SCIENTIFIC DISCUSSION

This module reflects the initial scientific discussion and scientific discussion on procedures, which have been finalised before 30 November 2004. For scientific information on procedures after this date please refer to module 8B.

1. Introduction

Voisin Consulting S.A.R.L. The applicant has applied in February 2001 via the Central Procedure for marketing authorisation of Trisenox for the treatment of relapsed acute promyelocytic leukemia in adult patients, characterised by the presence of the t(15;17) translocation and/or the presence of the PML/RAR-α gene.

Acute promyelocytic leukemia

Acute promyelocytic leukemia (APL) is a rare disease, accounting for 10-15% of all the acute myelogenous leukemia in adults.

APL is characterised by the accumulation of clonal hemopoietic precursors blocked at specific stages of development. APL is a rare disease with an incidence of 1,000 to 1,500 newly diagnosed patients a year in the United States and 700 to 800 in the European Union.

APL is classified under the French-American-British (FAB) morphological scheme as subtype M3 of acute myeloid leukemia (AML). The bone marrow morphology is characterised by greater than 30% blasts and abnormal promyelocytes; multiple Auer bodies, sometimes in bundles, heavy granulation obscuring the basophilic cytoplasm, and strong positive cytochemistry.

The disease involves a typical balanced translocation involving chromosomes 15 and 17 (t15:17) although exceedingly rare variants of this leukemia show balanced translocations of chromosomes 11/17 and 5/17. As a result of these translocations APL blasts invariably synthesize aberrant fusion forms of the retinoic receptor type alpha, PML-RARα in the case of t15:17, PLZF-RARα and NPM-RARα in the case of t11:17 and t5:17, respectively. In addition the breakpoint of the t15:17 chromosomal translocation is heterogeneous, leading to at least three molecular types of PML-RARα fusion proteins. The PML/RARα fusion protein is considered to have an important role in APL pathogenesis by causing a maturation block at the promyelocyte stage of myeloid differentiation.

These molecular defects allow the classification of APL patients in two categories: all-trans-retinoic acid (ATRA)-sensitive and ATRA-resistant. Patients with the t15:17 and t5:17 translocations are ATRA-sensitive, and those with the t11:17 translocation are resistant.

Among other actions, this mutant protein disaggregates PML Oncogenic Domains (PODs), which are spherical nuclear bodies that are attached to nuclear matrix. This disorganisation of the PODs is also thought to play a crucial role in APL pathogenesis by causing inhibition of apoptosis mechanisms. The t(15;17) translocation may be evidenced with reverse transcriptase-polymerase chain reaction (RT-PCR) using specific PML and RARα oligonucleotides. Depending of the RT-PCR technique used, its sensitivity level may vary between $1/10^4$ and $1/10^6$ cells.

Hence the hallmark of APL is the presence of abnormal promyelocytes either circulating in the peripheral blood or present in bone marrow. Because of the presence of these differentiated leukaemic promyelocytes, APL is biologically distinct from other types of AML and is associated with coagulation abnormalities including disseminated intravascular coagulation (DIC), plasmin-dependent fibrinogenolysis, and diffuse proteolysis. Coagulopathy may be responsible for massive fatal haemorrhages potentially exacerbated by chemotherapy, adding to the mortality associated with induction therapy especially in patients with high white blood cell count (WBC) at diagnosis.

Treatment options

The treatment of newly diagnosed APL patients consists of two phases: Induction phase to obtain remission (defined by bone marrow clearance), then a consolidation phase. This initial treatment is currently followed by a maintenance treatment.

All-trans retinoic acid (ATRA) is considered as the APL standard treatment. The current therapeutic approach is ATRA in combination with several cycles of anthracycline-based chemotherapy. This combination has significantly improved results compared to conventional chemotherapy alone.

With first line treatment combining ATRA and anthracycline based chemotherapy (CT), about 90% of the patients achieve complete response (CR). Molecular complete remission rate is 15 to 20 %, and

depends on the time-schedule of the RT-PCR analysis and its sensitivity level, and about 30 % subsequently relapse. ATRA can also be used at several phases of APL treatment: as the maintenance of treatment, ATRA possibly combined to chemotherapy can further reduce the risk of relapse to about 15 %.
Most of APL patients who have a first relapse after treatment with CT alone can achieve a second CR with ATRA. Patients who have relapsed after first line treatment including ATRA can achieve a second CR with ATRA; the response rate depends on the interval between the last administration of ATRA and the time of relapse. Results appear to be better when the interval is longer, but are difficult to evaluate precisely as CT is often rapidly added to ATRA in such circumstances. Salvage therapy with ATRA alone (as reported from MSK historical series) offers results inferior to those of CT. When CT is added to ATRA, toxicity is increased with up to 10 % toxic death in large published series.
With various chemotherapy regimens, patients can achieve CR (55 to 86%) according to the number of previous relapse(s), but toxicity is very high, and intensive chemotherapy cannot be administered in all cases.
So, in patients with APL relapse, there is a clear need for treatment that would allow to achieve CR with tolerable toxicity, or without the toxicity of concomitant CT.

Arsenic trioxide
Although As_2O_3 is a well-known poison, it has been in medical use for a long time. In 1865, arsenic compounds, in the form of potassium arsenic, were already described for the treatment of chronic myelogenous leukaemia. Because of its chronic toxicity, this treatment was replaced by the chemotherapeutic agent busulfan in the middle of the 20^{th} century. After a large scale clinical screening, therapeutic effects were identified in some human cancers such as leukaemia, oesophageal carcinoma, and lymphoma. The mechanisms of action underlying the apoptogenic and cyto-differentiating action of Trisenox are still largely unknown.
Trisenox contains arsenic trioxyde as active substance. The rationale for the development of arsenic trioxide (ATO) is based on both pharmacology data and previous human experience (with another ATO compound) conducted in China. ATO seems to have concentration-dependent dual effects on leukaemic cells: induction of apoptosis at relatively high concentrations (0.5 to 2.0 μM/L) and partial differentiation at lower concentrations (0.1 to 0.5 μM/l). The reported results in this series of APL patients that have relapsed after ATRA-anthracycline/cytarabine combination treatment suggested a high rate of CR, comparable to those obtained with chemotherapy.
The clinical development program was designed to evaluate the safety and efficacy of ATO for the re-induction of remission and for consolidation in APL patients who were refractory to, or had relapsed from, retinoid and anthracycline chemotherapy, or for whom anthracycline-based chemotherapy was contraindicated.

2. Chemical, pharmaceutical and biological aspects

TRISENOX 1 mg/ml, concentrate for solution for infusion, is presented as a sterile, clear, colourless aqueous solution contained in a 10 ml clear sealed type borosilicate glass ampoule. The product is terminally sterilised.
The manufacturing process of the product involves compounding filtration, filling and terminal sterilisation.

Composition
The finished product is presented as sterile pyrogen-free solution for injection. Apart from the active ingredient ATO, the other excipients are water for injections, adjusted with sodium hydroxide and hydrochloric acid to a pH of 8. The solution is contained in a Type I borosilicate glass ampoule.

Active substance
Arsenic trioxide As_2O_3 is a non-stoichiometric solid, prepared by roasting arsenic-containing ores and is purified by a re-sublimation process. The 'cubic crystal form' is used. It is sparingly soluble in water although the solubility increases at pH 12.0. On dissolution in water it undergoes hydrolysis to arsenious acid (pKa_1=9.2), one representation of which may be

$$As_2O_3 + 3H_20 \rightarrow 2\ As(OH)_3.$$

However, depending on pH, temperature, ionic strength etc., a number of arsenic-containing species could also be present in equilibrium in solution, possibly as the sodium salts under the slightly alkaline conditions of the finished product. Therefore, the precise definition of the active substance or substances, which are present in solution in the product, would be difficult both qualitatively and especially quantitatively. 'Arsenic trioxide' may be regarded as the 'active substance' by conventional agreement in this particular case, although it is accepted that there may be other arsenic-containing species which are eventually present in solution in the finished product and which may be ultimately responsible for the pharmacological activity in the proposed therapeutic indication.

Specification

The active substance specification for arsenic trioxide As_2O_3 is relevant for a substance to be used in parenteral products and comprises tests for physical appearance, identity, As (III) assay clarity of solution, residue on ignition, content of As (V), related substances (antimony, lead, etc.) particle size, loss on drying, microbial bioburden and endotoxin.

Batch analyses confirm satisfactory uniformity and compliance with the agreed, justified specification.

Stability

Three batches of active substance have been studied under normal long-term test conditions (25 ± 2°C/60 ± 5% RH, 100 ml glass bottles with polypropylene closures) and accelerated conditions (40 ± 2°C/75 ± 5% RH). Characteristics studied were physical appearance, assay, As (V) content, loss on drying and, particle size distribution. (Bioburden and endotoxins were checked annually). A photostability study was conducted on one sample with the conclusion that the active component is not light sensitive. The claim for retesting after one year when stored in tightly closed glass bottles between 15 and 30°C is sufficiently justified.

Other ingredients

The other ingredients of the formulation, hydrochloric acid, sodium hydroxide, water for injections all complies with PhEur monographs. There are no ingredients of animal origin which give rise to concerns related to TSE.

Product development and finished product

The choice of dosage form and formulation of the product was influenced by the unknown oral bioavailability of As_2O_3, the data reported in the clinical literature, and the limited solubility of As_2O_3 in water.

The manufacturing process itself is a simple dissolution/mixing and sterilisation procedure. The filled ampoules are terminally sterilised in a steam autoclave, subjected to a dye immersion test, visually inspected, labelled, packaged and placed in quarantine.

The in-process controls, including all the test limits, are all satisfactory.

Six lots, three of 50 litres and three of 300 litres, have been used for validation. Biological indicators are included in each sterilisation cycle. The cycle, including autoclave loading patterns, has been validated. The validation of the manufacturing process appears to have been thorough and the details provided indicate that the process is under control.

Product Specification

The finished product specification at release comprises tests for physical appearance (clear, colourless solution), As (III) identification and assay, As(V) assay, particulate matter, volume in container, sterility and endotoxins.

The development of analytical methods applied to the finished product is fully described; reference is made, when possible, to PhEur methods. Full details have been provided for eight batches of finished product. They all complied with the specifications.

Stability of the Product

Five lots of finished product have been placed on stability. Stability trials were done in the commercial primary packaging in accordance with ICH testing conditions for narmal long test and accelerated conditions.

Parameters tested were product appearance, As (III) and As (V) assay, pH, container appearance and particulate matter (visual); particulate matter (NIAC), sterility and endotoxins are tested yearly. Specifications and methods are identical to those used for release.

Results on assay of As (V) show that the amount of this impurity remains constant at levels which do not raise a toxicological concern in the context of use of the product.
A photostability study was conducted in the commercial ampoule and in an ampoule with a secondary carton package. The finished product is not light sensitive. A freeze-thaw study indicated that the finished product is not adversely affected by multiple cycles of freezing and thawing.
In general, no significant changes were observed in product quality during storage, and the shelf life as defined in the SPC is justified by the accumulated stability results. No specific labelling requirements are needed with regard to temperature.

In-use stability of the finished product
The stability and compatibility of the finished product relevant to the context of clinical use has been studied in 5% dextrose, 0.9% sodium chloride and Ringer's lactate solutions for infusion in their commercial containers. The parameters tested over 72 hours at room temperature (~25°C) and in a refrigerator (~4°C) were appearance, assay of As(III), As(V), UV spectroscopy and particulate matter. Adequate methods (anion-exchange HPLC and atomic absorption spectroscopy) were developed and validated to quantify As(III) and total arsenic. On the basis of the results, an in-use shelf-life of 24 hours (aseptic preparation and administration) and 48 hours under refrigeration are proposed and this is reflected in the SPC.

Discussion on chemical, pharmaceutical and biological aspects
It sometimes happens that the molecular species responsible for the pharmacological activity of a product is not the same as the active substance stated to be present in the product (this may follow from biotransformation *in vivo* for example). However, it is desirable to define the strength of the product as far as possible in terms of those substances that are actually present in the product, and which lead to its pharmacological activity. In this case, the precise qualitative and quantitative definition of those arsenic species actually present in solution in the finished product has been a matter of debate. The chemistry of arsenic compounds is not simple. However, it has been accepted by conventional agreement in this case that 'arsenic trioxide' may be regarded as the 'active substance' in the product, although as above, it is accepted that there may well be other species present in solution which may be responsible for the activity in the clinical indication.
The purity of ATO and the control of the manufacturing process and finished product indicate reliable reproducibility of the product and indicate in turn a reliable performance in the clinic.

3. Toxico-pharmacological aspects

The majority of preclinical data on toxicity and pharmacokinetics comes from published scientific and medical information, from formal reviews by official agencies (WHO and US EPA) as part of their evaluation of the risk to man of arsenical contamination of water and air. Whether these published works comply with GLP cannot be ascertained. However, the consistency of the results, the fact that they were obtained from peer-reviewed scientific publications, and that reviews by eminent experts have reached similar conclusions indicates that the available knowledge about the mechanisms of action of arsenical compounds, the doses or concentrations required and the toxic effects produced can be accepted with confidence.

Pharmacodynamics
Based on *in vitro* studies in cellular models of APL (the immortalized cell lines NB4 and UF-1), ATO has strong apoptogenic and anti-proliferative effects on APL cells. It does not trigger apoptosis in the PML-RARα-negative cell lines, possibly implying that the oncogene PML-RARα (the product of the t15:17 chromosomal translocation) may be a primary target for the activity of ATO and its degradation a first important step in the process of apoptosis.
In APL cells, the expression of PML-RARα results in delocalisation of the normal PML protein from nuclear bodies (NB) to other areas of the nucleus. Treatment of APL blasts with ATRA results in progressive degradation of PML-RARα and relocalisation of the PML protein into NBs. A similar effect is observed after treatment of APL cells with ATO. The apoptogenic and cyto-differentiating effects of ATO in the ATRA-resistant MR2 cell line indicate that the compound acts on APL cells regardless of their sensitivity to retinoids. ATO-induced apoptosis is not due to chelation of thiol

groups in proteins. Indeed, while ATO causes apoptosis in ATRA-sensitive NB4 cells, phenylarsine oxide reduces the intracellular concentration of NADH, but does not induce programmed cell death.
A cellular environment with low levels of gluthathione peroxidase (GPX) seems to be favourable for the apoptogenic activity of ATO. The GPX inhibitor mercaptosuccinic acid sensitizes cells to the apoptogenic action of ATO, while raising GPX by pre-treatment with selenite makes NB4 cells relatively resistant to the arsenical. Regardless of the upstream molecular determinants, ATO causes programmed cell death through a mitochondrial pathway, which involves cytochrome-c release from the mitochondria into the cytosol, activation of terminal caspases (such as caspase-3 and caspase-7), nuclear fragmentation, and DNA degradation.
ATO activity is not limited to APL and extends to other types of leukemia and solid tumors. *In vitro*, it inhibits, the growth of a panel of megakaryocytic leukemia cells and induces apoptosis. The PML-RAR-alpha negative HL-60 myeloblastic cell line shows much lower sensitivity to ATO. ATO and melarsoprol (an organic arsenical) cause apoptosis in a panel of human myeloma cells. Exposure times between 24 and 48 hours are necessary to inhibit the growth and induce apoptosis of target cells.
The cytotoxic activity of ATO has been tested on the NIH panel of neoplastic cell lines (60 cell lines representing different types of leukemia and solid cancer). LOX IMVI (melanoma), HOP-92 and NCI-H226 (lung carcinoma) and CAKI-1 (renal carcinoma) were the most sensitive lines.
Recent in vivo and in vitro studies have described the effects of ATO on neuroblastoma tumors:

- ATO (1.5 µM to 5µM) was able to induce cell death of different cultured human NB cell lines (IMR-32, LAN-1, LAN-2, SK-N-BE, SH-SY5Y, and SMS-MSN).
- ATO was also able to repress in vivo growth of xenotransplanted human NB cells: nude mice were injected with SK-N-BE cells and received ATO (200 to 400 µg). These mice showed a dose dependent inhibition of tumor growth.

Interferon alpha (IFN) interacts synergistically with ATO and causes cytotoxicity in adult T-cell leukemia/lymphoma (ATL) cells.
There is very little need to support the anti-leukemic activity of ATO in pre-clinical *in vivo* animal models of APL since convincing data in humans is already available. However, animal models are valuable insofar as they may give information about the *in vivo* mechanisms of action underlying the anti-leukemic activity of the arsenical. A single *in vivo* study using grafts of leukemic cells from PML-RARα transgenic animals found an approximately two-fold increase in the survival of animals after i.p. injection of the arsenical. This effect is of the same order of magnitude as after treatment with ATRA. Interestingly, co-administration of ATO and ATRA results in a strong synergistic effect in terms of survival, suggesting a complementary pharmacological action.
ATO (1-2 µM) is effective in vitro against various human myeloid leukaemia cells known to be resistant to a number of apoptotic stimuli, inducing pre-apoptotic mitochondrial events, cytosolic cytochrome c accumulation, caspase activities, and hyperacetylation of histones. ATO mediates also anti-myeloma activity both directly on tumor cells and indirectly to overcome myeloma growth and survival factors in the bone marrow microenvironment.
Another study demonstrated that ATO can induce apoptosis of clinically acquired RA-resistant APL cells in vitro and in vivo.

Pharmacokinetics
Most of the preclinical data about the pharmacokinetics of ATO in animals come from published information on inorganic forms of arsenic. The justification for this unusual application is that it was considered unethical and unnecessary to undertake extensive animal studies just to obtain information on what was already known for humans.
Inorganic arsenic compounds show good oral absorption (90% of a single oral dose), which aids extrapolation from the results of oral to parenteral studies. Arsenic distributes rapidly to red cells and to tissues, is retained in skin and hair longer than other tissues, but does not markedly accumulate in any organs or tissues of any species. The pattern of tissue distribution in the mouse is similar to that in humans, who show the highest levels in the kidney and spleen.
The distribution of arsenic in the rat is quite different from other animal species. In rat, a large majority of the arsenic becomes bound to haemoglobin in red blood cells, and very little reaches other tissues. For this reason, the rat is probably not an appropriate toxicokinetic model for distribution, metabolism, or excretion of arsenic by humans.
In mice the highest concentrations of arsenic a few hours after I.V. arsenite were in the liver, kidneys, bile, lung, spleen, epididymis, and gastrointestinal tract. At 72 hours post-dose, the highest

concentrations were in the skin, hair, epididymis, liver, and stomach. At 30 days, the maximum concentrations were in skin, hair, and the lens of the eye.
In pregnant mice, rats, hamsters, and primates, inorganic arsenicals cross the placental barrier when given orally or by injection, and arsenic is generally distributed in the late-term fetus in a manner similar to the adult animal.
The metabolism of arsenic has been extensively studied in humans and animals. There is metabolism to mono- and dimethyl derivatives, and some generation of dimethyl arsine by bacterial action in the colon. The effect of hepatic damage has not been investigated. Trivalent inorganic arsenic is metabolised by methylation to methylarsonic acid (MAA) and rapidly to dimethlyarsinic acid (DMAA). The metabolic detoxification requires hepatic methylases that are not involved in conventional pathways of drug metabolism and clearance.
Since the methyl derivatives of arsenic appear to be less toxic than inorganic arsenic, and since methylation tends to result in lower tissue retention of inorganic arsenic, the methylation process is usually viewed as a detoxification mechanism. Because methylation is an enzymatic process, an important issue is the dose of arsenic that saturates the methylation capacity of an organism. Limited data from studies in humans suggest that methylation may begin to become limiting at doses of about 0.2-1 mg/day. However, with these few observations, the dose rate at which methylation capacity becomes saturated cannot be precisely defined.
Elimination of arsenic following parenteral exposure of animals is similar to that seen following oral exposure. The major pathway of elimination in all species was renal, fecal excretion accounting for <10%. Excretion in urine was initially quite rapid in most species ($t_{1/2}$ 2 - 6 hours, followed by a slower $t_{1/2}$ of 15 days). In rabbits and mice, urinary excretion within eight hours usually accounts for about 50-80 % of the dose. Somewhat lower levels are excreted in the urine of marmoset monkeys probably because of the absence of methylation in these species. Whole-body clearance studies in mice indicate that the clearance of arsenate is lower (65 % removed within 24 hours) than that of arsenite is about (86% removed at 24 hours). Inorganic arsenic is initially excreted in urine in trivalent and pentavalent forms, and the organic methylated metabolite DMAA is the most prevalent form during the slower phase.

Toxicology

Single-dose toxicity
A single-dose toxicity study of ATO has not been carried out. However, a review of the Registry of Toxic Effects Chemical Substances (RTECS) shows that ATO has been extensively evaluated in acute toxicity studies. There is a remarkable consistency among various species, regardless of the administration route, in both lowest lethal dose and LD50 values for ATO. The LD50 (mg/kg) in mice were 10.7 i.v., 9.8-12.3 s.c., 11.0-11.8 i.p. and 39.4 p.o.

Repeated dose toxicity
Repeated toxicity studies were performed in four animal species commonly used in chronic toxicity studies. The low oral doses ranged between 0.1 and 5 mg/kg/day, and the i.p. doses were between 1.3 and 2 mg/kg/day. All results indicate that the low doses cause no important toxicity. The principal toxic actions of chronically administered arsenics are lesions of the skin, damage to the liver and kidneys, peripheral neuropathy, and some depression of hematopoiesis. In rodents, the NOEL was about 1-1.7 mg/kg/day for up to two years, and in man it is probably about 0.5-1 mg/kg/day.
The systemic toxic effects of arsenic exposure in animals were similar to those in humans. NOEL values, determined from subchronic exposure in mice (0.8 mg/kg/day), chronic exposure in rats (1.7 mg/kg/day), and dogs (0.3 mg/kg/day; 1.25 mg/kg/day), and the minimal toxicities in adolescent monkeys receiving 3.75 mg/kg/day, provide a safety factor of approximately 2 or more for i.v. administration of 0.15 mg/kg/day ATO in humans.

Reproductive toxicology
Reproductive toxicology studies were not conducted by the Applicant and are normally not required for anticancer agents such as ATO. However, inorganic arsenicals are teratogenic and cause maternal and fetal toxicity in the mouse and rat but not in the rabbit. They do not affect female fertility; male fertility has not been formally tested. The NOEL for developmental effects is 1-5 mg/kg/day in rodents. Postnatal development of the mouse was affected by prenatal exposure to 2 mg/kg/day.

Genotoxicity

Arsenite and ATO can be mutagenic in mammalian cells in culture. The mechanism of genotoxicity is not clear, as the conventional Ames' test has usually given a negative result. The effects indicate chromosomal damage and deletions but not simple point mutations.

In vivo, a significant dose-related increase in micronuclei was observed in the mouse bone marrow micronucleus test up to 5 mg/kg i.p., but no effect in a male dominant lethal test, indicating that there is no heritable risk. The compound seems to cause DNA damage by several mechanisms and affects DNA repair mechanisms in mammalian cells.

Carcinogenicity

Carcinogenicity studies were not conducted by the Applicant and are normally not required for anticancer agents such as ATO. ATO is a proven human carcinogen under certain circumstances of chronic exposure. There may be a threshold dose below which it does not have that action.

Local tolerance

The local tolerance to intravenous injections of ATO was studied in a repeated dose sub-acute study in dogs. There were no clinical observations of inflammation at the injection site. No necrosis or inflammatory cells were observed in the skin surrounding the injection site on histopathological analysis of the area around the injection site.

Environmental risk assessment/Ecotoxicity

Considering of the administered doses of Trisenox and the rarity of the disease to be treated, its medical use should not add appreciably to the environmental burden of arsenic. There is no reason to expect any risk to the community, to people in close proximity to patients or to other biota in the environment.

Discussion on pre-clinical and toxicological aspects

The applicant has presented *in vitro* and *in vivo* data demonstrating that ATO has anti-leukaemic activity in appropriate experimental models of APL. The one *in vivo* study indicates that ATO has a selective effect on leukaemic cells and does not seem to cause overt toxicity in animals. This results in a significant increase in the lifespan of ATO-treated animals compared to vehicle-treated animals. It would be important to strengthen these data in other in vivo models of APL.

The mechanisms of action underlying the apoptogenic and cyto-differentiating action of ATO are still largely unknown. It seems that the pharmacological activity is not related to its ability to interfere with intracellular sulfhydryl groups, and this indicates dissociation between the molecular mechanisms of the anti-leukaemic and toxic action of arsenicals. Furthermore, the anti-leukaemic activity of ATO is different from that of organic arsenicals, such as melarsoprol. As to the molecular determinants of the drug's apoptogenic activity, some results indicate a possible mediation by oxygen peroxides, which may damage mitochondria and lead to the activation of a classical apoptotic program. Whether the anti-leukaemic action of ATO is specific for APL and involves targeting upon PML-RAR-alpha remains to be clarified.

No formal safety pharmacology investigation has been carried out. Toxic effects of arsenic inhalation or administration are also well known. Levels of significant exposure for each route of arsenic exposure are available in a broad range of already published reviews and articles. An analysis of the toxic effects of arsenic is complicated by the fact that arsenic can exist in several different valence states and many different inorganic and organic compounds. However, even if data on arsenic inhalation or oral exposure are required to fully understand the toxic potential of arsenic, those effects are not totally the same after i.v. administration.

The absence of experimental safety study does not permit to evaluate all the general effects that may be potentially induced by the i.v. administration of ATO. However, a various number of published clinical data are focused on this subject, in particular on cardiovascular, respiratory or renal disorders.

In conclusion, Arsenic is a well-known and described product. Simultaneous exposure to arsenic and other genotoxic compounds may increase the DNA damage and the carcinogenic risk. This calls for caution in clinical application of sodium arsenite.

4. Clinical aspects

The application included clinical data from two main clinical trials (PLRXAS01 and 97-66) and trial extensions (PLRXAS02 and 98-13). A total of 52 patients with APL entered the treatment phase of the studies (Table 1). Data from two supportive studies with a total of 58 patients with advanced haematological (n=24 with 3 patients already recruited in the main studies) or non-haematological malignancies (n=34) were also provided. The safety population consists of 107 patients from the main studies and 141 patients enrolled in compassionate use studies.

Table 1: Clinical Studies

Trial (extension)	Population Design	N (extension)
97-66 (98-13)	Phase I/II: Relapsed or refractory APL.	12 (7)
PLRXAS01 (PLRXAS02)	Phase II (multicentre, non-randomised): Relapsed or refractory APL.	40 (18)
98-23	Phase I/II (dose ranging , PK): Advanced haematologic malignancies	24
98-46	Phase I (dose ranging, PK): Advanced solid tumours	34

Clinical pharmacology

Pharmacodynamics

Data from a pharmacodynamic study on APL patients treated with ATO were provided. The study was conducted since arsenic is known to increase the QT electrocardiographic (ECG) interval, which may lead to a very serious, sometimes even fatal, type of arrhythmia. The study focussed on the ECG alterations induced by the arsenical in patients treated with a typical cycle. Data were available for a total of 99 patients included in the clinical trials. The results in terms of anti-tumour activity of ATO are provided in the Clinical Efficacy section of this report.

A total of about 1000 ECG were taken at the beginning of the treatment (baseline ECG), during the induction phase and during the consolidation phase. The following primary parameters were taken into consideration: QTc interval at baseline, maximum QTc interval during the infusion, maximum QTc interval during the steady-state phase of infusion, difference from baseline in QTc interval, maximum change in QTc interval from baseline observed during the steady-state phase of infusion and difference from baseline in the heart rate. QTc intervals were stratified as normal, borderline or prolonged.

The results were analyzed as a whole or stratified according to sex and fertility status, as well as for age distribution.

After the first course of ATO, there was a statistically significant QTc prolongation in both males and females. The steady-state QTc prolongation is longer in men than women and steady-state values are attained sooner - as indicated by the half-life - in women than men. QTc interval prolongation was comparable for both sexes during the second course of arsenic therapy. These discrepancies are currently unexplained. No significant differences were observed in any of the parameters taken into consideration for the various age-groups. Surprisingly, the QT prolongation appeared to decline with increasing dosage, although this effect was not statistically significant. In evaluating these data, it must also be noted that the administered dose is just a rough proxy for ATO plasma levels, a more important parameter that was not determined in this study.

After treatment with ATO, heart rate increases from baseline level. The increase was not dependent on gender or dosage. A second course of ATO only influenced the heart rate increase in females in whom a second course of ATO was associated with a lower elevation of heart rate than during the first course.

Pharmacokinetics

Pharmacokinetics of arsenic was investigated in relapsed or refractory APL patients (study 97-66) as well as in two studies conducted in patients with advanced haematological or non-haematological malignancies.

Total elemental arsenic concentrations including inorganic arsenic, its methylated metabolites, and other forms of organic arsenic were determined in both plasma and red blood cells (RBCs) blood fractions, with a lower limit of quantification at 5 ng/ml (0.07 μM). In addition urine samples were collected at Day 1, at Day 2 and Day 3, and at the first day of subsequent weeks during induction. The lower limit of quantification was 2 ng/ml.
The 12 patients with APL enrolled in the 97-66 study were included in this pharmacological evaluation (Table 2). One paediatric patient received 5 mg/dose (0.17 mg/kg/dose), 6 patients including one paediatric patient received 10 mg/dose (0.06 to 0.18 mg/kg/dose), 1 patient received 15 mg/dose (0.16 mg/kg/dose), and 2 patients received 0.15 mg/kg/dose (14.3 and 15 mg/dose, respectively). ATO was administered over 2-hour, 3-hour, or 4-hour time periods.

Table 2: Study 97-66: Plasma Pharmacokinetic Parameters after a 4 hr I.V. Infusion (day 1)

	dose					
Age	mg	Mg/kg	t_{max} (hr)	C_{max} (ng/ml)	$T_{1/2}$ (hr)	AUC_{0-24hr}
36	10	0.18	4	26	19	473
45	10	0.12	4	19	175	381
30	10	0.18	2	31	27	434
62	10	0.10	2	22	149	495
25	10	0.06	4	20	NA	430
75	15	0.20	1	48	39	691
40	15	0.16	1	26	78	413
13	10	0.18	2	18	197	337
9	5	0.17	8	21	133	457
70	15	0.16	4	40	196	631
28	14.3	0.15	2	28	70	411
23	15	0.15	4	30	18	250
		Mean±SD	3.2±1.9	27.4±9	100±72	450±119

In the nine APL patients treated with 0.12 to 0.18 mg/kg/dose, the maximal concentration (C_{max}) after a 4-hour i.v. infusion was comprised between 18 and 40 ng/ml (0.24-0.53 μM) in the plasma fraction and between 16 and 35 ng/ml (0.21-0.47 μM) in the RBCs fraction. The time at maximal concentration (t_{max}) was 1 to 4 hours and the terminal half-life ($t_{1/2}$) was comprise between 19 and 197 hours in the plasma fraction and between 6 and 98 hours in the RBCs fraction.
There was a progressive increase in plasma arsenic concentration with daily dosing. Concentrations after one week of treatment were approximately 2-5 times the C_{max} on the first day of dosing. They reached a steady state comprised between approximately 50 and 125 ng/ml (0.67-1.7 μM/l) during the second week of treatment and thus might be considered as a good parameter for safety monitoring and overdose diagnosis.
One patient treated at 15 mg/dose (0.16 mg/kg/dose) presented a continuously progressive increase until approximately 200 ng/ml (2.7 μM) after one month of dosing. This patient required haemodialysis for the management of renal insufficiency from Day 14 of ATO administration.
Inorganic arsenic (trivalent forms) is metabolised by methylation to methylarsonic acid (MAA) and to dimethylarsinic acid (DMAA, also known as cacodylic acid). The main site of methylation *in vivo* is the liver, but it also occurs in the kidney and blood. Pentavalent arsenic does not undergo methylation; it is therefore eliminated by excretion in urine as unchanged compound or by transformation to trivalent arsenic.
Complete urine samples were collected over a 24-hour period from seven patients with cancer following daily doses of ATO: The amount of total arsenic detected in urine during a 24-hour period following the first dose approximated 10% of the administered dose.
The amount of arsenic recovered in urine increased with repeated daily dosing. With a urinary excretion half-life for arsenic of 3 to 5 days, it is estimated that 18-30 days would be required to recover 100% of a single dose. When urine samples were collected on days beyond when plasma concentrations appeared to have achieved steady-state (8 to 10 days), the average peak quantity of arsenic excreted in 24 hours was 60% of the administered daily dose. Based on PK and clinical observations, a 2-week or 3-week free interval has to be managed between two consecutive ATO cycles.
In the hematological cancer patients (Study 98-23) after 1 to 4 h i.v. infusion of doses from 0.1 to 0.3 mg/kg, the t_{max} of total arsenic in plasma and RBC ranged from 1 to 4 h and the C_{max} from 11 to 53

ng/mL, with a slightly wider range in RBC (10 to 74 ng/mL). The average C_{max} and AUC_{0-8h} values for plasma and RBC showed a dose-response relationship.
In patients with solid tumours (Study 98-46) treated with one daily 2-h i.v. Trisenox infusion at doses of 0.5 to 0.35 mg/kg, total arsenic measured on the first day of dosing peaked at or near the end of the infusion and then slowly declined. Average plasma and RBC C_{max} and AUC_{0-8h} for Regimen A (blood collections before, during and after the first infusion on day 1, and at the beginning and end of the day 2 infusion in the first cycle) essentially increased with the dose, suggesting a dose-concentration relationship, while for Regimen B (blood collections over 96 h during and after a single infusion) mean plasma and RBC arsenic concentrations increased over the course of the treatment, achieving a steady state on day 10.
Data on the tissue distribution of arsenic come from suicide victims; the highest concentrations were found in the liver and kidneys, with uniformly lower concentrations in muscle, heart, spleen, pancreas, and lungs. Arsenic is known to accumulate in skin, hair, nails, and brain and binds to blood and tissue proteins.
Inorganic arsenic species cross the placenta when given to pregnant rodents and non-human primates. ATO is excreted in human milk.
Interactions between ATO and oxidatively biotransformed drugs are unlikely because the methyltransferases involved in arsenic metabolism are not members of the cytochrome P-450 family. ATO did not cause concentration-dependent inhibition of any of the enzymes examined (CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP 3A4/5 and CYP4A9/11) and would therefore not be expected to inhibit the clearance of drugs that are metabolised primarily by these enzymes, at least at concentrations lower than 15,000 ng/ml.

Discussion on clinical Pharmacology
Arsenic is known to increase the QT electrocardiographic interval, which may eventually lead to a very serious, and sometimes fatal, type of arrhythmia. The results presented are in line with what is known from the literature on the cardiotoxicity of arsenic. Some uncertainties remain as to the reasons why the sex differences in QTc interval prolongation are observed only during the first course of treatment with ATO. Also, the inverse relationship between dose administered and QTc interval prolongation is unexplained. Correlative studies involving measurements of ATO plasma levels and QTc interval prolongation remain of interest.
The clinical pharmacokinetics of intravenous ATO has been evaluated in patients with relapsed or refractory APL (study 97-66) and patients with advanced hematological (study 98-23-PK) and non-hematological malignancies (study 98-46-PK). In no population was the pharmacokinetics of trivalent arsenic, the active species of Trisenox, adequately characterized because the analytical method did not distinguish between the different forms of arsenic. Additionally, no formal pharmacokinetic analysis was planned and only descriptive statistics have been provided. Nevertheless on the first day of dosing there appeared to be some dose-relation for mean exposure to the drug. Exposure to the drug increases with daily dosing, approaching a steady state by approximately two weeks, in terms of 0 and 4h plasma and RBC concentrations. Unfortunately pharmacokinetic parameters were not calculated at steady state. So it is not possible to ascertain whether the pharmacokinetic behaviour of ATO is linear after repeated dosing.
No pharmacokinetic studies have formally investigated elderly patients and patients with hepatic or renal impairment. The SPC cautions about the use of this drug in paediatric patients. The SPC also cautions about the use of Trisenox in patients with hepatic and renal impairment as its safety and effectiveness have not been studied. However, the fact that renal excretion is the main route of elimination of arsenic and the case of progressive drug accumulation in one patient who required haemodialysis for the management of renal insufficiency suggests that a survey on the effect of renal impairment on arsenic disposition would be useful (see Follow-up measures/Specific obligations).

Clinical Efficacy
The main clinical efficacy data submitted consisted of data derived from two non-randomised studies, which recruited a total of 52 patients with APL who had relapsed after retinoid and anthracycline chemotherapy, or for who anthracycline-based chemotherapy was contraindicated.

Main Studies
The two main studies submitted had similar design. Study 97-66 was a non-randomised study titled "Pharmacokinetics of Arsenic in Patients with Relapsed or Refractory Acute Promyelocytic Leukemia". Study PLRXAS01 was a non-randomised study titled "Multicenter Study of ATO in Relapsed or Refractory Acute Promyelocytic Leukemia".

Methods
Selection criteria for the studies included diagnosis of APL by bone marrow morphology during the present relapse (with confirmation by cytogenetics, FISH or RT-PCR after registration of the patient into the trial) and relapse from or resistance to standard antileukaemic therapy. Relapse from or resistance to standard antileukaemic therapy was defined as at least one course of induction chemotherapy using an anthracycline antibiotic and at least one course of induction or maintenance therapy using either all-*trans* retinoic acid or 9-*cis* retinoic acid.
The Phase I/II study 97-66 was conducted at the Memorial Sloan-Kettering Cancer Center and the Phase II PLRXAS01 study was conducted at nine centres in the United States.
There were differences in the dose regimen (reflecting the range of doses explored in the Phase I/II study) aims of one of the two studies. In the Phase I/II study, the dosing consisted of 10mg/dose, as derived from the Shanghai series (Shen Z-X *et al.*, 1997) and was escalated to 15 mg/dose. One paediatric patient received 5 mg/dose. To accommodate paediatric patients, dosing was lastly changed to fixed 0.15-mg/kg/dose regimens.

Induction therapy. The dose of 0.15 mg/kg/day was used for the PLRXAS01 study and ATO was administered until bone marrow remission was observed (see definition of CR) for up to a maximum of 60 doses (but only three patients received more than 49 doses). Review of the available data had suggested that dosing for up to 60 doses might be required to induce bone marrow remission, and that a consolidation course following CR during the initial treatment might prolong remission. Induction treatment was to be discontinued when the patient met the criteria for bone marrow remission, if substantial toxicity, overdose, or disease progression occurred, or after 60 doses had been administered.

Consolidation treatment. Patients who achieved clinical CR were eligible to receive one course of consolidation treatment with ATO, beginning 3 to 4 weeks after completion of the induction therapy. One course of consolidation treatment was defined as ATO at the same dose as administered during induction, on weekdays only, continuous daily, or some combination thereof, until the cumulative total of 25 treatments was reached. All therapy during consolidation was to be completed within 5 weeks (≤35 days). Patients who failed to reach CR after the induction period were not eligible for further treatment on this study.
Patients who remained in CR after the consolidation treatment were eligible to participate to extensions of the corresponding protocols (98-13 and PLRXAS02, respectively). Patients were allowed to receive up to an additional 4 courses of maintenance therapy at the same dose and schedule as they had received for consolidation.
Dose modification and protocol treatment discontinuation
Phase II protocol treatment was to be discontinued if symptoms suggesting potentially serious acute arsenic toxicity appeared (overdose), progressive disease(hyperleukocytosis by itself was not to be considered evidence for disease progression in the absence of abnormal promyelocytes) or significant toxicity. Significant toxicity: Treatment with ATO could be interrupted, adjusted, or discontinued before the scheduled end of therapy at any time that a toxicity Grade 3 or greater on the National Cancer Institute common toxicity criteria, version 1 (NCI-CTC), was observed. Treatment was to be interrupted in the case of significant:

- hepatotoxicity (defined as an increase in serum bilirubin, SGOT or alkaline phosphatase to >5 times baseline values),
- nephrotoxicity (defined as serum creatinine >4 times the upper limit of normal),
- significant neurological impairment (defined as somnolence, seizures, or impaired mentation),
- severe peripheral neuropathy or any non-hematologic Grade 4 toxicity.

Patients who experienced such adverse events classified as probably drug related could resume treatment only after resolution of the toxicity or (recovery to baseline status). In such cases, treatment

was to resume at 50% of the preceding daily dose. If the toxicity did not recur within 3 days of restarting treatment at the reduced dose, the daily dose could be escalated back to 100% of the original dose. Patients who experienced a recurrence of toxicity were to be removed from the study and not retreated. Patients with therapy interrupted for conditions that were not considered probably related to ATO treatment could resume treatment at the full dose at the discretion of the investigator.
At the earliest signs of ATRA-like syndrome, patients were to be treated with a short course of high dose corticosteroids (10 mg of dexamethasone intravenously twice per day for 3 days).
Objectives
The primary objective of study PLRXS01 was to determine the rate and duration of complete remission and the safety profile of ATO for the treatment of patients with APL who had relapsed or become refractory to conventional anti-leukaemic therapy. Secondary objectives were to determine the overall, failure-free and relapse-free survival. The primary objective of study 97-66 was to evaluate the safety and potential efficacy of ATO in patients with relapsed or refractory acute promyelocytic leukemia. A secondary objective was to evaluate the pharmacokinetics of As2O3.
Clinical *Complete Remission* was defined as cellular bone marrow aspirate with <5% blasts (and absence of leukemic cells), peripheral blood leukocyte count ≥3,000/mm^3 or absolute neutrophil count ≥1,500/mm^3, and platelet count ≥100,000/mm^3 . The date of CR was the last of the dates on which these criteria were met. In addition to the conventional criteria for CR described above, bone marrow cells were assessed for t(15:17) by conventional cytogenetics and/or by molecular cytogenetics using RT-PCR for PML/RAR-α or FISH. Disappearance of t(15;17) was considered supportive evidence for CR. Abnormalities confined to reports of cytogenetic or molecular studies, absent these clinical abnormalities, were not considered clinical relapse. Failure-free survival was calculated as the time from first ATO to relapse or death, whichever first; relapse-free survival was calculated as the time from CR to relapse or death, whichever first. Follow-up is planned to continue until at least five years after the beginning of induction therapy.
Evaluations
Evaluations of bone marrow aspirates were to be performed at least once on or before day 28, then weekly until bone marrow CR, before beginning consolidation treatment, between 7 and 21 days after completion of consolidation treatment and then approximately once every 3 months during the first year after achieving CR. Bone marrow morphology, differential count, and conventional cytogenetics were to be performed at the study sites; aliquots of the bone marrow samples were also to be sent for PML/RARα RT-PCR testing, which was conducted in a central laboratory.
In the main clinical studies, adverse events were classified as serious if they were fatal, immediately life-threatening, permanently or significantly disabling or required or prolonged hospitalization. The medical monitor who designated events as serious or not based on criteria stated in the protocols reviewed adverse events. EKGs were obtained at baseline and weekly or twice weekly during treatment as ATO was known to cause prolongation of the QT interval. Physical examinations and vital signs were required at baseline and twice each week during induction therapy with ATO. These were also obtained at the end of treatment and during follow-up. During consolidation treatment, physical examinations and vital signs were measured weekly. Standard clinical laboratory evaluations were performed at baseline, during treatment with ATO and at the final visit.
Sample size
The sample-size of study PLRXAS01 was calculated assuming a 50% CR for ATO and aiming to exclude a CR of 33% or lower, based on a one-sided lower confidence limit (alpha=0.05). All patients should have received at least one dose of ATO to be included in the efficacy analysis. The proportion of CR is reported together with 2-sided exact binomial 95% confidence intervals for the proportion.

Results
Participant flow, recruitment and baseline data
Tables 3-5 summarise the baseline characteristics and flow of participants in the two trials according to the different stages of ATO treatment. All selected patients started protocol treatment and received ATO for at least two weeks, except one patient from the 97-66 study who received ATO only for five days.
Study 97-66 was conducted during the 8 months from October 16, 1997 to June 5, 1998. Twelve patients entered and 11 completed induction therapy; these 11 patients entered and completed consolidation therapy. One patient died due to cerebral haemorrhage on the 6th day of induction. Two additional 97-66 patients died within 30 days after last study treatment (both due to cerebral

haemorrhage after APL recurrence and in one patient in the context of chronic renal failure requiring dialysis). The last follow-up contact for the 6 surviving patients was July 18, 2000.
The Phase II PLRXAS01 study was conducted during the 15 months from April 22, 1998 to July 5, 1999. The last follow-up contact for the 26 surviving patients was on July 28, 2000. Forty patients entered and 34 completed induction therapy; 30 patients entered and 28 completed consolidation therapy. Of the 34 patients from PLRXAS01 eligible for the consolidation phase six did not start it (two received stem cell transplantation), two had refused further treatment for personal reasons (non related adverse events), one had persistent worsening of peripheral neuropathy, and one had disease-related seizure and pulmonary hemorrhage). Conversely, 2/6 patients entered consolidation treatment without having achieved CR during induction. Two PLRXAS01 patients died during induction (one on the 39th day due to GI bleed, hypotension and stroke; one on the 16th day due to cerebral haemorrhage). One additional patient died during consolidation (due to pulmonary *Aspergillus* infection).
Two PLRXAS01 patients eligible for the consolidation phase received Stem Cell Transplant (SCT). Four other patients were eligible for consolidation but did not receive it; two patients for personal reasons, one patients due to persistent worsening of peripheral neuropathy, and one patient due to disease related seizure activity and pulmonary haemorrhage. Two patients entered consolidation treatment without having achieved CR during induction. All patients started protocol treatment and were included in the efficacy analysis.

Table 3. Baseline demographic and clinical characteristics

Demographics	**97-66 N=12**	**PLRXAS01 N=40**	**Total N=52(%)**
Age			
<18	2	5	7 (13%)
18 to 59	7	27	34 (65%)
≥60	3	8	11 (21%)
Gender			
Female	4	24	28 (54%)
Male	8	16	24 (46%)
Weight			
<75 kg	4	13	17 (33%)
75 to 100 kg	6	18	24 (46%)
>100 kg	2	9	11 (21%)
Months since diagnosis			
Mean	26.2	22.7	23.5
Median	21.1	18.1	18.4
Range	11.9-61.1	9.0-53.8	9.0-61.1
Prior BMT			
No	10 (83%)	35 (88%)	45 (87%)
Yes	2 (17%)	5 (13%)	7 (13%)
No. of prior regimens			
1	3	19	22 (42%)
2	3	17	20 (38%)
>2	6	4	10 (20%)

Table 4. Induction and consolidation treatment: Number of patients by treatment course and reason for protocol treatment discontinuation

	Induction Course		Consolidation Course	
Study	**97-66**	**PLRXAS01**	**97-66**	**PLRXAS01**
	n=12	**n=40**		
No. started	12	40	11	30
Completed	11	34	11	28
Early discontinuation	1	6	0	2
Fatal SAE	1	2		1
Non-fatal SAE		2		
Non-serious AE				1
Progressive disease		2		

Table 5. Maintenance treatment: Number of patients by number of maintenance courses completed (extension studies)

No. of courses completed	**98-13 n=7**	**PLRXAS02 n=18**	**Total n=25**
1	-	5	5
2	2	6	8
3	4	-	4
4	1	7	5

Clinical Efficacy Results

The main clinical efficacy results presented are summarised in table 6 for individual studies as well as for the merged data set (N=52). No patient was excluded from the main efficacy analysis.

CR rate: In the phase II study, 34/40 (85%) patients achieved a clinical CR. Overall, 45/52 (87%), APL patients enrolled in the two pivotal studies achieved a clinical CR. The median time to clinical CR was 57 days. At the time of last follow-up (July 28, 2000), 24 of 45 (55%) patients with CR remained free of disease. Sixteen had undergone allogeneic SCT (14 while in remission and 2 after further relapse). Three had undergone autologous SCT while in remission. Thirty-one of 45 (69%) patients with CR were alive at last contact with median follow-up of 18.5 months (range 11.7 to 32.4). The response rate seemed consistent across different patient subgroups although the power to detect any differences was small due to the small sample size and definitive conclusions could not be drawn. Overall, 5/7 paediatric patients (<18 years) and 2/4 (< 12 years) achieved CR. There were only 11 elderly patients and 9/11 (>60 years) and 7/8 (≥65) years achieved CR. CR was observed in 5/7 patients that had relapsed after prior SCT and in 40/45 without prior SCT. No difference was observed in CR rate by time from last ATRA therapy (6 or 12-months cut-points). CR was observed in 20/22 in patients who had received only one prior treatment regimen versus 25/30 in the remaining group.

Forty-five patients were evaluated for conversion to normal cytogenetics: 41 of them actually converted to normal karyotype and became negative for t(15:17). RT-PCR for PML-RARα was used to document CR in the 45 patients with CR: 35 patients in clinical CR (78% of CR patients) were negative for RT-PCR at the sensitivity level of 10^{-4}. The median time to cytogenetic remission was longer than the median time to clinical CR. Seven patients (16%) did not obtain molecular remission, and 3 were not evaluated for PML/RARα (2 patients who were not evaluated did not receive consolidation and all 3 received bone marrow transplants).

Overall survival: At last follow-up, 32 of 52 patients were alive with a median follow-up time of 18.3 months (range 12 to 32). The Kaplan-Meier estimate for 18-month survival was 66% (Table 6). Figure 1 shows the overall survival for the combined pivotal studies. A separate analysis of survival censoring the 19 SCT patients at the time of transplants showed similar results. Overall, 31/45 (69%)

patients with CR were alive at last contact, with median follow-up of 18.5 months (range 11.7 - 32.4). The estimated one-year survival was 57% in patients with prior SCT and 73% in those without prior SCT. The estimated 18-month survival for patients with one prior regimen was 86% compared to 55% and 50% for patients with two and more than two prior regimens.

Relapse-free survival: 21/45 patients who achieved a CR have relapsed or died. Two of these patients relapsed after stem cell transplantation. The 1-year Kaplan-Meier estimate of relapse-free survival was 64%, censoring follow-up at the time of stem cell transplantation (Table 6, Figure 2).

Failure-free survival: At the time of last follow-up, 27/52 patients had either relapsed or died (1-year failure-free survival = 62%).

Table 6 Summary of primary and secondary efficacy results

	97-66 n=12	**PLRXAS01 n=40**	**Total n=52**
No. of patients with CR	11 (92%)	34 (85%)	45 (87%)
95 % CI for CR rate	[62%, 100%]	[70%, 94%]	[74%, 94%]
1-year OS	75%	70%	71%
18-month OS	67%	66%	66%
1-year RFS	55%	71%	64%*
18-month RFS	36%	58%	NA

*censoring at BMT
Abbreviations: OS, overall survival; RFS, relapse-free survival; NA not available.

Figure 1. Overall survival

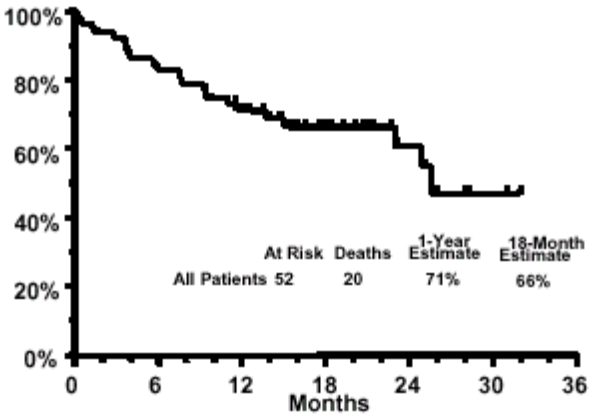
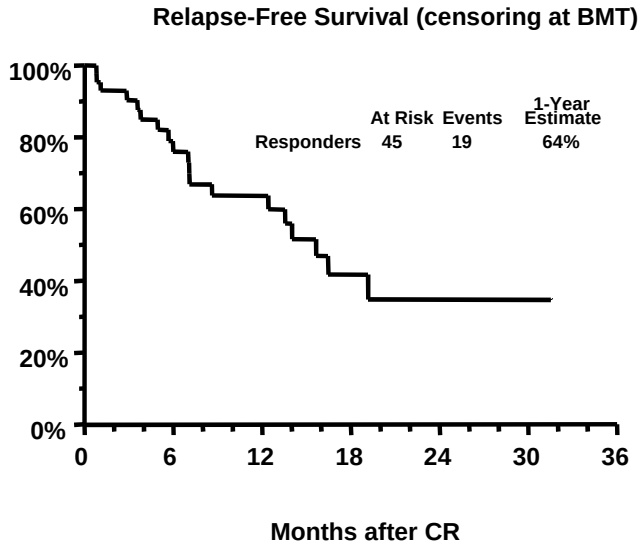


Figure 2. Relapse-free survival



Clinical Safety

The safety database consists of the data collected for 251 patients treated with ATO, divided into 3 populations for assessment (n=52, 58 and 141 patients based on the main clinical studies in APL, on clinical trials in other malignancies and from the compassionate use programme, respectively). Patients who started treatment were considered evaluable for safety and all patients were included in the clinical safety analysis.

Patient exposure

Exposure data is summarised in tables 7-8. All patients but one received ATO for at least two weeks. Combining both main studies, the median cumulative number of doses administered during induction was 31.5 (range, 5-60). The median cumulative doses during consolidation was 25 (range, 14-42), including the patient who received re-induction instead of consolidation. The median cumulative dose administered per patient during induction was 4.81 mg/kg (range, 0.75-9.) or 390 mg (range, 75-820). The median cumulative dose administered during consolidation was 3.74 mg/kg (range, 1.53-6) or 288 mg (range, 91.5-506). For the 25 patients who received treatment in the extension protocols the median cumulative number of doses administered during the extension treatment was 9.4 (range, 3-15). The total median cumulative doses, including induction, consolidation and extension treatment for 52 patients in the pivotal and extension studies was 11.7 mg/kg (range, 0.75-27.9) or 905 mg (range, 75-2886), including the patient who received re-induction instead of consolidation.

Table 7. Patient exposure in the main studies

Treatment Phase		Induction N=52	Consolidation N=41	Maintenance N=25	I, C or M* N=52
First to last dose (days)	Median	34.0	30.0	173.0	114.0
	Min Max	5.0 63.0	18.0 51.0	25.0 291.0	5.0 455.0
Cum. dose (mg/kg)	Mean	4.81	3.69	9.51	12.29
	Median	4.67	3.74	9.40	11.66
	Min Max	0.75 9.01	1.53 6.05	3.72 15.30	0.75 27.91
Cum. dose (mg)	Median	390.00	288.00	750.00	905.96
	Min Max	75.00 819.83	91.50 506.14	275.00 1910.50	75.00 2885.60
No. of doses	Mean	32.6	25.0	65.7	84.0
	Median	31.5	25.0	73.0	78.0
	Min Max	5.0 60.0	14.0 42.0	25.0 100.0	5.0 184.0
>1 Dose	n %	52 (100%)	41 (100%)	25 (100%)	52 (100%)
>25 Doses	n %	44 (85%)	2 (5%)	20 (80%)	50 (96%)
>30 Doses	n %	27 (52%)	1 (2%)	20 (80%)	47 (90%)
>45 Doses	n %	4 (8%)		20 (80%)	39 (75%)
>60 Doses	n %			13 (52%)	32 (62%)
>75 Doses	n %			9 (36%)	27 (52%)
>90 Doses	n %			8 (32%)	19 (37%)
>145 Doses	n %				11 (21%)
>190 Doses	n %				1 (2%)

* Induction, consolidation or maintenance course(s).

Table 8: Summary of patient exposure in clinical trials

	APL studies		Extension studies		Other hematologic malignancies	Advanced solid tumors
Study	***97-66***	***PLRXAS01***	***98-13***	***PLRXAS02***	***98-23***	***98-46***
N	12	40	7	18	24	34
No. of days between 1st and last dose	97	89.5	180	108.5	30	22
Median	(5-114)	(14-133)	(96-243)	(25-291)	(2-187)	(3-131)
Mean number of doses administered	27.6	34.2	68	64	27	8.4
Days	(5-39)	(14-60)	(40-93)	(25-100)	(2-75)	(3-25)
Median total cumulative dose of ATO	582.5 mg	679.3 mg	775 mg	668 mg	375.75	131 mg
Mg	(75-870)	(115.5-1181.2)	(440-1060)	(275-1911)	(19.8-1155)	(31.8-630)

Withdrawals due to Adverse Events in Pivotal Studies

The only patient who discontinued treatment from study 97-66 died on day 6 from subarachnoid hemorrhage and cardiac arrest which were considered unrelated to the ATO treatment. Six patients were withdrawn from study PLRXAS01 due to adverse events, four of which were serious. Three patients were withdrawn due to events that proved fatal within a few days after withdrawal.

Two coagulopathies associated with APL (GI bleeding leading to hypotension and stroke in one patient and an intracranial hemorrhage in another patient). The third death resulted from pulmonary aspergillosis that was present at the start of the study in a patient who had bone marrow and leukocyte remission but no platelet recovery due to graft-versus-host-disease from a prior bone marrow transplantation. None of the three deaths were considered related to ATO.

One patient with diffuse alveolar hemorrhage necessitating intubation and ventilation discontinued protocol treatment. The event was considered probably related to APL but possibly related to ATO. The remaining two adverse events that lead to discontinuation were a Grade 3 neuropathy and a severe bone marrow necrosis with bone pain.

Adverse events

Frequently occurring adverse events and adverse drug reactions for the main clinical studies are summarised in tables 9-10.

All 107 patients experienced some drug-related adverse events. The most common adverse events (occurring in 20 patients or more) were fatigue (76), edema (67), nausea (64), hyperglycemia (56), headache (52), pyrexia (50), diarrhea (44), vomiting (44), cough (43), dyspnea (41), tachycardia (39), hypokalemia (35), dermatitis (34), pruritus (31), sore throat (31), abdominal pain (30), insomnia (29), rigors (29), constipation (27), parasthesia (27), dizziness (27), arthralgia (25), blood magnesium decreased (24), weight gain (23), anxiety (21), anorexia (20, hypertension (20). The most common drug-related AE were hyperglycemia, fatigue, nausea, headache, hypokalemia, and parasthesia.

Sixty-three patients (59%) experienced severe or life threatening (grade 3 and 4) treatment-related adverse events. The most common were hyperglycemia (12), dyspnea (11), hypokalemia (10), neutropenia (7), thrombocytopenia (7), fatigue (7), increased ALT (6), and hypoxia (6) (see Appendix 2 Part I page 690-693). Thirty-seven (35%) experienced possibly, probably or related severe or life-threatening (grade 3 and 4) treatment-emergent AE. Hypokalemia (10), hyperglycemia (10), neutropenia (6), and increased ALT were the most common drug-related, severe and life-threatening adverse events.

The proportion of patients experiencing toxicity was similar in different age, gender, ethnic and weight groups. The number of prior regimens received or prior BMT did not increase the number of adverse events experienced by patients treated with ATO.

Table 9. Study 97-66: Frequently Occurring Adverse Events: Number (%) of Patients with Events, Drug-Related Events, and Severe/LT Events

Body System:	Group Term	Number of Patients (%) with Event		
		All Events	DR	Severe/LT
		12 (100%)	11 (92%)	10 (83%)
Metabolism & Nutr.	Hyperglycemia NOS	12	11	3
	Hypokalemia	7	4	3
	Hypocalcemia	6		1
Nervous System	Headache NOS	9	3	
General Disorders:	Fatigue	8	1	
	Pyrexia	8		
	Edema peripheral	5		
GI Disorders	Nausea	8	2	
	Diarrhea NOS	7	2	1
	Vomiting NOS	6		
	Abdominal pain NOS	5		
Cardiac	Tachycardia NOS	7	1	1
Investigations	Blood magnesium decreased	6	3	1
Respiratory	Dyspnea NOS	5	2	2
Psychiatric Disorder	Anxiety NEC	5		
Blood and Lymphatic	Thrombocytopenia	5		1

Abbreviations: DR = Considered possibly or probably related to study drug by the investigator; LT = NCI Grade 4.

Table 10. Study PLRXAS01: Frequently Occurring Adverse Events: Number (%) of Patientswith Events, Drug-Related Events, and Severe/LT Events

System Organ Class:	Group Term	Number of Patients (%) with Event		
		All Events	DR	Severe/LT
		40 (100%)	40 (100%)	27 (68%)
GI Disorders	Nausea	30 (75%)	19 (48%)	
	Vomiting NOS	23 (58%)	8 (20%)	
	Diarrhea NOS	21 (53%)	8 (20%)	
	Sore throat NOS	16 (40%)	1 (3%)	
	Abdominal pain NOS	15 (38%)	2 (5%)	3 (8%)
General Disorders:	Fatigue	25 (63%)	10 (25%)	2 (5%)
	Pyrexia	25 (63%)	7 (18%)	2 (5%)
	Edema NOS	16 (40%)	3 (8%)	
	Rigors	15 (38%)	2 (5%)	
Respiratory	Cough	26 (65%)	1 (3%)	
	Dyspnea NOS	15 (38%)	4 (10%)	4 (10%)
Nervous System	Headache NOS	24 (60%)	14 (35%)	1 (3%)
	Insomnia NEC	17 (43%)	1 (3%)	1 (3%)
Skin & SC disorders	Dermatitis NOS	17 (43%)	7 (18%)	
Metabolism & Nutr.	Hypokalemia	20 (50%)	15 (38%)	5 (13%)
	Hyperglycemia NOS	18 (45%)	16 (40%)	5 (13%)
Cardiac	Tachycardia NOS	22 (55%)	5 (13%)	

Abbreviations: see Table 9.

Serious Adverse Events

In patients with haematological malignancies, 37/107 patients (34%) experienced 61 related serious adverse effects.

Non-fatal SAEs in Main Clinical Studies

Besides non-fatal serious adverse events that were not unexpected in this population, due to their underlying disease, detailed safety data on the recurrence of pulmonary or thoracic symptoms, EKG changes, neurological toxicities, and myelosuppression was presented. Many patients experienced various thoracic symptoms including respiratory failure, with or without pulmonary hemorrhages, with or without hypotension and renal failure, with or without worsening DIC. Some patients had pleural or pericardial effusions. These usually occurred during the first three weeks of ATO treatment. In the minority of these patients, these events were classified as ATRA-like syndrome ("arsenic syndrome"). It is clear that ATRA-like syndrome may occur under ATO treatment in APL patients.

ECG changes: ECG changes with or without prolonged QT interval were reported as serious adverse events possibly or probably related to ATO in three patients enrolled in the main studies. One patient had concomitant palpitations subsequently diagnosed as Torsade de Pointes (see further).

Prolonged QT/QTc interval: An important adverse event attributed to ATO is prolongation of the QT/QTc interval seen on electrocardiogram (ECG). Two patients from the pivotal PLRXAS01 study and two patients from the SAE only population presented QT prolongation under ATO administration. In one patient, QT interval was normal at baseline and had increase starting on day 7 with maximum values on day 21 and persistent prolonged QT until day 111. In another patient, a prolonged QT was noted at day 30 on routine ECG. This patient experienced a brief, spontaneously resolving episode of torsade de pointes detected on telemetry monitoring. The patient went onto consolidation treatment without further evidence of QT-prolongation.

In fact, QT prolongation has been associated with torsade de pointes, a polymorphic ventricular tachycardia that occurs in the setting of an abnormally long QT interval. This ventricular arrhythmia has been associated with episodes of syncope and sudden cardiac death. The most common cause for torsade de pointes is administration of drugs that prolong the QT interval. Congenital cases (Long-QT Syndrome [LQTS]) have been described and relatively large numbers of individuals have been identified who carry silent mutations on LQTS genes. These patients may be especially sensitive to drugs that affect potassium currents as these channels are affected by the abnormal genes. There are a number of baseline characteristics that identify a patient at increased risk for torsade de pointes. The following are risk factors associated with drug-induced torsade de pointes: female gender,

hypokalemia, hypomagnesemia, bradycardia, diuretic use, recent conversion from atrial fibrillation, congestive heart failure or cardiomyopathy, QT-prolongation during drug administration, baseline prolonged-QT. Other causes of prolonged QT such as hypocalcemia or hypothyroidism are rare causes. There are a number of drugs commonly used in cancer patients undergoing induction chemotherapy, which are known to cause prolongation of QT, and other commonly used drugs which affect metabolism of drugs with a potential to cause prolonged QT. The treatment for torsade de pointes is careful attention to fluid and electrolyte balance and administration of potassium and magnesium to maintain this electrolyte well in the normal range. Cautious patient management should minimize the patient's risk of developing torsade de pointes. Management entails ECG monitoring of QT prolongation at regular intervals, withholding drug when a threshold of >460 msec has been reached, monitoring electrolytes and maintaining potassium and magnesium levels within the normal range and judicious use of concomitant medications.

An expert cardiology review of approximately 1,000 ECGs obtained in 99 patients receiving infusions of ATO confirmed that treatment with arsenic gradually prolongs the QT interval until steady state is reached. In this group, mean QTc prolongation was 47 plus or minus 5 msec and the half-time for approach to steady state was 6 plus or minus 2 days. QTc prolongation resolved after discontinuation of arsenic infusions. Infusions of arsenic seemed to have no effect on AV conduction or intraventricular conduction except in a subject who developed transient atrial fibrillation with a right bundle branch block and slow ventricular response in the setting of severe hyperkalemia. Heart rate was increased by approximately 10 beats during therapy compared to baseline. This effect was noted immediately after the first infusion and did not increase further as therapy progressed.

Peripheral neuropathy: Peripheral neurological events were reported in the pivotal and supportive populations. Twenty-three patients reported paresthesias, most of which were mild to moderate. Mild and moderate peripheral neuropathic events were reported which resolved upon cessation of the drug. Neuropathic events were observed as follows: paresthesias (32), hypoesthesia (8), abnormal gait (5), peripheral neuropathy (5), and one patient each reported hyperesthesia, hyporeflexia, myasthenic syndrome, unspecified neurological symptoms and peripheral motor neuropathy. Two patients had severe paresthesias (1 hand and foot and the other associated with muscle weakness), which resulted in discontinuation early from study drug treatment. One APL patient who discontinued early during consolidation went on to the extension study and received further ATO treatments, which were intermittently held while his neuropathy improved and then restarted. There were no grade 3 or 4 peripheral neuropathic adverse events reported. Seizures were reported in two patients in the PLRXAS01 study (one patient at day 1 and 3 of ATO therapy and again later at day 28 and 31 in the context of a severe Gram-negative sepsis; in another patient, seizure activity occurred while the patient was under mechanical ventilation at day 32 of ATO treatment, leading to its discontinuation). Of note, peripheral neuropathy was not graded as a serious adverse event, except in one case of presumptive neuropathic pain (although neurologic examinations did not confirm this).

Myelosuppression: Chronic exposure to arsenic is known to cause myelodysplastic features in the bone marrow associated with cytopenia. Such an effect is hard to assess in patients with acute leukemia during the first induction cycle but not during consolidation and maintenance cycles. The analysis of individual WBC counts before and during the consolidation and maintenance cycles (data not shown) showed that approximately 40% of these treatment courses were associated with a significant decrease in WBC. Treatment-related neutropenia was usually mild. Febrile neutropenia was observed in patient (at the end of the consolidation cycle).

Non-fatal SAEs in supportive Studies

In study 98-23 (patients with advanced hematologic malignancies), non-fatal serious adverse events were mainly non-documented or documented infections in 10 patients. One patient experienced hemolysis on day 16, following a RBC transfusion. Two patients (1062 and 1069) had abdominal symptoms related to an underlying chronic lymphocytic leukaemia in one patient and to a *Clostridium difficile* infection in the other one. These events were not related to ATO treatment. One patient experienced an asymptomatic QT prolongation (570 ms) associated with a decrease in serum potassium to 2.9, on day 36. This event was considered possibly related to ATO (1107). In 7 cases, ATO was discontinued due to adverse events.

In study 98-46 (patients with advanced solid tumors), non-fatal adverse events were mainly related to cancer progression (10 events in 10 patients). One patient (1080) with a history of adult onset DM presented a severe hyperglycemia with serum glucose 621 mg/dL related to ATO. A 75-year-old

patient experienced severe fatigue and severe increased ALT related to ATO. In 3 cases, ATO was discontinued due to adverse event.
Among the 141 patients treated in compassionate use, 25 patients had a total of 29 serious adverse events. Thirteen patients died. Study drug was discontinued in 16 patients due to SAE. Fifteen patients experienced related SAE including fatal atra syndrome (1), fatal sepsis (1), diarrhea and abdominal pain (1), fatal pain hypotension and hypoxia (1), dyspnea (1), bone pain and neutropenic fever (1), grade 3 neutropenia (1), neuropathy (2), worsening headache (1), fatal cerebral edema (1), fatal CNS infarct (1), QTc prolongation (1), myocardial injury (1), spontaneous abortion (1).

Deaths
In the 97-66 study, one patient died due to cerebral haemorrhage on day 6 after receiving 5 doses of ATO. Two additional patients died within 30 days after last study treatment, both in the context of APL progression after one induction and one consolidation ATO cycle. No deaths were considered to be related to ATO and were instead considered consistent with the known complications of APL. In the PLRXAS01 study, no patients died while receiving study treatment, but three patients were withdrawn due to SAEs that proved fatal within a few days after the withdrawals. Two of the three deaths were due to coagulopathies (one GI bleed leading to hypotension and stroke in one patient and one intracranial haemorrhage in another patient) associated with APL. Neither of these patients had achieved CR before the time of death. The third death resulted from pulmonary aspergillosis that was present at study start in a patient who had bone marrow and leukocyte remission, but no platelet recovery due to GVHD from a prior BMT. Again, none of these deaths was considered to be related to ATO treatment.

Concerning supportive studies, five deaths have been reported on-study in study 98-23. All were considered to be related to the underlying disease progression (1 refractory APL, 1 AML, 3 non-Hodgkin lymphomas). No deaths on-study or within 30 days after last study drug have been reported in study 98-46.

Table 11 : Number of patients with possibly or probably related SAEs.

System Organ Class	Preferred Term	97-66 N=12	98-13 N=7	AS01 N=40	AS02 N=18	98-23 N=24	98-46 N=34	Total N=107
All Body Systems	All terms	6	1	19	5	7	4	37
Blood and lymphatic system disorders	All terms		1	5	2			8
	Febrile neutropenia			1				1
	Leucocytosis NOS			1				1
	Leucopenia NOS		1					1
	Neutropenia		1	3	2			6
	Thrombocytopenia			1	1			2
Metabolism and nutrition disorders	All terms	4		7	1	3	1	15
	Hyperglycaemia NOS	2		4	1	2	1	10
	Hypermagnesaemia					1		1
	Hypernatraemia	1						1
	Hypokalaemia	3		5		2		10
	Ketoacidosis	1						1
Nervous system disorders	All terms			2				2
	Paraesthesia NEC			2				2
Cardiac disorders	All terms	1		1				2
	Pericardial effusion			1				1
	Tachycardia NOS	1						1
Vascular disorders	All terms			1				1
	Vasculitis NOS			1				1
Respiratory, thoracic and mediastinal disorders	All terms			4		1	1	6
	Dyspnoea NOS			1		1	1	3
	Hypoxia			1				1
	Pleural effusion			1				1
	Pleuritic pain			2				2
	Pulmonary alveolar haemorrhage			1				1
Gastrointestinal disorders	All terms	1		1				2
	Abd pain upper			1				1
	Diarrhoea NOS	1						1
Skin & subcutaneous tissue disorders	All terms			1		1		2
	Erythema NEC			1				1
	Pruritus NOS					1		1
Musculoskeletal, connective tissue and bone disorders	All terms			4				4
	Arthralgia			2				2
	Back pain			1				1
	Bone pain			2				2
	Bone pain aggravated			1				1
	Myalgia			1				1
	Pain in limb			1				1
General disorders and administration site conditions	All terms	1		2	1	2	1	7
	Chest pain NEC			1				1
	Fatigue					2		2
	Fatigue aggravated						1	1
	Pain NOS	1						1
	Pyrexia			1	1			2
Investigations	All terms	2		5	1	2	2	12
	ALT increased			3		1	2	6
	AST increased	1		1				2
	Biopsy bone marrow abnormal			1				1
	Blood bilirubin increased	1						1
	Blood magnesium decreased	1						1
	ECG QT prolonged			1	1	1		3

Clinical laboratory evaluations

Hematology and coagulation profile: Given the hematological perturbations related to the presence of the underlying disease, it is difficult to assess the hematological consequences of ATO treatment in patients enrolled in the pivotal studies. Hyperleukocytosis that occurred during induction cycle in APL patients only and putative myelosuppression that might occurred after prolonged ATO administration

in all patients are discussed above. Coagulation disorders occurring during the induction cycle in APL patients were considered as clearly related to the underlying leukemia.

Clinical chemistry: The most recurrent and relevant abnormalities are hypokalemia, hyperglycemia and increased liver enzymes levels. These abnormalities are not unexpected in such a complicated patient population. However, the recurrence of hyperglycemia and mild hepatic toxicity during the second consolidation cycle has to be noted.
Hepatic toxicity including increased total bilirubin, SGOT, SGPT, and/or alkaline phosphatase values may be considered to be related to ATO administration. However, no patient met the protocol-defined criteria for significant hepatic toxicity (5 x baseline for SGOT, bilirubin, or alkaline phosphatase) and there was no treatment discontinuation for this reason. Of note the elevation was transient and reversible in the majority of patients with either treatment or the discontinuation of ATO.

Vital signs and weight: Weight gain (23%) and hypotension (20%) were usually observed in APL patients. Both symptoms may be related to the differentiation syndrome ("arsenic syndrome"), as observed under ATRA therapy (ATRA syndrome). This syndrome is discussed in the following section.

Hyperleukocytosis and arsenic syndrome (differentiation syndrome): Two syndromes (hyperleukocytosis and differentiation syndrome) have been associated with ATO and other differentiation agents in the treatment of APL that are thought to confer increased morbidity. Differentiation syndrome (ATRA syndrome and arsenic syndrome) are often linked to a clinical syndrome manifested by fever, weight gain, hypotension, dyspnea, pulmonary infiltrates, and pleural or pericardial effusions with or without marked increase in WBC. This syndrome is considered as a clinical consequence of APL cell activation during the differentiation process. Recommended treatment is to initiate high dose steroids (dexamethasone 10 mg twice a day for 3 to 5 days) at the first suspected sign or symptom of the syndrome. Cytotoxic chemotherapeutic intervention and/or leukopheresis may be indicated in cases of life-threatening hyperleukocytosis. Hyperleukocytosis is not a prerequisite for differentiation syndrome, nor does every patient with hyperleukocytosis develop a clinical differentiation syndrome. As mentioned before, the presence of a differentiation syndrome requiring the introduction of steroid therapy may be difficult to diagnose, especially in patients without associated hyperleukocytosis. Many signs may be confounded with infection-related symptoms which theoretically contra-indicate steroid administration. Furthermore, the duration of the differentiation process that may reach 3 or 4 weeks and therefore may result in prolonged steroid administration in these patients.
Four patients had SAEs reported as differentiation syndrome during ATO treatment. Twelve patients had hyperleukocytosis, 4 of whom had values above 100,000/μL. One patient treated under the NCI compassionate use program died from cerebral infarct and had concomitant hyperleukocytosis. No markers to identify patients at higher risk for developing either differentiation syndrome or hyperleukocytosis were found.

Coagulation disorders: Other adverse events associated with APL are coagulation disorders including DIC. This syndrome is characteristic of APL and also thought to be worse in patients with treatment-induced hyperleukocytosis. The majority (41 of 52) pivotal patients had symptomatic DIC at baseline. Treatment with ATO did not appear to exacerbate the incidence of DIC. Mortality in the pivotal studies from DIC associated hemorrhage was approximately 11%, consistent with the 10 to 15% early mortality reported in the literature.

Late adverse events
Relationship between chronic arsenic ingestion and the development of skin and internal cancers has been reported. A recent study from Taiwan reported a dose-response relationship between the long-term arsenic exposure to high-inorganic arsenic artesian well water and the incidence of lung, bladder, and all sites cancers. In multivariate analysis, a cumulative arsenic exposure $\geq$ 20 mg/liter x duration of drinking artesian well water (year) was predictive of cancer development. Over the 7-year follow-up of this study, such a cumulative arsenic exposure corresponds to a cumulative ingested dose of 25 to 50 gr.
A quantitative assessment performed by the EPA using Taiwan data showed that for the U.S. population, the risk of developing skin cancer from lifetime exposure to ingested arsenic of 1 ug/kg/day ranges from 1 to 2 individuals per 1000. Assuming a 70 year lifetime and an average body weight of 70 kg, a cumulative exposure to arsenic of 1800 mg is associated with a risk of skin cancer

in 1-2 individuals per 1000. Other studies have suggested an elevated, but not quantifiable, risk for cancer of internal organs from exposure to arsenic.
The median cumulative dose of ATO for APL patients treated with induction, consolidation, and maintenance was 906 mg (range 75 to 2886 mg). One milligram of ATO contains 0.757 mg of arsenic, so the median exposure to arsenic was 686 mg (range 57 to 2185 mg). The maximum arsenic exposure is approximately equal to the cumulative dose of arsenic associated with an incidence of 1 to 2 cases of skin cancer per 1,000 people.
It was therefore considered very unlikely that the extent of exposure to ATO in these patients might represent a significant factor for further cancer development and the demonstrated benefit for APL patients receiving ATO therapy clearly outweighs the potential risk for subsequent cancer.

Drug interactions
No formal drug interaction studies have been performed. Drugs that can potentially bind to arsenic like N-acetyl cysteine, chelators or antioxidants like vitamin E may block the activity of ATO.

Discussion on clinical safety

Although the safety database is based on a small number of patients, the data presented are sufficient to assess the safety profile of ATO. The duration of exposure and the median total cumulative dose of ATO reported were higher for APL-patients than for non-APL patients.
The toxicity observed was not unexpected with ATO. The main toxicity included metabolism disorder including hyperglycemia and hypokalaemia, respiratory disorder and ALT increase. Seven patients (13%) were withdrawn due to adverse effect. No patient died from toxicity. Twenty six patients (44.8%) experienced nonfatal serious adverse events. Only three cases were treatment-related serious adverse events.
Ten patients (17%) have discontinued ATO treatment due to adverse events. In the main studies, only 3 patients (5.7%) were withdrawn from studies due to related adverse effects. In this context of relapsed or refractory leukemia, this percentage is low.
Particular caution is requested in patients with renal failure receiving ATO, as renal excretion is the main route of elimination of arsenic. Recommendations on monitoring of the patient are included in the SPC.
QT prolongation was a serious concern. Additional data regarding the predictability and the degree of QT prolongation and torsade de pointes were requested. The applicant provided data from previously treated patients (survivors from pivotal study and patients from ongoing studies) with cardiac toxicity. The applicant also submitted a US “dear health provider” on QT prolongation and risk of torsade de pointes in March 2001, due to a recent publication of Unnikrishnan reporting 3 cases of QT interval prolongation with torsade de pointes. Two patients died from cardiac arrythmia. These cardiac toxicities were collected with another ATO. The applicant reminded that 40% of patients treated with ATO experienced at least one QT interval prolongation greater than 500msec, with one non fatal case of torsade de pointes. Prolongation of QTc was observed between 1 and 5 weeks of daily ATO infusion, and returned to baseline by the end of 8 weeks after Trisenox infusion. This last information was added in section 4.4 of the SmPC.
Adequate recommendations are provided in the SPC including monitoring of electrocardiogram, electrolytes and renal function (see *Special precautions for use*). Recommendations for monitoring and treating hyperleukocytosis and leukocyte activation syndrome (APL differentiation syndrome) have also been provided in the SPC, section 4.4.

5. Overall conclusions, benefit/risk assessment and recommendation

Quality

The important quality characteristics of ATO are well defined and controlled, and the product is formulated, manufactured and controlled in a way that is characteristic of a solution for injection. The specifications and batch analytical results indicate a consistent product with uniform clinical performance from batch to batch. There are no outstanding quality issues, which have a negative impact on the benefit/risk balance.

Preclinical pharmacology and toxicology

There are no substantial problems in toxicology, since the effects of ATO have been studied for a long time. The compound's safety profile and the doses proposed for this specific therapy are not of particular concern as long as the recommendations of the SPC are carefully followed. A better understanding of the ATO mechanism of action could help optimise the dose, since the one now proposed is based on empirical criteria.

Efficacy

The dose used (0.15 mg/kg/d) was approximately 50% of the maximum tolerated dose. Intravenous infusion of 10 mg ATO daily for one to two months was observed to induce significant CR of APL, and there was no cross-resistance between ATO and other anti-leukaemic agents, including ATRA. Nevertheless, there is no conclusive data to establish that the chosen dose and duration of treatment are the most effective and safe ones. The rationale for restricting the number of treatment days to 50 courses is that only four patients received more than 44 doses in the 2 clinical trials. It is possible to conclude that if a patient does not achieve a bone marrow remission within 50 days then it is unlikely that further dosing will be effective (see SPC, section 4.2, *Posology and method of administration*).
Eleven of 12 and 34 of 40 patients achieved CR :overall CR rate: 87%. The CR rate observed with ATO was comparable to that of 90% with ATRA combined with intensive timed sequential chemotherapy, reported in a recent study in patients that had relapsed from prior chemotherapy (11 patients) or ATRA/chemotherapy (39 patients) after a median first complete remission of 17 months (Thomas et *al.*, 2000). The patient population in the Thomas paper was in 1st relapse, had age $\leq$ 65, and did not have a prior marrow transplant. In the ATO patients, ½ the patients were in 2nd or subsequent relapse, 15% were over age 65, and 13% had a prior transplant before ATO treatment.The efficacy of ATO in terms of a high rate of CR in relapsed APL patients is promising. However, the induction of remission with ATO is oftenwas not sufficient to obtain molecular remission in 22% of cases (16% excluding patients that were not evaluated) and its better efficacy in combination with ATRA or chemotherapy should be tested in terms of rate of molecular remission and duration of clinical remission.
Now it is known that after CR in relapsed patients, chemotherapy in combination with ATO as post-remission therapy gives better survival than ATO alone. This is in line with the observation that remission induction with ATO is not sufficient in most cases to obtain a molecular remission, as judged by reverse-transcriptase polymerase chain reaction for PML-RAR alpha fusion transcripts. The in vivo effect of ATO seems to be related to the expression of APL-specific PML-RAR alpha oncoprotein, and a synergistic effect between ATO and ATRA has been shown in the APL mouse model. Like with ATRA in APL, early relapses within a few months after ATO treatment were not infrequent (10% within six months), possibly, indicating that resistance to ATO. can emerge rapidly. In the follow-up of relapsed cases over 7 to 48 months, the estimated disease-free survival (DFS) rate at two years was 41%, and the median DFS was 17 months. Patients with white blood cell (WBC) count below 10 x 109/L at relapse had better survival than those with WBC count over 10 x 109/L.
The follow-up is still too short to draw firm conclusions, and there is no clear evidence that despite the high remission rate achieved with ATO in relapsed APL patients, this single agent can guarantee sustained long-term remission. Several competing strategies have successful in the treatment of clinical or molecular relapse in APL including combinations of ATRA and chemotherapy, autologous and allogeneic transplantation and, more recently, monoclonal antibodies against CD33. Therefore, prospective clinical trials based on ATO in combination with ATRA or chemotherapy must formally provide evidence of prolonged clinical activity as measured by molecular monitoring and duration of clinical remission. It is acknowledged that randomised studies in this population are difficult to carry out due to the rarity of APL, especially in relapse situations as the occurrence of relapse has recently decreased because of new therapeutic strategies (ATRA plus anthracycline-based chemotherapy). The applicant has committed to evaluate the efficacy and safety of ATO in frontline APL in a randomised controlled clinical trial. This will help to further define therapeutic strategy with ATO, and controlled study in relapse situation does not seem necessary.
In conclusion, the results in terms of clinical efficacy presented so far in relapsed APL patients are promising. The applicant has committed to undertake, among others, randomised clinical trials to evaluate the role of combining ATO with ATRA *versus* ATRA alone in the treatment of newly

diagnosed APL patients who are not eligible for the current therapeutic regimens based on ATRA and chemotherapy.

Safety

The safety profile of ATO in the data presented showed adverse effects that were not unexpected: metabolism disorders (hyperglycemia, hypokalemia), respiratory disorders (hypoxia, dyspnea), hematological disorders (neutropenia, thrombocytopenia, and differentiation syndrome), peripheral neuropathy, and increased alanine aminotransferase.
A particular concern with ATO treatment was QT prolongation: 40 % of patients treated with ATO experienced at least one QT interval prolongation greater than 500 ms, with one non fatal case of TDP. Furthermore, QT interval prolongation with torsades de pointes (TDP) were recently reported in ATO treated patients (Unnikrishnan *et al*., Blood 2001; 97). The mechanism of QTc prolongation remains unknown. The applicant has committed to provide a pharmacokinetic study in which blood samples will be collected at specified times in conjunction with serial ECG's to adequately characterise the concentration of ATO and its methylated metabolites versus time profile and QT measurements. In the lack of clear predictive and prognosis factors, regular assessments of QT intervals are necessary, and constant ECG monitoring is possibly required at the beginning of induction treatment: such recommendations are included in the SPC.
Concerning treatment-related deaths, no fatal drug reactions were observed with in ATO studies (despite a more heavily pre-treated population) whilst this was close to 10% in patients treated with ATRA+chemotherapy, in the same setting.
In conclusion, in the particular context of the proposed indication, Trisenox presented an acceptable safety profile, even if some particular concerns remained in the monitoring some adverse events, i.e: differentiation syndrome, respiratory disorders, and increased liver enzymes.

Benefit/risk assessment

The mainstay of APL management is currently based on ATRA plus chemotherapy, which has been shown to be superior to either chemotherapy or ATRA alone. However, a small proportion of patients (approximately 15%) are refractory to ATRA plus chemotherapy-induced remission and 30-40% experience relapse a number of years after remission. Thus a proportion of APL patients would benefit from active alternative treatment.
Following initial assessment, the CPMP's concerns related mainly to the lack of control group in the submitted clinical studies, the QT interval prolongation, the selected dosage, and to the lack of pharmacokinetic and safety data in specific populations (patients with renal failure or liver impairment).
Despite the lack of randomised controlled studies, the CR observed with ATO in the clinical trials presented shows that Trisenox has at least similar clinical efficacy to that reported in published series that investigated treatment with ATRA+chemotherapy in patients with relapsed disease. However, the relatively short follow-up for the ATO trials does not guarantee that long-term remission is maintained in a high proportion of patients. Also, the induction of clinical remission with ATO was often not sufficient to obtain molecular remission in 22% of cases (16% excluding patients that were not evaluated) and therefore, combination treatment of ATO with ATRA or chemotherapy should be tested in order to improve the rate of molecular remission and duration of clinical remission. Because of the few patients studied or the lack of evidence of particular benefit, the efficacy-safety profile of ATO in certain subgroups, such as patients with early relapse after ATRA, with contra-indications to anthracycline, with prior or eligible for SCT, and elderly patients, is still not defined. Other strategies, such as combinations of ATRA and intensive chemotherapy, autologous and allogeneic BMT, and monoclonal antibodies against CD33, are now available for the treatment of clinical and molecular relapses and some of these alternative approaches should be compared with ATO, even in patients apparently suitable for ATO treatment.
ATO was associated with potentially serious toxicity and vigilant monitoring is warranted. Compared to existing regimens, however, ATO showed a more favourable toxicity profile in terms of treatment-related deaths. These were observed in up to 10% of patients in the published series, whilst none were observed in the trials with Trisenox.
Overall, the activity observed with ATO is promising in terms of clinical efficacy in the proposed indication, which represents a disease setting in which so far patients could only benefit of few treatment options. Due to the rarity of the indication, it is considered that the applicant cannot

reasonably be expected to provide comprehensive evidence in respect of the particular therapeutic indication in relapsed disease. To this end, the Applicant has committed to complete an agreed programme of studies within a specified timeframe, the results of which shall form the basis of an annual reassessment of the benefit/risk profile.
The programme of studies includes:

- Phase I Study of Trisenox (arsenic trioxide) injection in Patients with Hepatocellular Carcinoma
- Phase III randomized study of concurrent tretinoin and chemotherapy with or without arsenic trioxide (As2O3) as initial consolidation therapy followed by maintenance with intermittent tretinoin therapy versus intermittent tretinoin plus mercaptopurine and methotrexate for patients with untreated acute promyelocytic leukemia.
- Phase II Study of Arsenic Trioxide in Patients with APL in Molecular Relapse

In addition, the applicant has committed to specifically address several issues within the context of ongoing or new clinical trials:

- To present a survey of Trisenox injection in Adult Cancer Patients with Renal Dysfunction
- To study the relationship between pharmacokinetics and QT prolongation in APL patients
- To assess the value of ATO in consolidation treatment for patients at high-risk of relapse
- To provide valuable prospective information on the therapeutic implications of a positive or negative RT-PCR
- To evaluate the value of combining ATO with ATRA *versus* ATRA alone in the treatment of newly diagnosed APL patients who are not eligible for the current therapeutic regimens based on ATRA and chemotherapy

Recommendation

Based on the CPMP review of data on quality, safety and efficacy, the CPMP considered by consensus that the benefit/risk profile of Trisenox for induction of remission and consolidation in adult patients with relapsed/refractory acute promyelocytic leukaemia (APL), characterised by the presence of the t(15;17) translocation and/or the presence of the Pro-Myelocytic Leukaemia/Retinoic-Acid-Receptor-alpha (PML/RAR-alpha) gene (previous treatment should have included a retinoid and chemotherapy), was favourable and therefore recommended the granting of the marketing authorisation under exceptional circumstances, to be reviewed annually.