

# Emergence of an unrelated highly aberrant clone in an AML patient at relapse four months after peripheral blood stem cell transplantation

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We report a case of AML-M1 with 5q aberration at diagnosis. The patient was treated with high-dose chemotherapy (HDCT). After remission induction, he received allogeneic peripheral blood stem cell transplantation (PBSCT) from an HLA-match donor brother. The successive follow-up conventional cytogenetics investigations in remission after HDCT and PBSCT revealed cytogenetic remission. The most interesting observation in this case is that relapsed marrow revealed the emergence of an entirely new, highly aberrant, unrelated clone with unusual translocations t(6;17)(p23;p11.2), +8, der(8)dup inv(8)(q23qter), t(10;19)(q26;q13.3) 4½ months after PBSCT. Our findings suggest the possibility of a mutagenic effect of HDCT and myeloablative intense chemotherapy before PBSCT that could have induced a genetic lesion in the recipient's genetically unstable stem cells in an environment of immunosuppression. The highly complex nature of the clone and the rapid clonal evolution indicates the possibility of selective pressure with proliferative advantage.

**Key words:** Acute myeloid leukemia, emergence, intense chemotherapy, mutagenic effects, peripheral blood stem cell transplantation, unusual aberrant clone

## Introduction

Extensive cytogenetics investigations have been carried out to evaluate the genotoxic effects of various anticancer drugs.<sup>[1-3]</sup> High-dose chemotherapy (HDCT) as an induction therapy, followed by consolidation, or HDCT as a conditioning regimen before transplantation is the general practice for disease management in patients with acute myeloid leukemia (AML). The mutagenic and carcinogenic effects of alkylating agents have been documented *in vitro* and/or *in vivo* in human bone marrow cells.<sup>[4-7]</sup> The therapy-related malignancies

like post-BMT or post-chemotherapy/radiation-induced leukemia frequently show aberrations of chromosomes 5, 7, 11q23, and 21q22 at early stages, probably due to genetic damage to the residual malignant cells.<sup>[6-9]</sup>

We report here a case of AML-M1 with 5q aberration at diagnosis. The patient was treated with HDCT followed first by peripheral blood stem cell transplantation (PBSCT) and later on by bone marrow stem cell transplantation. We performed prospective cytogenetics and VNTR studies at diagnosis and at regular intervals in remission after HDCT transplantation and at relapse. Our sequential cytogenetics studies revealed the emergence of a new, unusual, aberrant clone in relapse within a short interval of 30 days after PBSCT-induced cytogenetic remission. This warrants the genotoxic effect of chemotherapeutic drugs, which probably target genetically susceptible stem cells.

## Case Report

A 17-year-old boy was admitted in Tata Memorial Hospital with complaints of anemia, vomiting, and distension of the abdomen. His hematological findings were as follows: WBC  $23 \times 10^9/l$ , platelets  $112 \times 10^9/l$ , and Hb 7.66 gms/dl. Bone marrow aspirate (BMA) showed 90% blasts with cytoplasmic granules and Auer rods. The patient was diagnosed as AML with FAB M1 subtype. Immunophenotype screening of blasts by flow cytometry showed positive CD13 69%, CD33 74%, CD34 27%, and HLA DR 82%; it showed negative CD14, CD 36, as well as B- and T-cell markers.

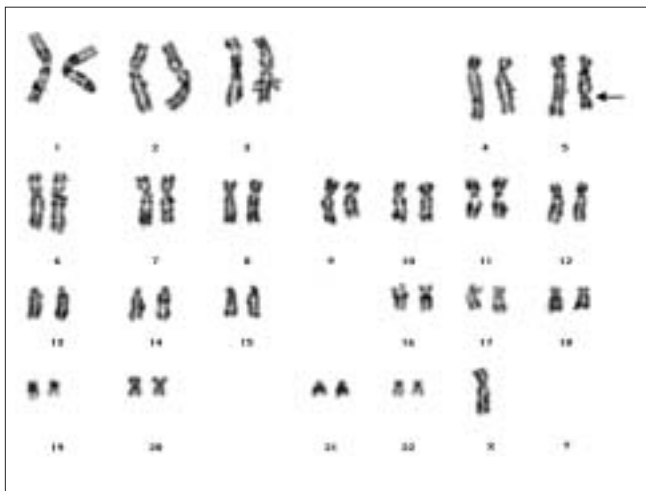
Conventional cytogenetics was carried out on unstimulated bone marrow cells. The BMA cells were harvested after 30 min and/or 24 h of incubation. Cytogenetic analysis at diagnosis revealed normal karyotypes as well as abnormal karyotypes [Table 1]. Abnormal karyotype was defined as 46,XY,dic(5)(q22). Loss of Y was detected as a random aberration [Table 1, Figure 1]. VNTR study using markers D17S250 and D17S579 was performed for chimerism analysis of post-transplanted recipient marrow. The PCR product length of donor and recipient DNA revealed polymorphism [Figure 2]. The patient was immediately put on primary HDCT. HDCT, consisting of idarubicin 200 mg/m<sup>2</sup> for 3 days and cytarabine 100 mg/m<sup>2</sup> for 7 days, was administered. Thereafter, he was reinduced with cytarabine 20 gm/m<sup>2</sup> for 5 days followed by consolidation of Ara-C for 4 days in two courses. Hematopathology of post-chemotherapy BMA (3 months after HDCT) showed 6% blasts. Cytogenetic analysis revealed a major clone

of normal karyotypes, 46,XY (13 cells) and a minor clone of 46,XY,dic(5q) (2 cells) [Table 1]. Four months after diagnosis the patient underwent allogeneic peripheral stem cell transplantation from his HLA-matched donor brother. Before transplantation the patient received a myeloablative regimen which included busulphan 16 mg/kg for 4 days and cyclophosphamide 128 mg/kg for 2 days. The patient also received GVHD (graft vs. host disease) prophylaxis with cyclosporin, methotrexate (600 mg/m<sup>2</sup>), and GM-CSF (5 µg/kg). On day 7 of transplantation, he developed grade I skin GVHD and upper GI GVHD. He was treated with methylprednisolone for 7 days. His bone marrow examination showed hypocellular marrow with 1% blasts. Cytogenetic evaluation on the 28<sup>th</sup> day after PBSCT showed a normal karyotype, 46,XY [Table 1]. Thereafter, the patient remained in remission for 4½ months. VNTR analysis revealed donor chimerism [Figure 2]. A month later, the patient presented to the clinic with fever, cough, and splenomegaly. Peripheral blood showed WBC 17.4 × 10<sup>9</sup>/l, platelets 41.1 × 10<sup>9</sup>/l, and 30% blasts. Bone marrow examination revealed 38% myeloid blasts by morphology, indicating relapse. Simultaneously, cytogenetic analysis in marrow cells identified a new hyperdiploid clone with the unusual translocations t(6;17)(p23;p11.2) and t(10;19)(q26.1;q13.3), along with trisomy 8 and der(8)dup inv(8)(q23qter) [Table1 and Figure 3]. VNTR analysis showed recipient chimerism in the bone marrow cells [Figure 2]. FISH was performed on a fixed cytogenetic preparation of bone marrow cells at relapse, using Vysis

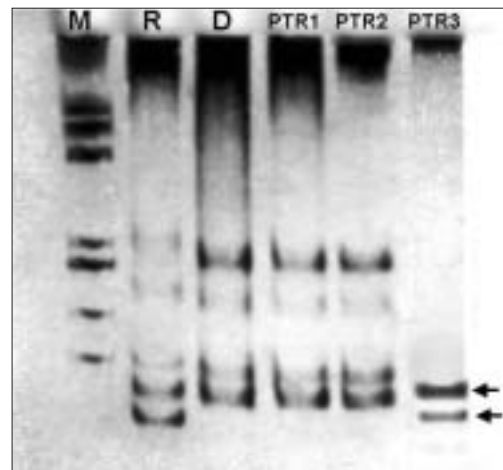
**Table 1: Sequential conventional cytogenetic analysis at diagnosis, in remission, and at relapse**

Status	Karyotype
At diagnosis	46,XY[10]/46,XY,dic(5q22)[3], 45,X,-Y,dic(5q22) [1]
Post-chemo remission	46,XY[13]/46,XY,dic(5q22) [2]
Post-PBSCT remission (28 <sup>th</sup> day)	46,XY [20]
Post-PBSCT remission (105 <sup>th</sup> day)	46,XY [15]
Post-PBSCT relapse (137 <sup>th</sup> day)	47,XY,t(6;17)(p23;p11.2), +8, der(8)dup inv(8)(q23qter), t(10;19)(q26.1;q13.3) [20]
Post-PBSCT relapse (179 <sup>th</sup> day)	47,XY,t(6;17)(p23;p11.2), +8, der(8)dup inv(8)(q23qter), t(10;19)(q26.1;q13.3) [20]
Post-BMT remission (on 28 <sup>th</sup> day)	46,XY [20]

[ ]: No. of cells



**Figure 1: Karyotype, 45,X,-Y,dic(5q22) at diagnosis**



**Figure 2: VNTR analysis by PCR. Tracks are (M) molecular weight marker ØX174, (R) recipient DNA, (D) donor DNA, (PTR1) (1<sup>st</sup> post-transplant 1<sup>st</sup> remission marrow), and (PTR2) (1<sup>st</sup> post-transplant 2<sup>nd</sup> remission marrow), (PTR3) (1<sup>st</sup> post-transplant relapse marrow)**

Inc. CEP 8, 10, 19q subtelomeric probe (TelVysion) and locus-specific MYC break apart rearrangement probe of locus 8q24. Interphase and metaphase FISH analysis revealed trisomy 8 [Figure 4A] and confirmed t(10;19) [Figure 4B]. FISH for trisomy 8 and t(10;19) was also done on a fixed cytogenetic preparation of bone marrow cells at diagnosis to rule out the presence of masked clones of +8, t(10;19) that might have been missed. The analysis revealed absence of trisomy 8 and t(10;19) at diagnosis. The patient was treated with standard recommended doses of fluradibine, idarubicine, Ara-C, and Myelotarg for 4 days. However, the patient failed to respond to chemotherapy. His marrow examination showed 66% blasts. Repeat cytogenetics analysis

revealed a similar karyotype picture to that noted before: 47, XY, t(6;17), +8, der(8)dup inv(8)(q23qter),t(10;19). The patient underwent a second transplant, i.e., bone marrow transplant from the same HLA-match brother 52 days after the first relapse. He received melphalan 90 mg/m<sup>2</sup> as a nonmyeloablative conditioning regimen. He was also given GVHD prophylaxis which included cyclosporin and methotrexate. A month later, after BMT, hematological evaluation of BMA showed hypocellular marrow with no blasts. Cytogenetic analysis also revealed a normal karyotype pattern, 46,XY. However, the patient developed GVHD within a short period. He had febrile neutropenia and flaring of fungal infection. He was given platelet transfusion for 6 weeks. His CT scan showed multiple enhancing regions in the right hemisphere and the cerebellum. The patient died within 4 months of the second transplantation.

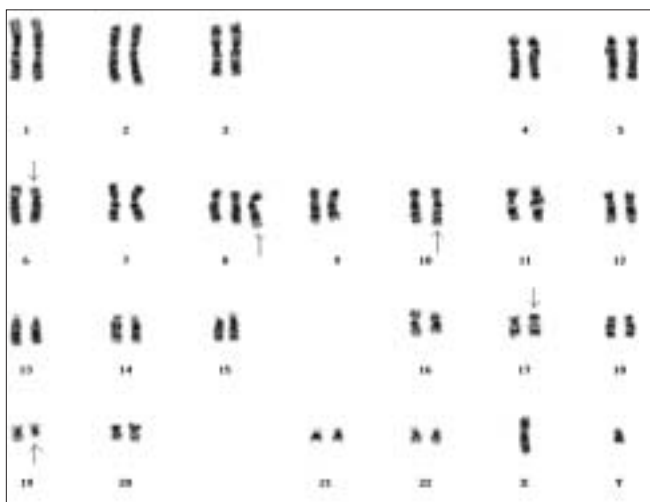


Figure 3: Karyotype, 47, XY,t(6;17)(p23;p11.2), +8,dup inv(8)(q23qter), t(10;19)(q26.1;q13.3) at relapse

## Discussion

The present case of AML FAB M1 had a minor clone of dic(5q22) as a primary aberration at diagnosis, which is a rare finding. Aberrations of 5q frequently occur as secondary aberrations in patients with AML.<sup>[10,11]</sup> Except MDS, aberrations of 5q generally occur as a part of the complex karyotype in *de novo* AML.<sup>[6,11,12]</sup> Post-chemotherapy remission marrow showed the persistence of a dic(5q) clone along with a major normal diploid clone. Later on, successive follow-up cytogenetic

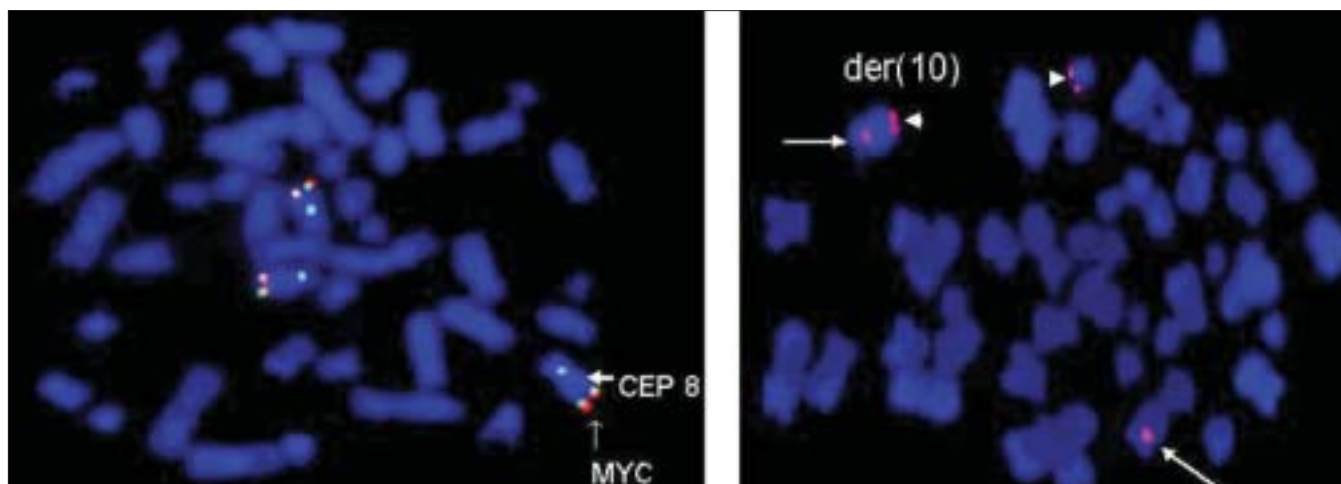


Figure 4: (A) FISH with CEP 8 (aqua) and LSI dual-color MYC probe on metaphase cell shows aqua signal of CEP 8 and orange/green signal of MYC at locus 8q24: signal pattern is indicative of trisomy 8. (B) FISH with CEP 10 (orange) and subtelomeric 19q probe (orange) on metaphase cell shows orange signal of CEP 10 at centromeric region and orange signal of subtelomeric 19q on normal 19 homologue and at 10q26, which is indicative of t(10;19)(q26.1;q13.3)

studies after PBSCT revealed a normal diploid karyotype, indicating successful peripheral stem cell transplantation with rejuvenation of normal hematopoiesis, which was supported by VNTR analysis that revealed donor genotype.

The induction of chromosomal damage has generally been reported after 3 years, and latest after more than 10 years, in long-term survivors after total body radiation and/or intense chemotherapy as a conditioning regimen before transplantation.<sup>[9,13-15]</sup> Some of these studies showed the persistence of therapy-induced aberrations in complete remission, which did not affect the prognosis of the patient.<sup>[13-16]</sup> Overall, these reported studies have demonstrated that genotoxic induction of radiation and chemotherapy is a slow process, requiring a long period for the development and clonal evolution of the genetically abnormal clone.

In view of these literature reports, our case of AML M1, in which the emergence of an unrelated, highly aberrant clone of multiple aberrations occurred within a short period of 30 days after cytogenetic remission followed by myeloablative allogeneic PBSCT, is a very unusual one. Prebet *et al.* have reported an MDS case who developed Ph 3 months after nonmyeloablative allogeneic PBSCT, which is another rare report.<sup>[16]</sup>

Our previous sequential studies in chronic myeloid leukemia (CML)-BP (blastic phase) has demonstrated the genotoxic properties of busulphan in comparison with hydroxyurea (HU) as displayed by the occurrence of a higher frequency of chromosomal abnormalities in CML-BP who were treated with busulphan/HU during the chronic phase.<sup>[5]</sup> In the present case, the genotoxic effect of alkylating drugs, including GVHD prophylaxis to induce multiple clonal aberrations in a short interval, is unusual. The mutagenic effect of a myeloablative intense chemotherapy regimen in an environment of immunosuppression might have induced genomic damage and triggered new aberrations in the recipient's stem cells, which are supposed to be genetically unstable. This may suggest the possibility of the origin of a genetically damaged donor stem cell clone which remained dormant during chemotherapy and later on recurred and evolved with selective pressure in an environment of immunosuppression. However, the VNTR pattern of relapsed marrow does not support the donor

origin and excludes the possibility of secondary leukemia of donor origin.

There is only one case of non-B, non-T-ALL involving t(10;19)(q26;q13) reported in the literature.<sup>[17]</sup> Translocation in chromosome 19, involving band q13, a locus of *bcl3*, has been demonstrated commonly in B-CLL<sup>[18]</sup> and in malignant brain tumor.<sup>[19]</sup>

Recurrent aberrations of 6p23 and 17p11.2 are not uncommon in AML.<sup>[12]</sup> However, t(6;17)(p23;p11.2) and t(10;19)(q26;q13) are unusual. Both 6p23 and 17p11.2 loci harbor important genes involved in carcinogenesis. These are, namely, murine Friend leukemia integration site homologue, *DEK* oncogene at 6p23 and signal transducer pseudogene, rhabdomyosarcoma-like pseudogene, and mitotic spindle assembly regulator gene at 17p11. Patients with complex karyotype and trisomy 8 usually have a very poor outcome.<sup>[20]</sup>

We conclude that in the present case with a sole 5q aberration at diagnosis, the emergence of a new, highly malignant, unrelated clone of multiple aberrations within a short period in relapsed PBSCT marrow is an unusual and very rare finding. It is probably a result of the mutagenic and immunosuppressive effect of a HDCT and PBSCT conditioning regimen that could have induced genetic toxicity in the recipient's genetically unstable stem cells. The highly complex nature of the clone and the rapid clonal evolution also raises the possibility of selective pressure with proliferative advantage.

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