Cancer Therapy: Preclinical

Cellular Uptake of Imatinib into Leukemic Cells Is Independent of Human Organic Cation Transporter 1 (OCT1)

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Abstract

Purpose: In addition to mutated BCR-ABL1 kinase, the organic cation transporter 1 (OCT1, encoded by SLC22A1) has been considered to contribute to imatinib resistance in patients with chronic myeloid leukemia (CML). As data are conflicting as to whether OCT1 transports imatinib and may serve as a clinical biomarker, we used a combination of different approaches including animal experiments to elucidate comprehensively the impact of OCT1 on cellular imatinib uptake.

Experimental Design: Transport of imatinib was studied using OCT1-expressing Xenopus oocytes, mammalian cell lines (HEK293, MDCK, V79) stably expressing OCT1, human leukemic cells, and Oct1-knockout mice. OCT1 mRNA and protein expression were analyzed in leukemic cells from patients with imatinib-naive CML as well as in cell lines.

Results: Transport and inhibition studies showed that overexpression of functional OCT1 protein in Xenopus oocytes or mammalian cell lines did not lead to an increased cellular accumulation of imatinib. The CML cell lines (K562, Meg-01, LAMA84) and leukemic cells from patients expressed neither OCT1 mRNA nor protein as demonstrated by immunoblotting and immunofluorescence microscopy, yet they showed a considerable imatinib uptake. Oct1 deficiency in mice had no influence on plasma and hepatic imatinib concentrations.

Conclusions: These data clearly demonstrate that cellular uptake of imatinib is independent of OCT1, and therefore OCT1 is apparently not a valid biomarker for imatinib resistance. Clin Cancer Res; 1–10. ©2013 AACR.

Introduction

Chronic myeloid leukemia (CML) is a malignancy of the hematopoietic system perpetuated by a population of leukemic stem cells with an acquired BCR-ABL1 fusion gene (1). The encoded chimeric p210BCR-ABL1 protein has a constitutively active tyrosine kinase domain, which activates signaling pathways essential for the pathogenesis of CML (2). Imatinib is a potent inhibitor of BCR-ABL1 in vitro and in vivo (3). Since 1998, imatinib is used in the clinic and is a highly effective therapy for Philadelphia chromosome-positive CML in patients in the chronic phase (CP; ref. 4). More than 95% of patients achieve complete hematologic response and more than 80% complete cytogenetic remission (5, 6). However, a proportion of patients fail or do not respond well to initial imatinib therapy, whereas other patients relapse due to acquired resistance (7, 8).

Imatinib resistance is caused by several mechanisms, the most frequent one being the clonal evolution of mutated BCR-ABL1 kinases that are more resistant toward inhibition by imatinib (7, 8). In addition, human drug transporters are increasingly recognized as important determinants for achieving sufficiently high intracellular drug concentrations (9, 10). While imatinib can be effluxed from cells by the ATP-dependent transporters ABCB1 (MDR1, P-glycoprotein) and ABCG2 (BCRP; ref. 11), it is less clear how imatinib, which is highly charged at physiological pH, is taken up into cells. Previous studies have indicated that intracellular imatinib uptake into leukemic cell lines, including CCRF-CEM (12) and K562 (13), is a temperature-dependent active transport mechanism. On the basis of the inhibition of cellular imatinib uptake by certain agents, such as verapamil and prazosin, human organic cation transporter 1 (OCT1, gene symbol

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Translational Relevance

Imatinib therapy is highly effective in treatment of chronic myeloid leukemia (CML). Organic cation transporter 1 (OCT1) has been suggested to contribute to imatinib resistance. However, in vitro transport data are conflicting, data of OCT1 protein expression on CD34+ leukemic cells are missing, and studies investigating the impact of OCT1 genetics, mRNA levels, and/or pharmacokinetics on imatinib response are inconsistent. To address the question whether OCT1 is a valid biomarker for imatinib response, we used an array of complementary methods that generated data signifying that OCT1 does not transport imatinib. We found that overexpression of functional OCT1 does not increase imatinib accumulation, leukemic cells lack OCT1 protein and either endogenously or after short hairpin RNA (shRNA) knockdown accumulate imatinib, and cellular imatinib uptake is not affected in OCT1-knockout mice. Although SLC22A1 mRNA is barely detectable in CD34+ leukemic cells, we identified more than 20 other SLC transporters with considerable higher mRNA expression as novel candidates for imatinib uptake.

Materials and Methods

A detailed description of the materials and methods is given in the Supplementary Data.

Study cohorts

CD34+ cells were isolated from peripheral blood samples from 4 newly diagnosed patients with CP-CML (Philadelphia chromosome–positive, Ph+) and from 4 Ph-negative (Ph−) non-CML donors by magnetic sorting as described (20). The investigation was approved by the ethical review board of the state Baden-Württemberg, Germany. Informed consent was obtained from patients. In addition, whole blood or bone marrow samples were acquired from 22 newly diagnosed patients with CP-CML Ph+ (Kiel cohort; 11 females, 11 males, median age, 64 years; range, 37–88 yrs) before imatinib therapy, having a mean BCR-ABL1/ABL1 ratio of 0.73 ± 0.33. The investigation followed the Declaration of Helsinki and was approved by the local ethics committee of the University of Kiel. Written informed consent was obtained from all patients.

Leukemic cell lines

The human CML cell lines K562 (21) and Meg-01 (22) and 9 different acute myeloid leukemia (AML) cell lines (23) were from American Type Culture Collection and the LAMA-84 (24) CML cell line was from German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany). Cell lines were cultivated in RPMI-1640 medium (Biochrom) with 10% fetal calf serum and glutamine.

OCT1-expressing cell lines

Five mammalian cell lines transfected with human OCT1/SLC22A1 cDNA were generated and cultured, expressing high levels of functional OCT1 using the well-established OCT probe substrates 1-methyl-4-phenylpyridinium (MPP) or tetraethyl ammonium (TEA): (i) human embryonic kidney 293 cells (HEK-OCT1; ref. 25), (ii) HEK293 OCT1-p.408V cells (see Supplementary Text and Fig. S4A), (iii) Chinese hamster ovary cells (CHO-OCT1; refs. 26, 27), (iv) Madin–Darby canine kidney cells (MDCK-OCT1; ref. 28), and (v) Chinese hamster lung fibroblasts (V79-OCT1; refs. 28, 29). HEK293 cells expressing mouse Oct1 (mOct1) or Oct2 (mOct2), generated and cultured as described, also had high Oct activity (30).

Transport studies

To assess imatinib uptake by OCT1-expressing oocytes, OCT1-expressing cell lines, and CML cell lines, we used 2 OCT1 probe substrates (MPP, TEA) and 3 OCT1 inhibitors [tetrabutyl ammonium (TBuA), prazosin, decynium22 (ref. 31)] to comprehensively analyze OCT1 function. Imatinib concentrations used for transport studies were not higher than 10 μmol/L that are similar to steady-state imatinib plasma levels in patients with CML (32), thereby reflecting the in vivo situation.

Pharmacokinetic experiments with mice

Female Oct1/2(+/−) and wild-type (WT) mice of the same genetic background (FVB) between 9 and 14 weeks of age were used (Taconic). Imatinib (50 mg/kg, 5 mg/mL in glucose 5%; ref. 33) and as control the OCT1 substrate metformin (5 mg/kg, 0.5 mg/mL in NaCl 0.9%; ref. 34) were injected into the tail vein of mice anesthetized with isoflurane. Mice were sacrificed at different time points by cervical dislocation. Livers were removed immediately, rapidly frozen, and stored at −80°C until analysis. Blood was collected by heart puncture and diluted 1:50 with 0.3 mol/L EDTA (pH 7.4). Plasma was collected by centrifugation...
Plasma and liver concentrations of imatinib and metformin were measured by liquid chromatography/tandem mass spectrometric (LC/MS-MS) analysis using a triple quadrupole mass spectrometer coupled to an HPLC system (Supplementary Data; ref. 35). Similar studies were conducted with oral imatinib (50 mg/kg) in WT mice and age-matched Oct1 (−/−) mice, also on an FVB background, kindly provided by Dr. Alfred Schinkel (Netherlands Cancer Institute, Amsterdam, the Netherlands; ref. 34). Experiments were approved by the local authorities of Baden-Württemberg (Regierungspräsidium Tübingen, Germany) and the Institutional Animal Care and Use Committee (St. Jude Children’s Research Hospital, Memphis, USA).

RNA isolation and quantification

See Supplementary Data for detailed description.

Flow cytometry and confocal laser scanning microscopy

CML cell lines, primary CD34+ from CP-CML Ph+ and Ph− non-CML donors, cultured as described (20), and transfected HEK cells were immunolabeled with a previously validated OCT1-specific polyclonal rabbit antiserum, able to distinguish graded levels of cellular OCT1 protein (27), and analyzed by flow cytometry (FACSCalibur, BD Biosciences) or observed by confocal laser scanning microscopy (TCS NT Confocal System, Leica Microsystems). See Supplementary Data for further details.

Immunoblot analysis

Isolation of membrane fractions, immunoblotting, and deglycosylation with peptide N-glycosidase F were conducted as described (27). OCT1 was detected with the previously described OCT1 antiserum (1:3,000 dilution; ref. 27).

Statistical analysis

Statistical significance was tested using the Student t test and Prism 5.0 (GraphPad Software). P < 0.05 was considered statistically significant.

Results

Imatinib inhibits transport of OCT1 probe substrate

We initially determined whether imatinib interacts with OCT1. Transport of the OCT1 probe substrate MPP into OCT1-expressing cells was potently inhibited by imatinib (IC50 = 0.095 μmol/L; Fig. 1A).

Figure 1. Assessment of imatinib as inhibitor and substrate of OCT1. A, uptake (1-second incubation) of OCT1 probe substrate [3H]MPP (0.1 μmol/L) into CHO-OCT1 cells in the presence of different imatinib concentrations. Data are normalized to uptake measured in the absence of imatinib and given as means ± SD from 3 experiments, each conducted in quadruplicate. B, uptake of OCT1 probe substrate [3H]MPP (12 nmol/L) or of [14C]imatinib (1 μmol/L) into OCT1-expressing or nonexpressing oocytes was measured after 30 minutes. Uptake was measured in the absence (−) or presence (+) of the OCT1 inhibitor tetrabutyl ammonium (TBuA, 1 mmol/L). Data are normalized means ± SE of 5 separate experiments, in each of which 7 to 10 oocytes were analyzed per experimental condition. C, uptake of OCT1 probe substrate [3H]TEA (100 μmol/L; means ± SD of 3 determinations) or of imatinib (2 μmol/L; mean ± SE of 3 determinations conducted in triplicate) into OCT1-expressing HEK cells or vector-transfected HEK cells (no OCT1 expression) was measured after 10 minutes. Uptake was measured in the absence (−) or presence (+) of the OCT1 inhibitor decynium 22 (5 μmol/L). D, time-dependent uptake of the main metabolite N-desmethyl imatinib (10 μmol/L) into OCT1-expressing HEK cells and vector-transfected control cells (Co). Data are mean ± SD of 3 determinations.
OCT1 does not transport imatinib in vitro and in mice

Inhibition of transport of a probe substrate by a specific compound does not provide any clues as to whether this compound is transported (36). To elucidate whether OCT1 transports imatinib, we used complementary approaches, that is, OCT1-expressing cells and mice with a genetic deletion of the Oct1 transporter. First, imatinib transport was assessed in Xenopus oocytes injected with OCT1/SLC22A1 cRNA (Fig. 1B). In this established model (26), the uptake of the OCT1 probe substrate MPP was increased 8-fold in the presence of OCT1 and completely blocked by the OCT1 inhibitor TBuA (31). In contrast, imatinib uptake was not different between nonexpressing and OCT1-expressing oocytes in the absence or presence of TBuA. Second, imatinib transport was evaluated using previously characterized OCT1-expressing cell lines, which show high and saturable uptake of probe substrates (25, 28). Imatinib transport was not different between the OCT1-expressing HEK, MDCK, and V79 cells and respective controls, whereas, as expected, uptake of the OCT1 probe substrate TEA was considerably higher into the OCT1-expressing cells versus controls (Fig. 1C; Supplementary Fig. S1). Moreover, imatinib uptake was not inhibited by the OCT1 inhibitor decynium22 but uptake of the probe substrate TEA was inhibited (Fig. 1C). The pharmacologically less active primary metabolite of imatinib, N-desethyl imatinib (37), significantly inhibited uptake of the OCT1 probe substrate TEA by 90.7% ± 1.0% (1.93 nmol/mg protein/10 minutes without vs. 0.18 nmol/mg protein/10 minutes with 50 μmol/L N-desethyl imatinib; n = 3); however, N-desethyl imatinib was not transported (Fig. 1D). Third, we used mice with a genetic deletion of the Oct1 transporter to assess imatinib hepatic uptake. Oct1(−/−) and Oct1/2 (−/−) knockout mice are the standard model to study the hepatic uptake of organic cations as shown for several compounds like TEA and metformin (34,38) and confirmed by our data (Fig. 2B). At 10 minutes after i.v. injection, imatinib plasma and hepatic concentrations were similar in knockout and WT mice (Fig. 2). Similarly after oral administration, Oct1 deficiency did not affect imatinib plasma and hepatic concentrations (Fig. 2). Our studies using cells expressing functionally active mOCT1 or mOCT2 (30) also confirm that imatinib is not transported by mouse Oct (Supplementary Fig. S2). Taken together, these data clearly indicate that neither imatinib nor its primary metabolite is transported by OCT1.

Cellular imatinib uptake is independent of OCT1 expression

The BCR-ABL1–positive human CML cell lines K562, Meg-01, and LAMA-84 are commonly used to study the effect of imatinib on cellular functions, such as proliferation or apoptosis, implying that imatinib is taken up into the cells. Imatinib uptake, particularly into the K562 cells, has been attributed to OCT1-dependent transport (13, 39). On the basis of our in vitro transporter studies we hypothesized that imatinib uptake into the CML cell lines is not due to OCT1. To elucidate this in more detail, we analyzed OCT1 expression on transcript, protein, and functional level as well as imatinib uptake in the CML cell lines compared with HEK-OCT1 and vector-transfected control HEK cells. Firstly, OCT1/SLC22A1 mRNA levels were quantified by TaqMan technology (Fig. 3A). High levels of OCT1/SLC22A1 mRNA were only detected in OCT1-expressing HEK cells, whereas OCT1/SLC22A1 transcripts were barely detectable in the different CML cell lines and in vector-transfected HEK cells, the levels being at least 50,000-fold lower than in HEK-OCT1 cells. We next investigated whether OCT1 is expressed on the protein level by quantifying cellular immunostaining using flow cytometry (Fig. 3B). Accordant with the mRNA data, fluorescence was highest in HEK-OCT1 cells and considerably lower in the CML cell lines. Subcellular OCT1 localization was analyzed by confocal laser scanning microscopy (Fig. 3C). Only the HEK-OCT1 cells were intensely stained in the plasma membrane and in intracellular vesicles. In contrast, staining intensity was very weak in the CML cell lines and only intracellular punctuate staining was observed. This staining in the CML cell lines may be due to cross-reactivity of the antisera with a protein other than OCT1 because in immunoblot analyses a band of about 70 kDa was detected in all 3 CML cell lines that remained unchanged after deglycosylation (Fig. 3D). As expected (25, 27), OCT1 was detected in membrane fractions from HEK-OCT1, but not from vector-transfected HEK cells, and deglycosylation reduced the apparent molecular mass of OCT1 to about 45 kDa. To
further confirm that the CML cell lines do not express a functional OCT1 protein, we measured uptake of the probe substrate TEA (Fig. 4A). Only the HEK-OCT1 cells showed significant TEA transport, as expected (25), whereas TEA transport was virtually absent in the CML cell lines (Fig. 4A) and vector-transfected HEK cells (Fig. 1C). Moreover, OCT1-dependent TEA uptake was significantly reduced in the presence of the inhibitors prazosin and decynium22 (Fig. 4A).

Next, we assessed imatinib transport after 10 minutes (initial uptake phase, Fig. 4B–D) and 120-minute incubation (Supplementary Fig. S3). Imatinib was taken up by the CML cell lines, the HEK-OCT1 cells and, remarkably, to the same extent also by the vector-transfected HEK cells (Figs. 4B and 1D, Supplementary Fig. S3A). Imatinib uptake was not altered in HEK293-p.408V cell line (Supplementary Fig. S4C). Imatinib uptake into the CML cell lines was significantly inhibited by prazosin (Fig. 4C), which had been used by White and colleagues to attribute imatinib uptake by K562 cells to OCT1 activity (13, 39). Notably, imatinib uptake into the HEK-OCT1 and vector-transfected HEK cells was not inhibited by prazosin after 10 minutes (Fig. 4C). After 120 minutes, cellular imatinib uptake by HEK cells was reduced by prazosin but was not OCT1-dependent as HEK cells and controls showed similar accumulation (Supplementary Fig. S3B). Moreover, imatinib uptake into the CML cell lines and the HEK-OCT1 cells was not inhibited by decynium22 after 10 minutes (Fig. 4D) using inhibitor concentrations sufficient to inhibit uptake of the OCT1 substrate TEA into HEK-OCT1 cells (Figs. 4A and 1C). Similar to the results with prazosin, a reduction of cellular imatinib accumulation by HEK cells by decynium22 after 120 minutes was independent of OCT1 expression (Supplementary Fig. S3C).

Taken together, these data indicate that imatinib uptake by the CML cell lines (K562, LAMA-84, Meg-01) and by HEK-OCT1 and vector-transfected HEK cells is independent of OCT1.

To test a potential role of OCT1 in cells with a leukemic background, we also used a panel of AML cells, some of which had increased OCT1/SLC22A1 mRNA levels compared with K562 cells (Fig. 5A). However, variability in mRNA was not predictive of imatinib uptake (Fig. 5B). A subsequent OCT1/SLC22A1 knockdown, using various shRNA constructs conducted in MV4-11 cells, the cell line with the highest intrinsic uptake of imatinib

Figure 3. Analysis of OCT1 expression in CML cell lines (Meg-01, LAMA-84, K562) and HEK cells. A, OCT1/SLC22A1 transcript levels were determined by real-time quantitative PCR in the CML cell lines as well as in OCT1-expressing HEK cells and vector-transfected control cells (Co) indicating extremely low OCT1 expression in CML cell lines. B, different cell lines were incubated with the OCT1 antiserum (27, 28) and then analyzed by flow cytometry confirming very low expression in CML cells. C, representative confocal laser scanning micrographs of the cells used for flow cytometric analysis. Green fluorescence, staining with the OCT1 antiserum; blue fluorescence, staining of nuclei. Bars, 10 µm. Data are mean ± SE of 3 independent preparations. D, membrane fractions from the different cell lines were analyzed for OCT1 protein content by immunoblot analysis using the OCT1 antiserum, which has been shown to distinguish graded levels of cellular OCT1 protein (27). Two micrograms protein and 20 µg protein were loaded from the HEK and the CML cell lines, respectively. Treatment of membrane fractions with PNGase F resulted in about 45 kDa band only in the HEK-OCT1 cells representing deglycosylated OCT1 protein.
(Fig. 5B), demonstrated that transporter expression levels could be reduced compared with a scrambled shRNA (Fig. 5C) but had no influence on the uptake of imatinib (Fig. 5D).

**OCT1 protein is not expressed in CD34⁺ CML cells**

To further support our hypothesis that OCT1 is not a determinant of cellular imatinib uptake, we investigated OCT1 expression in primary CD34⁺ CML cells as these are the target cells of imatinib therapy (40). Comparable to the CML cell lines (Fig. 3A), very low OCT1/SLC22A1 mRNA levels were determined in the CD34⁺ cells as well as in mononuclear cells from the CML Kiel study cohort (Fig. 6A). In comparison and previously described (27), OCT1/SLC22A1 mRNA levels in liver samples were high and exceeded those of the CML cells by about 500-fold. Again, comparable to the CML cell lines (Fig. 3C), no immunostaining was observed in the plasma membrane of CD34⁺ cells (Fig. 6B).

**SLC drug transporter expression in CD34⁺ CML cells**

Expression profiling of 55 SLC drug transporters, considered to be important for drug uptake by the PharmaADME Consortium (Supplementary Table S3), in the CD34⁺ cells by TaqMan assays indicated considerable expression of 21 transporters at least 20-fold higher compared with the expression of OCT1/SLC22A1 (Fig. 6C).

**Discussion**

The impact of the uptake transporter OCT1 in determining response to imatinib treatment is a topic of ongoing debate, with OCT1 genetics, OCT1/SLC22A1 mRNA levels, and cellular imatinib uptake each suggested to play a role in some studies but not in others (Supplementary Table S1). However, the essential question whether OCT1 transports imatinib remains open. Moreover, expression of OCT1 protein on CD34⁺ CML cells has not been studied. Therefore, we systematically investigated the role of OCT1 in imatinib transport by using different complementary experimental strategies including material of patients with CML.

The notion that OCT1 is the major uptake transporter for imatinib originates from in vitro studies showing that certain agents known to inhibit OCT1 also inhibited imatinib uptake into leukemic cells (12, 13). Because these kind of experiments do not prove validly that imatinib is actually transported by OCT1, we used various OCT1-expressing cell models well-established to study OCT1-mediated transport (25–28). Our finding that OCT1 expression in oocytes did not promote imatinib uptake, despite a significant uptake of an OCT1 probe substrate, confirms a previous report (15) and indicates that OCT1 is not involved in imatinib transport. This observation was corroborated by our studies with OCT1-transfected mammalian cells. As we recently demonstrated (25, 28) and also validated in the present study, these OCT1 transfectants show a substantial uptake of known OCT1 substrates such as TEA and express high levels of OCT1 protein. The uptake of the probe substrate TEA is almost completely abolished by the established OCT1 inhibitors prazosin or decynium22 (31). Yet, imatinib uptake into the OCT1-transfected HEK cells did not differ from that into vector-transfected control cells and could not be inhibited by prazosin or decynium22 after 10 minutes, which is the initial phase of OCT1-dependent uptake. These
results demonstrate that substantial overexpression of functional OCT1 protein does not result in imatinib transport. Our data seem at odds with 2 other studies showing a modest increase of cellular imatinib accumulation by about 1.5-fold (14) and about 1.2-fold (15) in OCT1-transfected HEK and KCL22 cells, respectively, compared with vector-transfected cells. While Wang and colleagues (14) discuss that their results support their previous work that OCT1 is an imatinib transporter (12), Hu and colleagues rather conclude that imatinib is only a weak OCT1 substrate (15). Of note, in both studies, OCT1 expression was only assessed on mRNA but not on protein level and imatinib uptake was not measured in the presence of OCT1 inhibitors. It is therefore ambiguous whether the slight increase of intracellular imatinib accumulation (14, 15) is actually due to OCT1 function or to differential expression of other transporters of relevance to imatinib (9).

In a very recent study, the KCL22-OCT1–transfected cells were re-evaluated and confirmed to express OCT1 protein (16). Moreover, OCT1-dependent imatinib transport was defined as the amantadine-inhibitable portion of cellular imatinib uptake. Although amantadine is a potent OCT1 inhibitor (31), it may also interact with other SLC uptake transporters (41) so that amantadine-inhibitable imatinib uptake may reflect activity of other transporters than OCT1 (42) in KCL22-OCT1 cells. To overcome such limitations, ideally, uptake studies should be conducted using a cell line with negligible background activity subsequently used for overexpression (43). However, this is neither the case for the KCL22 cells (14, 16) nor for the HEK, MDCK, or V79 cells, all showing high imatinib uptake already into control cells. Thus, different complementary approaches are required, as we did in our present work, to validly assess the role of a candidate transporter in substrate uptake.

In addition to the studies with OCT1-expressing cells, we also determined the effect of the absence of OCT1 in vivo using knockout mice. OCT1 is highly expressed in human and murine liver and a major determinant of hepatic accumulation of organic cations (27, 38, 44, 45). However, hepatic accumulation of imatinib, either given i.v. or orally, was independent from the presence of OCT1 further supporting that OCT1 does not mediate imatinib transport. These findings raise an important question: is the accumulation of imatinib into CML cells from patients and into CML cell lines indeed due to OCT1 function? We used a similar experimental approach to White and colleagues,
who defined the portion of imatinib accumulation after 120 minutes that is inhibited by prazosin as a measure for OCT1 activity (13, 39). We confirmed their findings that CML K562 cells accumulate imatinib and that this accumulation is indeed inhibited by prazosin when measured after 120 minutes. However, as discussed above, imatinib also accumulated into the vector-transfected control and OCT1-transfected HEK cells to a similar extent. Thus, the observation that prazosin and decynium22 reduced intracellular imatinib accumulation after 120 minutes in control and OCT1-transfected HEK cells indicates that other uptake proteins than OCT1 substantially contribute to imatinib cellular accumulation and the prazosin inhibitor assay does not reflect OCT1 activity. Therefore, additional studies using the prazosin inhibitor assay to elucidate an association with clinical outcome in patients with CML will not validly answer the question whether imatinib response depends on OCT1.

Another key finding of our study is that neither the CML cell lines nor the CD34⁺ CML cells express OCT1 protein (Figs. 3C and 6B), corroborating the fact that prazosin inhibition of imatinib uptake is not OCT1-dependent.

Integrating our results from all different complementary approaches, that is, (i) no enhancement of imatinib uptake despite considerable overexpression of OCT1 in different cell systems, (ii) imatinib uptake into CML cell lines
although OCT1 protein is not expressed, (iii) no effect of endogenous OCT1 knockdown on imatinib uptake in leukemic cells, and (iv) lack of involvement of OCT1 by OCT1-knockout mice studies, we conclude that OCT1 does not transport imatinib.

Beyond that and in line with previous reports (46), OCT1 mRNA expression was barely detectable in mononuclear cells or CD34+ cells from patients with CML and was in the range of expression levels observed in the CML cell lines (Fig. 6A). We therefore hypothesized that transporters other than OCT1 are involved in imatinib uptake into CD34+ cells as target cells of imatinib therapy (40). On the transcriptional level, we identified more than 20 SLC transporters as potential new candidates mediating cellular imatinib uptake because they are expressed at considerably higher levels than OCT1 in CD34+ cells. In-depth functional characterization of these transporter candidates warrants further investigation, which is beyond the scope of this study.

Finally, it may be argued that our results showing OCT1-independent cellular imatinib uptake are inconsistent with those studies reporting associations between OCT1 genetics or OCT1/SLC22A1 mRNA levels and clinical outcome of imatinib therapy (Supplementary Table S1). One explanation may be that BCR-ABL1 might reduce OCT1/SLC22A1 mRNA levels (47) so that the poor response to imatinib therapy of patients with low OCT1/SLC22A1 mRNA levels is due to presence of the BCR-ABL1 oncprotein rather than to OCT1 functioning as an imatinib uptake transporter. This is consistent with the finding that the OCT1/SLC22A1 mRNA level apparently does not independently predict clinical outcome, once the BCR-ABL1 mRNA level has been taken into account (17). Another explanation may be that OCT1/SLC22A1 expression is a composite surrogate for the expression of several transporters that are relevant to the intracellular uptake and retention of imatinib as discussed by Hu and colleagues (15). Similarly, OCT1 genetic variants may not be the causative variants for treatment failure to imatinib, but may be linked to variants in other genes relevant for imatinib action. We believe that our assumption is not in contrast to a very recent work indicating that specific OCT1 variants (p.M420del, p.M408V) may alter imatinib efficacy (16) as a linkage of these variants to other genes cannot be excluded and an underlying molecular mechanism for these candidate variants has not been provided so far.

In summary, we conclude from our current work that OCT1 does not transport imatinib and that imatinib accumulation into leukemic cells occurs independently from OCT1. The mechanisms responsible for imatinib uptake into leukemic cells are still elusive.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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