STUDY SYNOPSIS

INTERNATIONAL PRINCIPAL INVESTIGATORS
Dr. Anna Maria Testi, Department of Cellular Biotechnologies and Hematology, University La Sapienza, Via Benevento 6, 00161 Rome, Italy. Phone: +39.0649977422; fax+39.0644241984; E-mail: testi@bce.uniroma1.it

Prof. Franco Locatelli, Department of Pediatric Hematology and Oncology, IRCCS Bambino Gesù Children’s Hospital, Piazza Sant’Onofrio 4, 00185 Rome, Italy. Phone: +39 06 6859 2678; Fax: +39 06 6859 2292. E-mail: franco.locatelli@opbg.net

Prof. Gertjan Kaspers, Pediatric Oncology/Hematology, VU University Medical Center, De Boekeelaan 1117, NL-1081HV Amsterdam, The Netherlands. Phone: +31 20 4442420; Fax: +31 20 4442918. E-mail: GJL.Kaspers@vumc.nl

TITLE OF STUDY
A multicentre study combining arsenic trioxide (ATO) and all-trans retinoic acid (ATRA) for patients with newly diagnosed, standard-risk acute promyelocytic leukemia

CONDITION
Newly diagnosed standard-risk acute promyelocytic leukemia (APL/AML M3)

PRIMARY OBJECTIVE
To validate the efficacy in terms of event-free survival of a treatment combining arsenic trioxide (ATO) and all-trans retinoic acid (ATRA) in newly diagnosed APL standard-risk children and adolescents

SECONDARY OBJECTIVE
• To evaluate the short- and long-term toxicity profile of ATO in pediatric patients
• To compare the clearance kinetics of minimal residual disease (MRD) with that of the previous AIDA-like protocols, COG protocol and ICC APL Study 01
• To compare the duration of hospitalization and quality of life with the previous AIDA-like protocols and ICC APL study 01
• To prospectively evaluate the impact of FLT3-ITD on this patient population

TREATMENT PLAN
Treatment plan:
Arsenic trioxide (ATO) and ATRA

Duration of study recruitment:
36 months

Follow-up per patient:
5 years

KEY INCLUSION AND EXCLUSION CRITERIA
Key inclusion criteria:
- Newly diagnosed APL confirmed by the presence of PML/RARα, NPM1-RARα or NUMA-RARα fusion
- Age <18 years
- WBC at diagnosis ≤10 x 10⁹/L
- Bilirubin serum levels ≤3 mg/dL
- Creatinine serum levels ≤2 times the normal value for age
- Written informed consent by parents or legal guardians
**Exclusion criteria**
- Patients with a clinical diagnosis of APL but subsequently found to have PLZF-RARα fusion or lacking PML-RARα, NPM1-RARα or NUMA-RARα rearrangement should be withdrawn from the study and treated on an alternative protocol.
- WBC at diagnosis >10 x 10^9/L
- Significant arrhythmias, ECG abnormalities (see below), other cardiac contraindications (L-FeV < 50%) or neuropathy
- Concurrent active malignancy
- Uncontrolled life-threatening infections
- Pregnant or lactating females
- Patients who had received alternative therapy (APL not initially suspected; ATRA and/or ATO not available)

*ECG abnormalities:
  - Congenital long QT syndrome
  - History or presence of significant ventricular or atrial tachyarrhythmia
  - Clinically significant resting bradycardia (<50 beats per minute)
  - QTc > 450 msec documented during screening EKG

**TREATMENT**

**Induction**
ATO 0.15 mg/kg iv over 2 hours daily starting on day 1. If acute vasomotor reaction occurs, the infusion duration may be prolonged to 4 hours. ATO will be continued until achievement of hematological remission or for a maximum of 45 days. The first bone marrow aspirate to document achievement of hematological remission will be performed on day +28 and then repeated weekly whenever indicated/necessary.

ATRA 25 mg/m²/day will be administered orally in two equally divided doses and rounded to the nearest 10 mg increment, starting on day 1. ATRA treatment will be continued until hematological remission for a maximum of 45 days.

**Consolidation**
ATO 0.15 mg/kg iv over 2 hours daily for 5 days every week. Treatment will be continued for 4 weeks on and 4 weeks off, for a total of 4 cycles.

ATRA 25 mg/m²/day will be administered orally in two equally divided doses and rounded to the nearest 10 mg increment. Treatment will be administered for 2 weeks on 2 weeks off and for a total of 7 cycles.

A lumbar puncture with intrathecal injection of Ara-C (dose chosen according to patient’s age) will be performed at the beginning of the first and 3 course of consolidation therapy.

**Concomitant therapies**
- Prednisone 0.5 mg/kg/day from day 1 day +21 of induction to prevent differentiation syndrome (once known as ATRA syndrome). In case it will occur, Dexamethasone at 10 mg/m²/day in 3 divided doses will be employed until resolution of symptoms.
- Platelet concentrate transfusions to maintain platelets >30x10^9/l during the first 10 days. After day 10, platelets concentrates will be transfused when platelets count is <20x10^9/l or in presence of hemorrhagic symptoms.
- Packed red cell concentrates must be transfused to maintain Hb levels >8 g/dl.
- Supplemental electrolytes administered intravenously, to maintain potassium concentrations above 4 mEq/l and magnesium concentrations above 1.8 mg/dl (0.74 mmol/l) in order to reduce the risk of cardiac arrhythmia.

**Concomitant therapies in case of leukocytosis**
Guidelines for administering hydroxyurea (HU) in patients who will develop leukocytosis after initiation of therapy, are detailed in the table below:

<table>
<thead>
<tr>
<th>WBC 10 – 50 x 10^9/L</th>
<th>HU 20 - 30 mg/kg per day in 2 divided doses</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC &gt; 50 x 10^9/L</td>
<td>HU 40 – 60 mg/kg per day in 2 divided doses</td>
</tr>
</tbody>
</table>

HU must be discontinued when the WBC count will decrease to <10x10^9/L.
ASSESSMENT OF RESPONSE

One or more bone marrow (BM) aspirates will be carried out after induction therapy, prior to the first block of consolidation therapy to document the achievement of morphological complete remission (CR). BM aspirates will be repeated also after the 3rd consolidation course to document the achievement of molecular remission, after treatment discontinuation and then at 3 months, 6 months, 9 months and 12 months after treatment discontinuation.

Definitions

All responses may be hematological, cytogenetic or molecular.

**Complete remission (CR)**
- 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 > 1.0 x 10^9/l and the platelet count > 80 x 10^9/l.
- Cytogenetic Remission – disappearance of the diagnostic clonal abnormality [i.e. t(15;17)]
- Molecular Remission – absence of PML/RARα or NPM1-RARα or NUMA-RARα fusion transcripts in bone marrow by RQ-PCR, with an assay sensitivity of at least 10^-4.

Treatment failure
- Early death (ED) – any death occurring within 14 days from diagnosis from any cause
- Induction Death (ID) – any death occurring after 14 days from diagnosis, but before achieving CR
- Death in CR – any death occurring in patients who are in CR
- Resistant/Refractory Disease (RD) – persistent morphological evidence of APL at the end of induction
- Molecular Resistant/Refractory Disease (mRD) – persistence of the hybrid transcripts in bone marrow cells at the end of the 3rd consolidation course. Molecular resistance will always be confirmed in two consecutive marrow samples taken 2 weeks apart
- 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 transcripts in two successive samples in patients previously in molecular remission

STATISTICAL ANALYSIS

Efficacy / test accuracy:
The primary endpoint of the study is to validate the efficacy (measured as event-free survival probability) of ATO+ATRA treatment in childhood APL. This efficacy endpoint includes the following events: no achievement of hematological complete remission after induction therapy; no achievement of molecular remission after three consolidation courses (molecular resistance); relapse (hematological/molecular); death due to any cause.

Description of the primary efficacy / test accuracy analysis and population:
The goal is to demonstrate an equivalence of this treatment which does not contain cytostatic agents compared to the standard treatment combining ATRA and chemotherapy (i.e. ICC APL Study 01). The primary efficacy analysis will be performed in the intention-to-treat population. Further exploratory efficacy analyses may be performed in the per-protocol population.

The treatment in an individual patient will be terminated in case of:
- Normal treatment completion
- Grade 3-4 toxicity unresponsive to dose reduction
- Failure to achieve hematological CR at the end of induction therapy
- Failure to achieve molecular CR after three consolidation courses
- Relapse (hematological/molecular)
- 3 months delay among each programmed treatment cycle
- Major protocol violation
- Subject withdrew consent
- Lost to follow up
- Death
- Investigator’s opinion that therapy is not beneficial
- Ineligibility (PML-RARA RT-PCR negative or not evaluable at diagnosis)
Acute promyelocytic leukemia (APL) is a variant of acute myeloid leukemia (AML) characterized by consistent clinical, morphological, and genetic features. The disease-peculiar translocation (15;17) leads to the formation of the fusion gene PML-RAR alpha (PML-RARA). Typical APL features include a striking sensitivity to anthracyclines and the response in vitro and in vivo to differentiation therapy with all-trans retinoic acid (ATRA). The initial historical treatment was based on anthracyclines in combination with ARA-C. In the 1980s, the introduction of ATRA for the treatment of adults and children, led to an increase of remission rates up to 90%, together with a reduction of morbidity and mortality originally associated mostly with the typical APL coagulopathy at initial presentation (2-8). Nevertheless, still up to 10% of patients experience therapy-related mortality, and side-effects on the short- and long-term are significant.

### 1. RELEVANCE

#### 1.3 PREVALENCE, INCIDENCE, MORTALITY

Acute promyelocytic leukemia (APL) is a variant of acute myeloid leukemia (AML) characterized by consistent clinical, morphological, and genetic features. The disease-peculiar translocation (15;17) leads to the formation of the fusion gene PML-RAR alpha (PML-RARA). Typical APL features include a striking sensitivity to anthracyclines and the response in vitro and in vivo to differentiation therapy with all-trans retinoic acid (ATRA). The initial historical treatment was based on anthracyclines in combination with ARA-C. In the 1980s, the introduction of ATRA for the treatment of adults and children, led to an increase of remission rates up to 90%, together with a reduction of morbidity and mortality originally associated mostly with the typical APL coagulopathy at initial presentation (2-8). Nevertheless, still up to 10% of patients experience therapy-related mortality, and side-effects on the short- and long-term are significant.

#### 1.2. BURDEN OF THE DISEASE

As a result of risk-adapted therapy currently approximately 90% of the patients with newly-diagnosed APL can be cured from their disease. Despite the dramatic progress achieved in front-line therapy of APL with the ATRA plus IDArubicin (AIDA)-based regimen, relapses still occur in approximately 15% of patients. Moreover, these regimes are associated with significant toxicity due to severe myelosuppression frequently resulting in life-threatening infections, and with serious, although infrequent, late complications such as cardiomyopathy and the occurrence of secondary MDS (9,10). Furthermore, the current intensive standard therapy requires hospitalization of the patients including considerable financial resources and diminished quality-of-life.

#### 1.3 IMPROVEMENT OF THERAPY / IMPACT OF THE TRIAL

**Novelty:** Current conventional chemotherapy-based treatment can cure the majority of APL patients. Based on the APL0406 study in non-high-risk APL carried out in adults by the Italian-German collaboration we propose to validate the ATO/ATRA combination in pediatric patients. All major European cooperative groups have expressed their interest to join this effort.

**Clinical impact:** Available data in adults indicate that at least standard-risk APL patients may be cured without chemotherapy (with ATO/ATRA only), which is a pivotal milestone in the treatment of APL (1). However, this approach has been tested in a limited number of pediatric patients. The retrospective analyses of a single-center pediatric experience, showed that the application of ATRA and ATO as induction and consolidation therapy for newly diagnosed children with APL, resulted in excellent outcomes and improved the long-term prognosis (CR 95.3%; EFS 92.5%). ATO was well tolerated and was devoid of major acute side-effects (11).

If the proposed study will meet the endpoint of being validate in children, this will result in a change of current clinical practice in APL therapy.

**Patient benefit:** Through this approach, we expect less toxicity and mortality with an improved quality of life in patients treated with ATO/ATRA compared to the historical controls treated according to the ICC APL 01 Study.

**Socioeconomic impact:** From the start of consolidation, patients will be treated on an outpatient basis. Additionally, we expect less toxicity in these patients leading to less hospitalization.

### 2. EVIDENCE

<table>
<thead>
<tr>
<th>Secondary endpoints:</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Rate of hematological CR after induction</td>
</tr>
<tr>
<td>- Rate of molecular CR after induction</td>
</tr>
<tr>
<td>- Rate of early death during induction</td>
</tr>
<tr>
<td>- Probability of overall survival (OS) 2 years after treatment discontinuation</td>
</tr>
<tr>
<td>- Cumulative incidence of relapse (CIR) at 2 years</td>
</tr>
<tr>
<td>- Incidence of hematological and non-hematological toxicity (CTC-NCI grading)</td>
</tr>
<tr>
<td>- Rate of molecular remission after 3 consolidation cycles</td>
</tr>
<tr>
<td>- Assessment of PML/RARA transcript level reduction during treatment</td>
</tr>
<tr>
<td>- Quality of life and cost-effectiveness</td>
</tr>
<tr>
<td>- Total hospitalization days during therapy</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>STUDY DESIGN</th>
</tr>
</thead>
<tbody>
<tr>
<td>International, multi-center study</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TRIAL DURATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recruitment period (months): 36</td>
</tr>
<tr>
<td>Duration of the entire trial (months): 60</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PARTICIPATING CENTERS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Approximately 100 Institutions across different European and non-European countries.</td>
</tr>
</tbody>
</table>
Following the demonstration of its striking activity in relapsed patients (1-20-19), ATO had been licensed in the USA and Europe for the treatment of relapsed and refractory APL. Following this experience, several investigators have successfully explored the effect of ATO in newly diagnosed APL patients (20-24). In fact, recently the APL0406 study by the GIMEMA-AMLSG-SAL groups provided evidence that the combination of ATO and ATRA is at least as effective as the AIDA regimen in adults with non-high-risk APL, while sparing mainly hematological toxicity. This approach will be tested in children aged less than 18 years with APL in this intergroup study comprising the most important pediatric European groups, as well as other groups from South America and Middle-East.

### 3. STATISTICAL ANALYSIS

All patients enrolled in the study will be analyzed, following an intention-to-treat principle. Characteristics of patients will be summarized by cross-tabulations (categorical variables), quartiles (median etc; for ordinal factors) or by standard positional and variation parameters (mean, standard deviation; for continuous variables). Non-parametric tests will be applied for comparisons between groups (Chi-Squared and Fisher Exact test for categorical variables, Mann-Whitney and Kruskal-Wallis test for continuous variables). Survival estimates (OS, EFS and DFS) will be calculated by the Kaplan-Meier Product Limit estimator. Cumulative Incidence curves (e.g. for relapse rate) will be estimated using the proper non-parametric method.

### 4. ETHICAL CONSIDERATIONS

Based on the available data there is enough evidence to assume that this novel ATO/ATRA-based regimen is at least non-inferior to standard AIDA-based treatment in standard-risk APL patients. Given the toxicity of conventional chemotherapy, APL children might highly benefit from this new approach. Nevertheless, this has not been studied so far in pediatric patients within a prospective trial. Therefore, the study coordinators will periodically review the EFS rate during the course of the study. Accordingly, we will appoint a data safety monitoring board consisting of 3 independent experts in the field.

### 6. TRIAL MANAGEMENT

#### 6.1. MAJOR PARTICIPANTS

<table>
<thead>
<tr>
<th>#</th>
<th>Name</th>
<th>Affiliation</th>
<th>Responsibility/Role</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Prof. Franco Locatelli</td>
<td>Ospedale Pediatrico Bambino Gesù, Roma</td>
<td>AIEOP, International PIs</td>
</tr>
<tr>
<td></td>
<td>Dr. Anna Maria Testi</td>
<td>La Sapienza University of Roma</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Prof. Gertjan Kaspers</td>
<td>VU University Med. Center, Amsterdam</td>
<td>DCOG, International PI</td>
</tr>
<tr>
<td>3</td>
<td>Prof. Anne Auvrignon</td>
<td>Hopital Trousseau, Paris</td>
<td>FRALLE/LAME, Co-PI</td>
</tr>
<tr>
<td>4</td>
<td>Prof. Brenda Gibson</td>
<td>University of Glasgow</td>
<td>CCLG, Co-PIs</td>
</tr>
<tr>
<td></td>
<td>Dr. Owen Smith</td>
<td>University of Dublin</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Prof. Dirk Reinhardt</td>
<td>University of Essen</td>
<td>G-BFM, Co-Pls</td>
</tr>
<tr>
<td></td>
<td>Prof. Ursula Creutzig</td>
<td>Medical High School, Hannover</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Prof. Michael Dworzak</td>
<td>Sant’Anna Children Hospital, Wien</td>
<td>A-BFM, Co-PI</td>
</tr>
<tr>
<td>7</td>
<td>Prof. Henrik Hasle</td>
<td>University Hospital, Skejby, Aarhus, Denmark</td>
<td>NOPHO, Co-PI</td>
</tr>
</tbody>
</table>

#### 6.2 TRIAL-SUPPORTING FACILITIES

The trial will be sponsored by AIEOP and data will be collected through the CINECA data warehouse. National groups will provide co-sponsors that will have contracts with AIEOP, describing duties and responsibilities. Individual groups and countries may decide to apply this protocol as treatment guideline, since it could be regarded as best available treatment.

Reference List

17. G. Lazo et al., Cancer 97, 2218 (2003).