European recommendation for salvage therapy of relapsed acute promyelocytic leukemia (APL) including arsenic trioxide (ATO)

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2. Summary

Relapses of APL after modern front-line therapy have become rare and the published data are limited.

This recommendation for the management of relapsed APL including arsenic trioxide (ATO) is based on a series of meetings of an European APL group of experts between February of 2005 and November of 2007. The most important goal is to keep the toxicity of therapy as low as possible and the efficacy as high as possible.

Induction and consolidation therapy with arsenic trioxide (ATO) is strongly recommended. For postconsolidation therapy several options can be offered.

Acute promyelocytic leukemia is defined by a characteristic morphology (AML FAB M3/M3v), by the specific translocation t(15;17) and its molecular correlates (PML/RARα and RARα/PML) and by unique therapeutic requirements. Thereby it can be separated from all other forms of acute leukemia.
By all-trans retinoic acid (ATRA) in combination with chemotherapy cure rates of 70 to 85% can be reached. On average, about 10% of patients still die in the early phase of the treatment and up to 30% relapse. Molecular monitoring of the minimal residual disease (MRD) by qualitative nested RT-PCR and quantitative REAL-time PCR of PML/RARα allows to follow the individual kinetics of MRD and to identify patients with an imminent hematological relapse.

A standardized treatment for patients with relapsed APL has not yet been established. ATO exerts a dose dependent dual effect on APL blasts, apoptosis at higher and partial differentiation at lower concentrations. With ATO monotherapy remission rates over 80% were achieved in patients with relapsed APL and long-lasting molecular remissions are described. The drug was mostly well tolerated and the chemotherapy associated cytopenia and risk of infections was avoided. ATO was also successfully administered before allogeneic and autologous transplantation. ATO is approved for the treatment of relapsed and refractory APL in Europe and in the USA.

In the present recommendation of the European APL Group for relapsed APL, induction and consoldation therapy is based on ATO. In the consolidation cycle, ATO should be combined with ATRA.

Suitable patients for ATO therapy are:

- Patients in first or subsequent hematological or molecular relapse of APL
- Persistence of positive RT-PCR after front-line induction and consolidation therapy
- No complete hematological remission after first line therapy (rare cases)

According to the literature postconsolidation therapy after ATO has a positive impact on the outcome. There are several options for postconsolidation therapy.

Factors which have influence on the treatment decision in the individual case are:

(1.) the eligibility for allogeneic transplantation, (2.) the eligibility for autologous transplantation, (3.) the PCR status after consolidation and during follow up (RT-PCR of PML/RARα, sensitivity 10^{-4}), (4.) – in patients not qualifying for transplantation - the individual conditions to receive further ATO in combination with other drugs or chemo-therapy. (5.) age, clinical condition, and other considerations, at the discretion of the centre.

Allogeneic transplantation (PBSCT) can be considered in suitable patients with an available donor. In patients with a positive PCR after consolidation, one cycle of intensive chemotherapy (e.g. high dose ara-C/ mitoxantrone - HAM) before transplantation can be considered. Patients with a negative PCR result are to be transplanted without preceding chemotherapy as soon as possible.

Autologous PBSCT can be considered in patients with negative RT-PCR in the bone marrow before transplantation. In case of a negative PCR result, stem cells can be mobilized with standard dose of G-CSF alone. Optional chemotherapy can be indicated for two reasons: in order to mobilize stem cells and, furthermore, to reach a negative PCR in the positive cases.
If chemotherapy is administered, the intensity of the chemotherapy is recommended to be scheduled individually according to the PCR status after ATO and to the patient's age. (e.g. HAM with a single ara-C dose of 3 g/m² in case of a positive PCR in patients less than 60 years, HAM with 1 g/m² ara-C in case of a negative PCR in patients less than 60 years of age, and in all older patients regardless of the PCR status.)

To patients who are **not eligible for** allogeneic or autologous **transplantation** (e.g. persistent PCR-positivity in the marrow prior to transplantation, too old, no stem cells collected, contraindications against intensive chemotherapy), postconsolidation therapy of six short ATO cycles can be offered. These ATO cycles should be supplemented by ATRA ± mylotarg (GO). Other options are maintenance therapy with ATRA and low dose chemotherapy (MTX and 6-MP) or intensive chemotherapy (HAM).
Mylotarg monotherapy or other experimental therapy may be considered in advanced cases not qualifying for any of these options.

Genetic confirmation of the relapse (e.g. by RT-PCR of PML/RARα, FISH, cytogenetics, anti-PML pattern) is **mandatory**. The identification of the breakpoint by RT-PCR of PML/RARα is strongly recommended. The assessment of the molecular status by RT-PCR of PML/RARα after consolidation is **mandatory**. Further molecular monitoring is recommended to assess the efficacy of the single treatment steps and of the remission status.

2.1 Synopsis

European recommendation for salvage therapy of relapsed acute promyelocytic leukemia (APL) including arsenic trioxide (ATO).	
European APL Group of Experts	(Members in alphabetical order) Sergio Amadori — Italy Thomas Buechner — Germany Alan Burnett — Great Britain Giuseppe Cimino — Italy Hartmut Doehner — Germany Pierre Fenaux — France Eva Lengfelder — Germany Francesco Lo-Coco — Italy Bob Lowenberg — The Netherlands Miguel Sanz — Spain
Writing committee	Priv.-Doz. Dr. Eva Lengfelder — Germany Prof. Francesco Lo-Coco — Italy Prof. Pierre Fenaux — France Prof. Miguel Sanz — Spain
Adult APL patients qualifying for salvage therapy	Patients in first or subsequent hematological or molecular relapse of APL Persistence of positive PCR after front-line consolidation therapy No complete hematological remission after front-line therapy (rare cases). Age over 18 years, no upper age limit.
Contraindications against ATO and special warnings	**No ATO therapy:** Hypersensitivity to arsenic or any of the excipients, absolute QTc interval before the beginning of therapy with ATO ≥460 msec (with balanced electrolytes and no additional medication that prolong the QT interval), pregnancy, severe psychiatric disorder, physical weakness requiring care. **ATO only with close monitoring of the parameters:** Cardiac insufficiency NYHA stage III and IV, refractory kidney function disorder (serum creatinine ≥2 mg/100 ml), severe liver function disorder (bilirubin ≥2mg/100 ml; ALAT/ASAT ≥3-times norm)
Recommended diagnostic measures for confirmation of relapse and for the assessment of the remission status	The confirmation of relapse by genetic diagnosis (e.g. RT-PCR of PML/RARα, FISH, cytogenetics, anti-PML pattern) is **mandatory**. The identification of the breakpoint by RT-PCR of PML/RARα is strongly recommended. The assessment of the molecular status by RT-PCR of PML/RARα after consolidation is **mandatory**. Follow up PCRs with qualitative nested RT-PCR and quantitative REAL-time PCR of PML/RARα are recommended.

Recommended treatment *(see also overview, Paragraph 2.2)*	**Induction and consolidation therapy:** One induction cycle with ATO and one consolidation cycle with ATO. The ATO consolidation course should be combined with ATRA **Postconsolidation therapy:** **Options for patients qualifying for transplantation** • **Allogeneic transplantation** (PBSCT) can be considered in suitable patients with a related donor (or an unrelated donor, if this is in agreement with the respective national transplantation concept). The administration of chemotherapy preceeding allogeneic transplantation is stratified according to the PCR status after ATO: Intensive chemotherapy (HAM) should be considered in PCR positive patients according to the individual situation. No chemotherapy is to be given in PCR negative patients. • **Autologous PBSCT** can be considered in patients with negative PCR in the bone marrow before transplantation. The indication of chemotherapy and the intensity is recommended according to the PCR status of PML/RARα (sensitivity 10^{-4}) after ATO and to patient's age (HAM with either 3 or 1 g/m²). **Options for patients not eligible for transplantation** • **Postconsolidation therapy with six short cycles of ATO plus ATRA.** • **Postconsolidation therapy with six short cycles of ATO plus ATRA. plus mylotarg (GO).** • **ATRA plus low dose maintenance chemotherapy (MTX + 6-MP).** • **Intensive chemotherapy with high dose ara-C/ mitoxantrone (HAM ± ATRA).** **Monitoring of MRD** is mandatory after the consolidation with ATO and recommended after each treatment step. In case of poor response, patients can be treated further with new drugs and investigational therapy (e.g. with mylotarg or other experimental therapy).
	Optional **CNS prophylaxis** based on intrathecal MTX can be given at the end of the induction cycle, when the complete hematopoietic recovery has been observed. A second CNS treatment can be administered during or after the consolidation cycle.

2.2 European recommendation for salvage therapy in relapsed APL

HLA typing & Donor search

Induction

ATO

Consolidation

ATO + ATRA

Molecular assessment (RT-PCR)

Available therapeutic options

1. **Intensification therapy**
   a. Auto PBSCT
   b. Allo PBSCT
   c. ATRA + HD/CHT ± Auto PBSCT
2. **Maintenance therapy**
   a. ATRA + LD/CHT
   b. ATRA + ATO ± GO

Criteria for choice

1. PCR status: Auto PBSCT can be considered only in PCR -ve cases
2. Donor availability
3. Age, clinical condition, and other considerations, at centre discretion, can determine the option of either intensification or maintenance therapy.

3. Background

3.1 Biology and clinical course of APL

Acute promyelocytic leukemia (APL) includes 5-10% of cases of acute myeloid leukemia (AML). It is usually readily distinguishable from all other forms of acute leukemia. The APL blasts are characterized by a typical morphology (AML FAB M3/M3v) and a pathognomonic chromosome translocation t(15;17), which at a molecular level corresponds to the fusion genes PML/RARα and RARα/PML. A particular feature of APL is severe coagulopathy, which is associated with a high mortality rate [10,20].
By all-trans retinoic acid (ATRA) in combination with efficient chemotherapy 70 to 85% of patients with newly diagnosed APL are cured. About 10% of patients still die in the early phase of the induction therapy and up to 30% suffer a relapse in dependence on their risk profile [11,19,21,35].
The monitoring of minimal residual disease (MRD) has clinical significance in APL, as the persistence or the recurrence of a positive RT-PCR (sensitivity 10^{-4}) of PML/RARα in hematological remission is associated with an early relapse [5].
In the case of a relapse it is very likely that remission can be achieved again. Because of the relatively rare APL relapses and the limited comparability of the different types of treatment, hitherto there is no uniform concept for the relapse treatment of this form of leukemia [22].

3.2 Overview of the options for treating relapses of APL

The objectives of relapse treatment are the induction and the stabilization of renewed remission.
The chemotherapy regimens usually used in AML relapse in combination with ATRA have been shown to be effective in the induction of remission of relapsed APL. In general, however, they are no longer curative [2,44].
For younger patients with an available donor, allogeneic peripheral blood stem cell (PBSCT) or bone marrow transplantation (BMT) represents a curative therapeutic approach. Autologous PBSCT or BMT offers the possibility of sustained remission, perhaps even of a cure if patients are transplanted in molecular remission (i.e. PCR-negative for PML/RARα in the marrow) [24,25,29,30,36].
Arsenic trioxide (ATO) with APL blasts dose-dependently induces differentiation and apoptosis. In APL patients with relapse, remission rates of over 80% and sustained molecular remission could be achieved [16,23,40,41].
On small series, good efficacy of anti-CD33 antibodies, of liposomal ATRA, of synthetic retinoids and of gemtuzumab ozogamicin (GO, mylotarg) has been described [7,8,15,26,43].

3.3 Results with conventional treatment strategies in relapsed APL

The primary objective of relapse therapy in APL is the renewed induction of remission with as low a toxicity as possible. In a relapse of APL, anthracyclines and medium or high doses of ara-C, partly in combination with etoposide, have proved to be effective [44]. These regimens have generally been combined with ATRA and in patients in relapse they have achieved similar remission rates as in primary therapy of around 90% [2,44]. However, in general these remissions were no longer stable.
Allogeneic PBSCT with a transplant of a compatible related or unrelated donor has a confirmed curative potential in patients with relapsed APL. In general, before an allogeneic transplant it is endeavored to achieve molecular remission. However, it was also observed that some patients, who were positive before allogeneic transplantation, became negative after

transplantation. Hitherto, an unambiguous assessment of the influence of the PCR status on the prognosis before the allogeneic transplant has not been possible due to the lack of a sufficient quantity of molecular data [25].
As a result, in patients who do not have a donor available, long-term remission or even a cure could be induced by autologous SCT, provided that patients are transplanted in molecular remission [30]. According to a recent survey of the EBMT registry, allogeneic transplant was associated with greater anti-leukemic efficacy as compared to auto SCT but also with a higher transplant-associated mortality. As a consequence, the benefit of the allogeneic transplant was partly offset [29,36].
According to the results of the largest study published so far on 50 patients with relapsed APL, who in accordance with a protocol carried out in France were given intensive chemotherapy consisting of medium-high dosed ara-C, mitoxantron, etoposide and ATRA, the 3-year survival rate after allogeneic transplantation was only 11%. Eight out of 11 patients died in remission either of infections or of a GVHD. The median survival of these patients was just 8.2 months. Of 34 patients who were planned to receive an autologous transplant, in the end only 22 patients had a transplant performed, with the consequences of the toxicity of chemotherapy being a main reason for preventing the transplant from being carried out.
These results indicate that a high toxicity of chemotherapy has a negative effect on the feasibility and the results of transplantation [44].

3.4 Arsenic trioxide

Arsenic derivatives have already been used for more than 2000 years in Chinese medicine for the treatment of malignant and inflammatory diseases. Results in APL with arsenic trioxide [37,40,41] and tetra-arsenic tetra-sulfide [27] have been published recently. Arsenic trioxide, - As_2O_3 - (ATO) has recently been licensed in the USA and Europe for the treatment of relapsed and refractory APL under the name TRISENOX. The mechanism of action of ATO in APL is complex and not yet known in detail. By in vitro studies a dual effect has been shown. At a high concentration (0.5 to 2.0 µmol/L) in the first instance ATO induces apoptosis. Lower concentrations (0.1 to 0.5 µmol/L) lead to partial differentiation of the leukemic cells. Antiangiogenic effects are also being discussed [3,4,34,37].
The results of clinical trials of relapsed APL indicate a high sensitivity of APL blasts to ATO. However, in most trials the observation period is short and the number of patients included is limited [6,12,16,18,23,28,31,32,37, 38,40,41]. In these studies, hematological and molecular remission rates between 80 and 100% have been achieved. In most studies an absolute ATO dose of 10 mg per day or 0.15 mg/kg per day for up to 60 days was administered by intravenous infusion. The post-remission therapy was carried out in various ways either with further cycles of ATO, chemotherapy or a combination of the two.
The first published study with a longer observation period over 7 to 48 months includes 33 Chinese patients with relapsed APL. The survival rate after one year was 63.6%, and 41.6% after two years. At the time of the assessment, the median term of survival without disease was 17 months. Factors that had a positive effect on the remission period were a leukocyte count of less than 10000/µl at the start of the treatment with ATO ($p=0.038$) and the combination of ATO with chemotherapy in the post-remission therapy compared with ATO therapy alone ($p=0.01$). Nevertheless, sustained remissions of up to 41+ months with ATO alone have been observed [31].
In the American licensing study for ATO, molecular monitoring was carried out in 40 of the 52 patients who were included. The rate of molecular remissions after an induction cycle with ATO was 50% and after consolidation with a further ATO cycle it was 83%. The 3-year survival rate was 50%. Sub-group analyses after 4.2 years showed a relapse-free survival of

22% if an ATO monotherapy was used for induction and consolidation and of 86% if an autologous or allogeneic transplant followed ATO [6,40,41 updated].
Potentially dangerous side-effects of ATO are a prolongation of the QT interval in the ECG with the possible consequence of life-threatening ventricular tachycardia and the APL differentiation syndrome, which occurs in about 25% of patients [41,45]. If the detailed recommendations in the product information on TRISENOX are adhered to, the side-effects can generally be avoided or controlled. Particularly important are close ECG and electrolyte monitoring in patients with a raised cardiac risk and the early use of corticosteroids at the first signs of the differentiation syndrome.

In summary, the results of therapy with ATO indicate that in the treatment of APL, this agent has high anti-leukemic efficacy (hematological CR 80 – 100%, molecular remission over 80%) accompanied by relatively low toxicity. The anti-leukemic efficacy is also confirmed by the observation of sustained molecular remissions after ATO monotherapy. With additional chemotherapy and also with an autologous or allogeneic transplant, the relapse-free survival could be extended compared with ATO therapy alone. The results also indicate that with the use of ATO for the induction of remission, the toxicity of chemotherapy can be avoided and as a result a positive effect on the relapse-free survival after transplantation can be achieved.

3.5 Combination of ATO with other drugs

Synergistic effects of ATO and ATRA have been demonstrated in vitro [13]. Some patients who relapsed after ATO had another remission after ATO and ATRA [1]. In a randomized study, which included a total of 50 patients, in a comparison between ATO vs. ATO plus ATRA for the induction of remission in relapsed APL, no advantage was demonstrated for the combination in terms of survival and relapse-free survival [33]. Other studies have shown a benefit of the combination of ATO and ATRA. A Chinese group has recently reported a clinical study in 224 APL patients (156 newly diagnosed patients, 68 relapsed patients) in which the combination of ATO and low-dose ATRA was compared to ATO alone or standard-dose ATRA alone. ATO plus ATRA resulted in a significantly better clinical outcome, improving not only the therapeutic response, but also reducing the ATRA related toxic effects [46]. In a randomized study including newly diagnosed APL patients, the leukemic burden reflected by changes of the PML/RARα transcripts decreased more significantly in combined treatment than with ATO or ATRA monotherapy [39].
The immunotherapeutic agent mylotarg (GO) combines a humanised anti-CD33 monoclonal antibody with calicheamicin, a cytotoxic agent closely related to anthracyclines. Its use in APL has recently been associated with high activity both at onset of disease and at relapse [8,26]. In particular, Lo Coco et al. [26] recently showed that GO monotherapy given at a monthly dose of 6 mg/m^2 was able to induce a durable molecular remission in APL patients treated for 1st, 2nd or even more advanced molecular relapse. The high effectiveness of GO in APL may be explained by several factors such as by the homogeneous expression of CD33 coupled to the significantly lower level of the multidrug resistance gp170 in leukemic blasts. In addition, GO may represent a non cross-resistant drug with respect to ATO and ATRA, as recently suggested by Takeshita et al. who detected activity of GO on ATRA- and ATO-resistant APL cell lines [42]. The feasibility of a treatment scheme in which ATO, ATRA and GO are combined as recently been reported [9].

3.6 Summary and conclusions

The results with various treatment approaches indicate that it is possible in different ways to treat successfully relapses in APL. The relative rarity of relapses and the small numbers of

patients in the series published, often with only a short observation period, make it difficult to compare the individual therapeutic approaches.
ATO (TRISENOX) is now the most frequently used agent for recurrent disease and has been licensed in the USA and Europe for the treatment of APL patients at relapse. Against the background of the relatively high toxicity of chemotherapy and the relatively good tolerability of ATO with a high rate of efficacy, the question arises as to what extent the use of traditional chemotherapy or its intensification can be dispensed with if ATO is used. This applies in particular also to allogeneic or autologous transplantation.
It is the objective of the present European recommendation for relapsed APL to use the therapeutic strategy most suited for the individual patient including ATO, within an established approach.

Criteria that may play a part in deciding the postconsolidation therapy following ATO for the individual are:
1) the feasibility of an allogeneic PBSCT
2) the feasibility of an autologous PBSCT
3) the PCR status of the patient after consolidation
4) the pretreatment of the patient and the feasibility of further ATO or chemotherapy
5) age, clinical condition, and other considerations, at the discretion of the centre.

Achieving molecular remission (negative nested RT-PCR of PML/RARα, sensitivity 10^{-4}) by ATO should enable patients eligible for **allogeneic PBSCT** to dispense with chemotherapy before the transplant (avoiding toxicity) and consider intensive chemotherapy only to PCR-positive patients with the objective in this way of achieving molecular remission.
In patients in whom an **autologous PBSCT** is planned**,** the intensity of the chemotherapy needed for collecting stem cells could be stratified according to the results of the PCR. Here, in the case of molecular remission after ATO it would be a matter of mobilizing stem cells with G-CSF or of choosing a lower dose of chemotherapy, and in the case of a positive PCR of choosing a higher dose of chemotherapy (HAM with ara-C individual dose 3 vs. 1g/m²).
In patients who **do not qualify for any transplantation approach**, options for postconsolidation therapy are ATO combined with either ATRA or with the combination of ATRA and mylotarg (GO). Alternatives are maintenance therapy with methotrexate (MTX) and 6-mercaptopurin (MP) plus ATRA or chemotherapy with high dose ara-C and mitoxantrone.
Concomitant molecular monitoring by means of qualitative and quantitative PCR enables the individual kinetics of the MRD to be recorded as a function of the individual therapy stages and the stratification of the therapy to be organized accordingly.

Patients with advanced disease in whom no PCR-negativity has been achieved, or who relapse molecularly or hematologically again, can be treated further with mylotarg monotherapy or other experimental therapy.

4. Patients

4.1 Adult APL patients qualifying for salvage therapy

Patients in first or subsequent hematological relapse of APL
Patients in first or subsequent molecular relapse of APL
Persistence of positive RT-PCR after front-line consolidation therapy
No complete hematological remission after front-line therapy (rare cases)
Age over 18 years with no upper age limit

4.2 Evidence of a relapse of APL

(see also section 8)

Morphological relapse

Evidence of APL in the bone marrow (more than 5% blasts)
The confirmation of relapse by genetic diagnosis is **mandatory**
(e.g. RT-PCR of PML/RARα, FISH, cytogenetic evidence of translocation t(15;17), anti-PML pattern)**.**
The identification of the breakpoint by RT-PCR of PML/RARα is strongly recommended.

Molecular relapse

Reappearance of PCR positivity of PML/RARα (sensitivity 10^{-4})
The positivity has to be confirmed by low sensitivity PCR (sensitivity 10^{-3} to 10^{-4}) in two successive marrow samples taken 2 weeks apart in order to minimize false positivity.

Note: Positive RT-PCR of RARα/PML with a negative RT-PCR of PML/RARα is not considered as an indication for salvage therapy. Patients with this constellation should be closely monitored.

5. Treatment

5.1 Arsenic trioxide: application and side-effects

License

Arsenic trioxide (TRISENOX) is licensed in Europe and the USA for the treatment of relapsed and refractory APL.

Presentation

ATO is available in dissolved form in vials. One vial contains 10 mg ATO in 10 ml solution (corresponding to a concentrate of 1 mg ATO per 1 ml solution). ATO must be dissolved with 100 to 200 ml 5% glucose solution or 0.9% saline solution immediately after it has been removed from the vial. The prepared solution should be infused for 1 to 2 hours after it has been made up. If vasomotor reactions occur, the infusion should take place over 4 hours.

Side-effects
Detailed information on the pharmacological properties of ATO including the side-effect profile is contained in the "Summary of Product Characteristics of TRISENOX". Of the undesirable effects, particular emphasis is to be given to the APL differentiation syndrome and a prolongation of the QT interval in the ECG, which can lead to ventricular tachycardia (torsades de pointes) with a fatal outcome. In this context, possible interaction with other drugs must also be taken into account. For this reason, close monitoring of the ECG and of the electrolytes is necessary (see page 23). The K^+ and Mg^{++} levels should be in the high-normal range. In general, less threatening side-effects include hyperglycemia, headache, gastrointestinal disorders, neuropathy, skin disorders, arthralgia and bone pain as well as fatigue and insomnia.
An adequate supply of vitamins should be ensured, as severe neurological disorders (neuropathy, cramps) have been reported due to a thiamine deficiency under ATO. N-acetyl cysteine (NAC) should not be administered as concomitant medication, as in vitro it has led to an increase in glutathione concentration, which reduces the effect of ATO.

Pregnancy and lactation
In animal studies ATO has been shown to be embryotoxic and teratogenic. For this reason, during treatment with ATO, effective contraceptive measures must be taken in men and women of child-bearing potential. As ATO can be secreted in the mother's milk, breast-feeding should not be performed during treatment with ATO.

5.2 Contraindications against ATO and special warnings

No ATO therapy:
Hypersensitivity to arsenic or any of the excipients
Absolute QTc interval before the beginning of therapy with ATO ≥460 msec
(with balanced electrolytes and no additional medication that prolong the QT interval)
Pregnancy
Severe psychiatric disorder, physical weakness requiring care.

ATO only with close monitoring of the parameters:
Cardiac insufficiency NYHA stage III and IV
Refractory kidney function disorder (serum creatinine ≥2 mg/100 ml)
Severe liver function disorder (bilirubin ≥2mg/100 ml;
ALAT/ASAT ≥3-times norm)

The special warnings for use and further recommendations are described in detail in the "Summary of Product Characteristics of TRISENOX".

5.3 Recommendation for remission induction, consolidation and postconsolidation therapy of relapsed APL

(Overview in section 2.2)

1. **Remission induction with ATO monotherapy is recommended.**
   For the consolidation course, ATO is recommended to be combined with ATRA.

2. **The postconsolidation therapy after ATO includes several options. The most suitable therapy should be chosen for the individual patient:**

Options for patients qualifying for transplantation

- **ATO followed by allogeneic stem cell transplantation:**
  can be considered in suitable patients with a related donor (or an unrelated donor, if this is in agreement with the respective national transplantation concept).
- **ATO followed by autologous stem cell transplantation:**
  can be considered in patients with negative PCR in bone marrow before transplantation.

Options for patients not eligible for transplantation

- **ATO based postconsolidation therapy** supplemented by other drugs (either ATRA or ATRA plus mylotarg).
- **Chemotherapy: low dose chemotherapy** (maintenance therapy with MTX + 6-MP + ATRA), **or intensive Chemotherapy** (HAM ± ATRA, in particular, if the patients have not received high dose ara-C during front-line therapy).

3. **Patients not sufficiently responding to the ATO-based approaches can be treated** with new drugs and experimental therapy (e.g. with mylotarg or other therapy).

5.3.1 Induction of remission and consolidation with ATO in patients with hematological or molecular relapse of APL

5.3.1.1 Induction therapy with ATO

- **Dosage: ATO 0.15 mg/kg** body weight per day as an intravenous infusion over 1 to 2 hours.
- **<u>Hematological relapse:</u> ATO daily** until complete hematological remission or to a maximum of 60 days.
- **<u>Molecular relapse:</u>** ATO is administered on five consecutive days by two days without therapy over a total of five weeks (=25 days of therapy), to enable therapy on an outpatient basis:

  ATO (0.15 mg/kg/d) days 1 – 5, 8 – 12, 15 – 19, 22 – 26, 29 – 33

In patients with **hematological relaps**e, bone marrow aspiration is recommended, if the criteria of CR are reached in the peripheral blood or on day 60 of ATO, or earlier at individual needs. If it remains unclear, whether a complete hematological remission was reached at day 60, an additional examination of the bone marrow should be performed two weeks later. In patients, who do not enter hematological remission after that time, new drugs and experimental therapy can be considered.
In patients with **molecular relapse**, bone marrow biopsy can be done after the induction

course to evaluate the possible early molecular response by RT-PCR.
RT-PCR of PML/RARα is **mandatory** after the consolidation with ATO in hematological and in molecular relapse.
(For definitions of hematological and molecular remission see section 9, for molecular monitoring see section 7).

5.3.1.2 Consolidation therapy with ATO plus ATRA (hematological and molecular relapse)

- The consolidation therapy with ATO and ATRA should be started **four weeks after the end of the induction cycle with ATO.**

  To enable consolidation therapy on an outpatient basis, ATO is administered on **five consecutive days** by two days without therapy over a total of **five weeks** (=25 days of therapy). ATRA is given continuously.

ATO (0.15 mg/kg/d)	**days 1 - 5, 8 - 12, 15 - 19, 22 - 26, 29 - 33**
ATRA (45 mg/m²/d)	**days 1 - 33**

The assessment of the molecular status is **mandatory after consolidation.**

5.3.1.4 CNS prophylaxis

Optional **CNS prophylaxis** with a single dose methotrexate 15 mg intrathecal can be given at the end of the induction cycle, once the complete hematopoietic recovery has been observed. The addition of other drugs is possible. (e.g. methotrexate 15 mg, ara-C 40 mg, dexamethasone 4 mg; or MTX 12 mg and methylprednisolone 40 mg, according to the national attitude.)
A second identical CNS treatment can be administered during or after the consolidation cycle.

5.3.2 Options for postconsolidation therapy
(Overview in section 2.2):

5.3.2.1 Transplantation

- **Allogeneic transplantation**
  Patients with an available related donor (or an unrelated donor, if this is in agreement with the respective national transplantation concept) and no contraindications against transplantation (old age, co-morbidity etc.) can be given an **allogeneic PBSCT**.

  If the PCR is negative after ATO consolidation, the allogeneic PBSCT is carried out without further chemotherapy.

  If the PCR is positive after ATO consolidation, the allogeneic PBSCT can be carried out without further chemotherapy. However, the administration of an intensive chemotherapy (HAM cycle with high dose ara-C) should be considered (and be discussed with the transplantation team) before the allogeneic PBSCT with the aim of

achieving PCR negativity. After the administration of intensive chemotherapy (e.g. HAM), the transplantation should take place even if the PCR is positive.

Autologous transplantation

- Patients with a negative RT-PCR before transplantation and no contraindications (old age, co-morbidity etc.) can be given an **autologous PBSCT**.

  If PCR is negative after the second ATO cycle, stem cells can be mobilized with standard dose of G-CSF or alternatively after one course of HAM (ara-C individual dose 1g/m^2) and G-CSF.
  (Or no new mobilization, if stem cells are available after previous therapy.)

  If PCR is positive after the second ATO cycle, an additional course of chemotherapy can be given as an attempt to achieve molecular remission (which is the prerequisite to perform autologous transplantation): Age adapted HAM (ara-C individual dose 3g/m^2 (age <60 years) or 1g/m^2 (age >60 years), (HAM regimen: see section 5.3.2.2, paragraph 4)

5.3.2.2 Postconsolidation therapy with ATO and/or chemotherapy

Other options for postconsolidation therapy, in particular for patients not qualifying for allogeneic or autologous transplantation (allogeneic transplantation not possible, patients with persistent PCR-positivity in the marrow not qualifying for autologous transplantation, no stem cells collected, too old, contraindications against intensive chemotherapy) are as follows:

1. **ATO plus ATRA**
2. **ATO plus ATRA plus mylotarg (GO)**
3. **ATRA plus MTX plus 6-MP**
4. **Intensive chemotherapy (high dose ara-C/ mitoxantrone, HAM) ± ATRA**

1. ATO plus ATRA

One monthly course of ATO + ATRA includes:
ATO 0.15 mg/kg, days 1-5 and 8-12
ATRA 45 mg/m^2/d days 1-14
no therapy days 15–28, start of the next cycle day 29,
every 4 weeks for 6 courses.
After 6 courses, continue with ATRA 45 mg/m^2/d 15 days every 3 months to complete 2 years of maintenance.

2. ATO plus ATRA plus GO

One monthly course of ATO + ATRA + GO includes:
ATO 0.15 mg/kg, days 1-5 and 8-12
ATRA 45 mg/m^2 days 1-14
GO 6 mg/m^2 day 1
no therapy days 15-28, start of the next cycle day 29.
every 4 weeks for 6 courses.

3. ATRA plus MTX and 6-MP

Continuous 6-MP (90 mg/m^2/d),
MTX (15 mg/m^2/week) orally and
ATRA 45 mg/m^2 days 1-14, during 2 years.
Half dose of MTX and 6-MP if WBC counts are 2500 to 3500 or platelets 120000 to 100000/µl, stop during 10 days, if WBC counts are <2500 or platelets <100000/µl.

4. Intensive chemotherapy ± ATRA

High dose ara-C/ mitoxantrone (HAM):

Ara-C: 3 (1) g/m^2 12h intravenously (infusion over three hours) day 1,2,3
Mitoxantrone: 10 mg/m^2/day intravenously (infusion over 60 minutes) day 3,4,5.

- In patients less than 60 years of age with positive PCR, the recommended ara-C dose is 3g/m².
- In patients less than 60 years of age with negative PCR and in all patients over 60 years of age, irrespective of the PCR status the recommended ara-C dose is 1g/m².

5.4 Procedure with the APL differentiation syndrome and with hyperleukocytosis

If an APL differentiation syndrome occurs, or if one is suspected, or if the WBC counts are ≥10000/µl, **10 mg dexamethasone should be administered intravenously twice a day** without delay, while continuing with ATO. Dexamethasone is administered until the symptoms disappear, but for three days at least. If the symptoms reappear after ending the corticosteroids, dexamethasone is used again.
According to current knowledge, interrupting ATO therapy is recommended only, if there is no improvement with dexamethasone and if there are life-threatening side-effects.
Hydroxyurea is recommended if the WBC counts exceed 10000/µl or if the WBC counts double rapidly. Hydroxyurea should be started with 2 x 500 mg/day. Dose increase of hydroxyurea should be performed according to individual needs. The use of other cytoreductive drugs is at the discretion of the treating physician.

5.5 Supportive therapy

Supportive therapy with blood products and antibiotics is to be carried out in acute leukemias in accordance with the normal procedures.
Particular attention must be paid to the increased tendency to bleeding due to clotting disorders and thrombocytopenia that often occurs also in a relapse of APL. The main factors here are usually the marked lowering of fibrinogen or of the prothrombin time (Quick's value). The clotting disorders can be improved within a few days with arsenic. In the same way as with the experiences with ATRA, it is assumed that particularly in the initial phase of the therapy, there is a need to replace clotting factors (fresh plasma or individual factors).
Therefore, until the fibrinogen and Quick's value have stabilized, it is recommended that clotting and the thrombocyte count should be checked at least once a day. Until clotting has normalized, the thombocyte count should be maintained at between 30,000 and 50,000/µl by thrombocyte substitution. There are no confirmed recommendations for the administration of heparin or epsilon-aminocaproic acid.

5.6 Dose modification and stopping treatment with ATO

For information regarding modifying the dose and stopping treatment, and also for possible interactions with other medicinal products, see the details in the **Summary of Product Characteristics of TRISENOX**.
The following points in particular should be stressed:

1.) Depending on the individual situation, with any toxicity of WHO grade ≥3, the treatment must either be stopped altogether or interrupted.
2.) If ATO is used again, the dosage should only be 50% of the initial dose.
If the QT interval is prolonged, close monitoring of the ECG and of the electrolytes is necessary. Depending on the individual risk/benefit ratio, an interruption of ATO is indicated until the QTc interval is less than 460 msec (for details see the Summary of Product Characteristics of TRISENOX). The time between discontinuing ATO and normalization of the QTc interval may be several days. The concomitant administration of other substances that prolong the QT interval must absolutely be avoided. In the case of renal insufficiency, delayed elimination must be taken into account.
3.) On clinical signs of overdose, dimercaprol and penicillamine are available.

6. Recommended clinical examinations

(for details of molecular monitoring with RT-PCR see section 7)

6.1 Diagnostics before induction of remission

Results of clinical examinations (weight and height, signs of bleeding, size of spleen and liver, lymph nodes, extramedullary infiltrations), performance status
Blood picture (Hb, hematocrit, leukocytes, thrombocytes), differential blood picture
Bone marrow morphology and genetic confirmation of relapse (e.g. by RT-PCR, cytogenetics, FISH or PML-pattern)
The identification of the breakpoint by RT-PCR of PML/RARα is strongly recommended.
Blood chemistry parameters (creatinine, uric acid, Na, K, Ca, Mg, bilirubin, AP, GOT, GPT, CHE, LDH, serum protein, blood glucose)
Clotting status (Quick's test, fibrinogen)
Urine test
Chest x-ray
If needed, sonography or computer tomography
Electrocardiogramm (ECG),
(long-term ECG over 24 hours in the case of previous cardiac disorders)
Echocardiography
Bacteriology, mycology if necessary (throat swab or mouth rinsings, mid-stream urine or catheter urine, anal swab and swabbing of any open skin infections).

6.2 Diagnostics during treatment with ATO

Blood picture, daily checks at the beginning of ATO in particular if the leukocyte count increases rapidly or there is a need to replace erythrocyte concentrates or thrombocyte concentrates, thereafter every other day.
Checking of clotting status in the initial phase until clotting has normalized, every day, then twice a week

Electrolytes (sodium, potassium, magnesium), urea, creatinine at least three times a week, daily if necessary (particular caution in patients with prolongation of the QT interval)
Liver values (AP, γGT, ALAT, ASAT, CHE) at least twice a week, more frequently if required by the individual
LDH until normalization, twice a week
ECG as needed by the individual, daily in the case of changes (particularly a prolongation of the QT interval)
Blood glucose checks at least twice a week, more frequently if needed.

6.3 Diagnostics before the ATO consolidation and before further therapy

Clinical status, blood picture and blood chemistry findings, clotting according to the time before the induction therapy
Bone marrow aspiration (determination of the blast content), RT-PCR (quantitative and qualitative) (recommended)
ECG
Echocardiogram if needed by the individual.

6.4 Diagnostics for confirmation of remission and during remission

Confirmation of remission after ATO consolidation by bone marrow morphology and RT-PCR is mandatory *(see section 7)***.**

Recommended diagnostics in remission:
At monthly intervals:
Clinical status, blood picture, differential blood picture, blood chemistry (creatinine, uric acid, potassium, sodium, calcium, bilirubin, AP, GOT, GPT, CHE, LDH, total protein), clotting (Quick, fibrinogen),
Every three months: Bone marrow puncture (morphology, RT-PCR).

7 Molecular monitoring

- **Before the start of induction therapy with ATO,** the identification of the breakpoint by RT-PCR of PML/RARα is **strongly recommended**.
- RT-PCR after induction therapy can be considered individually.
- **The assessment of the molecular status is mandatory after consolidation** (bone marrow aspiration **approximately two weeks** after the end of the consolidation cycle).
- **Follow up PCRs are recommended** parallel with the hematological assessment (qualitative nested RT-PCR and quantitative REAL-Time PCR, bone marrow aspiration):
  a) two to four weeks after completion of the respective postconsolidation therapy in particular after the last ATO course, after intensive chemotherapy, after allogeneic or autologous transplantation.
  b) during remission every three months until occurrence of relapse, or until 3 years in remission.

After ATO consolidation or in cases who convert from negativity to positivity, the positivity has to be confirmed by low sensitivity PCR (sensitivity 10^{-3} to 10^{-4}) in two successive marrow samples taken **two weeks apart** in order to minimize false positivity.

Note: with negative qualitative PCR, but with an increase in the transcripts in the quantitative PCR, more frequent controls of the PCR are essential. The therapy stratification, however, should be based only on the results of the qualitative RT-PCR of PML/RARα (sensitivity 10^4).

8. Storage of material and laboratories for confirmative diagnostics

Providing the addresses of the laboratories available for confirmative diagnostic measures is in the responsibility of each country.
The storage of viable leukemic cells before the start of induction therapy and during follow up is strongly recommended (at the treating center or at the diagnostic laboratories).

9. Parameters and definitions to assess the response

Complete hematological remission:
Bone marrow: normocellular (2+) with <5% blasts
Peripheral blood: neutrophils ≥1500/µl
thrombocytes ≥100,000/µl

Hypoplasia without persisting leukemia
Bone marrow blasts <5%, hypocellular, peripheral remission criteria not reached

Definition of hematological relapse
Reinfiltration of the bone marrow with ≥5% leukemic blasts
or extramedullary relapse

Definition of molecular remission
Negativity of the RT-PCR of PML/RARα (sensitivity 10^{-4})

Definition of molecular relapse
Reappearance of the positivity of the RT-PCR of PML/RARα (sensitivity 10^{-4})
The positivity has to be confirmed by low sensitivity PCR (sensitivity 10^{-3} to 10^{-4}) in two successive marrow samples taken 2 weeks apart in order to minimize the risk of false positivity.

9. References

1) Au WY, Chim CS, Lie AK, Liang R, Kwong YL: Combined arsenic trioxide and all-trans retinoic acid treatment for acute promyelocytic leukemia recurring from previous relapses successfully treated using arsenic trioxide. Br J Haematol 2002;117:130-132.

2) Castagnola C, Lunghi M, Corso A, Tajana M, Zappasodi P, Dabusti M, Lazzarino M, Bernasconi C: Management of acute promyelocytic leukemia relapse in the ATRA era. Haematologica 1998;83:714-717.

3) Chen GX, Zhu J, Shi XG, Ni JH, Zhong HJ, Si GY, Jin XL, Tang W, Li XS, Xong SM, Shen ZX, Sun GL, Ma J, Zhang P, Zhang TD, Gazin C, Naoe T, Chen SJ, Wang ZY, Chen Z: In vitro studies on cellular and molecular mechanisms of arsenic trioxide (As_2O_3) in the treatment of acute promyelocytic leukemia: As_2O_3 induces NB_4 apoptosis with down-regulation of bcl-2 expression and modulation of PML-RARa/Pml proteins. Blood 1996;88:1052-1061.

4) Chen GQ, Shi XG, Tang W, Xiong SM, Zhu J, Cai X, Han ZG, Ni JH, Dhi GY, Jia PM, Liu MM, He KL, Niu C MA J, Thang P, Zhang TD, Paul P, Naoe T, Kitamura K, Miller W, Waxman S, Wang ZY, de The H, Chen SJ, Chen Z: Use of Trioxide (As_20_3) in the treatment of acute promyelocytic leukemia. (APL): I. As_2O_3 exerts dose-dependent dual effects on APL cells. Blood 1997;89:3345-3353.

5) Diverio D, Rossi V, Avvisati G, De Santis S, Pistilli A, Pana F, Saglio G, Martinelli G, Petti MC, Santoro A, Pellici PG, Mandelli F, Biondi A, Lo Coco F for the GIMEMA and AIEOP Cooperative Group: Early detection of relapse by prospective reverse transcriptase-polymerase chain reaction analysis of the PML/RARα fusion gene in patients with acute promyelocytic leukemia enrolled in the GIMEMA-AIEOP multicenter „AIDA“ trial. Blood 1998;92:784-789.

6) Dombret H, Fenaux P, Soignet SL, Tallman MS: Established treatment of patients with acute promyelocytic leukemia, and the introduction of arsenic trioxide as a novel therapy. Sem Hematol 2002;39 (Suppl.1):8-13.

7) Douer D, Estey E, Santillana S, Bennett JM, Lopez-Bernstein G, Boehm K, and Williams T: Treatment of newly diagnosed and relapsed acute promyelocytic leukemia with intravenous liposomal all-trans retinoic acid. Blood 2001;97: 73-80.

8) Estey EH, Giles FJ, Beran M, O'Brien S, Pierce SA, Faderl SH, Cortes JE, Kantarjian HM: Experience with gemtuzumab ozogamycin ("mylotarg") and all-trans retinoic acid in untreated acute promyelocytic leukemia. Blood 2002;99:4222-4224.

9) Estey H, Garcia-Manero G, Ferrajoli A, Faderl F, Verstovsek S, Jones D, kantarijan H: Use of all-trans retinoic acid plus arsenic trioxide as an alternative to chemotherapy in untreated acute promyelocytic leukemia. T. Blood 2006;107:3469-3473.

10) Fenaux P, Degos L: Differentiation therapy for acute promyelocytic leukemia. N Engl J Med 1997;337:1076-1077.

11) Fenaux P, Chastang C, Chevret S, Sanz M, Dombret H, Archimbaud E, Fey M, Rayon C, Huguet F, Sotto J, Gardin C, Makhoul PC, Travade P, Solary E, Fegueux N, Bordessoule D, San Miguel J, Link H, Desablens B, Stamatoullas A, Deconinck E, Maloisel F, Castaigne S, Preudhomme C, Degos L: A randomized comparison of all transretinoic acid (ATRA) followed by chemotherapy and ATRA plus chemotherapy and the role of maintenance therapy in newly diagnosed acute promyelocytic leukemia. Blood 1999;94:1192-1200.

12) George B, Mathews V, Balasubramanian P, Shaji RV, Chandy M, Srivasta A: Duration of remission in patients with acute promyelocytic leukemia primarily treated with arsenic trioxide. Blood 2002; 100: (50nd Annual Meeting of the American Society of Hematology), Abstract 1324.

13) Gianni M, Koken MH, Chelbi-Alix MK, et al. Combined arsenic and retinoic acid treatment enhances differentiation and apoptosis in arsenic-resistant NB4 cells. Blood 1999;91:4300–4310.

14) Jing Y, Wang L, Xia L, Chen GQ, Chen Z, Miller WH, Waxman S: Combined effect of all-trans retinoic acid and arsenic trioxide in acute promyelocytic leukemia cells in vitro and in vivo. Blood 2001;97:264-269.

15) Jurcic JG, DeBlasio T, Dumont L, Yao TJ, Scheinberg DA: Molecular remission induction with retinoic acid and anti-CD33 monoclonal antibody HuM195 in acute promyelocytic leukemia. Clin Cancer Res 2000;6:372-380.

16) Kwong YL, au WY, Chim CS, Pang A, Suen C, Liang R: arsenic trioxide- and idarubicin-induced remission in relapsed acute promyelocytic leukemia: Clinical, pathological and molecular features of a pilot study. Am J Hematol 2001;66:274-279.

17) Lallemand-Breitenbach V, Guillemin MC, Janin A, et al. Retinoic acid and arsenic synergize to eradicate leukemic cells in a mouse model of acute promyelocytic leukemia. J Exp Med 1999;189: 1043–1052.

18) Lazo G, Kantarjian H, Estey E, Thomas D, O'Brien S, Cortes J: Use of arsenic trioxide (As_2O_3) in the treatment of patients with acute promyelocytic leukemia. Cancer 2003;97:2218-2224.

19) Lengfelder E, Reichert A, Schoch C, Haase D, Haferlach T, Löffler H, Staib P, Heyll A, Seifarth W, Saussele S, Fonatsch Ch, Gassmann W, Ludwig WD, Hochhaus A, Beelen D, Aul C, Sauerland MC, Heinecke A, Hehlmann R, Wörmann B, Hiddemann W, Büchner T: Double induction strategy including high dose cytarabine in combination with all-trans retinoic acid: Effects in patients with newly diagnosed acute promyelocytic leukemia. Leukemia 2000;14:1362-1370.

20) Lengfelder E, Hehlmann R, Büchner Th: Aktuelle Therapiekonzepte bei akuter Promyelozytenleukämie. Dtsch Med Wschr 2001;126:1073-1079.

21) Lengfelder E, Saussele S, Haferlach T, Schoch C, Weisser A, Ludwig WD, Staib P, Heinecke A, Sauerland M, Berdel W, Wörmann B, Hehlmann R, Hiddemann W, Büchner Th for the AML Cooperative Group (AMLCG). Do patients with acute promyelocytic leukemia (APL) really have a good prognosis? Risk factors for early death and complications in the era of all-trans retinoic acid (ATRA). Results of the AMLCG. Onkologie 2002;25 (Sonderheft 4), 19 (Abstract 66).

22) Lengfelder E, Gnad U, Büchner Th, Hehlmann R: Treatment of Relapsed Acute Promyelocytic Leukemia. Onkologie 2003;26:373-379.

23) Leoni F, Gianfaldoni G, Annunciata M, Fanci R, Ciolli S, Nozzoli C, Ferrara F: Arsenic trioxide therapy for relapsed acute promyelocytic leukemia: A bridge to transplantation. Haematologica 2002;87:485-489.

24) Lo-Coco F, Diverio D, Avvisati G, Petti MC, Meloni G, Pogliani EM, Biondi A, Rossi G, Carlo-Stella C, Selleri C, Martino B, Specchia G, Mandelli F: Therapy of molecular relapse in acute promyelocytic leukemia. Blood 1999;94:2225-2229.

25) Lo-Coco F, Romano A, Mengarelli A. Allogeneic stem cell transplantation for advanced acute promyelocytic leukemia: results in patients treated in second molecular remission or with molecularly persistent disease. Leukemia. 2003;17:1930-1933.

26) Lo-Coco F, Cimino G, Breccia M, et al. Gentuzumab ozogamicin (Mylotarg) as a single agent for molecularly relapsed acute promyelocytic leucemia. Blood 2004;104:1995-1999.

27) Lu DP, Qiu JY, Wang Q, Liu KY, Liu YR, Chen SS: Tetra-arsenic tetra-sulfide for the treatment of acute promyelocytic leukemia: A pilot report. Blood 2002:99;3136-3143.

28) Matthews V, Balasubramanian P, Shaji JV, George B, Chandy M, Srivastava A: Arsenic trioxide in the treatment of newly diagnosed acute promyelocytic leukemia: A single center experience. Am J Hematol 2002;70:292-299.

29) Mandelli F, Labopoin M, Granena A, Iriondo A, Prentice G, Bacigalupo A, Sierra J, Meloni G, Frassoni F, Goldman J, Gratwohl A, and Gorin NC on behalf of the Working Party on Acute Leukemia of the European Cooperative Group for Bone Marrow Transplantation (EBMT): European survey of bone marrow transplantation in acute promyelocytic leukemia (M3). Bone Marrow Transplant 1994;14:293-298.

30) Meloni G, Diviero D, Vignetti M, Avvisati G, Capria S, Petti MC, Mandelli F, Lo Coco F: Autologous bone marrow transplantation for acute promyelocytic leukemia in second remission: Prognostic relevance of

pretransplant minimal residual disease assessment by reverse transcription polymerase chain reaction of the PML/RARα fusion gene. Blood 1997;90:1321-1325.

31) Niu C, Yan H, Yu T, Sun HP, Liu JX, Li XS, Wu W, Zhang FQ, Chen Y, Zhou L, Li JM, Zeng XY, Yang RR, Yuan MM, Ren MY, Gu FY, Cao Q, Gu BW, Su XY, Chen GQ, Xiong SM, Zhang TD, Waxman S, Wang ZY, Chen Z, Hu J, Shen ZY, Chen SJ: Studies on treatment of acute promyelocytic leukemia with arsenic trioxide: remission induction, follow-up, and molecular monitoring in 11 newly diagnosed and 47 relapsed acute promyelocytic leukemia patients. Blood 1999;94:3315-3324.

32) Ohnishi K, Yoshida H, Shigeno K, Nakamura S, Fujisawa S, Naito K, Shinijo K, Fujita Y, Matsui H, Sahara N, Takeshita A, Sahoh H, Terada H, Ohno R. Arsenic trioxide therapy for relapsed or refractory Japanese patients with acute promyelocytic leukemia: need for careful electrocardiogram monitoring. Leukemia 2002;16:617-622.

33) Raffoux E, Rousselot P, Poupon J, Daniel MT, Cassinat B, Delarue R, Taksin AL, Réa D, Buzyn A, Tibi A, Lebbé G, Cimerman P, Chomienne C, Fermand JP, de Thé H, Degos L, Hermine O, Dombret H: Combined treatment with arsenic trioxide and all-trans retinoic acid in patients with relapsed acute promyelocytic leukemia. J Clin Oncol 2003;21:2326-2334.

34) Roboz GJ, Dias S, Lam G, Lane WJ, Soignet SL, Warrell RP, Jr, and Rafii S: Arsenic trioxide induces dose- and time-dependent apoptosis of endothelium and may exert an antileukemic effect via inhibition of angiogenesis. Blood 2000 96: 1525-1530.

35) Sanz M, Martín G, González M, León A, Rayón C, Rivas C, Colomer D, Amutio E, Capote F, Milone G, de la Serna J, Román J, Barragán E, Bergua J, Escoda L, Parody R, Negri S, Calasanz M, Bolufer P. Risk-adapted treatment of acute promyelocytic leukemia with all-*trans*-retinoic acid and anthracycline monochemotherapy: a multicenter study by the PETHEMA group. Blood 2004;103:1237-1243.

36) Sanz MA, Labopin M, Gorin NC, de la Rubia J, Arcese W, Meloni G, Bacigalupo A, Alessandrino P, Carreras E, Iriondo A, Novitzky N, Jacobs P, Bandini G, Lo-Coco F, Frassoni F, Rocha V, on behalf of the Acute Leukemia Working Party of EBMT. Hemotopoietic stem cell transplantation for adults with acute promyelocytic leukemia in the ATRA era: a survey of the European Cooperative Group for Blood and Marrow Transplantation. Bone Marrow Transplantation 2007;39:461-469.

37) Shen ZX, Chen GQ, Ni JH, Li XS, Xiong SM, Qiu QY, Zhu J, Tang W, Sun GL, Yang KQ, Chen Y, Li Z, Fang ZW, Wang YT, Ma J, Zhang P, Zhang TD, Chen SJ, Chen Z, Wang ZY: Use of arsenic trioxide (As_2O_3) in the treatment of acute promyelocytic leukemia (APL). II. Clinical efficacy and pharmacokinetics in relapsed patients. Blood 1997;89:3354-3360.

38) Shen Y, Shen Z-X, Yan H, Chen J, Zeng XY, Li JM, Li XS, Wu W, Xiong SM, Zhao WL, Tang W, Wu F, Liu YF, Niu C., Wang ZY, Chen SJ, Chen Z: Studies on the clinical efficacy and pharmacokinetics of low-dose arsenic trioxide in the treatment of relapsed acute promyelocytic leukemia: a comparison with conventional dosage. Leukemia 2001;15:735-741.

39) Shen ZX, Shi ZZ, Fang J, Gu BW, Li JM, Zhu JM, Shi JY, Zheng PZ, Yan H, Liu YF, Chen Y, Wu W, Tang W, Waxman S, de Thé H, Wang ZY, Chen SJ, Chen Z: All-trans retinoic acid/ As_2O_3 combination yields a high quality remission and survival in newly diagnosed acute promyelocytic leukemia. PNAS 2004;101:5328-5335.

40) Soignet SL, Maslak P, Wang ZG, Jhanwar S, Calleja E, Dardashti LJ, Corso D, deBlasio A, Gabrilove J, Scheinberg DA, Pandolfi PP, Warrell RP: Complete remission after treatment of acute promyelocytic leukemia with arsenic trioxide. N Engl J Med 1998;339:1341-1348.

41) Soignet SL, Frankel SR, Douer D, Tallman MS, Kantarjian H, Calleja E, Stone RM, Kalaycio M, Scheinberg DA, Steinherz P, Sievers EL, Coutré S, Dahlberg S, Ellison R, and Raymond P. Warrell, Jr. United States Multicenter Study of Arsenic Trioxide in Relapsed Acute Promyelocytic Leukemia. J Clin Oncol 2001;19:3852-3860.

42) Takeshita A, Shinjo K, Naito K, et al.: Efficacy of gemtuzumab ozogamicin on ATRA- and arsenic-resistant acute promyelocytic leukemia (APL) cells. Leukemia 19: 1306–1311, 2005

43) Tobita T, Takeshita A, Kitamura K, Ohnishi K, Yanagi M, Hiraoka A, Karasuno T, Takeuchi M, Miyawaki S, Ueda R, Naoe T, and Ohno R: Treatment With a New Synthetic Retinoid, Am80, of Acute Promyelocytic Leukemia Relapsed From Complete Remission Induced by All-trans Retinoic Acid. Blood 1997 90: 967-973.

44) Thomas X, Dombret H, Cordonnier C, Pigneux A, Gardin C, Guerci A, Vekhoff A, Sadoun A, Stamatoullas A, Fegueux N, Maloisel F, Cahn JY, Reman O, Gratecos N, Berthou C, Huguet F, Kotoucek P, Travade P, Buzyn A, de Revel T, Vilque JP, Naccache P, Chomienne C, Degos L, Fenaux P, for the APL Study Group: Treatment of relapsing acute promyelocytic leukemia by all-trans retinoic acid therapy followed by timed sequential chemotherapy and stem cell transplantation. Leukemia 2000;14:1006-1013.

45) Unnikrishnan D, Dutcher JP, Varsnhneya N, Lucariello R, Api M, Garl S, Wiernik HP, Chiaramida S: Torsades de pointes in 3 patients with leukemia treated with arsenic trioxide. Blood 2001;97:1514-1516.

46) Wang G, Li W, Cui J, et al.: An efficient therapeutic approach to patients with acute promyelocytic leukemia using a combination of arsenic trioxide with low-dose all-trans retinoic acid. Hematol Oncol 2004;22:63–71.