Abstract

Purpose: The ATP-binding cassette protein ABCG2 (breast cancer resistance protein) effluxes some of the photosensitizers used in photodynamic therapy (PDT) and, thus, may confer resistance to this treatment modality. Tyrosine kinase inhibitors (TKI) can block the function of ABCG2. Therefore, we tested the effects of the TKI imatinib mesylate (Gleevec) on photosensitizer accumulation and in vitro and in vivo PDT efficacy.

Experimental Design: Energy-dependent photosensitizer efflux and imatinib mesylate's effects on intracellular accumulation of clinically used second- and first-generation photosensitizers were studied by flow cytometry in murine and human cells with and without ABCG2 expression. Effects of ABCG2 inhibition on PDT were examined in vitro using cell viability assays and in vivo measuring photosensitizer accumulation and time to regrowth in a RIF-1 tumor model.

Results: Energy-dependent efflux of 2-(1-hexyloxyethyl)-2-devinylpyropheophorbide-a (HPPH, Photoclor), endogenous protoporphyrin IX (PpIX) synthesized from 5-aminolevulinic acid, and the benzoporphyrin derivative monoacoid ring A (BPD-MA, Verteporfin) was shown in ABCG2+ cell lines, but the first-generation multimeric photosensitizer porfimer sodium (Photofrin) and a novel derivative of HPPH conjugated to galactose were minimally transported. Imatinib mesylate increased accumulation of HPPH, PpIX, and BPD-MA from 1.3- to 6-fold in ABCG2+ cells, but not in ABCG2– cells, and enhanced PDT efficacy both in vitro and in vivo.

Conclusions: Second-generation clinical photosensitizers are transported out of cells by ABCG2, and this effect can be abrogated by coadministration of imatinib mesylate. By increasing intracellular photosensitizer levels in ABCG2+ tumors, imatinib mesylate or other ABCG2 transport inhibitors may enhance efficacy and selectivity of clinical PDT.

Photodynamic therapy (PDT) is used for the treatment of many cancers. Photosensitizers are taken up by tumor cells and then activated by light (1), generating reactive oxygen species that cause cell death by necrosis or apoptosis (2). Expression of ATP-binding cassette (ABC) transport proteins renders tumor cells resistant to chemotherapy drugs that are substrates of these proteins (3), and the effect of these transporters on intracellular photosensitizer accumulation has been examined as a potential cause of resistance to PDT. The ABC family transport protein that has been most thoroughly investigated is ABCB1, or P-glycoprotein, but photosensitizers were found not to be substrates for this pump (4–8), nor were they substrates for ABC1, or multidrug resistance-associated protein-1 (8). In contrast, another ABC family transport protein, ABCG2 or breast cancer resistance protein, has been found to transport some photosensitizers and to decrease intracellular photosensitizer accumulation (8). Jonker et al. (9) showed that ABCG2 knock-out mice were photosensitive because of increased protoporphyrin IX (PpIX) levels. Robey et al. found that phophorbid e a is a specific substrate for ABCG2 (10), and that ABCG2 also transports pyropheophorbide-a methyl ester, chlorin e6, and 5-aminolevulinic acid–induced PpIX, but not hematoporphyrin IX, meso-tetra(3-hydroxyphenyl)porphyrin or meso-tetra(3-hydroxyphenyl)chlorin (8).

Tyrosine kinase inhibitors (TKI), including imatinib mesylate (Gleevec) and gefitinib (Iressa), are novel agents in cancer treatment that have been found to reverse resistance to chemotherapy drugs by blocking their efflux by ABCG2 (9, 11–14). In this study, we used the TKI imatinib mesylate to overcome resistance to PDT.

Materials and Methods

Reagents. 5-Aminolevulinic acid hydrochloride and cyclosporine A (CsA) were purchased from Sigma-Aldrich (St. Louis, MO). 2-[1-Hexyloxyethyl]-2-devinyl pyropheophorbide-a (HPPH, Photoclor) and HPPH-galactose conjugate were synthesized at the Roswell Park Cancer Institute. Details of the preparation and characterization of the HPPH-galactose conjugate are detailed elsewhere. Porfimer sodium...
(Photofrin) was obtained from Axcan Scandinavian, Inc. (Birmingham, AL). Benzoporphyrin derivative monoacid ring A (BPD-MA) was manufactured by QIT Inc., (Vancouver, Canada). Imatinib mesylate (Gleevec) was provided by Novartis Pharmaceuticals (Basel, Switzerland) and fumitremorgin C (FTC) by Dr. Susan Bates (NIH, Bethesda, MD). Gefitinib (Iressa) was manufactured by AstraZeneca (Bristol, England).

**Cell lines.** FaDu human hypopharyngeal squamous cell carcinoma, RIF-1 murine radiation-induced fibrosarcoma, and Colo 26 murine colon carcinoma cells were obtained from the American Type Culture Collection (ATCC; Manassas, VA); the Colo 26 cells were more than passage 20. BCC-1/KMC, a human basal cell carcinoma cell line (15), was provided by Dr. Tak-Wah Wong (National Cheng Kung University Hospital, Tainan, Taiwan). HEK-293 (transformed human embryonic kidney) cells transfected with either an empty pcDNA3 vector or a pcDNA3 vector containing full-length ABCG2 (HEK-293 pcDNA or HEK-293 482R) were generously provided by Dr. Susan Bates.

FaDu cells were grown in Eagle’s MEM supplemented with 10% fetal bovine serum, 1 mmol/L L-glutamine, 1% penicillin-streptomycin, and 1 mmol/L MEM sodium pyruvate. RIF-1 cells were grown in MEM-a, and BCC-1 cells and Colo 26 cells were grown in RPMI 1640; both media were supplemented with 10% fetal bovine serum, 2 mmol/L L-glutamine, and 1% penicillin-streptomycin. HEK-293 pcDNA and HEK-293 R482 cells were grown in Eagle’s MEM supplemented with 10% fetal bovine serum, 2 mmol/L L-glutamine, 1% penicillin-streptomycin, and 2 mg/mL G-418.

**Western blot analysis.** Aliquots of cell extracts were separated on 8% SDS-polyacrylamide gels. Protein was prepared in 30 µg quantities from all cell lines, except for HEK-293 482R cells, from which 2 µg protein were used. Proteins were transferred to Protran membranes (Schleicher & Schuell, Riviera Beach, FL), and the membranes were reacted with antibodies to ABCB1, ABCB1, and ABCG2 (RXP-53; Alexis Biochemicals, San Diego, CA) and β-actin (Sigma-Aldrich). Reaction with horseradish peroxidase–labeled secondary antibodies (ICN Biomedicals, Inc., Aurora, OH) was done in PBS containing 0.1% Tween 20 and 5% milk. Immune complexes were visualized by an enhanced chemiluminescence reaction (Amersham Biosciences, Piscataway, NJ). The enhanced chemiluminescence images were recorded on X-ray films with various exposure lengths.

**Photosensitizer accumulation and efflux assays.** Cells were plated in six-well plates at a density of 3 to 5 × 10^4 cells per well and incubated overnight. To study photosensitizer accumulation, cells were exposed to ABCG2 modulators, including 10 µmol/L imatinib mesylate, FTC (16) and CsA (17), and 5 µmol/L gefitinib, for 1 h before the addition of photosensitizers, which included HPPH (0.4–0.8 µmol/L), BPD-MA (0.2 µmol/L), HPPH-lactose (0.8 µmol/L), and Photofrin (2 µg/mL) in complete medium with 10% FCS, and 6-aminolevulinic acid (ALA; 0.4–0.8 µmol/mL) in 1% FCS medium) because 1-h preincubation with modulator replicates the conditions for clinical modulation and enhances the effect of some, although not all, modulators (18). Cells were cultured for an additional 4 h, then washed with cold culture medium and with PBS, and harvested using trypsin (0.25%)-EDTA (1 mmol/L), followed by addition of cold 10% FCS media. For uptake studies, the cells were washed with, and resuspended in, cold PBS and were kept on ice. Photosensitizer levels were immediately measured by flow cytometry as described below. For photosensitizer efflux assays, following trypanosinization and washing, the time 0 samples were treated as described above for uptake. The other aliquots were resuspended in photosensitizer- and modulator-free medium and placed at 4°C or 37°C for 1 h. Cells were then washed once with cold PBS, resuspended in cold PBS, and placed on ice. Photosensitizer levels were immediately measured by flow cytometry. Samples were analyzed using a FACSCalibur flow cytometer with dual laser excitation (488 and 635 nm). HPPH and HPPH-galactose were measured in the FL-4 channel (652-658 nm emission), and BPD-MA, Photofrin, and PdX were measured in the FL-3 channel (>670 nm emission). Excitation powers and detector gains were kept constant between experiments. Histograms measuring intracellular photosensitizer levels were generated using WinList software (Verity Software House, Topsham, ME). Results are expressed as the geometric mean of arbitrary fluorescence units ± SEM of three to four separate experiments. The HPPH enhancement ratio was calculated as the (mean HPPH fluorescence + imatinib mesylate)/(mean HPPH fluorescence without imatinib mesylate). The enhancement ratio data were fit with a three-parameter single rectangular hyperbola (SigmaPlot regression wizard; Systat Software, San Jose, CA).

**Phototoxicity assays.** Cells were plated in 96-well plates at a density of 1 × 10^5 cells per well. After overnight incubation, they were exposed to the photosensitizers HPPH (0.4 or 0.8 µmol/L), HPPH-galactose (0.8 µmol/L), BPD-MA (0.2 µmol/L), or Photofrin (2 µg/mL) in complete medium with 10% FCS, or Ala (0.4 or 0.8 mmol/L) in medium with 1% FCS, for 4 h with or without ABCG2 modulators added 1 h before the photosensitizers. In some experiments, Photofrin with or without ABCG2 modulators was added 25 h before irradiation, and 1 h before irradiation, cells were washed with complete medium without modulators to allow the release of loosely bound monomers (19). The ABCG2 modulators included imatinib mesylate, FTC, and CsA at 10 µmol/L and gefitinib at 5 µmol/L. Cells were irradiated with a filtered xenon arc lamp (600-700 nm) at a fluence rate of 14 mW/cm² for HPPH and BPD-MA or with a red light (570-700 nm; ref. 20) at a fluence rate of 6.3 mW/cm² for Ala and Photofrin. After irradiation, cells were washed with PBS. Fresh medium with 20% FCS was added, and cells were returned to the incubator. Cell viability was evaluated by the 1,3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay 48 h after irradiation. Enhancement of phototoxicity was defined as the ratios of the light doses giving a 0.5 surviving fraction (LD50) with and without imatinib mesylate. In some cases, an estimated LD50 was extrapolated from a single exponential fit to the survival data using the SigmaPlot regression wizard, with the requirement that R² > 0.9.

**In vivo HPPH levels and photodynamic therapy.** Eight-week-old female C3H/HeJ mice were injected intradermally with 4 × 10^5 RIF-1 tumor cells. When the tumors reached a diameter of 4 mm, groups of five mice received tail vein injections of 0.2 µmol/kg body weight HPPH alone or HPPH together with four doses of imatinib mesylate. Tumors were irradiated with 665 nm light 24 h after the HPPH, according to our standard protocol (21). Imatinib mesylate was given by oral gavage in two doses of 200 mg/kg body weight starting 2 h before the HPPH and 12 h later, followed by two doses of 100 mg/kg body weight 18 and 26 h later; the last dose was 2 h before photoradiation. The doses and intervals were chosen on the basis of prior studies demonstrating that 200 mg/kg every 12 h or 160 mg/kg every 8 h for 15 days was well tolerated (22), and that peak mouse plasma levels occurred 2 h after p.o. administration with a plasma half-life of about 4 to 5 h (23). Oral gavage was used to replicate the clinical administration of imatinib, as has been done by other investigators in mouse models (22–24). All animal experiments were approved by the Institutional Animal Care and Use Committee at Roswell Park Cancer Institute.

To determine photosensitizer levels, samples of tumor, skin, and muscle tissue were harvested 24 h after the HPPH administration and dissolved in Solvable (Perkin-Elmer, Boston, MA) at 53°C overnight. HPPH levels were measured by fluorometry (18). Briefly, the Solvable extract was diluted 1:1 with PBS, the photosensitizer levels were determined by fluorometry, and concentrations were extrapolated from standard curves. For PDT, groups of five tumor-bearing mice received HPPH or HPPH preceded by imatinib mesylate, as above. After 24 h, the tumors were treated with 665 nm light from an argon ion laser-pumped dye laser (Spectra Physics, Mountain View CA) with a fluence of 72 J/cm² delivered at a rate of 14 mW/cm² using a light field of 1 cm diameter. Additional control mice received no treatment on imatinib mesylate alone without HPPH. Tumors were measured every 1 to 3 days, and mice were sacrificed when tumor volumes exceeded 400 mm³.

4 X. Zheng, et al., submitted for publication.
**Statistical analyses.** Efflux and uptake data were compared using the two-sided Student's t test (Excel). P values <0.05 were considered significant. The Kaplan-Meier tumor response curves were compared using a log-rank test to generate P values (MedCalc Statistical Software, Mariakerke, Belgium).

**Results**

**HPPH is an ABCG2 substrate.** We studied two human and two mouse cell lines with a range of ABCG2 expression and a control cell line transfected with ABCG2. By Western blotting, expression of ABCG2 was high in BCC-1 cells derived from a human basal cell carcinoma, but was not found in the human head and neck squamous cell carcinoma line FaDu (Fig. 1A). ABCG2 also was high in murine RIF-1 fibrosarcoma and moderate in murine Colo 26 colon carcinoma cells. By densitometry, ABCG2 expression was 6- to 15-fold higher in HEK-293 cells transfected with ABCG2 (HEK-293 R482) compared with BCC-1, RIF-1, and Colo 26 cells; and it was absent in HEK-293 pcDNA controls transfected with an empty vector (Fig. 1A). The transporters ABCB1 (P-glycoprotein) and ABCC1 (multidrug resistance-associated protein-1) were not expressed in any of these cell lines (data not shown). After 4-h uptakes at 37°C, we determined the presence of active photosensitizer transport out of the cells by measuring temperature-dependent efflux at 4°C or 37°C using flow cytometry to quantify intracellular photosensitizer content. There were wide variations in initial HPPH uptake. The cell lines with large ABCG2 expression tended to have low HPPH accumulation (Fig. 1B). No significant changes in intracellular HPPH content were found in any of the cell lines after 1 h at 4°C relative to the initial content following uptake (0-h group; Fig. 1B). Similarly, after 1 h efflux at 37°C, there were no significant decreases in intracellular HPPH in FaDu or HEK-293 pcDNA cells, which lack ABCG2 expression. In contrast, after 1 h at 37°C, there was significant loss of HPPH content in Colo 26, RIF-1, and BCC-1 cells (P < 0.01). There was no measurable change in HEK-293 R482 cells, which had very low uptake, much less than the pcDNA control. Although immunologically detected ABCG2 does not always correlate with functional activity (25), it is likely that high transport activity in HEK-293 R482 cell limits the amount of photosensitizer accumulation and the amount available for efflux.

**ABCG2 modulators increase photosensitizer accumulation in vitro.** We evaluated the effects of different ABCG2 modulators on intracellular HPPH accumulation in RIF-1 cells, comparing cells with and without the modulator, as summarized in Fig. 2A. The ABCG2-specific inhibitor FTC (10 μmol/L) and the broad-spectrum inhibitor cyclosporine A (CSA, 10 μmol/L) produced 6-fold and 4.5-fold increases, respectively. The two TKIs imatinib mesylate (10 μmol/L) and gefitinib (5 μmol/L) caused 5-fold enhancement; gefitinib was limited to 5 μmol/L by its direct cell toxicity. All increases were strongly significant (P < 0.001). Imatinib mesylate at 10 μmol/L significantly increased intracellular HPPH levels in all of the ABCG2+ cell lines (P < 0.01 or P < 0.001), but not in ABCG2– FaDu or HEK pcDNA (Fig. 2B). Enhancement ratios were 1.6, 5.6, 2.4, and 4.9 for Colo 26, RIF-1, BCC-1, and HEK-293 428R, respectively (Fig. 2B). Although the modulators were given 1 h before the photosensitizer, we also found comparable enhancements when the TKI and HPPH were added together (data not shown). An imatinib mesylate dose-response study in ABCG2+ cells showed that 10 μmol/L imatinib mesylate gave >90% of maximum enhancement in Colo 26, RIF-1, and BCC-1 cells, and that 50% of maximum enhancement was reached at 1, 2, and 0.5 μmol/L, respectively (Fig. 2C). The HEK-293 428R cells only reached about 64% of estimated maximal inhibition with 10 μmol/L imatinib mesylate (Fig. 2C), but the level of transporter in these transfected cells is much higher than is likely to be found in carcinomas. Extrapolation of the rectangular hyperbola fit of the HEK-293 428R data suggests that 95% inhibition would be reached at 75 μmol/L imatinib mesylate.

Imatinib mesylate also increased intracellular levels of two other second-generation photosensitizers, ALA-induced PpIX, and BPD-MA, as shown in Fig. 2D for PpIX in Colo 26, RIF-1, and BCC-1 cells (left axis) and for BPD-MA in RIF-1 cells (right axis). PpIX levels were enhanced by factors of 2.5, 2, and 2.5 in Colo 26, RIF-1, and BCC-1 cells, respectively. The maximum PpIX levels varied, reflecting differences between cell lines in the kinetics of the heme biosynthetic pathway (26). The low level of PpIX in RIF-1 fibrosarcoma cells is expected because ALA does not cause significant PpIX accumulation in mesenchymally derived cells (26). In addition, the overall lower fluorescence of the PpIX compared with BPD-MA and HPPH reflects the much smaller absorption of red light by the porphyrin compared with the two chlorins, and the inefficient capture of >670 nm porphyrin fluorescence by the FL3 detection window of the flow cytometer. BPD-MA accumulation in RIF-1 cells was enhanced by a factor of 1.3. The greater effect of imatinib mesylate on HPPH (Fig. 2B) compared with
BPD-MA in the same RIF-1 cell line suggests that BPD-MA is less affected by ABCG2; similar variations were found by Robey et al. (8). Imatinib mesylate had no effect in FaDu cells (data not shown).

**ABCG2 modulators increase phototoxicity in vitro.** Consistent with higher photosensitizer levels, increases in phototoxicity were observed in the presence of ABCG2 modulators in ABCG2+ cells. Treatment conditions were chosen to cause low phototoxicity in the absence of modulators to more clearly appreciate the effects of the modulators. The data are normalized to the dark controls, so dark toxicities are not apparent. The dark toxicities were <10% except for gefinitib, which had ~20% dark toxicity at 5 μmol/L. In general, the HPPH phototoxicity data were linear in the semilog presentations in Fig. 3 and were well fit by single exponentials, with $R^2 > 0.9$ (data not shown). The effects of the different ABCG2 modulators on HPPH phototoxicity were compared in RIF-1 cells (Fig. 3, top left). Imatinib mesylate (solid line) and the other agents (dashed lines) comparably increased HPPH-PDT phototoxicity in a light dose-dependent fashion. The estimated light dose causing 50% phototoxicity (LD$_{50}$) increased by a factor of 14 using an extrapolated LD$_{50}$ of 10 J/cm$^2$ for RIF-1 cells without modulator. HEK-293 pCDNA cells were more sensitive to HPPH-PDT than HEK-293 R482 cells by a factor of 14 in the LD$_{50}$, Treatment with 10 μmol/L imatinib mesylate decreased the estimated LD$_{50}$ by 7-fold from about 4.2 to 0.6 J/cm$^2$ in HEK-293 R482 cells, but had no effect on the sensitivity of HEK-293 pDNA cells to HPPH-PDT (Fig. 3, top right). Imatinib mesylate increased HPPH- and ALA/PpIX-PDT phototoxicity in ABCG2+ Colo 26 (Fig. 3, middle left) and in human basal cell carcinoma BCC-1 cells (Fig. 3, middle right). Because of the high HPPH uptake in BCC-1, only a 0.4 μmol/L HPPH dose was used in these cells. The estimated LD$_{50}$ for HPPH decreased by factors of 6 in Colo 26 and 5 in BCC-1 cells. Neither 10 nor 20 μmol/L imatinib mesylate affected HPPH phototoxicity in the human squamous carcinoma line FaDu, which lacks ABCG2 expression (Fig. 3, bottom left). Finally, imatinib mesylate also increased the phototoxicity of BPD-MA (shown for RIF-1 cells in Fig. 3, bottom right), with a 2-fold decrease in LD$_{50}$.

**Imatinib mesylate increases HPPH levels and enhances the efficacy of HPPH-PDT in vivo.** In mice bearing s.c. RIF-1 tumors, imatinib mesylate increased median HPPH levels in the tumors 1.8-fold ($P = 0.001$), but did not have a significant effect on levels in skin ($P = 0.074$). Median HPPH levels in muscle increased 3-fold ($P < 0.001$), but median photosensitizer content in the muscle was only 6% or 10% of that in tumors ($P < 0.001$), without or with imatinib mesylate, respectively (Fig. 4A). The higher tumor HPPH levels correlated with enhanced in vivo PDT efficacy. Groups of mice were treated with low-dose PDT using 0.2 μmol/kg HPPH, followed 24 h later by 72 J/cm$^2$, 665-nm light at 14 mW/cm$^2$. This dose of HPPH was half of what is used with curative intent treatment of this tumor (21), and it was chosen to better delineate the effects of ABCG2 inhibition and to represent cases of suboptimal PDT doses. In the presence of imatinib mesylate, the time for 50% of the tumors to grow to 400 mm$^3$ doubled to 12.5 days versus 6 days with HPPH-PDT alone (Fig. 4B); the difference was strongly significant ($P < 0.001$). Compared with full-dose HPPH-PDT (0.4 μmol/kg), the low photosensitizer dose had minimal effect on the mouse skin adjacent to the tumors in the HPPH group. Imatinib mesylate + HPPH-PDT caused no additional normal skin damage by visual inspection. The brief course of imatinib mesylate alone had no effects on either the tumor or adjacent skin and caused no observable toxicity.

**ABCG2 substrate specificity of photosensitizers depends on their structures.** Although other factors may be involved, the variations in efflux between different photosensitizers found
by ourselves and others (8) are consistent with a structure dependence of the ABCG2 transporter. We tested whether more complex photosensitizer structures were less effectively transported. We examined both HPPH modified by conjugation with galactose and the Food and Drug Administration–approved first-generation multimeric agent Photofrin (Fig. 5A).

In RIF-1 cells, HPPH-galactose had a 28% temperature-dependent efflux (Fig. 5B, left axis), compared with a 62% loss for HPPH alone (Fig. 1). The imatinib mesylate–mediated 10% enhancement was not significant ($P = 0.32$) and was minimal compared with the >5-fold enhancement for unconjugated HPPH (Fig. 2). We examined the effect of temperature on retained Photofrin. Using both 4-h (data not shown) and 24-h incubations (Fig. 5B, right axis), we found no significant decrease in intracellular Photofrin levels after 1 h at either 4°C or 37°C. Imatinib mesylate caused a 27% and 39% increase in Photofrin uptake in RIF-1 cells with 4- and 24-h incubations, respectively (data not shown). However, Photofrin is a mixture of multimeric photosensitizers (27) that are considered to be the clinically active fraction, together with monomers that are poorly retained in cells and tissues and have little biological efficacy, but contribute to the fluorescence of the agent (19, 28). Because only these inactive monomers are likely ABCG2 substrates, imatinib mesylate–dependent effects on intracellular fluorescence have uncertain relevance. In phototoxicity experiments, imatinib mesylate did not increase the killing of either HPPH-galactose- or Photofrin-PDT (Fig. 5C).

Thus, attachment of galactose to a pyropheophorbide that is a good substrate for ABCG2 greatly reduces the transport effects. Similarly, the efflux and phototoxicity data suggest that multimeric photosensitizers are not transported by ABCG2.

### Discussion

We have shown structure-specific active transport of three clinically used second-generation photosensitizers by ABCG2, and inhibition of ABCG2-mediated photosensitizer transport and enhancement of both in vitro and in vivo PDT through the administration of the TKI imatinib mesylate. We found that TKIs increase intracellular photosensitizer accumulation and enhance phototoxicity in ABCG2+ cells. TKIs previously have been found to inhibit ABCG2-mediated transport of chemotherapy drugs and sensitize cells to chemotherapy (11–13), but our present study provides the first demonstration that a
clinically applicable TKI, imatinib mesylate, selectively increases accumulation of photosensitizer and enhances efficacy of both in vitro and in vivo PDT in ABCG2+ tumor cells. ABCG2+ cells, including Colo 26, RIF-1, BCC-1, and ABCG2-transfected HEK-293 cells, exhibited decreased intracellular levels of HPPH, ALA/PpIX, and BPD-MA and resistance to PDT with these agents. In contrast, transport of these photosensitizers and resistance to PDT was not found in FaDucells, which do not express ABCG2, or in plasmid-transfected HEK-293 cells. The extent of PDT resistance was quite significant, representing as much as a factor of 14 in the LD50 light dose. In our experiments, we varied light rather than photosensitizer dose because the lack of drug-light reciprocity due to sensitizer photobleaching can cause artifacts at low photosensitizer concentrations (29) and also because clinically, a fixed dose of the photosensitizer is given, whereas the physician can alter the light dose.

Because HPPH is a derivative of pyropheophorbide-a, the results for this agent, which is in promising phase II trials (30, 31), are consistent with the recent report of Robey et al. that pyropheophorbide-a is a substrate of ABCG2 (8). As shown in Fig. 2, HPPH and PpIX were more effectively transported than BPD-MA. A photosensitizer-dependent variation is consistent with the results of Robey et al. (8), although the liposomal formulation of BPD-MA also might affect uptake and intracellular distribution. The amount of HPPH transport was not directly proportional to the expression of ABCG2 measured by Western blot analysis, as exemplified by BCC-1 cells, which had higher levels of ABCG2 expression but exhibited a lesser degree of HPPH transport than the other cell lines with ABCG2 expression. Discordance between expression and function of ABCG2 has been previously shown in cancer cells (25). RIF-1 is commonly used in preclinical models for PDT. However, its very active transport system means that it should be used with caution in screening new agents. We note that Colo 26 cells reproducibly become ABCG2+ after about 20 passages; early passage cells are ABCG2- by Western blot.

![Fig. 5.](image-url)

**A** Structures of Photofrin and HPPH-galactose. **B**, temperature-dependent efflux of HPPH-galactose and effect of 10 μmol/L imatinib mesylate (left); and temperature-dependent efflux of Photofrin (right) in RIF-1 cells. Columns, uptake and efflux data are mean of at least three experiments, each comprising two replicates; bars, SE. **C**, survival of RIF-1 cells did not differ following treatment with 1.6 μmol/L HPPH-galactose with (△) or without (○) pretreatment with 10 μmol/L imatinib mesylate nor with 2 μg/mL Photofrin with (▲) or without (▼) pretreatment with 10 μmol/L imatinib mesylate. Points, phototoxicity data means of at least three experiments, each comprising at least six replicates; bars, SE.
The mechanism(s) by which imatinib mesylate and other TKIs inhibit transport of ABCG2 substrates are being studied. Houghton et al. (12) and Jordanides et al. (32) found that imatinib mesylate inhibits ABCG2 function, but is not an ABCG2 substrate (12). Conversely, Burger et al. (33) found imatinib mesylate to be an ABCG2 substrate that inhibits pump activity by competitive inhibition, whereas Ozvegy-Laczka et al. (11) showed that imatinib mesylate inhibits ABCG2 ATPase activity, possibly consistent with it not being a substrate. Finally, Nakanishi et al. (34) found that imatinib mesylate decreases expression of ABCG2 protein, but not mRNA, in bcr-abl+ cells through inhibition of the phosphoinositide-3-kinase–Akt pathway; this mechanism also might apply in malignant cells with other aberrant signaling mechanisms.

PDT acts directly on tumor cells and, in many cases, by shutting down the microvasculature feeding the tumor (2). Treatment selectivity is based on higher photosensitizer levels within the target than in surrounding normal tissues, and ABCG2 expression in tumors (35, 36) and on endothelial cells (35, 37, 38) can decrease both efficacy and selectivity. To our knowledge, the extent of ABCG2 expression on tumor and normal vasculature has not been compared or quantitated. Additional studies would be welcome because microvascular transport in tumors could play a substantial role in PDT outcomes. It is possible that ABCG2 inhibition would enhance vascular-targeted PDT.

In addition to baseline ABCG2 expression, hypoxia, which is very common in tumors, has been found to up-regulate expression of ABCG2 and to increase cell survival by decreasing intracellular accumulation of heme and other porphyrins (39). Therefore, hypoxia may inhibit PDT not only because the photodynamic process requires oxygen (2), but also through ABCG2-mediated decreases in intracellular photosensitizer levels. Importantly, ABCG2+ cancer stem cells (e.g., refs. 40–42) are expected to be relatively resistant to PDT with photosensitizers that are substrates for the ABCG2 transporter, and they may be responsible for late tumor recurrences (40, 41). Although ABCG2-mediated transport might be overcome by administering higher photosensitizer doses and/or treating at times when high circulating levels of the drug drive it into the tumor, this approach may cause unacceptable normal tissue damage. Thus, with photosensitizers that are ABCG2 substrates, inhibiting transport is likely to be a more successful approach to enhancing clinical PDT.

Administration of imatinib mesylate or other ABCG2 inhibitors in conjunction with PDT has significant potential for enhancing the efficacy of this therapeutic modality in the treatment of tumors that express ABCG2, including gastrointestinal, genitourinary, lung, and head and neck cancers (35, 36). Because transporter inhibition is only necessary during the interval between photosensitizer dosing and photolumination (0.5–48 h), toxicities should be minimal in relation to those associated with chronic administration of the TKI. In mice, HPPH has a circulating half-life of 12 h, and the tumor levels track the plasma levels (43), whereas the half-life of imatinib mesylate is ~4 to 5 h, and plasma level decrease to <1% in 12 h (23). To maintain tumor levels of imatinib mesylate before and during PDT, the last two imatinib mesylate doses were given every 6 h with the final dose timed to give peak plasma levels at the time when the mice received PDT. However, the in vivo studies were aimed as a proof of principle, and we did not attempt to optimize the imatinib dosing.

In contrast to the 4- to 5-h half-life in mice, the plasma half-life in humans is about 12 h (44), facilitating the use of the agent. With 400- and 600-mg doses of imatinib mesylate, Gambacorti-Passerini et al. (44) found mean peak plasma concentrations of 4 and 13 μmol/L, well within the effective range of imatinib mesylate in the in vitro experiments (Figs. 2 and 3). Pump inhibition may allow lower photosensitizer doses and may improve selectivity and decrease normal tissue damage. Imatinib mesylate may also increase the levels of endogenous porphyrins such as PpIX in ABCG2-expressing tumors, potentially enhancing diagnosis with devices that measure endogenous fluorescence, such as laser-induced fluorescence endoscopy (45). Finally, it is evident that ABCG2 transport is an important, previously unconsidered factor for the design of new photosensitizers. It is not surprising that multimeric Photofrin is not a substrate. With newer, monomeric agents, carbohydrate conjugation to a pyropheophorbide molecule blocks transport, as do the modifications in meso-tetra(3-hydroxyphenyl) porphyrin and meso-tetra(3-hydroxyphenyl) chlorin (8).

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The Tyrosine Kinase Inhibitor Imatinib Mesylate Enhances the Efficacy of Photodynamic Therapy by Inhibiting ABCG2

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