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Disappearance of PML/RARα acute promyelocytic leukemia-associated transcript during consolidation chemotherapy

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Abstract

Background and Objective. **Acute promyelocytic leukemia (APL) (M3 according to FAB classification) is a subtype of acute myelogenous leukemia characterized by a specific t(15;17) (q22;q12) chromosomal translocation. The majority of APL patients achieve morphologic remission after induction chemotherapy. They can be followed from this point by cytogenetic and molecular analysis of the persistence of the PML/RARα transcript. In order to determine the influence of successive courses of consolidation chemotherapy on clinical and molecular outcome, APL patients treated with all-trans retinoic acid (ATRA) and chemotherapy (AIDA-GIMEMA-LAP0493 protocol) were investigated.**

Design and Methods. **Twenty-four APL patients (pts) (15 males; 9 females) were studied by RT-PCR and cytogenetic analysis at diagnosis, after induction chemotherapy, at each point after any of three consolidation courses, and every 3 months during the first years of maintenance therapy. The median follow-up was 24 months (mths) (range 7-40 mths).**

Results. **All pts achieved hematologic remission after induction chemotherapy. Our results demonstrate that the majority (87%) of APL patients were still molecularly positive for the APL associated transcript after induction chemotherapy, while the majority (80%) of APL patients became PCR⁻ after the second consolidation chemotherapy.**

Interpretation and Conclusions. **The role of the third consolidation chemotherapy course in converting patients with persistent molecular evidence of disease from PCR⁺ to PCR⁻ was minimal. We confirm the validity of molecular follow-up after single courses of chemotherapy in monitoring the role of molecular remission.**

Key words: acute promyelocytic leukemia, karyotype, PML/RARα rearrangement, minimal residual disease

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Acute promyelocytic leukemia (APL) (M3 according to the FAB classification) is a subtype of acute myelogenous leukemia characterized by a specific t(15;17) (q22;q12) chromosomal translocation.[1] At the molecular level, the t(15;17) translocation results in the fusion of two genes, the promyelocytic leukemia gene (PML) on chromosome 15 and the nuclear retinoic acid receptor-α (RARα) on chromosome 17. A hybrid PML/RARα mRNA is produced and translocated in a chimeric protein involved in APL pathogenesis.[2] The expression of PML/RARα fusion mRNA provides a potential molecular marker that can be detected in leukemic cells from patients with APL.[3,4] Using PML and RARα sequence specific primers, we and others[5-7] have developed reverse transcription-polymerase chain reaction (RT-PCR) assays, with higher sensitivity than conventional cytogenetic analysis, for detection of the PML/RARα transcript in leukemic cells obtained from patients. We applied this method to a series of 24 consecutive newly diagnosed APL patients entered in a pilot study named AIDA (LAP0493)[8] who received a combination of all-trans retinoic acid (ATRA) and idarubicin (IDA) followed by 3 courses of intensive chemotherapy as consolidation. The aims of our study were: 1) to determine the influence, if any, of successive courses of chemotherapy on the disappearance of PML/RARα transcript; 2) to confirm the relationship between cytogenetic versus molecular analysis during the different chemotherapy courses of the protocol; 3) to confirm the role of the PML/RAR-α transcript in the clinical course of the disease.

Materials and Methods

Twenty-four patients (15 males, 9 females) affected by acute promyelocytic leukemia (APL) received the AIDA-GIMEMA LAP0493[8] protocol of induction chemotherapy, including ATRA and anthracycline (idarubicin). ATRA was administered orally beginning on the first day of induction at the dosage of 45 mg/m²/d until complete remission; idarubicin was administered intravenously at the dosage of 12 mg/m²/d on days 2, 4, 6 and 8.

Patients who achieved complete remission (CR) were consolidated with 3 courses of chemotherapy

without ATRA but including cytosine arabinoside, idarubicin, mitoxantrone, etoposide and 6-thioguanine. After consolidation, patients were submitted, on the basis of their molecular status ($PCR^{+/-}$ for PML/RARα hybrid gene), either to four arm maintenance chemotherapy or, if eligible, to allogeneic transplantation (BMT).[8]

At diagnosis and during any chemotherapy course, BM aspirates were obtained, after informed consent, and used for cytogenetic and molecular analysis.[4,5] Cytogenetic studies were performed on short-term cultures without stimulation, as reported elsewhere.[9] Total RNA, for the RT-PCR analysis, from Ficoll-Hypaque-separated mononuclear cells from the BM aspirates, was extracted using Chomczynsky and Sacchi's method[10] and subjected to RT-PCR performed as described elsewhere.[6,9,11-13] In brief, PCR amplification was performed by using R8 and R5 antisense primers and M2, M4 sense primers for 35 cycles. After the second run, 10 µL of the PCR mixture was run on 2% Nusieve agarose gel, stained with ethidium bromide and visualized with an ultraviolet lamp.[6]

Results

In all 24 patients, karyotypic analysis on BM aspirates was performed at diagnosis and confirmed the presence of the t(15;17) translocation. At diagnosis, we were able to detect the presence of PML/RARα transcript[9] (18 BCR-1; 1 BCR-2; 5 BCR-3) by RT-PCR analysis in all the patients. A schematic representation of the molecular and cytogenetic analyses is reported in Figure 1 and Table 1.

During maintenance, the patients were studied every 3 months during the first year. The median follow-up was 24 months (range 7-40). Nineteen patients (79%) achieved complete clinical remission (CCR), 3 (12%) died [2 (8%) due to refractory APL relapse; 1 (4%) in CR during consolidation therapy due to a secondary sarcoma] and 2 (8%) obtained CR after BMT following APL relapse (Table 2). After induction chemotherapy, 13 pts (54%) were cytogenetically (Cy) positive ($^{+}$), while 11 pts (46%) were negative ($^{-}$) for the karyotypic hallmark [t(15;17)]. At the same time most of our patients were still PCR positive: 21 (87%) vs. 3 (13%). After the first consolidation course, 3/23 (13%) evaluable pts were Cy^{+} vs. 20/23 (87%) pts who were Cy^{-}. At the same point, 8/23 (35%) were PCR^{+} vs. 15/23 (65%) who were PCR^{-}. One patient was not evaluable because he was HCV^{+}. After the second consolidation course 1/20 cytogenetic evaluable patients was positive; at the same point, 2 of the same 20 patients were PCR^{+}. Two other patients, who were

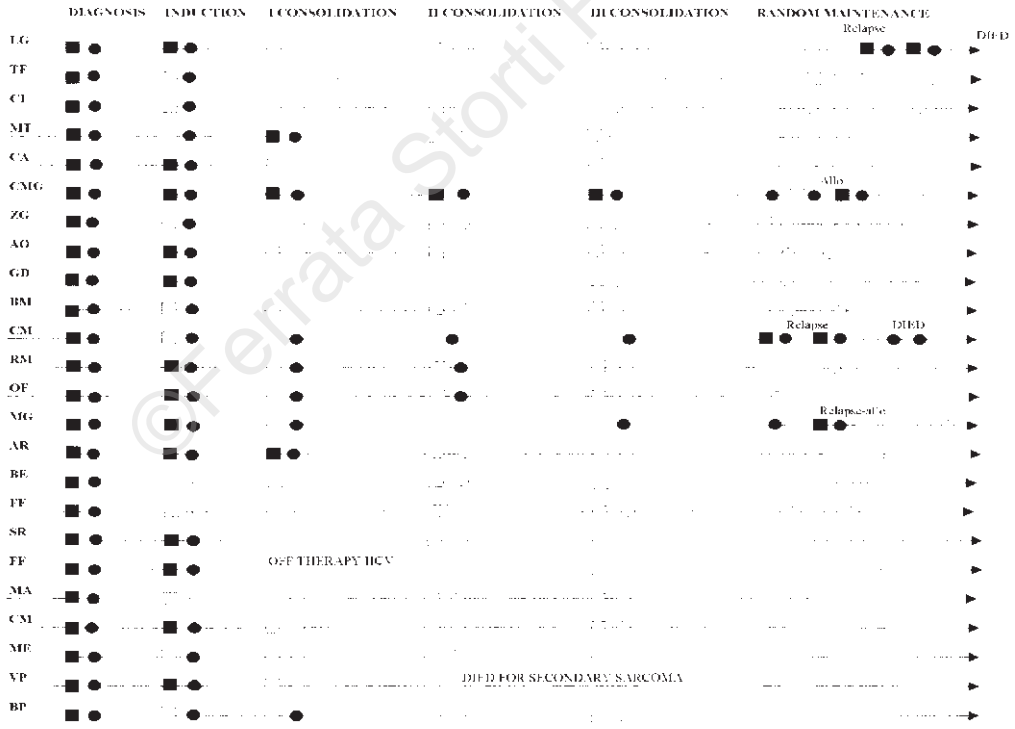

Figure 1. Display of RT-PCR results of all tested patients. The figure depicts the data of patients after the achievement of clinical remission (CR). The time of follow-up is denoted in months. Each box represents an RT-PCR assay performed at the indicated time after achievement of CR (number near the boxes). Open boxes (○) represent samples that were negative for the presence of a PML/RARα transcript by RT-PCR analysis. Full boxes (●) represent samples that were positive for the presence of a PML/RARα transcript by RT-PCR analysis. Arrows indicate the time of CR. Only samples with adequate RNA quality for amplification of control RNA were included.

Table 1. Schematic representation of molecular and cytogenetic analysis.

		Consolidation		
	Induction	*I*	*II*	*III*
Cytogenetic$^+$	13 (54%)	3 (13%)	1 (5%)	1 (5%)
Cytogenetic$^-$	11 (46%)	20 (87%)	10 (95%)	20 (95%)
Total evaluable patients	24	23	20	21
PML/RARα$^+$	21 (87%)	8 (35%)	4 (19%)	3 (13%)
PML/RARα$^-$	3 (13%)	15 (65%)	17 (81%)	20 (87%)
Total evaluable patients	24	23	21	23

positive after the second consolidation course and not cytogenetically evaluable, were still PCR$^+$.

Finally, after the third consolidation course, only one patient remained Cy$^+$ and then subsequently relapsed. At the same point, 3 patients were persistently PCR$^+$. All three PCR$^+$ patients relapsed, while one PCR$^-$ patient, after the three courses of chemotherapy, became PCR$^+$ and then relapsed during maintenance chemotherapy: 2 died of APL, while 2 received a transplant from a sibling HLA compatible donor and are now in CR and PCR–.

Discussion

Several studies have demonstrated that the persistence of PML/RARα transcript in early post-remission APL samples is associated with an early clinical relapse, usually within 6 months.[6,14-17] On the other hand, long term survival of APL patients occurs when cells carrying the specific PML/RARα rearrangement have been eradicated, indicating that PCR negativity should be considered the therapeutic goal in these patients.[13,18] Less information is present in literature on the role of different drugs and successive courses of chemotherapy on the cleavage of the PML/RARα transcript. Most of the published studies applied RT-PCR technology only at the end of chemotherapy, usually at the end of the last consolidation course, to assess further therapeutic intervention (maintenance therapy or autologous or allogeneic BMT).[8,16]

Induction therapy of APL with the AIDA protocol yields a high percentage of clinical CRs.[16] Our results further strengthen the clinical relevance of cytogenetic and PCR monitoring studies in APL. We confirm this observation: most of the patients with APL in CCR after induction chemotherapy have residual disease detectable by RT-PCR, despite being Cy$^-$. This suggests that eradication of the leukemic clone in most of these patients has not been achieved by induction chemotherapy and that further consolidation and maintenance is still necessary to obtain molecular remission.

Table 2. Patients' characteristics.

Pt.	*Age (yrs)*	*Sex*	*Follow-up (months)*	*Breakpoint*	*BMT*	*Status*
LG	53	M	16	BCR1	no	Died of APL relapse
TF	45	M	39	BCR1	no	CR
CI	32	M	34	BCR1	no	CR
MT	49	F	33	BCR1	no	CR
CA	55	M	32	BCR1	no	CR
CMG	37	M	32	BCR1	Allo	CR after ABMT
ZG	50	M	31	BCR1	no	CR
AO	30	F	30	BCR1	no	CR
GD	45	M	29	BCR2	no	CR
BM	32	F	27	BCR3	no	CR
CM	41	F	12	BCR3	no	Died of APL relapse
RM	15	F	25	BCR3	no	CR
OF	30	M	25	BCR1	no	CR
MG	34	M	24	BCR3	Allo	CR after AMBT
AR	41	F	19	BCR1	no	CR
BE	45	F	7	BCR1	no	CR
FF	33	M	18	BCR3	no	CR
SR	26	F	40	BCR1	no	CR
FF	27	F	20	BCR1	no	CR
MA	59	M	15	BCR1	no	CR
CM	17	M	18	BCR1	no	CR
ME	50	M	11	BCR1	no	CR
VP	43	M	12	BCR1	no	Died of osteosarcoma
BP	46	M	7	BCR1	no	CR

Our results also showed that the majority of our patients achieved molecular remission after the course of second chemotherapy, as reported elsewhere.[16] This observation could be explained in at least two ways: i) *resistance* to induction chemotherapy could be an *intrinsic* aspect of intra-individual diversity in this kind of leukemia, possibly associated with genetic variability (or the multistep carcinogenesis process) from patient to patient; ii) alternatively, varying sensitivities of leukemic cells to the drugs could be associated with different degrees of achievement of molecular remission status. In both cases, early detection of *resistant* patients could help anticipate therapeutic decisions, such as the use of ATRA not only in induction but also in consolidation, or referral to allogeneic or autologous BMT procedures.[19,20]

Furthermore, the observation that the majority of patients achieved molecular remission after the second course of consolidation therapy, could suggest that less post-remission treatment might be considered for these patients.[16]

Recently, some patients, enrolled in the AIDA protocol were treated for molecular relapse (defined as 2 consecutive PCR$^+$ after the end of consolidation). They were given new induction therapy with 30 days of

ATRA 45 mg/m^2 followed by Ara-C and mitoxantrone as consolidation and then further consolidation by autologous BMT, as reported elsewhere.[16,19] These reported studies confirm that APL patients who relapse, at least at molecular level, are easily rescued with second remission and could become long-term survivors. Finally, we confirm the validity of molecular follow-up after any course of consolidation chemotherapy in the management of APL patients.

Contributions and Acknowledgments

GM was the principal investigator: he designed the study, was responsible for ethical approval of the program, funding and direct supervision. EO performed the experiments, NT was responsible for cytogenetic analysis, GV was responsible for the clinical assessment of the patients, DD and GDE set up PCR procedures, FM and ST critically revised the manuscript and gave the final approval for publication.

The order of authorship reflects the significance of each of the author's contribution to the study.

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Disclosures

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