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# **The role of ABCG2 in modulating responses to anti-cancer photodynamic therapy**

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## **Abstract**

The ATP-binding cassette (ABC) superfamily G member 2 (ABCG2) transmembrane protein transporter is known for conferring resistance to treatment in cancers.

Photodynamic therapy (PDT) is a promising anti-cancer method involving the use of light-activated photosensitisers to precisely induce oxidative stress and cell death in cancers. ABCG2 can efflux photosensitisers from out of cells, reducing the capacity of PDT and limiting the efficacy of treatment. Many studies have attempted to elucidate the relationship between the expression of ABCG2 in cancers, its effect on the cellular retention of photosensitisers and its impact on PDT. This review looks at the studies which investigate the effect of ABCG2 on a range of different photosensitisers in different pre-clinical models of cancer. This work also evaluates the approaches that are being investigated to address the role of ABCG2 in PDT with an outlook on potential clinical validation.

## **Keywords**

Photodynamic Therapy, Photosensitisers, ABCG2, BCRP

## 1 Introduction

Anti-cancer Photodynamic Therapy (PDT) involves the administration of a tumour-retaining photosensitiser that is activated by light of a specific wavelength from a ground to an excited state of energy. The activated and unstable photosensitiser subsequently transfers the additional energy to oxygen to produce reactive oxygen species (ROS) [1–3]. Cytotoxic ROS cause tumour destruction through different mechanisms including apoptosis, necrosis, destruction of tumour vasculature, inflammation and the induction of the innate immune system [4]. The type of tumour and cell death elicited depends on the type of photosensitiser used, the type of cell and tissue under investigation, the intracellular localisation of the photosensitiser and the total dose of light administered [3]. Unlike systemic chemo- and biological therapy, PDT is site-specific and light irradiation is localised to the area of tumour growth [5,6]. In recent decades, many different types and classes of photosensitisers have been investigated including Haematoporphyrin derivatives, Porfimer Sodium (Photofrin), Aminolevulinic acid (ALA), meta(tetrahydroxyphenyl)chlorin (mTHPC), mono-L-aspartyl chlorin e6 (NPe6) and Hypericin [7]. Clinically, PDT can be applied as a standalone method of treatment or in adjunct with surgery or chemotherapy [8,9].

The ATP-binding cassette (ABC) superfamily G member 2 (ABCG2) transporter, also known as the breast cancer resistance protein (BCRP), is predominantly located in the cell membrane. The primary role of ABCG2 is to actively pump out endogenous metabolites, xenobiotics and toxins from cells or into intracellular compartments such as the endoplasmic reticulum for elimination [10,11]. In cancer research, ABCG2 has long been identified as one of the major contributors to multidrug resistance and treatment failure. Chemotherapeutic compounds such as doxorubicin, methotrexate,

mitoxantrone and topotecan have been identified as substrates of ABCG2 activity [12]. Clinically, high ABCG2 expression correlates with poor prognosis in acute myeloid leukaemia patients, as indicated by poor complete response, disease-free survival and overall survival [13]. Additionally, key genetic alterations such as the R482G 'gain-of-function' mutation have been identified in increasing drug efflux activity [11,14–17].

Research into ABCG2 has focused on identifying its role in conferring resistance to treatment. Many investigations have been conducted in an effort to reverse drug resistance and improve chemosensitivity. This includes the use of structurally-diverse small molecule inhibitors to bind and antagonise ABCG2 efflux pump activity, targeting ABCG2 protein translation through RNA-interfering retrovirus. This modulates intracellular signalling pathways that regulate ABCG2 activity, circumventing resistance by using compounds that are poor ABCG2 substrates [11,18–21].

Many studies have been conducted to investigate the interplay between ABCG2, the pharmacokinetics of various photosensitisers and the effect they have on the efficiency of PDT. Compared to other well-documented multidrug resistant proteins such as P-glycoprotein and Multidrug resistance-associated protein 1, ABCG2 has been found to have the largest impact on influencing the accumulation of photosensitisers and impeding PDT (Figure 1) [22]. All the studies evaluated in this review have been summarised in Table 1. This review has been categorised according to the different types of photosensitisers and will highlight the key developments and findings in understanding the role of ABCG2 in PDT. Studies

looking at techniques which attempt to overcome the PDT-limiting effect of ABCG2 will also be discussed in this review.

## **2 ABCG2 influences the accumulation of photosensitisers and PDT**

### **2.1 Pheophorbide-a**

Pheophorbide-a (PPa) is a photosensitiser derived from the metabolism and breakdown of chlorophyll. PPa possess efficient PDT-mediating properties with promising potential clinical application [23]. Jonker *et al.* conducted the earliest study looking at the effect of ABCG2 and intratumoural accumulation of photosensitisers [24]. In this *in vivo* investigation, *abcg2*-knockdown mice were generated and fed a chlorophyll-rich diet using alfalfa leaf concentrate. Under ambient lighting conditions, *abcg2*-knockdown mice were found to be hypersensitive to the diet as characterised by epidermal phototoxicity. Plasma levels of PPa were found to be significantly higher in these mice when compared to both wild-type mice on the chlorophyll-rich diet and *abcg2*-knockdown mice on a normal diet. The authors confirmed the transportation of PPa by ABCG2 by incubating ABCG2-overexpressing MEF3.8 (murine fibroblast) cells with PPa. An 18-fold reduction in intracellular PPa levels was observed in ABCG2-overexpressing cells when compared to parental cells, which could be reversed by Ko143 (a small molecule ABCG2 inhibitor). Kim *et al.* investigated the influence of ABCG2 in PPa treated SW480 and HT29 colorectal cancer cell lines [25]. PPa uptake and retention was found to be higher in SW480 cells as compared to HT29, which correlated with the low and high ABCG2 protein expression levels respectively. SW480 cells were found to be more sensitive to PPa-PDT than HT29 cells. To confirm the involvement of ABCG2, Ko143 pre-treated HT29 cells, prior to PPa-PDT, were found to be more sensitive to PDT than non-Ko143 pre-treated cells. ABCG2-overexpressing SW480 cells were found to be more

resistant to PPa-PDT than parental cells. Intracellular levels of ROS correlated with cell survival, with low levels being detected in resistant cells. *In vivo* analysis confirmed these findings: ABCG2-overexpressing SW480 xenografts were resistant to PPa-PDT as characterised by the continuous growth in sizes of xenografts following PDT. HT29 xenograft bearing mice treated with Ko143 and PPa-PDT were found to have a larger reduction in tumour growth when compared to PPa-PDT only treated mice. The overexpression and oncogenic activation of the transmembrane tyrosine kinase linked receptor cMET has been linked to the promotion and development of tumour growth through the stimulation of various downstream signalling pathways, including the cancer-progressing PI3K/Akt pathway [26,27]. Increased PI3K/Akt signalling has previously been found to increase the expression of ABCG2 [28,29]. Jung *et al.* found doxorubicin-resistant A2780 ovarian cancer cells to be more resistant to PPa-PDT when compared to their non-doxorubicin-resistant counterpart [30]. ABCG2 was the only gene among the 50 sampled ABC transporters in which expression was elevated more than 50% in the doxorubicin-resistant A2780 cells via both genetic and protein expression analysis. This study found an increase in cMET expression and PI3K/Akt signalling which could have contributed to increased ABCG2 expression. The inhibition of PI3K/Akt and repression of cMET lead to the suppression of ABCG2 which resulted in increased uptake of PPa, increased ROS production and improved responses to PPa-PDT in doxorubicin-resistant cells. Ko143 treatment improved sensitivity to PPa-PDT doxorubicin-resistant cells. Pan *et al.* investigated the responses of different human glioma cell lines to PDT, mediated by methyl ester pyropheophorbide-a (MPPa) [31]. The authors observed significant differences in sensitivity to MPPa-PDT between the different glioma cells lines. U251 and A172 cell lines were found to be the least and most resistant to treatment respectively. U251 cells had high intracellular

accumulation of MPPa and low ABCG2 expression levels. The inverse was found in A172 cells. Incubation with the ABCG2 inhibitor fumitremorgin C (FTC) reversed the effects of ABCG2 in A172 cells as shown by an increase in MPPa accumulation, increased ROS production and overall increased PDT cytotoxicity. In this study, the authors highlighted the heterogeneity in ABCG2 expression and varying degrees of inherent resistance to PDT between different cell lines of the same type of cancer. In comparison, the study by Tao *et al.* demonstrated acquired resistance in MG63 and HOS human osteosarcoma cell lines subjected to multiple cycles of MPPa-PDT and establishing PDT resistant cells [32]. Protein expression analysis showed a significant increase in ABCG2 expression in resistant cells when compared to parental cells. Intracellular accumulation of MPPa and generation of ROS was found to be lower in PDT resistant cells. Resistant cells were more capable of invasion, migration and colony formation following PDT. Tracy *et al.* investigated the influence of ABCG2 on the photosensitiser 2-[1-hexyloxyethyl]-2-devinyl pyropheophorbide-a (HPPa) and subsequent efficacy of PDT in patient-derived primary cultures of lung carcinoma, healthy lung epithelial and stromal cells [33]. High ABCG2 expressing carcinoma cells were found to eliminate intracellular HPPa when compared to low ABCG2 expressing epithelial cells. HPPa was found to be lost from fibroblasts even in the absence of ABCG2, suggesting selectivity for epithelial cells. The authors confirmed the activity of ABCG2 in a co-culture model comprising of high ABCG2 expressing carcinoma cells and low ABCG2 expressing tumour fibroblasts in the presence and absence of imatinib mesylate (Gleevec, ABCG2 inhibitor). Imatinib mesylate was found to prevent the loss of HPPa from carcinoma cells and an enhanced retention of HPPa was seen in carcinoma cells over the tumour fibroblasts.



The transcriptional factor Nrf2 plays a pivotal role in protecting cells against harmful stimulants by promoting the expression of cytoprotective genes. Oncogenic activation and gain of Nrf2 function can promote tumour survival, protect against oxidative stress and confer chemoresistance. Nrf2 has previously been shown to induce the upregulation of ABCG2 in cancers as a mechanism of resistance to treatment [34,35]. To highlight the interplay between Nrf2 and ABCG2 and its effect on PPa-PDT, Choi *et al.* investigated Nrf2 signalling in PPa treated carcinoma cell lines from different cancers [36]. *Nrf2*-knockdown MDA-MB-231 breast carcinoma cells were generated and found to have elevated ROS generation and increased intracellular uptake of PPa when compared to control cells. This resulted in enhanced cytotoxicity in *Nrf2*-knockdown cells following PPa-PDT. ABCG2 expression was found to be significantly reduced in *Nrf2*-knockdown cells. To demonstrate the relationship between Nrf2 and ABCG2, Ko143 treated *Nrf2*-knockdown and control cells were found to have no differences in PPa accumulation, ROS generation or PPa-PDT cytotoxicity. These findings were further confirmed in *Nrf2*-knockdown and control subtypes of HT29 colorectal carcinoma, MCF7 breast carcinoma, HCT116 colorectal carcinoma, A498 renal carcinoma and A172 glioblastoma cell lines. Tian *et al.* also investigated the relationship between Nrf2 signalling, ABCG2 and sensitivity to PDT [37]. An increase in Nrf2 and phosphorylated Nrf2 expression was observed in A2780 and SKOV3 human ovarian cancer cell lines subjected to MPPa-PDT. siRNA knockdown of Nrf2 improved responses to MPPa-PDT as shown by increased cytotoxicity, apoptotic activity and increased ROS generation. SKOV3 cells exhibited higher resistance to MPPa-PDT and the expression levels of phosphorylated Nrf2 and ABCG2 were found to be higher in SKOV3 cells when compared to A2780 cells. This suggests a possible interaction between Nrf2 and ABCG2 as a mechanism of resistance to MPPa-PDT.

## 2.2 Aminolevulinic acid/Protoporphyrin IX

The photosensitiser Protoporphyrin IX (PpIX) is an endogenous metabolite involved in the mitochondrial haem biosynthesis pathway. PpIX is a precursor to haem, a vital co-factor of haemoglobin that is required for the transportation of oxygen. The enzyme ferrochelatase is responsible for the efficient conversion of PpIX to haem. PpIX is produced from endogenous ALA under normal physiological conditions; a negative feedback loop can detect levels of haem production and inhibit the synthesis of ALA. This in turn prevents the accumulation of excess PpIX which can lead to phototoxicity. Exogenously administered ALA, can overcome the negative feedback mechanism and increase the production of PpIX in cells [38,39]. PpIX is known for its beneficial applications and therapeutic doses are clinically used for PDT (specifically for dermatological lesions), fluorescent diagnosis and fluorescence-guided surgery [40,41]. Many studies have conducted which have addressed the relationship between ABCG2 and ALA/PpIX under various experimental conditions and using different models.

Barron *et al.* investigated the accumulation of exogenous ALA-produced PpIX and PDT in correlation with ABCG2 in multiple cell lines [42]. The different cell lines were found to exhibit cell-specific differences in the retention of endogenous PpIX when co-treated with Ko143. Cell lines were found to have increased intracellular PpIX levels in the presence of Ko143. Protein expression analysis found higher expression of ABCG2 in cells with increased intracellular PpIX levels in the presence of Ko143. Low ABCG2 expression was observed in cells that were not influenced in the retention of PpIX in the presence of Ko143. This correlated with the efficacy of PpIX mediated PDT, whereby ABCG2 inhibition through Ko143 enhanced PDT

cytotoxicity. Bebes *et al.* looked at the accumulation of PpIX and PDT in relation to the expression of ABCG2 between differentiated and proliferating HaCaT keratinocyte cells [43]. Differentiated HaCaT cells (low ABCG2 protein expression) were able to accumulate more ALA-derived PpIX when compared to proliferating HaCaT cells (high ABCG2 protein expression). Ko143 was found to antagonise ABCG2 activity and increase PpIX accumulation which was noticeable in the high ABCG2 expressing proliferating cells. Additionally, Ko143 improved PDT cytotoxicity in combined Ko143 and ALA/PpIX-PDT treated cells.

Ogino *et al.* investigated the effects of foetal bovine serum (FBS) in cell culturing medium on ABCG2 activity [44]. T24 urothelial carcinoma cells were treated with ALA in FBS and FBS-free cell media and intracellular levels of PpIX were found to be significantly higher in FBS-free media cultured cells. A time- and ALA dose-dependant accumulation of PpIX was observed in cells cultured in FBS-free media. In the presence of FBS, the amount of extracellular PpIX was higher than intracellular PpIX. To confirm the activity of ABCG2 in eliminating PpIX in the presence of FBS, cells were treated with FTC and a significant increase in intracellular PpIX levels was observed. Similar effects were observed with cell media supplemented with bovine serum albumin. This study highlights the requirement of serum proteins to facilitate ABCG2 activity in eliminating intracellular PpIX. Hagiya *et al.* studied ALA/PpIX-PDT in different gastric cancer cell lines and the correlation between sensitivity to PDT and ABCG2 expression [45]. Of the various cell lines investigated, NKPS and MKN45 cells were found to be the most and least resistant to ALA/PpIX-PDT respectively. Intracellular levels of PpIX were also found to be lowest and highest in these particular cell lines respectively. Genetic and protein expression analysis showed the expression of ABCG2 in NKPS cells which was not

observed MKN45 cells. To confirm the activity of ABCG2, *abcg2*-knockdown NKPS cells were generated and found to have higher intracellular accumulation of PpIX when treated with ALA, and increased sensitivity to ALA/PpIX-PDT when compared to parental cells. Similar findings were observed in FTC-treated NKPS cells. The study by Sun *et al.* investigated gefitinib as a potential ABCG2 inhibitor in ALA/PpIX treated glioma cell lines [46]. Gefitinib was found to significantly increase intracellular PpIX levels in all cell lines. Gefitinib treatment reduced ABCG2 cellular membrane protein expression and also reduced ABCG2 gene expression. The addition of Gefitinib improved cellular responses to ALA/PpIX-PDT through increased cytotoxicity. Teshigawara *et al.* investigated ABCG2 and ALA/PpIX-PDT in several ovarian clear-cell carcinoma cell lines [47]. The different cell lines exhibited different levels of sensitivity to ALA/PpIX-PDT. This correlated with PpIX accumulation and ABCG2 gene expression, where resistant cells had lower intracellular PpIX levels and high ABCG2 expression. FTC treatment improved ALA/PpIX-PDT and increased PpIX accumulation in resistant cells. Nakayama *et al.* studied PpIX accumulation and ALA-PDT in dormant cells within a 3D spheroidal cell model [48]. Briefly, 3D spheroids of PC3 prostate cancer cells were generated and the presence of dormant cells was confirmed (characterised by the absence of cell proliferation, no cell death, metabolic suppression and the ability to recover to an active status). PpIX accumulation was found to be higher in dormant cells resulting in increased sensitivity to ALA/PpIX-PDT when compared to non-dormant active cells. Protein expression analysis showed an increase in PEPT1 (ALA influx transporter) and downregulation of ABCG2 in dormant cells. To confirm the effect of dormancy on improving PpIX accumulation and ALA/PpIX-PDT, metabolically active cell cultures were treated with methotrexate or cycloheximide to induce a dormant-like status

through cell cycle arrest. Following ALA/PpIX-PDT, ABCG2 expression was downregulated, PpIX accumulation had increased and cell viability had decreased.

ALA-derived PpIX fluorescence has previously been identified and shown as an effective method for mediating fluorescence-guided surgery in patients with malignant glioma [49]. However, the therapeutic efficacy of ALA/PpIX-PDT in gliomas is currently not well understood. Briel-Pump *et al.* investigated ALA/PpIX-PDT in multiple medulloblastoma and U373 glioblastoma cell lines [50]. A dose- and time-dependent accumulation of PpIX was observed in all cell lines; however, overall accumulation of PpIX was lower in medulloblastoma cell lines as compared to U373 cells. U373 cells were more sensitive to ALA/PpIX-PDT. ABCG2 protein expression was detected in all medulloblastoma cell lines with no expression found in U373 cells.

The mitochondria play a key role in the production of endogenous PpIX by facilitating the haem biosynthesis pathway. The PpIX-converting enzyme ferrochelatase is located in the inner membranes of the mitochondria [51]. Palasuberniam *et al.* looked at the uptake and intracellular distribution of PpIX and ALA/PpIX-PDT in a panel of breast cancer cell lines (oestrogen receptor positive (ER+), human epidermal growth factor receptor 2 positive (HER2+) and triple negative breast cancer (TNBC)) [52]. TNBC cells were found to have lower intracellular PpIX accumulation and localisation in mitochondria when compared to ER+ and HER2+ cells. The levels of intracellular PpIX also correlated with ALA/PpIX-PDT cell viability; TNBC cells were found to be greatly resistant to PDT. Ko143 mediated ABCG2 inhibition reversed the effects by enhancing PpIX uptake, increasing PpIX mitochondrial accumulation and improving responses to PDT in TNBC cells. Ko143

treatment had little effect on PpIX production and ALA/PpIX-PDT in ER+ and HER2+ cells.

### 2.3 Hypericin

Hypericin is a naturally occurring compound and the main chemical constituent in the St. John's wort flowering plant (*Hypericum perforatum*) [53,54]. Low doses of hypericin have been used for the treatment of mild to moderate depression and seasonal affective disorder [53,55]. Hypericin has also been evaluated for its anti-microbial, anti-cancer, fluorescent and photodynamic properties [56,57]. As a photosensitiser, hypericin exhibits bright fluorescence, potent ROS generation and minimal toxicity in the dark [53,55,56,58]. Hypericin's pre-clinical success as an emerging photosensitiser for PDT has transitioned its investigation into a current ongoing phase III multicenter clinical trial (*clinicaltrials.gov: NCT02448381*)[59].

Jendželovský *et al.* first reported the interaction between hypericin and ABCG2 in HT29 cells [60]. An increase in ABCG2 expression was observed in cells treated with hypericin. Pre-treating cells with the ABCG2 inhibitor FTC affected its function and improved intracellular hypericin accumulation. Proadifen was also found to suppress ABCG2 activity in a similar fashion. Inhibition and suppression of ABCG2 activity subsequently enhanced hypericin-PDT induced oxidative stress and apoptosis. A follow up study by Šemeláková *et al.* evaluated the impact of non-activated hypericin and hypericin-PDT on the expression and activity of ABCG2 in HT29 cells [61]. In the presence of Ko143, the uptake of hypericin was found to be increased due to the inhibited activity of ABCG2. The incubation of cells with hypericin alone resulted in increased ABCG2 gene and protein expression. Following light irradiation and hypericin-PDT, the expression of ABCG2 had substantially reduced. The study

demonstrated an increase in ABCG2 expression following incubation with hypericin. A reduction in ABCG2 expression is only observed during hypericin-PDT. Therefore, the incubation period is essential to find a balance between optimal intracellular levels of hypericin and loss of hypericin via ABCG2. Recently, Jendželovský *et al.* further confirmed the strong affinity of ABCG2 for hypericin in the acute promyelocytic leukaemia cell line HL60 [62]. ABCG2-overexpressing HL60 cells were generated and found to have a substantially increased ability to eliminate hypericin when compared to the parental cell line. ABCG2-overexpressing cells were significantly more resistant to hypericin-PDT and no apoptotic activity was observed following treatment. Ko143 reversed the effects of ABCG2 overexpression in cells leading to an increased accumulation of hypericin and induction of apoptosis, as characterised by increased annexin V staining and uptake of propidium iodide.

Khot *et al.* investigated the differences in responses to hypericin-PDT between 2D monolayer and 3D spheroidal models of HT29 and HCT116 colorectal cancer cell lines [63]. The authors found 3D spheroidal models of both cell lines to be significantly more resistant to Hypericin-PDT when compared to their respective 2D cell cultures. Protein expression analysis showed higher ABCG2 expression in 3D spheroidal models and co-treatment with Ko143 was found to moderately improve sensitivity in 3D spheroidal models of both cell lines to Hypericin-PDT. 5-lipoxygenase (5-LOX) is responsible for the production of lipid signalling molecules known as eicosanoids and leukotrienes from arachidonic acid [64]. 5-LOX plays a role in mediating pathological inflammation and its upregulation and metabolic activity has been associated with tumourigenesis in different types of cancers [65]. Kuchárová *et al.* evaluated the interplay between the 5-LOX pathway and ABCG2 and their effect on hypericin-PDT [66]. Synergistic reduction in cell viability was

observed in HT29 and MCF7 cells treated with hypericin-PDT and the 5-LOX pathway inhibitor MK-886. Increased intracellular accumulation of hypericin was found in cells co-treated with hypericin and MK-886, as compared to hypericin-only treated cells. Incubating cells with hypericin was reported to induce an increase in ABCG2 protein expression; however, MK-886 was reported to reduce ABCG2 protein expression even in the presence of hypericin. This study highlights a possible influencing mechanism whereby inhibiting the 5-LOX pathway can hinder ABCG2 activity and expression.

Biteghe *et al.* investigated the expression of ABCG2 in melanoma cells co-treated with dacarbazine and hypericin-PDT [67]. An increase in ABCG2 expression was observed in cells treated with dacarbazine alone. Of the two melanoma cell lines evaluated, UCT Mel-1 pigmented cells showed a reduction in ABCG2 expression following combined treatment (hypericin and dacarbazine) or hypericin alone. A375 unpigmented cells showed an increase in ABCG2 expression in the presence of hypericin. Hypericin was found to localise to the perinuclear region and hypericin-PDT was found to enhance the cytotoxicity of dacarbazine. No significant difference in reduction in cell viability was observed between combined treated and hypericin-PDT alone treated cell cultures. Combined treatment was also found to abrogate the self-renewal capacity of cell cultures. In this study, ABCG2 was not found to influence hypericin-PDT, especially in A375 unpigmented cells where an increase in ABCG2 expression did not result in increased resistance.

#### **2.4 Porfimer Sodium (Photofrin)**

Clinical PDT was first approved in Canada in 1993 to treat bladder cancers and Photofrin was the first clinically approved photosensitiser [1]. Photofrin is a first



generation photosensitiser and is still being used for PDT to treat lung, bladder and cervical cancer, and other forms of solid cancers. Due to the popularity of Photofrin as an agent for PDT, it is commercially available in Canada, Japan, USA and many European countries [68].

During PDT, light is required to activate the photosensitiser and subsequently generate ROS. Sonodynamic therapy (SDT) involves the use of low intensity ultrasound, to activate the photosensitiser and generate ROS in the presence of molecular oxygen [69]. Xu *et al.* studied the impact of Photofrin-mediated SDT with respect to ABCG2 in CD133+ glioma stem cells (GSC) and CD133- U251 glioma cells [70]. The high expression of ABCG2 has previously been identified as a mechanism of chemoresistance and a biomarker of GSC [71]. The uptake and intracellular retention of Photofrin was found to be significantly higher in U251 cells as compared to GSC. This resulted in higher resistance to Photofrin-SDT, reduced apoptosis and reduced ROS generation in GSC. ABCG2 expression was found to be significantly higher (around 6-fold) in GSC when compared to U251 cells.

## **2.5 Chlorin e6**

Since the identification of haematoporphyrin derivatives in the mid-20<sup>th</sup> century as dynamic photosensitisers for mediating PDT, a large proportion of research in PDT has been occupied by the identification of new porphyrin photosensitisers.

Alternative classes and types of photosensitisers have emerged as promising candidates, including chlorins and bacteriochlorins [72]. Chlorin e6 (Ce6) is an example of an ideal photosensitiser due to its high efficiency in singlet oxygen generation and activation by near-infrared wavelengths, which is suitable for penetration into deep tissues [73–77].

As described above, ABCG2 is recognised as a possible biomarker of GSC. Abdel Gaber *et al.* investigated whether the inhibition of ABCG2 could improve Ce6-PDT in glioma cell lines [78]. The authors found rapid elimination of Ce6 from ABCG2-overexpressing cells in the presence of FBS in the cell culturing media. In comparison, intracellular uptake and retention of Ce6 was higher in the absence of FBS. Ko143 was found to reduce the rate at which Ce6 was eliminated via ABCG2 and improved cytotoxic responses to Ce6-PDT. The authors found an increase in ABCG2 protein expression in 3D spheroidal models of U87 cells when compared to 2D monolayer cell cultures. 3D spheroids were also found to have reduced Ce6 accumulation and were significantly more resistant to Ce6-PDT.

## **2.6 Benzoporphyrin Derivative**

The photosensitiser Benzoporphyrin Derivative (BpD) has been the subject of many previous PDT investigations, gaining FDA approval in 2000 for clinical applications [79]. Huang *et al.* found a liposomal formulation of BpD to be an efficient mediator of PDT in MIA PaCa-2 pancreatic cancer cells [80]. Following a sub-lethal dose of PDT, ABCG2 protein expression decreased by up to 70% within 6 hours and was sustained for up to 24 hours. This reduction in ABCG2 was found to improve the uptake and retention of irinotecan in cells.

## **2.7 Other photosensitisers**

### **2.7.1 PPa, MPPa, Ce6, ALA/PpIX, HpIX, mTHPP and mTHPC**

Robey *et al.* conducted one of the earliest studies investigating the relationship between the intracellular accumulation of various photosensitisers, ABCG2 expression and PDT [22]. The authors found intracellular levels of PPa, MPPa, Ce6

and PpIX (generated from exogenous ALA) to be significantly lower in ABCG2-overexpressing bronchoalveolar carcinoma cells when compared to the parental cell line. FTC attenuated the efflux activity of ABCG2 by increasing the intracellular retention of these photosensitisers. Unlike the aforementioned photosensitisers, intracellular accumulation of Haematoporphyrin IX (HpIX), meso-tetra(3-hydroxyphenyl) porphyrin (mTHPP) and meso-tetra(3-hydroxyphenyl) chlorin (mTHPC) remained unchanged between the ABCG2-overexpressing and parental cells. In addition, FTC did not influence the intracellular levels of HpIX, mTHPP or mTHPC. Intracellular levels of the different photosensitisers in ABCG2-overexpressing cells correlated with sensitivity to PDT. Resistance to treatment was observed in PPa, MPPa, Ce6 and ALA/PpIX treated cells but not in mTHPC treated cells. This study successfully showed differences in the affinity of different chemically structured photosensitisers as substrates for ABCG2 efflux activity. These differences critically influence intracellular retention of the photosensitisers and sensitivity to PDT.

### **2.7.2 HPPa, Galactose-conjugated HPPa, ALA/PpIX and BpD**

Liu *et al.* investigated different photosensitisers in ABCG2-expressing (ABCG2+) and non-expressing (ABCG2-) cell lines [81]. Endogenous PpIX (generated from exogenous ALA), BpD and HPPa were shown to be substrates of ABCG2 as they were found to be eliminated from ABCG2+ but not from ABCG2- cells. ABCG2 inhibition through FTC or imatinib mesylate improved the intracellular retention of photosensitisers and improved PDT cytotoxicity in ABCG2+ cells *in vitro* and murine RIF1 fibrosarcoma (ABCG2+) tumour xenografts *in vivo*. The authors attempted to improve the pharmacokinetics of HPPa through a chemical structural modification. The conjugation of galactose to HPPa significantly improved intracellular retention of

the photosensitiser and PDT when compared to HPPa alone in RIF1 cells. The addition of imatinib mesylate did not influence the effects of the HPPa galactose conjugate, suggesting that this modification can bypass ABCG2 transport activity.

### **2.7.3 Photofrin and NPe6**

Usuda *et al.* investigated the retention of Photofrin and mono-L-aspartyl chlorin e6 (NPe6) and PDT in *abcg2*-transfected A431 epidermoid carcinoma cells [82].

Intracellular accumulation of Photofrin was found to be substantially lower in ABCG2-overexpressing cells when compared to the parental cell line. No difference in intracellular NPe6 levels was found between the two cell types suggesting that Photofrin is an ABCG2 substrate and NPe6 is not. Cell survival following PDT correlated with these findings where resistance to treatment was observed in Photofrin-PDT treated ABCG2-overexpressing cells. FTC reversed the effects of ABCG2 and improved sensitivity to Photofrin-PDT. No difference in sensitivity to NPe6-PDT was found between ABCG2-overexpressing and parental cells and the addition of FTC to NPe6-PDT did not influence sensitivity. In addition to investigating the *in vitro* differences in PDT efficacy between Photofrin and NPe6 between *abcg2*-transfected and parental cells, the authors evaluated the correlation between ABCG2 expression and responses to Photofrin and NPe6-PDT in centrally located early lung cancer patients. ABCG2 expression in patient tumours were scored via immunohistochemical staining analysis as 'moderate expression' [BCRP(1+)] or 'high expression' [BCRP(2+)]. A complete response rate of 73.6% (42/57) was observed in patients treated with Photofrin-PDT. 66.7% (18/27) of those scored as BCRP(2+) showed complete response. In comparison, a complete response rate of 91.6% (22/24) was seen in NPe6-PDT treated patients with 100% (18/18) scored as BCRP(2+) showing complete response [82,83].

### **3 ABCG2 mediated reduction of ROS toxicity via NFκB**

The Nuclear Factor kappa-B (NFκB) protein complex is comprised of transcriptional factors that collectively play a central role in regulating cellular responses during inflammation, cell growth and survival [84]. During PDT, NFκB has been implicated in aiding PDT by provoking an immune response to treatment and mediating oxidative stress [85]. This involves the regulation of inflammatory cytokines IL-1, IL-2 and IL-6, and activation of T-cells and macrophage inflammatory protein-1 [86,87]. ABCG2 has previously been shown to play a protective role against oxidative stress by impeding the production of ROS. This is potentially achieved by hindering the NFκB signalling pathway and inhibiting ROS-induced secretion of pro-inflammatory cytokines [88,89]. Aside from PDT applications, ABCG2 has been found to play a protective role in reducing oxidative stress in corneal epithelium progenitor cells [90]. Inhibiting ABCG2 activity resulted in increased generation of ROS. Similarly, ABCG2 protects cardiomyocytes against oxidative stress–induced hypertrophy, by increasing and stabilising intracellular levels of the anti-oxidant molecule, glutathione [91]. From the above, it can be postulated that ABCG2 does not directly eliminate ROS, but rather remove ROS-generating molecules and through activating anti-oxidant signalling pathways. This function of ABCG2 in the context of PDT is not well understood and requires further exploration.

### **4 Overcoming ABCG2 mediated resistance to PDT**

#### **4.1 Molecular Inhibition of ABCG2**

Targeting ABCG2 and reversing the transporter activity has been studied extensively in an effort to improve the therapeutic index of chemotherapeutics.

The fungal toxin FTC was the one of the first compounds to be identified as a specific and potent ABCG2 inhibitor. However, FTC was found to induce strong neurotoxicity *in vivo*, preventing further investigation or potential clinical translation [92]. Ko143 was designed as a less toxic synthetic analogue of FTC and has been used extensively in ABCG2-related studies, especially PDT (Table 1). Clinically approved small-molecule receptor tyrosine multikinase inhibitors such as sorafenib, gefitinib and imatinib have also shown success in inhibiting ABCG2 [20]. In a clinical setting, the administration of an ABCG2 inhibitor in adjunct to PDT is an attractive method to improve the outcome of treatment. ABCG2 inhibitors maybe given to patients between the administration of photosensitisers and photo-illumination, or simultaneously with photosensitisers [81]. Robey *et al.* have recently reviewed the challenges of clinically exploiting ABCG2 and raised a few important considerations [93]. ABCG2 plays a vital role in normal physiological functions such as the transport of metabolites in the gastrointestinal tract, across the blood-brain barrier and in the kidneys. In addition to the impairment of these normal functions, the increased uptake of photosensitisers in normal tissues via ABCG2 inhibition could result in phototoxicity. A large proportion of studies investigating the inhibition of ABCG2 to improve PDT have been restricted to *in vitro* investigation. Further *in vivo* studies with more effectual clinical translation need to be conducted to successfully show that intervening ABCG2 activity will improve response to PDT in patients.

## **4.2 Modifying and repackaging photosensitisers**

### **4.2.1 Structurally modifying photosensitisers**

A limitation of pharmacological compounds for cancer therapeutic applications is the molecular mechanisms of resistance which can impair and limit the efficacy of treatment. In recent years, much effort and emphasis has been placed on identifying

ways to overcome resistance. One concept is the modification and reformulation of existing therapeutics. This includes the conjugation of compounds onto therapeutics, the incorporation of targeting moieties and repackaging therapeutics in drug-delivering liposomal vesicles and nanomedicines [94–97]. Zheng *et al.* report on the conjugation of carbohydrate moieties to HPPa [98]. The conjugation of galactose to HPPa (HPPa-Gal) was found to improve intracellular uptake and retention in ABCG2-expressing RIF1 cells when compared to free HPPa. This resulted in more efficient PDT and increased cell death in HPPa-Gal treated cells. No difference in photosensitiser uptake was observed in HPPa-Gal and free HPPa treated Colon26 cells (negligible ABCG2 expression). *In vivo* analysis showed elevated light dose-dependent activity of HPPa-Gal in comparison to free HPPa in RIF1 tumour xenograft bearing mice. A larger proportion of mice in the HPPa-Gal-PDT treated group were cured of their tumours. Toxicity analysis showed little to no skin phototoxicity in HPPa-Gal treated mice. HPPa treated mice experienced strong erythema, possibly due to a large amount of HPPa being eliminated and not retained within xenografts. Morgan *et al.* conducted a similar study investigating structural modifications to HPPa and affinity for ABCG2 using carbohydrate conjugations [99]. The authors found HPPa-Gal was retained to a higher degree in ABCG2-expressing HEK293 cells when compared to free HPPa. Imatinib mesylate was found to further enhance intracellular retention of HPPa-Gal. PDT cytotoxicity was evaluated in 4T1 breast cancer cells *in vitro* and *in vivo*, and HPPa-Gal-PDT treated cells were found to be the most sensitive to treatment. A side population of high ABCG2 expressing cells were identified in an overall low ABCG2 expressing tumour xenograft. These cells possess stem-like properties and could potentially initiate tumour regrowth following PDT. Although resistant to HPPa-PDT, these stem-like cells were also found to be sensitive to HPPa-Gal-PDT.

Rapozzi *et al.* designed and evaluated a photosensitiser conjugate consisting of PPa conjugated to an androgen receptor ligand through a small pegylated linker [100]. Overexpression of the androgen receptor in prostate cancer makes it a promising candidate for developing targeting therapeutics and a biomarker for drug development [101]. Intracellular retention of the PPa conjugate was found to be higher in PC3 prostate cancer cells than the free PPa. The ABCG2 inhibitor reserpine was found to increase free intracellular PPa levels but did not influence the accumulation of the androgen receptor-binding PPa conjugate. This suggests that the conjugate is not a substrate for ABCG2 efflux activity. Following light irradiation, higher phototoxicity was observed in PPa conjugate treated cells when compared to free PPa treated cells. These studies show that through structurally modifying photosensitisers, their affinity for ABCG2 efflux activity is reduced, whilst their photodynamic properties are retained.

#### **4.2.2 Photosensitiser nanoparticle reformulation**

Indocyanine green (ICG) is a well-documented photosensitive agent with multiple clinical applications including fluorescent cholangiography, angiography and the detection of sentinel lymph nodes [102,103]. ICG can also be used for PDT and generates ROS by near-infrared irradiation. Li *et al.* investigated PDT using ICG covalently conjugated to gold nanospheres [104]. SKOV3 and CT26 cells were treated with ICG-conjugates or free ICG and, following light irradiation, higher toxicity was observed in ICG-conjugate treated cell cultures. An increase in Nrf2 expression was detected following PDT using both free and ICG-conjugates as a response to the production of ROS. As highlighted earlier, Nrf2 can induce the upregulation of ABCG2. Following PDT, an increase in ABCG2 was found in free ICG treated cells



and decreased following subsequent rounds of light treatment as free ICG was rapidly eliminated from cells. ABCG2 expression remained stable in cells treated with ICG conjugated nanospheres. Intracellular ICG fluorescence was also seen to be higher, suggesting that the nanosystem provided a way for ICG to escape ABCG2-mediated elimination. Roh *et al.* investigated the efficacy between Ce6 and Ce6-encapsulated polymeric nanoparticles functionalised with polyethylene glycol and polyethylenimine in AsPC-1 (moderate ABCG2 expression), MIA PaCa-2 (negligible ABCG2 expression) and *abcg2*-transfected MIA PaCa-2 (high ABCG2 expression) pancreatic cancer cell lines [105]. Intracellular retention of free Ce6 correlated with ABCG2 expression where low, moderate and high Ce6 levels were found in *abcg2*-transfected MIA PaCa-2, AsPC-1 and MIA PaCa-2 cells respectively. The opposite was observed in cells treated with Ce6-nanoparticles, with highest Ce6 levels in *abcg2*-transfected cells, suggesting that the nanoparticle reformulation can overcome ABCG2 activity. PDT induced cytotoxicity correlated with intracellular Ce6 levels. Nanoparticle reformulation of Ce6 significantly enhanced phototoxicity, improved ROS generation and improved sensitivity to treatment, especially in *abcg2*-transfected cells. Ko143 was found to further enhance phototoxicity in Ce6-nanoparticle treated cells. *In vivo* findings also showed Ce6-nanoparticle-PDT to be the most effective in reducing tumour size in AsPC-1 tumour xenograft bearing mice. Recently, Baglo *et al.* developed nanoliposomal formulations of phospholipids conjugated to BpD and investigated PDT in differential ABCG2-expressing cell cultures [106]. ABCG2 overexpressing MCF7 cells were found to rapidly eliminate BpD when compared to parental cells. FTC improved the retention of BpD, confirming ABCG2 activity. In comparison, the nanoliposomal formulation of BpD improved the uptake of BpD and no difference was observed in the presence of FTC. This further translated into PDT efficacy, with nanoliposomal-BpD-PDT treated

ABCG2 overexpressing MCF7 cells experiencing the highest phototoxicity.

Nanoparticle reformulations are an effective way of masking photosensitisers, bypassing ABCG2 efflux activity, and improving intracellular retention and overall PDT activity.

### **4.3 Hyperthermia and ABCG2 expression in PDT**

A recent study by Kurokawa *et al.* investigated the influence of hyperthermia in improving PDT through downregulating the expression of ABCG2 [107].

Hyperthermia (1 hour at 42°C) was found to improve the uptake and retention of haematoporphyrin dihydrochloride (HpD) in cancer-like mutant variants of the rat gastric epithelial cell line RGM-1. Hyperthermia-mediated increased intracellular accumulation of HpD resulted in increased HpD fluorescence and generation of mitochondrial ROS. Hyperthermia and PDT synergistically reduced cell viability when compared to either method alone. The increase in ROS via hyperthermia also led to an upregulation of the heme carrier protein-1 (HCP-1) and downregulation of ABCG2 protein expression, which was reversed by the ROS scavenger N-acetyl-L-cysteine. Hyperthermia can improve PDT by downregulating ABCG2 and upregulating HCP-1 by mitochondrial ROS. This study is a single example and further validation is required in different cancer models to prove the credibility of hyperthermia as an effective method for improving PDT efficiency. This method would be ideally suited to combined thermal ablation (tissue temperature should be maintained between 41–44°C) and PDT in the clinical setting.

### **4.4 Non-ABCG2 substrate photosensitisers**

The relationship between ABCG2 and its affinity for photosensitisers has predominantly been studied through experimental analysis. Studies have shown that

popular photosensitisers such as PPa, ALA/PpIX, Ce6 and hypericin are subject to ABCG2 activity; however, non-ABCG2 substrate photosensitisers such as mTHPC and NPe6 have shown to be, in part, unaffected by the presence of ABCG2 [22,33,82,83]. The studies by Robey *et al.* and Usuda *et al.* identified mTHPC and NPe6 as potential photosensitisers to overcome ABCG2-mediated resistance to PDT. Although this requires further validation, the clinical implication of this finding means stratifying patients according to ABCG2 expression levels and using non-ABCG2 substrate photosensitisers for PDT in high ABCG2 expressing cancers to improve the likelihood of eradicating tumours through bypassing ABCG2 resistance. Berg and colleagues are recognised as pioneering the concept of photochemical internalisation (PCI). Briefly, a therapeutic agent is packaged with a photosensitiser into endocytic vesicles. Upon light irradiation, ROS generated by the photosensitiser ruptures the membrane of the vesicle and releases the therapeutic agent at the site of interest [108]. The photosensitisers di-sulfonated meso-tetraphenylporphine (TPPS<sub>2a</sub>), di-sulfonated mesotetraphenylchlorin (TPCS<sub>2a</sub>) and di-sulfonated aluminiumphthalocyanine (AlPcS<sub>2a</sub>) are commonly used in PCI and have been reported as non-ABCG2 substrate photosensitisers [109]. All three photosensitisers were found to be retained in ABCG2-expressing MA11 breast cancer cells. Co-incubation with FTC did not influence intracellular photosensitiser levels and cells were found to be sensitive to PDT, irrespective of FTC mediated ABCG2 inhibition. In contrast, low levels of photosensitiser was found in PPa treated MA11 cells and FTC was required to improve PPa retention and sensitivity to PDT. The same group did a follow-up study to examine the resensitisation of PDT-resistant cells using the non-ABCG2 substrate photosensitiser TPCS<sub>2a</sub> [110]. MA11 cells were subjected to repeated cycles of PPa-PDT and surviving cells were selected to generate a PPa-PDT resistant cell line. Protein expression confirmed increased ABCG2 expression

in resistant cells when compared to parental cells. PPa-PDT resistant cells were subjected to TPCS<sub>2a</sub>-PDT and were found to respond to treatment.

Patient selection is key to identify those that will effectively respond to PDT. ABCG2 expression has been identified as a marker of cancer stem-like cells and an influencing factor of resistance to PDT [111]. Administering large doses of ABCG2-substrate photosensitisers and prolonging treatment times might overcome ABCG2-mediated efflux in tumours to some degree. This approach would also greatly reduce the therapeutic index and risk unacceptable adverse photosensitivity [81]. In these cases, co-treatment with an ABCG2 inhibitor or use of a non-ABCG2 substrate photosensitiser could potentially be successful approaches to enhancing clinical PDT.

## **5 The protective role of ABCG2 against phototoxicity and protoporphyria**

Under normal physiology, ABCG2 plays a protective role in maintaining intracellular levels of haem. Jonker *et al.* found that improper ABCG2 function resulted in excess accumulation of photosensitive compounds leading to cutaneous protoporphyria [24]. Under stressful and harmful conditions such as hypoxia, ABCG2 regulates toxic levels of cellular porphyrins and haem, aiding cell survival [112–114]. Endogenous PpIX levels are continuously regulated by ABCG2, to prevent a build-up of the photosensitiser that can lead to phototoxicity [115]. Kobuchi *et al.* found ABCG2 distributed in the mitochondrial fraction to play a role in regulating endogenous PpIX levels [116]. Mitochondrial ABCG2 can transport PpIX from the mitochondria to the cytosol and ABCG2 localised to the plasma membrane can eliminate PpIX from cells. Blocking ABCG2 activity increases intracellular accumulation of PpIX.

ABCG2 is therefore vital for regulating cellular homeostasis of porphyrins, to prevent phototoxicity [117]. This is confirmed by Tamura *et al.* that found genetic polymorphisms resulting in ABCG2 inactivity or the inhibition of wild-type ABCG2, resulted in increased intracellular accumulation of porphyrins and acute photosensitivity in non-cancerous cells [118]. From a clinical standpoint, adverse cutaneous phototoxicity has previously been observed in patients treated with the ABCG2 inhibitor, imatinib mesylate [119]. To achieve maximum therapeutic benefit, it is important to find a balance between PDT and adverse protoporphyria. The normal physiological activity of ABCG2 is vital to prevent phototoxicity, yet it can hinder the retention of photosensitisers and affect PDT.

## **6 Conclusion**

This review elucidates the impact of the transmembrane protein ABCG2 on the pharmacokinetics of photosensitisers and the efficacy of PDT. The role of ABCG2 in conferring chemoresistance has been well-documented and extends to PDT. Many studies have demonstrated different methods for overcoming ABCG2-mediated resistance to PDT. This includes co-treatment with ABCG2 inhibitors, using non-ABCG2 substrate photosensitisers and the modification of ABCG2 substrate photosensitisers to reduce binding affinity. The role of ABCG2 in protecting against phototoxicity is necessary in normal tissues, yet can hinder PDT in cancers. The studies described here are primarily pre-clinical evaluations. Careful consideration and further validation of the most effective method for downplaying ABCG2 in cancers for PDT is required prior to clinical applications.

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## 8 Disclosure

The authors declare no conflict of interest.

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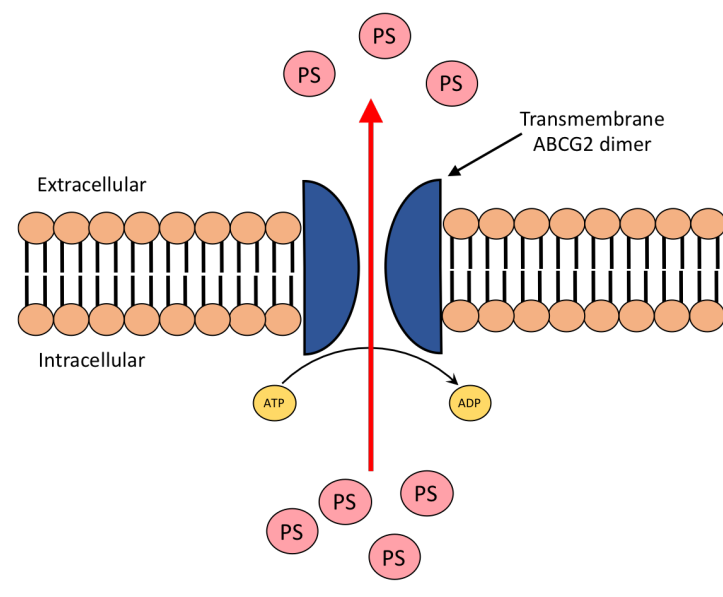
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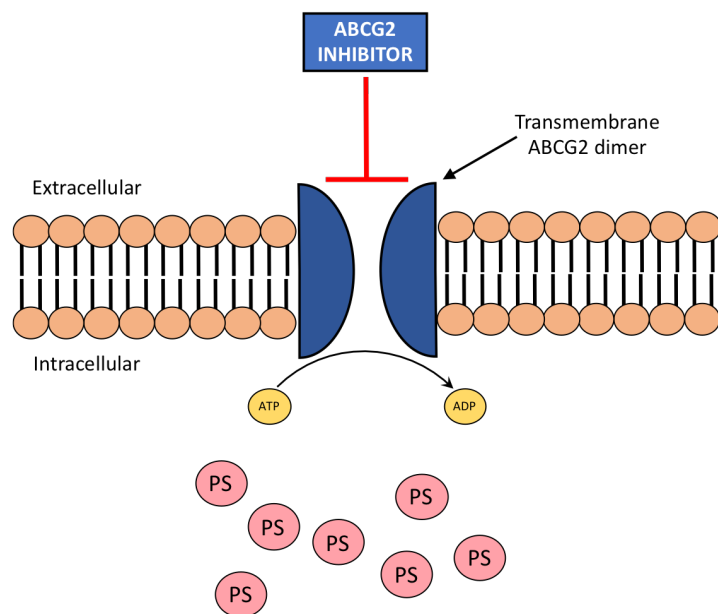
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**FIGURE 1**  
**NO INHIBITION**



**ABCG2 INHIBITION**



**Figure 1. Graphical representation of the photosensitiser efflux activity of ABCG2**

The dimerising transmembrane ATP-dependant ABCG2 efflux pump eliminates the intracellular accumulation of photosensitisers (PS) out of cells. ABCG2 inhibitors can directly bind and antagonise the activity of ABCG2 which improves the intracellular retention of photosensitisers for PDT.



**Table 1.** An overview of the studies included in this review

Photosensitisers	Experimental models	ABCG2 inhibitors	Outcomes of study	Reference
PPa	<i>abcg2</i> knockdown mice <i>in vivo</i> and fibroblast cell line <i>in vitro</i>	Ko143	<i>abcg2</i> knockdown mice experienced phototoxicity. ABCG2 overexpression reduced PS accumulation. Ko143 reversed effects of ABCG2.	Jonker 2002 [24]
	Colorectal cancer cell lines <i>in vitro</i> and tumour xenografts <i>in vivo</i>	Ko143	PS uptake, ROS generation and PDT correlated with ABCG2 expression. Ko143 reversed effects of ABCG2.	Kim 2015 [25]
	Ovarian cancer cell line <i>in vitro</i>	Ko143	PI3K/Akt inhibition and cMET repression lead to ABCG2 suppression, increased PS uptake, increased ROS generation and improved PDT. Ko143 reversed effects of ABCG2.	Jung 2015 [30]
	Various carcinoma cell lines <i>in vitro</i>	Ko143	<i>Nrf2</i> knockdown increased PS uptake, increased ROS generation and improved PDT. <i>Nrf2</i> knockdown lead to ABCG2 suppression. Ko143 did not affect ABCG2 activity in <i>Nrf2</i> knockdown cells.	Choi 2014 [36]
	Prostate cancer cell line <i>in vitro</i>	Reserpine	Androgen receptor ligand-conjugated PS improved PS retention and PDT.	Rapozzi 2015 [97]

			Reserpine improved retention of free PS	
MPPa	Osteosarcoma cell lines <i>in vitro</i>	-	PDT resistant cells (selected through repeated treatment) showed increased ABCG2 expression, reduced PS uptake and reduced ROS generation. PDT resistant cells were capable of invasion, migration and formed colonies after treatment.	Tao 2017 [32]
	Glioma cell lines <i>in vitro</i>	FTC	Varying levels of sensitivity to PDT between different cell lines. This correlated with PS accumulation and ABCG2 expression. FTC inhibited ABCG2 activity.	Pan 2017 [31]
	Ovarian cancer cell lines <i>in vitro</i>	-	PDT induced an increase in Nrf2 expression. <i>Nrf2</i> knockdown increased ROS generation and improved PDT.	Tian 2017 [37]
HPPa	Patient-derived primary lung carcinoma, healthy lung epithelial and stromal cells <i>in vitro</i>	Imatinib Mesylate	Low PS accumulation in high ABCG2 expressing cells. Imatinib Mesylate reversed effects of ABCG2.	Tracy 2011 [33]
	Fibrosarcoma and colon carcinoma cell lines <i>in vitro</i> and <i>in vivo</i>	-	Conjugation of galactose improved PS uptake and PDT <i>in vitro</i> and <i>in vivo</i> . Galactose conjugation improved toxicity of PS.	Zheng 2009 [95]

	Various cell lines (cancer and non-cancer) <i>in vitro</i> and <i>in vivo</i>	Imatinib Mesylate	Conjugation of galactose improved PS uptake <i>in vitro</i> and PDT <i>in vitro</i> and <i>in vivo</i> . Imatinib Mesylate enhanced PS uptake and retention	Morgan 2010 [96]
ALA/PpIX	Various cell lines (cancer and non-cancer) <i>in vitro</i>	Ko143	PS uptake and PDT correlated with ABCG2 expression. Ko143 reversed effects of ABCG2.	Barron 2013 [42]
	Keratinocyte cell line <i>in vitro</i>	Ko143	PS uptake and PDT correlated with ABCG2 expression. Ko143 reversed effects of ABCG2.	Bebes 2011 [43]
	Urothelial carcinoma cell line <i>in vitro</i>	FTC	FBS in cell media facilitated ABCG2 mediated elimination of PS. FTC reversed effects of ABCG2.	Ogino 2011 [44]
	Gastric cancer cell lines <i>in vitro</i>	FTC	Varying levels of sensitivity to PDT between different cell lines. This correlated with PS accumulation and ABCG2 expression. <i>abcg2</i> -knockdown increased PS uptake and improved PDT. FTC reversed effects of ABCG2.	Hagiya 2012 [45]
	Glioma cell lines <i>in vitro</i>	Gefitinib	Gefitinib increased PS uptake, reduced ABCG2 expression and improved PDT.	Sun 2013 [46]
	Ovarian clear-cell carcinoma cell lines <i>in vitro</i>	FTC	Varying levels of sensitivity to PDT between different cell lines. This correlated with PS accumulation and	Teshigawara 2018 [47]

			ABCG2 expression. FTC reversed effects of ABCG2.	
	Prostate cancer cell line <i>in vitro</i>	-	PS accumulation and PDT was enhanced in dormant cells. PEPT1 was upregulated and ABCG2 was downregulated in dormant cells.	Nakayama 2016 [48]
	Brain cancer cell lines <i>in vitro</i>	-	Glioblastoma cells had higher PS uptake, lower ABCG2 expression and were more sensitive to PDT than medulloblastoma cell lines.	Briel-Pump 2018 [50]
	Breast cancer and normal cell lines <i>in vitro</i>	Ko143	TNBC cells had lower PS accumulation and mitochondrial localisation and higher resistance to PDT than ER+ and HER2+ cells. Ko143 reversed effects of ABCG2.	Palasuberniam 2015 [52]
Hypericin	Colorectal cancer cell line <i>in vitro</i>	<i>Proadifen*</i> and FTC	The uptake of PS increased ABCG2 expression. Proadifen and FTC reduced ABCG2 activity resulting in increased PS uptake, enhanced oxidative stress and improved PDT.	Jendželovský 2009 [60]
	Colorectal cancer cell line <i>in vitro</i>	Ko143	Ko143 treatment increased PS uptake. Incubation with PS increased ABCG2 expression. ABCG2 expression	Šemeláková 2016 [61]

			reduced following PDT.	
	Acute promyelocytic leukemia cell line <i>in vitro</i>	Ko143	ABCG2-overexpressing cells had reduced PS uptake, increased PDT resistance and no apoptotic activity following PDT. Ko143 reversed effects of ABCG2.	Jendželovský 2019 [62]
	Colorectal cancer cell lines <i>in vitro</i>	Ko143	3D spheroids were more resistant to PDT than 2D cell cultures. ABCG2 protein expression was higher in 3D spheroids.	Khot 2018 [63]
	Various cancer cell lines <i>in vitro</i>	-	MK-886 (5-LOX inhibitor) can synergistically increase PS uptake, reduce ABCG2 expression and enhance PDT.	Kuchárová 2015 [66]
	Melanoma cell lines <i>in vitro</i>	-	Uncorrelated ABCG2 expression was found between different cell lines incubated with PS. PS enhanced Dacarbazine cytotoxicity and abrogate self-renewal capacity of cells. ABCG2 expression did not influence PDT.	Biteghe 2017 [67]
Photofrin	Glioma cell lines <i>in vitro</i>		High ABCG2 expressing glioma stem cells had lower PS uptake, higher SDT resistance, reduced apoptosis and reduced ROS generation as	Xu 2012 [70]

			compared to low ABCG2 expressing glioma cells.	
Ce6	Glioma cell lines <i>in vitro</i>	Ko143	FBS in cell media facilitated ABCG2 mediated elimination of PS. Ko143 reversed effects of ABCG2. 3D spheroids were more resistant to PDT than 2D cell cultures. ABCG2 protein expression and PS accumulation was higher and lower in 3D spheroids respectively.	Abdel Gaber 2018 [78]
	Pancreatic cancer cell lines <i>in vitro</i> and <i>in vivo</i>	Ko143	Nanoparticle reformulation of PS improved PS uptake, improved ROS generation and improved PDT. Ko143 further enhanced nanoparticle PDT.	Roh 2017 [102]
BpD	Pancreatic cancer cell line <i>in vitro</i>	-	Sub-lethal PDT reduced ABCG2 expression and improved the uptake of irinotecan	Huang 2016 [80]
	Breast cancer cell lines <i>in vitro</i>	FTC	Nanoliposomal repackaging of PS improved cellular retention and PDT. FTC reversed effects of ABCG2.	Baglo 2019 [103]
PPa, MPPa, Ce6, ALA/PpIX, HpIX, mTHPP and mTHPC	Various cell lines (cancer and non-cancer) <i>in vitro</i>	FTC	Varying degrees of intracellular retention of different PS was observed in ABCG2-overexpressing cells. FTC modulated ABCG2 activity. Intracellular	Robey 2005 [22]

			concentrations of PS correlated with sensitivity to PDT	
HPPa, Galactose-conjugated HPPa, ALA/PpIX and BpD	Various cell lines (cancer and non-cancer) <i>in vitro</i> and murine fibrosarcoma tumour xenografts <i>in vivo</i>	FTC and Imatinib Mesylate	PS retention was low in ABCG2-positive cells. ABCG2 inhibitors improved PS retention and PDT <i>in vitro and in vivo</i> . Galactose conjugation improved PS retention and PDT.	Liu 2007 [81]
Photofrin and NPe6	Epidermoid carcinoma cell line <i>in vitro</i> and patient tumour tissue for IHC staining	FTC	Photofrin retention was low and PDT resistance was seen in ABCG2-overexpressing cells. FTC reversed effects of ABCG2. ABCG2 expression did not influence NPe6 retention and PDT. NPe6-PDT was more efficient in high ABCG2 expressing patient tumours.	Usuda 2010 [82]
Indocyanine green	Various cancer cell lines <i>in vitro</i>	-	PS conjugated to gold nanospheres improved cellular retention and PDT.	Li 2017 [101]
HpD	Rat gastric epithelial cell line <i>in vitro</i>	-	Hyperthermia improved PS uptake, increased ROS generation and improved PDT.	Kurokawa 2019 [104]
TPPS <sub>2a</sub> , TPCS <sub>2a</sub> , AIPcS <sub>2a</sub> and PPa	Breast cancer cell line <i>in vitro</i>	FTC	All PS except PPa were found to be non-ABCG2 substrates	Selbo 2012 [106]
TPCS <sub>2a</sub> and PPa	Breast cancer cell line <i>in vitro</i>	-	High ABCG2 expressing cells that are resistant to PPa (ABCG2 substrate)-PDT	Olsen 2017 [107]

			were sensitive to TPCS <sub>2a</sub> (non-ABCG2 substrate)-PDT	
<p>Abbreviations: <b>PDT</b>: Photodynamic Therapy; <b>PS</b>: Photosensitiser; <b>NPe6</b>: Mono-L-aspartyl chlorin e6; <b>FTC</b>: Fumitremorgin C; <b>ROS</b>: Reactive Oxygen Species; <b>ABCG2</b>: ATP-binding cassette superfamily G member 2; <b>FBS</b>: Foetal Bovine Serum; <b>PEPT1</b>: Peptide transporter 1; <b>TNBC</b>: Triple Negative Breast Cancer; <b>ER+</b>: Oestrogen Receptor Positive; <b>HER2+</b>: Human Epidermal Growth Factor Receptor 2 Positive; <b>5-LOX</b>: 5-lipoxygenase; <b>SDT</b>: Sonodynamic Therapy; <b>PPa</b>: Pheophorbide-a; <b>MPPa</b>: Pyropheophorbide-a methyl ester; <b>HPPa</b>: 2-[1-hexyloxyethyl]-2-devinyl pyropheophorbide-a; <b>ALA/PpIX</b>: Aminolevulinic acid/Protoporphyrin IX; <b>Ce6</b>: Chlorin e6; <b>BpD</b>: Benzoporphyrin Derivative; <b>HpIX</b>: Haematoporphyrin IX; <b>mTHPP</b>: meso-tetra(3-hydroxyphenyl) porphyrin; <b>mTHPC</b>: meso-tetra(3-hydroxyphenyl) chlorin; <b>IHC</b>: Immunohistochemistry; <b>HpD</b>: haematoporphyrin dihydrochloride; <b>TPPS<sub>2a</sub></b>: Di-sulfonated meso-tetraphenylporphine; <b>TPCS<sub>2a</sub></b>: Di-sulfonated meso-tetraphenylchlorin; <b>AIPcS<sub>2a</sub></b>: Di-sulfonated aluminiumphthalocyanine;</p>				
<p>*Proadifen did not directly inhibit ABCG2 but was found to suppress ABCG2 activity.</p>				