mutation c.430_431insG leading to a premature stop codon Val144GlyfsX5 inherited from his father and a missense mutation c.389T>G generating a non-conservative amino acid substitution Leu130Arg inherited from his mother. This Leu130Arg substitution is a novel variation that was not detected in 90 healthy controls, so excluding the possibility of a polymorphic change. Both parents and one brother, heterozygous carriers of the c.430_431insG, had no detectable clinical phenotype (Figure 1).

In the second kindred, the patient, a 7-year old boy, was diagnosed at the age of 21 months after a persistent neutropenia and a long history of severe recurrent infections. Bone marrow aspiration showed myeloid dysplasia and arrest at myelocyte stage. Cytogenetic analysis was normal. He was started on G-CSF therapy with an average dose of 5 µg/kg once a day in order to maintain neutrophil count between 1.5 to 3.0×10^9/L. Patient development has been delayed since infancy, with mental and psychomotor retardation, serious walking impairment, and severe bilateral myopia. No episode of seizure was reported in this patient. The sequencing analysis of *HAX1* gene identified the homozygous mutation c.409C>T within exon 3, resulting in a premature stop codon p.Gln137X. This mutation is novel and heterozygous carrier status was confirmed in healthy parents and his sister. No consanguinity was reported among parents, but their origins are from the same geographical area.

So far only 10 *HAX1* mutations are described. The known *HAX1* mutations reported up to date, included the new mutations that we have described, are listed in Table 1.

As shown in the Table, analysis of the patients' genotypes and phenotypes revealed a striking correlation: mutations affecting transcript variant 1 only were associated with SCN, whereas mutations affecting both transcript variants 1 and 2 caused SCN and neurological symptoms, including epilepsy and neurodevelopmental delay.[7] This correlation is confirmed also in our patients. In fact, all the mutations are founded within exon 3, affecting transcript variants 1 and 2 of *HAX1* gene; both patients presented neurodevelopment abnormalities with mental and psychomotor retardation.

Our study describing the first 2 Italian patients with SCN due to *HAX1* mutations indicates that mutations of this gene are not limited to patients with specific ethnic origin. *HAX1* is a ubiquitously expressed gene but its mutations are relatively uncommon. Given this, the description of all new patients and the determination of whether the type of mutation impacts on phenotype and/or susceptibility to leukemic transformation, adds new and precious information needed to better characterize the clinical features of SCN-*HAX1* mutated patients and the role of HAX1 protein.

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References

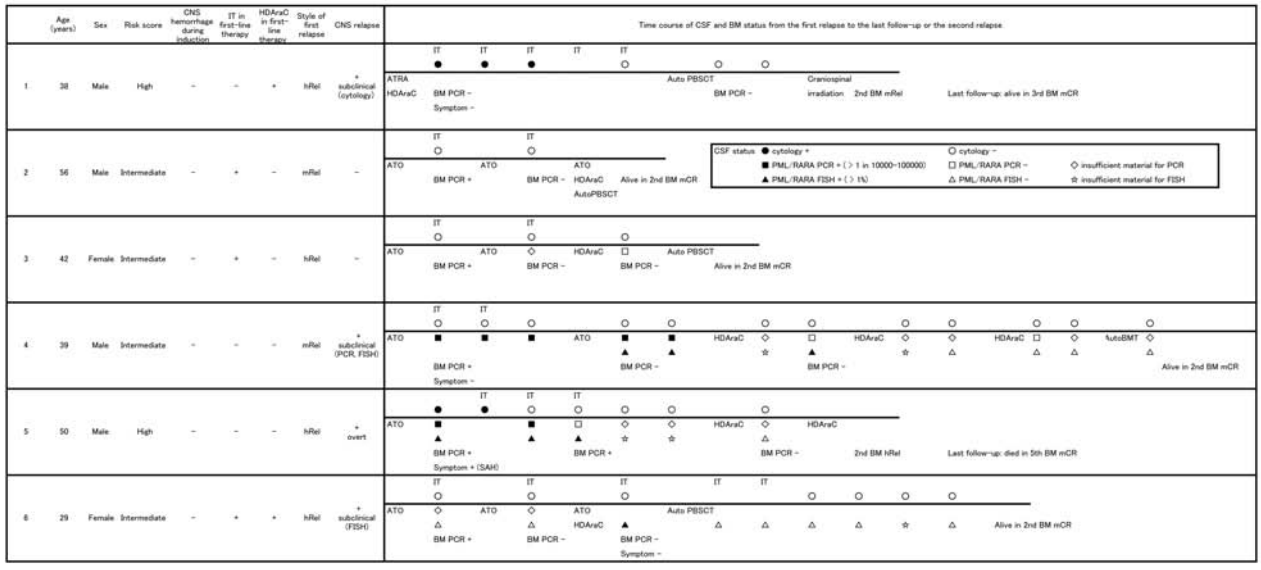
1. Klein C. Molecular basis of congenital neutropenia. Haematologica. 2009;94:(10)1333-6.
2. Horwitz M, Duan Z, Korkmaz B, Lee HH, Mealiffe ME, Salipante SJ. Neutrophil elastase in cyclic and severe congenital neutropenia. Blood. 2007;109(5):1817-24.
3. Klein C, Grudzien M, Appaswamy G, Germeshausen M, Sandrock I, Schaffer AA, et al. HAX1 deficiency causes autosomal recessive severe congenital neutropenia (Kostmann disease). Nat Genet. 2007;39(1):86-92.
4. Germeshausen M, Grudzien M, Zeidler C, Abdollahpour H, Yetgin S, Rezaei N, et al. Novel HAX1 Mutations in patients with severe congenital neutropenia reveal isoform-dependent genotype-phenotype associations. Blood. 2008;111(10):4954-7.
5. Smith BN, Ancljff PJ, Pizzey A, Khwaja A, Linch DC, Gale R. Homozygous HAX1 mutations in severe congenital neutropenia patients with sporadic disease: a novel mutation in two unrelated British kindreds. Br J Haematol. 2008;144(5):762-70.
6. Ishikawa N, Okada S, Miki M, Shirao K, Kihara H, Tsumura M, et al. Neurodevelopmental abnormalities associated with severe congenital neutropenia due to the R86X mutation in the HAX1 gene. J Med Genet. 2008;45(12):802-7.
7. Carlsson G, Elinder G, Malmgren H, Trebinska A, Grzybowska E, Dahl N, et al. Compound heterozygous hax1 Mutations in a Swedish patient with severe congenital neutropenia and no neurodevelopmental abnormalities. Pediatr Blood Cancer. 2009;53 (6):1143-6.
8. Lanciotti M, Caridi G, Rosano C, Pigullo S, Lanza T, Dufour C. Severe congenital neutropenia: a negative synergistic effect of multiple mutations of ELA2 gene. Br J Haematol. 2009;146(5): 573-582.

Molecular or cytogenetic monitoring and preemptive therapy for central nervous system relapse of acute promyelocytic leukemia

We read with great interest the article by Montesinos *et al.*[1] concerning central nervous system (CNS) relapse of acute promyelocytic leukemia (APL). They reported a low incidence of CNS involvement at first relapse in APL patients following therapy without CNS prophylaxis.[1] The optimal management of APL relapse in CNS has taken on increasing significance.[2] Here we report our experience concerning CNS relapse of APL and introduce a new approach with molecular or cytogenetic monitoring in cerebrospinal fluid (CSF) as contrasted with the observations by Montesinos *et al.*[1]

Since 2005, we experience of 6 patients with first relapse of APL at The University of Tokyo Hospital. These patients received different first-line therapies with or without prophylactic intrathecal chemotherapy (IT) and high-dose cytarabine (HDAraC), which are effective for CNS leukemia. All these first-line therapies included all-trans retinoic acid (ATRA) and anthracycline. Patients' characteristics are shown in Table 1. The patients with relapsed APL received arsenic trioxide and prophylactic IT except case 1, who received ATRA,

Table 1. Characteristics of patients with first relapse of acute promyelocytic leukemia at our institute.



CNS: central nervous system; IT: intrathecal chemotherapy; HDAraC: high-dose cytarabine; CSF: cerebrospinal fluid; BM: bone marrow; hRel: hematologic relapse; mRel: molecular relapse; PCR: polymerase chain reaction; FISH: fluorescence in situ hybridization; ATRA: all-trans retinoic acid; auto PBSCT: autologous peripheral blood stem cell transplantation; mCR: molecular complete remission; ATO: arsenic trioxide; auto BMT: autologous bone marrow transplantation; SAH: subarachnoid hemorrhage.

HDAraC, and prophylactic IT. Autologous hematopoietic stem cell transplantation was performed after achieving molecular complete remission.

Early detection of CNS invasion of malignant cells by polymerase chain reaction (PCR) or fluorescence *in situ* hybridization (FISH) has been adopted in some cases with CNS lymphoma or acute lymphoblastic leukemia.[3-5] Although an article reported that the introduction of routine lumbar puncture detected subclinical CNS leukemia in some newly diagnosed APL cases,[6] monitoring by PCR or FISH in CSF of APL patients has never been described. We evaluated the PCR and/or FISH monitoring in cases 3-6 (Table 1). None of the 6 cases showed CNS involvement at the moment of first relapse and all the cases initially had isolated hematologic or molecular bone marrow relapse. However, 4 out of 6 cases subsequently showed overt or subclinical CNS relapse during salvage therapy for first relapse of APL. All the cases with CNS relapse were with intermediate- or high-risk score,[7] which was consistent with the observations by Montesinos *et al.*[1] Case 5 showed simultaneous appearance of CNS involvement of APL and subarachnoid hemorrhage, as we described previously.[8] He received therapeutic IT repeatedly until all markers of CNS relapse, including cytology, PCR, and FISH, turned negative. Surprisingly, in the other 3 cases, we detected subclinical CNS relapse at the moment of routine prophylactic IT. For these patients, we provided preemptive IT or HDAraC repeatedly until the markers of CNS relapse turned negative. Although cytological examination detected subclinical CNS relapse in case 1, cytology remained negative in cases 4 and 6 when PCR and/or FISH markers turned positive. In addition, because cytology turned negative earlier than PCR and FISH markers in case 5, these novel monitoring methods were also useful for determining when to finish therapy for overt CNS relapse. Therefore, PCR and/or FISH were more sensitive than conventional cytological monitoring for detecting CNS relapse and residual disease during the preemptive therapy. On the other hand, it has been mentioned that the PCR approach in CSF has some pitfalls.[3,9] It is sometimes difficult to obtain sufficient DNA for PCR when cell counts are low, and false-positive results may be obtained owing to contamination with blood in the lumbar puncture procedure.[3,9] In fact, we could not obtain sufficient DNA for PCR in some CSF samples (Table 1). It is noteworthy that FISH analysis was positive in spite of negative PCR result in some samples of cases 4 and 5. This indicates that PCR results were false-negative because of insufficient material, suggesting superiority of FISH to PCR in monitoring CNS relapse in CSF samples when cell counts are low. False-positive results owing to contamination with blood were not suggested in our PCR and FISH analyses.

Montesinos *et al.*[1] did not mention whether their patients with isolated first bone marrow relapse experienced subsequent CNS relapse.

We consider this information to be indispensable because our observations indicated that subsequent CNS relapses during salvage therapy of relapsed APL are highly common, even if an incidence of CNS involvement at the moment of first relapse is low. We prevented and managed overt CNS relapses by adopting molecular monitoring and prophylactic or preemptive IT. Because overt CNS relapse can cause neurological symptoms and is difficult to cure, early detection is important. Therefore, we believe that these strategies should be considered for patients with relapsed APL, as contrasted with the undetermined role of CNS prophylaxis for patients with newly diagnosed APL.

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References

1. Montesinos P, Diaz-Mediavilla J, Deben G, Prates V, Tormo M, Rubio V, et al. Central nervous system involvement at first relapse in patients with acute promyelocytic leukemia treated with all-trans retinoic acid and anthracycline monochemotherapy without intrathecal orophylaxis. Haematologica. 2009;94(9): 1242-9.
2. Nagai S, Takahashi T, Kurokawa M. Beneficial and adverse effects of molecularly targeted therapies for acute promyelocytic leukemia in central nervous system. CNS Neurol Disord Drug Targets. 2009 [Epub ahead of print].
3. Gleissner B, Siehl J, Korfel A, Reinhardt R, Thiel E. CSF evaluation in primary CNS lymphoma patients by PCR of the CDR III IgH genes. Neurology. 2002;58(3):390-6.
4. de Haas V, Vet RJ, Verhagen OJ, Kroes W, van den Berg H, van der Schoot CE. Early detection of central nervous system relapse by polymerase chain reaction in children with B-precursor acute lymphoblastic leukemia. Ann Hematol. 2002;81(1):59-61.
5. van Oostenbrugge RJ, Hopman AH, Arends JW, Ramaekers FC, Twijnstra A. Treatment of leptomeningeal metastases evaluated by interphase cytogenetics. J Clin Oncol. 2000;18(10):2053-8.
6. Breccia M, Carmosino I, Diverio D, De Santis S, De Propris MS, Romano A, et al. Early detection of meningeal localization in acute promyelocytic leukaemia patients with high presenting leucocyte count. Br J Haematol. 2003;120(2):266-70.
7. Sanz MA, Lo Coco F, Martin G, Avvisati G, Rayon C, Barbui T, et al. Definition of relapse risk and role of nonanthracycline drugs for consolidation in patients with acute promyelocytic leukemia: a joint study of the PETHEMA and GIMEMA cooperative groups. Blood. 2000;96(4):1247-53.
8. Nagai S, Asai T, Watanabe T, Oshima K, Hangaishi A, Kanda Y, et al. Simultaneous appearance of central nervous system relapse and subarachnoid hemorrhage during the treatment for acute promyelocytic leukemia. Ann Hematol. 2008;87(7):593-5.
9. DeAngelis LM, Cairncross JG. A better way to find tumor in the CSF? Neurology. 2002; 58(3):339-40.

Mobilization of *PML/RARα* negative peripheral blood stem cells with a combination of G-CSF and CXCR4 blockade in relapsed acute promyelocytic leukemia pre-treated with arsenic trioxide

Very recently Montesinos *et al*. reported on the incidence of central nervous system (CNS) involvement at first relapse in patients with acute promyelocytic leukemia (APL) who had been treated with all-*trans* retinoic acid (ATRA) and anthracycline monochemotherapy without intrathecal prophylaxis.[1] Although this study showed a relatively low incidence of CNS involvement at first relapse, controversy over treatment options remains. The introduction of ATRA and more recently arsenic trioxide (ATO) has changed treatment options and outcome for APL.[2-3] In the setting of relapsed APL, ATO is currently regarded as the preferential remission induction therapy. However, for patients achieving complete remission (CR) thereafter, appropriate consolidation strategies have not yet been defined.[4] Autologous hematopoietic stem cell transplantation (HSCT) is one treatment option in relapsed APL. Here, we report a patient who had been diagnosed with relapsed APL involving the CNS and who achieved a second CR after ATO salvage therapy. Mobilization of peripheral blood stem cells (PBSC) was accomplished using a combination of granulocyte-colony stimulating factor (G-CSF) and CXCR4 blockade.

A 40-year old woman experienced extramedullary relapse of APL while on maintenance therapy after having achieved CR with ATRA containing induction chemotherapy. Due to multilocular CNS manifestation as well as molecular bone marrow involvement, ATO was started (5 cycles, 0.15 mg/kg, day 1-13) in parallel with local irradiation. Liposomal cytarabine (7 applications, 50 mg absolute/week) was applied intrathecally in order to treat meningeosis. After a molecular analysis of the bone marrow had shown negativity for *PML-RARα* transcripts after ATO and intrathecal therapy, G-CSF mobilization was started out of steady state in order to collect PBSC for autologous HSCT. While the WBC peaked at 31 Gpt/L, only 6/μL $CD34^+$ cells could be measured in the peripheral blood. The corresponding apheresis yield was only 0.9×10^6/kg $CD34^+$ PBSC. In order to achieve a target of $>2\times10^6$/kg $CD34^+$ PBSC, the patient received the CXCR4 antagonist AMD3100 subcutaneously at a dose of 240 μg/kg ten hours prior to the next apheresis in addition to G-CSF within a compassionate use program. CXCR4 blockade led to an increase in WBC (44 Gpt/L) and $CD34^+$ count (9/μL) with a subsequent harvest of 1.2×10^6/kg $CD34^+$ PBSC. Interestingly, both apheresis products were found to be *PML-RARα*-PCR negative (Figure 1). Sensitivity of nested PCR for *PML-RARα* was achieved according to the minimal target sensitivity of 10^{-4}.[5] Three weeks later, myeloablative conditioning containing 12 Gy total body irradiation (day -6 to -4) and 120 mg/kg of intravenous cyclophosphamide (day -3 to -2) was performed and followed by reinfusion of PBSC on day 0. Fast and stable trilineage engraftment was documented with neutrophils >0.5 Gpt/L and platelets > 50 Gpt/L on day +14 and +16, respectively. Three years later (day +1,144 after autologous HSCT) the patient remains in complete hematologic remission without clinical signs of extramedullary disease.

Arsenic trioxide has recently been shown to play an emerging role in relapsed and refractory APL with the majority of patients achieving a complete molecular remission.[3,6] Following molecular CR after ATO treatment, subsequent collection of PBSC and autologous HSCT after myeloablative chemotherapy is recommended but discussed controversially with regard to the best consolidation strategy.[4,7] Harvesting a satisfactory amount of $CD34^+$ PBSC after repetitive chemotherapy regimens might be challenging. Sequential therapy with ATO might even decrease the hematopoietic capacity. Application of AMD3100 in addition to G-CSF displays a possible option to compensate for poor HSC mobilization. Albeit, leukemic blasts are known to express CXCR4 and could, therefore, become potential targets of AMD3100.[8] Data in a murine model suggest that AMD3100 administration leads to an increased time-dependent mobilization of APL blasts by interrupting the CXCR4-SDF-1 axis.[9] But for AML in general no clinical trials exist in order to confirm or disprove whether mobilizing leukemic stem cells reflect a relevant problem in this setting. DiPersio *et al*. advised caution and stated that AMD3100 might not be intended for mobilization and harvest in patients with leukemia.[10]

Our limited experience in this patient suggests at least that in case of molecular remission, no apparent mobilization of *PML-RARα* positive cells occurred. Whether differ-