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Chemotherapy-free treatment for acute promyelocytic leukemia: the pediatric view of a revolutionary tale

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The addition of all-trans retinoic acid (ATRA) to the standard anthracycline-base chemotherapy has revolutionized the treatment of acute promyelocytic leukemia (APL) over the last decades, becoming a model for precision medicine. The protocols based on the combination of ATRA and chemotherapy allowed obtaining excellent response rates both for children and adults. However, the persistence of anthracycline chemotherapy as a backbone was a matter of concern for both acute and long-term complications. Efforts in reducing anthracycline cumulative dose or even eliminating anthracycline have been pursued in more recent pediatric protocols thanks to the introduction of arsenic trioxide (ATO). The impressive results of the ATRA/ATO combinations led to the introduction of protocols completely chemotherapy-free for standard-risk adult patients as the standard of care, whereas pediatric chemo-free protocols are still currently under evaluation. In this Review, we will critically retrace the history of this unique revolution in precision medicine, discussing the peculiar advantages for pediatric patients with APL.

KEYWORDS

acute promyelocytic leukemia, pediatric, all-trans retinoic acid, arsenic trioxide, gemtuzumab-ozogamicin.

Introduction

Acute promyelocytic leukemia (APL) is a distinct subtype of acute myeloid leukemia (AML) accounting for 5-10% of all pediatric AML (1). Its incidence varies among geographical areas, with a higher prevalence in most Latino/Hispanic countries (2). APL is characterized by the typical balanced t(15;17) (q22;q21) translocation, recurrent in 95-

98% of the cases (3). This translocation joins the promyelocytic leukemia (PML) gene located on chromosome 15 to the retinoic acid receptor alpha (RARα) gene on chromosome 17, resulting in an oncogenic fusion gene (PML-RARα) (4). In the remaining cases, other RARα rearrangements, cryptic insertions, or insertional viral mutagenesis have been described (5, 6). Pediatric APL is diagnosed mainly in late childhood, more than half of APL cases being diagnosed in children aged more than 10, while APL in infants is exceptionally rare (7). Females seem to be predominant in children compared to adults and the incidence of hyperleukocytosis, defined as white blood cells (WBC) >10 x 10^9/L, is higher (8). Of note, despite these differences, pediatric APL has been treated with adult protocols for a long time. For many years, APL was considered as one of the most malignant forms of acute leukemia due to the very rapid fatal course secondary to associated severe coagulopathy in many patients. These early complications are associated with a high WBC count at diagnosis. For this reason, the risk assessment of APL is based on the number of blasts at diagnosis with patients with more or less than 10 x 10^9/L WBC being considered as high and low risk, respectively (9).

In the last few years, APL treatment experienced a dramatic revolution. First, the introduction of all-trans retinoic acid (ATRA) in the chemotherapy protocols led to excellent response and survival rates in adults. This approach was subsequently applied to children with outstanding results (10). Second, arsenic trioxide acid (ATO) was introduced in the treatment of adult APL leading to the institution of completely chemotherapy-free protocols. In this view, a seminal randomized study by Lo-Coco and colleagues compared ATRA plus arsenic trioxide (ATO) vs all-trans retinoic acid (ATRA) plus anthracyclines as induction and consolidation treatment in the frontline setting for adult patients with low-risk APL. Although outcomes in both randomization arms were excellent, a statistically significant better event-free survival (EFS) and overall survival (OS) probability was observed in patients who received ATRA plus ATO alone (11). After these results, also pediatric hematologists started to adopt chemo-free treatments consisting only of ATRA and ATO with the addition of new target therapies, such as gemtuzumab-ozogamicin (GO) (12). In this review, we aim to critically review the development of this revolutionary approach with a particular focus on children.

Drugs employed in chemotherapy-free regimens

All-trans retinoic acid

ATRA is an isomer of the 13-*cis* retinoic acid (RA) approved, at the time of discovery, for skin diseases such as psoriasis or acne (13). It was demonstrated in the early 80ies as an *in-vitro* differentiating agent for promyeloblasts (13). In the middle '80, an adult patient with APL treated with RA who obtained complete remission (CR) was described (14). ATRA was subsequently further tested, showing a higher differentiating capacity compared to the isomer 13-cis RA. ATRA was first used to treat a 5-year-old child with newly diagnosed APL in critical conditions, achieving CR and subsequently the treatment was extended to more patients and to the treatment of relapsed APL (15, 16). The mechanism of action of ATRA was later discovered, alongside the leukemogenic mechanism resulting from the PML-RARα translocation (17). In detail, the PML/RARα aberrant protein can form large homodimers repressing the expression of promyelocytes differentiating genes binding to the so-called retinoic acid response elements (12). Moreover, PML-RARα can also repress the RA downstream promoter *via* modifications in the DNA methylating enzymes (18). ATRA acts on both mechanisms, since on the one hand, induces PML/RARα aberrant protein degradation and on the other can relieve the transcriptional repression (19, 20) (Figure 1). A peculiar feature of ATRA treatment in children is the possible onset of a complication known as *pseudotumor cerebri* (21). ATRA can induce the production of cerebrospinal fluid and inhibits its reabsorption, resulting into increased endocranial pressure. This clinical manifestation can occur in 15% of pediatric patients, a proportion significantly higher than that of adults, presenting with headache, vision alterations, and cranial nerve dysfunction (22). In about 30% of cases, the discontinuation of ATRA is sufficient for the resolution of symptoms; for the remaining cases, medications including mannitol, acetazolamide, or topiramates may be used. Known risk factors for *pseudotumor cerebri* are the high doses of ATRA and obesity, defined as a body mass index higher than 28 kg/m^2. Differentiation syndrome is another life-threatening condition that can occur following ATRA treatment in 5-20% of children. It is characterized by fever, hypotension, weight gain, pleural effusion, respiratory impairment, and renal failure, due to endothelial damage following promyelocyte differentiation. This complication can be responsible for early deaths if not promptly recognized and treated using corticosteroids. Of note, administration of ATRA after continuous exposure to the drug enhances its elimination, potentially impairing its efficacy, while intermittent schedule permits to achieve relatively high plasma drug exposure over the course of therapy (23). Intermittent schedule was also associated with better tolerability, especially in children, reducing the risk of ATRA-related neurotoxicity (24).

Arsenic trioxide

ATO is a very old drug, already mentioned by Hippocrates for the treatment of skin ulcers, and used at the beginning of the 19th century to treat several diseases. However, its use was abandoned for severe toxicities until the early 20th century. In the 70ies, ATO was found to possess anticancer proprieties and was thus used to treat several tumors, including APL (25). Initial results were extremely encouraging with CR rate ranging from 65% in the first experiences to 90% in larger cohorts after the optimization of drug formulation (26–28). Recently, an oral formulation of ATO named Realgar-Indigo naturalis formula (RIF) was tested with similar clinical effects but easier use (29). ATO mechanism of action was later elucidated, although still not completely. Interestingly, ATO exerts two different effects based on its concentration. A high concentration of the drug induces apoptosis, while a low concentration induces differentiation of the promyelocytes (27). Both effects were shown to be restricted to cells carrying PML-RARα and wild-type PML, suggesting that ATO directly targets PML (30). Functional studies revealed that ATO can also modulate

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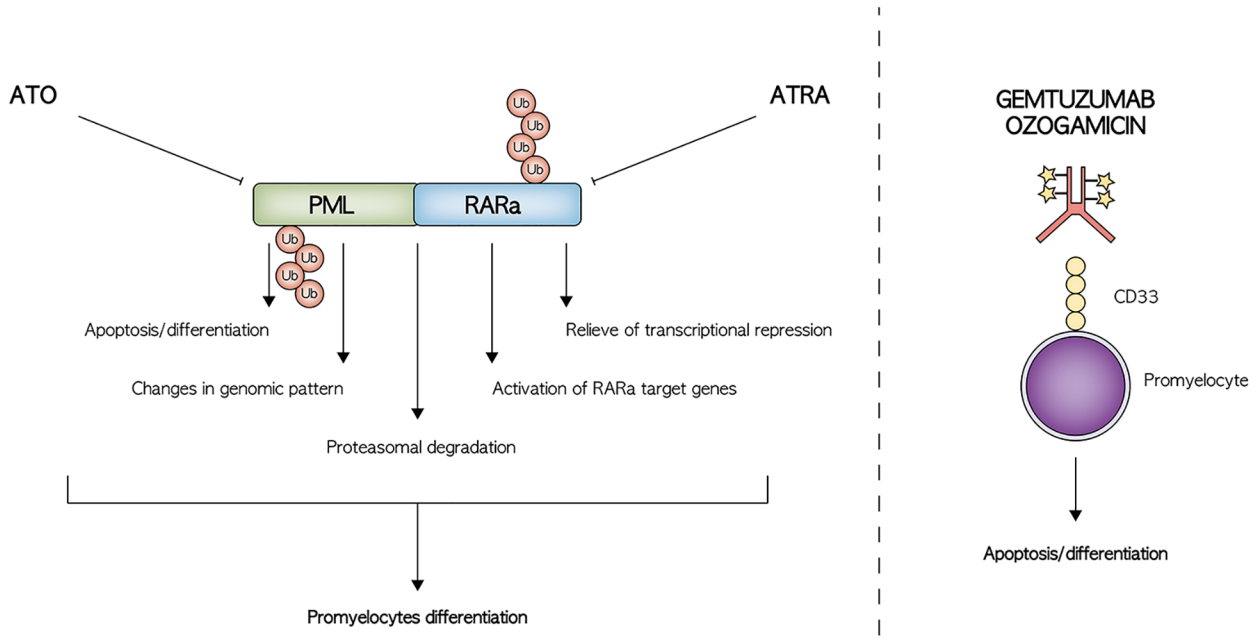

FIGURE 1
Schematic representation of the mechanism of action of the main drugs employed in the treatment of APL.

several genes regulated by ATRA, but its main function seems to be the induction of a deep change of proteome pattern in many intracellular pathways (19) (Figure 1). ATO toxicity profile includes differentiation syndrome and prolongation of the QT/QTc interval, which can occur in a variable percentage of cases between 10% and 17% (31, 32). This latter is potentially fatal, leading to *torsade de pointes*-type ventricular arrhythmia. Continuous cardiac monitoring should be performed on each patient and ATO discontinued when QTc interval increases over 450 msec. Of note, prolonged QTc has been described often without a clinical correlation and thus the indication of treatment discontinuation over 450 msec is a matter of debate (33). ATO can also results in hepatotoxicity with an increase in serum bilirubin, transaminases, or alkaline phosphatase and may require temporary suspension of the drug.

Gemtuzumab-ozogamicin

GO is a recombinant humanized monoclonal antibody, directed against CD33, conjugated with a cytotoxic antibiotic called calicheamicin (34). Treatment with GO has been associated with an increased risk of hepatotoxicity and hepatic veno-occlusive disease, especially following HSCT, and other non-specific adverse events, such as myelosuppression, thrombocytopenia and tumor lysis syndrome (35, 36). CD33 is highly expressed on APL blasts and shows a highly homogeneous expression pattern, representing an optimal therapeutic target (37). Many clinical trials conducted both in children and adults have shown the efficacy of this antibody-drug conjugate in the setting of AML (38). Clinical data for GO in APL were first reported in early 2000. A patient with relapsed APL after upfront treatment with ATRA/ATO received GO, achieving CR (39). After this first case, many others were reported with encouraging results (40–42). *In vitro* studies later confirmed the anti-leukemic effect of GO against ATRA- and ATO-resistant APL cell lines (43).

Adult data on chemotherapy-free treatment for APL

As already mentioned, specific therapeutic strategies for pediatric APL have been initially derived from adult trials. Chemotherapy has been historically used for the treatment of APL, and anthracyclines were recognized as the best suited class of drugs as early as 1970 (Figure 2). Chemotherapy with anthracyclines and cytarabine was considered the only treatment option for adult patients with APL until the late 1980s, when the introduction of all-*trans* retinoic acid (ATRA) led to an increase of remission rates up to 90-95%, together with a reduction of morbidity and mortality, mostly associated with severe coagulopathy (12, 44). Despite the dramatic improvements achieved in frontline therapy of APL with ATRA plus anthracycline-based regimens, relapses still occurred at a rate of approximately 10-20% (45). Moreover, these regimens were associated with significant toxicities due to severe myelosuppression, frequently associated with life-threatening infections and potentially serious late effects, including development of secondary MDS/AML and anthracycline-related myocardiopathy (46–50). The application of ATO since the early 1990s further improved the clinical outcome of refractory or relapsed, as well as newly of newly diagnosed APL[45]. In the randomized phase 3 APL0406 clinical trial conducted in 263 adults with newly diagnosed low- or intermediate-risk APL enrolled

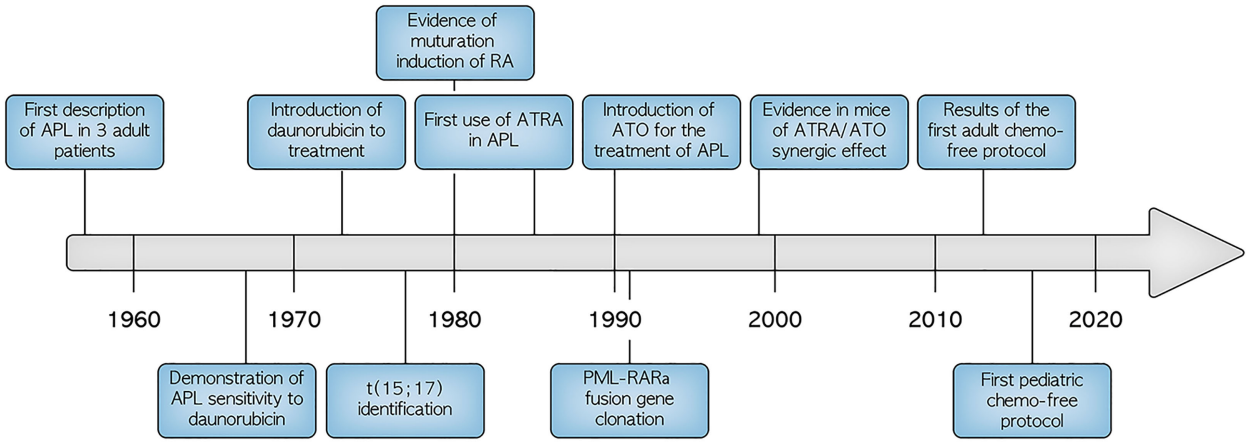

FIGURE 2
Timeline of the main steps in the development of chemo-free protocols for pediatric APL.

between 2007 and 2013, Lo Coco and colleagues compared induction and consolidation with ATRA and ATO vs ATRA and idarubicin plus maintenance with chemotherapy and ATRA. Low risk patients were defined as a white-cell count of no more than 10×10^9/L and a platelet count of more than 40×10^9/L at presentation, and intermediate risk level as a white-cell count lower than 10×10^9/L and a platelet count of no more than 40×10^9/L liter at presentation (11). The combination of ATRA and ATO resulted into statistically significant better EFS and OS rates at 50 months compared to the standard ATRA plus idarubicin therapy, 97.3% and 99.2% vs 80% and 92.6% respectively, with significantly reduced cumulative incidence of relapse and lower toxicity (11, 51). These data indicate that at least SR APL in adult patients can be cured with ATRA/ATO only and without chemotherapy. Notably, patients did not receive intrathecal therapy and, despite this, did not show increased incidence of CNS relapse. Recently, the AML17 randomized trial enrolled 235 patients including the ones with HR features in which GO was given at diagnosis to control the initial high WBC count if patients were randomized in the ATRA/ATO group. Compared to a standard ATRA and idarubicin regimen, ATRA/ATO resulted in higher EFS and reduced relapse rate at 4 years with no-difference in OS and quality of life (52). Long-term follow-up studies demonstrated the durability of the response and good short- and long-term tolerability without significant adverse effects (53–55).

Results of ATRA-based regimens in pediatric APL

The combination of ATRA and chemotherapy in adults offered more efficacious results compared to pediatric studies with chemotherapy-only intensive regimens (56, 57). The use of ATRA in pediatric APL has thus been proposed as a potentially very effective approach since the beginning of 90s, with encouraging results in first small pediatric cohorts (9, 58, 59). Anthracycline-based regimens, often including high cumulative doses, were historically considered the mainstay of treatment for pediatric APL similar to adults, but their late effects have always represented a major and peculiar concern in children. Anthracyclines are known to cause a unique dose-dependent cumulative cardiotoxicity, with an increased risk of congestive heart failure in cancer survivors who received anthracyclines in their chemotherapeutic regimens. This dose-limiting late cardiotoxicity can cause severe morbidity and mortality, despite the progress in monitoring and preventing anthracycline cardiotoxicity. This is particularly important in in patients with long life expectancy, considering that the incidence of cardiac abnormalities increases with the time (23). These findings, associated with the promising results of the frontline administration of ATRA, led to a trend of anthracycline dose reduction in recent pediatric protocols in favor of ATRA administration along all the treatment. On the other hand, ATRA-related side effects, particularly *pseudotumor cerebri*, represented a peculiar concern in pediatric patients (60). The first large cohort of pediatric APL homogeneously diagnosed with genetic evidence of t(15;17) in blasts treated with ATRA-based regimen was derived from a multicenter Italian study by GIMEMA in association with AIEOP (AIDA-0493). The trial evaluated the use of ATRA combined with chemotherapy in newly diagnosed APL, enrolling also patients younger than 18 years. The treatment protocol consisted in ATRA and idarubicin induction therapy followed by three consolidation courses of anthracyclines. This resulted in the administration of high cumulative anthracycline dosage (650 mg/m^2 of anthracycline daunorubicin equivalence). Molecular response was assessed by RT-PCR evaluating the PML-RARα transcript. Patients in complete molecular remission were randomized to either of four maintenance therapy arms, including 6-mercaptopurine and methotrexate, ATRA, chemotherapy with 6-mercaptopurine and methotrexate alternated to ATRA and no therapy. In children, ATRA was administered until the achievement of complete remission and for a maximum of 90 days, at a dose of 25 mg/m2/die in two doses, previously described as effective in adult population (61), with the aim of reducing the incidence of *pseudotumor cerebri*. Of the 107 eligible children who

received induction with ATRA, 103 (96%) achieved a complete molecular remission. At the end of consolidation, ninety-four patients were evaluable for molecular analysis and 91 (97%) presented a complete molecular remission. The 10-year OS and EFS were 89% and 76%, respectively, and a WBC count greater than $10x10^9$/L at diagnosis resulted the strongest prognostic predictor on EFS (59% vs 83%) (62). Similar to the Italian experience, the national Spanish transplantation network (PETHEMA) reported outcomes of 66 children with molecularly-proven APL, who received induction therapy with ATRA and idarubicin and consolidation with three courses of anthracycline chemotherapy only, with a slightly reinforced doses of idarubicin in intermediate and high-risk patients. Maintenance therapy consisted of ATRA and oral chemotherapy. A high CR rate was reported (92%) with manageable toxicity and favorable long-term outcome (DFS 82% and OS 87% at 5 years). The high incidence of hyperleukocytosis in children with APL was confirmed to be associated with a higher risk of relapse (63). The North American Intergroup trial (INT0129) included children with previously untreated newly diagnosed APL, randomly assigned to receive daunorubicin plus cytarabine or ATRA as induction therapy. Similar to the previous studies, patients received ATRA until CR, or for a maximum of 90 days. Patients in CR after induction received two cycles of consolidation therapy with daunorubicin and cytarabine. A second randomization after consolidation assigned patients to receive a maintenance therapy with ATRA or to observation. The study compared rates of CR, DFS, OS and toxicity in patients who received ATRA for induction and/or maintenance compared to conventional chemotherapy. Fifty-three patients were evaluated, and a significant difference was shown in term of 5-year DFS from time of CR for patients who were randomized to ATRA, for induction or maintenance or both, compared to patients who never received ATRA (48% vs 0%) (64). These favorable results were confirmed in a European multicenter study including two consecutive trials (APL 93 and APL 2000) comparing disease characteristics and outcomes specifically in different age ranges, particularly children (age < 4 and 4-12 years), adolescents (13 to 18 years), and adults (> 18 years). All enrolled patients received ATRA in the induction phase, as monotherapy or in combination with chemotherapy. Interestingly, adolescents and children age > 4 years treated with ATRA and chemotherapy had outcomes comparable to those of adults, while younger children (12 patients) seemed to experience higher relapse rate (52% relapse compared to 18% in patients of 5-18 years), suggesting a less effective action of ATRA-based regimens in this particular category of patients (65). Subsequent protocols tried to adopt an anthracycline-sparing approach, as in the three consecutive AML-BFM 93/98/2004 trials, in which reduced cumulative anthracycline dose (350 mg/m^2) was employed, combined with cytarabine and ATRA, with clinical outcomes achieved comparable to those reported in the AIDA 0493 protocol (66). A second multicenter Italian study (AIDA-2000) enrolled pediatric patients receiving the same induction therapy (ATRA 25 mg/m^2/day and idarubicin) followed by a risk-adapted consolidation (67). Low- and intermediate-risk children, defined as in Lo Coco and colleagues, received three less intensive anthracycline-based courses plus ATRA, while for high-risk patients, consolidation was the same of AIDA-0493. Maintenance therapy consisted of standard daily 6-mercaptopurine and weekly methotrexate given for two years, plus ATRA administered for fifteen days every three months. The efficacy of ATRA plus idarubicin as induction was confirmed, and, for low/intermediate risk children, the anthracycline-based plus ATRA consolidation was equally effective as the previous cytarabine-containing regimen of AIDA-0493 (7, 68).

A more recent international study (ICC-APL-01) enrolled 258 European and South American children with newly diagnosed APL receiving induction treatment with ATRA and 3 doses of idarubicin. The primary aims of the trial were to reduce the cumulative anthracycline doses, while maintaining an excellent response and to investigate the efficacy of a risk-adapted consolidation therapy based on the initial WBC count. The therapeutical protocol included ATRA in induction, consolidation, and maintenance. For both standard- and high-risk patients, induction consisted of oral ATRA with pediatric dose of 25 mg/m2 per day, administered for 30 consecutive days and 3 doses of idarubicin. After induction, standard and high-risk patients received 2 or 3 consolidation blocks, respectively. Maintenance therapy included ATRA cycles, given for the first 14 days every 12 weeks with low-dose chemotherapy (oral 6-mercaptopurine daily and methotrexate weekly) and was given to all patients for 2 years. The cumulative dose of daunorubicin-equivalent anthracyclines administered in standard and high-risk patients was 355 mg/m^2 and 405 mg/m^2, respectively. The other main objective of the study was to evaluate systematically at different time points the PML-RARα transcript by PCR in order to investigate the value of MRD for predicting relapse and to guide the therapy. In fact, this approach permitted to treat with a third consolidation block, equal to that of the HR group, those SR patients who were positive for the PML-RARα transcript at the end of the second consolidation block. The transcript was re-evaluated at the end of consolidation therapy before the start of maintenance therapy. Resistant disease was defined as positive PCR after third consolidation block and was treated with salvage therapy consisting of ATO, GO and ATRA and, if refractory, with allogeneic hematopoietic stem cell transplant (HSCT). Results showed that complete molecular remission was obtained in 97% of patients, while 5-year OS and EFS were 94.6% and 79.9%, respectively in the whole cohort, 98.4% and 89.4% in standard risk patients and 84.3% and 74.2% in high-risk patients. These results were comparable to previous studies without any stratification of treatment on the basis of MRD and including higher dose of chemotherapy, confirming the effectiveness of ATRA combined with a risk-adapted consolidation strategy (69).

The management of CNS prophylaxis in pediatric ATRA based protocols varies among different studies. The incidence of CNS relapse was not homogeneously reported, and therefore any comparison regarding the efficacy of ATRA-based treatment and CNS prophylaxis could not be performed. In the protocols in which it is reported, CNS relapse is under 1% (63, 66). Heterogeneous approaches to CNS disease prophylaxis have been proposed in the different protocols described, with a wide range of strategies from no prophylaxis to several intrathecal therapies and cranial irradiation. Details of adopted prophylaxis regimens are reported in Table 1.

Preliminary data on the use of the combination of ATRA and ATO in pediatric APL

Studies in adult APL showed that with the combination of ATRA and ATO leads to excellent results with lower toxicities compared to the therapy based on chemotherapy with ATRA (11). In the pediatric setting, the use of ATO was initially introduced for the treatment of patients with refractory/relapsed PML-RARα positive APL, with promising results (70, 71). The combination ATRA/ATO was then translated to the first-line therapy. A Chinese single-center study reported data about the use of ATO combined with ATRA as induction and consolidation therapy in combination with chemotherapy in newly diagnosed childhood APL Forty-three children, eleven of which classified in the HR group, treated with ATRA/ATO were compared with 25 pediatric patients (2 HR) previously treated with ATRA alone as induction therapy. The ATRA/ATO group achieved complete remission (CR) rates of 95.4% compared to 80% in ATRA-alone cohort. With a median follow-up time of 75 months, EFS and OS were 92.5% and 95.3% respectively in the ATRA/ATO group, significantly higher compared with the ATRA group. Of note, one patient had CNS relapse during consolidation chemotherapy (72). The COG AAML0631 trial enrolled 101 patients (66 SR and 35 HR). Both groups received ATRA only induction therapy, consolidation with ATRA/ATO and chemotherapy and maintenance with ATRA plus chemotherapy. The only difference in the two groups was the number of chemotherapy consolidation cycles, 2 versus 3. As a result, the cumulative anthracycline dose was 335 mg/m^2 of cumulative daunorubicin equivalents for SR patients and 385 mg/m^2 in the HR group. In the HR group 0.8% of patients died during induction, due to coagulopathy and differentiation syndrome, while no patients experienced an early death event in the SR group. Of the three relapses recorded, one in the SR group was a combined CNS and bone marrow, while in the HR group one was combined and one an isolated bone marrow relapse. The 3-year OS and EFS rate of 98% and 95% respectively were achieved for SR patients, while in the HR group the 3-year OS and EFS were 86% and 83%, respectively (73). Therefore, EFS for SR patients was noninferior to that of patients in the AIDA 0493 historical control, which used a significantly higher anthracycline dose and did not include ATO consolidation (73). A first international series of 11 pediatric patients treated with ATRA/ATO only in induction and consolidation phases was described by the AML-BFM group. All patients were treated according to a protocol similar to the adult APL0406, with lower ATRA dose, delayed start of ATO and an intermittent schedule. In all cases a CR was obtained after a median time of 10 weeks, without early death events (31). A chemotherapy-free approach was applied in 18 Italian children with newly diagnosed APL, of whom 89% were SR, using a modified APL0406 schedule with lower ATRA dose. All patients achieved molecular CR and were alive at a median follow-up of 24 months, with good tolerance to the therapy (32). These results were confirmed in a French cohort of 21 children, with all patients achieving molecular remission with a median of 8 weeks. OS and EFS of 100% were achieved after a median follow up of 17 months. Notably, the French protocol include two doses of idarubicin in HR patients (74). Comparable results were reported in another Chinese cohort of 17 pediatric patients with newly diagnosed APL treated with ATRA/ATO only, showing an OS and EFS of 94.1% and 100%, respectively. One patient in this protocol died during induction due to intracranial hemorrhage (75). Preliminary results on 27 children were reported from the Japanese protocol JPLSG AML-P13. Patients received 2 courses of induction consisting of ATRA, cytarabine, and daunorubicin. Patients with hematological CR were considered SR and received 3 intensification cycles of ATRA/ATO, followed by ATRA maintenance therapy, while patients non in CR were considered HR and received intensified schedule of ATO, followed by GO and a maintenance of 6-mercaptopurine and methotrexate. Molecular remission rate was 100% in both groups after intensification and no patient experienced relapse. The 3-year EFS rate and 3-year OS rate in full analysis set were 96.3% and 100.0%, respectively (76). Interestingly, the ATRA/ATO combined approach was also applied as a stand-alone therapy in children with relapsed APL with promising results in several case reports (77).

Clinical trial assessing the combination of ATRA and ATO for first line treatment of pediatric APL

Given the revolutionary results of the adult trial regarding the treatment of SR APL with chemo-free regimens and the encouraging preliminary data on the use of ATRA/ATO in children, two clinical trials have been performed in pediatric patients assessing the combination of ATRA and ATO alone for the treatment of newly diagnosed APL. The CCLG-APL2016 multicenter trial enrolled 193 children from 38 Chinese centers. In SR patients, induction, consolidation and maintenance therapy were performed using ATRA/ATO only, while for HR patients low dose anthracycline (200-300 mg/m^2 of cumulative daunorubicin equivalents) was added in the induction and consolidation phase. Interestingly, ATO was administered either intravenously or with an oral formulation depending on the investigator's choice. In 107 patients allocated to the SR group, the 2-year OS and EFS were 99% and 97% respectively, whereas in the high-risk group the corresponding value were was 95% and 90%. Only one patient in the HR group had combined CNS and bone marrow relapse despite the absence of intrathecal prophylaxis in the protocol. ATRA/ATO regimen was well tolerated without treatment-related death, and no retention of ATO in plasma, urine, hair, and nail was detected 6 months after the cessation of treatment (78). 154 pediatric patients from 85 pediatric oncology centers were enrolled in the COG AAML1331 trial and compared to an historical control group from the AAML0631 study. Patients in the SR received ATRA/ATO only as induction and consolidation therapy, while HR patients received 4 doses of idarubicin during induction and 14 days of dexamethasone therapy to prevent differentiation syndrome. No maintenance was administered in any patient. Therapy was well tolerated in both risk groups; 98 children were included in the SR group, with 98% 2-year EFS rate and 99% 2-year OS rate. The HR

TABLE 1 Summary of pediatric studies using ATRA/ATO combination.

Reference	Enrollment period	Treatment for SR and HR	ATRA dose	ATO dose	Cumulative Anthracycline dose	Intrathecal CNS prophylaxis	Patients, n	EDR	CR end induction	CR end consolidation	OS	EFS	Median follow-up
Testi 2005, AIDA 0493, GIMEMA-AIEOP	1993-2000	Induction: ATRA + IDA. Consolidation: 3 chemotherapy courses. Maintenance (*random*): chemo vs ATRA vs chemo-ATRA vs no therapy	25 mg/m^2/day	/	650 mg/m^2	/	107	3.7%	96% (hematological)	97% (molecular)	89%	76%	79
Ortega 2005, PETHEMA	1996-2004	Induction: ATRA + IDA. Consolidation: 3 chemotherapy courses. Maintenance: chemo + ATRA (increased idarubicin dose in HR patients)	25 mg/m^2/day	/	650 mg/m2 (+20 mg/m2 idarubicin in HR)	/	66	7.6%	92% (hematological)	97% (molecular)	87%	[DFS] 82%	39
Gregory 2009, INT0129, CCG-POG	1992-1995	Induction (*random*): chemo (DNR + ARA-C) vs ATRA. Consolidation 2 courses. Maintenance (*random*): ATRA vs no therapy	ATRA 45 mg/m^2/day	/	225-495 mg/m2	/	53 (27 ATRA / 26 chemo)	3.7% (ATRA) vs 11.5 % (chemo)	81% (ATRA) vs 65% (chemo) (hematological)	/	69.6% ATRA: 73% Chemo: 65%	[DFS] 41% ATRA: 49% Chemo: 29%	60
Bally 2012, European APL group, APL 93 and 2000 trials	1993-2004	*APL 93 trial.* Induction: risk stratification according to WBC count (< / > 5x10^9/L); in low risk (*random*): ATRA followed by chemotherapy (DNR + ARA-C) vs early addition of chemotherapy. Consolidation: 2 chemotherapy courses. Maintenance (*random*) ATRA vs chemo vs ATRA + chemo vs no therapy [for 2 years] *APL 2000 trial.* Induction: risk stratification = APL 93; in low risk [*random*): with or without ARA-C. ARA-C dose doubled in high risk	ATRA 45 mg/m^2/day	/	495 mg/m2	*APL 2000 trial:* MTX and ARA-C x1 during induction and x4 during consolidation (if WBC > 10x 10^9/L)	84 (26 ≤12 y/o / 58 13-18 y/o)	/	92% (≤12 y/o) 100% (13-18 years) (morphological)	/	80,4% (≤12 y/o) 93.6% (13-18 years)	/	60
Creutzig 2010, AML-BFM, AML-BFM 93-	1993-2007	ATRA during induction, consolidation and maintenance. Patients	25 mg/m^2/day	/	300 mg/m2 (trial 93), 320–350 mg/m2 (trial 98), and	ARA-C x11 + cranial	81	7.4%	93%	/	89 (5 y) and 82 (10 y)	73 (5 y) and 65 (10 y)	120

(Continued)

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TABLE 1 Continued

Reference	Enrollment period	Treatment for SR and HR	ATRA dose	ATO dose	Cumulative Anthracycline dose	Intrathecal CNS pro-phylaxis	Patients, n	EDR	CR end induction	CR end con-solidation	OS	EFS	Median follow-up
98-2004 protocol		received uniform AML-BFM SR therapy (not stratified by WBC count): induction [ARA-C, DNR and VP-16 - ADE), consolidation (2 cycles of 6-week therapy with seven different drugs or two blocks AI (ARA-C + idarubicin) + hAM (HD-ARA-C + mitoxantrone); intensification (HD ARA-C + VP-16 + cranial irradiation); maintenance: oral chemo			350–410 mg/m2 (trial 2004).	irradiation after intensification							
Lo-Coco 2010, Testi 2010, GIMEMA-AIEOP AIDA 2000	2000-2009	Induction = AIDA 0493 Consolidation: risk-adapted according to Sainz criteria: low and intermediate risk: less intensive anthracycline-based courses plus ATRA high risk patients = AIDA 0493 (3 chemotherapy courses) + ATRA Maintenance: daily 6-MP and weekly MTX for 2 years + ATRA for 15 days every 3 months	25 mg/m^2/day	/	650 mg/m2	/	123	0%	100% (hematological)	/	96% LR/IR: 95.6% HR: 96.8%	82.5% LR/IR: 82.7% HR: 82.3%	72
Testi 2018, International Consortium for Childhood APL , ICC-APL-01	2008-2017	Patients stratified into SR/HR according to the baseline WBC count </> $10x10^9$/L Induction: SR and HR ATRA + idarubicin. Consolidation: SR (2 courses) and HR (3 courses) + ATRA [Course 1: ARA-C and MTZ; Course 2: idarubicin; Course 3: HD-ARA-C and idarubicin]. Maintenance:	25 mg/m^2/day	/	SR: 355 mg/m2 HR: 405 mg/m2	ARA-C at the start of each consolidation block	258	3.1%	97% (hematological)	94% (molecular)	94.6% SR: 98.4 HR: 89.4 0-5y: 100 6-12y: 94.3 13-18y: 93	79.9% SR: 84.3 HR: 74.2 0-5y: 78 6-12y: 80.9 13-18y: 79.4	52

(Continued)

10.3389/fonc.2023.1135350

TABLE 1 Continued

Reference	Enrollment period	Treatment for SR and HR	ATRA dose	ATO dose	Cumulative Anthracycline dose	Intrathecal CNS prophylaxis	Patients, n	EDR	CR end induction	CR end consolidation	OS	EFS	Median follow-up
		oral 6-MP daily and MTX weekly + ATRA for 14 days every 12 weeks); 2 years.											
Cheng 2013, The Affiliated People's Hospital of Peking University, Beijing, China	1998-2011	Induction ATRA/ATO Consolidation with at least two cycles comprising daunorubicin, Ara-c, Vp16, homoharringtonine and ATRA/ATO. Maintenance with ATRA and mercaptopurine	20–40 mg/m^2/day	0.16 mg/kg/day	<350 mg/m^2	MTX, dexamethasone and ARA-C (if WBC > 10x10^9/L or CNS involvement)	43	4,7%	95,3% (hematological)	95,3% (hematological)	95,2%	92,5%	75
Kutny 2017, COG AAML0631Trial	2009-2012	Induction: ATRA only Consolidation ATRA/ATO and 2 chemotherapy cycles in SR or 3 cycles in HR Maintenance: ATRA plus chemotherapy.	25 mg/m^2/day	0.15 mg/kg/day	SR: 335 mg/m^2 HR: 385 mg/m^2	ARA-C SR: x3 HR: x4	SR: 66 HR: 35	0%	81% (hematological)	100% (molecular)	SR: 98% HR: 86%	SR: 95% HR: 83%	44,8
Creutzig 2017, BFM	2013-2016	Modified APL0406 schedule: A later start of ATO on day 10, lower ATRA dose and a 1- week break of ATRA after the first 14 days.	25 mg/m^2/day	0.15 mg/kg/day	0	ARA-C every 4 weeks starting at day 10 or after blast cell reduction	11	0%	100% (molecular)	100% (molecular)	/	/	29
Strocchio 2018, AIEOP	2014-2017	Modified APL0406 schedule: lower ATRA dose	25 mg/m^2/day	0.15 mg/kg/day	0	/	18	0%	100% (hematological)	100% (molecular)	/	/	24
Garcia Spezza 2019, French SFCE	2015-2018	SR: induction ATRA/ATO HR patients: idarubicin added at days 1 and 3.	25 mg/m^2/day	0.15 mg/kg/day	120 mg/m^2	/	21	0%	100% (molecular)	100% (molecular)	100%	100%	17
Li 2021, Yinzhou Hospita,, Ningbo Medical Center, Lihuili	2008-2018	Induction and consolidation with ATRA/ATO only	25 mg/m^2/day	0.16 mg/kg/day	0	MTX, dexamethasone and ARA-C CR (SR: x4; HR x6)	197	5,9%	94,1% (molecular)	94,1% (molecular)	94,1%	100%	49

(Continued)

10.3389/fonc.2023.1135350

TABLE 1 Continued

Reference	Enrollment period	Treatment for SR and HR	ATRA dose	ATO dose	Cumulative Anthracycline dose	Intrathecal CNS prophylaxis	Patients, n	EDR	CR end induction	CR end consolidation	OS	EFS	Median follow-up
Hospital ,Hwa Mei Hospital													
Hiroyuki 2022, preliminary results of the JPLSG AML-P13 Study	2014-2018	SR: induction consisting of ATRA, cytarabine, and daunorubicin, consolidation with ATRA/ATO, maintenance with intermittent ATRA HR (failure to achieve hematological remission after induction): induction consisting of ATRA, cytarabine, and daunorubicin, consolidation with intensified schedule of ATO, followed by gemtuzumab ozogamicin, maintenance with 6-mercaptopurine and methotrexate	/	/	/	/	25	/	92% (hematological)	100% (molecular)	100%	96,3 %	36
Zheng 2022, CCLG-APL2016 Protocol	2016-2018	SR: induction, consolidation and maintenance with ATRA/ATO only HR: low dose anthracycline added in induction and consolidation	25 mg/m^2/day	0.16 mg/kg/day	SR: 0 HR: 200-300 mg/m^2	/	SR: 107 HR: 86	SR: 1% HR: 5%	/	SR: 99% (molecular) HR: 95% (molecular)	SR: 99% HR: 95%	SR: 97% HR: 90%	28,9
Kutny 2022, COG AAML1331 Trial	2015-2019	SR: induction and consolidation with ATRA/ATO only, no maintenance HR: idarubicin added during induction, no maintenance	25 mg/m^2/day	0.16 mg/kg/day	SR: 0 HR: 240 mg/ m^2	MTX, hydrocortisone and ARA-C twice weekly until CSF negative plus one week.. Additional doses (x6) during consolidation. Only in patients with CNS leukemia or hemorrhage	SR: 98 HR: 56	SR: 1,0% HR: 0%	100% (molecular)	100% (molecular)	SR: 99% HR: 100%	SR: 98% HR: 96,6%	24,7

"/" means that the data was not reported.

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group had a 2-year EFS and OS rate of 96.6% and 100%, respectively. Despite using intrathecal therapy only in patients with CNS leukemia or hemorrhage (see Table 1), only one patient in the SR group experienced a combined bone marrow and CNS leukemia, confirming that pediatric APL patients who received arsenic trioxide may not require intrathecal treatment (68). These outcomes were noninferior compared to the historical comparator group treated with a combination of ATRA/ATO and chemotherapy (73). The advantages of the AAML1331 regimen compared to AAML0631 included shorter treatment duration, lower exposure to anthracycline and intrathecal chemotherapy, and fewer days of hospitalization (68). Two trials are currently ongoing assessing the application of totally chemo-free treatment protocols for newly diagnosed pediatric APL. The ICC APL 02 international trial (NCT04793919) applies an induction phase with ATRA/ATO alone for both SR and HR patients and a consolidation composed of four 28-day long ATO and seven 14-day long ATRA courses. For HR patients only, two doses of GO at 3 mg/m^2 are administered on days 2 and 4 of induction therapy. A chemo-free treatment protocol is under investigation as a Phase II study at MD Anderson (NCT01409161) as well. Patients, regardless of the risk group, receive as induction ATRA/ATO, and only high-risk patients receive GO once at weeks 1 and 4 at 9 mg/m^2. Patients achieving CR receive as consolidation ATO during weeks 1-4, 9-12, 17-20, and 25-28 and ATRA for 2 weeks on and 2 weeks off for a total of 4 courses. This intermittent schedule ensures the efficacy of ATRA limiting the increasing drug elimination typical of continuous administration and lowering the risk of neurotoxicity.

Conclusions

Treatment for APL is nowadays considered a prototypal model of precision medicine. As for other subtypes of pediatric acute leukemias (79), APL, in the past a highly fatal disease especially in the first few days after diagnosis, slowly became highly curable. This success is due to the development and application to the patient bedside of translational research and well-designed clinical trials. Translation of adult data to the pediatric population has been successful in pediatric APL; however, some differences persist with particular consideration to the long-term sequelae. Pediatric patients strongly benefit from chemo-free protocols considering their longer life-expectancy. Despite the impressive 2-year outcome reported in the CCLG-APL2016 and the COG AAML1331 trials for chemo-free treatment of SR patients, longer follow-up data are needed. Definitive data are also needed for the use of GO in the treatment of APL, with a particular focus on systemic short- and long-term toxicities. Moreover, the implementation of new drugs, such as the oral formulation of ATO, will further improve the quality of life of patients with APL during treatment. The ongoing pediatric ATRA/ATO trials, the ICC APL 02 and the MD Anderson study employing only ATRA/ATO protocols with the addition of GO in the HR group will provide important information regarding the effectiveness of chemo-free protocols even for HR patients and the real incidence of long-term sequelae.

Author contributions

RM designed the review. EM, DL and FB wrote the original draft. EM and FB designed the table. DL designed the figures APe, APr, FL and RM critically reviewed the manuscript. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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