

Case Report

Novel Chromosomal Aberration as Evidence of Clonal Evolution in a Case of Relapsed Acute Myeloid Leukemia

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Acute myeloid leukemia (AML) is a heterogeneous group of diseases with a multitude of molecular genetic aberrations and variable clinical outcome. Clonal chromosomal abnormalities have been identified in over 50% of AML cases, and have been regarded as one of the most important prognostic markers. We present a case of a 28-year-old Caucasian woman with AML without maturation, diploid karyotype, that was resistant to multiple chemotherapies and relapsed after matched unrelated stem cell transplantation. Conventional cytogenetic analysis performed on bone marrow specimens revealed 46,XX,t(2;16)(p21;p11.2),t(11;14)(p13;p11.2). The t(11;14)(p13;p11.2) was confirmed by fluorescence in situ hybridization using a whole chromosome paint probe for chromosome 11. Morphologically, the bone marrow was hypercellular with trilineage hypoplasia and 84% blasts. Flow cytometry analysis showed that the blasts were of myeloid immunophenotype. Molecular studies detected internal tandem duplication of the *FLT3* gene and a mutation in exon 12 of the *NPM1* gene. The patient then received monotherapy with AC220, achieved a brief remission, and died of relapsed disease 23 months after initial diagnosis. This is the first report of this novel clonal chromosome aberration as evidence of clonal evolution in AML.

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INTRODUCTION

Acute myeloid leukemia (AML) is a clonal hematopoietic stem cell disorder that is characterized by an excessive accumulation of immature myeloid blasts in the bone marrow and defective production of normal blood cells, which results in fatal infection, bleeding, and organ failure due to leukemic infiltration.¹ It has been well-recognized that AML is a heterogeneous group of diseases with a multitude of molecular genetic aberrations and variable clinical outcome. Clonal chromosomal abnormalities have been identified in over 50% of AML cases, and have been regarded as one of the most important prognostic markers.¹ Cytogenetic results have been integrated as an important part in the diagnosis, classification, risk stratification, treatment decision, and monitoring responses to therapy in the management of AML patients.

Numerous recurrent cytogenetic abnormalities have been described. Common cytogenetic aberrations involving chromosomes 2, 11, 14 and 16 include t(1;11)(q21;q23), t(2;3)(p11-23;q23-38), t(2;11)(p21;q23), t(4;11)(q21;q23), t(5;11)(q31;q23), t(5;11)(q35;p15), t(7;11)(p15;p15), t(9;11)(p22;q23), inv(11)(p15q22), t(11;20)(p15;q11), t(11;17)(q23;q12), t(11;17)(q23;q21), t(11;17)(q23;q25),

t(11;19)(q23;q13.1), inv(16)(p13q22), and t(16;21)(p11;q22).² We present a case of a 28-year-old Caucasian woman with AML with novel clonal cytogenetic abnormalities, 46,XX, t(2;16)(p21;p11.2),t(11;14)(p13;p11.2), identified during relapsed disease.

CASE REPORT

The patient was a 28-year-old Caucasian woman. In November 2009, she presented with leukocytosis and was diagnosed with AML without maturation (M1), diploid karyotype, positive for *FLT3* internal tandem duplication (ITD). She was treated with cytarabine, anthracycline and midostaurin, and achieved complete remission. She further received consolidation therapy with high-dose cytarabine. Unfortunately, in February 2010, she developed relapsed disease. Her salvage therapy included mitoxantrone, etoposide, cytarabine and sorafenib. She achieved second complete remission, and received matched, unrelated stem cell transplantation in June 2010. In September 2010, she had a second relapse, and came to our institution for further treatment options.

Upon presentation at our institution, her complete blood count revealed white blood cell 25.4 K/ μ L (reference range, 4.0 – 11.0 K/ μ L), hemoglobin 9.3 g/dL (reference range, 12.0 – 16.0 g/dL), platelet 9 K/ μ L (reference range, 140 – 440 K/ μ L), with 81% blasts identified on the peripheral blood

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smear. She had an elevated serum lactate dehydrogenase level (2016 IU/L; reference range, 313-618 IU/L) and an elevated serum β 2-microglobulin level (7.9 mg/L; reference range, 0.7 – 1.8 mg/L). Bone marrow biopsy demonstrated a hypercellular marrow with trilineage hypoplasia and markedly increased immature cells (84% blasts, **Figure 1**). Flow cytometry analysis demonstrated a population of blasts that were positive for CD13, CD33, CD34, CD38, CD64 (partial), CD117, HLA-DR, TdT (partial), myeloperoxidase, and negative for CD2, CD3, cytoplasmic CD3, CD5, CD7, CD10, CD14, CD19, CD20, CD41, CD56, consistent with myeloid immunophenotype. Karyotypic analysis showed 46,XX,t(2;16)(p21;p11.2),t(11;14)(p13;p11.2)[16]/46,XX[4] (**Figure 2**). The translocation (11;14) was confirmed by fluorescence in situ hybridization (FISH) using Cytocell whole chromosome paint (wcp) probe for chromosome 11

(**Figure 3**). Molecular studies detected *NPM1* exon 12 mutation in addition to *FLT3* ITD. No mutation was identified in *NRAS*, *KRAS*, *KIT* or *CEBPA* genes. Multiplex reverse transcription-polymerase chain reaction for the detection of 11 different leukemia-associated fusion transcripts, including b3a2, b2a2 and e1a2 transcripts of BCR-ABL1/t(9;22)(q34;q11.2), short and long forms of PML-RARA/t(15;17)(q22;q21), A and D forms of CBFMYH11/inv(16)(p13;q22) or t(16;16)(p13;q22), RUNX1-RUNX1T1/t(8;21)(q22;q22), E2A-PBX1/t(1;19)(q23;p13), MLL-AF4/t(4;11)(q12;q23) and TEL-AML1/t(12;21)(p12;q22), was negative. She received monotherapy with AC220 (a *FLT3* inhibitor), and achieved a brief remission. Unfortunately, she relapsed again in September 2011, and deceased a month later (23 months after initial diagnosis of AML).

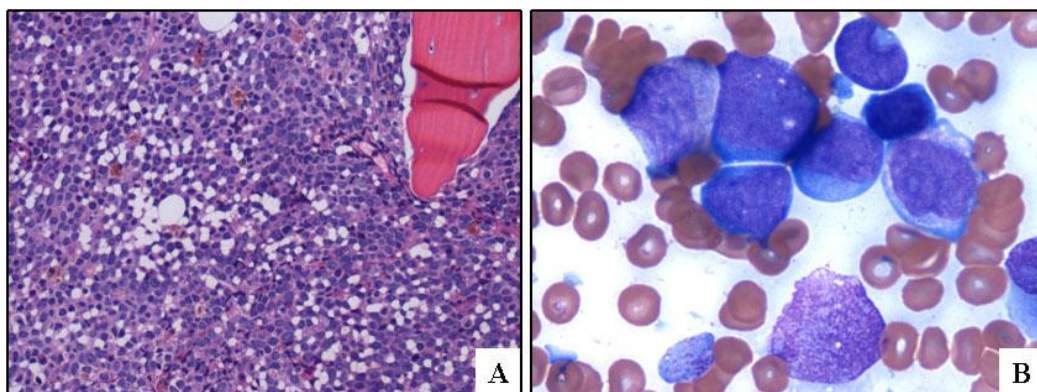


Figure 1.

A. Bone marrow core biopsy demonstrates a hypercellular marrow with trilineage hypoplasia and markedly increased immature cells (H&E, x200).

B. Bone marrow aspirate smear shows a cluster of blasts (Gimesa, x1000).

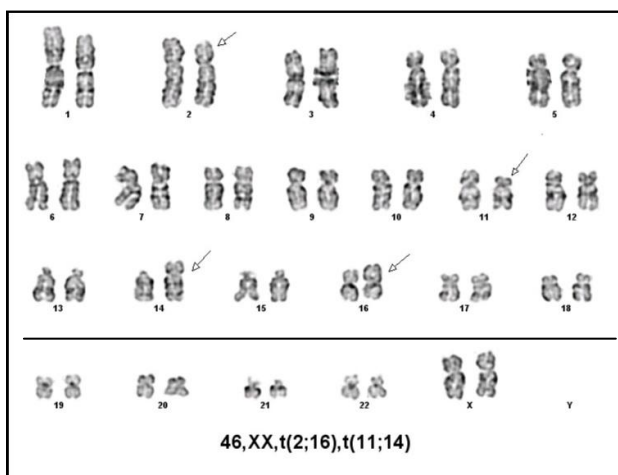


Figure 2. A representative karyotype and metaphase cell shows 46,XX,t(2;16)(p21;p11.2),t(11;14)(p13;p11.2). The arrows indicate translocations between chromosomes 2p21 and 16p11.2, and between 11p13 and 14p11.2, respectively.

DISCUSSION

Cytogenetic abnormalities have been regarded as one of the most important prognostic factors in AML. Clonal chromosomal aberrations have been detected in 52-59% of adult AML and 68-77% of pediatric AML, with +8,

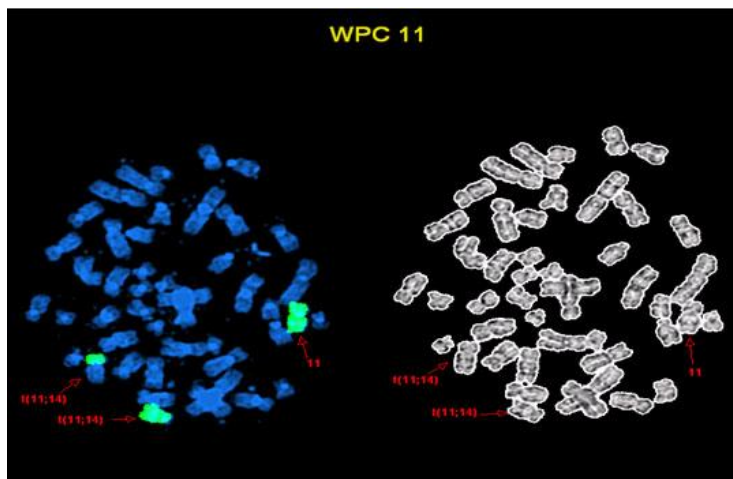


Figure 3. Fluorescence in situ hybridization using Cytocell whole chromosome painting probe for chromosome 11 on a G-banded metaphase spread confirms the t(11;14), which indicates: ish t(11;14)(p13;p11.2)(wcp11+).

-7/del(7q), +21, -5/del(5q) being the most common.² We report a case of AML with a novel cytogenetic abnormality comprising two translocations between chromosomes 2 and 16, and chromosomes 11 and 14, respectively.

Leukemogenesis can be modeled as a multi-step process that involves mutations or other genetic alterations of two broadly defined complementary classes of molecules. Class I mutations involve genes encoding receptor tyrosine kinases (e.g. *FLT3*, *KIT*) or their downstream effectors (e.g. *RAS*) that activate signal transduction pathways, providing a proliferative and/or survival advantage to the tumor cells. Class II mutations involve genes that affect transcriptional factors or components of the transcriptional co-activation complex (e.g. core binding factors, *CEBPA*, *NPM1*), resulting in impaired myeloid differentiation.³ Mutations within each of these complementary groups occur infrequently in the same tumor, whereas mutations between the two complementary groups often occur synergistically in the same AML patient.³ The patient described here had a *FLT3* mutation (ITD) at initial diagnosis. The *NPM1* status was unknown. Upon presentation to our institution at relapse, she showed both *FLT3* ITD and *NPM1* exon 12 mutations, which may play a synergistic role in the development and progression of the leukemia. Interestingly, the patient had a diploid karyotype at initial diagnosis of AML. However, karyotypic analysis of three relapsed bone marrow specimens revealed clonal changes 46,XX,t(2;16)(p21;p11.2),t(11;14)(p13;p11.2) in 6 of 8, 16 of 20, and 19 of 20 metaphases, respectively. The presence of t(11;14) was further confirmed by FISH using a WCP 11 probe. Neither of these two translocations has been reported previously. This is the first report of this novel clonal chromosomal aberration in AML. The possible impact of the patient's prior chemotherapy on this clonal evolution is implicated.

A number of genes located at chromosome 2p21 have been reported to be associated with the development of cancers. *EMLA* (echinoderm microtubule associated protein like 4), *TACSTD1* (tumor-associated calcium signal transducer 1), and *MTA3* (metastasis associated 1 family, member 3) are involved in cell-to-cell adhesion. *MSH2* (human mutS homolog 2) plays a role in DNA mismatch repair.⁴ Genes at 16p11 that have been implicated in tumor development include *EIF3C* (eukaryotic translation initiation factor 3, subunit C) that plays a role in mediating protein translation initiation, *FUS* (fusion involved in t(12;16) in malignant liposarcoma) that is a mRNA-binding protein with a suggested role in mRNA metabolism, *MAPK3* (mitogen-activated protein kinase 3), and *MVP* (major vault protein). It is worth mentioning that vault proteins are cytoplasmic organelles which mediate bidirectional nucleocytoplasmic

transport of a variety of substances, including cytotoxic drugs and have been implicated in multidrug-resistance to chemotherapy.⁵ Genes at 11p13 with potential role in leukemogenesis include *CD44* that plays a role in cell adhesion and traffic and transmission of growth signals mediating hematopoiesis and apoptosis, *LMO2* (LIM domain only 2) that interacts with TAL1 and GATA1 and suppresses myeloid differentiation, and *WT1* (Wilm's tumor suppressor gene) that functions as a potential transcriptional regulator for genes involved in cellular growth and metabolism and is expressed in 75-100% AML. *WT1* mutations have been reported in approximately 10% of AML with normal karyotype, and have been associated with younger age, higher blast count in peripheral blood, *FLT3* ITD, and a possible adverse clinical outcome.^{6,7}

In summary, we report the first case of a relapsed AML with a novel cytogenetic abnormality, 46,XX,t(2;16)(p21;p11.2),t(11;14)(p13;p11.2), in a young patient with immature phenotype, high blast count, *FLT3* ITD, *NPM1* mutation, multi-chemo resistance, and a poor clinical outcome. A possible impact of the patient's prior chemotherapy on this clonal evolution is implicated.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest to disclose.

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