# Pharmacogenetics and cost-effectiveness of systemic treatment in soft tissue sarcoma

Verboom, M.C.

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# Influence of *CYP2C8* polymorphisms on imatinib steady-state trough level in chronic myeloid leukemia and gastro-intestinal stromal tumor patients

Michiel Verboom, Loes Visser, Sander Kouwen, Jesse Swen, Jeroen Diepstraten, Ward Posthuma, Hans Gelderblom, Daniëlle van Lammeren, Erik Wilms

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## Abstract

Imatinib trough levels have been associated with its clinical effects. During chronic use of imatinib CYP2C8 becomes an important metabolizing enzyme due to cytochrome P450 3A4 (CYP3A4) auto-inhibition. Single Nucleotide Polymorphisms (SNPs) in CYP2C8 may affect imatinib trough levels. This study investigates the effect of common CYP2C8 polymorphisms (*1B (rs7909236), *1C (rs17110453), *3 (rs11572080 and rs10509681), and *4 (rs1058930)) on steady state trough levels of imatinib during chronic imatinib use in 43 patients with chronic myeloid leukemia (CML) or gastrointestinal stromal tumors (GIST). Standardized imatinib trough levels did not show a significant difference between wild type and variant groups for any tested SNPs, but an association with age was found with older patients having higher trough levels. This suggests that common CYP2C8 SNPs have no effect on the pharmacokinetics of imatinib.

## Introduction

The tyrosine kinase inhibitor imatinib has dramatically improved the treatment of patients with Chronic Myeloid Leukemia (CML) or with Gastrointestinal Stromal Tumors (GIST).[1,2] However, clinical response varies significantly between patients. This short communication aims to address the influence of several common SNPs in *CYP2C8* as potential pharmacogenetic biomarkers of this inter patient variability.

Clinical response of imatinib has been shown to be influenced by both patient and tumor factors. Associations with plasma trough level have also been reported and large inter-individual differences in trough levels have been described.[3,4] Imatinib is primarily metabolized to its active metabolite, N-demethylated piperizine derivative, by cytochrome P450 (CYP) 3A4 and CYP3A5. Chronic use of imatinib results in reduced CYP3A4 and CYP3A5 activity through auto-inhibition. When this occurs, CYP2C8 becomes an important metabolizer of imatinib.[5] The *CYP2C8* gene has several so called functional single nucleotide polymorphisms (SNPs), one of which has shown in *in vitro* tests to have a gain-of-function effect on imatinib, but which results in reduced enzyme activity in *in vitro* tests with other drugs.[6,7] The influence of CYP2C8 polymorphisms on imatinib pharmacokinetics has not yet been studied *in* vivo before. Therefore, this study aims to investigate the relationship between CYP2C8 polymorphisms and pharmacokinetics of imatinib, in patients who have used this drug for at least 30 days, which is long enough for CYP2C8 to have become the primary metabolizer.



## Material and methods

In a prospective study patients from three Dutch hospitals were included. Inclusion criteria were a confirmed diagnosis of CML or GIST, continuous imatinib usage of at least 30 days and written informed consent. In case of suspected non-adherence to imatinib (at the physician's discretion) or if the time of imatinib intake was not precisely known or was within 3 hours of sampling, the patient was excluded. During routine blood sampling for standard care, additional blood samples were taken for imatinib plasma concentration determination and for CYP2C8 genotyping. The study protocol was approved by the local science or ethics commission at each study site.

The plasma concentration of imatinib was determined using a validated LC-MS/MS assay in a single laboratory. These concentrations were recalculated into trough levels using the following formulas:

$$Conc_{24h} = Conc_{measured}\ x\ 0{,}5\ \left(\frac{24-interval}{t^{1/2}}\right)\ Conc_{12h} = Conc_{measured}\ x\ 0{,}5\ \left(\frac{12-interval}{t^{1/2}}\right).$$[8]

The calculated trough levels were standardized to a once-daily dose of 400 mg imatinib by dividing the calculated concentration with the daily dose and then multiplying it by 400. SNPs in *CYP2C8* were selected with a minimum minor allele frequency (MAF) of 0.02 in Caucasians. For the genotyping of SNPs *CYP2C8 *1C - rs17110453, *3 - rs10509681, *3 - rs11572080, *4 - rs1058930*, commercially available Taqman assays were used (Applied biosystems/Thermo Fisher Scientific, Waltham, MA, USA). For *CYP2C8 *1B - rs7909236* genotyping custom TaqMan assay was used (forward: GTATTGGATTGGAGCCCAGGTATTT, reverse: TGTTTCTCCATCATCACAGCACAT; Probes, VIC labeled: AAGTCCCTGGTTGTTCCA, FAM labeled: TCCCTGGTTTTTCCA). The genotyping results were tested for deviation of the Hardy Weinberg equilibrium, to exclude non-normally distributed genetic variation. As standardized trough levels were non-normally distributed, non-parametric tests were used for univariate analysis. For testing the association of standardized trough levels and patient characteristics the Spearman's rho and the Mann–Whitney U test were used, and for genotypes the latter test as well. When testing for differences between wild type and variant alleles, patients with heterozygous and homozygous variant genotypes were grouped together due to the paucity of the latter group in the patient cohort. A multivariate linear regression analysis was performed using the significantly associated patient characteristics and each SNP. A p value of less than 0.05 was considered statistically significant. SPSS version 22 was used (IBM Corp., Armonk, NY, USA).

## Results

From June 2014 to February 2015, 47 consecutive patients were included, of which 4 patients were excluded due incomplete sampling or samples taken within 3 hours in imatinib intake. Table 1a shows the characteristics of 43 included patients and Table 1b the genotyping results. The only significant difference between patients with CML and GIST was sex (p=0.009), all genotype results did not differ significantly (data not shown). All SNPs were in HWE (data not shown). Table 2 shows the association of standardized trough level with patient characteristics and with SNP genotypes. None of the tested SNPs were significantly associated. Only age showed an association, with older patients having higher trough levels (r=0.359, p=0.018). Figure 1 shows the distribution of the standardized trough level per genotype for each SNP. Table 3 shows the results of multivariate regression analyses of the standardized trough level with age and each genotype. This confirms the effect of age regardless of CYP2C8 genotype.

**Table 1a:** patient characteristics
**Table 1b:** genotyping results

| | | All patients | CML | GIST |
|---|---|---|---|---|
| **Patients N** | | | | |
| Age | median (range), in years | 63 (36 - 83) | 62 (36 - 83) | 63 (47 - 76) |
| Weight | median (range), in kg | 78 (51 - 108) | 75 (51 - 100) | 84 (62 - 108) |
| Standardized trough level | median (range), in µgl/L | 1029 (444 - 2790) | 1086 (444 - 2430) | 1028 (603 – 2790) |
| Sex | male (%) | 24 (56) | 8 (36) | 16 (76) |
| | female (%) | 19 (44) | 14 (64) | 5 (24) |
| Race | Caucasian (%) | 37 (86) | 19 (86) | 18 (86) |
| | other (%) | 6 (14) | 3 (14) | 3 (14) |
| ***CYP2C8* SNPs** | | | | |
| *1B (rs7909236, G/T) | wild-type (%) | 26 (60) | 13 (59) | 13 (62) |
| | variant (%) | 17 (40) | 9 (41) | 8 (38) |
| *1C (rs17110453, A/C) | wild-type (%) | 33 (77) | 16 (73) | 17 (81) |
| | variant (%) | 10† (23) | 6 (27) | 4† (19) |
| *3 (rs11572080, C/T) | wild-type (%) | 31 (72) | 14 (64) | 17 (81) |
| | variant (%) | 12 (28) | 8 (36) | 4 (19) |
| *3 (rs10509681, T/C) | wild-type (%) | 31 (72) | 14 (64) | 17 (81) |
| | variant (%) | 12† (28) | 8† (36) | 4 (19) |
| *4 (rs1058930, G/C) | wild-type (%) | 39 (91) | 20 (91) | 19 (90) |
| | variant (%) | 4 ( 9) | 2 ( 9) | 2 (10) |

Table 1a: patient characteristics, CML = chronic myeloid leukemia, GIST = gastrointestinal stromal tumor
Table 1b: genotyping results, all patients were heterozygote for the variant allele, except for †, where one patient was homozygous for the variant allele

**Table 2:** associations with standardized imatinib trough level

| | r | p value |
|---|---|---|
| Age | 0,359 | **0.018** |
| Weight | -0,221 | 0.155 |
| Sex | -0,224 | 0.142 |
| Race | -0,059 | 0.700 |
| Disease | 0,022 | 0.884 |
| | | |
| *1B (rs7909236) | -0,152 | 0.320 |
| *1C (rs17110453) | -0,162 | 0.287 |
| *3 (rs11572080) | -0,116 | 0.448 |
| *3 (rs10509681) | -0,116 | 0.448 |
| *4 (rs1058930) | -0,115 | 0.452 |

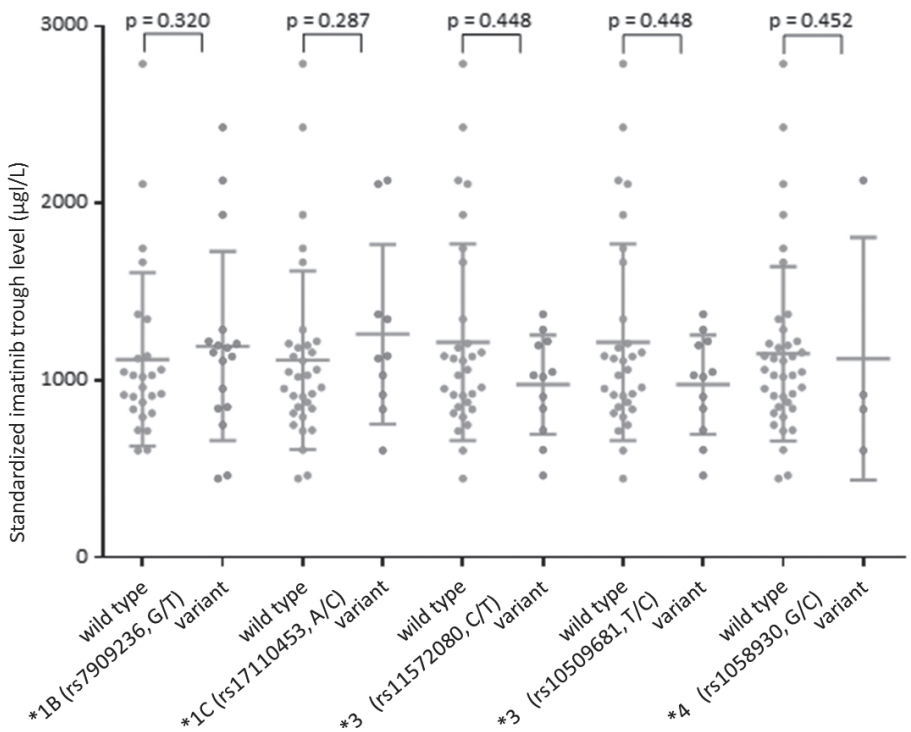
Univariate associations with imatinib trough level standardized for a daily dose of 400mg, r notes effect size, only age is significantly associated

**Table 3:** regression analysis of standardized imatinib trough level with age and SNP

| | Beta | p value |
|---|---|---|
| *Age* | 0.434 | **0.004** |
| **1B (rs7909236)* | 0.114 | 0.428 |
| | | |
| *Age* | 0.418 | **0.005** |
| **1C (rs17110453)* | 0.105 | 0.465 |
| | | |
| *Age* | 0.394 | **0.010** |
| **3 (rs11572080)* | -0.117 | 0.428 |
| | | |
| *Age* | 0.394 | **0.010** |
| **3 (rs10509681)* | -0.117 | 0.428 |
| | | |
| *Age* | 0.425 | **0.005** |
| **4 (rs1058930)* | 0.023 | 0.876 |

Multivariate regression analysis of imatinib trough level standardized for a daily dose of 400mg with age and SNP, Beta denotes effect size

**Figure 1:** Distribution of standardized imatinib trough level per SNP




Distribution of standardized imatinib trough level per SNP for wild type and variant allele groups, above each SNP the p-value of the Mann-Whitney U test is shown, the horizontal line denotes the mean and the vertical line the standard deviation.

## Discussion and conclusion

This study shows no statistically significant difference in standardized imatinib trough level between wild-type CYP2C8 and variant allele groups in patients with CML or GIST, who have used imatinib for at least 30 days. While *in vitro* studies of variant allele groups *CYP2C8 *2, *3, *4* have shown a reduced activity relative to wild type CYP2C8, an effect on metabolic clearance has not been seen *in vivo*.[7]

Based on a previous study, it is assumed that after auto-inhibition of the primary CYP3A4 metabolic pathway imatinib is metabolized by CYP2C8.[5] Possibly, polymorphisms of other CYP enzymes and transporters outweigh the effects of CYP2C8 SNPs on imatinib trough level.[9,10] Furthermore, by the time a slow-acting CYP2C8 becomes an imatinib metabolizer other CYP enzymes may also come into play, such as CYP2C9, CYP2C19, CYP2D6, or CYP1A2, and diminish CYP2C8 SNPs effects.[9] The association of age and increased imatinib standardized trough level was in line with a previously reported

weak correlation, but these authors considered this effect not likely to be to be clinically relevant due to large inter patient variability.[11]

The calculation of the imatinib trough level is one of this study's limitations. The formula uses a fixed elimination constant which makes it highly dependent on the interval between the intake and sampling of imatinib. Incorrect registration may thus influence the calculated trough level. Future studies may yield more precise results if the investigated drug is taken during clinical supervision. Furthermore, this study has a relative small number of patients, so only strong associations are likely to show in the present data.

In conclusion, this study suggests common *CYP2C8 SNPs *1B, *1C, *3 (rs10509681 and rs11572080)* and **4* have no effect on the pharmacokinetics of steady state imatinib in patients with GIST or CML, but age does show an association.

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