Original Study

Effect of *ABCG2*, *OCT1*, and *ABCB1* (*MDR1*) Gene Expression on Treatment-Free Remission in a EURO-SKI Subtrial

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Abstract

Whithin the EURO-SKI trial, 132 chronic phase CML patients discontinued imatinib treatment. RNA was isolated from peripheral blood in order to analyze the expression of MDR1, ABCG2 and OCT1. ABCG2 was predictive for treatment-free remission in Cox regression analysis. High transcript levels of the ABCG2 efflux transporter (>4.5‰) were associated with a twofold higher risk of relapse.

Introduction: Tyrosine kinase inhibitors (TKIs) can safely be discontinued in chronic myeloid leukemia (CML) patients with sustained deep molecular response. *ABCG2* (breast cancer resistance protein), *OCT1* (organic cation transporter 1), and *ABCB1* (multidrug resistance protein 1) gene products are known to play a crucial role in acquired pharmacogenetic TKI resistance. Their influence on treatment-free remission (TFR) has not yet been investigated. **Materials and Methods:** RNA was isolated on the last day of TKI intake from peripheral blood leukocytes of 132 chronic phase CML patients who discontinued TKI treatment within the European Stop Tyrosine Kinase Inhibitor Study trial. Plasmid standards were designed including subgenic inserts of OCT1, ABCG2, and ABCB1 together with *GUSB* as reference gene. For expression analyses, quantitative real-time polymerase chain reaction was used. Multiple Cox regression analysis was performed. In addition, gene expression cutoffs for patient risk stratification were investigated. **Results:** The TFR rate of 132 patients, 12 months after TKI discontinuation, was 54% (95% confidence interval [CI], 46%-62%). ABCG2 expression (‰) was retained as the only significant variable ($P = .02$; hazard ratio, 1.04; 95% CI, 1.01-1.07) in

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multiple Cox regression analysis. Only for the ABCG2 efflux transporter, a significant cutoff was found ($P = .04$). Patients with an ABCG2/GUSB transcript level >4.5‰ (n = 93) showed a 12-month TFR rate of 47% (95% CI, 37%-57%), whereas patients with low ABCG2 expression (≤4.5‰; n = 39) had a 12-month TFR rate of 72% (95% CI, 55%-82%). **Conclusion:** In this study, we investigated the effect of pharmacogenetics in the context of a CML treatment discontinuation trial. The transcript levels of the efflux transporter ABCG2 predicted TFR after TKI discontinuation.

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Introduction

Tyrosine kinase inhibitor (TKI) discontinuation in chronic myeloid leukemia (CML) constitutes an important pillar in future CML treatment.[1] Several studies showed that TKIs can safely be discontinued in CML patients with sustained deep molecular response (DMR).[2-4] So far, DMR and treatment duration were shown to be predictive for successful treatment-free remission (TFR) whereas age, risk scores, and sex were not known to have an influence.[3] In addition, molecular levels of DMR (molecular response 4-log reduction [MR^4], molecular response 4.5-log reduction) were not reported to be predictive for TFR.[2,5] Until now, TFR biomarker research focused mainly on immune surveillance and immune exhaustion. Natural killer cells and CD86+ cells proved to be predictive for relapse-free survival.[6-8] However, the effect of pharmacogenetic factors has not yet been analyzed in the recent context of discontinuation trials.

The breast cancer resistance protein (*ABCG2*), organic cation transporter 1 (*OCT1*), and multidrug resistance protein (*ABCB1*) gene products are known to play a crucial role in acquired pharmacokinetic drug resistance and DMR in nilotinib, imatinib, and dasatinib treatment of CML patients.[9,10] The human organic cation transporter 1 (OCT1, human organic cation transporter 1, or solute carrier family 22 member 1) is involved in the absorption, distribution, and elimination of endogenous compounds, toxins, and other xenobiotics that are positively charged at physiological pH.[11] OCT1 activity was shown to predict overall survival and TKI response in CML patients.[12,13] Some studies showed that its gene expression predicts TKI response[14 pp 1,15] but the results could not be reproduced in several other studies.[16,17] Thus, this correlation is still a matter of debate. ABCB1 (P-glycoprotein, multidrug resistance protein 1 [MDR1]) and ABCG2 (breast cancer resistance protein) are 2 members of the adenosine triphosphate-binding cassette family of membrane transporters, prominent for their role in multidrug resistance. Imatinib, dasatinib, and nilotinib were shown to be substrates as well as competitive inhibitors in different extents.[18-23] ABCB1 expression has been reported to predict TKI response.[23-25 pp 1] A recent meta-analysis showed that ABCG2 polymorphisms were potential CML response predictors.[26] In this study, we aimed to investigate whether aberrant gene expression of these influx and efflux channels predispose for CML relapse after TKI discontinuation.

Methods

Samples and Study Design

This analysis included 132 CML patients from the European Stop Tyrosine Kinase Inhibitor Study (EURO-SKI) trial, a prospective multicenter TKI discontinuation trial (NCT01596114).[3] In accordance with the Declaration of Helsinki, written informed consent was obtained from all patients. According to the EURO-SKI inclusion criteria, patients with MR^4 duration of at least 1 year and TKI treatment of at least 3 years were enrolled. TFR is on the basis of survival without loss of major molecular response and is expressed as molecular relapse-free survival rate in the Kaplan−Meier analysis.

Gene expression analyses were performed on leukocyte RNA isolated from peripheral blood samples of patients screened in our center and 10 healthy individuals (male n = 5; female n = 5) served as controls. Blood samples were collected at the last day of TKI intake (baseline).

Molecular Analysis

The gene expression levels of breakpoint cluster region (BCR)-Abelson murine leukemia viral oncogene homolog (ABL) and ABL1 were determined using quantitative real-time polymerase chain reaction (qRT-PCR) from total leukocyte RNA of peripheral blood samples. BCR-ABL transcript levels were measured using standard plasmid dilutions, as described previously.[27] Ratios derived from BCR-ABL/ABL1 were converted to the International Scale.

The qRT-PCR reactions for quantifying *ABCB1*, *ABCG2*, *OCT1*, and *GUSB* (beta-glucuronidase 1, reference gene) consisted of (per 20-µL reaction mix): 4 µL LightCycler 480 Probes Master Mix (Roche Diagnostics, Indianapolis, IN), 0.5 µL reverse primer, 0.5 µL forward primer, 0.25 µL anchor probe, 0.25 µL sensor probe (TIB Molbiol, Berlin, Germany), and 2 µL cDNA or plasmid dilution (see Supplemental Table 1 in the online version: primer sequences). The cycle settings were the following: 10 minutes denaturation at 95°C, 40 cycles of 10-second denaturation at 95°C, 20 seconds annealing at 60°C, and 30 seconds elongation at 72°C.

Cloning of Plasmid Standards

The plasmid was cloned by transforming and cultivating *Escherichia coli* one shot Top10F' (Invitrogen, Carlsbad, CA). The preparation of the plasmid DNA was done using HiSpeed Plasmid Maxi Kit (Qiagen, Hilden, Germany). Plasmid linearization was performed using the Xbal restriction enzyme. For absolute quantification of the ABCB1, ABCG2, and OCT1 transcript levels, the 5-log series of plasmid dilutions were amplified using qRT-PCR. For this reason, the pEX-A2 plasmid design (ID: 3321214, Eurofins, Luxembourg) contained an 861-base pair insert, on the basis of