

## CLINICAL CASE

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# Clonal heterogeneity in a patient with acute promyelocytic leukemia

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### Summary

A 18-year-old man was diagnosed with acute promyelocytic leukemia (APL). The conventional cytogenetic analysis revealed normal karyotype 46, XY, t(15; 17). Reverse transcriptase polymerase chain reaction (RT-PCR) identified PML-RARa chimeric transcripts. Complete remission (CR) was attained with 3 induction courses of Ara-C, daunorubicin and all-trans retinoic acid (ATRA). Three years later the patient relapsed. The blasts in bone marrow aspirate at relapse had AML-M3 morphology, and RT-PCR was positive for PML-RARa tran-

scripts. The patient was treated with ATRA and daunorubicin without success. Two months later the blasts in bone marrow aspirate showed AML-M2 morphology, the karyotype was 47, XY, +8 and RT-PCR revealed the presence of AML1-ETO transcripts and absence of PML-RARa transcripts. The patient attained second CR with 3 induction courses – a course with Ara-C and daunorubicin and 2 courses with idarubicin, Ara-C and etoposide.

**Key words:** karyotype, leukemia, morphology, relapse, remission, transcripts

### Introduction

Acute promyelocytic leukemia is characterized by specific clinical, cytogenetic and molecular features. The t(15; 17) chromosomal translocation that fuses the PML and RARa genes and creates the chimeric PML-RARa gene is a genetic hallmark of APL. The molecular abnormality is presented in the leukemic cells of all patients and the blasts exhibit unique sensitivity to the differentiating action of ATRA [1]. Because of this sensitivity to ATRA, APL patients with t(15; 17) are regarded as a favorable group among acute leukemia patients with CR rates of 80-90%.

However, a subset of APL patients may present

atypical cytogenetic or molecular features at different stages of the disease, with an incidence ranging from 29% to 43% [2,3].

The balanced translocation between chromosomes 8 and 21 that fuses the AML1 and ETO genes and results in a AML1-ETO gene is typically detected in patients with acute myeloid leukemia (AML)-M2 according to the French-American-British (FAB) classification and more rarely in patients with AML-M4 [4].

Trisomy 8 was the most frequent secondary change reported in many studies – in about 25% from t(15; 17) positive APL cases [5,6].

We report on a patient with clonal switch to AML1-ETO-positive PML-RARa-negative AML-M2 with trisomy 8 as a sole cytogenetic abnormality, which was observed shortly after the relapse of primary PML-RARa-positive APL.

### Case presentation

In August 1998, an 18-year-old male presented with epistaxis, gingival bleeding and petechiae. The hemoglobin level was 76 g/l, the platelet count  $10 \times 10^9/l$

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and the white blood cell count  $5.1 \times 10^9/l$ , with 57% blasts.

The diagnosis was made by standard morphological examination and cytochemical analysis of blood and bone marrow smears according to the criteria established by the FAB classification.

The metaphases chromosomes were prepared from bone marrow cells after 24 h *in vitro* cultures without stimulation, followed by routine harvesting and G-banding. The karyotypes were reported according to the ISCN [7].

RT-PCR for *PML-RARa* and *AML1-ETO* were performed according to the recommendations and following the protocols of BIOMED-1 Concerted Action [8].

The bone marrow aspirate was hypercellular with 83% blasts with typical AML-M3 morphology (Figure 1). The conventional cytogenetic analysis revealed karyotype 46, XY, t(15; 17); RT-PCR identified *PML-RARa* chimeric transcripts.

CR was attained with 3 induction courses of Ara-C (200 mg/m<sup>2</sup>, days 1-7) + daunorubicin (45 mg/m<sup>2</sup>, days 1-3) and ATRA (45 mg/m<sup>2</sup>/d) for 60 days in January 1999. The patient remained in CR until January 2002 with maintenance therapy with ATRA 45 mg/m<sup>2</sup>, 15 days every 3rd month.

In January 2002, the patient relapsed. The bone marrow aspirate showed 34% blasts with M3 morphology. RT-PCR was positive for *PML-RARa* transcripts. The patient was treated with ATRA (45 mg/m<sup>2</sup>/d) and daunorubicin (45 mg/m<sup>2</sup> for 4 days) without success.

In March 2002, the bone marrow aspirate contained 54% blasts with AML-M2 morphology (Figure 2); the karyotype was 47, XY, +8. RT-PCR re-

vealed the presence of *AML1-ETO* transcripts and absence of *PML-RARa* transcripts.

All previously taken samples were reexamined by RT-PCR for the presence of *AML1-ETO* transcripts. Negative results were observed in diagnostic samples as well as in samples taken during the remission period, but the samples from January 2002 were positive for *AML1-ETO* transcripts.

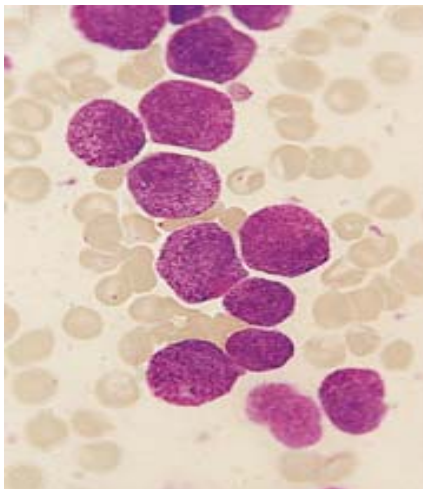
The patient attained second CR with 3 induction courses – a course of Ara-C (200 mg/m<sup>2</sup>, days 1-7) + daunorubicin (45 mg/m<sup>2</sup>, days 1-3) and 2 courses with idarubicin (12 mg/m<sup>2</sup>, days 1-3) + Ara-C (200 mg/m<sup>2</sup>, days 1-7) + etoposide (75 mg/m<sup>2</sup>, days 1-7), in July 2002. The maintenance therapy included idarubicin (12 mg/m<sup>2</sup>, day 1) + Ara-C (200 mg/m<sup>2</sup>, days 1-5) every month, and ATRA 45 mg/m<sup>2</sup>, 15 days every 3rd month. The molecular follow-up detected persistence of *AML1-ETO* and absence of *PML-RARa* transcripts.

The patient relapsed again in September 2003 with AML-M2 and died 2 months later.

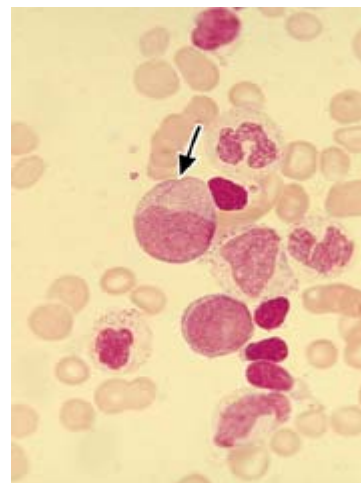
## Discussion

Although a majority of APL cases are characterized by t(15; 17), leading to the formation of *PML-RARa* fusion gene, additional cytogenetic or molecular abnormalities have been reported in some cases.

Co-expression of both markers (*PML-RARa* and *AML1-ETO*) is a rare finding in AML and only few such patients have been reported. In some cases *PML-RARa* and *AML1-ETO* were detected at the onset of the disease in cases of *de novo* chimeric M3: M2



**Figure 1.** Bone marrow aspirate in AML-M3 at diagnosis (May-Grunwald-Giemsa  $\times 1400$ ).



**Figure 2.** Bone marrow aspirate in AML-M2 at relapse with an Auer rod (arrow) (May-Grunwald-Giemsa  $\times 1400$ ).

AML [9-12] or simultaneously in the one and same AML-M2 cells [13]. In others, *AML1-ETO* transcripts appeared as an additional molecular abnormality during the relapse in *PML-RARa*-positive APL patients [12,14].

Relapse of APL after successful ATRA/chemotherapy treatment with a different subtype of AML is a rare event.

In our patient only one leukemic population with AML-M3 characteristics (blasts with typical M3 morphology, t(15; 17), *PML-RARa* transcripts) was established at diagnosis. Three years later the patient relapsed. The relapse was with AML-M3 characteristics. Soon after the unsuccessful treatment of relapse of primary *PML-RARa*-positive APL, a distinct leukemic population with AML-M2 characteristics (blasts with AML-M2 morphology, *AML1-ETO* transcripts) was established. The karyotype was 47, XY, +8 with *PML-RARa*-negative blasts.

This clonal heterogeneity at relapse is difficult to interpret and raises several questions:

1. Whether the two molecular abnormalities existed simultaneously at disease presentation or *AML1-ETO* transcripts appeared in addition to *PML-RARa* later, at the relapse of the primary APL.

2. Whether the relapse represents a single leukemia cell clone bearing both molecular abnormalities or two distinct clones characterized by one of the markers each.

3. Whether the clone, which emerged at relapse, represents a second leukemia.

It might be supposed that the *AML1-ETO*+ clone existed at diagnosis in a very low number of cells as a silent "preleukemic clone" without final transformation to malignancy until a second crucial molecular event drove one of these cells to overt leukemia [15]. This possibility is supported by the observation that low number of *AML1-ETO*+ cells are frequently generated by permanent mutagenesis in a significant proportion of adults without AML [16].

In our patients the relapse was characterized by the presence of both fusion transcripts. However, afterwards *PML-RARa* transcripts disappeared and *AML1-ETO* remained the only molecular finding. Thus it seems improbable that a malignant cell bears simultaneously both abnormalities, as it was reported in another case earlier [13], and we suppose that the detected *AML1-ETO* signal originated from a new distinct clone.

The absence of *AML1-ETO* transcripts at diagnosis and in the samples taken during the first remission as well as the different morphology of the leukemic population (M2 versus M3), the cytogenet-

ic findings [+8 versus t(15; 17)] and the presence of *AML1-ETO* transcripts at relapse support the hypothesis that the relapse represented two distinct clones characterized by one of the markers each. It might be speculated that the combined ATRA/chemotherapy at relapse eliminated or reduced the APL clone but this therapy was ineffective for the progression of the M2 clone.

It might be argued that our patient developed a second AML-M2 simultaneously and independently. What could be the cause of this second leukemia?

1. Therapy-related secondary leukemia?

It is reasonable to speculate that the secondary leukemia may be therapy-related. Firstly, the patient was exposed to drugs classically implicated in the pathogenesis of therapy-related (t-) AML. Furthermore, the patient was found to carry trisomy 8 at relapse, which is one of the most common unbalanced chromosomal abnormalities observed in t-myelodysplasia and t-AML [17].

2. Clonal switch?

The second leukemia might represent a clonal switch in the primary leukemia clone, since the consecutive cytological and molecular genetic examination revealed a shift from a composite M3: M2 morphology with co-expression of both *PML-RARa* and *AML1-ETO* transcripts towards a characteristic M2 morphology with persistence of *AML1-ETO*.

In conclusion, this case provides additional evidence for the multistep mechanism of progression of the disease, as well as the close association between molecular and morphological abnormalities.

The co-existence of t(15; 17) with other specific translocations may provide useful information on the level of cells from which leukemias originate.

Finally, the co-existence of different specific translocations offers some data with prognostic relevance.

## References

1. Grignani F, Fagioli M, Alcalay M et al. Acute promyelocytic leukemia: from genetics to treatment. *Blood* 1994; 83: 10-25.
2. Grimwade D, Howe K, Langabeer S et al. Establishing the presence of the t(15; 17) in suspected acute promyelocytic leukemia: cytogenetic, molecular and PML immunofluorescence assessment of patients entered into the MRC ATRA trial. MRC Adult Leukemia Working party. *Br J Haematol* 1996; 94: 557-573.
3. Slack JL, Arthur DC, Lawrence D et al. Secondary cytogenetic changes in acute promyelocytic leukemia – prognostic importance in patients treated with chemotherapy alone and association with the intron 3 breakpoint of the PML

- gene: a Cancer and Leukemia Group B study. *J Clin Oncol* 1997; 15: 1786-1795.
4. Ferrara F, Del Vacchio L. Acute myeloid leukemia with t(8; 21)/AML1/ETO: a distinct biological and clinical entity. *Haematologica* 2002; 87: 306-319.
  5. Schoch C, Haase D, Haferlach T et al. Incidence and implication of additional chromosome aberrations in acute promyelocytic leukemia with translocation (15; 17)(q22; q21): a report on 50 patients. *Br J Haematol* 1996; 94: 493-500.
  6. Sessarego M, Fugazza G, Bruzzone R et al. High frequency of trisomy 8 in acute promyelocytic leukemia: a fluorescence in situ hybridization study. *Cancer Genet Cytogenet* 1997; 97: 161-164.
  7. Mitelman F (ed). An international system for human cytogenetic nomenclature. Switzerland, Basel: S. Karger Publ Inc, 1995.
  8. Van Dongen JJ, Macintyre EA, Gabert JA et al. Standardized RT-PCR analysis of fusion gene transcripts from chromosome aberrations in acute leukemia for detection of minimal residual disease. Report of BIOMED-1 Concerted Action; investigation of minimal residual disease in acute leukemia. *Leukemia* 1999; 13: 1901-1928.
  9. Bonomi R, Giordano H, del Pilar Moreno M et al. Simultaneous PML/RARa and AML1/ETO expression with t(15; 17) at onset and relapse with only t(8; 21) in acute promyelocytic leukemia patient. *Cancer Genet Cytogenet* 2000; 123: 41-43.
  10. Bonomi R, Giordano H, Moreno P et al. Chimeric M3: M2 case of acute promyelocytic leukemia (APL): differential responses to all-trans retinoic acid (ATRA) and chemotherapy. *Blood* 1998; 92 (Suppl 1): 604a (abstr 2492).
  11. Stavroyanni N, Kalmantis T, Yataganas X. Simultaneous PML/RARa and AML1/ETO gene rearrangements in a patients with acute myeloid leukemia. *Leukemia* 1999; 13: 1294-1295.
  12. Xu L, Zhao WL, Xiong SM et al. Molecular cytogenetic characterization and clinical relevance of additional complex and/or variant chromosome abnormalities in acute promyelocytic leukemia. *Leukemia* 2001; 15: 1359-1368.
  13. Varella-Garcia M, Brizard F, Roche J et al. AML1/ETO and PML/RARa rearrangements in a case of AML-M2 acute myeloblastic leukemia with t(15; 17). *Leuk Lymphoma* 1999; 33: 403-406.
  14. Niu C, Yan H, Yu T et al. Studies on treatment of acute promyelocytic leukemia with arsenic trioxide: remission induction, follow up and molecular monitoring in 11 newly diagnosed and 47 relapsed acute promyelocytic leukemia. *Blood* 1999; 94: 3315-3324.
  15. Janz S, Potter M, Rabkin CS. Lymphoma and leukemia-associated chromosomal translocations in healthy individuals. *Genes Chromosomes Cancer* 2003; 36: 211-223.
  16. Basecke J, Cepek L, Mannhalter C et al. Transcription of AML1/ETO in bone marrow and cord blood of individuals without acute myelogenous leukemia. *Blood* 2002; 100: 2267-2268.
  17. Stavroyanni N, Yataganas X, Abazis D et al. Acute promyelocytic leukemia relapsing into FAB-M2 acute myeloid leukemia with trisomy 8. *Cancer Genet Cytogenet* 2000; 117: 82-83.