Acute Myeloid Leukemia and Myelodysplastic Syndromes Following Essential Thrombocytopenia Treated With Hydroxyurea: High Proportion of Cases With 17p Deletion

By Yvon Sterkers, Claude Preudhomme, Jean-Luc Lai, Jean-Loup Demory, Marie-Thérèse Caulier, Eric Wattel, Dominique Bordessoule, Francis Bauters, and Pierre Fenaux

Treatment with alkylating agents or radiophosphorous (32P) has been shown to carry a certain leukemogenic risk in myeloproliferative disorders (MPDs), including essential thrombocytopenia (ET). The leukemogenic risk associated with treatment with hydroxyurea in ET, on the other hand, is generally considered to be relatively low. Between 1970 and 1991, we diagnosed ET in 357 patients, who were monitored until 1996. One or several therapeutic agents had been administered to 326 patients, including hydroxyurea (HU) in 251 (as only treatment in 201), pipobroman in 43, busulfan in 41, and 32P in 40. With a median follow-up duration of 98 months, 17 patients (4.5%) had progressed to acute myeloid leukemia (AML; six cases) or myelodysplastic syndrome (MDS; 11 cases). Fourteen of these patients had received HU, as sole treatment in seven cases, and preceded or followed by other treatment in seven cases, mainly pipobroman (five cases). The remaining three leukemic progressions occurred in patients treated with 32P (two cases) and busulfan (one case). The incidence of AML and MDS after treatment, using 32P alone and 32P with other agents, busulfan alone and with other agents, HU alone and with others, and pipobroman alone and with others, was 7% and 9%, 3% and 61%, 3.5% and 14%, and 0% and 16%, respectively. Thirteen of 17 patients who progressed to AML or MDS had success-ful and molecular characteristics of the 17p deletion. Four of these patients had received no HU and progressed to AML or MDS had no 17p deletion. A review of the literature found cytogenetic analysis in 35 cases of AML and MDS occurring after ET, 11 of whom had been treated with HU alone. Five of 35 patients had rearrangements that resulted in 17p deletion. Four of them had been treated with HU alone. These results show that treatment with HU alone is associated with a leukemic risk of approximately 3.5%. A high proportion of AML and MDS occurring in ET treated with HU (alone or possibly followed by pipobroman) have morphologic, cytogenetic, and molecular characteristics of the 17p- syndrome. These findings suggest that widespread and prolonged use of HU in ET may have to be reconsidered in some situations, such as asymptomatic ET.

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ESSENTIAL THROMBOCYTHEMIA (ET) is a clonal myeloproliferative disorder (MPD) characterized by a persistent increase in platelet counts. Progression of ET to acute myeloid leukemia (AML), preceded or not by myelodysplastic syndrome (MDS), has been observed in 3% to 4% of cases, and, until recently, mostly in patients who had received treatment with radiophosphorous (32P) or alkylating agents, especially busulfan.2-15 Hydroxyurea (HU), which is considered to have relatively low leukemogenic potential, has therefore been widely used in ET in recent years. No characteristic morphologic or cytogenetic features of AML and MDS occurring during the course of ET have been described, by comparison to other therapy-related cases of AML or MDS.2-15

Recently, we and others reported, in AML and MDS, a strong correlation between 17p deletion, resulting from unbalanced translocations between 17p and another chromosome or less often from monosomy 17 or i(17q), typical dysgranulopoiesis combining pseudo Pelger Huet hypolobulation and small vacuoles in neutrophils, and p53 mutation.16,17 This correlation suggested that a new morphologic-cytogenetic-molecular entity or syndrome could be described in MDS and AML. Approximately 30% of patients with these characteristics had received chemotherapy for a prior neoplasm.16

We reviewed cases of AML and MDS occurring in 357 ET patients diagnosed from 1970 to 1991 in two hematologic centers and monitored until August 1996. Seventeen cases of AML and MDS were observed, generally in patients treated exclusively or predominantly with HU. Seven of them had 17p deletions and other characteristics of the 17p- syndrome.

MATERIALS AND METHODS

Patients. Between 1970 and January 1991, the diagnosis of ET was made at two institutions (Centre Hospitalier Universitaire [CHU], Lille, and Hopital St Philibert, Lomme, France) in 357 patients, according to the following criteria: (1) platelet count greater than 700 × 10^9/L on two different counts separated by a 1-month interval; (2) no known cause for reactive thrombocytosis, ie, no iron deficiency based on normal serum iron level and transferrin-binding capacity, absence of transferrin or of an underlying neoplasm; and (3) exclusion of inflammatory disease based on normal values of erythrocyte sedimentation rate, protein electrophoresis, and serum fibrinogen level; absence of splenectomy; absence of myelofibrosis when a leukoerythroblastic reaction or morphologic abnormalities of erythrocytes was present; absence of Philadelphia chromosome T3; and absence of monosomy 17 or i(17q).
phia chromosome when hematologic data were compatible with chronic myelogenous leukemia (CML) with thrombocytthemic onset; and absence of MDS, such as acquired idiopathic sideroblastic anemia or the 5q-syndrome, both of which can be associated with thrombocytosis.

The Lille center started to diagnose ET patients in 1970, and the Lomme center in 1982. Characteristics of the 147 patients diagnosed in Lille before July 1987, and their follow-up data to July 1988, have been previously reported. Follow-up data of patients from the two centers were analyzed on the reference date of August 31, 1996.

Treatment. Cytoreductive therapy was generally started when patients met one of the following criteria: platelet count greater than $1 \times 10^{10}/L$; age older than 65 years; symptomatic disease; and presence of another major risk factor for vascular disease (diabetes mellitus, severe hypertension, hypercholesterolemia, or heavy smoking), or of previous symptomatic artery disease (involving the coronary, cerebral or legs arteries).

Until 1980, first-line therapy generally consisted of busulfan (6 mg/d) or $^{32}$P (0.1 mCi/kg). After 1980, first-line therapy was generally with HU (at the starting dose of 1.5 g/d), except in some elderly patients who still received $^{32}$P. In patients in whom HU did not allow permanent control of platelet counts at less than $0.5 \times 10^{10}/L$, HU was generally replaced by pipobroman (Vercyte; Abbott, Rungis, France) at the starting dose of 1 mg/kg/day. Indeed, after several of our ET patients had experienced thrombotic episodes at platelet counts of 0.5 to 0.6 $\times 10^{10}/L$, we had aimed, in patients in whom treatment indicated, to maintain platelet counts at less than $0.5 \times 10^{10}/L$.

Overall, 326 of 357 patients had received at least one cytoreductive agent, including $^{32}$P in 40 patients, busulfan in 41, HU in 251, pipobroman in 43, and other drugs in five.

Methods. Cytogenetic analysis was performed with conventional banding techniques, and abnormalities were classified according to the International system for cytogenetic nomenclature. MDS and AML were classified according to French-American-British (FAB) criteria. Bone marrow smears obtained at diagnosis of MDS and AML were reviewed, with particular emphasis on analysis of myelodysplastic features.

Analysis of p53 mutations was made by single-strand conformation polymorphism (SSCP) analysis of exons 4 to 10 of the p53 gene and/or by immunocytochemical analysis of p53 protein using monoclonal antibodies, as previously described. Statistical analysis were performed with the chi-square test or Fisher’s exact test.

RESULTS

Clinical and hematologic features of cases of AML and MDS occurring after ET. With a median follow-up duration of 98 months (range, 22 to 265), 17 (4.5%) patients had progressed to AML (six cases) or MDS (11 cases). Fourteen cases were diagnosed among 334 patients monitored in Lille, and three among 23 patients monitored in Lomme. The characteristics and outcome of those patients are listed in Table 1. Fourteen patients had received HU, during a median period of 53 months (range, 3 to 96); seven of them had received HU alone, and seven had also received other treatments, including pipobroman in five (during a median period of 48 months; range, 4 to 76), $^{32}$P in one, melphalan in one, and busulfan in one (one patient had received three successive treatment regimens). The remaining three patients had received no HU, but had received $^{32}$P alone in two cases and busulfan alone in one case.

The median age of the 17 patients at diagnosis of ET was 62 years (range, 30 to 75), and there were 11 men and six women. The median interval between diagnosis of ET and diagnosis of AML or MDS was 84 months (range, 55 to 147). Six patients had AML not preceded by a phase of MDS, including five with M2 and one with M4 AML. Eleven patients had MDS, including refractory anemia (RA) in two cases, refractory anemia with excess of blasts (RAEB) in one, RAEB in transformation (RAEB-T) in six, and chronic myelomonocytic leukemia (CMML) in two. After the diagnosis of AML or MDS was made, two patients received intensive chemotherapy and one received low-dose cytarabine, with no response. Two patients were allografted as first-line therapy: one relapsed after 3 months and died, and one remained in complete remission after 72 months. The remaining patients were treated symptomatically. All patients except the allografted patient who was still in complete remission had died 1 to 36 months (median, 6) after the diagnosis of MDS or AML.

Cytogenetic and molecular features of AML and MDS occurring after ET. Thirteen of 17 patients had successful cytogenetic analysis at the time of progression to AML or MDS. Seven of them (41% of the 17 patients, and 54% of the karyotyped cases) had 17p deletions, resulting from unbalanced translocation between 17p and another chromosome in three cases (chromosome 5 in two and undetermined chromosome in one), del 17p in one, i(17q) in one, and monosomy 17 in the two remaining patients. Six of seven patients with a 17p deletion had additional cytogenetic abnormalities, involving in particular chromosome 5 and/or 7 (Table 1). Typical dysgranulopoiesis, including pseudo Pelger Huet hypolobulation and small vacuoles in greater than 5% neutrophils, as previously described,16,17 was seen in all of them. p53 mutation by SSCP analysis and/or p53 overexpression by immunocytochemistry (which in our experience in MDS and AML is always associated with the presence of a p53 missense mutation)20 was demonstrated in the six assessable cases.

The remaining patients had normal cytogenetics (two cases), complex cytogenetic findings without 17p involvement, where chromosome 5 but not 7 was involved (three cases), isolated acquired trisomy 21 (one case), or were not karyotyped (four cases). None of the 10 patients without 17p deletion had dysgranulopoiesis typical of 17p deletion cases and, among them, none of the four who were tested had a p53 mutation and/or overexpression.

Nine of 17 patients who progressed to AML or MDS had been karyotyped at diagnosis of ET, and cytogenetic findings were normal in all nine. They included five patients who had 17p deletions at the time of progression.

Incidence and features of AML and MDS according to cytoreductive treatment. The incidence of progression to AML and MDS according to cytoreductive treatment is shown in Table 2. It was 7.5% after $^{32}$P (7% after $^{32}$P alone), 5% after busulfan (3% after busulfan alone), 5.5% after HU (3.5% after HU alone), and 12% after pipobroman (zero of 12 patients treated with pipobroman alone). Differences were not significant among the four agents when used alone. However, progression was significantly more frequent after HU combined with other agents (seven of 50) than after HU alone (seven of 201, $P = .01$) and after one of the three other agents used alone (three of 76, $P = .04$).

The seven patients who progressed to AML and MDS with a
17p deletion had all received HU, as the sole cytoreductive agent in three of them, preceded or followed by pipobroman in three, and by busulfan and 32P in one case each. The median duration of treatment with HU in those seven patients had been 57 months. The three cases of AML or MDS that occurred in patients who had not received HU showed no 17p rearrangement. Thus, four cases of AML and MDS with 17p deletion occurred among 50 patients treated with HU and other drugs, as compared with three cases among 201 patients treated with HU alone (P = .03) and none among 106 patients who received no HU (P = .02).

**DISCUSSION**

The incidence of AML and MDS during the course of ET observed in this study where prolonged follow-up data were available in most patients (4.5%), was similar to that previously reported in other series of ET. Indeed, by combining the 14 published series (to our knowledge) of ET that included more than 30 patients,2-15 an incidence of progression to MDS or AML of 3.5% was found.

Most of the patients who developed MDS or AML in our study had received HU, generally for prolonged periods, and HU was the only cytoreductive agent used in seven of them. The incidence of MDS and AML in patients who had received HU alone was not significantly different than that of patients who had received busulfan alone or 32P alone, two treatments that are known to be leukemogenic. Most of our ET patients diagnosed before 1980 had received busulfan or 32P. After 1980, to avoid the leukemogenic effects of busulfan and 32P, patients were generally treated with HU. However, in Lille, we observed only one progression to AML among the first 147 patients monitored until 1988,5 whereas 13 new cases were diagnosed between 1988 and 1996.

These findings confirm that HU has some leukemogenic
potential in ET. HU is a nonalkylating myelosuppressive agent that inhibits DNA synthesis by inhibition of ribonucleoside diphosphate reductase, but also inhibits DNA repair.\cite{21} The leukemogenic potential of HU has been mainly studied in polycythemia vera (PV). In PV treated with HU alone, the incidence of progression to AML had initially been reported to be only 1% to 3% in two cohorts of approximately 100 cases followed over a median of 5 years.\cite{22,22} This was less than the 6% to 10% and 12% to 13% reported after ^32^P and chlorambucil, respectively, by the PV Study Group (PVSG) and other groups.\cite{24,25} On the other hand, the incidence of progression to AML in PV treated with HU alone was 8% at 12 years and 5.9% at 8.5 years in two recently published large series with prolonged follow-up evaluation\cite{26,27} as compared with 1.5% after phlebotomy alone.\cite{27} To our knowledge, after excluding cases with Philadelphia chromosome at diagnosis, 103 cases of acute leukemia and MDS occurring during the course of ET have been reported.\cite{2,15,16,60} Seven of them were acute lymphocytic leukemia and the remaining cases were AML or MDS. At least 34 of them had been treated with HU, and 19 with HU alone (Table 3). By combining published series of ET in which treatment and evolution were available,\cite{2,15} we found that 10 of 293 patients (3.4%) treated with HU alone had progressed to acute leukemia, a figure similar to our results.

In PV, long-term results of a large study that randomized patients to receive, after a first course of ^32^P, maintenance with HU versus no maintenance (and additional ^32^P if required) showed a significantly higher incidence of AML in the group maintained with HU, although the other group had received higher cumulative doses of ^32^P.\cite{28} In another study, leukemias in ET treated with HU occurred mainly in patients who, because of incomplete response to this drug, were switched to alkylating agents.\cite{29} Thus, HU also appears to increase the leukemic risk of other cytoreductive treatments administered in PV and ET. Our findings of a significantly higher incidence of evolution to AML and MDS in patients treated with HU and other agents as compared with HU alone or with other agents alone are in agreement with those reports.

Five of 17 patients reported here had, in addition to HU, received pipobroman, a bromide derivative of piperazine, which, although its formula is close to that of the alkylating agents, mainly appears to act as a metabolic competitor of pyrimidine bases.\cite{61} Pipobroman, in our experience and that of some other groups, often allows better control of platelet counts than HU.\cite{5,62} Three of the five MDS and AML cases occurring in patients who had received pipobroman had 17p deletions. The incidence of AML and MDS in patients who had received pipobroman was relatively high (12%), but AML and MDS occurred in patients who had also received other drugs, and none of the 12 patients treated with pipobroman alone had leukemic evolution. This suggests that the leukemic risk of pipobroman is found mainly in patients who also received other drugs, principally HU in our experience. Pipobroman has not been widely used in ET, and the risk of leukemia with this drug has not been previously well defined. In two series of 21 and 24 ET patients treated with pipobroman, no progression to AML had occurred, but the follow-up duration was relatively short.\cite{63,64} In PV, the risk of leukemia after pipobroman was considered to be 6% and 9% after 5 and 7 years of treatment, respectively, in one series,\cite{65} and 4.5% in another.\cite{66} Recently, in a randomized prospective study, it was evaluated at 8% after 12 years, similar to that observed with HU.\cite{26}

Our study also found that approximately 40% of the AML and MDS cases occurring in ET treated predominantly with HU had 17p deletions and other characteristics of what we and others described as the 17p− syndrome, ie, typical dysgranulopoiesis and p53 mutation.\cite{16,17} The seven ET patients who progressed to AML or MDS with a 17p deletion presented here had all received HU, which, in three of them, was the only antineoplastic agent used, whereas three other cases had also received pipobroman. Of the 103 cases of transformed ET reported until now, to our knowledge, 35 had cytogenetic analysis, and 17p deletion was found in five patients.\cite{4,13,42,51} Three of these five patients had monosomy 17 and additional complex cytogenetic findings, one had i(17q) (Table 4), and the last patient had deletion of chromosome 17.\cite{51} Four of these five patients had received HU as the sole antineoplastic agent, during 17, 24, 64, and 92 months, respectively (Table 4), and the last patient\cite{51} had received busulfan and low-dose cytarabine. At transformation, they were classified as M2 AML, acute undifferentiated leukemia, Mo AML, and RAEB-T, respectively, in four cases (Table 4), and not classified in the last case.\cite{51} No mention of dysgranulopoiesis was made in the five case reports. Patients with 17p deletions after treatment with HU alone constituted four of the 11 reported cases of ET that progressed to MDS or AML after HU alone and where karyotype was available (Table 3).

Morphologic, cytogenetic, and molecular features of the 17p− syndrome have not only been reported in AML and MDS, but also in CML in blast crisis. Indeed, in blast crisis CML, Sessarego et al\cite{67} found a strong correlation between pseudo Pelger Hüet hypolobulation and 17p deletion, often resulting from i(17q). Furthermore, in blast crisis CML, a correlation

<table>
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<tr>
<th>Table 2. Evolution to AML and MDS According to Cytoreductive Agents Received in 357 ET Patients</th>
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<tr>
<td><strong>Agent</strong></td>
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<tr>
<td><strong>32P</strong></td>
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<tr>
<td>Alone</td>
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<tr>
<td>And other agents*</td>
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<tr>
<td>Total</td>
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<tr>
<td>Busulfan</td>
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<td>And other agents*</td>
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<tr>
<td>Total</td>
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<tr>
<td>HU</td>
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<tr>
<td>Alone</td>
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<td>And other agents†</td>
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<tr>
<td>Total</td>
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<tr>
<td>Pipobroman</td>
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<tr>
<td>Alone</td>
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<tr>
<td>And other agents*</td>
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<tr>
<td>Total</td>
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<tr>
<td>Untreated</td>
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</table>

*Generally HU.  
†Generally pipobroman.
between p53 mutation and 17p deletion has been reported. Because blast crisis is the “natural” evolution of CML, one could suggest that blast crisis of other myeloproliferative disorders with features of the 17p syndrome could also result from their natural evolution. However, in untreated ET, leukemic evolution appears to be rare: only two of 46 evolutions of ET to acute leukemia recorded in large series of patients (Table 3) occurred in untreated patients.

The relatively high incidence of chromosome 17 involvement in MDS and AML following ET treated by HU in the literature and our findings suggests that prolonged use of HU in ET may lead to or at least increase the risk of MDS and AML with loss of 17p chromosomal material and p53 mutation. The incidence was particularly high (four of 50 cases) in patients treated with HU and other drugs. In particular, three cases occurred in patients who had received HU and pipobroman. Thus, a role for pipobroman in the pathogenesis of MDS and AML with 17p deletion, which could be additive to that of HU, is also possible in ET. Recently, Gaidano et al also found p53 mutations in four of 10 cases of AML following ET, in a study so far published only in abstract form. No data on treatment received by those patients was available, but they came from centers in which HU is generally used as first-line therapy in ET. Data on dysgranulopoiesis and karyotype at the time of progression to AML were not available. However, because of the high correlation between 17p deletion and p53 mutations observed in myeloid malignancies, it is probable that several of those patients had 17p deletions.

Overall, our findings in a large series of patients, with prolonged follow-up evaluation, confirm a certain leukemogenic potential for HU in ET. The use of HU probably also increases the leukemic risk of other cytoreductive treatments given in ET. We also found that MDS and AML occurring after treatment with HU (and possibly pipobroman) often had a 17p deletion, confirming a few previously published case reports. However, our findings need to be confirmed. We therefore encourage the publication of all cases of AML and MDS occurring during the course of ET treated with HU, with special emphasis on their morphologic and cytogenetic characteristics. Description of larger numbers of MDS and AML with 17p deletions after HU could lead to reconsideration of the widespread use of this drug and to more limited use, for instance, in

<table>
<thead>
<tr>
<th>First Author (Reference)</th>
<th>FAB Type</th>
<th>Treatment: Months</th>
<th>Karyotype</th>
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<tbody>
<tr>
<td>Furgerson30</td>
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<td>HU: 126</td>
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<td>HU: 47</td>
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<td>M2 AML</td>
<td>HU: 17</td>
<td>44, XX, –5, –7, + der(7)</td>
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<td>Murphy29</td>
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<td>46, XX, add(6)(p11),der(12)(q;16)(p11;p13),add(18)(p11), der(20)(q12;20)(q13;q11)</td>
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<td>Groupe Francais de Cytogenetique Hematologue12</td>
<td>UAL</td>
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<td>46, XX;i(17q)</td>
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<td>ALL</td>
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<td>M2 AML</td>
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<td>43,X,–Y,–7,t(7;8)(q34;22),t(7;12)(q34;13),–11,dic(12;14)</td>
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<td>Murphy29</td>
<td>ND</td>
<td>HU</td>
<td>ND</td>
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<td>HU and others drugs (15 cases)</td>
<td>UAL</td>
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<td>CMML</td>
<td>HU: 38, 6MP</td>
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<td>M4 AML</td>
<td>HU: 22, MEL</td>
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<tr>
<td>1 case</td>
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<td>HU + 35P</td>
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</tr>
<tr>
<td>Murphy29</td>
<td>5 cases</td>
<td>HU + 35P</td>
<td>ND</td>
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</tbody>
</table>

Abbreviations: UAL, undifferentiated acute leukemia; MDS, MDS of unspecified type; ALL, acute lymphocytic leukemia; ANLL, acute nonlymphocytic leukemia; CMML, chronic myelomonocytic leukemia; ND, not determined; MEL, melphalan; 6MP, 6-mercaptopurine.
asymptomatic ET. It would also possibly encourage larger use of drugs that are probably nonleukemogenic, such as interferon and anagrelide, when treatment is required in ET. From a more fundamental viewpoint, description of other cases of MDS and AML with 17p deletion after HU would also point to a possible relationship between HU and genes located in chromosome 17 (including the p53 gene), whose disruption could participate in the leukemogenic process.

REFERENCES

Acute Myeloid Leukemia and Myelodysplastic Syndromes Following Essential Thrombocythemia Treated With Hydroxyurea: High Proportion of Cases With 17p Deletion

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