Current first- and second-line treatment options in acute promyelocytic leukemia

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Practice points

- Acute promyelocytic leukemia (APL) is a rare subtype of acute myeloid leukemia characterized by a specific genetic hallmark which is the chromosomal translocation t(15;17) that leads to the production of PML–RARA oncoprotein.
- Consumptive coagulopathy is the main clinical feature at diagnosis, and leads to high risk of fatal hemorrhages. For this reason, APL diagnosis represents a clinical emergency, which requires immediate antileukemic treatment and transfusional support, even if disease is only suspected after morphological or clinical assessment.
- All-trans retinoic acid (ATRA) introduction dramatically improved APL patients survival: its association with anthracyclines led to a complete remission rate above 80%. Cytarabine addition provided benefits for high-risk patients.
- Arsenic trioxide (ATO) and ATRA association demonstrated to be highly effective especially in low/intermediate-risk patients, reducing cumulative incidence of relapse rates and toxicity related to treatment. Chemotherapy addition during induction phase could give an advantage to high-risk patients.
- Molecular monitoring of minimal residual disease has to be performed for 2–3 years after the end of consolidation courses. It has been demonstrated that treating patients in molecular relapse rather than hematological, gives a survival advantage and reduces reinduction treatment toxicity.
- ATO and ATRA association as salvage therapy for relapsed APL has to be chosen for patients with no prior exposure to this regimen or long CR1 after ATO-ATRA first-line treatment. Early relapse needs to be treated with the addition of chemotherapy.
- Autologous stem cell transplantation has to be considered as a suitable option for patient in CR2 with good performance status, even if ATO and ATRA prolonged treatment without intensification showed encouraging results.
- Allogeneic stem cell transplantation is indicated for patients who failed to achieve CR2 or who experienced relapse after CR2.

Outcome of acute promyelocytic leukemia (APL) has remarkably improved during the last 30 years, especially after the identification of *PML–RARA* oncogene as a key in the pathogenesis of APL and all-trans retinoic acid as therapeutic agent. Arsenic trioxide has been recently demonstrated to be the most effective single antileukemic agent and it has also showed synergistic action when combined with all-trans retinoic acid, decreasing relapse rate especially in low/intermediate-risk settings. Therapeutic advances led to complete remission rates of more than 90%, modifying disease history. In relapse setting, arsenic trioxide-based regimens showed efficacy for the achievement of second molecular complete remission. The most challenging issue in APL management remains the significant early deaths rate, nowadays the principal reason for treatment failure.

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KEYWORDS

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Acute promyelocytic leukemia (APL) is a rare subtype of acute myeloid leukemia (AML) characterized by distinctive morphological, biologic and clinical features. Cytological aspects of APL can be summarized in two main subtypes of presentation: a classical hypergranular one, characterized by numerous coarse cytoplasmic granules and Auer bodies, and a microgranular variant, in which leukemic cells present a basophilic cytoplasm containing only few or no granules [1]. The disease presents a driving chromosomal translocation $t(15;17)$ leading to the production of *PML-RARA*, a fusion oncogene that has a repressor role toward the activation of hormone-dependent cell differentiation pathways [2]. Variant translocations involve 1–2% of APL cases, presenting abnormal rearrangements such as *PLZF/RARA*, *NMP/RARA*, *NUMA/RARA*, *STAT5B/RARA*, *PRKAR1a/RARA*, *BCOR/RARA* and *FIP1L1/RARA* [3,4]. Hemorrhagic diathesis, prominent at diagnosis, represents the principal cause of early severe complications in these patients [5,6].

Early management

APL onset has to be considered a medical emergency: current international recommendations and guidelines suggest to immediately start treatment with all-trans retinoic acid (ATRA) once APL diagnosis is suspected by recognition of its typical clinical and morphological aspects [7,8]. Massive transfusion support has to be started simultaneously with antileukemic treatment, and should aim to maintain platelet and fibrinogen levels over $30\text{--}50 \times 10^9/\text{l}$ and 1.5 g/l , respectively.

Once diagnosis is confirmed through identification of the pathognomonic chromosomal translocation and the *PML-RARA* fusion gene, patients must undergo the most appropriate induction therapy, according to disease characteristics and clinical conditions.

A simple but efficient tool driving clinician in the choice for the optimal treatment strategy is Sanz's risk stratification: low-risk patients are defined by a white blood cell count (WBC) $<10 \times 10^9/\text{l}$ and platelet count $>40 \times 10^9/\text{l}$, intermediate risk characterizes patients presenting a WBC $\leq 10 \times 10^9/\text{l}$ and platelet count $\leq 40 \times 10^9/\text{l}$, high-risk identifies patients with a WBC $\geq 10 \times 10^9/\text{l}$ [9]. We recently identified immunophenotypic positivity for CD2, CD15, CD34, CD56 and the *FLT3-ITD* mutations as features capable to recognize patients with high

risk of relapse [10]. *FLT3-ITD* prognostic impact still remains controversial, with conflicting data reported in literature. A meta-analysis described worst outcomes in terms of overall survival (OS) and disease-free survival (DFS) for *FLT3-ITD* mutated APL patients treated with ATRA and chemotherapy if compared with unmutated patients [11]. More recent studies reported no association between relapse-free survival (RFS) and *FLT3-ITD* status in patients treated with ATO-based frontline regimens, suggesting that this treatment could overcome the unfavorable influence of this feature [12,13].

First-line treatment• **ATRA & chemotherapy**

ATRA is a compound capable of inducing terminal differentiation of clonal APL promyelocytes to mature granulocytes. It has been introduced in clinical studies from late 1980s, demonstrating a high activity in relapsed/refractory patients and successively in first line of treatment, both as a single agent and associated to chemotherapy. As single agent, complete remission (CR) rates ranged from 70 to 85%, but a high percentage of patients did not maintain response and relapsed [14,15]. Clinical trials started to investigate possible advantages deriving from frontline combination of ATRA and chemotherapy: evidences of outcome improvements with simultaneous administration of ATRA, cytarabine and daunorubicin as induction treatment were reported by Fenaux and colleagues [16]. Anthracyclines role were explored by both the Gruppo Italiano per le Malattie Ematologiche dell' Adulto (GIMEMA) and the Programa Espanol para el Tratamiento de las Hemopatias Malignas del Adulto (PETHEMA): two prospective trials demonstrated ATRA plus IDArubicin (AIDA) followed by consolidation and maintenance schedules of treatment-induced high and more lasting CR rates ranging from 89 to 95% [17,18]. PETHEMA and GIMEMA also modified AIDA protocol by adopting risk-adapted strategies: this approach led to less intensified schedules for low-intermediate risk patients, reducing related toxicity (especially myelosuppression) without decreasing CR rates. The Spanish LPA-99 trial proposed three consolidation courses based on ATRA and dose intensified anthracycline for intermediate- and high-risk patients: results showed improvement in DFS and OS, with an important reduction of cumulative incidence of relapse (CIR; 9 vs

20%) [19]. The Italian AIDA-2000 trial considered intensified treatment for high-risk patients by adding cytarabine and ATRA to anthracycline consolidation chemotherapy and ATRA for all risks: CIR rates showed a significant improvement if compared with previous AIDA-0493 trial, especially for high-risk patients (9.3 vs 49.7%) [20]. This approach was adopted also by PETHEMA LPA-2005 trial and confirmed an advantage in relapse rate at 3 years (14 vs 27% reported in LPA-99); this result suggests a synergistic effect of cytarabine when combined to ATRA [21,22]. These findings were subsequently explored and confirmed by different multicenter studies conducted worldwide, which contributed to the success of this treatment protocol [23,24]. The results of trials employing ATRA and chemotherapy in frontline treatment are shown in **Table 1**.

Risk-adapted strategies contributed to improve outcomes for APL patients treated with ATRA and chemotherapy. Notwithstanding, different issues in this setting remain unsolved. It remains still unclear as to which anthracycline has to be the choice, since no prospective study has ever compared idarubicin and daunorubicin. A recent retrospective study showed similar molecular and hematological relapse rate but higher daunorubicin-related toxicity [25]. Another open issue remains the cytarabine role in induction treatment. The British Medical Research Council AML15 trial described higher toxicity without any significant survival advantage when cytarabine was administered [23]. In high-risk setting,

the French group showed similar CR rates but an increased risk of relapse when cytarabine was not integrated in induction and consolidation courses, comparing to regimens containing this antileukemic agent [26]. At the present time, a common strategy is the use of cytarabine for high-risk patients during consolidation therapy.

As for other subtypes of AML, extramedullary disease and particularly CNS involvement has been described. PETHEMA group analyzed CNS relapses in LPA96 and LPA99 trials, reporting 2% of incidence: in multivariate analysis, emerged as favoring factors CNS hemorrhage at presentation and Sanz risk score (high risks presented a prevalence of 5.5%). Some authors emphasize the driving role of molecular cerebral spinal fluid examination, underlining the higher sensibility of this method if compared with cytology [27]. Owing to association of CNS relapses to high-risk patients and the not negligible incidence of this disease localization in APL, different authors and working groups suggested to offer intrathecal prophylaxis to high-risk patients, but only after the achievement of hematologic CR [28–30].

Finally, the role of maintenance therapy after the achievement of molecular CR (mCR), especially for low–intermediate-risk patients, remains under investigation. A French study described a survival advantage for patients receiving maintenance therapy, especially for those receiving ATRA and low-dose chemotherapy in combination [16]. This evidence was reported also from the US North Intergroup

Table 1. Results from trials evaluating outcomes of all-trans retinoic acid and chemotherapy regimens as first-line treatment.

Study (year)	Patients (n)	Median follow-up (months)	CR (%)	OS (%)	CIR (%)	Data interpretation	Ref.
Mandelli <i>et al.</i> (1997)	240	12	95	87	NA	AIDA + CHT consolidation + maintenance as a new standard of frontline treatment	[17]
Fenaux <i>et al.</i> (1999)	576	NA	93	NA	NA	ATRA + CHT is better than ATRA -> CHT	[16]
Sanz <i>et al.</i> (1999)	136	NA	89	82	NA		[18]
Sanz <i>et al.</i> (2004)	561	82	91	83	11	Dose intensified anthracycline reduced incidence of relapse in int.- and high-risk setting	[19]
	107 low risk	82	96	89	4		
	313 int. risk	82	94	88	7		
	140 high risk	82	81	68	27		
Lo-Coco <i>et al.</i> (2010)	445	59	95	87	11	Cytarabine and ATRA added to consolidation courses reduced incidence of relapse in high-risk setting	[20]
Sanz <i>et al.</i> (2010)	402	28	92	88	9		[21]
	83 low risk	28	99	96	6		
	191 int. risk	28	95	91	8		
	98 high risk	28	83	79	14		

AIDA: ATRA plus idarubicin; ATRA: All-trans retinoic acid; CHT: Chemotherapy; CIR: Cumulative incidence of relapse; CR: Complete remission; Int.: Intermediate; OS: Overall survival.

in a trial offering maintenance treatment with ATRA after CR achievement post-ATRA and chemotherapy regimens [31]. A GIMEMA trial randomized patients that achieved mCR to receive maintenance therapy with ATRA as single agent, ATRA and low-dose chemotherapy, only low-dose chemotherapy; a fourth observational arm included patients who did not receive any maintenance treatment. This study was unable to detect any survival improvement of maintenance arms compared with the observational one [32]. In line with these findings, the Japan Adult Leukaemia Study Group (JALSG) conducted a randomized trial which described no DFS advantage after maintenance treatment for patients in mCR [33].

• Arsenic trioxide-based regimens

Arsenic trioxide (ATO) employment in APL treatment started in the 1970s. ATO was first tested as antileukemic treatment by Chinese investigators, showing potent activity: Sun *et al.* reported a CR rate of 66% and a 10-year survival rate of 30% for APL patients treated with intravenous ATO as single agent, while Zhang and colleagues used ATO in 30 newly diagnosed and 42 relapsed patients, reporting CR rates of 73 and 52%, respectively [34,35]. In 1997, Shen *et al.* described outcomes for ATO-based treatment in 15 relapsed APL patients: 14 patients achieved a CR, in ten cases receiving ATO as single agent [36]. These CR rates were confirmed by following studies, in which this compound was tested as single agent in a small numbers of relapsed patients [37,38]. Two prospective trials, conducted in India and Iran, used ATO as single agent for induction and consolidation treatment. Both trials were reported with a long-term follow-up: the Indian study described 5-year OS and DFS of 74.2 and 80.2%, respectively, while the Iranian group reported 5-year DFS and OS of 66.7 and 64.4%. Both studies described higher relapse rates in high-risk patients [39,40].

ATO acts inducing cellular apoptosis and degradation of *PML/RARA* proteins targeting multiple intracellular pathways (induction of reactive oxygen species (ROS), caspase activation, *NF-κB* inhibition, *BCL-2* downregulation) [41]. Inorganic arsenic is methylated in liver, allowing the production of monomethylarsonic acid and dimethylarsinic acid. It has been demonstrated that those metabolites, especially monomethylarsonic acid, possess more cytotoxic power than the original compound and are also active against

some types of lymphoma cells [42]. Laboratory models showed synergistic action of ATRA and ATO, inducing granuloblastic differentiation and leading to the eradication of leukemic cells through retinoic acid receptor- α degradation [43,44]. Combination of ATRA and ATO as induction treatment was investigated for the first time by a Shanghai group, which randomized newly diagnosed patients to ATO or ATRA single agent treatment or the combination of the two compounds, followed by postinduction chemotherapy. The three arms showed similar CR rates ranging from 90 to 95.2%, but patients in the combination arm presented an advantage for what concerns time to CR achievement, platelet recovery and DFS [45]. The Australasian group performed the APML4 trial, treating newly diagnosed APL patients with ATRA, ATO and idarubicin as induction therapy, ATO plus ATRA for two consolidation cycles and ATRA plus low-dose chemotherapy as 2-year maintenance therapy. Investigators performed a historical comparison with the previous APML3 trial, in which induction and consolidation treatment did not include ATO. The results showed a better outcome in terms of DFS but no OS advantage. A recent update reported 5-year OS and event-free survival (EFS) of 94 and 90% (87 and 83% in high-risk setting, respectively) [46,47].

• ATO & ATRA (chemo-free strategy)

The first trial adopting induction and consolidation therapy based on ATRA and ATO was conducted at the MD Anderson Cancer Center. High-risk patients also received a single dose of gemtuzumab ozogamicin (GO) at the beginning of the induction cycle. Overall CR rate was 89%, with a significant advantage for low-risk patients if compared with high-risk (96 vs 79%, respectively), while estimated OS at 3 years was 85%. Earliest complications such as intracranial hemorrhage and differentiation syndrome (DS) were most common in high-risk group and represented the principal reasons for treatment failure. The study enrolled 82 patients and only three relapses were reported, all concerning the high-risk group [48,49]. These results suggested that chemotherapy-free approach could give a survival advantage to low- and intermediate-risk groups, while patients presenting with hyperleukocytosis could still benefit from the use of cytotoxic agents in induction phase.

For this reason, the GIMEMA and the German groups, AMLSG and SAL, designed the Phase III,

multicenter trial, namely APL0406, comparing ATRA and ATO with classic AIDA schedule (ATRA and idarubicin) in low- and intermediate-risk patients, in order to demonstrate the noninferiority of chemotherapy-free regimen. This study enrolled 276 patients; results were published in 2013 and followed by a final update in 2016, referring to an average follow-up of 40.6 months. OS (99.2 vs 92.6% $p = 0.0013$), EFS (97.3 vs 80% $p < 0.001$) and CIR (1.9 vs 13.9% $p = 0.0073$) were superior for ATRA and ATO arm as compared with chemotherapy induction in this subset of patients (Table 2). Two cases of molecular resistance after consolidation and two cases of therapy-related myeloid neoplasms were reported in AIDA group. Toxicity profile referred to ATRA and ATO administration principally consisted in elevated liver enzymes and QTc prolongation, side effects managed in all cases with temporary therapy discontinuation [50,51]. These findings led to the update of several international guidelines and recommendations, indicating ATRA and ATO frontline regimen as a possible new standard approach for low- intermediate-risk patients [8,9]. A recent randomized trial comparing ATO and ATRA with AIDA regimen named AML17 was conducted by the UK National Cancer Research Institute investigators, enrolling 235 patients. Unlike APL0406 trial, this study included also high-risk patients for whom treatment protocol provided the administration of GO during the first days of induction cycle. Other differences between the two studies regarded the absence of any maintenance treatment in AIDA arm and a less frequent ATO administration schedule, in order to improve patients' compliance. CR rates after induction treatment were 89 and 94% for AIDA and ATRA-ATO arms, respectively. Investigators reported similar 4-year OS rates of 89 and 93%, while 4-year EFS and CIR highlighted an advantage for chemo-free regimen (EFS 70 vs 91%, $p = 0.002$; CIR 18 vs 1%, $p = 0.0007$ for AIDA and ATRA-ATO arms, respectively). In the high-risk population, no significant differences were reported in terms of OS (87 vs 84%, $p = 0.5$) and EFS (87 vs 64%, $p = 0.07$) for AIDA and ATRA-ATO arms, respectively, suggesting that ATRA-ATO treatment could be a successful approach also in this setting if at least one dose of a single chemotherapeutic agent is added in induction phase. Toxicity profiles were similar to APL0406 study reports, with liver toxicity and QTc prolongation as the most common side effects [52]. The results of the main studies

employing ATO-based regimens in first line of treatment are summarized in Table 1 [39,53–56].

Several features related to ATRA and ATO regimen administration still need to be clarified, and should represent future key points of investigation in next clinical trials.

As discussed before, different studies explored the possibility to add other antileukemic agents in combination with ATRA and ATO (idarubicin in APML4 and GO in AML17), reporting promising results in high-risk patients. However, randomized trials need to be performed in order to clarify as to which agent and schedule should be employed.

A common complication associated to frontline treatment with ATRA and/or ATO treatment is DS. Respiratory distress, fever, weight gain, pleuro-pericardial effusions, pulmonary infiltrates and acute kidney injury characterize this life-threatening condition. DS often progresses rapidly, sometimes because its diagnosis can be misleading due to specific signs and symptoms at presentation. In ATRA and ATO setting, this complication has been reported in a range between 2 and 31% of patients [74,75]. Other trials evaluated DS incidence in both ATRA and chemotherapy and ATRA as single agent arms, reporting a diminished percentage of comparison in the combination arm (9% vs 18–25%) [76]. To avoid the onset of DS, most induction treatment regimens nowadays include steroids administration, a clinical practice that has been shown to be useful especially in patients with hyperleukocytosis at presentation [77,78]. As reported in different works, this approach seems to have reduced mortality rate associated to DS, which nowadays involves 1–2% of cases [20,21]. In APL0406 trial, beyond steroids prophylactic administration in ATRA-ATO arm, hyperleukocytosis was also managed with the addition of hydroxyurea, an intervention capable to neutralize this complication in all reported cases [50].

Nowadays no clinical trial has clearly demonstrated as to which approach, between prophylactic or pre-emptive steroids administration, provides better outcomes.

Despite low toxicity profile associated to ATO administration, some side effects often require temporary drug suspension or dose modification. These events frequently occur when QTc prolongation is detected, a consequence of ATO's effect on cardiac repolarization [79]. A recent US study analyzed electrocardiograms from 113 patients undergoing ATO treatment, reporting

Table 2. Results from trials evaluating different reinduction and consolidation strategies in acute promyelocytic leukemia relapsed patients (molecular relapse/hematologic relapse) after frontline all-trans retinoic acid plus chemotherapy.

Study (year)	Patients (n)	Median follow-up (months)	Treatment schedule	CR2 (%)	OS (%)	TRM (%)	Relapse (%)	DFS (%)	Ref.
Shen <i>et al.</i> (1997)	15	17	ATO ± CHT	93	>80	NA	29	NA	[36]
Niu <i>et al.</i> (1999)	47	24	ATO ± CHT	85	50	NA	52	42	[37]
Soignet <i>et al.</i> (2001)	40	17	ATO (+allo [14 patients]/auto-SCT [3 patients])	85	66	NA	NA	56	[57]
Ohnishi <i>et al.</i> (2002)	14	17	ATO + CHT (+allo-SCT [2 patients])	79	NA	NA	43	NA	[38]
Lazo <i>et al.</i> (2003)	12	24	ATO + CHT (+allo-SCT [1 patient])	100	80	NA	25	67	[58]
Raffoux <i>et al.</i> (2003)	20	21	ATO ± ATRA (+allo [7 patients]/auto-SCT [1 patient])	80	59	NA	25	59	[59]
Shigeno <i>et al.</i> (2005)	34	30	ATO ± CHT + ATRA (+allo [9 patients]/auto-SCT [1 patient])	91	56	NA	20	EFS 17%	[60]
Thomas <i>et al.</i> (2006)	50	30	ATRA + CHT	90	51	NA	NA	47	[61]
	25		ATO	84	77	NA	NA	90	
Alimoghaddam <i>et al.</i> (2011)	31	32	ATO	77	81	NA	29	55	[62]
Lou <i>et al.</i> (2014)	64								[63]
	(12 MR)	27	ATO ± ATRA (+1 auto-SCT)	92	100	NA	17	81	
	(52 HR)	38	ATO ± ATRA ± CHT (+allo [4 patients]/auto-SCT [2 patients])	89	72	NA	40	57	
De Botton <i>et al.</i> (2005)	122	84	ATRA + CHT	NA					[64]
	(23)		+Allo-SCT		52	39	4	93	
	(50)		+Auto-SCT		60	6	18	79	
	(49)		+Other consolidation treatment		40	NA	53	38	
Sanz <i>et al.</i> (2007)	332	42	ATRA + CHT	NA					[65]
	(195)		+Allo-SCT		NA	24	17	59	
	(137)		+Auto-SCT		NA	16	37	51	
Kohnno <i>et al.</i> (2008)	15	48	Auto-SCT	NA	76	0	20	69	[66]
	13		Allo-SCT		46	38	8	46	
Thirugnanam <i>et al.</i> (2009)	37	32	ATO/ATO + ATRA/ATO + ATRA + CHT	89					[67]
	(14)		+Auto-SCT		100	0	7	EFS 83%	
	(19)		+ATO ± ATRA		39	0	63	EFS 35%	
Ferrara <i>et al.</i> (2010)	13	25	Auto-SCT	NA	77	0	23	75	[68]
Ramadan <i>et al.</i> (2012)	31	55	Allo-SCT	NA	45	19.6	38	50	[69]
Yanada <i>et al.</i> (2013)	35	59	ATO + auto-SCT (23 patients)	81	77	0	13	EFS 65%	[70]
Pemmaraju <i>et al.</i> (2013)	16	122	CHT consolidation	NA	40	NA	19	NA	[71]
	7	74	Auto-SCT		86	10	0	EFS 69	
	14	118	Allo-SCT		49	29	14	EFS 41	
Fujita <i>et al.</i> (2013)	30	87	CHT consolidation	NA	77	NA	51	EFS 51	[72]
	6	84	Auto-SCT		83	0	59	EFS 42	
	21	84	Allo-SCT		76	19	10	EFS 71	
Holter Chakrabarty <i>et al.</i> (2014)	223	115	Allo-SCT	NA	54	31	18	50	[73]
	62	72	Auto-SCT		75	7	30	63	

APL: Acute promyelocytic leukemia; ATO: Arsenic trioxide; ATRA: All-trans retinoic acid; CHT: Chemotherapy; DFS: Disease-free survival; EFS: Event-free survival; HR: Hematologic relapse; MR: Molecular relapse; NA: Not available; OS: Overall survival; SCT: Stem cell transplantation; TRM: Treatment-related mortality.

that two-thirds of patients showed QTc prolongation when calculated with Bazett correction formula, widely used by clinicians. However, no clinically significant cardiac arrhythmias were diagnosed in those patients [80]. The authors concluded suggesting the utilization of different formulas (such as Fridericia or Hodges) for QTc

calculation and the modification of QT limit for ATO withdrawal to 500 ms [81].

As a result of ATO increased employment, actually explored also in high-risk patients, an unsolved issue is represented by CNS prophylaxis and its indications in ATO-based regimens. A pharmacodynamic study revealed the presence

of inorganic arsenic and its methylated metabolites, both with demonstrated antileukemic activity, in cerebral spinal fluid samples from three APL patients treated with intravenous ATO [82]. These evidences suggested that the amount of active drug passing the blood–brain barrier could provide a satisfactory antirelapse activity, but these findings need to be confirmed in larger studies.

Last, an innovation for this treatment regimen could be represented by oral ATO preparations. Several studies explored this possibility reporting high efficacy, favorable toxic profile, lower costs and improvement for patients' quality of life [83,84]. Randomized trials are needed to promote its inclusion in clinical practice.

• Treatment of variant translocations

Patients with clinical and morphological features of APL may not exhibit an identifiable t(15;17) by cytogenetic studies. In these cases, a compound or cryptic *PML–RARA* rearrangement can be observed at molecular analysis [3]. However, some of t(15;17) negative cases of APL are related to rare variant rearrangements that involve *RARA*. Most of these variant translocations, such as *NUMA/RARA*, *PRKAR1a/RARA* and *FIPIL1/RARA*, showed high sensitivity to standard APL treatment regimens. *NPM/RARA* and *BCOR/RARA* translocations demonstrated a good response to ATRA and ATO too, but also a higher tendency to relapse [85,86]. *ZBTB16/RARA* variant, the most common variant translocation, did not show high sensitivity to ATRA [87]. Patients with this translocation can show an initial response to ATRA, which is able to trigger cells differentiation but is not to fully eradicate leukemic cells and to permit the achievement of a CR [88]. Some authors described also a resistance toward ATO, which is supposed to be conferred by absent activity of ROS toward *ZBTB16/RARA* [89]. The European Working Party described 11 cases of *ZBTB16/RARA*-positive APL: ten patients were treated with different chemotherapy regimens in association with ATRA, and all achieved a CR. Five patients are still alive at a median follow-up of 28 months; two of them received an allogeneic stem cell transplantation (SCT) [3]. Similar to *ZBTB16/RARA*, also *STAT5B/RARA* cases showed poor sensitivity to ATRA [90]. In conclusion, patients presenting a variant translocation conferring resistance to conventional APL treatment may benefit

from an antileukemic treatment based on the combination of chemotherapy and ATRA.

Second-line treatment

• ATO-based salvage therapy, role of SCT

ATRA and ATO combination for newly diagnosed APL patients have permitted the achievement of postinduction CR rates up to 90% and cure rates of approximately 80%. Relapses after ATO-based treatment occurred in a very small amount of cases, while about 20% of patients treated with ATRA and chemotherapy approach experienced disease relapse [91].

Several studies through years underlined the importance of monitoring minimal residual disease (MRD) in order to detect molecular relapse as soon as possible, which is defined as the identification of two positive samples with reverse transcription-PCR (RT-PCR) in marrow samples collected at least 2 weeks apart after consolidation courses. Detection of molecular relapse allows early therapeutic intervention, which determined, in ATRA plus chemotherapy setting, an increase in OS if compared with patients treated in hematological relapse [92,93]. A retrospective analysis explored the outcome of 64 *PML/RARA*-positive APL patients in first hematological (81%) or molecular (19%) relapse treated with ATO. The investigators reported an advantage in terms of 3-year RFS and OS for molecular relapse group if compared with hematological relapse group (RFS: 81.5 vs 57% and OS 100 vs 72%, respectively) [63].

Recently published data from European LeukemiaNet registry on 155 patients in first relapse treated in reinduction with ATO, documented an advantage for patients treated in molecular relapse only in terms of DS and infections rates, if compared with patients treated in hematological relapse. No survival differences were reported. All patients presented *PML/RARA* transcript [94].

Several reports describe that molecular monitoring of MRD seems to give an advantage to patients. Expert opinions underline the necessity to perform a longitudinal monitoring through RT-PCR every 3 months for the first 2 years after the end of consolidation courses. It is debatable whether low- and intermediate-risk patients treated with ATO and ATRA regimen need to be monitored for MRD because of the very low rate of relapse in this setting. No clear indications have been provided about monitoring duration in APL relapsed patients [29,49,95].

International guidelines suggests to use ATO plus ATRA treatment for patients with no prior exposure to ATO in first line and also for either late (>6 months) or early (<6 months) relapses after ATO-containing regimens, but in the last case NCCN guidelines recommend to add an anthracycline for two cycles of salvage therapy [8]. Patients achieving a second mCR should receive intensification treatment such as autologous SCT, or continue consolidation treatment with ATO and ATRA regimen. Due to lack of prospective studies comparing these two postremission approaches, clinicians should consider individual patient characteristics, such as age and/or performance status, to select the appropriate choice. Patients who failed to achieve a second mCR should receive an allogeneic SCT or, if this procedure could not be performed, be addressed to investigational approaches [7,8].

ATO administration as salvage therapy in relapsed APL was approved after the publication of the results of a trial conducted by the US Intergroup, which reported a CR rate of 85% and an 18-month RFS of 56%, in 40 patients in first or second relapse [57]. A literature review described outcomes of 304 relapsed patients treated with ATO as reinduction therapy between 1997 and 2011. Approximately 40% of treated patients were in second or more advanced relapse, and 59 of them received autologous or allogeneic SCT as postinduction therapy. CR rate was 86%, with an mCR rate of 52% and an estimated 24-month survival ranging from 50 to 81% [96]. Different authors documented better outcomes in this setting when ATRA was combined to ATO: in a meta-analysis, Wang and colleagues reported 2-year CR and DFS rates of 70 and 85%, with few cases of early death [97]. Our group described outcomes of a small cohort of patients who received prolonged treatment with ATRA and ATO showing a durable CR rate of 88% without SCT intensification after a noteworthy follow-up (ranging from 11 to 50 months) [98].

Postconsolidation therapy has to be selected between autologous or allogeneic SCT (intensified approach), or other treatment like maintenance with ATO-based regimens. Despite several experiences regarding this topic have been reported in literature, no randomized trials directly comparing these different treatment options are available, and this approach remained a matter of discussion. A retrospective review compared autologous versus allogeneic

SCT in APL relapsed patients, describing 7-year RFS and OS of 92 and 52% for allogeneic and 79 and 60% for autologous, but with a treatment related mortality (TRM) of 39 and 6%, respectively [64]. A retrospective analysis based on the Center for International Blood and Marrow Transplant Research registry, reported outcomes from 294 patients in CR2 treated with allogeneic (79%) or autologous (21%) SCT: 5-year DFS and OS and 3-year TRM documented an advantage for autologous SCT (63, 75 and 2%, respectively) when compared with allogeneic (50, 54 and 30%) [73]. Gorin from European Society for Blood and Marrow Transplantation recently stated that, lacking strong evidences supporting allogeneic in this setting, autologous SCT should be preferred for patients in second CR (CR2) [99].

A retrospective multicenter experience from Italian group reported a 5-year OS of 45% and a TRM of 19% in CR2 or in third CR (CR3) patients receiving allogeneic SCT [69]. A Phase II study conducted in Japan evaluated the efficacy of ATO as reinduction and consolidation treatment followed by autologous SCT in 35 relapsed patients: 5-year EFS and OS reported were 65 and 77% [70]. The Indian group compared outcomes of 37 relapsed patients who received ATO-ATRA (19 patients) or auto-SCT (14 patients) as CR2 consolidation regimen: 5-year EFS (34.5 vs 83.3%, respectively, $p < 0.001$) and OS (38.5 vs 100%, respectively, $p < 0.001$) were superior in the second arm [67]. A retrospective study, collected from European Society for Blood and Marrow Transplantation and Center for International Blood and Marrow Transplant Research registries, evaluated outcome for 207 patients in CR2 treated with ATO alone (32%) or with autologous SCT intensification (68%): 5-year OS showed an advantage for auto-SCT group (78 vs 48%, respectively, $p < 0.001$) [100]. These evidences were confirmed also from European LeukemiaNet registry, which reported a 3-year OS of 77% for the autologous group vs 59% of ATO alone [57]. The results of trials employing ATO-based regimens in second line of treatment and comparing allo- and auto-SCT are listed in **Table 3** [36,37,57–63,65,66,68–73,101].

These findings support recommendations for autologous SCT in CR2 for patients in good performance status. Patients relapsed after a long-term CR could take advantage from postconsolidation ATO and ATRA. Due to high TRM, allogeneic

Table 3. Results from trials evaluating outcomes of arsenic trioxide-based regimens as first-line treatment.

Study (year)	Patients (n)	Median age (years)	Median follow-up (months)	Sanz risk		Treatment schedule	CR (%)	OS (%)	Relapse (%)	DFS (%)	Ref.
				Low/int.	High						
Shen <i>et al.</i> (2004)	61	30.5	18	47	14	ATRA	95	85	26.3	70	[45]
		39.5				ATO	90	90	11.1	88	
		34				ATRA + ATO	95.2	95.2	0	100	
Estey <i>et al.</i> (2006)	44	45	16	25	19	ATRA + ATO (+GO for high risk)	89	86	8	86	[48]
Ravandi <i>et al.</i> (2009)	82	42	99	56	26	ATRA + ATO (+GO for high risk)	92	85	4	NA	[49]
Dai <i>et al.</i> (2009)	162	34	30	123	39	ATRA + CHT	90.3	NA	22.2	72.4	[53]
		32				ATRA + CHT (+ATO consolidation)	90.3	NA	5	93.8	
						ATRA + ATO	93.3	NA	4.8	92.6	
Mathews <i>et al.</i> (2010)	72	28	60	22	50	ATO	86	74.2	NA	80	[39]
Gore <i>et al.</i> (2010)	45	50	31	31	14	ATRA + CHT (+ATO consolidation)	91	88	2.2	90	[54]
Powell <i>et al.</i> (2010)	481	NA	54	368	113	ATRA + CHT	90	86	NA	70	[55]
						ATRA + CHT (+2 ATO cycles)	90	81	NA	90	
Ghavamzadeh <i>et al.</i> (2011)	197	29	38	160	37	ATO	86.3	64.4	25.3	66.7	[40]
Lou <i>et al.</i> (2013)	137	38.4	35	92	45	ATRA + ATO	93.4	98 (high) 99 (low/int.)	4	88 (high) 98 (low/int.)	[56]
Zhu <i>et al.</i> (2013)	231	36	39	185	46	Oral arsenic ATO	99.1 97.4	99.1 96.6	0.8 0.8	98.1 95.5	[102]
Iland <i>et al.</i> (2015)	124	44	50	99	24	ATRA + ATO + CHT	95	94	1.7	95	[47]
Burnett <i>et al.</i> (2015)	235	47	30.5	178	57	ATRA + CHT	89	89	18	NA	[52]
						ATRA + ATO	94	93	1	NA	
Platzbecker <i>et al.</i> (2016)	266	46.6	40.6	266	0	ATRA + CHT	97	92.6	13.9	82.6	[51]
						ATRA + ATO	100	99.2	1.9	97.3	

ATO: Arsenic trioxide; ATRA: All-trans retinoic acid; CHT: Chemotherapy; CR: Complete remission; DFS: Disease-free survival; GO: Gemtuzumab ozogamicin; Int.: Intermediate; NA: Not available.

SCT finds an indication only for patients unable to achieve a CR2 or in subsequent CR.

• New treatment strategies

Different authors reported efficacy of oral arsenic formulation in APL patients [102,103]. Zhu and colleagues conducted a randomized trial comparing oral tetra-arsenic tetra sulphide (indigo realgar) with intravenous ATO in 231 newly diagnosed patients, receiving induction, consolidation and maintenance therapy with ATRA associated to ATO or indigo realgar. A recent update with a median follow-up of 61 months reported similar 7-year CIR, EFS and OS rates between indigo realgar and ATO groups (4.7 and 5.3%; 93.7 and 89.4%; 95.4 and 90.9%, respectively) [104].

Tamibarotene is a synthetic retinoid that showed capacity of differentiation and

affinity with retinoic acid receptor ten times more potent than ATRA [105,106]. The Japanese group investigated about the use of tamibarotene as maintenance therapy, compared with ATRA. No significant differences in the two arms were reported, even if tamibarotene seems to be more efficacious in high-risk patients [107]. Tamibarotene has been also tested in relapsed patient after ATO and ATRA treatment, inducing a response in 64% of patients [108]. Future trials evaluating feasibility and safety of a possible combination with ATO are needed.

Future perspective

Clinical management of APL patients has dramatically improved during last years due to introduction of ATO-based frontline regimens, which reduced relapse rates and the incidence of

related deaths. Despite this, several challenges still have to be faced, that will probably represent key points to be solved in next years.

Early mortality rates, still ranging between 18 and 30%, as reported by different registries, did not show significant improvements during last years [109–112]. The major cause of death is still represented by severe hemorrhages usually developed before obtaining a diagnosis, an issue related to a clinical presentation that can be misunderstood in peripheral institutions with no specialized department. These findings documented that early deaths in APL are underestimated in clinical trials probably because enrolled patients are strongly selected but suggested that clinical skills in diagnosis and management of APL patients in emergency departments has to be ameliorated through specific educational programs.

Several evidences published demonstrated improved clinical outcome, reduced toxicity in low- and intermediate-risk patients. However, we still do not know if high-risk patients could take advantage from ATO-based regimens, due to the low number of patients enrolled in clinical trials. GO and other antileukemic drugs have been often added to conventional treatment and tested in this setting, with unclear benefits. Novel agents resulted to increase ATO activity: bortezomib, a proteasome inhibitor, could enhance ATO activity through the downregulation of NF- κ B pathway and induction of ROS generation in blast cells, as demonstrated *in vitro* and *in vivo* in animal models. A Phase II trial enrolled 16 relapsed APL patients who received ATO-bortezomib association: treatment was well tolerated and all patients achieved molecular remission, which was sustained at a median follow-up of 447 days [113]. Other studies evaluated possible clinical use for new small molecules: a recent study reported antileukemic activity of PRIMA-1 compound through *p53* reactivation and caspase-mediated apoptosis [114]. Fucoidan, a natural compound derived from a marine algae, showed capacity to increase apoptosis and enhance differentiation of blast cells when associated to ATO and ATRA *in vitro* and *in vivo* mice models [115]. A hyaluronan/zinc oxide nanocomposite was recently tested in a subset of cancer cells, in order to demonstrate antineoplastic activity. In HL-60 APL cells it showed an important proapoptotic function, increasing caspase activity [116].

These new molecules have to be investigated in order to assess safety and feasibility of

a possible association with ATO and ATRA, especially for high-risk and relapsed patients.

Despite high efficacy of chemo-free approach, a small number of patients still experience relapse after ATO and ATRA treatment. Different authors performed mutational analysis of neoplastic cells in APL newly diagnosed and relapsed patients, reporting recurrent alterations in *FLT3*, *WT1*, *NRAS* and *KRAS* in the first category and mutations in *PML* and *RARA* genes in the second group. Furthermore, two novel loss-of-function mutations involving *ARID1B* and *ARID1A* genes were discovered in relapsed group [117]. Chendamarai and colleagues described a mechanism of environment mediated drug resistance to ATO in APL leukemic cells through the upregulation of adhesion and cytokine genes at gene expression profile analysis [118].

These data suggest that biological and mutational profile of relapsed APL leukemic cells need to be further explored, looking for possible targets of new specific drugs.

Conclusion

Clinical management and outcome of APL patients have deeply changed after the introduction of ATRA as a treatment option in association with chemotherapy. Disease knowledge and baseline characteristic analysis have allowed the adoption of risk-adapted therapeutic approaches, to de-escalate chemotherapy treatment, in order to reduce drugs-related morbidity. Inclusion of ATO for newly diagnosed APL patients has improved long-term outcome and reduced toxicity. Several trials demonstrated the efficacy of chemo-free regimen based on ATO and ATRA combination in low/intermediate-risk setting. Further studies are needed to better understand if a similar approach can be safe and effective also for high-risk patients. New target drugs could enhance ATO and ATRA activity and find a therapeutic role in high-risk and relapsed patients.

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