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.

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

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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/cyttoplasmic 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 176C911 and RT 10012, 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 ▲.
nal 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: on one of the normal chromosome 8 and one each on der(8) and on der(16) (Figure 2A); the RT 100 gave two signals, on one 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. 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 AM L-M 4. 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/m² i.v. every day for three days and cytosine arabinoside 100 mg/m² 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 AM L.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.

<table>
<thead>
<tr>
<th>Pts</th>
<th>Years</th>
<th>Sex</th>
<th>Prior tumor</th>
<th>Chemotherapy</th>
<th>Cumulative Dose (mg)</th>
<th>Interval between the onset of treatment for the primary tumor and AML diagnosis (mo.)</th>
<th>FAB</th>
<th>Prior MDS (mos.)</th>
<th>Chemotherapy</th>
<th>Resp.</th>
<th>Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>29/F</td>
<td></td>
<td>Glioblastoma</td>
<td>Nitrosur.</td>
<td>N.D.</td>
<td>23</td>
<td>M4</td>
<td>No</td>
<td>Dauno, Ara-C, Cy</td>
<td>HD</td>
<td>&lt;1</td>
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<tr>
<td>2</td>
<td>92/M</td>
<td></td>
<td>Merkel’s carcinoma</td>
<td>Doxo, Cy, VCR</td>
<td>N.D.</td>
<td>8</td>
<td>M4</td>
<td>Yes</td>
<td>No</td>
<td>N.K.</td>
<td>&lt;1</td>
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<tr>
<td>3</td>
<td>38/F</td>
<td></td>
<td>HD</td>
<td>Mechlor, VCR, Procar., Doxo, Bleo, Vinbl.</td>
<td>4.8; 11.2; 2.2; 200; 80; 48</td>
<td>7</td>
<td>M4</td>
<td>No</td>
<td>High-dose Ara-C</td>
<td>HD</td>
<td>&lt;1</td>
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<tr>
<td>4</td>
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<td>Ovarian cancer</td>
<td>Yes</td>
<td>N.K.</td>
<td>N.K.</td>
<td>M4</td>
<td>No</td>
<td>N.K</td>
<td>N.K.</td>
<td>N.K</td>
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<td>5</td>
<td>64/F</td>
<td></td>
<td>Breast cancer</td>
<td>5-FU, 4-Epi-Doxo</td>
<td>3,000; 330; 3000</td>
<td>9</td>
<td>M4</td>
<td>No</td>
<td>Etopos, Ara-C</td>
<td>HD</td>
<td>&lt;1</td>
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<tr>
<td>6</td>
<td>53/M</td>
<td></td>
<td>NHL</td>
<td>Mercapt., MTX, Cy, Dauno, VCR, Asp., BCNU, Daunorubicin</td>
<td>10,400; 700; 7,800; 900; 90,000; 640</td>
<td>23</td>
<td>M5b</td>
<td>No</td>
<td>Amsa, Ara-C</td>
<td>HD</td>
<td>&lt;1</td>
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<tr>
<td>7</td>
<td>12/M</td>
<td></td>
<td>Accidental prenatal X-ray exposure</td>
<td>Adramycin, Cisplatinum</td>
<td>450; 1,080**</td>
<td>144</td>
<td>M4</td>
<td>No</td>
<td>High-dose Ara-c</td>
<td>CR</td>
<td>6→</td>
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<tr>
<td>8</td>
<td>19/M</td>
<td></td>
<td>Osteosarcoma</td>
<td>Adramycin, Cisplatinum</td>
<td>450; 1,080**</td>
<td>21</td>
<td>M5a</td>
<td>No</td>
<td>Ida Etopos, Ara-C</td>
<td>CR</td>
<td>10*→→</td>
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<tr>
<td>9</td>
<td>45/F</td>
<td></td>
<td>Breast cancer</td>
<td>EVCF (3 courses), FEC (3 courses)</td>
<td>20</td>
<td>M5a</td>
<td>No</td>
<td>Mito, Ara-C</td>
<td>HD</td>
<td>&lt;1</td>
<td></td>
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<tr>
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<td>65/F</td>
<td></td>
<td>NHL</td>
<td>CLB, Cy, Flu</td>
<td>1,260; 9,720; 810</td>
<td>37</td>
<td>M4</td>
<td>No</td>
<td>6-TG Ida Ara-C</td>
<td>HD</td>
<td>&lt;4</td>
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</tbody>
</table>

Resp. = response; Surv. = survival; mo. = months; NHL = non-Hodgkin’s lymphoma; Nitrosur. = nitrosurea; Doxo. = doxorubicin; Cy= cyclophosphamide; VCR= vincristine; Mechlor. = mechloretamine; 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/m²; FEC = 5-FU 100 mg/m²; Cy = 200 mg/m²; CLB = chlorambucil; ND = not determined; N.K. = not known; HD = hypoplastic death; CR = complete remission; Etopos = etoposide; Amsa = amasiticine; Mito = mitoxantrone; 6-TG = 6-thioguanine; Ida = idarubicin; ** = mg/m²; * = allogeneic bone marrow transplantation from identical sibling; → = alive and well.
anthraccline and pulsed cyclophosphamide regimen with or without radiotherapy or in radiotherapy or in therapy with alkylating agents (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, 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, suggesting a low expression or instability of the fused transcripts.

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. 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, 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/m² 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 and in hairy cell leukemia patients given cladribine the occurrence of AML was not greater than with other treatments. Scattered t-AML cases have, however, been described recently in patients receiving a purine analog as first-line treatment or after a previous therapy including alkylating agents or anthracyclines. Some of these patients had chromosome 5, 7 and 8 abnormalities, which are typically seen after treatment with alkylating agents; not all of them, howev-
References


