LETTER TO THE EDITOR

ASXL1, TP53 and IKZF3 mutations are present in the chronic phase and blast crisis of chronic myeloid leukemia

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The mechanism of transformation from chronic phase (CP) to blast crisis (BC) chronic myeloid leukemia (CML) is heterogeneous and poorly understood.1 The most frequently genetic aberration observed at this advanced stage includes a second Philadelphia (Ph) chromosome, trisomy 8, isochromosome 17 and trisomy 19, alone or in various combinations and complex aberrations.1 Clonal cytogenetic evolution appears to be the most consistent predictor of blast transformation, present in up to 80% of patients, until now.2 At present, little is known about the molecular mechanisms underlying the mutational profiling at CP-CML disease progression and only limited changes occurring during clonal evolution at BC have been described.3 We read with great interest a series of manuscripts reporting somatic mutations in patients with CML in both the CP and BC.4–8 Mutations in genes such as TP53, NPM1, IKZF1, RUNX1 and ASXL1 have been described in CML progression. However, all these studies have analyzed a limited number of genes and mainly focused on BC phase. Interestingly, Soverini et al.7 performed a massive parallel sequencing at three different stages (diagnosis, major molecular response and disease progression) of a patient who developed a lymphoid BC. In this study, IDH2 R140Q was detected in a very low number of cases, and the mutation was only observed in BC. To investigate the genetic changes associated with CML progression, we performed whole-exome sequencing (WES) of an individual patient at three different phases: CP, complete cytogenetic remission and BC. The patient was a 65-year-old man diagnosed with a Ph+ CML and presented with a hypercellular bone marrow (BM) containing 2% blast cells. Karyotype analysis showed the Ph chromosome in all analyzed metaphases at diagnosis. The patient was treated with imatinib (400 mg/day) achieving, along the treatment, complete hematological response after 1 month and complete cytogenetic response (CCyR) after 12 months. Conversely, he never achieved major molecular response during this period. Unfortunately, after 14 months from diagnosis, the patient progressed to a myeloid BC that did not respond to a second-line treatment (Dasatinib + Idarubicin-AraC) and died of the disease 18 months after diagnosis. At that time, the G-banding analysis showed a complex karyotype (Supplementary Figure 1). To perform the WES technique, DNA samples were obtained from the normal tissue (oral mucosa) and BM blasts at diagnosis (CP), at CCyR and at the time of disease progression (BC) from the index CML patient. The preparation of shotgun libraries from the leukemic (CP, CCyR and BC) and non-leukemic genomic DNA followed by an in-solution exome capture was performed with the use of a commercial platform (Agilent) covering 50 Mb of coding exons (~1.60% of the genome). After massive parallel sequencing using the Genome Analyzer Ix (Illumina), candidate somatic mutations were identified using RUBioSeq software.9 The bioinformatics analysis and the filtering steps to identify the coding variants are detailed in the Supplementary Material.
clonality and clonal evolution patterns during the CML progression. In this sense, 93% of the selected SNSs and indels that were present in the CP were also seen in BC. In fact, the percentages of reads of the mutant alleles identified for the most relevant genes were the same (around 50%) both at CP and at BC (Figure 1B). As expected, these data suggest that the same clone was present in CP and BC.

Interestingly, TP53 mutation (p.G244S) is present in all the stages, pinpointing its potential role as a tumor-initiating event. In contrast, ASXL1 (p.G679*), UBE2G2 (p.D35V), ZEB2 (p.L420R) and IKZF3 (p.E318K) were present in CP and BC, also suggesting that these alterations are initiating events and co-occur with the BCR/ABL1 fusion gene (Figure 1A). All these SNSs were validated using PCR amplification and direct DNA sequencing of the same samples that were subjected to WES. Finally, SNSs unique to the BC sample (ARSD, FRG2C and AADACL3 mutations) may contribute to leukemic transformation; however, we were not able to validate these two mutations by Sanger sequencing due to the limitations to leukemic transformation; however, we were not able to validate these two mutations by Sanger sequencing due to the limitations to leukemic transformation. In addition, this genetic alteration has been found also in 19% of patients with blast-phase myeloproliferative neoplasm, suggesting a potential pathogenic role in the myeloid lineage. To date, mutations in other members apart from IKZF1 have never been described in human leukemia. In order to explore this genetic feature, we screened for mutations in IKZF3 gene in the 26 samples using conventional PCR and Sanger sequencing. IKZF3 were also mutated in 8% of CP and BC/NCgR samples, suggesting a potential role of this gene in myeloid leukemia (Table 1).

In conclusion, WES allowed the identification of a large number of mutated genes, even at the CP of CML, which harbor prognostic and predictive significance, such as ASXL1 and TP53, both found mutated at CP and BC. The study of the mutation profile through the course of the disease indicated that, at least in this patient, the number and the type of mutations were similar at CP and BC. In addition, we identified for the first time deleterious mutations in

### Table 1. Chromosomal and molecular features in 13 CP and BC/NCgR paired samples

| Id | Type | Age (years) | Follow-up (months) | Karyotype | Mutational status |
|----|------|-------------|--------------------|-----------|------------------|
| 1a | CP   | 65/M        | 18                 | 45,Xt(9;22)(q34q11.1),rob(13;14)(q10;q10)10/| c.2035G>T p.G679* |
| BC |      |             |                    | 46,Xt(9;22)(q34q11.2),rob(13;14)(q10;q10)10/ | c.2035G>T p.G679* |
| 2  | CP   | 31/F        | 6                  | 46,XX,t(9;22)(q34q11.2)[20] | c.2498_2501del p.S833E |
| BC |      |             |                    | 46,XX,t(9;22)(q34q11.2)[20] | c.2498_2501del p.S833E |
| 3  | CP   | 70/M        | 3                  | 46,Xt(9;22)(q34q11.2)[20] | wt |
| BC |      |             |                    | 46,Xt(9;22)(q34q11.2)[20] | wt |
| 4  | CP   | 41/F        | 5                  | 46,XX,t(9;22)(q34q11.2)[20] | wt |
| BC |      |             |                    | 46,XX,t(9;22)(q34q11.2)[20] | wt |
| 5  | CP   | 54/M        | 5                  | 46,XX,t(9;22)(q34q11.2)[20] | wt |
| BC |      |             |                    | 46,XX,t(9;22)(q34q11.2)[20] | wt |
| 6  | CP   | 85/M        | 7                  | 46,XX,t(9;22)(q34q11.2)[20] | wt |
| BC |      |             |                    | 46,XX,t(9;22)(q34q11.2)[20] | wt |
| 7  | CP   | 36/M        | 8                  | 46,XX,t(9;22)(q34q11.2)[20] | wt |
| BC |      |             |                    | 46,XX,t(9;22)(q34q11.2)[20] | wt |
| 8  | CP   | 34/M        | 12                 | 46,XX,t(9;22)(q34q11.2)[20] | c.71C |
| BC |      |             |                    | 46,XX,t(9;22)(q34q11.2)[20] | c.71C |
| 9  | CP   | 8/M         | 4                  | 46,XX,t(9;22)(q34q11.2)[20] | c.71C |
| BC |      |             |                    | 46,XX,t(9;22)(q34q11.2)[20] | c.71C |
| 10 | CP   | 44/F        | 24                 | 46,XX,t(9;22)(q34q11.2)[20] | c.71C |
| BC |      |             |                    | 46,XX,t(9;22)(q34q11.2)[20] | c.71C |
| 11 | CP   | 73/F        | 20                 | 46,XX,t(9;22)(q34q11.2)[20] | c.71C |
| BC |      |             |                    | 46,XX,t(9;22)(q34q11.2)[20] | c.71C |
| 12 | CP   | 60/F        | 14                 | 46,XX,t(9;22)(q34q11.2)[20] | c.71C |
| BC |      |             |                    | 46,XX,t(9;22)(q34q11.2)[20] | c.71C |
| 13 | CP   | 69/F        | 3                  | 46,XX,t(9;22)(q34q11.2)[20] | c.71C |
| BC |      |             |                    | 46,XX,t(9;22)(q34q11.2)[20] | c.71C |
| 14 | CP   | NA          | 24                 | 45,XX,t(9;22)(q34q11.2)[20] | c.71C |

Abbreviations: BC, blast crisis; CP, chronic phase; F, female; M, male; NCgR, no cytogenetic response; wt, wild type. Whole-exome sequencing analyzed patient.
IKZF3, UBE2G2 and ZEB2 in CML. Although current diagnostic procedures recommend the study of ABL1 mutations in non-responder patients, our data suggest that sequencing a wider panel of genes could be also beneficial in the clinical management of these patients.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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