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CORRESPONDENCE

UGT1A1 genotype does not affect tyrosine kinase inhibitors efficacy and safety in chronic myeloid leukemia

To the Editor:

Gilbert's syndrome (GS) is a condition characterized by intermittent unconjugated hyperbilirubinemia without structural liver damage, affecting about 10% of the caucasian population. It is an autosomal recessive disorder, mainly associated with variations in uridine-50-diphosphate (UDP)-glucuronosyltransferase gene (UGT1A1). In Caucasians, the most common variation is the TATA box polymorphism, in which an insertion of an additional TA-repeat into the promoter region of the gene results in a A(TA) 7TAA sequence, that differs from the more prevalent A(TA) 6TAA. Gilbert's syndrome is a benign condition which does not lead to liver inflammation, cellular destruction, fibrosis, or cirrhosis but is merely clinically characterized by intermittent episodes of uncomplicated unconjugated hyperbilirubinemia.[1]

The effect of GS on liver complication and outcome of hematological malignancies has been scarcely investigated, with only a previous study in a series of children diagnosed with acute lymphoblastic leukemia,[2] a series of adult patients with Hodgkin lymphoma[3] and some anecdotal case reports in acute leukemia patients.[4]

Tyrosine-kinase inhibitors (TKIs) are currently used for the treatment of chronic myeloid leukemia (CML). Nilotinib inhibits bilirubin metabolism via UGT1A1, thereby increasing bilirubin levels; not surprisingly, GS has been associated with nilotinib-induced hyperbilirubinemia in patients affected by CML.[5-7] Similar increases in bilirubin were also reported in patients with GS treated with imatinib or dasatinib.[8]

The aim of the present study is to assess if in CML-chronic phase (CML-CP) patients treated with either first- or second-generation TKIs, GS has an impact on clinical outcomes, assessed by cytogenetic/molecular response rates and progression-free survival (PFS), as well as on hematological or extra-hematological toxicities.

We retrospectively collected data on CML-CP patients consecutively treated with TKIs at our institution between February 2002 and November 2018. All the following data were collected at baseline before TKI initiation: sociodemographic and hematological variables, disease risk scores (Sokal, Eutos, Hasford, and ELTS), and TKI starting dose. Monitoring and responses evaluation followed the current European LeukemiaNet recommendations.[9] Progression-free survival was calculated from the start of first-line TKI to any of the following events: primary or secondary resistance, and/or discontinuation due to intolerance. Hematological and extra-hematological toxicities were graded according to the Common Toxicity Criteria - Adverse Events (CTCAE) version 5.0.

Gilbert's syndrome genotype was investigated by polymerase chain reaction (PCR) amplification of a region encompassing the TATA-box of the UGT1A1 gene (forward primer: 5′-GTC ACG TGA CAC AGT CAA AC-3′: reverse primer: 5′-TTT GCT CCT GCC AGA GGT-3′; annealing temperature: 62°C. RefSeq UGT1A1 NG_033238.1). DNA fragment length analysis was performed by 12% polyacrylamide gel electrophoresis (PAGE): a 98 bp fragment indicating the presence of the (TA)6TAA wild-type allele, while a 100 bp fragment accounts for the (TA)7TAA allele (c.-41_-40dupTA). The more rare (TA)8TAA allele (c.-43_-40dupTATA) could be as well evidenced by this technique as a 102 bp PCR product, but it was not detected in any of the patient studied.

Clinical and sociodemographic characteristics were described using absolute and relative frequencies. We evaluated time to resistance (truncated at 10 years) according to GS genotype by calculating the Kaplan-Meier function and by fitting univariate and multivariable Cox models adjusted for gender, age, and line of therapy. *P* values were obtained from post-estimation global Wald tests. Statistical analysis was performed with Stata 15 (StataCorp. 2017).

One hundred and five CML-CP patients consecutively treated with either first- or second-generation TKIs were evaluated. Clinical-demographic data at diagnosis are showed in Table S1. In particular, concomitant drugs were reported in 49 patients (46.7%), with seven patients taking more than five medications. Gilbert's syndrome genotypes were distributed as follows: 17 (16.2%) patients were 7/7, 44 (41.9%) 6/7 and the remaining cases were wild-type.

The majority of the patients were treated with imatinib (69.5%), following by nilotinib (17.1%) and dasatinib (13.3%). Complete cytogenetic response (CCyR) was obtained in 79 (75.2%) patients, of whom 55 (52.4%) were within 3 months of treatment. Among 7/7, 6/7 and 6/6 genotypes, CCyR was achieved by 12 (70.6%), 31 (70.4%) and 29 (65.9%) patients, respectively. Major molecular response (MMR) was obtained in 73 patients (69.5%); among them eight (47%) were 7/7, 23 (52.3%) 6/7 and 20 (45.4%) 6/6. Deep molecular response (DMR) was achieved in 51 (48.6%) patients, of whom 8 (47.1%), 23 (52.3%) and 20 (45.4%) showed a 7/7, 6/7, or 6/6 genotype, respectively. Interestingly, none of these differences was statistically significant.

Forty-seven patients switched to second-line therapy, of whom 18 (38.3%) for primary resistance, 14 (29.8%) for secondary resistance and 15 (14.3%) for intolerance or unacceptable toxicity.

Among patients who experienced primary resistance, two (22.2%) were 7/7, seven (36.8%) were 6/7 and nine (45%) wild-type. A similar distribution was also recorded among patients who switched for toxicity.

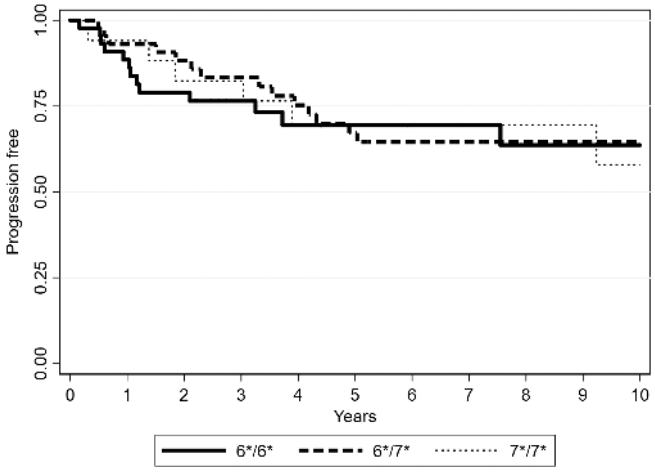

FIGURE 1 Progression-free survival (Kaplan-Meier estimate) according to Gilbert's syndrome genotype

Progression-free survival did not differ according to GS genotype either in the univariate (P = .98) or in the adjusted Cox analysis (P = .77) subgroups (Figure 1). Between second-line treated patients, 35 (74.5%) achieved an MMR and 17 (36.2%) a DMR. In this subset of patients, no differences were observed between GS genotypes and second-line MMR and/or DMR achievement.

Ten patients (9.5%) were in the third-line of treatment, mainly for secondary resistance; among them, two (20%), four (40%) and four (40%) were 7/7, 6/7 and 7/7, respectively.

Hematological toxicity was reported in 26 (24.8%) patients and extra-hematological toxicities in 64 (61%) (Table S2). Hyperbilirubinemia was reported in 26 (40.6%) patients, of whom nine (34.6%) reached grade 3/4. Among the latter, 55.6% of the cases belonged to the homozygous genotype subgroup. However, no significant difference in hematological as well as extra-hematological toxicity was found according to GS genotype (Table S2).

Hyperbilirubinemia is the most frequent laboratory adverse event observed with nilotinib, but in most cases does not represent a true hepato-cellular toxicity.[10] Nilotinib and to a lesser extent other TKIs have the potential to inhibit UGT1A1, which may contribute to TKI-related unconjugated hyperbilirubinemia.

Gilbert's syndrome is often underestimated in clinical practice, mainly because the specific laboratory text is performed only when bilirubin levels are significantly increased. However, this condition can be present also in patients with normal or borderline bilirubin levels.

In our case series GS was detected in a higher percentage of patients than expected (about 16.2% with homozygous genotype *vs* 10% in the general population).

Even though homozygous patients showed a higher rate of grade 3/4 hyperbilirubinemia, GS did not affect response rate and outcome in CML patients. This datum was further confirmed with all the three TKIs used in first-line, that is, imatinib, dasatinib, and nilotinib. Indeed, comparing patients with or without GS, no difference was observed in terms of achievement of CCyR, MMR, DMR, hematological or extra-hematological toxicity, regardless of the specific TKI used.

In conclusion, considering the concomitant occurrence of other non-TKI-related causes of bilirubin increase, GS should not represent an absolute contraindication to nilotinib therapy, as TKIs choice should be based on other concomitant variables.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of Good Clinical Practice. At their first admission to our hospitals, all subjects gave written informed consent to retrospectively collect clinical data and leave leftovers from the routine diagnostic samples for further no-profit studies, in accordance with the Declaration of Helsinki. Accordingly, a new submission to the ethics committee for the present study was not required.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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Isolated isochromosomes i(X)(p10) and idic(X)(q13) are associated with myeloid malignancies and dysplastic features

To the Editor:

An isodicentric X chromosome, with a breakpoint in Xq13 named idic(X)(q13), is a rare but recurrent abnormality found in about 1% of myelodysplastic syndromes (MDS), especially in old females. Most cases with idic(X)(q13) are MDS, but a number of acute myeloid leukemias (AML), usually secondary to MDS, have also been reported, and a few cases of myeloproliferative neoplasms (MPN).[1] The idic(X)(q13) is frequently the only cytogenetic abnormality, albeit one or more copies of the idic, beside a normal X chromosome being present in the same cells. This suggests that idic(X)(q13) may be implicated in the early process of the disease. The presence of idic(X)(q13) is specifically associated with MDS in the WHO classification. Its clonal detection by conventional cytogenetics is sufficient to diagnose MDS, in case of cytopenias without morphological dysplasia.[2] Conversely, the isochromosome of the short arm of chromosome X named i(X)(p10) is a very rare abnormality. It is observed in some cases of myeloid or lymphoid malignancies, both as a unique abnormality, and as part of a more complex karyotype, in female, and less frequently in male patients.

Myelodysplastic syndromes evolution is heterogeneous with transformation to AML in about 30% of cases. An International Prognostic Scoring System (IPSS) is commonly used to predict leukemic evolution of primary untreated adults and to adapt therapy. Revised (R) in 2012,[3] this score includes bone marrow blast percentage, hemoglobin level, absolute neutrophil count, platelet count and cytogenetics. There is no definite prognostic impact from Idic(X)(q13), and is considered as another abnormality in the intermediate group of the IPSS-R.

Recently, exome and genome-wide sequencing of MDS cases revealed a great diversity of genomic aberrations, with more than 25 recurrent mutations.[4] Several genes involved in pre-messenger RNA splicing have been shown to be mutated in half of MDS patients, revealing a new leukemic pathway involving spliceosome dysfunction.

To characterize the clinical and genetic profile of myeloid neoplasms associated with idic(X)(q13) and i(X)(p10), we performed a retrospective study, between 2002 and 2017, of 45 patients with myeloid neoplasm who presented these abnormalities. All patients gave their informed consent in agreement with the Helsinki declaration; and the Institutional Ethics Committee at Avicenne Hospital approved this study. The Idic(X)(q13) chromosome was found in 33 cases, and the i(X)(p10) in 12 patients who were all female. Ten cases were associated with other chromosomal abnormalities mainly involving chromosomes five and seven. The analysis was then restricted to 35 patients with an isolated X chromosome abnormality. In the majority of cases (n = 29), the X chromosome abnormality was detected at diagnosis. Isochromosomes could result from transverse, instead of longitudinal misdivision of the centromere; another mechanism could be chromatid exchange involving two homologous chromosomes. In both scenarios, it leads to the loss of the long arm and gain of the short arm of chromosome X, resulting in a state of genetic imbalance. It is possible that the frequency of i(X)(p10) was previously underestimated in the literature. The cytogenetic distinction between del(Xq) and i(Xp) is difficult due to the similarity of Xp- and q-arm banding patterns, extending from the centromere to band Xq24 (Figure 1).

A morphological centralized review of the bone marrow smear was performed by two cytologists. The revised diagnosis was MDS (n = 25) [MDS with ring sideroblasts (n = 1), MDS with uni (n = 1) or multilineage dysplasia (n = 10) and with ring sideroblasts (n = 2), MDS with excess blasts −1 (n = 6), MDS with excess blasts 2 (n = 3), MDS with myelofibrosis (n = 2)] or AML with multilineage dysplasia (n = 2), chronic myelomonocytic leukemia (n = 4), atypical chronic leukemia (n = 1) or unclassifiable cytopenia (n = 3). The majority of patients presented marked dysplastic features (>10%) in one or more of the major lineages. No specific feature was observed.

Some patients had a history of previous neoplasia or myeloid neoplasm, including four solid tumors (breast cancer n = 3), two AML with normal karyotype and NPM1 mutation, one acute lymphoblastic leukemia, one lymphoma, one chronic myelocytic leukemia on complete remission, two immune thrombocytopenias and two monoclonal gammopathies.

Nineteen analyzed patients presented a median of 2.9 mutations in the 27 genes analyzed by Next Generation Sequencing. The most