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ORIGINAL ARTICLE

A. Venditti · A. Tamburini · F. Buccisano
M.T. Scimò · G. Del Poeta · L. Maurillo · M.C. Cox
E. Abruzzese · M. Tribalto · M. Masi · S. Amadori

A phase-II trial of all *trans* retinoic acid and low-dose cytosine arabinoside for the treatment of high-risk myelodysplastic syndromes

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Abstract Twenty-two patients with high-risk myelodysplastic syndrome (HRMDS) were treated with a 10-day course of oral all *trans* retinoic acid (45 mg/m^2) and s.c. low-dose cytosine arabinoside (LDARAc) given at the dose of 20 mg twice per day. The courses were repeated monthly until response or progression; in the case of response, the therapy was administered until relapse. Morphologic diagnoses were refractory anemia with excess blasts (RAEB) in nine, RAEB in transformation (RAEB-t) in nine, and chronic myelomonocytic leukemia (CMMoL) in four patients; in all cases, bone-marrow blast infiltration was greater than 10% (median 20%, range 12–30%). When the international prognostic scoring system was applied, all the cases qualified as intermediate/high-risk categories. Nineteen patients were males and three were females; the median age was 69 years (range 25–90 years); three patients had previously been treated with conventional chemotherapy, and one of them had also undergone autologous bone-marrow transplantation. The criteria of response were defined as follows: (1) complete response: normalization of blood counts and bone-marrow blasts (<5%), and (2) partial response: decrease in bone-marrow blast infiltration by 50%, and two of the following parameters – improvement in hemoglobin level by 1.5 g/dl or decrease by 50% in transfusional requirement, increase by 50% in absolute neutrophil count, and increase by 50% in platelet count. Overall, 7 (32%) of 22 patients achieved a response, with 5 (23%) being classified as complete responders and 2 (9%) as partial responders. Fifteen (68%) patients did not achieve any response, and 14 died of progressive disease or infectious disease. The overall median survival was 8 months (range 1–27 months), whereas the median survival of responders was 16 months (range 8–27 months); the median duration of response was 11 months (range 2–21 months). Moderate to severe hematological toxicity and infections were the most common side effects. In conclusion, it seems that the association of ATRA and LDARA-C may be effective in approximately 30% of HRMDS patients. Optimizing this approach might be pursued by selecting, on a biological basis, those cases more likely to respond or by incorporating other differentiating agents or growth factors.

Key words HRMDS · ATRA · LDARAc

A. Venditti (✉) · A. Tamburini · F. Buccisano · M.T. Scimò
G. Del Poeta · L. Maurillo · M.C. Cox · E. Abruzzese
M. Tribalto · M. Masi · S. Amadori
Hematology, University oTor Vergata, St. Eugenio Hospital,
Piazzale Umanesimo 10, I-00144 Rome, Italy
e-mail: avenditti@pelagus.it
Tel.: +39-6-510002504
Fax: +39-6-5915965

Introduction

Myelodysplastic syndromes (MDS) comprise a heterogeneous group of clonal disorders characterized by ineffective hematopoiesis, leading to pancytopenia and variable outcome [1, 2]. These disorders share the progressive evolution of a monoclonal population of hematopoietic cells to a preleukemic status and eventually overt acute leukemia [3, 4]. Among these, high-risk MDS (HRMDS) patients have a very poor prognosis, and the only therapeutic option capable of significantly prolonging survival is autologous and allogeneic stem cell transplantation [5, 6, 7]. Outside the setting of transplant procedures, intensive chemotherapy has proven to be an effective treatment for HRMDS; in fact acute myeloid leukemia (AML)-like protocols achieved results clearly indicating that patients with HRMDS have the same chances to respond as AML patients [8, 9]. Due to the inadequacy of the current treatment modalities, the making-decision process is extremely difficult for those patients with HRMDS who are not suitable to receive intensive chemotherapy and/or stem cell transplanta-

tion because of age and/or poor performance status. In this context, a number of therapeutic attempts have been made, including attenuated dose of chemotherapy [10–12], differentiating agents [13–15], and growth factors [16–18], but no evidence that these approaches can alter the natural history of the disease has been generated. Our previous experience in the treatment of "poor risk" AML [19] and recent experimental evidence suggesting the role of all-*trans* retinoic acid (ATRA) as a down-modulator of mechanisms of chemoresistance [20, 21] have prompted us to investigate the effects of an association of low-dose cytosine arabinoside (LDARA-C) and ATRA in 22 patients with HRMDS. The objectives of the study were to determine the response rate, duration of response, and toxicity of this combination in a group of MDS patients with a very unfavorable prognosis.

Patients and methods

Patients

Between January 1995 and May 1999, 22 patients with primary HRMDS were treated with the combination of LDARA-C and ATRA. Morphologic diagnoses, as evaluated by three independent pathologists, were refractory anemia with excess blasts (RAEB) in nine, RAEB in transformation (RAEB-t) in nine, and chronic myelomonocytic leukemia (CMMoL) in four patients; in all cases, bone-marrow blast infiltration was greater than 10% (median 20%, range 12–29%). When the international prognostic scoring system (IPSS) was applied [22], the cases qualified as intermediate-2 (9) or high-risk (10) categories. In three cases, although the cytogenetics remained unknown because of technical failure, based on the bone-marrow blast infiltration and the number of cytopenias, the score was 1.5 or greater; thus, they belonged to the intermediate-2 group, at least. Nineteen patients were males and three were females; the median age was 69 years (range 25–90 years). All the patients were considered ineligible for intensive chemotherapy because of either advanced age (15 aged ≥ 67 years), poor clinical conditions (WHO >2), or the presence of other concomitant diseases associated with organ failure. Two patients with RAEB-t, aged 44 years and 56 years, were treated in relapse occurring after intensive chemotherapy. A 25-year-old male with resistant CMMoL was entered into the protocol after intensive chemotherapy and autologous bone-marrow transplantation (Table 1).

Table 1 Clinical characteristics of the patients. *RAEB* refractory anemia with excess blasts; *RAEB-t* RAEB in transformation; *CMMoL* chronic myelomonocytic leukemia; *RBC* red blood cell; *PLT* platelet; *AuBMT* autologous bone-marrow transplantation

No. of patients	22
Male/female ratio	19/3
Age (years)	
Median	69
Range	25–90
Diagnosis	
RAEB	9
RAEB-t	9
CMMoL	4
Bone-marrow blast infiltration	
Median	20%
Range	12–30%
Karyotype	
Normal	12 (54%)
Abnormal	7 (32%)
Inadequate	3 (14%)
International prognostic scoring system	
Intermediate-2	12
High	10
RBC transfusion-dependent	
No.	14
%	64
PLT transfusion-dependent	
No.	6
%	27
Prior therapy (no. of patients)	
None	19
Intensive chemotherapy	2
AuBMT	1

Treatment schedule

The median time from diagnosis or documentation of resistance/relapse after previous chemotherapy to the beginning of ATRA plus LDARA-C was 2 weeks (range 1–6 weeks). Treatment consisted of oral ATRA at 45 mg/m^2 from day 1 to day 10, and 20 mg of subcutaneous ARA-C administered twice a day from day 1 to day 10. The therapy was delivered on an outpatient basis with self-administration of s.c. ARA-C. The courses were repeated monthly until response or progression; in case of response, the therapy was continued until relapse. At least four cycles of therapy were to be given before evaluating the treatment response. During the study period, supportive care with packed red cells (PRCs), platelets (PLTs), and antibiotic was given when indicated. Monitoring of patients included physical examination, full blood cell counts, hepatic and renal functions, cholesterol and triglyceride levels. Bone-marrow status was assessed before each course of therapy.

Criteria of response

Criteria of response were defined as follows:

1. Complete response. Normalization of blood counts and bone-marrow blasts ($<5\%$)
2. Partial response. Decrease in bone-marrow blast infiltration by 50% at least, and two of the following parameters: (i) improvement in hemoglobin (Hb) level by 1.5 g/dl or decrease by 50% in transfusional requirement, (ii) increase by 50% at least in absolute neutrophil count (ANC) and, (iii) increase by 50% at least in platelet count (PM). Although not included among the criteria of response, even the improvement of dysplasia was evaluated.

Cytogenetics

Chromosome preparation and banding was performed on bone-marrow specimens, culture preparations, or both, according to established procedures [23]. Karyotypes were made according to the guidelines of the International System for Human Chromosome Nomenclature of 1991 [24].

Statistical analysis

The Kaplan-Meier product-limit method was used for the estimation of actuarial curves. The differences between curves were tested using the long-rank test. Overall survival (OS) was calculated from the beginning of the therapy to the date of last follow-up, or death. Disease-free survival (DFS) was calculated from the date of response (complete or partial) to the date of last follow-up, relapse or death. Relationships between response to therapy and patient's characteristics were estimated using the Fisher's exact test.

Results

All 22 patients were evaluable for response to therapy (Table 2) and toxicity (Table 3). Overall, seven patients (32%) achieved a response, with five (23%) being classified as complete responders and two (9%) as partial responders. Among the seven responders, two had RAEB, three RAEB-t and two CMMoL; five carried a normal karyotype, whereas one had a del(5q) associated with monosomy of chromosome 7; in the remaining one, cytogenetics was not evaluable. On a morphological basis, partial responders and two of the complete responders still had signs of erythroid and granuloblastic dysplasia in the bone marrow. The median Hb level at diagnosis was 9.8 g/l (range 8.6–11.7 g/l), the median ANC 0.9×10^9/l (range $0.57–10 \times 10^9$/l), and the median PLT count 48×10^9/l (range $5.7–170 \times 10^9$/l). Before the therapy, none of the patients was dependent on PRC or PLT transfusion, except for one who had a requirement of 5–7 PLT units per week. After he achieved a partial response, the number of PLTs remained stable around the value of 30×10^9/l and the patient needed support no more. A median number of two courses (range 1–4) was needed to achieve a response, whereas the median time to response was 2 months (range 2–24 months). The median duration of survival and response was 16 months (range 8–27 months) and 11 months (range 2–21 months), respectively. One patient died of pneumonia in partial remission. Six (86%) patients relapsed and five succumbed because of progressive disease, whereas the remainder is still on palliative therapy.

Fifteen (68%) patients failed to achieve any response and progressed to overt leukemia or died of complications. Seven of them had RAEB, six RAEB-t, and two CMMoL; seven carried a normal karyotype, whereas four had a complex karyotype and two had a monosomy 7. In three cases, cytogenctics was not assessed due to technical failure. The median Hb level at diagnosis was 8 g/l (range 6–10.2 g/l), the median ANC 0.7×10^9/l (range $0.06–2.4 \times 10^9$/l), and the median PLT count 47×10^9/l (range $9–190 \times 10^9$/l). Fourteen patients were dependent on PRC transfusions before and during the therapy, with a median requirement of 2 PRC units per month (range 1–5). Five patients required a PLT support (median 8 units per month, range 3–10), which remained unaltered during the therapy. The median survival for this group of patients was 6 months (range 1–19 months); 11 died of progressive disease, 2 of pulmonary infections and 2 are still alive and on palliative treatment.

The probability of achieving a response was significantly associated with minimal or absent pre-therapy transfusional needs ($P = 0.031$), and this was, in fact, the main feature differentiating clinically the group of responders from that of non-responders. Other factors, such as French–American–British (FAB) subtype, bone-marrow blast infiltration and cytogenetics, were also considered but found not to significantly influence response to treatment.

Therapy-associated toxicity for all patients is detailed in Table 3. Toxicity was mainly hematological; in particular, 17 patients experienced a WHO > grade 2 thrombocytopenia, 12 patients had grade-3 anemia and 14 patients had a WHO > grade 2 neutropenia. Seven patients had infectious episodes (WHO > grade 2). In two patients, a significant increase in triglyceride level was observed, whereas erythema and cheilitis was encountered in one case. Neither oral mucositis nor gastrointestinal complications were observed.

Table 2 Response to therapy

	Total	Responders	Non-responders
Patients	22	7 (32%)	15 (68%)
Complete remission	–	5 (23%)	–
Partial remission	–	2 (9%)	–
Median survival (months)	8	16	6*
Median duration of disease-free survival (months)	–	11	–

* P value = 0.021

Table 3 Toxicity and side effects evaluated by World Health Organization criteria

Toxicity	No. of patients (%)	WHO grade			
		1	2	3	4
Anemia	19 (86)	3	2	12	2
Neutropenia	18 (82)	2	2	6	8
Thrombocytopenia	20 (91)	2	1	6	11
Infections	13 (59)	1	5	3	4
Hypertriglyceridemia	2 (9)	1	1	–	–
Cutaneous	1 (4)	–	2	–	–
Nausea	16 (73)	16	–	–	–

Discussion

The therapeutic management of HRMDS still remains problematic, especially in those patients not suitable for aggressive chemotherapy because of age, poor performance status, or severe concomitant diseases. Available in vitro data have shown an interaction between ATRA and ARA-C, with a significant increase in killing of cultured AML cells [25]. The ARA-C-sensitizing action of ATRA seems to be mediated by downregulation of bcl-2 [21, 26], which in turn causes the leukemic cells be less resistant to oxidative stress [26]. Moreover, the addition of retinoids to AML cultures is associated with a down-modulation of the MDRI protein [20]. With such experimental data in mind and following our previous experience in

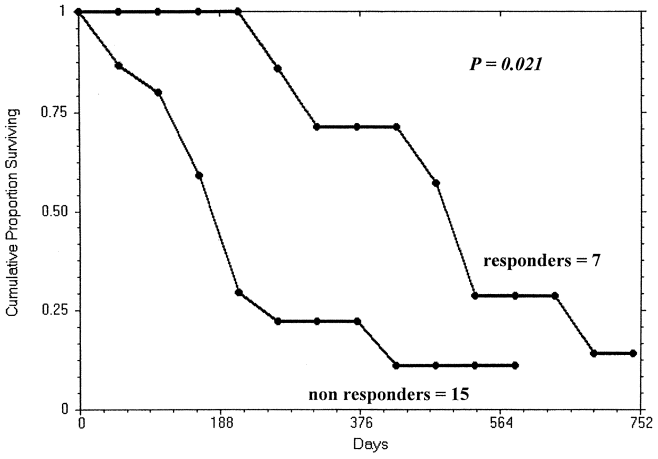

Fig. 1 The figure shows the overall survival of responders relative to non-responder patients

the treatment of poor risk AML [19], 22 patients with HRMDS, who would have otherwise been given only supportive therapy, were treated with a combination of ATRA and LDARA-C.

The results of our study indicate that the association of ATRA and LDARA-C can induce a response in approximately 30% of HRMDS patients. In particular, 23% (5 of 22) patients entered CR and 9% (2 of 22) PR. Importantly, the duration of survival was significantly prolonged in responders relative to non-responders ($P = 0.021$) (Fig. 1). Minimal/absent pretherapy transfusional support was the sole parameter significantly associated with the probability of achieving a response ($P = 0.031$). Although the interval of time between the onset of disease and the first visit to our institute was not known, we can speculate that the importance of transfusional needs reflects the duration of disease, which in turn may represent, by itself, a poor prognostic indicator. In this view, all patients in our study had primary HRMDS, and the median time elapsed from diagnosis to initiation of therapy was 2 weeks with no difference between responders and non-responders.

Studies intended to assess the role of ATRA as a single agent in MDS have provided disappointing results, with occasional responses of short duration [13, 14, 15]. However, LDARA-C has been widely used in a number of clinical trials with controversial findings. Although in an initial report a promising response rate of 71% was observed [27], subsequent trials could not reproduce the same results, with responses ranging between 25% and 45% [10, 11, 12]. In a comprehensive analysis of the published literature [11], the CR and PR rates with LDARA-C alone were 17% and 19%, respectively, with a median survival of 15 months. More recently, LDARA-C has been combined with growth factors in a number of studies. Among them, the association of LDARA-C with GM-CSF or interleukin-3 (IL-3) was investigated in a large randomized phase-III study by the EORTC leukemia Cooperative Group [28]. The study [28], including 201 patients with HRMDS, concluded that no statistically significant differences existed with regard to treatment response, between LDARA-C alone, LDARA-C plus granulocyte–macrophage/colony-stimulating factor (GM-CSF), and LDARA-C plus IL-3 (49%, 28%, and 45%, respectively). No differences between the three alternatives were observed in terms of survival, except for a trend in favor of LDARA-C plus IL-3.

In general, the literature provides poorly comparable results owing to the different criteria for definition of response, routes of administration, dosages, duration of treatment, and the inclusion of all FAB subtypes. Thus, the clear identification of specific subsets of patients who can benefit from the use of LDARA-C is not possible. In this view, despite a response rate reminiscent of those reported in the literature, our study population was highly homogeneous and included HRMDS patients with very poor prognosis and with no other options than supportive care; in addition, we used very stringent criteria for defining PR. Notably, although the natural history of the disease appears unaltered, the median survival of responders is significantly longer than that for non-responders (16 months vs 6 months) ($P = 0.021$) (Fig. 1). Our results are at variance with those of Nair et al. [29], who were unable to show any beneficial effect with the association of ATRA plus LDARA-C. Apart from the smaller number of patients, in Nair's study, ATRA was given in a continuous fashion for 90 days and then on alternate days until day 275. The continuous ATRA treatment is associated with an accelerated clearance of the drug, resulting in a low plasma concentration; this may partially explain the inferior results achieved in that study.

Generally, toxicity was acceptable (Table 3), and no instances of ATRA syndrome were reported. WHO grade 3–4 neutropenia was observed in 14 patients; in 7 it was febrile and required systemic administration of antibiotics. However, hospitalization was not needed and the infectious complications were managed in the outpatient clinic. Eleven cases of thrombocytopenia grade 4 were observed, but they were not associated with relevant clinical manifestations. Although the treatment was well tolerated, it appears that the hematological toxicity was more pronounced than our previous experience in the treatment of poor-risk AML with the same schedule [19]. Finally, all patients stayed in their own home during the treatment and were able to self-administer subcutaneous ARA-C.

In conclusion, our results suggest that approximately 30% of HRMDS patients can benefit from the combination of ATRA and LDARA-C when no other conventional therapeutic options are available. Optimizing this approach might be pursued by selecting the best candidates on a biological basis and/or by

incorporating other differentiating agents or growth factors. The question of whether the combination is indeed superior to LDARA-C needs to be addressed in a large randomized phase-III study.

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