Dasatinib Cellular Uptake and Efflux in Chronic Myeloid Leukemia Cells: Therapeutic Implications

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Abstract

Purpose: The organic cation transporter OCT-1 mediates active transport of imatinib. We recently showed that low OCT-1 activity is a major contributor to suboptimal response in chronic myeloid leukemia (CML) patients treated with imatinib. The relevance of OCT-1 activity and efflux pumps in determining intracellular uptake and retention (IUR) of dasatinib was assessed.

Experimental Design: The effect of OCT inhibitors on [^{14}C]dasatinib and [^{14}C]imatinib IUR was compared using peripheral blood mononuclear cells from newly diagnosed CML patients. The role of efflux transporters was studied using ABCB1- and ABCG2-overexpressing cell lines and relevant inhibitors.

Results: Unlike imatinib, there was no significant difference in the dasatinib IUR at 37°C and 4°C ($P = 0.8$), and OCT-1 inhibitors including prazosin did not reduce dasatinib IUR significantly. In CML mononuclear cells, prazosin inhibitable IUR was significantly higher for imatinib than dasatinib (6.38 versus 1.48 ng/200,000 cells; $P = 0.002$; $n = 11$). Patients with high OCT-1 activity based on their imatinib uptake had $IC_{50}^{dasatinib}$ values equivalent to patients with low OCT-1 activity. Dasatinib IUR was significantly lower in ABCB1-overexpressing cell lines compared with parental cell lines ($P < 0.05$). PSC833 (ABCB1 inhibitor) significantly increased the dasatinib IUR ($P < 0.05$) and reduced $IC_{50}^{dasatinib}$ (from 100 to 8 nmol/L) in K562-DOX cell line. The ABCG2 inhibitor Ko143 significantly increased dasatinib IUR in ABCG2-overexpressing cell lines and reduced $IC_{50}^{dasatinib}$.

Conclusion: Unlike imatinib, dasatinib cellular uptake is not significantly affected by OCT-1 activity, so that expression and function of OCT-1 is unlikely to affect response to dasatinib. Dasatinib is a substrate of both efflux proteins, ABCB1 and ABCG2.

Imatinib mesylate (Glivec; Novartis Pharmaceutical) is the first line of treatment for newly diagnosed chronic-phase chronic myeloid leukemia (CML-CP) patients. However, up to 15% to 25% of newly diagnosed CML-CP patients have primary resistance (failure to achieve any level of cytogenetic response at 6 months, lack of major cytogenetic response at 12 months, and absence of complete cytogenetic response at 18 months) to imatinib at 400 mg/d (1–3). Currently, mechanisms of primary imatinib resistance are not well defined, but kinase domain mutations do not appear to be an important cause (4). We have shown previously that the intrinsic sensitivity of imatinib (*in vitro* concentration of imatinib required to inhibit phosphorylation of the adaptor protein Crkl by 50%; $IC_{50}^{imatinib}$) is related to the intracellular uptake and retention (IUR) of imatinib, but $IC_{50}^{nilotinib}$ is not correlated with nilotinib IUR (5). The organic cation transporter OCT-1 is an important mediator of active imatinib influx (5, 6). We have shown recently that the functional activity of the OCT-1 protein is a major determinant of molecular response to imatinib. Furthermore, the majority of CML patients who have suboptimal response to imatinib have low OCT-1 activity (7).

Dasatinib (Sprycel; formerly BMS-354825; Bristol-Myers Squibb) is a second-generation ABL and Src kinase inhibitor. In *in vitro* assay, it is approximately 300 times more potent than imatinib and active against most imatinib-resistant BCR-ABL mutants, except the T315I (8). In this current study, we have examined cellular influx and efflux of dasatinib and compared them with those already identified for imatinib. Understanding the dasatinib cellular transport mechanisms may facilitate the development of more effective treatment strategies based on transporter function.

Patients, Materials and Methods

Cell lines. KU812 and K562 (BCR-ABL$^+$) cell lines were obtained from the American Type Culture Collection. K562-DOX (ABCB1-overexpressing cell line) and VBL-100 (ABCB1-overexpressing variant of CCRF-CEM) were provided by Prof. Leonie Ashman (University of

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Received 12/13/07; revised 3/16/08; accepted 3/18/08.

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

Requests for reprints: Timothy P. Hughes, Division of Haematology, Institute of Medical and Veterinary Science, Frome Road, Adelaide, South Australia 5000, Australia. Phone: 61-8-82223330; Fax: 61-8-82223139; E-mail: Timothy.hughes@imvs.sa.gov.au.

doi:10.1158/1078-0432.CCR-07-5095

Newcastle). MEF3.8 and the ABCG2-overexpressing variant MEF-BCRP1 were kindly provided by Dr. John Allen (Centenary Institute).

All cell lines (except Mef3.8 and Mef-BCRP1) were cultured in RPMI 1640 supplemented by 10% FCS, L-glutamine (2 mmol/L; SAFC Bioscience), and penicillin/streptomycin (Sigma-Aldrich). Mef3.8 and Mef-BCRP1 cell lines were cultured in DMEM supplemented by 10% FCS, L-glutamine (2 mmol/L), and penicillin/streptomycin.

Patient samples. Blood was collected from CML-CP patients at presentation to the Royal Adelaide Hospital Haematology Clinic. All samples were collected with informed consent and importantly all samples were collected before commencement of imatinib therapy.

Mononuclear cells (MNC) were isolated from blood using Lymphoprep (Axis-Shield PoC) density gradient centrifugation. Experiments were done on cryopreserved cells following controlled rate freezing and storage in liquid nitrogen.

Kinase inhibitors. Dasatinib and [^{14}C]dasatinib were kindly provided by Bristol-Myers Squibb. [^{14}C]dasatinib was dissolved in ethanol at 1 mg/mL. Nonradiolabeled dasatinib was prepared at concentration of 10 mmol/L in DMSO (Merck K GaA).

Imatinib mesylate (Glivec) together with [^{14}C]imatinib were kindly provided by Novartis Pharmaceuticals. Stock solutions of imatinib were prepared at 10 and 1 mmol/L in distilled water, sterile filtered, and stored at 4°C.

Drugs and inhibitors. The OCT inhibitors prazosin, progesterone (OCT-1 and OCT-3 inhibitors), procainamide (OCT-1 and OCT-2 inhibitors), *N*-methyl-nicotinamide (OCT-2, inhibitor), and corticosterone (OCT-3 inhibitor; Sigma Aldrich) were used at 100, 10, 100, 1,000, and 10 μmol/L, respectively. The ABCB1 inhibitor PSC833 (provided by Novartis Pharmaceuticals) and the fumitremorgin C analogue Ko143 (ABCG2 inhibitor was supplied by Dr. John Allen) were used at 10 and 0.5 μmol/L, respectively.

Western blot analysis. Western blot analysis for phosphorylated Crkl was done as described previously (9). Briefly, 2×10^6 patient MNC or 2×10^5 cells of BCR-ABL+ cell lines (K562, K562-DOX, and K562-ABCG2) were cultured in increasing concentrations of dasatinib (0-1,000 nmol/L) for 2 h. Cells were then washed with cold PBS and lysed in 20 μL Laemmli's buffer by boiling for 12 min. Protein lysates (10 μL equivalents to 1×10^6 patient MNC and 1×10^5 cells of cell lines) were resolved on a SDS/10% (w/v) polyacrylamide gel and electrophoretically transferred to polyvinylidene difluoride membrane (GE Healthcare). Following blocking, the membrane was probed with anti-Crkl antibody C20 (Santa Cruz Biotechnology), detected with ECF substrate (GE Healthcare), and analyzed by Fluor Imager analysis (Molecular Dynamics). Signals were quantified using Image Quant software (Molecular Dynamics), and the ratio of phosphorylated Crkl to total Crkl was determined. IC_{50} values were determined as the concentration of drug required to reduce phosphorylation of the adaptor protein Crkl (phosphorylated Crkl) by 50%.

Radiolabeled drug uptake and retention assay. The IUR assay was done as described previously (5). In brief, 2×10^5 cells were incubated at 37°C (unless specified otherwise) with varying concentrations of [^{14}C]dasatinib or [^{14}C]imatinib (0-2 μmol/L). Isotopes were resuspended to 1 mg/mL and specific activities were 3.3 MBq/mg for [^{14}C]STI571 and 1.18 MBq/mg for [^{14}C]dasatinib. After incubation, the cellular and aqueous phases were separated, and incorporation was determined using a Top Count Microplate Beta Scintillation counter (Perkin-Elmer) following the addition of Microscint 20 (Perkin-Elmer) scintillation fluid.

IUR assays were done in triplicate and repeated if the assay results were not concordant. K562 cells were used as a control for reproducibility of the assay, and if the results fell outside of the mean ± 2 SD, then the assay was repeated.

Overexpression of ABCG2 in the K562 cell line. K562 cells were transfected with pcDNA3-BCRP (ref. 10; kindly provided by Prof. Douglas Ross) using electroporation, selected with geneticin (G418, 500 μg/mL; Life Technologies/Invitrogen), and subcloned by limiting dilution in 96-well flat-bottomed culture plates. Positive subclones were identified by PCR screening, and overexpression of BCRP (ABCG2 protein) was assessed by using flow cytometry (anti-hBCRP1/ABCG2; R&D Systems).

Statistical analysis. The Mann-Whitney rank-sum or *t* tests were used to assess the difference between groups. *P* values < 0.05 were considered significant.

Results

Dasatinib cellular uptake kinetics. To assess the effect of duration of culture on dasatinib IUR, K562 cells (2×10^5) were cultured with [^{14}C]dasatinib (2 μmol/L) over a dynamic range of 5 minutes to 2 hours at 37°C and 4°C. Maximum dasatinib cellular uptake was achieved within 5 minutes at both temperatures, and there was minimal variation between 5 minutes and 2 hours (Supplementary Fig. S1A and S1B). Similarly findings were shown at lower concentration of dasatinib (0.5 and 1 μmol/L) and in HL-60 cell line (data not shown). For all subsequent experiments, the IUR assay was done over a 2-hour period to enable direct comparison of the IUR of dasatinib with the $IC_{50}^{dasatinib}$.

Dasatinib IUR was assessed in K562 cells (37°C and 4°C) and KU812 cells (37°C) over a range of dasatinib concentration (100-2,000 nmol/L). Dasatinib IUR at 15 minutes and at 2 hours was linear over the concentrations used for the study,

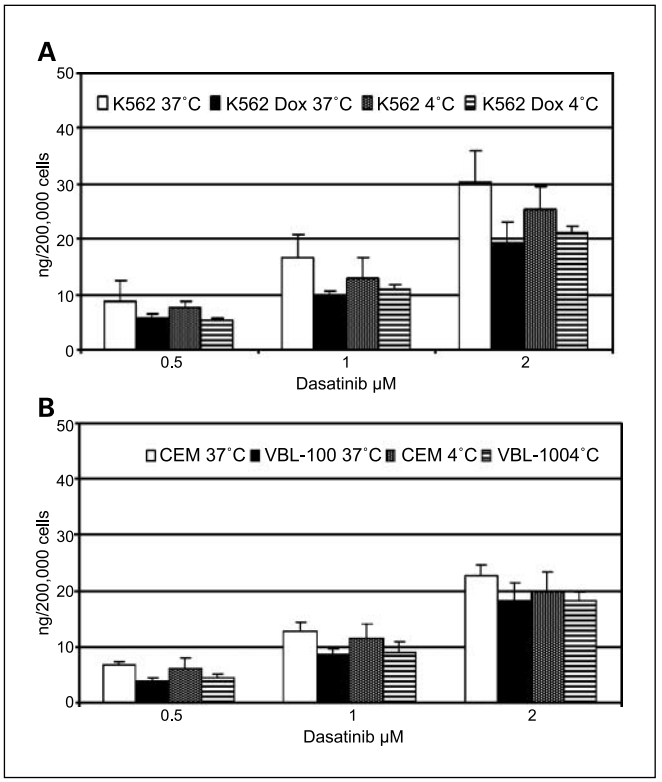

Fig. 1. Comparison of dasatinib IUR at 37°C and 4°C in ABCB1-overexpressing cell lines compared with their parental cell lines. *A,* at 37°C, dasatinib IUR (0.5, 1, and 2 μmol/L) is significantly lower ($P = 0.002$, 0.003, and 0.002, respectively) in K562-DOX compared with the parental cell line K562 ($n = 5$). However, at 4°C, there is no significant difference in IUR ($P = 0.41$ and 0.18 at 1 and 2 μmol/L dasatinib, respectively), except at 0.5 μmol/L ($P = 0.02$; $n = 4$). *B,* at 37°C, dasatinib IUR (0.5, 1, and 2 μmol/L) is significantly lower ($P < 0.001$, $P < 0.001$, and $P = 0.03$, respectively) in VBL-100 compared with the parental cell line CCRF-CEM ($n = 5$). However, at 4°C, there is no significant difference ($P = 0.23$, 0.15, and 0.38 at 0.5, 1, and 2 μmol/L dasatinib, respectively; $n = 3$) in dasatinib IUR between the two cell lines.

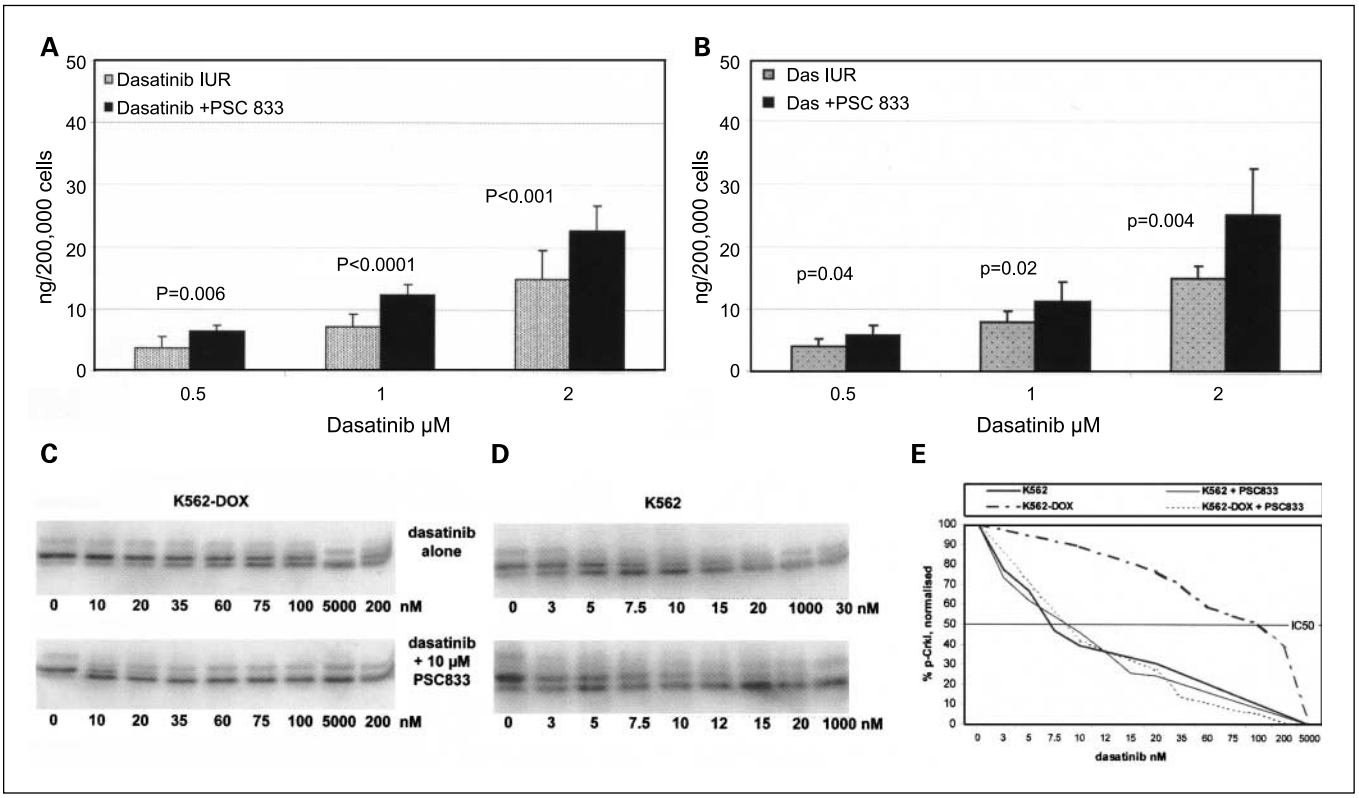

Fig. 2. Effect of PSC-833 (ABCB1 inhibitor) on dasatinib IUR in K562-DOX and VBL-100 cell lines. PSC-833 significantly increased dasatinib IUR in (*A*) K562-DOX cell line ($P = 0.006$, $P < 0.001$, and $P < 0.001$ at 0.5, 1, and 2 μmol/L dasatinib, $n = 5$) and (*B*) VBL-100 cell line ($P = 0.04$, 0.02, and 0.004 at 0.5, 1, and 2 μmol/L dasatinib; $n = 5$). *C*, Western blot analysis. *i* and *ii*, Western blots; *iii*, densitometry analysis of blots *i* and *ii*. $IC_{50}^{dasatinib}$ in K562-DOX cell line is higher than K562 (100 versus 7 nmol/L) and PSC833 reduced $IC_{50}^{dasatinib}$ in K562-DOX (100 to 8 nmol/L; *i* and *iii*) but not in K562 cell line (7 versus 8 nmol/L; *ii* and *iii*).

suggesting that dasatinib cellular uptake is predominantly by diffusion (Supplementary Fig. S1C and S1D).

Effect of temperature on dasatinib IUR. It has been shown previously that the uptake of imatinib is temperature dependent, suggesting that the transport process is primarily active (5, 6). To assess the temperature dependence of dasatinib uptake, assays were done at 4°C and 37°C in MNC from CML patients ($n = 10$) and in cell lines. In contrast to the findings with imatinib, there was no significant difference in the dasatinib IUR at 37°C and 4°C (Supplementary Table S1).

Role of ABCB1 in dasatinib cellular efflux. K562-DOX (BCR-ABL+) and VBL-100 (BCR-ABL-) ABCB1-overexpressing cell lines along with their respective parental cell lines (K562 and CCRF-CEM) were used for assessing the role of ABCB1 in dasatinib cellular efflux. High expression of ABCB1 in K562-Dox cell line was shown by flow cytometry (CD243-PE; Beckman Coulter; Supplementary Fig. S2A and S2B).

The dasatinib IUR at 37°C was significantly lower in K562-DOX ($P = 0.002$, 0.003, and 0.002 at 0.5, 1, and 2 μmol/L, respectively) and VBL-100 ($P < 0.001$, $P < 0.001$, and $P = 0.03$) than in the respective parental cell lines (Fig. 1A and B). However, at 4°C, there was no significant difference observed ($P > 0.05$; Fig. 1A and B). PSC833, an analogue of cyclosporine and a potent ABCB1 inhibitor (11), significantly increased dasatinib IUR in K562-DOX ($P = 0.006$, $P < 0.001$, and $P < 0.001$ at 0.5, 1, and 2 μmol/L, respectively) and VBL-100 cell lines ($P = 0.04$, 0.02, and 0.004; Fig. 2A and B) but not in the parental cell lines K562 and CEM ($P = 0.3$ and 0.6).

Effect of inhibition of ABCB1 on the $IC_{50}^{dasatinib}$. The $IC_{50}^{dasatinib}$ was assessed in K562 and K562-DOX with and without the addition of PSC833. In the absence of PSC833, $IC_{50}^{dasatinib}$ was a markedly higher in K562-DOX compared with K562 (100 versus 7 nmol/L). The addition of PSC833 reduced the $IC_{50}^{dasatinib}$ for K562-DOX to 8 nmol/L (Fig. 2C, *i* and *iii*) but did not change significantly in K562 cells (7 versus 8 nmol/L; Fig. 2C, *ii* and *iii*). These data indicate that dasatinib is transported by ABCB1 and that high expression levels of this protein can translate to significant changes in $IC_{50}^{dasatinib}$, which can be modulated by the use of ABCB1 inhibitors.

Role of ABCG2 in dasatinib cellular efflux. Interactions of ABCG2 with imatinib have been noted previously (12–14). To assess the effect of ABCG2 on the intracellular concentration of dasatinib, the IUR assay was done in Mef3.8 (mouse embryonic fibroblast), Mef-BCRP1 (ABCG2-overexpressing variant), and K562 and K562-ABCG2 cell lines with and without Ko143 (ABCG2 inhibitor). ABCG2 overexpression was confirmed by flow cytometry (Supplementary Fig. S2C and S2D).

The dasatinib IUR (2 μmol/L) was significantly lower in the Mef-BCRP1 cell line compared with parental cell line (21.96 versus 40.03 ng/200,000 cells; $P = 0.016$) and Ko143 significantly increased the dasatinib IUR in the Mef-BCRP1 cell line (21.96 versus 45.67 ng/200,000 cells; $P = 0.007$; Fig. 3A) but not in the parental cell line (40.03 versus 52.88 ng/200,000 cells; $P = 0.1$). Similarly, dasatinib IUR was lower, but not significantly so, in the K562-ABCG2 cell line compared

with the parental K562 (23.52 versus 26.26 ng/200,000 cells; $P = 0.1$), and Ko143 significantly increased the dasatinib IUR in the K562-ABCG2 cell line (23.52 versus 30.29 ng/200,000 cells; $P = 0.03$; Fig. 3B) but not in parental cell line (26.26 versus 25.23 ng/200,000 cells; $P = 0.78$).

In keeping with these data, the $IC_{50}^{dasatinib}$ was higher in the K562-ABCG2 cell line compared with K562 (25 versus 11 nmol/L). Ko143 reduced the $IC_{50}^{dasatinib}$ in K562-ABCG2 (25 versus 10.5 nmol/L; Fig. 3C, *i* and *iii*) but did not change the $IC_{50}^{dasatinib}$ in K562 (11 versus 11.5 nmol/L; Fig. 3C, *ii* and *iii*).

These data suggest that expression of high levels of ABCG2 protein reduce dasatinib IUR, resulting in an increase in the $IC_{50}^{dasatinib}$. We have shown that this effect can modulated using ABCG2 inhibitors.

Effect of OCT inhibitors on dasatinib cellular uptake. To assess the role of OCT on dasatinib cellular uptake, the dasatinib IUR was compared with the imatinib IUR in the MNC of CML-CP patients in the presence and absence of known OCT inhibitors. There was no significant difference between the uptake of imatinib (2 μmol/L) and dasatinib (2 μmol/L) in CML MNC (mean imatinib IUR, 20.4 ng/200,000 cells; mean dasatinib IUR, 16.20 ng/200,000 cells; $P = 0.1$; Fig. 4A) over the 2-hour period. The addition of prazosin, an inhibitor of both OCT-1 (IC_{50}, 1.8 μmol/L) and OCT-3 (IC_{50}, 13 μmol/L), did not reduce the dasatinib IUR significantly (at 2 μmol/L and 250 nmol/L; $P = 0.6$ and 0.23, respectively) nor interpatient variability (Fig. 4A-C; Table 1). However, in keeping with our previous findings, prazosin significantly reduced the IUR of imatinib to 13.33 ng/200,000 cells ($P = 0.005$) and reduced interpatient variability (Fig. 4A-C; Table 1). In addition, procainamide, progesterone, corticosterone, and *N*-methyl-nicotinamide did not reduce the dasatinib IUR in KU812 cells ($P = 0.4$, 0.9, 0.8, and 0.1, respectively) and CML MNC cells (Table 1).

[^{14}C]dasatinib has low specific activity. Therefore, at lower concentration, subtle effect of OCT inhibitors on dasatinib IUR could be missed; hence, dasatinib IUR was studied at 2 μmol/L. However, therapeutically achievable dasatinib concentration is 100 to 180 nmol/L (15, 16); hence, we have also compared the effect of OCT inhibitors at lower concentration. Prazosin, procainamide, and other OCT inhibitors did not reduce dasatinib IUR at 250 nmol/L in MNC of CML patients ($n = 5$; Table 1). Similarly, prazosin and procainamide did not reduce dasatinib IUR in KU812 cell line at 100 nmol/L ($P = 0.9$ and 0.76, respectively), 200 nmol/L ($P = 0.29$ and 0.50, respectively), and 2 μmol/L ($P = 0.21$ and 0.72, respectively; Fig. 4D and E).

We recently defined the OCT-1 activity (prazosin inhibitable imatinib IUR) as the difference between imatinib IUR in the presence and absence of prazosin and showed that patients with high OCT-1 activity (≥7.2 ng/200,000 cells) have superior molecular responses to imatinib compared with patients with low OCT-1 activity (<7.2 ng/200,000 cells; ref. 7). In this current analysis, we show that prazosin inhibitable IUR was significantly higher for imatinib compared with dasatinib (6.38 versus 1.48 ng/200,000 cells; $P = 0.002$; $n = 11$; Fig. 4B and C).

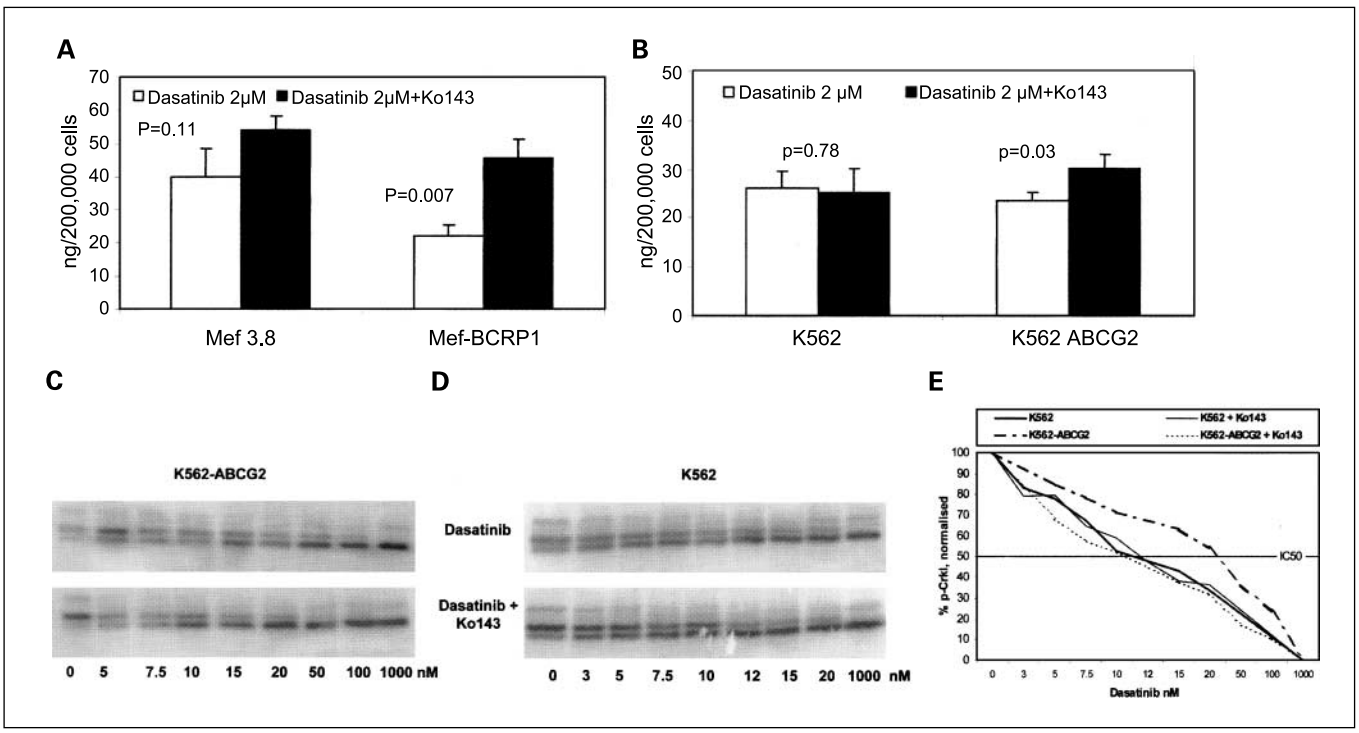

Fig. 3. Effect of ABCG2 protein overexpression on dasatinib IUR. *A,* dasatinib IUR is significantly less in Mef-BCRP1 (21.96 versus 40.03 ng/200,000 cells; $P = 0.016$) compared with the parental cell line (Mef3.8) and Ko143 (ABCG2 inhibitor) significantly increased dasatinib IUR in Mef-BCRP1 cells ($P = 0.007$) but not in the parental cell line ($n = 5$). *B,* dasatinib IUR is lower in K562-ABCG2 (23.52 versus 26.62 ng/200,000 cells; $P = 0.1$) cells compared with the parental cell line, and Ko143 significantly increased dasatinib IUR in K562-ABCG2 cell line ($P = 0.03$) but not in parental cell line ($n = 4$). *C,* Western blot analysis. *i* and *ii,* Western blots; *iii,* densitometry analysis of blots *i* and *ii*. $IC_{50}^{dasatinib}$ is markedly higher in K562-ABCG2 cell line compared with K562 (25 versus 11 nmol/L) and Ko143 reduced $IC_{50}^{dasatinib}$ in K562-ABCG2 cell line (25 versus 10.5 nmol/L; *i* and *iii*) but not in parental cell line (11 versus 11.5 nmol/L; *ii* and *iii*).

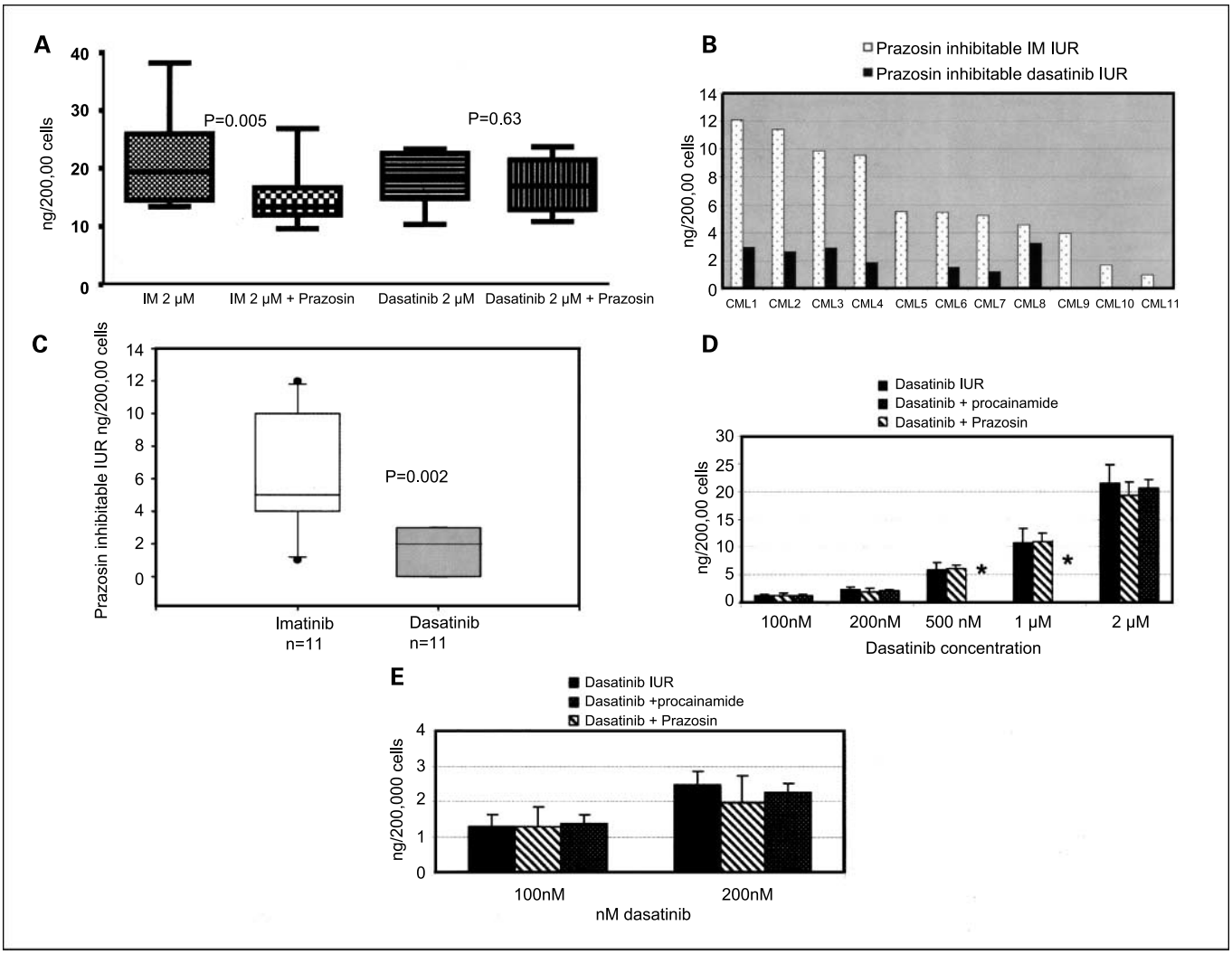

Fig. 4. Effect of OCT inhibitors on dasatinib and imatinib IUR. *A,* in MNC of newly diagnosed CML-CP patients (n = 11), prazosin (potent OCT-1 and weak OCT-3 inhibitor) significantly reduced imatinib IUR and reduced interpatient variability; however, it neither reduced dasatinib IUR nor variability in dasatinib IUR. *B,* there is significant interpatient variation in prazosin inhibitable (OCT-1 activity dependent) imatinib IUR. However, prazosin inhibitable dasatinib IUR is minimal with less interpatient variation. *C,* prazosin inhibitable (OCT-1 activity dependent) imatinib IUR is significantly higher than prazosin inhibitable dasatinib IUR (P = 0.002). *D* and *E,* KU812 cell line: prazosin did not reduce dasatinib IUR significantly at 100 nmol/L (1.2 ± 0.3 versus 1.3 ± 0.5 ng/200,000 cells; P = 0.9), 200 nmol/L (2.4 ± 0.41 versus 1.9 ± 0.8 ng/200,000 cells; P = 0.3), 500 nmol/L (5.82 ± 1.2 versus 5.94 ± 0.56 ng/200,000 cells; P = 0.8), 1 μmol/L (11.33 ± 2.7 versus 11.28 ± 1.6 ng/200,000 cells; P = 0.9), and 2 μmol/L (21.3 ± 3.3 versus 18.74 ± 2.61 ng/200,000 cells; P = 0.21). Similarly, procainamide (OCT-1 and OCT-2 inhibitors) did not reduce dasatinib IUR at 100 nmol/L (1.2 ± 0.3 versus 1.3 ± 0.25 ng/200,000 cells; P = 0.7), 200 nmol/L (2.4 ± 0.41 versus 2.2 ± 0.2 ng/200,000 cells; P = 0.5), and 2 μmol/L (21.3 ± 3.3 versus 20.5 ± 1.6 ng/200,000 cells; P = 0.4). *, effect of procainamide on dasatinib IUR was not assessed at 0.5 and 1 μmol/L.

We have shown previously that patients with low $IC_{50}^{imatinib}$ (below median) have significantly higher OCT-1 activity than patients with high $IC_{50}^{imatinib}$ (7). However, $IC_{50}^{dasatinib}$ in patients with high OCT-1 (n = 12) and low OCT-1 (n = 14) activity (2.0 versus 2.5 nmol/L; P = 0.3) was not significantly different in CML-CP patients. As shown in Fig. 5A, imatinib IUR was significantly higher in patients with high OCT-1 activity compared with patients with low OCT-1 activity; however, there was no significant difference in dasatinib IUR between the two groups (Fig. 5B).

Correlation between dasatinib IUR and $IC_{50}^{dasatinib}$. We have performed $IC_{50}^{dasatinib}$ and dasatinib IUR in MNC of newly diagnosed CML-CP patients (n = 18) before treatment. The median $IC_{50}^{dasatinib}$ was 2.20 nmol/L and median dasatinib IUR was 16.33 ng/200,000 cells at 2 μmol/L dasatinib. In contrast to previous observations with imatinib, there was no correlation between dasatinib IUR and $IC_{50}^{dasatinib}$, suggesting that in newly diagnosed CML-CP patients dasatinib IUR is a not a major contributor to the interpatient variability observed in the $IC_{50}^{dasatinib}$.

Discussion

Significant numbers of newly diagnosed CML-CP patients respond to imatinib; however, responses are variable, with up to 30% failing to achieve major molecular responses by 24 months (17). We have shown previously that $IC_{50}^{imatinib}$ in MNC of CML-CP patients at diagnosis predicts molecular response in newly diagnosed CML patients treated with imatinib (9). We (5) and Thomas et al. (6) have shown that imatinib uptake is an active process and that the major active protein involved in imatinib uptake is OCT-1. We also showed

Table 1. Effect of OCT inhibitors on [^{14}C]dasatinib (250 nmol/L and 2 μmol/L) and [^{14}C]imatinib (2 μmol/L) IUR in MNC of CML-CP patients

OCT inhibitors	Dasatinib IUR at 250 nmol/L (ng/200,000 cells)		Dasatinib IUR at 2 μmol/L (ng/200,000 cells)		Imatinib IUR at 2 μmol/L (ng/200,000 cells)	
	Mean ± SD (*n*)	*P*	Mean ± SD (*n*)	*P*	Mean ± SD (*n*)	*P*
No inhibitor	3.22 ± 0.56 (5)		16.20 ± 4.57 (11)		20.4 ± 7.0 (11)	
Prazosin	2.83 ± 0.33 (5)	0.23	15.34 ± 3.80 (11)	0.6	13.3 ± 2.7 (11)	0.005
Procainamide	2.61 ± 0.72 (5)	0.18	13.98 ± 4.11 (10)	0.2	14.73 ± 4.06 (10)	0.04
Progesterone	2.58 ± 0.42 (5)	0.07	15.04 ± 5.16 (10)	0.5	19.46 ± 7.33 (10)	0.3
Corticosterone	3.19 ± 0.65 (5)	0.94	17.28 ± 5.15 (10)	0.5	20.25 ± 8.66 (10)	0.3
Nicotinamide	—	—	13.20 ± 2.15* (8)	0.2	14.19 ± 2.2† (5)	0.1

NOTE: Effect of inhibitors were compared with no inhibitor control.
*Effect of nicotinamide on dasatinib IUR was assessed in eight patients and the mean value of dasatinib IUR with and without nicotinamide was not significantly different (15.51 versus 13.20 ng/200,000 cells).
† Effect of nicotinamide on imatinib IUR was assessed in five patients and the mean value of imatinib IUR with and without nicotinamide was not significantly different (17.24 versus 14.19 ng/200,000 cells).

that OCT-1-mediated cellular uptake is key determinant of imatinib IUR and interpatient variation in $IC_{50}^{imatinib}$ (5). In our recent article, we have shown that most patients who have suboptimal molecular response to standard dose of imatinib have low OCT-1 activity measured in MNC at diagnosis (*in vitro* assay calculated as [^{14}C]imatinib IUR with and without prazosin; ref. 7). In a randomized phase II trial, dasatinib was more effective than high-dose imatinib (800 mg/d) in patients resistant to conventional dose of imatinib (400-600 mg/d; ref. 18). Here, we have assessed the effect of OCT-1 activity on dasatinib cellular uptake.

In this study, we have shown that dasatinib cellular uptake is rapid, occurring within 5 minutes. We (5) and others (6) have shown previously that the intracellular concentration of imatinib achieved over a 2-hour period is significantly lower at 4°C than at 37°C. In contrast, this current study revealed no significant difference in the intracellular concentration of dasatinib achieved between 37°C and 4°C in MNC of CML patients and cell lines. Dasatinib IUR was linear over the range of drug concentrations tested at both temperatures. These findings suggest that, in contrast to imatinib, dasatinib cellular uptake is predominantly a passive process.

As reported previously (5, 6), the OCT-1 inhibitor prazosin significantly reduce the imatinib IUR and remove the observed interpatient variability. However, the addition of prazosin did not reduce dasatinib IUR (P = 0.63) nor reduce interpatient variability. Furthermore, the prazosin inhibitable IUR (OCT-1 activity) was significantly higher for imatinib compared with dasatinib (P = 0.002). In contrast to imatinib, dasatinib IUR was not significantly higher in patients with high OCT-1 activity (imatinib) compared with patients with low OCT-1 activity (imatinib). Unlike $IC_{50}^{imatinib}$ (7), $IC_{50}^{dasatinib}$ was not significantly different in patients with high and low OCT-1 activity. These data suggest that, in contrast to imatinib (5, 6), dasatinib cellular uptake is not OCT-1 dependent. Using a panel of OCT inhibitors (OCT-1, OCT-2, and OCT-3 inhibitors), we have also shown that dasatinib cellular uptake is not OCT-2 or OCT-3 dependent.

We have shown previously that OCT-1 mRNA level correlates with OCT-1 activity (r = 0.378; P = 0.002) and patients with high OCT-1 activity have superior molecular responses to imatinib compared with patients with low OCT-1 activity, but this difference could be partially overcome by the use of higher doses of imatinib (7). Unlike imatinib, dasatinib cellular uptake is not significantly affected by OCT-1 activity, so that expression and function of OCT-1 is unlikely affect response to dasatinib.

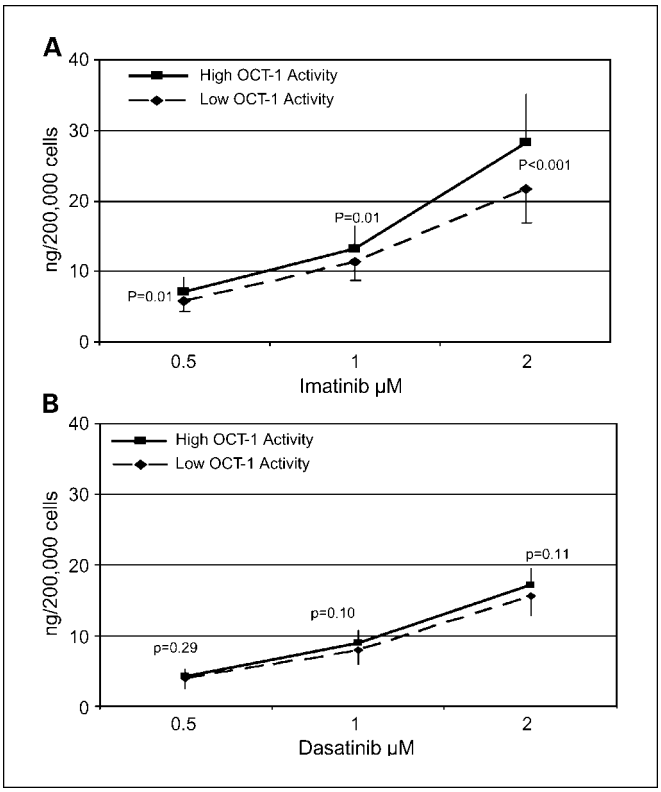

Fig. 5. Effect of OCT-1 activity on imatinib and dasatinib IUR in MNC of CML-CP patients. *A,* dasatinib IUR is not significantly different between patients with high OCT-1 activity (≥7.2 ng/200,000 cells; n = 12) and low OCT-1 activity (<7.2 ng/200,000 cells; n = 14). *B,* imatinib IUR (0.5, 1, and 2 μmol/L) is significantly higher in patients with high OCT-1 activity compared with patients with low OCT-1 activity.

We (5) have shown previously that $IC_{50}^{imatinib}$ correlates strongly with imatinib IUR; however, there is no correlation between nilotinib IUR and $IC_{50}^{nilotinib}$. We now show that there is also no correlation between dasatinib IUR and $IC_{50}^{dasatinib}$ in MNC of CML patients. This may suggest that, in newly diagnosed CML-CP patients, variations in dasatinib IUR may not be an important determinant of intrinsic sensitivity to dasatinib ($IC_{50}^{dasatinib}$). From a clinical perspective, it is important to recognize that the median $IC_{50}^{dasatinib}$ in newly diagnosed CML-CP patients is 2.20 ± 0.84 nmol/L, which is significantly below the dasatinib concentration achieved in patients (15). Hence, unlike imatinib, interpatient variability in $IC_{50}^{dasatinib}$ and IUR is unlikely to be clinically significant for newly diagnosed CML patients receiving dasatinib. These findings are based on MNC and not on leukemic progenitor or stem cells. Similar studies in more primitive stem cells are in progress at our centre.

Efflux proteins belong to the family of ATP-binding cassette transporter, comprising seven subfamilies (ABCA-ABCG), and encoded by 48 genes. ABCB1, ABCC1, and ABCG2 are expressed at high levels on hematopoietic primitive cells (19, 20). There are multiple studies showing that imatinib is a substrate for ABCB1 (6, 21–24). Here, we show that the dasatinib IUR is significantly lower in ABCB1-overexpressing cell lines compared with their parental cell lines. We have also shown that PSC833 significantly increases the dasatinib IUR in an ABCB1-overexpressing cell lines and reduces the $IC_{50}^{dasatinib}$ in K562-DOX cell line. This data suggest that dasatinib is a substrate for ABCB1.

There are several clinical implications of these findings. Dasatinib induces rapid hematologic and cytogenetic response in patients with Philadelphia chromosome positive (Ph^+) acute lymphoblastic leukemia, who are resistant or intolerant to imatinib (25). However, ATP-binding cassette transporters are highly expressed in brain and testes; hence, achieving adequate concentrations of dasatinib in testes and the central nervous system to control or prevent relapse at these extramedullary sites may be difficult. This could potentially be enhanced by drugs that inhibit ABCB1 activity, such as cyclosporine (26) or the proton pump inhibitors (27). ABCB1 is also expressed in gastrointestinal tract; similar considerations apply to gastrointestinal uptake. Polymorphisms or mutations that affect ABCB1 activity and overexpression of ABCB1 may all be relevant to the clinical efficacy of dasatinib. However, the importance of ABCB1 expression in the gastrointestinal tract remains uncertain. Recently, Kamath et al. (28) reported that there was no significant difference in the amount of dasatinib unabsorbed in gastrointestinal tract in P-glycoprotein (ABCB1) knockout mice and wild-type mice.

ABCG2 (BCRP1) is highly expressed on normal hematopoietic stem cells (29) and CML $CD34^+$ cells (14). The role of ABCG2 in imatinib transport is controversial. Houghton et al. (13) and Jordanides et al. (14) reported that imatinib is a potent inhibitor but not a substrate of ABCG2, whereas Burger et al. (12) reported that imatinib is a substrate of ABCG2. Here, we have shown that at 37°C dasatinib IUR is lower in ABCG2-overexpressing cell lines compared with the parental line, and Ko143 (ABCG2 inhibitor) significantly increased dasatinib IUR in Mef-BCRP1 and K562-ABCG2 cell lines. The $IC_{50}^{dasatinib}$ was higher in K562-ABCG2 compared with K562 and was reduced by Ko143, suggesting that dasatinib is also transported by ABCG2.

ABCG2 protein is susceptible to mutation at amino acid position R482, which affects functional characteristic of protein (30) and may lead to discrepancy between protein expression and activity. We are currently addressing if mutation in ABCG2 protein and protein expression level affect the dasatinib cellular efflux.

Knowledge of the cellular uptake/efflux pathways of the tyrosine kinase inhibitor and activity/expression of these pumps in individual patients may help us to individualize treatment. Currently, in newly diagnosed CML-CP patients, apart from clinical assessment (stage, duration of disease, prior treatment, and Sokal score), we also evaluate IC_{50} and IUR of three tyrosine kinase inhibitors (imatinib, dasatinib, and nilotinib), OCT-1 activity (imatinib), and OCT-1 mRNA level.

In summary, we have shown for the first time that dasatinib is a substrate of the ABCB1 and ABCG2 transporters in leukemic cells. We have also shown that dasatinib cellular influx is predominantly passive and not OCT-1, OCT-2, or OCT-3 dependent. We have suggested previously that OCT-1 activity should be considered in determining the optimal dose of imatinib for newly diagnosed patients. Our findings here provide evidence that consideration of OCT-1 activity is not likely to be relevant to dasatinib. However, these findings will need to be confirmed in a prospective study.

Disclosure of Potential Conflicts of Interest

D.L. White has received commercial research support from Novartis and Bristol-Myers Squibb and honoraria from Novartis. T.P. Hughes has received commercial research grants and honoraria from and consulted with Bristol-Myers Squibb and Novartis.

Acknowledgments

We thank Prof. Douglas D. Ross (University of Maryland School of Medicine, Greenebaum Cancer Center) for kindly providing the ABCG2 plasmids and Prof. Andrew Somogyi (Discipline of Pharmacology, University of Adelaide) for discussion and suggestions.

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Clin Cancer Res 2008;14:3881-3888.