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Long-term Persistence of Dysplastic Features in Patients with Acute Promyelocytic Leukemia Treated with All-trans Retinoic Acid and Arsenic Trioxide

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To the editor.

Current treatment for standard-risk (SR) acute promyelocytic leukemia (APL) is based on the combination of all-*trans* retinoic acid (ATRA) and arsenic trioxide (ATO), which allows to achieve complete remission in virtually all patients.[1] Long-term results of the APL0406 trial and others have shown prolonged survival and lower rates of adverse effects, long-term toxicity, and secondary neoplasia with ATRA/ATO when compared to ATRA-chemotherapy (e.g., AIDA) approaches.[2,3] Since the chemo-free treatment was approved about a decade ago, real-life data on long-term follow-up of patients undergoing ATRA/ATO treatment have recently become available.[4]

Despite ATRA and ATO may induce morphological dysplasia during treatment (https://imagebank.hematology.org/image/60848/arsenicinduced-dysplasia-in-apl), to our knowledge studies on morphologic changes in bone marrow (BM) progenitor cells during follow-up of ATRA-ATO therapy, in patients achieving morphological and molecular remission, have not been reported. Therefore, taking into account the potential dysplastic effects of ATO treatment,[5] we systematically assessed the rate of bone marrow (BM) dysplastic changes by leveraging the BM slide archives of APL patients treated at Tor Vergata University Hospital, Rome, Italy.

Thus, we gathered BM slides of patients treated with ATRA-ATO at our Center and evaluated the dysplastic features or unexpected morphologic changes at different time points after ATO treatment. Longitudinal BM evaluations were used to verify the persistence of morphological alterations. As a control, we analyzed the bone marrow smears of patients treated with the AIDA chemotherapy combination.[6]

This study enrolled 11 adult patients diagnosed with APL from October 2010 to May 2020, for whom longitudinal BM slides were available. BM evaluations were performed 3 months after the end of consolidation for both protocols and then 12 months (median, range: 11-15 months) after consolidation or maintenance for ATRA/ATO and AIDA regimens, respectively. The morphological BM revision was independently performed by two experienced morphologists (E.S. and S.F.). The evaluation of dysplastic features was performed according to established criteria.[7,8] The grade of dysplasia was defined as follows: absent (grade 0): no dysplastic features; mild (grade 1): dysplastic features present in <10% of one cell lineage; moderate (grade 2): dysplastic features in 10-20% of one lineage; severe (grade 3): dysplastic features in > 20% of a single cell lineage. To evaluate dysplasia (cut-off ≥10% per cell lineage), at least 100 granulocytes, 100 erythroid precursors, and 30 megakaryocytes were evaluated. Unconventional features, such as the presence of eosinophils, mastocytes, promonocytes, and blasts, were also considered.

For statistical analysis, continuous variables from patients treated with ATRA-ATO versus AIDA were compared using the Student t-test or Mann-Whitney U test, as appropriate. Continuous variables observed at different time points (pairing post-consolidation and follow-up samples) following ATRA-ATO were compared using the Student t-test or Wilcoxon test, as appropriate, after checking for a normal distribution of variables.

Overall, patients' median age at APL onset was 52 years (range: 36-71), with a 1.2 male-to-female ratio. Ten patients (91%) presented a classical APL morphology, and only one (9%) had a microgranular type. *PML::RARA* isoforms were BCR1 in 6 patients (55%), whereas the remaining had the BCR3 isoform (45%). According to Sanz's risk score, 8 patients (73%) were diagnosed with low-risk, 2 (18%) intermediate-risk, and one (9%) with high-risk APL. Overall, 8 patients (73%) received ATRA/ATO, and 3 were treated with the AIDA protocol (27%). Patient characteristics and results are summarized in **Table 1**.

Table 1. Patient characteristics and results.

	ATRA-ATO (n=8 pts)	AIDA (n=3 pts)	
Median age (years)	54.5 (36-71)	43 (38-60)	
Sex (male/female)	3/5	3/0	
APL morphological variant	8 classical	2 classical 1 microgranular	
PML/RARα isoform	4 BCR1 4 BCR3	2 BCR1 1 BCR3	
Risk category	6 Low 2 Intermediate	2 Low 1 High	
Dysplastic changes after consolidation (median %, range)			**p-value**
Myeloid	20 (16-35)	12 (5-19)	0.123
Erythroid	15 (4-19)	8 (7-14)	0.252
Megakaryocytic	23 (9-35)	21.5 (20-23)	0.883
Promonocytes	1.5 (0-4)	0 (0-0.5)	0.083
Blasts	2 (1-4)	1 (0-2)	0.170
Dysplastic changes at 12-month follow-up (median %, range)			**p-value**
Myeloid	17.5 (12-35)	20 (5-26)	1.000
Erythroid	14.5 (7-20)	9 (6-11)	0.089
Megakaryocytic	19 (15-35)	10 (7-10)	0.026
Promonocytes	1.25 (0.5-8)	0 (0-0.5)	0.030
Blasts	2.25 (0.5-4)	0 (0-2)	0.061

In the whole group, a moderate grade of dysplasia was observed at the consolidation time point in both the erythroid (15% vs. 8% in the ATRA/ATO and AIDA group, respectively) and granulocytic (20% and 12% in the ATRA/ATO and AIDA group, respectively) lineages. Meanwhile, a severe grade of dysplasia was observed for the megakaryocytic lineage (23% vs. 21,5% in the ATRA/ATO and AIDA groups, respectively).

Focusing the analysis on different treatment protocols adopted, at the end of consolidation, at least a moderate grade of dysplasia was observed in all hematopoietic lineages for both treatment protocols, except for the erythroid lineage treated with AIDA (in which dysplasia was mild). Furthermore, we observed a higher proportion of promonocytes [1,5% vs. 0% (p=0.083), respectively] and mastocytes [1% vs. 0% (p=0.084) in ATRA/ATO vs. AIDA treatment groups, respectively (**Table 1**).

At the 12-month follow-up time point, the grading of dysplasia was moderate for all three cell lineages for the ATRA-ATO group. At the same time, it was moderate for granulocytic lineage and mild for both erythroid and megakaryocytic lineages in the AIDA group. In particular, the rate of megakaryocytic dysplasia was significantly higher in ATRA-ATO vs AIDA treatment groups (19% vs 10%, respectively, p=0.026). Similarly, we confirmed an increased proportion of promonocytes in the ATRA-ATO vs AIDA treatment groups at the same time point (1.25% vs 0%, p=0.030). Furthermore, there was a trend for increased erythroid dysplasia (14.5% vs. 9%, p=0.089) and of the proportion of myeloblasts e (median 2.25% vs. 0%, p=0.061) in the ATRA-ATO vs. AIDA group, respectively. Of note is that all patients were alive at a median of 45 months of follow-up from diagnosis (range 33-112 months), in complete molecular remission, and with no alterations of blood cell counts, thereby configuring such cases as idiopathic dysplasia of unknown significance (IDUS), a condition characterized by the presence of dysplastic bone marrow features, without any significant cytopenia.[9]

An important observation highlighted in our study is that while both ATRA-ATO and AIDA combinations induce treatment-related BM morphologic changes early in the treatment course (e.g., the end of consolidation), dysplastic features were mostly evident at long-term follow-up after ATRA-ATO exposure. Particularly, ATRA-ATO-treated patients presented moderate dysplasia of granulocytic and erythroid lineages and moderate/severe megakaryocytic dysplasia at both time points (**Figure 1**). These observations suggested that arsenic exposure may generate a characteristic IDUS form, which we will call “AIDUS” (ATO-induced dysplasia of uncertain significance).

As limitations of the study, we acknowledge the small sample size, the retrospective nature of the analysis, and the lack of cytogenetic and/or molecular data, which were not collected during patients' follow-up, lacking cytopenia, in the absence of suspicion of evolution into a secondary myeloid neoplasm.

However, our results may emphasize the need for careful morphological evaluation of long-term BM smears in patients exposed to ATRA/ATO, which may

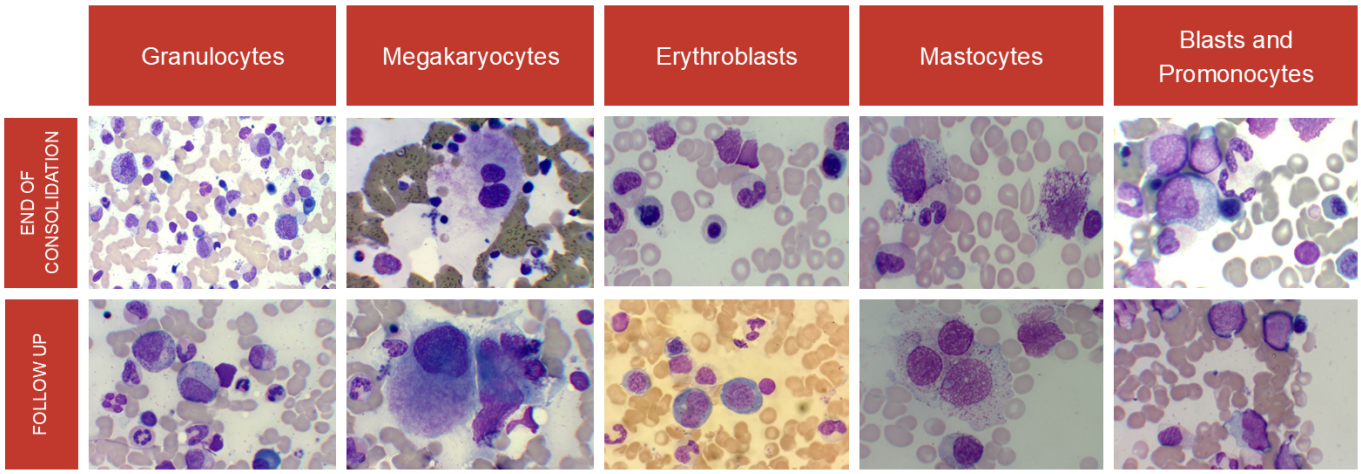

Figure 1. Post-arsenic dysplasia. In the figure, the top and low rows show the end of consolidation and follow-up pictures from 8 patients treated with ATRA/ATO. Each column illustrates examples of dysplasia in different lineages as well as mastocytes, promonocytes, and blasts. Images are taken by means of optical microscopy, May–Grünwald stain, oil immersion 100x.

cause alarm and misdiagnoses of MDS in case of co-occurrence of not-related, poorly-investigated cytopenia. Indeed, long-term follow-up of the APL0406 trial has confirmed no deaths related to a secondary myeloid neoplasm in the ATO arm, compared to two cases (1 AML and 1 MDS) in the AIDA group.[3] Furthermore, to our knowledge, no cases of secondary myeloid neoplasms have been diagnosed after the exclusive ATRA-ATO combination, despite several events reported after chemotherapy-based treatment.[10-15] In this line, cytopenias during follow-up appear to be very rare in patients treated with ATRA-ATO.[16]

Notably, the last ELN guidelines, given the very low probability of relapse for non–high–risk patients, discourage prolonged BM minimal residual disease assessments in patients treated with ATRA-ATO[17]. IDUS after ATRA/ATO even begs the question of the actual need for long-term BM evaluations in patients with standard-risk APL treated with ATRA-ATO.

In conclusion, our findings of myelodysplastic changes in patients with APL treated with ATRA/ATO are intriguing but clinically non-significant to date. They do not indicate BM follow-up studies, which may turn out to be a confounding factor during patients' follow-up. However, considering the inherent poisoning nature of arsenic on bone marrow and the lack of data on very long-term effects, prolonged follow-up of morphological peripheral blood smears could be considered.[18-20]

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Data availability statement. The data supporting Table 1 are not publicly available to protect patient privacy. However, the corresponding author can provide access upon request.

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