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LETTER TO THE EDITOR

Rapid conversion of chronic myeloid leukemia to chronic myelomonocytic leukemia in a patient on imatinib therapy

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BCR-ABL1, the product of the Philadelphia chromosome (Ph), is sufficient for inducing the chronic phase of chronic myeloid leukemia (CML).[1] Tyrosine kinase inhibitors (TKIs) suppress the Ph^+ cell clone, restoring polyclonal hematopoiesis.[2] However, clonal cytogenetic abnormalities in Ph^- cells become detectable in some patients achieving a cytogenetic response to TKIs, and slow evolution to myelodysplasia or acute myeloid leukemia (AML) has been observed.[2,3] In rare cases, identical abnormalities were demonstrated both in Ph^+ and Ph^- cells, but most cases are consistent with independently acquired abnormalities, although an undetected common ancestral event cannot be excluded (reviewed in Loriaux and Deininger[4]). The clinical conundrum of coexisting leukemic disorders is that TKI suppression of CML may unmask a different, more aggressive disease. Here, we report a patient who after starting imatinib rapidly converted from CML to fatal chronic myelomonocytic leukemia (CMML), demonstrating that this is not a theoretical consideration. Whole-exome sequencing (WES) and genotyping of individual colonies revealed the clonal architecture during disease evolution and implicated *TET2* and *ASXL1* variants as early or germline events.

CASE DESCRIPTION

A 77-year-old man presented with fever and 16 kg weight loss. Clinical examination was unremarkable without splenomegaly. The white blood cell (WBC) count was 270 000/μl, with a myeloid left shift; hemoglobin was 9 g/dl and platelets were 55 000/μl (Supplementary Table 1). Bone marrow (BM) biopsy was 90% cellular with a left shift (Figures 1a and b). BM metaphase karyotyping was 46XY, t(9;22)(q34;q11.2)[20] and blood *BCR-ABL1* mRNA (e13a2) was 10% on the international scale (IS). The patient

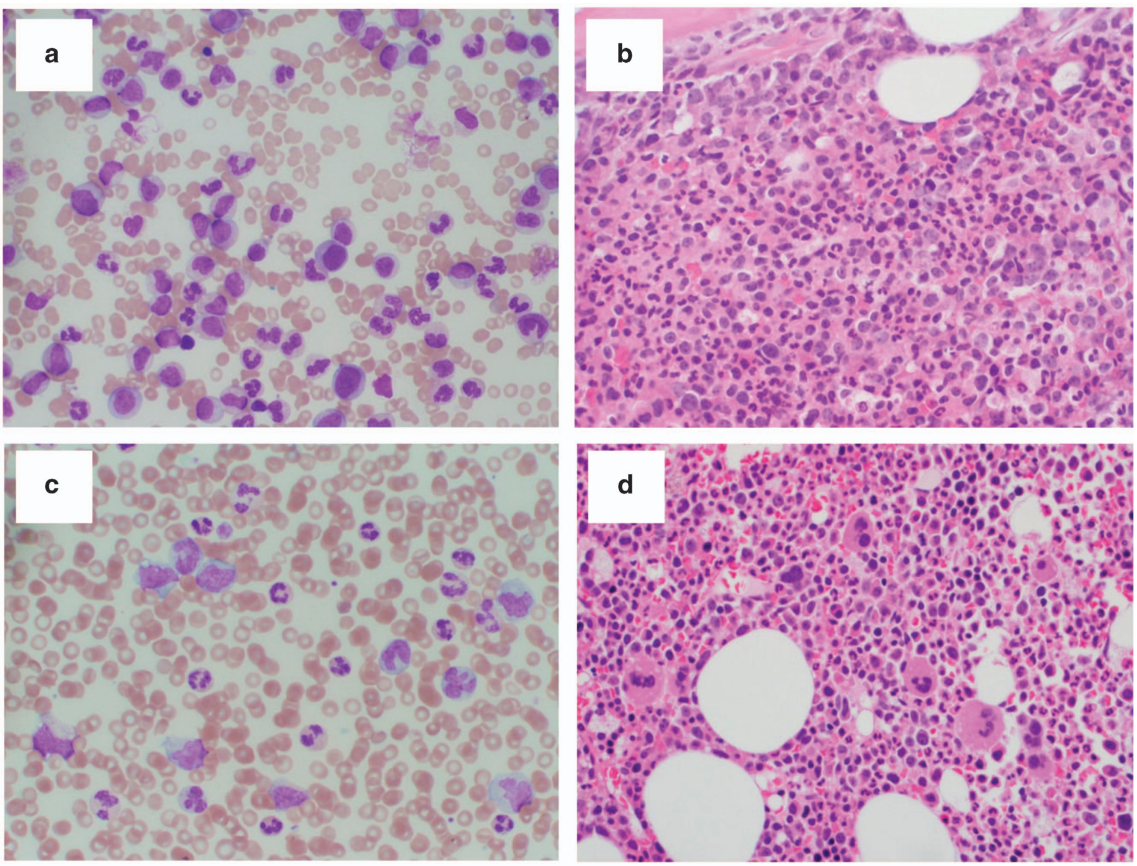

Figure 1. Blood and BM morphology. (**a**) Peripheral blood smear at CML diagnosis demonstrating marked leukocytosis with granulocytic left shift and decreased platelets. (**b**) BM biopsy at CML diagnosis shows hypercellularity with granulocytic hyperplasia. (**c**) Peripheral blood smear on day 92 of imatinib therapy showing leukocytosis with monocytosis and (**d**) corresponding BM biopsy showing hypercellular BM with occasional hypolobated megakaryocytes.

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was started on 400 mg imatinib (considered day 1) and stayed on the same dose throughout the treatment. On day 67, partial hematological response was demonstrated, but a rise of monocytes was noted (Supplementary Table 1). At day 92, the WBC count rose to 73 000/μl, monocytes were 19%, hemoglobin was 9 g/dl and platelets were 80 000/μl. BM histology showed increased monocytes (Figures 1c and d), karyotyping was 46XY,[30] and *BCR-ABL1* was 0.12% IS. Sequencing was negative for *BCR-ABL1* kinase domain mutations. A diagnosis of CMML was established. 5-Azacytidine was added, with initial improvement of blood counts. The subsequent clinical course was complicated by sepsis; the patient declined further leukemia therapy and passed away.

SOMATIC MUTATIONS ASSOCIATED WITH PHENOTYPIC CONVERSION TO CMML

We performed WES (average read depth: 61×) on blood $CD14^+$ cells from day 92, with $CD3^+$ cells from the diagnostic sample as constitutional control. We identified four somatic single-nucleotide variants (SNVs; $EZH2^{I669M}$, $KRAS^{G12R}$, $MSLN^{P462H}$ and $NTRK3^{V443I}$), all of which were confirmed by Sanger sequencing (Supplementary Table 2). Sequenom MassARRAY (Agena Bioscience, San Diego, CA, USA) identified the same mutations in the day 67, 78, 92 and 124 samples, but not the diagnostic sample (Supplementary Table 3). In addition, we identified one nonsense variant in *ASXL1* (c.24422delC → p.P808fs*10) and two nonsense variants in *TET2* (*TET2* c.1219delT → p.S407fs*20; c.4932delA → p.Y1645fs*50). Across all samples, including $CD3^+$ and diagnostic $CD14^+$ cells, *ASXL1* c.24422delC and *TET2* c.4932delA were detected at ~50%, whereas *TET2* c.1219delT was detected at ~30% (Supplementary Table 3). *ASXL1* c.24422delC and *TET2* c.1219delT are listed in catalogue of somatic mutations in cancer and have been confirmed as somatic, whereas our findings are consistent with germline mutations or acquisition by a multipotent hematopoietic stem cell. Although *TET2* c4932delA has not been reported in the catalogue of somatic mutations in cancer (COSMIC), a very similar variant (COSM4170135, c.4928delC, p.P1644fs*51) has. WES of the diagnostic sample at an average depth of 319× failed to identify additional mutations specific to the CML clone, but confirmed the presence of low-level $EZH2^{I669M}$, $KRAS^{G12R}$, $MSLN^{P462H}$ and $NTRK3^{V443I}$.

CLONAL ARCHITECTURE AND EVOLUTION

To unravel clonal relationships, we plated $CD34^+$ cells from diagnosis, day 67 and 78 in colony assays (no viable cells available from days 92 and 124). Both DNA and RNA were extracted from ~100 single colonies, and analyzed for $EZH2^{I669M}$, $KRAS^{G12R}$, $MSLN^{P462H}$ and $NTRK3^{V443I}$ by MassARRAY, and for *BCR-ABL1* messenger RNA (mRNA) by quantitative reverse transcription PCR. In the diagnostic sample, 92/100 colonies were informative for BCR-ABL1 and all for DNA mutational analysis. Only 38% of informative colonies were *BCR-ABL1*-positive. This is unusual, as myeloid colonies from newly diagnosed CML patients are almost exclusively *BCR-ABL1*-positive,[5] but is consistent with the low *BCR-ABL1* expression (10% IS). Altogether 14% of colonies were positive for at least one of the four somatic SNVs, all of which were *BCR-ABL1*-negative; 43% were wild type (Figure 2a). Genotypes included $KRAS^{G12R}$, $KRAS^{G12R}/MSLN^{P462H}/NTRK3^{V443I}$ and $KRAS^{G12R}/MSLN^{P462H}/NTRK3^{V443I}/EZH2^{I669M}$ (Figure 2a). The failure of WES and MassARRAY to detect the SNVs in the diagnostic sample likely reflects their lower sensitivity; alternatively, *in vitro* culture with cytokines may favor CMML colonies owing to their GM-CSF (granulocyte-macrophage colony-stimulating factor) hypersensitivity.[6] No *BCR-ABL1*-positive colonies were detected on day 67, indicating effective CML therapy; however, 51/105 colonies (49%) were positive for at least one of the four SNVs. Most of the genotypes followed the patterns of the diagnostic sample (Figure 2a, lower panel); two colonies were $KRAS^{G12R}/NTRK3^{V443I}/EZH2^{I669M}$ and one was heterozygous $MSLN^{P462H}$. Despite the increase of colonies with at least one SNV, the ratio between the various SNVs remained largely stable (Figure 2a). On day 78, colonies positive for all four SNVs were dominant; two colonies were $KRAS^{G12R}/NTRK3^{V443I}/EZH2^{I669M}$, two were heterozygous $MSLN^{P462H}$ only, and one was heterozygous for $KRAS^{G12R}/NTRK3^{V443I}/EZH2^{I669M}$ and homozygous for $MSLN^{P462H}$ (Figure 2a). Altogether, these data are consistent with somatic acquisition of $KRAS^{G12R}$, followed by $NTRK3^{V443I}$ and $MSLN^{P462H}$, and finally $EZH2^{I669M}$ (Figure 2b). Whether $MSLN^{P462H}$ and $NTRK3^{V443I}$ were acquired successively or simultaneously cannot be distinguished. $KRAS^{G12R}/NTRK3^{V443I}/EZH2^{I669M}$ colonies could reflect loss of the mutant *MSLN* allele in a side clone, or a sequencing error, which would also explain detection of $MSLN^{P462H}$ as the only SNV in two colonies. As colony assays may skew clonal ratios present *in vivo*, we quantified $KRAS^{G12R}$, $MSLN^{P462H}$, $NTRK3^{V443I}$ and $EZH2^{I669M}$ by pyrosequencing of MNCs from all five samples (Figure 2c). None of the SNVs was detected at diagnosis. $KRAS^{G12R}$ occurred with at least 10% higher allelic frequency than the other SNVs in all subsequent samples, consistent with the initial acquisition. $EZH2^{I669M}$ was 10–15% higher than $MSLN^{P462H}$. This is at variance with the colony data suggesting $EZH2^{I669M}$ was acquired last, but could be explained by $MSLN^{P462H}$ loss from the clone harboring all four mutations (Figure 2b), via deletion or acquired uniparental disomy of the non-mutated allele. As shown in Figure 2a, we observed such a clone at low frequency in the colony assays (two colonies in the day 67 and 78 samples). Discrepancies in clonal representation between colony and sequencing data were also demonstrated in AML.[7]

Although the presentation of our patient was consistent with CML, low hemoglobin and platelets were unusual in the absence of other high-risk CML features. On imatinib, the clinical phenotype rapidly morphed to CMML and four somatic point mutations were detected by WES of $CD14^+$ cells on day 67. Although neither MassARRAY nor pyrosequencing had identified any of these variants at diagnosis, their rapid appearance suggested they predated imatinib therapy, which was confirmed by colony sequencing. *BCR-ABL1* and the CMML-related point mutations may have arisen independently in different hematopoietic stem cells or may share a common abnormal ancestor. The latter is suggested by nonsense SNVs in *ASXL1* and *TET2*, with identical allelic ratios in $CD3^+$ and $CD14^+$ cells, and MNCs, and in all sequential samples, consistent with their presence in the germline or in a pluripotent hematopoietic stem cell. Somatic mutations in CMML T cells have been reported, although typically at a lower allelic ratio than in the myeloid lineage.[8] Verification would require an alternative source of germline DNA, which is not available. Irrespective of this limitation, it is likely that $TET2^{S407fs*20}$ and $ASXL1^{P808fs*10}$ contribute to CMML in this patient, as they have been reported as validated somatic variants in catalogue of somatic mutations in cancer. Moreover, we have shown that *TET2* (~50%) and *ASXL1* (~40%) are among the most commonly mutated genes in CMML, whereas the frequency of *NRAS* or *KRAS* mutations is only 10–20%.[9] Another study identified *TET2* mutations as founder mutations in CMML,[8] while $KRAS^{G12R}$ was as a secondary event, and *EZH2* mutations are acquired late.[9] This suggests that RAS^{G12R} and $EZH2^{I669M}$ were acquired by *TET2/ASXL1*-mutant cells. $EZH2^{I669M}$ is a recurrent CMML mutation and associated with poor outcome.[10] Neither $MSLN^{P462H}$ nor $NTRK3^{V443I}$ have been described in CMML or other cancers. *MSLN* encodes a precursor of two proteins, megakaryocyte potentiation factor, which enhances cytokine effects on megakaryocytes, and mesothelin, a cell adhesion molecule.[11,12] p.P462T was previously reported in esophageal carcinoma.[13] Mutations of *NTRK3* (also known as TrkC) have been described in medulloblastoma and other cancers.[14] In AML cell lines, *NTRK3* enhances proliferation and inhibits apoptosis through activation of PI3K/AKT and

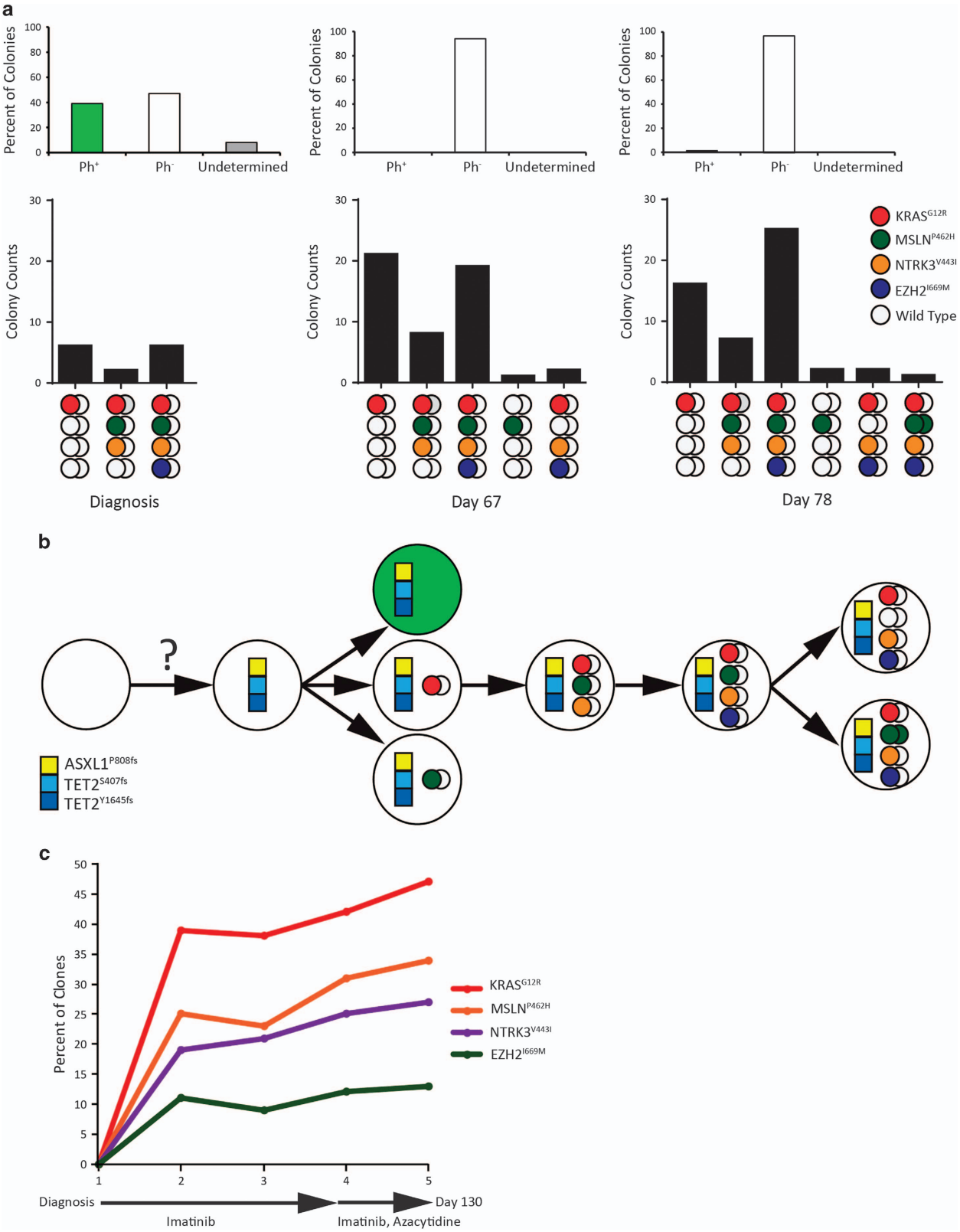

Figure 2. Genotyping of successive blood samples. (**a**) Colony genotyping at diagnosis, day 67 and day 78 (upper panel). Proportion of BCR-ABL1-positive (green), BCR-ABL1-negative (white) and BCR-ABL1-undetermined colonies (gray; lower panel). Numbers of colonies with *KRAS*^{G12R}, *MSLN*^{P462H}, *NTRK3*^{V443I} and *EZH2*^{I669M} alleles. Gray circles represent the nonmutant alleles. (**b**) Clonal architecture and evolution. The squares denote SNVs that were either present IN myeloid as well as T cells, suggesting they were germline variants or acquired by a progenitor cell with multilineage potential. Circles represent the four somatic SNVs, with color coding as in the previous panel. (**c**) Quantification of somatic SNVs by pyrosequencing.

AKT/mTOR.[15] Functional characterization will be required to determine whether *MSLN*^{P462H} and *NTRK3*^{V443I} contribute to disease progression or are bystanders.

Secondary Ph$^-$ leukemia after treatment for CML is rare. Prior to the introduction of imatinib, such cases were ascribed to cytotoxic chemotherapy. In the era of TKIs, effective suppression of the highly proliferative Ph$^+$ clone may lead to rapid expansion of a previously unrecognized leukemic clone, as in our patient. It is conceivable that a nonspecific agent, such as hydroxyurea, might have been a better option compared with the imatinib/5-azacytidine combination, illustrating the challenges of applying targeted therapy to clonally complex myeloproliferative neoplasms.

CONFLICT OF INTEREST

MWD is on the advisory board and is a consultant for Ariad, Incyte, Novartis and Pfizer, and serves on the advisory board for CTI BioPharma Corp. His laboratory receives research funding from Bristol-Myers Squibb, Celgene, Gilead and Novartis. The remaining authors declare no conflict of interest.

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Supplementary Information accompanies this paper on the Leukemia website (http://www.nature.com/leu)