Outcome of patient with high-risk chronic myelomonocytic leukemia, treated with decitabine prior to transformation to acute myeloid leukemia: A case report

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Abstract. The present study describes a patient with high-risk chronic myelomonocytic leukemia (CMML), for whom decitabine therapy achieved partial remission, prior to a sudden transformation to acute myeloid leukemia (AML) and an inferior outcome. The 53-year-old male reported easily bruising for 5 months. Examination indicated a diagnosis of CMML. Chromosome analysis identified a 48, XY, +8, +21 karyotype, classifying the patient as high-risk, according to a clinical/molecular CPSS (CPSS-Mol) model. Gene sequencing detected a mutation in DNA methyltransferase 3α, which is relatively rarely identified in CMML and has recently been reported to have an independent prognostic impact on overall survival time. Partial remission was achieved with decitabine treatment, and hematologic improvement was observed subsequent to 2 cycles of treatment. However, a sudden transformation to AML led to fatality of the patient. This case suggests that decitabine may be an effective therapeutic for high-risk CMML; however, the response may be temporary, and the ultimate outcome may be extremely poor. Therefore, novel treatment strategies of CMML, including combination therapies with decitabine, or targeted drugs, including Janus kinase inhibitors or granulocyte-macrophage colony stimulating factor monoclonal antibodies, require investigation.

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Introduction

Chronic myelomonocytic leukemia (CMML) is a form of myeloid neoplasm with dysplastic and proliferative features. It is defined by the presence of persistent peripheral blood (PB) monocytosis ($\geq 1 \times 10^9$//), when monocytes account for $\geq 10\%$ of the White Blood Cell (WBC) count (1). Other disorders, including Philadelphia (Ph) chromosome, BCR, RhoGEF and GTPase activating protein/proto-oncogene tyrosine-protein kinase ABL1 (BCR-ABL1) fusion gene, and rearrangements of platelet derived growth factor receptor α (PDGFRα), platelet derived growth factor receptor β (PDGFRβ) and fibroblast growth factor receptor 1 (FGFR1), require exclusion prior to diagnosis (2). The French-American-British (FAB) classification (3) and World Health Organization (WHO) (4) have classified CMML into 2 subtypes (CMML-I and -II), according to leukocyte count and the proportion of bone marrow (BM) blasts, respectively.

Due to the heterogeneity of the disease, the clinical course and outcomes of patients with CMML are variable (5). A number of prognostic studies have been performed, in which cytogenetic and molecular abnormalities were indicated to be important prognostic factors. Cytogenetic abnormalities, including trisomy 8, complex karyotypes, monosomy 7, del7q, trisomy 21, and -Y, have been identified to have prognostic value (6). Patients with trisomy 8, complex karyotypes, monosomy 7 and del7q are classified as high-risk according to the majority of prognostic models (7), and exhibit a higher leukemic transformation rate and shorter overall survival time (6-8). A number of molecular abnormalities are commonly exhibited by patients with CMML, including mutations in additional sex combs like transcriptional regulator 1 (ASXL1), Tet methylcytosine dioxygenase 2 (TET2), serine and arginine rich splicing factor 2 (SRSF2), NRAS proto-oncogene, GTPase (NRAS), runt related transcription factor 1 (RUNX1), SET binding protein 1 (SETBP1), Cbl proto-oncogene (CBL), JAK2 and RUNX1 are common, while mutations in RUNX1, NRAS, SETBP1 and ASXL1, are independently associated with inferior overall survival (OS) time (9).

The present case study describes a high-risk patient with CMML, who achieved PR for 2 months with decitabine therapy. However, the patient demonstrated a sudden transformation

to AML and eventually an inferior outcome, indicating that decitabine treatment as a single agent may not be satisfactory for CMML cases that have a tendency to transform to AML. This highlights the requirement to discover novel treatment strategies for CMML.

Case report

In September 2015, a 53-year-old man was admitted in The First Affiliated Hospital of Lanzhou University (Lanzhou, China), having bruised easily for ~5 months. PB analysis revealed a white blood cell count (WBC) of $35.07x10^9$ cells/l, a monocyte level of $4.46x10^9$ cells/l, a hemoglobin (Hb) level of 74 g/l and a platelet count of $44.0x10^9$ cells/l. A bone marrow aspirate examination using Wright-Giemsa stain revealed a cell proportion of 6.5% myeloblasts, 2.5% promyelocyte, 10.5% promonocytes and 20% immature monocytes. (Fig. 1), resulting in a diagnosis of CMML according to the 2008 WHO myelodysplastic (MDS)/myeloproliferative neoplasms classification system (2). Immunophenotyping analysis revealed that the blasts were positive for cytoplasmic myeloperoxidase antigens and cluster of differentiation (CD)64, 11c, 13, 11b and 33, and negative for CD7, 117, 3, 16, 20, 10, 19, 56, 4 and 34, and cytoplasmic CD79a and CD3 antigens, which were detected on the surface of the monoblasts by multiparameter flow cytometry. A total of 2 ml bone marrow was extracted using a heparin anticoagulation solution (25 IU heparin sodium; BD Biosciences, Franklin Lakes, NJ, USA) at 15-25°C for 30 min. The concentration of marrow mononuclear cells specimen was adjusted to $5x10^9$ cells/l, and fluorescent labeling antibody reagents, FITC, PE, PerCP or APC (all from BD Biosciences) were added. The solutions were protected from light and incubated for 15 min. A total of 2 ml Red Blood Cell Lysis Buffer was added to each sample and incubated for 10 min at room temperature in darkness. The sampled were then centrifuged at 40 x g for 6 min, and the supernatant was discarded. The remaining sample was washed twice in PBS, and cells resuspended in PBS for detection using a FACSCalibur full automatic multicolor analysis flow cytometer system (BD Biosciences) using FlowJo version 10.2 software (FlowJo LLC, Ashland, OR, USA).

Giemsa (G) banding analysis required preparation by adding 3 ml 0.25% trypsin (BD Biosciences) solution to 45 ml 0.85% saline, with a pH of 6.8-7.2, and warmed to 37° using a water bath. Chromosome specimens were digested in trypsin solution at 37°C for 2-3 min, then swiftly rinsed with 0.85% saline to terminate trypsin activity. This was followed by Giemsa staining (5 ml; BD Biosciences) for 15 min at 32°C and then the slides were rinsed with tap water and air dried. The staining was observed under a low power light microscope (magnification, x10) and the metaphase of mitosis was observed. Then an oil objective (magnification, x100) was used to observe Giemsa (G) banding. G banding analysis did not detect Ph chromosome.

Total RNA was isolated from the bone marrow aspirate using TRIzol (Thermo Fisher Scientific, Inc., Waltham, MA, USA, according to the manufactuerer's protocol. Reverse transcription of was performed using a reverse transcription kit (Takara Bio, Inc., Otsu, Japan). The temperature protocol was as follows: 37°C for 15 min, 85°C for 5 sec and kept at 4°C until use. The fluorophore used for the reverse transcription-quantitative polymerase chain reaction (RT-qPCR; Takara Biotechnology Co., Ltd., Dalian, China) was Dalian's Tap enzyme (Takara Bio, Inc.). The target gene primers were as follows: BCR-ABL, bcr-abl, forward, 5'-AGGGTG CACAGCCGCAACGGC-3', reverse 5'-GGCTTCACTCAG ACCCTGAGG-3'. The reference gene was β-actin. The reference gene primers were as follows: β-actin, forward, 5'-GGAGATTACTGCCCTGGCTCCTA-3' and reverse, 5'-GACTCATCGTACTCCTGCTTGCTG-3'. The thermocycling conditions were as follows: 95°C for 30 sec, then 40 cycles at 95°C for 5 sec and 60°C for 20 sec. The results were expressed as $2^{-\Delta\Delta Cq}$ (10). The quantity of BCR-ABL transcript was normalized to the ABL expression level. The copy number of BCR-ABL fusion gene and the copy number of ABL were calculated, and the result was expressed as the ratio of BCR-ABL copy number to ABL copy number. RT-qPCR did not detect BCR-ABL1 gene fusion for this patient.

Fluorescence in situ hybridization demonstrated that PDGFRα, PDGFRβ and FGFR1 were not rearranged (Fig. 2). The method was performed using 4 genes locus specific probes for ASS, PDGFRα, PDGFRβ and FGFR, localized at 9q34, 4q12, 5q32-33 and 8p12, respectively (all from Kindstar Global, Hester Clinical Inspection, Beijing, China). Bone marrow blood (3 ml) was extracted and the sampled centrifuged at 250 x g, room temperaure for 8 min and the supernatant discarded. Potassium chloride (5 ml) was added and the samples incubated at 37°C for 20 min. The chromosome specimens stored at -20°C, were then fixed with methanol and glacial acetic acid (3:1) for 30 min at 37°C. The slide obtained from the previous step were bathed in 2x SSC solution (2x SSC solution configuration method: Nacl 175.5 g, trisodium citrate 88.2 g, add 1,000 ml ddH$_2$O, PH adjusted to 5.3. Add ddH$_2$O and diluted with 1:9, adjusted pH to 7.0) at 37°C for 10 min, so as to increase cell permeability, simulate cell physiological environment and ensure the stability of the test substances, prior to graded dehydration in 70, 85 and 100% ethanol at room temperature. Denaturation took place in 70% formamide and 2x SSC, at 72°C for 3 min. A total of 10 µl probe mixture, with 5 µl probe, 3 µl DNA specimen, 0.5 µl salmon sperm DNA and 1.5 µl H$_2$O was incubated for 10 min in a water bath at 72°C, then placed in an iced water bath for 5 min, and finally a 37°C water bath for 5 min. The specimens were mounted onto glass slides using RubberCement (Kindstar Global, Beijing, China) and placed at 37°C overnight for hybridization. Specimens were washed for 5 min at 72°C in 0.4x SSC and then for 2 min with 0.1% TritonX-100 at room temperature. DAPI (Kindstar Global) was added to PBS and a 10 µg/ml DAPI solution was prepared, and the specimens incubated at 37°C for 15 min. Fluorescence hybridization signals of interphase cells were observed under the excitation of UV/Texas red/FITC trichromatic filters with an Olympus BX60 fluorescence microscope (Olympus Corporation, Tokyo, Japan). A total of 400 probe mixture cells were analyzed at one time. Image acquisition was achieved using an autosomal auto analysis system (Kindstar Global). This allowed a CMML diagnosis to be made. In addition, conventional cytogenetic analysis performed using the G banding technique
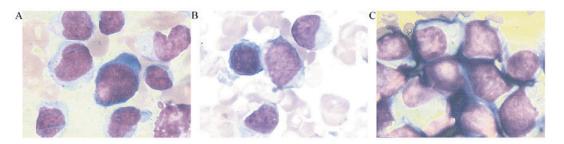
Figure 1. Normocellular bone marrow aspirate of the subject patient, containing (A) myeloblasts, promonocytes and immature monocytes. (B) Following 3 cycles of decitabine treatment, the proportion of bone marrow blasts was <5%, and (C) the level of monoblasts increased to 59%. Magnification, x100.
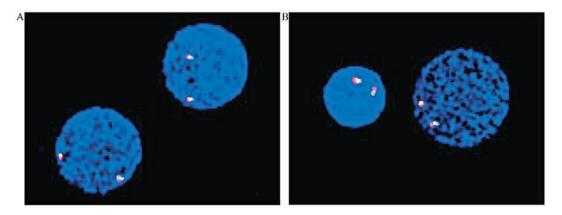

Figure 2. Fluorescence in situ hybridization analysis demonstrated no evidence of (A) PDGFRα or (B) PDGFRβ rearrangement. PDGFRα, platelet derived growth factor receptor α; PDGFRβ, platelet derived growth factor β.

revealed a 48, XY, +8 and +21 karyotype in all 20 metaphase cells (Fig. 3).

Meanwhile, gene sequencing using a polymerase chain reaction binding-based sequencing method, performed externally (Shanghai Di Shuo Becken Ltd Medical Examination, Shanghai, China), detected a missense mutation in DNMT3A (Fig. 4A) and nonsense mutations in TET2 (Fig. 4B). There was no evidence of mutations in SF3B1, SRSF2, ASXL1, RUNX1, FLT3-ITD, C-kit/D816V, NPM1 or CEBPA. According to these cytogenetic abnormalities, the case was defined as high-risk CMML.

The patient was treated with decitabine, administered at 25 mg daily for 5 consecutive days every 28 days. The patient underwent 3 courses of this regimen. The BM response was examined following each treatment cycle. Following 2 cycles of therapy, the patient achieved partial remission, classified according to the modified International Working Group response criteria in myelodysplastic syndromes (MDS) (11). The level of BM blasts had decreased to <5% and the percentage reduction was >50%. Furthermore, the patient's diagnosis was altered from CMML-II to CMML-I according to the FAB subtype criteria. Following 3 cycles of therapy, the patient maintained PR and displayed hematologic improvement (HI), including erythroid and platelet responses (11). The majority of parameters had improved, including an increase in Hb level from 56-168 g/l and an increase in platelet level from 2.1×10^{10}-6.2×10^{10}/l. BM aspirate analysis revealed a proportion of 2.5% myeloblasts and 0% promonocytes (Fig. 1B).

Subsequent to 3 cycles of decitabine therapy, the level of lactate dehydrogenase progressively increased from
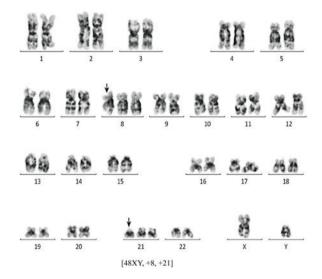

Figure 3. Representation of the 48, XY, +8, +21 karyotype.

267 to 11,347 U/l and absolute monocytosis (AMC) was detected (from 0.32×10^9 to 1.86×10^9 cells/l), potentially indicating disease progression. PB examination confirmed a WBC of 13.4×10^9/l, 81 g Hb/l, 32×10^9 platelets/l and 11347 U lactate dehydrogenase/l. BM aspirate analysis revealed 73.5% myeloblasts and 59% monoblasts (Fig. 1C). BM biopsy demonstrated a trend towards AML-M5. A diagnosis of disease transformation to AML-M5 was made, and fatality of the patient occurred 1 month after AML transformation, and prior to any AML treatment.
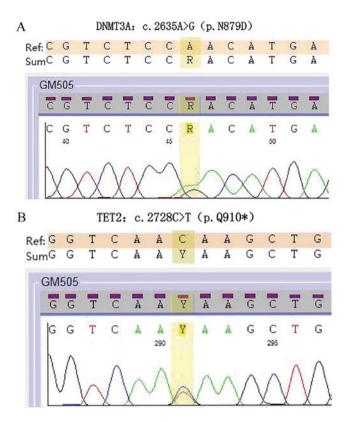
Figure 4. Results of gene sequencing. (A) A missense mutation in DNMT3A was detected, and (B) a nonsense mutation in TET2 was detected. DNMT3A, DNA methyltransferase 3α; TET2, Tet methylcytosine dioxygenase 2.

Discussion

CMML is a clonal stem cell disorder, with characteristics of myeloproliferative and myelodysplastic neoplasms (1). Due to the heterogeneity of the disease, it is associated with variable clinical courses and outcomes, and an inherent tendency to transform to AML. CMML-I has a 14% chance and CMML-II has a 24% chance of developing into AML within 2 years, increasing to 18 and 63% after 5 years, respectively (4). To date, a number of clinical parameters have been reported to be associated with poor survival time of patients with CMML, including age, sex, Eastern Cooperative Oncology Group performance status (12), Hb level, WBC count, number of circulating immature myeloid cells, proportion of BM blasts, karyotype and β2-microglobulin/lactate dehydrogenase levels (13,14). Furthermore, previous reports have demonstrated that a high proportion of BM blasts, elevated lactate dehydrogenase, male sex and a low Hb level were independent prognostic factors (15). Most recently, cytogenetic status and specific gene mutations have been identified as important prognostic factors, and have been incorporated into the CMML risk stratification system (7,16).

In the present case, a 48, XY, +8, +21 karyotype was detected by conventional cytogenetic analysis. Clonal cytogenetic abnormalities have been reported in >30% CMML cases (17). Multiple prognostic models have been defined to stratify CMML patients into different risk categories, based on the Global MD Anderson Prognostic Scoring system (G-MDAPS) (8,18), Spanish cytogenetic risk stratification system (14) and CMML-specific prognostic scoring system

(CPSS) (18,19). These systems stratify patients based on cytogenetic abnormalities and are effective in predicting survival and leukemic transformation (17). Furthermore, certain prognostic models, including The Groupe Francophone des Myelodysplasies (GFM) (15) and Mayo Molecular Model (MMM) (15) integrate gene mutation into risk assessment, which significantly improves the risk stratification of CMML. In an international collaborative study, Elena *et al* (9) proposed a specific prognostic score based on 260 CMML patients, called the CPSS-Mol (9). This score integrated karyotype with genetic mutation, red blood cell transfusion dependence, WBC count and percentage of BM blasts. It stratified CMML patients into 4 different risk groups that had significantly different median OS times and incidences of leukemic progression (9). The patient discussed in the present study exhibited cytogenetic abnormalities of trisomy 8 and trisomy 21, which are classified as high-risk factors according to the majority of prognostic models, including the CPSS-Mol. Such cases are considered to have a high leukemic transformation rate and shorter OS time.

Molecular abnormalities are identified in >90% CMML patients (20). Genetic sequencing identified DNMT3A and TET2 mutations in the patient discussed in the present study. These genes are involved in epigenetic regulation and DNA methylation. Mutations in DNMT3A, located on chromosome 2q23.3, are identified in 2-5% patients with CMML (21). Considering this low frequency, its prognostic value has not been investigated. Itzykson *et al* (16) reported that DNMT3A does not impact OS or leukemia-free survival (LFS). In addition, Jaiswal *et al* (22) indicated that DNMT3A is associated with age-associated clonal hematopoiesis and increased overall mortality. However, a recent study by Patnaik *et al* (21) indicated that DNMT3A mutations are independent prognostic factors of an inferior OS time. Patients with DNMT3A mutations were demonstrated to be more likely to exhibit low hemoglobin levels, high monocyte counts, a high proportion of bone marrow blasts and abnormal karyotypes. Considering rare DNMT3A mutations have an independent prognostic impact on survival, the gene may be integrated into CMML prognostic models.

TET2 is a member of the TET family, and is located on chromosome 4q24. Mutations in this gene are identified in >60% CMML patients (15). However, to the best of our knowledge, it has not been associated with OS nor LFS, and the prognostic value of TET2 mutations in CMML remains controversial. Previous research has suggested that TET2 mutations have an adverse effect in patients with CMML (23,24). However, other studies have suggested a relatively good prognosis for patients with CMML patients carrying TET2 mutations. For example, Patnaik *et al* (25) demonstrated that the presence of clonal TET2 mutations in the absence of clonal ASXL1 mutations has a favorable effect on OS time. In addition, mutations in TET2 are associated with an improved response to hypomethylating agents (HMAs) (26). However, the prognostic impact of TET2 mutation remains unclear. Furthermore, although the interaction between TET2 and ASXL1 has been studied, the association between TET2 and other genes, including DNMT3A, remain unknown. Therefore, a more comprehensive genomic analysis is required in to study the interaction between TET2 and DNMT3A, and to confirm the exact role of these genes.

Due to the heterogeneity of CMML, there exists no standard therapy. Available treatments include chemotherapy, allogeneic stem cell transplantation (HCT) and HMAs (decitabine and azacitidine) (15). Chemotherapies, including etoposide, cytarabine, topotecan and lonafarnib, have been reported to have poor response rates and severe toxicities (15). Although HCT currently remains the only potentially curative therapeutic strategy for CMML, in the case of old age, poor cytogenetics or high-risk classification, this treatment is not effective (27-29). HMAs, which are approved by the Food and Drug Administration (FDA), remain the safest and most efficient mode of therapy, and the main mode of treatment for high risk CMML (30). Compared with HCT, HMAs are often considered as a first line treatment for patients with CMML-II a high proportion of BM blasts (31). Kantarjian *et al* (32) demonstrated that decitabine treatment was associated with a survival advantage in patients with high-risk MDS compared with intensive chemotherapy. Although these studied (30,31) confirmed the therapeutic effect of HMA in CMML. However the overall response rate (ORR) and complete remission rate (CR) are low, previous studies have indicated that the ORR of patients to HMAs is ~30-40%, with CR only 15% (15,33). In addition, the responses may not be sustained, and can lead to poor OS time and transformation to high risk phenotypes. However, one study described a patient with AML, transformed from CMML, who reached complete remission with decitabine combined with a low dose of cytarabine, aclarubicin and granulocyte colony-stimulating factor (34). Although the results did not have statistical significance, they may offer insight into potential therapeutic strategies for AML, transformed from CMML.

The case described in the present study is relatively rarely observed. However, previous research has demonstrated that loss of response to decitabine in patients who had reached remission from CMML is not uncommonly. For example, Padron *et al* (35) discussed that while the use of decitabine in CMML is FDA-approved, but HMA cannot offer meaningful potential to change the natural history of the disease. In addition, The effectiveness of HMAs is temporary and survival after loss of response is dismal (15). Patnaik *et al* (15) demonstrated that the overall response rates of HMA are about 30-40%, with complete remission rates of 15%. However, the responses are generally not sustained, and the OS time, subsequent to loss of response, is often poor. The results inferred from the present study corroborate these conclusions. Thus, investigation into novel strategies to treat such patients are urgently required.

A number of targeted therapy drugs are currently in preclinical studies, including anti-granulocyte-macrophage colony stimulating factor (GM-CSF) antibodies (36) and JAK inhibitors (35,37). Early studies have demonstrated that GM-CSF hypersensitivity is a hallmark of juvenile myelomonocytic leukemia (JMML) (38). A previous study demonstrated that the majority of CMML cases exhibit GM-CSF hypersensitivity, indicating a potential therapeutic target in reducing CMML cell proliferation via GM-CSF neutralization with targeted anti-GM-CSF monoclonal antibodies or JAK inhibitors (36). Padron *et al* (36) demonstrated that GM-CSF-dependent phosphorylated-signal transducer and activator of transcription sensitivity has therapeutic potential in CMML. A phase I trial of ruxolitinib, JAK1/2 inhibitor, conducted by Padron *et al* (35), identified that ruxolitinib is as safe to administer as pacritinib and momelotinib, with good tolerance and minimal toxicity. In addition, the drugs demonstrated a broad range of activity in hematologic, spleen and symptom response, and correlative analysis highlighted the ruxolitinib-associated cytokine depletion in CMML (35). In addition, a previous study identified that treatment with JAK inhibitors may be downregulated in several genes, including the inflammatory response in myeloproliferative neoplasms (39).

However, the current treatment regime for CMML, and the available therapy for AML transformed from CMML is limited. Based on its aforementioned success, decitabine requires further investigation to be used in combination therapies for CMML. Considering decitabine can temporarily improve CMML and that HCT remains the only potentially curative therapeutic strategy, we suggest that timely hematopoietic stem cell transplantation is necessary. Novel targeted therapies, including JAK inhibitors and targeted anti-GM-CSF monoclonal antibodies, are being developed. These are promising for use in patients with high-risk CMML, which has a tendency to transform to AML.

Conclusion

In the patient presented in the present study, the trajectory of a sudden transformation from CMML, with a high risk of molecular and cytogenetic abnormalities, into AML was observed during decitabine treatment. Although the patient achieved partial remission, an aggressive clinical course and inferior outcome followed, indicating that the identified molecular and cytogenetic abnormalities may have accelerated the course of disease and resulted in its rapid progression. Thus, decitabine as a single agent was unable to produce satisfactory results in high-risk CMML, and novel targeted treatment strategies, are urgently required.

Availability of data and materials

The analyzed data sets generated during the study are available from the corresponding author, on reasonable request.

Author contributions

HL contributed to the conception of the study, and was a major contributor in writing the manuscript. JC designed the study and conducted the analysis. LZ helped perform the analysis and discussions. QX, MMX and SLZ were responsible for data acquisition. BL interpreted the results. The final version of the manuscript was read and approved by all authors.

Ethics approval and consent to participate

The patient provided informed consent for participation in the present study.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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