CHILDHOOD ACUTE PROMYELOCYTIC LEUKEMIA

INTERNATIONAL SPONSOR: AIEOP
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ICC APL Study 02

Treatment study for children and adolescents with Acute Promyelocytic Leukemia

Open label, prospective multicenter trial

Version 4.0, dated September 25, 2024

Sponsor Code: ICC APL Study 02

EU CT Number: 2024-518669-83-00
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Trial Title: Treatment study for children and adolescents with Acute Promyelocytic Leukemia

Trial Number: ICC APL Study 02

Protocol Version: Version 4.0, dated September 25, 2024

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LOCAL SIGNATURE

Trial Title: Treatment study for children and adolescents with Acute Promyelocytic Leukemia

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I herewith certify that I agree to adhere to the trial protocol and to all documents referenced in the trial protocol.

	Date	**Signature**
Principal Investigator (site):	_____	_____
	Full Name	_____
	Organization/ Department	_____

TABLE OF CONTENTS

1. SYNOPSIS

1.1 TREATMENT SYNOPSIS FOR STANDARD-RISK (SR) PATIENTS

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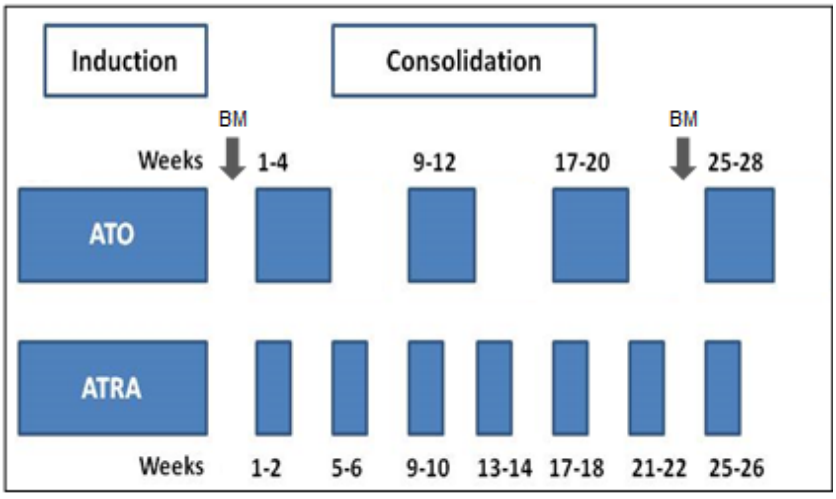
TITLE OF STUDY	A multicenter study combining arsenic trioxide (ATO) and all-trans retinoic acid (ATRA) for patients with newly diagnosed standard-risk acute promyelocytic leukemia
CONDITION	Newly diagnosed standard-risk acute promyelocytic leukemia (APL/AML M3)
PRIMARY OBJECTIVE	To evaluate the efficacy 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.
PRIMARY OBJECTIVE	- To evaluate 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 when combined with ATRA 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 estimate the cumulative incidence of both molecular and hematological relapse - To calculate the probability of overall survival and the early death rate - To prospectively evaluate the impact of FLT3-ITD in this patient population - To compare the duration of hospitalization and quality of life with the previous AIDA-like protocols and ICC APL study 01
KEY INCLUSION AND EXCLUSION	Key inclusion criteria:

CRITERIA	- Newly diagnosed APL confirmed by the presence of PML/RARα fusion gene - Age <18 years - WBC less than $10x10^9$/L at presentation before start of treatment - Written informed consent by parents or legal guardians - If applicable, female participants must have negative pregnancy test by beta-HCG dosing. - Patients of child-bearing or child-fathering potential must be willing to adapt their own conduct so as not to procreate during the study participation and must contact their physician to identify the most appropriate strategy for this purpose starting from the time of enrolment and for 3 months after receiving the last drug dose infusion. <u>Exclusion criteria</u> - Patients with a clinical diagnosis of APL but subsequently found to lack PML/RARα rearrangement should be withdrawn from the study and treated with an alternative protocol - Significant liver dysfunction (bilirubin serum levels >3 mg/dL, ALT/AST serum levels greater than 5 times the normal values) - Creatinine serum levels >2 times the normal value for age - Significant arrhythmias, EKG abnormalities (*see below), other cardiac contraindications (L-FEV < 50% or LV-FS <28%) - Neuropathy grade 2 or greater - 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) * EKG 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 PLAN	<u>Treatment plan</u>: Arsenic trioxide (ATO) and all-trans retinoic acid (ATRA) <u>Duration of study recruitment</u>: 72 months <u>Minimum follow-up per patient</u>: 2 years 

	Induction ATO 0.15 mg/kg/day i.v. over 2 hours daily starting on day 1 until achievement of CR and anyway for no more than 60 days. ATO can be discontinued during induction only after at least 28 days of treatment and when an absolute neutrophil count (ANC) greater than 1 x 10^9/L and a platelet (PLT) count greater than 100x109/L is achieved. If acute vasomotor reaction occurs, the infusion duration may be prolonged to 4 hours. The first bone marrow (BM) aspirate to document the achievement of hematological remission will be performed on day +35 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, starting on day 1. ATRA treatment will be continued until hematological remission for a maximum of 60 days and a minimum of 28 days. Consolidation There will be a 2-week interval between the end of induction therapy and the beginning of the first consolidation course. ATO will be administered at the dosage of 0.15 mg/kg/day i.v. 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/m^2/day will be administered orally in two equally divided doses and rounded to the nearest 10 mg. Treatment will be administered for 2 weeks on 2 weeks off and for a total of 7 cycles.
TREATMENT OTHERWISE	Concomitant therapies Prednisone 0.5 mg/kg/day from day 1 to day 15 of induction to prevent differentiation syndrome (once known as ATRA syndrome). In case this complication occurs, dexamethasone at 10 mg/m^2/day in 2-3 divided doses will be employed until resolution of symptoms. Platelet concentrate transfusions to maintain platelets >50 x 10^9/L during the first 10 days. After day 10, platelets concentrates will be transfused when either the platelet count is <20 x 10^9/L or in presence of hemorrhagic symptoms. Fresh frozen plasma or fibrinogen concentrates to maintain fibrinogen levels above 150 mg/dL. 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 sustained leukocytosis (e.g., for at least 3 days) after initiation of therapy, are detailed as follows: - WBC 20 - 50 x 10^9/L: HU 20 - 30 mg/kg per day in 2 divided doses - WBC > 50 x 10^9/L: HU 40 - 60 mg/kg per day in 2 divided doses HU must be discontinued when the WBC count will decrease back to <10 x 10^9/L.

ASSESSMENT OF RESPONSE	One or more BM aspirates will be carried out after induction therapy, prior to the first block of consolidation therapy, to document the achievement of hematological 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 - Complete remission (CR and CRi) ▪ Hematological Complete Remission – the bone marrow is regenerating normal hematopoietic cells and contains < 5% blast cells by morphology. The ANC in the peripheral blood should be > 1.0 x 109/L and the PLT count > 100 x 109/L. CR indicates a hematological remission with no signs of leukemia elsewhere. CRi indicates a CR except that peripheral blood neutrophils and/or platelets do not meet the criteria as defined above. ▪ Molecular Remission – absence of PML/RARα fusion transcript in BM 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 (maximum of 60 days) ▪ 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 a second consecutive marrow sample taken 2 weeks apart ▪ Hematological Relapse – reappearance of promyeloblasts/abnormal promyelocytes (> 5%) in the bone marrow ▪ Molecular relapse – reappearance of the transcripts in two successive samples taken at least 2 weeks apart 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 -EFS- probability) of ATO+ATRA treatment in SR childhood APL. This efficacy endpoint includes the following events: no achievement of hematological CR/CRi after induction therapy (resistant/refractory disease); 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 (e.g. ICC APL Study 01 or other internationally used protocols). 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. Safety

	The treatment in an individual patient will be terminated in case of: - Treatment completion - Grade 4 toxicity unresponsive to dose reduction - Failure to achieve hematological CR/CRi at the end of induction therapy - Failure to achieve molecular CR after three consolidation courses - Relapse (hematological/molecular) - 3-month delay among each programmed treatment cycle - Major protocol violation - Withdrawal of consent by parents or legal guardians - Lost to follow-up - Death - Investigator's opinion that therapy is not beneficial - Ineligibility (PML/RARα RT-PCR negative or not evaluable at diagnosis) <u>Secondary/exploratory endpoints:</u> - Rate of hematological CR/CRi after induction - Rate of molecular CR after induction - Level of MRD after induction - Rate of early and aplastic death during induction - Rate of overall survival (OS) at 2 years after treatment discontinuation - Cumulative incidence of either hematological or molecular relapse (CIR) at 2 years - Incidence of hematological and non-hematological toxicity (CTC-NCI grading) - Rate of molecular remission after 3 consolidation cycles - Assessment of PML/RARα transcript level reduction during treatment - Quality of life and cost-effectiveness - Total hospitalization days during therapy
STUDY DESIGN	International, multicenter study, aimed at recruiting at least 46 SR patients
TRIAL DURATION	Recruitment period (months): 72 Duration of the entire trial (months): 96 End of study: the last follow-up visit (so that 2 years after the end of treatment) of the last patient
PARTICIPATING CENTERS	Approximately 100 Institutions across different European Countries.

1.2 TREATMENT SYNOPSIS FOR HIGH-RISK (HR) PATIENTS

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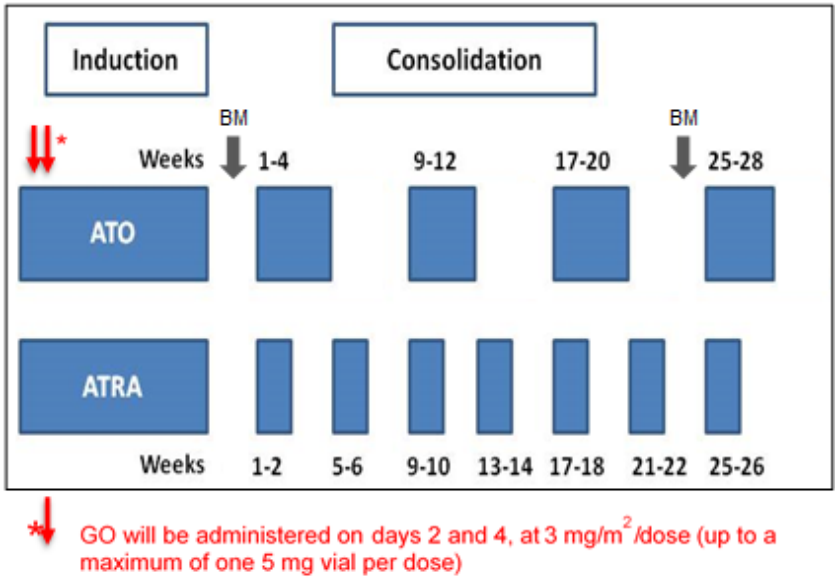
TITLE OF STUDY	A multicenter study with a safety run-in phase combining Gemtuzumab ozogamicin (GO), arsenic trioxide (ATO) and ATRA for patients with newly diagnosed high-risk acute promyelocytic leukemia
CONDITION	Newly diagnosed high-risk acute promyelocytic leukemia (APL/AML M3)
PRIMARY OBJECTIVE	- To evaluate the efficacy in terms of event-free survival of a treatment combining arsenic trioxide (ATO), all-trans retinoic acid (ATRA) and Gemtuzumab ozogamicin (GO) in newly diagnosed APL high-risk children and adolescents
SECONDARY OBJECTIVE	- To evaluate the short- and long-term toxicity profile of ATO when combined with ATRA plus GO in pediatric patients - To compare the clearance kinetics of minimal residual disease (MRD) with that of the previous AIDA-like protocols, COG protocols and ICC APL Study 01 - To estimate the cumulative incidence of both molecular and hematological relapse - To calculate the probability of overall survival and the early death rate - To prospectively evaluate the impact of FLT3-ITD in this patient population - To compare the duration of hospitalization and quality of life with the previous AIDA-like protocols and ICC APL study 01
KEY INCLUSION AND EXCLUSION CRITERIA	Key inclusion criteria - Newly diagnosed APL confirmed by the presence of PML/RARα fusion gene - Age <18 years - WBC more than 10x109/L at presentation before start of treatment - Written informed consent by parents or legal guardians - If applicable, female participants must have a negative pregnancy test by beta-HCG dosing. - Patients of child-bearing or child-fathering potential must be willing to adapt their own conduct so as not to procreate during the study participation and must contact their physician to identify the most appropriate approach strategy for this purpose starting from the time of enrolment and for 3 months after receiving the last drug dose. Exclusion criteria - Patients with a clinical diagnosis of APL but subsequently found to lack PML/RARα rearrangement should be withdrawn from the study and treated with an alternative protocol - Significant liver dysfunction (bilirubin serum levels >3 mg/dL, ALT/AST serum levels greater than 5 times the normal values) - Creatinine serum levels >2 times the normal value for age

	- Significant arrhythmias, EKG abnormalities (*see below), other cardiac contraindications (L-FEV < 50% or LV-FS <28%) - Neuropathy grade 2 or greater - 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) *EKG 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 PLAN	Treatment plan: Arsenic trioxide (ATO), all-trans retinoic acid (ATRA) and Gemtuzumab ozogamicin (GO) Duration of study recruitment: 72 months Minimum follow-up per patient: 2 years  Induction ATO will be administrated at the dosage of 0.15 mg/kg/day i.v. over 2 hours daily starting on day 1 until achievement of CR and anyway for no more than 60 days. ATO can be discontinued during induction only after at least 28 days of treatment and when an ANC greater than $1x10^9$/L and a PLT count greater than 100 $x10^9$/L is achieved. If acute vasomotor reaction occurs, the infusion duration may be prolonged to 4 hours. The first BM aspirate to document achievement of hematological remission will be performed on day 35 and then repeated weekly whenever indicated/necessary. ATRA 25 mg/m^2/day will be administered orally in two equally divided doses and rounded to the nearest 10 mg, starting on day 1. ATRA treatment will be continued until hematological remission for a maximum of 60 days, and a minimum of 28 days. GO will be administered on days 2 and 4, at 3 mg/m^2/dose i.v. (up to a

	maximum of one 5 mg vial per dose). In case, for logistic reasons, the patient is unable to receive the first dosage of GO within the first 72 hours from diagnosis, the possibility of administering a single dose of anthracycline (e.g., Idarubicin 12 mg/m^2 as prolonged i.v. infusion) in this time interval is left to the treating physician's decision. GO will be distributed to the Centers participating into the study by the pharmacy unit of the Ospedale Bambino Gesù of Rome, Italy. The drug will be supplied free-of-charge by the Pfizer Company. Consolidation There will be a 2-week interval between the end of induction therapy and the beginning of the first consolidation course. ATO will be administered at the dosage of 0.15 mg/kg/day i.v. 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/m^2/day will be administered orally in two equally divided doses. Treatment will be administered for 2 weeks on 2 weeks off and for a total of 7 cycles.
TREATMENT OTHERWISE	Intrathecal therapy A therapeutic lumbar puncture with intrathecal injection of Methotrexate, Methylprednisolone and Cytarabine (doses chosen according to patient's age) will be performed at the beginning of the 1st and 3rd course of consolidation therapy. Concomitant therapies Prednisone 0.5 mg/kg/day from day 1 to day 15 of induction to prevent differentiation syndrome (once known as ATRA syndrome). In case this complication occurs, dexamethasone at 10 mg/m2/day in 2-3 divided doses will be employed until resolution of symptoms. Platelet concentrate transfusions to maintain platelets >50x109/L during the first 10 days. After day 10, platelets concentrates will be transfused when platelet count is <20 x 10^9/L or in presence of hemorrhagic symptoms. Fresh frozen plasma or fibrinogen concentrates to maintain fibrinogen levels above 150 mg/dL. 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). Concomitant therapies in case of leukocytosis Guidelines for administering hydroxyurea (HU) in patients who have sustained leukocytosis (e.g., for at least 3 days) at time of or after initiation of therapy, are as follows: - WBC 20 – 50 x 10^9/L: HU 20 - 30 mg/kg per day in 2 divided doses - WBC >50 x 10^9/L: HU 40 - 60 mg/kg per day in 2 divided doses HU must be discontinued when the WBC count will decrease back to <10 x 10^9/L.
ASSESSMENT OF	One or more BM aspirates will be carried out after induction therapy, prior

RESPONSE	to the first block of consolidation therapy to document the achievement of hematological 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 - Complete remission (CR and CRi) ▪ Hematological Complete Remission – the BM is regenerating normal hematopoietic cells and contains <5% blast cells by morphology. The ANC in the peripheral blood should be > 1.0 x 10^9/L and the PLT count >100 x 10^9/L. CR indicates a hematological remission with no signs of leukemia elsewhere. A CRi indicates a CR except that peripheral blood neutrophils and/or PLT do not meet the criteria as defined above. ▪ Molecular Remission – absence of PML/RARα fusion transcript 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 (maximum of 60 days) ▪ 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 a second consecutive marrow sample taken 2 weeks apart ▪ Hematological Relapse – reappearance of promyeloblasts/abnormal promyelocytes (>5%) in the bone marrow ▪ Molecular relapse – reappearance of the transcripts in two successive samples taken at least 2 weeks apart 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 GO+ATO+ATRA treatment in childhood APL. This cumulative efficacy endpoint includes the following events: no achievement of hematological CR/CRi after induction therapy; no achievement of molecular remission after three consolidation cycles (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 classical cytostatic agents compared to the standard treatment combining ATRA and chemotherapy (e.g., ICC APL Study 01 or other international used protocols). In addition, it has been agreed with COG that the data will be compared to that of COG AAML 1331, in which a very similar approach is applied except that idarubicin is given instead of GO at induction (12 mg/m2/dose on days 1-3-5-7). The primary efficacy analysis will be performed in the intent-to-treat population. Further exploratory efficacy analyses may be performed in the per-protocol population.

	Safety The treatment in an individual patient will be terminated in case of: - Normal treatment completion - Grade 4 toxicity unresponsive to dose reduction - Failure to achieve hematological CR/CRi at the end of induction therapy - Failure to achieve molecular CR after three consolidation courses - Relapse (hematological/molecular) - months delay among each programmed treatment cycle - Major protocol violation - Withdrawal of consent by parents or legal guardians - Lost to follow-up - Death - Investigator's opinion that therapy is not beneficial - Ineligibility (PML/RARα RT-PCR negative or unevaluable at diagnosis) Secondary/exploratory endpoints: - Rate of hematological CR/CRi after induction - Rate of molecular CR after induction - Level of MRD after induction - Rate of early and aplastic death during induction - Rate of overall survival (OS) 2 years after treatment discontinuation - Cumulative incidence of either hematological or molecular relapse (CIR) at 2 years - Incidence of hematological and non-hematological toxicity (CTC-NCI grading) - Rate of molecular remission after 3 consolidation cycles - Assessment of PML/RARα transcript level reduction during treatment - Quality of life and cost-effectiveness - Total hospitalization days during therapy
STUDY DESIGN	International, multicenter study, after a safety run-in phase enrolling 6 patients; a Data and Safety Monitoring Committee (DSMC) will be installed and will review the toxicity data in these 6 patients before study itself is initiated. At least 43 HR patients will be included in the trial.
TRIAL DURATION	Recruitment period (months): 72 Duration of the entire trial (months): 96 End of study: the last follow-up visit (so that 2 years after the end of treatment) of the last patient
PARTICIPATING CENTERS	Approximatively 100 Institutions across different European Countries.

2. VISIT SCHEDULE

Table 1. Visit schedule for both SR and HR patients enrolled into the study

	Diagnosis	During Induction	Day 35 Induction Response	Prior Cons. 1	Prior Cons. 2	Prior Cons. 3	After Cons. 3	Prior Cons. 4	After Cons. 4	End of Treatment	Follow-Up Visit Until End of Trial
Physical examination	*incl. comorbidities	daily, including body weight	*	*	*	*	*	*	*	*	every 3 months until 1 year and every 6 months until 2 year, after treatment discontinuation
Vital signs and Performance status score	*	daily; during GO infusion vital signs must be monitored during infusion and for 4 hours after its completion	*	*	*	*	*	*	*	*	every 3 months until 1 year and every 6 months until 2 year, after treatment discontinuation
Blood counts (1)	*	daily during 1st week, afterwards at least 3 times/week	*	*	*	*	*	*	*	*	every 3 months until 1 year and every 6 months until 2 year, after treatment discontinuation
BM for morphology	*	-	*(7)	-	-	-	*	-	-	*	every 3 months until 1 year after treatment discontinuation (9)
BM for molecular biology (2)	*	-	*	-	-	-	*	-	-	*	every 3 months until 1 year after treatment discontinuation (9)
BM for cytogenetics	*	-	-	-	-	-	-	-	-	-	-
BM for immunophenotyping (3)	*	-	-	-	-	-	-	-	-	-	-
Coagulation tests (4)	*	daily until normalization, 2 times/week thereafter	*	*	*	*	*	-	-	*	-
Serum biochemistry (5)	*	2-3 times/week during the first 3 weeks, then weekly until CR	*	*	*	*	*	-	-	*	-
Electrocardiogram	*	biweekly until CR	-	*(8)	*(8)	*(8)	*	-	-	*	-

Echocardiography (6)	*	-	-	*	*	*	*	-	-	*	-
Urine analysis	*	-	-	-	-	-	-	-	-	-	-
Hepatitis and HIV serology	*	-	-	-	-	-	-	-	-	-	-
Pregnancy test (if appropriate)	*	-	-	-	-	-	-	-	-	-	-
Quality of Life Evaluation	*	-	*	*	*	*	*	-	-	*	every 6 months
Safety reporting (AEs, SAEs reporting)	All AEs end SAEs, irrespective of causality, will be collected between the time of informed consent signature until 30 days after the last dose of study drug product GO and other drugs administered in ICC-APL-02 protocol such as ATO, ATRA Only AEs and SAEs related to any study procedure or GO and other drugs administered in ICC-APL-02 protocol such as ATO, ATRA will be collected between the time of informed consent signature until end of study										

Legend:

"(*)" to be performed and "(-)" not to be performed

(1) hemoglobin, leukocytes, platelets, neutrophils, blasts

(2) PML-RARA (PML-RARA incl. isoforms, FLT3-ITD, FLT3-TKD only at diagnose)

(3) HLA-DR, CD2, CD7, CD9, CD11b, CD13, CD14, CD15, CD19, CD33, CD34, CD56, CD117

(4) Quick/PT, aPTT, fibrinogen, factor XIII, AT III, and D-dimers

(5) glucose, creatinine, uric acid, bilirubin, transaminases, alkaline phosphatase, LDH, sodium, potassium, calcium, phosphorus, magnesium, total proteins, albumin, cholesterol and triglycerides

(6) incl. L-VEF

(7) if not evaluable, repeat bone marrow on a serial basis until achievement of CR or failure

(8) EKG have to be performed weekly during ATO consolidation treatment

(9) any time for those patients with features suggestive for relapse

3. SUMMARY

TREATMENT STUDY FOR CHILDREN AND ADOLESCENTS WITH ACUTE PROMYELOCYTIC LEUKEMIA

Acute promyelocytic leukemia (APL) in children has become a highly curable disease with the combination of all-trans retinoic acid (ATRA) and anthracycline-based chemotherapy with an overall remission rates equal to or higher than 98% and cure rates now exceeding 80%[1-9].

Based on data coming from adults indicating that at least standard-risk APL patients may be cured without chemotherapy (e.g., with a treatment combining arsenic trioxide (ATO) and ATRA only)[10-12], this ICC APL 02 study was designed with the aim of validating the efficacy of a treatment combining:

- ATO and ATRA in newly diagnosed APL standard-risk (SR) children and adolescents and
- ATO, ATRA and Gemtuzumab ozogamicin (GO) in newly diagnosed APL high-risk (HR) children and adolescents.

Following one induction course of treatment combining ATO and ATRA +/- GO depending on risk stratification, patients will receive 4 ATO/ATRA based consolidation blocks. This is the first pediatric trial delivering a non-chemotherapy-based treatment for children with APL, being the whole treatment based on the use of ATRA, ATO (and GO in HR patients). The aim of the study is to demonstrate at least an equivalent efficacy and safety of this treatment not containing cytostatic agents compared to the standard protocols combining ATRA and chemotherapy (e.g., ICC APL Study 01).

The trial is open to all patients with a diagnosis of acute promyelocytic leukemia (APL) who are PCR-positive for the PML-RARα transcript and less than 18 years of age.

This will be an international study, comprising the most important pediatric European groups, expecting to recruit 46 and 43 patients in SR and HR arms, respectively, in 5 years. The duration of study recruitment will be 72 months with a minimum follow-up per patient of 2 years.

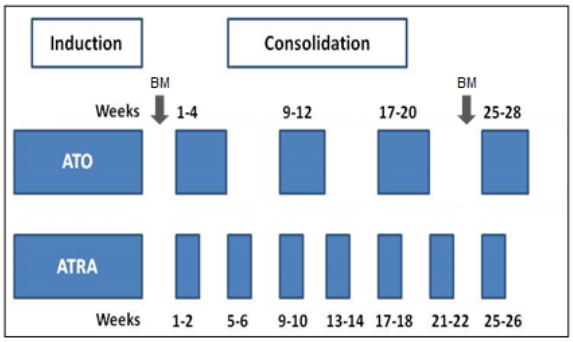
The evaluation of hematological CR will be carried out after induction therapy, prior to the first block of consolidation therapy. MRD results after induction will not have an impact on subsequent therapy. By contrast, MRD results after the third consolidation course will influence the subsequent treatment, MRD-positive patients being eligible to rescue treatment, including hematopoietic stem cell transplantation (HSCT). BM aspirates will be repeated after the end of therapy, and 3 months, 6 months, 9 months and 12 months after treatment discontinuation.

This is a collaborative international study in APL in children and adolescents aimed at providing information about procedures for the entry, treatment and follow-up of pediatric patients with APL. It is not intended that this document 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 protocol before starting the treatment.

4. OUTLINE

SR patients



HR patients

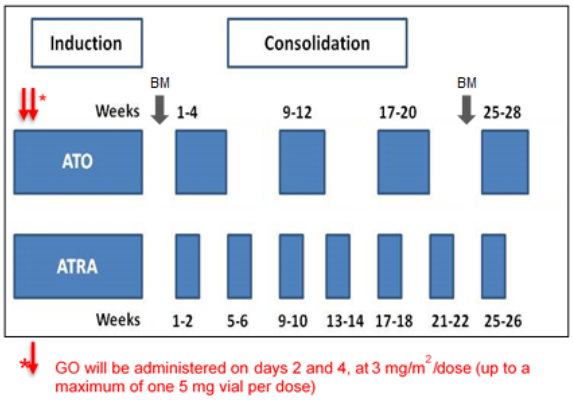


Figure 1. Treatment for PML-RARα+ APL in children and adolescents (patients with morphological APL must initially be treated according to this protocol, but patients who are subsequently found to lack PML-RARα fusion gene should thereafter be treated on their national standard AML protocol).

5. INTERNATIONAL STUDY MANAGEMENT

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<td colspan="3">Each Country will identify a Lab with a person responsible for molecular diagnosis and monitoring</td>
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6. 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
CNS	central nervous system
CR	complete remission
CRi	complete remission with incomplete hematologic recovery
DSMC	Data and Safety Monitoring Committee
DIC	disseminated intravascular coagulation
ED	early death
EFS	event-free survival
FLT3-ITD	internal tandem duplication of FLT3 gene
FFP	fresh-frozen plasma
FISH	fluorescence *in situ* hybridization
GO	Gemtuzumab ozogamicin (Mylotarg®)
HR	high-risk
ICC APL	international consortium for childhood APL
IDA	idarubicin
IT	intrathecal
MTX	methotrexate
MPDN	methylprednisolone
MRD	minimal residual disease
OS	overall survival
PB	peripheral blood
PJP	Pneumocystis jirovecii 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			
SOS	sinusoidal obstruction syndrome			
SR	standard risk			
WBC	white	blood	cell	count

7. ETHICAL CONSIDERATIONS

The ICC APL Study 02 must be approved by the national and Local Ethical Committee (LEC) at each treatment center, 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 02 will be conducted in accordance with the EU Directive for Good Clinical Practice in Clinical Trials.

Based on the currently available literature data, there is enough evidence to assume that this novel ATO/ATRA±GO-based regimen is at least non-inferior to standard AIDA-based and ICC APL Study 01 treatment in HR APL patients. Given the toxicity of conventional chemotherapy, APL children might highly benefit from this new approach, but no prospective trial is currently available. Therefore, the Principal Investigators of the study will periodically review the EFS rate during the course of the study. Accordingly, we will appoint a data safety monitoring committee (DSMC) consisting of 3 independent experts in the field.

8. OBJECTIVES

8.1 PRIMARY OBJECTIVE

- To assess, in an international pediatric study, the efficacy, in terms of event-free survival, of a combination of ATO and ATRA in newly diagnosed SR APL children and adolescents and to explore the safety and efficacy of a combination therapy comprising ATRA/ATO + GO in HR APL.

8.2 SECONDARY OBJECTIVES

- To evaluate the short- and long-term toxicity profile of ATO in pediatric patients, when combined with ATRA (SR APL) or ATRA plus GO (HR APL)
- 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 estimate the cumulative incidence of both molecular and hematological relapse
- To calculate the probability of overall survival and the early death rate
- To prospectively evaluate the impact of FLT3-ITD on this patient population
- To compare the duration of hospitalization and quality of life with those of the previous AIDA-like protocols and ICC APL study 01

9. ENDPOINTS

The primary endpoint of the study is event-free survival (EFS). This cumulative 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, including early death, at 2 years from diagnosis. We aim at reaching a 3-year EFS probability of 90% (95% CI: 84.1-95.9%) and 80% (95% CI: 72.1-87.9%) in SR and HR patients, respectively.

Secondary end-points will be:

- Rate of hematological CR after induction
- Rate of early and aplastic death during induction
- Overall survival (OS)
- Cumulative incidence of either hematological and molecular relapse (CIR)
- Incidence of hematological and non-hematological toxicity
- Kinetics of MRD clearance
- Rate of molecular remission after 3 consolidation cycles
- Assessment of PML/RARα transcript level reduction during treatment
- Toxicity - hematological and non-hematological
- Supportive care requirements
- Total hospitalization days during therapy and health economic impact

10. STUDY DESIGN

The ICC APL Study 02 is an international, multicenter non-randomized study delivering risk-stratified treatment based on the ICC APL 01 Study and experiences from adults on the efficacy of the combination of ATRA plus ATO in SR patients with APL.

The study is aimed at recruiting 46 SR patients and 43 HR patients. With regards to the part of the study for HR patients, this will start after a safety run-in phase enrolling 6 patients; a DSMC will be installed and will review the toxicity data in these 6 patients before the continuation of the study. This is the first pediatric trial delivering a non-chemotherapy-based treatment for children with APL, being the whole treatment based on the use of ATRA ATO +/- GO.

1. All patients who will enter the study must be PCR positive for the PML-RARα transcript. 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 according to the modified Sanz criteria[13]; standard risk (SR) - WBC < 10 x 10^9/L: high risk (HR) - WBC $\geq$ 10 x 10^9/L.
3. The combination ATRA plus ATO is included in induction and consolidation. No maintenance therapy will be delivered.
4. The induction is represented by one course combining ATRA and ATO. Two doses of GO will be administered to HR patients at the beginning of induction (e.g., on day +2 and +4).
5. Both SR and HR patients will receive 4 ATO/7 ATRA blocks in consolidation.
6. The evaluation of hematological response will be carried out after induction therapy, prior to the first block of consolidation therapy. MRD results will be evaluated only for research purposes and will not have an impact on subsequent therapy.
7. HR patients will receive a therapeutic lumbar puncture with intrathecal injection of Methotrexate, Methylprednisolone, and Cytarabine (doses chosen according to patient's age) at the beginning of the 1st and 3rd course of consolidation therapy. SR patients will not receive intrathecal prophylaxis during this treatment protocol.
8. Concomitant therapies will be delivered in specific cases like hydroxyurea (HU) in patients who will develop leukocytosis after initiation of therapy or prednisone during induction therapy to prevent differentiation syndrome.
9. In case of failure to achieve molecular CR after the third consolidation course or in case of relapse (hematological/molecular), patients will be eligible for a salvage regimen[14].

11. JUSTIFICATION OF STUDY DESIGN AND TREATMENT SCHEDULES

11.1 BACKGROUND

Acute promyelocytic leukemia (APL) is a subtype of acute myeloid leukemia (AML) characterized by consistent clinical, morphologic, and genetic features. According to the FAB classification, APL is designated as "M3 leukemia" and assigned to the WHO defined type of AML with recurrent cytogenetic abnormalities, "acute promyelocytic leukemia with PML/RARα"[15]. Detection of the PML-RARα fusion is carried out by fluorescence *in situ* hybridization (FISH) or RT-PCR, while the t(15;17)(q22;q12) anomaly is detectable by conventional karyotyping. APL is often clinically characterized by the presence of coagulation abnormalities, including mostly hyperfibrinolysis, disseminated intravascular coagulation (DIC), and unspecific proteolysis. In a significant proportion of cases, mutations of the FLT3 gene with an internal tandem duplication (ITD) of the juxtamembranous domain and point mutations in the tyrosine kinase domain II can be detected, although their independent prognostic impact remains controversial. Both mutations are correlated with higher leukocyte counts, while a FLT3-ITD mutation is associated with the M3variant (M3v) subtype and the S-type PML/RARα protein. APL represents approximately 4-8% of pediatric AML[16], with a higher incidence in children of Hispanic and Mediterranean origin. Indeed, while in the United States, as in Central and Northern Europe, the percentage of APL patients is 5-7% of all pediatric AML cases, a higher frequency (even up to 15-20%) is reported in children of Latino/Hispanic descent. In the AML Berlin-Frankfurt-Münster (BFM) studies, 8-10 APL children were registered per year, compared to 16-18 pediatric patients treated, each year, within the Gruppo Italiano Malattie Ematologiche dell'Adulto (GIMEMA)-Associazione Italiana Ematologia Oncologia Pediatrica (AIEOP) AIDA trials[17].

The median age at presentation is similar to that of other AML subtypes (9-10 years), but APL has rarely been reported in the first 2 years 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 [1]. As compared to the adult disease, secondary APL is much less frequent in children. Some studies have indicated that the female sex is predominant among children, but not in adults.

As mentioned above, 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%)[18], 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 latter subtype and that involving NuMA[19] as a result of t(11;17)(q13;q21) are also sensitive to retinoic acid; by contrast, 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].

Pediatric patients appear to present more commonly with hyperleukocytosis, as compared to their adult counterparts [5,17]. Approximately 35-40% of children with APL fall within a HR group defined by a presenting WBC ≥10 x 10^9/L, which is often associated with M3v morphology, presence of FLT3 length mutations and predicts for poorer outcome[5,6,16,20]. This is due to both an increased risk of induction death, particularly as a result of hemorrhage, and a significantly higher rate of relapse[6-8,16].

Specific therapeutic strategies for pediatric APL have been derived from adult trials that included children. Most of these approaches are based on the combination of ATRA and anthracycline-containing chemotherapy[21].

While until the late 1980s, chemotherapy with anthracyclines and cytarabine was the only treatment option for patients with APL, the introduction of ATRA for the treatment of adults and children, led to an increase of remission rates up to 98%, together with a reduction of morbidity and mortality, mostly associated with early fatal coagulopathy[7,17].

RT-PCR, besides offering the advantage of defining the PML/RARα rearrangement at diagnosis, enables sensitive detection of minimal residual disease (MRD) during treatment and follow-up[22-26]. The information derived from MRD monitoring can help in the definition of a relapse risk stratification[27], and can be translated into operational therapeutic decisions, such as the institution of salvage treatment at the time of PCR-detected minimal disease recurrence[28-31].

Despite the dramatic improvements achieved in frontline therapy of APL with ATRA plus anthracycline-based regimens, relapses still occur in approximately 20% of patients. Moreover, these regimens are associated with significant toxicities due to severe myelosuppression frequently associated with life-threatening infections and potentially serious late effects, including development of secondary MDS/AML and anthracycline-related myocardiopathy.

In a recent randomized clinical trial conducted in adults in SR APL (WBC at diagnosis <10 x 10^9/L, APL0406 trial), a combination of arsenic trioxide (ATO) and ATRA has been shown to result into better survival and EFS rates with significantly lower toxicity, compared to the standard ATRA + idarubicin (AIDA) therapy[10-12,32]. These data, obtained in the adult setting, indicate that at least SR APL patients can be cured without chemotherapy (with ATO/ATRA only). However, a treatment approach with frontline ATO has been tested in a limited number of pediatric patients.

In the last pediatric experience conducted by the ICC, the objectives were not only to deliver risk-stratified treatment based according to the modified Sanz criteria [13] and to monitor MRD by RQ-PCR for PML-RARα and adjust treatment accordingly, but also to reduce the cumulative anthracycline dosage.

Inspired by both the results of ICC APL01 and the adult APL0406 trial we intend to perform the first pediatric chemotherapy-free approach testing the combination of ATRA/ATO +/- GO based on risk-stratification, expecting less severe hematologic toxicity and treatment-related mortality, thus resulting in an improved outcome for these children.

11.2 EXPERIENCE FROM ICC APL 01

The ICC APL 01 international study was open to accrual since January 2008. Both European and non-European centers participated into the study.

Treatment was stratified according to the risk group (SR or HR, based on WBC count at diagnosis < or $\geq$10 x 10^9/L) and MRD level during post-remissional treatment. SR patients received induction (ATRA 25 mg/m^2/day plus idarubicin 12 mg/m^2/day on days 3-5-7) followed by two consolidation courses (I: ATRA, intermediate-dose cytarabine, mitoxantrone; II: ATRA plus idarubicin). HR patients received ATRA plus idarubicin at the same doses in induction, followed by 3 consolidation courses (I and II identical to SR patients, III: ATRA, intermediate-dose cytarabine, idarubicin). This therapy was followed by 2 years of maintenance treatment (ATRA given every 3 months, methotrexate weekly and daily 6-mercaptopurine). The total anthracycline dose was 355 mg/m^2 and 405 mg/m^2 for SR and HR patients respectively, these values being significantly lower than those employed in other successful trials on childhood APL.

Treatment was guided by BM MRD monitoring at the end of consolidation and every 3 months during maintenance therapy and for 1 year after maintenance completion. SR

patients with persistent molecular disease after consolidation were allocated to receive a third course of treatment identical to that given to HR patients. Children with persistent MRD after 3 courses, and those with molecular or frank relapse during or after treatment, were eligible for salvage treatment, based on the use of ATO.

At the 2015 meeting of the American Society of Hematology (ASH 2015), Dr. Testi on behalf of the whole group reported on 240 patients (58% SR, 42% HR) enrolled into the ICC APL01 study. Early death rate was 3%. Eighteen patients experienced disease relapse (either molecular or hematological). The 3-year probability of EFS was 81% for all patients (82% and 78% in SR and HR patients, respectively; p 0.07). Data on MRD monitoring and the impact of persistent MRD on final outcome are still under evaluation.

The ICC APL study 01, besides being an example of successful international collaboration in childhood leukemia, produced an outcome comparable to that of children treated with AIDA 2000 Protocol, which employed higher anthracycline doses (650 mg/m^2 daunorubicin-equivalent)[32]. These results confirm those reported by Creutzig et al., delivering reduced anthracyclines (cumulative dose 350 mg/m^2) with extended ATRA and cytarabine.[33]

11.3 ARSENIC TRIOXIDE (ATO)

Current treatment strategies for childhood APL aim at decreasing the incidence of relapse and chemotherapeutic toxicity. The introduction of ATRA has been crucial for both antileukemic efficacy in APL and for reducing the early death rate. More recently, ATO, first introduced for treatment of patients with refractory/relapsed PML-RARα positive APL [34-37], has been shown to be highly effective for achieving high cure rates in association with reduced toxicity in adults with APL.

A dual mechanism of action has been described: at low concentrations, ATO exerts a partial differentiating effect by inducing the SUMOylation of both PML-RARα and PML leading to the degradation through the proteasome pathway; at high concentrations, ATO induces apoptosis through caspase activation, reactive oxygen species (ROS) production, and induction of mitochondria mediated intrinsic apoptotic pathway.

Although some relapsed 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 [35]. 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. The main experiences with the use of ATO as a first line treatment in pediatric APL and related results in terms of outcome are shown in the following table:

Table 2. Experiences with the use of ATO in pediatric APL

Author	Year	N. pts	Age (median value/range)	Induction	CR (%)	Post-induct.	Outcome
Zhang L, et al. Pediatr Blood Cancer. 2008 [38]	1999-2012	65	13	ATRA±ATO	90.8	CHT	5-y EFS 77.5% 5-y OS 88.9%
Zhou H, et al. Blood. 2010[39]	2001-2011	19	4-15	ATO	89.5	ATO	5-y EFS 72.7% 5-y OS 83.9%%

Wang H et al. Int J Hematol. 2010[40]	2000-2011	35	NA	ATO±ATRA	85.7	CHT	5-y EFS 78.3% 5-y OS 82.7%
Zhang L, et al. Int J Hematol. 2011[41]	2003-2012	37	2-14	ATRA±ATO	94.6	CHT	5-y EFS 79.2% 5-y OS 91.5%
Cheng Y et al. Eur J Hematol, 2013[42]	1998-2011	43	2-17	ATRA+ATO	95.3	ATO+CHT	6-yEFS 92.5% 6-yOS 97.1%

As mentioned above, in a recent randomized clinical trial in adult patients affected by SR APL (WBC at diagnosis < 10 x 10^9/L, APL0406 trial), Lo Coco et al. showed that a combination of ATO and ATRA offers better survival rates with significantly lower toxicity and better Quality of Life, as compared to the standard ATRA + idarubicin (AIDA) therapy [10]. This study suggests that APL is curable without conventional chemotherapy. These clinical results support previously reported clinical and experimental evidence indicating that ATRA and ATO act synergistically to eradicate APL.

Data from this randomized study urged the ICC Consortium on APL to develop a front-line protocol without conventional chemotherapy, the ICC APL Study 02, in which SR patients will be treated with ATRA plus ATO, and HR patients with ATRA, ATO and GO.

The ICC-APL Study 02 protocol is based on the conventional continuous dosing schedule of ATO as currently recommended by the manufacturer.

11.4 GEMTUZUMAB OZOGAMICIN (MYLOTARG®)

Anti-CD33 targeted immunotherapy, with Gemtuzumab ozogamicin (GO) may have a role as innovative treatment in APL[43-45]. 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.

Side effects of GO are myelosuppression, allergic reactions and liver toxicity including sinusoidal obstruction syndrome (SOS). GO has been shown to elicit a significant antileukemic activity in AML, being especially active in APL, due to the high expression of the CD33 antigen on APL cells[36,43,45].

12. INCLUSION AND EXCLUSION CRITERIA

12.1 INCLUSION CRITERIA

- Newly diagnosed APL confirmed by the presence of PML/RARα fusion gene
- Age <18 years
- Written informed consent by parents or legal guardians
- If applicable, female participants must have a negative pregnancy test by beta-HCG dosing.
- Patients of child-bearing or child-fathering potential must be willing to adapt their own conduct so as not to procreate during the study participation and must contact their physician to identify the most appropriate approach strategy for this purpose starting from the time of enrolment and for 3 months after receiving the last drug dose

12.2 EXCLUSION CRITERIA

- Patients with a clinical diagnosis of APL but subsequently found to lack PML/RARα rearrangement should be withdrawn from the study and treated on an alternative protocol
- Significant liver dysfunction (bilirubin serum levels >3 mg/dL, ALT/AST serum levels greater than 5 times the normal values)
- Creatinine serum levels >2 times the normal value for age
- Significant arrhythmias, EKG abnormalities (*see below), other cardiac contraindications (L-FEV <50% or LV-FS <28%)
- Neuropathy
- Concurrent active malignancy
- Uncontrolled life-threatening infections
- Pregnant or lactating female
- 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

13. INVESTIGATIONS AT DIAGNOSIS AND DURING TREATMENT

13.1 BONE MARROW ASPIRATE

Treatment and study entry should be based on suspicion of M3 or M3v morphology; since APL is a hematological emergency, treatment should not be postponed to 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 fusion gene should be treated on the national standard AML protocol.

Immunophenotyping of BM cells will be investigated only at the time of the diagnosis.

Bone Marrow Cytogenetic Analysis including FISH will be performed at the time of diagnosis. Cytogenetics will be carried out locally. If this is not possible, it can be performed by the Centralized Lab, shipping a diagnostic bone marrow sample (3-5 ml) in heparinized culture medium or in lithium heparin.

Molecular diagnosis and monitoring

Molecular Analysis at Diagnosis

A paired BM 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 (for more details see the laboratory manual).

Diagnostic samples required:

- Bone marrow: 2-3 mL in heparinized culture medium for cytogenetic analysis
- Bone marrow: 3-5 mL in sodium citrate or EDTA for molecular analysis
- Peripheral blood: 10-20 mL in sodium citrate or EDTA for molecular analysis

A patient with suspected APL should be registered in the online system and with the national trials office by the standard route. If online registration with CINECA is not possible, please register the patient on paper 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.

E-mail: madivona@gmail.com - tiziana.ottone@uniroma2.it

Phone: +390620902169-3800 - +390672596278

Fax: +390620902169 - +390620903800

Contact Name: to be specified Country by Country

13.2 PHYSICAL EXAMINATIONS

Physical examination, including weight and height, must be performed and documented in the medical records at diagnosis.

Physical examination, including body weight, needs to be repeated daily during induction, at day 35 after induction, at day 1 of every consolidation course, at the end of treatment, every 3 months until 1 year after treatment discontinuation, and at any occurrence of toxicity or clinical problems. If relevant signs of toxicity or clinical problems become evident, they need to be documented as AEs or SAEs according to the indications reported in Section 22 and in the SAE & Expected SAR Form Completion Guidelines contained in the Investigator Site File (ISF).

For patients receiving Gemtuzumab ozogamicin, vital signs must be monitored during the infusion and for 4 hours following its completion.

13.3 PREGNANCY TESTING AND BIRTH CONTROL

For all female patients with childbearing potential, a serum or urine pregnancy test, will be performed prior to starting study therapy. Following a negative pregnancy result at diagnosis, appropriate approach for patients of childbearing potential must be commenced.

Patients of child-bearing or child-fathering potential must be willing to adapt their own conduct so as not to procreate during the study participation and must contact their physician to identify the most appropriate approach strategy for this purpose starting from the time of enrolment and for 3 months after receiving the last drug dose such as abstinence from sexual intercourse or such as other methods, of which the scientific limitations will be explained, such as, specifically:

- progestogen-only oral hormonal contraception, where inhibition of ovulation is not the primary mode of action;
- male or female condom with or without spermicide;
- cap, diaphragm or sponge with spermicide.

13.4 OTHER MEDICAL ASSESSMENTS

At diagnosis, a work-up must be performed, including complete blood count (CBC), serum biochemistry (glucose, creatinine, uric acid, bilirubin, transaminases, alkaline phosphatase, LDH, sodium, potassium, calcium, phosphorus, magnesium, total proteins, albumin, cholesterol and triglycerides…), coagulation screening including D-dimers and fibrinogen, electrocardiogram, echocardiogram, urine analysis, infection screening, and pregnancy test (if appropriate), as specified in Table 1.

During the treatment and follow-up period, assessments will be performed at the time-points specified in the Schedule of Assessments (Table 1).

14. PROCEDURES FOR ENTRY INTO THE STUDY AND DATA RECORDING

14.1 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 the study will be notified to CINECA.

14.2 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 two study-specific forms, such as **Patient Information Sheet** and **Consent Form.**

14.3 DIAGNOSTIC MATERIAL

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

14.3.1 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:

See details Country-specific

Contact Name: Prof. Alessandra Biffi

Address: Dipartimento di salute della donna e del bambino azienda ospedaliera - Università di Padova. U. O. C. Oncoematologia pediatrica Via Giustiniani, 3

City: Padova

Postcode: 35128

Tel: +39 049 8211468 - +39 049 8211465

Fax: +39 049 8211462 - +39 049 8211465

Email: alessandra.biffi@unipd.it

14.3.2 Cytogenetics

Cytogenetics will be carried out locally. If this is not possible, it can be performed by the Centralized Lab, shipping a diagnostic bone marrow sample (3-5 mL) in heparinized culture medium or in lithium heparin.

In case of the latter, contact details for the national cytogenetic laboratory are given below:

See details Country-specific

Contact Name: **Prof. Maria Teresa Voso**

Address: Laboratorio di Diagnostica Avanzata Oncoematologica piano 1-settore E, stanza 39, Fondazione Policlinico di Tor Vergata, V.le Oxford, 81, 00133 Roma (Italy)

E-mail: voso@med.uniroma2.it, madivona@gmail.com, tiziana.ottone@uniroma2.it.

Phone: +390620902169-3800 or +390672596278

Fax +390620902169-3800

14.3.3 Molecular Analysis

In addition to PML/RARα transcript analysis, patients will be investigated for the presence of a FLT3 mutation (although FLT3 status does not influence patient management in the study, it may predict the occurrence of early complications).

Contact Name: **Prof. Maria Teresa Voso**

Address: Laboratorio di Diagnostica Avanzata Oncoematologica piano 1-settore E, stanza 39, Fondazione Policlinico di Tor Vergata, V.le Oxford, 81, 00133 Roma (Italy)

E-mail: voso@med.uniroma2.it, madivona@gmail.com, tiziana.ottone@uniroma2.it.

Phone: +390620902169-3800 or +390672596278

Fax +390620902169-3800

14.3.4 Immunophenotyping

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

See details Country-specific

Contact Name: **Prof. Alessandra Biffi**

Address: Dipartimento di salute della donna e del bambino azienda ospedaliera - Università di Padova. U. O. C. Oncoematologia pediatrica Via Giustiniani, 3

City: Padova

Postcode: 35128

Tel: +39 049 8211468 - +39 049 8211465

Fax: +39 049 8211462 - +39 049 8211465

Email: alessandra.biffi@unipd.it

14.4 DATA RECORDING GUIDELINES

14.4.1 Patient Registration

a. Fill in immediately the patient registration form, National Center and National laboratory will 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, 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.

15. RISK GROUP STRATIFICATION, ASSESSMENT OF RESPONSE AND DEFINITION

15.1 RISK GROUP ASSIGNMENT

Standard Risk Patients (SR) are defined as those patients with APL and a WBC less than 10 x 10^9/L at presentation before start of treatment.

High Risk Patients (HR) are defined as those patients with APL, with the highest pre-treatment WBC count equal to or greater than 10 x 10^9/L at presentation.

15.2 ASSESSMENT OF RESPONSE

A bone marrow aspirate will be carried out after induction therapy, prior to the first block of consolidation therapy, to document the achievement of hematological CR. MRD results on this sample will not have an impact on subsequent therapy. BM aspirates will be repeated after the 3rd consolidation cycle, after the end of therapy, and 3 months, 6 months, 9 months and 12 months after treatment discontinuation.

15.3 DEFINITIONS

All responses may be hematological or molecular.

Remission

- Hematological Complete Remission – BM is regenerating normal hematopoietic cells and contains < 5% blast cells by morphology. The ANC in peripheral blood should be > 1.0 x 109/L and the PLT count > 100 x 109/L. CR (complete remission) indicates a hematological remission with no signs of leukemia elsewhere. CRi (complete remission with incomplete hematologic recovery) indicates a CR except that peripheral blood neutrophils and/or PLT do not meet the criteria as defined above.
- Molecular Remission – absence of PML/RARα fusion transcript in BM 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/CRi – any death occurring in patients who are in CR/CRi
- Resistant/Refractory Disease (RD) – persistent morphological evidence of APL at the end of induction (maximum of 60 days)
- Molecular Resistant/Refractory Disease (mRD) – persistence of the hybrid transcripts in BM cells at the end of the 3rd consolidation cycle. Molecular resistance will always be confirmed in a second consecutive marrow sample taken 2 weeks apart
- Hematological Relapse – reappearance of promyeloblasts/abnormal promyelocytes (>5%) in BM
- Molecular relapse – reappearance of the transcripts in two successive samples taken 2 weeks apart in patients previously in molecular remission.

16. INDUCTION THERAPY

This study is only for children and adolescents with PML/RARα fusion transcript, since patients with APL FAB-M3 but lacking one of these rearrangements would be undertreated by the ICC APL 02 protocol. Because APL is a hematological emergency, ATRA should be commenced as soon as the diagnosis is suspected. Study entry should not be postponed to wait a molecular or cytogenetic confirmation of the diagnosis of APL. Patients subsequently found to lack PML/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 prior to treatment: less than 10 x 10^9/L
- High Risk (HR) – WBC prior to treatment: equal to or greater than 10 x 10^9/L

16.1 STANDARD RISK

INDUCTION THERAPY FOR SR																			
Days	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	→	**28**	→	**60**
ATRA																		
ATO																		

ATRA	Starting on day 1 until achievement of CR and anyway for no more than 60 days. ATRA will be discontinued during induction only after at least 28 days of treatment and when an ANC greater than 1 x 10^9/L and a PLT count greater than 100 x 10^9/L is achieved	max 60 days
ATO	Starting on day 1 until achievement of CR and anyway for no more than 60 days. ATO will be discontinued during induction only after at least 28 days of treatment and if an ANC greater than 1 x 10^9/L and a PLT count greater than 100 x 10^9/L is achieved	

Drug	**Dose**	**Route**	**Administration**	**Comments**
ATRA	25 mg/m^2	p.o. in two equally divided doses and rounded to the nearest 10 mg	Daily	Starting on day 1 until achievement of CR and anyway for no more than 60 days. ATRA will be discontinued during induction only after at least 28 days of treatment and when an ANC greater than 1 x 10^9/L and a PLT count greater than 100 x 10^9/L is achieved
ATO	0.15 mg/kg	i.v. over 2 h	daily	Starting on day 1 until achievement of CR and anyway for no more than 60 days. ATO will be discontinued during induction only after at least 28 days of treatment and if an ANC greater than 1 x 10^9/L and a PLT count greater than 100 x 10^9/L is achieved

Patients with SR APL will not receive any lumbar puncture during their treatment.

16.2 HIGH RISK

INDUCTION THERAPY FOR HR																		
Days	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**→**	**28**	**→**	**60**
ATRA	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
ATO	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
GO		■		■														

ATRA	Starting on day 1 until achievement of CR and anyway for no more than 60 days. ATRA will be discontinued during induction only after at least 28 days of treatment and when an ANC greater than 1 x 10^9/L and a PLT count greater than 100 x 10^9/L is achieved	max 60 days
ATO	Starting on day 1 until achievement of CR and anyway for no more than 60 days. ATO will be discontinued during induction only after at least 28 days of treatment and if an ANC greater than 1 x 10^9/L and a PLT count greater than 100 x 10^9/L is achieved	
GO	Gemtuzumab ozogamicin on day 2, 4	

Drug	Dose	Route	Administration	Comments
ATRA	25 mg/m^2	p.o. in two equally divided doses and rounded to the nearest 10 mg	Daily	Starting on day 1 until achievement of CR and anyway for no more than 60 days. ATRA will be discontinued during induction only after at least 28 days of treatment and when an ANC greater than 1 x 10^9/L and a PLT count greater than 100 x 10^9/L is achieved
ATO	0.15 mg/kg	i.v. over 2 hours	daily	Starting on day 1 until achievement of CR and anyway for no more than 60 days. ATO will be discontinued during induction only after at least 28 days of treatment and if an ANC greater than 1 x 10^9/L and a PLT count greater than 100 x 10^9/L is achieved
GO	3 mg/m^2 (up to a maximum of one 5 mg vial)	i.v. over 2 hours	day 2, 4	The drug, supplied free-of-charge by the Pfizer company, will be distributed across Europe by the pharmacy unit of the Bambino Gesù Children's Hospital in Rome. In case, for logistic reasons, the patient is unable to receive the first dosage of GO within the first 72 hours from diagnosis, the possibility of administering a single dose of anthracyclines (e.g. Idarubicin 12 mg/m^2 as prolonged i.v.

				infusion) in this time interval is left to the treating physician's decision.

ATRA 25 mg/m^2/day per os in two equally divided doses and rounded to the nearest 10 mg should be started as soon as the diagnosis is suspected.

All patients who will enter the study must be PCR positive for the PML-RARα transcript. 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.

ATRA Dosing Table for APL Study 02 – dose 25 mg/m^2/day			
BSA (m^2)	**ATRA dose to be given**	**Ideal total daily dose**	**Variance (%) from ideal dose**
≤ 0.60	10 mg daily	up to 15 mg	-33 to -23
0.61 to 1.00	10 mg twice a day	>15 to 25 mg	+25 to -20
1.01 to 1.40	20 mg each morning and 10 mg at night	>25 to 35 mg	+7 to -14
1.41 to 1.80	20 mg twice a day	>35 to 45 mg	+12 to -12
1.81 to 2.20	30 mg each morning and 20 mg at night	>45 to 55 mg	+10 to -10
2.21 to 2.40	30 mg twice a day	>55 to 60 mg	+8 to 0

ATO 0.15 mg/kg i.v. over 2 hours daily starting on day 1. If acute vasomotor reaction occurs, the infusion duration may be prolonged to 4 hours.

ATRA and ATO will be discontinued during induction only after at least 28 days of treatment and if an ANC greater than 1x10^9/L and a PLT count greater than 100x10^9/L is achieved.

HR patients will receive a Gemtuzumab ozogamicin (GO) dose of:

- 3 mg/m^2 for patients with BSA greater than or equal to 0.6 m^2 (up to a maximum of one 5 mg vial) and
- infused over a 2-hour period after premedication with paracetamol, methylprednisolone and i.v. chlorphenamine 1 hour before starting the GO infusion. Paracetamol may be repeated as necessary in the event of fever and chills. Vital signs must be monitored during the infusion and for 4 hours following its completion.

For HR patients, GO, supplied free-of-charge by the Pfizer company, will be distributed across Europe by the pharmacy unit of the IRCCS Bambino Gesù Children's Hospital in Rome.

In case, for logistic reasons, the patient is unable to receive the first dosage of GO within the first 72 hours from diagnosis, the possibility of administering a single dose of anthracyclines (e.g. Idarubicin 12 mg/m2 as prolonged i.v. infusion) in this time interval is left to the treating physician's decision.

The first BM aspirate to document the achievement of hematological remission will be performed on day 35 and then repeated weekly whenever indicated/necessary.

Lumbar puncture will not be performed at initial diagnosis because of the risk of bleeding and the low incidence of CNS involvement in APL. Two therapeutic lumbar punctures will be administered in HR patients at the beginning of the 1st and 3rd consolidation course (doses chosen according to patient's age, see paragraph 17.2).

Note the precautions to be taken during ATRA and ATO therapy - refer to previous and subsequent sections for treatment modifications, which may be required and ensure familiarity with this section before commencing treatment.

16.3 CONCOMITANT THERAPIES

- Prednisone 0.5 mg/kg/day from day 1 to day 15 of induction to prevent differentiation syndrome (once known as ATRA syndrome). In case this complication occurs, Dexamethasone at 10 mg/m^2/day in 2-3 divided doses will be employed until resolution of symptoms.
- Platelet concentrate transfusions to maintain platelets >50 x 10^9/L during the first 10 days. After day 10, platelets concentrates will be transfused when platelet count is <20 x 10^9/L or in presence of hemorrhagic symptoms.
- Fresh frozen plasma or fibrinogen concentrates to maintain fibrinogen levels above 150 mg/dL.
- 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.

16.4 CONCOMITANT THERAPIES IN CASE OF HYPERLEUKOCYTOSIS

Guidelines for administering hydroxyurea (HU) in patients who will develop sustained leukocytosis (e.g., for at least 3 days) after initiation of therapy, are detailed in the table below:

- WBC 20 – 50 x 10^9/L: HU 20 - 30 mg/kg/day, in 2 doses
- WBC >50 x 10^9/L: HU 40 – 60 mg/kg/day, in 2 doses

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

16.5 TREATMENT MODIFICATION DURING ATRA THERAPY

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

16.5.1 Differentiation Syndrome

- Incidence: 5-20% of patients given ATRA

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[46].

No single feature is diagnostic of the syndrome, but suggestive features include the 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 differentiation syndrome (e.g., unexplained respiratory

distress), and prior to development of a full-blown syndrome, the following measures should be immediately undertaken:

- temporary (e.g. until disappearance of symptoms and signs) discontinuation of treatment
- prompt initiation of Dexamethasone at 10 mg/m2/dose i.v, in 2-3 divided doses until disappearance of symptoms and signs, and for a minimum of 3 days
- furosemide, when clinically required

In case of diagnosis of a severe APL differentiation syndrome the administration of APL therapy should be stopped in time. Early transfer of patients to an ICU for improved monitoring of vital signs should be considered.

16.5.2 Pseudotumor Cerebri

- Incidence: 10-20% of patients given ATRA

This complication is more common in children than adults. As in idiopathic intracranial hypertension, these patients have signs and symptoms of intracranial hypertension, including headache, pulsatile tinnitus, transient visual obscurations, ophthalmoplegia, and occasionally visual loss, but have no other identified cause for intracranial hypertension. Intracranial hypertension associated with ATRA usually presents between 3 to 17 days after onset of ATRA therapy. The pathophysiology of ATRA-induced intracranial hypertension is unknown, but is likely related to intracranial hypertension due to vitamin A toxicity. It has to be mentioned that 10-20% of pediatric patients undergoing ATRA therapy develop severe headache without evidence of intracranial hypertension.

In patients developing *pseudotumor cerebri*, it is often necessary to temporarily discontinue ATRA treatment and to administer opiates. Acetazolamide may improve symptoms.

16.5.3 Hepatotoxicity

This is defined as an increase in serum bilirubin, AST/ALT, or alkaline phosphatase >5 times the normal upper level and may require at physician discretion a temporary suspension of ATRA and ATO.

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 differentiation 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 severe signs and symptoms of ATRA toxicity, please contact the study coordinators for discussing the therapeutic decisions. The occurrence of APL differentiation syndrome during induction is not a contraindication to use ATRA during consolidation or maintenance.

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

16.6 ATO TOXICITY PROFILE

Although quite well tolerated, when administered as a single agent, ATO has a recognized toxicity profile. It is contraindicated in patients with hypersensitivity to arsenic or any of the excipients in the product.

The most prominent adverse events with ATO in APL have included weight gain and fluid retention, leukocytosis, differentiation syndrome, liver toxicity (increased serum levels of ALT and AST) and prolongation of the QTc interval on the electrocardiogram.

Peripheral neuropathy, hyperglycemia, and cutaneous reactions have also been described.

16.6.1 Monitoring patients

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

- APL differentiation syndrome
- Prolongation of the QT/QTc interval seen on the EKG
- Leukocytosis

16.6.2 APL Differentiation Syndrome

APL differentiation syndrome is characterized by fever, dyspnea, weight gain, pulmonary infiltrates and pleural or pericardial effusions, with or without leukocytosis and can be fatal. High-dose dexamethasone 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, 10 mg/m2/day in 2-3 divided doses
- and continued until disappearance of symptoms and signs, or for a minimum of 3 days.

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

16.6.3 Electrocardiogram (EKG) 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 [47]. 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 (e.g., quinidine, amiodarone, sotalol, dofetilide), antipsychotics (e.g., thiordazine), antidepressants (e.g., amitriptyline), some macrolides (e.g., erythromycin), some antihistamines (e.g., terfinadine and astemizole), and some quinolone antibiotics (e.g., sparfloxacine). Other risk factors include congestive heart failure, administration of potassium-wasting diuretics, amphotericin B or other conditions that result in hypokalemia or hypomagnesemia.

EKG and Electrolyte Monitoring Recommendations: Prior to initiating therapy with ATO, a 12 lead EKG 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 (EKG). For QTc greater than 450 msec[48], corrective measures must be completed and the QTc reassessed with serial EKG's prior to

considering using ATO. During therapy with ATO, potassium concentrations must be maintained above 4 mEq/L and magnesium concentrations must be maintained above 1.8 mg/dL. Patients who reach an absolute QT interval value >450 msec must be reassessed and immediate action should be 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 450 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 at least twice weekly, and more frequently for clinically unstable patients, during induction and consolidation.

16.6.4 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.

A list of drugs to avoid and drugs to use with caution is available at https://crediblemeds.org/, an up-to-date resource maintained by the Arizona Center for Education and Research on Therapeutics (AzCERT).

As it may be necessary to give some of these drugs at the same time as ATO, extra monitoring may be advisable.

16.6.5 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 Terminology Criteria for Adverse Events (CTCAE), Version 5.0 (except for QTc prolongation, for which the risk/benefit of continuing versus suspending ATO therapy must be considered, see also previous paragraph) 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. Continuation of treatment in patients who experience a recurrence of toxicity must be discussed with the study coordination.

- Laboratory Tests: The patient's electrolyte and glucose 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.

16.6.6 Hyperleukocytosis

Treatment with ATO has been associated with the development of hyperleukocytosis (>20 x 10^9/L and rising counts) in around half of patients treated in hematologic relapse. Patients

developing hyperleukocytosis should be observed carefully for development of differentiation syndrome. ATO-induced hyperleukocytosis is not considered an indication for chemotherapy (other than hydroxyurea) or leukapheresis, which could potentially exacerbate the coagulopathy. Indication for treatment with hydroxyurea are reported above.

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.

16.6.7 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 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 or medical products known to cause hypokalemia or hypomagnesemia. The influence of ATO on the efficacy of other antileukemia medicinal products is unknown.

16.7 ADMINISTRATION OF GEMTUZUMAB OZOGAMICIN (GO, Mylotarg®)

GO will be supplied free-of-charge by Pfizer. The drug will be distributed across Europe to the Centers of the participating Countries by the pharmacy unit of the IRCCS Bambino Gesù Children's Hospital in Rome (responsible: Dr. Tiziana Corsetti).

GO should not be given to patients whose liver function tests exceed 4 times the upper limit of normal.

Patients with a WBC greater than 20 x 10^9/L at the time of GO administration can be treated with rasburicase because of the potential risk of tumor lysis syndrome.

Patients should not be given azole antifungal drugs until day 5 after the administration of GO. For patients given prophylactic azole treatment, this should be discontinued 5 days before the administration of GO.

Patients will receive a GO dose of:

- 3 mg/m^2 for patients with BSA greater than or equal to 0.6 m^2 (up to a maximum of one 5 mg vial) and
- Infant patients < 1 year of age: 0.1 mg/kg for patients with BSA less than 0.6 m^2 (Guest EM, Aplenc R, Sung L, Raimondi SC, Hirsch BA, Alonzo TA, Gerbing RB, Wang YJ, Kahwash SB, Heerema-McKenney A, Meshinchi S, Gamis AS. Gemtuzumab ozogamicin in infants with AML: results from the Children's Oncology Group trials AAML03P1 and AAML0531. Blood. 2017 Aug 17;130(7):943-945)
- infused over a 2-hour period after premedication with paracetamol, methylprednisolone and i.v. chlorphenamine 1 hour before starting the GO infusion. Paracetamol may be repeated as necessary in the event of fever and chills. Vital signs must be monitored during the infusion and for 4 hours following its completion.

Vials of Gemtuzumab ozogamicin lyophilized powder for injection must be stored in the refrigerator at 2 to 8 °C (36 to 46 °F) and protected from light.

Prior to reconstitution, allow the drug product vials to reach ambient temperature (e.g. approximately 5 minutes). After reconstitution and dilution, chemical and physical in-use

stability of the Gemtuzumab ozogamicin has been demonstrated for 6 hours at 2 to 25 °C (36 to 77 °F).

Gemtuzumab ozogamicin lyophilized powder for injection vials should be stored in a refrigerator at 2 to 8°C (36 to 46°F) and protected from light.

Before reconstitution, allow the drug vials to reach room temperature (e.g. approximately 5 minutes). After reconstitution and dilution, chemical and physical in-use stability of Gemtuzumab ozogamicin has been demonstrated for 6 hours at 2 to 25°C (36 to 77°F).

After reconstitution, the original vial may be stored for up to 16 hours in a refrigerator (2°C - 8°C) or up to 3 hours at room temperature (below 30°C).

The diluted solution can be stored for up to 18 hours in a refrigerator (2° to 8°C) and for up to 6 hours at room temperature (below 30°C). Allowable storage time at room temperature (below 30°C) includes time required for preparation of the diluted solution, equilibration, if necessary, and administration to the patient. The maximum time from preparation of the diluted solution to administration should not exceed 24 hours. Keep away from light and do not freeze. It is recommended that the infusion container is made of polyvinyl chloride (PVC) with DEHP, ethylene vinyl acetate (EVA) or polyolefin (polypropylene and/or polyethylene).

It is necessary to filter the diluted solution. An inline 0.2-micron polyethersulfone (PES) filter with low protein binding capacity should be used for the infusion of MYLOTARG.

Syringe doses should use small bore (microbore) infusion lines with a 0.2-micron polyethersulfone (PES) in-line filter with low protein binding.

Direct sunlight, direct window light and ultraviolet light should be avoided during drug storage, dose preparation, transportation, and infusion. Minimize exposure of vials and dosing solutions to room light during storage and infusion. All dosing solution preparations should take place in a biologic safety hood with the fluorescent light off. During transport of the dosing solution, the infusion bag should be protected from any potential exposure to light by placing in a closed container. During drug infusion, make sure the i.v. bag is protected from light with UV protective covering. The infusion line does not need to be protected from light.

17. CONSOLIDATION THERAPY

There will be a 2-week interval between the end of induction therapy and the beginning of the first consolidation course

17.1 CONSOLIDATION THERAPY FOR SR AND HR

	week	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
ATO	day	1-5								1-5								1-5								1-5			
ATRA	day	1-7				1-7				1-7				1-7				1-7				1-7				1-7			
		1st consolidation ↑ **I.T. ***								**2nd consolidation**								**3rd consolidation** ↑ **I.T. ***								**4th consolidation**			

* only for HR patients

Drug	Dose	Route	Administration	Comments
ATRA	25 mg/m^2	p.o. in two equally divided doses and rounded to the nearest 10 mg	daily	Treatment will be administered for 2 weeks on 2 weeks off and for a total of 7 cycles.
ATO	0.15 mg/kg	i.v. over 2 h	daily for 5 days every week	Treatment will be continued for 4 weeks on and 4 weeks off, for a total of 4 cycles.
Intrathecal Methotrexate, Methylprednisolone, Cytarabine	According to age (see 17.2)	intrathecal	at the beginning of the 1st and 3rd course of consolidation only for HR patients	

There will be a 2-week interval between the end of induction therapy and the beginning of the first consolidation course

A therapeutic lumbar puncture with intrathecal injection of Methotrexate, Methylprednisolone, Cytarabine (doses chosen according to patient's age) will be performed at the beginning of the first and third course of consolidation therapy in HR children.

17.2 INTRATHECAL THERAPY

A therapeutic lumbar puncture with intrathecal injection of **Methotrexate**, **Methylprednisolone**, **Cytarabine** (doses chosen according to patient's age as shown in the following table) will be performed at the beginning of the first and third course of consolidation therapy in HR patients[49,50].

AGE (years)	METHOTREXATE	METHYLPREDNISOLONE	CYTARABINE
<1	6 mg	4 mg	16 mg
≥1 - <2	8 mg	6 mg	20 mg
≥2 - <3	10 mg	8 mg	26 mg
≥3	12 mg	10 mg	30 mg

18. SUPPORTIVE CARE

The induction course and consolidation phases of therapy are intensive and can be associated with a risk of infection and hemorrhage. Local supportive care policies should be adopted to minimize treatment complications; they 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 - e.g., two determinations of ≥38°C 30 minutes apart or a single determination ≥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 given with caution during ATRA therapy. ATRA is metabolized by cytochrome P450 and azoles inhibit cytochrome P450.
- G-CSF therapy should **not** be given routinely
- ATRA is teratogenic and effective contraception must be used in appropriate patients.

Coagulopathy

In the absence of treatment, it can be rapidly fatal and, even after initiation of therapy, it remains the major cause of early mortality in spite of recent advances in APL treatment. The pathogenesis of hemorrhagic complications of APL is complex and in particular includes factors of blood coagulation and fibrinolysis[51]. The coagulopathy is biochemically relevant, manifesting as a severe hypofibrinogenemia, increased levels of fibrin degradation products, a prolonged prothrombin time, a prolonged partial thromboplastin and thrombin time and thrombocytopenia. The highest risk for early death and hemorrhagic complications has been reported in the first 4 days of therapy but may extend until day 30[52]. The introduction of ATRA and ATO in the management of APL significantly improved clinical outcomes mainly due to reduced hemorrhagic-related complications and early deaths. For this reason, ATRA should be initiated at first suspicion of APL without waiting for confirmation of diagnosis. In addition to administration of ATRA, it is of the utmost importance to rapidly provide adequate supportive therapy in order to mitigate the risk of hemorrhagic complications at diagnosis and during the first days of induction treatment. Thrombocytopenia and coagulopathy should be carefully and frequently monitored and promptly corrected. Rigorous attention to hemostatic support in this phase is critical, especially as the beneficial effect of ATRA on correction of coagulopathy may take several days after ATRA initiation. Key measures for hemostatic support during the diagnostic evaluation and during the first days of induction therapy should include:

- frequent blood examinations, including blood count and coagulation tests (of note, as a result of coagulopathy, multiple examinations may be required during one day);
- aggressive hemostatic/transfusional support aimed at maintaining platelet count >50×10^9/L during the first 10 days and >20×10^9/L after the first 10 days or in presence of hemorrhagic signs (of note, as a result of coagulopathy, multiple platelet transfusions may be required to meet these parameters during the first days of treatment);
- administration of fibrinogen is recommended if fibrinogen levels are below 150 mg/dL. In case of unavailability of pure fibrinogen preparation, a substitution with fresh frozen plasma (FFP) or fibrinogen is indicated. FFP transfusions should be aimed at maintaining fibrinogen level >150 mg/dL, and PT and PTT near normal levels (of note, as a result of coagulopathy, multiple fresh frozen plasma transfusions or fibrinogen concentrate administrations may be required to meet

these parameters during the first days of treatment). The prophylactic administration of heparin and the use of antifibrinolytic agents (e.g. tranexamic acid) have no proven benefit;
- any invasive procedure (including the insertion of central venous catheters) should be delayed after the resolution of coagulopathy.
- lumbar puncture will not be performed at initial diagnosis because of the risk of bleeding and the low incidence of CNS involvement in APL.

It must be noted that treatment with ATRA and ATO can result in a reversion of the clotting disorder into a thrombophilic constellation with thromboembolic complications.

19. ATO MANUFACTURING AND LABELING

TRISENOXTM should be diluted with 100 to 250 mL 5% dextrose injection, USP or 0.9% Sodium Chloride injection, USP, using proper aseptic technique, immediately after withdrawal from the ampule. The TRISENOXTM ampule is single-use and does not contain any preservatives. Do not mix TRISENOXTM with other medications.

After dilution in intravenous solutions, TRISENOXTM is chemically and physically stable for 24 hours at 15-30°C and 48 hours at refrigerated (2-8°C) temperatures. 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°C, unless dilution has taken place in controlled and validated aseptic conditions.

19.1 Dosage and Administration

TRISENOXTM is recommended to be given according to the schedule described below: the same dose is recommended for children, adults, and elderly.

TRISENOXTM should be administered intravenously over 2 hours. The infusion duration may extend up to 4 hours if acute vasomotor reactions are observed. A central venous catheter is not required.

19.2 Dispensing

TRISENOXTM (arsenic trioxide) injection is supplied in 10 ampoule packs, each ampoule containing 10 ml of a sterile, clear, colorless solution (1 mg arsenic trioxide/1 mL). To be stored at 25°C (77°F); excursions permitted to 15-30°C (59-86°F). Do not freeze. Do not use beyond expiration date printed on the label.

20. 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 https://apl.cineca.org/study/APL02/

21. STATISTICAL CONSIDERATIONS

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 and EFS) 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 and considering the competitive risk (e.g. death in CR).

21.1 Efficacy / test accuracy

The primary endpoint of the study is to validate the efficacy (measured as EFS probability) of ATO+ATRA+/-GO treatment in childhood APL. This cumulative efficacy endpoint includes the following events: no achievement of hematological complete remission after induction therapy; no achievement of molecular remission after three consolidation cycles (molecular resistance); relapse (hematological/molecular); death.

21.2 Description of the primary efficacy / test accuracy analysis and population

The goal is to demonstrate the safety and efficacy of this treatment which does not contain cytostatic agents compared to the standard treatment combining ATRA and chemotherapy (e.g. ICC APL Study 01). In addition, it has been agreed with COG that the data will be compared to that of COG AAML 1331, in which a very similar approach is applied except that idarubicin is given instead of GO at induction (12 mg/m^2/dose on days 1-3-5-7). The primary efficacy analysis will be performed in the intent-to-treat population. Further exploratory efficacy analyses may be performed in the per-protocol population. We aim at obtaining a 3-year EFS probability of at least 90% and 88% for SR and HR patients, respectively. With a type I error probability of 5%, a power of 80% and a non-inferiority limit of 10%, planned accrual is 46 SR patients and 43 HR patients.

The treatment in an individual patient will be terminated in case of:

- Normal treatment completion
- Grade 4 toxicity unresponsive to dose reduction
- Failure to achieve molecular CR/CRi after 4 cycles of ATO (after 3rd consolidation); these patients will be eligible for the salvage regimen, currently being developed
- Relapse (hematological/molecular); these patients will be eligible for the salvage regimen, currently being developed
- 3 months delay among each programmed treatment cycle
- Major protocol violation
- Withdrawal of consent by parents or legal guardians
- Lost to follow up
- Death
- Investigator's opinion that therapy is not beneficial
- Ineligibility (PML/RARα negative or not evaluable at diagnosis)

21.3 Secondary endpoints

- Rate of hematological CR/CRi after induction
- Rate of molecular CR/CRi after induction
- Rate of early death during induction

- Rate of overall survival (OS) at 3 years
- Rate of cumulative incidence of relapse (CIR) at 3 years
- Incidence of hematological and non-hematological toxicity (CTC-NCI grading)
- Rate of molecular remission after 3 consolidation cycles
- Assessment of PML/RARA transcript level reduction during treatment
- Quality of life and cost-effectiveness
- Total hospitalization days during therapy

An interim safety analysis will be performed when 40 patients (with at least 15 HR patients) will be enrolled.

22. ADVERSE EVENT REPORTING

The collection and reporting of Adverse Events (AEs) will be in accordance with the Medicines for Human Use Clinical Trials Regulations 2004 and its subsequent amendments, or the equivalent appropriate legislation in the participating member state. The Investigator should assess the seriousness and causality (relatedness) of all AEs experienced by the patient (this should be documented in the source data) with reference to the Investigator Brochure or Summary of Product Characteristics as appropriate.

22.1 Data Safety and Monitoring Committee

The DSMC will consist of 2 members of the scientific community and one biometrician/ statistician not involved in this trial. The committee will meet to review study data with the study coordinators of the trial and review all Serious Adverse Reactions (SARs) and Suspected Unexpected Serious Adverse Reactions (SUSARs). Meetings will take place approximately every 6-12 months depending on the number of Adverse Reactions. The DSMC will give recommendations about the continuation of the trial and/or about necessary trial amendments**.**

Proposed members of the DMSC are:

Dr. Martin Zimmermann: University of Hannover, Germany; Zimmermann.Martin@mh-hannover.de

Dr. Edward Anders Kolb: Nemours Center for Cancer and Blood Disorders, Nemours/A. I. duPont Hospital for Children, Wilmington; Edward.Kolb@nemours.org

Dr. Martin Tallman: Leukemia Service, Memorial Sloan Kettering Cancer Center, New York; TallmanM@mskcc.org

22.2 Adverse Event (AE)

Any untoward medical occurrence after exposure to trial intervention, which does not necessarily have a causal relationship with the trial intervention.

Please note this does not include abnormal laboratory findings. An abnormal laboratory value is only considered to be an AE if the abnormality:

- results in patient early discontinuation from the study treatment
- requires treatment, modification or interruption of dose, or any other therapeutic intervention, or is judged to be of significant clinical importance.

22.3 Serious Adverse Event (SAE)

Any adverse event [appearance of (or worsening of any pre-existing)] which meets any one of the following criteria:

- Results in death
- Is life-threatening*
- Requires hospitalization** or prolongation of existing inpatients' hospitalization
- Results in persistent or significant disability or incapacity
- Is a congenital anomaly/birth defect
- Or is otherwise considered medically significant by the Investigator***

Comments:

All AEs that do not meet any of the criteria for serious should be considered as non-serious AEs. The terms "severe" and "serious" are not synonymous: severity refers to the intensity of an AE (mild, moderate, severe), without correlation to its medical significance. "Serious"

is a regulatory definition based on subject or event outcome or action criteria usually associated with events that pose a threat to the subject's life or vital functions. Seriousness serves as the guide for defining regulatory reporting obligations.

Severity and seriousness should be independently assessed when recording AEs and SAEs on the Clinical Report Forms (CRF).

* Life threatening in the definition of a SAE refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe.

** Hospitalization is defined as an unplanned, formal inpatient admission, even if the hospitalization is a precautionary measure for continued observation. Thus, hospitalization for protocol treatment (e.g. line insertion), elective procedures (unless brought forward because of worsening symptoms), or for social reasons (e.g. respite care), are not regarded as an SAE.

*** Medical judgment should be exercised in deciding whether an AE is serious in other situations. Important AEs that are not immediately life threatening or do not result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above, should be considered serious.

22.4 Suspected Unexpected Serious Adverse Reaction (SUSAR)

A SAR that is unexpected e.g. the nature, or severity of the event is not consistent with the applicable product information.

A SUSAR should meet the definition of an AR, UAR and SAR.

22.5 Unexpected Adverse Reaction (UAR)

An AR, the nature or severity of which is not consistent with the applicable product information (e.g. Investigator Brochure for an unapproved IMP or (compendium of) Summary of Product Characteristics (SPC) for a licensed product).

When the outcome of an AR is not consistent with the applicable product information the AR should be considered unexpected.

22.6 SAE Reporting

For more detailed instructions on SAE reporting refer to the *SAE Reporting Manual v 1.0* contained in the Investigator Site File (ISF).

AEs defined as serious and which require reporting as a SAE should be reported on an SAE Form. When completing the form, the Investigator will be asked to define the causality and the severity of the AE which should be documented using the CTCAE version 5.0.

The AE grading (severity) scale found in the NCI CTCAE v5.0 will be used for AE reporting. The NCI CTCAE v5.0 can be found:

https://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_50

Table 3. Adverse Event Grading Scale of Severity

Grade	Severity	Description[a]
1	Mild (apply event-specific NCI CTCAE grading criteria)	Asymptomatic or mild symptoms; clinical or diagnostic observations only;

		intervention not indicated.
2	Moderate; minimal (apply event-specific NCI CTCAE grading criteria)	Local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL*.
3	Severe (apply event-specific NCI CTCAE grading criteria)	Medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL**.
4	Very severe, life-threatening, or disabling (apply event-specific NCI CTCAE grading criteria)	Life-threatening consequences; urgent intervention indicated.
5	Death related to AE	

[a] Use these alternative definitions for Grade 1, 2, 3, and 4 events when the observed or reported AE is not in the NCI CTCAE listing.

Activities of Daily Living (ADL):

*Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.

**Self-care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

SAEs that are judged to be at least possibly related to the Investigational Medicinal Product (IMP) (must still be reported in an expedited manner irrespective of how long after IMP administration the reaction occurred.

In case of web system/eCRF malfunctioning, the Investigator (or delegate) must complete, date and sign a paper SAE Form to manually record data. The form should be sent together with a SAE Cover Sheet to the ICC APL02 Central Pharmacovigilance as soon as possible and no later than 24 hours after first becoming aware of the event.

To report a SAE, send or fax the SAE Form with an SAE Cover Sheet to:

ICC APL02 Central Pharmacovigilance

e-mail: ICC.APL02.pharmacovigilance@opbg.net or +39 0668594552

On receipt, the ICC APL02 Central Pharmacovigilance will allocate each SAE a unique reference number. This number will be transcribed onto the SAE Cover Sheet which will then be sent or faxed back to the site as proof of receipt. If confirmation of receipt is not received within 1 working day please contact the ICC APL02 Central Pharmacovigilance. The SAE reference number should be quoted on all correspondence and follow-up reports regarding the SAE. The SAE Cover Sheet completed by the ICC APL02 Central Pharmacovigilance should be filed with the SAE Form in the ISF.

For SAE Forms completed by someone other than the Investigator, the Investigator will be required to countersign the original SAE Form to confirm agreement with the causality and severity assessments. The form should then be returned to the ICC APL02 Central Pharmacovigilance by e-mail and the original copy kept in the ISF. Investigators should also report SAEs to their own Trust in accordance with local practice.

22.6.1 Provision of follow-up information

Patients should be followed up until resolution or stabilization of the event. Follow-up information should be provided on a new SAE Form (refer to the Serious adverse event (SAE) Reporting manual V 1.0 for further information).

On receipt of an SAE Form, seriousness and causality will be determined independently by a Clinical Coordinator. An SAE judged by the Investigator or Clinical Coordinator to have a reasonable causal relationship with the trial medication will be regarded as a Serious Adverse Reaction (SAR). The Clinical Coordinator will also assess all SARs for expectedness. If the event meets the definition of a SAR that is unexpected (i.e. is not defined in the Reference Safety Information (taken from the Summary of Product Characteristics) for any patient group) it will be classified as a Suspected Unexpected Serious Adverse Reaction (SUSAR).

22.7 Reporting to the Competent Authority and main Research Ethics Committee

22.7.1 Suspected Unexpected Serious Adverse Reactions (SUSAR)

The ICC APL02 Central Pharmacovigilance will report a minimal data set of all individual events categorized as a fatal or life threatening SUSAR to National Competent Authorities (through Eudravigilance) and main National Ethics Committee within 7 days. Detailed follow-up information will be provided within an additional 8 days. All other events categorized as SUSARs will be reported within 15 days.

22.7.2 Serious Adverse Reactions (SAR)

The ICC APL02 Central Pharmacovigilance will include details of all SAEs, SARs (including SUSARs) in a Development Safety Update Report (DSUR) produced annually from the date of the first Clinical Trial Authorization received for the trial to the submission of the End of Trial Declaration. The National Coordinating Centers will be responsible for forwarding this report to the relevant Competent Authority, National ECs and Investigators

22.7.3 Adverse Events (AE)

Details of all AEs will be reported to the Competent Authorities on request.

22.7.4 Other safety issues identified during the course of the trial

The Competent Authorities and National Ethics Committees will be notified immediately if a significant safety issue is identified during the course of the trial.

22.7.5 Investigators

Details of all SUSARs and any other safety issue which arises during the course of the trial will be reported to the Principal Investigator in charge for Pharmacovigilance. A copy of any such correspondence should be filed in the ISF.

22.8 Data Safety and Monitoring Committee

The independent DSMC will review all SUSARs and SARs occurred during the clinical trial.

22.9 Electronic Case report form (eCRF)

All data of the participants have to be recorded in electronic CRFs.

The Investigator is responsible for all data of the participant to be documented in the electronic Case Report Form (eCRF) exclusively designed for the study immediately, correctly and completely.

Corrections in the eCRF are to be conducted only by authorized personnel and to be justified. The former database entry will remain retrievable. All dates and corrections are recorded automatically concerning date, time point and person.

22.10 Data Storage

22.10.1 Responsibilities of the Sponsor

As required by law, all important study documents have to be stored by the sponsor for at least 10 years after the clinical trial was finished or stopped.

22.10.2 Responsibilities of the Investigator

All documents, which are related to the clinical study and to the distribution of the study medication (e.g. CRFs, written informed consent forms, study medication lists and other relevant material), have to be stored for at least 10 years.

Source data like patients' charts, laboratory analyzes, and other original data have to be stored for the longest possible time that is usual practice at the investigator's site, at least for 10 years.

22.10.3 Privacy and confidentiality

Recording, storage, disclosure, and analysis of personal data of the participants within this clinical study are in accordance with legal requirements. The participant has to agree on the handling of his/her data within the informed consent form. The participant has to be informed about:

Data are recorded electronically in eCRFs, will be handled confidentially, and disclosed to others (sponsor, local and federal authorities, independent ethical committee) only pseudonymized.

Persons who are authorized by the sponsor and the authorities to monitor and inspect the clinical study can have insight into participant related data.

These persons have to handle the data confidentially. The clinical investigator is dispensed from his/her medical confidentiality towards these persons.

The written consent for data recording and documentation during this clinical study is irreversible. When a participant withdraws the written consent, all data which are documented so far can be used pseudonymized to analyze the effect of the study medication if needed

23. PREMATURE DISCONTINUATION OF THE CLINICAL TRIAL

The sponsor has the right to discontinue the study due to relevant medical or administrative reasons. Participants who still receive medication during the time of discontinuation will undergo a final visit which has to be documented.

Possible reasons for discontinuation by the sponsor are:

- failure in recruiting patients;
- data quality is insufficient;
- unforeseen circumstances at the study site that make the continuation of the study impossible;
- occurrence of unjustifiable risks or toxicity;
- new scientific knowledge that does not justify continuation of the clinical study.
- Study discontinuation will be decided by the coordinating investigators in cooperation with the sponsor.

24. END OF STUDY

The end of the study is identified as the last follow-up visit (so that 2 years after the end of treatment) of the last patient.

25. 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 steering 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 enrolls at least 5 patients in the study, and anyone else who made an important contribution to the study.

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DECLARATION OF HELSINKI

WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI

Recommendations guiding physicians

in biomedical research involving human subjects

Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964 and amended by the:

29th WMA General Assembly, Tokyo, Japan, October 1975

35th WMA General Assembly, Venice, Italy, October 1983

41st WMA General Assembly, Hong Kong, September 1989

48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996

52nd WMA General Assembly, Edinburgh, Scotland, October 2000

53rd WMA General Assembly, Washington DC, USA, October 2002 (Note of Clari6cation added)

55th WMA General Assembly, Tokyo, Japan, October 2004 (Note of Clari6cation added)

59th WMA General Assembly, Seoul, Republic of Korea, October 2008

64th WMA General Assembly, Fortaleza, Brazil, October 2013

Preamble

1. The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, including research on identificable human material and data. The Declaration is intended to be read as a whole and each of its constituent paragraphs should be applied with consideration of all other relevant paragraphs.

2. Consistent with the mandate of the WMA, the Declaration is addressed primarily to physicians. The WMA encourages others who are involved in medical research involving human subjects to adopt these principles.

General Principles

3. The Declaration of Geneva of the WMA binds the physician with the words, “The health of my patient will be my first consideration,” and the International Code of Medical Ethics declares that, “A physician shall act in the patient’s best interest when providing medical care.”

4. It is the duty of the physician to promote and safeguard the health, well-being and rights of patients, including those who are involved in medical research. The physician’s knowledge and conscience are dedicated to the fulfilment of this duty.

5. Medical progress is based on research that ultimately must include studies involving human subjects.

6. The primary purpose of medical research involving human subjects is to understand the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best proven interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.

7. Medical research is subject to ethical standards that promote and ensure respect for all human subjects and protect their health and rights.

8. While the primary purpose of medical research is to generate new knowledge, this goal can never take precedence over the rights and interests of individual research subjects.

9. It is the duty of physicians who are involved in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects. The responsibility for the protection of research subjects must always rest with the physician or other health care professionals and never with the research subjects, even though they have given consent.

10. Physicians must consider the ethical, legal and regulatory norms and standards for research involving human subjects in their own countries as well as applicable international norms and standards. No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.

11. Medical research should be conducted in a manner that minimises possible harm to the environment.

12. Medical research involving human subjects must be conducted only by individuals with the appropriate ethics and scientiific education, training and qualifications. Research on patients or healthy volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional.

13. Groups that are underrepresented in medical research should be provided appropriate access to participation in research.

14. Physicians who combine medical research with medical care should involve their patients in research only to the extent that this is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the patients who serve as research subjects.

15. Appropriate compensation and treatment for subjects who are harmed as a result of participating in research must be ensured.

Risks, Burdens and Benefits

16. In medical practice and in medical research, most interventions involve risks and burdens. Medical research involving human subjects may only be conducted if the importance of the objective outweighs the risks and burdens to the research subjects.

17. All medical research involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and groups involved in the research in comparison with foreseeable benefits to them and to other individuals or groups affected by the condition under investigation. Measures to minimise the risks must be implemented. The risks must be continuously monitored, assessed and documented by the researcher.

18. Physicians may not be involved in a research study involving human subjects unless they are confident that the risks have been adequately assessed and can be satisfactorily managed. When the risks are found to outweigh the potential benefits or when there is conclusive proof of definitive outcomes, physicians must assess whether to continue, modify or immediately stop the study.

Vulnerable Groups and Individuals

19. Some groups and individuals are particularly vulnerable and may have an increased likelihood of being wronged or of incurring additional harm. All vulnerable groups and individuals should receive specifically considered protection.

20. Medical research with a vulnerable group is only justified if the research is responsive to the health needs or priorities of this group and the research cannot be carried out in a non-vulnerable group. In addition, this group should stand to benefit from the knowledge, practices or interventions that result from the research. Scientific Requirements and Research Protocols

21. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.

22. The design and performance of each research study involving human subjects must be clearly described and justified in a research protocol.

The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information regarding funding, sponsors, institutional affiliations, potential conflicts of interest, incentives for subjects and information regarding provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study.

In clinical trials, the protocol must also describe appropriate arrangements for post-trial provisions.

Research Ethics Committees

23. The research protocol must be submitted for consideration, comment, guidance and approval to the concerned research ethics committee before the study begins. This committee must be transparent in its functioning, must be independent of the researcher, the sponsor and any other undue influence and must be duly qualified. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as applicable international norms and standards but these must not be allowed to reduce or eliminate any of the protections for research subjects set forth in this Declaration.

The committee must have the right to monitor ongoing studies. The researcher must provide monitoring information to the committee, especially information about any serious adverse events. No amendment to the protocol may be made without consideration and approval by the committee. After the end of the study, the researchers must submit a final report to the committee containing a summary of the study's findings and conclusions.

Privacy and Confidentiality

24. Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information.

Informed Consent

25. Participation by individuals capable of giving informed consent as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no individual capable of giving informed consent may be enrolled in a research study unless he or she freely agrees.

26. In medical research involving human subjects capable of giving informed consent, each potential subject must be adequately informed of the aims, methods, sources of funding,

any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail, post-study provisions and any other relevant aspects of the study. The potential subject must be informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal. Special attention should be given to the specific information needs of individual potential subjects as well as to the methods used to deliver the information. After ensuring that the potential subject has understood the information, the physician or another appropriately qualified individual must then seek the potential subject's freely-given informed consent, preferably in writing. If the consent cannot be expressed in writing, the non-written consent must be formally documented and witnessed. All medical research subjects should be given the option of being informed about the general outcome and results of the study.

27. When seeking informed consent for participation in a research study the physician must be particularly cautious if the potential subject is in a dependent relationship with the physician or may consent under duress. In such situations the informed consent must be sought by an appropriately qualified individual who is completely independent of this relationship.

28. For a potential research subject who is incapable of giving informed consent, the physician must seek informed consent from the legally authorised representative. These individuals must not be included in a research study that has no likelihood of benefit for them unless it is intended to promote the health of the group represented by the potential subject, the research cannot instead be performed with persons capable of providing informed consent, and the research entails only minimal risk and minimal burden.

29. When a potential research subject who is deemed incapable of giving informed consent is able to give assent to decisions about participation in research, the physician must seek that assent in addition to the consent of the legally authorised representative. The potential subject's dissent should be respected.

30. Research involving subjects who are physically or mentally incapable of giving consent, for example, unconscious patients, may be done only if the physical or mental condition that prevents giving informed consent is a necessary characteristic of the research group. In such circumstances the physician must seek informed consent from the legally authorised representative. If no such representative is available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee. Consent to remain in the research must be obtained as soon as possible from the subject or a legally authorised representative.

31. The physician must fully inform the patient which aspects of their care are related to the research. The refusal of a patient to participate in a study or the patient's decision to withdraw from the study must never adversely affect the patient-physician relationship.

32. For medical research using identifiable human material or data, such as research on material or data contained in biobanks or similar repositories, physicians must seek informed consent for its collection, storage and/or reuse. There may be exceptional situations where consent would be impossible or impracticable to obtain for such research. In such situations the research may be done only after consideration and approval of a research ethics committee.

Use of Placebo

33. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best proven intervention(s), except in the following circumstances:

Where no proven intervention exists, the use of placebo, or no intervention, is acceptable; or

Where for compelling and scientifically sound methodological reasons the use of any intervention less effective than the best proven one, the use of placebo, or no intervention is necessary to determine the efficacy or safety of an intervention

and the patients who receive any intervention less effective than the best proven one, placebo, or no intervention will not be subject to additional risks of serious or irreversible harm as a result of not receiving the best proven intervention.

Extreme care must be taken to avoid abuse of this option.

Post-Trial Provisions

34. In advance of a clinical trial, sponsors, researchers and host country governments should make provisions for post-trial access for all participants who still need an intervention identified as beneficial in the trial. This information must also be disclosed to participants during the informed consent process.

Research Registration and Publication and Dissemination of Results

35. Every research study involving human subjects must be registered in a publicly accessible database before recruitment of the first subject.

36. Researchers, authors, sponsors, editors and publishers all have ethical obligations with regard to the publication and dissemination of the results of research. Researchers have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. All parties should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results must be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest must be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

Unproven Interventions in Clinical Practice

37. In the treatment of an individual patient, where proven interventions do not exist or other known interventions have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorised representative, may use an unproven intervention if in the physician’s judgement it offers hope of saving life, re-establishing health or alleviating suffering. This intervention should subsequently be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information must be recorded and, where appropriate, made publicly available.

APPENDIX A – LIST OF MSC PROTOCOL CHANGES

MSC	Current Protocol Version	Content	Text of protocol
France	**Version 1.2_FRA, dated January 7, 2021**	Section 1.1 TREATMENT SYNOPSIS FOR STANDARD-RISK (SR) PATIENTS. Key inclusion and exclusion criteria AND Section 1.2 TREATMENT SYNOPSIS FOR HIGH-RISK (HR) PATIENTS. Key inclusion and exclusion criteria AND Section 12 INCLUSION AND EXCLUSION CRITERIA 12.2 Exclusion Criteria Specific exclusion criteria: - bilirubin exclusion criteria expressed in unit of the International System - the creatinine clearance instead to assess renal function - Exclusion criteria associated to the risk of QT prolongation - Contraindications of medicinal products (GO,	<u>Added:</u> - Significant liver dysfunction (bilirubin serum levels >51 µmol/L, ALT/AST serum levels greater than 5 times the normal values) - Creatinine serum levels >2 times the normal value for age or abnormal renal function defined as calculated creatinine clearance < 90 ml/min/1.73m² - Significant arrhythmias, EKG abnormalities (*see below), other cardiac contraindications (L-FEV <50% or LV-FS <28%) or other risks factors as: ▪ heart disease: myocardial ischemia or infarction, congestive heart failure, left ventricular hypertrophy, cardiomyopathy, conduction disorder in the 6 months prior to inclusion ▪ electrolyte abnormalities (hypokalemia with serum potassium concentrations <3 mEq/L, hypomagnesemia with serum magnesium concentrations <1.2 mg/dL, hypocalcemia with serum calcium concentrations <8 mg/dL) which cannot be corrected with supplemental electrolytes administration - Concerning Trioxyde Arsenic: Hypersensitivity to the active substance or to any of the following excipient (Sodium hydroxide, Hydrochloric acid (for pH adjustment), Water for injections) - Concerning all trans retinoic acid: Hypersensitivity to tretinoin, retinoids, soya, peanut or any of the following excipient of the capsule contents (yellow beeswax, hydrogenated soya-bean oil, partially hydrogenated soya-bean oil, soya-bean oil) of the capsule shell (gelatin, glycerol (E 422), karion 83: Sorbitol, Mannitol, Starch (maize), titanium dioxide (E 171), iron oxide yellow (E 172), iron oxide red (E 172). Combination with vitamin A, tetracyclines, retinoids is also contraindicated. * ECG abnormalities: - have QT/QTc interval > 470 ms (for women) and > 450 ms (for men) on the ECG - History of arrhythmia (especially ventricular arrhythmias, atrial fibrillation or recent recovery from atrial fibrillation)

		ATO and ATRA)	
		Section 1.1 TREATMENT SYNOPSIS FOR STANDARD-RISK (SR) PATIENTS. TREATMENT OTHERWISE AND Section 1.2 TREATMENT SYNOPSIS FOR HIGH-RISK (HR) PATIENTS. TREATMENT OTHERWISE AND Section 16.3 CONCOMITANT THERAPIES Contraindicated concomitant therapies	Added: Combination with vitamin A, tetracyclines, retinoids is contraindicated with the use of all trans retinoic acid.
		Section 1.1 TREATMENT SYNOPSIS FOR STANDARD-RISK (SR) PATIENTS. STATISTICAL ANALYSIS AND Section 1.2 TREATMENT SYNOPSIS FOR HIGH-RISK (HR) PATIENTS. STATISTICAL ANALYSIS AND	Changed: Normal treatment completion Incidence and severity of hematological (grade 4) and non-hematological toxicity (grade 3 and 4) (CTC-NCI grading)

		Section 21 STATISTICAL CONSIDERATIONS Differentiation of hematological and non-hematological severity.	
		Table 1. Visit Schedule for both SR and HR patients enrolled into the Study Addition of Peripheral blood samples for molecular biology	<u>Added:</u> - Peripheral blood for molecular biology: Visit Schedule for both SR and HR patients enrolled into the Study at diagnosis and at relapse
		Section 12. INCLUSION AND EXCLUSION CRITERIA Subsection 12.2 Inclusion Criteria Defining the “women of child bearing potential “ according to the CTFG	<u>Added:</u> - A woman is considered of childbearing potential (WOCBP), e.g. fertile, following menarche and until becoming post-menopausal unless permanently sterile. Permanent sterilisation methods include hysterectomy, bilateral salpingectomy and bilateral oophorectomy. A postmenopausal state is defined as no menses for 12 months without an alternative medical cause. A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a post-menopausal state in women not using hormonal contraception or hormonal replacement therapy. However in the absence of 12 months of amenorrhea, a single FSH measurement is insufficient. A man is considered fertile after puberty unless permanently sterile by bilateral orchidectomy - With their physician, they must agree on the most appropriate approach for birth control from the time of enrollment in this study and for 3 months after receiving the latest infusion.

		Section 13.
		INVESTIGATIONS AT DIAGNOSIS AND DURING TREATMENT
		Subsection 13.1
		Bone Marrow Aspirate
		Specification of the maximum volume of blood
			<u>Changed:</u>
			- Bone marrow: 2-3 ml in EDTA for molecular analysis
			- Peripheral blood: from 4.5 to 20 ml depending on the patient's weight in EDTA for molecular analysis:
			  - Below 10kg: 4.5 ml maximum
			  - 10 - 20 kg: 7.5 ml
			  - 20 - 30 kg: 10 ml

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<td>samples authorized at each visit, and at each cycle according to the weight (recommended by European regulations).</td>
<td>
- 30kg and more: 20 ml
- The volume of each sample will not exceed 1% of the total blood volume, in accordance with the recommendations "Ethical considerations for clinical trials on medicinal products conducted with minors".
- Volume corresponding to weight of patients are described in the following table:
<table>
<tr><th>Body weight (kg)</th><th>Circulating total blood volume (ml)</th><th>Maximum allowable sample volume at single time(ml)-1%of total blood volume</th></tr>
<tr><td>2.5 -5</td><td>250 -500</td><td>2.5 –5</td></tr>
<tr><td>5-12</td><td>480 -960</td><td>4.8 -9.6</td></tr>
<tr><td>20-30</td><td>1600 -2400</td><td>16–24</td></tr>
<tr><td>30-70</td><td>2400 -5600</td><td>24 –56</td></tr>
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- Cytogenetics will be carried out locally. If this is not possible, it can be performed by the Centralized Lab, shipping a diagnostic bone marrow sample (3-5 ml) in heparinized culture medium or in lithium heparin.
- (for more details see the laboratory manual).
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<td>Section 15

RISK GROUP STRATIFICATION, ASSESSMENT OF RESPONSE AND DEFINITION

<u>Added:</u>

Subsection 15.4</td>
<td>
- The sponsor has the right to discontinue the study due to relevant medical or administrative reasons. Participants who still receive medication during the time of discontinuation will undergo a final visit which has to be documented. Possible reasons for discontinuation by the sponsor are:
- failure in recruiting patients;
- data quality is insufficient;

unforeseen circumstances at the study site
that make the continuation of the study
impossible;

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		Stopping rules Subsection 15.4-1 Final stop to dosing and termination of the trial Subsection 15.4-3 Stopping within the run-in cohort of HR patients Subsection 15.4-4 Stopping within the cohort of HR patients during induction and consolidation phases Subsection 15.4-5 Stopping within the cohort of SR patients during induction and consolidation phases Specification of the stopping criteria for a subject and for cohorts in accordance with the GCP (ICH E6, section 6.4.6) and the recommendations of the "guideline on strategies to identify and mitigate risks for first-in-human and early clinical trials with investigational medicinal products"	- occurrence of unjustifiable risks or toxicity; - new scientific knowledge that does not justify. ▪ continuation of the clinical study - Study discontinuation will be decided by the coordinating investigators in cooperation with the sponsor - The run-in phase for HR patients will be permanently stopped if, during the induction phase, one of the following events occurs: ▪ Death in ≥2 out of 6 patients enrolled in the run- in phase ▪ Failure to achieve hematological remission at the end of induction therapy in ≥2 out of 6 patients enrolled in the run-in phase ▪ Grade 4 non hematologic toxicities that put the patients at risk of life or of permanent sequelae and not responsive to specific treatment in ≥2 out of the 6 patients enrolled in the run-in phase. - The HR arm will be permanently stopped if one of the following events occurs: - during the induction phase: ▪ More than 7 patients will die of treatment-related causes ▪ More than 5 patients will require Intensive Care Unit admission for differentiation syndrome - during the consolidation phase: ▪ More than 12 patients will experience a relapse (either hematological or molecular) ▪ More than 5 patients will die of treatment-related causes - More than 10 patients will experience a torsade-de-pointes The SR arm will be permanently stopped if one of the following events occurs: - during the induction phase: ▪ More than 2 patients will die of treatment-related causes ▪ More than 5 patients will require Intensive Care Unit admission for differentiation syndrome - during the consolidation phase: ▪ More than 7 patients will experience a relapse (either hematological or molecular) ▪ More than 5 patients will die of treatment-related causes ▪ More than 10 patients will experience a torsade-de-pointes

		Section 22 ADVERSE EVENT REPORTING Reporting to the Competent Authority and main Research Ethics Committee Description of the plan for rapid communication of serious adverse events and suspected unexpected serious adverse reactions (SUSARs) between the sponsor, the investigators of all sites and the patients in accordance with the "Guideline on strategies to identify and mitigate risks for first-in-human and early clinical trials with investigational medicinal products"	<u>Added:</u> The ICC APL02 Central Pharmacovigilance will provide the report of any SUSAR to the National Coordinating Centres, which will be responsible for forwarding this report to the relevant Competent Authority, National ECs and Investigators. The investigators will inform the patients included in the trial of any Suspected Unexpected Serious Adverse Reaction documenting it in medical records. In accordance to the information acquired with the SUSARs, the ICF will be amended and the patients should renew their participation to the trial by signing the new ICF. The National Coordinating Centres relevant Competent Authority, NationalECs and Investigators, which will inform the patients included in the trial.
Netherlands	Version 2.0 dated December 21, 2022	See Appendix Netherlands	Dutch appendix_V1.0 dd 04-05-23
Czech Republic	Version 1.1, dated November 08, 2022	Section 1.0 SYNOPSIS Subsection 1.1 Treatment synopsis for standard-risk (SR) patients AND Subsection 1.2 Treatment synopsis for high-risk (HR) patients	<u>Added in the Consolidated Protocol, not applicable in the Czech protocol:</u> • WBC less than 10x109/L at presentation before start of treatment. • WBC more than 10x109/L at presentation before start of treatment

		Key inclusion criteria	
		Section 7 Ethical considerations	Changed: **Czech protocol**: ICC APL Study 02 will be conducted in accordance with the Declaration of Helsinki, the Guideline for Good Clinical Practice E6 (R2) and applicable legislation **Consolidated protocol**: ICC APL Study 02 will be conducted in accordance with the EU Directive for Good Clinical Practice in Clinical Trials
		Section 11 JUSTIFICATION OF STUDY DESIGN AND TREATMENT SCHEDULES Section 11.3 Arsenic Trioxide (ATO)	Removed in the Consolidated Protocol. Applicable for the Czech protocol: ▪ ATO treatment will take place during hospitalizations in an infusion for 1-2 hours, patients will be monitored for at least 2 hours after the infusion. ▪ Due to the risk of QTc interval prolongation, both regular ECG monitoring and caution should be exercised when administering concomitant medication that may prolong the QTc interval. SmPC recommendations must be followed in the treatment of ATO.
		Section 11 JUSTIFICATION OF STUDY DESIGN AND TREATMENT SCHEDULES Subsection 11.4.1 Toxicity management	Removed in the Consolidated Protocol, Applicable for the Czech protocol: ▪ According to available data, the toxicity of Gemtuzumab ozogamicin in the pediatric population should not be significantly different from that in adult patients. We also have experience with this product in children in off-label submission. The expected toxicity and management of this medicinal product are governed by the SmPC of this medicinal product.
		Section 12 INCLUSION AND EXCLUSION CRITERIA Subsection 12.2 Exclusion Criteria	Changed: **Czech protocol:** Patients of child-bearing or child-fathering potential must be willing to compliance with highly reliable contraception and must contact their physician. With their physician, they must agree on the most appropriate approach for birth control from the time of enrollment in this study and for 3 months after receiving the latest infusion. **Consolidated Protocol:** Patients of child-bearing or child-fathering potential must be willing to adapt their own conduct so as not to procreate during the study participation and must contact their physician to identify the most appropriate approach strategy for this purpose starting from the time of enrolment and for 3 months after receiving the last drug

			dose Removed in the Consolidated Protocol, Applicable for the Czech protocol: ▪ Patients must not take part in other clinical trials at the same time ▪ Patients with contraindications for study treatment according to the relevant SmPCs will not be included in the study
		Section 13 INVESTIGATIONS AT DIAGNOSIS AND DURING TREATMENT Subsection 13.1 Bone Marrow Aspirate	Added in the Consolidated Protocol, not applicable in the Czech protocol: ▪ Cytogenetics will be carried out locally. If this is not possible, it can be performed by the Centralized Lab, shipping a diagnostic bone marrow sample (3-5 ml) in heparinized culture medium or in lithium heparin. ▪ (for more details see the laboratory manual). Changed: **Czech protocol**: Bone marrow: 2-3 ml in EDTA for molecular analysis **Consolidated Protocol:** Bone marrow: 3-5 mL in sodium citrate or EDTA for molecular analysis **Czech protocol**: Peripheral blood: 10-20 ml in EDTA for molecular analysis **Consolidated Protocol** Peripheral blood: 10-20 mL in sodium citrate or EDTA for molecular analysis
		Section 13 INVESTIGATIONS AT DIAGNOSIS AND DURING TREATMENT Added: Subsection 13.3	Applicable for the Czech protocol: - A female is considered of childbearing potential (WOCBP), e.g. fertile, following menarche and until becoming post-menopausal unless permanently sterile. Permanent sterilisation methods include hysterectomy, bilateral salpingectomy and bilateral oophorectomy. - For Cytarabine all female with childbearing potential must use effective contraception during treatment and for up to 6 months after treatment according to SmPC of

		Pregnancy Testing and Birth Control <u>Added:</u> Subsection 13.3.1 Birth control methods which may be considered as highly effective <u>Added:</u> Subsection 13.3.2 Acceptable birth control methods which may not be considered as highly effective <u>Added:</u> Subsection 13.3.3 Assessment of pharmacokinetic interaction between the IMP and hormonal contraceptives and recommendations on the use of hormonal Contraceptives <u>Added:</u> Subsection 13.3.4 Birth control methods which are considered unacceptable in clinical trials	Cytarabine. - For Tretinoin is absolutely essential that every female with childbearing potential use effective contraception during treatment and for one month after treatment has stopped. A pregnancy test must be performed every month throughout the treatment according to SmPC of Tretinoin. - For Methotrexate female with childbearing potential must use effective contraception during treatment and for at least 6 months thereafter. As a precautionary measure, it is recommended that sexually active men or their partners use reliable contraception during treatment of the man and for at least 6 months after stopping methotrexate. Men must not donate sperm during treatment with methotrexate and for 6 months after discontinuation of methotrexate. According to SmPC of Methotrexate. - For Arsenic trioxide female with childbearing potential must use effective contraception during therapy and for 6 months after. Advise males with female sexual partners of reproductive potential to use effective contraception during therapy and for 3 months after. According to SmPC of Arsenic trioxide - For Methylprednisolone female with childbearing potential must use effective contraception during therapy according to SmPC of Methylprednisolone. - For Mylotarg (Gemtuzumab ozogamicin) female with childbearing potential or partners of female with childbearing potential have to use 2 effective methods of contraception during treatment with MYLOTARG and for at least 7 months for female or 4 months for men after the last dose. - Methods that can achieve a failure rate of less than 1% per year when used consistently and correctly are considered as highly effective birth control methods. - Possible contraception methods for females: • combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation (Hormonal contraception may be susceptible to interaction with the IMP, which may reduce the efficacy of the contraception method): • oral • intravaginal • transdermal • progestogen-only hormonal contraception associated with inhibition of ovulation (Hormonal contraception may be susceptible to interaction with the IMP, which may reduce the efficacy of the contraception method): • oral

			• injectable • implantable • intrauterine device (IUD) • intrauterine hormone-releasing system (IUS) • bilateral tubal occlusion • vasectomised partner • sexual abstinence - Possible contraception methods for males: • vasectomised partner • sexual abstinence - Acceptable birth control methods that result in a failure rate of more than 1% per year include: • progestogen-only oral hormonal • contraception, where inhibition of ovulation is not the primary mode of action • male or female condom with or without spermicide • cap, diaphragm or sponge with spermicide - A combination of male condom with either cap, diaphragm or sponge with spermicide (double barrier methods) are also considered acceptable, but not highly effective, birth control methods - For hormonal contraception methods, caution should be taken to possible interaction with used IMP. Interaction with the IMP leading to reduced efficacy of the hormonal contraception method can occur due to e.g. increased metabolism (enzyme induction). As a general rule, use of hormonal contraception is not recommended if a clinically relevant interaction with contraceptive steroids has been observed or is suspected. If an interaction with contraceptive steroids has been observed or is suspected, but the effect is considered to be of limited clinical significance, the hormonal contraception method must be supplemented with a barrier method (preferably male condom). Periodic abstinence (calendar, symptothermal, post-ovulation methods), withdrawal (coitus interruptus), spermicides only, and lactational amenorrhoea method (LAM) are not acceptable methods of contraception. Female condom and male condom should not be used together

		Section 14 PROCEDURES FOR ENTRY INTO THE STUDY AND DATA RECORDING Subsection 14.3.2 Cytogenetics	<u>Changed:</u> **Czech protocol**:Cytogenetics will be carried out locally and reviewed nationally or nationally centralized. **Consolidated Protocol**: Cytogenetics will be carried out locally. If this is not possible, it can be performed by the Centralized Lab, shipping a diagnostic bone marrow sample (3-5 mL) in heparinized culture medium or in lithium heparin.
		Section 16 INDUCTION THERAPY Subsection 16.6 ATO toxicity profile <u>Added:</u> Subsection 16.8 prohibited concomitant medication:	<u>Removed in the Consolidated Protocol. Applicable for the Czech protocol:</u> - The ECG will be monitored twice a week during induction treatment, in patients where the QTc interval has not been significantly prolonged, it is possible to reduce the frequency of ECG monitoring during consolidation to once a week. <u>Applicable for the Czech protocol:</u> - It is forbidden to administer other anti-leukemia (non-study) treatment. Any supportive treatment should always be given with individual consideration of the risks and benefits to the patient according to the specific SmPC of each study product. Intrathecal treatment will be performed according to standard recommendations and in accordance with the SmPC of the products used. The medicines administered, their dosage and number are the international standard of treatment.
		Section 17 CONSOLIDATION THERAPY Subsection 17.2 Intrathecal therapy	<u>Applicable for the Czech protocol:</u> - Intrathecal treatment will be performed according to standard recommendations and in accordance with the SmPC of the products used. The medicines administered, their dosage and number are the international standard of treatment.
		Section 21 Statistical considerations	<u>Added in the Consolidated Protocol, not applicable for the Czech protocol:</u> - An interim safety analysis will be performed when 40 patients (with at least 15 HR patients) will be enrolled

		Section 22 Adverse event reporting Subsection 22.2 AND Subsection 22.3	<u>Changed:</u> **Czech protocol** All medical occurrences which meet the definition of an AE should be recorded. **Consolidated Protocol** Any untoward medical occurrence after exposure to trial intervention, which does not necessarily have a causal relationship with the trial intervention. **Czech protocol** Any untoward medical occurrence or effect that at any dose: **Consolidated Protocol**: Any untoward medical occurrence after exposure to trial intervention, which does not necessarily have a causal relationship with the trial intervention **Czech protocol** The term severe is often used to describe the intensity (severity) of a specific event. This is not the same as serious, which is based on patients/event outcome or action criteria. **Consolidated Protocol**: All AEs that do not meet any of the criteria for serious should be considered as non-serious AEs. The terms “severe” and “serious” are not synonymous: severity refers to the intensity of an AE (mild, moderate, severe), without correlation to its medical significance. “Serious” is a regulatory definition based on subject or event outcome or action criteria usually associated with events that pose a threat to the subject’s life or vital functions. Seriousness serves as the guide for defining regulatory reporting obligations. Severity and seriousness should be independently assessed when recording AEs and SAEs on the Clinical Report Forms (CRF). <u>Removed in the Consolidated Protocol. Applicable for the Czech protocol:</u> - Serious Adverse Reaction (SAR: An Adverse Reaction which also meets the definition of a Serious Adverse Event

		Added: Subsection 23.2 Premature discontinuation of the clinical trial by patient or parent/ legal guardian	Applicable for the Czech protocol: - Premature discontinuation of a patient from the study is possible at the discretion of the patient or his legal representatives. Due to the inappropriate toxicity of the treatment at the discretion of the investigator. In such cases, the patient will receive standard treatment or other possible treatment in agreement with the national acute myeloid leukemia treatment center. Pregnancy is also a reason for early termination of study treatment. Patients who terminate study treatment early remain in the study to monitor safety, efficacy, and other parameters of the study treatment.
Sweden	Version 1.1, dated 12-Dec-2018	See Appendix Sweden	Appendix A for Sweden version 1.1 dd 01-SEP-2021

The following changes approved in Protocol v 3.0 of 27 July 2023 are not present in the versions of the Current Protocols of France, Sweden, Czech Republic and Netherlands.

Protocol version	Protocol section	Description of changes
3.0, dated July 27, 2023	**STEERING COMMITTEE / INTERNATIONAL PRINCIPAL INVESTIGATORS**	Prof. Franco Locatelli […] Catholic University of the Sacred Heart, Rome […] Prof.ssa Maria Teresa Voso, Laboratorio di Diagnostica Avanzata Oncoematologica, Fondazione Policlinico di Tor Vergata, V.le Oxford, 81, 00133 Roma (Italy), phone: +390620902169-3800 or +390672596278, fax +390620902169-3800, E-mail voso@med.uniroma2.it
	SYNOPSIS TREATMENT SYNOPSIS FOR STANDARD-RISK (SR) PATIENTS	Prof. Franco Locatelli […] Catholic University of the Sacred Heart, Rome […] Prof.ssa Maria Teresa Voso, Laboratorio di Diagnostica Avanzata Oncoematologica, Fondazione Policlinico di Tor Vergata, V.le Oxford, 81, 00133 Roma (Italy), phone: +390620902169-3800 or +390672596278, fax +390620902169-3800, E-mail voso@med.uniroma2.it

		[…] Patients of child-bearing or child-fathering potential must be willing to adapt their own conduct so as not to procreate during the study participation and must contact their physician to identify the most appropriate strategy for this purpose starting from the time of enrolment and for 3 months after receiving the last drug dose […] WBC less than $10x10^9$/L at presentation before start of treatment […] *Figure: Added BM at the end of Induction* […] ATRA 25 mg/m^2/day will be administered […] rounded to the nearest 10 mg, starting on day 1.
	SYNOPSIS TREATMENT SYNOPSIS FOR HIGH-RISK (HR) PATIENTS	Prof. Franco Locatelli […] Catholic University of the Sacred Heart, Rome […] Prof.ssa Maria Teresa Voso, Laboratorio di Diagnostica Avanzata Oncoematologica, Fondazione Policlinico di Tor Vergata, V.le Oxford, 81, 00133 Roma (Italy), phone: +390620902169-3800 or +390672596278, fax +390620902169-3800, E-mail voso@med.uniroma2.it […] Patients of child-bearing or child-fathering potential must be willing to adapt their own conduct so as not to procreate during the study participation and must contact their physician to identify the most appropriate strategy for this purpose starting from the time of enrolment and for 3 months after receiving the last drug dose […] WBC more than $10x10^9$/L at presentation before start of treatment […] *Figure: Added BM at the end of Induction* […] ATRA 25 mg/m2/day will be administered […] rounded to the nearest 10 mg, starting on day 1.

	Table 1. Visit schedule for both SR and HR patients enrolled into the study	Safety reporting (AEs, SAEs reporting) - All AEs end SAEs, irrespective of causality, will be collected between the time of informed consent signature until 30 days after the last dose of study drug product GO and other drugs administred in ICC-APL-02 protocol such as ATO, ATRA - Only AEs and SAEs related to any any study procedure or GO and other drugs administred in ICC-APL-02 protocol such as ATO, ATRA will be collected between the time of informed consent signature until end of study
	OUTLINE	*Figure: Added BM at the end of Induction of bot SR and HR patients*
	INTERNATIONAL STUDY MANAGEMENT	**STEERING COMMITTEE / INTERNATIONAL PRINCIPAL INVESTIGATORS** Prof. Franco Locatelli […] Catholic University of the Sacred Heart, Rome […] Prof.ssa Maria Teresa Voso, Laboratorio di Diagnostica Avanzata Oncoematologica, Fondazione Policlinico di Tor Vergata, V.le Oxford, 81, 00133 Roma (Italy), phone: +390620902169-3800 or +390672596278, fax +390620902169-3800, E-mail voso@med.uniroma2.it […] **DATA MANAGEMENT** OPBG Dipartimento di Onco-Ematologia e Terapia Cellulare e Genica Ospedale Pediatrico Bambino Gesù, IRCCS Piazza di Sant'Onofrio, 4 0165 Roma, Italy Phone 06 6859 3697 Fax 06 6859 2292
	STUDY DESIGN	before the ~~phase II~~ *continuation of the* study ~~itself is initiated~~

	INCLUSION AND EXCLUSION CRITERIA INCLUSION CRITERIA	Patients of child-bearing or child-fathering potential must be willing to adapt their own conduct so as not to procreate during the study participation and must contact their physician to identify the most appropriate strategy for this purpose starting from the time of enrolment and for 3 months after receiving the last drug dose
	PROCEDURES FOR ENTRY INTO THE STUDY AND DATA RECORDING Cytogenetics	Cytogenetics will be carried out locally. If this is not possible, it can be performed by the Centralized Lab, shipping a diagnostic bone marrow sample (3-5 ml) in heparinized culture medium or in lithium heparin. […] Contact Name: Prof Maria Teresa Voso Address: Laboratorio di Diagnostica Avanzata Oncoematologica piano 1-settore E, stanza 39,Fondazione Policlinico di Tor Vergata, V.le Oxford, 81, 00133 Roma (Italy) e-mail: voso@med.uniroma2.it, madivona@gmail.com,
	PROCEDURES FOR ENTRY INTO THE STUDY AND DATA RECORDING Molecular analysis	Contact Name: Prof Maria Teresa Voso Address: Laboratorio di Diagnostica Avanzata Oncoematologica piano 1-settore E, stanza 39,Fondazione Policlinico di Tor Vergata, V.le Oxford, 81, 00133 Roma (Italy) e-mail: voso@med.uniroma2.it, madivona@gmail.com,
	INDUCTION THERAPY STANDARD RISK	ATRA 25 mg/m^2 p.o. in two equally divided doses and rounded to the nearest 10 mg
	INDUCTION THERAPY HIGH RISK	GO Gemtuzumab-Ozogamycin 3 mg/m^2 (up to a maximum of one 5 mg vial) […] Lumbar puncture […] at the beginning of the 1st and 3rd consolidation course (doses chosen according to patient's age, see paragraph 17.2).
	INDUCTION THERAPY ATO TOXICITY	Treatment with ATO must be interrupted […] (CTCAE), Version 5.0 (except for QTc prolongation, for which the risk/benefit of continuing versus suspending ATO therapy must be considered, see also previous paragraph)

	PROFILE	
	INDUCTION THERAPY ADMINISTRATION OF GEMTUZUMAB OZOGAMICIN (GO, MYLOTARG®)	Patients will receive a GO dose of: - 3 mg/m2 for patients with BSA greater than or equal to 0.6 m2 (up to a maximum of one 5 mg vial) and - Infant patients < 1 year of age: 0.1 mg/kg for patients with BSA less than 0.6 m2 (Guest EM, Aplenc R, Sung L, Raimondi SC, Hirsch BA, Alonzo TA, Gerbing RB, Wang YJ, Kahwash SB, Heerema-McKenney A, Meshinchi S, Gamis AS. Gemtuzumab ozogamicin in infants with AML: results from the Children's Oncology Group trials AAML03P1 and AAML0531. Blood. 2017 Aug 17;130(7):943-945) infused over a 2-hour period after 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. Vital signs must be monitored during the infusion and for 4 hours following its completion. […] Gemtuzumab ozogamicin lyophilized powder for injection vials should be stored in a refrigerator at 2 to 8°C (36 to 46°F) and protected from light. Before reconstitution, allow the drug vials to reach room temperature (i.e. approximately 5 minutes). After reconstitution and dilution, chemical and physical in-use stability of gemtuzumab ozogamicin has been demonstrated for 6 hours at 2 to 25°C (36 to 77°F). […] It is necessary to filter the diluted solution. An inline 0.2 micron polyethersulfone (PES) filter with low protein binding capacity should be used for the infusion of MYLOTARG. Syringe doses should use small bore (microbore) infusion lines with a 0.2 micron polyethersulfone (PES) in-line filter with low protein binding.
	CONSOLIDATION THERAPY CONSOLIDATION THERAPY FOR SR AND HR	ATRA 25 mg/m^2 p.o. in two equally divided doses and rounded to the nearest 10 mg
	SUPPORTIVE CARE	*Added "Coagulopathy" paragraph*
	ADVERSE EVENT REPORTING	**Adverse Event (AE)** Any untoward medical occurrence after exposure to trial intervention, which does not necessarily have a causal

		relationship with the trial intervention. […] - results in patient early discontinuation from the study treatment - requires treatment, modification or interruption of dose, or any other therapeutic intervention, or is judged to be of significant clinical importance. […] **Serious Adverse Event (SAE)** Any adverse event [appearance of (or worsening of any pre-existing)] which meets any one of the following criteria: […] Comments: […] **SAE Reporting** For more detailed instructions on SAE reporting refer to the SAE Reporting Manual v 1.0"" contained in the Investigator Site File (ISF). […] The AE grading (severity) scale found in the NCI CTCAE v5.0 will be used for AE reporting. The NCI CTCAE v5.0 can be found: https://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_50 […] *Added "Adverse Event Grading Scale of Severity" section*
	REFERENCES	52. Hambley BC, Norsworthy KJ, Jasem J, et al. Fibrinogen consumption and use of heparin are risk factors for delayed bleeding during acute promyelocytic leukemia induction. Leuk Res. 2019;83:106174.
	DECLARATION OF HELSINKI	*Updated to Declaration of Helsinki 2013*