective toxicity, cytotoxicity, drug–drug interactions, metabolic activation and also enables analyses of pharmacokinetic parameters (e.g., MDR1 and MRP1 influence) (Li 2009).

The major advantages of the IdMOC system include the ability to do high-throughput screening assays, retaining the homogeneity of the individual culture conditions of each cell line and the possibility of inter-cell metabolite interactions between each organ-representative. The model also functions as a low cost alternative for screening with an adaptable microenvironment to optimise growth conditions for each individual cell type. A major problem with this system, however, is the elevated risk for cross contamination. Another restriction lies in the disparity of the metabolic needs of the various cultures, potentially causing saturation or starvation of some of the cells receiving universal medium (Li et al. 2012; Uzgare and Li 2013). Time-dependent interactions between the organs are also not simulated, reducing this model's capability of giving exact quantitative results (Sung et al. 2013).

The application of the IdMOC system in MDR studies was suggested by Uzgare and Li (2013) in a review of the IdMOC model. Targeted disruption of specific transporters responsible for MDR (e.g., MDR1, MRP1, etc.) and the pharmacokinetic consequences thereof can be investigated in this model because of the incorporation of liver cells (Uzgare and Li 2013). A cytotoxicity study on tamoxifen was completed using the IdMOC system incorporating primary cells of different origin namely hepatocytes (liver), cortical cells (kidney), small airway epithelial cells (lung), astrocytes (central nervous system), aortic endothelial cells (blood vessels) and the MCF-7 human breast adenocarcinoma line. This allowed for the effective quantification of cytotoxicity in normal cells compared to cancer cells. Each individual organ system provided results comparable to those seen in literature during human trial cytotoxicity studies, thus validating this model (Li et al. 2011; Uzgare and Li 2013).

Microfluidic channel-based systems (tumour-on-a-chip)

The idea of microfluidic systems was conceptualised around the design of a system encapsulating complicated, time intensive and expensive laboratory protocols into a relatively inexpensive, portable cell culture model that involves the manipulation of relatively small amounts of fluid (microlitre or picolitre) in a channel-based system on a chip (El-Ali et al. 2006; Whitesides 2006). Microfluidic systems were designed to overcome the absence of pharmacokinetic features in in vitro cell-based models as well as to refine, automate and reduce the associated costs of current laboratory practises (Guo et al. 2012). The incorporation of multiple barriers commonly affecting drug delivery into organ/tissue compartments, as well as an interconnected microfluidic channel arrangement acting as a vascular circulating system, allowed for testing the compounds' permeability capabilities in the in vitro setting. Culture medium acts as a blood surrogate in the microfluidic channels, delivering important nutrients into the cells, while carrying metabolites away from the cells. The microfluidic channel model is scaled according to human physiological parameters, which provides a realistic degree of drug exposure to each specific physiological barrier. This also allows a better understanding of pharmacokinetic interactions at each of the barriers, thus increasing predictive capabilities during extrapolation to the in vivo situation (Dittrich and Manz 2006). Microfluidic chips containing cell cultures are equipped with microphysiometers capable of measuring numerous metabolic parameters (e.g., oxygen consumption, glucose uptake, lactate production) by means of microsensors (Weltin et al. 2014). The incorporation of multiple-well plates (e.g., 96-well plates) also makes this model a viable option for high-throughput screening (Weibel and Whitesides 2006). Its dynamic nature ensures the real-time monitoring of cell culture parameters needed for drug development, while minimising standard deviations of results due to the concentrated amounts of reagent used in these studies. Human cell lines can be used in these systems, each chosen to have the desired characteristics of the designated organ portrayed in the model (e.g., MDR cancer combined with a normal epithelial cell line). The model can be manipulated to test permeability potential through different barriers by sequential organisation of the tissue chambers (e.g., intestinal lumen before the cancer cells) (Ghaemmaghami et al. 2012; Kashaninejad et al. 2016). Microfluidic based systems also have the capability to present drug synergistic effects that cannot be seen in traditional in vitro studies and the replicated vascular system allows for different dose exposure parameters, giving results similar to those in vivo. Currently, there are vast selections of these chips, each containing unique mechanisms and added protocols. Organ systems included in this design is lung, liver, intestinal, urinary, breast, brain, and bone marrow tumours (Bhise et al. 2014; Caplin et al. 2015; Van der Helm et al. 2016).

Different types of microfluidic channel systems have been developed and used in studies as briefly described below. The next step in developing advanced organ-on-a-chip systems is to incorporate multiple organ cell cultures in the same system. This creates an in vivo like microphysiological system that represents all of the major tissues responsible for metabolic processes, as well as influencing drug pharmacokinetics and pharmacodynamics (Maschmeyer et al. 2015). One example of a multi-organ co-culture as part of a tumour-on-a-chip design included HepG2/C3A (hepatocellular carcinoma/liver), MEG-01 (megakaryoblast/bone marrow), MES-SA (drug sensitive uterine sarcoma) and MES-SA/DX-5 (MDR uterine sarcoma) cell lines. The HepG2/

C3A cell line was included to view the effect of the metabolism of doxorubicin on the less active doxorubicinol. The MEG-01 cell line represented the thrombocytopenic effect of this drug due to bone marrow suppression, while the cancer cell line MES-SA/DX-5 was transfected to overexpress P-gp, thereby inducing MDR. This P-gp overexpression allowed the researchers to determine if the required concentration of drug needed to have cytotoxic effects on the resistant cancer cells, was in an acceptable range to avoid systemic toxicity (Tatosian and Shuler 2009).

The lung-on-a-chip microfluidic device is a novel biomimetic device developed to improve the model by incorporating key characteristics of tissues that were absent from the traditional microfluidic channel system. The lung-on-a-chip microfluidic device was designed to accommodate an ECM coating (consisting of collagen and fibronectin), whilst human alveolar epithelial cells and human pulmonary microvascular endothelial cells were cultured to confluent monolayers on opposing membrane sides, which allowed airflow between the barriers. This airflow allowed for an air–liquid interface similar to the alveolar airspace found in human lungs. The compartmentalised design enabled controlled delivery of nutrients to the different cell cultures. Two vacuum inducing micro-chambers were installed to mimic sub-atmospheric pressure driven stretching of the membranes to replicate normal breathing. In this model, the researchers attempted to replicate the major factors of pulmonary physiology to evaluate how drug compounds may act in these systems and to compare the outcomes to actual in vivo results (Huh et al. 2010).

In another application, polydimethylsiloxane (PDMS) round bottom micro-wells were constructed as microchip bases and hydrostatic pressure was used to guide the introduced cancer cell suspension to the membranes. Gravitational forces kept the cells in place and forced the formation of 3D cell culture constructs. The mesothelioma cell line (H2052) and primary cell lines from patients (NSCLC, squamous carcinoma and pericytes) were introduced into the model as mono- and co-cultures to test the effect of stromal cells on MDR, using cisplatin as chemotherapeutic compound. This system allowed viable assays, but a concentration gradient generator was proposed for future, more realistic studies (Ruppen et al. 2015).

It is evident that the microfluidic channel-based system incorporates conventional cell models, which presents its own range of drawbacks including cell changes and differentiations through passages and physiological structure differences compared to cells within in vivo organs. Even though the microfluidic system allows the culturing of various cell types, the formation of native connections between the different cell types, as it occurs in vivo, is prohibited. Additionally, adapting this model for high-throughput screening studies is difficult and expensive because of the expertise needed to maintain viability and sterility for all the different cell cultures in play (Kashaninejad et al. 2016).

3D spheroid cell cultures

Complex 3D spheroid cell cultures are being incorporated into modern in vitro screening models, as an attempt to bridge the gap between traditional flat culture shortcomings and the limitations experienced with in vivo studies (Imamura et al. 2015; Lee et al. 2009). This design more realistically mimics the physiological characteristics of in vivo tumours by allowing cell–cell interaction, as well as better communication within the microenvironment (e.g., extracellular matrix). Intercellular communication includes the activation of signalling pathways, which mediates a cascade of effects including molecular, subcellular and cellular changes. These changes can include alterations in protein/gene expression, molecular exchanges, enhanced cellular transport effects (endocytosis/exocytosis) and cellular behavioural changes (e.g., apoptosis, proliferation, cell shape, motility) (Elliott and Yuan 2011; Kriston-Vizi and Flotow 2017).

3D spheroid cell culture is seen as the first and oldest 3D cell culture model construct and dates back to the 1970′s. This model depends on the tumour cells' capability to differentiate into heterogeneous multicellular spheroids by either introducing them into an ECM as a scaffold-dependant (i.e., scaffold-based) construct or by introducing external factors to enforce spheroid growth (i.e., non-scaffold based). Scaffold-based cell culture models include matrix-on-top, matrix-embedded, matrix-encapsulation, spinner flasks and the micro-patterned plate technique. Non-scaffold-based cell culture models employ ultra-low attachment plates, hanging drop, microgravity (rotating wall-vessels/bioreactors or clinostat), spontaneous aggregation, magnetic levitation and 3D bioprinting techniques (Moon et al. 2009; Nath and Devi 2016). After the cells start piling up on each other in 3D spheroid cultures, they develop surface receptors known as integrins, which are used to anchor themselves onto the ECM and thereby sustaining tumour growth and enhancing cell–cell communication (Antoni et al. 2015; Breslin and O'Driscoll 2013).

The connection of these cells to the microenvironment initiates increased protein and gene expression levels of MDR precursors, which are generally limited in conventional models. Changes that were found to occur in these cell structures included metabolic changes, increased responses to cellular stress, cellular structural changes, increased signal transduction, slower proliferation cycles (quiescent cells), limited drug surface contact, inhibited apoptotic precursors and elevated expression of cellular transporter proteins, all playing a role in the increased resistance of these cell models to cancer treatment (Nath and Devi 2016). Barrera-Rodríguez

and Fuentes (2015) compared the drug resistance properties of monolayer and spheroid models of two NSCLC cell lines (INER-37 and INER-51). The 3D spheroids of both cell lines were less sensitive to etoposide, teniposide, 5-fluorouracil, doxorubicin, cytosine-arabinofuranoside, methotrexate and camptothecin, than the monolayer cultures. Moreover, the expression of some MDR-associated genes (MDR1, MRP1, Topo IIα, Topo IIβ, GST-μ and Topo I) was higher in the INER-37 spheroids, than in the 2D monolayers. Ultimately, it was found that the 3D models conferred MDR in cells through numerous gene expression changes, most notably the induction of MDR transporters (e.g., P-gp).

3D spheroids more accurately predict the true in vivo fate of a drug, not only as a result of ABC efflux transporter expression, but also due to proper cell-to-cell contact organisation and communication. The capability of cells in a spheroid to use cadherin molecules and integrin surface receptors to mediate resistance against drug treatment, allowed for the design of effective MDR cell culture models (Barrera-Rodríguez and Fuentes 2015; Desoize and Jardillier 2000; Shield et al. 2009). The expression of epidermal growth factor receptor (EGFR) and β-III tubulin in a 3D and 2D cultured prostate cancer cell line (DU145) was influenced by the matrix type and this correlated with the resistance level towards rapamycin and docetaxel (Edmondson et al. 2016). The major advantage of a 3D spheroid cell culture model is the formation of the in vivo-like necrotic centres (although recent publications showed some spheroid systems to be devoid of necrotic centres) and an overall heterogeneity (Wrzesinski and Fey 2018). In most larger 3D cell spheroids (i.e., exceeding 150 μm in diameter), the nutritional starvation and hypoxic conditions of the tumour centre causes necrosis accompanied by micro-metastatic behaviour, thus allowing the investigation of anticancer compound evasion mechanisms, as well as the overall influence that these physiological changes may have on tumour biology (Hirschhaeuser et al. 2010; LaBarbera et al. 2012; Ma et al. 2012).

The superiority of this model lies in its ability to shy away from the exponential growth phase priority that 2D cell cultures exhibit, by re-directing energy to develop advanced tumour-like structures (Wrzesinski et al. 2014). This mechanism of sophisticated self-assembly occurs because the cells reach a "dynamic equilibrium", and this phenomenon essentially bridges the gap between in vivo and in vitro screening (Wrzesinski et al. 2014). 3D cell models have been shown to have greater chemo- and radiotherapy resistance compared to their 2D counterparts and they show similar characteristics to those expressed by native cancers, including lactate accumulation, oxygen consumption/hypoxia and proliferation. It has also been suggested that 3D cell culture models lead to more aggressive cancer phenotypes with sustained proliferative signalling, inducing angiogenesis, resisting cell death, genome instability and mutations, and deregulating cellular energetics (Longati et al. 2013). The 3D spheroids can be used to determine lethal doses closely related to in vivo values (Fey and Wrzesinski 2012). Given that not all the cells in a spheroid are exposed to a drug at the same time and do not have the same proliferation rates, using spheroids will contribute to a deeper understanding of the mechanistic properties of cancer treatments (Lovitt et al. 2014).

Unfortunately, 3D cell culture operating techniques can be time consuming and the reproducibility of some techniques are questionable (Vinci et al. 2012). Furthermore, if the size of the spheroid is greater than 150 μm, necrosis of the core can occur due to inadequate nutrient and oxygen diffusion (Chatzinikolaidou 2016), although this is greatly dependent on the type of spheroid system being used (Wrzesinski and Fey 2018) and considering that a necrotic core is not necessarily a limitation of a cancer-specific model (Riffle et al. 2017; Staudacher et al. 2018). Drug penetration can also be restricted by the size of the spheroid, diffusion through the ECM and cell-to-cell and cell–matrix adhesion, influencing the effect of chemotherapy (Longati et al. 2013).

In vivo models for multidrug resistant cancer treatment screening

Although in vitro cell culture-based models are fundamental building-blocks in preclinical cancer research, in vivo animal models are still used as the decisive factor in the preclinical anticancer drug screening process before further clinical trials in humans may be conducted (ICH 1998; FDA 2008). In vivo animal models resemble in part the in vivo responses and physiological conditions in humans (Stakleff and Von Gruenigen 2003), providing predictive means to study patient responses to anticancer drugs. Mice are the most commonly used animal model in cancer treatment research as their genome is well-characterised, making the manipulation of their genes much easier with the established genetic engineering techniques already available. Furthermore, their small size and short development period alleviates excessive breeding and maintenance costs in comparison to larger animals (Cheon and Orsulic 2011; Rangarajan and Weinberg 2003; Roper and Hung 2012). The most frequently used mouse cancer models involve xenografts, where human cell-derived tumours or human tumour explants (patient-derived) are implanted into immunocompromised mice, and genetically engineered mouse models (GEMMs) that are genetically manipulated to form spontaneous tumours. As both these models have been applied in cancer research to study efflux transporter-related MDR, they will be discussed in the following sections. However, it is important to mention that other rodent models also exist, but have not necessarily

been commonly utilised for efflux transporter-related MDR cancer studies.

Mouse allografts involve the injection of immunocompetent mice with murine cancer cells or tumours. This model eliminates the bias of xenografts, which use only immunocompromised mice (Dranoff 2012; Sharpless and DePinho 2006). However, the interspecies differences between human and mouse cells result in poor translation between the drug efficacy in allograft models compared to humans, as comprehensively reviewed by Rangarajan and Weinberg (2003). Despite such discrepancies, mouse allografts have been used to generate orthoallobanks of NSCLC for preclinical drug screening (Ambrogio et al. 2014), as well as basal cell carcinoma models for carcinogenesis and drug pathway studies (Wang et al. 2011).

By exposing rodents to carcinogens such as dimethylbenz(a)anthracene (DMBA), *N*-methyl-*N*-nitrosourea (MNU) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), spontaneous carcinogen-induced tumours can develop (Rao and Reddy 1980; Schuller et al. 1993). However, the locality of tumour formation is not always constant or controllable. In addition, there is the unpredictable tumour formation rate and the adverse effects (cancer-unrelated) of such chemical substances on overall tissues (Qiu and Su 2013).

In vivo hollow fibre assay models

In 1995, the NCI developed the hollow fibre assay (HFA) for the preliminary in vivo screening of anticancer drugs as a means to bridge the gap between in vitro cell-based assays and in vivo xenograft models (Hollingshead et al. 1995). This assay entails the culturing of 12 human cancer cell lines (of 6 different histologies) in highly permeable biocompatible polyvinylidene fluoride (PVDF) hollow fibres, followed by implanting them into nude mice at both subcutaneous and intraperitoneal locations. After anticancer drug exposure for 4–6 days, the fibres are removed and cytotoxicity effects detected with colorimetric tetrazolium-based dye (i.e., MTT) (Decker et al. 2004; Hollingshead et al. 1995). The majority of HFA-based data have been used in prioritising anticancer drugs with a high probability of antitumour activity in follow-up in vivo xenograft models. By implementing a few modifications to the original NCI protocol such as extending the drug exposure period, using immunocompetent mice or conducting other end-point measurements, several studies have proven the potential of the HFA to study antitumour vasculature drugs and tumour angiogenesis (Phillips et al. 1998; Shnyder et al. 2005), DNA damaging agents (Veiga et al. 2011), proteasome-inhibiting drugs (Bachmann et al. 2016) and the antitumour effects of active plant constituents (Liu et al. 2014; Mi et al. 2009). The antitumour activity determined with the HFA has been found to positively correlate with that of xenograft models (Johnson et al. 2001; Lee and Rhee 2005).

Xenograft cancer models

Xenograft cancer models are based on the implantation of a tumour(s) into immunocompromised mice, which ensures that no rejection of the induced tumour occurs (Gould et al. 2015). Such immunocompromised mice can either be athymic nude mice, with a mutation in the *Foxn1* gene (Fogh et al. 1977), or severely compromised immunodeficient (SCID) mice, with a nucleotide polymorphism in the *Prkdc* gene causing abnormalities to or complete absence of T- and B-lymphocytes (Cekanova and Rathore 2014; Morton and Houghton 2007). The tumour implantations can either be done orthotopically or heterotopically (Schuh 2004; Shaw et al. 2004). Orthotopic models are formed when the tumour is directly implanted into its organ of origin, whereas heterotopic models are placed directly underneath the skin (subcutaneous). As subcutaneous (heterotopic) xenografts can be observed with the naked eye in hairless (nude) mice and the procedure is much easier and less invasive than orthotopic surgeries, this model is frequently used for rapid cytotoxic or cytostatic anticancer drug screening (Killion et al. 1998; Kumar et al. 2016). In contrast, orthotopic tumours provide a more appropriate measure to study the tumour at its primary site of origin and to evaluate possible metastasis and invasion (Kumar et al. 2016). However, orthotopic models have to be excised from killed mice to measure the tumour volume, and therefore, only end-point measurements can be taken. Xenograft cancer models can be classified as either cell-derived or patient-derived, as discussed in the following sections, with the focus on efflux transporter-based models used for anticancer drug screening applications.

Cell-derived xenografts

The transplantation of in vitro cultured human cancer cell lines into mice with compromised immune systems is the traditional way to generate a xenograft. The specific type of induced cancer, the exact amount of cells required to form a solid tumour and the time it takes for tumour formation, all depend on the specific cancer cell line used (Dipersio 1981; Fogh et al. 1977; Schuh 2004). Given that a considerable number of established cell lines are available for transplantation, a wide range of human cancer types can be studied. Nonetheless, the continuous passaging of the in vitro cell lines can lead to drifted cells with different genetic characteristics than their primary cancer tissue of origin (Haddad and Yee 2008; Hughes et al. 2007). In addition, the homogeneity of an injected cell mass does not recapitulate the complexity of a tumour with evolved cells in symbiotic growth (Hanahan and Weinberg 2011). The implantation

of tumour cells in different sites than where they normally occur as a disease in humans can also contribute to discrepancies in translation (Junttila and De Sauvage 2013). As previously mentioned, the use of immunocompromised mice can also provide misleading results as not all cancers arise in immunodeficient patients (Frese and Tuveson 2007; Sharpless and DePinho 2006). Even though cell-derived xenografts (CDXs) have several limitations, this in vivo model is still frequently used for preclinical anticancer treatment screening.

Overall, for the screening of anticancer drugs against MDR tumours associated with efflux transporter up-regulation, human cancer cells showing efflux transporter overexpression are injected either subcutaneously (used predominantly) or orthotopically into immunocompromised mice generating a CDX. After the allocated drug treatment period, the cell-based tumour growth is evaluated for subsequent changes to the tumour size and volume. This is followed by immunohistochemical (IHC) analysis or western blot and quantitative reverse transcription polymerase chain reaction (RT-qPCR) experiments to identify the transporter protein expression levels. Using these end-point measurements, CDXs can provide valuable information regarding the efflux transporter proteins responsible for drug resistance in specific cancers (Katayama et al. 2016; Redaelli et al. 2015; Yang et al. 2015), the modulation effect of drug combinations on efflux transporter-related MDR in cancer (Argov et al. 2009; Chakravarty et al. 2016; Li et al. 2016) and the potential of herbal medicine to re-sensitise tumours towards anticancer drugs (Zhou et al. 2015).

Using in vivo CDXs in conjunction with in vitro cytotoxicity assays, several studies have proposed the combinational therapy of drugs as an effective regiment to overcome cancer MDR. Table 2 provides a broad overview of a range of CDXs used for efflux transporter-based anticancer drug screening. Argov et al. (2009) studied the modulation effect of fluoxetine (Prozac) on the moderate drug resistance of doxorubicin in subcutaneous human colon cancer (HCT-15) CDXs expressing only P-gp. Fluoxetine was found to reverse moderate MDR in vitro by inhibiting the P-gp efflux pump and improving doxorubicin intracellular accumulation. Tumour progression was drastically delayed when fluoxetine was combined with doxorubicin in vivo, which proved the combination therapy to be as efficient as bevacizumab treatment. Chakravarty et al. (2016) used the aggressive MDR breast cancer cell line, MCF-7/Dox, in orthotopic xenografts to investigate the anticancer efficacy of the clinically approved antiretroviral drug, nelfinavir. Multiple exposures to nelfinavir in vitro enhanced intracellular doxorubicin levels and inhibited P-gp expression and efflux. The co-exposure to nelfinavir and doxorubicin resulted in a 65–80% reduction of in vivo tumour growth, in comparison to each drug alone. As this breast cancer model overexpressed P-gp, nelfinavir was shown to counteract MDR by inhibiting P-gp activity. Another study utilised human embryonic kidney cells (HEK293) transfected with the ABCG2 transporter, also known as BCRP, to generate ABCG2-expressing xenografts (Arnaud et al. 2011). These MDR xenografts were implemented to assess the potential of MBLI-87, a non-toxic ABCG2-specific inhibitor, to increase irinotecan accumulation by modulating ABCG2-related drug resistance. The MBLI-87 inhibitor was able to re-sensitise the resistant tumours to irinotecan therapy, although it had no significant effect when treatments were discontinued for 14 days and reconstituted thereafter. This incident was similar to clinical findings also stating premature discontinuation to be a cause of survival failure (Stein et al. 2008). Cell-derived xenografts have also been used to evaluate the anticancer efficacy of drug-loaded nanoparticles against efflux transporter-related cancer MDR. Patil et al. (2010) used poly(D,L-lactide-*co*-glycolide) (PLGA) and polyethyleneimine (PEI) to induce gene silencing in P-gp overexpressing xenograft models (JC cells used). Using nanoparticles encapsulating PLGA-PEI and paclitaxel, with the surface functionalised with biotin for specific tumour targeting, the efficacy of paclitaxel to inhibit in vivo tumour growth was significantly higher compared to treatments without gene silencing. Therefore, the use of dual-agent nanoparticles focusing on gene silencing and cytotoxic drug encapsulation shows potential to defeat cancer MDR, especially when the particle surface is coated with specific ligands commonly recognised by cancer cell receptors, such as biotin (Patil et al. 2010).

Patient-derived xenografts

In an attempt to improve on the shortcomings of CDXs, the direct transplantation of a patient's primary tumour material as fresh explants into immunocompromised mice has been developed (Fiebig and Burger 2002; Morton and Houghton 2007; Williams et al. 2013). These models, known as patient-derived xenografts (PDXs) or tumour grafts, more closely recapitulate the parental tumour behaviour, histology, genetic diversity, and metastatic patterns (DeRose et al. 2011; Marangoni et al. 2007; Tentler et al. 2012). Bearing in mind that the tumour is directly transplanted into the murine host, the in vitro manipulation of the tumour is completely eliminated, ensuring the preservation of the original tumour heterogeneity. With the genomic analysis of 1075 established PDXs and corresponding cell lines, Gao et al. (2015) demonstrated that several signalling pathways were over- or under-represented in cell lines, whereas matching PDXs showed accurate representation thereof. Moreover, only minimum genetic drift occurred between the passages of a particular PDX, in contrast to the loss of certain developmental pathways in the cell lines upon continuous in vitro

culturing. Such similarities between PDXs and the original tumours were also observed for human breast cancer models (Marangoni et al. 2007). This explains the deferral of the in vitro NCI-60 cell panels, used for more than 25 years as the golden standard for anticancer drug screening, in favour of PDX collections that closely mimic the human tumour drug response (Ledford 2016). The concept of using PDXs as mouse surrogates (newly referred to as "avatars") in chemotherapeutic research has evolved into a promising practice to improve patient treatment regimens. A patient's tumour is used to generate a number of PDXs in various murine hosts and these xenografts are then studied concurrently or ahead of the actual patient treatment program (White 2013). This "avatar" approach can help guide the therapeutic management of the clinical trial and introduce key features necessary for the development of personalised medicine (Richmond and Su 2008). Patient-derived xenografts have also been shown to accurately portray the biodistribution of anticancer drugs when coupled to radiolabelled tracers and examined with micropositron emission tomography (microPET) imaging (Gangloff et al. 2005).

Patient-derived xenografts are more commonly implanted subcutaneously, but orthotopic and mammary fat pad implantations have also been done before (Marangoni et al. 2007; Williams et al. 2013). The development of validated PDX models is a long-term process with high costs. Just the average tumour latency, from tumour implantation to graft appearance, takes up to 12 months. Sadly, patients with rapidly growing tumours will most probably not benefit from their "avatar" models, established for their personalised therapy, as these model development programs are too time-consuming. Furthermore, the engraftment into an immunocompromised host has the potential to select for a more aggressive phenotype than originally present in the human patient (Marangoni et al. 2007). The lack of an intact immune system in the immunocompromised murine host hinders the ability to study cancer-related immune responses in PDXs (Dey et al. 2013; Malaney et al. 2014). However, this limitation has been addressed with bioengineering approaches using GEMMs (discussed in the following section), immune cells and fibroblasts to establish an immunocompetent mouse model (Shultz et al. 2012).

Irrespective of the abovementioned disadvantages of PDXs, these models can provide predictive preclinical means to evaluate patient drug responses, especially those related to efflux transporters involved in cancer MDR (Table 2). Earlier research conducted by Arvelo et al. (1995) investigated the potential of the P-gp inhibitor, verapamil, to counter MDR in SCLC PDXs. By combining verapamil with CyCAV, a multifaceted chemotherapeutic dosing consisting of cyclophosphamide, cisplatin, doxorubicin (adriamycin) and etoposide, the drug efficacy was improved against the resistant tumours. Therefore, verapamil inhibited the P-gp efflux transporter responsible for MDR in SCLCs. A recent study investigated the role of the transporter ABCB5 in the drug resistance of neuroendocrine skin cancer or Merkel cell carcinoma (MCC) (Kleffel et al. 2016). After the treatment of intraperitoneal MCC PDXs with carboplatin or etoposide, the expression level of ABCB5 was eightfold higher than the control PDXs. But, when these anticancer drugs were combined with an ABCB5 blocking antibody, tumour growth was significantly reduced and tumour apoptosis drastically enhanced. These results clearly showed the potential of ABCB5-targeted drug resistance reversal therapies to enhance the success of MCC treatment in the clinic.

Genetically engineered mouse models

Genetically engineered mouse models (GEMMs) are the most advanced in vivo models used for human cancer research. These models involve the genetic manipulation of cancer-related genes for drug efficacy screening, studying the interactions between chemotherapeutic agents as well as research on the role of these target genes in tumour biology and tumorigenesis (Sharpless and DePinho 2006). For the purpose of this review, GEMMs can be categorised as either transgenic or endogenous (Frese and Tuveson 2007; Van Miltenburg and Jonkers 2012). In essence, transgenic GEMMs are generated through the microinjection of cDNA with specific promoters into the nuclei of fertilised oocytes, which induces the expression of targeted oncogenes (Lee 2014; Tuveson and Jacks 2002). However, the expression level of such oncogenes may not always be predictable or accurately controlled, as apoptosis or senescence may also result from such manipulations (Sarkisian et al. 2007). Endogenous GEMMs, on the other hand, can either lose the ability to express oncogenes through the disruption or inactivation of the target allele (through knockout techniques) or express modified regions of the target allele by the insertion of the desired mutation into the target allele (knock-in approaches) (Lee 2014). Yet, several of these allele disruption approaches can cause embryonic lethality or developmental defects as these mutations are present in the whole mouse (Lee et al. 1992). Through the use of conditional alleles requiring site-specific recombinases to induce mutations, the locality of mutations can be restricted to specific tissues (Sharpless and DePinho 2006).

The use of GEMMs in cancer research harbours a number of improvements on other model pitfalls. As GEMMs are generated in the presence of an intact immune system (Legrand et al. 2009), the inadequacy of xenografts to study cancer-related immune responses is overcome (Dranoff 2012). Furthermore, the histopathological and molecular analyses of GEMMs have shown the accurate recapitulation of essential characteristics of human tumours, which is not always the case for CDXs. Liu et al. (2007) developed a mouse model

for human BRCA1-associated breast cancer through the simultaneous deletion of the *Brca1* and *p53* alleles. These tumours were found to closely resemble human basal-like breast cancers based on expansive growth, the expression of basal epithelial biomarkers and genomic instability. A year later, Rottenberg and colleagues used the same breast cancer GEMMs (*K14cre*;*Brca1*$^{F/F}$;*p53*$^{F/F}$) to investigate the mechanism involved in the acquired resistance of the PARP inhibitor, AZD2281 (Rottenberg et al. 2008). The overexpression of the P-gp efflux transporter was the observed mechanism of acquired resistance as the P-gp inhibitor, tariquidar, successfully reversed the resistance. By breeding mice carrying *Abcg2*-deleted alleles with the *K14cre*;*Brca1*$^{F/F}$;*p53*$^{F/F}$ GEMM, Zander et al. (2010) confirmed that the ABCG2 transporter confers resistance to the topoisomerase I inhibitor, topotecan, in vivo. Therefore, this specific model proves to be a valuable predictor of human BRCA1-mutated breast cancers in preclinical trials.

Knockout GEMMs have been used to investigate the role of efflux transporters in anticancer drug failure and cancer MDR. Table 2 provides evidence of several knockout models. An *Mrp4* knockout mouse model showed that MRP4 restricts topotecan drug distribution and its overexpression caused resistance to topotecan (Leggas et al. 2004). Marchetti et al. (2014) used *Mdr1a/1b*$^{-/-}$ (P-gp) knockout mice to study the pharmacokinetics of the C-4 methyl carbonate paclitaxel analogue, BMS-275183, in the presence of the proton pump inhibitor, pantoprazole. After an exposure period of 6 h, the apparent oral bioavailability of *Mdr1a/1b*$^{-/-}$ mice in comparison to wild-type mice was 97% and 63%, respectively. Significant increases were also observed in the absolute brain concentrations of BMS-275183 in knockout mice, compared to control groups. The in vivo double-knockout GEMMs confirmed the affinity of BMS-275183 for the P-gp efflux transporter. Not only knockout approaches are used; knock-in models can also provide information on efflux transporters. Gu et al. (2009) created a mouse model by inserting a firefly luciferase (*fLUC*) gene into the murine *mdr1a* genetic locus. This model proved to faithfully show basal *mdr1a* expression, as well as induced expression after xenobiotic treatment. Changes in luminescence intensities were observed after treatment with paclitaxel, docetaxel and pregnenolone-16α-carbonitrile. These correlated to the statistically significant induction of *mdr1a.fLUC* expression in drug-exposed mice compared to controls, demonstrating the feasibility of linking the conditional knock-in system with a luminescent reporter gene for potential in vivo tumour bio-imaging.

Improvements for preclinical MDR treatment screening

For the past 30–40 years, the NCI made significant contributions to the list of standard drugs available for chemotherapy. The majority of these drugs were tested on the NCI-60 cell panel, even though today researchers are sceptic about using only conventional 2D flat cultures for anticancer efficacy screening. Such scepticism can be substantiated by the proven discrepancies in conventional 2D cell culture growth patterns, when compared to human tumour tissues. Calitz et al. (2018) and Wrzesinski et al. (2014) clearly noted differences occurring between cells cultured in 2D and 3D. Conventional 2D cultures require synthetic growth surfaces with artificial nutrient sources, while regular sub-culturing (e.g., trypsinisation) of the cell population is necessary to ensure the availability of sufficient growth space and nutrients. Therefore, 2D cell cultures are in a constant state of proliferation and active cell growth to recover from sub-culturing. In contrast, dynamic 3D spheroids have reached a state of "dynamic equilibrium" after about 18 days' post-trypsinisation (Wrzesinski and Fey 2013). During this stage, these spheroids start to physiologically mature and form functional structures and characteristics of the original tumour tissue. Given that the dynamic spheroids are allowed to completely recover from laboratory practices (e.g., trypsinisation), the formation of an ECM is an important feature of such 3D cultures. An ECM ensures proper cell-to-cell communication, which is crucial to represent true human tumours. This explains why the NCI recently shifted its preclinical screening focus to the use of other models, such as PDXs (Ledford 2016). As part of the NCI Precision Oncology Initiative^{SM} Resource, the NCI is developing a patient-derived model repository (PDMR) (https://pdmr.cancer.gov) which serves as a platform for the comparative assessment of cancer drug treatments across PDXs, in vitro patient-derived tumour cell cultures, cancer associated fibroblasts and patient-derived organoids.

Considering that each in vitro and in vivo model discussed in this paper has its own advantages and limitations, no single model can comprehensively elucidate on the entire efflux transporter-facilitated drug resistance mechanism of cancer as yet. In fact, a combination of both in vitro and in vivo models would enhance the preclinical decision-making of a chosen drug for further human clinical trials, where the advantages of a particular model overshadow the shortcomings of the other model. For example, preliminary screening of various drug targets at a wide range of concentrations for anticancer efficacy using conventional 2D flat cell cultures, followed by further in depth screening of the most potent compounds (as

determined by 2D screening) using advanced 3D cancer spheroids. From this, only the most promising drug targets with the least detrimental side-effects may be tested in PDXs, after which one drug is selected for human trials. This approach can greatly decrease the number of animals needed for research purposes, potentially decreasing drug development costs and addressing the ethical issues of animal-based research. It may also increase the success rate of clinical trials, since more accurate pre-screening was possible. It is also suggested that clinicians and academic researchers collaborate continuously, to ensure early communication and advice on relevant findings.

It should be kept in mind that human tumours form complex structures, interact with other normal tissues and do not exist as single cell types found in monoculture models. Therefore, several future directions to overcome cancer MDR should be aimed at developing techniques to produce co-cultures containing different cancer cell types. By simultaneously co-culturing with different cell lines, a better representation of the tumour microenvironment can be obtained. High-throughput hanging drop technology has been used to successfully produce a 3D co-culture of DLD1 colon cancer cells with mouse NIH3T3 fibroblasts, for the validation of RNAi therapy (Thoma et al. 2013). Another study co-cultured NSCLC cells with T-lymphocytes, using scaffolds, to develop a 3D model for the exploration of tumour-immune responses (Alonso-Nocelo et al. 2016). Fu et al. (2019) found tumour-associated macrophages (THP-1) to induce autophagy in co-cultured hepatocarcinoma cell lines (SMMC-7721 and Huh-7), contributing to oxaliplatin resistance. Therefore, the use of in vitro 3D co-cultures in general can provide a more realistic portrayal of the tumour-related responses associated with anticancer drug treatments.

Adding to the heterogeneity and complexity of a human tumour is the fact that tumours can contain their own subpopulation of stem cells responsible for cellular "plasticity". These cancer stem cells (CSCs) can shift as a response to external/internal stimuli from a quiescent state to actively proliferating; from chemosensitive to chemoresistant (by overexpressing ABC efflux transporters) and from an epithelial to a mesenchymal phenotype (see Fiori et al. (2017) and Zakaria et al. (2017) for a comprehensive discussion on CSC biology). The hypothesis is that these CSCs hold the key to cancer recurrence (after remission has been medically confirmed) or the emergence of the same type of cancer in various organs. Several studies refer to these CSCs as circulating tumour cells that configure the metastasis of a cancer (Cayrefourcq et al. 2015). Therefore, by targeting CSCs specifically, improved anticancer treatment strategies can evolve for clinical applications against multidrug resistant and metastatic cancer. 3D cell culture models have been shown as suitable for use in CSC research. Stankevicius et al. (2017) used scaffold-attached laminin rich ECM and scaffold-free multicellular spheroid models (liquid-overlay technique) to investigate CSC marker expression, associated with self-renewal and epithelial to mesenchymal transition, of human colorectal carcinoma (CRC) cells (DLD1 and HT29). In comparison to 2D culture conditions, the CRC cells showed higher expression of the CSC-associated genes when cultured in 3D. This is in accordance with the findings of Reynolds et al. (2017), who found that CSC content was the highest in 3D embedded spheroid models compared to 2D cultures.

To this end, complex 3D cell culture-based models continuously demonstrate truthful resemblances to the in vivo or human-like environment in which tumours can arise. Seeing as tumour complexity can add to the long list of tumour properties portrayed in 3D models, this approach can contribute to this approach becoming the gold standard in efflux transporter-based MDR screening of anticancer drugs.

Conclusions

The causative effect of drug efflux transporters, especially those of the ABC-superfamily, to the occurrence of MDR in cancer is widely recognised. A collection of therapeutic strategies exists to combat efflux-based MDR mechanisms in cancer including the co-administration of efflux transporter inhibitors/modulators, RNAi therapy, metronomic chemotherapy combined with immunotherapy, to name but a few. The preclinical screening of such therapeutic strategies form a fundamental part of the drug development process and both in vitro and in vivo models are used for this purpose; each with their own strengths and limitations. In vitro models applied can range from easily maintained conventional 2D flat cultures (having poor translatability towards in vivo tissues) to more intricate 3D culture systems that closely imitate the true in vivo microenvironment. In vivo animal models predominantly used for efflux transporter-based MDR treatment screening, involve the grafting of either cell-derived or patient-derived tumours into immunodeficient rodents to generate xenografts. Although predictive patient drug responses can be obtained from xenografting, the model development is extremely time-consuming and the absence of a fully functioning immune system in the murine host restricts studies on cancer immune responses. By genetically manipulating cancer-associated genes in mouse models, accurate representations of human tumour properties can be achieved while using models with intact immune systems.

Given that cancer research to overcome efflux transporter-driven MDR is a multifaceted approach, no particular model should be used in solitary. In fact, the utilisation of a complete "package" of preclinical screening models would provide a better understanding and refinement of which

therapeutic strategies to explore in further effective clinical trials. It is clear, however, that current models are still not optimal for high-throughput drug development as implementation has been slow. Most models are developed for basic research and never advance beyond this. This highlights the need to not only develop better models, but to also streamline the current technology available.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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