
Clinical and Laboratory Observations

Novel Translocation in Acute Megakaryoblastic Leukemia (AML-M7)

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Summary: The authors report a unique translocation in a patient with M7 acute myeloid leukemia and review the literature. A 22-month-old girl without Down syndrome was diagnosed with acute myeloid leukemia, subtype M7 (AML-M7), and died with relapsed disease following bone marrow transplantation. Tumor cells were evaluated using cytogenetics (including spectral karyotyping), immunohistochemistry, and flow cytometry. The patient was found to have a previously unreported complex translocation as follows: 50,XX,der(1)t(1;5)(p36?.1;p15?.1),del(5)(p15?.1),+6,+der(6;7)(?;?),der(7)t(6;7)(?;p22)[2],der(9)t(6;9)(?;p21)t(9;14)(q34;q11.2-q13),+10,t(12;16)(p13;q24),-14[2],del(14)(q13)[2],+der(19)t(1;19)(?;p13.3),+22[cp 4]. AML-M7 in non-Down syndrome patients is a rare disease that requires improved prognostic markers.

Key Words: Acute myeloid leukemia—Cytogenetics—Megakaryocytic leukemia—Spectral karyotyping—Translocation.

Chromosome abnormalities have helped to identify the prognosis of patients with acute megakaryoblastic leukemia (AML). Megakaryoblastic leukemia was first described as a subtype of acute myelogenous leukemia in 1931 (1) and was considered an undifferentiated variant of acute myeloid leukemia until 1985. Acute megakaryoblastic leukemia subtype M7 is a relatively rare form of leukemia, found in 7% to 10% of all pediatric patients with AML (1–3). There are now well-established immunohistochemi-

cal identification criteria to diagnose AML-M7, including the presence of CD41, CD42b, and CD61; however, cytogenetic evaluation of AML-M7 is still evolving (1–5).

AML-M7 is marked by a younger age of onset than other subtypes of AML, as well as a poor response to therapy (1,3). Stem cell transplantation in first remission is often considered (5). In contrast, patients with AML-M7 and Down syndrome often have excellent outcomes (1–3).

Chromosomal analysis has provided improved therapeutic stratification among patients with other subtypes of AML (6–8). Since AML-M7 is a rare diagnosis in children without Down syndrome and more information to assess prognosis is needed, we present a novel karyotype for a patient with AML-M7 who died 18 months following diagnosis.

CASE REPORT

A 22-month-old white girl was referred for evaluation of new-onset rash and bruising. No overt bleeding was reported. A distant cousin had been diagnosed with leukemia, but there was no other family history of malignancy diagnosed at an unusually young age. There was no family history of bleeding disorders. There were no known parental or fetal exposures to known carcinogens. On physical examination the patient was a phenotypically normal female without stigmata of Down syndrome. She had bruises over her trunk and extremities and petechiae on the dorsal surfaces of both hands. Small (<1 cm) lymph nodes were found in the cervical, axillary, and inguinal regions. No hepatosplenomegaly was detected. Initial laboratory studies revealed hemoglobin of 10.2 g/dL, a platelet count of $22 \times 10^3/\mu\text{L}$, and a white blood cell count of $18.3 \times 10^3/\mu\text{L}$ with a differential consisting of 24% neutrophils, 2% bands, 3% monocytes, and 71% lymphocytes. Initial review of the peripheral smear showed no abnormal white cells. Other laboratory studies, including serum electrolytes, tests of liver function, and urine levels of homovanillic acid and vanillylmandelic acid, were normal.

The patient appeared to have immune thrombocytopenia purpura, but since she was mildly anemic, a bone marrow

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aspirate was performed. The initial marrow was difficult to aspirate, a "dry pull," and yielded few cells for evaluation; however, the bone marrow biopsy showed cells suspicious for malignancy (bone marrow 1, Table 1). Follow-up bone marrow aspirates and core biopsies were performed and evaluated for histology, immunophenotype, and cytogenetic studies, including spectral karyotyping (bone marrow 2, Table 1). In these bone marrow samples, blasts represented 5% to 20% of the nucleated cells and expressed markers of megakaryocytic differentiation. Thus, the initial diagnosis was a myelodysplastic syndrome (refractory anemia with excess blasts). A repeat bone marrow evaluation 1 week later demonstrated that the percentage of blasts in the bone marrow increased; blasts also became apparent in the peripheral blood. A diagnosis of acute megakaryoblastic leukemia (FAB AML-M7) was established.

The patient received induction chemotherapy with daunomycin (45 mg/m² per day for 3 days), Ara-C (1,000 mg/m² per dose q12h for 14 doses), and thioguanine (100 mg/m² per day), followed at week 4 by a second course of Ara-C, as above. Bone marrow aspirate following marrow recovery showed a normal female karyotype (bone marrow 4, Table 1). Beginning at week 8, the patient received consolidation with etoposide (100 mg/m² per day for 5 days) and mitoxantrone (10 mg/m² per day for 4 days). Six

months after diagnosis, the patient received an allogeneic marrow graft that was T-cell-depleted by elutriation and augmented by CD34-positive selection. The conditioning regimen was cyclophosphamide (50 mg/kg per day for 4 days), total body irradiation (300 cGy/d for 4 days with partial lung shielding 1 day), and methylprednisolone (10 mg/kg per day divided TID for 3 days). The patient experienced grade 2 acute graft-versus-host disease of the skin that was treated by a pulse of high-dose steroid followed by taper. Ten months from diagnosis, a marrow biopsy was cellular with increased immature myeloid elements and scattered atypical micromegakaryocytes and infiltrated with 5% blasts. Eleven months from diagnosis, the patient was found to have circulating blasts. At that time her immune suppression was withdrawn and she received donor lymphocytes to induce a graft-versus-leukemia effect, which was unsuccessful. She died of sepsis 20 months after initial presentation.

METHODS

Histologic and Immunophenotypic Analysis

Hematoxylin and eosin-stained histologic sections were prepared from bone marrow core biopsy specimens. Aspirate smears and core biopsy touch preparations were stained

TABLE 1. Summary of peripheral blood and bone marrow specimen analysis and results

Sample source	Timing from start of therapy	Working diagnosis	G-banding	SKY
Bone marrow 1 ("dry pulls")	Day-16	Marrow dysplasia	50,XX,del(5)(p15.2),+6,del(6)(q21q26),t(9;14)(q34;q13),+10,+22,+mar[8]/46,XX[4]	ND
Bone marrow 2 (touch preps, dissociated core biopsies)	Day-15	Myelodysplastic syndrome	50,XX,del(5)(p15.2),+6,del(6)(q21q26),t(9;14)(q34;q13),+10,+22,+mar[9]/46,XX[6]	50,XX,der(1)t(1;5)(p36?.1;p15?.1),del(5)(p15?.1),+6,+der(6;7)(?;?),der(7)t(6;7)(?;p22),der(9)t(6;9)(?;p21)t(9;14)(q34;q11.2),+10,t(12;16)(p13;q24),-14,del(14)(q23),+der(19)t(1;19)(?;p13.3),+22[2],44,XX,del(5)(p15.1),-13,-20[1],44,XX,del(5)(p15.1),-6,-13[1],46,XX[4]
Bone marrow 3 (aspirated marrow and core biopsies) Peripheral blood	Day-9	AML-M7	ND	Bone marrow-ND Peripheral blood (unstimulated): 49,XX,der(1)t(1;5)(p36?.1;p15?.1),del(5)(p15?.1),+6,+der(6;7)(?;?),der(7)t(6;7)(?;p22),-8,der(9)t(6;9)(?;p21)t(9;14)(q34;q11.2),+10,t(12;16)(p13;q24),-14,del(14)(q23),+der(19)t(1;19)(?;p13.3),+22[1],50,X,-X,der(1)t(1;5)(p36?.1;p15?.1),del(5)(p15?.1),+6,+der(6;7)(?;?),der(7)t(6;7)(?;p22),der(9)t(6;9)(?;p21)t(9;14)(q34;q11.2),+10,t(12;16)(p13;q24),-14,del(14)(q23),+der(19)t(1;19)(?;p13.3),+22[1],46,XX [3]
Bone marrow 4 (aspirate and core biopsy)	Day 28	AML-M7/Remission marrow	46,XX[15]	ND

ND, not done due to inadequate sample.

with Wright-Giemsa stain. Immunohistochemical stains were performed using formalin-fixed, decalcified, paraffin-embedded tissue sections, an avidin-biotin-peroxidase complex (ABC) method, and an automated immunostainer (Ventana-Biotech, Tucson, AZ). The antibodies were specific for CD45RB (Leukocyte Common Antigen), CD20 (L26), CD45RO (UCHL-1), kappa and lambda immunoglobulin light chains, factor VIII-related antigen, desmin, cytokeratin, and synaptophysin. Immunophenotypic analysis by flow cytometry was performed on bone marrow aspirates according to standard methods. The panel of antibodies used included those reactive with CD45, CD7, CD10, CD19, CD33, CD34, CD41, CD42b, CD61, and HLA-DR.

Cytogenetic Analysis

Bone marrow cells from a biopsy (bone marrow 3, Table 1) were harvested directly (without culturing) and after 24 hours in culture. G-banding was performed using standard cytogenetic techniques. Twenty-seven dividing cells were analyzed. Two-color FISH painting was performed, following manufacturer's instructions, using labeled chromosome 9 and 14 libraries obtained from Vysis (Downer's Grove, IL). Fifteen metaphase cells were examined.

Spectral Karyotyping

Metaphase spreads were prepared from peripheral blood and bone marrow cells prior to treatment. The peripheral blood was cultured unstimulated for 24 hours and 4 days. The bone marrow was also cultured unstimulated for 24 hours. Five metaphase spreads derived from peripheral blood and seven spreads obtained from bone marrow were analyzed by spectral karyotyping (SKY) (9).

Preparation of SKY probes, methodology for slide pretreatment, hybridization, detection, and imaging were previously described (10). Prior to SKY analysis, the slides were pretreated with pepsin to remove excess cytoplasm and postfixed in 1% formaldehyde in 1× PBS/50 mmol/L MgCl₂. The slides were then denatured for 1.5 minutes in 70% formamide/2× SSC at 80°C and hybridized with SKY probes for 72 hours. Following hybridization, the indirect labels (biotin and digoxigenin) were detected with Avidin-Cy5 (Vector, Burlingame, VT), mouse anti-digoxin (Sigma Chemical Co., St. Louis, MO), and sheep anti-mouse-Cy5.5 (Amersham Life Sciences, Arlington Heights, IL). Subsequently, the slides were counter-stained with DAPI and covered with para-phenylene-diamine antifade solution (Sigma).

SKY images were acquired with the SD200 SpectraCube system and analyzed using SKYView v1.2 software (Applied Spectral Imaging, Carlsbad, CA) (9). Five images of G-banded metaphase spreads were acquired using a Leica DMRBE-microscope using a custom-designed filter TR-2 (Chroma Technology, Brattleboro, VT), a green filter, and a charge-cooled-device camera (Photometrics).

RESULTS

Diagnosis of AML-M7

Histologic and immunophenotypic analyses established a diagnosis of acute megakaryoblastic leukemia. Scattered throughout the marrow were immature-appearing medium to large cells, with fine chromatin, indistinct to discernible nucleoli, and pale eosinophilic cytoplasm. In aspirate smears and touch preparations, megakaryocytes were dysmorphic with hypolobated nuclei. Erythroid and myeloid precursors showed megaloblastoid features. Lymphocytes were small and mature. Occasional blasts showed cytoplasmic blebs. Auer rods were not seen. A peripheral blood smear 2 weeks after presentation showed blasts that were morphologically consistent with those seen in the bone marrow (not shown).

Immunophenotypic analysis by flow cytometry demonstrated a blast population with myeloid characteristics based on the expression of CD33. The cells weakly expressed CD45 and co-expressed markers of megakaryocytic differentiation: CD41, CD42b, and CD61. The blasts failed to express CD7, CD10, CD19, CD34, or HLA-DR (not shown).

Bone Marrow Reveals Clonal Rearrangements With Standard G-banded Karyotype and FISH Analysis

G-banding (bone marrows 1 and 2, Table 1) demonstrated trisomy for chromosomes 6, 10, and 22 and multiple structural rearrangements (Fig. 1). The combined G-banding and FISH results indicated a deletion in the short arm of chromosome 5, a deletion in the long arm of chromosome 6, and a translocation between the long arms of chromosomes 9 and 14. There was also an unidentified small marker chromosome, later identified by SKY as der(19). Ten cells (37%) had a normal female karyotype (not shown). Two-color FISH with whole chromosome 9 and 14 libraries revealed 5 cells with a normal fluorescence pattern and 10 with an abnormal pattern. In the abnormal cells, one chromosome 9 and one chromosome 14 were painted entirely (Fig. 1). One chromosome 9 had chromosome 14 fluorescence at the bottom of the long arm. There was a small (G-sized) chromosome that painted entirely with 14. A B-group-sized chromosome painted with 14 for part of its short arm. These results were interpreted as showing a reciprocal 9;14 translocation and probably a 5;14 translocation as well. G-banding of the bone marrow specimen from day 28 revealed only normal cells, consistent with remission (bone marrow 4, Table 1).

Spectral Karyotyping Identifies Additional Reciprocal and Unbalanced Translocations

To characterize the marker chromosome and identify rearrangements not detected by G-banding or FISH (11), SKY was performed (Table 1). The mitotic index on all cytogenetic preparations was low, and all analyzable spreads were imaged. SKY analysis of the five peripheral blood cells

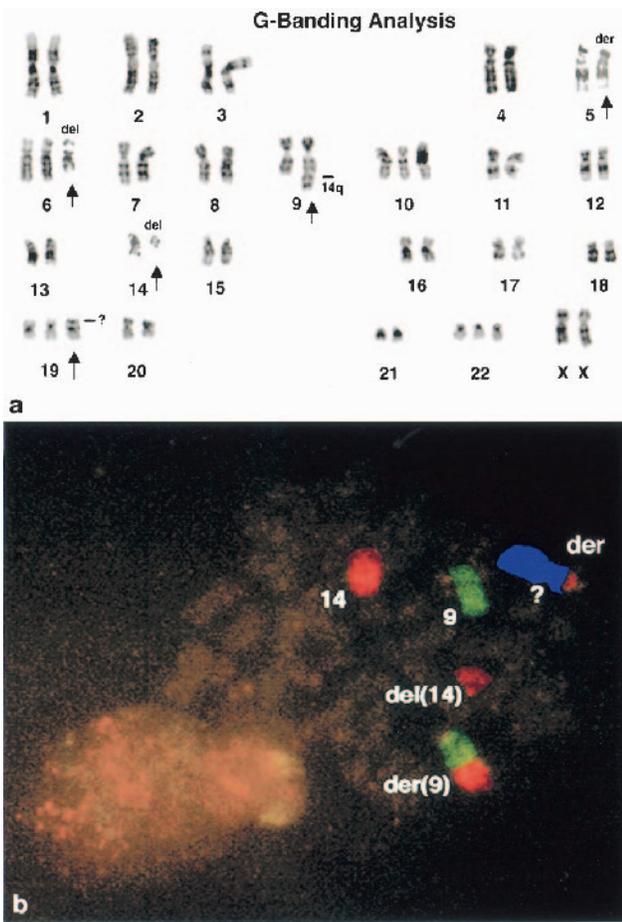


FIG. 1. Comparison of G-banded karyotype and FISH analysis. The upper panel (a) shows the karyotype of the patient with AML-M7 following G-banded analysis. The arrows indicate the numeric and structural aberrations found. Note the normal-appearing chromosomes 12 and 16. In the original karyotype a marker chromosome is indicated. Subsequent to SKY, the marker is placed as der(19). The lower panel (b) shows two-color FISH of the bone marrow cells. Chromosome 9 and 14 libraries were used; these appear green and red, respectively. One normal 9 and one normal 14 are seen, as well as a small 14 (“del 14”). The chromosome labeled “der(9)” shows a 9;14 translocation. The chromosome labeled “?” fluoresced only at the tip of the p arm. The unfluoresced portion is pseudocolored blue.

FIG. 2. SKY analysis of a bone marrow cell from a patient with AML-M7. *Left panel:* RGB display of overlapping metaphase spreads that has been hybridized with a SKY probe and comes from our patient with AML-M7. The chromosomal aberrations are identified by arrows. *Right panel:* Panel summarizing the structural aberrations detected by SKY analysis with the RGB display chromosome on the left, the SKY classification color in the center, and the G-band chromosome on the right. The upper row shows a balanced translocation discerned by SKY analysis, t(12;16)(p13;q24), which was not detected by G-banding. The second row presents normal copies of chromosomes 1 and 5 and shows the deletion 5p and unbalanced translocation der(1)t(1;5). The third row shows the unbalanced translocation der(9)t(6;9;14); the material derived from chromosome 6 (red classification color-fused to 9p/white) was not detected by G-banding. The fourth row shows the unbalanced derived chromosomes involving chromosomes 6 and 7. The last row shows the unbalanced translocation der(19)t(1;19), which also was undetected by G-banding.

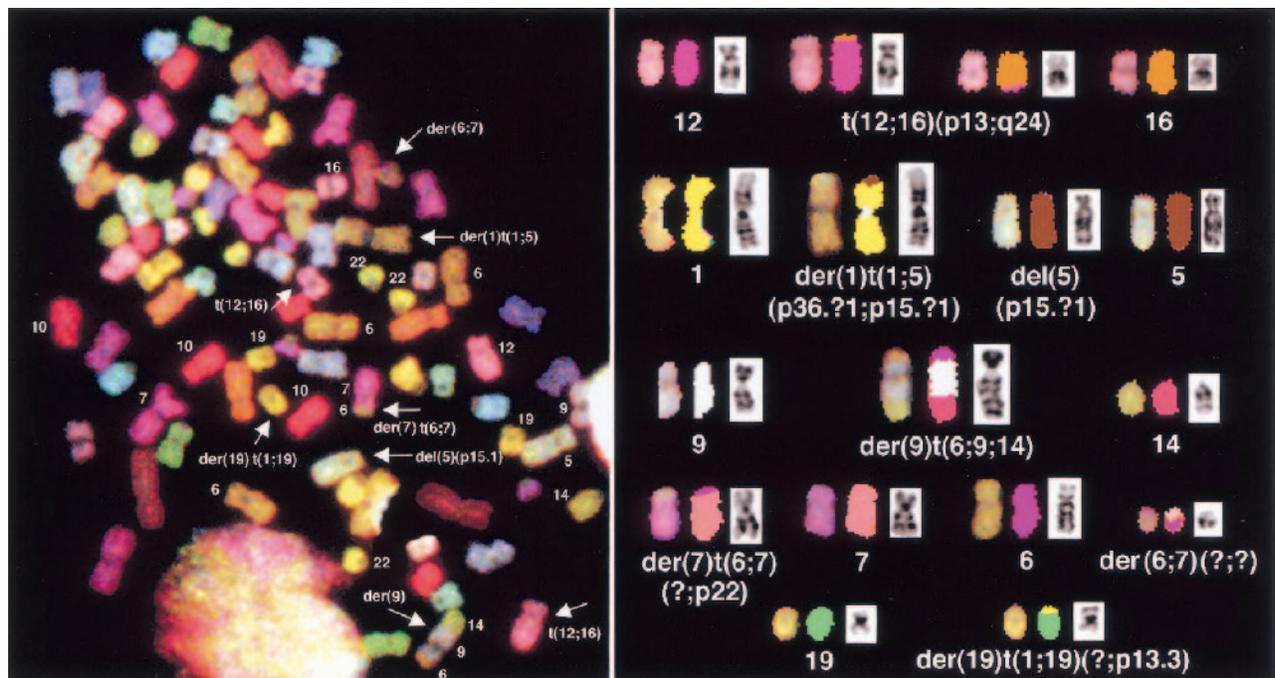


TABLE 2. Comparison of karyotypic abnormalities in our case compared with reported cases of AML-M7

	Karyotypic abnormalities in this case										
	+6	+10	+22	5p15	7p22	9p21	9q34	12p13	14q11	16q24	19p13
Occurrences in 60 AML-M7 reports (16)	18	7	9	2	1	5	3	2	1	1	3
Percentage of 60 total cases (%)	30	12	15	3	2	8	5	3	2	2	5

disclosed two cell populations. The first group had two metaphase spreads with eight structural aberrations. These included a del(5) and 7 translocated chromosomes, as well as trisomies for chromosomes 6, 10, and 22 (Fig. 2). The second group consisted of three normal metaphase spreads (46,XX). Analysis of the eight bone marrow cells (bone marrow 2, Table 1) revealed three different cell populations. The first clone had two cells with the identical abnormal chromosomes as seen in the peripheral blood specimen. The composite karyotype for this abnormal clone was 50,XX,der(1)t(1;5)(p36?.1;p15?.1),del(5)(p15?.1),+6,+der(6;7)(?:?),der(7)t(6;7)(?:p22)[2],der(9)t(6;9)(?:p21)t(9;14)(q34;q11.2),+10,t(12;16)(p13;q24),14[2],del(14)(q23)[2],+der(19)t(1;19)(?:p13.3),+22 [cp 4] (see Fig. 2). The aberrations shown in bold were not detected by G-banding and were identified by SKY analysis. Only one of the structural aberrations was definitively shown to be a reciprocal translocation, t(12;16). It is possible that the der(1)t(1;5) and del(5)(p15.1) are also reciprocal translocation partners. However, at this level of chromosome extension (approximately 400 band level), it is impossible to discern if there is a portion of chromosome 1 fused to the terminal end of the short arm of the aberrant chromosome 5. The small piece of chromosome 14 fused to the short arm of chromosome 5, as seen by FISH analysis, was not found by SKY analysis. The second clone had two cells that contained a deletion within chromosome 5: its karyotype is 46,XX,del(5)(p15.1)[2],-13,-20[1]. -6,-13[1]. The third clone had four spreads that were normal (46,XX).

DISCUSSION

Our patient's initial presentation was marked by bone marrow that was difficult to aspirate and the absence of circulating leukemic cells. The malignancy progressed rapidly with the appearance of peripheral blasts and an increased number of blasts in bone marrow. Diagnosis was established as AML-M7 by light microscopy, flow cytometry, and immunohistochemistry. We performed a complete cytogenetic evaluation and found a complex, previously undescribed karyotype in two evaluated clonal populations. A summary of the observed breakpoints and numerical changes in our patient is 1p36.1, 5p15.1, 7p22, 9p21, 9q34, 12p13, 14q11.2 (SKY) but 14q13 by G-bands, 16q24, 19p13.3 and +6, +10, +22. None of these changes was reported in a large series of children with AML-MDS (12). Our patient lacked monosomy 7, a common chromosomal change in MDS. However, it is possible that the rearranged chromosome 7 in our patient disrupted key genes for the oncogenesis of AML-M7.

This child also had an unusual third chromosomal abnormality found by SKY analysis in her bone marrow but not in peripheral blood consisting of a deletion of part (p15.1) of chromosome 5. This clone suggested the existence of a stable abnormal cell of origin from which her leukemia may have originated and involving genes at 5p15.1 in either a deletion or a translocation, possibly with 14q as seen by FISH or with another piece of a chromosome too small to detect with our techniques. Unfortunately, in-

TABLE 3. Breakpoints identified from this AML-M7 karyotype implicated in hematologic malignancy

Chromosome and breakpoint involved	Genes implicated	Diseases	Study
Chromosome 19			
t(17;19)(q23;p13)	Gene fusion E2A-HLF	ALL	Hunger et al. (31)
t(1;19)(q23;p13)	Gene fusion E2A-PBX1	ALL	Mellentin et al. (32)
t(7;19)(q35;p13)	Activated gene LYL1	ALL	Cleary et al. (33)
t(11;19)(q23;p13.3)	Gene fusion MLL-ENL	ALL/AML	Tkachuk et al. (34)
Chromosome 16			
t(16;21)(q24;q22)	Gene fusion AML1-MTG16	AML	Gamou et al. (17)
Chromosome 12			
t(12;21)(p13;q22)	Gene fusion TEL-AML-1	ALL	Golub et al. (35); Romana et al. (36)
t(12;22)(p13;q11)	Gene fusion TEL-MN1	AML	Buijs et al. (37)
t(5;12)(q33;p13)	Gene fusion TEL-PDGFRB	CMMML	Golub et al. (38)
Chromosome 14			
t(8;14)(q24;q11)	Activation of MYC	Burkitts	Taub et al. (39)
Chromosome 9			
t(9;22)(q34;q11)	Gene fusion BCR-ABL	CML/AML/ALL	Goff et al. (40); de Klein et al. (41)
t(7;9)(q34;q34)	Activated gene TAN1 (chr9)	T-cell ALL	Ellisen et al. (42)

adequate tumor sample prevented further characterization. To fully discern this karyotype required the complementary techniques of G-banding, FISH, and SKY.

We searched the Cancer Genome Anatomy Project database (<http://cgap.nci.nih.gov/Chromosomes/Mitelman>) for all cases of AML-M7 with these abnormalities or breakpoints. The karyotypic abnormalities observed in our patient that have been identified in other patients with AML-M7 include the numeric aberrations +6, +10, +22, and the breakpoints 5p15, 7p22, 9p21, 9q34, 12p13, 14q11, 16q24, and 19p13 (Table 2). We found that 60 cases of AML-M7 were reported that included at least one of the abnormalities found in our patient (Table 2). One third of these cases contained additions of chromosome 6. Approximately one sixth of these cases contained additional chromosome 10 or 22. Only one of the abnormalities, 1p36.1, in our patient was not previously reported in patients with AML-M7.

Karyotype abnormalities in patients with AML-M7 cover a large range of chromosomes and range from translocations to multiple trisomies or deletions (13–15). Many of these breakpoints occur in patients with other types of leukemia and lymphoma (Table 3) (31–42). The t(12;16)(p13;q24) might represent an undescribed TEL fusion partner located at 16q24. There are hundreds of reported cases involving 16q24 in malignancy and many in leukemia/lymphoma (16). Rearrangements of 16q24 described in leukemia and lymphoma include t(16;21)(q24;q22) (AML), t(16;22)(q24;q11) (CML,ph+), t(2;16)(p11;q24) (NHL), and t(8;16)(q22;q24) (AML). In AML/MDS there is a report of a balanced translocation t(16;21)(q24;q22). In AML, the gene at the 16q24 breakpoint is MTG16 (17–19).

Numeric abnormalities of chromosomes in AML-M7 include -7, -5, +6, +8, +10, +12, +21, and +22 (2,5,14,15,20–25). In addition, multiple studies have demonstrated a link between AML-M7 and Down syndrome (26–29). The t(1;22)(p13;q13) has been frequently observed in infants without Down syndrome with megakaryocytic AML-M7 (3,30). Our patient did not have either physical or karyotypic evidence of trisomy 21.

Many other karyotype abnormalities have been reported in patients with AML-M7 that were not observed in our patient, including t(17p) (20), t(15;17) (13), t(X;6)(p11.21;q23) (23), t(11;14) (p13;q11) (24), t(1;15) (q10;q10) (15), and t(6;13) (p23;q14) (25). The latter, a child whose disease progressed from MDS-RAEBIT to AML-M7, was found to have numerous cytogenetic abnormalities including, but not limited to, t(6;13) (p23;q24), +6, +21 (25).

We report a patient who evolved from MDS/RAEBIT to AML-M7. Further classification and identification of prognostic chromosomal changes for MDS, RAEBIT, and AML-M7 require a larger database of karyotypic findings. Increasing the database for these rare diseases could contribute to stratification and therapeutic advances for these patients.

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