Treatment of progression of Philadelphia-negative myeloproliferative neoplasms to myelodysplastic syndrome or acute myeloid leukemia by azacitidine: a report on 54 cases on the behalf of the Groupe Francophone des Myelodysplasies (GFM)

*Sylvain Thepot,1,2 *Raphael Itzykson,1 Valerie Seegers,3 Emmanuel Raffoux,4 Bruno Quesnel,5 Yasmine Chait,6 Lucile Sorin,7 Francois Dreyfus,8 Thomas Cluzeau,9 Jacques Delaunay,10 Laurence Sanhes,11 Virginie Eclache,12 Caroline Dartigues,13 Pascal Turlure,14 Stephanie Harel,15 Celia Salanoubat,16 Jean-Kacibadijan,17 Pierre Fenaux,1,2 and Lionel Adès1,2

1 Service d’Hematologie Clinique, Hôpital Avicenne, Assistance Publique–Hôpitaux de Paris (AP-HP), Université Paris 13, Bobigny, France; 2Inserm U848, Villejuif, France; 3Département d’Informatique Medecale, Hôpital Saint Louis AP-HP, Université Paris 7, Paris, France; 4Service d’Hematologie Adulte, Hôpital Saint Louis AP-HP, Université Paris 7, Paris, France; 5Service d’Hematologie Clinique, Hôpital Huriet, Centre Hospitalier Universitaire (CHU) de Lille, Université Lille 2, Lille, France; 6Service d’Oncologie-hématologie, Groupe Hospitalier Intercommunal, Montfermeil, France; 7Service d’Hematologie clinique, Hôpital Purpan, CHU de Toulouse, Université Paul Sabatier, Toulouse, France; 8Service d’Hematologie Clinique, Hôpital Cochin APHP, Université Paris 5, Paris, France; 9Service d’Hematologie Clinique, Hôpital l’Archet, Université de Nice, Nice, France; 10Service d’Hematologie Clinique, Hôpital Cochin, Université Paris 5, Paris, France; 11Service d’Hematologie, Centre Hospitalier Universitaire de Perpignan, Perpignan, France; 12Laboratoire d’Hematologie, Hôpital Avicenne, Université Paris 13, Bobigny, France; 13Service d’Hematologie, Hôpital Bretonneau, Université de Tours, Tours, France; 14Service d’Hematologie, Hôpital Dupuytren, Université de Limoges, Limoges, France; 15Service d’Hematologie Adulte, Hôpital Necker AP-HP, Université Paris 5, Paris, France; 16Service d’Hematologie, Centre Hospitalier Sud Francillien, Corbeil Essonne, France; and 17Centre d’Investigation Clinique, Hôpital Saint Louis AP-HP, Université Paris 7, Paris, France

Transformation of Philadelphia (Ph)–negative myeloproliferative neoplasms (MPNs) to myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML) is associated with poor response to chemotherapy and short survival. Fifty-four patients with Ph-negative MPN (including 21 essential thrombocytopenia [ET], 21 polycythemia vera [PV], 7 primary myelofibrosis, and 5 unclassified MPN) who had progressed to AML (n = 26) or MDS (n = 28) were treated with azacitidine in a patient-named program. Overall response rate was 52% (24% complete response [CR], 11% partial response [PR], 8% marrow CR or CR with incomplete recovery of cytopenias, 9% hematologic improvement) and median response duration was 9 months. Prognostic factors were for overall response the underlying MPN (71% vs 33% responses in ET and PV, respectively; \( P = .016 \)); prognostic factors for CR achievement were the underlying MPN (14% CR for PV vs 43% for ET; \( P = .040 \)) and World Health Organization classification at transformation (36% vs 12% CR in MDS and AML, respectively, \( P = .038 \)). Recurrence of chronic phase features of the initial MPN was observed in 39% of the responders. Median overall survival was 11 months. Azacitidine gives encouraging results in Ph-negative MPN having progressed to AML or MDS, but response duration is short, and consolidation treatments have to be evaluated. (Blood. 2010; 116(19):3735-3742)

Introduction

Philadelphia (Ph)–negative myeloproliferative neoplasm (MPN) progression to acute myeloid leukemia (AML) is preceded or not by myelodysplastic syndromes (MDS) in 5%–10% of the cases after 10 years and probably at an even higher incidence with longer follow-up.1,4 Those transformations are associated with poor response to chemotherapy (including anthracycline/cytosine arabinoside–based intensive chemotherapy [IC] or lower dose regimens), to a high risk of relapse after allogeneic stem cell transplantation (SCT), resulting in poor survival in most cases.5,6 Aberrant cytosine-phosphate-guanosine island methylation of tumor suppressor genes plays a key role in the progression of MDS.7 In MPN, the mechanisms underlying progression to MDS and AML are poorly known, although hypermethylation of p15INK4b and p16INK4a genes has been described during the leukemic evolution of myeloid metaplasia.8 The hypomethylating agent azacitidine has shown significant survival benefit in higher risk MDS.9,10 In MPN, azacitidine appears to have limited efficacy in primary myelofibrosis (PMF) and myelofibrosis occurring during the evolution of essential thrombocythemia (ET) or polycythemia vera (PV) despite the induction of global hypomethylation.11,12 However, to our knowledge, response to azacitidine has not been evaluated in series of MDS and AML occurring during the course of Ph-negative MPN. We report here our experience with azacitidine treatment in 54 patients with MDS or AML secondary to MPN.

Methods

Patients

After approval of azacitidine by the US Food and Drug Administration (FDA) for the treatment of MDS in 2004 and before approval by the European Agency for the Evaluation of Medicinal Products (EMEA) at the end of 2008, the French health agency (Agence Française de Sécurité Sanitaire des Produits de Santé [AFSSAPS]) opened a compassionate patient-named program (Autorisation Temporaire d’Utilization) of azacitidine in higher risk MDS and poor-risk AML, in cooperation with the Groupe Francophone des Myélodysplasies (GFM). All patients with...
international prognostic scoring system intermediate 2 or high-risk MDS (de novo or therapy related) could be included in this program. For untreated AML, patients could only enter the program if they were considered “untut” for IC, based on age (generally > 65 years), comorbidities, or on characteristics associated with poor response to IC (AML after MDS or MPN, complex cytogenetic findings). All applications were reviewed by AFSSAPS (including review by one expert in the field) for approval, and patient informed consent was required before inclusion. A short case report form was sent to the treating physician after inclusion.

Between September 2004 and January 2009, 931 patients were included in this program; 735 of them, who had received ≥ 1 cycle of azacitidine and were treated in centers that had accepted to send data for all their patients included in the program to GFM, were considered evaluable (centers that did not send information on all their patients were considered not evaluable, to avoid any bias). All participating centers approved this study. Of those 735 patients, 54 with MDS or AML after Ph-negative MPN formed the basis of the present study. The study was conducted according to the Declaration of Helsinki.

Diagnosis and classification of MDS and AML was made according to the World Health Organization (WHO) classification. However, because azacitidine has also been approved by FDA and EMEA in refractory anemia with excess blasts in transformation (RAEB-t), considered as AML in the WHO classification but as MDS in the French-American-British (FAB) classification, analysis of results was also made with FAB criteria for AML and MDS. Although most initial diagnoses of MPN have been made before the Janus kinase-2 (JAK2) V617F era, using both the Polycythemia Vera Study Group and WHO 2001 criteria, JAK2 mutational status, available in 41 patients during MPN chronic phase, was considered evaluable in 24 patients (59%; Table 1). During MPN chronic phase, JAK2 V617F positive in 24 patients (59%; Table 1). During MPN chronic phase, JAK2 V617F positive in 24 patients (59%; Table 1). During MPN chronic phase, JAK2 V617F positive in 24 patients (59%; Table 1). During MPN chronic phase, JAK2 V617F positive in 24 patients (59%; Table 1). During MPN chronic phase, JAK2 V617F positive in 24 patients (59%; Table 1). During MPN chronic phase, JAK2 V617F positive in 24 patients (59%; Table 1). During MPN chronic phase, JAK2 V617F positive in 24 patients (59%; Table 1). During MPN chronic phase, JAK2 V617F positive in 24 patients (59%; Table 1).

Table 1. Patient characteristics and treatment results according to initial MPN

<table>
<thead>
<tr>
<th>All patients</th>
<th>PV</th>
<th>ET</th>
<th>PMF</th>
<th>MPNu</th>
<th>Overall P</th>
<th>P (ET vs PV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>54</td>
<td>21</td>
<td>21</td>
<td>7</td>
<td>5</td>
<td>NS</td>
</tr>
<tr>
<td>Median age, y</td>
<td>68.5</td>
<td>66</td>
<td>71</td>
<td>73</td>
<td>71</td>
<td>NS</td>
</tr>
<tr>
<td>JAK2 V617F positive in chronic phase, n/N (%)</td>
<td>24/41 (59)</td>
<td>12/14 (86)</td>
<td>9/16 (56)</td>
<td>2/7 (29)</td>
<td>1/4 (25)</td>
<td>.023</td>
</tr>
<tr>
<td>Median time to transformation, mo</td>
<td>71</td>
<td>154</td>
<td>70</td>
<td>19</td>
<td>19</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>AML at transformation, n (%)</td>
<td>26 (48)</td>
<td>13 (62)</td>
<td>10 (48)</td>
<td>3 (43)</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td>MDS at transformation, n (%)</td>
<td>28 (52)</td>
<td>8 (38)</td>
<td>11 (52)</td>
<td>4 (57)</td>
<td>5 (100)</td>
<td>NS</td>
</tr>
<tr>
<td>Complex karyotype at transformation, n (%)</td>
<td>16 (30)</td>
<td>8 (44)</td>
<td>6 (33)</td>
<td>2 (33)</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td>Overall response to azacitidine, n (%)</td>
<td>28 (52)</td>
<td>7 (33)</td>
<td>15 (71)</td>
<td>4 (57)</td>
<td>2 (40)</td>
<td>NS</td>
</tr>
<tr>
<td>Restoration of chronic phase MPN features after azacitidine, n (%)</td>
<td>11 (20)</td>
<td>3 (14)</td>
<td>8 (38)</td>
<td>0</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td>Median survival from onset of azacitidine, mo</td>
<td>11</td>
<td>7</td>
<td>16</td>
<td>8</td>
<td>10</td>
<td>NS</td>
</tr>
</tbody>
</table>

PV indicates polycythemia vera; ET, essential thrombocythemia; PMF, primary myelofibrosis; MPNu, myeloproliferative neoplasm unclassified; NS, not significant; JAK2, Janus kinase-2; AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; and MPN, myeloproliferative neoplasm.

**Treatment**

Azacitidine was to be administered subcutaneously at the approved FDA/EMEA schedule (75 mg/m^2/d during 7 days every 28 days) for ≥ 4 cycles. Dose reductions were made in case of grade ≥ 2 side effects according to National Cancer Institute toxicity criteria (Common Terminology Criteria for Adverse Events v3.0), except for neutropenia or thrombocytopenia or both. Delays in cycles were recommended in case of grade 4 cytopenias, until recovery above baseline values or on the physician’s decision. All responders after 4-6 cycles of azacitidine were to continue treatment until progression. Transfusion thresholds, in agreement with AFSSAPS were as follows. Recommendations for red blood cell transfusions were hemoglobin (Hb) level of < 8 g/dL or higher (9-10 g/dL) in case of severe infection, underlying cardiac or pulmonary disease, or severe symptoms of anemia; recommendations for prophylactic platelet transfusions were platelet count < 20 x 10^9/L or less in case of fever, rapid platelet decrease, mucositis, or concomitant coagulopathy.

**Assessment of response**

Response was evaluated after 4-6 cycles by blood count, bone marrow aspirate, and cytogenetic studies. Response was defined for MDS as achievement of complete response (CR), partial response (PR), marrow CR (mCR) or hematologic improvement (HI) according to the International Working Group (IWG) 2006 criteria; for AML as achievement of CR, PR, or CRi (ie, CR with incomplete recovery of cytopenias) according to IWG-AML 2003 criteria. Patients in CR/CRI were allowed to have persistent blood or marrow features or both of MPN, provided the blast percentage and peripheral blood cytopenias satisfied response criteria.

Complete cytogenetic response was defined by the disappearance of all chromosomal abnormalities without appearance of new ones and partial cytogenetic response by at least a 50% reduction of the number of mitoses with any chromosomal abnormality, in agreement with IWG 2006 criteria for MDS. Recurrence of initial features of MPN was defined by a Hb level > 17 g/dL in men and > 15 g/dL in women if associated with an increase of ≥ 0.2 g/L from a person’s baseline value, or a sustained platelet count > 450 x 10^9/L, in agreement with WHO 2008 criteria for MPN classification.

**Data collection and statistical analysis**

Because the case report form sent to treating centers on inclusion was relatively short, missing data were subsequently collected by clinical research associates of the GFM. Prognostic factors of response were analyzed in the whole population and separately in MDS and AML by univariate and multivariate analyses (the latter with the use of a logistic regression model). Characteristics were compared by chi-square or nonparametric tests (Fisher exact test) for qualitative variables, and quantitative variables were compared by Wilcoxon and Kruskal-Wallis tests. Censored end points were estimated by the nonparametric Kaplan-Meier method, then compared between groups by the log-rank test. Analyses were made at the reference date of December 1, 2009, with R software 2.8.1 (The R Project).

**Results**

**Pretreatment characteristics of the patient population**

The study population consisted of 34 men and 20 women, with a median age of 69.5 years (range, 37-89 years) at inclusion in the azacitidine program. The initial MPN was PV in 21 patients (39%), ET in 21 patients (39%), PMF in 7 patients (13%), and unclassified MPN in 5 patients (9%). JAK2 V617F mutational status, available in 41 patients during MPN chronic phase, was positive in 24 patients (59%; Table 1). During MPN chronic phase, 50 of 54 patients had received cytoreductive therapy, whereas 4 patients had only received antiaggregating or anticoagulant...
therapy. Cyto-reductive therapy had been hydroxyurea (HU) in 34 patients, pipobroman in 20 patients (11 of whom had also received HU before or after pipobroman; Table 2). Four patients with 4 PV had received radiophosphorus (P32), 2 patients with PV had received interferon alfa, and 3 patients with atypical CML had received imatinib mesylate.

Median time from diagnosis of MPN to MDS/AML was 71 months (range, 3-398 months) in the whole cohort, 89 months for patients with AML, and 58 months for patients with MDS (P = .006). Eight patients had already been treated after transformation by IC followed by allogeneic SCT in 3 patients, IC alone in 2 patients, and low-dose cytosine arabinoside in 3 patients. At the onset of azacitidine, 5 patients were refractory and 3 patients were in relapse after those treatments.

At inclusion in the azacitidine program, 26 patients (48%) had AML, including 9 with RAEB-t (17%), and 28 patients (52%) had MDS, including 9 (17%) with RAEB-1 and 19 (35%) with RAEB-2.

Eight patients (15%) had cytogenetic failure, 10 (19%) had normal karyotype, and 36 (67%) had 1 or several clonal abnormalities: 12 patients had 1 abnormality, including isolated −7/−7q− in 5 patients and isolated +8 in 2 patients; 8 patients had 2 abnormalities; and 16 patients (30%) had a complex karyotype (≥3 abnormalities) that included −7/del 7q in 9 patients, and del 5q/−5 in 11 patients, with a median number of 5.5 abnormalities (range, 3-9 abnormalities). Chromosome 17 abnormalities were observed in 11 patients (del 17p in 8 patients and −17 in 3 patients). In MDS (n = 28), the International Prognostic Scoring System was int-1 in 2 patients (7%), int-2 in 12 patients (43%), and high in 10 patients (36%), whereas the remaining 4 patients with MDS had cytogenetic failure (Table 3). Four of the 5 unclassified MPN had normal karyotype (and 1 cytogenetic failure) during MPN phase and 3 had monosomy 7 at transformation. Finally, the 15 patients with JAK2-V617F tested at the time of transformation still had JAK2-V617F mutation, whereas the remaining 9 were not tested.

**Response to azacitidine**

The median number of cycles of azacitidine administered was 6 (range, 1-28 cycles). Thirty-five (65%) patients received azacitidine at the FDA/EMEA-approved schedule (75 mg/m²/d during 7 days every 28 days), whereas 19 patients (35%) received an attenuated dosing or schedule (100 mg/d during 7 days or 75 mg/m²/d during 5 days). The main reason to reduce the number of days to 5 per cycle was the difficulty for some centers to administer the drug during weekends, whereas the main reason to use a flat dose of 100 mg/d was patients’ older age or frailty or both (including renal failure, observed in 5 cases). Thirteen patients (24%) received <4 cycles of azacitidine because of early death in 9, restoration of MPN features in 2 patients, intercurrent illness in 1 patient, and 1 patient’s decision 1; whereas 41 patients (76%) received ≥4 cycles.

As seen in Table 4, response was achieved in 28 of 54 patients (52%), including 13 CR (24%), 1 CRi (2%), 3 mCR (6%), 6 PR (11%), and 5 stable disease with hematologic improvement (9%; HI-E = 3 and HI-P = 2). Four of the 11 patients with baseline cytogenetic abnormality who achieved CR had cytogenetic evaluation at response, and 3 of those 4 patients achieved complete cytogenetic response.

With a median follow-up of 17 months after response evaluation, 23 of the 28 responders had relapsed after a median of 6 months (range, 1.5-22 months), and 6 patients were still responding after a median of 15 months (range, 10 to 34 months). Median response duration was 9 months. One of the complete responders received allogeneic SCT and was alive in CR after 14 months.

**Prognostic factors of response**

The underlying MPN was the only prognostic factor for overall response rate (ORR), with a higher ORR in patients with ET (71%) than for patients with PV (33%; P = .016; Table 1). In the cases of PMF, 4 responses, including 1 CR, 1 PR, and 2 HI, were

---

**Table 2. Clinical characteristics and treatment of MPN before transformation**

<table>
<thead>
<tr>
<th>Karyotype</th>
<th>ET</th>
<th>PV</th>
<th>PMF</th>
<th>MPNu†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Favorable*</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Intermediate*</td>
<td>10 (38)</td>
<td>7 (33)</td>
<td>11 (52)</td>
<td>12 (40)</td>
</tr>
<tr>
<td>Unfavorable*</td>
<td>12 (46)</td>
<td>7 (33)</td>
<td>11 (52)</td>
<td>12 (40)</td>
</tr>
</tbody>
</table>

**Table 3. Patient characteristics at transformation**

<table>
<thead>
<tr>
<th>Karyotype</th>
<th>AML (n = 26), n (%)</th>
<th>MDS (n = 28), n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Favorable*</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Intermediate*</td>
<td>10 (38)</td>
<td>12 (46)</td>
</tr>
<tr>
<td>Unfavorable*</td>
<td>12 (46)</td>
<td>14 (54)</td>
</tr>
</tbody>
</table>

**WHO classification**

- Marrow blasts > 30%: 17 (65)
- Marrow blasts 20%-30% (RAEB-1): 9 (35)
- RAEB-1: 9 (32)
- RAEB-2: 19 (68)

**IPSS**

- Low: 1 (20)
- Intermediate 1: 2 (7)
- Intermediate 2: 12 (43)
- High: 10 (36)
- NE‡: 4 (14)

---

HU indicates hydroxyurea; ET, essential thrombocythemia; PV, polycythemia vera; PMF, primary myelofibrosis; and MPNu, myeloproliferative neoplasm unclassified.

*During MPN phase.

†Three of the patients with MPNu received imatinib.

‡Medical Research Council classification.

§International Prognostic Scoring System classification.

¶Cytogenetic failure.
observed. Other pretreatment factors, including age, sex, white blood cell count, percentage of bone marrow blasts, JAK2 mutational status, and karyotype, were not significantly correlated with ORR. In AML, the ORR was 38%, including 12% CR, 4% CRi, and 23% PR. In MDS, the ORR was 64%, including 36% CR, 11% mCR, and 21% stable disease with HI. The comparison between ORR obtained in AML and MDS was difficult, because of somewhat different response criteria in those 2 categories (Table 4). When classifying patients with the FAB criteria, the ORR was 59% in MDS and 35% in AML (P = NS; details not shown).

Prognostic factors of CR achievement were the type of underlying MPN (14% CR for PV vs 43% for ET; P = .040) and WHO classification at transformation (12% CR in patients with AML vs 36% in MDS; P = .038; the comparison being possible because of similar CR criteria in AML and MDS). When adjusted on underlying MPN, WHO classification at transformation became even more significant (P = .007).

Prognostic factors of response duration were the presence of JAK2 V617F mutation (median of 11 months vs 5 months for JAK2 wild type; P = .005) and the recurrence of chronic phase features of MPN at response (median of 9 vs 5 months in the absence of such recurrence; P = .005). Other pretreatment characteristics were not prognostic of response duration. In particular, median response duration was 9 months in AML (range, 1.6 to 17 months) compared with 6 months in MDS (range, 1.2 to 34 months; P = NS).

Recurrence of chronic phase features of underlying MPN

A somewhat unexpected finding was that, in 11 patients (20%), corresponding to 39% of the responders, and including 8 of the 21 (38%) patients with initial ET and 3 of the 21 patients (14%) with initial PV (P = .80), response to azacitidine was associated with recurrence of chronic phase features of the initial MPN, ie, thrombocytopenia in 8 and polycythemia in 3. This response was seen after 1-5 cycles (median, 3 cycles) and required restarting cytoreductive therapy with HU or interferon in 4 of them. No such evolution was observed in the 4 patients with PMF who responded. In 2 patients, recurrence of chronic phase MPN features lead to early discontinuation of azacitidine after 3 courses (Table 5). At the onset of azacitidine, 3 of the 11 patients with recurrence of chronic phase MPN features had AML and 8 had MDS. JAK2 V617F mutation was present during the chronic MPN phase in 5 of the 9 tested patients, during transformation in 3 of 5 tested patients, and still positive in both patients tested during the second chronic MPN phase.

According to IWG response criteria, 9 of the 11 patients with recurrence of chronic phase MPN features had achieved CR (including 2 cytogenetic CR) and 2 HI (patients 14 and 33 in Table 3). By univariate analysis, no prognostic factor of recurrence of chronic phase MPN features (including age, sex, marrow blast percentage, white blood cell count, platelet count, JAK2 mutation status, previous MPN type) was found in the whole patient population and in responders to azacitidine. Nine of the 11 patients relapsed to AML/MDS after a median time of 8.6 months (range, 1.6-22 months), whereas 2 remained responders: 1 after ≥33 months, treated by interferon and 1 who received an allograft (with reduced-intensity conditioning regimen) in CR after 4 cycles of azacitidine, still alive in CR 14 months after transplantation.

Overall survival

With a median follow-up of 20 months (range, 3 to ≥39 months) from onset of azacitidine, 44 patients (81%) had died, 30 (68%) from overt disease progression, 5 (11%) from bleeding (including 3 during azacitidine treatment), 8 (18%) from sepsis (including 1 during azacitidine treatment), 1 (4%) from thrombosis without recurrence of PV, and 1 (4%) from cardiac failure. Ten patients (19%) were still alive after ≥3 to ≥39 months, including 4 patients in CR and 2 in persisting HI.

Median OS was 11 months (Figure 1), 8 months in AML and 14 months in MDS (P = NS). According to the FAB classification, median OS was 8 months in AML and 13 months in MDS (P = NS).

OS did not significantly differ between patients with ET and PV as initial MPN (median, 16 vs 7 months, respectively; P = .150; Figure 2). When adjusted on initial MPN (ET vs PV), OS, however, became significantly better for patients with MDS than for patients with AML (P = .01). In patients with initial PMF, median survival was 8 months. Other pretreatment characteristics including age, sex, platelet count, circulating blast count, Hb level, percentage of bone marrow blasts karyotype, and JAK2 mutational status were not predictive of OS. In multivariate analysis, adverse cytogenetics (including complex karyotype, −7 or del 7q) and higher marrow percentage, white blood cell count, platelet count, JAK2 V617F mutation status, previous MPN type) was found in the whole patient population and in responders to azacitidine. Nine of the 11 patients relapsed to AML/MDS after a median time of 8.6 months (range, 1.6-22 months), whereas 2 remained responders: 1 after ≥33 months, treated by interferon and 1 who received an allograft (with reduced-intensity conditioning regimen) in CR after 4 cycles of azacitidine, still alive in CR 14 months after transplantation.

Overall survival

With a median follow-up of 20 months (range, 3 to ≥39 months) from onset of azacitidine, 44 patients (81%) had died, 30 (68%) from overt disease progression, 5 (11%) from bleeding (including 3 during azacitidine treatment), 8 (18%) from sepsis (including 1 during azacitidine treatment), 1 (4%) from thrombosis without recurrence of PV, and 1 (4%) from cardiac failure. Ten patients (19%) were still alive after ≥3 to ≥39 months, including 4 patients in CR and 2 in persisting HI.

Median OS was 11 months (Figure 1), 8 months in AML and 14 months in MDS (P = NS). According to the FAB classification, median OS was 8 months in AML and 13 months in MDS (P = NS).

OS did not significantly differ between patients with ET and PV as initial MPN (median, 16 vs 7 months, respectively; P = .150; Figure 2). When adjusted on initial MPN (ET vs PV), OS, however, became significantly better for patients with MDS than for patients with AML (P = .01). In patients with initial PMF, median survival was 8 months. Other pretreatment characteristics including age, sex, platelet count, circulating blast count, Hb level, percentage of bone marrow blasts karyotype, and JAK2 mutational status were not predictive of OS. In multivariate analysis, adverse cytogenetics (including complex karyotype, −7 or del 7q) and higher marrow
Table 5. Baseline characteristics and their evolution in patients who restored chronic phase MPN features during azacytidine treatment

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age, y</th>
<th>Initial MPN status</th>
<th>Time to progression, y</th>
<th>Marrow blasts, %*</th>
<th>Hb level at inclusion, g/L</th>
<th>Plt count at inclusion, × 10^9/L</th>
<th>Cytogenetics*</th>
<th>Response</th>
<th>Hb level at evaluation, g/L†</th>
<th>Plt count at evaluation, × 10^9/L†</th>
<th>Cytoreductive therapy‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>60</td>
<td>PV</td>
<td>7.8</td>
<td>AML</td>
<td>8§</td>
<td>8.4</td>
<td>20</td>
<td>CRi</td>
<td>17.1</td>
<td>300</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>61</td>
<td>PV</td>
<td>22.2</td>
<td>RAEB-2</td>
<td>10</td>
<td>8.0</td>
<td>70</td>
<td>CR</td>
<td>12.1</td>
<td>900</td>
<td>IFN</td>
</tr>
<tr>
<td>10</td>
<td>71</td>
<td>PV</td>
<td>11</td>
<td>RAEB-2</td>
<td>17</td>
<td>8.8</td>
<td>147</td>
<td>CR</td>
<td>17.0</td>
<td>325</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>82</td>
<td>ET</td>
<td>8.9</td>
<td>AML</td>
<td>34</td>
<td>8.4</td>
<td>22</td>
<td>Failure</td>
<td>10.2</td>
<td>658</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>75</td>
<td>ET</td>
<td>14.4</td>
<td>RAEB-2</td>
<td>12</td>
<td>10.1</td>
<td>478</td>
<td>CR</td>
<td>14.5</td>
<td>871</td>
<td>BU</td>
</tr>
<tr>
<td>19</td>
<td>85</td>
<td>ET</td>
<td>16</td>
<td>RAEB-2</td>
<td>11</td>
<td>9.5</td>
<td>258</td>
<td>CR</td>
<td>10.6</td>
<td>790</td>
<td>HU</td>
</tr>
<tr>
<td>24</td>
<td>87</td>
<td>ET</td>
<td>15.3</td>
<td>RAEB-1</td>
<td>7</td>
<td>9.6</td>
<td>48</td>
<td>Failure</td>
<td>15.9</td>
<td>485</td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>59</td>
<td>ET</td>
<td>2.3</td>
<td>RAEB-1</td>
<td>18</td>
<td>8.0</td>
<td>135</td>
<td>CR</td>
<td>14.4</td>
<td>641</td>
<td>HU</td>
</tr>
<tr>
<td>44</td>
<td>61</td>
<td>ET</td>
<td>13.8</td>
<td>RAEB-1</td>
<td>8</td>
<td>9.1</td>
<td>82</td>
<td>CR</td>
<td>15.3</td>
<td>547</td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>81</td>
<td>ET</td>
<td>2.4</td>
<td>RAEB-2</td>
<td>10</td>
<td>7.4</td>
<td>58</td>
<td>CR</td>
<td>13.4</td>
<td>591</td>
<td>HU</td>
</tr>
<tr>
<td>50</td>
<td>61</td>
<td>ET</td>
<td>6.8</td>
<td>AML</td>
<td>26</td>
<td>10</td>
<td>128</td>
<td>CR</td>
<td>15.1</td>
<td>808</td>
<td></td>
</tr>
</tbody>
</table>

MPN indicates myeloproliferative neoplasm; JAK2, Janus kinase-2; WHO, World Health Organization; Hb, hemoglobin; Plt, platelet, PV, polycythemia vera; WT, wild type; AML, acute myeloid leukemia; CRi, complete response with incomplete recovery of cytopenias; RAEB-2, refractory anemia with excess blasts 2; CR, complete response; IFN, interferon; ET, essential thrombocythemia; NA, not assessed; AML, acute myeloid leukemia; HI, hematologic improvement; BU, oral busulfan; HU, hydroxyurea; and RAEB-1, refractory anemia with excess blasts 1.

*At inclusion.
†At response.
‡After recurrence of chronic phase MPN features.
§This patient was classified as AML on the basis of 31% circulating blasts.
blast percentage (as continuous variable) were associated with worse OS ($P = .03$ for both parameters).

**Side effects of treatment**

Grade 3-4 hematologic toxicities were the main side effects of azacitidine, mainly during the first 4 cycles of azacitidine: 23 patients (43%) and 3 patients (6%) had infection and bleeding requiring hospitalization, with a fatal outcome in 4 and 2 cases, respectively. Nonhematologic side effects included grade 3 skin rash in 1 patient, leading to early azacitidine discontinuation. Grade 1-2 nonhematologic side effects mainly included diarrhea, constipation, and local side effects at injection sites, as previously described for azacitidine.11,12

**Discussion**

This is, to our knowledge, the first report of a relatively large cohort of patients with Ph-negative MPN treated by a hypomethylating agent after having progressed to MDS/AML. We observed a 64% response rate to azacitidine in patients who had progressed to MDS and 38% in patients who had progressed to AML. The response rate was significantly higher in patients with ET than for patients with PV as initial MPN and, in close to 40% of the responders, response was associated with recurrence of chronic phase MPN features (thrombocytosis in ET and erythrocytosis in PV; Table 5).

The response rate to azacitidine observed in this series of MDS secondary to MPN was similar to that observed in MDS as a whole,3,10 although the CR rate was somewhat higher in the present report, whereas response duration was somewhat shorter (median of 9 months compared with 13.6 months in AZA 001 study and 21 months in CALGB 9221 study, but the last study included a high proportion of lower risk MDS).

The only significant prognostic factor for ORR was the type of underlying MPN. Indeed, patients evolving from ET responded better than patients evolving from PV while in transformed PMF; the number of patients was small to conclude (4 of the 7 patients responded). Patients with MDS at transformation and patients with ET as primary MPN had significantly higher CR rates than patients with AML at transformation and PV as primary MPN, respectively. In agreement with previous studies of azacitidine in MDS,10,23 complex karyotype was not a poor predictive factor for response. This is particularly important to consider in transformations of MPN, which often carry complex chromosomal abnormalities.

The response rates and survival we observed appeared better than those generally reported with other treatments in MDS/AML occurring during the course of MPN. Indeed, ≥ 4 series, totaling ~ 200 AML/MDS after MPN treated with low-dose chemotherapy, IC or best supportive care only have been reported,5,24-26 all of them with poor results (Table 6). In particular, IC gave a CR rate of 46%, but remissions were short, and median progression-free survival was only 5 months in the M.D. Anderson study.26 In the absence of subsequent allogeneic SCT, all previously reported remissions were short, whereas median response duration in our series was somewhat longer, 9 months, after a median follow-up of 20 months. Moreover, the median survival of 11 months observed in our study was somewhat higher than in those series, where it ranged from 2.6 to 5 months with chemotherapy or supportive care.5,25,26

**Table 6. Outcome of MPN after MDS/AML transformation in literature series**

<table>
<thead>
<tr>
<th>Study</th>
<th>Sample size</th>
<th>Treatment</th>
<th>CR/CRi, %</th>
<th>Median survival with specific treatment, mo</th>
<th>Overall median survival, mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cervantes et al, 199114</td>
<td>13</td>
<td>Not detailed</td>
<td></td>
<td></td>
<td>3.0</td>
</tr>
<tr>
<td>Passamonti, 20055</td>
<td>23</td>
<td>Best supportive care (n = 7)</td>
<td></td>
<td>2.5</td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Low dose Ara-C (n = 4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>6-Thioguanine (n = 4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Intensive chemotherapy (n = 8)</td>
<td>13</td>
<td>5.6</td>
<td></td>
</tr>
<tr>
<td>Mesa et al, 200525</td>
<td>91</td>
<td>Best supportive care (n = 48)</td>
<td></td>
<td>2.0</td>
<td>2.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oral busulfan/etoposide (n = 4)</td>
<td>0</td>
<td>2.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vindesine (n = 9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Other (n = 6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Intensive chemotherapy (n = 24)</td>
<td>0</td>
<td>3.9</td>
<td></td>
</tr>
<tr>
<td>Tam et al, 200826</td>
<td>74</td>
<td>Best supportive care (n = 19)</td>
<td></td>
<td>6.0</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gemtuzumab (n = 4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Azacytidine (n = 3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dasatinib (n = 2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Other (n = 3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Intensive chemotherapy (n = 41)</td>
<td>46</td>
<td>7.0</td>
<td></td>
</tr>
</tbody>
</table>

A total of 201 patients were evaluated.

MPN indicates myeloproliferative neoplasm; AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; CR, complete response; CRi, complete response with incomplete recovery of cytopenias; and Ara-C, cytosine arabinoside.
Interestingly, decitabine, another demethylating agent, also provided better survival than high-dose chemotherapy in blast phase of CML.27,28

In our study, 4 of the 7 cases of transformed PMF responded, and their median survival was 8 months. Those findings in patients with blastic rather than fibrotic progression of PMF do not contradict the disappointing results obtained with hypomethylating agents in primary or secondary myelofibrosis.11,12

Only 1 patient received allogeneic SCT after azacitidine, a small number largely related to the median age of our cohort (69.5 years), making difficult to evaluate this procedure after azacitidine.

Importantly, we observed in 39% of the responders reappearance of features of chronic phase MPN, including thrombocytosis in patients with ET and erythrocytosis in patients with PV. Those responses tended to occur early after treatment onset. To our knowledge, this type of evolution had only been reported in blast crisis of CML treated with IC or decitabine.27,28 In Ph-negative MPN having progressed to MDS/AML and treated successfully with chemotherapy, narrow histologic features of chronic phase MPN or of a JAK2 V617F clone have been reported29 but not to our knowledge recurrence of thrombocytosis or erythrocytosis. In 24 cases of leukemic transformation of PMF who received AML-like IC at the Mayo Clinic, 10 had after treatment bone marrow biopsy findings of chronic phase disease without increase of blast percentage.25 As in our experience, patients who reverted to chronic phase disease finally relapsed in the absence of allogeneic SCT. Those findings suggest that azacitidine may in some patients with AML/MDS transformation of MPN reverse the disease course to a chronic phase rather than suppress the MPN clone. A similar mechanism of action of azacitidine has also been suggested in de novo MDS, and it could implicate gene hypomethylation.29,30

Blastic progression of Ph-negative MPN is multifactorial but may indeed involve aberrant methylation of tumor suppressor genes,31 including the negative regulators of JAK– signal transducer and activator of transcription (STAT) signaling, suppressor of cytokine signaling-1 (SOCS-1), and suppressor of cytokine signaling-3 (SOCS-3).8,32,33 Such epigenetic deregulation may occur early during MPN progression, as for example, methylation of the CXCR4 chemokine promoter recently involved in the constitutive mobilization of CD34+ in PMF and reversed by azacitidine in vitro.34 Likewise, JAK2 was recently reported to directly interfere with chromatin assembly in the nucleus.35

Altogether, our results suggest that azacitidine can induce a substantial response rate with relatively low toxicity in Ph-negative MPN having progressed to AML/MDS.26 Most responses are however short, and consolidation treatments, particularly allogeneic SCT or combination with other agents, are required in those patients for whom prognosis remains very poor.

Acknowledgments

We thank investigators of GFM group (Groupe Francophone des Myelodysplasies) who enrolled patients in this study and the French health agency (AFSSAPS).

This work was supported in part by an unrestricted research grant from Celgene to the GFM group.

Authorship

Contribution: S.T. and R.I. collected the data and wrote the manuscript; V.S. performed statistical analysis; L.A. and P.F. designed the study and wrote the manuscript; and E.R., B.Q., Y.C., L. Sorin, F.D., T.C., J.D., L. Sanhes, V.E., C.D., P.T., S.H., C.S., J.-J.K., P.F., and L.A. enrolled the patients and collected the data.

Conflict-of-interest disclosure: P.F. has received research funding from Celgene and Amgen. The remaining authors declare no competing financial interests.

Correspondence: Lionel Ades, AP-HP, Hôpital Avicenne, Service d’Hematologie Clinique, Paris 13 University, 125 rue de Stalingrad, 93009 Bobigny, France; e-mail: lionel.ades@avc.aphp.fr.

References


Treatment of progression of Philadelphia-negative myeloproliferative neoplasms to myelodysplastic syndrome or acute myeloid leukemia by azacitidine: a report on 54 cases on the behalf of the Groupe Francophone des Myelodysplasies (GFM)

Sylvain Thepot, Raphael Itzykson, Valerie Seegers, Emmanuel Raffoux, Bruno Quesnel, Yasmine Chait, Lucile Sorin, Francois Dreyfus, Thomas Cluzeau, Jacques Delaunay, Laurence Sanhes, Virginie Eclache, Caroline Dartigeas, Pascal Turlure, Stephanie Harel, Celia Salanoubat, Jean-Jacques Kiladjian, Pierre Fenaux and Lionel Adès

Updated information and services can be found at:
http://www.bloodjournal.org/content/116/19/3735.full.html

Articles on similar topics can be found in the following Blood collections
- Clinical Trials and Observations (4507 articles)
- Free Research Articles (4400 articles)
- Myeloid Neoplasia (1645 articles)

Information about reproducing this article in parts or in its entirety may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#repub_requests

Information about ordering reprints may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#reprints

Information about subscriptions and ASH membership may be found online at:
http://www.bloodjournal.org/site/subscriptions/index.xhtml