ACUTE PROMYELOCYTIC LEUKEMIA - TOWARDS A CHEMOTHERAPY-FREE APPROACH TO CURE IN ALL PATIENTS

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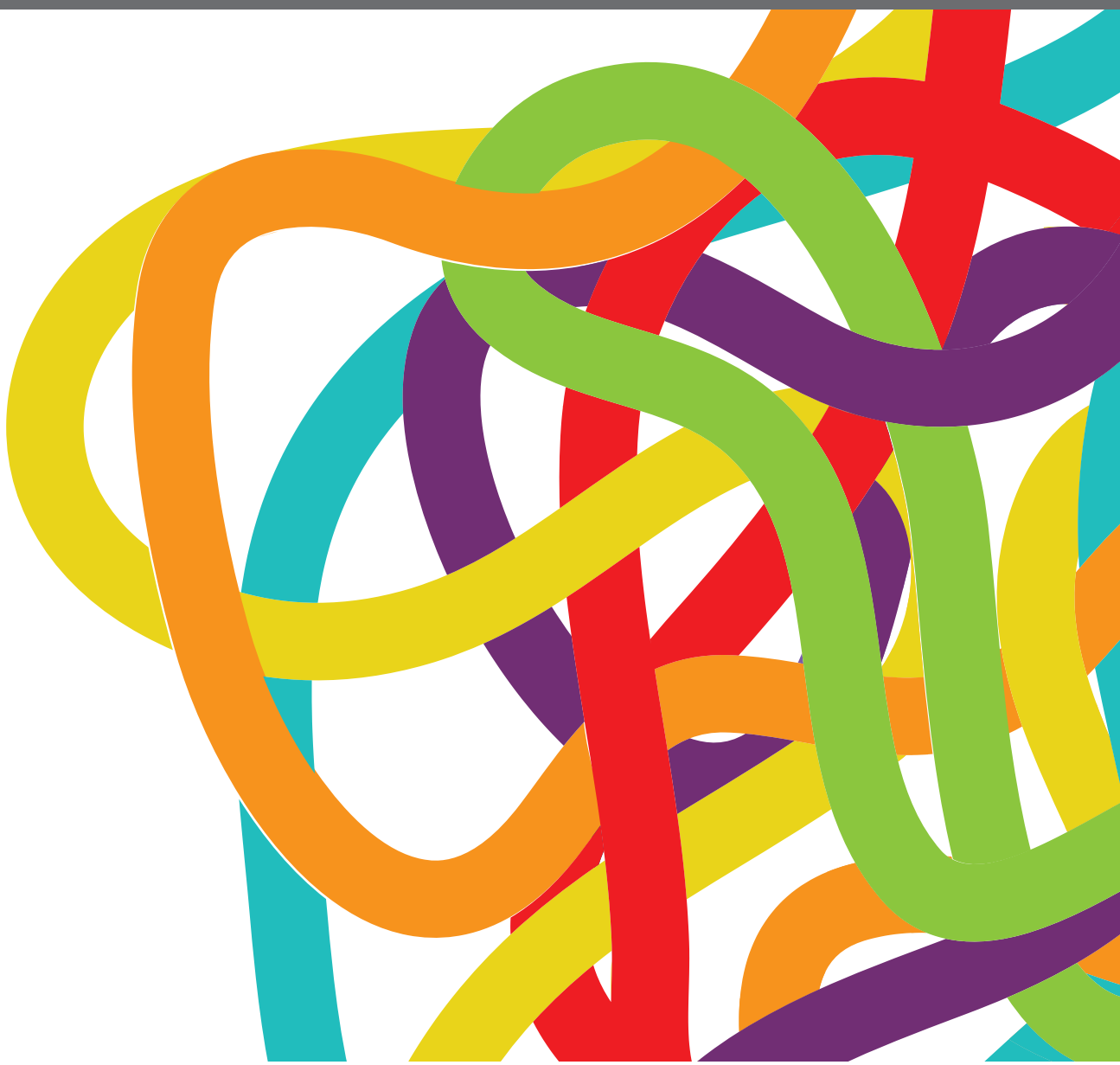

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ACUTE PROMYELOCYTIC LEUKEMIA - TOWARDS A CHEMOTHERAPY-FREE APPROACH TO CURE IN ALL PATIENTS

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EDITORIAL
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Editorial: Acute Promyelocytic Leukemia — Towards A Chemotherapy-Free Approach to Cure in All Patients

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Keywords: acute promyelocytic leukemia, arsenic trioxide, chemotherapy-free, tetraarsenic tetrasulfide, oral arsenic trioxide

Editorial on the Research Topic

Acute Promyelocytic Leukemia - Towards a Chemotherapy-Free Approach to Cure in All Patients

Acute promyelocytic leukemia (APL) is a subtype of acute myeloid leukemia (AML) characterized by t(15;17)(q24;21) and the fusion gene *PML-RARA*. With optimal supportive care and frontline use of all-trans retinoic acid (ATRA) and chemotherapy, first complete remission (CR1) rates of more than 90% and long-term survival of more 80% can be achieved. Regimens that include As_2O_3, ATRA and chemotherapy result in a CR rare of 90-100% and OS between 86-97% (1–3). Frontline treatment of APL has evolved rapidly. An emerging theme is the incorporation of As_2O_3 early in the treatment algorithm, starting from induction to consolidation.

Various forms of arsenic were used in China for over 5000 years. Arsenic first appeared in Western Medicine in the eighteenth century. In hematology, oral arsenic was first reported in the treatment of chronic myeloid leukemia from the 1860s to 1920s in Germany and Boston City (4). This treatment was phased out following World War II with the development of alkylating chemotherapy and radiotherapy. Intravenous pure As_2O_3 solution was first used in Harbin, China in 1973. Its mechanism of action, pharmacokinetics and clinical efficacy was extensively published in 1996. In this Research Topic collection, Kumana et al. gave a historical account on the development of pure oral arsenic trioxide that was invented and patented in Hong Kong. With memories of the Fowler's solution, an oral As_2O_3 formulation or the "modern" liquor arsenicalis was revived in 1998 in Hong Kong. In Hong Kong, China, an oral preparation of As_2O_3 (oral-As_2O_3) was developed, and was shown to be efficacious for APL in first relapse (R1), inducing second complete remission (CR2) in more than 90% of patients (5). Further, in an effort to prevent relapses, oral-As_2O_3 was used during induction and maintenance of CR1 (6, 7). This strategy resulted in favorable overall-survival (OS) and leukemia-free-survival (LFS). This 1mg/ml oral-As_2O_3 solution has a bioavailability comparable with that of i.v. As_2O_3 (8). Oral arsenic trioxide (Arsenol ®) from Hong Kong was also the first oral preparation of pure arsenic trioxide produced under the Good Manufacturing Practice (GMP) standards. On the other hand, Realgar-Indigo naturalis formula (RIF) was developed in the 1980s entirely based on traditional Chinese Medicine (TCM) concepts and comprises realgar, *Indigo naturalis*, *Salvia miltiiorrhiza* and *Radix pseudostellariae* (9). Tetraarsenic tetrasulphide (As_4S_4), indirubin and tanshinone IIA are the

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active ingredients of RIF with *in-vitro* synergism (9). In this Research Topic, Zhu and Lou et al. described the all oral, chemotherapy-free model in the frontline management of APL highlighting the applications of RIF and summarized the clinical data and excellent outcome of patients treated with RIF-based induction. Zhu also highlighted a major obstacle to cure in APL, that is, early deaths. In addition, multi-centre clinical trials in patients treated with ATRA, arsenic trioxide and anthracyclines reported a relatively low incidence of early deaths of 3-10% (1, 2). Xu and Huang discuss the evolution of various therapeutic approaches from hospital-based induction and consolidation to home-based oral As_2O_3-based therapy especially during consolidation and maintenance. Other oral formulations of As_2O_3 are also being investigated with the hope of eventually developing an all oral, effective regimen in standard-risk APL (10).

With regard to the therapeutic application of intravenous arsenic trioxide, Russell and Dillon summarize the United Kingdom's NCRI AML17 experience using the attenuated arsenic trioxide regimen in newly diagnosed APL. The treatment of "high-risk" APL remains a topic of contention as As_2O_3 is not yet approved for this indication. An important exploratory study by the MD Anderson Cancer Center using ATRA-As_2O_3 with or without gemtuzumab ozogamacin (GO) suggested that an essentially chemotherapy-free regimen might be feasible for the upfront treatment of APL (11). The NCRI AML17 trial built on these findings to investigate the de-intensification of treatment by randomizing patients irrespective of their risk status between As_2O_3-ATRA and the ATRA-idarubicin (AIDA) regimen. The AML17 trial included a total of 57 high-risk patients and their overall survival at 4 years was not significantly different from standard risk patients, being 95% in standard-risk compared with 87% in high-risk patients. Of the 28 high-risk patients in the As_2O_3 arm of AML17 who received the planned induction of ATRA, As_2O_3 and GO, the 4-year survival was 89% (12).

Last, but not least, Sanz et al. reappraised the role of hematopoietic stem cell transplantation (HSCT) in the current era of arsenic trioxide. As_2O_3-based regimens are currently regarded as the first option for relapsed APL. The selection of the most appropriate post-remission treatment option for patients in second CR (CR2), including the use of HSCT, depends on several variables, such as pre-transplant molecular status, duration of first remission, age, and donor availability. Despite modest evidence from retrospective studies, autologous HSCT has been the preferred option for consolidation in patients achieving molecularly negative CR2. The suitability of HSCT as compared with other non-HSCT alternatives has recently engendered much interest, thereby necessitating prospective controlled studies to define the role of HSCT in APL.

With the above backdrop, this special Research Topic provides an overview on the biology of APL and highlights perspectives on the past, present, and future treatment approaches in APL. This topic plays an important role in addressing the development of As_2O_3 –based therapy in improving the outcome of APL, from once the most fatal to currently the most curable leukemia.

AUTHOR CONTRIBUTIONS

HG wrote and approved the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The University of Hong Kong currently holds two United States (US) patents (7,521,071 B2 and 8,906,422 B2), one Japanese patent (4786341) and one European patent (EP 1562616 B1) for the use of oral arsenic

trioxide in the treatment of leukemias and lymphomas. HG is employed by the University of Hong Kong.

The remaining 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.

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MINI REVIEW
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Resurrection of Oral Arsenic Trioxide for Treating Acute Promyelocytic Leukaemia: A Historical Account From Bedside to Bench to Bedside

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Various forms of arsenic were used in China and elsewhere for over 5,000 years. Following the initial success of intravenous arsenic trioxide (i.v. As_2O_3), we revived an oral formulation of pure As_2O_3 in 1998 for the treatment of acute promyelocytic leukemia (APL). We were the first to produce a 1 mg/ml oral-As_2O_3 solution and showed that it had comparable bioavailability to i.v. As_2O_3. Moreover, we also reported that intracellular arsenic concentrations were considerably higher than the corresponding plasma values. Our oral-As_2O_3 was patented internationally and registered in Hong Kong for the treatment of APL. Safety, tolerability and clinical efficacy was confirmed in long-term follow-up studies. We have extended the use of oral-As_2O_3 to frontline induction of newly diagnosed APL. With these findings, we are moving toward an era of completely oral and chemotherapy-free management of APL.

Keywords: oral arsenic trioxide, acute promyelocitic leukaemia, history, pharmacokinetics, clinical applications

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INTRODUCTION AND INITIAL OBSERVATIONS

Over many centuries and even millennia, numerous accounts have attested that imbibing arsenicals was a powerful means of poisoning as well as a purported remedy for treating many diseases (1–4). Arsenic first appeared in Western Medicine in the eighteenth century. It was first patented in 1771 by Thomas Wilson for the treatment of malaria and agues. Thomas Fowler from Edinburgh subsequently produced a 1% solution of potassium arsenite, known as "Fowler's solution" (1) From the 1830s to the 1930s, oral arsenic was predominantly used for the management of syphilis, parasitic infestations, chronic skin conditions, and asthma (4). In Hematology, oral arsenic was first reported in the treatment of chronic myeloid leukemia from the 1860s to 1920s in Germany and Boston (1, 4). This practice was phased out following World War II with the development of alkylating chemotherapy and radiotherapy. Oral Fowler's solution, known as "liquor arsenicalis" was produced in Queen Mary Hospital, Hong Kong until the mid-1950s when its use as an anti-leukemic agent was replaced by chemotherapy and radiotherapy (1).

Pure intravenous pure As_2O_3 solution was first used in Harbin, China in 1973. Data on the mechanism, pharmacokinetics, and clinical efficacy were extensively published in 1996. Similar treatment results were confirmed around the world (5–7). Moreover, as the Chinese had described using intravenous (i.v.) treatment, the Food and Drug Administration (FDA) in the US agreed to license an American company to produce an i.v. formulation of As_2O_3 (Trisenox®) for treating APL. Treatment with Trisenox® was inconvenient, cumbersome and prohibitively expensive. Depending on the source, current monthly costs of i.v.-As_2O_3-based regimens typically used

during induction or re-induction of APL may amount to ∼10,000–11,000 U.S. dollars, though more affordable generic formulations are increasingly available (8, 9). Moreover, besides the burdensome quality of life impairments and medication costs of such recurrent i.v. treatment, patients inevitably incurred additional expenses. The latter would be to cover the costs of hospital admissions or day-care attendances, medical and nursing staff, i.v. infusion equipment and fluids, as well as to deal with infusion site-related complications. In addition, the patients would incur necessary travel expenses and loss of earnings due to absence from work.

With memories of Fowler's solution, we revived oral-As_2O_3 or the "modern" liquor arsenicalis in 1998 as a means of treating APL patients. This stemmed from two sets of key historical observations. Both of them could be regarded as bedside experiences and inferences that led to laboratory testing and bench-side work, the fruits of which were eventually passed on to patients at the bedside:

1. Meticulously chronicled medical records of Hong Kong CML patients cared for in the 1950s, consistently detailed objective benefits after treatment with Fowler's solution. Accordingly, researchers set out to reinvestigate a possible role for oral-As_2O_3 as part of the modern management of APL patients. Treating patients with an oral As_2O_3 formulation manufactured in accordance with Good Manufacturing Practice (GMP) could therefore have the potential to confer important benefits with a degree of confidence and safety that was never attained by Fowler's solution.
2. Meanwhile, important bedside observations and correlations arose from advances in molecular genetics, *in-vitro* studies describing As_2O_3 induced apoptosis and differentiation of APL cells, and an understanding of chromosomal mutations that accompany aging and disease (10, 11). Notably, APL is almost always associated with the specific chromosomal translocations, *t*(15;17)(q24;q21), and there was mounting evidence that their presence identified patients who respond much more favorably to treatments based on arsenic and all-*trans*-retinoic acid (ATRA) than to conventional treatment (12, 13).

In contrast to using the i.v. route, treatment with a safe and reliable oral-As_2O_3 formulation whose production conformed to GMP standards, obviously had the potential to vastly improve quality of life and treatment affordability for APL patients. At the same time, it could also harness the newly realized benefits of avoiding conventional chemotherapy. Another important advantageous distinguishing feature of oral as opposed to i.v. dosing was that it appeared to be less cardiotoxic. Potentially and actually fatal cardiac arrhythmias associated with excessive electrocardiographic QTc interval prolongation were a recognized feature of parenteral treatment (14–16).

REDEVELOPING AN ORAL FORMULATION OF ARSENIC TRIOXIDE IN HONG KONG

The primary objective of this initiative was to determine whether the systemic bioavailability of an in-house locally developed oral As_2O_3 formulation would be comparable to that following commercially available i.v. dosing, when administered to patients with relapsed/refractory APL or acute myeloid leukemia. A secondary objective was to ascertain the extent of arsenic accumulation in the non-cellular and cellular components of blood. However, redeveloping such an oral formulation and determining its systemic bioavailability whilst ensuring acceptability for clinical use in very sick patients posed a number of significant challenges. These challenges and how they were addressed are listed:

1. In the absence of any readily available commercially produced pharmaceutical grade As_2O_3 powder, a good quality substitute was eventually sourced from Sigma (U.S.A.).
2. Being a sparingly soluble powder, a suspension was prepared in sterile water and subjected to manipulation of its pH to produce a clear colorless solution with a pH 7.2 that contained 1 mg/ml of As_2O_3. Contrary to recommendations in available pharmacopeias, fungicide was not added as the entire preparation process was conducted in a pharmaceutical isolator. Samples subsequently submitted to microbiology and chemical testing yielded no fungi and the solute concentration remained unchanged; its shelf-life exceeded 6 months and very likely extended to more than 1 year.
3. As a means of investigating As_2O_3 bioavailability in fairly sick hospitalized patients, the study protocol was necessarily unconventional and did not entail tolerability testing, randomization, a crossover design, or any form of blinding. In all, 9 patients (aged 17–67 years; mean weight of 58 Kg; 6 men and 3 women) were recruited using predefined inclusion/exclusion criteria and asked to refrain from seafood (an arsenic source) in the preceding week. None of them had antecedent renal or liver function test abnormalities. Each patient received a 10 mg i.v. infusion of As_2O_3 over 1 h on day 1, followed by a 10 mg oral dose 24 h later. Venous blood samples were drawn from each patient, just before initiating i.v. dosing and at predefined times over the next 48 h. To maximize retrieval of potentially useful data from each sample, aliquots of whole blood and freshly separated plasma were stored and subsequently analyzed in batches using well-established methods.
4. Institutional Ethics Committee approval of the proposed unconventional bioavailability study protocol was achieved after drawing attention to several compelling issues. First, all the patients would have relapsed or refractory disease that was an indication for i.v. As_2O_3 treatment and second, they would have to give written informed consent. The committee was also informed that recruiting healthy volunteers to take oral and i.v. arsenic to conduct a formal bioavailability study would prove daunting and nor could it reveal how the oral-As_2O_3 would be tolerated by diseased patients.
5. Not surprisingly, publication of the study findings based on such an unconventional protocol was treated with skepticism by many journals. One journal editor however, appreciated the special circumstances constraining administration of arsenic to very sick patients and saw fit to allow publication of the study findings, despite advice to the contrary from some reviewers.

ORAL ARSENIC TRIOXIDE BIOAVAILABILITY DETERMINATION AND FINDINGS

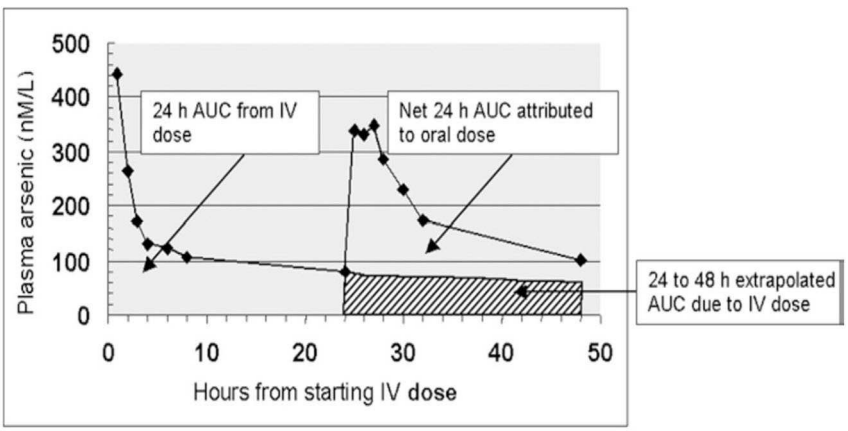
For each patient, systemic bioavailability following i.v. and oral treatment involved comparison of corresponding area under the curve (AUC) for arsenic concentration vs. time plots attributable to each form of dosing (17). The relevant AUCs were derived using standard computer software incorporating the trapezoidal rule.

Figure 1 shows plasma arsenic concentrations (in excess of basal levels) prevailing between 0 and 48 h in one representative patient (17). It illustrates how i.v. and oral bioavailability (AUC over the first 24 h following each dose) was inferred, assuming first order terminal elimination of arsenic. The net attributable 0–24 h AUC after oral dosing was taken to be the difference between the gross 24–48 h AUC and the extrapolated 24–48 h AUC attributed to i.v. dosing. In the same way, plasma and whole blood arsenic concentration AUCs were computed for all 9 patients, and the ensuing mean ± standard error of mean (SEM) results and 95% confidence intervals (C.I.) were calculated.

CLINICAL DEVELOPMENT

These pharmacokinetic studies indicated that, first, the oral formulation and i.v. dosing achieved comparable systemic bioavailability, and secondly, arsenic concentrations in the cellular component of blood were considerably higher than in plasma. Since then, the main finding of this study, namely that orally administered (though differently formulated) As_2O_3 attains virtually the same systemic bioavailability as i.v. dosing was confirmed by others (18, 19). The latter researchers also pointed out that compared to i.v. dosing, oral dosing was well-tolerated, more convenient, and equally safe. The observation that arsenic attains higher concentrations in the cellular components of blood than in plasma has also been reported (20). Furthermore, due to arsenic existing as III and V forms, its speciation, pharmacokinetics, and metabolic profiling is complex and confusing (10, 19–22). Yet, based on the latter available pharmacokinetic findings, there are reasonable grounds for accepting that the terminal phase of arsenic elimination from plasma approximates to a first order process, and supports extrapolation of 24–48 h AUCs attributable to i.v. dosing. As indicated by the CIs detailed in **Figure 1B**, there was substantial inter-patient variation in both plasma and whole blood AUCs. Though not tabulated for individual patients, up to ∼5 and 10-fold variations, respectively, were encountered between individuals, whilst inter-day variation was much less marked. Interestingly, since considerable inter-patient plasma level/AUC variations have also been encountered after i.v. dosing (21, 22), differences in arsenic absorption capacity from the gut is unlikely to be the main explanation. Thus, conceivable reasons for such variations include saturable tissue binding of As_2O_3, dietary indiscretions by individual patients, and inherent disease state/physiological differences. It is nevertheless evident that repeated courses of oral As_2O_3

A



B

		Day 1 (0-24 h) AUC attributed to IV Dose	Day 2 (24-48 h) AUC attributed to Oral Dose
Plasma:	Mean ± SEM	2673 ± 262	2640 ± 343
	95% CI	1839-3507	1850-3430
Whole Blood:	Mean ± SEM	3702 ± 483	
	95% CI	2587-4816	#

#Calculation of valid mean, SEM, and 95% CI values for all 9 patients was not feasible, as one had received emergency packed cell and platelet transfusions

FIGURE 1 | (A) Area under the curve (AUC) of arsenic levels attributed to intravenous (i.v.) and oral dosing with arsenic trioxide in a single patient; **(B)** Area Under the Curve (AUC) of Arsenic Concentrations (nanomolar-hours) [adapted from Kumana et al. (17) with permission].

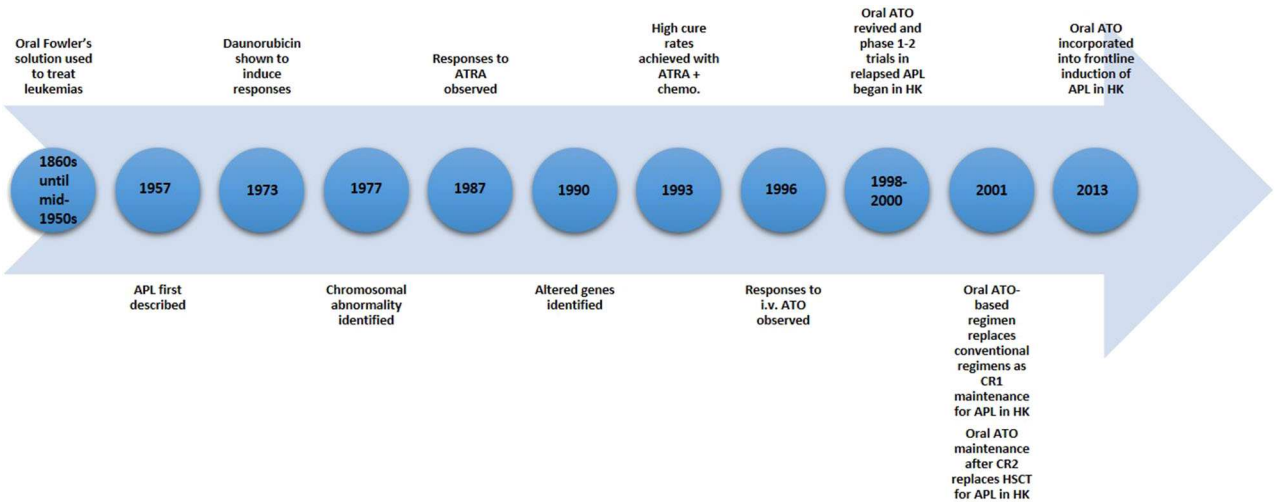


FIGURE 2 | Timeline of advances in acute promyelocytic leukaemia (APL) treatment and the development of oral arsenic trioxide (ATO) in Hong Kong. ATRA, all-*trans*-retinoic acid; chemo., chemotherapy; HK, Hong Kong; i.v., intravenous; CR1, first completeremission; CR2, second complete remission; HSCT, hematopoietic stem cell transplantation.

with these types of therapeutic doses are safe (18, 19, 23). The optimal dose of oral-As_2O_3 we suggest is 10 mg (0.15-0.2 mg/kg) per day in adult patients (≥50 kg) with normal renal function. In adults weighing less than 50 kg and in pediatric patients, oral-As_2O_3 solutions at 0.15 mg/kg are advised. With plasma and intracellular arsenic level monitoring, we were also able to administer oral-As_2O_3 safely at a lower doses for patients with end-stage renal failure or patients dialysis (24, 25).

Interestingly, standard i.v.-As_2O_3 dosing regimens have been repeatedly incriminated as a cause of cardiac arrhythmias and sudden death, possibly associated with electrocardiographic QTc interval prolongation induced by arsenic (14–16). The latter phenomena have been linked to excessive levels of plasma arsenic. Oral dosing seems to mitigate this cardiac risk, possibly because arsenic's entry into the circulation from the gut is much more gradual and the peak concentrations attained are consequently much lower (26). Based on having demonstrated this particular likely pharmacokinetic advantage of the aforementioned oral-As_2O_3 formulation, in 2009 its inventors were granted a U.S. patent for treating APL patients. Thereafter, patents for this formulation were also granted by the European Union, China, and Japan. Nevertheless, it should be noted that there has been a single case report of such cardiac arrhythmias occurring transiently after oral therapy of a patient with known chemotherapy-induced dilated cardiomylopathy (27).

A plethora of well-conducted phase 2 studies followed. Our group demonstrated excellent long-term outcomes in APL patients treated with oral-As_2O_3-based regimens (28–31). In a 15-year prospective follow-up study in 73 patients with relapsed APL, idarubicin (6 mg/m^2/day for 5 days) plus oral-As_2O_3 (10 mg/day), all-trans-retinoic acid (45 mg/m^2/day) and ascorbic acid (1 g/day) (AAA) for 42 days resulted in a 100% molecular remission rate (28). Ascorbic acid was used in the AAA regimen due to its synergism with As_2O_3 which has been shown *in-vitro* and clinically (30, 32, 33). Following second complete remission (CR2), 2 monthly cycles of idarubicin (6 mg/m^2/day for 3 days) plus AAA for 7 days followed by 12 cycles of AAA maintenance (given for 2 weeks every 2 months for 2 years) resulted in 5-year and 10-year overall survival (OS) of 79.5 and 67.3%, respectively (28). Importantly, this was achieved without hematopoietic stem cell transplantation (HSCT) in CR2. This shows that prolonged AAA maintenance is an effective post-remission strategy following CR2, obviating the need for HSCT, a procedure still considered a standard for managing such patients in many places around the world. We then moved AAA forward as post-remission maintenance following CR1, which resulted in a 5-year leukemia-free survival (LFS) and OS of 90% and 97%, respectively (29–31). Most recently, we incorporated AAA (given for 42 days) into frontline induction for newly diagnosed APL with daunorubicin (50 mg/m^2/day for 3 days) followed by 2 cycles of consolidation with daunorubicin (50 mg/m^2/day for 2 days) and cytarabine (100 mg/m^2/day for 5 days) and 2 years of AAA maintenance. Both LFS and OS were 100% at 5 years (29). In patients aged 70 or above or those with medical co-morbidities, chemotherapy was omitted and patients were treated with an entirely oral regimen comprising 42 days of AAA and no relapses have been observed so far (29). With AAA-based regimens, outcome for both newly diagnosed and relapsed APL were independent of the conventional risk scores. With LFS plateauing 2 years after completion of maintenance both in CR1 or CR2, long-term molecular monitoring is not necessary 2 years beyond completion of AAA maintenance following CR1 or CR2 (28, 29). Even in conventional high-risk patients, our strategy of incorporating oral-As_2O_3 to frontline induction achieved excellent long-term outcomes similar to those achieved in low-risk patients. It remains to be seen whether maintenance is

necessary in low-risk patients treated with frontline oral-As_2O_3-based induction. We are currently testing frontline induction with AAA in APL (ClinicalTrials.gov Identifier: NCT03624270) in a risk-adapted manner incorporating a chemotherapy-free approach.

In our oral-As_2O_3 studies, both short-term and long-term cardiac safety was confirmed. QTc prolongation, ventricular arrhythmias and cardiac failure were not observed. QTc prolongation occurred in 16% of patients given i.v. As_2O_3 which was significantly higher than that observed with our regimen (12, 34, 35). We have demonstrated lower peak plasma arsenic levels with oral-As_2O_3 dosing that probably accounted for the cardiac safety. Drug-induced transaminitis (Grade 1–2: 31%; Grade 3–4: 26%) were all reversible with transient dose reductions or interupptions (29). Upon normalization of liver enzymes, all patients were able to tolerate oral-As_2O_3 at 10 mg/day without recurrence of transaminitis. Our regimen of AAA showed similar or lower rates of hepatoxicity than with i.v. As_2O_3-ATRA treatment, the latter being associated with transaminitis that occurred in 44–71% of patients (12, 34, 35). Acyclovir prophylaxis is used universally in all patients on oral-As_2O_3 due to the risk of herpes zoster that was demonstrated in our earlier studies (36). Differentiation syndrome (DS) occurred in 26 and 12% in patients with newly diagnosed APL and APL in first relapse (R1), respectively, whilst no induction deaths were observed. With early recognition, cytoreduction and the use of dexemathasone, interruption of oral-As_2O_3 or ATRA was not necessary in our studies. Other common side-effects of the AAA regimen included headache (Grade 1–2: 32%) and upper gastrointestinal upset (Grade 1–2: 11%) most of which were either self-limiting or controlled with simple analgesics and antacids.

In our studies involving relapsed APL patients receiving oral-As_2O_3, as with i.v.-As_2O_3 treatment – there was a high risk of central nervous system disease in those with severe relapses (28, 37). We also showed that after an oral administration, meaningful cerebrospinal fluid (CSF) levels of arsenic were achieved implying its benefit in the prophylaxis or treatment of central nervous system (CNS) disease (38, 39). CSF and plasma arsenic levels were linearly correlated with CSF arsenic levels at about 18% of the levels in plasma levels (38). Frontline use in newly diagnosed APL may ameliorate the risk. Since 2013, none of our patients treated with frontline oral-As_2O_3 had evidence of CNS relapse (29). There is limited data on CSF arsenic levels in patients treated with i.v. As_2O_3. The concurrent use of intravenous mannitol may significantly increase CSF arsenic levels comparable to those in blood, thus providing the prospect of managing CNS APL effectively (40).

OTHER ORAL ARSENIC PREPARATIONS

Realgar-Indigo naturalis formula (RIF) was developed in the 1980s entirely based on traditional Chinese Medicine (TCM) concepts and comprises realgar, *Indigo naturalis*, *Salvia miltiiorrhiza* and *Radix psudostellariae* (40) and was launched in Mainland China in 2009. Tetraarsenic tetrasulphide (As_4S_4), indirubin and tanshinone IIA are the active ingredients of RIF, which show in-*vitro* synergism (40, 41). RIF showed comparable efficacy to i.v. As_2O_3 (42, 43), together with improved quality of life and reduced costs. Hepatotoxicity is frequently reported and occurs in about 58–65% (Grade 1–2: 49–55%; Grade 3–4: 9–10%) of patients treated with RIF plus ATRA (42, 43). Another common toxicity of RIF is diarrhoea which occurs in about 15% of patients. Prolonged QTc intervals are uncommon with RIF at dosages of 60 mg/kg/day but occurs in about 24% of patients given a high dose of 7.5 g/day (44). Clinically significant arrhythmias are nevertheless rare. Groups in Australia (ANZCTR registration number: ACTRN12616001022459 and the United States (ClinicalTrials.gov Identifier: NCT03048344) have developed novel formulations of oral As_2O_3. For instance, ORH-2014 was recently shown to be safe in patients with leukaemia and had comparable bioavailability to i.v. As_2O_3 (19).

CONCLUSION

The history of APL and development of arsenic trioxide has formed a paradigm for targeted therapy in cancer (**Figure 2**). There is every reason to believe that the form of oral treatment described above can offer therapeutic benefits equivalent to dosing with i.v. As_2O_3, with the added advantages of far superior convenience (enabling injection free home treatment with outpatient supervision), greater affordability, and a lower risk of cardiac incidents. Moreover, though reverting to oral treatment can be considered as only a small incremental step in the overall context of treating haematogical malignancies such as APL, for individual patients it can nevertheless be regarded as an important and life-changing advance. A commercially available oral-As_2O_3 in the not-too-distant future will pave the way for this inexpensive and convenient form of As_2O_3 to be available worldwide.

AUTHOR CONTRIBUTIONS

CK, Y-LK, and HG: conception, manuscript writing, and final approval of manuscript. RM: manuscript writing and final approval of manuscript. All authors: contributed to the article and approved the submitted version.

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Conflict of Interest: The University of Hong Kong currently holds two United States (US) patents (7,521,071 B2 and 8,906,422 B2), one Japanese patent (4786341) and one European patent (EP 1562616 B1) for the use of oral-As_2O_3 in the treatment of leukemias and lymphomas. HG, CK, and Y-LK are employed by or associated with the University of Hong Kong.

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Therapeutic Approaches for Acute Promyelocytic Leukaemia: Moving Towards an Orally Chemotherapy-Free Era

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The treatment of acute promyelocytic leukaemia (APL) has evolved dramatically over the past several decades, making the disease a highly curable form of acute leukaemia. The discoveries of all-*trans* retinoic acid (ATRA) and arsenic trioxide (ATO) were landmark events, leading to historic revolutions in the treatment of APL. One major change was from chemotherapy-based to chemotherapy-free treatment regimens, and the combination of ATRA plus ATO without chemotherapy has been recommended as the standard therapy for non-high-risk APL. The other major change was from the intravenous administration of medicine in the hospital to a largely home-based oral approach, which is a more cost-effective and convenient treatment model. In this review, we focus on the evolution of therapeutic approaches for APL, as well as the challenges that remain with the current approaches.

Keywords: acute promyelocytic leukaemia, oral, chemotherapy-free, all-*trans* retinoic acid, Realgar-Indigo naturalis formula

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INTRODUCTION

Acute promyelocytic leukaemia (APL) was associated with a severe bleeding tendency and an extremely poor prognosis in history (1). In the last thirty years, the therapeutic outcomes of APL have markedly improved. The combination of all-*trans* retinoic acid (ATRA) and arsenic trioxide (ATO) is reported to result in complete remission (CR) rates exceeding 90% and overall survival (OS) rates of 85–99% (2–8).

In the evolution of therapeutic approaches for APL, the first historical milestone was chemotherapy based on anthracyclines (9). The use of daunorubicin in induction therapy improved CR rate from 13 to 55% in APL (9). However, the median duration of remission remained poor, ranging from 11 to 29 months (10, 11). Subsequently, the first therapeutic results of ATRA and ATO were published in 1988–1996 (12, 13). Besides, various oral arsenic formulations were also introducted (14). These represent the three landmarks that contributed to two revolutions in the treatment of APL: from chemotherapy-based to chemotherapy-free regimens and from the intravenous administration of medicine in a hospital to a largely home-based oral approach. These evolutions have made the treatment of APL more efficient, convenient and affordable. Here, we

review the significant changes in treatment approaches for APL, as well as the challenges that remain with the current approaches.

THE FIRST REVOLUTION: FROM CHEMOTHERAPY-BASED TO CHEMOTHERAPY-FREE REGIMENS

The adoption of ATRA and ATO as treatments constituted a landmark in the development of targeted therapy for APL. In the 1980s, leukaemic promyelocytes were found to possess the unique capability to undergo differentiation when exposed to ATRA (12). Later, ATO was found to be capable of eradicating APL-initiating cells, resulting in a curative effect (15).

Improved Remission Rates and Survival Outcomes With the Combination of ATRA/ATO

In the pre-ATRA period, anthracycline-based chemotherapy regimen with or without cytosine arabinoside (Ara-C) was applied in newly diagnosed APL patients. With anthracycline alone, complete remissions were in about 50–70% of patients and median duration of remission was 6.5–26 months (9, 16). With anthracycline plus Ara-C regimen, CR rates ranged from 68 to 72% (17, 18). In the chemotherapy alone era, only 35–45% of patients achieved a cure with anthracycline-based consolidation therapy (19).

When ATRA was introduced as an APL differentiation therapy, the treatment paradigm evolved to ATRA-based regimens (combined ATRA and chemotherapy). The ATRA-based regimens in the induction phase produced improved CR rates over 90% (20–22). After obtaining CR, consolidation therapy composed of chemotherapy with or without ATRA was administered, and the long-term disease-free survival (DFS) with this treatment paradigm was estimated to range from 68.5 to 85.6% (20–23).

The introduction of the ATRA/ATO combination as a synergistic therapy created new possibilities for the treatment of APL. In the initial attempt, combined ATRA/ATO for the induction of remission followed by consolidation chemotherapy resulted in a CR rate of 95.2% and a DFS rate of 100% with a median follow-up of 18 months (24). In subsequent studies, the combination of ATRA and ATO was applied as both induction and consolidation therapy, which resulted in CR rates of 89%-92% and an OS rate of 85% (2, 3).

Optimal Place of Combined ATRA/ATO in Newly Diagnosed APL

The APML4 study combined ATRA with ATO and idarubicin in induction therapy and used ATRA-ATO as consolidation therapy in 124 patients with all-risk groups. The survival outcomes were inspiring, with 5-year OS of 94% and EFS of 90% (7). The AML17 trial included all risk groups for newly diagnosed APL and compared a chemotherapy-free ATRA-ATO treatment regimen with the standard chemotherapy-based regimen (ATRA and idarubicin). Burnett et al. showed that the CR rated were comparable between the two groups, but ATRA-ATO group was associated with a better 4-year EFS (91.0 vs. 70.0%, $P = 0.002$) (6).

In newly diagnosed non-high-risk APL patients, a randomized multi-centre trial conducted by Lo-Coco et al. in 2013 compared ATRA-ATO combination therapy with ATRA–idarubicin therapy for induction and consolidation therapy in newly diagnosed patients and showed similar CR rates (100 vs. 95%) but improved 2-year EFS in the former (97 vs. 86%) (5). In 2017, after a prolonged follow-up among the extended therapy patients, the EFS at 50 months for patients in the ATRA-ATO versus ATRA-chemotherapy arms were 97.3 vs. 80.0% (25). Based on the above results, the combined use of ATO and ATRA as a first-line treatment for non-high-risk APL has been adopted by the National Comprehensive Cancer Network (NCCN) guidelines (26) and the European Leukaemia Net (ELN) recommendations (27).

A chemotherapy-free protocol has also been studied in newly diagnosed high-risk APL patients. Researchers from the University of Texas MD Anderson Cancer Center reported a CR rate of 96% and a 5-year EFS of 81% in high-risk patients treated with ATO, ATRA plus gemtuzumab ozogamycin [GO] for induction and ATRA/ATO for consolidation therapy (8). More recently, the SWOG Cancer Research Network has applied the chemotherapy-free combination of ATRA, ATO and GO for induction therapy followed by chemotherapy included for consolidation treatment in high-risk patients. Overall, 86% of the patients achieved CR, and the 3-year EFS was 78%, with the largest sample size (N = 70) of high-risk patients (28). Currently, there is no definitely recommended treatment option for high-risk patients (26, 27).

Reduced Chemotherapy-Related Toxicity in the ATRA/ATO Period

The synergistic activity of combined ATRA and ATO has been indicated in the treatment of APL, thereby lessening the need for chemotherapy and reducing the risk of chemotherapy-related toxicities. Specifically, such a chemotherapy-free regimen is likely to reduce the risk of myelosuppression-related complications, cardiotoxicity associated with anthracycline exposure, and secondary malignancies associated with the use of ATRA plus traditional chemotherapy (29). In a previous study, 17% cases treated with ATRA plus idarubicin developed secondary malignancies (30).

Although ATRA plus ATO was associated with more frequent increases in liver enzymes and QTc prolongation, these effects were reversible and manageable with reduced doses or the temporary discontinuation of the drug (5). We should note that chronic arsenic exposure has potential risk of causing cancer, but there is no clinical evidence.

THE SECOND REVOLUTION: FROM INTRAVENOUS ADMINISTRATION TO AN ORAL APPROACH

Intravenous ATO is inconvenient, involves frequent hospitalization and requires the maintenance of vascular access. Thus, an oral arsenic drug has been anticipated for a long time; this oral approach could facilitate a largely home-based protocol and further promote the quality of life of APL patients.

In China, the study of oral arsenic formulations has a long history. There are three main inorganic arsenic forms, namely, red arsenic (tetra-arsenic tetra-sulfide, As_4S_4, which is isolated from realgar), yellow arsenic (As_2S_3, also known as orpiment), and white arsenic (arsenic trioxide, As_2O_3, which is made by burning realgar or orpiment) (31). Beginning in the 1970s, arsenic oxide (As_2O_3) was used to treat APL (1). In the decades that followed, different oral arsenic formulations have been tried, and excellent outcomes in APL have been reported (32, 33).

Realgar-Indigo naturalis formula (RIF) is a drug compound containing 30 mg of realgar, 125 mg of Indigo naturalis, 50 mg of Radix salviae miltiorrhizae, 45 mg of Radix pseudostellariae, and 20 mg of garment film in one pill. Based on its clinical results (34) and the anti-APL activity *in vitro* and *in vivo* (35), RIF has been approved by the Chinese FDA and has been commercialized and commonly available in China since 2009.

Comparable Efficacy Between RIF and Intravenous Arsenic Formulations in Newly Diagnosed APL

Chemotherapy Included Protocol in Consolidation Period

Huang's group led a multi-centre, randomized controlled phase III study (APL07) comparing oral RIF (60 mg/kg) and ATO (0.16 mg/kg) in newly diagnosed APL patients as both induction and maintenance therapies from November 2007 through September 2011 (36). All patients received three courses of consolidation chemotherapy and maintenance treatment with sequential ATRA followed by either RIF or ATO for two years. With a median follow-up of 39 months, the 2-year DFS was 98.1% in the RIF group and 95.5% in the ATO group. No significant difference was noted between the RIF and ATO groups with regard to the CR rate (99.1 vs. 97.2%; $P = 0.62$). In 2016, Huang's group updated the follow-up data and reported that the estimated 7-year EFS rates were similar between the RIF and ATO groups (93.7 vs. 89.4%, $P = 0.37$). In addition, the estimated 7-year incidences of relapse and EFS were also similar between the high-risk and non-high-risk groups (2.4 vs. 5.0%, $P = 0.55$; 91.2% vs. 91.5%, $P = 0.74$) (37).

In the treatment of paediatric APL, another randomized, multi-centre non-inferiority trial was conducted in China to determine whether intravenous ATO can be replaced by oral RIF (38). A total of 82 patients who were 16 years old or younger were randomly assigned to the ATO (n = 42) or RIF (n = 40) group. In this trial, patients received three courses of consolidation therapy containing ATRA, ATO and low-intensity chemotherapy with mitoxantrone; cytarabine was added for high-risk patients. The estimated 5-year EFS was 100% in both groups after a median 3-year follow-up.

RIF and ATRA Without Chemotherapy

Huang et al. conducted a single-centre pilot study to evaluate the efficacy of oral arsenic and ATRA without chemotherapy in newly diagnosed non-high-risk APL patients. A total of 20 consecutive patients were given oral arsenic RIF (60 mg/kg) and ATRA (25 mg/m^2) as induction therapy and then RIF on a schedule of 4 weeks on and 4 weeks off and ATRA on a schedule of 2 weeks on and 2 weeks off for 7 months as post-remission therapy (39). All patients achieved haematologic complete remission after a median time of 29.5 days. The rate of complete molecular remission was 65% at 3 months and 100% at 6 months (39). This preliminary result is encouraging and provides initial evidence for the success of a largely home-based treatment protocol for the treatment of non-high-risk APL.

Subsequently, these findings were confirmed by a multi-centre, non-inferiority, open-label, randomized, controlled phase 3 trial at 14 centres in China (40). In this trial, RIF (60 mg/kg/d) or arsenic trioxide (0.15 mg/kg/d) and ATRA (25 mg/m^2/d) were administered until CR was obtained. The consolidation therapy was RIF (60 mg/kg/d) or intravenous arsenic trioxide (0.15 mg/kg/d) in one 4 weeks on and 4 weeks off regimen for four cycles and ATRA (25 mg/m^2/d) in one 2 weeks on and 2 weeks off regimen for seven cycles. The estimated 2-year EFS and OS were 97 vs. 94% ($P = 0.49$) and 100 vs. 94% ($P = 0.049$), respectively. This study has suggested that non-high-risk APL can be cured using oral arsenic plus ATRA without conventional chemotherapy.

Huang's group has tried to extend the outpatient model to newly diagnosed high-risk APL patients (41). A total of 20 patients were included in a single-centre cohort study. All subjects received oral arsenic RIF (60 mg/kg/d) and ATRA (25 mg/m^2/d) as induction therapy until CR was achieved. Hydroxyurea (Hu, 3 g/d) or Hu (3 g/d) plus cytarabine (200 mg/d) was used to reduce the leukaemia burden if patients' WBC counts were (10–20) $\times$ 10^9/L or over 20 $\times$ 10^9/L before induction until the WBC count was lower than 10 $\times$ 10^9/L. The consolidation therapy was consistent with that in non-high-risk cases (39), including RIF in a 4 weeks on and 4 weeks off regimen for four cycles and ATRA in a 2 weeks on and 2 weeks off regimen for seven cycles. With a median follow-up of 33 months, 20 patients (100%) achieved a CR after a median time of 30 days. The 3-year estimated OS and EFS were 100% and 89.4%, respectively.

Evidence of Oral As_2O_3 Solution in Relapsed or Newly Diagnosed APL

More recently, the use of oral As_2O_3 solution in reinduction/maintenance regimens in relapsed APL, as a front-line treatment in newly diagnosed APL and in maintenance regimens after first CR have been reported by Gill's team with encouraging outcomes (42–44).

Since 2002, Gill et al. have conducted a prospective study among APL patients who have experienced their first relapse and used protocols that involve oral As_2O_3 reinduction followed by As_2O_3 maintenance. In detail, the reinduction therapy comprised oral As_2O_3, ATRA and ascorbic acid (AAA) on days 1–28 and idarubicin for 5 days. After achieving CR2, patients received consolidation therapy with idarubicin. After the completion of consolidation, maintenance therapy with the AAA regimen (2 weeks every 2 months for 2 years per protocol) was administered. Finally, all 73 patients obtained CR2 after oral As_2O_3-based reinduction and received oral As_2O_3 based maintenance. The 5-year and 10-year OS rates in the cohort were 79.5 and 67.3%, respectively (42). This cohort study showed that oral As_2O_3-based reinduction remained effective despite previous exposures, and it was an effective maintenance therapy for patients in CR2.

Subsequently, Gill's team brought oral As_2O_3 incorporation into front-line treatment in newly diagnosed APL patients. Sixty-two consecutive patients received the AAA regimen with additional daunorubicin in younger patients for induction therapy. In the comparator group, 37 subjects received similar consolidation and maintenance therapies but did not receive oral As_2O_3 for induction therapy. The CR rates were both 100%, and the 5-year OS rates were comparable (100 vs. 96.9%, $P = 0.21$) in the arsenic and non–arsenic induction subgroups. However, the 5-year DFS in the arsenic induction subgroup was significantly superior to that of the non–arsenic induction subgroup (100 vs. 90.5%, $P = 0.03$) (43).

Recently, the role of As_2O_3 in the maintenance of CR1 in APL has also been demonstrated. A total of 129 consecutive adult patients achieved CR1 with conventional induction and consolidation. Then, they underwent AAA maintenance for 2 years. At a median follow-up of 100 months, the 10-year DFS and OS were estimated to be 85 and 87%, respectively. This analysis indicated that AAA maintenance therapy is an effective choice for long-term survival in all risk categories of APL (44).

Reduced Cost, Shorter Hospitalization, and Improved Quality of Life With Oral Forms

Jiang et al. conducted a retrospective study and compared the medical costs and length of hospital stay between oral RIF plus ATRA and intravenous ATO plus ATRA as the first-line treatment in APL patients involved in the clinical trial APL07 at Peking University People's Hospital. The median total medical costs were significantly lower in the RIF group ($13,183.49) than in the ATO group ($24,136.98) ($P < 0.0001$). The median total length of hospital stay in the RIF group was also obviously shorter (48 days) than that in the ATO group (54 days) ($P < 0.0001$) (45). This was also consistent with the results in the paediatric cohort, with results of 67.8 vs. 43.9 days and 68.1 vs. 48.1 days for non-high-risk and high-risk patients in the ATO and RIF groups, respectively.

Oral arsenic formulations can be administered outside the hospital, which reduces the need for hospital visits, resulting in a superior quality of life than that associated with intravenous ATO (46). In our single-centre pilot study, patients received RIF plus ATRA as induction and post-remission treatment. They resumed their usual lifestyle during post-remission therapy and rated their quality of life as nearly normal on the FACT-G questionnaire (39). In the respective cohorts from Beijing, most patients in the RIF group could resume their work or study status with a relatively better quality of life during maintenance therapy after consolidation chemotherapy (45).

CHALLENGES AND UNRESOLVED ISSUES

First, there is concern that the combination of two differentiating drugs without chemotherapy might lead to an increased risk of leucocytosis and differentiation syndrome. It has been reported that 35%-47% of non-high-risk adults develop leucocytosis after chemotherapy-free induction therapy, while 24% develop leucocytosis after ATRA plus chemotherapy induction (5, 25, 39, 47). In paediatric APL patients, the incidence of leucocytosis is reported to be much higher, reaching more than 90% (48). In addition, different kinetics of WBC proliferation were observed during induction with oral arsenic plus ATRA and ATO plus ATRA. A higher WBC count was observed in the RIF group than in the ATO group after 10 days of treatment (9.22×10^9/L vs. 4.10×10^9/L, $P = 0.015$) (49). Currently, prompt supportive measures such as cytoreductive chemotherapy and prophylactic corticosteroids have been proven to be useful to minimize the impact of leucocytosis (47).

In addition, CNS relapse is uncommon in patients with APL treated with traditional ATRA plus chemotherapy regimens. However, there are no formal data reporting its incidence and suggesting the need for CNS prophylaxis in the chemotherapy-free era (27, 50). It is necessary to prolong the follow-up time to determine the incidence and to conduct a randomized study to identify the optimal prophylactic strategy.

Challenges remain regarding the therapeutic use of chemotherapy-free regimens for high-risk APL. As mentioned in the updated ELN guidelines, two potential options for high-risk patients are ATRA plus ATO with the addition of appropriate cytoreductive chemotherapy and conventional ATRA plus chemotherapy. The use of chemotherapy-free or minimal chemotherapy needs to be explored in more randomized trials to enable a comparison with conventional treatment in patients in separate high-risk categories.

In summary, APL used to have a high mortality rate; however, the majority of patients can be cured at present. The emergence of ATRA and arsenic represents the beginning of targeted therapy for APL, and the advent of oral arsenic formulations allows a largely home-based oral protocol. Both therapeutic revolutions have made the treatment of APL more efficient, convenient, and affordable. In the future, challenges regarding the appropriate sequence of chemotherapy-free regimens in high-risk patients and the long-term efficacy in the new era need to be addressed.

AUTHOR CONTRIBUTIONS

X-JH designed the review. Z-LX and X-JH wrote the manuscript and gave final approval for the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2020.586004/full#supplementary-material

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UK Experience of an Alternative ATO Dosing Regimen in APL

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The introduction of all-trans retinoic acid (ATRA), and more recently of arsenic trioxide (ATO) in the treatment of Acute Promyelocytic Leukaemia (APL), has been instrumental in achieving the high cure rates recently reported. For the majority of patients, it is now possible to successfully treat this disease "chemo-free" without the use of cytotoxic chemotherapy as reflected in current clinical guidelines. The Sanz risk score developed by the GIMEMA and PETHEMA groups categorizes patients into three risk groups—low, intermediate, and high and correlates with relapse-free survival (RFS). Low- and intermediate-risk APL are now often considered together as 'standard-risk' defined by a white blood cell count (WBC) of less than 10 x 10^9/L. High-risk APL has a WBC greater than 10 x 10^9/L. In the UK our approach for patients with standard risk APL is to treat with ATRA and ATO without the use of cytotoxic chemotherapy. This approach is based on results from two large randomized clinical trials. The GIMEMA APL0406 trial showed an overall survival advantage compared to anthracycline-based chemotherapy plus ATRA. The UK NCRI AML17 trial which used an attenuated dose of ATO demonstrated a significant reduction in relapse and improved relapse-free survival. In the UK, the National Institute for Clinical Excellence approved both ATO plus ATRA regimens for re-imbursement for standard risk Acute Promyelocytic Leukaemia (APL). We use the AML17 schedule in standard-risk patients upfront and also in patients with relapsed Acute Promyelocytic Leukaemia (APL) previously treated with chemotherapy or in those with molecular persistence. The treatment of high-risk Acute Promyelocytic Leukaemia (APL) remains an area of contention as ATO is not approved for this indication. These patients have a greater risk of complications during remission induction with ATO including differentiation syndrome. The optimal approach is to incorporate chemotherapy early into the treatment schedule with either Gemtuzumab Ozogamicin (GO) as in the high-risk arm of the NCRI AML17 trial and MD Anderson Cancer Centre studies or Idarubicin as in the Australian APML4 study.

Keywords: acute promyelocytic leukaemia (APL), arsenic trioxide, minimal residual disease, gemtuzumab ozagamicin, NCRI AML17

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PRE-ATO ERA STUDIES IN NCRI TRIALS

Following the MRC AML 12 trial, the combination of ATRA and chemotherapy became the standard induction treatment in newly diagnosed patients with APL in the UK (1). De-intensification of therapy became the priority for subsequent trials and the question of the optimal chemotherapy backbone to combine with ATRA was the subject of the NCRI AML15

trial. In that trial the standard MRC-based chemotherapy previously used in the AML12 trial comprising two courses of induction with daunorubicin, AraC and etoposide (ADE) with ATRA followed by consolidation with courses of MACE then MiDAC was randomized against the anthracycline and ATRA regimen (AIDA) developed by the PETHEMA and GIMEMA groups (2, 3). AIDA, which consisted of idarubicin and ATRA induction followed by 3 cycles of anthracycline-based consolidation followed by maintenance for 2 years, proved as effective and less myelosuppressive than MRC combination chemotherapy approach (CR rate 93% in both arms, 5 year overall survival of 84% vs 83%). The AIDA regimen proved less toxic with fewer deaths in remission, reduced supportive care requirements and reduced in-patient stay (4).

In the relapsed setting arsenic trioxide (ATO)had been successfully used as salvage therapy showing satisfactory outcomes in a number of studies (5–8) leading to its regulatory approval for relapsed APL in 2000 in the USA and a year later in Europe. In the AML15 trial, centralized sequential MRD monitoring with a sensitivity of up to $1{:}10^5$ to $1{:}10^6$ for good-quality samples had been introduced with samples taken after each course of chemotherapy. MRD was used to direct pre-emptive ATO therapy in patients with molecular persistence of disease or molecular relapse. This application of centralized MRD monitoring and pre-emptive therapy with ATO was associated with a significant reduction in the rate of hematological relapse when compared with the previous MRC AML12 trial (5% vs 12% at 3 years, $P = .02$) (9). The experience of ATO as salvage therapy paved the way for its evaluation as upfront therapy in the subsequent UK NCRI AML17 trial.

AML17 EXPERIENCE USING ATTENUATED ATO IN NEWLY DIAGNOSED APL

An important exploratory study by the MD Anderson Cancer Center using ATRA-ATO with or without GO suggested that an essentially chemotherapy-free regime might be feasible for the upfront treatment of APL (10). The NCRI AML17 trial built on these findings to investigate the de-intensification of treatment by randomizing patients irrespective of their risk status between ATO-ATRA and the AIDA regimen. For high risk patients GO was also administered on day 1 in the ATO arm (11). The Italian and German collaboration (GIMEMA-AMLSG-SAL) performed the first randomized trial randomizing ATO-ATRA against the AIDA regimen (APL 0406 trial) and showed that ATO-ATRA resulted in superior overall survival (12, 13). Unlike the AML17 trial this study was confined to standard risk patients only excluding the 25% of patients with high risk disease

The ATO schedule used in AML17 was different to that used in the APL 0406 trial. The protocol was based upon previous experience of ATO in a multi-center European study of 104 patients with myelodysplastic syndrome and utilized a higher loading ATO dose in week 1 of induction and consolidation but less frequent dosing in subsequent weeks resulting in overall significantly lower ATO exposure and was well tolerated (11). As previously reported in detail (14) In AML17, ATRA was given in a daily divided oral dose of 45 mg/m^2 until remission, or until day 60, and then in a 2 weeks on-2 weeks off schedule. ATO was given as a 1-h IV infusion using a loading dose of 0·3 mg/kg on days 1 to 5 of each induction and consolidation course, and then at a dose of 0·25 mg/kg twice weekly in weeks 2 to 8 of course 1 and weeks 2 to 4 of courses two to five (see **Figure 1**) (14). This contrasts with the daily ATO dosing schedule used in the APL 0406 trial, in the Australian APML4 study (15) and in the MD Anderson studies (10) which consist of ATO 0.15 mg/kg during induction until remission is achieved, typically 5 to 6 weeks. This is followed by four consolidation cycles, with a cycle length of 8 weeks with ATO being administered during weeks 1 to 4 at 0.15 mg/kg/d for 5 days per week. Thus the AML17 schedule offers a twice weekly schedule of ATO infusions during weeks 2 to 8 of induction and weeks 2 to 4 of the four courses of consolidation.

In AML17 high-risk patients also received a single dose of GO (6 mg/m^2) on day 1 with the aim of preventing the leukocytosis occurring during ATO-based induction which increases the risk of differentiation syndrome. In addition 7 out of 86 standard risk patients in the ATO-ATRA arm received GO because of a rising white cell count. For patients randomized to AIDA, idarubicin was given intravenously at 12 mg/m^2 on days 2, 4, 6, and 8 of course 1, and then at 5 mg/m^2 on days 1 to 4 of course 2; mitoxantrone at 10 mg/m^2 on days 1 to 4 of course 3, and idarubicin at 12 mg/m^2 on day 1 of the final course. During induction treatment, ATO could be discontinued temporarily in the presence of differentiation syndrome, QT prolongation on ECG, or hepatotoxicity. Routine prophylaxis for differentiation

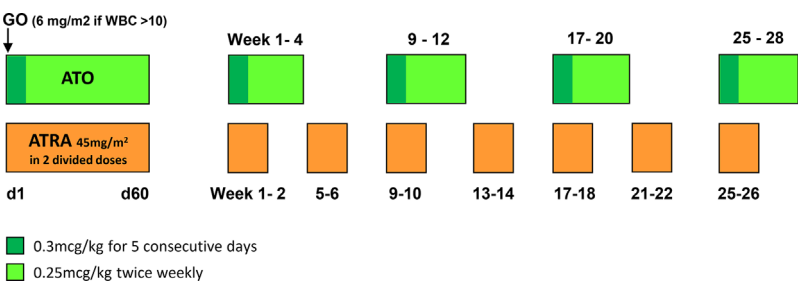

FIGURE 1 | ATO plus ATRA dose schedule.

syndrome was not recommended in the protocol, but prompt use of dexamethasone was suggested on clinical suspicion of differentiation syndrome.

MRD monitoring by reverse-transcription-quantitative PCR (RT-qPCR) was performed using bone marrow aspirates after each course of ATO and then 3 monthly for 3 years. The AIDA arm in AML17 had been de-intensified due to the omission of maintenance, the value of which had previously been questioned (16). Furthermore secondary AML had emerged as an important cause of treatment failure in the NCRI AML15 trial where 2 years of maintenance had been used (4).

As previously reported (14) from 05/2009 to 10/2013, 235 adult patients were randomized in the trial, the median age was 47 years (16–77 years); 57 had high risk APL of whom 30 received ATO and of these 28 received GO and 2 received idarubicin when GO was not available. 49 patients were over 60 years at diagnosis. Compliance with treatment was excellent at 99% in course 1 of ATO/ATRA and treatment was well tolerated with 60 day mortality of 5% overall and 1% in standard risk patients. During course 1 hyperbilirubinemia, and cardiac events were more common with ATRA and idarubicin, but there was no difference in raised liver enzyme levels between the two arms. Overall, liver toxicity appeared less frequent than in the GIMEMA-AMLSG-SAL protocol where grade 3–4 liver toxicity was reported in 63% of patients treated with ATRA-ATO (3). Differentiation syndrome was the most frequent complication seen and was reported in 25% of patients in the ATO-ATRA arm (23/86 standard risk and 7/30 high risk patients) which was not different to that reported in the AIDA arm. Cardiac events were more frequently reported with ATO-ATRA with grades 1 to 4 toxicity in 22% of patients and 3 of the 6 deaths by day 60 in that arm were cardiac events.

The results have been reported in detail by Burnett et al. (14). To summarize in terms of response, ATO-ATRA resulted in CR rate of 94% with no significant difference in the rate of complete remission (CR) between arms (ATO-ATRA 94%, AIDA 89%; HR 0.54 (0.21–1.34), p=0.18). Molecular complete remission (CR_{MRD-}) was achieved in 91% of patients with ATO-ATRA and 88% with AIDA (p=0.43).No differences were seen in 5 year overall survival and was 92% (ATO-ATRA arm) vs 86% (AIDA arm) (HR 0.71; 95% CI, 0.33–1.50; p=0.4) (14). Of the patients who became MRD negative, molecular relapse free survival was significantly improved at 5 years being 97% with ATO-ATRA compared to 78% with AIDA (HR 0.25 (0.12–0.52) p=0.0002). No patient treated with ATO-ATRA who became molecularly negative actually relapsed whereas among AIDA treated patients the 5-year cumulative incidence of relapse was 20%. This resulted in a superior relapse free survival for ATO-ATRA (96% vs 86%, HR 0.43 (0.18–1.03) p=0.06). The reduction in relapse for ATRA-ATO was seen in both high and standard risk patients. Other advantages seen with ATO-ATRA included a reduced requirement for supportive care including less time spent in the hospital (33 versus 27 days in course 1). There were no reported cases of secondary AML reported in the ATO-ATRA arm of AML17 or indeed in the APL0406 trial (13, 14).

The lack of overall survival benefit for ATO-ATRA in AML17 contrasts with the outcomes reported in the GIMEMA-AMLSG-SAL APL0406trial (3, 13). One explanation for the lack of survival benefit in AML17 was that it was related to high compliance with centralized MRD monitoring resulting in the effective salvage of AIDA-treated patients which frequently took place at the time of molecular relapse. However, despite this lack of survival benefit the attenuated schedule of ATO did result in a similar response rate and RFS benefit as the conventional dose regimen but with lower drug acquisition costs and reduced burden of ATO administration which amounted to just 63 doses (14). These findings led to the approval of the attenuated AML17 schedule for re-imbursement by the National Institute for Clinical Excellence (NICE) in the UK. Also to its and its recommendation as an option for the upfront treatment of standard risk APL, in the ELN APL guidelines (17) and in the 2019 NCCN APL guidelines (18).

EXPERIENCE OF ATTENUATED ATO DOSING IN HIGH-RISK AND RELAPSED APL

As previously discussed high risk APL patients were not included in the APL 0406 trial and ATO is not currently approved by the European Medicines Agency (EMA) for this group and is not reimbursed in the UK. It is generally agreed that cytotoxic chemotherapy should be administered with ATRA-ATO in high risk patients as the WBC may rapidly rise further after the initiation of ATRA with the risks of complications including differentiation syndrome. A number of approaches have been used. These include combining ATRA-ATO with GO as used in AML17 and by the MD Anderson Cancer Centre (10, 19) or with idarubicin as used in the Australian APML4 trial (15). The AML17 trial included a total of 57 high risk patients and their overall survival at 4 years was not significantly different from standard risk patients being 95% (95% CI 86%–98%) in standard risk compared with 87% (95% CI, 68%–95%) in high-risk patients Of the 28 high-risk patients in the ATO arm of AML17 who received the planned induction of ATRA, ATO and GO the 4 year survival was 89% (95% CI 70%–96%) (14). Unfortunately due to drug supply problems AML17 included only a relatively small sample size of high-risk patients and a prospective clinical trial (APOLLO trial) has been launched in Europe to verify whether the ATRA-ATO approach can be extended to the high-risk group. The NCRI AML17 result in high risk APL is however supported by a report from the MD Anderson Cancer Centre who also observed a low relapse risk in 187 standard- and high-risk (n=54) APL patients treated with ATO-ATRA, with the addition of GO (9 mg/m^2)in the high risk group (19). The 5-year relapse-free and overall survival rates were 96%, and 88%, respectively. The Australian APML4 trial with idarubicin, ATRA and ATO treated 23 high-risk patients with a 5-year DFS of 95% and OS 87% (15).

The current approach to high-risk APL in the UK is with AIDA as ATO is not commissioned or reimbursed in this group. Recent guidance develop during the COVID pandemic has amended this recommendation to permit the use of ATO-

ATRA for consolidation after receiving the first course of AIDA induction, the aim being to minimize neutropenia, hospital admission and the risk of nocosomial infection during subsequent courses of therapy. There were concerns that upfront ATO may be problematic for high-risk patients during the COVID pandemic because of the added risk of COVID-related pulmonary complications interacting with differentiation syndrome, moreover, the signs and symptoms of the two conditions show significant overlap which may create diagnostic uncertainty. However, the recent finding that dexamethasone is an effective treatment for COVID pneumonitis as well as its established efficacy in APL differentiation syndrome could simplify management decisions in this situation.

The attenuated ATO schedule has also been shown to be effective in patients relapsing after chemotherapy (20). Current recommendations for patients relapsing after initial treatment with chemotherapy are to re-induce with ATO-ATRA followed by consolidation with autologous transplantation in molecular CR if achieved (17). In AML17 a total of 189 patients were treated with AIDA, of whom 32 relapsed. These patients were treated with ATO-ATRA, receiving a median of 4 cycles (range, 1–5). Of the 31 patients treated, all achieved CR_{MRD-}. Only 5 patients received additional chemotherapy therapy and that was after achieving molecular remission with ATO. Only 13 of 32 patients were transplanted in second remission (10 autograft, 3 allograft), including 4 of the 5 patients with CNS disease present at relapse. Of the 18 patients treated with ATO-ATRA who were not transplanted and did not receive any additional chemotherapy the 5-year overall survival was 88%. A similar approach of treating relapse after chemotherapy with prolonged ATO-ATRA therapy was reported by Cicconi et al. (21). They observed 5 relapses out of 20 patients who had achieved a complete molecular response to salvage ATO-ATRA. These results suggest that treatment of relapse with prolonged ATO-ATRA is an option for patients achieving molecular remission with ATO -ATRA who did not have CNS disease at relapse especially in patients who have had a long first remission (21).

MRD MONITORING IN APL IN ATO ERA

Molecular monitoring still plays an important role in patients treated with upfront ATO-ATRA however clinicians should be aware that its role, and consequently the recommended monitoring schedule, is different to that in patients treated with ATRA-CHT.

Most, but not all, patients treated with either ATO-ATRA or ATO-CHT achieve CR_{MRD-}. In AML17, MRD-positivity at the end of treatment was seen in 3/106 (2.8%) patients who received at least one full course of ATO-ATRA and in 1/119 (1.3%) treated with ATRA-CHT (14). In contrast no patients in APL0406 had MRD-positivity after third consolidation (n=75 and 70 for ATRA-ATO and ATRA-CHT respectively) (13, 22). It is recommended that molecular monitoring should be performed until CR_{MRD-} is documented (17). This requires that both *PML-RARA* and reciprocal *RARA-PML* transcripts are undetected in two consecutive, technically adequate bone marrow aspirate samples.

The time to achievement of molecular negativity may differ between patients treated with ATO-ATRA and ATO-CHT. In AML17, the median time to CR_{MRD-} was 111 days with ATRA-ATO and 83 days with ATRA-CHT. The proportion of patients achieving molecular negativity by day 60 was also lower in patients treated with ATO-ATRA (56% vs 73%, p=0.03). The kinetics of molecular clearance did not differ between ATO-ATRA and ATO-CHT in APL0406, however fewer patients were evaluated in this analysis. It is not recommended to modify treatment for patients who have MRD-positivity after the first or second cycle of ATRA-ATO (17). For the small proportion of patients who have molecular persistence beyond the end of the third cycle, expert advice should be sought.

Finally, and perhaps most importantly, the rate of relapse is much lower with ATRA-ATO compared to ATRA-CHT as outlined above. In APL0406 only 2/75 (2.6%) patients in the ATO-ATRA arm relapsed and in AML17 there were no relapses in patients who had achieved CR_{MRD-}. Therefore, sequential monitoring is not recommended for standard-risk patients who have been treated with frontline ATO-ATRA and who have achieved a documented CR_{MRD-} as the very low risk of relapse does not justify repeated bone marrow examinations (17). Patients with high-risk APL treated with ATO-based protocols should still receive molecular monitoring as the rate of relapse remains somewhat uncertain due to the relatively small number of patients treated in clinical trials to date. Patients treated with ATO-based salvage therapies following relapse after frontline ATRA-CHT treatment should continue to receive molecular monitoring as they remain at elevated risk of subsequent relapse, and pre-emptive treatment at molecular relapse may improve outcome (10, 14). For these patients, bone marrow monitoring every three months for at least two years after the completion of treatment is recommended (17).

CONCLUSIONS

The attenuated ATO dosing schedule offers advantages of convenience for patients compared with conventional dosing regimen with evidence of reduced hepatotoxicity. Although no direct comparison has been made with the conventional dose regimen the attenuated schedule proved effective as first line therapy in both standard risk disease and also in high risk patients and following relapse after chemotherapy. The regimen offers advantage in that it reduces both the frequency of administration and the acquisition costs of ATO in UK.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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The History of the Chemo-Free Model in the Treatment of Acute Promyelocytic Leukemia

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Acute promyelocytic leukemia (APL) has become a highly curable disease after four decades of endeavors. Thanks to the efforts of investigators throughout the world, the chemo-free concept has become a reality for both low- and high-risk patients. All-trans retinoic acid (ATRA) plus arsenic trioxide (ATO) without chemotherapy has become a first-line treatment for newly diagnosed APL and has been adopted in guidelines or expert recommendations from the NCCN and ELN and in China. Though the regimen has achieved great success, challenges still exist. The rate of early death still has not diminished significantly and is a major obstacle to curing all patients. Leukocytosis is the most important factor for ED, and completely abandoning chemotherapy is dangerous for certain patients in practice. To narrow the gap between guidelines and practice, this review aims to examine the history of the chemo-free model for the treatment of APL in the arsenic-alone era (1974–2002) and the arsenic plus ATRA era (2002–present) and provide practical considerations regarding early death.

Keywords: acute promyelocytic leukemia, ATRA, ATO, chemotherapy, early death

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THE EVOLUTION OF THE CHEMO-FREE ERA

Arsenic Monotherapy

Although the term "chemo-free" was introduced for APL in 2011, the history of chemo-free practices can be traced back four decades (**Figure 1**). The chemo-free era can be characterized into two phases: the arsenic monotherapy phase (1974–2002) and the arsenic plus ATRA combination phase (2002–now). Sun et al. from Haerbin, China, reported long-term follow-up results after one injection of ATO-containing monotherapy in 32 patients with newly diagnosed APL between 1974 and 1985 (1). The complete remission (CR) and partial remission rates were 50 and 19%, respectively, and the 5-year overall survival (OS) was 50%. This result was subsequently confirmed by using pure ATO alone in an extension study including 124 patients from the same group (2). Lu et al. first reported the excellent results of a pilot study of 19 patients using oral tetra-arsenic tetra-sulfide (As4S4) alone; the authors reported a CR rate of 100% and a 3-year disease-free survival (DFS) of 76.6% (3). However, the total course of arsenic was >3 years in the above studies,

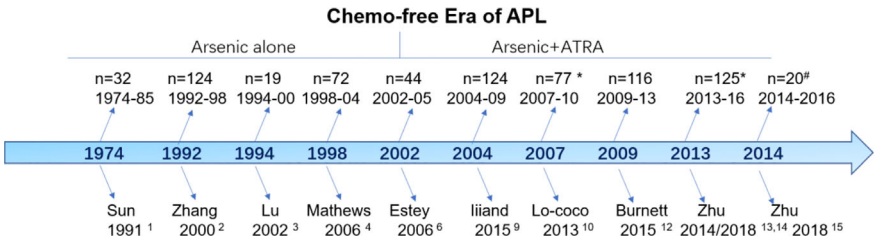

FIGURE 1 | The history of the chemo-free model for newly diagnosed acute promyelocytic leukemia.

which affected the quality of life of the patients. Another two studies from India and Iran shortened the postremission course of ATO to 28 weeks (7 months) and reported similar results (CR rate of 86% and 3-year OS of 86%) (4, 5). The 7-month postremission usage of ATO in the above two protocols provided evidence to design the later arsenic plus ATRA-based chemo-free model.

ATO Plus ATRA

In 2002, Estey et al. from MD Anderson Cancer Center was the first to investigate the chemo-free model using ATO plus ATRA (CD33-antibody gemtuzumab ozogamicin (GO) for cytoreduction during induction) during induction and postremission treatment (6). Postinduction treatment consisted of four courses of ATO (daily for 5 days/week for 4 weeks every other month; total of 80 doses) and ATRA (2 weeks on/2 weeks off for 7 months). The study included 82 patients, and the CR rate was 92%. The early death rate was 9%, and the estimated 3-year OS was 85%. This result was further confirmed by updated results from the long-term follow-up of the same group, which provided the basis for the subsequent APL0406 study (7, 8). Moreover, IIiand et al. reported an excellent outcome using ATO and ATRA for induction and consolidation, but the inclusion of idarubicin during induction and low-dose cytotoxic agents during maintenance caused this regimen to slightly deviate from the chemo-free goal (9).

Based on the study by Estey et al., Lo-Coco et al. conducted a randomized noninferiority trial, APL0406, using ATRA plus ATO vs. ATRA plus idarubicin for patients with newly diagnosed, non-high-risk (now low-risk) APL (10, 11). The ATRA plus ATO group showed a CR rate of 100% and a 2-year OS of 99% with a median follow-up of 34.4 months. The NCRI AML17 trial aimed to investigate the de-intensification of treatment by randomizing patients irrespective of their risk status between a chemotherapy-free approach (ATO-ATRA) and the ATRA-Chemotherapy (AIDA) regimen (12). ATO was given intravenously at 0·3 mg/kg on days 1–5 of each course, and at 0·25 mg/kg twice weekly in weeks 2–8 of course 1 and weeks 2–4 of courses two to five, the usage of ATO was different from that of the APL0406 study. Gemtuzumab Ozogamicin (GO) was also administered on day1 in the ATO-ATRA arm for high risk patients. CR rate of 94% and 5 year OS was 92% in ATO-ATRA arm.[12] This study and subsequent update results support ATRA and ATO as first-line treatment not only for low-risk but also for high-risk patients.

Oral Arsenic Plus ATRA

The next goal was to realize a completely oral, chemo-free model for APL, named the postremission outpatient model. Based on the APL0406 study (10), Zhu et al. first performed a pilot study in 20 low-risk patients using only the oral Realgar-Indigo naturalis formula (RIF) and ATRA without chemotherapy during induction and outpatient-based postremission (13). All patients achieved CR and were still alive and in CR at a median follow-up of 77 months. Subsequently, we demonstrated the noninferiority between oral RIF-ATRA and IV ATO-ATRA in a randomized controlled trial (14). In the end, we extended this concept to high-risk patients, while only incorporating minimal chemotherapy, between April 2014 and September 2016 (15). All 20 patients achieved CR, and the 3-year OS and EFS rates were 100 and 89.4%, respectively (15).

Oral ATO is another arsenic that was first revived by a group from Hong Kong, who thereafter completed a series of clinical trials on this issue (16–18). Recently, Gill et al., in a 15-year prospective follow-up study in 73 patients with relapsed APL, reported 5-year and 10-year OS of 79.5 and 67.3%, respectively (17). Most recently, the same group using oral ATO, ATRA and chemotherapy, reported that both LFS and OS were 100% at 5 years (18). The above studies also inspired interest in the research and development of oral ATO in the USA and Australia. One oral arsenic, named ORH-2014, has completed a phase 1 open-label, dose-escalating study which indicate that ORH-2014 at 15 mg is safe, bioavailable, and provides the required arsenic exposure compared to intravenous ATO at the approved dose (0.15 mg/kg) (19). Moreover, the dose of 10mg is recommended in the future phase 2 and phase 3 trials. Oral ATO developed in Australia is also being evaluated by the ALLG phase I study (APML5) (ACTRN12616001022459).

Early Death Is the Major Obstacle to Curing All Patients

Early death (ED) is commonly defined as death from any cause within 30 days of diagnosis[12] or at any time during induction (10, 11). Details about this definition have been systematically reviewed in recent years (19–33). As a result of selection bias, clinical trial data have underestimated the impact of ED, but a series of epidemiologic studies revealed that a significant proportion of patients continue to suffer early death (27–29). Encouragingly, however, newer epidemiologic studies now suggest that ED rates may be improving (30–33). According to the US SEER database, ED rates

have improved over time (2000–2004, 25.3%; 2005–2009, 20.6%; 2010–2014, 17.1%) in the ATRA plus chemotherapy era (33).

Whether the ED rate can be further reduced in the ATRA plus ATO era remains uncertain. The most important studies of the most representative groups (PETHEMA, GIMEMA, European APL, MRC, etc.) have reported ED rates of around 5% for more than two decades in the ATRA plus chemotherapy era (34–36). Zhu et al. reported that the ED rate in the ATRA plus ATO group was 5.5% (n = 758) based on the data from three large centers in China, which excluded the patients who died without receiving treatment (37). It seems that no difference of ED rate exists between ATRA plus chemotherapy model and ATRA plus arsenic model. Whether ED rate is different between the two models in the population-based study need to be investigated in the future.

Toxicity of Arsenic and ATRA

The common toxicity of ATO plus ATRA or oral RIF plus ATRA had been systematically reviewed by us recently (38). Liver damage, gastrointestinal toxicity, and headache are common (>10%), while prolongation of the QTc interval and rash are rare (<5%), which is unpredictable before treatment and difficult to perform preemptive therapy. The most important and sometime fatal adverse effect before treatment or during induction therapy with arsenic plus ATRA is leukocytosis, defined as a white blood cell (WBC) count over 10×10^9/L. Lou et al. reported that pretreatment WBCs of $10–50 \times 10^9$/L and $>50 \times 10^9$/L had early death rates of 8.7 and 41.2%, respectively (39). Yoon et al. recently reported that progressive hyperleukocytosis is a relevant predictive marker for differentiation syndrome, early death and subsequent relapse in patients with APL (40). Patients with a WBC before treatment of $10–43 \times 10^9$/L that increased to a WBCmax $>43 \times 10^9$/L experienced an increased risk of early death (33.3%). The multivariate analysis revealed that a WBCmax $>43 \times 10^9$/L correlated significantly with both early death and differentiation syndrome. Similarly, Therefore, timely minimization of leukocytosis is urgent, and successful prevention of the occurrence of leukocytosis is better.

CONCLUSION

The history of APL treatment is almost miraculous. After four decades of endeavors, APL has evolved from a highly fatal disease into a highly curable disease. A chemo-free treatment using only ATRA and ATO in non-high-risk patients was easily applied in clinical practice and is now recommended by current guidelines (41–46). A complete oral and chemo-free model using oral arsenic and ATRA further simplified the procedures and made home-based treatment a reality for more patients.

Apart from ED, the relapse is another major challenge of APL, especially in high-risk patients (27). Until now, no consensus molecular cytogenetic abnormalities at the time of diagnosis can reliably predict the relapse, but monitoring PML-RARA transcripts after treatment is a confidential tool to predict relapse. Currently, ATO plus ATRA is the first choice for the first relapse of APL after front treatment with ATRA plus chemotherapy or ATRA plus ATO. Autologous HSCT remains an appropriate option for younger patients in molecular remission and allogeneic HSCT reserved for patients with persistent molecular positive or with higher degrees of relapse (43).

From the perspective of history, the story of struggling with APL is nearing its end, and this successful model is expected to be attempted on other malignances.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

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Hypertriglyceridemia in Newly Diagnosed Acute Promyelocytic Leukemia

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The primary aim of the present retrospective study was to investigate lipid profiles and kinetics in acute promyelocytic leukemia (APL) patients. We analyzed 402 newly diagnosed APL patients and 201 non-APL patients with acute myeloid leukemia (as control). Incidence of hypertriglyceridemia in APL patients and non-APL patients was 55.82% and 28.4% ($p = 0.0003$). The initial levels of triglycerides, total cholesterol, high-density lipoprotein cholesterol and low-density lipoprotein cholesterol were higher in APL patients than in control (all $p < 0.0001$). In APL patients, triglyceride levels were significantly increased during induction treatment with all-trans retinoic acid and arsenic. Multivariable analysis showed that age, being overweight (body mass index ≥25) and APL were independent risk factors for hypertriglyceridemia in all patients before treatment. High triglyceride levels were not significantly associated with disease-free survival or overall survival in the APL patients. In summary, in the current study triglyceride levels were significantly elevated in APL patients before treatment, and they increased during induction treatment, but there were no significant corresponding effects on survival.

Keywords: acute promyelocytic leukemia, hypertriglyceridemia, all-trans retinoic acid, body mass index, peroxisome proliferator-activated receptor gamma

INTRODUCTION

Dyslipidemia is reportedly often detected in cancer patients, and it has recently attracted increased attention due to its potential prognostic value of cancer and mortality from cardiovascular diseases (1–3). In recent years, progress has been made in the study of dyslipidemia in patients with hematological malignancies. In some studies, there have been lower levels of total cholesterol (TC), high-density lipoprotein cholesterol (HDL), and low-density lipoprotein cholesterol (LDL), and higher triglyceride (TG) levels in patients with hematological malignancies (4–6). Small sample sizes and a lack of subgroup analyses may be the cause of the inconsistent results. As early as 1997 Estey et al. (7) reported that the incidence of obesity in acute promyelocytic leukemia (APL) patients was significantly higher than that in other types of acute myelocytic leukemia (AML). Many subsequent studies also indicated that obesity may be associated with APL (8) and differentiation syndrome (9), and that it may be have substantial adverse effects on clinical outcomes (10–12). Mazzarella et al. (13) recently reported that obesity was a

dependent risk factor for APL. However, the correlation between dyslipidemia and APL has not been reported. Therefore, a study investigating a homogeneous leukemia type with a uniform treatment protocol and an adequate sample size is required.

In this retrospective study, we aimed to conduct a comprehensive study of lipid metabolism in 402 APL patients and a uniform protocol with all-trans retinoic acid (ATRA) and arsenic combination treatment to assess dyslipidemia during treatment and a 1-year follow-up period.

MATERIALS AND METHODS

Patients and Sample Collection

A total of 603 leukemia patients were included in this retrospective study, 402 APL patients and 201 non-APL acute myelocytic leukemia patients (non-APL patients). APL patients aged over 14 years who were admitted to the clinic center affiliated with the First Affiliated Hospital of Zhejiang University School of Medicine from January 2011 to December 2019 were included. For comparisons, we collected the data of AML patients without APL in our hospital from January 2017 to December 2017. The study was approved by the institutional Ethics Committee and it was conducted in accordance with the Declaration of Helsinki.

APL Patients were treated primarily in accordance with the Shanghai APL protocol (14). We, routinely, took orally ATRA at 25 mg/m^2/day and were intravenous injected arsenic trioxide 10 mg/day, until complete remission was achieved. APL patients underwent 3 courses of consolidation therapy (homoharringtonine/Mitox/daunorubicin and cytarabine) and sequentially underwent a course of ATRA combined with arsenic trioxide, 14 days apart for 24 months. For the non-APL patients, 3 days of idarubicin (10 mg/m^2/day) and 7 days of cytarabine (100 mg/m^2/day) were performed as induction therapy. When complete remission was achieved, patients received several courses of conventional chemotherapy including treatment of cytarabine every 12 h for 6 days (1.5–3.0 g/m^2), homoharringtonine for 3 days (2 mg/m^2/day), cytarabine for 7 days (75 mg/m^2 twice a day) and aclarubicin for 7 days (12 mg/m^2/day); daunorubicin for 3 days (45 mg/m^2/day) and cytarabine for 7 days (100 mg/m^2/day); idarubicin for 7 days (6–8 mg/m^2/day) and aclarubicin for 5 days (20 mg/m^2/day).

Demographic and clinical data that were obtained from medical records included: age, sex, height, weight, body mass index (BMI), WBC, hemoglobin count, platelet counts, albumin, alanine transaminase, aspartate transaminase, serum creatinine, uric acid, lactate dehydrogenase, Sanz's risk score (15), PML-RARa transcript isoform and additional cytogenetic aberrations. The concentration of total TG, TC, HDL, and LDL and serum glucose were measured in all patients before the initiation of chemotherapy, twice a week during the first 4 weeks of induction chemotherapy, before the second course of the treatment, and during the third, sixth, and twelfth months. Blood samples were collected and stored in tubes containing ethylene diamine tetraacetic acid, and plasma levels of fasting TC, TG, HDL, and LDL *via* enzymatic method (Boehringer Mannheim, Mannheim, Germany). The lipid abnormality status was determined according to the criteria described by the expert panel of the National Cholesterol Education Program Adult Treatment Panel III Third Report (16). The upper limits of normal TG, TC and LDL were 1.7 mmol/L (150 mg/dL), 6.1 mmol/L (234.6 mg/dL) and 4.0 mmol/L (152.9 mg/dL), respectively. The lower limit of normal HDL was 0.96 mmol/L (55.4 mg/dL). The reporting recommendations for tumor marker prognostic studies (REMARK) guidelines were used as reference (17). We collected a total of 25,783 samples.

Definition of Variable

According to the 2019 ESC/EAS Guidelines, hypertriglyceridemia is defined as 1.7 mmol/L (150 mg/dL) (18). In TG-based analysis, the patients were categorized into two major groups: high triglyceride group (HTG group, TG ≥ 1.7 mmol/L) and non HTG group (TG < 1.7 mmol/L). Patients were categorized into underweight/normal (BMI < 25) and overweight (BMI ≥25) in accordance with the current World Health Organization criteria. The initial WBC counts of APL patients were evaluated and adjusted. WBC counts ≤10 × 10^9/L were considered as low risk and > 10 × 10^9/L were considered high risk (19). Disease-free survival (DFS) was only used in analyses of patients who achieved complete remission and it was measured from the date of achievement of remission until the date of relapse or death from any cause; at last follow-up, patients with unknown relapse or death were removed on the date of their last examination. Overall survival (OS) was applied to all patients and it was defined as the length of time from the date of diagnosis to the death from any cause; patients whose death was at last follow-up were removed on the last date that they were known to have been alive.

Statistical Analysis

Data are presented as median and absolute range (non-normally distributed data) or frequencies. The Shapiro-Wilk test was used to assess the normality of data distributions. The Wilcoxon Mann-Whitney test was used to compare the distribution of numerical variables. The χ^2 test was used in qualitative variables. The relationships between clinical factors and dyslipidemia were assessed using univariable and multivariable logistic regression models. All multivariable analyses were adjusted by sex and age. The Kaplan–Meier method was used to estimate univariate survival curves and the differences between curves were analyzed *via* the log-rank test. Multivariable Cox proportional hazard regression models were used to assess the prognostic impact of hypertriglyceridemia with regard to OS and DFS. Statistical analyses were performed using SPSS software, version 23.0, and $p < 0.05$ was considered statistically significant.

RESULTS

Patient Characteristics

Our study included 402 APL patients and 201 non-APL patients. 22.1% (71/321) of the APL patients were overweight, and 16.4%

(33/201) of the non-APL patients were overweight (chi square test, $p = 0.1$); the median BMI was 22.83 ± 3.32 kg/m^2 in APL patients and 22.59 ± 3.08 kg/m^2 in non-APL patients (Mann–Whitney test, $p = 0.426$). The initial concentration of TG, TC, HDL, and LDL in the APL patients were significantly higher than those in control (Mann–Whitney test, $p < 0.001$, < 0.001, $= 0.002$, < 0.001). The proportions of the APL and non-APL patients who had hypertriglyceridemia before treatment were 55.8% and 28.4%, respectively (chi square test, $p < 0.001$). Detailed characteristics of the study population are shown in **Table 1**.

Relationships Between Hypertriglyceridemia and Clinical Factors

We used univariable and multivariable h to explore the correlation between the hypertriglyceridemia and other clinical factors. In univariable analysis BMI and leukemia type were both risk factors for hypertriglyceridemia ($p < 0.001$). The results indicated that being overweight (BMI ≥ 25) (odds ratio [OR] 1.160, 95% confidence interval [CI] 1.087–1.238, $p < 0.001$) and leukemia type (OR 3.558, 95% CI 2.312–5.477, $p < 0.001$) were associated with hypertriglyceridemia. In APL patients hypertriglyceridemia was significantly associated with age (OR 1.026, 95% CI 1.008–1.044, $p = 0.004$), being overweight (OR 1.149, 95% CI 1.053–1.254, $p = 0.002$), and higher WBC count (OR 1.022, 95% CI 1.005–1.040, $p = 0.011$). While PML-RARa transcript isoform ($p = 0.185$) and abnormal karyotype ($p = 0.907$) were not significantly associated with hypertriglyceridemia. Compared with non-APL patients, APL patients were younger (OR 0.971, 95% CI 0.954–0.988, $p = 0.001$), higher hypertriglyceridemia (OR 1.828, 95% CI 1.383–2.415, $p < 0.001$), and had lower white blood cells (OR 0.977, 95% CI 0.967–0.986, $p < 0.001$) and platelets (OR 0.981, 95% CI 0.975–0.987, $p < 0.001$) before treatment. Detailed results of logistic regression modelsing are shown in **Table 2**.

Lipid Kinetics

Changes of TG levels during induction treatment are shown in **Figure 1**. TG concentrations were higher in APL patients than in non-APL patients at every timepoint investigated ($p \leq 0.001$). The concentration of TG in APL patients continued to increase and peaked on day 10 (median 2.93, range 0.71–11.4, $p < 0.001$). In contrast, the concentration of TG in the non-APL patients followed a valley curve and reached the nadir on the day 18 (median 1.27, range 0.25–10.32, $p < 0.001$). Additionally, the TG concentration of the APL patients was higher than that of the non-APL patients at every timepoint ($p \leq 0.001$). Furthermore, the median TG value in APL patients was higher than the upper limit of normal, and the corresponding value in the non-APL patients was lower than the upper limit of normal.

TABLE 1 | Characteristics of the Study Population According to the Type of Leukemia.

	Non-APL	APL	P-value
Age (years), range	48.59(18–83)	42.9 (14–84)	<0.001
Gender			0.21
Male, No. (%)	95(47.3)	212(52.7)	
Female, No. (%)	106(52.7)	190(47.3)	
Height (cm), range	164.59(146–185)	164.62(148–184)	0.968
Weight (kg), range	61.38(35.5–100)	62.16(38–101)	0.461
BMI(kg/m2), range	22.59(0.5–98.8)	22.83(0.2–117.2)	0.426
Underweight/normal, BMI<25, No. (%)	168(83.6)	250(77.9)	0.1
Overweight/obese, BMI≥25, No. (%)	33(16.4)	71(22.1)	
WBC(10 ×109/L), range	35.55(0.5–98.8)	12.70(0.2–117.2)	<0.001
≥10 ×109/L, No. (%) 97(48.3)		118(29.5)	<0.001
<10 ×109/L, No. (%) 104(51.7)		282(70.5)	
HBG*(g/L), range	90.96(48–161)	88.59(41–157)	0.325
PLT*(10 ×109/L), range	62.31(6–361)	36.65(4–225)	<0.001
ALB*(g/L), range	40.47(28.5–53.4)	42.48(25.3–66.8)	0.095
ALT*(U/L), range	27.99(5–389)	32.68(4–560)	0.068
AST*(U/L), range	27.17(7–539)	31.49(6–367)	0.062
Cr*(μmol/L), range	63.74(32–170)	64.96(18–322)	0.315
UA*(μmol/L), range	270.21(96–578)	235.44(36–561)	0.288
LDH*(IU/L), range	708.38(129–1025)	465.49(115–4462)	<0.001
TG*(mmol/L), range	1.57(0.44–10.39)	2.29(0.43–20.86)	<0.001
HTG group TG≥1.70, No. (%)	57(28.4)	211(55.8)	<0.001
TC*(mmol/L), range	3.45±1.09(0.96–7.13)	4.28(1.96–7.68)	<0.001
HDL*(mmol/L), range	0.86(0.23–2.44)	0.97(0.15–4.27)	0.002
LDL*(mmol/L), range	1.84(0.11–3.67)	2.28(0.11–4.99)	<0.001
Glucose*(mmol/L), range	5.67(2.14–14.51)	6.65(3.35–33.71)	<0.001

APL, acute promyelocytic leukemia; non-APL, acute myeloid leukemia exclude of APL; BMI, body mass index; WBC, white blood cell; HBG, Hemoglobin count; PLT, platelet count; ALB, albumin; ALT, alanine transaminase; AST, aspartate transaminase; Cr, serum creatinine; UA, uric acid; LDH, lactate dehydrogenas; TG, triglyceride; HTG group, high triglyceride group ≥ 1.70 (mmol/L); TC, cholesterol; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol.

TABLE 2 | Logistic regression models evaluating the associations between clinical variables and hypertriglyceridemia in APL and non-APL patients.

All patients		**Univariable analysis**	**P-value**	**Multivariable analysis**	**P-value**
Dependent variable	**Independent variable**	**OR (95% CI)**		**OR (95% CI)**	
HTG	Age	1.009(0.998–1.020)	0.130	1.016(1.003–1.029)	0.019
	Gender	1.246(0.889–1.747)	0.202	1.143(0.767–1.705)	0.512
	BMI	1.168(1.097–1.242)	<0.001	1.160(1.087–1.238)	<0.001
	Leukemia type	3.185(2.154–4.710)	<0.001	3.558(2.312–5.477)	<0.001
Leukemia type (non-APL)	Age	1.025(1.013–1.038)	<0.001	1.030(1.012–1.048)	0.001
	Gender	1.259(0.872–1.816)	0.219	1.543(0.906–2.628)	0.110
	BMI	0.980(0.924–1.040)	0.507	0.994(0.910–1.086)	0.897
	TG	0.555(0.446–0.689)	<0.001	0.547(0.414–0.723)	<0.001
	WBC	1.020(1.013–1.027)	<0.001	1.024(1.014–1.034)	<0.001
	PLT	1.014(1.010–1.019)	<0.001	1.019(1.013–1.026)	<0.001
	LDH	1.001(1.000–1.001)	0.001	1.000(1.000–1.001)	0.161
APL patients		**Univariable analysis**	**P-value**	**Multivariable analysis**	**P-value**
HTG	Age	1.021(1.007–1.035)	0.002	1.026(1.008–1.044)	0.004
	Gender	1.564(1.039–2.354)	0.032	1.222(0.715–2.088)	0.463
	BMI	1.165(1.080–1.257)	<0.001	1.149(1.053–1.254)	0.002
	WBC	1.026(1.012–1.040)	<0.001	1.022(1.005–1.040)	0.011
	LDH	1.001(1.000–1.002)	0.001	1.001(1.000–1.001)	0.122
	transcript isoform				

APL, acute promyelocytic leukemia; non-APL, acute myeloid leukemia exclude of APL; HTG group, high triglyceride group ≥ 1.70 (mmol/L); BMI, body mass index; WBC, white blood cell; TG, triglyceride concentration; PLT, platelet count; LDH, lactate dehydrogenase; OR, odds ratios; CI, confidence interval; PML-RARα, promyelocytic leukemia-retinoic acid receptor alpha.

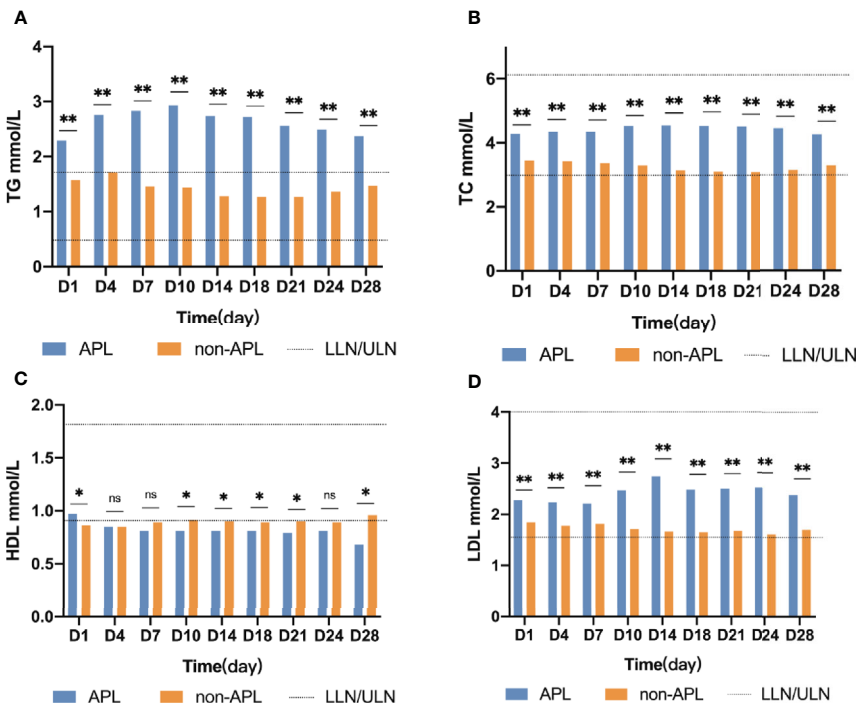

FIGURE 1 | Lipid kinetics. Significant difference (*P < 0.05,**P < 0.0001); n.s., not significant. APL, acute promyelocytic leukemia; non-APL, acute myeloid leukemia exclude of APL; TG, triglyceride; TC, cholesterol; HDL, high-density lipoprotein cholesterol; LDH, low-density lipoprotein cholesterol; ULN, upper limits of normal; LLN, lower limits of normal.

In non-APL patients TC, LDL, and serum glucose concentrations were significantly lower than those of APL patients, at all timepoints investigated ($p < 0.05$). However, the median TC, LDL, and glucose values in APL or non-APL patients at each time point were within the normal range. The detailed information is shown in **Figure 1**.

There was no significant difference in TG between APL and non-APL patients after 3 months, although the median was outside the normal range. At 12-month follow-up, there were 46.4% (83/179) of APL patients and 49.4% (88/178) of non-APL patients were hypertriglyceridemic. More detailed information pertaining to TG and hypertriglyceridemia is shown in **Figure 2**.

Associations Between Hypertriglyceridemia and Survival in APL Patients

The median follow-up time for the 353 surviving patients was 44 months (ranges 5–105 months). All APL patients experienced hematologic remission before maintenance therapy. 21 (early death rate 5.22%) patients died during induction therapy, 7 died after disease relapse, 3 survived after relapse, and 21 (5.2%) missed the follow-up. The 3-year DFS and OS rates were 92.65% and 93.12%, respectively. Neither DFS nor OS differed significantly in APL patients with and without hypertriglyceridemia (DFS hazard ratio [HR] 0.580, 95% CI 0.257–1.307, $p = 0.097$; OS HR 0.486, 95% CI 0.202–1.174, $p = 1$, **Figure 3**).

DISCUSSION

To the best of our knowledge the current study constitutes the first clinical evidence that TG levels are elevated in APL patients at the time of initial diagnosis compared with non-APL patients, and TG levels increased during induction therapy with ATRA and arsenic. Additionally, our results suggest that APL and being obesity (BMI ≧ 25) are risk factors for hypertriglyceridemia but hypertriglyceridemia is not significantly associated with DFS or OS in APL patients.

Chinese research (Chinese Chronic Diseases and Risk Factors Surveillance Research, n = 163,641, from 2013–2014) showed that the prevalence of hypertriglyceridemia was 25.8% in healthy adults which is higher than the 13.1% incidence reported in 2012 (20). In the USA, data from the National Health and Nutrition Examination Surveys showed that 47% of all adults in 2010 had hypertriglyceridemia (21). European data from 2016 show that 27% of all adults have a nonfasting triglyceride level >2.0mmol/l (22). Unsurprisingly, associations between hypertriglyceridemia and cancer have been reported in several studies (2, 3). Combined treatment with asparaginase and corticosteroids leads to hypertriglyceridemia in up to 67% of patients receiving treatment for acute lymphoblastic leukemia (5, 23). The hypertriglyceridemia incidence in APL patients is still unknown; our study found that the incidence of hypertriglyceridemia in Chinese APL patients before treatment was as high as 55.8%. After 1 year of follow-up, the incidence of hypertriglyceridemia in APL was still 46.4%.

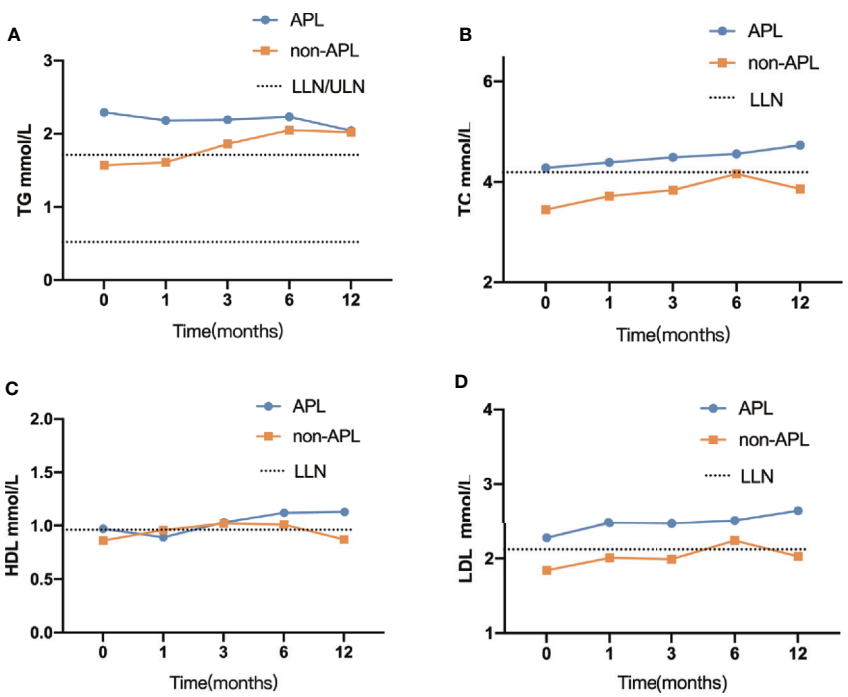

FIGURE 2 | Comparison between the lipid profile at each time point. **(A)** TG, triglyceride **(B)** TC, cholesterol **(C)** HDL, high-density lipoprotein cholesterol **(D)** LDL, low-density lipoprotein cholesterol; APL, acute promyelocytic leukemia; non-APL, acute myeloid leukemia exclude of APL; ULN, upper limits of normal; LLN, lower limits of normal.

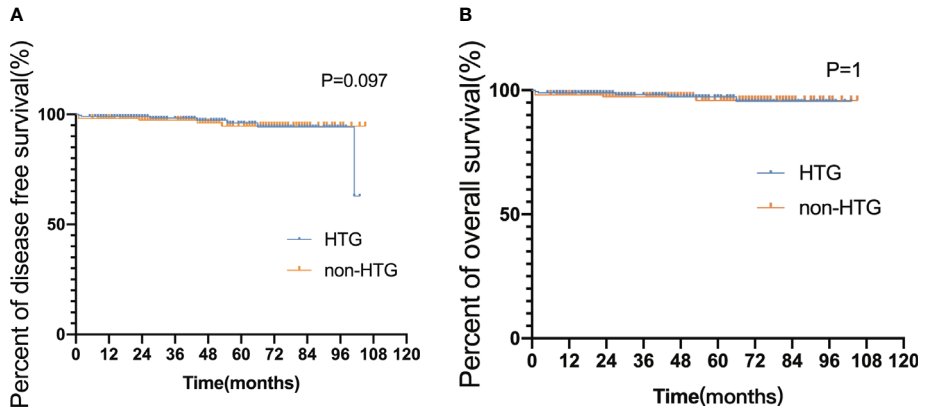

FIGURE 3 | Disease-free survival and overall survival in HTG and non-HTG patients with APL. **(A)** Disease-free survival and **(B)** overall survival HTG group, high triglyceride group ≥ 1.70 (mmol/L); non-HTG group, triglyceride < 1.7 (mmol/L); Significant difference ($P < 0.05$).

We speculated that high TG levels—a risk factor for atherosclerotic cardiovascular disease—would lead to adverse prognoses. Large observational, epidemiological, genetic, and Mendelian randomization studies support the hypothesis that elevated blood triglyceride levels are independently associated with increased risk of atherosclerosis and coronary artery disease (22, 24, 25). TG levels > 1,000 mg/dL (11.4 mmol/L) can also induce acute pancreatitis (26). In the current study, only one APL patient had a lipid profile of 20.86 mmol/L. That patient did not develop acute pancreatitis during the course of treatment.

The mechanism of hypertriglyceridemia development in APL patients has not been completely clarified (27). We concluded that hypertriglyceridemia may be associated with APL as well as being overweight for three possible reasons. One pertains directly to being overweight, another pertains to the PML/RARα fusion protein, and the last involves the induction of abnormal lipid metabolism in APL patients by ATRA therapy.

With regard to being overweight, in a population-based cross-sectional study obesity was a significant risk factor for APL (13). The risk was particularly high in the APL subtype, with an estimated 44% HR increase per additional 5 kg/m^2. Obesity is also associated with differentiation syndrome (9), relapse (10), and poor survival (11). Genetic up-regulation of pro-inflammatory factors related to direct growth promotion, generation of genotoxic oxidative stress, immune modulation (28, 29), and metabolic components of endogenous agonists for peroxisome proliferator-activated receptors (PPAR) (30, 31) may be involved in this clinical phenomenon in APL. While the pathophysiology of hypertriglyceridemia remains poorly understood (32), the mechanism of hypertriglyceridemia in the setting of obesity has been linked to insulin resistance. Not all obesity patients are insulin resistant (33), thus he interconnections involved need to be further explored.

With respect to the PML/RARα fusion protein, galectin-12 is selectively overexpressed in APL cells (34), and this overexpression is mediated by PPARγ (27). RAR activation leads to increased secretion and decreased catabolism of TG-rich particles, causing the accumulation of TG in the plasma and a secondary decrease in HDL-C levels. Because PML protein was initially described as a tumor suppressor, studies investigating PML have mainly focused on its roles in apoptosis, cell cycle regulation (35), tumorigenesis (36) and tumor metastasis (37). A growing number of studies have also observed an association between PML protein and metabolism. PML is upregulated in metastatic breast cancer and non-metastatic breast cancer with a poor prognosis, and studies indicate that PML may be involved in expression of the stem cell factor SOX9 and associated initiation of breast cancer (37). Carracedo and Pandolfi et al. (35) reported that hepatic PML protein levels are increased in obese individuals, suggesting that PML may be involved in hepatic function. Analyses of the microarray data in PML-depleted Human Umbilical Vein Endothelial Cells suggest that PML is involved in the regulation of a large number of metabolic genes (38, 39). The rearrangement of glucose and fatty acid metabolic gene expression in PML knockout mice, reportedly resulted in an increased metabolic rate and counteracted Western diet-induced obesity symptoms (40). In a previous study there was a negative correlation between PML-RARα expression and PPARγ in APL cells (41). Subsequent experiments suggest that the metabolic stress sensor Tribbles homolog 3 may inhibit the activity of PPARγ by interfering with interaction between PPARγ and RXR, and promoting the ubiquitination and degradation of PPARγ. The synergistic effect of PML-RARα and elevated Tribbles homolog 3 can inhibit the activity of PPAR and cause abnormal lipid metabolism in newly diagnosed APL patients. APL patients. Therefore, PML can be used as a nutritional sensor to maintain metabolic homeostasis.

Abnormal lipid metabolism in APL patients was induced by ATRA therapy. In APL patients ATRA reportedly stimulates the synthesis of cholesterol and triglycerides in the liver, elevating blood lipid levels (42). *In vitro* experiment indicated that TG levels in NB4 APL cell line treated with ATRA were significantly higher than TG levels prior to treatment (27). The G0/G1 switch

gene 2 (G0S2) is also a direct PPARγ target gene and may be involved in the adipocyte differentiation (43). In another study adipose triglyceride lipase was a rate-limiting factor in the inhibition of TG metabolism by G0S2, which partially mediates the therapeutic effects of ATRA in APL by increasing TG levels (44). In addition, the transcription factor forkhead box O1 (FOXO1) is stimulated by retinoid therapy and is inactivated *via* phosphorylation of insulin. This inactivation leads to decreased gluconeogenesis and the release of very low-density lipoprotein by the liver after feeding. FOXO1 stimulates microsomal triglyceride transfer protein and apolipoprotein C-III, which lead to the activation of particle assembly and inhibition of lipoprotein lipase, the two —which are both causes of increased plasma TG (45). Interestingly, the retinoid effects on FOXO1-dependent increases in apolipoprotein C-III are inhibited by PPARγ (46).

Treatment for hypertriglyceridemia includes the management of lifestyle and secondary factors, and pharmacotherapy. The guidelines recommend that if the patient has conditions, such as type 2 diabetes, obesity, alcohol overuse, hypothyroidism, pregnancy, hepatosteatosis, renal failure, or concomitant drug use, the primary disease should be treated. The management of mild-to-moderate hypertriglyceridemia (< 10 mmol/L) should follow recommended guidelines with an initial emphasis on diet and exercise after these secondary conditions have been addressed. The potential benefits of using related inhibitors such as PPARγ agonizing thiazolidinediones require further investigation. Thiazolidinediones (rosiglitazone, pioglitazone) are oral insulin-sensitizing medications used in type 2 diabetes mellitus that can reduce glucose with a minimal risk of hypoglycemia and potential anti-atherosclerotic effects. Some studies suggested that thiazolidinediones can inhibit the proliferation of HL-60 cell lines (47). *In vitro*, pioglitazone induces chronic myelogenous leukemia cells to exit the quiescent state, thereby making them sensitive to the effects of imatinib. After promising results in a case series (48) and a single-arm phase 2 study (49), combination treatment with pioglitazone and imatinib is now under prospective randomized investigation (ClinicalTrials.gov Identifier NCT02767063). Whether it is necessary to use thiazolidinediones in combination in APL requires rigorous investigation.

As time went on, the blood lipid levels were similar in approximately 1 year. Gastrointestinal side effects after chemotherapy in non-APL patients may reduce their intake, but APL induction therapy has little effect on diet. The reason for this is unclear. After only one year of follow-up, the incidence of hypertriglyceridemia in APL was still 46.4%. This may be because they were still on retinoic acid maintenance treatment, or because many APL patients are prone to hypertriglyceridemia due to lifestyle habits such as excessive nutrient intake, low levels of exercise, and relatively low levels of participation in social activities. Our results suggested that elevated TG did not result in shorter DFS or OS under this protocol. Notably in this regard, the complications of associated with hypertriglyceridemia take a long time to manifest. Hypertriglyceridemia remains an important clinical phenomenon, and lipid metabolism is very important in APL.

There are several limitations to this study. The follow-up period was relatively short, limiting the conclusions that could be drawn about many common complications associated with hypertriglyceridemia such as arteriosclerosis and coronary heart disease. In addition, with the prolonged follow-up time, there are more data on blood lipid loss.

In summary, there are evidently significant associations between body weight and hypertriglyceridemia in APL patients, and potential relationships between them require further investigation. Hypertriglyceridemia may be related to the pathogenesis of APL and this may have implications with respect to treatment with ATRA. Relationships between APL, ATRA treatment, and lipid metabolism require further investigation, and such research may ultimately result in improved prognoses.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Research Ethics Committee of the first affiliated Hospital, College of Medcine, Zhejiang University. Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

HZ contributed to the conception and design of the article. JS and YL wrote the manuscript. JJ revised the manuscript. All authors contributed to article revision, read, and approved the submitted version.

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Oral Realgar-Indigo Naturalis Formula Plus Retinoic Acid for Acute Promyelocytic Leukemia

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Treatment paradigm of acute promyelocytic leukemia (APL) is by no mean the most remarkable story of cancer therapy. Recently, the advent of oral arsenic formulations (oral-arsenic trioxide and Realgar-Indigo Naturalis formula (RIF)) based regimens may provide a therapeutic advance by curing APL with two oral agents. Indeed, the oral RIF plus all-trans-retinoic acid (ATRA) without chemotherapy display highly efficacy in patients with APL. The safety profile of RIF plus ATRA make possible to treat APL patients in a home-based manner during postremission therapy. To our knowledge, RIF was the first commercially available oral arsenic agent approved in China. The RIF plus ATRA regimens are becoming a preferred frontline care for APL in China. In this review, we will discuss the history, current evidences and challengers of RIF-based strategies in APL. More and more APL patients may enjoy a cure with a normal quality-of-life after induction in the near future.

Keywords: realgar-indigo naturalis formula, all-trans retinoic acid, PML-RARA, arsenic trioxide, chemotherapy-free

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INTRODUCTION

Acute promyelocytic leukemia (APL) was first described in 1923 by Swiss hematologist Dr. Albert Alder (1). In the 1950s, LK Hillestad and J Bernard further described a case series and recognized as a clinical entity of APL by its unique morphologic characteristics with prominent granules and hemorrhagic diathesis features (2, 3). Importantly, the initial breakthrough was the uncover of the unique chromosomal aberration t(15, 17) (q22;q12) in APL by Dr. J Rowley in 1970s (4). However, it was not until 1990, the landmark molecular fusion of the promyelocytic leukemia (PML) and retinoic acid receptor alpha (RARA) gene was identified (5). Given the central role of PML-RARA in the leukemogenesis of APL, the chimeric oncoprotein was considered an idea molecular target for antileukemic therapy.

Surprising, the targeted therapy to PML-RARA for APL was developed by an empirical-based way. As early as in 1985, all-trans-retinoic acid (ATRA) was first administrated in the induction treatment of APL by Dr. Wang ZY and his team from Shanghai Institute of Hematology (6). The combination of ATRA and anthracycline-based protocol significantly improved the long-term outcome of APL (7). In 1970s, another miracle agent, intravenous arsenic trioxide (ATO) was observed preliminary evidence of efficacy in APL patients by Dr. Zhang TD et al. from Harbin Medical University (8). Subsequent studies by Dr. Chen Z et al. confirmed the high efficacy and

safety of intravenous ATO for APL, initially in relapsed patients and then for newly diagnosed patients (9–11).

Along with the clinical progress, basic investigators have revealed that both ATO and ATRA directly targeted the PML-RARA, which trigger the degradation of the PML-RARA fusion oncoprotein (12). Additionally promising, ATO was found to target PML protein directly (13). The targeted therapy model assumed to eradicate the APL-initiating cells and ultimately reduce the need for chemotherapy (14). In line with this preclinical model, the follow-up trials demonstrated that the ATO plus ATRA targeted approach could definitively cure APL (14). Thus, ATRA and arsenic were defined as a "magic bullet" for APL.

ORAL REALGAR-INDIGO NATURALIS FORMULA DEVELOPMENT AND PILOT STUDIES

Realgar (contains approximately 90% As_4S_4), an oral form of arsenic compound, is an agent originally used as traditional Chinese medicine. As early as in 1980s, Dr. Huang SL et al. initially developed a Realgar-Indigo Naturalis Formula (RIF) for APL. RIF (250 mg per pill) was the combination of four natural products: Realgar (30 mg per pill), Indigo naturalis (125 mg per pill), Radix salviae miltiorrhizae (50mg per pill) and Radix pseudostellariae (45 mg per pill).

Similar to ATO, As_4S_4 induces the ubiquitination and degradation of PML-RARA, and relocalization of PML protein in primary APL cells was observed. Interestingly, the four components of RIF could make synergistic effect and strengthen the antileukemia effect (15). Clinical data accumulated over the last decades of RIF-based therapy in APL. Here, **Table 1** summarized the main publication data of RIF-based treatment for APL.

In 1995, Dr. Huang SL et al. undertook a pilot study of APL patients treated by oral RIF based induction in six Chinese centers from 1988 to 1993 (16). Forty-three patients were newly diagnosed APL and 17 were relapsed APL. Patients received RIF till completely remission (CR) or 60 days. The dose was started with 15 pills daily and gradually ramp-up to 30 pills daily in a week. Glucocorticoid or low-dose chemotherapy was allowed. The overall CR rate was 98.3%. All patients have received RIF more than 30 days achieved CR. Moreover, the side effects of RIF appeared generally manageable. The frequent main side effects were epigastria discomfort, abdominal distention, mild diarrhea, skin rash, and liver enzyme increase. This is the first study reported the efficacy and safety of RIF-based therapy in APL. However, the postremission therapy was not clearly defined and long-term outcomes were not reported.

Subsequently, a team led by Dr. Lu DP from Peking University, first isolated highly purified crystalline As_4S_4 from natural Realgar. A total of 129 APL patients were enrolled during 1994–2000 (17). During induction, As_4S_4 was orally administered at a dosage of 50 mg/kg/day (four times daily) until CR. After CR1, patients received As_4S_4 single agent for postremission therapy. The results were quite encouraging. In

TABLE 1 | Summary of the main publications of Realgar-Indigo Naturalis Formula (RIF)-based regimens in the treatment of acute promyelocytic leukemia (APL).

Study	Trials	Period	No. of patients	High-risk	Age, median (range)	Induction	Postremission	Chem-free	CR (%)	Median follow-up (m)	Long-term outcomes
Huang et al. (16)	Retro	1988–1993	60 (R/R=17)	NA	31(5–72)	RIF	Chem	No	98.3	NA	NA
Lu et al. (17)	Retro	1994–2000	129 (CR1=123; R/R=7)	NA	NA	As4S4	As_4S_4	Yes	100	23	6-year DFS= 87.4%
Lin et al. (18)	Pros; phase 2	2002–2004	61	NA	35(23–47)	RIF	NA	No	96.7	NA	NA
Zhu et al. (19)	Pros; phase 3	2007–2011	114	21	33(15–60)	RIF+ATRA	RIF/ATRA/Chem	No	99.1	61	7-year EFS=93.7%; 7-year OS=95.4%
Yang MH et al. (20)	Pros; phase 3	2011–2017	40	8	9.9 (2–16)	RIF+ATRA	RIF/ATRA/Chem	No	100	36	5-year EFS = 100%; 5-year OS =100%
Zhu et al. (21)	Pros; phase 2	2013–2014	20	0	35(20–58)	RIF+ATRA	RIF/ATRA	Yes	100	14	NA
Zhu et al. (22)	Pros; phase 3	2014–2015	69	0	34 (24–47)	RIF+ATRA	RIF/ATRA	Yes	100	32	2-year EFS= 97%
Zhu et al. (23)	Pros; phase 2	2014–2016	20	20	36(16–61)	RIF+ATRA	RIF/ATRA	Yes	100	33	3-year OS =100%; 3-year EFS 89.4%
Lou et al. (24)	Retro	2015–2019	142	28	41(14–81)	ATO/RIF+ATRA	RIF/ATRA	Yes	NA	20.7	2-year DFS =99%

Chem, Chemotherapy; CR, complete remission; DFS, disease-free survival; EFS, event-free survival; m, month; NA, not available; No., number; OS, overall survival; Pros, Prospective; Retro, Retrospective; RFS, relapse-free survival; RIF, Realgar-Indigo Naturalis Formula.

the newly diagnosed group (n=15), the 3-year disease-free survival (DFS) rates was 76.6%. In the CR1 group (n=99), the 6-year DFS rates were 87.4%. The data suggested that oral As_4S_4 single agent could cure more than 70% of APL patients. This seems consistent with APL patients using single intravenous ATO regimen.

In terms of toxicity, As_4S_4 was usually well tolerated, such as asymptomatic QTc prolongation (33%), transient elevation in liver enzyme levels (10.5%), mild nausea (3.2%), skin itching (3.2%). No myelosuppression occurred during postremission therapy. Pharmacokinetic and pharmacodynamic analysis of As_4S_4 was performed in seven patients. Both blood and urinary arsenic levels eliminated after discontinuation of As_4S_4.

RANDOMIZED TRIALS: ORAL REALGAR-INDIGO NATURALIS FORMULA VERSUS ALL-TRANS-RETINOIC ACID

To further evaluate the effective and side effects of oral RIF in APL, the Chinese APL Cooperative Group conducted a prospective Phase II trial to compare the efficacy and safety of RIF versus ATRA based induction therapy in patients with newly diagnosed APL (18). During induction, patients received RIF (dose ramp up from 15 pills/day to 30 pills/day in 10 days), or ATRA at 25 mg/m^2/day (in two divided doses). Low intensive chemotherapy was used for cytoreduction. By intention-to- treat (ITT) analysis, the CR rate was 80.8% in RIF group versus 75.7% in the ATRA group ($P > 0.05$). The trial suggested that RIF was an alternative choice of APL. The main limitation of the study was not available of long-term outcomes.

RANDOMIZED TRIALS: ORAL REALGAR-INDIGO NATURALIS FORMULA PLUS ALL-TRANS-RETINOIC ACID VERSUS INTRAVENOUS ARSENIC TRIOXIDE PLUS ALL-TRANS-RETINOIC ACID

Based on the phase II trial, the Chinese APL Cooperative Group further conducted a phase III multicenter, randomized, prospective study between 2007 and 2011 (ChiCTR-TRC-12002151), which compared RIF plus ATRA versus ATO plus ATRA based regiment in front-line therapy of all-risk APL (19). During induction, patients were given oral RIF (60 mg/kg/day, orally in three divided doses) or ATO (0.16 mg/kg) combined with ATRA (25 mg/m^2/day, orally in two divided doses). On achieving CR1, patients received three cycles of chemotherapy and maintenance with sequential RIF or ATO plus ATRA for 2 years. The study was a noninferiority design and the primary end point was DFS.

A total of 242 patients were enrolled at seven centers. The 2-year DFS was 98.1% in the RIF group versus 95.5% in the ATO group and confirmed the noninferiority ($P < 0.001$). Similar hematologic and non-hematologic adverse events were reported. Moreover, pharmacodynamics analysis showed oral RIF were similar to intravenous ATO. Thus, this is the first study demonstrate that an oral RIF and intravenous ATO have broadly similar efficacy and safety. In addition, the updated results revealed the estimated 7-year EFS and OS rates were similar between the RIF and ATO groups (94% versus 89%, $P = 0.37$; 95% versus. 91%, $P = 0.31$, respectively) (25). The estimated 7-year EFS and OS were also similar between the high-risk and non-high-risk groups (25).

Since the oral RIF plus ATRA may be easier for pediatric patients to take, the South China Children Leukemia Group (SCCLG) conducted a randomized study to compare the efficacy, safety and the number of hospital days between RIF or f intravenous ATO-based therapies in pediatric APL (20). The induction and consolidation treatment contained ATO or RIF plus ATRA and low intensity chemotherapy. The RIF was given at a dose of 135 mg/kg/day (≯30 pills/day). Intravenously ATO was given at a dose of 0.16 mg/kg/day (≯10 mg/day). The results showed the 5-year EFS was 100% in both groups. The data suggested that oral RIF was similar efficacy and safe with intravenous ATO in pediatric APL patients, with the advantage of reducing hospital stay days.

Base on the phase II trial and the phase III trial, the therapeutic effect of RIF in APL is well established. The Chinese Food and Drug Administration approved RIF as the first-line treatment for APL. RIF was implemented into the treatment algorithm and widely used for APL in China. The optimal recommended dose of RIF was 60 mg/kg/day in adult.

CHEMOTHERAPY-FREE APPROACH: ORAL REALGAR-INDIGO NATURALIS FORMULA PLUS ALL-TRANS-RETINOIC ACID

Although the Chinese APL07 trial achieved excellent long-term outcomes in newly diagnosed APL, the three cycles of consolidation chemotherapy have been associated with myelosuppresion, risk of cardiovascular complications and the development of therapy-related myeloid neoplasms. In 2002, Estey and coworkers from M.D. Anderson Cancer Center published the results of the combined ATRA with intravenous ATO regimen without chemotherapy regimen (only plus gemtuzumab ozogamycin for high risk patients) in newly diagnosed APL (26). The trial suggested that APL patients could be cured by targeted therapy alone. In 2013, the landmark APL0406 trial demonstrated the superior efficacy, safety and quality of life for ATO-ATRA in comparison with the ATRA plus chemotherapy regimen in non-high risk APL patients (27, 28). Thus, the chemotherapy-free approach was moving to the frontline in APL treatment.

Based on the previous intravenous ATO and RIF data, it is logical to apply oral RIF plus ATRA chemotherapy-free in newly diagnosed APL patients. Zhu HH et al. conducted a single arm pilot study of using the oral RIF plus ATRA protocol in non-high risk patients (26). The trial enrolled 20 patients. Oral RIF was administered (60 mg/kg/day) and ATRA (25 mg/m^2/day) as induction therapy. Postremission therapy schedule was RIF with

a 4 weeks on and 4 weeks off and ATRA 2 weeks on and 2 weeks off for 7 months. All patients achieved molecular CR. No patients relapsed during the cut-off date of last follow-up (20).

Subsequently, a multicenter, non-inferiority, randomized, controlled phase 3 trial were conducted at 14 centers in China (22). The trial compared directly of oral RIF plus ATRA with intravenous ATO plus ATRA in newly diagnosed non-high-risk APL patients. The primary outcome was EFS at 2 years. A total of 109 patients were assigned to RIF-ATRA group (n=72) or intravenous ATO-ATRA group (n=37). The 2-year EFS was 97% versus 94%. The non-inferiority outcome was also confirmed.

CHEMOTHERAPY-FREE APPROACH IN HIGH-RISK ACUTE PROMYELOCYTIC LEUKEMIA

Moreover, based on the excellent outcomes of chemotherapy-free approaches in non-high risk APL patients, this raises the question as to whether is it possible for de-escalation or even elimination of chemotherapy for the treatment of high-risk APL patients. Indeed, Zhu et al. conducted an earlier pilot single arm trial with RIF plus ATRA in 20 high-risk patients (23). Minimal cytotoxic agents were allowed during induction. The consolidation approach was RIF (60 mg/kg daily) in a 4-week on and 4-week off regimen for four cycles and ATRA (25 mg/m^2 daily) in a 2-week on and 2-week off regimen for seven cycles, which was the same postremission schedule as in non-high risk group. The data demonstrated that the 3-year estimated OS and EFS are 100% and 89.4%. Although longer-term follow-up, and more patients needed, this study favored the option of chemotherapy-free regimens in high-risk APL patients.

HOME-BASED MANAGEMENT OF PATIENTS DURING POSTREMISSION THERAPY

Oral RIF and ATRA allow home treatment for APL patients during postremission therapy. The regimen may reduce the cost, hospital/clinic visit and health care resources. It is more convenient for both patients and medical staff, especially in the season while COVID-19 became epidemic. In our experience, the safety profile of oral RIF plus ATRA was favorable during postremission therapy. The side effects are usually mild, such as associated with grade 1/2 neutropenia, gastrointestinal toxicity, and edema. Patients usually come in every 2–4 weeks for blood tests and every three months for bone marrow aspiration (24, 29). Majority of patients could go back to work or school with maintaining normal lives.

CRITICAL ISSUES IN THE CURRENT CHEMOTHERAPY-FREE ERA

However, despite the promising activity of oral arsenic plus ATRA chemotherapy-free approach, several challenges remain. First, early death before or during induction remain the most critical challenge in APL. Early diagnosis, intensive supportive therapy and risk-adapted approach may reduce the early complications during induction. For high-risk patients, anthracyclines, or gemtuzumab ozogamycin may still be necessary to control hyperleukocytosis.

Second, although the main ingredient of RIF is As_4S_4, RIF is a compound preparation. Usually, patients need to take about 12–15 pills every day. It would be easy to take if RIF pills were modified by improving producing technology.

Our goal is to cure APL with treatments that are broadly available. RIF is not accessible outside China yet. Fortunately, trials of other oral arsenic formulations are ongoing. Kwong et al. from University of Hong Kong initially use oral-ATO in first relapse, and then in frontline of APL treatment (30). Oral-ATO may be rational to replace the intravenous ATO with emerging evidence increased. Such as, Ravandi et al. conducted a phase I clinical trials of oral-ATO (ORH-2014, NCT03048344) in patients with advanced hematologic malignancies. The Australasian Group also conducted a phase I trial (ACTRN12616001022459) using oral-ATO in APL patients.

Finally, data of chemotherapy-free approach in high-risk patients are still limited. Near future, well-designed clinical trials may possible to optimize the dose and schedule strategy of RIF plus ATRA protocol. The ongoing trials (APOLLO, NCT02688140 by the European intergroup; NCT03624270 by the University of Hong Kong, ChiCTR1900023309 in China) are evaluating the chemotherapy-free approaches in high-risk patients. The results of these trials are very expected indeed.

CONCLUSIONS AND PROSPECTIVE

More than 30 years of experience with RIF, and 10 years since the approval of RIF by the Chinese FDA, the efficacy and safety of RIF-based regiments has been well established (31). Till now, over 8,000 patients with APL have been treated with oral RIF in China. Overall, oral arsenic, a gift from nature, may representative the first oral arsenical formula applied in APL. Near one century after first describe of APL, we believe the treatment of APL is moving toward an entirely oral chemotherapy-free approach among most patients. The oral arsenic plus ATRA will ultimately make treatment safer, less financial burden, and more accessible to patients.

AUTHOR CONTRIBUTIONS

LJ, MF, JJ, and ZH conceptualized the study, wrote the manuscript, and gave the final approval of manuscript. All authors contributed to the article and approved the submitted version.

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REVIEW
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Evolving Chemotherapy Free Regimens for Acute Promyelocytic Leukemia

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With the treatment advances over the last three decades, acute promyelocytic leukemia (APL) has evolved from being the most malignant form of acute leukemia to a leukemia with excellent long term survival rates. In the present review, we have summarized data leading to the development of the currently used treatment regimens for APL, which incorporate either none or minimal chemotherapeutic drugs. We have discussed the historical aspects of APL treatment along with the challenges associated with chemotherapy-based approaches and our experience with the use of single agent arsenic trioxide (ATO) which was one of the first successful, non-chemotherapy approaches used for APL. Subsequently, we have reviewed the data from major clinical trials in low-intermediate risk APL and high risk APL which guide the current clinical practice in APL management. With accumulating data on oral ATO, we postulate that the treatment for low-intermediate risk APL will be a completely oral ATO + ATRA regimen in the future. While for high-risk APL, we believe that minimal anthracycline use with ATO + ATRA might become the standard of care soon. A number of promising non-chemotherapy drugs with pre-clinical data would merit clinical testing in the high risk and relapsed setting, with potential to translate to a complete oral chemotherapy free combination regimen in combination with ATO and ATRA.

Keywords: differentiation therapy, arsenic trioxide, acute promyelocytic leukemia, all-trans retinoid acid (ATRA), non-chemotherapeutic treatment

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INTRODUCTION

Acute promyelocytic leukemia (APL) is a distinct form of acute myeloid leukemia (AML) characterized by t(15;17), a reciprocal translocation leading to a fusion transcript *PML-RARA*. This leads to a block in differentiation of the leukemic cells at the stage of promyelocytes.

APL was first described in 1957 by a Norwegian physician in a case series involving three patients, all of whom were treated with steroids and died. It was noted that APL has a very rapid fatal course and has a severe bleeding tendency making it the "most malignant form of acute leukemia" (1). Subsequently, daunorubicin was noted to result in remission in almost 70% patients with median remission duration of about 2 years. The responses and survival rates, at those times, were similar to those in other patients with a diagnosis of AML (2, 3). However, unique to this subtype of AML was the exacerbation of a coagulopathy, associated with fatality in a proportion of cases, after initiation of chemotherapy (4).

All-Trans Retinoic Acid

The advent of all-trans retinoic acid (ATRA) has revolutionized the treatment in APL with high complete remission (CR) rates without myelosuppression, reduced length of hospitalization, dramatic reduction in coagulopathy, reduced transfusion requirements, and reduction in early treatment related mortality (5). However, despite high CR rates, none of the patients were cured with single agent ATRA (6). It was subsequently demonstrated through a series of landmark studies that a combination of chemotherapy and ATRA used upfront during induction, along with a risk-adapted consolidation approach resulted in excellent long-term survival rates exceeding 80% (7–11).

Arsenic Trioxide

The single most potent therapy for APL both in relapsed as well as upfront therapy for APL is arsenic trioxide (ATO) (12, 13). Historically, arsenic compounds have been used for treating various ailments, like chronic myeloid leukemia, trypanosomiasis, and dermatological conditions including syphilis (14). In China, at the Harbin Medical University, "Ai ling No 1", an arsenic-based traditional Chinese recipe, was evaluated methodically for its therapeutic role in various malignancies (15). This preparation was called 713 (for the year and month of the initiation of this study). They studied more than 1,000 patients with various malignancies and noted that this preparation was maximally beneficial for the treatment of patients with APL. Subsequent studies also confirmed these observations (4). Single agent ATO was shown to result not only in excellent CR rates but also that these remissions were durable, in patients with APL relapsing after ATRA+chemotherapy (12). The dose used was 10 mg daily for adults till achievement of CR. This dose was derived from experience with the doses used in Chinese native medicines and not formal Phase I clinical trials. Subsequently, it was found that ATO was active in APL in doses ranging from 0.06 mg/kg to 0.2 mg/kg and that a dose of 0.15 mg/kg/day can be used in children (16). Various strategies for treating APL incorporating ATO include: upfront use of single agent ATO, use of ATO after achieving remission with ATRA+chemotherapy, use of ATO +ATRA induction followed by chemotherapy consolidation, and use of ATO+ATRA and chemotherapy in induction (17). The long-term cure rates with a combination of ATO and ATRA in low-intermediate risk APL have exceeded 90%–95%, and hence this forms the current standard of care for treating low-intermediate risk APL (18, 19).

Need for Non-Chemotherapy Approaches

Elimination of cytotoxic chemotherapy has potential advantages of reduction in myelosuppression and resulting infections and bleeding, reduction in early risk of hemorrhagic events partly attributed to release of procoagulants after destruction of APL cells, and reduction in risk of long-term complications like cardiotoxicity and secondary myeloid neoplasms (17). Additionally, the health resource utilization and treatment costs are also significantly lower with non-chemotherapy approaches in APL (20). This is of particular significance for low-middle income countries wherein the resources are limited and there is an increasing burden of antimicrobial drug resistance.

OUR EARLY EXPERIENCE WITH NON-CHEMOTHERAPY REGIMEN–SINGLE AGENT ATO

At our institution, two patients with APL who relapsed following chemotherapy (one of them also received ATRA) were advised palliative care. They subsequently chose to take alternative medications containing arsenic (Ayurvedic preparation from Vaidya Balendu Prakash at Dehradun, India) and went into durable remissions. They were administered this preparation continuously and in one case, this was continued beyond 5 years. Of these, one was noted to have severe arsenic keratosis and subsequently died of secondary squamous cell carcinoma (4, 21).

Following this, treatment with single agent ATO for APL was evaluated in the setting of a clinical trial since 1998 at our center. Our hospital pharmacy manufactured the intravenous ATO in-house with appropriate quality control measures. This manufacturing process was subsequently transferred to the industry in the year 2001 (Intas Pharmaceuticals Ltd, Matoda, Gujarat, India). We demonstrated that single agent ATO led to durable remissions in newly diagnosed APL with 5-year overall survival of 74.2 ± 5.2%. The toxicity profile was acceptable with mild reversible toxicity in the majority. There were no sudden cardiac deaths or acute hepatic failure. There were no long-term toxicities in terms of cardiac dysfunction or second malignancies. Additionally, seven patients in our cohort attained normal parenthood after completing ATO treatment (22–24).

In addition to efficacy and safety data, we also reported the pattern of leukocyte recovery following single agent ATO therapy in APL. With ATO treatment, about $1/3^{rd}$ patients have initial prolonged leukopenia followed by gradual normalization of the counts while the remaining $2/3^{rd}$ patients have an initial leukocytic response. We used hydroxyurea (or anthracycline if WBC counts increased despite hydroxyurea) to manage the leukocytosis. The leukocytosis is usually followed by a leukopenic phase of variable duration following which there is recovery to normal WBC counts (21). We reported, for the first time, that FLT3 activating mutations and secondary cytogenetic changes were not associated with an adverse impact on clinical outcome in APL treated with ATO (23, 25).

We also studied the arsenic levels in hair and nail samples of patients and control subjects. We noted no significant difference in the arsenic retention between controls and patients who had completed treatment at least 2 years ago. Also, immediately at the end of treatment, the arsenic levels were less than the lower limit of the normal range defined by the Agency for Toxic Substances and Disease Registry (ATSDR), Atlanta, Georgia (24).

VARIOUS STRATEGIES OF USING ATO IN APL

Upfront use of single agent ATO has been shown to result in excellent long term survival rates in patients having WBC counts less than 5×10^9/L and platelet count $>20 \times 10^9$/L, whereas 5-year overall survival for the high risk patients was 63% (24). Also

multiple courses of ATO consolidation after single agent ATO induction has been shown to improve the disease-free survival in APL (26).

In the North American Leukemia Intergroup study C9710, addition of ATO in consolidation following ATRA + chemotherapy induction, resulted in improved event-free survival (80% versus 63% at 3 years, p 0.001) however the overall survival was similar (86% versus 81% at 3 years, p 0.059) (27).

Pre-clinical studies showed synergy between ATO and ATRA both in *in vitro* experiments with APL cell lines as well as in animal model experiments with a possible benefit of sequential therapy over concurrent treatment. These have further formed the rationale for early clinical studies using the chemotherapy free combination of ATO and ATRA (28, 29).

A combination of ATO and ATRA used during induction followed by chemotherapy-based consolidation has been shown to be better than either agent used alone in terms of the time to CR, time to platelet recovery, fold change in the *PML-RARA* transcripts and also the long-term relapse risk (30).

Use of ATO-ATRA along with idarubicin during induction followed by ATO+ATRA consolidation and maintenance with ATRA/6-Mercaptopurine/Methotrexate resulted in excellent long-term survival exceeding 90% in the Australian APML4 study, with reduction in anthracycline exposure to a large extent (31).

LOW AND INTERMEDIATE RISK APL

Low-intermediate risk APL is defined as APL with initial white blood cell count $\leq 10 \times 10^9$/L) (32). Randomized clinical trials (GIMEMA-AMLSG-SAL APL0406 and UK NCRI AML17) have demonstrated the superiority of combination therapy with ATO and ATRA (ATO + ATRA) over conventional therapy with ATRA and chemotherapy (ATRA + chemotherapy) in low-intermediate risk APL (18, 19).

APL0406 (ClinicalTrials.gov Identifier: NCT00482833)

In the APL0406 trial, with the median follow-up being 40.6 months, treatment with ATO + ATRA resulted in significant improvement in the overall survival in patients with low-intermediate risk APL as compared to ATRA + chemotherapy treatment (99.2% versus 92.6% at 50 months, p = 0.0073). The updated results from the APL0406 study showed an increasing benefit over time with ATO + ATRA as compared to ATRA + chemotherapy. At a median follow up of 66.4 months, the 6-year event-free survival was significantly better with ATO + ATRA as compared to ATRA + chemotherapy (96.6% versus 77.4%, p 0.0002). The cumulative incidence of relapse was significantly lower with ATO + ATRA as compared to ATRA + chemotherapy (1.7% versus 15.5%, p 0.02).

The proportion of patients with grade 3–4 thrombocytopenia and neutropenia for more than 15 days, those with infections and fever of unknown origin, and those with grade 3–4 gastrointestinal toxicity was lower with ATO + ATRA as compared to ATRA + chemotherapy. The proportion of patients with grade 3–4 hepatotoxicity and QTc prolongation during induction, and those with all grades of neurotoxicity during consolidation was greater with ATO + ATRA as compared to ATRA + chemotherapy. The toxicity resolved in all patients with temporary discontinuation of therapy except one patient who required ATO's permanent discontinuation. During induction, there were four deaths in the ATRA + chemotherapy group (1 cardiovascular, 1 ARDS, 1 ischemic stroke, and 1 respiratory disease) with none in the ATO + ATRA group. Post-induction, there were five deaths while in remission in the ATRA + chemotherapy group, of which two had therapy-related myeloid neoplasm, while two died while in remission in the ATO + ATRA group of which one had colon carcinoma. Additionally, there were four deaths due to relapse in the ATRA + chemotherapy group (19, 33).

UK NCRI AML17 (International Standard Randomized Controlled Trial Number ISRCTN55675535)

The UK NCRI AML17 trial primarily looked at the quality of life measured using the European Organization for Research and Treatment of Cancer (EORTC) QLQ-C30 global health status in APL patients treated with ATO + ATRA versus ATRA + chemotherapy. They had used an attenuated ATO schedule with ATO 0.3 mg/kg IV for 5 days in week 1 followed by 0.25 mg/kg IV twice a week from week 2 onward during both induction and consolidation in variance with the daily ATO used in APL0406 trial (**Table 1**). They did not report a significant difference in the quality of life between the patients treated in the two groups studied. In fact, for cognitive and role functioning domains, significant benefits were recorded in favor of ATO + ATRA. Most patients (93%) with high risk APL in the ATO + ATRA group received gemtuzumab ozogamicin. In the updated results from the AML17 trial, at a median follow-up of 67.4 months, frank relapses were significantly lower with ATO (1% versus 5% at 5 years, p 0.005) resulting in improved relapse-free survival irrespective of the risk group (for low risk 95% versus 87%, p 0.3, while for high risk 100% versus 83%, p 0.03).

The 60-day mortality was similar with ATRA + chemotherapy and ATO + ATRA (9% versus 5%, p 0.22). The causes of death in the ATO + ATRA group were: 3 cardiac events, 1 renal failure, 1 infection, and 1 due to several causes, while for 11 patients in the ATRA + chemotherapy group were: 3 hemorrhages, 3 infections, 2 pulmonary causes, 1 renal cause, and 2 progressive disease. The incidence of therapy-related myeloid neoplasm was 6%, with no case seen after ATO + ATRA. Of 32 patients (including 17 with a molecular relapse) who relapsed on ATRA + chemotherapy group, 31 patients attained molecular CR with ATO and had 5 year survival of 88% with a median follow-up of 44.9 months. The highly effective salvage therapy with ATO and minimal residual disease monitoring resulted in a lack of overall survival benefit between the two arms of the AML17 trial in variance with the APL0406 trial. Hyperbilirubinemia, cardiac events, gastrointestinal events, and alopecia were more frequent in the ATRA + chemotherapy arm during treatment course 1. The proportion of

TABLE 1 | Different treatment schedules and survival rates for major trials on APL.

Study	Induction	Consolidation/ Maintenance	Toxicity	Survival data	Cumulative ATO and anthracycline dose
APL0406 (low-intermediate risk) (19, 33) ATO+ATRA arm	ATO 0.15mg/kg IV + ATRA 45mg/m^2 PO – maximum of 60 days (median time to CR – 32 days)	ATO 0.15mg/kg IV for 5 days per week, 4 weeks on 4 weeks off, for 4 courses and ATRA 45mg/m^2 PO daily 2 weeks on and 2 weeks off, for 7 courses	No induction deaths; 2 deaths while in remission post induction	At 50 months, OS of 99.2% (95%CI: 97.7 to 100)	16.8mg/kg to 21mg/kg ATO
APL0406 (low-intermediate risk) (19, 33) ATRA+CHT arm	Idarubicin 12mg/m^2 IV on days 2,4, 6, and 8 along with daily ATRA 45mg//m^2 PO for up to 60 days	Idarubicin 5mg/m2/day IV for days 1 to 4 (first cycle), mitoxantrone 10mg/m2/day IV on days 1-5 (second cycle) and idarubicin 12mg/m2 IV on day 1(third cycle). ATRA 45mg/m2/day PO from day 1 to day 15 during each consolidation cycle. Maintenance – 6-MP 50mg/m2/day, MTX 15mg/m2/week alternating with ATRA 45mg/m2/day given for 15 days every 3 months	4 deaths during induction and 5 while in remission post induction	At 50 months, OS of 92.6% (95%CI: 87.9 to 97.5)	80mg/m2 of idarubicin and 50mg/m2 of mitoxantrone
UK AML 17 (all risk groups) (18, 34) ATO+ATRA arm	ATO 0.3mg/kg IV for 5 days in week 1 followed by 0.25mg/kg twice a week for week 2 to 8.ATRA 45mg/m2 PO daily up to 60 days GO 6mg/m2 single dose within 4 days for high risk.	Course 2 to 5: ATO 0.3mg/kg IV for 5 days in week 1 followed by 0.25mg/kg twice a week for week 2 to 4. ATRA 45mg//m2 PO 2 weeks on and 2 weeks off.	60-day mortality – 5%, Therapy related myeloid neoplasms - none	5-year OS 92%	17mg/kg ATO
UK AML 17 (all risk groups) (18, 34) ATRA+CHT arm	Idarubicin 12mg/m2 IV on days 2,4, 6, and 8 along with daily ATRA 45mg/m2 PO for up to 60 days	Idarubicin 5mg/m2/day for days 1 to 4 (first cycle), mitoxantrone 10mg/m2/day on days 1-5 (second cycle) and idarubicin 12mg/m2 on day 1(third cycle). ATRA 45mg/m2/day from day 1 to day 15 during each consolidation cycle.	60-day mortality – 9%; Therapy related myeloid neoplasms – 6%	5-year OS 86%	80mg/m2 of idarubicin and 50mg/m2 of mitoxantrone
APML4 study (All risk groups) (35)	ATRA 45mg/ m2 days 1-36. Idarubicin 12mg/ m2 for age 1 to 60 years on day 2, 4, 6, and 8 ATO 0.15mg/kg IV days 9 to 36	ATRA 45mg/ m2 and ATO 0.15mg/kg IV from day 1 to 28 in cycle 1. ATRA 45mg/ m2 on days 1-7, 15-21, 29-35 and ATO 0.15mg/kg IV 5 days in a week for 5 weeks in cycle 2. 8 cycles of maintenance: ATRA 45mg/ m2 days 1-14 along with MTX 5-15mg/ m2 and 6-MP 50-90 mg/ m2 on days 15-90.	Early death rate - 3.2%; No therapy related myeloid neoplasm	5-year OS 96% (95%CI: 90-99) for low-intermediate risk and 87% (95%CI: 65-96) for high risk	12.15mg/kg ATO 48mg/m2 of idarubicin
MDACC (All risk groups) (36, 37)	ATO 0.15mg/kg IV + ATRA 45mg/m2 PO – till CR (median of 30 days) GO 9mg/m2 or Idarubicin 12mg/m2 on day 1 in case of high risk	ATO 0.15mg/kg IV 5 days per week, 4 weeks on 4 weeks off, for 4 courses and ATRA 45mg/m2 PO daily 2 weeks on and 2 weeks off for 7 courses	Induction mortality - 4%	5 year OS 89% for low risk and 86% for high risk	16.5mg/kg ATO 12mg/m2 idarubicin
SWOG 0535 (high risk) (38)	ATO 0.15mg/kg IV + ATRA 45mg/m2 PO – till CR (median of 39.5 days) GO 9mg/m2 on day 1	ATO 0.15mg/kg IV for 25 days (Cycle 1 and 2). Daunorubicin 50mg/m2 for 3 days and ATRA 45mg/m2 for 7 days (cycle 3 and 4) and GO 9mg/m2 on day 1 (cycle 5 and 6) Maintenance 1 year : ATRA 45mg/m2 for 7days (every 14 days) with 6-MP 60mg/m2 and MTX 20mg/m2 weekly	6-week mortality – 11%	3 year OS of 86% (95%CI: 75 to 92)	13.5mg/kg ATO 300mg/m2 daunorubicin

(APL, acute promyelocytic leukemia; CHT, chemotherapy; CR, complete remission; ATO, arsenic trioxide; ATRA, all-trans retinoic acid; GO, gemtuzumab ozogamicin; 6-MP, 6-mercaptopurine; MTX, methotrexate; OS, overall survival).

patients with high alanine transaminase (ALT) levels between the two groups was similar. After treatment course 1, the grade 3–4 ALT elevation was more common with ATO + ATRA than ATRA + chemotherapy (20% grade 3 and 5% grade 4 versus 8% grade 3 and 2% grade 4). After course 2, liver toxicities did not differ between the 2 groups while cardiotoxicity was more frequent with ATO + ATRA (7% grade 1–2, 3% grade 3, and none grade 4). Treatment discontinuation was required for 2 patients on ATO+ ATRA while on induction and for 6 while on consolidation (as per clinician's decision, with 2 having QTc prolongation). ATO + ATRA was associated with lesser days of hospitalization, lower transfusion, and intravenous antibiotic requirement as compared to ATRA + chemotherapy during the first two courses of therapy (18, 34).

HIGH RISK APL

High risk APL is defined as APL with initial white blood cell count >10 × 10^9/L) (32). In high-risk APL, broadly two approaches—either with minimal anthracycline use during induction or with the use of gemtuzumab ozogamicin, have been combined with ATO + ATRA.

APML4 Study (Australian New Zealand Clinical Trials Registry, number ACTRN12605000070639)

The APML4 study was a non-randomized phase 2 trial which looked at the freedom from relapse and early death with the addition of ATO to ATRA and idarubicin in induction with an ATO + ATRA consolidation. Thus, there was minimal use of anthracycline limited to induction therapy alone. However after two cycles of consolidation, patients received maintenance therapy with ATRA, 6-mercaptopurine, and methotrexate for 2 years. The results were compared with that of the earlier APML3 study which had excluded ATO. The early death rate was lower (3.2% versus 7.1%) however this did not achieve statistical significance. Among the 124 patients studied, 23 were high risk as per the Sanz risk stratification. Among the high risk patients, on comparison with the APML3 results, the freedom from relapse was significantly better (2-year rate of 95% versus 69%; p 0.024) however the overall survival was similar (2-year OS 87% versus 87%; p 0.40). For high risk disease, the cumulative incidence of relapse was 5% at 5 years compared to 31% at 2 years in the APML3 study. Also there were no deaths in remission during consolidation. There were no instances of secondary myelodysplasia or leukemia reported (35).

Gemtuzumab Ozogamicin

Addition of gemtuzumab ozogamicin (GO) to the combination of ATO + ATRA in induction is a promising approach which has been evaluated in multiple studies (36–38). In the MD Anderson Cancer Center (MDACC) trial (ClinicalTrials.gov Identifier: NCT01409161), of the 187 patients studied, 54 were high risk. The induction mortality was 4%. The CR rate in low risk as well as high risk patients was 96% with a median time for attaining CR of 30 (17–80) days. Among the 54 high risk patients, 45 received GO 9mg/m^2 on day 1 while the remaining received idarubicin 12 mg/m^2 on day 1 due to lack of availability of GO. The 5-year EFS and OS for the high risk group was 81% and 86% respectively. Five patients with high risk disease developed disease relapse. Treatment related adverse events included hepatotoxicity grade 3 and above in 14% patients and infections grade 3 and above in 23.5% patients.

Similarly, as mentioned earlier in the UK NCRI AML17 study GO was added to ATO+ATRA in the high risk subset and was associated with a 100% relapse-free survival, however the study was not powered to address efficacy in this subset.

In the SWOG study (ClinicalTrials.gov Identifier: NCT00551460), patients with high risk APL were treated with ATO + ATRA and GO in induction, followed by consolidation with two cycles of ATO, two cycles of ATRA with chemotherapy (daunorubicin) and two cycles of GO. They also included 1 year of maintenance with ATRA, 6-mercaptopurine, and methotrexate. They studied 70 patients with high risk APL. The 6-week mortality rate was 11% and 86% patients achieved CR at the end of induction. Treatment related adverse events during induction included about elevated liver enzymes grade 3 and above in 16% patients and infections grade 3 and above in 20%. The 3 year EFS was 78% (95% confidence interval: 67% to 86%). About 37% patients in remission were not able to complete the planned post-remission therapy including 12% due to adverse events.

ORAL ATO

Three oral formulations of ATO are being developed. One is a liquid formulation and has been shown to have comparable efficacy similar to IV ATO. The safety and efficacy of this formulation has been established in frontline setting given after ATRA and chemotherapy (39). The second one is oral arsenic realgar-Indigo naturalis formula (RIF) given as 60 mg/kg bodyweight daily. In non-high risk patients, this formulation is non-inferior to intravenous ATO with a similar adverse effect profile (40). Thirdly, ORH-2014 is another oral formulation for which pharmacokinetic and safety data is available (41).

OTHER POTENTIAL NON-CHEMOTHERAPY OPTIONS IN APL

Targeting Microenvironment Mediated Resistance to ATO by Downregulating the NK- kB Pathway

We have been using ATO-based therapy for upfront treatment of APL for the last two decades (24). We have shown that there is a significant contribution of microenvironment mediated resistance to ATO at relapse (42) and demonstrated the importance of the NF-kB pathway in mediating this resistance. We further demonstrated a

synergistic effect of bortezomib, a proteasome inhibitor which is known to downregulate the NF-kB pathway, and ATO in overcoming ATO resistance in pre-clinical studies. This synergy is due to downregulation of the NF-kB pathway, increased generation of reactive oxygen species in malignant cells, and an increased unfolded protein response. Since ATO is known to cause PML-RARA degradation through the proteasome, a theoretical concern with the use of a proteasome inhibitor with ATO was that whether the proteasome inhibitor would inhibit the degradation of the PML-RARA oncoprotein. However, we noted that with the combination of ATO and bortezomib, the PML-RARA oncoprotein is cleared through an alternative p62-dependent autophagy pathway (43). This work has been translated to a phase 2 clinical trial for relapsed APL wherein we have demonstrated that a combination of ATO, ATRA, and anthracycline with the addition of bortezomib is safe (44) (**Figure 1**).

Targeting Epigenetic Resistance to ATRA by Activating MEK/ERK Signaling

Drug resistance to ATRA is mediated by mutations in the ligand binding domain of PML-RARA or epigenetic modifications in the RARA promoter preventing expression of genes targeted by retinoic acid. The permissiveness of the RAR promoter is restored by activating the MEK/ERK signaling pathway (45).

It has also been demonstrated that the *in vitro* resistance to ATRA in APL cell lines can be overcome by use of glycogen synthase kinase-3beta inhibitors including lithium chloride by restoring ATRA-induced differentiation. This effect is abolished by inhibition of the MEK/ERK1/2 pathway. In *in vivo* mouse models, lithium chloride combined with ATRA resulted in a significant survival advantage as compared to ATRA alone (46).

Targeting Energy Metabolism and Anti-Apoptotic Pathways

We have previously shown that there exists a significant change in the energy metabolism pathways in APL cell lines resistant to ATO (47). This forms a rationale for targeting APL with energy metabolism inhibitors.

The vitamin E derivative (+) α-tocopheryl succinate acts by inhibiting the mitochondrial respiratory chain complex I. It exerts pro-apoptotic effects in various tumors. In a mouse model of APL, this compound has been shown to be as effective as ATO or ATRA (48).

Also, targeting anti-apoptotic pathways can be potentially explored in APL. In fact, primary APL samples have been shown to be significantly more sensitive to venetoclax than non-APL AML samples (49). The therapeutic role of mitocans, such as venetoclax, in APL remains to be explored.

Synergistically Enhancing Differentiation

Valproic acid also has been shown to induce differentiation in APL and has synergy with ATRA in preclinical models (50). Also, 1alpha, 25-dihydroxyvitamin D3 and vitamin K2 derivatives have been shown to augment retinoic acid induced differentiation of APL cell lines (51, 52).

WAY FORWARD

Table 1 summarizes the major clinical trials in APL which have explored treatment approaches either without or with minimal chemotherapy. Non-chemotherapy approach consisting of ATO + ATRA is the current standard of care for treating low-

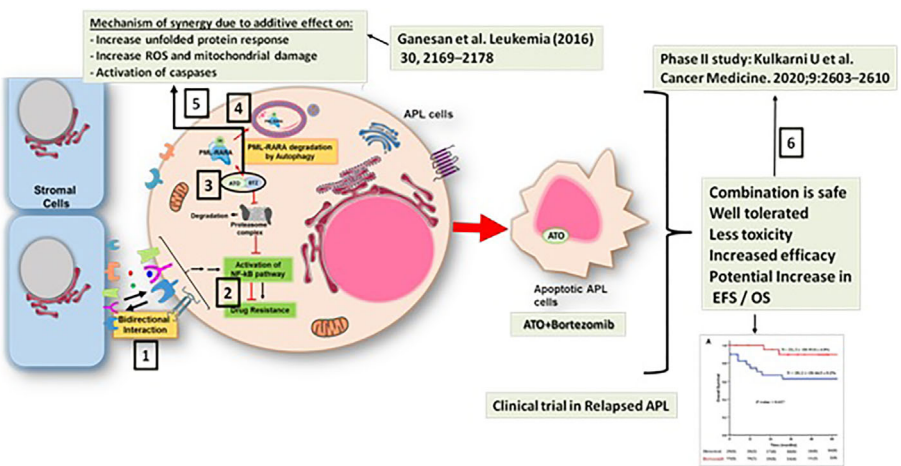

FIGURE 1 | Synergy between arsenic trioxide (ATO) and bortezomib in the treatment of acute promyelocytic leukemia. 1. There is a significant contribution of microenvironment mediated resistance to ATO at relapse (41). 2. This resistance is mediated predominantly by the NF-kB pathway (41). 3. Preclinical studies show a synergistic effect of bortezomib and ATO in overcoming ATO resistance (42). 4. With the combination of ATO and bortezomib, the PML-RARA oncoprotein is cleared through a p62-dependent autophagy pathway (42). 5. The mechanism of the synergy between ATO and bortezomib involved downregulation of the NF-kB pathway, increased unfolded protein response, and an increased generation of reactive oxygen species in the malignant cell (42). 6. This work has been translated to a phase 2 clinical trial for relapsed APL wherein we have demonstrated that the combination of ATO, ATRA, and anthracycline with the addition of bortezomib is safe (43).

TABLE 2 | Anticipated treatment algorithm for APL in future.

Treatment phase	Low-intermediate risk APL	High risk APL
Induction	Oral ATO + oral ATRA • Dose and schedule optimization and possible addition of other non-chemotherapy agents to further reduce cumulative dose of ATO and ATRA and their side effects	Oral ATO + oral ATRA + either of the following: • Minimal anthracycline, • Gemtuzumab ozogamicin (targeting high CD33 expression on APL cells), • Bortezomib (targeting microenvironment mediated ATO resistance *via* NF-kB signaling), potential for potent oral proteasome inhibitors to be evaluated here • Glycogen synthase kinase -3beta inhibitors like lithium chloride (targeting epigenetic modification of the RARA promoter *via* MEK/ERK signaling), • Vitamin E or venetoclax (targeting energy metabolism or anti-apoptotic pathways), • Valproic acid or 1alpha, 25-dihydroxyvitamin D3 (synergistic differentiating activity with ATRA)
Consolidation	Oral ATO + oral ATRA	Oral ATO + oral ATRA

(APL, acute promyelocytic leukemia; ATO, arsenic trioxide; ATRA, all-trans retinoic acid).

intermediate risk APL based on evidence from randomized clinical trials. For high risk disease, the TUD-APOLLO-064 randomized controlled trial is evaluating ATO + ATRA + idarubin-based induction regimen for high risk APL patients with ATO + ATRA consolidation as post-remission strategy with event-free survival as the primary outcome and standard AIDA regimen as the comparator. The results of this study are expected in the near future and are eagerly awaited.

Table 2 shows the anticipated treatment algorithm for APL in the future. With long term safety and efficacy data with oral ATO, we could possibly have a completely oral regimen of ATO + ATRA for low-intermediate risk APL. For high risk APL, if randomized data (TUD-APOLLO-064, ClinicalTrials.gov Identifier: NCT02688140) show superiority over conventional AIDA regimen, ATO + ATRA with minimal anthracycline use could be the standard therapy in the near future as shown in the anticipated treatment algorithm in **Table 2**. Other potential drugs that can be explored in the setting of high risk or relapsed disease have been shown in **Table 2**. Targeting microenvironment mediated drug resistance *via* the NF-kB signaling using bortezomib is an approach which we are currently exploring (43, 44). Combining glycogen synthase kinase-3beta inhibitors like lithium chloride with ATRA for targeting epigenetic mechanisms of resistance to ATRA *via* MEK/ERK signaling is another interesting approach. Also, lithium chloride is known to induce autophagy which is also a key component of ATRA induced differentiation in APL (46, 53). Targeting energy metabolism and antiapoptotic pathways using drugs like vitamin E and venetoclax respectively also could have potential therapeutic significance in high risk or relapsed APL, as could agents like vitamin D and Valproic acid showing synergy in differentiation in APL (48–51). All these approaches could pave the way for completely non-chemotherapy approaches in the high risk and relapse settings as well.

Over the last few decades, APL has transformed from being the "most malignant form of acute leukemia" to the "most curable form of acute leukemia" (54). Other than development of non-chemotherapy approaches, the current controversies in this field include optimal management of early deaths related to coagulopathy (like the role of recombinant thrombomodulin), the optimal dosing of ATRA and ATO, the optimal therapy for high-risk APL, the role for intrathecal prophylaxis, the role of prophylactic corticosteroids during induction therapy, and the need for maintenance therapy after consolidation (55, 56). Limitations of the present review are that we have not covered these controversies in the management of APL other than the non-chemotherapy approaches and also that we did not have a pre-defined search strategy for literature review.

AUTHOR CONTRIBUTIONS

UK wrote the manuscript. VM conceptualized and reviewed the manuscript. All authors contributed to the article and approved the submitted version.

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REVIEW
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Role of Hematopoietic Stem Cell Transplantation in Acute Promyelocytic Leukemia

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The indication of hematopoietic stem cell transplantation (HSCT) in acute promyelocytic leukemia (APL) has evolved historically from a widespread use in front-line therapy during the pre-ATRA era to a virtual rejection of this indication for patients treated with modern treatments. HSCT in first complete remission could only be considered for an extremely small fraction of patients with persistent MRD at the end of consolidation or for those who relapse. In the pre-ATO era, relapsed patients were usually treated with readministration of ATRA and chemotherapy as salvage therapy, generally containing high-dose cytarabine and an anthracycline, followed by further post-remission chemotherapy and/or HSCT. ATO-based regimens are presently regarded as the first option for relapsed APL. The selection of the most appropriate post-remission treatment option for patients in second CR (CR2), as well as the modality of HSCT when indicated, depends on several variables, such as pre-transplant molecular status, duration of first remission, age, and donor availability. Although with a moderate level of evidence, based on recent retrospective studies, autologous HSCT would be at present the preferred option for consolidation for patients in molecular CR2. Allogeneic HSCT could be considered in patients with a very early relapse or those beyond CR2. Nevertheless, the superiority of HSCT as consolidation over other alternatives without transplantation has recently been questioned in some studies, which justify a prospective controlled study to resolve this still controversial issue.

Keywords: acute promyelocytic leukaemia, hematopoietic (stem) cell transplantation (HCT), all-trans retinoic acid (ATRA), arsenic trioxide, relapse

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INTRODUCTION

Modern treatment approaches for patients with newly diagnosed APL, using the combination of all-trans retinoic acid (ATRA) with either arsenic trioxide (ATO), chemotherapy or both, result in 90% to 95% complete remission (CR) rates with virtual absence of primary resistance, and 85% to 90% rates of long-term survival (1). Different to most subtypes of acute myeloid leukemia, these outstanding results in APL have been obtained without consolidating patients in first CR (CR1) with hematopoietic stem cell transplantation (HSCT). In this context, HSCT has virtually ceased to play

any role in CR1 and relegated to consolidate patients in CR2 or beyond after salvage therapy for relapsed APL (1–3).

In this review article, we will discuss the clinical outcomes of HSCT in patients with APL and discuss the issues related to this indication, including the preferred choice of donor, source of hematopoietic stem cells, and conditioning regimen, among other controversial matter. We will also address the main prognostic factors for transplant outcomes that have been identified. We aim to provide insight into decision making regarding the optimal use of HSCT in patients with APL.

HSCT HAS NO ROLE IN FRONT-LINE THERAPY FOR NEWLY DIAGNOSED APL

The indication of HSCT in patients with APL has evolved historically from a widespread use of this procedure in front-line therapy during the pre-ATRA era to a virtual rejection of this indication when patients are treated with modern treatments containing ATRA. Except for the beginning of the ATRA era, in which many groups still continued to indicate an HSCT in CR1 (4, 5), this has gradually been abandoned and explicitly rejected by the European LeukemiaNet (ELN) recommendations (1, 2) and the National Comprehensive Cancer Network (NCCN) guidelines (3).

Currently, the general consensus is that HSCT has no role patients in CR1, even in high-risk patients.

HSCT AS CONSOLIDATION THERAPY IS BETTER THAN NON-HSCT IN RELAPSED APL

Only a few small retrospective and uncontrolled studies have compared the therapeutic efficacy of HSCT versus consolidation without transplantation in relapsed patients with APL (**Table 1**). Most of these studies showed that transplanted patients had a higher event-free survival (EFS), ranging from 61% to 83% for auto-HSCT (6, 7, 9) and from 41% to 71% for allo-HSCT (6, 9, 10), compared to those not transplanted, ranging from 30% to 45% (6, 7, 10). It should be noted, however, that these differences were statistically significant in only two of these studies (6, 7).

The superiority of transplantation versus non-transplantation was even more apparent for overall survival (OS), which ranged from 60% to 100% for auto-HSCT (6–11) and 49% to 79% for allo-HSCT (6, 8–10), while it was 39% to 75% for those not transplanted (6–11). Except from two small studies (9, 10), the remaining ones showed a significantly higher OS (6–8, 11). Not without reason, it can be argued that differences in survival outcomes could be explained, at least in part, by a selection bias, since transplantation is ruled out in a sizable proportion of patients because they are considered clinically unfit. However, this explanation would not be enough, since practically all the studies have shown a higher relapse rate in patients undergoing consolidation treatment without transplantation (6, 7, 10), including a retrospective ELN study in relapsed APL treated with ATO-based regimens (8). It should be noted, however, that some groups have recently reported prolonged remissions in series of patients relapsing after ATRA plus chemotherapy treated with ATO plus ATRA without transplant.

Based on the previously mentioned studies, the ELN recommendations (1) and the NCCN guidelines (3) consider transplantation as the best option to consolidate patients in CR2 or beyond after salvage therapy for relapsed APL. This is, however, an unresolved issue, since some recent reports question the need for transplantation, at least in patients who achieve molecular remission with ATO and ATRA (1) or ATO, ATRA, mitoxantrone and bortezomib (12). Therefore, a prospective controlled study is warranted to address this still controversial issue.

TABLE 1 | Hematopoietic stem cell transplantation (autologous and/or allogeneic) compared with non-transplantation in relapsed APL.

Reference	Study period	Salvage therapy	No. of patients			Event-free survival	Overall survival	Relapse rate	Time
			Auto	Allo	Non-HSCT				
De Botton et al. (6)	1992-2001	ATRA+CHT	50	23	49	61 vs 52 vs 30 P = 0.002	60 vs 52 vs 40 P = 0.005	87 vs 92 vs 38[a] P = 0.005	7-year
Thirugnanam et al. (7)	1998-2006	ATO-based	14	–	19	83 vs 34 P = 0.001	100 vs 39 P = 0.001	7 vs 63[b] P < 0.0001	5-year
Lengfelder et al. (8)	2003-2011	ATO-based	60	33	55	NA	77 vs 79 vs 59 P = 0.09	37 vs 39 vs 59 P = 0.05	3-year
Pemmaraju et al. (9)	1980-2010	Miscellaneous	10	17	16	69 vs 41 vs NA P = 0.45	86 vs 49 vs 40 P = 0.48	NA	7-year
Fujita et al. (10)	1997-2002	ATRA+CHT	6	21	30	42 vs 71 vs 45 NS	83 vs 76 vs 75 NS	58 vs 10 vs 51[c] P = 0.007	7-year
Ganzel et al. (11)	<2000-2011	ATO-based	140	–	67	NA	78 vs 42 P < 0.001	NA	5-year

NA, not available; NS, not significant.

[a]*Relapse-free survival.*

[b]*Crude relapse rate.*

[c]*Allo vs auto: P = 0.007; allo vs non-HSCT: P = 0.009.*

THE CHOICE OF AUTOLOGOUS OR ALLOGENEIC HSCT

Once it has been shown that consolidation with HSCT, either auto-HSCT or allo-HSCT, results in better survival outcomes than non-transplant strategies for patients in CR2 or beyond, the next question that arises is the appropriate type of transplant to be done. The choice of autologous or allogeneic transplantation should be based on the modality that has shown higher efficacy in this setting. Again a few small, retrospective and uncontrolled studies have compared the therapeutic efficacy of auto-HSCT and allo-HSCT in relapsed patients with APL (**Table 2**). None of these studies was able to demonstrate superiority of one modality of HSCT over the other in EFS, except from an analysis of the Acute Leukemia Working Party (ALWP) of the European Blood and Marrow Transplantation (EBMT) that has been recently published (16). In this large study that included 341 and 228 APL patients in CR2 who underwent auto-HSCT and allo-HSCT, respectively, EFS was significantly higher in the former group (75% vs 55%). Auto-HSCT was also superior to allo-HSCT in terms of OS, not only in this study, but also in other large studies (6, 15). The most likely explanations for finding a clearer benefit in OS than in EFS could be, on the one hand, because the potential benefit of a graft-versus-leukemia (GVL) effect in patients undergoing allo-HSCT would be widely counterbalanced by a higher transplant related mortality (TRM) systematically reported in that setting compared with patients undergoing auto-HSCT (5, 6, 9, 10, 13–15). In fact, no study was able to demonstrate a significant reduction in the relapse rate, while the two largest studies reported a significantly higher rate of TRM in the allo-HSCT (15). On the other hand, a second relapse after auto-HSCT is probably a clinical situation with a higher chance of subsequent salvage as compared to a second relapse after allo-HSCT. This would also explain that, in most series, the increase in the OS rate with respect to the EFS is significantly greater in patients undergoing auto-HSCT compared to those undergoing allo-HSCT (9, 10, 15).

In addition to comparative studies providing evidence that auto-HSCT is a better treatment strategy than allo-HSCT in relapsed APL (**Table 2**), other large case series have also demonstrated the outstanding efficacy and feasibility of that procedure after salvage therapy with ATO-based treatment (17), improving the outcomes of those with ATRA plus chemotherapy-based approaches (18).

THE CHOICE OF STEM CELL SOURCE AND CONDITIONING REGIMEN

As far as we know, there are no prospective and controlled studies comparing results between peripheral blood and bone marrow as a source of hematopoietic progenitors in the specific context of APL. However, it is a fact that peripheral blood has been by far the preferred stem cell source used in most studies analyzing auto-HSCT in this disease (**Table 3**). Except for the previous EBMT study conducted between 1993 and 2003 (5), in which the proportion of patients transplanted with peripheral blood was 53%, all the remaining comparative studies reported percentages in the narrow range between 86% and 100%, including the last EBMT study in which the proportion increased to 92% (16). It should be noted, however, that the few retrospective studies that compared bone marrow with peripheral blood showed that the stem cell source did not

TABLE 2 | Autologous versus allogeneic hematopoietic stem cell transplantation in relapsed APL.

Reference	Study period	Salvage therapy	No. of patients		Event-free survival	Overall survival	Relapse rate	TRM rate	Time
			Auto	Allo					
Sanz et al. (5)	1993-2003	ATRA+CHT	195	137	51 vs 59[a]	NA	37 vs 17 P = NA	16 vs 24 P = NA	5-year
De Botton et al. (6)	1992-2001	ATRA+CHT	50	23	61 vs 52 P = 0.11	60 vs 52 P = 0.04	79 vs 92[a] P = 0.19	6 vs 39 P = NA	7-year
Kohno et al. (13)	1999-2004	Miscellaneous	15	13	69 vs 46[b] P = 0.4	76 vs 46 P = 0.2	21 vs 9 P = NS	20 vs 46 P = NA	4-year
Lengfelder et al. (8)	2003-2011	ATO-based	60	33	NA	77 vs 79 NS	37 vs 39 NS	NA	3-year
Pemmaraju et al. (9)	1980-2010	Miscellaneous	10	17	69 vs 41 P = 0.45	86 vs 49 P = NS	30 vs 18[c] P = NA	20 vs 47 P = NA	7-year
Fujita et al. (10)	1997-2002	ATRA+CHT	6	21	42 vs 71 NS	83 vs 76 NS	58 vs 10[b] P = 0.007	0 vs 19	7-year
Alimoghaddam et al. (14)	1989-2011	ATO-based	11	29	52 vs 62[b] P = 0.64	47 vs 66 NS	NA	0 vs 21[c]	5-year
Holter Chakrabarty et al. (15)	1995-2006		62	232	63 vs 50[b] P = 0.1	75 vs 54 P = 0.002	30 vs 18 P = 0.4	7 vs 31 P < 0.001	5-year
Sanz et al. (16)	2004-2018		341	228	75 vs 55 P = 0.001	82 vs 64 P = 0.001	23 vs 28 P = 0.28	3 vs 17 P = 0.001	2-year

NA, not available; NS, not significant.
[a]*Leukemia-free survival.*
[b]*Disease-free survival.*
[c]*Crude relapse rate.*

TABLE 3 | Stem cell source and conditioning intensity.

Reference	Auto-HSC					Allo-HSCT						
	No. of Patients	Source, %		Conditioning, %		No. of Patients	Source, %		Conditioning, %		Donor	
		BM	PB	MAC	RIC		BM	PB	MAC	RIC	MSD	Non-MSD
Sanz et al. (5) {Sanz:2007jv}	195	47	53			137	64	36			100	0
De Botton et al. (6)	50	14	86			23	87	13			78	22
Kohno et al. (13)	15	3	12	15	0	13	10	3	12	1	7	6
Thirugnanam et al. (7)	14	0	100	100	0	–	–	–				
Ferrara et al. (19)	13	0	100	100	0							
Lengfelder et al. (8)	60	NA	NA*	100	0	33	NA	NA	80	20	NA	NA
Pemmaraju et al. (9)	10	25	75	100	0	17	47	53	100		71	29
Fujita et al. (10)	6	0	100	100	0	21	71	19	90	10	38	62
Alimoghaddam et al. (14)	11	20	80	NA	NA	29			NA	NA	100	0
Ramadan et al. (20)	–	–	–	–	–	31	42	58	97	3	58	42
Holter Chakrabarty et al. (15)	62	12	88	89	8	232	66	34	92	7	53	47
Ganzel et al. (11)	140	NA	NA	NA	NA	–	–	–	–	–	–	–
Sanz et al. (16)	341	5	95	86	14	228	18	79	68	32	57	43
Yanada et al. (17)	35	0	100	100	0	–	–	–	–	–	–	–
Yanada et al. (18)	184	4	96	100	0	–	–	–	–	–	–	–
Yanada et al. (21)	443	4	96			–	–	–				

*PB in almost all cases.
NA, not available.

affect either of the outcomes (5, 21). In contrast, the most common stem cell source in the allo-HSCT setting was bone marrow (range, 64% to 87%), except for a couple of series that reported a lower proportion of 47% and 18% (9). Interestingly, most studies in patients with APL undergoing allo-HSCT include a very high proportion of those from matched sibling donor (MSD), sometimes up to 100%, which is significantly higher than would be due to the average availability of this type of donor (roughly 30%). In some studies, this reflects the eligibility criteria established for the study, which exclude transplants from alternative sources and donors, but in other studies it may reflect the attitude of many reluctant physicians to choose alternative donors when a MSD is not available. Probably also, the retrospective studies currently available in relapsed APL do not reflect the great advances and growing use of alternative donors that are taking place in the field of allo-HSCT. It is a fact that significant differences in survival in the past between MSD, matched unrelated mismatched related and unrelated donors are gradually being curtailed.

Regarding conditioning intensity, myeloablative conditioning regimens (MAC) were almost universally used for auto-HSCT, except a few patients who received reduced intensity regimen (RIC) (15). Although MAC was also the most common preparative regimen in allo-HSCT, RIC is increasingly used for older and medically unfit patients up to 32% (16). Unfortunately, data following RIC in APL are currently lacking.

As shown in **Table 4**, TBI-based regimens were preferred for auto-HSCT in the CIBMTR registry data (76%) (15) and in a single center study (10). In contrast, non-TBI-based regimens were preferred in most recent report of the EBMT registry (85%) (16), the Japan Adult Leukemia Study Group (100%) (17), and the Japanese Society for Hematopoietic Cell Transplantation (JSHCT) (96%) (21), as well as other single center studies (7, 13, 19). None of these studies with an appropriate sample size have addressed the comparison of results according to the conditioning regimen to draw significant conclusions.

In allo-HSCT, TBI and non-TBI-based regimens were equally distributed in the CIBMTR registry data (15) and the previous EBMT study (5). However, TBI-based regimens were preferred in in some studies (6, 10, 13) and non-TBI in others (9), including the most recent of the EBMT registry (16).

THE ROLE OF TRANSPLANTATION IN PATIENTS WITH MINIMAL RESIDUAL DISEASE POSITIVE AFTER SALVAGE THERAPY

It is generally accepted that patients undergoing transplantation with minimal residual disease (MRD) positive have a worse prognosis than in MRD-negative status. The clinical situation in which APL patients do not achieve MRD-negative status can occur in two different scenarios, one in which after first-line consolidation treatment the patient does not achieve molecular remission (primary molecular resistance or molecular persistence) and another in which the patient does not achieve molecular remission after salvage consolidation therapy (secondary molecular resistance). For the extremely low fraction of patients in the former scenario, given their poor prognosis, unless they are promptly managed aggressively prior to the occurrence of a hematologic relapse (22), allo-HSCT has traditionally been considered as the first option when patients are suitable for transplantation. It should be noted that molecular persistence at this point was already uncommon in early studies (3%-4%) (23), but has almost disappeared (<1%) in patients receiving state-of-the art treatments with either ATRA plus ATO (24) or ATRA plus chemotherapy-based approaches (25). As far

TABLE 4 | Conditioning regimens.

Reference	Auto-HSC						Allo-HSCT						
	TBI, %	Chemotherapy, %					TBI, %	Chemotherapy, %					
		BU/CY	BU/FLU	BU/MEL	FLU/MEL	Other		BU/CY	BU/FLU	BU/MEL	FLU/MEL	Other	
Sanz et al. (5)	29						53						
De Botton et al. (6)	56	34	0	8	2	0	74	24	0	0	0	0	
Ferrara et al. (19)	0	31	0	0	0	69							
Kohno et al. (13)	27	40	0	0	0	33	85	0	0	0	0	15	
Thirugnanam et al. (7)	0	100	0	0	0								
Pemmaraju et al. (9)	0	50	20	0	0	30	18	18	24	–	18	24	–
Fujita et al. (10)	100	–	–	–	–	–	68	32	–	–	–	–	
Alimoghaddam et al. (14)	–	–	–	–	–	100	–	90	10	–	–	–	–
Ramadan et al. (20)	–	–	–	–	–	–	50	50					
Holter Chakrabarty et al. (15)	76						50						
Sanz et al. (16) {Sanz:2020ve}	15	46	4	13	0	22	34	28	18	1	6	13	
Yanada et al. (17)	0			100									
Yanada et al. (18)	0	8		76		16							
Yanada et al. (21)	4	17		55		25*							

*BU/ETP/Ara-C (16%).

as we know, the only study whose objective was to analyze the outcomes in patients with molecular persistence at the end of first-line consolidations was reported in 2004 and with a very small sample size (22). Four patients undergoing allo-HSCT were alive in hematologic and molecular remission at 64, 92, 98 and 118 months, while three patients treated with chemotherapy followed by auto-HSCT were alive in hematologic and molecular remission at 64, 96 and 98 months. These three patients were in molecular remission at the time of auto-HSCT.

Regarding the second scenario, that is, in patients MRD-positive who underwent transplantation during CR2, data are even scarcer. Some studies in the past showed data that today could provide clues for the interpretation of more current, apparently paradoxical data. While Meloni et al. reported 7 patients with relapsed APL undergoing auto-HSCT being MRD-positive and all patients relapsed within 9 months of transplantation (26), two single case reports showed long-term molecular remission in patients transplanted in CR2 after receiving MRD-positive autografts (27, 28). It was subsequently described in four patients who had molecular evidence of disease in at least one of the harvested samples and remained MRD negative after auto-HSCT (29). This could be explained, among other hypothesis, by the non-clonogenic nature of the PML/RARA-positive cells present in the graft. In fact, the persistence of differentiating PML/RARA-positive cells and spontaneously cleared during follow-up is a common event in patients receiving ATRA. In addition, long-term hematopoiesis after autologous HSCT would be sustained by the subset of CD34+/CD38− progenitor cells administered, and these immature progenitors have been shown to lack the PML/RAR rearrangement in APL patients.

Some recent studies have analyzed transplant outcomes according to MRD status before transplantation. Despite the bias inherent in the generally recommended strategy of limiting autologous transplantation for MRD-negative patients, relegating allogeneic transplantation for MRD-positive patients, these studies have provided some interesting data. Surprisingly, as in the CIBMTR study (15), the most recent EBMT study also found that transplant outcomes in MRD-positive recipients were not statistically different between autologous and allogeneic HSCT (16). Although these data should be interpreted with caution for a number of reasons, such as sample size and selection bias, among others, both studies have confirmed that a proportion of MRD-positive recipients can achieve long-term disease control not only undergoing allo-HSCT, but surprisingly also after auto-HSCT. This interesting finding apparently contradicts the notion that performing auto-HSCT in patients MRD positive is hopelessly doomed to failure. A recent study of the JSHCT, in a large series of APL patients undergoing auto-HSCT, with 35 being MRD-positive and 293 MRD-negative pre-transplantation, reported no association between MRD status and TRM, relapse, and OS rates (21). The cumulative incidence of relapse at 5 years was 10.3% (95% CI 7.1–14.3%) for patients with negative PML-RARA, and 8.9% (95% CI 2.3–21.3%) for patients with positive PML-RARA at transplant. In contrast to the study by Meloni et al. (26), in which bone marrow was the stem cell source, the vast majority of the patients included in the IBMTR, EBMT and JSHCT studies used peripheral blood stem cells (15, 21). These findings raise the question of the feasibility of performing auto-HSCT in patients MRD-positive when using peripheral blood as a of stem cell source and deserve to be confirmed in prospective studies.

PROGNOSTIC FACTORS

A few registry-based studies have addressed a reliable analysis of prognostic factors associated to transplant outcomes in APL.

Although originally introduced for allo-HSCT in chronic myeloid leukemia (30), and later demonstrated its applicability to acute myeloid leukemia and other malignant and non-malignant diseases (22), the classical EBMT risk score has never been tested for patients with APL. However, some of the few available studies on prognostic factors in APL have demonstrated the predictive value of some of the factors considered in the EBMT risk score. Thus, age (> 40 years) and a shorter duration of CR1 adversely influenced overall mortality in the CIBMTR study (15, 21), while in the EBMT study (16), age assessed as a continuous variable (per 10 years) and also a shorter interval from diagnosis to transplant showed an adverse impact not only on TRM (only age), but also in relapse risk, leukemia-free and overall survival. In another retrospective pan-European study in patients receiving ATO as salvage therapy (8), in addition to molecular persistence, a shorter duration of CR1 (<18 months) had an adverse impact on transplant outcomes (LFS and OS) in multivariable analysis.

The stage of the disease as such, another factor considered in the EBMT risk score, has been little studied in APL. In fact, as far as we know, only a retrospective study carried out in five Italian transplant centers has addressed this issue in a relatively small series (20). The study reported the outcome of 31 APL patients who underwent allo-HSCT in CR2 (n=15) or beyond (n=16), with OS being worse in patients with more advanced disease, but at the limit of statistical significance (p = 0.05) in the univariate analysis, while relapse rate was not statistically significant. Although it is expected that other factors considered in the EBMT risk score, such as donor type and donor recipient sex combinations, could also be predictive of transplant outcomes in APL, we are not aware of any study that has analyzed these factors in this disease.

Regarding prognostic factors in auto-HSCT, in addition to the duration of CR1, it has recently been suggested that salvage therapy with ATO is associated with a delayed hematopoietic recovery after transplantation. This association was initially suggested in two cases (31), but later confirmed in a retrospective review of 58 APL patients undergoing auto-HSCT at 21 institutions in the United States and Japan (32). This study found that ATO exposure prior to hematopoietic progenitor cell collection has negative impact on hematopoietic recovery after auto-HSCT. Fortunately, this delay in hematopoietic recovery does not appear to have a significant impact on TRM and other transplant outcomes.

Patients with CNS or other extramedullary relapse have classically been associated with a poorer outcome than those with isolated bone marrow relapse (33). However, recent data have not been able to demonstrate a negative impact of extramedullary disease on transplant outcomes after salvage therapy (8, 11).

CONCLUSIONS

The high cure rate currently obtained in patients with APL using modern treatments with ATRA plus chemotherapy or ATRA plus ATO point out that there is no role for HSCT in front-line therapy. The indication of HSCT has been relegated as consolidation of relapsed patients who achieve second CR after salvage therapy whenever possible. Although with a moderate level of evidence, based on recent retrospective studies, autologous HSCT would be the preferred option for consolidation for patients in molecular CR2. Allogeneic HSCT could be considered in patients with a very early relapse or those beyond CR2. Nevertheless, the superiority of HSCT as consolidation over other alternatives without transplantation has recently been questioned in some studies, which justify a prospective controlled study to resolve this still controversial issue.

AUTHOR CONTRIBUTIONS

All authors have contributed equally. All authors contributed to the article and approved the submitted version.

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Efficacy and Tolerability of First Line Arsenic Trioxide in Combination With All-Trans Retinoic Acid in Patients With Acute Promyelocytic Leukemia: Real Life Experience

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Acute promyelocytic leukemia is a variant of acute myeloid leukemia characterized by t(15;17) and PML/RAR alfa fusion gene. The discovery of the molecular pathogenesis has led to entitle all-trans retinoic acid (ATRA) as the first targeted therapy for acute leukemia. It is usually associated to anthracycline-based chemotherapy with high response rates, but potential long-term sequelae including therapy-related malignancies have been observed. Arsenic trioxide (ATO) was added to obviate these complications and investigational trials aimed to a new strategy with the incorporation of arsenic trioxide (ATO) into initial therapy instead of chemotherapy in selected patients. ATRA plus ATO without chemotherapy was the first attempt to treat low and intermediate-risk patients with APL. Our study aims to describe a monocentric cohort of patients with newly diagnosed APL effectively treated with ATO plus ATRA underlying its efficacy together with the high grade of tolerability of this association. From January 2009 to December 2019 23 APL patients were diagnosed and treated with ATO plus ATRA regimen: 14 males and 9 females patients with a median age of 45 years (range 18-72), for the majority intermediate risk (15 patients, 65%). The treatment was well tolerated and all patients achieved molecular remission after a median time of 3 months (range 1-6 months). All patients proceeded to consolidation phase as outpatients, they maintained complete molecular response at a median time of 44 months (range 15-127) except for 1 patient. All but one patient are alive and in response at a median follow-up of 48 months (range 9-141) without late effects. ATO plus ATRA regimen shows advantages in comparison to chemotherapy; in fact it allowed to treat patients in which chemotherapy could even not be applicable and it did not show secondary hematological diseases. The association of ATO to ATRA as chemo-free regimen enabled to treat APL even without chemotherapy.

Keywords: acute promyelocytic leukemia, arsenic trioxide, all-trans retinoic acid, first-line, secondary leukemias

INTRODUCTION

In the setting of acute myeloid leukemia (AML) acute promyelocytic leukemia (APL) is a variant characterized by t(15;17) and PML/RAR alfa. All-trans retinoic acid (ATRA) was used as a targeted therapy on the basis of this molecular transcript and was usually associated to anthracycline-based chemotherapy (1). This treatment achieved overall remission rates of up to 95% and cure rates over 80% (2–4). Italian GIMEMA and Spanish PETHEMA trials demonstrated a high antileukemic efficacy of this protocol in terms of complete remission (CR) and disease-free survival rates (1). All these patients received induction according to AIDA schedule (5), 3 courses of consolidation and then maintenance. For consolidation, GIMEMA patients received 3 courses with idarubicin/cytarabine, mitoxantrone/etoposide, and idarubicin/cytarabine/thioguanine respectively, whereas PETHEMA patients received the same drugs and dose schedule of idarubicin and mitoxantrone without non-intercalating agents (3, 6). Data of the Spanish group indicated that, to reduce significantly toxicity, might be used in APL a less intensive consolidation and this seemed not to compromise the antileukemic effect. A minor role for cytarabine and etoposide was also suggested in the treatment of newly diagnosed PML/RAR alfa-positive APL patients.

In the long-term follow-up, some sequelae have been described. The 10-year cumulative incidence of deaths in CR, resulting mainly from myelosuppression, has supported the need for less intensive myelosuppressive treatments, particularly for consolidation therapy (7).

Late complications after chemotherapy included therapy-related malignancies as therapy-related acute myeloid leukemia (t-AML) and myelodysplastic syndrome (t-MDS). t-MDS and t-AML patients usually showed poor responses to conventional chemotherapy with overall median survivals of 10 months (range 7–22 months) (8).

Arsenic trioxide (ATO), very effective as a single agent, was initially used for the treatment of the relapsed patients after treatment with ATRA and chemotherapy (9, 10). ATO has a different toxicity profile than ATRA and chemotherapy, but experimental studies tried to incorporate ATO into initial therapy and to reduce or omit the use of chemotherapy in selected patients.

Analysis of the cooperative group showed 3-year Kaplan-Meier curves with the estimation of relapse-free survival (RFS) (1). The resulting predictive model for RFS showed the capacity of segregating patients into statistically different groups ($p<0.0001$): low-risk (white blood cells WBC count $\leq 10 \times 10^9$/L, platelet count $> 40 \times 10^9$/L), intermediate-risk (WBC count $\leq 10 \times 10^9$/L, platelets $\leq 40 \times 10^9$/L), and high-risk (WBC count $> 10 \times 10^9$/L) groups.

ATRA plus ATO without chemotherapy was an attempt in low and intermediate-risk APL patients; for high-risk APL triple therapy with limited anthracycline or gemtuzumab ozogamicin during induction was the principal option.

The introduction of arsenic trioxide (ATO) tried to obviate complications of chemotherapeutic agents in low and intermediate-risk groups. The use of ATO and ATRA showed excellent results in terms of CR and long-term leukemia free survival (11, 12).

Our study aims to describe a monocentric cohort of patients with newly diagnosed APL effectively treated with ATO plus ATRA analyzing efficacy and tolerability of this association.

MATERIALS AND METHODS

Patients with newly APL were diagnosed on the basis of morphologic features and confirmed by genetic and molecular characteristics. Translocation t(15,17) was detected by conventional karyotyping or fluorescence *in situ* hybridization (FISH) (13), *PML-RARA* fusion gene by means of reverse-transcriptase–polymerase-chain-reaction (RT-PCR) assay (14, 15).

The study was retrospective monocentric and it included all consecutive patients affected by APL and treated with ATRA plus ATO; few cases were treated also with idarubicin only in the induction phase.

According to Sanz's risk score (1) they were classified as low, intermediate or high-risk.

Patients receive ATRA plus ATO for induction and consolidation therapy or standard ATRA–idarubicin induction therapy followed by ATRA-ATO for consolidation.

Data on patients who were still alive and in first molecular CR were censored at the most recent follow-up visit. Overall survival and cumulative incidence of relapse were defined according to the NCI workshop definitions (16).

Guidelines for the prevention and management of coagulopathy, hyperleukocytosis, prolongation of the corrected QT (QTc) interval (17), and hematologic and non-hematologic toxic effects were predefined in the protocols. As prophylaxis for the differentiation syndrome (18), prednisone at a dose of 0.5 mg per kilogram of body weight per day was administered from day 1 until the end of induction therapy. For suspected differentiation syndrome, ATRA, ATO or both were temporarily discontinued and intravenous dexamethasone was administered at a dose of 10 mg every 12 hours until the disappearance of signs and symptoms for a minimum of 3 days. Common terminology criteria for adverse events (CTCAE) version 4.0 was used for toxicity assessment.

RESULTS

From January 2009 to December 2019 we diagnosed 23 APL patients who were treated with ATO plus ATRA regimen: 14 males and 9 females patients with a median age of 45 years (range 18-72), for the majority intermediate risk (15 patients, 65%) but 4 patients were low-risk and 4 patients high-risk. Patients in the high-risk category according to Sanz's criteria were treated with ATRA plus idarubicin (3 patients) and ATRA plus ATO with the addition of two doses of idarubicin (1 patient) in the induction. All the 23 patients were treated according to the regimen of ATO plus ATRA in the consolidation phase, included the 4 patients at high-risk, due to severe concomitant comorbidities.

The treatment of ATRA (started at the suspicion of APL) and ATO (started after a median of 2 days, range 1-5) was well tolerated: brief interruptions of ATO (≤ 3 days) were registered

in 11 patients (in 7 for QTc prolongation, 3 for malaise, 1 for hepatic toxicity of grade 2). A possible retinoic acid syndrome was described in 10 cases; even if in many cases it was only suspected ATRA was discontinued for a brief period, patients were treated with steroids and then ATRA was restarted without any complications.

During the induction phase, we also registered 8 episodes of fever, 3 mild hepatic toxicities of grade 1 or 2, 2 thrombotic events (1 deep vein thrombosis and 1 thrombophlebitis of the leg), 2 viral infections sustained by HSV, 1 ATRA myopathy of grade 2 and 1 skin rash of grade 1.

All patients achieved molecular remission after a median time of 3 months (range 1-6 months).

All patients followed consolidation treatment as outpatients; only 4 patients were hospitalized: 1 patient was admitted for the suspicion of benign intracranial hypertension, 1 patient for pneumonia before starting consolidation, 1 patient for fever and pericardial effusion and 1 patient performed cholecystectomy as inpatient in surgical ward. During consolidation only 3 patients (13%) reduced ATO dosage for QTc prolongation; in two patients we registered 2 different episodes of QTc prolongation.

Other adverse events were mild hepatotoxicity (5 patients: 3 of grade 2 and 2 of grade 1), infections (cystitis in 2 patients, FUO in 2 patients, herpes in 1 patient, diarrhea grade 1 in 1 patient), pitting edema with fluid retention (3 patients) and headache (2 patients). All these adverse events were easily managed on outpatient ward; there was no delay in the treatment.

All patients maintained molecular response for a median time of 44 months (range 15-127) except for 1 patient who relapsed after 11 months: he restarted ATO plus ATRA achieving a second but brief molecular response and central nervous system localization; stem cell transplantation was carried out but the patient died from transplant related complications. All but one patient are alive and in response at a median follow-up of 48 months (range 9-141) without late effects.

DISCUSSION

ATO plus ATRA association shows advantages in comparison to chemotherapy: patients were hospitalized only during the induction phase and the rate of hospitalization and complications was very low and highly manageable. This regimen also allowed to treat high-risk patients in which chemotherapy could not be applicable such as secondary APL (due to prior chemotherapy or immunosuppressive agents), intracerebral hemorrhage or high rate of hemorrhagic symptoms and massive pulmonary embolism (19).

In the management of APL, it should be considered also the incidence of t-MDS and t-AML, which was considerable after a high-dose chemotherapy for a prior cancer and low in the case of patients treated by conventional therapy for APL, as only few cases have been described in literature. Some studies hypothesized the role of alkylating agents and etoposide to induce t-MDS or t-AML, but it was also investigated the leukemic potential of other drugs as anthracyclines. The median period to diagnose t-MDS or t-AML was 34 months (range 25-40) and all patients presented chromosome abnormalities (mainly deletions or loss of the long arm of chromosome 5 and/or 7, or balanced translocations involving the 21q22 band) and poor prognosis with median survivals of 10 months (range 7-22) (8).

We already published a report (20) in which we compared ATO plus ATRA with a control cohort treated with chemotherapy plus ATRA according to AIDA-2000 (12). Data on the historical group showed median molecular response time of 85 months (range 16-296) but 3 patients developed t-MDS/AML after 3 years, 6 years and 23 years from the end of the maintenance phase respectively, and 1 patient showed myelodysplastic features at bone marrow cytogenetics such as del5 after 12 years from the end of the maintenance phase. Two patients with t-MDS died for progressive disease.

Considering our comparison, the approach with ATO plus ATRA allowed to reduce hospitalization, transfusion support, early complications mainly related to consolidation phase and late complications such as t-MDS/AML whose progression could be fatal.

Exciting results are coming from clinical trials testing oral arsenic formulation (21) and they seem to further extend the benefit from ATO plus ATRA combination retaining excellent activity with the simple oral combination of these powerful agents and more clinical trials are ongoing to validate them.

Studies on health-related quality-of-life outcomes demonstrated for this regimen a better profile in terms of fatigue (22), with APL patients safely back to their normal daily living.

The association of ATO to ATRA as chemo-free regimen enabled to treat acute leukemia even without chemotherapy.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

FA and SS designed the study, recorded clinical and biological data and wrote the manuscript. PC, FS, SG, LL, II, EM, NP, and LP contributed to the study design, recorded clinical and biological data and wrote the manuscript. All authors contributed to the article and approved the submitted version.

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The Combination of Gefitinib With ATRA and ATO Induces Myeloid Differentiation in Acute Promyelocytic Leukemia Resistant Cells

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In approximately 15% of patients with acute myeloid leukemia (AML), total and phosphorylated EGFR proteins have been reported to be increased compared to healthy CD34$^+$ samples. However, it is unclear if this subset of patients would benefit from EGFR signaling pharmacological inhibition. Pre-clinical studies on AML cells provided evidence on the pro-differentiation benefits of EGFR inhibitors when combined with ATRA or ATO *in vitro*. Despite the success of ATRA and ATO in the treatment of patients with acute promyelocytic leukemia (APL), therapy-associated resistance is observed in 5-10% of the cases, pointing to a clear need for new therapeutic strategies for those patients. In this context, the functional role of EGFR tyrosine-kinase inhibitors has never been evaluated in APL. Here, we investigated the EGFR pathway in primary samples along with functional *in vitro* and *in vivo* studies using several APL models. We observed that total and phosphorylated EGFR (Tyr992) was expressed in 28% and 19% of blast cells from APL patients, respectively, but not in healthy CD34$^+$ samples. Interestingly, the expression of the EGF was lower in APL plasma samples than in healthy controls. The EGFR ligand AREG was detected in 29% of APL patients at diagnosis, but not in control samples. *In vitro*, treatment with the EGFR inhibitor gefitinib (ZD1839) reduced cell proliferation and survival of NB4 (ATRA-sensitive) and NB4-R2 (ATRA-resistant) cells. Moreover, the combination of gefitinib with ATRA and ATO promoted myeloid cell differentiation in ATRA- and ATO-resistant APL cells. *In vivo*, the combination of gefitinib

and ATRA prolonged survival compared to gefitinib- or vehicle-treated leukemic mice in a syngeneic transplantation model, while the gain in survival did not reach statistical difference compared to treatment with ATRA alone. Our results suggest that gefitinib is a potential adjuvant agent that can mitigate ATRA and ATO resistance in APL cells. Therefore, our data indicate that repurposing FDA-approved tyrosine-kinase inhibitors could provide new perspectives into combination therapy to overcome drug resistance in APL patients.

Keywords: epidermal growth factor receptor (EGFR), erlotinib, gefitinib, all-trans retinoic acid (ATRA), acute promyelocytic leukemia (APL), ATRA-resistance, ATO-resistance, arsenic trioxide (ATO)

INTRODUCTION

The clinical introduction of *all-trans* retinoic acid (ATRA) and arsenic trioxide (ATO) revolutionized the treatment of acute promyelocytic leukemia (APL), leading to a disease-free survival rate of 80-90% (1). Nevertheless, 5-10% of APL patients still relapse due to ATRA or ATO resistance (2). Despite the cytotoxic activities of ATRA and ATO in APL cells, low doses of those agents result in induction of terminal myeloid cell differentiation (3, 4). In this context, previous reports demonstrated that inhibitors of the epidermal growth factor receptor (EGFR) increased ATRA and ATO-induced expression of the myeloid differentiation marker CD11b in AML cells (3–7). Nonetheless, the use of EGFR inhibitors in combination with standard therapy was not previously explored in APL cells resistant to ATRA and ATO.

Non-small cell lung cancer (NSCLC) demonstrated constitutive activation of the epidermal growth factor (EGF)/EGFR pathway, due to mutations on the *EGFR* (8). Although *EGFR* mutations are rare in AML (9–11), the level of EGF—the main EGFR ligand—was elevated in the urine of patients diagnosed with APL and decreased after ATRA-induced complete remission (12). Hence, it is conceivable that the activation of the EGF/EGFR signaling pathway could also confer APL leukemic cells with a survival advantage. However, the prevalence and clinical significance of EGFR and its interactors in APL patients remains unknown.

It has been well established that the distinct dimer interfaces formed between the extracellular domain of the EGF receptor and its respective ligands EGF and amphiregulin (AREG) differentially activate intracellular signaling cascades to regulate cell proliferation and differentiation (13). The EGFR tyrosine kinase inhibitors gefitinib (ZD1839) and erlotinib (CP-358774) are small-molecule compounds that prevent the binding of ATP to the intracellular domain of EGFR, thus impairing autophosphorylation and downstream signal transduction (14). The efficacy and safety of gefitinib and erlotinib as first-line therapies for NSCLC have been demonstrated in several clinical trials and retrospective studies (15). Although there is evidence of patients with co-occurrence of acute myeloid leukemia (AML) and NSCLC, which achieved complete hematological remission when treated with erlotinib monotherapy (16, 17), subsequent studies evaluating the response of AML patients to EGFR inhibitors alone could not corroborate these findings (18–20). In a phase II trial, 26/29 (90%) patients with refractory or relapsed AML who received erlotinib monotherapy discontinued treatment because of disease progression. Nevertheless, combination therapies between differentiation agents with EGFR inhibitors have not been evaluated in AML patients (18).

Here, we evaluated the effects of EGFR pharmacological inhibition in distinct APL models. Gefitinib monotherapy induced apoptosis and inhibited the proliferation of NB4 (ATRA-sensitive) and NB4-R2 (ATRA-resistant) APL cells. Additionally, the combination between gefitinib with ATRA and ATO rewired NB4-R2 and NB4 ATOr (ATO-resistant) cells into sensitivity to standard therapy for APL. *In vivo*, APL mice treated with ATRA alone or in combination with gefitinib exhibited increased overall survival in comparison with the vehicle-treated group.

MATERIAL AND METHODS

Chemicals

Gefitinib (#S1025) and erlotinib (#S7786) were purchased from Selleck Chemicals (Houston, TX, USA). ATRA and ATO were purchased from Sigma-Aldrich (St. Louis, MO, USA). Gefitinib, erlotinib, and ATRA were dissolved in dimethyl sulfoxide (DMSO). ATO was dissolved in NaOH (1 M). All compounds were stored at −20°C.

Cell Culture

The human APL cell lines NB4 (ATRA-sensitive), NB4-R2 (ATRA-resistant), NB4 ATOr (ATO-resistant), and NB4 clone 21 (parental line of NB4 ATOr) were cultured in Roswell Park Memorial Institute 1640 medium (Gibco, Rockville, MD, USA) with 2 mM L-glutamine (Invitrogen, Carlsbad, CA, USA) and 10% of fetal bovine serum (FBS; Vitrocell, Campinas, Brazil) at 37°C in a humidified atmosphere of 5% CO_2. Cell lines were tested and authenticated by STR DNA fingerprinting analysis (Laboratory of Biochemical Genetics, Department of Genetics, Medical School of Ribeirao Preto – University of Sao Paulo).

Patient Samples

Primary patient APL blasts, healthy $CD34^+$ cells, and plasma samples were collected from BM aspirates. Mononuclear cells were isolated by Ficoll density gradient centrifugation (Histopaque-1077; Sigma-Aldrich). $CD34^+$ cells were isolated

from the BM of healthy volunteers using the CD34 Microbead Kit (#130-046-703; Miltenyi Biotec, Auburn, CA, USA) according to the manufacturer's instructions. Plasma was obtained by centrifugation (500 g for 10 minutes) of heparinized BM aspirate and stored in aliquots at −80°C until use. BM $CD34^+$ cells or plasma samples from healthy donors were used as controls. The study was approved by the local Research Ethics Committee of the Medical School of Ribeirao Preto, University of Sao Paulo, Ribeirao Preto, Sao Paulo, Brazil (Reference: CAAE 05060818.9.0000.5440). All human samples were collected after obtaining written, informed consent from patients according to the recommendations of the Declaration of Helsinki.

Apoptosis Assay, Determination of 50% Effective Dose (ED_{50}) and Combination Index

To evaluate apoptosis, NB4 and NB4-R2 cells were seeded in 24-well plates at a density of 5×10^5/well and treated with ATO (1–4 μM), gefitinib (5–40 μM, alone or in combination with 2 μM of ATO), erlotinib (5–120 μM), or vehicle (DMSO, 0.01%) for 24 h. To detect apoptotic cells, the cells were washed and resuspended in 100 μL binding buffer, 3 μL Annexin V-fluorescein isothiocyanate (BD Biosciences, San Jose, CA, USA), and 3 μL propidium iodide (PI; 50 μg/ml), followed by an incubation in the dark for 20 min. Fluorescence was detected by flow cytometry on a FACSCalibur instrument (Becton Dickinson, San Jose, CA, USA) and analyzed with FlowJo software (Treestar, Ashland, OR, USA). A minimum of 10 000 events was acquired for each sample. The ED_{50} and combination index were calculated using CompuSyn software (CompuSyn, Paramus, NJ, USA); the latter is a quantitative measure of drug interaction, with a value < 1 or > 1 indicating synergism and antagonism, respectively, and a value of 1 indicating an additive effect (21).

Proliferation Assay

After 24 h of exposure to gefitinib, cells were washed with phosphate-buffered saline (PBS); 4 mL cold 70% ethanol was then added dropwise to the cell pellet while vortexing, followed by storage at −20°C for up to 15 days before staining. The cells were resuspended and washed with staining buffer (PBS with 1% FBS and 0.09% NaN_3), and 100 μL of cell suspension (1×10^7/ml) was transferred to a tube containing 5 μL of Ki-67-PE antibody (#12-5698-82, clone: SolA15; eBioscience, San Diego, CA, USA) or PE-conjugated IgG1as an isotype control. After incubation for 30 min, cells were washed twice, resuspended in staining buffer, and analyzed by flow cytometry on a FACSCalibur instrument (Becton Dickinson, San Jose, CA, USA) and analyzed with FlowJo software (Treestar, Ashland, OR, USA). A minimum of 10 000 events was acquired for each sample. Positivity is expressed as a percentage of positive cells and mean fluorescence intensity (MFI).

Differentiation Assay

For *in vitro* experiments, NB4, NB4-R2, NB4-ATOr, and NB4 clone 21 cells were collected 72 h after drug treatment, washed, and resuspended in 100 μL PBS and incubated with CD11b-PE (#347557, clone: D12), CD11c-APC (#559877, clone: B-ly6), CD15 (#562371, clone: 7C3.rMAb), and CD16 (#557758, clone: 3G8) (BD Biosciences). Cells obtained from BM, or the spleen of leukemia model mice were labeled with antibodies against CD11b-PE (#553311, clone: M1/70), CD117-FITC (#561680, clone: 2B8), Gr1-FITC (#551460, clone: 1A8; all from BD Biosciences), then collected and washed and resuspended in PBS. The percentage of positive cells and MFI were determined by flow cytometry.

Western Blotting

Whole-cell lysates were prepared with extraction buffer (10 mM EDTA, 100 mM Tris, 10 nM $Na_4P_2O_7$, 100 mM NaF, 10 mM Na_3VO_4, 2 mM phenylmethylsulfonyl fluoride, and 1% Triton X-100) followed by centrifugation at 10 000 × g for 20 min at 4°C. Protein concentration was determined with the Bradford assay and 50 μg of lysate was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis on a 10% polyacrylamide gel. The proteins were transferred to a polyvinylidene difluoride membrane (Amersham Hybond-P; GE Healthcare, Memphis, TN, USA) that was probed with antibodies against total EGFR (#2232, polyclonal, 1:1000) and phosphorylated (p-)EGFR (Tyr992) (#2235, polyclonal, 1:1000) (both from Cell Signaling Technology, Danvers, MA, USA); SYK (#1240, clone: 4D10, 1:1000; Santa Cruz Biotechnology, Santa Cruz, CA, USA); and β-actin (#A5441, clone: AC-15, 1:60 000; Sigma-Aldrich). Protein bands were visualized using SuperSignal West Dura Extended Duration Substrate (Thermo Fisher Scientific, Waltham, MA, USA) and the Gel Doc XR+ system (Bio-Rad, Hercules, CA, USA).

Enzyme-Linked Immunosorbent Assay

Plasma EGF and AREG concentrations were measured with the Human EGF Quantikine ELISA Kit (#DEGFR0) and Human Amphiregulin Quantikine ELISA Kit (#DAR00; both from R&D Systems, Minneapolis, MN, USA), respectively, according to the manufacturer's instructions.

PCR for Genotyping

DNA was isolated using the QIAamp DNA Mini Kit (Qiagen, Germantown, MD, USA), according to the manufacturer's instructions, and used as the template for PCR. The 25 μL reaction contained 2.5 μL of 5× reaction buffer, 2 μL of 25 mM $MgCl_2$, 3 μL of 10 mM dNTP mix, 2 μL of each primer (5 μM), and 0.2 μL GoTaq DNA polymerase (Promega, Madison, WI, USA). A 3 μL volume of diluted DNA sample (300 ng) was used for conventional PCR, and amplified products (20 μL) were visualized by electrophoresis with Tris–acetic acid–EDTA buffer on a 1.2% (w/v) agarose gel stained with ethidium bromide under ultraviolet light. PCR amplification was performed on a GeneAmp PCR System 9700 thermocycler (Applied Biosystems, Foster City, CA, USA) under the following conditions: 94°C for 5 min; 35 cycles of 94°C for 30 s, annealing at the melting temperature for 45 s, and 60°C for 30 s; and 60°C for 7 min. The following forward and reverse primers were used: PML, 5'-TCAAGATGGAGTCTGAGG AGG-3' and 5'-CTGCTGCTCTGGGTCTCAAT-3'; and β-actin, 5'-TCTTGATAGTTCGCCATGGAT-3' and 5'-GGTCATC TTTTCACGGTTGG-3'.

In Vivo Experiments

To investigate the *in vivo* effects of the EGFR inhibitors gefitinib or erlotinib as monotherapy or combined with ATRA, we used a syngeneic transplantation mouse model of APL with leukemia cells from human chorionic gonadotropin (hCG)–promyelocytic locus–retinoic acid receptor A (PML–RARA) transgenic mice (B6129 mixed background), as previously described (22, 23). The hCG-PML-RARA mice were kindly donated by Dr. Pier Paolo Pandolfi (Beth Israel Deaconess Medical Center, Harvard University) and maintained at the Laboratory of Experimental Animal Studies (Fundação Hemocentro de Ribeirão Preto–Ribeirão Preto, SP, Brazil).

8 to 12-week-old male wildtype (WT) littermates, weighing approximately 30 g each, were used as transplant recipients after lethal irradiation (7 Gy split into two doses from an X-ray source i.e., two 3.5 Gy doses, 4 h apart - RS200 from Rad Source Technologies, Inc., Georgia, USA). In the next day, the animals were exposed to a dose of 2% isoflurane for 5 min to induce anesthesia, and immediately afterward 4×10^6 viable leukemic blasts from hCG-PML-RARA mice (200 µL in PBS) were injected intravenously using a syringe with a 30-gauge disposable needle (BD Biosciences) through the retro-orbital sinus. After this time, the mice were monitored and assessed for engraftment analysis. The engraftment was confirmed by conventional PCR analysis of the DNA isolated from 100 µL of heparinized peripheral blood samples collected *via* submandibular vein by using a 5-mm lancet (Goldenrod Animal Lancet, Medipoint, Mineola, NY), under 2% isoflurane anesthesia.

This analysis was done once per week until the *PML–RARA* fusion gene expression was detected (**Figure 5A**). After molecular engraftment confirmation in peripheral blood samples, mice were randomly (by the physical method of paper sortition) assigned to treatment groups: gefitinib (100 mg/Kg/day; n=7) and vehicle (1:10 solution of DMSO : PBS; n=7) (**Figure S1A**); gefitinib (200 mg/Kg/day; n=5) and vehicle (n=6) (**Figure S1B**); erlotinib (200 mg/Kg/day; n=4) and vehicle (n=4) (**Figure 4B**); gefitinib (200 mg/kg/day; n=7), ATRA (2.5 mg/kg/day; n=7), gefitinib plus ATRA (n=9), or vehicle (n=5) (**Figure 4D**). Gefitinib (100 or 200 mg/kg/day), erlotinib (200 mg/kg/day), ATRA (2.5 mg/kg/day) diluted in 200 µL of PBS, or vehicle, were administered every day in the afternoon hours during the light cycle by intraperitoneal (i.p.) injection, for 15 consecutive days. The dose, drug administration route, and treatment period for EGFR inhibitors and ATRA have been established based on previous studies (24–28). Overall survival of mice was defined as the length of time from the start of treatment until the date of spontaneous death or euthanasia. All animal experiments were performed at the Laboratory of Experimental Animal Studies (Fundação Hemocentro de Ribeirão Preto – Ribeirão Preto, SP, Brazil).

Animal experiment protocol and experimental procedures were approved by the Animal Care and Use Committee of the Medical School of Ribeirao Preto of the University of Sao Paulo (Protocol no. #016/2016) and conformed to the rules and regulations of the National Council for Control of Animal Experimentation of Brazil (CONCEA). This manuscript was written following the ARRIVE reporting guideline for reporting animal research (29). A completed ARRIVE guidelines checklist is included in **Table S2**.

Statistical Analysis

Significant differences between groups were evaluated with the unpaired t-test or Kruskal–Wallis test, followed by Dunn's *post hoc* test. Bivariate correlation analysis with Spearman's test was performed to determine the correlation between BM plasma concentration of EGF or AREG and WBC count at the time of diagnosis. The log-rank test (with Kaplan–Meier curves) was used for overall survival analysis. Statistical analyses were performed using Prism v.7.03 software (GraphPad, La Jolla, CA, USA). The significance level was set as $P \leq 0.05$.

RESULTS

EGFR Protein Expression Is Only Detected in a Subset of APL Patients.

We evaluated EGFR and the non-receptor tyrosine kinase SYK [a potential off-target of EGFR inhibitors (24)] protein levels in bone marrow (BM) cells obtained from 21 patients diagnosed with APL and a pool of BM-derived $CD34^+$ cells isolated from six healthy subjects (controls). EGFR protein was expressed in 6/21 (28.5%) APL patients (**Figure 1A**), but not in control samples. Notably, 4/21 (19%) APL samples also showed positivity for p-EGFR (Tyr992) (**Figure 1A**), indicating activation of the EGF/EGFR signaling pathway. SYK protein expression was neither detected in APL nor control subjects (**Figures 1A** and **S1**). In addition, the *EGFR* gene expression (by real-time quantitative polymerase chain reaction) was not detected in any of these specimens (**Table S1**).

EGF and AREG Concentrations in BM Plasma Samples

Next, we sought to investigate whether the levels of EGF and AREG (EGFR ligands) measured in the plasma of APL patients correlates with the protein expression of EGFR on APL blasts. The EGF levels were lower in BM aspirates of APL patients at diagnosis (n=16) compared to healthy control subjects (n=15) (median concentration of 127.3 ± 149 *vs* 322.2 ± 136 pg ml^{-1}, *P<0.01*) (**Figure 1B**). AREG was detected in BM plasma of 5/17 APL patients at diagnosis but was absent in control samples (n=20) (**Figure 1C**). Among the five AREG-positive BM plasma samples from APL patients, four had a median AREG concentration of 126.8 ± 3.1 pg ml^{-1} (with an outlier sample displaying concentration >1000 pg ml^{-1}). No correlation was observed between EGF (n=14) or AREG (n=5) levels in BM plasma of APL patients and peripheral white blood cell (WBC) counts (EGF: $r^2 = 0.01$ – **Figure S2A**; AREG: $r^2 = 0.045$ – **Figure S2B**).

Effects of EGFR Inhibitors Alone or in Combination With ATO or ATRA on APL Cell Lines

We first evaluated EGFR and SYK protein levels in NB4 and NB4-R2 cells. As previously demonstrated, both APL cell lines are negative for EGFR (**Figure S3B**) but express SYK (**Figure S3A**), an off-target of EGFR inhibitors. The 50% effective dose

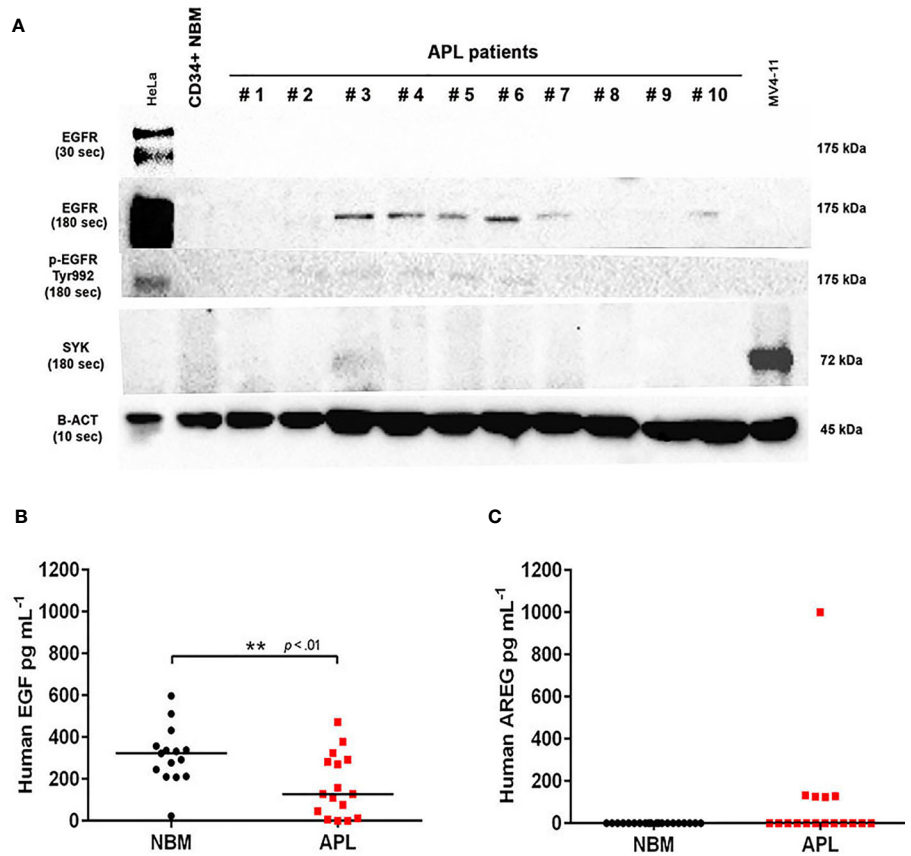

FIGURE 1 | EGFR, p-EGFR (Tyr992), SYK, EGF, and AREG protein expression in APL patient samples. **(A)** Western blotting analysis of EGFR, p-EGFR (Tyr992), SYK, and β-actin protein levels in CD34$^+$ cells isolated from normal BM of one healthy adult volunteer and 10 representative primary APL samples collected at diagnosis. HeLa cells served as a positive control to assess EGFR and p-EGFR (Tyr992) expression, and MV4-11 cell extracts were used as a positive control for SYK expression. EGFR and p-EGFR (Tyr992) were detectable when exposed for 180 seconds (sec). **(B)** EGF levels in BM plasma samples from healthy donors (n=15) and APL patients at diagnosis (n=16). **(C)** Plasma AREG levels in BM from healthy donors (n=20) and APL patients at diagnosis (n=17). (**) $p < 0.01$ (Mann-Whitney U-test).

(ED_{50}) values for the cytotoxic activities of gefitinib, erlotinib, and ATO, as well as the combination index (CI) values for gefitinib plus ATO in the two APL cell lines, are shown in **Table 1**. Since ATRA does not induce cytotoxicity at physiological concentrations after 24h in APL cell lines (30), we did not perform experiments using EGFR inhibitors combined with ATRA. Compared to the vehicle, gefitinib induced increased apoptosis in NB4 cells (**Figure 2A** and **Figure S4A**) and NB4-R2 cells (**Figure 2B** and **Figure S4B**). With a dose-dependent effect, the maximum cell death was observed at 40 μM for gefitinib treatment. Erlotinib had higher ED_{50} values in both NB4 and NB4-R2 cells compared with gefitinib (**Table 1** and **Figure S4C**); therefore, the analysis of synergism with ATO was performed using only the latter (**Table 1** and **Figures 3E, F**). Evaluation of Ki-67 staining (MFI) revealed that gefitinib was only able to impair APL cell proliferation at concentrations > 40 μM in NB4 and NB4-R2 cells (**Figures 2E–H**). In addition, treatment with gefitinib did not alter the proliferative rate of primary human (n=3; **Figure S5A**) or murine (**Figure S5B**) APL blast cells. We next evaluated

TABLE 1 | The 50% effective dose (ED_{50}) values of gefitinib, erlotinib, and arsenic trioxide (ATO) in NB4 and NB4-R2 cells, and combination index (CI) values of gefitinib plus ATO at different effective levels.

Cell type	ED50[1]			CI[1](Gefitinib+ATO)			
	Gefitinib(μM)	Erlotinib	ATO(μM)	at ED50	at ED75	at ED90	at ED95
NB4	22.08±5.22	71.66±2.97	2.28±0.14	1.51±0.25	1.42±0.23	1.40±0.29	1.42±0.34
NB4-R2	27.0±7.35	79.95±3.36	2.91±0.94	1.35±0.19	1.10±0.41	1.00±0.56	0.99±0.65

[1]*ED_{50} and CI values were calculated by using the CompuSyn software according to the Chou and Talalay method (21).*

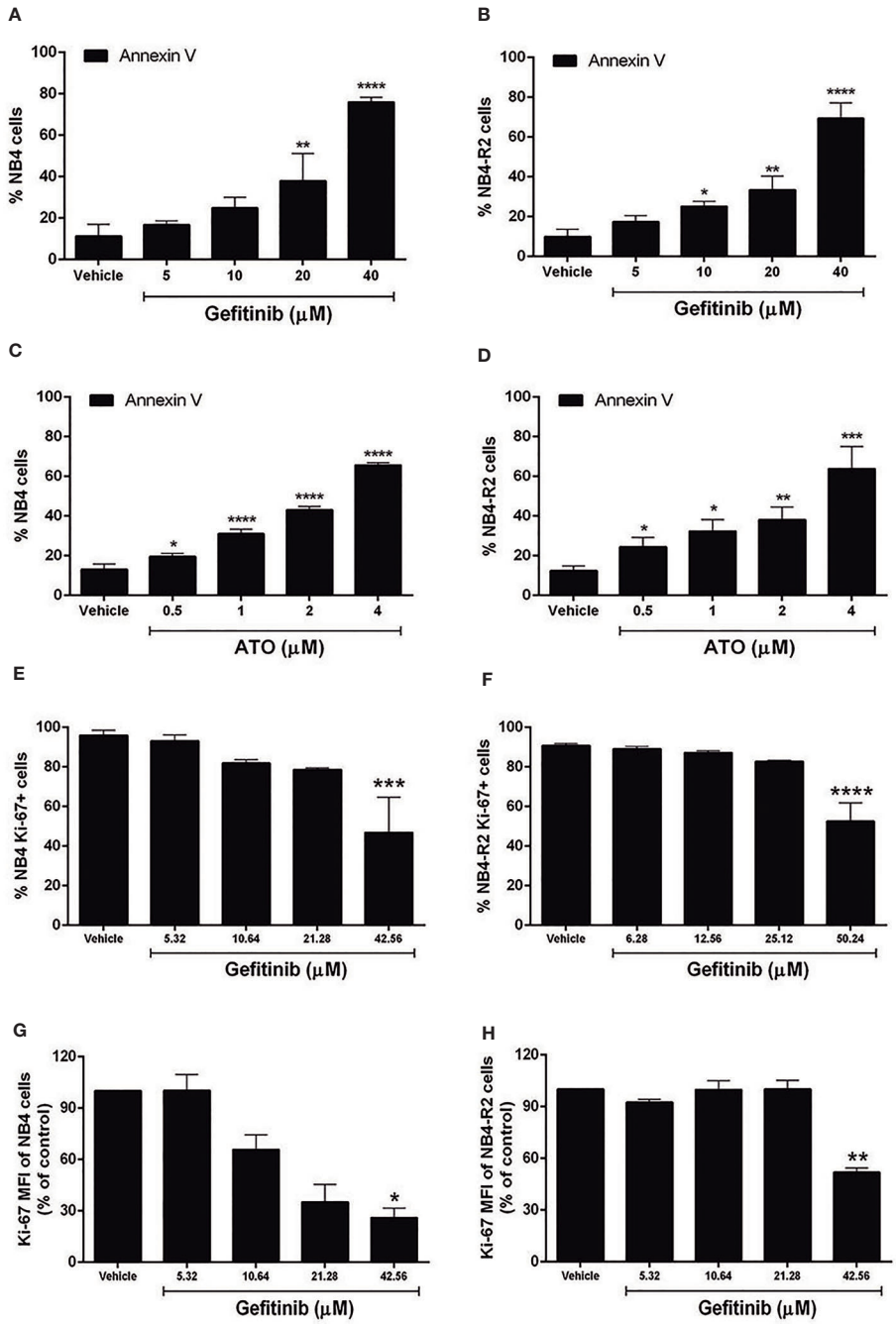

FIGURE 2 | Effects of gefitinib or ATO monotherapy on APL cell apoptosis and proliferation. Gefitinib (5, 10, 20, and 40 μM) **(A, B)** and ATO (0.5, 1, 2, and 4 μM) **(C, D)** treatment for 24 h decreased the fraction of apoptotic NB4 and NB4-R2 cells in a concentration-dependent manner, as determined by Annexin V/PI staining and flow cytometry analysis. Proliferation of NB4 **(E)** and NB4-R2 **(F)** cells was reduced at gefitinib concentrations higher than twice the ED_{50} after 24 h of treatment. **(G, H)** MFI values for NB4 and NB4-R2. Bar graphs show mean ± SD of at least three independent experiments. (*) $p < 0.05$, (**) $p < 0.01$, (***) $p < 0.001$, (****) $p < 0.0001$ (Kruskal–Wallis test, followed by Dunn's *post hoc* test).

myeloid differentiation using NB4, NB4-R2, and NB4 ATOr cells (including the respective parental NB4 cells – hereafter called NB4 clone 21) when treated with ATRA (0.01, 0.1 and 1 μM) and ATO (0.5 μM), in the presence or absence of gefitinib (10 μM). Gefitinib monotherapy did not induce APL cell differentiation (**Figures 3A–D**); however, the combined therapy of gefitinib plus ATRA and ATO enhanced APL cell differentiation, measured by the surface expression of the myeloid differentiation markers CD11b, CD11c, CD15, and CD16 (**Figures 4A–L** and **S6**).

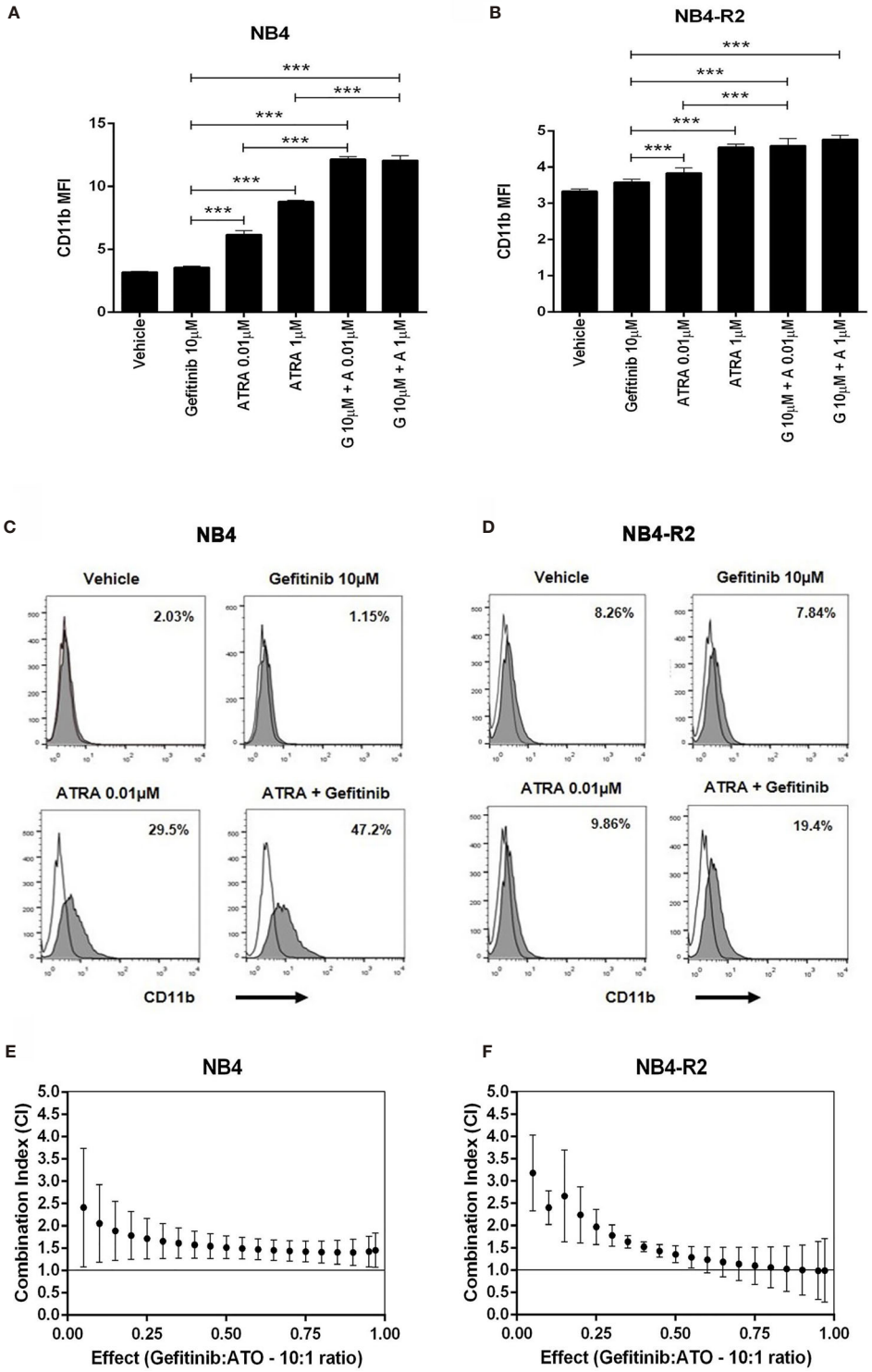

FIGURE 3 | The effect of gefitinib combined with ATRA or ATO on NB4 and NB4- R2 cells. **(A, B)** CD11b MFI of NB4 and NB4-R2 cells treated with ATRA alone (0.01 or 1 μM), gefitinib alone (10 μM), or ATRA plus gefitinib for 72 h. Gefitinib potentiated the myeloid differentiation-inducing effect of ATRA in NB4 **(A)** and NB4-R2 cells **(B)**; representative histograms with percentages for CD11b expression in NB4 **(C)** and NB4-R2 **(D)** cells are shown. Plots show the combination index (CI) versus the fractional effect of gefitinib plus ATO treatment at 10:1 constant ratio in NB4 **(E)** and NB4-R2 **(F)** cells. CI values were calculated by using the CompuSyn software according to the recommendations of Chou and Talalay (21). Data represent the mean ± SD of at least three independent experiments. (***) $p < 0.001$ (Kruskal– Wallis test, followed by Dunn's *post hoc* test).

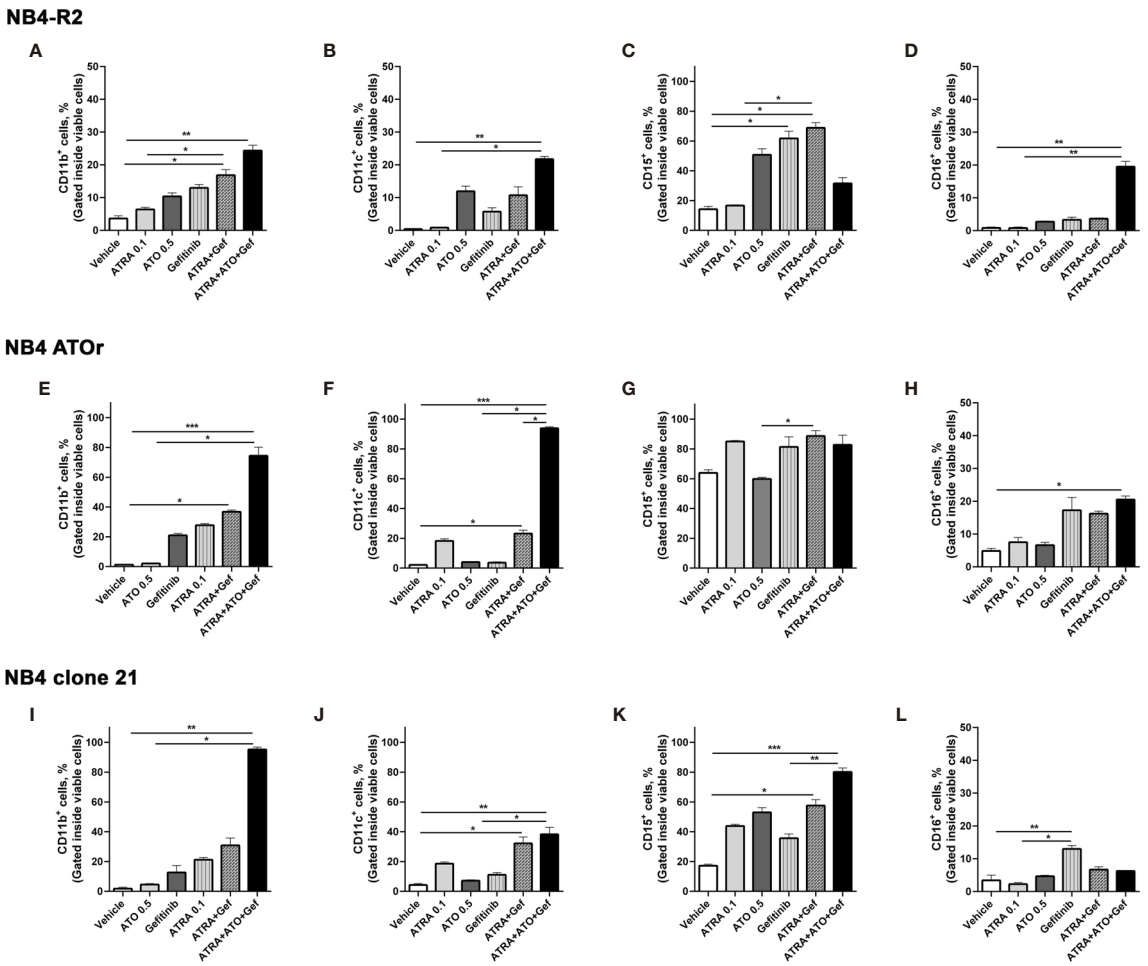

FIGURE 4 | Gefitinib therapy sensitizes APL-resistant cells to ATRA and ATO. The APL cell lines NB4-R2 **(A–D)**, NB4 ATOr **(E–H)**, and the respective parental NB4 clone 21 **(I–L)** were treated with ATRA alone (0.1 μM), ATO alone (0.5 μM), gefitinib alone (10 μM), ATRA plus gefitinib or ATRA and ATO plus gefitinib for 72 h and the expression of the myeloid differentiation markers CD11b, CD11c, CD15, and CD16 was measured by flow cytometry. Gefitinib potentiated the myeloid ATRA-induced differentiation in APL cells resistant to ATRA (NB4-R2) and ATO (NB4 ATOr), as well as in NB4 clone 21 cells. Data represent the mean ± SD of at least three independent experiments. (*) $p < 0.05$, (**) $p < 0.01$, (***) $p < 0.001$ (Kruskal–Wallis test, followed by Dunn's *post hoc* test).

Effects of EGFR Inhibitors in an APL Mouse Model

To investigate the *in vivo* effects of the ATRA/EGFR inhibitor combination, we first assessed the protein levels of Egfr in APL blasts from the transgenic hCG-PML-RARA mice (pool of leukemic blast cells, n=5; **Figure S3B**). No Egfr protein expression was detected in murine APL blasts, suggesting that any potential effects observed upon Egfr monotherapy in this model would be the result of off-target activity. APL transplanted mice were treated for 15 consecutive days with gefitinib (100 or 200 mg/kg/day) or erlotinib (200 mg/kg/day) after confirmation of APL engraftment by PCR analysis of the peripheral blood samples (**Figure 5A**). Mice treated with gefitinib (100 mg/kg/day) (median survival=38 days; 95% confidence interval - CI =34–45 days) (**Figures S7A, B**) or erlotinib monotherapy (200 mg/kg/day; median survival=51 days; 95% CI=44–57 days) (**Figure 5B**) exhibited no prolonged survival compared to the respective vehicle-treated controls (control group for gefitinib: median survival=41 days; 95% CI=35–46 days; control group for erlotinib: median survival=52 days; 95% CI=44–59 days). Treatment with gefitinib at the highest dose (200 mg/kg/day) exhibited a trend to prolong the survival of APL mice (median survival=56 days; 95% CI=47–65 days) (P>0.05).

Due to the increased survival observed in APL mice treated with 200 mg/kg/day of gefitinib, we evaluated if the combination with ATRA (2.5 mg/kg/day) could display additive or synergistic effects *in vivo*. Treatment with ATRA alone or in combination with gefitinib significantly increased the survival rate compared to gefitinib or vehicle (**Figure 5C**), although, there was no difference in survival between mice treated with ATRA alone *versus* ATRA plus gefitinib. Additionally, no differences were observed in spleen weight between the treatment groups (**Figure 5D**). Evaluation of BM and spleen cells regarding the quantification of $CD11b^{-}CD117^{+}$ (APL blasts) and

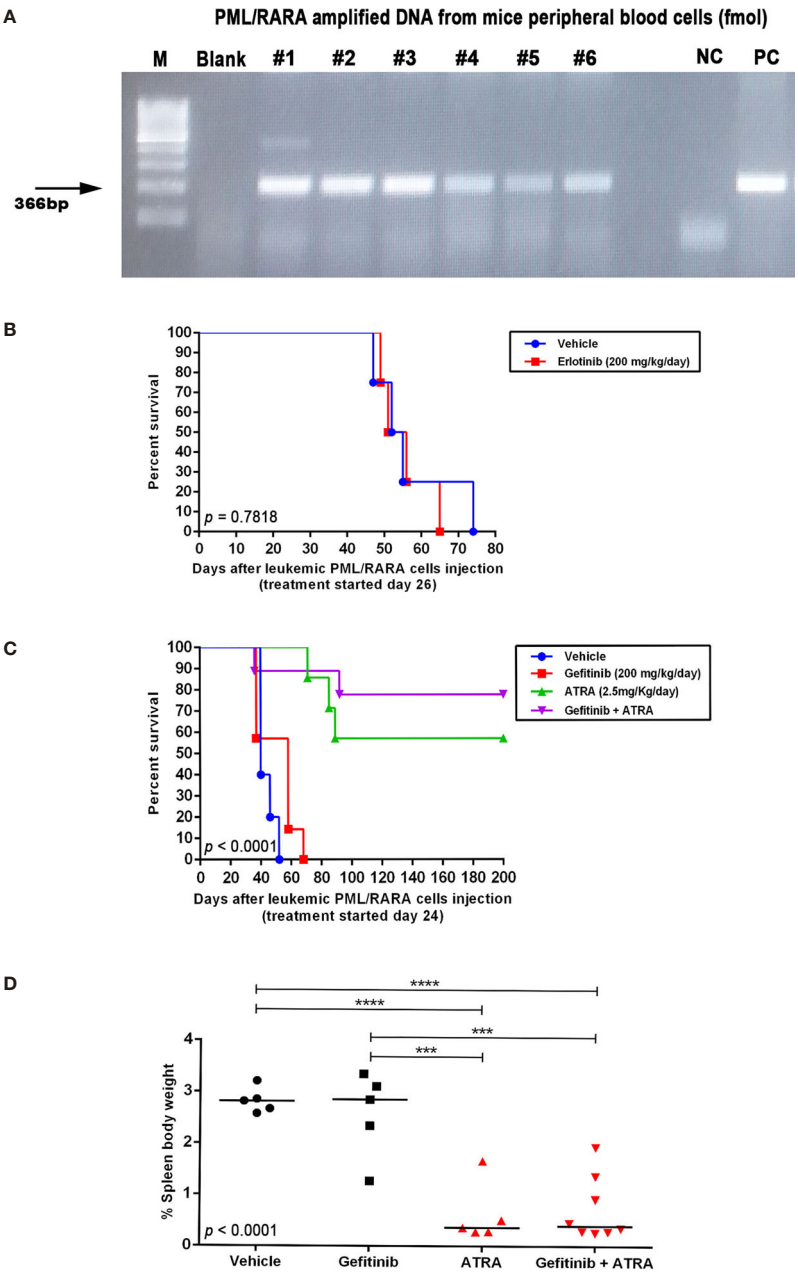

FIGURE 5 | *In vivo* effects of EGFR inhibitors alone or in combination with ATRA in an APL mouse model. **(A)** Expression of the PML–RARA fusion gene in peripheral blood of WT mice 3 weeks after transplantation of leukemic blasts from hCG-PML–RARA transgenic mice detected by conventional PCR. **(B)** Kaplan–Meier survival curves of mice treated with vehicle (DMSO; n=4) or erlotinib at 200 mg/kg/day (n=4). Survival **(C)** and spleen weight-to-body weight ratio **(D)** of mice treated with gefitinib [200 mg/kg/day; n=7 - **(C)** and n=5 - **(D)**], ATRA [2.5 mg/kg/day; n=7 - **(C)** and n=5 - **(D)**], gefitinib plus ATRA [n=9 - **(C)** and n=8 - **(D)**], or vehicle [n=5 - **(C, D)**]. Surviving mice at 200 days post transplantation were sacrificed. (***) $p < 0.001$, (****) $p < 0.0001$ (log-rank or Kruskal-Wallis test followed by Dunn's *post hoc* test).

$CD117^{-}CD11b^{+}/Gr1^{+}$ (myeloid mature) cells at the end of the experiment revealed no differences in the frequency of these two leukemic cell populations comparing treatment groups (**Figure S8**). These results suggest that at least *in vivo*, the combination gefitinib plus ATRA did not enhance the differentiation effect of ATRA monotherapy.

DISCUSSION

In the present study, consistent with previous findings (3, 4) we validated that the combination between gefitinib or erlotinib with ATRA and ATO enhanced the drug-induced myeloid differentiation in APL cells. Moreover, we demonstrate for the

first time that this combination was effective for ATRA- and ATO-resistant APL cells, most likely due to an off-target effect. Altogether, our results provide new insights into the ongoing challenge of developing therapies to overcome ATRA and ATO resistance in APL patients.

Despite the anti-leukemic activity of EGFR inhibitors, AML cell lines do not express EGFR (3, 4, 6, 24, 31), implying that gefitinib and erlotinib may act *via* EGFR-independent mechanisms in this malignancy. In this context, other tyrosine kinases have been identified as potential targets of gefitinib and erlotinib, including SYK (32), which predicts a favorable response to fms-like tyrosine kinase 3 (FLT3)-inhibitors in AML patients harboring mutations in the *FLT3* gene (33–35), with no *EGFR* expression. Notably, similarly to ATRA, the inhibition of SYK was reported to induce differentiation of primary APL blasts (32). The NB4 APL cell lines (including the resistant ones), present detectable expression of SYK, although they are negative for EGFR, suggesting that the mechanism underlying the gefitinib-induced APL sensitization to ATRA and ATO might be linked to off-targets downstream the SYK pathway, as demonstrated previously for other AML cell lines (32). Of note, SYK protein expression was not detected in our primary APL samples, raising the possibility of a broader than SYK spectrum of off-target effects upon EGFR inhibitor therapy in APL. Our findings highlight the relevance of repurposing the FDA-approved tyrosine kinase-targeted therapies to overcome the resistance of a specific subgroup of patients with APL who are unresponsive to standard treatment.

Previous *in vitro* studies have shown that differentiation, cell cycle arrest, and apoptosis are induced in AML cells in response to gefitinib and erlotinib, either alone, or in combination with ATRA or ATO (3, 4, 6, 7, 24, 31). Consistent with these findings, we observed that gefitinib enhanced apoptosis and suppressed proliferation in APL cell lines. Mechanistically, the activation of JNK (c-*jun* NH_2 terminal kinase), a molecular gefitinib off-target (36), plays a crucial role in the ATO-induced apoptosis of APL cells (37), partially explaining the mild cytotoxicity antagonism interaction between ATO and gefitinib.

The EGFR protein was expressed at a low level in 28% of APL BM samples at diagnosis, which was lower than the frequency reported in AML patients (89%). Besides the fact that APL represents a distinct subtype of AML, the difference in EGFR expression could also emanate from different detection methods (Western blot *versus* RPPA). Moreover, p-EGFR (Tyr992) was expressed in 4/6 EGFR-positive samples, suggesting that EGFR activation in APL is similar to other AML subtypes. There was no correlation between *EGFR* mRNA and protein levels, but such disparity has been reported for other genes in different tissues and may reflect post-translational modifications (38, 39). Notably, we found that plasma EGF concentrations were lower in the BM of APL patients compared to healthy controls, in contrast to the higher EGF levels reported in the urine of APL patients at diagnosis (12).

The clinical potential of EGFR inhibitors in AML was first revealed in patients with AML and concurrent NSCLC who achieved complete remission after gefitinib or erlotinib treatment (16, 17, 40). This motivated preclinical studies to assess the efficacy of EGFR inhibitors repurposed into AML clinics. In a mouse xenograft model of AML, erlotinib suppressed tumor growth and increased survival (24). In contrast, in our syngeneic APL mouse model, neither gefitinib nor erlotinib alone induced blast differentiation or prolonged survival. This was consistent with the lack of response observed in patients with advanced AML treated with gefitinib (19). In addition, other studies found that erlotinib monotherapy did not affect cell differentiation or disease remission in AML patients (18, 20). In the present work, the combination of gefitinib and ATRA extended survival in mice and reduced spleen weight-to-body weight ratio compared to gefitinib or vehicle but was not superior to ATRA monotherapy. One limitation of our study is the high sensitivity of hCG-PML/RARA leukemic cells to ATRA monotherapy, in contrast to APL patients, which in the event of relapse frequently show ATRA resistance. Further functional *in vivo* studies are necessary to verify the efficacy of EGFR or other tyrosine kinases inhibitors in APL models resistant to ATRA or ATO treatment.

Although the use of EGFR inhibitors did not prolong survival or increase myeloid differentiation in an APL mouse model sensitive to ATRA, gefitinib stimulated apoptosis, inhibited cell proliferation, and re-sensitized ATRA- and ATO-resistant APL cells to ATRA and ATO induced differentiation, respectively. These findings provide a basis for future studies to explore the potential role of tyrosine kinase-targeted selective therapies in combination with standard therapy, which could be exploited to reverse ATRA and ATO resistance in a subset of patients with APL. Finally, although some of the EGFR signaling components are expressed in APL patient blasts, further investigations are necessary to understand their biological implications on leukemia progression, since the effects of EGFR inhibitors seem to be a result of off-target activities.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Research Ethics Committee of the Medical School of Ribeirao Preto, University of Sao Paulo, Ribeirao Preto, Sao Paulo, Brazil (Reference: CAAE 05060818.9.0000.5440). The patients/participants provided their written informed consent to participate in this study. The animal study was reviewed and approved by Animal Care and Use Committee of the Medical School of Ribeirao Preto of the University of Sao Paulo (Protocol no. #016/2016).

AUTHOR CONTRIBUTIONS

Conceptualization, ER. *Methodology*, LA, IW, DP-M, CO, LC, APL, NA, SM, VD, MN, JA-F, DN, HP, RA-P, CB, PS, ASGL, CA. *Software*, LA, CB, PS. *Validation*, LA, IW, DP-M, CO, LC, APL. *Formal analysis*, LA, IW, DP-M, CO, ER. *Investigation*, LA, IW. *Resources*, ER. Data curation, LA, IW, DP-M, ER. *Writing—original draft preparation*, LA and ER. *Writing—review and editing*, ER, LA, DP-M, RA-P, JS, EA, TO, NN. *Supervision*, JS, EA, TO, NN. Revision of the manuscript. ER. Project administration, ER, PS, ASGL, CA. *Funding acquisition*, ER. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2021.686445/full#supplementary-material

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