INTERNATIONAL CONSORTIUM FOR CHILDHOOD ACUTE PROMYELOCYTIC LEUKEMIA

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Associazione Italiana di Ematologia ed Oncologia Pediatrica

ICC APL STUDY 01

Treatment study for children and adolescents with Acute Promyelocytic Leukemia

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TREATMENT STUDY FOR CHILDREN AND ADOLESCENTS WITH ACUTE PROMYELOCYTIC LEUKEMIA

SUMMARY

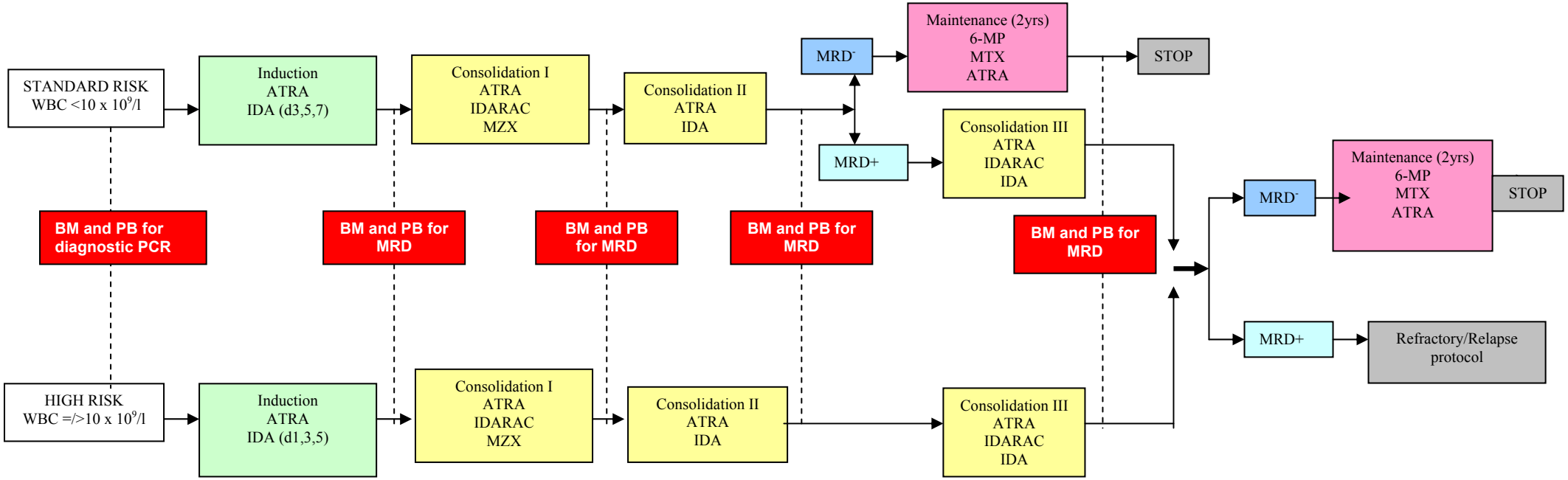
This study is open to all patients with a diagnosis of acute promyelocytic leukemia (APL) who are PCR positive for the PML-RARα transcript or rarer retinoid sensitive subtypes (i.e. NPM-RARα, NuMA-RARα) and less than 21 years of age (for AIEOP, see appendix A). APL is a rare disease with each national group recruiting small numbers of patients to their trials annually. Therefore this will be an international study expecting to recruit 60-70 patients per annum and a total of 300 patients in 5 years. The study aims to limit the use of anthracyclines and stratify treatment by risk group: standard risk – WBC $<10 \times 10^9$/l : high risk – WBC $\geq 10 \times 10^9$/l. All-*trans* retinoic acid (ATRA) is included in all phases of therapy and intermediate dose Ara-C (IDARAC) is given during consolidation treatment. Following one induction course of treatment standard risk patients have 2 consolidation blocks whilst high risk patients have 3 consolidation blocks.

The PML-RARα transcript will be monitored throughout and standard risk patients with detectable minimal residual disease by real time quantitative reverse transcriptase polymerase chain reaction (RQ-PCR$^+$) at the end of the second consolidation block will receive a third consolidation block identical to high risk patients. Patients who are RQ-PCR$^+$ for PML-RARα after completion of the third block of consolidation therapy will be candidates for refractory/relapse treatment, but will remain on study. Refractory/relapsed patients who remain RQ-PCR$^+$ for PML-RARα will be candidates for allogeneic bone marrow transplantation (allo-BMT), whilst those who become RQ-PCR$^-$ for PML-RARα will have individualised treatment with ongoing MRD monitoring.

These study guidelines are intended to describe a collaborative international study in APL in children and adolescents and to provide information about procedures for the entry, treatment and follow-up of patients. It is not intended that these guidelines be used as an aide-memoir or guide for the treatment of other patients. Every care has been taken in its drafting, but corrections and amendments may be necessary. Before entering patients into the study, clinicians must ensure that the study has received clearance from their Local Research Ethics Committee and any other necessary body.

Clinicians are asked to read the whole study guidelines before commencing treatment

FIGURE 1: Treatment for PML-RARα+ and all other retinoid sensitive subtypes of APL in children and adolescents (patients with morphological APL must initially be treated according to this protocol, but patients who are subsequently found to lack a retinoid sensitive gene fusion should thereafter be treated on their national standard AML protocol).



Section 7.5.1 and 7.5.2 describe specimens required at diagnosis and for MRD monitoring.

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1. ABBREVIATIONS

AD	aplastic death
APL	acute promyelocytic leukemia
APTT	activated prothrombin time
Ara-C	cytosine arabinoside, cytarabine
ATO	arsenic trioxide
ATRA	all-trans retinoic acid
BM	bone marrow
BMT	bone marrow transplantation
CB	cord blood
CNS	central nervous system
CR	complete remission
CRF	case record form
DFS	disease-free survival
ED	early death
EFS	event-free survival
EHD	early hemorrhagic death
EMR	extramedullary relapse
FFP	fresh-frozen plasma
FISH	fluorescence in-situ hybridisation
GO	gemtuzumab ozogamicin (Mylotarg®)
HR	high-risk
ICC APL	international consortium for childhood APL
IDA	idarubicin
IDARAC	intermediate dose ara-C
IT	intrathecal
LREC	local regulatory and ethical committee
MFD	matched family donor
6MP	mercaptopurine
MRC	Medical Research Council
MRD	minimal residual disease
MSD	matched sibling donor
MTX	methotrexate
MZX	mitoxantrone
OS	overall survival
PB	peripheral blood
PCP	pneumocystis carinii pneumonia
PCR	polymerase chain reaction
PML	promyelocytic leukemia
PT	prothrombin time
RARα	retinoic acid receptor alfa
RAS	retinoic acid syndrome
RD	resistant disease
RQ-PCR	real-time quantitative PCR
RR	relapse rate
SR	standard risk
6TG	thioguanine
VP16	etoposide
WBC	white blood cell count

2. ETHICAL CONSIDERATIONS

The ICC APL Study 01 must be approved by the national and local ethical committee (LREC) at each treatment centre, according to national requirements, before patients are entered.

The right of a patient (or guardian on their behalf) to refuse to participate in the study without giving reasons must be respected. After the patient has entered the study, the clinician is free to give alternative treatment to that specified in the study at any stage if he/she feels that it is in the patient's best interest, and the reason for doing so should be recorded. Similarly, the patient must remain free to withdraw at any time from study treatment without giving reasons and without prejudicing any further treatment. All patients who come off study therapy for whatever reason will still need to remain within the study for the purposes of follow-up and data analysis.

ICC APL Study 01 will be conducted in accordance with the EU Directive for Good Clinical Practice in Clinical Trials.

3. OBJECTIVES

3.1 Primary Objectives:

- to conduct an international pediatric study for APL based on the GIMEMA-AIEOP/AIDA 93 protocol (the study from the Italian GIMEMA -AIEOP group which has produced the best results in children with APL to date), with optimal outcome and less toxicity
- to reduce cumulative anthracycline dosage
- to deliver risk stratified treatment based on modified Sanz criteria
- to monitor minimal residual disease by RQ-PCR for PML-RARα and adjust treatment accordingly

3.1 Secondary Objectives:

- To monitor cardiotoxicity by echocardiography

3.3 Endpoints:

The main endpoints will be:

- Achievement of molecular complete remission (CRm) and reasons for failure
- Duration of remission, rates of molecular and frank relapse and deaths in first CR
- Overall survival
- Toxicity - hematological and non-hematological
- Supportive care requirements

4. STUDY DESIGN

The ICC APL Study 01 is a non-randomized study delivering risk stratified treatment based on the Gimema –AIEOP/AIDA 93 protocol.

1. All patients who enter the study must be PCR positive for the PML-RARα transcript (or have another retinoid sensitive subtype of APL, i.e. NPM-RARα or NuMA-RARα). However, treatment and study entry should be based on suspicion of M3 or M3v morphology. As APL is a hematological emergency, treatment should not wait for cytogenetic and/or molecular confirmation, and ATRA should be started as soon as the diagnosis is suspected.
2. Patients are risk-stratified at diagnosis by modified Sanz criteria; standard risk (SR) - WBC < 10 x 10^9/l: high risk (HR) - WBC =/> 10 x 10^9/l.
3. ATRA is included in induction, consolidation and maintenance therapy.
4. Following one induction course of treatment standard risk patients have 2 consolidation blocks whilst high-risk patients have 3 consolidation blocks.
5. Intermediate Dose Ara-C (IDARAC) is given during consolidation.
6. Standard risk patients who are RQ-PCR$^+$ for PML-RARα transcript at the end of the second consolidation block will receive a third consolidation block identical to the high risk patients.
7. Patients who are RQ-PCR$^+$ for PML-RARα transcript after completion of the third block of consolidation therapy will be candidates for the refractory /relapsed strategy involving arsenic trioxide+/- GO+/-ATRA.
8. Refractory/ relapsed patients who remain RQ-PCR$^+$ for PML-RARα transcript will be candidates for allo-BMT, whilst those who are RQ-PCR$^-$ for PML-RARα transcript will have individualised treatment with ongoing MRD monitoring.
9. Isolated CNS relapse will be treated with systemic and intrathecal chemotherapy.

5. JUSTIFICATION OF STUDY DESIGN AND TREATMENT SCHEDULES

5.1 Clinical and Molecular Characteristics of Pediatric Acute Promyelocytic Leukemia (APL):

Acute Promyelocytic Leukemia (APL), FAB classification M3 or M3v, represents approximately 4-8% of pediatric AML[1] but the incidence may be higher in children of Hispanic and Mediterranean origin. The median age at presentation is probably similar to that of other AML subtypes (7-9 years), but APL has rarely been reported in the first year of life. It can arise de novo or be therapy related (t-APL). The characteristics and outcome of t-APL appear similar to those of de novo APL[2].

APL is characterized by chromosomal rearrangements of 17q21 involving the gene encoding the Retinoic Acid Receptor Alpha (RARα), which is most commonly fused to the PML gene (ProMyelocytic Leukemia) as a result of the t(15;17)(q22;q21) translocation[3]. In a minority of cases (<5%), RARα is fused to an alternative partner, which in the pediatric setting is most commonly nucleophosmin (NPM1) resulting from the t(5;17)(q35;q21) translocation[4,5]. This subtype and that involving NuMA as a result of t(11;17)(q13;q21) are sensitive to retinoic acid; whereas APL involving PLZF and STAT5b as a result of t(11;17)(q23;q21) and interstitial deletion of chromosome 17, respectively are resistant to ATRA[3].

While the outcome of APL patients with the PML-RARα fusion treated with extended courses of ATRA in combination with anthracycline–based chemotherapy is generally favorable[6], pediatric patients appear to more commonly present with hyperleukocytosis, as compared to their adult counterparts[7]. Approximately 35-40% of children with APL fall within a high risk group defined by a presenting WBC ≥10 x 10^9/L, which is associated with M3v morphology, presence of FLT3 length mutations and predicts for poorer outcome[1,7]. This is due both to an increased risk of induction death, particularly as a result of hemorrhage, as well as a significantly higher rate of relapse[1,8, 9,10]. Using ATRA and anthracycline–based chemotherapy protocols followed by maintenance with ATRA, 6-MP and methotrexate, relapse rates for patients with a WBC <10 x 10^9/L at diagnosis are typically 10% or less, while rates may exceed 20% for patients with a WBC ≥10 x 10^9/L.

5.2 Experience from GIMEMA-AIEOP AIDA 93:

GIMEMA-AIEOP AIDA 93[10] recruited 983 patients with newly diagnosed APL between January 1993 and June 2000 of whom 124 (13%) were aged less then 18 years.

Induction chemotherapy consisted of Idarubicin 12mg/m^2/day x 4 given with ATRA 25 mg/m^2/day (compared to an adult dose of 45 mg/m^2/day) until remission or maximum of 90 days. Consolidation consisted of 3 monthly courses: Ara-C 1gm/m^2/day x 4 with Idarubicin 5mg/m^2/day x 4 (course 1), Mitoxantrone 10mg/m^2/day x 5 with VP-16 100mg/m^2/day x 5 (course 2), and Idarubicin 12mg/m^2/day x 1, Ara-C 150mg/m^2/8 hrly x 5 and 6-TG 70mg/m^2/8 hrly x 5 (course 3).

Molecular response by reverse transcriptase polymerase chain reaction (RT-PCR) was assessed following the third consolidation course and PCR-negative patients were initially randomized to one of four maintenance arms (6-MP+methotrexate; ATRA alone; ATRA+6-MP+methotrexate; no maintenance) but subsequently to ATRA alone or ATRA+6-MP+methotrexate.

One hundred and three (96%) of the 107 evaluable children achieved hematological complete remission (CR) and 4 died during induction (1 sepsis; 3 intracranial hemorrhage). Overt ATRA syndrome occurred in two cases and pseudotumor cerebri in 10 children. The overall survival (OS), disease-free-survival (DFS) and event-free-survival (EFS), at more than 10 years, were 89%, 78% and 76% respectively. The only significant predictor of hematological CR was the WBC; 100% vs 89% (p=0.01) for WBC less than $10x10^9$/L and equal to or greater than $10x10^9$/L respectively. A WBC at diagnosis of equal to or greater than $10x10^9$/L also had a significant impact on EFS (59% vs 83% at 10 years). Ninety-four children were evaluable for RT-PCR at the end of consolidation; of these, 91 (97%) tested PCR-negative and 3 PCR-positive. Children who were in molecular remission after consolidation therapy and randomized to receive ATRA and maintenance chemotherapy were significantly more likely to remain in molecular remission than those children who received ATRA alone (77% vs 42% respectively; p=0.0177).

These results demonstrate that the combined use of ATRA and anthracyclines is well tolerated and highly effective in children, therefore the ICC APL study will be based on the AIEOP-AIDA 93 trial. This study recognizes the apparent increase in ATRA-related side effects observed in children and accordingly a reduced dose of ATRA as compared to adults will be employed. It also aims to reduce the cumulative dose of anthracyclines. While a high cumulative dosage of anthracyclines (650 mg/m^2) was delivered in the AIDA protocol and no serious cardio-toxicity observed, long term survivors remain under evaluation. Although the benefit of Ara-C in consolidation remains unproven[11, 12,] it may have a role in patients with a high WBC, a commoner situation in childhood APL, and will be included in this study. The role of ATRA in the post-remission phase of patients with APL is also controversial. The combination of intermittent ATRA and chemotherapy may reduce rates of molecular and frank relapse and hence improve outcome, particularly for patients with a high WBC at presentation [6,12,13,14.]

5.3 Rationale for Molecular Diagnostics and Monitoring:

Determination of the underlying molecular lesion is critical for the appropriate clinical management of APL, because the PML-RARα fusion gene predicts a favorable response to molecularly targeted therapies in the form of ATRA and arsenic trioxide (ATO). Approximately 10% of APL cases with an underlying PML-RARα fusion have no detectable t(15;17) at diagnosis, either due to unsuccessful cytogenetic analysis, cryptic rearrangements or more complex rearrangements including simple variant translocations [5,15].

Patients in whom the PML- RARα fusion is documented molecularly, but the t(15;17) is not detected cytogenetically, have an equally favorable outcome to those with overt t(15;17)[16].

It is important to define PML- RARα isoform as a basis for subsequent MRD monitoring. The rare pediatric retinoid-sensitive subtypes of APL, with NPM1-RARA and NuMA-RARA fusions[4, 5 17], may not be sensitive to ATO[3].

5.3.1 MRD detection is an independent predictor of outcome in APL

There is evidence to suggest that more precise tailoring of therapy may be achieved through monitoring of minimal residual disease (MRD), which has been shown to provide an independent risk factor for relapse[18]. Using conventional "end-point" RT-PCR assays which achieve a sensitivity of approximately 1 cell in 10^4, Italian studies undertaken in children and adults in conjunction with the AIDA protocol have established that patients with PML- RARα transcripts still detectable at the end of consolidation (who account for ~5% of cases overall) or those subject to a later recurrence of PCR positivity (molecular relapse) are destined to undergo subsequent hematological relapse, which may however in both instances be averted by additional therapy[10,19,20,21].

In addition, analysis of children and adults treated in the UK Medical Research Council (MRC) ATRA trial showed that MRD monitoring can distinguish subgroups of patients at differing risk of subsequent relapse relatively early during their treatment course.

Patients with detectable fusion transcripts following 3 courses of treatment (who accounted for 10% of patients) had a significantly increased risk of subsequent relapse (57% vs 26% at 5 years, p=0.008) associated with poorer overall survival (57% vs 82%, p=0.03) compared to those testing PCR negative at the same stage[16].

These findings have been supported by a study from the German AML Cooperative Group using RQ-PCR, in which patients who failed to achieve a 3-log reduction in PML-RARα transcript level within the first 3-4 months of therapy had an increased risk of early relapse[22].

5.3.2 Real-time quantitative RT-PCR assays enhance MRD detection in APL

Whilst MRD monitoring using conventional nested RT-PCR has provided valuable prognostic information, the majority of relapses occur in patients in whom no disease-related transcripts are detectable at the end of consolidation[16,23,24,25,26]. This may be a reflection of the relatively limited sensitivity of conventional assays and/or variation in RNA quality/quantity due to poor sample quality and efficiency of the reverse transcription (RT) step.

A single MRD assessment at the end of therapy cannot be relied upon to identify all APL patients who are destined to relapse[23, 24].

In the Italian studies, patients with recurrent PCR positivity (i.e. molecular relapse - defined by two consecutive PCR positive results) were found to invariably relapse, whereas low rates of relapse were observed in patients with serial negative tests[19]. It is clear that reliability of MRD detection can be further improved using RQ-PCR approaches[22,27,28,29,30,31], which are marginally more sensitive than conventional nested RT-PCR assays for the PML-RARα fusion[32]. However, the major benefit afforded by RQ-PCR technology is the capacity to quantify fusion gene transcript levels in relation to expression of an endogenous control gene (e.g. ABL), thereby enabling rising or falling trends in normalized fusion transcript levels to be identified on serial sampling and to more readily identify poor quality samples that could potentially give rise to “false-negative” results.

RQ-PCR assays are rapid, facilitate high throughput sample analysis, are highly reproducible and readily standardized, thereby lending themselves to MRD assessment in multi-center clinical studies. Optimized RQ-PCR protocols for detection of the PML-RARα fusion gene and ubiquitously expressed control genes have been established by the Europe Against Cancer (EAC) Group[33], validated with respect to conventional RT-PCR assays[32] and used to determine treatment approach in the UK MRC AML15 trial.

5.3.3 The use of MRD–directed pre-emptive therapy

The successful use of molecular monitoring for MRD to predict impending relapse has led to enthusiasm for the concept of pre-emptive therapy, whereby patients are treated when they still have minimal residual disease, since this could potentially afford a better outcome as compared to retreating the patient when they have progressed to frank relapse. There is a particularly strong rationale for this in APL due to the well-recognized risk of induction death due to the associated coagulopathy; it would seem logical that early treatment when disease burden is relatively low would afford less risk of treatment-related complications as compared to re-induction in the presence of overt disease.

Indeed, preliminary evidence from the GIMEMA and PETHEMA groups suggests a survival benefit for pre-emptive therapy as compared to treatment at the point of frank relapse[20,25,34]. The ICC APL Study 01 will test the hypothesis that rigorous MRD monitoring using RQ-PCR coupled with pre-emptive therapy could enable additional therapy to be targeted specifically to the subgroup of patients who would otherwise be destined to relapse. Patients with persistent PCR positivity or molecular relapse will receive molecularly targeted therapy, with ATO and may proceed to stem cell transplantation. For patients achieving molecular remission and with a PCR negative stem cell harvest, good results have been achieved with autologous stem cell transplantation[35,36].

Allogeneic transplant is generally reserved for APL patients in whom harvest of PCR negative stem cells is unsuccessful or who fail to achieve second molecular remission.

The use of MRD monitoring to determine treatment approach is critically dependent upon standardization and rigorous quality control[37,38]. In particular, treatment modification based upon persistent PCR positivity following consolidation or molecular relapse should require the documentation of a PCR positive result in two successive samples with a rising PML-RARα transcript level and be subject to confirmation by a second laboratory in an independent sample[38].

Generally bone marrow is considered the "gold standard" for MRD assessment in APL[38]. In order to use MRD monitoring to reliably identify the majority of patients destined to relapse and to guide pre-emptive therapy, evidence to date suggests that PCR status of marrow should be determined at the end of consolidation and then 3-monthly thereafter for at least 2 years[38]. MRD monitoring is also valuable in guiding the need for additional therapy in the post-transplant setting[39.]

5.4 Arsenic Trioxide (ATO):

Despite its historical reputation as a toxin and a poison, ATO has been used therapeutically in a variety of diseases for many centuries. ATO has now emerged as the treatment of choice for patients with refractory/relapsed PML-RARα+ APL[40,41].

Although some patients can be successfully re-induced into a second remission with ATRA alone or in combination with chemotherapy, ATO offers the advantage of inducing molecular remission in the majority of patients after two cycles of therapy and without significant myelosuppression[41]. For children this is particularly attractive as it allows largely outpatient therapy and avoids further anthracycline exposure. However, there are limited clinical data on the pediatric use of ATO. In one study of 7 patients under 21 years of age (range 5–16 years) treated with ATO at the recommended dose of 0.15mg/kg/day, 5 patients achieved a complete response. ATO has also been evaluated as part of first-line therapy of APL in the recent US Intergroup trial C9710[42]. Safety and effectiveness in pediatric patients under 5 years of age has not been studied to any great extent.

The optimal dosing schedule for ATO is not known. At least three dosing regimens are currently in use: (i) continuous dosing at 0.15mg/kg/day, (ii) intermittent dosing at 0.15mg/kg/day for 5 days, off for two days, weekly x 4 with a two week break when daily ATRA is given and the cycle repeated x 2 and (iii) intermittent dosing starting with a loading dose of 0.3mg/kg for 5 days followed by 0.25 mg/kg twice weekly. These dosing schedules are comparable in terms of efficacy and toxicity profiles.[43,44]

The ICC-APL Study 01 protocol is based on the conventional continuous dosing schedule as currently recommended by the manufacturer,

Cephalon, particularly as most of the patient pharmacokinetic data and toxicity profiles of ATO gathered to date relate to this regimen. However, as the conventional daily dosing schedule is logistically difficult to deliver, alternative equivalent dosing schedules may be used in preference.

5.5 Gemtuzumab Ozogamicin (Mylotarg®):

Anti-CD33 targeted therapy, with gemtuzumab ozogamicin (GO) may have a particular role in APL. The conjugate contains calicheamicin, which belongs to the family of intercalating anthracyclines. The features of the linker, linking the antibody to the cytotoxic agent, make it possible that calicheamicin is released only intracellularly, thereby avoiding much of anthracycline-related toxicity.

However, GO has side-effects, such as myelosuppression, allergic reactions and liver toxicity including a veno-occlusive disease type of syndrome, called the sinusoidal obstruction syndrome (SOS). The latter side-effect is seen more frequently when GO is administered within 3-4 months before BMT, but also in the setting of BMT, SOS seems rare when lower doses of GO are being applied. While single-agent GO has significant antileukemic activity in AML in general, it seems especially active in APL, possibly because of the high CD33 expression typically seen on APL cells.[45,46,47]

6. INCLUSION AND EXCLUSION CRITERIA

6.1 Inclusion Criteria:

- Patients with a clinical diagnosis of initial APL and subsequently confirmed to have PML-RARα, NPM1-RARα or NUMA-RARα fusion. Whilst this study is only for ATRA-sensitive APL, APL is a hematological emergency and ATRA should be commenced as soon as the diagnosis is suspected. Study entry should not wait until the diagnosis of APL has been confirmed molecularly or cytogenetically
- Less than 21 years of age at initial diagnosis (for AIEOP, see appendix A)
- Considered suitable for anthracycline-based chemotherapy
- Written informed consent available
- Females of childbearing age must have a negative pregnancy test and subsequently must attempt to avoid pregnancy

6.2 Exclusion Criteria:

- Patients with a clinical diagnosis of APL but subsequently found to have PLZF-RARα fusion or lacking PML-RARα, NPM-RARα or NuMA-RARα rearrangement should be withdrawn from the study and treated on an alternative protocol.
- Refractory/relapsed APL (the guidelines in this protocol for that subgroup are intended for patients treated from initial diagnosis according to this protocol)
- Concurrent active malignancy
- Pregnant or lactating
- Physician and patient/guardian think that intensive chemotherapy is not an appropriate treatment option
- Patients who have received alternative chemotherapy for 7 days or longer without ATRA for any reason (either APL not initially suspected or ATRA not available).

7. INVESTIGATIONS AT DIAGNOSIS AND DURING TREATMENT

7.1 Bone Marrow Aspirate:

Treatment and study entry should be based on suspicion of M3 or M3v morphology; as APL is a hematological emergency, treatment should not wait for cytogenetic and/or molecular confirmation and ATRA should be started as soon as the diagnosis is suspected. Cases not confirmed to have PML-RARα, NPM-RARα or NuMA-RARα should be treated on the national standard AML protocol.

7.2 Immunophenotyping

7.3 Coagulation Screen to include d-dimers

7.4 Bone Marrow Cytogenetic Analysis including FISH

7.5 Molecular Diagnosis and Monitoring

7.5.1 Molecular Analysis at Diagnosis

A paired bone marrow and blood sample for molecular analysis (see below) is mandatory in order to define the underlying molecular subtype (including definition of breakpoint location) and also as a baseline for subsequent MRD monitoring.

Diagnostic samples required:

Marrow: **2-3mls in heparinized culture medium (AIEOP group: heparinized culture medium or Na citrate) for cytogenetic analysis**

Marrow: 2-3mls in EDTA (AIEOP group: Na citrate) for molecular analysis

Blood: 10-20mls in EDTA (AIEOP group: Na citrate) for molecular analysis

If you have a patient with suspected APL, please register him or her in the online system and with your national trials office by the standard route. If online registration with CINECA is not possible, please register the patient on paper (see Appendix VII) and fax the registration form to the International Data Center at +39 051 345759 and to the National trial office. Please notify your national reference laboratory as soon as possible to make arrangements for molecular analysis. Contact details for the national laboratory are given below:

Contact Name: Dr Daniela Diverio
Dipartimento di Biotenologie Cellulari ed Ematologia – Policlinico Umberto I
Address: Via Benevento, 6
City: Roma
Postcode: 00161

E-mail: diverio@bce.uniroma1.it
Mobile: +39 - 335 284403
Lab Tel no: +39 - (0)6 441639821
Lab Fax no:+39 - (0)6 441639820, 44241984

7.5.2 MRD Detection

Previous studies where MRD assessment has been shown to predict outcome have been based on PCR analysis of bone marrow (BM) samples; use of peripheral blood (PB) is not validated for this purpose. Therefore, BM should be taken at each MRD time-point, together with a paired peripheral blood (PB) sample, to determine concordance between results (see below for sample details). Preliminary results from the UK NCRI AML 15 Trial have shown that BM is a more sensitive and reliable indicator of presence of MRD[43]. **Heparin should be avoided in the sampling procedure, since it can interfere with PCR assays**. Therefore all samples should be taken with un-heparinized marrow needles into EDTA tubes.

Follow-up samples required:

Marrow (2-3mls first pull into EDTA or Na citrate) & peripheral blood (**10mls** into EDTA or Na citrate) at the following time-points:

1. On regeneration following induction – for FISH and PCR
2. On regeneration following consolidation I – for PCR
3. On regeneration following consolidation II – for PCR
4. On regeneration following consolidation III (where applicable) – for PCR
5. BM+PB samples three-monthly during maintenance, to be taken before ATRA – for PCR
6. BM+PB samples three monthly during the 12-month period following completion of maintenance – for PCR

All samples should be sent to the designated national reference laboratory, ensuring arrival within 24hrs to minimize RNA degradation in transit, which compromises assay sensitivity. For patients testing PCR positive following 2 courses of consolidation chemotherapy, the case should be discussed with the national clinical coordinator and the MRD coordinator before proceeding with an additional course of chemotherapy.

In order to ensure that underlying MRD is not missed due to poor quality samples, all follow-up marrow samples taken following completion of consolidation should afford a sensitivity of at least 1 in 1000.

If this is not achieved, the BM and PB sample should be repeated, but commencement of maintenance should not be delayed pending PCR results following the final consolidation course.

If PCR results from the reference laboratory indicate molecularly persistent disease (PCR positivity at end of consolidation) or molecular relapse the national coordinator should be notified by the responsible clinician and reference laboratory.

A second and separate set of paired BM and PB samples should be repeated as soon as possible (within 2 weeks) and sent to the national reference laboratory which will arrange to have the samples assayed in the designated international reference laboratory (see Page 5 - “International Study Management” and Appendix 1 – “Participating Clinical & Laboratory Groups”). Ideally, BM samples would also be taken for FISH analysis at this stage. The responsible clinician, clinical coordinator and MRD coordinator will be informed of the results; patients confirmed to test PCR positive on separate samples analyzed in at least two laboratories (or with evidence of residual disease as indicated by FISH) should be treated according to the refractory/relapse treatment guidelines (see section 14). Timing of MRD assessment for patients subject to relapse will be indicated by the study coordinators.

Left over cells/nucleic acid will be banked with appropriate consent at each designated national reference laboratory for use in future ethically approved studies.

8. PROCEDURES FOR ENTRY INTO THE STUDY AND DATA RECORDING

8.1 Center Registration:

The EU Directive on the Conduct of Clinical Trials places obligations on all investigators. In order to be registered as a trial center, investigators at each institution will be asked to confirm: (1) that they are familiar with the EU Directive on Good Clinical Practice in the Conduct of Clinical Trials, (2) that the study has national /local ethical approval as appropriate , (3) that the institution has accepted the responsibilities under their national research governance regulations, (4) that written consent has been obtained for each patient and a copy will be retained , (5) that they agree to report serious adverse events as set out in this protocol or in any subsequent guidance (6) that they agree to participate in random audit if requested, (7) that they will report data in a timely fashion, (8) that material to be stored for research is obtained using the trial consent documentation, (9) that consent for entry to the study will be notified to CINECA.

8.1.1 Patient recruitment

Patients may be recruited to this study only after a center is fully registered. This is the responsibility of the individual center and the national clinical trial center. Patients should be consented for entry into the study using the **Patient Information Sheet** and **Consent Form (see appendices).**

8.2 Diagnostic Material:

It is particularly important to define the cytogenetics and molecular characteristics of APL in each patient as this will be relevant to the treatment strategy.

8.2.1 Morphology

Central morphological review should be arranged by each participating national group, where this is standard practice, or, bone marrow and peripheral blood samples are centralized for morphological diagnosis. Contact details are given below:

Contact Name: Prof. Giuseppe Basso

Address: Dipartimento di Pediatria

Azienda Università Ospedale

Address: Via Giustiniani, 3

City: Padova

Postcode: 35128

Email: Giuseppe.basso@unipd.it

Tel: 049-8213523, 8211471

Fax: 049-8211462, 8211465

8.2.2 Cytogenetics

Cytogenetics will be carried out locally and reviewed nationally or nationally centralized. In case of the latter contact details for the national cytogenetic laboratory are given below:

Contact Name: Prof. Giuseppe Basso

Address: Dipartimento di Pediatria

Azienda Università Ospedale

Address: Via Giustiniani, 3

City: Padova

Postcode: 35128

Email: Giuseppe.basso@unipd.it

Tel: 049-8213523, 8211471

Fax: 049-8211462, 8211465

8.2.3 Molecular Analysis

Details of sample requirements for molecular diagnosis and MRD monitoring have been given previously (see section 7). In addition to PML-RARα transcript analysis, patients will be investigated for the presence of a FLT3 mutation if possible (although FLT3 status does not influence patient management in the study).

8.2.4 Immunophenotyping

This should be done locally and/or nationally. In case of the latter contact details are given below:

Contact Name: Prof. Giuseppe Basso

Address: Dipartimento di Pediatria

Azienda Università Ospedale

Address: Via Giustiniani, 3

City: Padova

Postcode: 35128

Email: Giuseppe.basso@unipd.it

Tel: 049-8213523, 8211471

Fax: 049-8211462, 8211465

8.3 Data Recording guidelines:

Patient Registration —a) fill inn immediately the patient registration form (please refer to Appendix VII). National Center and National laboratory wiil be notified automatically by the system.

b) fill in the Pathology – Diagnosis form, the Clinical examination and the Eligibility form within 1 month post-patient registration

Once a patient has been recruited into the study, it is very important to have full details of the subsequent course of events, even if study therapy has been abandoned. Although clinical decisions remain with the physician (see Section 2, Ethical Considerations), follow-up data must continue to be collected on such patients and study forms must be filled in, as far as possible, giving details of the therapy actually received and its outcome. All participating groups must agree to forward encoded (anonymous) data to CINECA.

9. RISK GROUP STRATIFICATION, ASSESSMENT OF RESPONSE AND DEFINITION

9.1 Risk Group Assignment:

9.1.1 Standard Risk Patients (SR) are defined as those patients with a WBC less than $10x10^9$/L at presentation.

9.1.2 High Risk Patients (HR) are defined as those patients whose highest pre-treatment WBC is equal to or greater than $10x10^9$/L at presentation.

9.2 Assessment of Response:

A bone marrow aspirate should be carried out after induction therapy, prior to the first block of consolidation therapy and should include material for molecular analysis and FISH. The BM aspiration should be done not earlier than 7-10 days after stopping ATRA. Primary resistance in APL is essentially unreported and patients should not be considered primary resistant on the basis of morphological, cytogenetic, FISH or PCR results following induction[48].

9.3 Definitions:

All responses may be hematological, cytogenetic or molecular.

9.3.1 Complete Remission

- **Hematological Remission** - the bone marrow is regenerating normal hematopoietic cells and contains <5% blast cells by morphology. The absolute neutrophil count in the peripheral blood should be $>1.0x10^9$/l and the platelet count $\geq 100x10^9$/l.
- **Cytogenetic Remission** – disappearance of the diagnostic clonal abnormality
- **Molecular Remission** - absence of PML-RARα fusion transcript in bone marrow by RQ-PCR, with an assay sensitivity of at least 10^{-4}.

9.3.2 Treatment Failure

- **Early Death (ED)** – any death occurring within 14 days from diagnosis from any cause.
- **Aplastic Death (AD)** – any death occurring after 14 days from diagnosis, but before achieving CR.
- **Death in CR**
- **Resistant/Refractory Disease (RD)** – persistent detection of morphological, cytogenetic and/ or molecular evidence of APL after consolidation therapy.

9.3.3 Relapse

- **Hematological Relapse** – reappearance of promyeloblasts/abnormal promyelocytes (>5%) in the bone marrow.
- **Cytogenetic Relapse** – reappearance of the cytogenetic abnormality t(15;17) after repeated negative cytogenetic analysis as determined by karyotype and/or FISH.
- **Molecular Relapse** – reappearance of the PML-RARα transcript in two successive samples in patients previously in molecular remission.

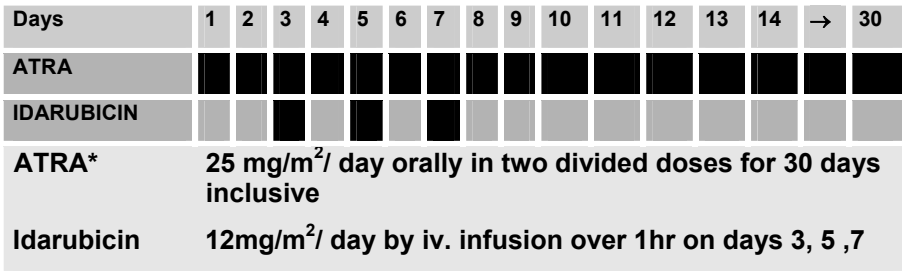
10. INDUCTION CHEMOTHERAPY

This study is only for children and adolescents with ATRA-sensitive APL (i.e. with PML-RARα, NPM-RARα or NuMA-RARα fusion transcript), since patients with APL FAB-M3 but lacking one of these rearrangements could be undertreated by the ICC APL 01 protocol. Because APL is a hematological emergency, ATRA should be commenced as soon as the diagnosis is suspected. Study entry should not wait until the diagnosis of APL has been confirmed molecularly or cytogenetically. Patients subsequently found to lack PML-RARα, NPM-RARα or NuMA-RARα by RT-PCR should not be treated on this study but according to the standard national AML protocol.

Patients should be risk stratified by their WBC for appropriate treatment allocation.

- Standard Risk (SR) – WBC: less than $10X10^9$/L
- High Risk (HR) – WBC: highest WBC prior to treatment equal to or greater than $10X10^9$/L

TABLE I : TABLE I - INDUCTION TREATMENT FOR SR PATIENTS



ATRA*	**25 mg/m^2/ day orally in two divided doses for 30 days inclusive**
Idarubicin	**12mg/m^2/ day by iv. infusion over 1hr on days 3, 5 ,7**

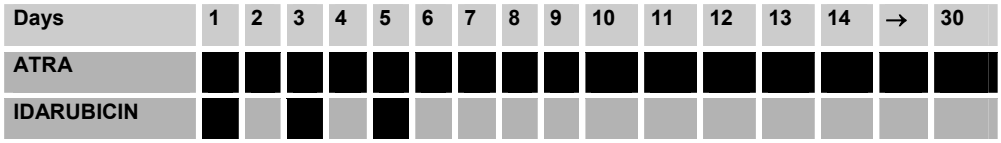
* *Please note that ATRA is all-trans retinoic acid (tretinoin) and will be generally referred to as ATRA throughout the text of this protocol.*

- Bone marrow assessment should be carried out following recovery of the neutrophil count to 1.0 x 10^9/l and the platelet count to 100 x 10^9/l, between 7-14 days after stopping ATRA, but not less than 7 days and should include material for minimal residual disease (BM and PB) monitoring. At this stage, BM morphology can be difficult to interpret and should not influence treatment. All patients should proceed to the first block of consolidation therapy irrespective of their morphological, cytogenetic or molecular status.

- Lumbar puncture should not be done at initial diagnosis because of the risk of bleeding and the low incidence of CNS involvement in APL.
- ATRA should be given for a total of 30 days.
- Note the precautions to be taken during ATRA therapy – refer to Section11.2 for treatment modifications which may be required and ensure familiarity with this section before commencing treatment.
- Note dose modifications which may be required for idarubicin. In the case of hepatic dysfunction and bilirubin 21 – 34 micromol/L (1.05 – 1.7 mg/dl), reduce idarubicin dose by 50%. If bilirubin > 34micromol/L (1.7 mg/dl) idarubicin is contraindicated.

In the case of renal impairment and serum creatinine 100 – 175 micromol/L (1.1 – 1.9 mg/dl) reduce idarubicin dose by 50%. A clinical decision is required on whether to dose the patient if the serum creatinine exceeds 175 micromol/L (1.9 mg/dl).

TABLE II: INDUCTION TREATMENT FOR HR PATIENTS



ATRA	**25 mg/m^2/ day orally in two divided doses for 30 days inclusive**
Idarubicin	**12mg/m^2/ day by iv. infusion over 1hr on days 1, 3, 5**

- Bone marrow assessment should be carried out following recovery of the neutrophil count to 1.0 x 10^9/l and the platelet count to 100 x 10^9/l, between 7-14 days after stopping ATRA, but not less than 7 days and should include material for minimal residual disease (BM and PB) monitoring. At this stage, BM morphology can be difficult to interpret and should not influence treatment. All patients proceed to the first block of consolidation therapy irrespective of morphological, cytogenetic or molecular status.
- Lumbar puncture should not be done at initial diagnosis because of the risk of bleeding and the low incidence of CNS involvement in APL.
- ATRA should be given for a total of 30 days.
- Note the following guidelines: Supportive Care during Induction therapy (section 10.1), Supportive Care Guidelines (section 13).
- Note the precautions to be taken during ATRA therapy – refer to Section 11.2 for treatment modifications which may be required and ensure familiarity with this section before commencing treatment.
- Note dose modifications which may be required for idarubicin. In the case of hepatic dysfunction and bilirubin 21 – 34 micromol/L (1.05 – 1.7 mg/dl), reduce idarubicin dose by 50%. If bilirubin > 34 micromol/L (1.7 mg/dl)idarubicin is contraindicated.

  In the case of renal impairment and serum creatinine 100 – 175 micromol/L reduce (1.1 – 1.9 mg/dl) idarubicin dose by 50%. A clinical decision is required on whether to dose the patient if the serum creatinine exceeds 175 micromol/L (1.9 mg/dl).

ATRA Dosing Table for APL Study 01 – dose 25mg/m^2/day				
Weight (kg)	SA (m^2)	ATRA dose to be given	Ideal total daily dose	Variance (%) from ideal dose
11 to 15	0.53 to 0.59	10mg daily	13 to 15mg	-23 to +33
16 to 29	0.62 to 1	10mg twice a day	16 to 25mg	+25 to -20
30 to 49	1.1 to 1.4	20mg each morning and 10mg at night	28 to 35mg	+7 to -14
50 to 69	1.5 to 1.8	20mg bd	38 to 45mg	+5 to +12
70 to 90	1.9 to 2.2	30mg each morning and 20mg at night	48 to 55mg	+4 to -10

10.1 Supportive Care During Induction:

1. ATRA Syndrome (Retinoic acid syndrome)

   Patients who present with a WBC ≥$10x10^9$/l should receive Dexamethasone 5 mg/m^2/dose (max single dose 10mg) 12-hourly iv. for the first 5 days of chemotherapy as prophylaxis against retinoic acid syndrome. Leukopheresis should not be performed due to risk of precipitating fatal hemorrhage.

2. Blood Product Support

   APL may be associated with a coagulopathy characterized by disseminated intravascular coagulation with secondary activation of the fibrinolytic system and hyperfibrinolysis. Early hemorrhagic death (EHD) (within the first 10-14 days of treatment) remains the main cause of death during induction therapy. ATRA significantly reduces EHD and improves the parameters of fibrinolysis with normalization of the d-dimers usually within the first 5-10 days after the start of chemotherapy.

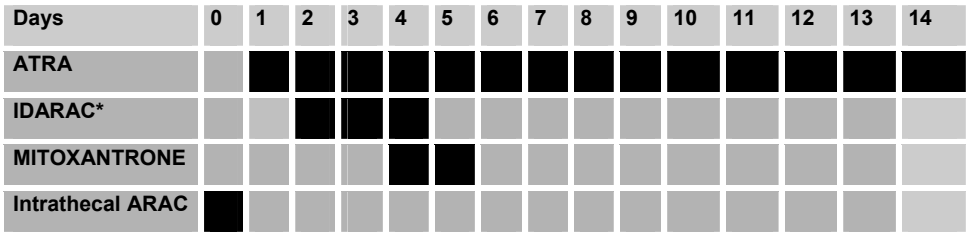
   a) During the first 10 days of induction therapy or until the coagulopathy has resolved.
      - Maintain platelet count above $50x10^9$/l.
      - Transfuse FFP and cryoprecipitate to correct PT, APTT and fibrinogen, maintaining a fibrinogen at least above 1.5 – 2.0 g/dl.

   b) After 10 days of induction therapy and ATRA or after the coagulopathy has resolved transfuse platelets and FFP according to national guidelines.

   c) Heparin and Tranexamic Acid should not be routinely used. Factor VIIa (Novoseven) may have a role in severe bleeding refractory to conventional treatment.

11. CONSOLIDATION CHEMOTHERAPY

TABLE III - CONSOLIDATION I FOR SR AND HR PATIENTS



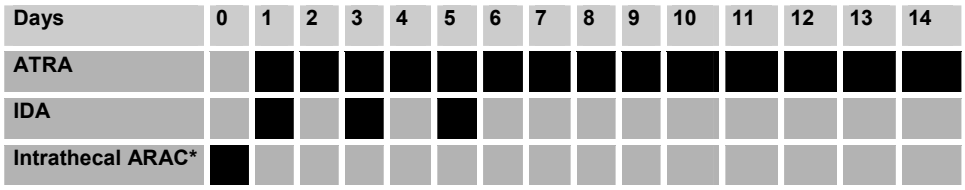
ATRA	**25 mg/m^2/day orally in two divided doses on day 1-14 inclusive**
IDARAC	**1 g/m^2 12 hrly by iv infusion over 3 hrs on day 2,3,4 (6 doses)**
MITOXANTRONE	**10 mg/m^2/day by iv infusion over 1hr on days 4, 5**
Intrathecal ARA-C	**dose adjusted by age (section 11.1) on day 0**

** Please note that Ara-C is cytarabine.*

- Consolidation I should start on recovery of the neutrophil count to 1.0 x 10^9/l and the platelet count to 100 x 10^9/l after induction therapy, but not earlier than 7 days after stopping ATRA.
- IT Ara-C should be given at the start of this block of therapy. The timing can be adjusted to allow this to be done simultaneously with the bone marrow following count recovery from the induction course of treatment. This is defined as a neutrophil count of 1.0 x 10^9/l and a platelet count of 100 x 10^9/l.
- If clinical condition and/or blood counts do not allow high dose chemotherapy to be given, start ATRA and give chemotherapy as soon afterwards as possible. If this takes more than 2 weeks, consult the P.I.
- Refer to ATRA dose modification guidelines (section 11.2) and Supportive Care guidelines (section 13).
- Prednisolone (0.5% Predsol) eye drops should be used during each course of IDARAC and be continued for 5 days after the course finishes – the dose is two drops into each eye every 2 hours.
- Note dose modifications which may be required for cytarabine. In the case of hepatic dysfunction and bilirubin > 34 micromol/L (1.7 mg/dl) each dose should be reduced by 50%. No dose reductions are required for renal impairment.
- Note dose modification which may be required for mitoxantrone. In the case of hepatic dysfunction and bilirubin > 60 micromol/L (3 mg/dl) the maximum dose should be 8mg/m^2.
- Bone marrow assessment should be carried out on count recovery and should include material (BM+PB) for minimal residual disease monitoring.

- The timing of IT Ara-C on day 0 of Consolidation II can be adjusted to allow this to be done simultaneously with the bone marrow following recovery from Consolidation I.

TABLE IV – CONSOLIDATION II FOR SR AND HR PATIENTS

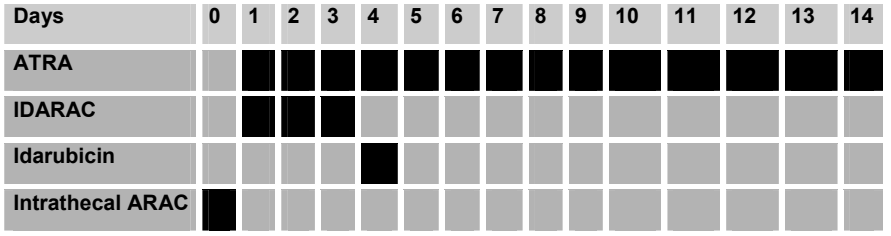


ATRA	**25 mg/m^2/day orally in two divided doses on days 1-14 inclusive**
Idarubicin	**5 mg/m^2/day by iv infusion over 1hr on days 1, 3, 5**
Intrathecal ARAC	**dose adjusted by age (section 11.1) on day 0**

* *Please note that Ara-C is cytarabine.*

- Consolidation II should start on recovery of the neutrophil count to 1.0 x 10^9/l and the platelet count to 100 x 10^9/l after consolidation I, but not earlier than 7 days after stopping ATRA.
- If clinical condition and/or blood counts do not allow high dose chemotherapy to be given, start ATRA and give chemotherapy as soon afterwards as possible. If this takes more than 2 weeks, consult the P.I.
- Refer to ATRA dose modification guidelines (section 11.2) and Supportive care guidelines (section 13).
- Note dose modifications which may be required for idarubicin. In the case of hepatic dysfunction and bilirubin 21 – 34 micromol/L (1.05 – 1.7 mg/dl), reduce idarubicin dose by 50%. If bilirubin > 34micromol/L (1.7 mg/dl) idarubicin is contraindicated.
- In the case of renal impairment and serum creatinine 100 – 175 micromol/L (1.1 – 1.9 mg/dl) reduce idarubicin dose by 50%. A clinical decision is required on whether to dose the patient if the serum creatinine exceeds 175 micromol/L (1.9 mg/dl)
- Bone marrow assessment should be carried out on count recovery and should include material (BM+PB) for minimal residual disease monitoring.
- The timing of IT Ara-C on day 0 of Consolidation III (or d1 of Maintenance for SR patients) can be adjusted to allow this to be done simultaneously with the bone marrow following recovery from Consolidation II.
- **Note: SR patients who are MRD negative after Consolidation II proceed to maintenance therapy. SR patients who are MRD positive after Consolidation II should receive Consolidation III.**

TABLE V – CONSOLIDATION III ONLY FOR HR PATIENTS AND SR PATIENTS MRD POSITIVE AFTER CONSOLIDATION COURSE II



ATRA	**25 mg/m^2/day orally in two divided doses on days 1-14 inclusive**
IDARAC*	**1 g/m^2 12 hrly by iv infusion over 3 hrs on day 1,2,3 (6 doses)**
Idarubicin	**10 mg/m^2/day by iv infusion over 1hr on day 4 only**
Intrathecal ARAC	**dose adjusted by age (section 11.1) on day 0**

** Please note that Ara-C is cytarabine.*

- Consolidation III should start on recovery of the neutrophil count to 1.0 x 10^9/l and the platelet count to 100 x 10^9/l after consolidation II, but not earlier than 7 days after stopping ATRA.
- If clinical condition and/or blood counts do not allow high dose chemotherapy to be given, start ATRA and give chemotherapy as soon afterwards as possible. If this takes more than 2 weeks, consult the P.I.
- Prednisolone (0.5% Predsol) eye drops should be used during each course of IDARAC and be continued for 5 days after the course finishes – the dose is two drops into each eye every 2 hours.
- Refer to ATRA dose modification guidelines (section 11.2) and Supportive care guidelines (section 13).
- Note dose modifications which may be required for cytarabine.In the case of hepatic dysfunction and bilirubin > 34 micromol/L (1.7 mg/dl) each dose should be reduced by 50%. No dose reductions are required for renal impairment .
- Note dose modifications which may be required for idarubicin.In the case of hepatic dysfunction and bilirubin 21 – 34 micromol/L (1.05 – 1.7 mg/dl),reduce idarubicin dose by 50%. If bilirubin > 34 micromol/L idarubicin is contraindicated.

  In the case of renal impairment and serum creatinine 100 – 175 micromol/L (1.1 – 1.9 mg/dl) reduce idarubicin dose by 50%. A clinical decision is required on whether to dose the patient if the serum creatinine exceeds 175 micromol/L (1.9 mg/dl)
- Bone marrow assessment should be carried out on count recovery and should include material (BM+PB) for minimal residual disease monitoring. The timing of IT Ara-C on day 1 of Maintenance Therapy can be adjusted

to allow this to be done simultaneously with the bone marrow following recovery from Consolidation III.

- **Note: MRD negative patients progress to maintenance therapy. MRD positive patients have failed first line treatment and should be treated according to the Refractory/Relapse guidelines.**

11.1 Inthrathecal Cytarabine (Ara-C) Doses Adjusted for Age:

AGE (years)	CYTARABINE
<1	15mg
1	20mg
2	25mg
3+	30mg

11.2 Treatment Modification during ATRA Therapy:

During induction treatment ATRA may be temporarily discontinued in the presence of one of the following complications: ATRA syndrome, pseudotumor cerebri or hepatotoxicity.

11.2.1 ATRA Syndrome (RAS) – incidence : 5-20%

Patients with hyperleukocytosis are at greatest risk of this complication, which can occur at any time from day 1 to day 35 after the start of induction therapy most commonly around day 7.

No one feature is diagnostic of the syndrome, but features include presence of: unexplained fever, weight gain, respiratory distress, interstitial pulmonary infiltrates, and pleural or pericardial effusion, with or without elevated WBC. However, at the earliest manifestations of suspected ATRA syndrome (e.g. unexplained respiratory distress), and prior to development of a full-blown syndrome, the following measures should be immediately undertaken:

- temporary discontinuation of ATRA treatment
- prompt initiation of dexamethasone, 5 mg/m^2 every 12 hours (max single dose 10mg) iv., until disappearance of symptoms and signs, and for a minimum of 3 days
- furosemide when clinically required

11.2.2 Pseudotumor Cerebri – incidence : 10-20%

This is commoner in children than adults and is defined as the presence of:

- severe headaches with nausea, vomiting, and visual disorders

It is often necessary to temporarily discontinue ATRA treatment and to administer opiates. Acetazolamide may improve symptoms at a dose of 8mg/kg three times a day orally or by iv

bolus for children up to the age of 12 years and 250-375mg three times a day for children over 12 years.

11.2.3 Hepatotoxicity:

This is defined as:

- an increase in serum bilirubin, AST/ALT, or alkaline phosphatase >5 times the normal upper level and may require a temporary suspension of the ATRA

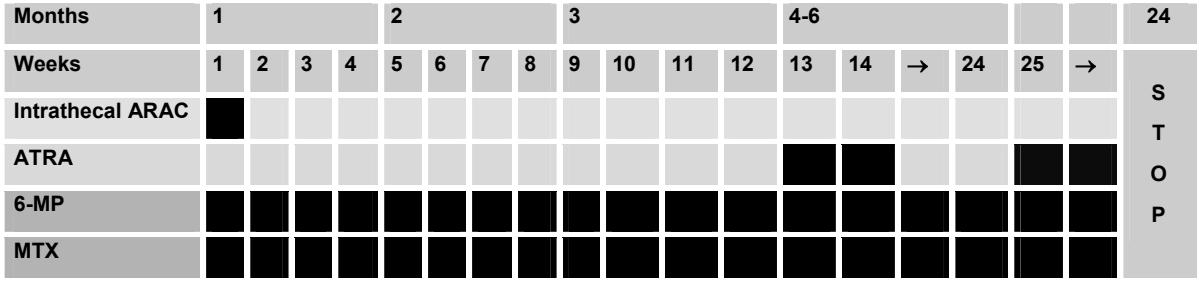
As soon as the symptoms and the patient's clinical condition improve, the treatment with ATRA can be resumed at 50% of the previous dose for the first 4 days after the disappearance of retinoic acid syndrome, amelioration of pseudotumor cerebri or when serum bilirubin, AST/ALT or alkaline phosphatase reduces to <4 times the normal upper level. Thereafter, in the absence of worsening of the previous toxicity, ATRA should be resumed at full dosage. In young patients, in whom it is not possible to resume ATRA at 50% of the previous dose because of the limitations of the preparation, ATRA should be restarted at full dose with caution.

In the case of reappearance of signs and symptoms of ATRA toxicity, the drug must be discontinued indefinitely during induction therapy. However, it should be included in subsequent consolidation courses. The occurrence of ATRA syndrome during induction is not a contra-indication to use of ATRA during consolidation or maintenance.

Other complications of ATRA include bone pain, dryness of skin, Sweets syndrome, hypercalcaemia, acute pancreatitis, thrombocytosis, headache, hypertriglyceridaemia, myalgia and cheilitis.

12. MAINTENANCE THERAPY

TABLE VI – MAINTENANCE THERAPY



Intrathecal ARAC	**dose adjusted by age (section 11.1) on day 1 only**
ATRA	**25 mg/m^2/day orally in two divided doses for the first 14 days in every 12 wks**
6-MP (mercaptopurine)	**50 mg/m^2/once daily orally**
MTX (methotrexate)	**25 mg/m^2/once weekly orally**

- Patients must be MRD negative before starting maintenance therapy.
- Maintenance treatment should start once the neutrophil count is 1.0 x 10^9/l and platelet count is 100 x 10^9/l but not earlier than 7 days after completing ATRA therapy. In case blood counts do not recover, contact the national clinical coordinator.
- **6-Mercaptopurine** (6-MP), 50 mg/m^2/day orally to be given as a single dose. Doses should be taken at least one hour after the evening meal without milk products. The dose will be adjusted according to the neutrophil and platelet count (dose adjustment as stated in section 12.1). Maintenance treatment must be continued for 2 years.
- **Methotrexate** (MTX), 25 mg/m^2 weekly orally to be given as a single dose. The dose will be adjusted according to the neutrophil and platelet count (dose adjustment as stated in section 12.1). Maintenance treatment must be continued for 2 years.
- **ATRA 25 mg**/m^2/day orally, to be given in two divided doses for the first 14 days in every 12 weeks, for the 2 years of maintenance. The first ATRA maintenance course will begin three months after recovery from the last consolidation course ie the first 12 week maintenance cycle will NOT include ATRA. MTX and 6-MP will be continued during ATRA therapy
- **Cotrimoxazole** as prophylaxis against Pneumocystis carinii pneumonia (PCP) should be given throughout maintenance therapy according to local policy.

Surface Area	Co-trimoxazole	Trimethoprim	Sulphamethoxazole
0.5-0.75 m^2	240 mg bd	40 mg bd	200 mg bd
0.76-1.0 m^2	360 mg bd	60 mg bd	300 mg bd
over 1 m^2	480 mg bd	80 mg bd	400 mg bd

bd = twice daily

Co-trimoxazole will be given orally on two consecutive days per week throughout maintenance treatment. If a child remains cytopenic after being off chemotherapy for 3 weeks or more, co-trimoxaxole should be stopped. Co-trimoxazole should be re-introduced when the child is back on optimal doses of 6 MP and MTX.

If cytopenias recur with the reintroduction of co-trimoxazole, the drug should be stopped for at least 2 months. The maintenance of optimal doses of 6MP and MTX should take precedence over continuing co-trimoxazole. However, it should be remembered, that if co-trimoxazole is stopped, the child is at increased risk of PCP and consideration should be given to the use of alternative prophylaxis, such as nebulized pentamidine or oral dapsone.

Modification of Maintenance Doses of both 6MP and MTX:

- WBC below 1.5 x 10^9/l: temporary discontinuation of maintenance
- WBC between 1.5 - 2.5 x 10^9/l: reduce doses by 50%
- WBC between 2.5 - 3.5 x 10^9/l: maintain doses, except if neutrophil count is below 0.5 x 10^9/l when maintenance should be temporarily discontinued
- WBC repeatedly above 3.5 x10^9/l, and neutrophils > 1.0 x 10^9/l, increase doses by 25%
- Platelets between 50 - 75 x 10^9/l: reduce doses by 50%
- Platelets <50 x 10^9/l: temporary discontinuation of maintenance

Minimal Residual Disease Monitoring During Maintenance Therapy

All patients should be monitored by 3-monthly paired marrow and blood samples during maintenance and for 1 year after completion of maintenance. Samples should be taken before ATRA therapy during each 12-weekly cycle. All samples should be sent to the designated national representative laboratory, ensuring arrival within 24hours to minimize RNA degradation on transit, which compromises assay sensitivity (see section 7.5.2). In some instances samples may need to be repeated earlier if the preceding sample affords suboptimal sensitivity (below 10^{-4}) or suggests possibility of low level MRD.

13. SUPPORTIVE CARE

The remission induction and consolidation phases of therapy are intensive and will be associated with a risk of infection and hemorrhage. Local supportive care policies should be in place to minimize treatment complications, which should include:

- Venous access via Central Venous catheter: insertion delayed until coagulopathy resolved.
- Control of nausea and vomiting
- Mouth care
- Response to a significant pyrexia — i.e. two readings of ≥38°C 30 minutes apart or a single reading ≥38.5°C.
- Antibiotic treatment of febrile episodes — including antibiotic choice(s) and monitoring, duration of therapy, and the treatment of non-response
- Antifungal prophylaxis – azoles should be avoided or at least given with caution during ATRA therapy. ATRA is metabolized by cytochrome P450 and azoles inhibit cytochrome P450.
- G-CSF therapy may be given as clinically indicated but it should **not** be given routinely
- Cotrimoxazole as prophylaxis against pneumocystis carinii pneumonia should be given throughout maintenance therapy.
- **ATRA** is teratogeneic and effective contraception must be used in appropriate patients.

14. REFRACTORY/RELAPSE THERAPY

Patients on this study will be regularly monitored for the PML-RARα fusion transcript which should identify those with molecularly resistant disease and therefore at high risk of relapse, and those in whom the PML-RARα fusion transcript reappears having been previously undetected on repeated testing; both situations should be detected in the absence of overt hematological disease. Without molecular monitoring and pre-emptive therapy, approximately 10-20% of children with APL treated on ATRA and anthracycline based chemotherapy will suffer a frank hematological relapse. Marrow relapse is much commoner than CNS relapse, which rarely occurs in isolation[49]. MRD monitoring should allow the identification of disease when the leukemic burden is still low when delivery of pre-emptive therapy using arsenic trioxide (ATO) is significantly less likely to induce hyperleukocytosis and the associated differentiation syndrome, as compared to treatment at time of hematologic relapse[43]. Moreover, GIMEMA and PETHEMA group studies have indicated a survival advantage for pre-emptive therapy over salvage treatment once the patient has clinically overt disease[20,25,34]. Therefore serial MRD monitoring by RQ-PCR, to enable delivery of pre-emptive therapy, will be adopted in this study.

14.1 Definitions:

Hematological relapse: re-appearance of promyeloblasts/abnormal promyelocytes (>5%) in the bone marrow.

Molecular relapse: for the purposes of this study this will be defined as the reappearance of the PML-RARα fusion transcript after repeatedly previously negative RQ-PCR assays, detected in two successive samples and confirmed in an independent laboratory (see sections 7.5.2 and 9.3.3).

Refractory disease: is defined as the persistent detection of morphological, cytogenetic or molecular evidence of APL after consolidation therapy.

14.2 Treatment of Refractory/Relapsed APL:

Patients with hematological or molecular refractory disease and those with molecular relapse will be treated according to the same refractory/relapse treatment guidelines. Patients in whom hematological/morphological, cytogenetic or molecular evidence of APL is persistently detected after consolidation therapy, and those with molecular relapse, will be treated with arsenic trioxide (ATO). ATO will be given initially on an inpatient basis because of potential toxicity. Those with frank relapse and a WBC>10x10^9/l will **in addition to ATO** receive GO (3mg/m^2) and at least two doses of Rasburicase on day 1. **A second dose of GO should be considered on day 4 if the WBC has not fallen below 10x10^9/l**. These patients require close clinical and biochemical monitoring for evidence of ATO toxicity, differentiation syndrome and/or tumour lysis syndrome.

14.2.1 ATO (Arsenic Trioxide) Toxicity Profile

The most prominent adverse events with ATO in APL have included weight gain and fluid retention, leukocytosis, differentiation syndrome, and prolongation of the QTc interval on the electrocardiogram.

Peripheral neuropathy, hyperglycaemia, and cutaneous reactions have also been described. A full toxicity profile is outlined in Appendix IV.

14.2.2 ATO Dosing Regimen

Please note that ATO is arsenic trioxide. The preparation to be used is Trisenox.®

- **Course 1:**

The standard dosing schedule (based on ideal body weight) for all children is 0.15mg/kg daily by intravenous infusion over 1-2 hours and continued for 50 days, at which stage disease status is assessed by bone marrow morphology and RQ-PCR. If acute vasomotor reactions occur, the infusion duration may be extended to 4 hours.

- ATRA Phase:

A period of at least three weeks will occur between ATO treatments, course 1 and 2. During this period ATRA, 25 mg/m^2/day will be administered orally in two equally divided doses. ATRA treatment will stop the day prior to the ATO course 2.

- **Course 2:**

All patients should receive a second course of ATO according to the standard schedule at a dose of 0.15mg/kg/day 3-4 weeks after completion of the first course. This should be given as a once daily intravenous infusion over 1-2 hours, 5 days per week, followed by 2 days of rest, for a maximum of 5 weeks (25 doses).

- **Dosing Schedule for ATO (Arsenic Trioxide):**

ATO COURSE 1	ATRA PHASE	ATO COURSE 2
0.15 mg/kg/day given daily for 50 days	At least 3 weeks rest from ATO – ATRA to be given at 25mg/m^2/d during this period before starting ATO course 2	0.15 mg/kg/day given 5 times per week (25 doses in total)

14.3 Monitoring patients:

Careful monitoring is required for the following complications that may occur in patients with relapse/refractory APL who are being treated with ATO and treatment should initially be given as an inpatient:

- Leukocyte Activation Syndrome (APL differentiation syndrome)
- Prolongation of the QT/QTc interval seen on the ECG
- Leukocytosis

Leukocyte Activation Syndrome (APL Differentiation Syndrome)

Twenty-five percent of patients with frank relapse of APL treated with ATO experience symptoms similar to the ATRA syndrome (RAS). RAS or APL differentiation syndrome is characterized by fever, dyspnoea, weight gain, pulmonary infiltrates and pleural or pericardial effusions, with or without leukocytosis and can be fatal. High-dose steroids given early at the first suspicion of the APL differentiation syndrome appear to mitigate signs and symptoms. Irrespective of the leukocyte count treatment should be initiated promptly with:

- dexamethasone, 5 mg/m^2/dose every 12 hours (max single dose 10mg).
- and continued until disappearance of symptoms and signs, or for a minimum of 3 days.

The majority of patients do not require termination of ATO therapy during treatment of the APL differentiation syndrome.

Prolongation of the QT/QTc Interval seen on the ECG

ATO can cause QT interval prolongation, complete atrioventricular block and can lead to torsade de pointes. Drugs that can prolong the QT interval should be avoided (for a list of drugs visit www.torsades.org and see Appendix IV) as should drugs and conditions that can lead to hypokalaemia and hypomagnesaemia.

In clinical trials, 40% of patients treated with ATO experienced at least one QT corrected (QTc) interval prolongation greater than 500 msec. Prolongation of the QTc was observed between 1 and 5 weeks after ATO infusion, and then returned to baseline by the end of 8 weeks after ATO Infusion[50.]

ECG and Electrolyte Monitoring Recommendations

Prior to initiating therapy with ATO, a 12 lead ECG must be performed and serum electrolytes (potassium, calcium and magnesium) and creatinine must be assessed; pre-existing electrolyte abnormalities must be corrected and, if possible, concomitant drugs known to prolong the QT interval should be discontinued. Patients with risk factors for

QTc prolongation or torsade de pointes should be monitored with continuous cardiac monitoring (ECG).

For QTc greater than 500 msec, corrective measures must be completed and the QTc reassessed with serial ECG's prior to ATO.
During therapy with ATO, potassium concentrations must be kept above 4mEq/l and magnesium concentrations above 1.8mg/dl (0.74mmol/l). Patients who reach an absolute QT interval value > 500 msec must be reassessed and immediate action taken to correct concomitant risk factors, if any, and the risk/benefit of continuing versus suspending ATO therapy must be considered.

If syncope, or a rapid or irregular heartbeat develops, the patient must be hospitalized and monitored continuously. Serum electrolytes must be monitored and abnormalities corrected, ATO therapy must be temporarily discontinued until the QTc interval shortens to below 460 msec and until the syncope and irregular heartbeat cease. There are no data on the effect of ATO on the QTc interval during infusion. Electrocardiograms must be obtained twice weekly, and more frequently for clinically unstable patients, during induction and consolidation.

Drugs to Avoid/Use with Caution

As noted above, it is advisable to avoid concomitant use of drugs known to prolong the QT interval. Please note that arsenic trioxide has no inhibitory activity on substrates of the major cytochrome P450 enzymes and therefore drugs that are substrates for these P450 enzymes are not expected to interact with ATO.

Drugs to AVOID include amiodarone, sotalol, antipsychotics, intravenous erythromycin, terfenadine, astemizole.

Drugs to use with caution include ketoconazole, itraconazole, voriconazole, fluconazole, oral erythromycin, clarithromycin, ciprofloxacin, high dose ondansetrone and intravenous pentamidine. As it may be necessary to give some of these drugs at the same time as ATO, extra monitoring may be advisable.

Leukocytosis

Induction treatment with ATO for APL patients in frank relapse has been associated with the development of hyperleukocytosis ($\geq$10 x 10^9/L) in approximately half of patients[49,51]. There appears to be no relationship between baseline WBC and the development of hyperleukocytosis or a correlation between baseline and peak WBC.
The leukocytosis resolves on continuation of ATO, normalizes with remission and no additional treatment is required, apart for careful observation for development of differentiation syndrome (see above).

14.4 Type of and Time to Transplantation:

The response to ATO should be assessed by RQ-PCR after Course 1 and again after Course 2. Those patients who are PCR negative in the marrow following Course 2 of ATO will have a further course of ATO, dose scheduling as in course 2 (0.15mg/kg/day x 5 days x 5 – according to the standard dosing schedule) plus gemtuzumab ozogamicin (GO) (3mg/m^2 on day 1 and again on day 15).

Patients in molecular remission will have ongoing MRD monitoring and each individual case should be discussed with the Trial Coordinator. As some of these children will be cured, transplantation will be reserved for those who experience a molecular relapse. Maintenance treatment must be considered in patients that remain PCR negative.

In those patients who again have molecular disease after the 2^{nd} course of ATO, treatment will be with a further course of ATO + GO (but at 6mg/m^2), and any additional treatment required to achieve molecular remission before proceeding to un-purged autologous BMT. Patients should have bone marrow harvested following this course providing they are in molecular remission. An aliquot of harvested stem cells (blood or marrow) should be sent for molecular analysis. Only patients in molecular remission with an adequate PCR negative harvest should be considered for autologous transplantation.

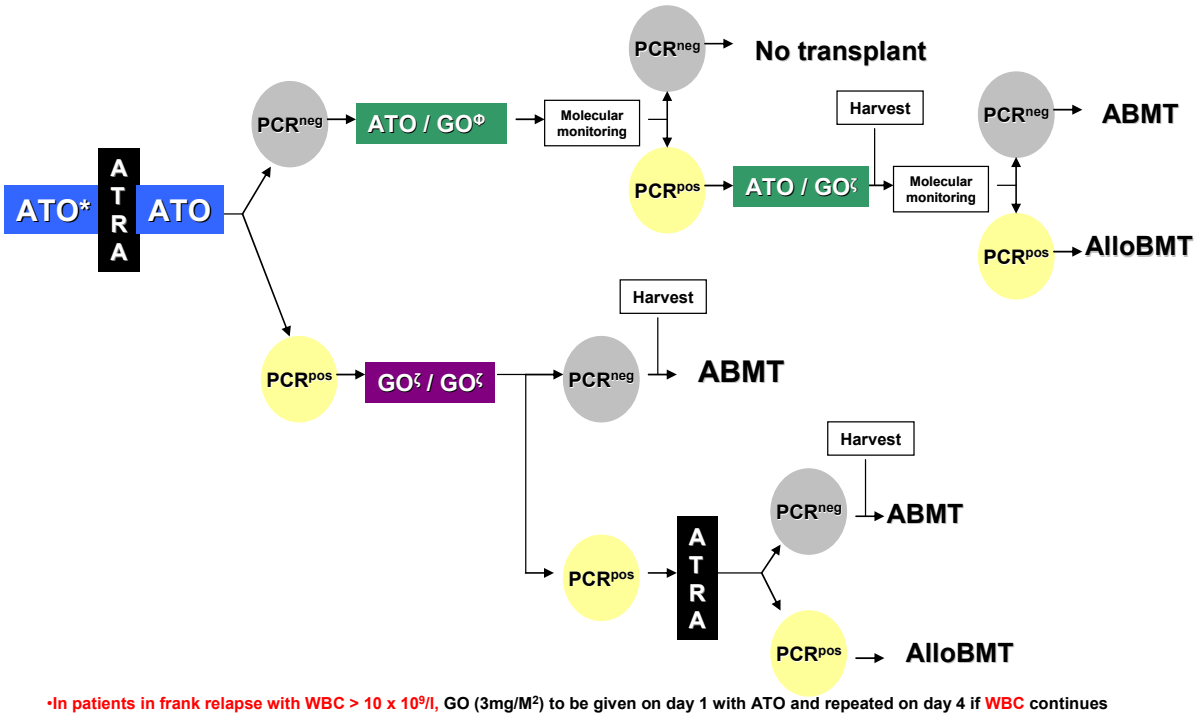
Those patients who continue to show persistent PCR positivity after the second block from course 1 of ATO treatment will be offered GO in accordance with previously published data[45-47]. GO will be administered twice at a dose of 6 mg/m^2 at a 15 day interval i.e. the second dose of GO at 6mg/m^2 will be given 15 days following the first dose.

The bone marrow (and blood) MRD status should be assessed on regeneration after the second dose of GO.

Patients who test PCR-negative after the second GO administration should proceed to ABMT, subject to obtaining a satisfactory PCR negative stem cell harvest. Patients who test PCR-positive after 2 courses of GO should receive ATRA 25mg/m^2/day orally in two divided doses until MRD negative, or for a maximum of 30 days.

Patients who test PCR negative after ATRA therapy proceed to ABMT. Patients who fail to achieve PCR-negativity or from whom a satisfactory PCR negative stem cell harvest cannot be obtained are candidates for alloBMT (see Figure 2).

FIGURE 2: Therapeutic Roadmap for children with APL who relapse or who are primary refractory



•In patients in frank relapse with WBC > 10 x 10^9/l, GO (3mg/M^2) to be given on day 1 with ATO and repeated on day 4 if WBC continues

•to be > 10x10^9/l Φ = GO at 3mg/m2, ζ = GO at 6mg/m2

14.5 Administration of gemtuzumab ozogamicin (GO, Mylotarg®):

GO should not be given to patients whose liver function tests exceed twice the upper limit of normal and should be given with rasburicase cover to those patients with a WBC greater than 10 x 10^9/l at the time of GO administration because of the risk of tumour lysis.

Patients should not be given azole antifungal drugs until day 5 after the administration of GO and for patients who have been taking a prophylactic azole this should be discontinued 5 days before the GO dose is due.

Patients will receive a GO dose of 3mg or 6mg protein/m^2 and will receive premedication with paracetamol, methylprednisolone and iv chlorphenamine 1 hour before starting the GO infusion. Paracetamol may be repeated as necessary in the event of fever and chills occurring. Vital signs must be monitored during the infusion and for 4 hours following its completion.

The reconstituted dose of GO should be diluted in 100ml sodium chloride 0.9% and infused over 2 hours. When aseptically prepared under controlled, validated conditions in the pharmacy, the diluted dose

may be stored for up to 16 hours at room temperature and must be protected from light (applies during infusion also).

It is important to note that the infusion must be completed within 20 hours of reconstitution.

14.6 Treatment of Extramedullary Relapse (EMR) +/- MRD positivity in BM:

Extramedullary relapse has been reported most often in the skin, CNS and testes. The majority of patients in whom there is a CNS component to relapse initially present with high risk disease (WBC >10x10^9/L)[52,53].

Moreover, the majority of patients with extramedullary disease will have morphological or molecular evidence of marrow involvement and should be treated as if they have MRD positivity in the BM. Approximately 10% of patients will have a CNS component at the time of relapse and therefore CNS involvement should be actively sought at this time.

14.7 Treatment for CNS relapse:

Patients with CNS relapse will receive both systemic and intrathecal treatment.

14.7.1 Triple intrathecal chemotherapy (see table below) should be given every 3 days until blasts clear from the CSF. A further two triple intrathecal injections should be given at 7 day intervals.

14.7.2 When CNS remission has been achieved systemic chemotherapy should begin. IT MTX, ara-C and hydrocortisone at age adjusted doses should be given on Day 1 of both induction and consolidation blocks. In case of combined systemic and CNS relapse, systemic chemotherapy should start at onset, with triple intrathecal and subsequent intrathecal triple therapy as described above.

<u>Induction</u>

- Ara-C 3g/m^2/day by iv infusion over 3 hours 12-hourly on days 1,3 and 5 (six doses). Remember prednisolone 0.5% eye drops - two drops into each eye every 2 hours, continuing for 5 days after the last cytarabine dose.
- GO will be given at a dose of 3mg protein /m^2 on day 1 of induction
- ATRA 25mg/m^2 / day orally in two divided doses on days 1-14 inclusive
- IT MTX, ara-C and Hydrocortisone : dose adjusted by age on day 1

<u>Consolidation</u>

- Ara-C 3g/m^2 /day by iv infusion over 3 hours 12-hourly on days 1,3 and 5 (six doses). Remember prednisolone 0.5%

eye drops - two drops into each eye every 2 hours, continuing for 5 days after the last cytarabine dose.

- GO will be given at a dose of 3mg protein /m^2 on day 1 of consolidation.
- ATRA 25mg/m^2 / day orally in two divided doses on days 1-14 inclusive
- IT MTX, ara-C and Hydrocortisone : dose adjusted by age on day 1

AGE (years)	METHOTREXATE	HYDROCORTISONE	CYTARABINE
<1	5.0mg	5.0mg	15mg
1	7.5mg	7.5mg	20mg
2	10mg	10mg	25mg
3+	12.5mg	12.5mg	30mg

Patients who test PCR-negative after consolidation should proceed to ABMT, subject to obtaining a satisfactory PCR negative stem cell harvest. Those patients who test PCR-positive after consolidation are candidates for allogeneic transplantation.

Patients who do not receive irradiation as part of their transplant procedure should receive cranial radiation at a dose of 2400 cGy.

15. DATA MANAGEMENT.

Remote data entry will be available by a web-based registration system housed in Bologna (Italy) at the organization CINECA. Data management coordinators will be informed about the procedure. Case record forms will be provided for national groups who wish to use paper forms instead of the web-based system. These forms should then be forwarded to CINECA by the central data office of each participating group. Each national group will be responsible for the organization of the collection of their national data prior to receipt by CINECA.

The website address of CINECA is www.apl.cineca.org

16. STATISTICAL CONSIDERATIONS

This concerns a non-randomized risk-group stratified therapeutic study limiting exposure to anthracyclines and using molecular monitoring of minimal residual disease to guide therapy. The study is planned to run for 5 years and follow up will be for a minimum of 10 years. It is expected that 60-70 patients (a total of 300) per year will be recruited. Endpoints of the study (section 3.3) will be determined in eligible patients, in whom the diagnosis has been molecularly confirmed and in whom molecular monitoring of MRD has been performed as prescribed in the study. It is anticipated that less than 10% of recruited patients will be ineligible for molecular monitoring because of an absence of a suitable specimen.

Stopping Rules:

An interim analysis on MRD status will be performed every 6 months after the start of the study. It will be based on the number of courses (denoted by T) which are required to produce MRD negativity and allow for the "uncertainty" about patients who are still undergoing treatment at the time of the interim analysis. The "event" for the survival analysis is achieving MRD negativity. The "critical" point is not being MRD negative after T=4 courses which would lead to entry into the Refractory/Relapse protocol.

Patients will be scored as follows at the time of the interim analysis:

- Patients who are already MRD negative have T=1, 2, 3 or 4 (and are not "censored").
- Patients that enter the Refractory/Relapse protocol are "censored" at T=4, implying that they are still MRD positive at this time point.
- Patients for whom the outcome is still unknown are censored at T=1, 2 or 3, depending on their stage in the treatment protocol.

Kaplan-Meier curves (+ 95% confidence interval) will be produced for SR, HR (as defined at diagnosis) and the total group.

The Kaplan-Meier probability at T=4 is the best estimate of the probability of entering the Refractory/Relapse protocol.

The trial is stopped if the probability of being MRD positive at T=4 in the total group is significantly greater than 10%, with a two-tailed 95% confidence interval above 10%.

The Data safety and monitoring committee will make recommendations about whether or not the study should stop if the MRD positivity probabilities at earlier T-values, and/or in the subgroups, are higher than expected.

Various outcomes for this stopping rule (based on 10,000 simulations) are given in the Table below:

Scenario	% MRD after1 course (T1)	% MRD after 2 courses (T2)	% MRD after 3 courses (T3)	% MRD after 4 courses (T4)	**Probability of early stopping (%)**	Mean duration of study (months)
1	76	20	08	05	**0.1**	62
2	80	40	20	10	**6.1**	60
3	80	50	25	12	**26**	54
4	90	60	30	15	**76**	39
5	90	70	40	20	**99.8**	20

Scenario 1 gives the MRD positivity percentages observed in the MRC AML15 trial for APL patients.

Scenario 2 gives the limits of acceptable MRD positivity percentages. In scenarios 3-5 the trial should be stopped. Note that the trial is stopped early in scenario 5.

Stopping rules will only apply to patients treated by groups to whom the EU Directive applies.

Data on MRD status will be made available to the Data Safety and Monitoring Committee at 6 monthly intervals along with the number of molecular and frank relapses and the probability of overall survival. These data will be compared to published data on incidences of molecular and frank relapse and overall survival. The MRD status will be considered in the context of the stopping rules.

17. STUDY GOVERNANCE AND ADVERSE EVENT REPORTING

The EU Directive on Good Clinical Practice in the Conduct of Clinical Trials requires the investigators to report unexpected Serious Adverse Events (SAEs). The study will be monitored by an independent Data Safety and Monitoring Committee.

17.1 Data Safety and Monitoring Committee:

Prof R. Foá
Prof S. Piantadosi
Prof M.S. Tallman

17.2 Adverse Event Reporting:

Principal Investigators from each participating institution have an obligation to report relevant and unexpected Serious Adverse Events (SAE) which occur in this study to their national clinical trials office for forwarding to CINECA in a timely manner. It is recognized that adverse events which may be life-threatening, are a normal consequence of APL or its effective treatment, and many clinical changes in the patient's condition are expected.

Definitions

For the purpose of this study a **Serious Adverse Event (SAE)** is defined as an event that:

- results in death
- is life threatening
- requires unexpected hospitalization or unexpected prolongation of existing hospitalization
- results in persistent or significant disability or incapacity

The following should NOT be reported as SAEs:

- hospitalization due to febrile neutropenia
- death following relapse (of course death must be recorded later-on)
- pre-existing toxicities which meet the criteria set out above; it is only the *development* of these toxicities after entering the study which should be reported.

Expected Serious Adverse Drug Reaction or Event (SSAR)

All complications as a result of severe bone marrow failure, the adverse reactions or events described in Appendix III and IV, and those described in the summary of the product characteristics for each protocol drug are 'expected', even if they result in death. These will therefore be categorized as SSARs

Adverse Events (AEs)

NCI Grade 3 or 4 toxicities should be reported on the toxicity form. There is no need to report Grade 1 and 2 toxicities.

17.2 Causality:

Investigators will be asked to record their opinion as to whether the SAE as defined above was related to the study medication. This will be further reviewed by the Study Management Group.

17.3 Collection of Data:

Preliminary discussion of the event may take place with the chief Investigator. SAEs should be recorded on the Adverse Event Form. SAEs in one participating country will be available to all participating groups and should initially be forwarded to the appropriate national clinical trials unit and then forwarded to the CINECA

17.4 Time of Report:

Any death that is clearly **not** due to, or associated with**,** persistent or progressive disease should be reported to the trial office within 24 hours.

17.5 Reporting to the Regulatory Authorities:

The national clinical coordinators or his/her nominee will review and record all SAEs. He/she will be responsible for reporting the events to the appropriate regulatory authorities and to the Study Steering Committee in the appropriate timelines

18. AUTHORSHIP GUIDELINES

A participating group/center agrees to provide data as required for proper analysis of this study and as made clear by case record forms (CRF's). Data submitted to the central organization (CINECA) will remain the property of the group (or individual center) that submitted it. However, it is agreed that the submitted data will be used for intergroup analyses of the study endpoints as agreed and stated in the protocol. Until the intergroup study endpoints have been reported and published in (a) peer-reviewed journal(s), no individual group or center should report or publish data related to the study endpoints. The (blinded) data, however, can be presented at regional and closed meetings including the annual I-BFM-SG meeting, provided the data are not made available publicly.

Data analyses and publications on aspects other than the study endpoints, but concerning the patients that enrolled in the collaborative study, are encouraged. It is also encouraged but not a requirement that such additional projects are being done in a collaborative setting.

Authorship will be granted to the members of the writing committee of this study, to the international coordinators of molecular diagnosis and monitoring, and to one clinical and one molecular diagnosis and monitoring coordinator of each participating group that enrols at least 5 patients in the study, and anyone else who made an important contribution to the study.

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APPENDIX II: ATRA Dosing and Administration

ATRA dosing table calculated at a dose of 25mg/m^2/day				
Weight (kg)	SA (m^2)	ATRA dose to be given	Ideal total daily dose	Variance (%) from ideal dose
11 to 15	0.53 to 0.59	10mg daily	13 to 15mg	-23 to +33
16 to 29	0.62 to 1	10mg twice a day	16 to 25mg	+25 to -20
30 to 49	1.1 to 1.4	20mg each morning and 10mg at night	28 to 35mg	+7 to -14
50 to 69	1.5 to 1.8	20mg bd	38 to 45mg	+5 to +12
70 to 90	1.9 to 2.2	30mg each morning and 20mg at night	48 to 55mg	+4 to -10

Tretinoin (ATRA) is only available as a 10mg capsule and the preparation is viscous and light sensitive making it difficult to give a fraction of a capsule.

- Dose banding by weight is suggested. The greatest variance from the ideal calculated dose is in small children. These children should start with a daily dose of 10mg and in the absence of toxicity consideration should be given to increasing the dose to 10mg bd or alternate day dosing with 10mg alternating with 20mg.

- Because of the limitation of the preparation it will not always be possible to split the dose into two equal doses. Where the dose cannot be equally split, it is suggested that the larger dose be given in the morning.

- Guidance on cutting open the ATRA capsules and administering ATRA for children too young to take capsules whole is given below.

- Giving ATRA by nasogastric tube is very difficult and the dose unreliable because the preparation sticks to the tube. However, in the rare situation where there is no alternative, such as for ventilated patients, a guideline on administration is given below .

- It is suggested that
  - topical vitamin E (or lip salve enriched with vitamin E) should be applied to the lips twice a day during ATRA therapy if cheilitis develops.
  - Direct sun exposure should be avoided whilst on ATRA therapy .
  - Exposure to vitamin A products should be avoided whilst on ATRA therapy.

<u>Guidelines for the cutting ATRA (Tretinoin, all-trans retinoic acid, Vesanoid®) capsules and administering ATRA.</u>

Tretinoin (ATRA) capsules will be given as 10mg capsules.

The following guidelines have been prepared to maximise the amount of drug recovered from the capsule and to minimise the risk of skin contamination, especially to women of childbearing age.

Gloves must be worn for this procedure.

1. Remove the number of capsules required for the dose from the bottle and place in a plastic medicine pot.

2. Assemble equipment:
- 1 pair non-sterile gloves
- Small pair sharp clean scissors (to be used only for this purpose)
- 1 dessert spoon
- 1 teaspoon
- 1 small tray
- Small portion of ice cream/yoghurt
- Kitchen roll, kept just for this purpose
- Cytotoxic waste bin
-
3. Put on gloves.

4. Place dessert spoon on clean surface.

5. Take a capsule between finger and thumb and hold upright firmly. With the scissors snip the tip off the capsule in to the tray to avoid any possible harm to the eyes.

6. Carefully squeeze the contents of the capsule on to the dessert spoon.

7. Discard empty capsule in cytotoxic waste bin.

8. Use kitchen roll to wipe any drug from gloves then dispose of kitchen roll immediately in the cytotoxic waste bin.

9. Repeat steps 5 to 8 for each capsule needed.

10. After all the required capsules have been snipped, use the teaspoon to place some soft ice cream or yoghurt on to the dessert spoon.

11. Using the teaspoon mix the ice cream/yoghurt and medicine together.

12. Give medicine to child.

13. Clean surfaces with kitchen roll and wash all equipment, including scissors, in hot soapy water.

14. Dispose of gloves in cytotoxic waste bin.

15. Wash hands thoroughly.

16. Return cytotoxic waste bin to hospital when full.

Guidelines for the administration of ATRA (Tretinoin, all-trans retinoic acid, Vesanoid®) by nasogastric tube.

The guidelines are based on the method reported by Shaw.[54.] However, this mode of administration should only be used in exceptional circumstances. The technique is challenging for professionals and would be unsuitable for parents during the maintenance phase of treatment and therefore the nasogastric route should only be used for intubated patients.

- The ATRA capsules should be cut open and the contents aspirated via a 19-gauge needle into a syringe primed with 1 mL of soybean oil.

- Small amounts of safflower oil (better taste than soybean oil) should be mixed with the remaining contents in the capsule to allow more of the active drug to be drawn into the syringe. The resulting mixture of ATRA and soybean oil should be uniformly stirred and placed in a glass syringe and administered via a nasogastric tube or orally for patients unable to swallow capsules or intubated.

- This formulation is stable for 24 hours after preparation, and can be used within that time period.

- An alternative approach might be the use of a relatively newly developed intravenous liposomal ATRA compound which has been studied mostly in adults with APL.[55,56] Liposomal ATRA is usually given at 90 mg/m^2 intravenously every other day for remission induction until complete remission (CR). It is manufactured in the US by Aronex Pharmaceuticals as Atragen (R). Since liposomal ATRA is expensive and not readily available in all countries, it is recommended only in patients who do not tolerate liquid ATRA, as described above, and in countries where the drug is available.

APPENDIX III: General Drug Details

- **Cytosine Arabinoside** — Ara-C, Cytarabine

  Cytarabine is available as a 20mg/ml injection for intravenous and intrathecal administration.

  Dose: 1000mg/m^2 intravenously over 3 hours in Consolidation I and III, and 3000mg/m^2 iv over 3 hours for refractory/relapsed therapy.

  Age-related dose 15mg, 20mg, 25mg or 30mg by intrathecal injection during Consolidation blocks and at the start of Maintenance. For intrathecal administration,only preservative-free drug must be used.

  Note that different doses are used in the case of CNS relapse.

  The vials of drug are stored at room temperature.

  In patients with impaired hepatic function (bilirubin >34umol/L) the dose should be reduced by 50%. No reductions are necessary for renal impairment.

  Side effects at the doses prescribed include bone marrow depression, nausea, diarrhoea, oral ulceration and hepatic dysfunction. A cytarabine syndrome has also been described. It is characterised by fever, myalgia, bone pain, occasional chest pains, maculopapular rash, conjunctivitis and malaise. It usually occurs 6-12 hours following administration, and is more common with higher doses.

- **Gemtuzumab Ozogamicin** — Mylotarg™ (CMA-676), Wyeth Genetic Institute

  Gemtuzumab ozogamicin (GO) for injection is supplied as an amber glass vial containing 5mg of GO lyophilized powder. This vial should be refrigerated (2-8ºC).

  <u>Preparation</u>

  The drug product is light sensitive and must be protected from direct and indirect sunlight and unshielded fluorescent light during the preparation and administration of the infusion. **All preparation should take place in a biologic safety hood with the fluorescent light off**. Reconstitute the contents of each vial with 5ml water for injection. Gently swirl each vial. Each vial should be inspected to ensure dissolution and for particulates. The final concentration of drug in the vial is 1mg/ml. This solution may be stored refrigerated (2-8° C) and protected from light for up to 8 hours. Reconstituted vials of drug should not be frozen.

  Before administration, withdraw the desired volume from each vial and inject into a 100ml bag of 0.9% Sodium Chloride. Place the 100-ml bag into an UV protectant bag. The resulting drug solution in the IV bag may be stored for up to 16 hours at room temperature.

Administration

DO NOT ADMINISTER AS AN INTRAVENOUS PUSH OR BOLUS
Once the reconstituted GO is diluted in 100ml sodium chloride 0.9% for infusion, the resulting solution should be infused over 2 hours. Prior to infusion inspect visually for particulate matter and discoloration.

A separate IV line equipped with a low protein-binding 1.2-micron terminal filter must be used for administration of the drug (see note**).** GO may be given peripherally or through a central line.

Premedication, with paracetamol, methylprednisolone and an antihistamine (such as chlorphenamine), should be given before each infusion to reduce the incidence of a post-infusion symptom complex. Vital signs should be monitored during infusion and for four hours following infusion.

Azole antifungals should not be given until day 5 after the administration of GO and for patients who have been taking a prophylactic azole this should be discontinued 5 days before the GO is due.

Instructions for Use, Handling and for Disposal

Procedures for handling and disposal of cytotoxic drugs should be applied.

Cautions

Hepatic Insufficiency: Patients with hepatic impairment will not be included in the clinical studies.

Renal Insufficiency: Patients with renal impairment will not be included in the clinical studies.

Note

The recommended in-line filter for GO administration is a 1.2-micron polyether sulfone (PES) filter, e.g. “intrapurlipid” (Braun product number 4099702). If that filter is not available, the following filters may be used: 0.22 micron PES, 0.20 micron cellulose acetate, 0.8 to 1.2 micron cellulose acetate/cellulose nitrate (mixed ester), or 1.2 micron acrylic copolymer.

- **Mitoxantrone**

Mitoxantrone is presented as a dark blue aqueous 2mg/ml solution in vials which are stored at room temperature.

Dose: 10mg/m^2 by intravenous infusion over 1 hour in Consolidation I.

In hepatic dysfunction with a bilirubin >60umol/L the maximum dose should be 8mg/m^2.

Side effects include tissue necrosis following extravasation outside a vein. It is myelosuppressive.It is probably slightly less cardiotoxic than daunorubicin but care should be taken to avoid low serum potassium levels. Anorexia, diarrhoea, stomatitis, fatigue and mild alopecia have also been described.

- **Idarubicin**

Idarubicin is available as vials containing 5mg or 10mg idarubicin in powder form,for reconstitution,stored at room temperature.

Dose: 12mg/m^2 by intravenous infusion over 1 hour in Standard Risk (SR) and High Risk (HR) Induction

5mg/m^2 by intravenous infusion over 1 hour in Consolidation II for SR and HR patients

10mg/m^2 by intravenous infusion over 1 hour in Consolidation III if applicable

In cases with hepatic dysfunction dose reduction is required: bilirubin 21 – 34umol/L reduce the dose by 50%. Greater rises contraindicate the administration. For renal impairment with a serum creatinine 100 – 175umol/L reduce the dose to 50%. Administration at higher creatinine levels is a clinical decision.

Side-effects

The major side effect is myelosuppression. Cardiac toxicity may occur, manifested by cardiac failure, arrhythmias or cardiomyopathies, either during therapy or several weeks later. The cumulative dose associated with cardiotoxicity is not known. Idarubicin may cause a red discoloration of the urine for 1-2 days after administration. Reversible alopecia will occur, and some nausea or vomiting and oral mucositis should be expected. Elevation of liver enzymes and bilirubin may occur in a minority of patients.

Idarubicin should not be given to patients with severe renal or liver impairment.

- **ATRA (Tretinoin,Vesanoid®)**

The most common adverse effect of ATRA is headache of mild to moderate severity. Younger (pediatric) patients appear to be more sensitive to this particular effect. Bone pain, occasionally requiring analgesic treatment, has also been observed. Biochemical abnormality of liver function has occasionally been reported, specifically raised transaminases, alkaline phosphatase and bilirubin, but these are reversible on stopping the drug.

The most serious adverse event has been a syndrome characterized by fever, respiratory distress and episodic hypotension, usually in association with leukocytosis ("ATRA Syndrome"). The onset of this syndrome has usually been in the first 1-2 weeks of drug treatment. Should this occur ATRA should be stopped. Some cases are reported to respond well to high-dose corticosteroid therapy, dexamethasone 5mg/m^2 (maximum dose 10mg) 12 hourly iv. for 3 or more days.

Prolonged ATRA treatment may cause dryness of the skin. ATRA is also believed to be highly teratogenic. Advice concerning contraception should be given to appropriate patients.

- **Intrathecal Methotrexate (MTX) and Intrathecal Hydrocortisone** are both used (together with intrathecal cytarabine, see above) in cases of CNS relapse.

  Intrathecal MTX dose: 5mg,7.5mg,10mg or 12.5mg depending on age. Vials of drug are stored at room temperature, protected from light and must be preservative–free for intrathecal use.

  Side effects of intrathecal MTX include headache, vertigo, ataxia, convulsions and abnormal vision.

  Intrathecal Hydrocortisone dose: 5mg, 7.5mg, 10mg or 12.5mg depending on age. Vials of drug are stored at room temperature. Preservative - free drug and diluent must be used for intrathecal preparation.

- **Methotrexate for Oral Use**

  Available as 2.5mg and 10mg scored tablets, stored at room temperature, protected from light. Methotrexate may be made up in liquid form to be taken orally for those patients unable to swallow tablets. Preparations once made up have a limited shelf life. Recommended concentration is 10mg in 5ml.

  Dose:25mg/m^2 once a week as a single dose, the dose is adjusted according to the protocol depending on the neutrophil and platelet count.

  Side effects include neurotoxicity, mucositis, liver dysfunction, bone marrow depression.

- **Mercaptopurine**

  Available as 10mg and 50mg scored tablets, stored at room temperature. Mercaptopurine may be made up in liquid form for those patients unable to swallow tablets. Preparations once made up have a limited shelf life. Recommended concentration is 100mg in 5ml.

  Dose: 50mg/m^2 daily as a single dose,the dose is adjusted according to the protocol,depending on the neutrophil and platelet count.

  Side effects include bone marrow depression and liver dysfunction.

- **Arsenic Trioxide** (Trisenox^{TM} – Cephalon)

  Trisenox solution for infusion contains 1mg/ml (arsenic trioxide). It is presented as a sterile, clear, aqueous solution in a single–use 10ml ampoule. ATO is a trivalent inorganic arsenical. The active substance is a white crystalline powder that is very poorly soluble in water.

  Trisenox must be diluted in 100-250 ml of glucose 5% or sodium chloride 0.9% immediately after withdrawal from the ampoule and must not be mixed with or concomitantly administered in the same intravenous line with other medicinal products.

Aseptic technique must be strictly observed throughout the handling of Trisenox since no preservation is present.
After dilution in intravenous solutions, Trisenox is chemically and physically stable for 24 hours at 15-30^{o} C and 48 hours at refrigerated temperatures (2-8^{o}). From a microbiological point of view, the product must be used immediately. If not used immediately in-use storage times and conditions prior to use are the responsibility of the user and would normally not be longer than 24 hours at 2-8^{o} C, unless dilution has taken place in controlled and validated aseptic conditions.

Trisenox is given as a slow infusion over 1-2 hours daily until bone marrow remission is achieved. The daily infusions should be given on an inpatient basis at the beginning of induction therapy, followed, when the acute symptoms of APL have resolved and the patient's condition is stable, by outpatient administration for the remaining induction and consolidation treatment period.

APPENDIX IV: ATO Toxicity Profile

ATO when administered as a single agent, although quite well tolerated has a recognized toxicity profile. It is contraindicated in patients with hypersensitivity to arsenic or any of the excipients in the product. There are a number of special warnings and special precautions for use listed below:

1. Clinically unstable APL patients are especially at risk and will require more frequent monitoring of electrolyte and glycemia levels as well as more frequent hematologic, hepatic, renal and coagulation parameter tests.

2. Leukocyte Activation Syndrome (APL Differentiation Syndrome):

   Twenty-five percent of patients with APL treated with ATO in hematologic relapse develop symptoms similar to a syndrome called the retinoic-acid-acute promyelocytic leukemia (RA-APL) or APL differentiation syndrome or RAS, characterized by fever, dyspnoea, weight gain, pulmonary infiltrates and pleural or pericardial effusions, with or without leukocytosis. This syndrome can be fatal. The management of the syndrome has not been fully studied, but high-dose steroids have been used at the first suspicion of the APL differentiation syndrome and appear to mitigate signs and symptoms. At the first signs that could suggest the syndrome (unexplained fever, dyspnoea and/or weight gain, abnormal chest auscultatory findings or radiographic abnormalities), high dose steroids - dexamethasone 5mg/m^2 12 hourly iv. (maximum dose 10mg) - must be immediately initiated, irrespective of the leukocyte count and continued for at least 3 days or longer until signs and symptoms have abated. The majority of patients do not require termination of ATO therapy during treatment of the APL differentiation syndrome. It is recommended that chemotherapy not be added to treatment with steroids since there is no experience of administration of both steroids and chemotherapy during treatment of the leukocyte activation syndrome due to ATO.

3. Electrocardiogram (ECG) Abnormalities:

   Arsenic trioxide can cause QT interval prolongation and complete atrioventricular block. QT prolongation can lead to a torsade de pointes-type ventricular arrhythmia, which can be fatal. The risk of torsade de pointes is related to the extent of QT prolongation and concomitant administration of QT prolonging medicinal products such as class Ia and III anti-arrhythmics (eg quinidine, amiodarone, sotalol, dofetilide), antipsychotics (eg thiordazine), antidepressants (eg amitriptyline), some macrolides (eg erythromycin), some antihistamines (eg terfinadine and astemizole), and some quinolone antibiotics (eg sparfloxacine). Other risk factors include congestive heart failure, administration of potassium-wasting diuretics, amphothericin B or other conditions that result in hypokalemia or hypomagnesemia and in these situations ATO should be avoided.

   ECG and Electrolyte Monitoring Recommendations: Prior to initiating therapy with ATO, a 12 lead ECG must be performed and serum electrolytes (potassium, calcium and magnesium) and creatinine must be assessed; pre-existing electrolyte abnormalities must be corrected and, if possible, medicinal products that are known to prolong the QT interval should be discontinued.

Patients with risk factors for QTc prolongation or torsade de pointes should be monitored with continuous cardiac monitoring (ECG). For QTc greater than 500 msec, corrective measures must be completed and the QTc reassessed with serial ECG's prior to considering using ATO. During therapy with ATO, potassium concentrations must be kept above 4mEq/l and magnesium concentrations must be kept above 1.8mg/dl. Patients who reach an absolute QT interval value >500 msec must be reassessed and immediate action taken to correct concomitant risk factors, if any while the risk/benefit of continuing versus suspending ATO therapy must be considered. If syncope, rapid or irregular heartbeat develops, the patient must be hospitalized and monitored continuously, serum electrolytes must be assessed. ATO therapy must be temporarily discontinued until the QTc interval regresses to below 460 msec, electrolyte abnormalities are corrected, and the syncope and irregular heartbeat cease. There are no data on the effect of ATO on the QTc interval during the infusion. Electrocardiograms must be obtained twice weekly, and more frequently for clinically unstable patients, during induction and consolidation.

4. Dose Modification:

Treatment with ATO must 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 2 is observed and judged to be possibly related to ATO treatment. Patients who experience such reactions that are considered ATO related must resume treatment only after resolution of the toxic event or after recovery to baseline status of the abnormality that prompted the interruption. In such cases, treatment must resume at 50% of the daily preceding dose. If the toxic event does not recur within 3 days of restarting treatment at the reduced dose, the daily dose can be escalated back to 100% of the original dose. Patients who experience a recurrence of toxicity must be removed from the treatment.

- *Laboratory Tests*: The patient's electrolyte and glycemia levels, as well as hematologic, hepatic, renal and coagulation parameter tests must be monitored at least twice weekly, and more frequently for clinically unstable patients during the induction phase and at least weekly during the consolidation phase.
- *Patients with renal or hepatic impairment*: Safety and effectiveness of ATO in patients with renal and hepatic impairment have not been studied. Particular caution is needed in patients with renal failure receiving ATO, as renal excretion is the main route of elimination of arsenic.

5. Hyperleukocytosis:

Treatment with ATO has been associated with the development of hyperleukocytosis ($\geq$10 x 10^9/l) in around half of patients treated in hematologic relapse. There did not appear to be a relationship between baseline white blood cell (WBC) counts and development of hyperleukocytosis nor did there appear to be a correlation between baseline WBC count and peak WBC counts. Hyperleukocytosis was never treated with additional chemotherapy and resolved on continuation of ATO. Patients developing hyperleukocytosis should be observed carefully for development of differentiation syndrome. ATO-induced hyperleukocytosis is not considered an indication for chemotherapy or leukopheresis, which could potentially exacerbate the coagulopathy.

Since hyperleukocytosis is associated with differentiation of APL cells, this is not observed during consolidation courses of ATO once the patient is in clinical remission.

6. Interaction with other medical products and other forms of interaction:

No formal assessments of pharmacokinetic interactions between ATO and other therapeutic medicinal products have been conducted. QT/QTc prolongation is expected during treatment with ATO, and torsade de pointes and complete heart block have been reported. Patients who are receiving, or who have received, medicinal products known to cause hypokalemia or hypomagnesemia, such as diuretics or amphotericin B, may be at a higher risk for torsade de pointes. Caution is advised when ATO is co-administered with other medicinal products known to cause QT/QTc interval prolongation such as macrolide antibiotics, the antipsychotic thioridazine or medical products known to cause hypokalemia or hypomagnesemia. Additional information about QT prolonging medicinal agents is provided above in section 3. The influence of ATO on the efficacy of other antileukemia medicinal products is unknown.

APPENDIX V: Stem Cell Transplant Guidelines

See sections 14.4 to 14.7 for eligibility for SCT. Patients who achieve MRD negativity with ATO/GO/ATRA salvage therapy and an adequate PCR negative stem cell harvest are eligible to proceed to an autologous BMT procedure. Preparation will consist of oral Busulphan 4mg/kg/day (5mg/kg/day if less than 2 years of age) x 4 days or IV Busulphan (Busilvex) with dosing based on weight (see below), and Cyclophosphamide 50mg/kg/day x 4 days. Cyclophosphamide could be replaced with Melphalan 140mg/kg for those with significant cardiotoxicity pre BMT.

Weight	< 9kg	9 to < 16kg	16 to 23 kg	>23 to 34kg	>34 kg
Busilvex dose*	1.0 mg/kg	1.2 mg/kg	1.1mg/kg	0.95 mg/kg	0.8 mg/kg

** total dose given over 4 consecutive days every 6 hours for a total of 16 doses.*

Those patients with persistent MRD positivity will be considered for Allogeneic BMT using donor hierarchy given below:

Choice	**Family Donor**	**Unrelated Donor**	**Unrelated CB**
1st	MSD or matched CB		
2nd	Phenotypic MFD	10/10	6/6
3rd	5/6	9/10	≥5/6
4th	≤ 4/6**		≤ 4/6

** requires in vitro T-cell depletion

Preparative regimens will differ depending on donor type but will usually include:

- Busulphan 16-20 mg/kg, Cyclophosphamide 200mgkg;
- TBI 12 -14.4 Gy in 6-8 #s, Cyclophosphamide 120 mg/kg;
- Busulphan 16mg/kg, Cyclophosphamide 120mg/kg, Melphalan 140mg/kg;

Additional Campath or ATG may be given with non-sibling donors.

In the MRD positive setting an attempt should be made to augment GVL activity with early taper of immunosuppression +/- donor lymphocyte infusions.

Patients undergoing BMT after GO therapy should receive prophylactic defibrotide to reduce incidence of VOD.

Post-transplant MRD monitoring is recommended to guide the need for additional therapy.

APPENDIX VI: Guidelines for Cardiotoxicity Monitoring

Please use M-mode echo measurements according to the American Heart Association guidelines. Whenever possible the patient should be afebrile with a Hb of >9.0 g/dl (>5.5 mmol/l). An ECG should be monitored simultaneously with the M-mode echo. The blood pressure should be recorded either during the last part of the echocardiograph or immediately afterwards.

What to Measure:

Baseline echocardiogram	- apical 4 chamber view - apical 5 chamber view (with aorta) - short axis left ventricle (papillary muscle level) - short axis left ventricle (aortic valve/pulmonary artery level)
Baseline colour Doppler	- apical 4 chamber (mitral, tricuspid, aortic flows)
Pulsed Doppler	- aortic flow (measurement of Left Ventricular Ejection Time (LVET)) - mitral valve (tips of mitral valve leaflets)
M-mode	- left ventricle – parasternal axis

When to Measure:

If at all possible a baseline echocardiograph should be performed before the start of therapy, or within one week of the initiation of therapy. APL is a hematological emergency, treatment should not be postponed for any reason and ATRA should be started as soon as the diagnosis is suspected. Ideally repeat Echo 1 week prior to each consolidation course and 3 months after the completion of therapy. Echocardiography should be repeated 12 months from diagnosis and at 3 yearly intervals thereafter. If the Fractional Shortening (FS) is <28% repeat annually.

APPENDIX VII:

Patient Initials*:	First name: |__|__|__| Last name: |__|__|__|
Gender*:	M F
Date of Birth*:	_____/___/_____ (dd/mm/yyyy)
Second Malignant Neoplasia:	Yes No
if YES, specify previous neoplasm:	_____

National Medical Code Number or any administrative number allowing to identify your patient: **(Optional)**	_____

Has written consent been obtained?*
If Yes, signed by:
Date of signature:

Date of patient registration at the center:*	_____/___/_____ (dd/mm/yyyy)

Fields marked with * are mandatory.

Center: _____

Country: _____

APPENDIX VIII

CENTER PARTICIPATION FORM
Available at: www.alp.cineca.org

Please complete the following steps: 1) fill in the form 2) print the completed form and collect signatures 3) fax the form with the signatures to CINECA +39 051 6171 365	
We agree to:	
☐ comply with the protocol requirements	
☐ provide the necessary information through the CINECA Remote Data Entry System for central review in a timely manner	
☐ obtain the necessary ethical and regulatory approval required by our country prior to entry the first patient	
	Institutional Review Board (IRB) or Ethics Committee (EC) approval ☐ fax Date of approval: ___/___/____ (dd/mm/yyyy) **Health Authority and/or other applicable approval as required by national regulations** ☐ fax Date of approval: ___/___/____ (dd/mm/yyyy)
Participating Center:	
* Hospital/Institution name:	_____
* Department name:	_____
* Address:	_____
* Zip Code:	_____
* City:	_____
* Country:	_____

* Telephone:	_____
* FAX:	_____
*** Responsible clinician:**	
* Surname:	_____
* Forename:	_____
address (if different from above):	_____
* Telephone:	_____
* E-mail:	_____
Signature:	_____
Signature Date:	___/___/____ (dd/mm/yyyy)
Please click on the checkbox to fill in data:	

☐ **Responsible Biological Molecular Laboratory**

* Surname:	_____
* Forename:	_____
* Telephone:	_____
* E-mail:	_____
Signature:	_____
Signature Date:	___/___/____ (dd/mm/yyyy)

☐ **Responsible Cytogenetic Laboratory**

* Surname:	_____
* Forename:	_____
* Telephone:	_____
* E-mail:	_____
Signature:	_____
Signature Date:	___/___/____ (dd/mm/yyyy)

☐ **Responsible Morphological Laboratory**	
* Surname:	_____
* Forename:	_____
* Telephone:	_____
* E-mail:	_____
Signature:	_____
Signature Date:	___/___/____ (dd/mm/yyyy)

☐ **Responsible Data Manager**	
* Surname:	_____
* Forename:	_____
* Telephone:	_____
* E-mail:	_____
Signature:	_____
Signature Date:	___/___/____ (dd/mm/yyyy)

---------- ---------- ----------

Date (dd/mm/yyyy) Name Signature

APPENDIX IX:

Center	Patient Code	Initial	Date of birth	Reg. Date

SERIOUS ADVERSE EVENT			
Fields marked with * are mandatory.			
Date Section A filled in*:	___/__/_____ (dd/mm/yyyy)	Date of Onset of SAE*:	___/__/_____ (dd/mm/yyyy)
Serious Adverse Event description: **(In the on line form it is possible to browse into CTCAE data base)**	_____		
Reasons for seriousness*:	Life threatening Required (prolonged or unplanned) hospitalisation Resulted in severe or permanent disability/incapacity Unexpected grade III or IV toxicity Congenital anomaly/Birth defect Other reason Death		
if other specify:	_____		
Trial phase*:	Induction Consolidation I Consolidation II Consolidation III Maintenance Refractory/Relapse Induction Refractory/Relapse Consolidation Extramedullary Relapse Induction Extramedullary Relapse Consolidation Follow Up		Date of start of phase ___/__/_____ (dd/mm/yyyy)
Description of protocol therapy received			
1. Drug*:	ATRA IDA ID ARA-C MITOXANTRONE IT ARA-C 6-MP MTX ATO IT MTX HYDROCORTISONE		
Dose (Give Units):	_____	Indication:	_____
Frequency:	_____	Route:	_____

Date Started:	___/__/_____ (dd/mm/yyyy)	Date stopped/continuing:	___/__/_____ (dd/mm/yyyy)
Relation of drug to SAE*:	Unrelated Unlikely/remote Possible Probable Definite Can't be classified	If can't be classified, specify:	_____
Action taken*:	None Dose reduction Next course delay Temporarily discontinued Permanently discontinued Other	If other, specify:	_____
2. Drug:		ATRA IDA ID ARA-C MITOXANTRONE IT ARA-C 6-MP MTX ATO IT MTX HYDROCORTISONE	
Dose (Give Units):	_____	Indication:	_____
Frequency:	_____	Route:	_____
Date Started:	___/__/_____ (dd/mm/yyyy)	Date stopped/continuing:	___/__/_____ (dd/mm/yyyy)
Relation of drug to SAE*:	Unrelated Unlikely/remote Possible Probable Definite Can't be classified	If can't be classified, specify:	_____
Action taken*:	None Dose reduction Next course delay Temporarily discontinued Permanently discontinued Other	If other, specify:	_____
3. Drug:		ATRA IDA ID ARA-C MITOXANTRONE IT ARA-C 6-MP MTX ATO IT MTX HYDROCORTISONE	
Dose (Give Units):	_____	Indication:	_____

Frequency:	_____	Route:	_____
Date Started:	___/__/_____ (dd/mm/yyyy)	Date stopped/continuing:	___/__/_____ (dd/mm/yyyy)
Relation of drug to SAE*:	Unrelated Unlikely/remote Possible Probable Definite Can't be classified	If can't be classified, specify:	_____
Action taken*:	None Dose reduction Next course delay Temporarily discontinued Permanently discontinued Other	If other, specify:	_____
Description of concomitant therapy received			
	1. Drug:	_____	
Dose (Give Units):	_____	Indication:	_____
Frequency:	_____	Route:	_____
Date Started:	___/__/_____ (dd/mm/yyyy)	Date stopped/continuing:	___/__/_____ (dd/mm/yyyy)
Relation of drug to SAE*:	Unrelated Unlikely/remote Possible Probable Definite Can't be classified	If can't be classified, specify:	_____
Action taken*:	None Dose reduction Next course delay Temporarily discontinued Permanently discontinued Other	If other, specify:	_____
	2. Drug:	_____	
Dose (Give Units):	_____	Indication:	_____
Frequency:	_____	Route:	_____
Date Started:	___/__/_____ (dd/mm/yyyy)	Date stopped/continuing:	___/__/_____ (dd/mm/yyyy)
Relation of drug to SAE*:	Unrelated Unlikely/remote Possible Probable Definite Can't be classified	If can't be classified, specify:	_____
Action taken*:	None Dose reduction Next course delay Temporarily discontinued	If other, specify:	_____

	Permanently discontinued Other		
3. Drug:		_____	
Dose (Give Units):	_____	Indication:	_____
Frequency:	_____	Route:	_____
Date Started:	___/__/_____ (dd/mm/yyyy)	Date stopped/continuing:	___/__/_____ (dd/mm/yyyy)
Relation of drug to SAE*:	Unrelated Unlikely/remote Possible Probable Definite Can't be classified	If can't be classified, specify:	_____
Action taken*:	None Dose reduction Next course delay Temporarily discontinued Permanently discontinued Other	If other, specify:	_____
Serious Adverse Event Outcome			
Outcome:	Resolved Resolved with sequelae On-going Death	Date of resolution:	___/__/_____ (dd/mm/yyyy)
If Resolved with sequelae specify sequelae:		_____	
Date of Death:	___/__/_____ (dd/mm/yyyy)	Cause of Death:	☐ Tumor ☐ Toxicity ☐ Infection ☐ Surgery ☐ Other
Comments:		_____	
Classification:		SAE SUSAR SAR	

Fields marked with * are mandatory.

APPENDIX X:

ICC APL STUDY 01

Parent/guardian Information Sheet

Introduction

Your doctor will have explained that your child has a condition called Acute Promyelocytic Leukemia commonly referred to as APL. This is a malignancy of the bone marrow in which there is a deficiency of mature blood cells and an excess of immature cells called promyelocytes.

CLINICAL STUDIES:

Children with leukemia have been treated within clinical studies for many years and over the years this approach has led to a steady improvement in outcome. These studies generally involve all children in one country or a number of countries. APL is a rare form of leukemia and for this reason ICC APL Study 01 will involve children from many countries around the world. Clinical studies aim to find out the best treatment and do this by comparing one treatment against another, where the best treatment is not known, or by giving everyone, what is thought to be the best treatment, and comparing this with previous studies. ICC APL Study 01 will give every child with APL the same treatment, which is thought to be the current best.

BACKGROUND TO ICC APL STUDY 01:

- ICC APL Study 01 will use a protocol similar to that used in GIMEMA-AIEOP/AIDA 93 which has produced amongst the best results in children.

- It is known that how well a child with APL does in the long term depends on the number of leukemia cells found in the blood (WBC count) at diagnosis. Therefore children with a higher WBC (>$10x10^9$/L) will receive more treatment than those children with a lower WBC. All children will receive induction chemotherapy followed by consolidation treatment. Children with a higher WBC will receive 3 blocks of consolidation therapy and those with a lower WBC will receive 2 blocks of consolidation therapy.

- A group of drugs called anthracyclines or anthracycline–like drugs (Idarubicin, Mitoxantrone) are very important for the treatment of APL. However, these drugs, if given in high enough doses can cause damage to the heart muscles. Although the best dose of anthracyclines in APL is not known, ICC APL Study 01 will use a total dose which is less than that used by many national groups. It is hoped that this will decrease the risk of damaging the heart muscles without reducing the anti-leukemic effect.

- Following induction and consolidation therapy all patients will receive maintenance treatment with oral chemotherapy for two years.

- A drug called ATRA (all-trans retinoic acid) which is specifically effective in APL will be given in all phases of treatment.

- Using sophisticated tests it is possible to detect tiny amounts of APL disease when there are no abnormal cells visible in the bone marrow. Experience from other trials indicate that patients do best when treatment is increased or changed when tiny amounts of disease are present, and before there are any obvious changes in the bone marrow. ICC APL Study 01 aims to increase or change treatment if these sophisticated tests suggest that disease is present, when one would expect it to be absent. This will be done by regularly monitoring for the presence of disease by testing both blood and bone marrow samples. Details of these will be explained to you by your doctor.

- Children who do not clear disease or who show recurrence as measured by these sophisticated tests will have alternative treatment. This will initially be with a drug called Arsenic Trioxide (ATO) which is very effective in APL, followed by either Mylotarg, another effective drug in APL, or ATRA and an autologous or allogeneic bone marrow transplant. The precise treatment will depend on their response to ATO. These drugs all have side effects which will be explained to you by your doctor but are briefly listed below.

GENERAL INFORMATION:

1. Your child may or may not receive any direct benefit from taking part in the study. However, information obtained during the course of the study may help us to understand better your child's condition or illness. It may also help us in selecting treatment for future patients.

2. It is up to you to decide whether to allow your child to take part or not. If you do decide to take part, you are free to withdraw your child at any time and without giving a reason. This will not affect the standard of care your child will receive. Your doctor will not be upset if you decide that your child will not take part.

3. All the information collected about your child during the course of the research will be kept strictly confidential. Any published report of the research will not identify him/her.

4. Your doctor will tell you about the known side effects of the treatment.

5. Your GP will normally be informed that your child is taking part. If this is a problem for you or your child, you should discuss it with your study doctor.

6. Named information about your child may be passed to your National Government Registries and Regulatory Authorities. All personal details will be treated as strictly confidential by these organisations. The progress of this study will be regularly monitored by a Data and Ethics Committee, which is independent of all the study investigators. Their duties include ensuring that no unexpected side effects are happening.

7. Sometimes during the course of a study new information becomes available. Your doctor will talk to you about this and discuss with you whether you want your child to continue in the study. If you decide to withdraw your child, the study doctor will make arrangements for your child's care to continue. If you decide to allow your child to continue in the study you may be asked to sign an updated consent form.

8. This study has been accepted by a Multi Centre Research Ethics Committee and the Local Ethics Committee of your hospital.

SPECIFIC SIDE EFFECTS OF DRUGS:

Any treatment for leukemia will have potential side effects, which your doctor will explain to you.

All chemotherapy can result in nausea, vomiting, hair loss, and a sore mouth (mucositis). All patients who receive chemotherapy will experience a fall in their blood count and when their blood count is low they are at risk of serious infection. The drugs used in this protocol, which are anthracyclines or anthracycline - like drugs, Idarubicin and Mitoxantrone, may cause damage to the heart muscles. Patients will be regularly monitored by a scan called an echocardiogram to detect any changes, but this may not prevent it happening.

A number of the drugs used in this study have specific side effects which are well recognised and will be carefully monitored for.

ATRA

ATRA is a very effective drug in APL. Patients with APL are at risk of bleeding when they are first diagnosed and this risk is reduced by ATRA. In addition, patients who receive ATRA do better in the long term than those who do not. However, there are a number of specific side effects associated with ATRA which are described below.

- **ATRA Syndrome (RAS) – incidence : 5-20%**
  Patients may develop fever, weight gain, breathing problems and changes on a chest x-ray. It is likely that they will be treated by temporarily stopping the drug and giving steroids.

- **Pseudotumor Cerebri – incidence : 10-20%**
  Children may develop severe headaches with nausea, vomiting, and visual disorders. This is treated by stopping ATRA for a short period of time and by giving painkillers.

- **Liver Disturbance**
  Children may develop abnormal liver function tests whilst receiving ATRA. This will be carefully monitored and a decision about the best management will be made by your doctor.

ARSENIC TRIOXIDE

Arsenic can cause problems very similar to those described under ATRA Syndrome and can also cause abnormalities of conduction of the heart, if given with other drugs.

GEMTUZUMAB OZOGAMICIN (MYLOTARG®)

Mylotarg can lead to a low blood count, allergic reactions and liver abnormalities including a veno-occlusive disease type of syndrome, which is the term used to describe clotting of the blood vessels.

WHAT HAPPENS NOW?

Having had a talk with your child's treatment team and having read this study summary, you are requested to consent for your child to enter the study. You will also be asked permission for us to store some excess cells from the blood or bone marrow if there are some left over after the diagnostic tests. This will be used for future research.

IS YOUR CHILD ELIGIBLE FOR THIS STUDY?

The International Consortium for Childhood (ICC) APL Study 01 has been designed for patients between 0 and 21 years (for AIEOP see appendix A) with Acute Promyelocytic Leukemia.

WHAT ARE THE DISADVANTAGES OF TAKING PART?

Any treatment for Leukemia will have potential side effects, which your doctor will explain to you. It is not anticipated that taking part in this study will add extra side effects.

WHAT ARE THE POSSIBLE BENEFITS OF TAKING PART IN THE STUDY?

You will be advised by your doctor of why he/she considers that treatment is necessary for your child. There is no guarantee that your child's treatment will be successful. The study is continuously monitored by co-ordinators and an independent monitoring committee to ensure that outcome is as good as expected and that there are no unexpected harmful effects.

If new information becomes available, the study will stop. If this happens your child will be given whatever is then thought to be the best treatment.

WHAT IF SOMETHING GOES WRONG?

If your child is harmed by taking part in this study, there are no special compensation arrangements. If your child is harmed due to someone's negligence, then you may have grounds for legal action.

Regardless of this, if you have any cause to complain about any aspect of the way you or your child has been approached or your child treated during the course of their illness, you should go through the normal national complaints mechanisms. Your hospital will have a formal complaints procedure, which is available to you.

Clinical studies have been a great help in the improvement of treatment for all leukemias. The participation of previous patients has improved the treatment now available for your child, and it is important that the process continues.
We hope you will help future patients – and perhaps also your own child – by agreeing for your child to take part in this study, but please feel assured that you are under no pressure to do so. If you do give permission for your child to take part, you will be free to withdraw your child from the study at any time without giving a reason. Whatever you decide, we will obviously do our best for your child.

Thank you for taking the time to read this document and considering this study.

ICC APL STUDY 01

PATIENT 10 –18 YEARS INFORMATION SHEET

Introduction

Although you are not old enough to legally give consent (your permission) to have treatment, your doctor would like you to understand as much as possible about the treatment you need. Your doctor will talk to you to explain what needs to be done to get you better. You should read this leaflet, which contains a lot of useful information about your treatment, and talk to your Mum or Dad or guardian about what we need to do to cure your leukemia. If there is anything that you are not sure of do not be frightened to ask your doctor or your nurse.

Your doctor will have explained that you have a serious blood condition called Acute Promyelocytic Leukemia, APL for short. This is a cancer of the bone marrow in which there is a deficiency of mature blood cells and an excess of immature cells called promyelocytes.

CLINICAL STUDIES:

Children with leukemia have been treated with clinical studies for many years and over the years this has resulted in more children being cured of leukemia. Very few children get APL and, because of this, children from many countries will be treated exactly the same way on this study. Everyone will receive the same treatment, which is thought to be the best that there is.

ICC APL STUDY 01:

- Children treated on the ICC APL Study 01 will receive what doctors believe to be the best treatment available.
- You will receive a number of courses of chemotherapy given through a drip. This will take a number of months to complete. Afterwards you will receive tablets, called maintenance treatment, for two years.
- Using special tests it is possible to detect tiny amounts of your leukemia when no bad cells can be seen in the bone marrow. Doctors believe that it is best to treat you before abnormal cells start to be seen in the bone marrow. Therefore your blood and bone marrow will be regularly tested for tiny amounts of leukemia.

GENERAL INFORMATION:

1. You may or may not benefit from taking part in this study, but the information we get from the study may help us to better understand your leukemia. It may also help us in selecting treatment for future patients.

2. After talking to your parent(s) or guardian and the doctors and nurses, we would like you to be directly involved with the decision about whether to take part or not. Even if you do take part, after discussion with your parent(s) or guardian, you can stop being in the study at any time and without giving a reason. This will not affect the way you are looked after and your doctor will not be upset with you.

3. All the information collected about you during the course of the study will be kept strictly confidential. That means that no one except you, your parent(s) or guardian, and the staff involved with APL will be able to find out anything about what is wrong with you and what treatment you have. Any published medical report of the research will not identify you.

4. Your doctor will tell you about the known side effects of the treatment options mentioned in the information sheets about the study.

5. Your GP will normally be informed that you are taking part. If this is a problem for you, you should discuss it with your study doctor.

6. Named information about you may be passed to the national government registries and regulatory authorities in order to follow up your health status. All personal details will be treated as strictly confidential by these organisations.

7. To ensure that nobody is put in danger there are official bodies, who are not directly involved with APL, that keep an eye on what is happening to the patients in the study. These include a Data and Ethics Committee, which is independent of all the study investigators. Their duties include ensuring that no unexpected side effects are happening.

8. Sometimes during the course of a study new information becomes available. Your doctor will talk to you about this and discuss with you and your parent(s) or guardian whether you want to continue in the study. If you stop being in the study the study doctor will make sure that you still get the best treatment available. If you continue in the study you may be asked to sign an updated consent form.

It is important for you to know that if you are worried at any time or do not understand what is going on, that you should talk about your worries with your Mum or Dad or guardian. You can also talk about any problems with the doctors and nurses who are looking after you. After discussion with your parent(s) or guardian you can stop being in the study at anytime. This will not affect the care you get.

WHAT HAPPENS NOW?

Having had a talk with your doctors and nurses, your Mum or Dad or guardian and having read this study leaflet you will also be asked if it is alright for us to keep some of your leukemia cells, if there are any left over from the tests you had when you first came into hospital. They will be used for future research. If you are not sure what is going on at any time during your treatment, please ask. No one will be annoyed with you. It is important that you understand what is happening.

WHY ARE YOU ELIGIBLE FOR THIS TREATMENT?

The APL study has been designed for patients between 0 and 21 years (for AIEOP, see appendix A) with Acute Promyelocytic Leukemia.

WHAT ARE THE POSSIBLE BENEFITS OF TAKING PART IN THE STUDY?

You will get the best treatment presently available.

WHAT IF SOMETHING GOES WRONG?

If during the study something went wrong, you would have the same rights as any other patient having treatment in any hospital. If you feel that there is anything specifically to do with the study you want to complain about, you should discuss your worries with your Mum or Dad or guardian.

Thank you for taking the time to read this document and considering this study.

APPENDIX XI: Add-on Biological Studies

DOWNREGULATION OF THE INHIBITORY RECEPTOR SIRPα IN ACUTE PROMYELOCTIC LEUKEMIA (APL): ROLE OF ABERRANT PROMOTER HYPERMETHYLATION AND POTENTIAL FOR INNOVATIVE THERAPY.

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INTRODUCTION

Pediatric Acute Promyelocytic Leukemia

Acute Promyelocytic Leukemia (APL), FAB classification M3 or M3v, represents approximately 4-8% of pediatric AML[1] but the incidence may be higher in children of Hispanic and Mediterranean origin. The median age at presentation is probably similar to that of other AML subtypes (7-9 years), but APL has rarely been reported in the first year of life. It can arise de novo or be therapy related (t-APL). APL is characterised by chromosomal rearrangements of 17q21 involving the gene encoding the Retinoic Acid Receptor Alpha (RARα), which is most commonly fused to the PML gene (ProMyelocytic Leukemia) as a result of the t(15;17)(q22;q21) translocation[2]. In a minority of cases (<5%), RARα is fused to an alternative partner, which in the pediatric setting is most commonly nucleophosmin (NPM1) resulting from the t(5;17)(q35;q21) translocation[3,4]. This subtype and that involving NuMA as a result of t(11;17)(q13;q21) are sensitive to retinoic acid; whereas APL involving PLZF and STAT5b as a result of t(11;17)(q23;q21) and interstitial deletion of chromosome 17, respectively are resistant to ATRA[2]. While treatment outcome for APL patients with the PML-RARα fusion treated with extended courses of ATRA in combination with anthracycline–based chemotherapy is generally favourable[5], pediatric patients appear to present more commonly with hyperleucocytosis, as compared to their adult counterparts. Approximately 35-40% of children with APL fall within a high risk group defined by a presenting WBC ≥10 x 10^9/L, which is associated with M3v morphology and presence of FLT3 length mutations and predicts a poorer outcome. This is due both to an increased risk of induction death, particularly as a result of hemorrhage, as well as a significantly higher rate of relapse[1,6-8]. Using ATRA and anthracycline–based chemotherapy protocols followed by maintenance with ATRA, 6-MP and methotrexate, relapse rates for patients with a WBC <10 x 10^9/L at diagnosis are typically 10% or less, while rates may exceed 20% for patients with a WBC ≥10 x 10^9/L.

Signal regulatory protein α

Signal regulatory protein α (SIRPα) is a transmembrane receptor predominantly expressed on myeloid and neuronal cells. The extracellular domain of SIRPα interacts with its broadly expressed ligand CD47 which induces SIRPα mediated signaling. The cytoplasmic tail contains 2 immunoreceptor tyrosin-based inhibitory motifs (ITIMs) that upon phosphorylation recruit and activate the cytosolic tyrosine phosphatases SHP-1 and/or SHP-2. SIRPα signaling via ITIMs has been shown to negatively regulate many signaling pathways leading to reduction in tumor migration, survival and cell transformation[9]. Interestingly, a reduced SIRPα expression has been found in >70% of AML patients. Immature (M0, M1), myeloblastic (M2) and promyelocytic (M3) AMLs showed low SIRPα expression while monocytic AML cells (M4/M5) demonstrate relatively high or normal surface expression levels[10]. However it should be indicated that only low numbers (a total of 58 AML samples) were evaluated in that study. We have recently confirmed and extended this finding by Western blotting in AML cell lines **(Figure 1)** and primary AML samples, as well as by evaluating SIRPα mRNA levels in a cohort of 285 AML samples which included 19 adult APL samples (micro-array data provided by Valk *et a*l , NEJM, 2004)[11].

Importantly we have now obtained direct evidence that the relative lack of SIRPα on AML could cause a decreased level of growth control. In particular, the ligation of SIRPα on AML M2 t(8;21) myeloid cells by specific antibodies against SIRPα, has been shown to inhibit growth by promoting apoptosis (*Hubeek et al*, *manuscript in preparation*). This demonstrates that SIRPα-derived signals can directly control myeloid cell growth and survival and suggest that a reduction in SIRPα expression may contribute to the pathogenesis of AML, particularly in the immature FAB types.

We hypothesize that the observed down-regulation of SIRPα in APL might be the result of aberrant promoter hypermethylation, which has potential relevance for innovative therapy.

AIMS OF THE STUDY

We propose that SIRPα is downregulated in APL and that this involves hypermethylation of the SIRPα promoter.The aim of this project is to study the downregulation of the inhibitory myeloid receptor SIRPα in APL patient samples, which will be achieved by:

1) Evaluating SIRPα protein expression by immunocytochemistry.

2) Analysing the methylation status of the *PTPNS1* (the gene encoding SIRPα) promoter region using Methylation Specific PCR (MSP) and bisulfite sequencing.

RELEVANCE OF STUDY FOR APL PATIENTS

The effectiveness of retinoic acid in the treatment of APL has demonstrated that targeting the pathways that regulate growth and differentiation can be effective. The inhibitory myeloid receptor SIRPα has been shown to be downregulated in immature AML FAB types, including APL. Moreover, ligation of SIRPα with an agonistic antibody has been shown to inhibit growth.

We will investigate whether it is possible to re-express SIRPα in APL cells using clinically available compounds, e.g. the demethylating agent decitabine (DAC) and HDAC inhibitors. This would create a rational basis for the design of anti-leukemic therapies targeting SIRPα. It should be mentioned that these experiments will be part of a study that also includes a functional analysis of SIRPα in APL cell lines (e.g.NB4). In particular, we will investigate whether reconstitution of SIRPα expression in such APL cells will allow enhanced growth inhibitory signalling and will stimulate differentiation Finally, we will explore whether combinations of demethylating agents and/or HDAC inhibitors, SIRPα targeting (using agonistic antibodies), and ATRA provides a potential for better therapeutic effects in APL.

PRELIMINARY RESULTS IN OTHER SUBSETS OF LEUKEMIA

We have screened several leukemic cell lines and AML patient samples for SIRPα protein expression using Western blotting with a commercially available antibody. The AML cell lines KG1a (FAB M0) and Kasumi-1(FAB M2) expressed lower levels of the SIRPα protein than U937 cells (FAB M5). SIRPα expression was highest in the monoblastic cell line THP1. As expected, we did not observe SIRPα expression in the acute lymphoblastic cell lines Jurkat, 697 and Cem (**Figure 1**). SHP-1 and SHP-2 levels were comparable in all AML cell lines. Preliminary results in 25 pediatric AML samples also demonstrated that SIRPα expression was low (or even absent) in FAB M1 and 2 samples and high in FAB M4 and M5. In general, the expression of the SIRPα protein therefore appears to be in concordance with mRNA levels.

A

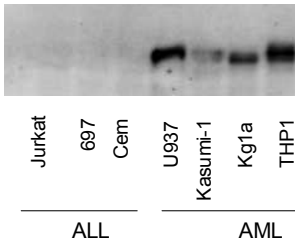

Figure 1: SIRPα protein expression in leukemic cell lines, detected by Western blotting.

Using MSP we evaluated the methylation pattern of the *PTPNS1* promoter in 3 leukemic cell lines (Kg1a, Kasumi-1 and HL60). MSP revealed partial methylation of the *PTPNS1* promoter region **(Figure 2)**. The MSP1 primer set showed both methylated and unmethylated bands in the leukemic cell lines. No methylation was detected using the MSP2 primer set. To further establish the methylation status of the *PTPNS1* gene we are currently performing bisulfite sequencing.

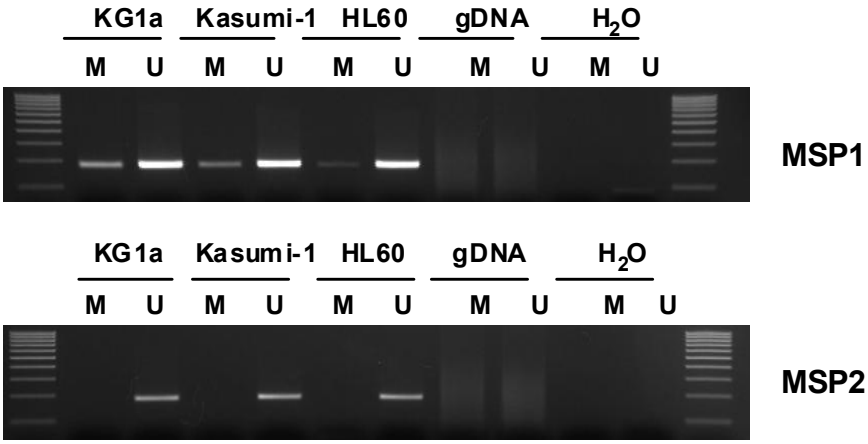

Figure 2: Analysis of the *PTPNS1* gene in AML cell lines, using 2 primer sets (MSP1 and MSP2). Genomic DNA and water served as negative controls. Lane M, amplified product with primers recognizing methylated *PTPNS1* and lane U, amplified product with primers recognizing unmethylated *PTPNS1*.

MATERIALS AND METHODS

Immunocytochemistry: The expression of SIRPα will be evaluated on cytospins by immunocytochemistry using the monoclonal antibody 1.23A that we have developed. We are currently optimizing the protocol.

DNA isolation and bisulfite treatment: Cells will be resuspended in an eppendorf vial containing 1ml HIRT buffer. Five μl proteinase K (20 mg/ml) will be added and the tubes will be incubated at 50°C for 2 hours, then at 37°C overnight. The cell solution will be split into two 500μl aliquots and 250μl phenol and 250μl chloroform will be added. The solution will be shaken vigorously for 15 s and centrifuged for 10 minutes at 14,000 g. The aqueous top layer will be removed and the phenol/chloroform extraction will be repeated. The aqueous top layer will be removed again, transferred into a clean tube and 500μl chloroform/isoamylalcohol mix (24:1) will be added. The tube will be mixed vigorously again and centrifuged for 10 minutes. The aqueous layer will be removed and the DNA will be precipitated in a 5 ml bijou containing 4 ml NaOAC/EtOH (1:24). DNA will be removed, washed with 1 ml of 70% EtOH and resuspended in TE buffer. DNA concentrations will be measured with spectrophotometer. 200-500 Ng of DNA will be treated with bisulfite using the EZ DNA Methylation Gold kit (Zymo Research).

Primer design and Methylation specific PCR (MSP): The SIRPα protein is encoded by the *PTPNS1* gene located on chromosome 20. A CpG island is present in the promoter region of this gene, which overlaps the first exon and the ATG start site. DNA methylation patterns of the *PTPNS1* promoter region will be determined by MSP[12] with specific primers designed for either methylated or unmethylated bisulphite treated DNA. Primers were designed for two regions in the *PTPNS1* promoter CpG island (MSP1 & MSP2; **Figure 3**). Human methylated DNA will be used as a positive control for methylated alleles of SIRPα and genomic cell line DNA will be used as a negative control for methylated alleles of SIRPα. PCR parameters for both regions (MSP1 and 2) are: 94°C 3 min (1 cycle); 94°C 30 s, 55°C 30 s (42 cycles); 72°C (1 cycle). If methylation is observed using MSP, the methylation will be further characterized by bisulfite sequencing.

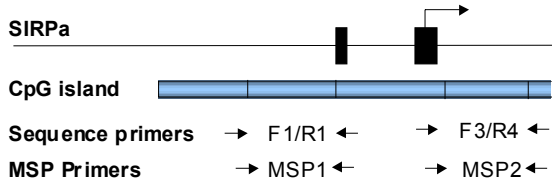

Figure 3: Schematic representation of the *PTPNS1* gene (encoding SIRPα). The blue bar indicates the CpG island. The black boxes indicate the first two exons and the arrow indicates the ATG transcription start site. Locations of the primers for methylation specific PCR (MSP) and bisulfite sequencing are indicated with arrows.

REQUESTED SAMPLES AND DATA

Number of cells per sample

We propose to study SIRPα expression and promoter methylation in 20-25 samples from pediatric APL patients. This number will allow a reliable estimation of the incidence of SIRPα downregulation in pediatric APL and the causal role of promoter hypermethylation. In order to perform immunocytochemistry and MSP/bisulfite sequencing, we request 6 cytospins and a cell pellet containing 2-5x10^6 cells for DNA isolation. This should be stored as cells or DNA locally or nationally until requested.

Clinical and cell-biological data

We would like to obtain clinical and cell-biological data for all included patient samples, consisting of: date of birth, date of diagnosis, WBC, sex, FAB-type, cytogenetic and molecular genetic data, date of CR, date and type of event, date of last follow-up.

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MOLECULAR MECHANISMS UNDERLYING THE PATHOGENESIS OF ACUTE PROMYELOCYTIC LEUKAEMIA IN CHILDREN

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BACKGROUND

Paediatric acute myeloid leukaemia (AML) is commonly associated with reciprocal balanced chromosomal translocations, which lead to the formation of chimaeric proteins that play a key role in mediating the leukaemic phenotype. Whilst a substantial body of information has been collected with respect to the functional activity of leukaemia-associated oncoproteins such as PML-RARα generated by the t(15;17) in acute promyelocytic leukaemia (APL), which is one of the commoner forms of AML arising in children, relatively little is known about the mechanisms that give rise to chromosomal translocations which represent a critical early step in leukaemogenesis. However, some insights have been gained through the investigation of therapy-related leukaemias, which invariably have *de novo* counterparts. For many years it has been appreciated that exposure to drugs that target DNA topoisomerase II predisposes to the development of secondary leukaemias characterised by balanced translocations, particularly involving *MLL* at 11q23, *NUP98* at 11p15, *AML1* at 21q22 and *RARA* at 17q21 – genes that are all recurrently involved in paediatric AML arising *de novo*. These observations have naturally implicated topoisomerase II in the DNA damage process, but how this occurs has been uncertain.

Topoisomerase II changes DNA topology, relaxing supercoiled DNA by transiently cleaving and re-ligating both strands of the double helix via the formation of a covalent cleavage intermediate. Many chemotherapeutic drugs that target topoisomerase II induce DNA damage by disrupting the cleavage-re-ligation equilibrium, increasing the concentration of DNA topoisomerase II covalent complexes. To investigate mechanisms underlying formation of the t(15;17) chromosomal translocation following exposure to drugs targeting topoisomerase II, such as mitoxantrone which has been particularly implicated in therapy-related APL (t-APL), we used functional *in vitro* assays which entail trapping of the cleavage complexes and mapping of cleavage sites at the sequence level. Interestingly, long-range PCR and sequence analysis revealed tight clustering of translocation breakpoints within an 8bp region of *PML* intron 6 in mitoxantrone-related cases (Figure 1), with scan statistics indicating that the observed pattern of breakpoint clustering was highly unlikely to have occurred by chance ($p<0.001$). Indeed, the functional assay demonstrated that the breakpoint hotspot region corresponded precisely to a preferential site of mitoxantrone-induced topoisomerase II-dependent DNA cleavage (Mistry *et al*, NEJM 2005). Similarly the observed translocation breakpoints within the *RARA* locus on chromosome 17 were also confirmed to be preferred sites of topoisomerase II mediated DNA damage induced by mitoxantrone.

The identification of the preferential mitoxantrone-induced topoisomerase II dependent cleavage site in the *PML* locus and an etoposide-induced breakpoint hotspot within *MLL*, provide a molecular explanation for the propensity to development of different subtypes of AML according to the nature of the particular chemotherapeutic agent used.

Whilst these studies clearly implicate DNA topoisomerase II in mediating DNA cleavage at translocation breakpoints in therapy-related APL, as opposed to a more indirect process involving induction of apoptotic nucleases, mechanisms underlying formation of reciprocal translocations in patients with *de novo* AML who account for the majority with the disease remain uncertain. There has been considerable interest as to whether environmental agents or other chemical exposures might be implicated, with attention focusing particularly upon naturally occurring and synthetic topoisomerase II inhibitors including flavonoids and benzene derivatives such as phenol and hydroquinones. The latter have a wide range of activities pertinent to leukaemogenesis, including induction of DNA damage. Widely varying levels of benzene metabolites are detected in normal individuals unexposed to benzene, reflecting differences in diet, smoking habits, gut flora and rates of metabolism – correlated in some instances with polymorphisms in genes encoding components of the detoxification pathways such as NAD(P)H:quinone oxidoreductase I (NQO1). Presence of the C609T polymorphism in *NQO1*, which decreases activity of the enzyme, is associated with an increased risk of infant leukaemias involving *MLL*, as well as therapy-related and *de novo* AML arising in adults, including cases associated with balanced translocations. Moreover, characterisation of translocation breakpoints at the genomic level in a limited number of paediatric AML cases coupled with analysis of neonatal Guthrie spots has indicated that some cases of childhood AML including APL arise *in utero*, suggesting that dietary or environmental exposures during pregnancy could be relevant to leukaemogenesis. Apart from benzene derivatives, many therapeutically, environmentally or dietary-derived topoisomerase II inhibitors are quinone containing compounds that are typically metabolized by NQO1; this raises the interesting possibility that topoisomerase II mediated DNA cleavage may also play a role in generating chromosomal translocations in cases considered to have *de novo* AML.

While APL accounts for 5-10% of AML cases presenting in Europe and the USA, recent epidemiological studies have highlighted marked variation in the prevalence of APL according to geography and ethnicity. Indeed in some Latin American countries APL accounts for up to a third of AML cases and has been observed to have a high prevalence in children. In this regard, previous studies have reported a different distribution of breakpoints within the *PML* locus on chromosome 15 in APL cases arising in some population groups as compared to those reported in European Caucasian populations. This suggests that a more comprehensive investigation is warranted, which could provide significant insights into the aetiology of APL.

In addition to the uncertainty regarding the mechanisms leading to DNA cleavage that is a key step in the genesis of chromosomal translocations associated with *de novo* AML, it is unclear whether abnormalities in DNA repair pathways play a role in predisposing individuals to developing the disease. Previously it has been shown that experimentally induced double-strand DNA breaks can be resolved giving rise to chromosomal translocations through non-homologous end joining (NHEJ) and annealing at homologous repeat sequences. Characterisation of genomic breakpoint junction regions in cases of secondary leukaemia with reciprocal translocations arising following exposure to agents targeting topoisomerase II, as well as those of *de novo* leukaemias, commonly reveals microhomologies and small deletions and duplications indicative of double-strand DNA break repair through the NHEJ pathway (Figure 2). Interestingly, using established *in vitro* assays for DNA double-strand break end-ligation efficiency and repair fidelity, it has been shown that a variety of leukaemic cell lines and primary AML blasts exhibit defective NHEJ activity with increased end-ligation efficiency and accompanying misrepair.

Data from the Rassool lab suggest that these phenomena are a response to increased rates of DNA damage in leukaemic cells in comparison to normal haematopoietic cells. However, this raises an important question as to whether misrepair of double-strand DNA breaks is an intrinsic property of transformed cells or actually whether it contributes to the generation of leukaemia-associated translocations. This proposal seeks to address this issue in the context of paediatric APL and also to investigate whether topoisomerase II plays a significant role in generating the t(15;17) for patients with no previous history of chemotherapeutic exposure.

PLAN OF INVESTIGATION

Characterisation of *PML-RARA* genomic breakpoint junctions in paediatric APL

PML breakpoint location will initially be determined by nested reverse transcriptase PCR (RT-PCR) using methods long-established in the Grimwade laboratory. Appropriate primer sets will be used to amplify the genomic junction region by long-range PCR. Breakpoint specific *PML* primers are used in combination with 8 different *RARA* primers covering the whole of *RARA* intron 2 that extends for 16.9kb. PCR products are sequenced using ABI3100 capillary sequencer. Using this approach, we have already successfully characterised der(15) and der(17) genomic junctions in over 40 cases of *de novo* APL and 23 cases of therapy-related APL presenting in adults. All breakpoints will be independently validated using short-range PCR to amplify *PML-RARA* and reciprocal *RARA-PML* genomic translocation breakpoints in fresh aliquots of DNA. Where possible, neonatal Guthrie cards will be sought to investigate whether the t(15;17) arose *in utero* and if so, the range of observed latency periods before presentation with overt leukaemia.

Determination of topoisomerase II dependent cleavage at identified t(15;17) translocation breakpoints in paediatric APL

This will be investigated by *in vitro* cleavage assays that are established in the Guy's laboratory (Mistry et al, NEJM 2005). Having identified genomic *PML-RARA* and *RARA-PML* junction sequences, substrates containing the normal homologues of the *PML* and *RARA* translocation breakpoints are 5'end-labelled and exposed to a range of naturally occurring topoisomerase II inhibitors (e.g. quercetin, genistein, catechin) in the absence or presence of human DNA topoisomerase IIα. In all cases, additional reactions will be carried out to evaluate the heat stability of the covalent complexes formed. Cleavage products are resolved in an 8% polyacrylamide-7.0M urea gel in parallel with dideoxy sequencing reactions primed at the same 5'end. Cleavage products are visualised by autoradiography and quantitated using PhosphoImager and IMAGEQUANT software.

Investigation of NHEJ activity in primary paediatric APL samples

To determine whether abnormal NHEJ activity may be a factor in the development of paediatric APL we propose to employ *in vitro* end-ligation efficiency and repair fidelity assays, in collaboration with Dr Feyruz Rassool, Baltimore. Previous work from Dr Rassool's laboratory has revealed increased and aberrant NHEJ activity in a variety of leukaemic cell lines in comparison to normal CD34+ selected cells and peripheral blood lymphocytes. Using the same experimental methods, our preliminary findings have revealed similar findings in the APL NB4 cell line that harbours the t(15;17).

To establish whether abnormal NHEJ is an intrinsic property of APL blasts or whether patients have a predisposition to development of the t(15;17) as a result of abnormal NHEJ activity, assays will be undertaken using nuclear extracts derived from ficoll-separated and magnetic bead (Miltenyi Biotech) selected cell populations derived from diagnostic samples, using CD33 and CD3 selection to enrich for APL blasts and T-lymphocytes as an internal control, respectively. T-lymphocytes will be expanded prior to nuclear extract preparation by phytohaemagglutinin (PHA) and IL2 stimulation; previous studies have shown that this does not affect NHEJ activity. Nuclear cell extracts are incubated in the presence of ATP with ^{32}P-labelled pUC18 DNA, which has been previously digested with *Eco*RI to create a double-strand break in the plasmid. ^{32}P-labelled products are deproteinised and analysed by agarose-gel electrophoresis. End-ligation efficiency is assessed by phosphorimaging and two-dimensional densitometry analysis. A DNA repair fidelity assay will also be carried out, which involves the incubation of nuclear cell extracts with *Eco*RI linearised pUC18 DNA in the presence of ATP, followed by transfection of *E.coli* DH5α with purified DNA from these assays. Results obtained in primary APL blasts, T-lymphocytes derived from APL patients and leukaemic cell lines will be compared with PHA/IL2 stimulated T-lymphocytes from normal healthy donors. These studies will be complemented by investigation of polymorphisms/mutations in genes encoding components of detoxification and DNA repair pathways, which could predispose to the development of APL in children.

APPENDIX

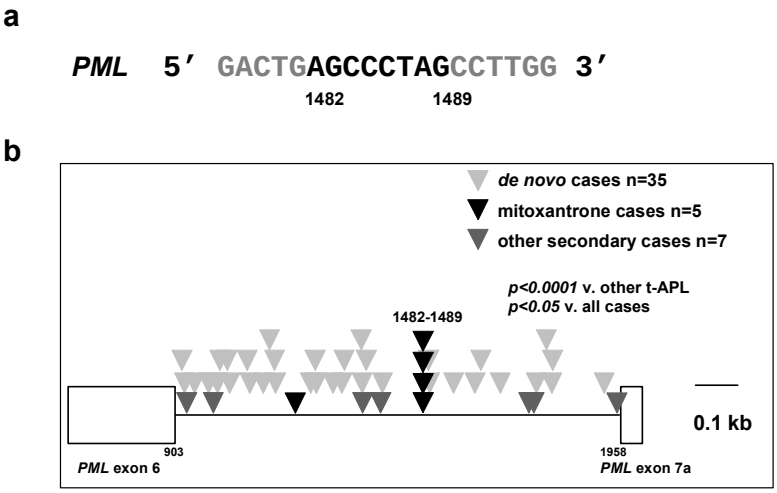

Figure 1: Identification of a breakpoint hotspot in *PML* intron 6 in mitoxantrone-related APL (from Mistry *et al*, NEJM 2005)

(a) *PML* intron 6 sequence encompassing mitoxantrone-associated translocation breakpoints, which clustered at positions 1482-1489 (black).

(b) *PML* intron 6 genomic translocation breakpoint distribution in *de novo* and secondary APL, showing translocation breakpoint hotspot in mitoxantrone-exposed cases.

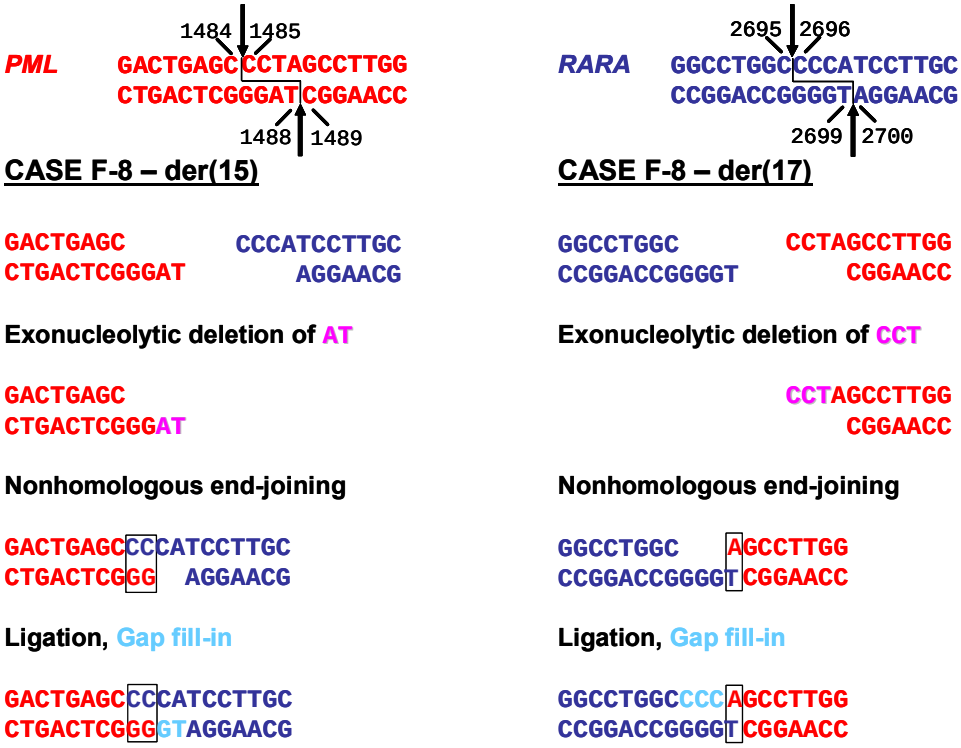

Figure 2

DNA topoisomerase II cleavage sites at *PML* position 1484 and *RARA* position 2695 with 4-base 5'overhangs, and processing to form der(15) and der(17) breakpoint junctions in a case of mitoxantrone-related APL (case F-8)

Native *PML* and *RARA* sequences are red and blue, respectively. The processing includes exonucleolytic nibbling to form two-base (der(15)) or single-base (der(17)) homologies and creation of both breakpoint junctions by error-prone NHEJ. In formation of the der(15), positions 1487-1488 on the antisense strand of *PML* are lost by exonucleolytic nibbling (pink) before NHEJ joins the indicated bases. Positions 1485-1487 on the sense strand of *PML* are lost by exonucleolytic nibbling (pink) and the der(17) forms by NHEJ. Template-directed polymerization of the relevant single-stranded overhangs fills in any gaps (light blue). Each *RARA* overhang is preserved completely.

APPENDIX XII:

ICC APL STUDY 01

PARENT/ GUARDIAN WRITTEN CONSENT FORM 1

(Please circle one)

1. Have you read the attached Information Sheet? **YES / NO**
2. Have you had an opportunity to discuss this study and ask questions? **YES / NO**
3. Have you received satisfactory answers to all of your questions? **YES / NO**
4. Have you received enough information about the study? **YES /NO**
5. To whom have you spoken? Dr. / Mr. / Ms._____
6. Do you understand that you are free to withdraw your child from the study: **YES / NO**

   *at any time

   *without having to give reasons

   *without affecting his/her future medical care?

7. Sections of the medical records relating to your child's participation in the study may be inspected by responsible individuals from the sponsor (to be decided),the national clinical trials unit, CINECA and Government Regulatory Authorities. Information contained within your child's medical record and records maintained by National Government Registries may also be used to follow up your child's health status. All personal details will be treated as STRICTLY CONFIDENTIAL by these individuals and organisations.

   Do you give your permission for these individuals to have access to your child's records? **YES / NO**

8. Do you agree for your child to participate in this study? **YES / NO**
9. Do you agree to your GP being informed of his/her participation in this study? **YES / NO**
10. Do you give permission for your child's left-over samples to be stored and used for future ethically approved studies? **YES / NO**

PARENT/GUARDIAN CONSENT FORM:

- I have been given a copy of the relevant Parent Information Sheet for this study. I have read it and understood it.
- I have discussed my child's participation in this Study with the Study Doctor and/or another person nominated by him/her.
- I have had the opportunity to ask the Study Doctor any questions and I have received satisfactory answers. If I want to ask any further questions I understand that I may contact the Study Doctor or his/her colleagues or staff.
- I understand that I must tell the Study Doctor (or his/her nominee) if I notice any unusual or unexpected symptoms or if my child's health changes.
- I understand that my child's medical records may need to be reviewed by the study sponsors (to be decided), the national clinical trials unit, CINECA or Government Regulatory Authorities. I also understand that information contained within my child's medical record and records maintained by National Government Registries may also be used to follow up my child's health status.
- I understand that I may withdraw my child from the study at any time, without giving a reason, and without affecting the standard of medical care my child will receive.

I voluntarily agree for my child to participate in this study/part of the study.

Name of patient

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Name of parent/guardian	**Signature**	**Date**

I certify that I have explained to the above patient's parent(s) or guardian the nature and purpose of this study, and the potential benefits and possible risks associated with participation in this study. I have answered all questions that have been raised.

----------	**----------**	**----------**
Name of Investigator	**Signature**	**Date**

ICC APL STUDY 01

PATIENT WRITTEN CONSENT FORM 1

(Please circle one)

1. Have you read the attached Information Sheet? **YES / NO**
2. Have you had an opportunity to discuss this study and ask questions? **YES / NO**
3. Have you received satisfactory answers to all of your questions? **YES / NO**
4. Have you received enough information about the study? **YES /NO**
5. To whom have you spoken? Dr. / Mr. / Ms._____
6. Do you understand that you are free to withdraw from the study: **YES / NO**

   *at any time

   *without having to give reasons

   *without affecting your future medical care?

7. Sections of your medical records relating to your participation in the study may be inspected by responsible individuals from the sponsor (AIEOP), the national clinical trials unit, CINECA and Government Regulatory Authorities. Information contained within your medical record and records maintained by National Government Registries may also be used to follow up your health status. All personal details will be treated as STRICTLY CONFIDENTIAL by these individuals and organisations.

   Do you give your permission for these individuals to have access to your records? **YES / NO**

8. Do you agree to participate in this study? **YES / NO**
9. Do you agree to your GP being informed of your participation in this study? **YES / NO**
10. Do you give permission for your left-over samples to be stored and used for future ethically approved studies? **YES / NO**

PATIENT CONSENT FORM:

- I have been given a copy of the relevant Patient Information Sheet for this study. I have read it and understood it.
- I have discussed my participation in this Study with the Study Doctor and/or another person nominated by him/her.
- I have had the opportunity to ask the Study Doctor any questions and I have received satisfactory answers. If I want to ask any further questions I understand that I may contact the Study Doctor or his/her colleagues or staff.
- I understand that I must tell the Study Doctor (or his/her nominee) if I notice any unusual or unexpected symptoms.
- I understand that my medical records may need to be reviewed by the study sponsors (AIEOP), the national clinical trials unit, CINECA or Government Regulatory Authorities. I also understand that information contained within my medical record and records maintained by National Government Registries may also be used to follow up my health status.
- I understand that I may withdraw from the study at any time, without giving a reason, and without affecting the standard of medical care that I will receive.

I voluntarily agree for to participate in this study/part of the study.

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Name of patient	**Signature**	**Date**

I certify that I have explained to the above patient the nature and purpose of this study, and the potential benefits and possible risks associated with participation in this study. I have answered all questions that have been raised.

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Name of Investigator	**Signature**	**Date**

Appendix A: AIEOP Group Specific Appendix

ICC APL Study 01
TREATMENT STUDY FOR CHILDREN AND ADOLESCENTS WITH ACUTE PROMYELOCYTIC LEUKEMIA

Introduction

All Italian investigators shall refer to this appendix as they are under the sponsorship of AIEOP. Administrative details and aspects of the protocol specific to AIEOP are included in this Group Specific Appendix.

Inclusion criteria

- Patients with a clinical diagnosis of initial APL and subsequently confirmed to have PML-RARα, NPM1-RARα or NUMA-RARα fusion. Whilst this study is only for ATRA-sensitive APL, APL is a hematological emergency and ATRA should be commenced as soon as the diagnosis is suspected. Study entry should not wait until the diagnosis has been confirmed molecularly or cytogenetically
- **Less than 18 years of age at initial diagnosis**
- Considered suitable for anthracycline-based chemotherapy
- Written informed consent available
- Females of childbearing age must have a negative pregnancy test and subsequently must attempt to avoid pregnancy

Diagnostic Material

Central morphological review is made as in the standard practice; all bone marrow and peripheral blood samples are centralized for morphological diagnosis as follows:

Contact Name: Prof. Giuseppe Basso
Address: Dipartimento di Pediatria, Azienda Universitaria Ospedale,

Via Giustiniani 3
City: Padova
Postcode: 35128
Email: giuseppe.basso@unipd.it
Tel: 049-8213523, 8211471

Cytogenetics

Cytogenetics will be carried out locally and centralized nationally, as in the standard practice, to the national cytogenetic laboratory, as follows:

Contact Name: Prof. Giuseppe Basso
Address: Dipartimento di Pediatria, Azienda Universitaria Ospedale,
Via Giustiniani 3
City: Padova
Postcode: 35128
Email: giuseppe.basso@unipd.it
Tel: 049-8213523, 8211471

Immunophenotyping

This should be done locally and and centralized nationally, as in the standard practice, to the national immunophenotyping laboratory, as follows:

Contact Name: Prof. Giuseppe Basso
Address: Dipartimento di Pediatria, Azienda Universitaria Ospedale,
Via Giustiniani 3
City: Padova
Postcode: 35128
Email: giuseppe.basso@unipd.it
Tel: 049-8213523, 8211471

Informed consent document

Documented informed consent must be obtained for all parent/guardian of the patients and for all patients aged > 10 years.
These should include:

- Appendix X: Parent/guardian Information Sheet
- Appendix X: Patient 10 – 18 years Information Sheet
- Appendix XII: Parent/guardian Written Consent Form 1
- Appendix XII: Patient Written Consent Form 1
- Appendix B: Informed Consent to the treatment of data according to – Deliberazione 24 Luglio 2008 - Linee guida per i trattamenti dei dati personali nell'ambito delle sperimentazioni cliniche di medicinali. (Deliberazione n. 52). (GU n. 190 del 14-8-2008)

Appendix B: AIEOP Group Specific Appendix

ICC APL Study 01
TREATMENT STUDY FOR CHILDREN AND ADOLESCENTS WITH ACUTE PROMYELOCYTIC LEUKEMIA

GARANTE PER LA PROTEZIONE DEI DATI PERSONALI

Deliberazione 24 luglio 2008

Linee guida per i trattamenti di dati personali nell'ambito delle sperimentazioni cliniche di medicinali. (Deliberazione n. 52). (GU n. 190 del 14-8-2008)

Allegato n. 1
Informativa e manifestazione del consenso al trattamento dei dati personali

Titolari del trattamento e relative finalita'

Il Centro di sperimentazione (Sezione di Ematologia, Dipartimento di Biotecnologie Cellulari ed Ematologia, Università La Sapienza, Roma) e l'Associazione Italiana di Ematologia ed Oncologia Pediatrica (AIEOP), che ha commissionato lo studio che Le e' stato descritto, ciascuno per gli ambiti di propria competenza e in accordo alle responsabilita' previste dalle norme della buona pratica clinica (decreto-legge n. 211/2003), tratteranno i dati personali di vostro/a figlio/a, in particolare quelli sulla salute e, soltanto nella misura in cui sono indispensabili in relazione all'obiettivo dello studio, altri dati relativi alla Sua origine, ai Suoi stili di vita e alla Sua vita sessuale (ecc.), esclusivamente in funzione della realizzazione dello studio e a fini di farmacovigilanza.
A tal fine i dati indicati saranno raccolti dal Centro di sperimentazione e trasmessi all'AIEOP e alle persone o societa' esterne che agiscono per loro conto, tra le quali il CINECA (Consorzio Universitario senza scopo di lucro).

Il trattamento dei dati personali relativi alla cartella clinica elettronica del paziente e' indispensabile allo svolgimento dello studio: il rifiuto di conferirli non consentira' a vostro/a figlio/a di parteciparvi.

Natura dei dati

Il medico che seguira' vostro/a figlio/a nello studio La/o identifichera' con un codice: i dati che La/o riguardano raccolti nel corso dello studio, ad eccezione del Suo nominativo, saranno trasmessi al CINECA, registrati, elaborati e conservati unitamente a tale codice, alla Sua data di nascita, al sesso, al Suo peso e alla Sua statura (tutti i dati personali relativi alla cartella clinica). Soltanto il medico e i soggetti autorizzati potranno collegare questo codice al Suo nominativo.

Modalita' del trattamento

I dati, trattati mediante strumenti anche elettronici, saranno diffusi solo in forma rigorosamente anonima, ad esempio attraverso pubblicazioni scientifiche, statistiche e convegni scientifici. La partecipazione di vostro/a figlio/a allo studio implica che, in conformita' alla normativa sulle sperimentazioni cliniche dei medicinali, il personale del CINECA, il Comitato Etico, e le autorita' sanitarie italiane e straniere potranno conoscere i dati che La/o riguardano, contenuti anche nella Sua documentazione clinica originale, con modalita' tali da garantire la riservatezza della Sua identita'.

Esercizio dei diritti

Potra' esercitare i diritti di cui all'art. 7 del Codice (es. accedere ai Suoi dati personali, integrarli, aggiornarli, rettificarli, opporsi al loro trattamento per motivi legittimi, ecc.) rivolgendosi direttamente al centro di sperimentazione (Dott.ssa Anna Maria Testi) o, per il suo tramite, all'AIEOP e al CINECA.
Potra' interrompere in ogni momento e senza fornire alcuna giustificazione la partecipazione di vostro/a figlio/a allo studio: in tal caso, i campioni biologici a Lei/Lui correlati verranno distrutti. Non saranno inoltre raccolti ulteriori dati che La/o riguardano, ferma restando l'utilizzazione di quelli eventualmente gia' raccolti per determinare, senza alterarli, i risultati della ricerca.

Consenso

Sottoscrivendo tale modulo acconsento al trattamento dei dati personali di mio/a figlio/a per gli scopi della ricerca nei limiti e con le modalita' indicate nell'informativa fornitami con il presente documento.

Nome e Cognome dell'interessato (in stampatello)…….

Firma dell'interessato……

Data ….…./…….