features. These translocations can be detected by reverse transcriptase-mediated PCR (RT-PCR), which becomes a sensitive, rapid and objective method for diagnosis (3). However, the classification of other categories is based on morphology of bone marrow (BM) cells and on the history of patients, although a number of genetic alterations, which are involved in the pathogenesis of AML and associated with the prognosis of patients, have been documented (4). Recently, it has been demonstrated that mutations of *FLT3*, *NPM1*, and *C/EBPA* genes are preferentially found in AML with normal cytogenetics and are highly implicated in the prognosis (5). The fourth edition of the WHO classification have included *NPM1* and *C/EBPA* mutations as provisional entities in AML-RGA, but not *FLT3* mutation because it is associated with a number of other entities (6). Furthermore, the AML-MLD category has been renamed as AML with myelodysplasia-related changes, in which myelodysplastic syndrome (MDS)-related cytogenetic abnormality, as well as previous history of MDS and MLD, has been included as a criteria for the diagnosis. However, it was suggested that AML is the consequence of two broad complementation classes of mutations: those that confer a proliferative and/or survival advantage to hematopoietic progenitors (class I mutation) and those that impair hematopoietic differentiation and confer properties of self-renewal (class II mutation) (7). In addition, clinical significance of genetic alterations in the setting of morphologic MLD remains unclear. Therefore, it is necessary to analyze genetic alterations comprehensively, taking them into account all together rather than individually to elucidate the genetic background and prognostic impact in AML (8).

It has been generally considered that *FLT3*, *cKIT*, and *N-RAS* mutations are class I mutations, and *C/EBPA* and *AML1* mutations, and *AML1/ETO*, *CBFB/MYH11*, *PML/RARA*, and *MLL* abnormalities are class II mutations, while overlap mutations of these mutations between class I and class II or within the same class in a clinical sample are not fully characterized, and the positions of *NPM1* mutation and the partial tandem duplication of the *MLL* gene (*MLL*-PTD) remain unclear. *TP53* mutations are reportedly infrequent but are associated with a poor prognosis in *de novo* AML (9–11). In addition, an association between *TP53* mutations and complex karyotype in therapy-related MDS and AML has been reported (12, 13). However, the position of *TP53* mutation remains unclear.

In this study, we comprehensively analyzed mutations of *FLT3*, *cKIT*, *N-RAS*, *C/EBPA*, *AML1*, *MLL*, *NPM1*, and *TP53* genes as well as cytogenetics in newly diagnosed *de novo* AML to disclose the feature of their overlap mutations. Furthermore, we examined the association of cooperative mutations with clinical characteristics and morphologic MLD of *de novo* AML.

Patients and methods

Patients and samples

The diagnosis of AML was based on the WHO classification. All BM smears from patients were evaluated by the authors according to the WHO criteria and morphological diagnosis was confirmed. The study population included 144 newly diagnosed *de novo* AML patients from January 1990 who were received the remission induction therapy in our institutes. We unselectively included all patients into the present study if their samples were available. The median age and WBC count at the diagnosis of the analyzed patients were 52 yr (range, 15–85 yr) and 10.3×10^9/L (range, $0.6–351 \times 10^9$/L), respectively. Twenty-one patients were of age 65 yr or older. Cytogenetic analysis revealed that a normal karyotype was found in 54 patients and an abnormal karyotype was in 90 patients including 19 t(8;21)(q22;q22), 3 inv(16)(p13q22), 14 t(15;17)(q22;q12) and 2 11q23 abnormalities. AML-MLD was identified in 34 patients, who did not have a history of MDS. BM samples from patients with AML were subjected to Ficoll-Hypaque (Pharmacia LKB, Uppsala, Sweden) density gradient centrifugation. Informed consent was obtained from all patients to use their samples for banking and molecular analysis, and approval was obtained from the ethics committee of Nagoya university school of medicine.

Therapy

Among the AML patients analyzed, patients younger than 65 yr old were treated with the AML protocols of the Japan Adult Leukemia Study Group or their modifications (14, 15). Briefly, the induction therapy consisted of cytarabine (Ara-C) and idarubicin (IDR) or Ara-C and daunorubicin (DNR). Patients who achieved complete remission (CR) subsequently received three courses of consolidation therapy consisted of high-dose Ara-C or four courses of consolidation consisted of Ara-C and mitoxantrone, Ara-C and DNR, Ara-C and aclarubicin, and Ara-C, etoposide, vincristine and vindesine. Patients aged 65 yr or older received the dose-reduced induction therapy consisted of Ara-C and IDR or Ara-C and DNR. For the consolidation therapy, four courses of the dose-reduced regimen were administered.

Cytogenetics analysis

The cytogenetic G-banding analysis was performed with standard methods. A complex karyotype was defined as

at least three unrelated chromosomal aberrations. Chimeric transcripts, *BCR/ABL*, *AML1/ETO*, *CBFB/MYH11*, *PML/RARA*, and *MLL/AF9*, were examined by real-time PCR as previously described (3).

Screening for mutations of *FLT3, cKIT, N-RAS, AML1, C/EBPA, TP53, MLL,* and *NPM1* genes

High-molecular-weight DNA and total RNA were extracted from the samples using standard methods. *FLT3* gene mutations of the internal tandem duplication in the juxtamembrane domain (FLT3/ITD) and deletion and point mutation in the kinase domain (FLT3/KDM), *NPM1* gene mutation of exon 12, *N-RAS* gene mutations of codons 12, 13, and 61 and *TP53* gene mutations of exons 5–8 were examined as reported and confirmed by the sequencing procedure (11, 16–19). The partial tandem duplication of the *MLL* gene (*MLL*-PTD) was examined by RT-PCR as described previously (17). Mutations of *AML1*, *C/EBPA* and exon 8, 10–11, and 17 of *cKIT* were screened by denaturing high performance liquid chromatography (DHPLC) analysis using the WAVE Maker System (Transgenomic Inc., San Jose, CA, USA) as reported (20–22). DHPLC gradients and temperatures were determined using WAVE Maker System software. When heterozygous profiles were identified by visual inspection of the chromatograms, mutations were confirmed by cloning and sequencing procedures as reported (20).

Statistical analysis

Differences in continuous variables were analyzed with the Mann–Whitney *U*-test for distribution among two groups or the Kruskal–Wallis test for distribution among more than two groups. Frequencies were analyzed using Fisher's exact test for 2×2 tables or Pearson's chi-squared test for larger tables. Multivariate analysis to identify risk factors for achieving CR was performed using the logistic-regression model. Survival probabilities were estimated by the Kaplan–Meier method, and differences in survival distributions were evaluated using the log-rank test. The prognostic significance of the clinical variables was assessed using the Cox proportional hazards model. These statistical analyses were performed with StatView-J 5.0 (Abacus Concepts Inc., Berkeley, CA, USA). For all analyses, the *P*-values were two-tailed, and a $P < 0.05$ was considered statistically significant.

Results

Mutations of *de novo* AML

Genetic alterations of AML patients according to cytogenetics are summarized in Table 1. At least one mutation in the *FLT3*, *cKIT*, *N-RAS*, *AML1*, *C/EBPA*, *MLL*, *NPM1, and TP53* genes was identified in 84 of the 144 AML patients (58.3%). *FLT3* mutation was the most frequently identified in entire AML patients (35/144, 24.3%), followed by *NPM1* (29/144, 20.1%) and *C/EBPA* (17/144, 11.8%) mutations. In *FLT3* mutation, FLT3/ITD and FLT3/KDM were identified in 28 (19.4%) and seven (4.9%) patients, respectively. No overlap mutation of FLT3/ITD and FLT3/KDM was observed. In cytogenetically normal AML, *NPM1* mutation was the most frequently identified (19/54, 35.2%), followed by FLT3/ITD (15/54, 27.8%) and *C/EBPA* (13/54, 24.1%) mutations. *FLT3* mutation was also frequently identified in AML with *PML/RARA*, *AML1/ETO*, or *CBFB/MYH11*, although *NPM1* and *C/EBPA* mutations were not. In contrast, *cKIT* mutation was not identified in cytogenetically normal AML, while it was frequently identified in AML with *AML1/ETO* (3/19, 15.8%) or *CBFB/MYH11* (1/3, 33.3%). When comparing cytogenetically normal and abnormal patients, *NPM1* and *C/EBPA* mutations were

Table 1 Genetic alterations of 144 *de novo* AML patients according to cytogenetics

	PML/RARA	AML1/ETO	CBFB/MYH11	MLL abnormalities	Normal	Other abnormalities	Complex	Total	*P*-value
Number	14	19	3	2	54	38	14	144	
Mutation (%)									
FLT3/ITD	3 (21.4)	0	1 (33.3)	0	15 (27.8)	8 (21.1)	1 (7.1)	28 (19.4)	NS
FLT3/KDM	0	2 (10.5)	0	0	3 (5.6)	2 (5.3)	0	7 (4.9)	NS
cKIT	0	3 (15.8)	1 (33.3)	0	0	4 (10.5)	0	8 (5.6)	0.0208
N-RAS	1 (7.1)	0	0	0	4 (7.4)	6 (15.8)	0	11 (7.6)	NS
TP53	0	1 (5.3)	0	0	1 (1.9)	1 (2.6)	8 (57.1)	11 (7.6)	<0.0001
NPM1	0	0	0	0	19 (35.2)	8 (21.1)	2 (14.3)	29 (20.1)	0.0076
MLL-PTD	0	1 (5.3)	1 (33.3)	1 (50)	5 (9.3)	3 (7.9)	2 (14.3)	13 (9.0)	NS
C/EBPA	0	0	0	0	13 (24.1)	2 (5.3)	2 (14.3)	17 (11.8)	0.0242
AML1	0	0	0	0	1 (1.9)	2 (5.3)	0	3 (2.1)	NS

Distributions of *cKIT, TP53, NPM1,* and *C/EBPA* mutations according to cytogenetics were significantly different.
AML, acute myeloid leukemia; NS, no significance.

frequently identified in cytogenetically normal patients (33.9% vs. 11.4%, $P = 0.0014$ and 23.2% vs. 4.5%, $P = 0.0011$, respectively). *TP53* mutation was identified in 11 AML patients (7.6%), and eight of them showed complex karyotype. As *AML1* mutation was identified in only three patients, further analysis is required to confirm the statistical significance of its distribution.

Overlap mutations

In the present study, we identified a total of 165 mutations in entire AML patients. Interestingly, 103 of the 165 mutations (62.4%) were overlapped with another mutations. *AML1* (3/3, 100%), FLT3/KDM (7/7, 100%), FLT3/ITD (24/28, 85.7%), *N-RAS* (9/11, 81.8%), and *NPM1* (21/29, 72.4%) mutations were frequently overlapped with another mutations. In contrast, overlap mutations of *PML/RARA* (3/14, 21.4%) and *AML1/ETO* (6/19, 31.6%) were relatively infrequent (Fig. 1A). The most frequent overlap mutation was *FLT3* and *NPM1*, which was observed in 16 of the 51 patients with overlap mutations, followed by *FLT3* and *MLL*-PTD (five patients), *FLT3* and *C/EBPA* (four patients), *FLT3* and *PML/RARA* (three patients), *cKIT* and *AML1/ETO* (three patients), *N-RAS* and *NPM1* (three patients) and *TP53* and *MLL*-PTD (three patients). Importantly, overlap pattern of mutations was not random as shown in Fig. 1(B). The overlap mutations of *FLT3* and *cKIT* were not identified. In addition, *PML/RARA*, *AML1/ETO*, *CBFB/MYH11*, and *MLL* gene abnormality and *AML1* and *C/EBPA* mutations were not overlapped each other. These results were consistent with the genetic model of class I and class II mutations. However, overlap patterns of *N-RAS*, *TP53*, *MLL*-PTD, and *NPM1* mutations seemed different from above mutations. *N-RAS* mutation was identified in nine patients, while each one of them were overlapped with FLT3/ITD and *cKIT* mutations, respectively. Likewise, overlap mutations of *TP53* and FLT3/ITD, *TP53* and *cKIT*, *MLL*-PTD and *AML1/ETO*, *MLL*-PTD and *CBFB/MYH11*, *MLL*-PTD and *MLL/ENL*, and *NPM1* and *C/EBPA* were found in each one patient. According to the general consideration that *N-RAS* and *TP53* mutations were the class I mutation, and *MLL*-PTD and *NPM1* mutations were the class II mutation, these overlap mutations seemed to irregularly occur within the same class. Taken together, overlap mutations in the same class were observed in seven patients, while five of them showed mutations in three different genes. In this study, 51 of the 144 patients (35.4%) revealed overlap mutations, and overlap mutations consisted of two or three genes were found in 46 and five patients, respectively. However, both class I and class II mutations were always included in all patients with three mutations (Table 2). Therefore, only two patients showed two mutations within the same class: one consisted of *N-RAS* and *cKIT* mutations and the other consisted of *MLL*-PTD and *MLL/ENL* (Table 2).

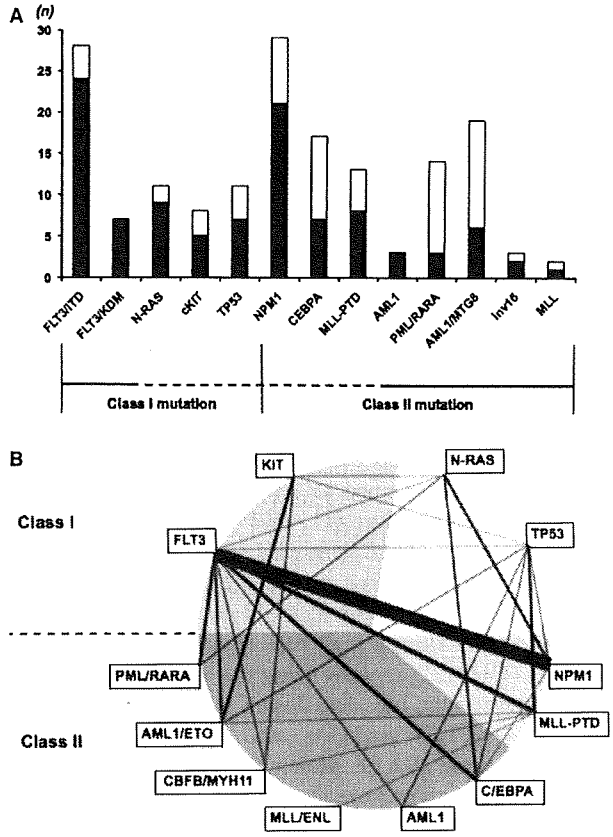

Figure 1 Prevalence and overlap pattern of class I and class II mutations in *de novo* AML. (A) We identified a total of 165 class I or class II mutations, 103 of which (62.4%) were overlapped with other mutations. Black and white bars indicate mutations with or without additional overlapped mutations, respectively. (B) Overlap pattern of each mutation. Red lines indicate mutations within the same class. Each line thickness represents the prevalence of overlap mutation. All FLT3/KDM overlapped with class II mutations, while two FLT3/ITD overlapped with class I mutations.

TP53 mutation was overlapped with a variety of gene mutations. Seven of the 11 *TP53* mutated cases showed overlap mutations: two was overlapped with *MLL*-PTD and each one with *NPM1*, *C/EBPA*, *AML1*, *cKIT*, and FLT3/ITD mutations. Cytogenetic analysis revealed that 10 *TP53* mutated cases had abnormal karyotypes: eight were complex karyotype, five of which had a deleted chromosome 17, and each one was 46XY, del(5q) and 46XY, t(8;21), del(9). Importantly, one case with *TP53* mutation and the karyotype 46XY, del(5q) harbored

Table 2 Characteristics of AML patients with overlap mutations within the same class

Age (yr)	Sex	WHO category	FAB	WBC ($\times 10^9$/L)	Mutation
Two mutations					
64	F	Not otherwise specified	M2	18.8	*cKIT, N-RAS*
46	F	Recurrent genetic abnormalities	M4	1.4	*MLL-PTD, MLL/ENL*
Three mutations					
76	M	Not otherwise specified	M4	47.5	*FLT3/ITD, TP53, MLL-PTD*
18	M	Myelodysplasia-related changes	M4	124.9	*FLT3/ITD, NPM1, C/EBPA*
50	F	Recurrent genetic abnormalities	M3	12.0	*FLT3/ITD, N-RAS, PML/RARA*
17	M	Recurrent genetic abnormalities	M2	11.0	*cKIT, TP53, AML1/ETO*
29	F	Recurrent genetic abnormalities	M4Eo	113.3	*FLT3/ITD, MLL-PTD, CBFB/MYH11*

AML, acute myeloid leukemia; WHO, World Health Organization.

FLT3/ITD, and one case with *TP53* mutation and the karyotype 46XY, t(8;21), del(9) harbored *cKIT* mutation. Although one case showed a normal karyotype, it was overlapped with the *AML1* mutation. Taken together, all *TP53* mutated cases had another genetic alterations (Fig. 2). On the other hand, a complex karyotype was found in 14 patients. Ten patients showed MLD, and eight of them had *TP53* mutation. The remaining four patients without MLD did not harbor *TP53* mutation, although three of them showed del(17) or del(17p). A genotype consisting of complex karyotype and *TP53* mutation was therefore specifically found in AML-MLD.

Prognostic implications of the mutational status

We analyzed prognostic implications of the mutational status in 130 AML patients except for acute promyelocytic leukemia. We divided AML patients into four groups: AML-MLD with or without complex karyotype and *TP53* mutation, AML with *AML1/ETO* or *CBFB/MYH11* (CBF-AML) and other type AML. Clinical characteristics of each group are shown in Table 3. Median age of the patients in CBF-AML was significantly younger than the other groups ($P = 0.012$), while there was no significant difference in median WBC count among 4 groups. The CR rate in AML-MLD with complex karyotype and *TP53* mutation (25%) was significantly lower than the other groups ($P = 0.0012$). Multivariate logistic-regression analysis revealed that WBC count (over 100×10^9/L) [odds ratio, 12.910 (95% CI: 3.101–53.742); $P = 0.0004$], wild-type *NPM1* [odds ratio, 10.640 (95% CI: 2.185–51.810); $P = 0.0034$), the genotype consisting of complex karyotype and *TP53* mutation [odds ratio, 8.755 (95% CI: 1.166–12.987); $P = 0.0271$], wild-type *C/EBPA* [odds ratio, 7.534 (95% CI: 1.111–51.103); $P = 0.0387$] and the presence of MLD [odds ratio, 3.891 (95% CI: 1.166–12.987); $P = 0.0271$] were independent unfavorable factors for achieving CR (Table 4). Furthermore, we analyzed prognostic implications of genetic alterations in addition to age, WBC count and existence of MLD. Multivariate Cox regression analysis with stepwise selection showed that the genotype consisting of complex karyotype and *TP53* mutation [odds ratio, 5.988 (95% CI: 2.681–13.333); $P < 0.0001$], not CBF-AML [odds ratio, 2.602 (95% CI: 1.107–6.116); $P = 0.0283$], FLT3/ITD [odds ratio, 1.843 (95% CI: 1.063–3.196); $P = 0.0294$] and age

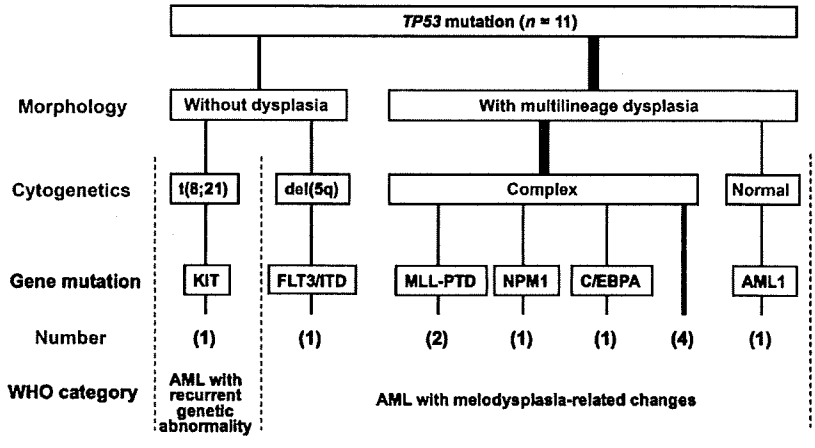

Figure 2 Association of *TP53* mutations with morphology, cytogenetics, and other mutations. *TP53* mutations were overlapped with a variety of mutations. Eight of the 11 *TP53* mutated cases showed complex karyotype, and all of them revealed morphologic MLD.

Table 3 Clinical characteristics of 130 AML patients except APL

	AML with MLD				
	With complex/ TP53 Mt.	Without complex/ TP53 Mt.	CBF-AML	Other AML	*P*-value
Number	8	26	22	74	
Age (yr)					
Median	60.5	55.5	**33.0**	53	0.012
Range	20–81	18–85	16–72	15–77	
WBC ($\times 10^9$/L)					
Median	6.6	16.9	10.3	22.9	NS
Range	1.2–22.4	0.8–139.5	3.1–113.3	0.9–351.0	
CR	**2**	19	20	60	0.0012
(%)	(25.0)	(73.1)	(90.9)	(81.1)	
Cytogenetics risk (%)					
High	8 (100)	3 (11.5)	–	10 (13.5)	
Complex	8 (100)	2 (7.7)	–	4 (5.4)	
Intermediate	–	23 (88.5)	–	64 (86.5)	
Normal	–	18 (69.2)	–	36 (48.6)	

Median age of the patients in CBF-AML was significantly younger than the other groups. The CR rate in AML-MLD with complex karyotype and *TP53* mutation was significantly lower than the other groups. Bold letters indicate significantly different groups.
AML, acute myeloid leukemia; APL, acute promyelocytic leukemia; MLD, multilineage dysplasia; CR, complete remission.

Table 4 Unfavorable risk factors for achieving CR in *de novo* AML except for APL

Factors	OR	95% CI	*P*-value
WBC count (>100 x 10^9/L)	12.910	3.101–53.742	0.0004
Wild-type *NPM1*	10.640	2.185–51.810	0.0034
Complex karyotype/TP53 mutation	8.755	1.098–69.821	0.0405
Wild-type *C/EBPA*	7.534	1.111–51.103	0.0387
Presence of MLD	3.891	1.166–12.987	0.0271

AML, acute myeloid leukemia; CR, complete remission; MLD, multilineage dysplasia.

Table 5 Unfavorable prognostic factors for overall survival in *de novo* AML except for APL

Factors	OR	95% CI	*P*-value
Complex karyotype/TP53 mutation	5.988	2.681–13.333	<0.0001
Not CBF AML	2.602	1.107–6.116	0.0283
FLT3/ITD	1.843	1.063–3.196	0.0294
Age (>60 yr)	1.764	1.086–2.865	0.0218

AML, acute myeloid leukemia.

(over 60 yr) [odds ratio, 1.764 (95% CI: 1.086–2.865); $P = 0.0218$] were poor prognostic factors for overall survival (Table 5). For disease-free survival, not CBF-AML [odds ratio, 2.475 (95% CI: 1.176–5.208); $P = 0.0169$] and *AML1* mutation [odds ratio, 8.134 (95% CI: 1.039–63.642); $P = 0.0453$] were identified to be poor prognostic factors.

Discussion

In this study, we comprehensively analyzed mutations in the *FLT3*, *cKIT*, *N-RAS*, *AML1*, *C/EBPA*, *MLL*, *NPM1*, *and TP53* genes as well as cytogenetics in 144 newly diagnosed *de novo* AML patients. We identified a total of 165 class I or class II mutations, 103 of which (62.4%) were overlapped with other analyzed mutations, supporting that multiple genetic alterations have been accumulated at the diagnosis of AML. However, overlap mutations of *PML/RARA* (3/14, 21.4%) and *AML1/ETO* (6/19, 31.6%) were infrequent. Furthermore, overlap mutations of *C/EBPA* mutations, which are frequently found in cytogenetically normal AML, were relatively infrequent (7/17, 41.2%). It is well known that *PML/RARA*, *AML1/ETO*, and *C/EBPA* mutations are associated with favorable prognosis, while recent reports revealed that additional class I mutations, such as *FLT3* and *cKIT* mutations, reduced their favorable prognosis. Our results further indicate that it is necessary to clarify whether there are unknown class I mutations overlapped with them, which influence their prognostic impacts.

We demonstrate here that most overlap mutations found in AML consisted of class I and class II mutations. Although overlap mutations within the same class were found in seven patients, five of them additionally had the other class mutation. These results suggest that most overlap mutations within the same class might be the consequence of acquiring an additional mutation after the completion both of class I and class II mutations. Therefore, it is possible that an unknown

mutation of the opposite class was acquired in two patients, whose mutations consisted of two mutations within the same class. On the other hand, it has been reported that mutations in genes functioning in different pathways can occur in the same cancer, while genes functioning in the same pathway are rarely mutated in the same sample (23). However, it was also observed that certain overlap mutational patterns violate these rules and demonstrate tissue-specific variations (23). In addition, we found that mutated genes, which overlapped with the same class mutations, were limited in *N-RAS*, *TP53*, *MLL*-PTD, and *NPM1*. As these mutations are sometimes acquired at the relapse, it is necessary to analyze whether these irregular overlap mutations cooperatively participate in the development of AML, and whether these mutations are acquired during the disease progression (18, 24).

We further demonstrate the characteristic cooperative features of *TP53* mutation in *de novo* AML. Ten of the 11 patients with *TP53* mutation showed cytogenetic abnormality, and six of them showed an additional mutation. Furthermore, additional mutations involved both class I and class II mutations. Because of the evidence that p53 mediates quiescence of normal hematopoietic stem cells, Pedersen-Bjergaard *et al.* (25) proposed that *TP53* mutation functionally represent a new class III mutation. On the other hand *TP53* has been known as a tumor suppressor gene of importance for genomic stability, and it has been suggested that leukemic progenitor accumulates additional chromosomal alterations after inactivation of *TP53* in the development of AML (26). Although it is necessary to clarify how *TP53* mutation biologically involves the accumulation of genetic alterations during the development of AML and how their interactions are associated with the leukemogenesis, the previous and our results collectively suggest that *TP53* mutation might be a distinguishable class of mutation from class I and class II mutations.

Haferlach *et al.* (27) reported that *TP53* mutations were strongly associated with the complex karyotype in AML, but the association with dysplastic morphology was not evaluated; however, our results indicate that this genotype is closely associated with dysplastic morphology regardless of the history of MDS. It has been reported that *TP53* mutation is associated with loss of chromosome band 17p13, where the *TP53* gene is located (9, 28). Deletion of 17p, which occurred by unbalanced translocation between chromosome 17p and another chromosome, monosomy 17 or i(17), is reportedly observed in 3–4% of AML and MDS and 10–15% of therapy-related MDS or AML (29–33). Furthermore, it has been reported that 17p deletion is closely associated with dysgranulopoiesis, *TP53* mutation and additional complex cytogenetic findings in AML and MDS (34, 35). In our study, deletion of 17p was identified in eight of entire 144 AML patients (5.6%), and all showed a complex karyotype. Of note is that five of eight patients with complex karyotype and *TP53* mutation showed 17p deletion, while the chromosome 17 was intact in the remaining three patients. Prognostic implication of the genotype consisting of a complex karyotype and *TP53* mutation is particularly notable as to be an independent unfavorable prognostic factor both for achieving CR and overall survival in entire *de novo* AML patients. These results collectively indicate that this genotype generates a disease entity in *de novo* AML.

Although several genetic alterations have been shown to be associated with MDS-related AML, morphologic evidence is recommended as the most available marker for the diagnosis of AML-MLD (36); however, diagnosis of dysplasia is difficult to qualify and/or quantify. Wandt *et al.* (37) demonstrated that MLD was associated with an unfavorable cytogenetic profile and that the presence of MLD was an unfavorable risk factor for achieving CR in univariate analysis but not a poor prognostic factor for either event-free or overall survival by analyzing a large number of AML patients both with and without a history of MDS. Consistently, the presence of MLD was identified to be an independent unfavorable risk factor for achieving CR by multivariate analysis, but not for long-term survival in our analysis of all AML patients. We could not identify the AML-MLD-specific genotype other than a complex karyotype and *TP53* mutation and there were some AML-MLD without any genetic alterations, indicating that AML-MLD is still genetically heterogeneous.

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Management of acute promyelocytic leukemia: recommendations from an expert panel on behalf of the European LeukemiaNet

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The introduction of all-*trans* retinoic acid (ATRA) and, more recently, arsenic trioxide (ATO) into the therapy of acute promyelocytic leukemia (APL) has revolutionized the management and outcome of this disease. Several treatment strategies using these agents, usually in combination with chemotherapy, but also without or with minimal use of cytotoxic agents, have provided excellent therapeutic results. Cure of APL patients, however, is also dependent on peculiar aspects related to the management and supportive measures that are crucial to counteract life-threatening complications associated with the disease biology and molecularly targeted treatment. The European LeukemiaNet recently appointed an international panel of experts to develop evidence- and expert opinion–based guidelines on the diagnosis and management of APL. Together with providing current indications on genetic diagnosis, modern risk-adapted front-line therapy and salvage treatment, the review contains specific recommendations for the identification and management of most important complications such as the bleeding disorder, APL differentiation syndrome, QT prolongation and other ATRA- and ATO-related toxicities, as well as for molecular assessment of response to treatment. Finally, the approach to special situations is also discussed, including management of APL in children, elderly patients, and pregnant women. (Blood. 2009;113:1875-1891)

1. Introduction

Although the real incidence of acute promyelocytic leukemia (APL) is unknown, it is a relatively rare hematologic malignancy. The number of newly diagnosed cases per year in the United States is estimated to be 600 to 800.[1,2] One of the most striking features of APL is its age-associated incidence rate. The disease is very uncommon in children less than 10 years of age. Its incidence increases steadily during the teen years, reaches a plateau during early adulthood, and remains constant until it decreases after age 60 years.[3] This is in marked contrast to other subtypes of acute myeloid leukemia (AML), where there is a steady rise to age 55 years, after which there is an exponential increase. There is also a suggestion in the literature that APL arising as a complication of previous exposure to chemotherapy (particularly drugs targeting topoisomerase II) or radiotherapy is becoming more prevalent, particularly in patients with a history of breast cancer.[4-6] With respect to the incidence of APL among ethnic groups, contradictory data regarding a presumed higher incidence of APL in persons from Mexico, Central and South America, Italy, and Spain have been reported in the literature.[2,7] Therefore, this epidemiologic issue is still a matter of controversy and deserves additional investigation.

The introduction of all-*trans* retinoic acid (ATRA; tretinoin) into the therapy of APL completely revolutionized the management and outcome of this disease. This agent represents one of the most spectacular advances in the treatment of human cancer, providing the first paradigm of molecularly targeted treatment. After the advent of ATRA, the introduction of arsenic trioxide (ATO), probably the most biologically active single drug in APL, has provided a valuable addition to the armamentarium and may have contributed to further improvements in the clinical outcome of this disease. Several treatment strategies using these agents, usually in combination with chemotherapy, have provided excellent therapeutic results with survival rates exceeding 70% in multicenter clinical trials. Cure of patients with APL depends not only on the effective use of combination therapy involving differentiating and classical cytotoxic agents, but also, critically, upon supportive care measures that take into particular account the biology of the disease and the complications associated with molecularly targeted therapies. Moreover, it is important to consider diagnostic suspicion of APL as a medical emergency (uncommon in AML) that requires several specific and simultaneous actions, including immediate commencement of ATRA therapy, prompt genetic diagnosis, and measures to counteract the coagulopathy.

Although there are some recent exhaustive reviews addressing the management of APL[8-13] and national guidelines from the United States and United Kingdom[14,15] on the management of AML that include some specific items on APL, no comprehensive yet succinct guidelines focused on APL have been produced. Therefore, the European LeukemiaNet appointed an international panel of experts to develop evidence- and expert opinion–based guidelines on the management of APL. The publication of guidelines for the management of challenging malignant diseases provides an excellent tool to spread knowledge of the optimum

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treatment of a given disease, but it is particularly useful in APL for some additional reasons: (1) APL is a rare disease and most patients are treated in institutions with limited experience; (2) the excellent outcome reported recently with current treatments may engender a sense of safety and complacency that may lead to underestimation of the importance of crucial aspects of APL management; and (3) some issues related to the treatment of APL are not well known, lack strong supporting data, and, therefore, involve the risk of adopting erroneous practices, which although appropriate and routinely used for the management of other AML subtypes are inappropriate for management of APL.

2. Methods

2.1. Composition of the panel

The panel included 12 members with recognized clinical and research expertise in APL, of whom 9 came from European Union countries (France, Germany, Italy, Spain, United Kingdom, and The Netherlands), 2 from the United States, and 1 from Japan.

2.2. Scope of the review

A computerized literature search of the PubMed, Cochrane, and Medline databases in the English language was conducted using the key words "acute promyelocytic leukemia" with subheadings "anthracycline," "all-*trans* retinoic acid," "arsenic trioxide," "retinoic acid/differentiation syndrome," "pregnancy," "disseminated intravascular coagulation" (DIC), "stem cell transplantation," and "febrile neutropenia." Relevant abstracts presented at the 2004, 2005, 2006, and 2007 meetings of the American Society of Hematology and the American Society of Clinical Oncology were also reviewed. The authors have substantial experience in the field. The levels of evidence and grading of recommendations were those defined in the "General Guidelines for Methodologies on Research and Evaluation of Traditional Medicine,"[16] which are based on those of the US Agency for Healthcare Research and Quality, formerly the Agency for Health Care Policy and Research (Appendix).

3. Approach to the patient with suspected APL

Although there is a general consensus on the need to confirm the diagnosis of APL at the genetic level, both differentiation and supportive therapy should be started before the results of genetic tests are available. In the majority of cases the diagnosis is suggested by the characteristic morphology of the leukemic population,[17,18] however, it is also important to consider the possibility of APL should any suspicion be raised based on immunophenotypic profile or presence of severe coagulopathy; in all such cases ATRA should be commenced immediately and continued until the diagnosis is confirmed or refuted at the genetic level.

3.1. Rapid confirmation of genetic diagnosis

Because the efficacy of differentiation treatment based on retinoids and/or arsenic derivatives is strictly dependent on the presence of the *PML/RARA* fusion in leukemia cells, genetic confirmation of this specific lesion is mandatory in all cases. Morphologic diagnosis of hypergranular (typical) APL is highly predictive of an underlying *PML/RARA* rearrangement, and immunophenotyping by multiparameter flow cytometry can improve the accuracy of diagnosis,[19,20] particularly in patients with morphologic features evoking a microgranular (variant) subtype. However, patients with morphologic and/or immunophenotypic features suggestive of APL without the *PML/RARA* rearrangement, and vice versa, have been described in the literature.[21-23]

3.1.1. Diagnostic workup and sample processing. Recommendations for diagnostic workup are summarized in Table 1.

All patients should have a marrow aspirate. This may be omitted only when the peripheral blast count is high and the patient is to be considered for palliative treatment only. A trephine biopsy is required only in the case of a dry marrow aspirate and where no abnormal cells are present in the peripheral blood (PB) to allow a morphologic and molecular diagnosis.

Morphologic studies require a Romanowsky-derived stain, such as Wright, Wright-Giemsa, or May-Grunwald-Giemsa stains, usually complemented by myeloperoxidase or Sudan black B stain. Immunophenotyping by multiparameter flow cytometry can increase the accuracy of a morphologic suspicion of *PML/RARA*-positive APL. Typically, *PML/RARA*-positive leukemia blasts show immunophenotypic features similar to those of normal promyelocytes ($CD34^{-/+}$ heterogeneous, $CD117^{-/+}$ dim, $HLADR^{-/+}$ dim, $CD13^{+/++}$, $CD11b^{-}$).[19] However, unlike their normal counterpart, PML/RARα-positive promyelocytes display abnormally low levels of CD15 ($CD15^{-/+}$ dim vs $CD15^{+++}$).[19,20] Blasts of the hypogranular variant form of APL (M3v) frequently coexpress the T lineage–affiliated marker CD2 with myeloid markers CD13 and CD33.[24-26]

Confirmation of genetic diagnosis is mandatory and should be performed, if possible, on leukemia cells from bone marrow (BM). The identification of the APL-specific genetic lesion in leukemic cells is feasible at chromosome, DNA, RNA, and protein levels with the use of conventional karyotyping, fluorescence in situ hybridization (FISH), reverse transcriptase polymerase chain reaction (RT-PCR), or anti-PML monoclonal antibodies, respectively. Each of these has advantages and disadvantages.

Karyotyping. Karyotyping on G-banded metaphases obtained from BM samples is usually performed by conventional methods on direct, 24-hour, and 48-hour cultures. Although highly specific, cytogenetic analysis is expensive, very time-consuming, needs good quality metaphases (lacking in up to 20%), and by definition fails to detect cases where the *PML-RARA* fusion results from cryptic rearrangements (false negatives). In addition, secondary chromosomal abnormalities seem not to have significant prognostic value in APL.[27,28] However, cytogenetics is potentially useful in the characterization of cases lacking the *PML-RARA* fusion. This may facilitate identification of rarer molecular subtypes of APL including those with t(11;17)(q23;q21), t(11;17)(q13;q21), and t(5;17)(q35;q21), leading to *PLZF-RARA*,[29] *NuMA-RARA*[30] and *NPM1-RARA*[31] fusions, respectively, as well as others more recently described.[32-34]

FISH analysis. FISH analysis of *PML/RARA* can be carried out using standard methods and commercially available fluorescently labeled probes. Although in some cases PB samples are suitable for study (particularly when hyperleukocytosis is present at diagnosis), FISH is preferably performed in BM samples. The protocol for FISH detection of *PML/RARA* has been reported in detail by Grimwade et al.[35] This methodology is highly specific and sensitive, and much less expensive and time-consuming than karyotyping. However, it is important to recognize the potential limitations of some probe sets used for molecular diagnostics. In particular, those that specifically detect the *RARA-PML* fusion gene on

Table 1. Diagnostic workup and supportive care

Recommendation	Level of evidence	Grade of recommendation
1.1. Once a diagnosis of APL is suspected, the disease should be managed as a medical emergency.	IV	C
1.2. Diagnosis should be confirmed by molecular detection of *PML-RARA* fusion (or rare molecular variants).	IIa	B
1.3. In addition to conventional karyotyping, FISH, and RT-PCR, immunostaining with anti-PML antibody can be used for rapid diagnosis of APL.	IIa	B
Management of coagulopathy		
1.4. Treatment with ATRA should be started immediately that a diagnosis of APL is suspected.	Ib	A
1.5. Liberally transfuse with fresh frozen plasma, fibrinogen, and/or cryoprecipitate and platelet transfusions to maintain the fibrinogen concentration and platelet count above 100-150 mg/dL and 30-50 $\times 10^9$/L, respectively.	IIb	B
1.6. The benefit of heparin, tranexamic acid, or other anticoagulant or antifibrinolytic therapy remains questionable and should not be used routinely outside the context of clinical trials.	IV	C
Management of hyperleukocytosis (WBC >10 × 10^9/L)		
1.7. Chemotherapy should be started without delay, even if the molecular results are still pending.	IV	C
1.8. Leukopheresis should be avoided due to risk of precipitating fatal hemorrhage.	III	B
1.9. Prophylactic steroids can be given, which may reduce the risk of APL differentiation syndrome.	IV	C
Management of APL differentiation syndrome		
1.10. Steroids (10 mg dexamethasone intravenously bd) should be started immediately at the earliest clinical suspicion of incipient APL differentiation syndrome. Once the syndrome has resolved, steroids can be discontinued and ATO/ATRA recommenced.	IIa	B
1.11. Temporary discontinuation of differentiation therapy (ATRA or ATO) is indicated only in case of severe APL differentiation syndrome.	IIa	B
Management of treatment with ATO		
1.12. Treatment with ATO should be restricted to cases confirmed to be *PML/RARA*-positive.	IIb	B
1.13. Treatment with ATO requires careful monitoring to maintain electrolytes in the normal range, keeping the serum potassium above 4.0 mEq/L and serum magnesium above 1.8 mg/dL.	IV	C
1.14. Treatment with ATO requires careful monitoring of the QT/QTc interval.*	IV	C

*If the QT (or QTc for patients with heart rate > 60 beats per minute) interval is prolonged longer than 500 msec, ATO should be withheld, the electrolytes (potassium and magnesium) repleted, and other medications that may cause prolonged QTc interval searched for and, ideally, discontinued. Once the QT/QTc returns to approximately 460 msec and the electrolytes are repleted, ATO may be resumed.

der(17) will not show fusion signals in the presence of nonreciprocal rearrangements where *RARA-PML* is deleted or where *PML-RARA* is formed as a result of an insertion. Small *PML-RARA* insertions can also be missed by FISH when using very large probes; in such cases it is more appropriate to use relatively small cosmid probes.[22] FISH provides no information about the isoform of *PML/RARA*, which is required for molecular monitoring of minimal residual disease. However, FISH can be useful in the investigation of suspected APL cases that lack a *PML-RARA* fusion, using *RARA* probes that span the breakpoint region to investigate for evidence of a *RARA* rearrangement, facilitating the identification of the fusion partner.

RT-PCR. RT-PCR analysis of *PML-RARA* is preferably carried out on RNA extracted from BM samples, although the fusion transcript is usually readily detectable in PB even in cases presenting with leukopenia. Standardized RT-PCR assays for detection of the *PML-RARA* fusion were established within the Biomed-1 Concerted Action.[36] RT-PCR probably provides the "gold standard" approach for confirming a diagnosis of APL. In addition to its high specificity and sensitivity, it is essential for defining *PML* breakpoint location thereby establishing the target for reliable monitoring of MRD. However, poor RNA yield (false negative), contamination and artifacts (false positives), and the relatively long turnaround time (~2 days) are the main drawbacks of this methodology. In addition, it is advisable that diagnostic and monitoring samples be analyzed in reference laboratories by well-trained personnel with considerable expertise in RT-PCR for *PML-RARA*.

Immunostaining with anti-PML monoclonal antibodies on dry smears of BM or PB (providing circulating blasts are present) is helpful to achieve a rapid diagnosis. This technique is highly specific for presence of an underlying PML-RARα fusion protein,[37-40] indicated by a microspeckled staining pattern (> 30 nuclear dots) in the nuclei of leukemic cells with the PML antibody, which detects both PML-RARα and the normal PML protein. The test will also be positive in those rare cases where atypical breakpoints occur within the *PML* locus, which could potentially be missed by standard PCR primers. In normal cells and blasts from other subtypes of leukemia (including APL molecular variants, eg, *PLZF-RARA* and *NPM1-RARA*) a wild-type PML staining pattern is observed with discrete nuclear dots (typically < 20/nucleus) that relate to organelles known as PML nuclear bodies.[22,23] Either indirect immunofluorescence or immunohistochemistry may be used. Results from the immunofluorescence assay can be achieved in only 2 hours. In light of its very convenient cost-benefit ratio, the assay is highly recommended for rapid confirmation of the diagnosis of APL and effectively identifying patients with the PML-RARA fusion, thereby predicting those likely to respond to molecularly targeted therapy with ATRA or ATO. This test is particularly valuable in small centers lacking access to a molecular diagnostics laboratory and in cases in which RNA is not available to confirm a diagnosis.

All of the aforementioned options are equally specific, but not equally reliable, methods to confirm the genetic diagnosis of APL. In particular, cytogenetics is much less efficient than the others. In terms of rapidity, specificity, and sensitivity, FISH and immunostaining with anti-PML monoclonal antibodies are highly efficient to confirm the diagnosis of APL. However, these techniques should not replace RT-PCR, which allows definition of the type of *PML-RARA* isoform and the target for MRD evaluation.

Sample processing and banking. A specific recommendation for sample banking of all AML cases at diagnosis has been made in the revised criteria for AML diagnosis and outcome evaluation.[41] The laboratory that will assume the responsibility for sample

banking (recommended), confirmation of diagnosis at the genetic level, and monitoring MRD by RT-PCR, should receive samples as follows:

FISH or immunostaining For FISH or immunostaining studies, 3 to 4 BM smears and 3 to 4 PB smears at diagnosis are required. Samples can be sent at ambient temperature. Smears that are not used immediately must be stored for banking at −20°C, covered by aluminum paper.

PML-RARA *analysis.* For RNA extraction and RT-PCR analysis of *PML-RARA*, one BM aspirate vial (2-3 mL) and one PB vial (20-30 mL), both in sodium citrate or ethylenediaminetetraacetic acid (EDTA), are required. Samples should be processed within 24 hours. Isolated mononuclear cells in guanidium isothiocyanate can be stored for banking at −20°C.

Karyotyping and FISH. For conventional karyotyping and FISH studies, BM aspirate (1-2 mL) should be collected in heparin and dispatched at ambient temperature. Samples will be processed on arrival for these diagnostic studies and pelleted nuclei fixed with methanol and acetic acid (3:1) can be stored at −20°C.

Other genetic studies. Mutations in the gene encoding the fms-like tyrosine kinase 3 (FLT3) are seen more frequently in APL than in other subtypes of AML.[42] However, although FLT3 mutations are associated with a higher white blood cell (WBC) count at presentation, they do not add to the specific diagnosis of APL or influence management, nor do they furnish important independent prognostic information.[43,44] Therefore, the inclusion of the analysis of these mutations in the routine work up of APL is not recommended on the basis of current evidence. Although APL has a characteristic gene expression signature on microarray[45,46] and this may provide a useful additional diagnostic tool in the future, for the time being this approach remains in the research arena.

3.2. Institution of supportive measures to counteract the coagulopathy

Intracerebral and pulmonary hemorrhages are relatively common life-threatening complications occurring while the characteristic coagulopathy of APL is active. These complications are not only the most frequent cause of death early during induction therapy but can also occur before the diagnosis of APL has been made and therapy started. Data about the proportion of patients developing such hemorrhages before starting induction therapy are extremely scarce in the literature. In general, major series have not provided details about patients considered not eligible for treatment because of poor clinical condition. The US Intergroup[47] and the Programa de Estudio y Tratamiento de las Hemopatías Malignas (PETHEMA) group[48] have reported around 5% of patients considered not eligible for induction therapy due to very poor clinical condition (Eastern Cooperative Oncology Group [ECOG] performance status >3), mostly due to lethal or life-threatening hemorrhages before starting therapy.[49] In this context, it is reasonable to recommend that supportive measures to counteract the coagulopathy should be instituted immediately the diagnosis of APL is considered and consist of fresh frozen plasma, fibrinogen and/or cryoprecipitate and platelet transfusions to maintain the fibrinogen concentration and platelet count above 100 to 150 mg/dL and 30 to 50 × 10^9/L, respectively, which should be monitored at least once a day (more frequently if required). Such replacement therapy should continue during induction therapy until disappearance of all clinical and laboratory signs of coagulopathy. Despite a need-adapted transfusion policy under strict monitoring of the coagulopathy, patients presenting some factors have a higher risk of developing a fatal hemorrhage. These factors are the following: patients with active bleeding,[50] hypofibrinogenemia (<100 mg/dL),[51] or increased levels of fibrin degradation products or D-dimers combined with prolonged prothrombin time or activated partial thromboplastin time,[48] as well as those presenting with increased WBC[52,53] or peripheral blast[48,50] counts, abnormal levels of creatinine,[48] or poor performance status.[51] Further investigation is warranted in these patients.

Central venous catheterization, lumbar puncture, and other invasive procedures (eg, bronchoscopy) should be avoided before and during remission induction therapy due to high risk of hemorrhagic complications. In addition, it has been also suggested that an exacerbation of the distinctive procoagulant state of APL may lead not only to increase the hemorrhagic risk but also to a higher incidence of thrombosis. The benefit of heparin, tranexamic acid, or other anticoagulant or antifibrinolytic therapy to attenuate the hemorrhagic risk remains questionable and these agents should not be used routinely outside the context of clinical trials. There are also case reports of the use of recombinant factor VIIa in the situation of severe life-threatening hemorrhage.[54,55]

3.3. Initiation of treatment with ATRA

Treatment with ATRA should be initiated without waiting for genetic confirmation of the diagnosis, preferably the same day that diagnosis is suspected. Although there is no evidence supporting this recommendation, it is reasonable to presume a favorable risk-benefit ratio associated with this approach; moreover, ATRA is unlikely to have any deleterious effect should genetic assessment fail to confirm the diagnosis of APL. ATRA is known to improve the biologic signs of APL coagulopathy rapidly; hence early initiation of this agent is likely to decrease the risk of severe bleeding. The supportive measures to be adopted upon institution of ATRA therapy are discussed below. Since APL treatment protocols are now specific for cases with documented *PML-RARA* fusion, for those presenting with low WBC count, administration of appropriate chemotherapy may be delayed until the genetic diagnosis is confirmed; however, in patients with hyperleukocytosis (eg, WBC > 10 × 10^9/L), due to the high risk of induction death and retinoic acid syndrome, chemotherapy should be started without delay even if the molecular results are still pending (see section 5.1.2).

4. Appropriate setting for the management of APL

The recommendations by the British Committee for Standards in Hematology (BCSH) in the recently published Guidelines on the management of acute myeloid leukemia in adults[15] can be generally assumed for the management of APL. However, the peculiarities of APL deserve some additional comments and specific recommendations. In brief, the BCSH recommends that patients with AML should be managed by a multidisciplinary team serving a population of no fewer than 500 000 and that acute leukemia induction therapy should only be carried out in those centers treating at least 5 patients per year with intensive chemotherapy. According to this recommendation, centers treating 5 to 10 AML patients per year would have the opportunity of treating no more than one APL patient every 2 years. Although this limits experience of APL management at smaller centers, it is clear that optimum clinical treatment is dependent upon rapid access to diagnosis and hospital facilities with ATRA and blood products. This highlights the importance of guidelines to raise clinical awareness of the rapid

actions needed for diagnosis and rigorous supportive care aspects to reduce the risk of induction death irrespective of the size of the treatment center.

5. Treatment of newly diagnosed patients

5.1. Supportive care

Recommendations for supportive care measures are summarized in Table 1.

5.1.1. Specific supportive measures. Besides the specific supportive measures intended to reduce morbidity and mortality associated with the characteristic coagulopathy that were described above (section 3.2), other measures should be instituted to reduce some clinical complications typically associated with the administration of ATRA and ATO, such as the APL differentiation syndrome (formerly retinoic acid syndrome) seen with both agents, and several electrolyte abnormalities and prolongation of the QT interval which can occur with ATO therapy.

5.1.1.1. Prevention and management of APL differentiation syndrome. Physicians caring for patients with APL treated with ATRA or ATO should be aware of early symptoms or signs suggestive of the APL differentiation syndrome. Diagnosis of this syndrome should be suspected clinically in the presence of one of the following symptoms and signs: dyspnea, unexplained fever, weight gain, peripheral edema, unexplained hypotension, acute renal failure or congestive heart failure, and particularly by a chest radiograph demonstrating interstitial pulmonary infiltrates, or pleuropericardial effusion. Because of the life-threatening nature of the full-blown syndrome, specific treatment with dexamethasone at a dose of 10 mg twice daily by intravenous injection should be started promptly at the very earliest symptom or sign. This policy is highly recommended even though none of the aforementioned signs and symptoms is pathognomonic of the syndrome, and they can be due to concurrent medical problems, such as bacteremia, sepsis, fungal infection or congestive heart failure. Temporary discontinuation of ATRA or ATO is indicated only in case of severe APL differentiation syndrome (ie, patients developing renal failure or requiring admission to the intensive care unit due to respiratory distress). Otherwise, these differentiating agents could be maintained unless progression to overt syndrome or lack of response to dexamethasone is observed. If a favorable response is obtained, dexamethasone should be maintained until complete disappearance of symptoms, and then ATRA or ATO should be resumed. While preemptive therapy with dexamethasone currently represents the standard approach to treat patients developing APL differentiation syndrome, there is at present no evidence that prophylactic corticosteroid is advantageous to reduce rates of morbidity and mortality associated with this syndrome. Nevertheless, in uncontrolled studies, a very low mortality or morbidity rate was reported as a result of differentiation syndrome after ATRA treatment when corticosteroids were administered prophylactically in patients presenting with WBC count greater than 5×10^9/L.[56,57]

5.1.1.2. Prolonged QT interval associated with ATO. Treatment with ATO is associated with several electrolyte abnormalities and QT prolongation that can lead to a torsade de pointes-type ventricular arrhythmia, which can be fatal.[58] This requires careful monitoring to maintain the serum potassium above 4.0 mEq/L and serum magnesium above 1.8 mg/dL, well above the lower limit of normal. If possible, drugs that are known to prolong the QT interval should be discontinued. In patients who reach an absolute QT interval value longer than 500 msec, ATO should be withheld, the electrolytes (potassium and magnesium) repleted, and other medications that may cause prolonged QT interval searched for and ideally discontinued. The risk/benefit of continuing versus suspending arsenic therapy should be considered in any case. If syncope, rapid or irregular heartbeat develops, the patient should be hospitalized for ECG and electrolyte monitoring and ATO therapy should be temporarily discontinued. Once the QT/QTc returns to approximately 460 msec, the electrolytes are repleted, and the syncope and irregular heartbeat cease, ATO may be resumed. In addition to prolongation of the QT/QTc interval, approximately 13% of patients treated with ATO may develop hypokalemia or hyperglycemia.

5.1.2. Other supportive measures. Apart from the aforementioned specific measures, general supportive care aspects in APL, the use of antibiotics, hematopoietic growth factors, prevention of tumor lysis syndrome, and transfusion policy, including red cell, granulocyte, and platelet transfusions (once the coagulopathy is under control), do not differ from those applied in patients with other subtypes of AML.[15]

However, leukapheresis is not recommended as part of initial therapy for APL patients presenting with elevated WBC, because this procedure may exacerbate the coagulopathy and was associated with a high risk of induction death in one series.[59] Early institution of chemotherapy in combination with ATRA accompanied by prophylactic steroids is the standard treatment approach in this life-threatening situation. In patients presenting with WBC count greater than 10×10^9/L, chemotherapy is generally started on day 1 within a few hours of the first dose of ATRA, aiming to control the coagulopathy while reducing the risk of APL differentiation syndrome which is particularly high in these patients. On the other hand, treatment-related hyperleukocytosis, which frequently develops during induction with ATO, can be safely managed with careful observation, checking in particular for evidence of emerging APL differentiation syndrome (see section 5.1.1.1, "Prevention and management of APL differentiation syndrome").[60] Approximately 50% of patients treated with ATO develop leukocytosis, with a peak WBC count at approximately 20 days after the first dose. In the situation of marked hyperleukocytosis after ATO, administration of hydroxyurea can be considered although its clinical benefit is unclear; however, such leukocytosis resolves at a median of 10.5 days after the peak, despite continuation of ATO.

5.2. Targeted treatment

Recommendations for management during and after induction and consolidation therapy are summarized in Table 2.

5.2.1. Induction therapy.

5.2.1.1. The current standard approach. The simultaneous administration of ATRA and anthracycline-based chemotherapy is currently considered the standard induction treatment in newly diagnosed patients with APL. This combination results in extremely high antileukemic efficacy, leading to complete remission (CR) in 90% to 95% of patients and primary resistance has been only reported in just a few anecdotal cases.[50,52,53,57] These data indicate that virtually all *PML-RARA*–positive APLs are sensitive to ATRA and anthracycline-based chemotherapy. Several other clinical trials conducted over the last decade have led to the optimization of ATRA and anthracycline-containing chemotherapy schedules.[9,10,12,13] Initial studies involving ATRA monotherapy, which also resulted in high rates of CR, highlighted the need for administering some type of consolidation chemotherapy to avoid disease relapse. Several subsequent studies and especially 2 randomized trials of the European APL group[61] and the North American

Table 2. Management during induction, consolidation therapy, and beyond

Recommendation	Level of evidence	Grade of recommendation
2.1. Eligible patients should be offered entry into a clinical trial.	IV	C
Induction therapy		
2.2. Induction therapy should consist of the administration of concomitant ATRA and anthracycline-based chemotherapy.	Ib	A
2.3. Standard induction therapy should not be modified based on the presence of leukemia cell characteristics that have variably been considered to predict a poorer prognosis (eg, secondary chromosomal abnormalities, *FLT3* mutations, CD56 expression, and BCR3 *PML-RARA* isoform).	IIa	B
2.4. ATO should be used as standard therapy in countries where pharmaceutical quality locally produced arsenic compounds provide a more affordable treatment approach than ATRA plus chemotherapy.	III	B
2.5. Treatment with ATRA should be continued until terminal differentiation of blasts and achievement of CR, which occurs in virtually all patients after conventional ATRA + anthracycline induction schedules.	IIa	B
2.6. Clinicians should refrain from making therapeutic modifications on the basis of incomplete blast maturation (differentiation) detected up to 50 days or more after the start of treatment by morphology, cytogenetics, or molecular assessment.	IV	C
Consolidation therapy		
2.7. Two or 3 courses of anthracycline-based chemotherapy are considered the standard approach for consolidation therapy.	Ib	A
2.8. The addition of ATRA to chemotherapy in consolidation seems to provide a clinical benefit.	IIb	B
2.9. Consolidation for high-risk patients younger than 60 years with WBC counts higher than 10×10^9/L should include at least one cycle of intermediate- or high-dose cytarabine.	IIb	B
2.10. ATO in consolidation should at present be restricted to investigation within clinical trials or those patients considered unfit for conventional chemotherapy.	IV	C
2.11. Molecular remission in the bone marrow should be assessed at completion of consolidation by RT-PCR assay affording a sensitivity of at least 1 in 10^4.	IIa	B
2.12. Patients with confirmed molecular persistence should be considered for allogeneic HSCT.	IV	C
2.13. For patients with molecular persistence who are not candidates for allogeneic HSCT, ATO or gemtuzumab ozogamicin may be considered.	IIa	B
Management after consolidation		
2.14. Maintenance therapy should be used for patients who have received an induction and consolidation treatment regimen wherein maintenance has shown a clinical benefit.	Ib	A
2.15. Because early treatment intervention in patients with evidence of MRD affords a better outcome than treatment in full-blown relapse, every 3 months MRD monitoring of bone marrow should be offered to all patients for up to 3 years after completion of consolidation therapy.	IIb	B
2.16. Bone marrow generally affords greater sensitivity for detection of MRD than blood and therefore is the sample type of choice for MRD monitoring to guide therapy.	IIa	B
2.17. For patients testing PCR-positive at any stage after completion of consolidation, it is recommended that a bone marrow is repeated for MRD assessment within 2 weeks and that samples are sent to the local laboratory, as well as to a reference laboratory for independent confirmation.	IV	C
2.18. CNS prophylaxis can be considered only for patients with hyperleukocytosis.	IV	C

Intergroup,[47] showed that patients receiving ATRA followed by chemotherapy had significantly better outcomes compared with patients treated with chemotherapy alone. In both studies, the CR and early death rates were not statistically different, but the relapse rate was significantly higher for the patients treated with chemotherapy alone. The outcomes with the sequential administration of ATRA followed by chemotherapy were subsequently improved on over the past decade when ATRA and chemotherapy were given simultaneously. This was clearly shown in a randomized study of the European APL group[52] comparing the sequential versus the simultaneous ATRA plus chemotherapy schedule, and further confirmed in other large multicenter trials.[53,57,62-65] Based on these studies, an evidence-based consensus has been reached to establish the combination of ATRA plus anthracycline-based chemotherapy as the current standard approach for newly diagnosed APL.

As to the type of anthracycline and whether it should be combined with other chemotherapy agents, both issues still remain controversial; at least as far as induction therapy is concerned. Comparable CR rates have been reported using ATRA plus daunorubicin and cytarabine[52,66] and ATRA plus idarubicin alone,[62,64] with no apparent advantage observed by adding other cytotoxic agents.[53] The only randomized trial reported so far[67] was unable to demonstrate differences in terms of CR and induction failure rates when comparing ATRA plus daunorubicin alone versus ATRA plus daunorubicin plus cytarabine for induction therapy. However, this study demonstrated an increased risk of relapse when cytarabine was omitted from both induction and consolidation therapy. A preliminary result of a second randomized trial has recently been reported which compared ATRA with idarubicin against ATRA with daunorubicin and cytarabine. There was no overall difference in response, relapse, or overall survival, but a small increase in deaths in remission was noted in the cytarabine-containing arm.[68] With respect to the type of anthracycline, idarubicin has shown a slight survival advantage compared with daunorubicin in conjunction with cytarabine only in younger AML patients.[69] In APL, no prospective studies have been conducted to assess the comparative value of both anthracyclines.

Exceptions to the use of the standard approach should be considered only for individual cases in which chemotherapy is contraindicated (eg, severe organ failure, anticoagulant therapy, patients older than 80 years), as well as in cases in which alternative options for induction therapy are dictated by socioeconomic factors or clinical trial design. However, modifications to the standard approach based on the presence of leukemia-cell characteristics that have variably been considered to predict a poorer

prognosis (eg, secondary chromosomal abnormalities, *FLT3* mutations, CD56, and BCR3 or short *PML-RARA* isoform) are not supported by the available data.

5.2.1.2. ATO as an alternative approach. After the successful results in the treatment of relapsed patients with APL first reported in China and then replicated in Western populations,[70,71] several trials have been designed to investigate the role of ATO in front-line therapy. As far as we know, only 4 relatively small and selected series using ATO from China,[72] Iran,[73] India,[74] and M.D. Anderson Cancer Center (Houston, TX)[75] have been published so far. The CR rate in these studies ranged from 86% to 95%. However, it should be noted that ATO was combined with ATRA[72,75] and/or chemotherapy[72,74] and/or gemtuzumab ozogamicin[75] in a variable proportion of patients, particularly those presenting with hyperleukocytosis.

Altogether, these promising results with ATO-based regimens indicate that appropriately designed comparisons with the standard ATRA plus anthracycline chemotherapy approach in terms of efficacy, safety, and cost-effectiveness are warranted. In the meantime, the use of ATO-based regimens should be restricted to patients included in clinical trials or for those in whom chemotherapy (especially anthracyclines) is contraindicated. Nevertheless, it should be remarked that, in countries where locally produced arsenic compounds provide a more affordable treatment approach than ATRA plus chemotherapy, arsenic-based regimens have been adopted as the standard of care. In this scenario, a cheaper ATO, produced under good quality control, would be effective in curing many APL patients.

5.2.1.3. Assessment of induction response. Results of morphologic, cytogenetic, and molecular evaluation should be cautiously interpreted at the end of induction therapy. Morphologic features in BM during differentiation therapy can lead to erroneously labeling some patients as resistant by inexperienced pathologists. In fact, potentially misleading cytomorphologic features due to incomplete blast maturation are occasionally seen even after several weeks from treatment initiation (up to 40-50 days). Similarly, a delayed differentiation of blasts can lead to the detection of cells displaying the t(15;17) by conventional cytogenetics or FISH, particularly when these tests are performed at an early time after induction. These morphologic and cytogenetic assessments should not lead to any treatment modification. Rather, treatment with ATRA should be continued allowing sufficient time for terminal differentiation of blasts to occur. As discussed above, CR is attained in virtually all patients with genetically proven *PML-RARA* APL. In this respect, a recent study by the PETHEMA group[48] found that 6 of the 7 patients who were registered by their physicians as having primary resistant disease among 739 patients treated with ATRA plus idarubicin had been assessed for response much earlier than is currently recommended (ie, while still pancytopenic, 18-33 days after chemotherapy).[8,41] Unfortunately, the premature administration of salvage therapy to these patients precluded the possibility to determine whether they genuinely exhibited primary resistance or whether in these instances there was merely a delay in terminal differentiation of APL blasts. In case of any doubt about the achievement of CR, it is recommended to repeat another BM assessment after an additional interval of 2 to 3 weeks while keeping the patient on ATRA, and meanwhile to refrain from new therapeutic interventions. Since the introduction of this strategy, no cases of resistant leukemia have been recorded among the last 350 patients enrolled in the PETHEMA studies.[48]

Molecular assessment by RT-PCR on regeneration after induction has no clinical relevance, because PCR positivity at this early time point may simply reflect delayed maturation instead of resistance. Therefore, clinicians should refrain from making therapeutic decisions on the basis of results at this time point. In sharp contrast, results of PCR analyses performed after completion of consolidation are relevant to determine the relapse risk in the individual patient (see section 5.2.2.6).

5.2.2. Consolidation therapy. The achievement of molecular remission rates of roughly 95% in patients receiving at least 2 further cycles of anthracycline-based chemotherapy after induction has led to the adoption of this strategy as the standard for consolidation.[8] However, some issues related to this phase of therapy remain controversial and will be discussed in detail.

*5.2.2.1. The role of all-*trans *retinoic acid.* The benefit provided by the addition of ATRA to chemotherapy for consolidation has not yet been demonstrated in randomized studies. Nevertheless, historical comparisons of consecutive trials carried out independently by the Gruppo Italiano Malattie Ematologiche dell'Adulto (GIMEMA)[76] and PETHEMA[57] groups showed a statistically significant improvement in outcomes when ATRA at standard dose (45 mg/m^2 per day for adults; 25 mg/m^2 per day for children) was given for 15 days in conjunction with chemotherapy, suggesting that ATRA contributes to reduction in relapse risk.

5.2.2.2. The role of cytarabine. From the first successful regimen using daunorubicin as monotherapy[77] to the present, the role of cytarabine in APL has remained controversial. None of the studies conducted in the pre-ATRA era, including a randomized one,[78] showed an advantage for addition of cytarabine to anthracyclines compared with using high-dose anthracyclines as single agents. With the incorporation of ATRA into most state-of-the-art regimens, the controversy about the role of cytarabine has remained unresolved. A recent randomized study by the European APL group[67] reported an increased risk of relapse when cytarabine was omitted from a schedule including daunorubicin. The conclusions of this interesting study, however, should be interpreted with caution. As a matter of fact, the results of this study might have been largely dependent on the particular choice and dose of anthracycline chemotherapy used (daunorubicin at a cumulative dose of 495 mg/m^2). Of note, a joint analysis of the PETHEMA and the European APL groups[79] demonstrated a significantly lower cumulative incidence of relapse in patients younger than 65 years with WBC counts less than 10×10^9/L at presentation who were treated with anthracycline monochemotherapy (ie, with no cytarabine) in the PETHEMA LPA99 trial compared with patients in the best arm of the European APL 2000 trial including cytarabine. However, a trend in favor of cytarabine administration was observed in the same joint study for high-risk patients with WBC counts higher than 10×10^9/L. A possible explanation for these discrepancies between chemotherapy regimens with or without cytarabine could be the different type and/or dosing schedule of the anthracyclines involved. Whereas the PETHEMA protocol used idarubicin (cumulative dose, 80-100 mg/m^2) and mitoxantrone (cumulative dose, 50 mg/m^2), the European APL protocol used daunorubicin (cumulative dose, 495 mg/m^2). Indeed, a previous joint analysis of the PETHEMA and GIMEMA group studies that used identical type and dosage schedules of anthracyclines showed no difference in outcome with the addition of other chemotherapy agents, including cytarabine, in the GIMEMA group study.[80] Similarly, the preliminary results of the MRC15 trial which were referred to earlier, did not show a benefit for the cytarabine containing arm irrespective of the white blood count.[60] However, in keeping with results of the joint analysis of the PETHEMA and European APL groups,[79] the most recent Italian study does suggest a benefit for using cytarabine in combination with ATRA in

consolidation in patients with high-risk disease (ie, presenting WBC $> 10 \times 10^9$/L).[76] Therefore, in summary, the majority of studies suggest a potential benefit in terms of reduction of relapse risk for the addition of at least one cycle of intermediate or high-dose cytarabine in patients younger than 60 years with WBC counts higher than 10×10^9/L but no difference in overall survival.

5.2.2.3. The role of ATO. The role of ATO in postinduction therapy for newly diagnosed APL patients has been explored not only to consolidate CR aiming to minimize or even eliminate chemotherapy,[72-75] but also to reinforce standard ATRA plus chemotherapy regimens. Results of the 4 studies using ATO for induction and postremission therapy showed a high antileukemic activity of this agent. However, as discussed above, the use ATO in front-line therapy should at present be restricted to investigation within clinical trials or the treatment of those patients considered unfit for conventional chemotherapy. Recently, the use of ATO to reinforce standard ATRA plus chemotherapy regimens has been supported by a large randomized study by the US Intergroup.[66] In this study, patients receiving 2 courses of 25 days of ATO (5 days a week for 5 weeks) immediately after the patient entered a CR and before the standard postremission regimen with 2 more courses of ATRA plus daunorubicin had a significantly better event-free and overall survival than those who received only ATRA plus chemotherapy. This interesting study, although providing high-level evidence for the use of ATO in consolidation, does not definitively clarify whether reinforcement of consolidation therapy with this agent improves the outcome of a given "standard therapy," because overall survival in the control arm was relatively low compared with rates reported by other groups using ATRA and anthracycline chemotherapy–based schedules. The results in the pediatric age group in the US Intergroup study were particularly disappointing compared with other standard approaches.[81-84] Future studies will address whether ATO can allow deintensification of APL therapy without compromising cure rates or indeed improve on outcomes currently achieved with optimal ATRA and anthracycline–based protocols.

5.2.2.4. The role of hematopoietic stem cell transplantation. The role of hematopoietic stem cell transplantation (HSCT) in front-line therapy of APL has changed dramatically during recent years. In fact, the high cure rate obtained using upfront ATRA and chemotherapy indicates that there is no role for routine use of HSCT for patients who are in the first molecular remission at the end of consolidation. For the small fraction of patients with persistent MRD at this time point, given the poor prognosis of this subset of patients,[85] allogeneic HSCT should be considered for those with a suitable human leukocyte antigen (HLA)–matched donor available. Because this group can progress rapidly to overt relapse, additional therapy (eg, ATO) can be used to reduce disease burden and ideally achieve molecular CR before transplantation. For the time being, nearly all experience in HSCT has been based on the use of ablative conditioning regimens. Data after reduced-intensity conditioning in this disease are currently lacking. For those who are not candidates for allogeneic HSCT because a suitable HLA-matched donor is not available or the overall performance and clinical condition render the patient unsuitable for transplantation, other experimental options such as ATO, gemtuzumab ozogamicin, or both should be considered. Provided that the patient achieves molecular CR in the marrow and has a PCR negative harvest autologous HSCT can be considered as consolidation therapy. Although good results have been achieved using this approach,[86,87] the role of transplant is uncertain, since it is possible that long-term remissions can also be achieved with multiple courses of ATO and/or gemtuzumab ozogamicin.

5.2.2.5. Risk-adapted consolidation. It is generally assumed that older patients are more vulnerable to chemotherapy toxicity and most protocols limit the age to receive full-dose chemotherapy not only for consolidation, but also for induction. Even with therapeutic approaches that have been demonstrated to have a relatively low toxicity, such as the PETHEMA treatment schedules, mortality rate in CR ranged from less than 1% in patients younger than 60 to 19% in patients older than 70 years.[88] Therefore, it is a reasonable option to design therapeutic strategies to reduce treatment-related morbidity and mortality in this setting. In addition, there is a tendency to design risk adapted strategies to modulate treatment intensity in consolidation according to predefined risk factors for relapse, particularly presenting WBC.[80] This tailored strategy seems to be an efficient approach to minimize toxicity while targeting more intensive therapy to those patients at most risk of relapse.

5.2.2.6. Molecular assessment at the end of consolidation and beyond. In contrast to the lack of clinical value of molecular assessment performed at the end of induction (see section 5.2.1.3), RT-PCR of *PML-RARA* in BM carried out on regeneration after the final course of chemotherapy is extremely relevant to determine the relapse risk in the individual patient.[23,89] The achievement of molecular remission at this time point represents a therapeutic milestone and is considered a major treatment objective as recommended by the International Working Group for the standardization of response criteria in AML.[41] The evidence supporting this statement was obtained using conventional nested RT-PCR assays, which afford a relatively low sensitivity, typically detecting 1 leukemic cell in 10^3 to 10^4 cells in BM. Real-time quantitative PCR (RQ-PCR) assays are marginally more sensitive and provide several advantages.[90] In particular, they are less prone to contamination, allow kinetics of disease response and relapse to be defined, and enable poor quality samples that could have given rise to "false negative" results according to conventional RT-PCR assays to be identified based upon the level of endogenous control gene transcripts (eg, *ABL*).[89] For patients testing PCR-positive at any stage after completion of consolidation, it is recommended that a BM is repeated for MRD assessment within 2 weeks and that samples are sent to the local laboratory, as well as to a reference laboratory for independent confirmation. It is now accepted that patients with persistent or recurrent disease at the molecular level confirmed in 2 consecutive low-sensitivity assays after completion of consolidation will invariably relapse unless additional therapy is given.[85,91] The optimal management for patients with documented molecular relapse is uncertain and has been already discussed in section 5.2.2.4, "The role of hematopoietic stem cell transplantation."

5.2.3. Maintenance therapy. Despite the benefit provided by ATRA-based maintenance therapy in 2 randomized studies,[47,52] the systematic use of postconsolidation therapy is still a controversial matter in patients achieving molecular remission at the end of consolidation. As the molecular status at the end of consolidation had not been tested in these studies, it might be possible that a proportion of patients with levels of residual disease within the PCR-detectable range benefited from maintenance. Two other randomized studies, however, have recently reported no benefit from 2 different maintenance regimens.[92,93] It should be noted, however, that these studies were carried out in patients testing negative for *PML/RARA* at the end of consolidation. The Italian study involving ATRA, 6-mercaptopurine, and methotrexate has been published only in abstract form to date[92] and the Japanese study,[93] using 6 courses of intensified maintenance chemotherapy

Table 3. Management of special situations

Recommendation	Level of evidence	Grade of recommendation
Older patients		
3.1. Elderly patients in good clinical condition should be managed with a treatment approach similar to that used in younger patients slightly attenuated in dose intensity.	IIa	B
Patients with severe comorbidities		
3.2. Older and younger patients with severe comorbidities unfit for chemotherapy (especially anthracyclines) are candidates to receive ATO-based treatment schedules.	III	B
Children		
3.3. ATRA at 25 mg/m^2 per day is the recommended dose in children and adolescents.	IIa	B
Pregnant women		
3.4. Management of APL in pregnancy requires the involvement of the patient, hematologist, obstetrician, and neonatologist.	III	B
3.5. Retinoids are highly teratogenic and should be avoided in the first trimester unless the patient decides to have a termination of pregnancy.	III	B
3.6. ATRA can be used in the second and third trimesters of pregnancy.	III	B
3.7. Arsenic derivatives are highly embryotoxic and are contraindicated at any stage of pregnancy.	IV	C
3.8. In patients presenting in the first trimester and not wishing to have a termination of pregnancy, induction therapy with daunorubicin alone can be offered.	IV	C
3.9. Although chemotherapy appears reasonably safe in the second and third trimesters of pregnancy, it is associated with an increased risk of abortions and premature delivery, and induction of labor between cycles of chemotherapy should be considered.	III	B
3.10. Stringent fetal monitoring, with particular emphasis on cardiac function, is recommended for patients receiving ATRA with or without chemotherapy during pregnancy.	IV	C
3.11. For deliveries before 36 weeks of gestation, antenatal corticosteroids before preterm delivery are recommended to reduce the risk of morbidity and mortality associated with respiratory distress syndrome.	IIb	B
3.12. After successful delivery, breastfeeding is contraindicated if chemotherapy or ATO is needed.	IV	C
3.13. Female patients with APL should be advised against conceiving while exposed to ATRA or ATO for consolidation and maintenance therapy.	IV	C
Management of therapy-related APL		
3.14. Patients with tAPL should the treated like those with de novo APL, but modifications may be necessary taking into account cardiac toxicity and prior anthracycline exposure.	III	B

without ATRA, showed no benefit in terms of reducing relapse rate and moreover was associated with a significantly poorer chance of survival. These conflicting data indicate that the relative benefit of maintenance depends upon the prior induction and consolidation therapy. Therefore, it is appropriate to use maintenance in conjunction with protocols in which it has been shown to confer benefit.

Despite the uncertainty of the benefit provided by maintenance therapy, it is a fact that several patients PCR-negative at the end of consolidation will ultimately relapse, especially among those presenting with WBC count higher than 10×10^9/L. Previous studies have shown that the majority of these patients can be identified by sequential MRD monitoring using qualitative or quantitative PCR, allowing the opportunity for preemptive therapy to prevent disease progression to overt morphologic relapse.[90,94,95]

5.3. Central nervous system prophylaxis

The central nervous system (CNS) is the commonest site of extramedullary disease in APL and at least 10% of hematologic relapses are accompanied by CNS involvement.[96] Therefore, the possibility of CNS disease should be considered in any APL patient with neurologic symptoms and be excluded in patients subject to relapse.

It was suggested, early in the ATRA era, that there was a possible association between development of extramedullary disease and the use of ATRA. However, a large study of the GIMEMA, carried out in patients treated with or without ATRA, failed to demonstrate this correlation.[97] Rather, it is conceivable that extramedullary relapses are more apparent, given the greater availability of molecularly targeted therapies and prolonged survival of patients. Because the majority of CNS relapses occur in patients presenting with hyperleukocytosis,[98] some strategies include CNS prophylaxis for patients in this particular high-risk setting. For such patients, it is advisable to postpone CNS prophylaxis until after the achievement of CR because lumbar puncture at presentation and during induction is extremely hazardous. However, the benefit of this policy has not been established. For patients without hyperleukocytosis, in whom the risk of CNS relapse is extremely low, there is a general consensus to avoid CNS prophylaxis.

6. Management of special situations

Recommendations for the management of special situations are summarized in Table 3.

6.1. Older patients

Unlike other forms of AML arising in older patients, APL is relatively uncommon in this age group and has a relatively favorable outcome. In fact, older patients with APL seem at least as responsive to therapy as do younger patients. In addition, older patients are more likely to present with low-risk features compared with younger patients.[88] To some extent, this observation may explain the relatively low relapse rate observed in patients more than 70 years of age receiving ATRA and moderately reduced anthracycline-based chemotherapy.[88,99] However, as mentioned in section 5.2.2.5 ("Risk-adapted consolidation"), it is generally assumed that older patients are more vulnerable to therapy-related

toxicity (higher rates of neutropenic sepsis and increased treatment-related mortality). Even with therapeutic approaches that have demonstrated a relatively low toxicity, such as the PETHEMA protocols, mortality rate in CR ranged from less than 1% in patients younger than 60 to 19% in patients older than 70 years.[88] Therefore, it is reasonable to design therapeutic strategies aiming to reduce morbidity and mortality due to chemotherapy in this setting and very especially for those unfit for more intensive therapy. For the frail patients who are considered unfit for conventional treatment, ATO with or without ATRA might be a reasonable alternative to the standard ATRA plus chemotherapy approach, although supporting scientific data currently are limited, particularly with respect to rates of remission and complications such as APL differentiation syndrome.[75]

6.2. Patients with severe comorbidities

Several alternative front-line treatment approaches have been designed to minimize the use of chemotherapy in APL. Most of these are based on the use of ATRA, ATO, and gemtuzumab ozogamicin, with minimal or no chemotherapy[75] (see section 5.2.1, "Induction therapy"). Although information on long-term outcome using these alternative approaches in unfit patients is lacking, they could reasonably be used for older and younger patients with severe comorbidities in which intensive chemotherapy is contraindicated (eg, patients with cardiac impairment or other severe organ dysfunction). As with conventional ATRA and anthracycline–based chemotherapy, the aim of treatment in such patients should be to achieve molecular remission, with MRD monitoring being used to guide the need for additional therapy.

6.3. Children

APL accounts for 4% to 8% of pediatric AML in the United States.[100] Higher proportions of APL in pediatric series have been reported from other areas of the world, especially from Italy and Central and South America,[101-103] but most of these studies share many methodologic limitations and deserve additional investigation. Compared with the disease in adults, APL diagnosed in childhood more frequently presents with hyperleukocytosis (approximately 40% in children vs 20%-25% in adults).[88] Information on the long-term health status of children treated with ATRA and anthracycline-based chemotherapy is still scarce. As far as we know, only 2 relatively small pediatric series from the German-Austrian-Swiss group[81] and the European APL group,[82] as well as 2 larger series from the GIMEMA[83] and PETHEMA[84] groups have published therapeutic results using a standard approach. Due to concern regarding the use of high dose of anthracyclines and their potential long-term cardiac toxicity, some attempts to simply reduce the exposure to these agents without any additional treatment modification have been made. In this respect, the preliminary analysis of the US Intergroup study of children treated with ATRA and reduced dose intensity of anthracycline-based chemotherapy have not been encouraging.[66] It is conceivable that, given the benefit demonstrated by the addition of ATO in adults, the same therapeutic measure may compensate for a relatively low dose intensity of chemotherapy in children.[66] Although this strategy has not been tested formally, ATO appears to be effective in pediatric APL as well.[104] Whereas in adults several attempts have been made to use ATO-based regimens to reduce chemotherapy, as far as we know, there is only very limited experience of this agent in children with newly diagnosed APL.[105]

Although some protocols include CNS prophylaxis, at least for patients with hyperleukocytosis, it should be noted that neither an increased incidence of CNS relapse nor a benefit of CNS prophylaxis has been established in children.

To decrease the frequency of side effects associated with induction therapy including ATRA, particularly severe headache and pseudotumor cerebri (PTC), which are both frequently observed in children, most investigators have used a reduced dose of ATRA (eg, 25 mg/m^2 instead of 45 mg/m^2) in the pediatric age group.[81-84] In this respect, the study by Castaigne et al[106] showed no difference in terms of pharmacokinetics, therapeutic efficacy, triggering of hyperleukocytosis, or retinoic acid syndrome with ATRA at 25 mg/m^2 per day compared with the standard dose of 45 mg/m^2 per day. The apparently lower incidence of PTC and headache, together with the excellent therapeutic results obtained with this reduced dose, suggests that 25 mg/m^2 per day is recommended as the standard dose in children and adolescents (see section 5.2.1, "Induction therapy"). Because headache is common during ATRA therapy, it is important to distinguish its etiology including PTC, CNS leukemia, or bleeding. The diagnosis of PTC is based on increased intracranial pressure with normal cerebrospinal fluid (CSF) composition and negative cerebral imaging studies (ie, computed tomography or magnetic resonance imaging scan). It is usually accompanied by papilledema, but this is not a requirement for the diagnosis of PTC.[107] In this situation, sustained elevations in CSF pressure should be documented through successive lumbar punctures or by prolonged intracranial pressure monitoring, if necessary.[108] Sometimes, the symptoms of PTC resolve with the initial "diagnostic" lumbar puncture. If this occurs, no further medical treatment is required. If symptoms persist, temporary discontinuation or dose reduction of ATRA, analgesics, and administration of steroids and acetazolamide are the mainstays of the medical treatment of PTC. Acetazolamide is administered in initial doses of 25 mg/kg per day and the dose titrated upward until clinical response is attained (maximum dose 100 mg/kg per day). Electrolytes must be monitored to evaluate for the development of hypokalemia and acidosis. If acetazolamide is ineffective, then prednisone can be given at a dose of 2 mg/kg per day for 2 weeks followed by a 2-week taper.[109]

6.4. Pregnant women

The incidence of APL during pregnancy is not well established and most published data are based on case reports or very small series, but it is accepted that it is infrequent. When it occurs, it is a challenging situation, that nevertheless carries a high chance of successful outcome for mother and baby after a decision-making process that requires the involvement of the patient, hematologist, obstetrician, and neonatologist, as was recently emphasized in the guidelines on the management of AML in the United Kingdom.[15] It is important to highlight that any delay in starting treatment can compromise the chance of successful remission in AML; this is particularly true in APL, which is considered a medical emergency. Apart from other considerations, given the teratogenic potential of chemotherapy, ATRA, and ATO on the fetus, the most important factors in helping to come to a decision are the gestational age and the attitude of the patient to risk, both for herself and the fetus. For practical purposes, in these guidelines we will address separately the management of APL in pregnant women presenting during the first trimester from disease arising during the second or third trimester.

6.4.1. Management of APL during the first trimester of pregnancy. Given the teratogenic potential of chemotherapy, ATRA and arsenic trioxide on the fetus, therapeutic options for patients diagnosed during the first trimester are extremely limited in terms of the chance of successful outcome for the fetus. Although specific information regarding teratogenicity of ATRA is lacking, this agent is considered highly teratogenic, certainly because this side effect has been clearly demonstrated with other retinoids and particularly with the closely related isotretinoin.[110] As a result, ATRA should be avoided during the first trimester. Administration of chemotherapy during the first trimester, although frequently safe, is also associated with fetal malformations, increased risk of abortion, and low birth weight.[111] Therefore, the crucial decision in a patient with APL during the first trimester is whether to continue with the pregnancy and receive anthracycline chemotherapy alone or commit to terminate the pregnancy once the patient is hemodynamically stable, in which case conventional treatment with ATRA and chemotherapy can be commenced immediately. If elective abortion is unacceptable, administration of chemotherapy alone, without ATRA, would be the only reasonable option that can be offered during the first trimester. There is some limited evidence that idarubicin, which is more lipophilic than other anthracyclines, favoring increased placental transfer, might be more toxic in pregnancy.[112] For this reason, it has been suggested that daunorubicin might be preferred because this agent is known to be effective in APL and there is more published experience of its use in pregnancy.[111] It should be noted that in case of using chemotherapy alone, there will be an increased risk of hemorrhage due to release of procoagulants and plasminogen activators from malignant promyelocytes. If remission is achieved with chemotherapy and the pregnancy is progressing normally, treatment with ATRA could be administered later during the second and third trimesters. The administration of additional courses of chemotherapy during the second and third trimesters will be discussed below.

Regarding ATO, given its high potential embryotoxicity, it cannot be recommended for use at any stage of pregnancy. In fact, as far as we know, this agent has not been used in pregnant humans with APL, probably because it has been shown to be highly embryotoxic in several animal models. Human data are limited to a few populations exposed to arsenic (from drinking water or from working in or living near metal smelters) in which low birth weight, spontaneous abortion, and stillbirth are reported.[113]

Female patients with APL treated conventionally should be routinely advised against conceiving while exposed to ATRA or ATO for consolidation and maintenance therapy.

6.4.2. Management of APL during the second and third trimesters of pregnancy. Despite the limited clinical experience, treatment with ATRA and anthracycline-based chemotherapy seem reasonably safe when applied to patients with APL presenting during the second or third trimester of pregnancy. Although no serious complications have been observed in the mother, the considerable literature on the effect of chemotherapy on the developing fetus, recently reviewed by Culligan and colleagues,[111] suggests that chemotherapy does not cause congenital malformation, but increases the risk of abortion, prematurity, low birth weight, neonatal neutropenia, and sepsis. For these reasons, there is reluctance to use chemotherapy before delivery. With regard to the administration of ATRA beyond the first trimester of pregnancy, it is considered relatively safe for mother and fetus. Although the experience is quite limited, there were no fetal malformations reported suggestive of retinoic acid embryopathy.[111] However, because some cases of reversible fetal arrhythmias and other cardiac complications at birth have been reported,[114,115] stringent fetal monitoring, with particular emphasis on cardiac function, is recommended for patients receiving ATRA alone or combined with chemotherapy during pregnancy.

Regarding maternal outcome after treatment with ATRA-containing regimens, there is no evidence that this would be different in pregnancy compared with nonpregnant patients. Therefore, keeping in mind the aforementioned adverse effects on the fetus of both components of treatment, ATRA and chemotherapy, the following strategies can be outlined.

(1) Sequential use of ATRA and chemotherapy. With this approach, patients would be treated with ATRA alone until CR, delaying the administration of chemotherapy until after birth, scheduled for when the fetus is deemed to be of sufficient maturity for elective delivery. A gestational age of at least 32 weeks is considered relatively safe when appropriate neonatal care is provided.[116] For deliveries before 36 weeks of gestation, antenatal corticosteroids before preterm delivery are recommended to reduce the risk of morbidity and mortality associated with respiratory distress syndrome.[117] It is assumed that vaginal delivery is associated with reduced risk of bleeding, and it is preferred (if not contraindicated) to cesarean section, which might be required for other reasons.[111]

Regarding maternal outcomes, the expected response rate with ATRA alone is not significantly different to ATRA plus chemotherapy in terms of CR rate, but it can have an unfavorable impact on the risk of relapse.[52] Perhaps, this theoretical disadvantage could be later counteracted with a reinforcement of postremission therapy. If this strategy is followed, the administration of chemotherapy should not be delayed excessively to avoid resistance and disease recurrence, which could compromise long-term outcome for the patient. It has been suggested that molecular assessment of response and subsequent RQ-PCR monitoring can be used to indicate the need for introduction of chemotherapy.[111] It should also be noted that using ATRA alone there is an increased risk of APL differentiation syndrome (approximately 25%).[47]

(2) Simultaneous administration of ATRA and chemotherapy. This approach, currently considered the standard treatment for patients in the nonpregnant state, provides the best chances of cure and is a clear option for high-risk patients with hyperleukocytosis and for those in which appropriate RQ-PCR monitoring is not realistic. As previously mentioned for the management of patients during the first trimester, daunorubicin might be preferred to idarubicin in pregnancy, but this is not so clear for patients with fetus in advanced gestational age.

After successful delivery, breastfeeding is contraindicated if chemotherapy or ATO is needed. The rest of management does not differ from other patients with APL.

6.5. Therapy-related APL

Scarce information is available about the true incidence of therapy-related APL (tAPL) because these patients are less likely to be entered into clinical trials and available estimates are subject to important methodologic limitations being based on retrospective series[4,5] or the experience of single referral centers.[118,119] In these studies, tAPL cases accounted for a range from less than 5% to 22% of all APL cases. A growing incidence of tAPL has been reported over the last few years paralleling the increased use of topoisomerase II–targeted drugs in both malignant and nonmalignant diseases. Breast carcinoma is by far the most frequent previous cancer, followed by lymphoma, with a large predominance of non-Hodgkin lymphoma compared with Hodgkin

Table 4. Management of relapse

Recommendation	Level of evidence	Grade of recommendation
4.1. For patients with confirmed molecular relapse (defined as 2 successive PCR-positive assays, with stable or rising *PML-RARA* transcript levels detected in independent samples analyzed in 2 laboratories) preemptive therapy has to be started promptly to prevent frank relapse.	IIa	B
4.2. Although ATRA in combination with chemotherapy can be used as salvage therapy, ATO-based regimens are presently regarded the first option for treatment of relapsed APL.	IV	C
4.3. Patients achieving second CR should receive intensification with SCT or chemotherapy, if possible.	IV	C
4.4. Allogeneic HSCT is recommended for patients failing to achieve a second molecular remission.	IV	C
4.5. Autologous HSCT is a valid option for patients without detectable MRD in the marrow and with an adequate PCR negative harvest.	IIa	B
4.6. For patients in whom HSCT is not feasible, the available options include repeated cycles of ATO with or without ATRA with or without chemotherapy.	IV	C
4.7. For patients with CNS relapse, induction treatment consists of weekly triple intrathecal therapy (ITT) with methotrexate, hydrocortisone, and cytarabine until complete clearance of blasts in the cerebrospinal fluid, followed by 6 to 10 more spaced out ITT treatments as consolidation. Systemic treatment should also be given.	IV	C

disease, whereas other tumor types were found with lower incidence.[5] The drugs most commonly implicated in tAPL are epirubicin and mitoxantrone, but several cases have been reported to follow exposure to radiotherapy alone.[6,120-122] Intriguingly, it has also been reported that some cases of secondary APL (sAPL) have arisen in patients whose primary tumor was treated by surgery without chemotherapy or radiotherapy exposure.[4,5] The latency period between chemotherapy exposure and onset of tAPL is relatively short (< 3 years) and typically occurs without a preceding myelodysplastic phase. Hematologic findings do not differ from those observed in de novo APL, as previously reported for other tAML with specific karyotype.[123,124] However, although the incidence of secondary chromosomal abnormalities seems similar to that observed in de novo APL, the involvement of chromosomes 5, 7, or 17 appears to be more common in tAPL patients compared with those with de novo APL.[5]

Regarding tAPL arising in patients treated for nonmalignant diseases, it should be noted that since the approval by the US Food and Drug Administration and the European Agency for the Evaluation of Medicinal Products in 2000 of mitoxantrone for treating aggressive forms of multiple sclerosis, cases of tAPL have been increasingly reported.[125] Review of published case reports indicates that APL with the t(15;17) is the predominant genetic subtype of tAML arising in this context, with risks of developing this complication estimated at approximately 1 in 400 patients with multiple sclerosis treated with mitoxantrone.[126]

Current data suggest that patients with tAPL have a relatively favorable prognosis, although the results of one study indicated a higher incidence of early death during treatment.[5] Although a more precise knowledge of the outcome of patients with tAPL treated with state-of-the-art therapy should be prospectively established, at present there is generally no reason to manage these patients in a manner different from those with de novo APL. However, in a significant number of patients with tAPL, the use of conventional anthracycline-based regimens is limited by previous anthracycline exposure and/or cardiac impairment induced by treatment of the primary condition. In such situations, ATO in combination with ATRA provides an option for consolidation after standard induction therapy or as first-line treatment using schedules such as those published by the M. D. Anderson group.[75]

6.6. Genetic variants of APL

Specific management of the rare genetic variants of APL cannot be recommended because the available evidence is mostly based on single case reports. Nevertheless, because the nature of the *RARA* fusion partner can be critical to determine the response to ATRA and ATO,[127] it is reasonable that, as a general rule, patients with ATRA-sensitive variants should be treated with standard protocols involving ATRA combined with anthracycline-based chemotherapy, while those with ATRA-resistant variants should be managed with AML-like approaches. In this regard, *NuMA-RARA*, *NPM1-RARA*, and *FIP1L1-RARA* are known to be ATRA-sensitive, while others are ATRA-resistant (*STAT5b-RARA*),[32] relatively resistant (*PLZF-RARA*), or their sensitivity to ATRA is unknown (*PRKAR1A-RARA*).[33] Sensitivity to ATO has not been documented outside *PML-RARA*-positive APL, except for *PLZF-RARA*-positive APL, which has been shown to be resistant.[128]

7. Management of relapse

Recommendations for the management of relapse are summarized in Table 4.

7.1. Molecular relapse

Two studies carried out in the pre-ATO era have suggested a benefit for preemptive therapy in patients who develop molecular relapse compared with treatment initiated at the time of frank hematologic relapse.[94,95] The benefit of early treatment intervention remains to be proven in the context of ATO-based relapse therapy, however the obvious risks of hemorrhagic death and development of APL differentiation syndrome when patients present with overt disease argue strongly in favor of starting therapy as early as possible in case of emergent relapse. This has provided the rationale for sequential MRD monitoring (by every 3 months BM assessment with assays achieving sensitivity of 1 in 10^4) after completion of therapy to allow early treatment intervention. This schedule of monitoring takes into account the maximum assay sensitivities achievable for detection of APL fusion transcripts[129] as well as the typical kinetics of disease relapse.[130] Because the majority of relapses occur within the first 3 years after completion of consolidation,[131] molecular monitoring can reasonably be discontinued after this period. The clinical utility of PB as a sample source for MRD detection remains to be established; although good concordance between blood and marrow PCR results has been reported during early stages of therapy,[132] analyses of paired molecular surveillance samples taken after completion of consolidation have shown an advantage for marrow, which on average affords 1.5-log greater sensitivity.[90,130] Recurrence of PCR positivity typically is apparent