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case of the month

Malignant Lymphomas

Translocation (8;16) in a patient with acute myelomonocytic leukemia, occurring after treatment with fludarabine for a low-grade non-Hodgkin's lymphoma

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ABSTRACT

We describe a 65-year old woman who developed a t(8;16)(p11;p13) positive acute myeloid leukemia (AML)-M4 without prior myelodysplasia 36 months after a low-grade non-Hodgkin's lymphoma treated with alkylating agents (chlorambucil and cyclophosphamide) and fludarabine, a purine analog with a significant activity in lymphoproliferative disorders. The t(8;16)(p11;p13) is present in 0.4% of AML of M4-M5 cytotype. In the present case it was identified by conventional cytogenetics; involvement of the MOZ and CBP genes was demonstrated by fluorescence *in situ* hybridization, but not by reverse transcription polymerase chain reaction. The patient died of sepsis after the first course of induction chemotherapy. This is the first t(8;16) AML-M4 arising after fludarabine treatment of which the leukemogenic role in our case is very difficult to ascertain. Most t(8;16) therapy-related-AML cases had received anthracyclines with or without cyclophosphamide; none was ever administered chlorambucil. Our patient was never given anthracyclines and the cumulative doses of chlorambucil and cyclophosphamide employed were low.

Key words: t(8;16), AML-M4, FISH, fludarabine

A t(8,16)(p11;p13) is a non-random chromosome abnormality observed in 0.4% of acute myeloid leukemia (AML) patients, mostly classified as M4-M5.[1] It is detected in both *de novo* and secondary AML and it is very specific, identifying a peculiar form of AML M4-M5 characterized by a mixture of monocytic and granulocytic enzymes due to a block in differentiation at the granulo-monocytic stage, by prominent blast cell erythrophagocytosis (76% of cases), by the frequent occurrence of extramedullary disease (62% of cases) and, finally, by a poor response to chemotherapy.[1,2] It has recently been shown that the rearrangement fuses the MOZ gene, mapped at band 8p11, with the CBP (CREB binding protein) gene located at band 16p13.[3] Although t(8;16) mainly occurs in *de novo* AML, as many as 20% of all cases are observed after treatment for another malignancy (t-AML)[4,5] These patients, who had been administered alkylating agents[6] or radiotherapy[7] or, in most cases, an anthracycline combined with pulsed cyclophosphamide (Cy) regimen,[8] develop an AML with clinical features similar to those observed in *de novo* cases. Herein we report a patient who developed a t(8;16)(p11;p13) M4 AML after treatment consisting at first of intermittent chlorambucil (CLB) and then of fludarabine combined with cyclophosphamide and dexamethasone.

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Case report

In September 1996 a 65-year old woman presented with generalized adenopathies. An abdominal ultrasonography confirmed the presence of multiple adenopathies. Her blood count was as follows: hemoglobin (Hb) 13.2 g/dL, white blood cells (WBC) 8.9×10^9/L, platelets (Plts) 191×10^9/L; leukocyte differential count showed neutrophils 53%, eosinophils 5%, lymphocytes 40%, and monocytes 1%. A bone marrow aspirate demonstrated 70% mature-appearing lymphocytes without any signs of dysplasia. The lymphocyte population was CD5, CD19 and CD23 positive on immunophenotyping. A lymph node biopsy allowed diagnosis of a non-Hodgkin's lymphoma, lymphocytic/B-cell chronic lymphocytic leukemia. She received intermittent CLB along with prednisone, which were stopped one year later because of a good partial response (CLB total dose: 1,260 mg). In November 1998, however, lymphoma progression occurred. The patient received six monthly courses of FluCyD[9] (fludarabine 25 mg/m^2/day, cyclophosphamide 250 mg/m^2/day and dexamethasone 20 mg/m^2/day over three days) which were well tolerated without any side effects; the last FluCyD course, only, was delayed as the WBC count was $<2.0\times10^9$/L. A complete remission (CR) was achieved.

In October 1999 the patient was still in CR from the lymphoma, according to morphology and immunophenotyping. Physical examination demonstrated liver enlargement (3 cm below costal margin), with mild splenomegaly (0.5 cm below costal margin), without any lymphadenopathy.

Hematologic examination

In October 1999 the analysis of the patient's peripheral blood showed the following: Hb 11.8 g/dL, WBC 1.8×10^9/L, Plts 136×10^9/L and leukocyte differential neutrophils 58%, eosinophils 4%, lymphocytes 32% and monocytes 5%. These values remained unchanged until January 2000, when induction chemotherapy was started.

Bone marrow morphology

In October 1999 a bone marrow biopsy revealed a normocellular marrow with mild dyserythropoiesis and with 40% large-sized blasts with a low nuclear/cytoplasmic ratio and with cytoplasm containing dispersed azurophilic granules. Erythrophagocytosis was evident. A bone marrow aspirate performed one month later showed a stable blast cell percentage that increased up to 70% two months later.

Immunophenotypic studies

Immunophenotypic analysis of the bone marrow blast cell population revealed positivity for CD14, CD15, CD33 and HLA-DR. The absolute number of CD4 positive peripheral lymphocytes was 219/μL.

Cytogenetics, fluorescence in situ hybridization (FISH) and reverse transcription polymerase chain reaction

Cytogenetics studies were carried out on bone marrow cells at diagnosis, one month and two months later using a trypsin-Giemsa banding technique. Metaphase cells were examined from short-term unstimulated cultures. Chromosome abnormalities were defined according to ISCN.[10]

FISH was performed on cytogenetic preparations. In order to demonstrate involvement of the MOZ and CBP genes we used the two probes YAC 176C9[11] and RT 100[12], both biotinylated (green signals). At diagnosis a mixture of cells with a nor-

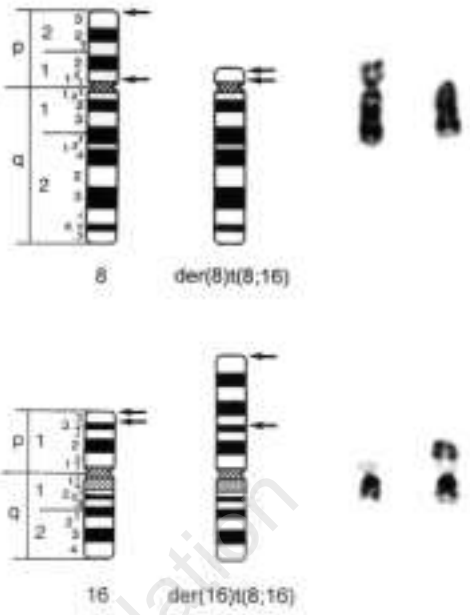

Figure 1. Schematic diagram representing the normal chromosomes 8, 16, der(8) and der(16). On the right side of each derivative chromosome, the chromosomes as seen in trypsin-Giemsa banding.

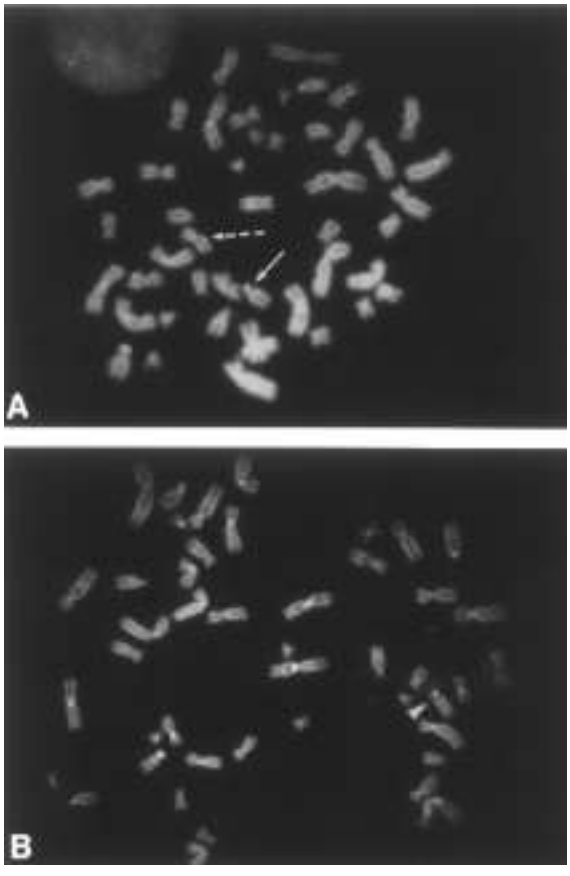

Figure 2. FISH analysis of a cell carrying the t(8;16). A) Hybridization with the biotinylated YAC 176C9 probe. Three green signals are observed: one on normal chromosome 8, one each on der(8), indicated by an arrow and on der(16) indicated by a segmented arrow. B) Hybridization with the biotinylated RT 100 probe (green signals). The probe is located on normal chromosome 16 and on der(8), indicated by ►.

mal chromosome pattern (thirty-four mitoses) and with the t(8;16)(p11;p13) (six mitoses) was noted (Figure 1). FISH identified the t(8;16) in ten out of the forty-six metaphases screened. In leukemic cells the YAC 176C9 probe (green signals), split by the translocation, gave three signals: one on the normal chromosome 8 and one each on der(8) and on der(16) (Figure 2A); the RT 100 gave two signals, one on normal chromosome 16 and the other on der(8) (Figure 2B). One month later the number of t(8;16) positive cells increased, as detected by both cytogenetics (thirty-three positive mitoses out of forty-two) and FISH (forty-six positive metaphases out of forty-eight). Two months later the t(8;16) was found in all the cells screened.

MOZ-CBP fusion transcript junction messages were amplified by nested PCR following reverse transcription, performed as already reported.[3,13] Neither MOZ-CBP nor CBP-MOZ transcripts were detected by the RT PCR assay.

Treatment and outcome

These findings allowed us to make a diagnosis of AML-M4. From October 1999 to January 2000 the patient received 6-thioguanine as monochemotherapy and supportive therapy in order to contain the bone marrow blast cell percentage. Subsequently, at the end of January 2000, she started chemotherapy with idarubicin 12 mg/m^2 i.v. every day for three days and cytosine-arabinoside 100 mg/m^2 i.v. every twelve hours for seven days. She died of sepsis during the cytopenic period.

Discussion

Up to now a t(8;16) has been observed in 44 patients with either *de novo* or secondary AML.[4] In the majority of these t-AML, with the prior cancer being a solid tumor slightly more often than a hematologic malignancy, leukemia developed after a short latency (usually less than three years), without a preceding myelodysplastic phase. Previous treatment mostly consisted in an

Table 1: t(8;16)(p11;p13) therapy-related AML: clinical characteristics.

Pts	*Years/ Sex*	*Prior tumor*	*Chemotherapy*	*Cumulative Dose (mg)*	*Interval between the onset of treatment for the primary tumor and AML diagnosis (mo.)*	*FAB*	*Prior MDS (mos.)*	*Chemotherapy*	*Resp.*	*Survival*
1	29/F	Glioblastoma	Nitrosur.	N.D.	23	M4	No	Dauno, Ara-C, Cy	HD	<1
2	92/M	Merkell's carcinoma	Doxo,Cy,VCR	N.D.	8	M4	Yes	No	N.K.	<1
3	38/F	HD	Mechlor.,VCR,Procar., Doxo.,Bleo.,Vinbl.	4.8; 11.2; 2,240; 200; 80; 48	7	M4	No	High-dose Ara-C	HD	<1
4	68/F	Ovarian cancer	Yes	N.K.	N.K.	M4	No	N.K.	N.K.	N.K.
5	64/F	Breast cancer	5-FU, 4-Epi-Doxo	3,000; 300; 3,000	9	M4	No	Etopos., Ara-C	HD	<1
6	53/M	NHL	Mercapt., MTX, Cy, Dauno, VCR, Asp., BCNU, Dactinomycin	10,400; 700; 7,800; 900; 29; 90,000IU; 320; 640	23	M5b	No	Amsa, Ara-C	HD	<1
7	12/M		Accidental prenatal X-ray exposure		144	M4	No	High-dose Ara-c	CR	6→
8	19/M	Osteosarcoma	Adriamycin, Cisplatinum	450; 1,080**	21	M5a	No	Ida Etopos. Ara-C	CR	10*→
9	45/F	Breast cancer	EVCF (3 courses), FEC (3 courses)		20	M5a	No	Mito. Ara-C Etopos., Dauno	HD	<1
10	65/F	NHL	CLB, Cy, Flu	1,260; 9,720; 810	37	M4	No	6-TG Ida Ara-C	HD	<4

*Resp.= response; Surv.= survival; mo.= months; NHL=non-Hodgkin's lymphoma; Nitrosur.= nitrosurea; Doxo.= doxorubicin; Cy= cyclophosphamide; VCR= vincristine; Mechlor.= mechlorethamine; Procar.=procarbazine; Bleo=bleomycin; Vinbl.=vinblastine; 5-FU=5-fluorouracil; 4-epi-doxo= 4-epidoxorubicin; Mercapt.=mercaptopurine; MTX=methotrexate; Dauno=daunorubicin; Asp=asparaginase; EVCF=epirubicin 50 mg/m^2, VCR 0.6 mg/m^2, Cy 200 mg/m^2, 5-FU 300 mg/m^2; FEC=5-FU 500 mg/m^2, Epirubicin 50 mg/m^2, Cy 300mg/m^2; CLB=chlorambucil; ND=not determined; N.K.=not known; HD=hypoplastic death; CR=complete remission; Etopos=etoposide; Amsa=amsacrine; Mito=mitoxantrone; 6TG=6-thioguanine; Ida=idarubicin; **=mg/m^2 *= allogeneic bone marrow transplantation from identical sibling; →=alive and well.*

anthracycline and pulsed cyclophosphamide regimen with or without radiotherapy[4,5,8] or in radiotherapy[7] or in therapy with alkylating agents[6] (Table 1). Our patient developed AML-M4 without a prior MDS thirty-five months after starting treatment for a non-Hodgkin's lymphoma. She, like nearly 80% of the cases in the literature, presented the t(8;16) as sole karyotype defect,[2] suggesting that the translocation is a primary abnormality. At diagnosis conventional cytogenetics identified the t(8;16) in six out of the thirty-four mitoses studied. FISH showed that the YAC 176C9 probe was split by the translocation and that the RT 100 probe was translocated onto chromosome 8 in ten out of forty-six mitoses (Figures 2A and 2B). In this way the involvement of MOZ and CBP was demonstrated. In contrast, RT PCR identified neither the MOZ-CBP nor the CBP-MOZ transcripts. This fact has already occurred in all but two of the cases reported in the literature,[3,13] suggesting a low expression or instability of the fused transcripts.[12]

It has been speculated that the CBP gene on 16p13.3 may be a target of topo II isomerase inhibitors (including anthracyclines and their derivatives, and epipodophyllotoxins) and that the MOZ gene on 8p11 may be a preferential target of anthracyclines often combined with pulsed Cy.[4] Our patient, however, never received anthracyclines but only intermittent CLB and subsequently six monthly courses of FluCyD. A causative role for CLB or Cy in our t(8;16) AML seems questionable. The cumulative dose of CLB was low (1,260 mg). None of the t(8;16) t-AML cases reported in the literature had ever received CLB (Table 1). Cy, less leukemogenic than other alkylating agents,[14] was given intermittently up to a total dose of 9,720 mg. Considering the t(8;16) t-AML reported, Cy was given to three such patients; its dose was unspecified in one case, 1,500 mg/m^2 in another one, and 7,800 mg (cumulative dose) in the last one. Fludarabine is a purine analog with a significant activity in lymphoproliferative disorders and its use is increasing. The risk of t-AML after purine analog treatment is still unassessed. In a large series of chronic lymphocytic leukemia patients receiving fludarabine regimens as initial therapy no case of t-AML was detected[15] and in hairy cell leukemia patients given cladrinine the occurrence of AML was not greater than with other treatments.[16] Scattered t-AML cases have, however, been described recently in patients receiving a purine analog as first-line treatment[17-20] or after a previous therapy including alkylating agents or anthracyclines.[21-24] Some of these patients had chromosome 5, 7 and 8 abnormalities, which are typically seen after treatment with alkylating agents;[6] not all of them, however, had received those drugs. The role of fludarabine in determining t(8;16) in our AML patient is very difficult to ascertain. Fludarabine might have interfered with DNA repair mechanisms,[25] or, alternatively, it might have promoted the t(8;16) M4-AML through its well recognized immunosuppressive effect due to T-lymphocyte depletion.[26] In this way fludarabine might have allowed the expansion of a neoplastic clone already present in the patient. This hypothesis might be supported by cytogenetic and FISH findings showing normal mitoses along with abnormal ones at the onset of the disease. The possibility, however, exists that a mixture of normal and abnormal metaphases derived from the detection of leukemia in an early phase. One month later the number of t(8;16) metaphases progressively increased and, on the last analysis, all the metaphases were abnormal. The mixture of normal and abnormal metaphases is typically seen in at least half *de novo* t(8;16) AML cases but in only one t-AML reported in the literature.[2]

To our knowledge this represents the first report of a t(8;16) in a case of t-AML following alkylating agents and fludarabine therapy for a non-Hodgkin's lymphoma. Whatever the mechanism of leukemogenesis in our patient, we suggest that attention should be paid to the occurrence of such a complication in patients receiving a purine analog especially if this is given after alkylating agents. Further work is necessary to determine the role and the molecular targets of purine analogs in t-MDS/t-AML development.

Contributions and Acknowledgments

PB designed and performed the experiments. PE, EO wrote the manuscript. PC helped with FISH experiments. SC helped with PCR analysis. MB helped with cytogenetic analysis. SG labeled the probes used in the FISH studies. EO, CA were responsibles for patient referral to our laboratory. GP helped with immunophenotypic studies. MC helped with cell cultures. ML and CB contributed to the review of the paper. All authors were equally responsible for conception and design of the study and appoved the final version of the paper.

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Disclosures

Conflict of interest: none.

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References

1. Mitelman F, Heim S. Quantitative acute leukemia cytogenetics. Gene Chromosome Canc 1992; 5:57-66.
2. Velloso ERP, Mecucci C, Michaux L, et al. Translocation t(8;16)(p11;p13) in acute non-lymphocytic leukemia: report on two new cases and review of the literature. Leuk Lymphoma 1996; 21:137-42.
3. Borrow J, Stanton VPJr, Andresen JM, et al. The translocation t(8;16)(p11;p13) of acute myeloid leukaemia fuses a putative acetyltransferase to the CREB-binding protein. Nat Genet 1996; 14:33-41.
4. Michallet AS, Lai JL, Bauters F, Fenaux P, Quesnel B. A new case of therapy-related acute myeloid leukaemia with t(8;16)(p11;p13). Br J Haematol 1998; 100:797-803.
5. Quesnel B, Kantarjian H, Pedersen Bjergaard J, et al. Therapy-related acute myeloid leukemia with t(8;21), inv(16), and t(8;16): a report on 25 cases and review of the literature. J Clin Oncol 1993; 11:2370-9.
6. Rowley JD, Vingon C, Gollin SM, Rosenberg CL, Wyandt HE, Milunsky A. Chromosomal translocations in secondary acute leukemias. N Engl J Med 1996; 334:601-3.
7. Savasan S, Mohamed AN, Lucas DR, Dugan MC, Ryan JR, Ravindranath Y. Acute myeloid leukaemia with t(8;16)(p11;p13) in a child after intrauterine X-ray exposure. Br J Haematol 1996; 94:702-4.
8. Demuynck H, Verhoef GEG, Zachèe P, et al. Therapy-related acute myeloid leukemia with t(8;16)(p11;p13) following anthracycline-based therapy for non-metastatic osteosarcoma. Cancer Genet Cytogen 1995; 82:103-5.
9. Lazzarino M, Orlandi E, Montillo M, et al. Fludarabine, cyclophosphamide, and dexamethasone (FluCyD) combination is effective in pretreated low-grade non-Hodgkin's lymphoma. Ann Oncol 1999; 10:59-64.
10. Mitelman F. An International System for Human Cytogenetic Nomenclature: Guidelines for Cancer Cytogenetics. Basel:Karger; 1995.
11. Chaffanet M, Mozziconacci MJ, Fernandez F, et al. A case of inv(8)(p11q24) associated with acute myeloid leukemia involves the MOZ and CBP gene in a masked t(8;16). Genes Chromosome Canc 1999; 26:161-5.
12. Giles RH, Dauwerse JG, Higgins C, et al. Detection of CBP rearrangements in acute myelogenous leukemia with t(8;16). Leukemia 1997; 11:2087-96.
13. Aguiar RCT, Chase A, Coulthard S, et al. Abnormalities of chromosome band 8p11 in leukemia: two clinical syndromes can be distinguished on the basis of MOZ involvement. Blood 1997; 90:3130-5.
14. Cuzick J, Erskine S, Edelman D. A comparison of the incidence of the myelodysplastic syndrome and acute myeloid leukaemia following melphalan and cyclophosphamide treatment for myelomatosis. Br J Cancer 1987; 55:523-9.
15. Keating MJ, O'Brien S, Lerner S, et al. Long-term follow-up of patients with chronic lymphocytic leukemia (CLL) receiving fludarabine regimens as initial therapy. Blood 1998; 92:1165-71.
16. Saven A, Burian C, Koziol JA, Piro LD. Long-term follow-up of patients with hairy cell leukemia after cladribine treatment. Blood 1998; 92:1918-26.
17. Cheson BD, Vena DA, Barrett J, Freidlin B. Second malignancies as a consequence of nucleoside analog therapy for chronic lymphoid leukemias. J Clin Oncol 1999; 17:2454-60.
18. Kroft SH, Tallman MS, Shaw JM, Tangaavelu M, Peterson LC. Myelodysplasia following treatment of chronic lymphocytic leukemia (CLL) with 2-chlorodeoxyadenosine (2-CdA). Leukemia 1997; 11:170.
19. Coso D, Costello R, Cohen-Valensi R, et al. Acute myeloid leukemia and myelodysplasia in patients with chronic lymphocytic leukemia receiving fludarabine as initial therapy. Ann Oncol 1999; 10:362-3.
20. Van den Neste E, Louviaux I, Michaux JL, et al. Myelodysplastic syndrome with monosomy 5 and/or 7 following therapy with 2-chloro-2'-deoxyadenosine. Br J Haematol 1999; 105:268-70.
21. Schlaifer D, Rigal-Huguet F, Pris J, Brousset P, Delannoy A, Bosly A. Secondary neoplasms in two patients treated with purine analogues. Nouv Rev Fr Hematol 1994; 36:341.
22. Frewin RJ, Provan D, Smith AG. Myelodysplasia occurring after fludarabine treatment for chronic lymphocytic leukemia. Clin Lab Haematol 1997; 19:151-2.
23. Laurencet FM, Zulian GB, Cabrol C, Betticher D. Myelodysplastic syndrome with biclonal monosomy 7 and trisomy 8 after treatment with cladribine (2-chloro-2'-deoxyadenosine) and involved field radiation therapy. Cancer Genet Cytogen 1997; 99:85-9.
24. Kong LR, Huang CE, Hakimian D, et al. Long-term follow-up and late complications of 2-chlorodeoxyadenosine in previously treated, advanced, indolent non-Hodgkin's lymphoma. Cancer 1998; 82:957-64.
25. Plunkett W, Gandhi V, Huang P, et al. Fludarabine: pharmacokinetics, mechanism of action, and rationales for combination therapies. Semin Oncol 1993; 20(Suppl. 7):2-12.
26. Cheson BD. Infectious and immunosuppressive complications of purine analogue therapy. J Clin Oncol 1995; 13:2431-48.