All-Trans Retinoic Acid in Patients With Myelodysplastic Syndromes: Results of a Pilot Study

By C. Aul, V. Runde, and N. Gattermann

Considering the beneficial effect of all-trans retinoic acid (ATRA) in acute promyelocytic leukemia (APL), it has been speculated that ATRA might also be useful for treating other hematologic malignancies. To test this hypothesis, we performed a dose-escalating 3-month-trial of ATRA in 15 patients with primary or secondary myelodysplastic syndromes (MDS). Morphologic diagnoses were refractory anemia (RA) in 4, RA with ring sideroblasts (RARS) in 2, RA with excess blasts (RAEB) in 7, and RAEB in transformation (RAEB/T) in 2 cases. Patients included were required to have one or more of the following criteria: transfusion-dependent anemia, pronounced neutropenia ($\leq 0.5 \times 10^9$/L) or thrombocytopenia ($\leq 20 \times 10^9$/L), or increasing blast cells in the peripheral blood or bone marrow. Therapy was started at an ATRA dose of 30 mg/m$^2$/d, administered orally as two doses of 15 mg/m$^2$/d every 12 hours. The retinoid dose was increased to 60 mg/m$^2$/d after 4 weeks and to 90 mg/m$^2$/d after 8 weeks. Among 14 patients assessable for response, none obtained a complete or partial remission. Three patients had a minor response, manifested by either reduction in transfusion requirements (2 patients) or increase in neutrophil and platelet counts (1 patient). During the study period, 5 patients progressed to more advanced stages of MDS or overt leukemia. Three patients with chromosomal abnormalities receiving ATRA for a period of 10 to 12 weeks retained their cytogenetic marker after completion of treatment. Side effects of ATRA primarily affected the skin and mucous membranes, with 13 of 15 patients having at least low-grade dermatologic toxicity. In 2 cases, treatment had to be prematurely stopped because of intolerable conjunctivitis or progressive neurologic symptoms. These data suggest that ATRA has little effect on MDS. The lack of response of MDS patients, as compared with those with APL, may be attributed to the absence of the t(15;17) translocation that seems to be a prerequisite for clinical efficacy of ATRA.

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glyceride levels, or neurologic abnormalities were not present at the start of treatment. BM aspirates and biopsies were routinely performed on days 1 and 90 of study and evaluated for blast cell population.

**Monitoring of patients.** Monitoring of patients included physical examination, complete blood cell counts, liver and renal function tests, cholesterol and triglyceride levels, urine analysis and coagulation tests before treatment and thereafter every 2 weeks during ATRA administration. BM aspirates and biopsies were routinely performed on days 1 and 90 of study and evaluated for blast cell count, presence of cytologic abnormalities, and cellularity. In 3 of 4 patients presenting with an abnormal karyotype, BM chromosomal studies were repeated at the end of the study. Cytogenetic analysis

### Table 1. Clinical and Hematologic Characteristics of the MDS Population Before Starting ATRA Treatment

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Diagnosis (FAB)</th>
<th>Peripheral Blood Counts (10^9/L)</th>
<th>BM Aspirate</th>
<th>Ring Sideroblasts (%)</th>
<th>Karyotype</th>
<th>RBC Before Therapy</th>
<th>Previous Pharmacologic Therapy</th>
<th>Time to Initiation of ATRA Therapy (mo)</th>
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<tr>
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<td>F</td>
<td>RA</td>
<td>9.8 8.4 19</td>
<td>3 3</td>
<td>46, XX</td>
<td>30 TAD</td>
<td></td>
<td></td>
<td>6</td>
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<tr>
<td>2</td>
<td>60</td>
<td>F</td>
<td>RA</td>
<td>3.9 2.5 103</td>
<td>4 0</td>
<td>46, XX</td>
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<td></td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>69</td>
<td>M</td>
<td>RA</td>
<td>2.1 0.4 92</td>
<td>4 2</td>
<td>46, XY</td>
<td>140 Epo/GM-CSF</td>
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<td>4 0</td>
<td>46, XX</td>
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<tr>
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<td>70</td>
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<td>RARS</td>
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<td>2 87</td>
<td>46, XY, 5q</td>
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<td></td>
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<td>6</td>
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<td>7</td>
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<td>RAEB</td>
<td>2.1 1.2 87</td>
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<td>0.5</td>
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<tr>
<td>8</td>
<td>76</td>
<td>F</td>
<td>RAEB</td>
<td>2.3 1.2 160</td>
<td>8 0</td>
<td>46, XX, 5q</td>
<td>7</td>
<td></td>
<td></td>
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<tr>
<td>9</td>
<td>67</td>
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<td>125 LD Ara-C</td>
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<tr>
<td>10</td>
<td>77</td>
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<td>RAEB</td>
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<td>RAEB</td>
<td>4.2 2.3 87</td>
<td>16 63</td>
<td>46, XY</td>
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<td>RAEB</td>
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<td>18 39</td>
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<tr>
<td>13</td>
<td>70</td>
<td>M</td>
<td>RAEB*</td>
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<td>18 0</td>
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<tr>
<td>14</td>
<td>54</td>
<td>M</td>
<td>RAEB/T</td>
<td>10.3 8.0 6</td>
<td>20 9</td>
<td>47, XY, +ring</td>
<td>24</td>
<td></td>
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</tr>
<tr>
<td>15</td>
<td>85</td>
<td>F</td>
<td>RAEB/T</td>
<td>1.4 0.5 189</td>
<td>28 8</td>
<td>46, XX</td>
<td>2</td>
<td></td>
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<td>0.5</td>
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Abbreviations: RBC, packed red blood cell transfusions; TAD, thioguanine, cytosine arabinoside, and daunorubicin; Epo, erythropoietin; GM-CSF, granulocyte-macrophage colony-stimulating factor; LD Ara-C, low-dose cytosine arabinoside.

* Secondary MDS after chemotherapy for multiple myeloma.

**Table 2. Duration, Dosage, and Outcome of MDS Treatment With ATRA**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>ATRA Dosages (mg/m²/d)</th>
<th>Cause of Study Discontinuation</th>
<th>Hematologic Response</th>
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<tbody>
<tr>
<td>1</td>
<td>4 3 4</td>
<td>--</td>
<td>MR; no transfusion requirement for 9 mo</td>
</tr>
<tr>
<td>2</td>
<td>4 3 4</td>
<td>--</td>
<td>Progressive disease (RAEB)</td>
</tr>
<tr>
<td>3</td>
<td>4 3 4</td>
<td>Headaches and confusion</td>
<td>Stable disease</td>
</tr>
<tr>
<td>4</td>
<td>4 3 4</td>
<td>--</td>
<td>Stable disease</td>
</tr>
<tr>
<td>5</td>
<td>4 3 4</td>
<td>--</td>
<td>MR; no transfusions for more than 12 mo</td>
</tr>
<tr>
<td>6</td>
<td>4 3 4</td>
<td>--</td>
<td>Stable disease</td>
</tr>
<tr>
<td>7</td>
<td>4 3 4</td>
<td>Death (sepsis)</td>
<td>Stable disease</td>
</tr>
<tr>
<td>8</td>
<td>4 3 4</td>
<td>Conjunctivitis</td>
<td>Not evaluable</td>
</tr>
<tr>
<td>9</td>
<td>4 3 4</td>
<td>--</td>
<td>Stable disease</td>
</tr>
<tr>
<td>10</td>
<td>4 3 4</td>
<td>--</td>
<td>MR; improvement in neutrophil and platelet counts</td>
</tr>
<tr>
<td>11</td>
<td>4 3 4</td>
<td>--</td>
<td>Progression to RAEB/T</td>
</tr>
<tr>
<td>12</td>
<td>4 3 4</td>
<td>--</td>
<td>Stable disease</td>
</tr>
<tr>
<td>13</td>
<td>4 3 4</td>
<td>--</td>
<td>Progressive disease (AML)</td>
</tr>
<tr>
<td>14</td>
<td>4 3 4</td>
<td>Death (AML)</td>
<td>Progressive disease (AML)</td>
</tr>
<tr>
<td>15</td>
<td>4 3 4</td>
<td>Progression to AML</td>
<td>Progressive disease (AML)</td>
</tr>
</tbody>
</table>
Fig 1. Transfusion requirements, neutrophil counts, platelet counts, and marrow blast cell percentages before and after administration of ATRA in 14 MDS patients evaluable for response. (I) RA; (II) RARS; (III) RAEB; (IV) RAEB-T.

was performed using standard Giemsa banding technique. A total of 10 to 40 metaphases was analyzed in each case. Side effects of ATRA therapy were evaluated and documented at 2-week intervals. Toxicity was graded according to the proposals of Miller et al24 and Meyenkens et al.25 Protocol treatment had to be stopped if patients developed leukocytosis greater than $20 \times 10^9/L$, transformed to AML, or experienced unacceptable side effects of ATRA. Our criteria for continuing dose escalation were principally geared to a preset grade of toxicity (grade III). However, with regard to skin and mucous membrane toxicities, patients' willingness to tolerate toxicities was overriding. Accordingly, patients with grade-III cheilitis or grade-III skin changes were not automatically taken off the study but asked whether they wanted to terminate or continue ATRA treatment. Patients with skin toxicities who wished to stay on the protocol were treated with emollients.

Response criteria. Complete remission (CR) was defined as a return of the following hematologic parameters to normal for a period of at least 8 weeks: hemoglobin concentration, white blood cell count (WBC), platelet count, and BM blast percentage. Partial remission (PR) was defined as a 50% or greater improvement in cytopenia and 50% or greater reduction in BM blasts, and disappearance of all transfusion requirements for at least 8 weeks. Minor response (MR) was defined as a 50% or greater improvement in cytopenia or a 50% or greater reduction in blast cell count or a 50% decrease in transfusion requirements for at least 8 weeks. Patients without improvement in cytopenia, BM blasts or transfusion requirements were regarded as having stable disease. Progressive disease was defined as worsening cytopenia or increasing BM blasts or increasing transfusion requirements.

RESULTS

Hematologic responses. A total of 10 out of 15 patients completed 3 months of ATRA therapy (Table 2). In 3 patients (nos. 3, 8, and 15), treatment had to be prematurely stopped after 1, 7, and 10 weeks because of side effects of ATRA and/or transition of MDS into overt AML; 2 additional patients (nos. 7 and 14; morphologic diagnoses, RAEB and RAEB/T, respectively) died after 8 and 10 weeks of therapy from septic complications and transformation of MDS to AML, respectively.

The effect of ATRA on transfusion requirements, neutrophil and platelet counts, and medullary blast cell infiltration is shown in Fig 1. None of the patients obtained a CR or PR. Three patients (nos. 1, 5, and 10) had MR. Of these, 1 patient with a diagnosis of RAEB (no. 10) showed a normalization of granulocyte values, significant increase in platelet counts (from $20 \times 10^9/\mu L$ to $53 \times 10^9/\mu L$), and slight reduction in medullary blast cell percentage (from 10% to 7%). The patient's anemia, however, remained unchanged over the course of study (Fig 2). After discontinuation of ATRA, the neutrophil and platelet count fell to pretreatment values.
In addition, 2 patients (nos. 1 and 5; morphologic diagnoses, RA and RARS, respectively) responded to ATRA treatment with a reduction in transfusion requirements. However, it should be noted that 1 of these patients (no. 1) had been treated with aggressive chemotherapy 9 weeks before starting the ATRA protocol (Fig 3). Therefore, it can not be excluded that the decrease in transfusion requirements, which lasted for 9 months, was primarily caused by the preceding chemotherapy and may not reflect a response to ATRA. In both patients, no improvement in other hematologic parameters was seen during ATRA treatment. Patient no. 5 received a second course of ATRA (outside the study protocol) after 14 months because his hemoglobin values deteriorated. He responded again with rising hemoglobin concentrations (Fig 4).

Of the patients studied, 6 were classified as having stable disease (nos. 3, 4, 6, 7, 9, and 12); 2 of them experienced a transient increase in both platelet and neutrophil counts for a period of 5 and 7 weeks, respectively. While on protocol, 5 patients showed an increasing proportion of medullary blast cells. Of these, 1 patient (no. 2), with an initial diagnosis of RA, progressed to RAEB; 1 patient with RAEB (no. 11) evolved to RAEB/T, and 3 patients (nos. 13, 14, and 15; among them 2 cases of RAEB/T) developed overt AML. ATRA treatment was terminated after 1 week in 1 patient (no. 8) because of severe conjunctivitis, and this patient's data were considered inevaluable.

Concerning BM morphology, we observed 1 patient with RARS (no. 6) in whom ATRA treatment was accompanied by a marked reduction in the proportion of ring sideroblasts (from 61% to 18%). However, this change did not translate into an increase in hemoglobin values. In other patients, dysplastic features of hematopoietic cells remained unchanged after therapy. None of our patients showed a significant decrease or increase in BM cellularity on trephine biopsies performed at the end of the study. Three patients with chromosomal abnormalities receiving ATRA for a period of 10 to 12 weeks (nos. 5, 13, and 14), retained their cytogenetic marker after completion of treatment.

Side effects. ATRA-related side effects are detailed in Table 3. Changes of the skin and mucous membranes were encountered in 13 of 15 patients (86%) and consisted of erythema and hyperkeratosis, cheilitis, rhagades, conjunctivitis, or nail dystrophy. One patient (no. 8) developed severe conjunctivitis within 7 days of initiation of therapy and had to be taken off the study. Three patients (nos. 4, 6, and 15) complained of myalgias that were associated with slight elevations of serum creatine kinase activity. At an ATRA dosage of 90 mg/m²/d, 1 of these patients (no. 4) developed a dolent tumor of the right biceps muscle at the time of completion of the study. The tumor gradually resolved in a few weeks time. In another patient (no. 3), ATRA treatment had to be stopped because of progressive neurologic symptoms (headaches and confusion). Other side effects observed in our patients included fatigue (2 cases), nausea and vomiting (1 case), diarrhea (1 case), and joint pain (2 cases). While on protocol, approximately 50% of the patients experienced transient asymptomatic elevations of serum triglycerides, whereas biochemical signs of liver cell damage or renal toxicity were not noted.

DISCUSSION

MDS are characterized by impaired proliferation and maturation of hematopoietic precursor cells, resulting in ineffective blood cell production. Complications of BM failure are important causes of death and often occur before transformation of MDS into AML. There has been much interest in applying differentiation-inducing agents in these disorders, particularly as the hematopoietic cells in MDS are arrested at a more mature developmental stage than cells from AML patients. When assessed by various in vitro systems, retinoids possess strong differentiation-inducing activity on cultured human MDS and AML cells. Clini-
In this study, 15 patients with various subtypes of MDS were treated with increasing doses of ATRA (30 to 90 mg/m²/d) over a maximum period of 3 months. The ATRA dosage was escalated because previous studies of 13-CRA in MDS suggested a correlation between the retinoid dose and clinical effectiveness. At the highest dose level, MDS patients in our study received twice the amount of ATRA required for successful treatment of APL. Except for 1 case in which ATRA treatment had to be stopped after 1 week because of unacceptable side effects, all patients received the test drug for at least 7 weeks, with 10 patients being treated...
over the full period of 3 months. Previous experience with 13-CRA in MDS has shown that hematologic improvement usually occurs after 3 weeks of therapy.\textsuperscript{31} Therefore, all patients evaluable in this study were treated long enough and with adequate intensity, thereby fulfilling the prerequisites for clinical efficacy of ATRA, provided that ATRA is at all active in MDS.

Contrary to two recently published case reports,\textsuperscript{22} we were unable to show a beneficial effect of ATRA in most of our MDS patients. Using rather stringent response criteria, none of our patients achieved CR or PR. There were only 3 MRs, manifested by either reduction in transfusion requirements (2 patients) or increase in neutrophil and platelet counts (1 patient). However, in 1 of these patients, hematologic improvement could not reliably be attributed to the administration of ATRA. Repeated karyotyping showed persistence of cytogenetic abnormalities in 3 patients examined. A total of 6 patients had stable disease, whereas 5 patients progressed to more advanced stages of MDS or to overt leukemia. We assume that our patients would have shown a similar clinical course if ATRA had not been administered.

Considering the different effects of ATRA in patients with MDS and APL, it is tempting to speculate that clinical activity of ATRA requires the presence of the \(t(15;17)\) translocation. This cytogenetic defect, present in 70\% to 100\% of patients with APL,\textsuperscript{32,33} is not found in MDS. Apart from APL, \(t(15;17)\) has occasionally been observed during promyelocytic blast crisis of chronic myeloid leukemia,\textsuperscript{34,35} and such patients were also shown to respond to ATRA.\textsuperscript{36} Recent molecular studies in APL have shown that the \(t(15;17)\) translocation results in rearrangements of the retinoic acid receptor gene (RAR\(\alpha\)), with expression of two aberrant forms of RAR\(\alpha\) transcripts.\textsuperscript{37,38} The abnormal RAR\(\alpha\) gene products are thought to inhibit the expression of retinoid acid target genes involved in granulopoietic differentiation. Although the precise mechanism of action of ATRA in APL is still unknown, pharmacologic doses of ATRA are consistently able to overcome the maturation arrest. As MDS lack the \(t(15;17)\) abnormality, it is not surprising that differentiation therapy with ATRA generally fails in these disorders.

The marginal hematologic responses that we achieved in some of our MDS patients might have been induced by other mechanisms, such as an antiproliferative effect on the preleukemic cell clone,\textsuperscript{39,40} stimulation of residual normal myeloid progenitor cells,\textsuperscript{41} or cytodifferentiating activities of ATRA not dependent on the presence of abnormal retinoic acid receptors.\textsuperscript{42}

Toxicities of ATRA in our trial were similar to those seen with 13-CRA in previous studies\textsuperscript{18,30} and primarily affected the skin and mucous membranes. Two patients had to be withdrawn from the protocol because of intolerable conjunctivitis and central nervous system (CNS) toxicity that manifested as progressive headaches and confusion. At the highest dose level, almost all patients had at least low-grade dermatologic toxicity partly combined with other side effects such as arthralgias, myalgias, neurologic symptoms, or pronounced elevations of triglyceride levels. Because of the advanced age of most MDS patients, long-term administra-
tion of high doses of ATRA aimed at increasing its efficacy does not appear realistic. This view is supported by a recent phase-I study in pediatric patients with cancer in which the maximum tolerated dose of ATRA was 60 mg/m²/d. It is noteworthy that, despite applying ATRA at doses as high as 90 mg/m²/d, none of our patients developed hematologic and clinical signs of the "retinoic acid syndrome," which has previously been described as a frequent and potentially life-threatening complication in patients with APL. Again, this points to a difference in ATRA treatment between MDS and APL.

Based on the data of this trial, we surmise that ATRA has little clinical effect on MDS. Recently, Ohno et al. published the results of a multinstitutional study of 23 MDS patients treated with ATRA at an oral dose of 45 mg/m²/d. Clinical results were very similar to ours, and ATRA was considered only minimally effective in MDS. Further studies will have to clarify whether treatment results can be improved by combining ATRA with other differentiation-inducing agents such as cytarabine, interferons, or hematopoietic growth factors which have shown synergistic effects with ATRA in vitro. Recently, 9-cis-retinoic acid (9-CRA) was found to be moderately more potent than ATRA as far as its effects on normal and leukemic hematopoiesis in vitro are concerned. Although both 9-CRA and ATRA can directly bind the RAR with similar affinity, 9-CRA alone directly binds to the retinoid X receptors (RXR), suggesting that biologic differences exist between these retinoids that might be exploited clinically. The 9-CRA-RXR complex might activate a unique set of differentiation-related genes that are not as efficiently transactivated by the ATRA-RAR complex. Although hematopoietic cells may be capable of metabolizing ATRA to 9-CRA, this may not be as efficient as exogenously providing the ligand to the cells. Therefore, even though our study could not show clinical efficacy of ATRA, other retinoids may still turn out to be useful in the treatment of MDS.

REFERENCES


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