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Clinico-biological features and outcome of acute promyelocytic leukemia patients with persistent polymerase chain reaction-detectable disease after AIDA front-line induction and consolidation therapy

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A B S T R A C T

Background and Objectives. Front-line treatment of acute promyelocytic leukemia (APL) with all-trans retinoic acid (ATRA) and chemotherapy results in molecular remission in approximately 95% of patients tested after consolidation. The small fraction of patients with persistence of molecular disease (i.e. those in whom polymerase chain reaction (PCR) is positive for PML/RARα) after such therapy are thought to have a dismal prognosis but this has not yet been investigated in detail.

Design and Methods. We analyzed the clinico-biological features at presentation of APL patients who showed PCR-detectable residual disease and compared them to those of patients achieving molecular remission after AIDA induction and consolidation. Furthermore, we report the outcome of patients with molecularly persistent disease treated with salvage therapy.

Results. Patients attaining molecular remission (n=650) and patients who tested PCR+ve at the end of consolidation (n=23) were not statistically significantly different as regards median age, white cell and platelet counts, morphologic subtype (M3 or M3v), fibrinogen levels or PML/RARα transcript type. As to treatment outcome after salvage therapy, 7 patients were treated before morphologic relapse [3 with chemotherapy and autologous stem cell transplantation (SCT) and 4 with allogeneic SCT], and are alive after 64-118 months. Of 16 patients treated at the time of morphologic relapse, only 2 patients are alive, both of whom had received an allogeneic SCT.

Interpretation and Conclusions. Our findings indicate that APL patients who are molecularly resistant to the AIDA protocol have no distinguishing features at presentation. Their outcome suggests the need for early therapeutic intervention with aggressive treatment prior to the occurrence of hematologic relapse.

Key words: acute promyelocytic leukemia, PML/RARα, persistent residual disease

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Nowaday, more than 95% of patients with acute promyelocytic leukemia (APL) treated with all-trans retinoic acid (ATRA) and combination chemotherapy regimens achieve molecular remission by the end of consolidation.[1-3] As shown by several studies on minimal residual disease (MRD) monitoring, sustained molecular remission in this disease is associated with long-term remission while persistence or recurrence of MRD after consolidation almost invarably predicts hematologic relapse.[3-14] The small fraction of patients who show persistence of polymerase chain reaction (PCR) positivity at this time point are commonly regarded as having a very aggressive subtype of disease that needs additional intensive treatment including allogeneic stem cell transplantation (SCT).[3] However, the clinico-biological features at diagnosis and the subsequent treatment outcome of these patients have never been reported.

In this study we analyzed the presenting features of 23 patients who tested PCR +ve and compared them to those of 650 patients attaining molecular remission at the end of consolidation following the AIDA 0493 protocol of the Italian GIMEMA group. We also report the outcome of those patients who received salvage therapy for molecular disease or subsequently developed hematologic relapse.

Design and Methods

A total of 910 patients with newly diagnosed APL were enrolled in the AIDA 0493 study[7] during the years 1993-2000. Accord-

ing to protocol requirements, the diagnosis of APL was confirmed in all patients at the genetic level by reverse transcription (RT)-PCR amplification of the PML/RARα hybrid and/or by karyotypic identification of the t(15;17) translocation. All eligible patients were treated with ATRA and idarubicin for induction followed by three polychemotherapy consolidation cycles as previously reported.[7] The degree of any coagulopathy was evaluated according to the published criteria,[14] and the grade of any hemorrhagic syndrome was evaluated on the basis of National Cancer Institute Common Toxicity Criteria (version 2.0). Relapse risk at the time of diagnosis was established according to the criteria described by Sanz *et al.*[15] Retinoic acid syndrome (RAS) was diagnosed and graded as reported by Frankel *et al.*[16] Central nervous system involvement was documented by lumbar puncture which was performed in symptomatic patients based on the presence of *related* symptoms (nausea, vomiting, headache).[17]

According to protocol design,[7] bone marrow samples were collected in all cases at the end of the 3rd consolidation cycle and analyzed by RT-PCR of the PML/RARα transcript in order to decide further therapy. Molecular persistence of residual disease was defined as positivity for PML/RARα detected in two successive marrow samples collected 2-4 weeks apart after the end of consolidation, using an RT-PCR assay with sensitivity of 10^{-4}. Patients in molecular remission were intended to receive maintenance treatment, while further intensive therapy was recommended for patients with PCR-detectable persistent disease after consolidation.[7] For molecular studies, cryopreserved GTC samples were sent in dry ice to two referral molecular biology laboratories (Hematology Dept., University *La Sapienza* of Rome and Clinica Pediatrica, University of Milan-Monza) where RT-PCR analyses were performed. The reagents and laboratory protocol used for RT-PCR of PML/RARα have been reported in detail elsewhere.[10] The sensitivity of the RT-PCR assay was determined by amplifying serially diluted RNA mixtures of a diagnostic sample with 100% of blasts and the t(15;17)-negative myeloid cell line GF-D8.

The PML/RARα transcript was still detectable in the presence of 0.1 ng total RNA, that is at a final dilution of 10^{-4}. Such a detection level was repeatedly obtained in several new experiments.[10]

Differences in the distribution of variables between groups of patients were analyzed by the χ^2 test (using Yates' correction) or Fisher's exact test, in the case of categorical data. In the case of continuous data, Student's t-test was used for variables with a normal distribution; otherwise the Kruskal-Wallis test was used.

Results

Clinico-biological characteristics at presentation

Of 673 patients who completed consolidation, 23 (3.4%) tested PCR positive and 650 PCR negative at this time point. In all the 23 PCR positive cases, molecular persistence of residual disease was confirmed in a new bone marrow sample collected within 4 weeks of the previous one that had been PCR+ve. The main clinico-biological features of both groups at presentation are reported in Table 1. Patients attaining molecular remission and those with persistent molecular disease after consolidation showed no statistically significant differences in age, white cell and platelet counts whatever cut-point was used, morphologic subtype (M3 vs. M3v), PML/RARα transcript type, fibrinogen levels or incidence of ATRA syndrome during induction.

Treatment outcome

Response to treatment and outcome are shown in Table 2. Seven of the 23 patients received immediate salvage therapy while still in hematologic remission with molecularly persistent residual disease, while 16 received salvage therapy at the time of hematologic relapse. There were no statistically significant differences in presenting white cell count, platelet counts or risk category distribution according to Sanz *et al.*[15] between the two groups. The time elapsed between confirmation of molecular persistence of residual disease and beginning treatment in the two groups was 8 months (range 2.5-15) and 5.1 months (range 2-8.5) for patients treated for molecular or hematologic relapse, respectively.

Four patients in the first group (those treated for molecular relapse) underwent allogeneic stem cell transplantation (SCT) and are alive in hematologic and molecular remission at 64, 92, 98 and 118 months, while 3 patients were treated with chemotherapy followed by autologous SCT and are alive in hematologic and molecular remission at 64, 96 and 98 months. These 3 patients were in 2nd molecular remission at the time of autologous SCT.

Sixteen patients had a hematologic relapse at a median time of 4 months (range 1-10) after confirmation of PCR-positivity at the end of consolidation and received salvage treatment for overt disease recurrence. Of these, 6 patients received chemotherapy alone, 2 received arsenic trioxide alone, and 1 patient ATRA alone as salvage therapy; all died of progressive disease. Five patients were treated by chemotherapy (4 cases) or ATRA alone (1 patient) followed by allogeneic SCT; of these, 3 died of progressive disease and 2 patients are alive in hematologic and molecular remis-

Table 1. Clinico-biological features at diagnosis of patients with persistent minimal residual disease (PCR positive) as compared to patients who entered molecular remission (PCR negative) after AIDA induction and consolidation.

		APL population (650)	*PCR+ (23)*	*p*
Age	Years	35.4	35.5	0.759
	range	1.9-73.9	12.8-62.5	
	Male	347 (53.4)	9 (39.1)	0.257
Sex (n, %)	Female	303 (46.6)	14 (60.9)	
	NA	–	–	
	M3	605 (94.2)	20 (87.0)	0.319
FAB (n, %)	M3v	37 (5.8)	3 (13.0)	
	NA	8	–	
	No	222 (34.5)	7 (31.8)	0.972
Hemorrhages (n, %)	Yes	421 (65.5)	15 (68.2)	
	NA	7	1	
	1	329 (57.1)	6 (31.6)	0.063
PML/RARα	2	40 (7.0)	3 (15.8)	
BCR type (n, %)	3	207 (35.9)	10 (52.6)	
	NA	74	4	
	No	595 (93.3)	20 (90.9)	1.000
ATRA syndrome (n, %)	Yes	43 (6.7)	2 (9.1)	
	NA	12	1	
WBC ($\times 10^9$/L)	N. of patients	650	23	0.1421
	median	2.75	5.8	
	range	0.30-180.0	0.30-96.7	
	N. of patients	640	22	0.6162
PLTS ($\times 10^9$/L)	median	23.0	23.5	
	range	1-241.0	10.0-84.0	
	N. of patients	628	22	
Fibrinogen (mg/dL)	median	149.0	117.0	0.0702
	range	0-998.0	65.0-337.0	

NA: not available.

sion at 62 and 74 months. Finally, 2 patients received chemotherapy followed by autologous SCT and both died of progressive disease. All patients undergoing either allogeneic or autologus SCT were in 2nd molecular remission at the time of transplantation. The type of chemotherapy used for salvage in each group is described in detail in Table 2. Disease progression was associated in this series with a high incidence of extramedullary leukemia infiltration. In fact, central nervous system involvement was documented in 8 patients while one patient had focal APL in the outer ear, as already reported.[18]

Discussion

In this study, we identified 23/673 (3.4%) patients with APL who tested PCR-positive for PML/RARα after front-line induction and consolidation therapy according to the AIDA protocol, confirming a very low frequency of resistant disease after this type of treatment. Unfortunately, we were not able to identify distinguishing presenting features in this small subset of patients. In fact, with the sole exception of slightly lower fibrinogen levels, no relevant differences were

Table 2. Type of treatment and outcome of patients receiving salvage therapy for molecularly persistent disease or clinical relapse.

N.	*Disease status at time of salvage treatment*	*Therapy*	*Outcome (months)*
3	molecular persistence	CHT* + autoBMT	3 pts in CCR (+64; +96; +98)
4	molecular persistence	alloBMT	4 pts in CCR (+64; +92; +98; +118)
9	hematologic relapse	CHT** alone	9 pts died of progressive disease
5	hematologic relapse	CHT° + alloSCT	3 pts died; 2 pts in CCR (+62; +74)
2	hematologic relapse	CHT°° + autoSCT	2 pts died of progressive disease (+14; +15)

**1 pt, Prot. 0191 (cytarabine + mitoxantrone); 2 pts, Prot. MEC (mitoxantrone + etoposide + cytarabine); °3 pts, Prot. 0191, 1 pt Prot. MEC; 1 pt ATRA; **4 pts, Prot. 0191; 2 pts, arsenic trioxide; 1 pt, Prot. MEC; 1 pt, ATRA+ idarubicin; 1 pt, ATRA. °°1 pt, Prot. MEC; 1 pt, Prot. ICE (idarubicin+cytarabine+etoposide);*

observed between the two groups of PCR positive and PCR negative patients.

As to outcome results, in spite of the low numbers analyzed, we observed that patients with molecularly resistant disease after combined ATRA and chemotherapy had a very poor prognosis unless they were given early salvage therapy including aggressive approaches. The comparative analysis of risk factors in patients treated for molecular or hematologic relapse showed no significant differences concerning the timing of therapeutic intervention. However, there was a longer time interval between confirmation of molecular resistance and beginning salvage in the patients treated for molecular relapse, so it cannot be excluded that these patients had a more indolent disease.

With respect to the type of salvage treatment, we observed a high incidence of disease progression and extramedullary infiltration in patients treated with chemotherapy with or without autologous SCT. By contrast, patients who received intensive chemotherapy before morphologic relapse and subsequently underwent SCT remained alive and disease-free. In the group of patients receiving salvage at the time of hematologic relapse, only those who underwent allogeneic SCT are still alive. Hence, autologous SCT was effective only for patients who received intensive chemotherapy before morphologic relapse. Significantly, the PCR status of these patients prior to autologous SCT indicated that all had achieved molecular remission prior to transplantation.

In conclusion, our study indicates that intensive treatment should be started immediately in APL patients who have molecularly resistant disease despite modern front-line induction and consolidation regimens. It is likely that patients with an HLA-identical sibling eligible for allogeneic SCT will benefit from this procedure, as it can result in prolonged remission. Additional clinical studies will be needed to establish whether alternative strategies, including non-cross-resistant chemotherapy, arsenic trioxide or anti-CD33 monoclonal antibody, could represent valid alternative approaches in the subset of molecularly resistant patients.

Contributions. MB and FLC designed the study, carried out the analysis and wrote the manuscript. D.Di and NIN carried out the experiments and contributed to data analysis. FL, DDa, AS, GV and FM contributed to the study design and critically reviewed the paper. MV and MCP performed statistical analyses and reviewed the manuscript. All authors approved the final version of the manuscript. The authors reported no conflict of interest.

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