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Treatment of Acute Promyelocytic Leukemia by Retinoids

P. Fenaux[1] (✉) · Z. Z. Wang[2] · L. Degos[3]

[1]Service d'Hématologie Clinique, Hôpital Avicenne, Paris 13 University, 93000 Bobigny, France
pierre.fenaux@avc.ap-hop-paris.fr

[2]Institute of Hematology, Shangai, China

[3]Institut d'Hématologie, Hôpital Saint Louis, Paris, France

Abstract We review the role of all-*trans* retinoic acid (ATRA) in the treatment of acute promyelocytic leukemia (APL). The combination of ATRA and conventional anthracycline-ARA-C chemotherapy (CT) has clearly demonstrated its superiority over CT alone (in terms of relapse and survival) in newly diagnosed APL. Combination treatment probably also reduces the incidence of initial failures, and complete remission (CR) rates greater than 90% are now regularly reported in large multicenter trials. Some randomized studies strongly suggest that prolonged maintenance treatment (for 1 or 2 years) with ATRA and low-dose CT, and possibly very early introduction of anthracycline CT during induction treatment, may reduce the incidence of relapse. With those treatments, the relapse risk appears to be only 10%–15%, although it remains greater in patients who initially have high white blood cell counts (often associated with variant M_3 morphology, short bcr_3 isoform, etc.) and patients with residual disease detectable by RT-PCR at the end of consolidation courses. In those patients, addition of arsenic derivatives to induction or consolidation treatment (or both treatments together) may prove useful and is currently being tested. ATRA syndrome (now generally called APL differentiation syndrome, as it is also seen with arsenic derivatives) remains the major side effect of ATRA treatment. It occurs in 10%–15% of patients and is currently fatal in at least 10% of them. Rapid onset of CT or high dose steroids (or both) should improve its outcome. A sizeable proportion of APL patients who relapse after ATRA and CT can be durably salvaged by the same treatment followed by allogeneic or autologous stem cell transplantation, provided the transplant (in the autologous setting) is RT-PCR-negative. However, in relapsing APL arsenic derivatives (mainly arsenic trioxide) are now considered to be the reference treatment. Some of the current issues with ATRA treatment in newly diagnosed APL include whether ATRA has a role during consolidation treatment and whether arabinoside (AraC) is required in addition to anthracyclines in the chemotherapy combined to ATRA.

Acute promyelocytic leukemia (APL) is a specific type of acute myeloid leukemia (AML) characterized by the morphology of blast cells (M_3 in the French–American–British classification of AML) [1, 2], the t(15;17) translocation [3] that fuses the PML gene on chromosome 15 to the retinoic acid receptor (RAR)

alpha gene on chromosome 17 [4, 5], and a specific type of coagulopathy [6, 7]. Until the late 1980s, intensive cytoreductive chemotherapy (CT), usually combining an anthracycline and cytosine arabinoside (AraC), was the only effective treatment of APL. Over the last 15 years, the advent of all-*trans* retinoic acid (ATRA), and more recently of arsenic trioxide, have greatly improved the therapeutic approach of APL.

1
Background: Results of Chemotherapy Alone in APL

1.1
Induction Chemotherapy

With anthracycline AraC regimens, complete remission (CR) rates of only 50%–60% had generally been reported in the 1970s, but results subsequently improved, and CR rates of 70%–0% were reported in the 1980s [8–13]. Failure to achieve CR was due, in early reports, to CNS bleeding during the first days of treatment in at least two-thirds of the cases. Sepsis during the phase of aplasia accounted for the majority of other failures. By contrast, resistant leukemia was generally seen in less than 10% of the patients, probably reflecting the high sensitivity of APL cells to anthracyclines. Several studies, including recent ones, have shown that total induction doses of daunorubicin (DNR) greater than 200 mg/m^2–250 mg/m^2 were required to obtain these results [8, 14, 15]. In addition, both in randomized and nonrandomized studies, there was no evidence that anthracycline–AraC combinations were superior to anthracyclines alone if the latter were given at high dose (e.g., at least 300 mg/m^2 during induction for DNR [16]). Idarubicin appeared to be at least as effective as DNR in APL.

Other induction drugs (6 thioguanine, VP 16) do not seem to bring any benefit during induction CT in APL. High-dose AraC has been suggested to improve results over conventional dose AraC in one study [19], but gave poorer results due to increased toxicity in other studies [16, 20].

1.2
Optimal Management of Coagulopathy During Treatment with Chemotherapy Alone

The bleeding diathesis in APL results from a combination of disseminated intravascular coagulation (DIC) due to the release of procoagulants from abnormal promyelocytes and also from excessive fibrinolysis and proteolysis, as blast cells also contain plasminogen activators and liposomal neutrophil

enzymes that may be released during cell lysis, and are able to cleave various substrates, including fibrinogen [6, 7].

Significant coagulopathy, present at diagnosis in 80% of cases of APL, is worsened (or triggered in the remaining patients) by the onset of CT. Intensive platelet support during CT, aiming at maintaining platelet counts above 50,000/mm^3, is crucial in the management of coagulopathy of APL, especially in patients presenting with hyperleukocytosis, who have an increased risk of early death due to bleeding whereas the role of other treatments, including heparin, antifibrinolytic agents, and fibrinogen concentrates, is unproven [23].

1.3 Post-induction Chemotherapy

Once CR has been achieved, APL, even when treated with CT alone, is associated with a lower risk of relapse than other types of AML treated identically [12, 16]. However, the optimal post-induction therapy remains controversial in APL. In AML as a whole, it has been shown that intensive consolidation CT, without maintenance, was at least equal or superior to milder consolidation courses followed by prolonged maintenance therapy. However, in APL, two studies have suggested that prolonged maintenance CT with 6 mercaptopurine (6MP) and methotrexate (MTX) could prolong remissions when compared to shorter consolidation regimens [15, 23], although those results were not confirmed in a large randomized GIMEMA (Gruppo Italiano Malattie Ematologiche dell'Adulto) trial [17].

1.4 Prognostic Factors of Treatment with Chemotherapy Alone in APL

In newly diagnosed APL treated with CT alone, patients older than 50 [15, 23] with hyperleukocytosis at diagnosis or major thrombocytopenia [23] had a higher risk of early death.

Shorter remissions were seen in patients with hyperleukocytosis [12] and in patients with a microgranular APL variant [15].

1.5 In Conclusion: Achievements and Limits of Chemotherapy Alone in APL

Published data suggest that anthracycline–AraC regimens with sufficient anthracycline dosage, associated to intensive platelet support during induction, yielded CR in 75%–80% of newly diagnosed APL patients, with a risk of early

death due to bleeding of about 10%–15%. With anthracycline-based consolidation and possibly maintenance CT, median CR duration ranged from 11 to 25 months so that, overall, only 35%–45% of the patients could be cured by CT alone. Patients presenting with high leukocyte counts, which account for 15%–20% of APL cases, had a particularly poor prognosis with CT alone, as their CR rate was only 50%–60%, and the risk of relapse was high.

2 First Results Obtained with ATRA Alone in APL

Discovery of the activity of ATRA in APL was made by Chinese investigators, especially the Shanghai group [25], and the drug was subsequently used in France and other Western countries. In the first reports of ATRA therapy published by the Shanghai group [25], the French group [26, 27], and then by other groups [28, 29], CR rates of about 90% were reported in newly diagnosed and first-relapse APL, generally with a 45 mg/m^2 daily dose of ATRA. The presence of Auer rods in neutrophils, the absence of aplasia, and the study of X chromosome-linked polymorphisms, in particular, showed that response was not obtained by cytotoxicity but by differentiation of APL blasts into neutrophils, leading to progressive replacement of leukemic hematopoiesis by normal polyclonal hematopoiesis [25, 26,29–32]. Rapid improvement of coagulopathy, instead of the initial worsening observed with conventional CT, was also seen.

These first reports, however, drew attention to two major drawbacks of ATRA treatment. The first was that, mainly in newly diagnosed APL, a rapid rise in leukocytes was seen in one-third to one-half of the patients, accompanied by clinical signs of "ATRA syndrome" which proved fatal in some patients [26, 33, 34]. Low-dose CT (with hydroxyurea or low dose AraC) did not succeed in lowering leukocyte counts and preventing the fatal outcome, whereas more intensive anthracycline-AraC CT was able to reduce leukocyte counts and allowed most patients to enter CR [33]. It was also shown that high-dose dexamethasone also had a favorable effect on "ATRA syndrome," now often called "APL differentiation syndrome." The second drawback of ATRA therapy was the development of resistance to this drug: patients who achieved CR with ATRA and received either ATRA alone or low-dose CT for maintenance therapy generally relapsed within a few months of CR achievement [26, 27, 32]. These findings prompted clinicians to administer treatment combining ATRA and intensive CT in APL.

3
ATRA Combined to Intensive Chemotherapy in Newly Diagnosed APL

Many studies including two randomized trials (the European APL 91 trial and a U.S. Intergroup study) have clearly demonstrated the superiority of combined treatment with ATRA and intensive CT over intensive CT alone in newly diagnosed APL.

3.1
Randomized Studies Demonstrate the Superiority of ATRA Chemotherapy Combinations over Chemotherapy Alone

The European trial (APL 91) compared CT alone (three intensive courses of DNR and AraC) and ATRA followed by the same CT in newly diagnosed APL between 1991 and 1992. In the ATRA group, the first CT course was rapidly added to ATRA if WBC counts were greater than 5,000/mm^3 at diagnosis, or increased during treatment. The trial was prematurely stopped after 18 months because event free survival (EFS) was significantly better in the ATRA group [36, 37]. The last interim analysis, performed 73 months after closing date of the study, confirmed the significantly higher actuarial EFS, lower relapse rate, and better survival in the ATRA group [38]. This trial also confirmed that the combination of ATRA and CT reduced the incidence of early relapses, occurring within 18 months of diagnosis, without increasing the incidence of later relapses by comparison with CT alone. The U.S. Intergroup study, randomizing ATRA followed by CT and CT alone in newly diagnosed APL, was performed between 1992 and 1995. Patients who achieved CR were further randomized between no maintenance and ATRA maintenance (see Sect. 4). The CR rate did not differ between patients who received ATRA for induction and those that did not. However, the incidence of relapse was significantly reduced in patients who received ATRA during induction compared to those who received CT alone, and those differences translated into survival differences [39, 41]. Also of note was that fewer relapses occurred with CT followed by ATRA, as compared to CT alone. All subsequent cooperative group studies have also shown superiority of ATRA CT combination over CT alone (47,109).

3.2
CR Rates Obtained with ATRA Combined to Chemotherapy

Although the above-mentioned randomized trials did not show significant differences in CR rates between patients treated with ATRA combined to CT compared to CT alone, it appears that the addition of ATRA to CT, in recent

experiences, has somewhat increased CR rates. Indeed, results of several recent European trials of ATRA combined to CT in newly diagnosed APL, which has included more than 1,500 evaluable patients, showed CR rates between of 92% and 91% [40]. These results, obtained on a multicenter basis, show that with better knowledge of the utilization of ATRA (and especially of the prophylaxis of its major side effect, i.e., ATRA syndrome) by clinicians, very high CR rates, above 90%, can be achieved by combining this drug to CT in newly diagnosed APL. By contrast, CR rates above 80% have rarely been reported in newly diagnosed APL treated with CT alone.

4 Consolidation and Maintenance Treatment with ATRA in APL

Two randomized studies strongly suggest a beneficial role for maintenance treatment in newly diagnosed APL treated with ATRA and consolidation CT. The U.S. Intergroup trial randomized patients who had received ATRA followed by three DNR-AraC courses to continuous maintenance with ATRA (45 mg/m^2 per day) during 1 year or no maintenance. The incidence of relapse was significantly lower in patients who received maintenance ATRA (10 of 46 cases) than in patients who received no maintenance (21 of 54 patients). Liver toxicity of the treatment was, however, relatively important. A benefit for ATRA maintenance was also found in patients who had received no ATRA during induction therapy. In addition, patients who received CT alone followed by maintenance with ATRA had a similar outcome to patients who received ATRA followed by CT, but no maintenance ATRA [39].

The European APL group (APL 93 trial) randomized patients who had achieved CR with a combination of ATRA and CT to receive no maintenance, maintenance with ATRA (45 mg/m^2 per day) 15 days every 3 months, continuous low-dose CT with 6MP and MTX or both during 2 years using a 2-by-2 factorial design [40]. The rationale for intermittent rather than continuous ATRA for maintenance was based on pharmacokinetic studies showing a progressive decrease of serum peak levels of ATRA after a few weeks of treatment, due to hypercatabolism of the drug [49–52], those studies showed that this hypercatabolism was reversible after a few weeks of drug discontinuation [51]. The rationale for low-dose maintenance CT was based on two nonrandomized studies (see previous paragraph). The incidence of relapse after 2 years was 25% in patients who received no maintenance ATRA vs 13% in those who received maintenance ATRA ($p = 0.02$), and 27% in patients who received no maintenance CT vs 11% in those who received maintenance CT ($p = 0.0003$). Furthermore, an additive effect of intermittent ATRA and low-dose CT in

reducing the risk of relapse was seen. Regarding survival, the effect was significant for maintenance with CT, but was only borderline for ATRA, at least with the current follow-up. In addition, patients presenting with WBC counts exceeding 10,000/mm^3, who remain at higher risk of relapse after ATRA and intensive CT, seemed to benefit particularly from maintenance with both CT and ATRA. Finally, liver toxicity (and other toxicities) was moderate with intermittent ATRA.

Those results have led most groups to use ATRA for maintenance treatment generally in combination with low-dose CT, especially MTX and 6MP. Long-term results of the APL 93 trial suggest that this type of maintenance treatment should be administered during at least 1 year. Indeed, in that trial, where maintenance treatment was scheduled for 2 years, the incidence of relapse was 45% in patients who discontinued treatment after less than 1 year (for reasons other than relapse) as compared to 16% in patients who received it during more than 1 year.

ATRA may also play a role when administered during consolidation courses, in combination with anthracycline-based CT. In the PETHEMA (Programa para el Tratamiento de Hemopatías Malignas) experience, reduction in the relapse rate was observed between the LPA (Leucemia Promielocitica Aguda) 96 and LPA 99 trials by addition of 15 days of ATRA during the three consolidation courses in the high- and intermediate-risk patients [52]. However, the addition of ATRA during consolidation courses in the LPA 99 trial was not randomized. It was also associated with an increase in the cumulative dose of anthracyclines, by comparison with the LPA 96 trial, rendering any straightforward explanation difficult.

5
Prognostic Factors in Patients Treated with ATRA and Chemotherapy

In spite of the improvement of outcome seen with the combination of ATRA and CT, some patients with newly diagnosed APL still do not achieve CR, and others still relapse.

5.1
Prognostic Factors of CR Achievement

Of APL patients, 5%–10% fail to achieve CR with ATRA and CT, almost exclusively due to early death, as resistance to ATRA is exceptional (probably less than 1/500) in cytogenetically t(15;17) or molecularly (PML-RAR alpha rearrangement) confirmed APL [40]. The three major causes of early death

are CNS bleeding, ATRA syndrome, and sepsis, the latter usually occurring later, during the phase of aplasia induced by CT. High WBC counts and older age appear to be the main risk factors of early death in APL.

5.2 Prognostic Factors of Relapse

With a combination of ATRA and CT followed by maintenance treatment, about 10%–15% of APL cases still relapse. Prognostic factors of relapse include pretreatment factors, and monitoring of minimal residual disease (mrd) by reverse transcriptase (RT)-PCR analysis of PML-RAR alpha rearrangement.

5.2.1 Pretreatment Factors

High WBC counts remain a risk factor of relapse in all reports [53], even if, as seen above, results of the European APL group suggest that maintenance treatment may particularly reduce the risk of relapse in this population [40]. Some of the other risk factors found in most studies—including morphological M3 variant, low platelet count, and expression of CD34 or CD2 on blast surface Fms-like tyrosine kinase 3 (FLT3) duplication—are generally correlated to high WBC counts [54–56]. On the other hand, a meta-analysis of GIMEMA and PETHEMA protocols in APL (both of which included maintenance with ATRA and low-dose CT) showed that WBC and platelet counts had independent prognostic value for relapse. Disease-free survival (DFS) at 4 years was 98% in patients with WBC counts less than 10,000/mm^3 and platelets greater than 40,000/mm^3, 90% in patients with platelets less than 40,000/mm^3 but WBC less than 10,000/mm^3, and only 68% in patients with WBC greater than 10,000/mm^3 [47].

Other factors independent of the WBC count, including CD13 and CD56 [57] expression, were associated with an increased risk of relapse in some studies, whereas cytogenetic abnormalities in addition to t(15;17) did not confer a higher relapse risk. Regarding the PML-RAR alpha breakpoint, the short isoform (S isoform or bcr_3) and possibly the rare bcr_2 (or V) breakpoint have poorer prognosis than bcr_1 (or L breakpoint); they are also usually correlated with high WBC counts and may not carry a poor prognostic value per se. The degree and rapidity of in vitro differentiation of APL blasts with ATRA may also have prognostic value [58].

5.2.2 Monitoring of Minimal Residual Disease

A clear correlation has been found in APL between detectable disease by RT-PCR and the risk of relapse. Patients with detectable PML-RAR alpha fusion messenger RNA (mRNA) by RT-PCR at the end of consolidation and perhaps, more importantly, patients with positive findings after a phase of negative results are at high risk of relapse [59–63]. It should be noted, however, that for unknown reasons, the PML-RAR fusion transcript appears to be easily degraded in bone marrow samples, often making results uninterpretable. In addition, in spite of consensus meetings [64], there may be a certain interlaboratory variation in the sensitivity of RT-PCR, especially due to the fact that some laboratories perform one-round PCR, and others two-round PCR. Therefore, comparison between successive samples rather than interpretation of one given sample is advised.

U.S. and Italian investigators have used an assay with consistent but only moderate sensitivity but close to 100% specificity [59, 60]. With this assay, a negative result had good but not perfect predictive value, whereas a positive test ("molecular relapse") was considered sufficiently reliable to institute treatment, including toxic treatment with autologous stem cell transplantation [59, 60].

Quantitative PCR techniques, especially with TaqMan probes, determine for each patient at a given point a quantity of fusion PML-RAR mRNA. This allows better analysis of an increase or decrease in successive samples, and should improve clinical interpretation of results and therapeutic decisions [61, 63].

5.3 Extramedullary Relapses

A relatively large number of cases of extramedullary relapses have been reported in APL treated with ATRA and CT, generally as single case reports [65]. Extramedullary sites were seen in 13 of the 97 relapses in a GIMEMA group study and 10 of the 169 relapses seen by the European APL 93, LPA 96, and LPA 99 trials [40, 65]. Sites of extramedullary relapse in those three studies mainly included the CNS and less often the skin or other organs. CNS relapse-was associated with marrow relapse in 8 of our 10 cases; it was only molecular, however, in half of those cases.

Although it was initially suggested that the use of ATRA could increase the incidence of extramedullary relapses in APL, this was not confirmed by subsequent studies. The latter in fact strongly supported the fact that it was

the prognostic improvement brought by ATRA that increased the number of surviving patients potentially at risk of extramedullary disease.

6
Unresolved Issues in the ATRA and Chemotherapy Combination Treatment of Newly Diagnosed APL

6.1
Duration and Dosing of ATRA During Induction Treatment

The optimal duration of ATRA during induction treatment of APL is not known. Most centers administer ATRA until achievement of hematological CR, which usually occurs after 40–60 days if ATRA is used alone and after less than 30 days if CT is combined to ATRA from the onset. A British Medical Research Council (MRC) randomized study compared a short course (5 days) of ATRA followed by CT to a long course of ATRA (until CR achievement) combined to the same CT in newly diagnosed APL. A better outcome was seen in the latter group, demonstrating that longer administration of ATRA during induction is required [43]. It is, however, unknown if discontinuation of ATRA before CR achievement (e.g., after 15 to 20 days of treatment) is sufficient to achieve full activity of the drug, in particular in terms of reduction of the incidence of relapses. Analysis of the outcome of patients who had early discontinuation of ATRA may be difficult to interpret in this context, as early discontinuation is generally due to the occurrence of ATRA syndrome, which may be per se a risk factor of relapse in APL [44].

Although ATRA is generally used at the dose of 45 mg/m^2 per day, it has been shown that lower doses, i.e., 25 mg/m^2 per day, gave similar CR results [45]. Those lower doses are often applied in children when severe headache, due to ATRA, develops [46]. It is not certain, however, if the additive or synergistic effect obtained with ATRA, at 45 mg/m^2 per day, and CT on reducing the incidence of relapses in APL would persist completely with lower doses of ATRA.

6.2
Scheduling of ATRA and Chemotherapy in APL

Most cooperative groups initially treated APL patients by ATRA alone, until CR achievement, and then introduced CT. The European group (APL 93 trial) randomized newly diagnosed APL patients with WBC counts 5,000/mm^3 between ATRA followed by CT (ATRACT) and ATRA plus CT (ATRA+CT, where CT was started on day 3 of ATRA treatment). The CR rate was similar

in the two groups, but relapses at 2 years were significantly less frequent in the ATRA+CT group [40]. This suggested that the "additive" or "synergistic" effect of ATRA and CT on reducing the incidence of relapse in APL was optimal when the two treatment modalities were administered together. In addition, there was a lower incidence of APL differentiation syndrome in the ATRA+CT group, as compared to the ATRA followed by CT group (see below).

6.3 Role of AraC in the Chemotherapy of APL

As previously seen, when APL is treated with CT alone, it is unclear if AraC has a beneficial role in addition to an anthracycline in the treatment of APL. When CT is combined to ATRA, this is also debated. Sanz et al. [47] treated, in the LPA 96 trial, newly diagnosed APL with ATRA and four successive courses of CT with idarubicin or mitoxantrone alone, followed by maintenance treatment with intermittent ATRA and low-dose continuous 6MP and MTX. The CR rate was 89% and the 4-year incidence of relapse was 79%. After the end of CT, 93% of the patients had converted to PCR-negative. Furthermore, consolidation CT courses were associated with no mortality, limited morbidity, and very few days in hospital. Those results were recently confirmed in the LPA 99 trial [52]. The low mortality in CR was in contrast with the 3.5% mortality (when unrelated causes of death in CR were excluded) observed by the European APL group after consolidation CT with two DNR-AraC courses [40]. Estey et al. [48] also obtained favorable results in APL using ATRA followed by idarubicin without AraC in 43 patients. However, the European APL group performed a study randomizing newly diagnosed APL patients with an initial WBC count of less than 10,000/mm^3 between treatment with ATRA+DNR and treatment with ATRA+DNR+AraC. All patients received maintenance treatment with continuous 6MP+MTX and intermittent ATRA. Early termination of the trial was made due to significantly higher relapse rates in patients treated without AraC. Differences with the (nonrandomized) Spanish LPA 96 and LAP 99 trials are difficult to explain. They may be due to the higher cumulative dose of anthracyclines used in the Spanish studies, to a possible superiority of idarubicin over daunorubicin in APL or to the fact that, as seen above, the addition of ATRA during consolidation courses may reduce the incidence of relapses.

The role of AraC in addition to ATRA and anthracycline therefore remains debated, at least concerning patients with no high pretreatment WBC counts. Indeed, in patients with WBC counts greater than 10,000/mm^3, the relapse rate was 23% in LPA 96 and 99, without AraC, whereas it was only 8% in the French APL 2000 trials, where patients received AraC (including high-dose

AraC). The GIMEMA group's results, although they were not randomized, also strongly supported a role for AraC in reducing the relapse rate in APL with WBC counts greater than 10,000/mm^3 [67]. Results of the German APL group also support those findings [68]. Therefore, in APL with high WBC counts, where the risk of relapse remains relatively important, the main issue may not be so much the role of AraC, which appears justified, but a possible interest of adding arsenic derivatives during consolidation courses.

7
Role of Retinoids in the Treatment of Relapsing APL

7.1
Retreatment with ATRA

In patients who relapse shortly after discontinuation of ATRA no response to ATRA, even at higher doses, is generally observed [32, 69, 70]. Pharmacokinetic studies have indeed shown that prolonged use of ATRA was associated with hypercatabolism of the drug through cytochrome P450 mechanisms, with dramatic reduction of the serum peak levels of ATRA [50, 51].

On the other hand, in the APL 91 European trial, all the 10 patients initially treated with ATRA who relapsed and were retreated with ATRA achieved a second CR. An explanation could be that all relapses, in patients initially treated with ATRA and intensive CT in this trial, occurred more than 6 months after CR achievement (i.e., more than 6 months after discontinuation of ATRA). Pharmacokinetic studies indeed suggest that the hypercatabolism of ATRA induced by treatment with this drug is reversible after a few weeks of ATRA discontinuation [52, 70]. Another reason is that in some of those patients, CT was relatively rapidly added to ATRA, generally due to increasing WBC counts, and that this possibly overcame partial resistance to ATRA. Recently, the European APL group completed a study of ATRA followed by intensive CT in 50 APL patients in first relapse who had received ATRA during their first line treatment. Of the 50, 45 achieved CR [71]. When performed after this intensive salvage treatment, autologous stem cell transplantation gave very favorable results if (this was generally the case) stem cells were collected in molecular CR. By contrast, allogeneic stem cell transplantation (SCT) after such an intensive regimen give a high incidence of toxic mortality [72]. These findings further support a role for arsenic trioxide, a nonmyelosuppressive agent capable of inducing molecular CR after two courses in most relapsing patients, as the first-line treatment of relapsing APL.

7.2 Other Forms of Retinoids

Another argument supporting the systematic use of arsenic derivatives in the first-line treatment of relapsing APL is that maintenance treatment with ATRA is used more often by clinical cooperative groups. Therefore a large proportion of first relapses now concern patients who are receiving or have recently received ATRA, and will probably show resistance to this drug. In addition, in some patients, resistance to ATRA is not due to hypercatabolism of the drug, but to the occurrence of point mutations in the DNA-binding domain of the RAR alpha gene [73]. Those mutations are associated to irreversible resistance to ATRA.

In those situations of resistance to ATRA, preliminary studies suggest that liposomal ATRA can still induce some responses [74]. One reason is that this mode of administration of ATRA does not seem to induce hypercatabolism of the drug and reduction of its plasma levels [48, 75]. Because it interacts with both RXR and RAR receptors, 9-*cis* retinoic acid (RA), does not induce its own catabolism to the same extent as ATRA. Favorable preliminary results with 9-*cis* RA have been published, but responses were seen in relapsing patients that were possibly not resistant to conventional ATRA [76, 77]. A synthetic retinoid Am80, approximately 10 times more potent than ATRA as an in vitro differentiation inducer, has been used in a cohort of relapsing APL patients and 58% achieved CR [78]. However, because all those patients had discontinued ATRA for at least 18 months, it is also unclear whether Am80 was superior to ATRA in this situation, and whether it was able to overcome resistance to ATRA.

8 APL Differentiation Syndrome (ATRA Syndrome) and Other Side Effects of ATRA

ATRA therapy is usually well-tolerated and its side effects moderate. ATRA has a few major side effects, however, dominated by ATRA syndrome (now rather called APL differentiation syndrome, as it can also be observed during treatment with arsenic derivatives).

8.1 APL Differentiation Syndrome (ATRA syndrome)

8.1.1 Incidence and Clinical Signs

A progressive and symptomless rise in WBC counts is frequently seen with ATRA treatment, but our group [26, 33] reported in some cases a rapid rise of WBC counts associated with cardiopulmonary and renal failure. Frankel et al. (1992) [34] then precisely described clinical symptoms of this "ATRA syndrome," and several large series of cases of this syndrome have been published [44, 108]. Clinical signs of ATRA syndrome combine fever, respiratory distress, weight gain, lower extremity edema, pleural or pericardial effusions, hypotension, and sometimes renal failure. These signs are preceded by increasing WBC counts in the majority of case, but some patients develop symptoms at normal WBC counts [69]. Of note is that some cases of ATRA syndrome can occur upon recovery from aplasia in patients who have received early CT and are still receiving ATRA [44].

ATRA syndrome occurred in 6%–27% of the patients in previous reports, and mortality of the syndrome ranged from 8%–15% [34, 37, 39, 109, 110]. In the European APL 93 trial, patients who survived ATRA syndrome had a higher risk of bone marrow relapse than patients who had no ATRA syndrome (32% vs 15% at 2 years). In another study, the occurrence of ATRA syndrome was associated with a possibly higher risk of subsequent extramedullary relapse [111]. Finally, of note is that ATRA syndrome has not been reported during maintenance treatment with ATRA. Therefore, patients who experience tretinoin syndrome during induction treatment may safety receive tretinoin for maintenance.

8.1.2 Pathophysiology of Hyperleukocytosis and ATRA Syndrome

The pathophysiology of hyperleukocytosis and ATRA syndrome is still not completely understood. ATRA syndrome is not due to leukostasis or thrombosis (or both) [34], and because its clinical signs are reminiscent of those observed in endotoxic shock and in adult respiratory distress syndrome (ARDS), a possible stimulatory effect of ATRA on cytokine expression by APL cells has been envisaged. Induction of interleukin (IL)-1 alpha and granulocyte colony-stimulating factor (G-CSF) secretion by APL cells under ATRA may contribute to hyperleukocytosis in vivo. On the other hand, the secretion of IL-1 alpha, IL-6, tumor necrosis factor (TNF) alpha, and IL-8—which are involved in leukocyte activation and adherence, and are implicated in the development of ARDS—could have a pathogenetic role in ATRA syndrome [112, 113].

More recently, it has been shown that ATRA induced aggregation of NB4 cells (an APL cell line). This process was mediated by the adhesion molecules lymphocyte function-associated antigen (LFA)1 and intercellular adhesion molecule (ICAM)2 and was reversed by addition of methylprednisolone [114]. These findings suggest that modification of the adhesive properties of APL cells by ATRA could play a role in ATRA syndrome.

8.1.3 Prophylaxis and Treatment

Once ATRA syndrome has developed, addition of low-dose CT is ineffective in lowering WBC counts, and leukapheresis is unable to reverse symptoms. Two different approaches aimed at preventing or treating early ATRA syndrome are proposed. One of them, mainly used by the European and Japanese groups [33, 37, 109], consists of adding CT from the onset of ATRA in patients presenting with high WBC counts (WBC greater than 5,000/mm^3 in the European trial, or greater than 3,000/mm^3 in the Japanese trials) or when increases in the WBC counts are seen with ATRA. This approach has been associated with a low incidence of fatal ATRA syndrome. A disadvantage of this approach is that about two-thirds of the patients treated with ATRA also received early CT. However, several reports have shown that the period of neutropenia and thrombocytopenia is significantly shorter in patients who receive CT while already on ATRA, by comparison with CT alone [37, 115]. Furthermore, intensive CT, if not administered early, would have to be administered later on, as consolidation treatment. The possibility, suggested by the European APL 93 trial, that early onset of CT (ATRA+CT) reduces the incidence of relapse, by comparison to ATRA followed by CT (ATRA CT), could be an additional argument for the early onset CT, even in the absence of high WBC counts. This attitude is now a standard approach for the Spanish PETHEMA and Italian GIMEMA groups. Also, of note is that, in APL 93 trial, there were significantly fewer cases of ATRA syndrome in patients who received ATRA+CT as compared to those treated by ATRA CT (y%) [44, 116].

By contrast, the usual U.S. approach is to prevent ATRA syndrome by high-dose intravenous corticosteroids (dexamethasone, 10 mg IV twice daily for 3 days or more) as soon as the first symptoms occur. This attitude proved effective in the U.S. Intergroup study, both for preventing ATRA syndrome and reducing its mortality [39].

Finally, there is a consensus concerning the fact that patients presenting with high WBC counts (e.g., more than 15,000–20,000/mm^3) will very often develop severe ATRA syndrome with ATRA alone, and require CT and intravenous dexamethasone from the onset of treatment. Some of these patients

even present with symptoms analogous to those of ATRA syndrome at diagnosis [117]. The same recommendations that apply to ATRA syndrome during treatment with ATRA apply to the similar syndrome observed after treatment with arsenic derivatives.

8.2 Coagulopathy and Thrombosis

Because the release of leukemic cell components during ATRA treatment is slow when compared to massive cell death induced by CT, no exacerbation of the bleeding tendency is observed in APL patients undergoing ATRA therapy. In the European APL 91 trial, median time to disappearance of significant coagulopathy was 6 days after CT alone and 3 days in the ATRA group ($p = 0.001$) [37]. ATRA therapy may be especially important in reducing the severity of the bleeding tendency in hyperleukocytic APL patients, a population still at a relatively high risk of early death with CT alone [6, 7, 118].

In APL patients treated with ATRA alone, primary fibrinogenolysis disappears during the first 5 days of treatment, while DIC and leukocyte-mediated proteolysis seem to persist during the first 2 or 3 weeks of ATRA therapy. This could lead to a transient period of hypercoagulability, which could explain the few well-documented cases of thromboembolic events in APL patients treated with ATRA [119–121].

8.3 Other Side Effects of ATRA

Dryness of lips and mucosae are usual but are reversible with symptomatic treatment. Increases in transaminases and triglycerides are common, but they have never required treatment discontinuation in our experience. Headache, due to intracranial hypertension, is generally moderate in adults but may be severe in children, and associated with signs of pseudotumor cerebri [46]. Lower ATRA doses (25 mg/m^2 per day) reduce this side effect in children and seem as effective as conventional doses of 45 mg/m^2 per day in inducing CR [46]. Isolated fever frequently develops in the absence of other signs of ATRA syndrome (or infection) and is reversible within 48 h of ATRA discontinuation [37, 109].

Other side effects, including bone marrow necrosis [11], hypercalcemia [122], erythema nodosum [123], marked basophilia [124, 125], severe myositis [126], Sweet syndrome [127, 128], Fournier's gangrene (necrotizing fasciitis of the penis and scrotum) [129, 130], thrombocytosis [131], and necrotizing vasculitis [132] have rarely been reported with ATRA treatment.

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