1. TITLE PAGE

FINAL CLINICAL STUDY REPORT

Study title: A Phase I/II study of Azacitidine (Vidaza®) in pediatric patients with relapsed high-grade pediatric MDS or JMML

Name of investigational product: Azacitidine (Vidaza®)

Indication studied: relapsed Myelodysplastic syndrome (MDS) and juvenile myelomonocytic leukemia (JMML)

Description: international, collaborative, prospective, open label, phase I/II study. This phase I/II study looks at two different disease indications (relapsed MDS and JMML). Each disease indication will be considered in independent treatment arms which will run in parallel with each other. One dose escalation will be allowed for each arm.

Due to the rarity of the two diseases being enrolled, this study will combine dose escalation in the phase I setting with efficacy based rules for discontinuing a study arm early or not, and declaring the study treatment in a given arm positive or not, in a phase II setting with regards to the primary endpoint, which is response rate.

Name of the sponsor: Erasmus MC, Rotterdam, The Netherlands

Protocol identification: EudraCT number 2010-022235-10

Development phase of study: Phase 1/2

Study initiation date / first subject visit: 13-06-2012 / 24 Jun 2013

Study completion date / last subject completed: 12 Feb 2020 / 28 Feb 2018

Name and affiliation of PI: prof. dr. M.M. van den Heuvel-Eibrink, Erasmus MC, & prof. dr. C.M. Zwaan, Erasmus MC,

Name of sponsor signatory: Erasmus MC, prof. Dr. C.M. Zwaan

The study was performed in compliance with the principles of Good Clinical Practice, including the archiving of essential documents.

Date of the report: 21 December 2021

2. SYNOPSIS

Main Study Objective	• To establish the recommended dose and preliminary efficacy of azacitidine in children with relapsed advanced MDS or JMML
Additional Study Objectives	• To determine the safety and tolerability of azacitidine in relapsed advanced MDS and JMML • To determine (preliminary) the haematological remission rate in these patients • To describe the durability of response, disease free and overall survival, including the number of patients undergoing stem-cell transplant after treatment with azacitidine • To describe the number of patients transforming into AML • To determine the plasma pharmacokinetic parameters of azacitidine • To study the pharmacodynamic effects of azacitidine in relapsed pediatric advanced MDS or JMML
Study Rationale	Myelodysplastic syndromes (MDS) and juvenile myelomonocytic leukemia (JMML) are rare malignant diseases of childhood. MDS in children can be divided in primary and secondary MDS. MDS after prior chemotherapy or radiation therapy, after prior acquired aplastic anemia, or in bone marrow failure disorders and familial diseases is generally classified as secondary MDS. So far, stem cell transplantation is the only curative treatment option for both MDS and JMML. No other agents are available to treat these diseases successfully, and HSCT results in approximately 50% survival only; hence there is clear unmet medical need. Over the past few years, we have increasing evidence that aberrant methylation contributes to the malignant phenotype of JMML and childhood advanced MDS. The demethylating agent azacitidine has been shown to improve survival and is authorized for adults with MDS, but so far no studies are available in children with MDS or JMML. In the current study we want to establish the recommended dose and preliminary efficacy of azacitidine, in children with primary advanced MDS or JMML in a pre-transplantation window at relapse. This study will provide a preliminary proof of concept whether a demethylating agent is able to induce responses in these diseases, and whether this agent indeed results in hypomethylation. Pharmacodynamic studies should provide further support for this proof of concept.
General Study Design	This is an international, collaborative, prospective, open label, phase I/II trial. The study will be conducted as an investigator-initiated study in a European network (EWOG-MDS and ITCC) with Erasmus MC acting as international sponsor, and with free drug provided by Celgene, who are also responsible for the PK-studies. Financial support is provided by the Go4Children foundation and Celgene. It needs to be mentioned that the HSCT procedure itself is not part of this protocol and should be performed under EWOG or institutional guidelines at the discretion of the principle investigator. We will however capture data to assess whether azacitidine influences outcome post-HSCT (follow-up for relapse/survival one year post-HSCT)

Study Population	In this study 2 disease indications will be included, stratum 1 and stratum 2: • Stratum 1: Relapsed patients with advanced primary MDS to bridge patients to a second HSCT. At relapse azacitidine may also be continued when a 2nd transplant is not feasible, as long as the patient benefits from treatment and in absence of major safety concerns. • Stratum 2: Relapsed patients with JMML to bridge patients to a second HSCT. Azacitidine may also be continued when a 2nd transplant is not feasible and as long as the patient benefits from treatment and in absence of major safety concerns. Inclusion criteria: • Diagnosis of relapsed advanced primary MDS or JMML, established at initial diagnosis by the diagnostic criteria as specified in the EWOG-MDS 2006 protocol (see appendix 1), and defined as: o Relapsed MDS: After a documented CR or PR, this designation is defined as - a reappearance of blasts in the peripheral blood, - or ≥5% blasts in the bone marrow not attributable to any other cause (e.g. bone marrow regeneration after consolidation therapy), and confirmed with flowcytometry. o Relapsed JMML: After a documented CR or PR, this designation is defined as - reappearance of organomegaly - in combination with elevated WBC with peripheral blood monocytosis (greater than $1x10^9$/l), - and/or the reappearance of a cytogenetic or molecular lesion indicative of prior disease. - In addition, clinical criteria may be used, which include objective parameters such as increase in spleen size of >50% from baseline, and/or the appearance of new skin lesions, and/or oxygen need, - and/or blast crises/transformation to AML. • 1 month to ≤ 18 years old • Lansky play score ≥ 60; or Karnofsky performance status ≥ 60 (appendix 2) • Life expectancy ≥ 3 months • Normal renal function defined as less than or equal to NCI-CTCAE grade 1 (max 1.5 x ULN). • Normal liver function defined as less than or equal to NCI-CTCAE grade 1 (max 2.5 x ULN for transaminases and bilirubin) • No chemotherapy within 3 weeks of start of study medication. For 6-MP or low-dose cytarabine in JMML patients 1 week wash-out time is sufficient. • For JMML patients: saturation >92% without additional supply of oxygen • For JMML patients: peripheral blood monocyte count greater than $1.0x10^9$/l • For relapsed patients following HSCT: recovery of all acute toxic effects of prior chemotherapy/stem-cell transplantation.

	• Able to comply with scheduled follow-up and with management of toxicity.
	• Reproductive Function
	  • Female patients of childbearing potential must have a negative urine or serum pregnancy test confirmed prior to enrollment.
	  • Female patients with infants must agree not to breastfeed their infants while on this study.
	  • Male and female patients of child-bearing potential must agree to use an *highly* effective method of contraception approved by the investigator during the study and for 90 days after the last dose of azacitidine.
	  • *Highly* effective methods of contraception include (but not exclusively) the following contraceptive methods:
	  • combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation
	  • progestogen-only hormonal contraception associated with inhibition of ovulation
	  • intrauterine device (IUD)
	  • intrauterine hormone-releasing system (IUS)
	  • sexual abstinence.
	• Written informed consent from patients or from parents or legal guardians for minor patients, according to local law and regulations.
	
	Exclusion criteria:
	
	• Other serious illnesses or medical conditions
	• Genetic abnormalities indicative of AML
	• JMML patients in whom a diagnosis of Noonan syndrome is suspected based on clinical history and/or presenting symptoms
	• Patients with secondary MDS with underlying bone-marrow failure syndromes or with familial MDS
	• Isolated extramedullary disease
	• Symptomatic CNS-involvement
	• Current uncontrolled infection
	• Cardiac toxicity (shortening fraction below 28%)
	• Concurrent treatment with any other anti-cancer therapy is not allowed
	• Pregnant or lactating patients
	• Patients who cannot be regularly followed up for psychological, social, familial or geographic reasons
	• Patient with expected non-compliance to toxicity management guidelines
	• Prior treatment with a demethylating agent
	• Allergy to azacitidine or mannitol.

Study Treatment	Azacitidine can either be administered IV or SC. In the USA azacitidine is approved for IV use, which is not the case in Europe. SC administration may be painful and induce skin reactions, and may therefore be difficult to apply in younger children.

There are strict time restrictions between dissolving azacitidine for IV use and the end of the infusion, which is logistically difficult in most centers. Therefore, sites are allowed to choose between IV and SC administration, whichever suits best for the site and/or the patient's preference (see administration guidelines below). Once a route of administration in a particular patient is chosen this should remain the same for that particular patient.

SC administration and time-lines between dissolving and administration:

- For immediate use of the SC administration the time between dissolving and administration is maximum 45 minutes, when the reconstituted product is kept at room temperature.
- Azacitidine suspension maintained stability for up to 22 hours when reconstituted with refrigerated (2°C to 8°C) water for injection, and then stored at 2°C to 8°C. After removal from refrigerated conditions, the suspension may be allowed to equilibrate to room temperature for up to 30 minutes prior to administration.

IV administration and time-lines between dissolving and administration:

Azacitidine will be supplied by Celgene as a sterile lyophilized powder containing 100 mg of azacitidine and 100 mg of mannitol per vial. For IV administration, vials containing 100 mg of freeze-dried azacitidine will be reconstituted to a concentration of 10mg/mL (with no additional dilutions) with 10mL of sterile water for injection. When needed, the reconstituted solution can be stored in the refrigerator set at 2°C to 8°C for up to 2 hours. Vigorously shake or roll the vial until all solids are dissolved. The solution should be clear. Parenteral drug product should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. The volume of azacitidine solution withdrawn from the reconstituted vial (10mg/mL) may be diluted with volumes less than 50 mL of either 0.9% Sodium Chloride injection of Lactated Ringer's injection. This will make it possible to achieve a final azacitidine concentration between 0.9 mg/mL and 4 mg/mL. The product should be warmed by hand for a period of 2 minutes before administration and IV administration should be completed within a 15 minute period after the hand warming has occurred.

Intravenous Solution Incompatibility: Azacitidine is incompatible with dextrose solutions, Hespan, or solutions that contain bicarbonate. These solutions have the potential to increase the rate of degradation of azacitidine and should therefore be avoided.

Solution Stability: Azacitidine reconstituted for IV administration should be administered within 45 minutes after reconstitution. If elapsed time is greater than 45 minutes, the reconstituted suspension should be discarded

	appropriately and a new dose prepared, as stability has not been confirmed for longer storage times, and hence no delayed administration is allowed. Dose: In children older than 1 year of age and a body weight > 10 kg dosing will be based on BSA, otherwise a mg/kg dose will be used. Two dose levels will be studied. One course is defined as 7 days of azacitdine, which is repeated once every 28 days. Children ≥1 year of age and ≥10 kg body weight: • Level -1 (dose reduction): 50 mg/m^2/day IV/SC x 7 days, one course every 28 days • Level 1: 75 mg/m^2/day IV/SC x 7 days, one course every 28 days • Level 2: 100 mg/m^2/day IV/SC x 7 days, one course every 28 days Children<1 year of age or <10 kg body weight: • Level -1 (dose reduction): 1,7 mg/kg/day IV/SC x 7 days, one course every 28 days • Level 1: 2,5 mg/kg/day IV/SC x 7 days, one course every 28 days • Level 2: 3,3 mg/kg/day IV/SC x 7 days, one course every 28 days In all patients, every effort should be made to transplant patients only after having received at least 3 courses of azacitidine (hence ~3 months of pre-HSCT treatment), or receive additional courses in case the donor search and HSCT preparations have not been finalized, and the patient benefits from treatment (investigator discretion). This 3 months period was chosen as in adult MDS the *median* time to respond to azacitidine was 3 months, and also because this complies with the usual preparation time for HSCT. In patients for whom no donor is available or who cannot be transplanted for other reasons, it is advised to treat with at least 6 courses of azacitidine (in absence of safety concerns) before it is decided to take patients off study for apparent lack of efficacy, based on the observation in adult MDS that it may take up to 6 courses before a response becomes evident. However, patients with progressive disease will be taken off study, and can either be transplanted directly in case a donor is available, or receive other chemotherapy. For advanced MDS patients progressive disease is defined as evolution to MDS-related AML (>30% bone marrow blasts). In JMML, progressive disease is defined as clinical progression including objective parameters such as increase in spleen size of >50% from baseline, and/or the appearance of new skin lesions, and/or oxygen need, and/or blast crises/transformation to AML. Patients who show benefit and for whom no donor is available or who cannot be transplanted for other reasons will be offered to continue azacitidine as long as they receive benefit, in absence of major safety

	concerns. Patients, for whom other more curative treatment options (i.e. a stem-cell transplant) will become available, will be taken off study.
Investigational medicinal product	Azacitidine is the investigational medicinal product in this study and will be provided by Celgene. Azacitidine is approved for SC administration by the EMA (Dec 17th, 2008) for use in adults with MDS, CMML or AML who cannot be transplanted. In 2004, the U.S. Food and Drug Administration approved azacitidine both for SC as well as for IV administration for treatment of adult patients with several types of MDS or CMML.
Study Design	This phase I/II study looks at two different disease indications (relapsed MDS and JMML). Each disease indication will be considered in independent treatment arms which will run in parallel with each other. One dose escalation will be allowed for each arm. Due to the rarity of the two diseases being enrolled, this study will combine dose escalation in the phase I setting with efficacy based rules for discontinuing a study arm early or not, and declaring the study treatment in a given arm positive or not, in a phase II setting with regards to the primary endpoint, which is response rate. As such, moving from stage 1 to stage 2 in this two stage design takes into account study treatment tolerability and response data: Each stage (stage one and stage two) will consist of a minimum of 3 patients per disease indication. Both study arms will follow a 2-stage design, with the option of a safety run-in in the case of the JMML arm. For the MDS arm, if there is at least one patient achieving response (defined as CR or PR) and there are no patients experiencing a dose-limiting toxicity among the three first patients in stage one, another three patients will be treated at the next higher dose level, if applicable. In case of 1 dose-limiting toxicity among the first three patients, the cohort will be expanded to 6 patients at the starting dose-level. If there is at least one out of six patients achieving response and no more than one patient experiences a dose-limiting toxicity in stage one, stage two shall open for enrolment. In case of no responses the arm shall be closed to enrollment. In case there is more than one dose-limiting toxicity in stage one, the dose is set to the previous level (if applicable), and stage 2 shall open for enrolment if at least one patient responded at that dose-level. Otherwise, the arm shall be closed to enrolment. The dose will be increased only if <2 of the 6 evaluable patients (30%, across stage-one and stage-two) achieve a response, and/or there are ≤ two dose-limiting toxicities; otherwise the therapy will be deemed unpromising for further consideration. For the JMML arm, the safety run-in will include 3 patients and the tolerability of the therapy will be considered using a classic 3+3 design. Should the therapy be considered tolerable, stage one shall enrol patients to a higher dose, or otherwise the patients in the safety run-in will be

	considered part of stage one. During stage-one, if ≥ 1 of the 3 evaluable patients for the primary endpoint achieve a response then stage two shall open to enrolment, or otherwise that arm shall be closed to enrolment. At the end of stage two, the therapy will be considered positive for possible further investigation if ≥ 2 of the 6 evaluable patients (30%, across stage-one and stage-two) achieve a response; or otherwise considered unpromising for further consideration.
Sample size	We will recruit a maximum of 12 patients in each stratum, and hence 24 patients in total. Including screen failures or drop-outs or in case of DLTs we may need to recruit a maximum of 28 patients. The study will last approximately 8 years from first patient first visit (FPFV) to last patient last visit (LPLV). The DSMB will confirm the RP2D based on the generated data before the expansion cohort in phase 2 is considered.
Guidelines for Dose Escalation of Azacitidine	Intra-patient dose-escalation in patients with relapsed MDS (stratum 1) who started at dose-level 1 may take place after the 3rd course, in patients not achieving CR but showing some response to azacitidine (i.e. stable disease or PR). For relapsed JMML patients in stratum 2, intra-patient dose escalation is allowed if the patient does not receive a PR after 1 course. Intra-patient dose-escalation may only take place in case of a favourable safety profile when treated at the lower dose. Following dose-escalation, patients will be treated at dose-level 2 with a 28-day interval.
Safety Assessment and Guidelines for Dose Reduction of Azacitidine	Toxicity monitoring will be done applying the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events Version 4.0 (NCI CTCAE). The NCI CTCAE v4.0 can be viewed on-line at the following NCI web site: http://ctep.cancer.gov/reporting/ctc.html. All adverse events (AE) must be forwarded to the sponsor. For serious adverse event (SAE) an expedited procedure is in place. Dose limiting toxicities (DLTs) are AEs as defined below and considered at least possibly drug-related, and will be limited to the *first course* of azacitidine. Non-hematologic DLTs: • Any ≥ grade 3 study drug related non-hematologic toxicity occurring in spite of appropriate medical management. • Any non-hematologic laboratory abnormality of Grade 4, or Grade 3 lasting ≥ 7 days, and requiring treatment discontinuation or interruption or dose-reduction in subsequent courses. • Any clinically-important toxicity of Grade ≥ 2 requiring treatment discontinuation or interruption ≥ 7 days or dose-reduction in subsequent courses.

	The following will <u>not</u> be considered DLTs: grade 3 nausea and/or vomiting that can be subsequently controlled, alopecia, drug fever, anorexia, and transient grade 3 transaminase elevations that return to ≤grade 1 within 7 days. <u>Hematologic DLTs:</u> It is anticipated that the underlying hematological disorder may result in severe myelosuppression and its associated complications. Therefore, myelosuppression/pancytopenia and grade 3 febrile neutropenia will not be considered DLTs. However, *prolonged* myelo-suppression will be considered a DLT in *responding patients* only. This is defined as grade 3 or 4 myelosuppression, which represents a worsening from baseline lasting more than 42 days with evidence of a hypocellular marrow (marrow cellularity less than 5%), and without evidence of persisting leukemia. DLTs may result in delay of subsequent treatment courses, or dose adjustments. <u>Dose reduction</u> All patients must have recovered from acute grade 3 or 4 side effects from previous courses of azacitidine before starting the 2nd or subsequent course, as specified in detail below: <u>Dose reduction for hematological toxicity</u> Hematological toxicity will be difficult to assess and to differentiate from the natural course of the underlying disorder. As the majority of patients will be treated prior to a stem cell transplant procedure we will usually NOT consider dose reduction for hematological toxicity. The only exception will be dose-reduction based on hematological toxicity in JMML patients who have achieved CR in prior courses, but who do not recover counts after 4 weeks, as well as in patients with relapsed disease (either JMML or MDS) in CR who will be treated with multiple courses of azacitidine because of lack of a re-transplantation option. Detailed guidelines are given below in the protocol in paragraph 4.4.3. <u>Dose reduction for non-hematological toxicity</u> For grade 3 or 4 non-hematological toxicity, which is at least potentially related to study treatment, and that is not clinically manageable with regular supportive care/medical management, treatment with azacitidine needs to be interrupted until the toxicity decreases to ≤ grade 1 (or ≤ grade 2, if this is baseline). Subsequent courses may then be given at the next lower dose-level. Please see details in paragraph 4.4.3 in the protocol. Alternatively, for clinically manageable toxicities which are at least potentially related to study treatment, and which are expected side-effects in case of intensive chemotherapy (febrile neutropenia, diarrhea, and mucositis), no dose-reduction is needed, unless this toxicity was considered too severe per investigator's discretion. When the toxicity was considered too severe by the

	investigator, subsequent courses may then be given at the next lower dose-level.

3. TABLE OF CONTENTS FOR THE INDIVIDUAL CLINICAL STUDY REPORT

4. LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

6-MP	6-Mercaptopurine
ALL	Acute lymphoblastic leukemia
AML	Acute myeloid leukemia
Ara-C	Cytarabine
ASR	Annual Safety Report
BLQ	Below the Limit of Quantification
BM	Bone marrow
BSA	Body surface area
BU	Busulfan
CBC	Complete blood count
CCR	Complete cytogenetic response
CMR	Complete molecular remission
CNS	Central nervous system
CR	Complete remission
CRF	Case report form
CY	Cyclophosphamide
DCOG-ECTC	Dutch Childhood Oncology Group-Early Clinical Trial Consortium
DNA	Deoxyribonucleic acid
DSMB	Data safety monitoring board
EBMT	European Blood and Marrow Transplantation
EFS	Event free survival
EMA	European Medicines Agency
ESR	Expedited Safety Report
EWOG-MDS	European Working Group of MDS in childhood
FTase	Farnesyl transferase
GCP	Good clinical practice
GFR	Glomerular Filtration Rate
GM-CSF	Granulocyte macrophage-colony stimulating factor
HbF	Fetal hemoglobin
HI	Hematological improvement
HSCT	Hematopoietic stem cell transplantation
IB	Investigator's Brochure
IEC	Independent ethics committee
ICH	International Conference on Harmonisation
IRB	Institutional review board
ITCC	Innovative Therapies for Children with Cancer Consortium
IV	Intravenous
Kg	Kilogram
JMML	Juvenile myelomonocytic leukemia
LLQ	Lower Limit of Quantification
MAPK	Mitogen-activated protein kinase
MDR-AML	Myelodysplasia-related AML
MDS	Myelodysplastic syndrome
MFD	Matched family donor
CTCAE	Common Terminology Criteria for Adverse Events

NF1	Neurofibromatosis type 1
OS	Overall survival
PB	Peripheral blood
PCR	Partial cytogenetic response
PD	Pharmacodynamics
PoD	Progression of Disease
PK	Pharmacokinetics
PR	Partial response
RAEB	Refractory cytopenia with excess blasts
RAEB-t	Refractory cytopenia with excess blasts in transformation
RARS	Refractory anemia with ringed sideroblasts
RC	Refractory cytopenia
SC	Subcutaneous
SPC	Summary of Product Characteristics
UD	Unrelated donor
ULN	Upper Limit of Normal
WHO	World Health Organization

5. ETHICS

5.1 Independent Ethics Committee (IEC) or Institutional Review Board (IRB)

This study and amendments were reviewed and approved by the ethical principles of the IRB/IEC. See Appendix 16.1.3 for a list of IEC and/or IRB.

5.2 Ethical Conduct of the Study

The study was conducted in accordance with the ethical principles that have their origins in the Declaration of Helsinki.

5.3 Patient Information and Consent

The informed consent forms (ICF) included all elements required by ICH, GCP and applicable regulatory requirements, and adhered to GCP and to the ethical principles that have their origin in the Declaration of Helsinki. The consent form contained a statement that the sponsor and regulatory authorities have direct access to subject records. ICFs were reviewed and approved by the IEC. Prior to the beginning of the study, the Investigator had the IRB/IEC's written approval/favorable opinion of the written informed consent form and any other information to be provided to the subjects. Freely given written informed consent was obtained from every subject or their legally acceptable representative prior to clinical trial participation, including informed consent for any screening procedures conducted to establish subject eligibility for the trial.

6. INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

For the EWOG-MDS group:

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Collaboration:

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- EWOG-MDS Group
  C.M. Niemeyer, Head of EWOG Coordinating Study Center, Freiburg
  M.M. van den Heuvel-Eibrink, Chair of EWOG-MDS
  F. Locatelli, former chair of the EWOG-MDS group

- Celgene
  E. Laille (Manager Clinical Pharmacology) for Central PK analysis coordination
  M.R. Rodriguez, Associate Manager Medical Affairs-IITs

SPONSOR:

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7. INTRODUCTION

In this study 2 different pediatric disease indications, MDS and JMML, were considered:

- Stratum 1:
  Relapsed patients with advanced MDS to bridge patients to a second HSCT. At relapse azacitidine may also be continued when a 2nd transplant is not feasible, as long as the patient benefits from treatment.

- Stratum 2:
  Relapsed patients with JMML to bridge patients to a second HSCT. Azacitidine may also be continued when a 2nd transplant is not feasible and as long as the patient benefits from treatment.

There is clear medical need in relapsed advanced MDS and relapsed JMML to control disease pre-HSCT without the disadvantages associated with intensive chemotherapy. So far no agents have been successfully applied in this window or are specifically registered for use in these disease conditions.

The demethylating agent azacitidine has been shown to improve survival and is authorized for adults with MDS, but so far no studies are available in children with MDS or JMML.

Based on adult data in MDS and the favorable safety profile we feel that a study in relapsed pediatric MDS and JMML is warranted. There are available pediatric safety data using azacitidine dosages that are much higher than proposed in this study, therefore we decided not to dose-reduce azacitidine in this study but to use a similar dose as has been shown to be safe and effective in adult MDS. Moreover, recently (2015 and 2016), case series have been published using these dosages in pediatric MDS and JMML. Apparently this dose results in adequate hypomethylation, whereas the leukemia studies in the past have focused on the use of azacitidine as a regular cytotoxic compound (hence MTD-based).

In the current study we wanted to establish the recommended dose and preliminary efficacy of azacitidine, in children with primary advanced MDS or JMML in a pre-transplantation window at relapse. This study would provide a preliminary proof of concept whether a demethylating agent is able to induce responses in these diseases, and whether this agent indeed results in hypomethylation. Pharmacodynamic studies should provide further support for this proof of concept.

Unfortunately, during the whole study period the inclusion rate was very low and in recent years the inclusion rate dropped even further. Especially for JMML patients a strong decline of inclusion was noticed, with a 5 year time period between the last two included JMML patients.

Possible explanations for the lack of inclusion of JMML patients are that the outcomes after transplantation for primary JMML patients have improved. As a result, fewer patients were available than anticipated when setting up the study. In addition, azacitidine is also freely available and commonly used for patients with JMML. Patients will therefore be less likely to travel to a study center to participate in the study.

As the results of the AZA-JMML-001 study presented at the ASCO 2019 showed that monotherapy with azacitidine was well tolerated in newly diagnosed JMML patients we found that the clinical equipoise is decreased to a level that the investment (time and costs) to include the remaining JMML patients is too large.

It was therefore decided, in consultation with the PIs, to stop the study prematurely on the 12th of February 2020. This had consequences for the analyses which will now be mostly descriptive.

8. STUDY OBJECTIVES

Main Study Objective

- To establish the recommended dose and preliminary efficacy of azacitidine in children with relapsed advanced MDS or JMML

Additional Study Objectives

- To determine the safety and tolerability of azacitidine in relapsed advanced MDS and JMML
- To determine (preliminary) the haematological remission rate in these patients
- To describe the durability of response, disease free and overall survival, including the number of patients undergoing stem-cell transplant after treatment with azacitidine
- To describe the number of patients transforming into AML
- To determine the plasma pharmacokinetic parameters of azacitidine
- To study the pharmacodynamic effects of azacitidine in relapsed pediatric advanced MDS or JMML

9. INVESTIGATIONAL PLAN

9.1 Overall Study Design and Plan-Description

This is an international, collaborative, prospective, open label, phase I/II trial. The study was conducted as an investigator-initiated study in a European network (EWOG-MDS and ITCC) with Erasmus MC acting as international sponsor, and with free drug provided by Celgene, who are also responsible for the PK-studies. Financial support is provided by the Go4Children foundation and Celgene.

This phase I/II study looks at two different disease indications (relapsed MDS and JMML). Each disease indication will be considered in independent treatment arms which will run in parallel with each other. One dose escalation will be allowed for each arm.

In children older than 1 year of age and a body weight > 10 kg dosing will be based on BSA, otherwise a mg/kg dose will be used. Two dose levels will be studied. One course is defined as 7 days of azacitdine, which is repeated once every 28 days.

Children ≥1 year of age and ≥10 kg body weight:

- Level -1 (dose reduction): 50 mg/m^2/day IV/SC x 7 days, one course every 28 days
- Level 1: 75 mg/m^2/day IV/SC x 7 days, one course every 28 days
- Level 2: 100 mg/m^2/day IV/SC x 7 days, one course every 28 days

Children<1 year of age or <10 kg body weight:

- Level -1 (dose reduction): 1,7 mg/kg/day IV/SC x 7 days, one course every 28 days
- Level 1: 2,5 mg/kg/day IV/SC x 7 days, one course every 28 days
- Level 2: 3,3 mg/kg/day IV/SC x 7 days, one course every 28 days

In all patients, every effort should have been made to transplant patients only after having received at least 3 courses of azacitidine (hence ~3 months of pre-HSCT treatment), or after receiving additional courses in case the donor search and HSCT preparations have not been finalized, and the patient benefited from treatment (investigator discretion). This 3 months period was chosen as in adult MDS the *median* time to respond to azacitidine was 3 months, and also because this complies with the usual preparation time for HSCT.

In patients for whom no donor was available or who could not be transplanted for other reasons, it was advised to treat with at least 6 courses of azacitidine (in absence of safety concerns) before it was decided to take patients off study for apparent lack of efficacy, based on the observation in adult MDS that it may take up to 6 courses before a response becomes evident. However, patients with progressive disease were taken off study, and could have either be transplanted directly in case a donor is available, or received other chemotherapy. For advanced MDS patients progressive disease is defined as evolution to MDS-related AML (>30% bone marrow blasts). In JMML, progressive disease is defined as clinical progression including objective parameters such as increase in spleen size of >50% from baseline, and/or the appearance of new skin lesions, and/or oxygen need, and/or blast crises/transformation to AML.

Patients who showed benefit and for whom no donor was available or who could not be transplanted for other reasons were offered to continue azacitidine as long as they receive benefit, in absence of major safety concerns.

Intra-patient dose-escalation in patients with relapsed MDS (stratum 1) who started at dose-level 1 took place after the 3rd course, in patients not achieving CR but showing some response to azacitidine (i.e. stable disease or PR).

For relapsed JMML patients in stratum 2, intra-patient dose escalation was allowed if the patient did not receive a PR after 1 course.

Intra-patient dose-escalation may have only taken place in case of a favorable safety profile when treated at the lower dose. Following dose-escalation, patients were treated at dose-level 2 with a 28-day interval.

Each stage (stage one and stage two) consisted of a minimum of 3 patients per disease indication. Both study arms followed a 2-stage design, with the option of a safety run-in in the case of the JMML arm.

For the MDS arm, if there was at least one patient achieving response (defined as CR or PR) and there were no patients experiencing a dose-limiting toxicity among the three first patients in stage one, another three patients were treated at the next higher dose level, if applicable. In case of 1 dose-limiting toxicity among the first three patients, the cohort was expanded to 6 patients at the starting dose-level. If there was at least one out of six patients achieving response and no more than one patient experiences a dose-limiting toxicity in stage one, stage two shall was opened for enrolment. In case of no responses the arm was closed to enrollment. In case there was more than one dose-limiting toxicity in stage one, the dose is set to the previous level (if applicable), and stage 2 was opened for enrolment if at least one patient responded at that dose-level. Otherwise, the arm was closed to enrolment.

The dose was increased only if <2 of the 6 evaluable patients (30%, across stage-one and stage-two) achieved a response, and/or there were ≤ two dose-limiting toxicities; otherwise the therapy would be deemed unpromising for further consideration.

For the JMML arm, the safety run-in included 3 patients and the tolerability of the therapy was considered using a classic 3+3 design. Should the therapy been considered tolerable, stage one should enroll patients to a higher dose, or otherwise the patients in the safety run-in will be considered part of stage one. During stage-one, if ≥ 1 of the 3 evaluable patients for the primary endpoint achieved a response then stage two should open to enrolment, or otherwise that arm was closed to enrolment. At the end of stage two, the therapy was considered positive for possible further investigation if ≥ 2 of the 6 evaluable patients (30%, across stage-one and stage-two) achieved a response; or otherwise considered unpromising for further consideration.

It needs to be mentioned that the HSCT procedure itself was not part of this protocol. We will however capture data to assess whether azacitidine influences outcome post-HSCT (follow-up for relapse/survival one year post-HSCT)

9.2 Discussion of Study Design, including the Choice of Control Groups

Discussion study design

Due to the rarity of the two diseases being enrolled, this study combined dose escalation in the phase I setting with efficacy based rules for discontinuing a study arm early or not, and declaring the study treatment in a given arm positive or not, in a phase II setting with regards to the primary endpoint, which

is response rate. As such, moving from stage 1 to stage 2 in this two stage design takes into account study treatment tolerability and response data.

Discussion starting dose

Pediatric trials usually start with 80% of the adult recommended dose. In adult MDS patients dose escalations from 75 up to 100 mg/m2 for 7 days with an interval of 28 days have generally been well-tolerated, although only a limited number of patients was treated at the 100 mg/m2 dose-level (n=19) (Kaminskas et al, 2005). These dose-levels were selected for inhibition of DNA methylation in-vitro, and as such do not exert major cytostatic effects. Higher dose-levels are associated with significant and dose-limiting bone marrow toxicity, which occurs only in about 10% of MDS patients using the lower dose-levels (Silverman et al, 2002). Other side effects at these dose-levels include gastro-intestinal toxicity (nausea, vomiting, diarrhea), injection site events, arthralgia, dizziness, dyspnea, cough and myalgia (Kaminskas et al, 2005).

As the preferred dose in adults (75 mg/m2 x 7 days) is not based on dose-limiting-toxicities (DLTs) but represent a 'biologically effective' dose, and the dose-levels used in adults are safe, the same dose-levels will be used for the pediatric study. This is also based on safety data available from treatment of leukemias in children, during which much higher dosages have been applied using azacitidine at MTD-levels as a cytostatic rather than a demethylating agent.

9.3 Selection of Study Population

9.3.1 Inclusion criteria

General conditions:

- Diagnosis of advanced primary relapsed MDS or JMML established at initial diagnosis by the diagnostic criteria as specified in the EWOG-MDS 2006 protocol (see appendix 1)
  - Relapsed MDS:
    After a documented CR or PR, this designation is defined as
    - a reappearance of blasts in the peripheral blood,
    - or ≥5% blasts in the bone marrow not attributable to any other cause (e.g. bone marrow regeneration after consolidation therapy), and confirmed with flowcytometry.
  - Relapsed JMML:
    After a documented CR or PR, this designation is defined as
    - reappearance of organomegaly
    - in combination with elevated WBC with peripheral blood monocytosis (greater than 1x109/l),
    - and/or the reappearance of a cytogenetic or molecular lesion indicative of prior disease.
    - In addition, clinical criteria may be used, which include objective parameters such as increase in spleen size of >50% from baseline, and/or the appearance of new skin lesions, and/or oxygen need,
    - and/or blast crises/transformation to AML.
- 1 month to ≤ 18 years old
- Lansky play score ≥ 60; or Karnofsky performance status ≥ 60 (appendix 2)
- Life expectancy ≥ 3 months

- Normal renal function defined as less than or equal to CTCAE grade 1 (max 1.5 x ULN).
- Normal liver function defined as less than or equal to CTCAE grade 1 (max 2.5 x ULN for transaminases and bilirubin)
- No chemotherapy within 3 weeks of start of study medication. For 6-MP or low-dose cytarabine in JMML patients 1 week wash-out time is sufficient.
- For JMML patients: saturation >92% without additional supply of oxygen
- For JMML patients: peripheral blood monocyte count > 1.0x109/l
- For relapsed patients following HSCT: recovery of all acute toxic effects of prior chemotherapy/stem-cell transplantation.
- Able to comply with scheduled follow-up and with management of toxicity.
- Reproductive Function
    - Female patients of childbearing potential must have a negative urine or serum pregnancy test confirmed prior to enrollment.
    - Female patients with infants must agree not to breastfeed their infants while on this study.
    - Male and female patients of child-bearing potential must agree to use an *highly* effective method of contraception approved by the investigator during the study and for 90 days after the last dose of azacitidine.
    - *Highly* effective methods of contraception include (but not exclusively) the following contraceptive methods:
- combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation
- progestogen-only hormonal contraception associated with inhibition of ovulation
- intrauterine device (IUD)
- intrauterine hormone-releasing system (IUS)
- sexual abstinence.
- Written informed consent from patients or from parents or legal guardians for minor patients, according to local law and regulations.

9.3.2 Exclusion criteria

- Prior or current history: o Other serious illnesses or medical conditions
    - Genetic abnormalities indicative of AML
- JMML patients in whom a diagnosis of Noonan syndrome is suspected based on clinical history and/or presenting symptoms
- Patients with secondary MDS with underlying bone-marrow failure syndromes or with familial MDS
- Isolated extramedullary disease
- Symptomatic CNS-involvement
- Current uncontrolled infection
- Cardiac toxicity (shortening fraction below 28%)
- Concurrent treatment with any other anti-cancer therapy is not allowed
- Pregnant or lactating patients
- Patients who cannot be regularly followed up for psychological, social, familial or geographic reasons
- Patient with expected non-compliance to toxicity management guidelines

- Prior treatment with a demethylating agent
- Allergy to azacitidine or mannitol.

9.3.3 Removal of patients from therapy or assessment

When applicable, patients with progressive disease were taken of study, and could be transplanted directly in case a donor is available, or receive other chemotherapy.

9.4 Treatments

9.4.1 Treatments administered

In this study both IV infusion or SC administration was allowed. Therefore sites were allowed to choose between IV and SC administration based on the site's and the patient's preference. However, the route of administration should always be the same in a given patient.

9.4.2 Identity of investigational product(s)

Azacitidine is also known as Vidaza®. Azacitidine is a white to off-white solid powder 100 mg single-use vials without preservatives, and the molecular weight is 244. The finished product is supplied in a sterile form for reconstitution as a suspension for subcutaneous (SC) injection, or reconstitution as a solution for intravenous (IV) infusion.

9.4.3 Method of assigning patients to treatment groups

Assignment was based on diagnosis (JMML vs MDS)

9.4.4 Selection of doses in the study

Dosing was based on body surface area in older children, however, in children below 1 year of age of less than 10 kg body weight dosing will be based on mg/kg. Two dose-levels will be studied. One course is defined as 7 days of azacitdine, which is repeated once every 28 days.

Children ≥1 year of age and ≥10 kg body weight:

- Level -1 (dose reduction): 50 mg/m2/day IV/SC x 7 days, one course every 28 days
- Level 1: 75 mg/m2/day IV/SC x 7 days, one course every 28 days
- Level 2: 100 mg/m2/day IV/SC x 7 days, one course every 28 days

Children<1 year of age or <10 kg body weight:

- Level -1 (dose reduction): 1,7 mg/kg/day IV/SC x 7 days, one course every 28 days
- Level 1: 2,5 mg/kg/day IV/SC x 7 days, one course every 28 days
- Level 2: 3,3 mg/kg/day IV/SC x 7 days, one course every 28 days

9.4.5 Selection and timing of dose for each patient

Rationale for the starting dose

is provided in paragraph 9.2

Intra-patient dose-escalation

Stratum 1: Intra-patient dose-escalation in patients with relapsed MDS who started at dose-level 1 may have taken place after the 3rd course, in patients not achieving CR but showing some response to azacitidine (hence a PR or stable disease). Patients should have been taken off study when they did not

show any response after the 6th course of treatment, or when there was clear progressive disease (for instance development of MDS-related AML).

Stratum 2: For relapsed JMML patients dose-escalation may have taken place after the 1st course in patients who did not achieve a PR. Note: in case JMML patients developed the need for supplemental oxygen therapy they should have come off study and be treated with regular chemotherapeutic alternatives such as cytarabine or FLAG.

Intra-patient dose-escalation may have only taken place in case of a favorable safety profile when treated at the lower dose as assessed by the local investigator.

Following dose-escalation, patients were treated at dose-level 2 with a 28-day interval, similar to the lower dose.

Dose reduction

All patients must have recovered from acute grade 3 or 4 side effects from previous courses of azacitidine before starting the 2nd or subsequent course, as specified in detail below.

Dose reduction for hematological toxicity

Hematological toxicity will be difficult to assess and to differentiate from the natural course of the underlying disorder. As the majority of patients will be treated prior to a stem cell transplant procedure dose reduction will usually NOT be considered for hematological toxicity. The only exception will be dose-reduction based on hematological toxicity in JMML patients who have achieved CR in prior courses but who do not recover counts after 4 weeks, and in patients with relapsed MDS, who will be treated with multiple courses of azacitidine because of lack of a re-transplantation option. Detailed guidelines are given below per stratum:

- *Stratum 1*:
  - In case patients are to be re-transplanted we will not take hematological toxicity into account and administer azacitidine in 28-day courses irrespective of counts, unless there is evidence of progression to MDS-related AML.
  - In case a re-transplantation in short term is not expected we will follow the guidelines as provided in the SPC for adults:

a) *Patients without reduced baseline blood counts (i.e. WBC > 3.0 x 109/l and ANC >1.5 x 109/l, and platelets > 75.0 x 109/l) prior to the first treatment:*

If hematological toxicity is observed following azacitidine treatment, the next course of azacitidine therapy should be delayed until the platelet count and the ANC have recovered. If recovery is achieved within 14 days (after day 28), no dose adjustment is necessary. However, if recovery has not been achieved within 14 days (after day 28), the dose should be reduced to 50% of the given dose in the prior course if the nadir ANC was below 1000x106 and/or platelets below 50X109/l. Following dose modifications, the course duration should return to 28 days.

b) *Patients with reduced baseline blood counts (i.e. WBC < 3.0 x 109/l or ANC < 1.5 x 109/l or platelets < 75.0 x 109/l) prior to the first treatment*

Following azacitidine treatment, if the decrease in WBC or ANC or platelets from that prior to treatment is less than 50%, or greater than 50% but with an improvement in any cell line differentiation, the next course should not be delayed and no dose adjustment made.

If the decrease in WBC or ANC or platelets is greater than 50% from that prior to treatment, with no improvement in cell line differentiation, the next course of azacitidine therapy should be delayed until the platelet count and the ANC have recovered. If recovery is achieved within 14 days (after day 28), no dose adjustment is necessary. However, if recovery has not been achieved within 14 days (after day 28), bone marrow cellularity should be determined. If the bone marrow cellularity is > 50%, no dose adjustments should be made. If bone marrow cellularity is ≤ 50%, treatment should be delayed and the dose reduced. In case of BM cellularity between 15-50% and recovery within 14 days (after day 28) a full dose will be given, in case of longer delay 50% dose will be administered. In case of cellularity below 15% and recovery within 14 days (after day 28), 100% dose will be given, if recovery takes longer 33% of the dose will be given.

- *Stratum 2*:
  • For patients with JMML, hematological toxicity will only be assessed in patients with complete remission, following the established response criteria for JMML as given elsewhere in this protocol (see 6.7). In case of lack of count recovery in patients with JMML in CR and in absence of leukemia relapse the next course should be given at the next lower dose-level. In case of insufficient response (PR or less) the next course will start at day 28 without consideration of the hematological parameters with the aim to improve response. Please consider the intra-patient dose-escalation guidelines for such patients.

Dose reduction for non-hematological toxicity

For grade 3 or 4 non-hematological toxicity, which is at least potentially related to study treatment, and that is not clinically manageable with regular supportive care/medical management, treatment needs to be interrupted until the toxicity decreases to ≤ grade 1 (or ≤ grade 2, if this is baseline). Subsequent courses may then be given at the next lower dose-level as defined elsewhere in the protocol.

Alternatively, for clinically manageable toxicities which are at least potentially related to study treatment, and which are expected side-effects in case of hematological malignancies (mainly febrile neutropenia), no dose-reduction is needed, unless this toxicity was considered too severe per investigator's discretion. When the toxicity was considered too severe by the investigator, subsequent courses may then be given at the next lower dose-level.

Renal impairment: No formal studies have been conducted in patients with decreased renal function. No specific modification to the starting dose is recommended in patients with renal impairment (e.g. baseline serum creatinine or blood urea nitrogen (BUN) ≥ 2-fold above upper limit of normal (ULN) or serum bicarbonate less than 20 mmol/l) prior to starting treatment; subsequent dose modifications should be based on hematology and renal laboratory values. If unexplained reductions in serum bicarbonate levels to less than 20 mmol/l occur, the dose should be reduced by 50% on the next course. If unexplained elevations in serum creatinine or BUN to ≥ 2-fold above baseline values and above ULN occur, the next course should be delayed until values return to normal or baseline and the dose should be reduced to the next lower dose level.

Hepatic impairment: No formal studies have been conducted in patients with hepatic impairment. No specific dose modification is recommended for patients with hepatic impairment prior to starting treatment; subsequent dose modifications should be based on hematology laboratory values.

9.4.6 Blinding

Not applicable

9.4.7 Prior and concomitant therapy

Anti-emetics

Azacitidine is moderately emetogenic. Therefore, standard anti-emetic therapy (such as a 5HT3 antagonist) was administered prior to therapy, per institutional protocol.

Administration related supportive care measures

Patients with JMML and high WBC may have required hyperhydration and tumor-lysis prevention measures as per institutional protocol.

Supportive Care

Blood Products

The choice of blood products (leucocyte reduction, irradiation) was be done according to institutional guidelines. Irradiation was recommended in the pre-transplantation window. CMV- and ParvoB19 negative patients should have received CMV/ParvoB19 negative blood products, again according to institutional guidelines.

Persistent bleeding may be attributable to thrombocytopenia and patients should have received platelet transfusions as quickly as possible. Prophylactic platelet transfusions may also been considered if the platelet count drops <10,000/ul and in case of symptoms.

Infection Prophylaxis

The use of antibacterial or antifungal prophylaxis was left to the discretion of the investigator and was in line with institutional guidelines.

Treatment of Fever and Neutropenia

Patients with neutropenia (ANC ≤ 500x106/l (or < 1,000 and falling) and fever should have empiric systemic antibiotics started immediately. Broad-spectrum antibiotics should have been initiated according to institutional guidelines, and should have covered major gram-negative pathogens, as well as alpha hemolytic streptococcus and staphylococci.

The persistence of fever during broad spectrum antibiotic coverage or the emergence of a new fever in neutropenic patients with negative blood cultures warrants the initiation of IV antifungal treatment, unless other causes are apparent.

Colony Stimulating Factors

G-CSF should have only be administrated in case of serious or life-threatening febrile neutropenia.

Highly effective methods of contraception

Male and female patients of child-bearing potential must have agreed to use an *highly* effective method of contraception approved by the investigator during the study and for 90 days after the last dose of azacitidine. *Highly* effective methods of contraception include (but not exclusively) the following contraceptive methods:

- combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation
- progestogen-only hormonal contraception associated with inhibition of ovulation
- intrauterine device (IUD)
- intrauterine hormone-releasing system (IUS)

- sexual abstinence.

Nutrition
Active measures should have been used to prevent weight loss of greater than 10% of pre-illness body weight. If possible, enteral feedings were preferred to parenteral.

Concomitant Therapy
No concomitant chemotherapy or other investigational therapy was allowed during the study other than prescribed in this protocol. If such therapy was needed per investigator discretion the patient should go off study.

Use of alternative medications (e.g. herbal or botanical) was not permitted during the entire study period.

9.4.8 Treatment compliance
Not applicable

9.5 Efficacy and Safety Variables

9.5.1 Efficacy and safety measurements assessed and flow chart
Adverse Event Monitoring and Reporting
The Site Investigator was responsible for monitoring the safety of patients who enroll in the study. All AEs occurring after any administration of azacitidine were followed until End of Treatment or resolution or to return to base-line values. If drug-related AEs were present at End of Treatment, early follow-up visits were required at a maximum interval of 4 weeks until all such AEs resolve to baseline or CTC Grade ≤ 1, or were deemed irreversible. The descriptions and grading scales found in the revised CTCAE version 4.0 were be used for adverse event reporting. A copy of the CTCAE version 4.0 can be downloaded from the CTEP website (http://ctep.cancer.gov/reporting/ctc.html).
Adverse events, as well as serious adverse events should have been reported until 30 days after the last administration of study medication, or until another treatment regimen was started, whichever occured first. However, in the case of AEs occurring later than this deadline but which were considered related to the study medication by the principle investigator, such AEs still need to have been reported.

Reporting Serious Adverse Events
All SAEs occurring during the study or within 30 days of the last administration of azacitidine treatment must have been reported to the coordinating investigators / sponsor within 24 hours of occurrence.

Reporting Requirements
Each AE was assessed to determine if it meets the criteria for SAEs. If an SAE occured, expedited reporting would follow local and international regulations, as appropriate.

Serious Adverse Event Reporting Requirements
All SAEs occurring during the study or within 30 days of the last administration of azacitidine treatment were reported to the coordinating investigators / sponsor. If an SAE occured, the sponsor Erasmus MC was notified within 24 hours of Investigator awareness of the event. In particular, if the SAE was fatal or life threatening, notification to the sponsor was made immediately, irrespective of the extent of available AE information. This timeframe also applies to additional new information (follow up) on previously forwarded SAE reports as well as to the initial and follow up reporting of exposure during pregnancy, exposure via breastfeeding, and occupational exposure cases.

For all SAEs, the Investigator was obligated to pursue and provide information to the sponsor in accordance with the timeframes for reporting specified above. In addition, an Investigator may have been requested by the sponsor to obtain specific additional follow up information in an expedited fashion. This information collected for SAEs was more detailed than that captured on the AE CRF. In general, this included a description of the AE in sufficient detail to allow for a complete medical assessment of the case and independent determination of possible causality. Information on other possible causes of the event, such as concomitant medications, vaccines, and/or illnesses should have been provided. In the case of a patient death, a summary of available autopsy findings must have been submitted as soon as possible to the sponsor or its designated representative.

Non Serious Adverse Event Reporting Requirements

All AEs were reported on the AE page(s) of the CRF. It should be noted that the form for collection of SAE information is not the same as the AE CRF. Where the same data are collected, the forms must be completed in a consistent manner. For example, the same AE term should have been used on both forms. AEs should have been reported using concise medical terminology on the CRFs as well as on the form for collection of SAE information.

Suspected Unexpected Serious Adverse Reaction Reporting Requirements

Suspected Unexpected Serious Adverse Reaction (SUSAR) is defined as a suspected Adverse Reaction that occurs in the trial and that is both unexpected and serious.
Suspected adverse reactions (AR) are those AEs of which a reasonable causal relationship to any dose administered of the investigational medicinal product and the event is suspected.
Unexpected adverse reactions are adverse reactions, of which the nature, or severity, is not consistent with the applicable product information (e.g. Investigator's Brochure for an unapproved IMP or SmPC for an authorized medicinal product). For this study the IB of Vidaza was used to assign expectedness. If a previously reported non-serious adverse reaction occured as a serious reaction, the reaction would been considered to be unexpected and reported as a SUSAR. Each fatal reaction was also reported as a SUSAR if not reported as fatal in the IB before.

Timelines for SUSAR reporting are as follows:

• Initial fatal or life-threatening SUSARs will be reported to the competent authorities as soon as possible but no later than 7 calendar days from initial receipt of SAE by Sponsor. A completed follow-up will be submitted within an additional 8 calendar days;
• All other SUSARs will be reported to the competent authorities as soon as possible but no later than 15 calendar days from initial receipt of SAE by Sponsor;
• SUSAR's will also be reported to regulatory authorities in accordance with applicable local regulations, within the same timelines;
• SUSAR's originating from other countries will be reported to the ethics committee and investigators via yearly DSUR reports.

An overview of all measurements are provided in Annex IIIa and Annex IIIb

9.5.2 Appropriateness of measurements

Screening measures conducted prior to enrollment constitute a standard battery of tests designed to thoroughly examine the potential subject for any medical issues.

9.5.3 Primary efficacy variable(s)

Preliminary Efficacy

• The primary efficacy variable is the overall response rate in the 2 strata, defined as the number of patients with either a CR or PR, as defined in paragraph 6.7 of the study protocol, over the total number of patients evaluable for the analysis. Patients discontinuation from study treatment without a disease assessment but with the cause for treatment discontinuation being disease progression were considered as having a disease progression for this analysis.

• Cytogenetic/Molecular Response Rate is defined as the proportion of patients achieving a cytogenetic (MDS) / molecular (JMML) response, i.e. number of responders over the number of patients included in the EE analysis population. The corresponding Clopper-Pearson 95% confidence interval shall be presented per disease strata and the per disease phase.

• Stem Cell Transplantation (HSCT) rate is calculated as the number of patients undergoing a HSCT post study drug administration over the number of subjects in the 1) the EE population, 2) the safety population. The corresponding Clopper-Pearson 95% confidence interval shall be presented per disease strata and per disease phase.

• Duration of Response (DoR) is defined as the time from first observed response until first observed disease progression thereafter. Only patients observed with a response will be included in the analysis of DoR. Due to the anticipated low number of patients expected to be observed as having a response, the median DoR time shall be calculated by the median time per patient who is responding, and also by means of the Kaplan-Meier method if more than 5 patients respond in any stratum. Median, minimum and maximum values were reported as well as the corresponding 95% confidence interval of the median per disease strata and per treatment phase.

• Progression Free Survival (PFS) is defined as the time elapsed from first study dose administration until first observed disease progression or death from any cause, whichever occurs first. Patients without an event are censored at the last evaluation date. The median OS time was be calculated using the Kaplan-Meier method. Median, minimum and maximum values will be reported as well as the corresponding 95% confidence interval of the median per disease strata and per treatment phase.

• Overall Survival (OS) is defined as the date from first study dose administration until death from any cause. Patients without an event are censored at the last evaluation date. The median OS time was calculated using the Kaplan-Meier method. Median, minimum and maximum values were reported as well as the corresponding 95% confidence interval of the median per disease strata and per treatment phase.

Pharmacokinetics

Pharmacokinetic parameters of azacitidine was calculated from plasma concentration-time profiles using non-compartmental methods, though compartmental analysis may be employed if appropriate. Plasma PK parameters includes, but is not limited to:

• Cmax: observed maximum plasma concentration

• Tmax: observed time to maximum plasma concentration

• AUC0-t: area under the plasma concentration time curve from time zero to the last quantifiable time point, calculated by the linear trapezoidal rule

• AUC0-∞: area under the plasma concentration time curve from time zero to infinity, calculated by the linear trapezoidal rule and extrapolated to infinity will be calculated according to the following equation: AUC0-∞ = AUC0 t + (Ct/λz), where Ct is the last quantifiable concentration

• λz: terminal phase rate constant, determined by linear regression of the terminal points of the log-linear plasma-concentration-time curve

• t½: terminal phase half-life, will be calculated according to the following equation: t½ = 0.693/λz

• CL: total clearance, calculated as Dose/AUC0-∞

• Vd: volume of distribution will be calculated according to the equation: Vd = (CL)/λz

By-subject listing of pharmacokinetic blood sample collection times, derived sampling time deviations, and PK parameters are be provided. Azacitidine plasma concentrations and resulting PK parameters are summarized using descriptive statistics (N, arithmetic mean, standard deviation, minimum, median, maximum, percent coefficient of variation, and geometric mean) for each treatment. Concentrations that are below the limit of quantitation (BLQ) were be treated as zero for the computation of descriptive statistics and listed with the lower limit of quantitation (LLQ) indicated. Missing concentrations will be omitted from the calculation of descriptive statistics.

Figures of mean azacitidine concentration-time data was illustrated for each treatment. Individual azacitidine subject concentration-time data for each treatment is graphically presented on linear and semi-logarithmic scales.

9.5.4 Drug concentration measurements

Summary of PK data

10 subjects worth of data were analyzed by route of administration, IV or subcutaneous (SC). Subject 9 has unusually high azacitidine concentrations and exposure parameters (Cmax more than 10-fold higher than highest Cmax observed in the other subjects and AUC more than 35-fold higher than highest AUC value observed in the other subjects) therefore summaries are provided excluding subject 9.

Table 1. IV PK Parameters by Individual

Subject	AUCt (hr*ng/ml)	AUC∞ (hr*ng/ml)	Cmax (ng/ml)	T1/2 (hr)	Tmax (hr)	CLss (L/hr)	Vz (L)
1	744.95	749.79	3310.00	0.33	0.08	147.10	69.09
4	272.27	273.55	1050.00	0.28	0.08	174.30	71.21
5	543.82	544.77	2300.00	0.50	0.08	165.10	118.81
6*	518.35	521.99	1620.00	0.29	0.08	148.01	62.41
7	708.74	NC	2270.00	NC	0.08	NC	NC
9**	26764.98	26768.70	42900.00	0.52	0.50	5.23	3.93
10	286.93	288.69	972.00	0.29	0.08	261.04	108.92

AUC∞ = area under the plasma concentration time curve from time zero to infinity; AUCt = area under the concentration time curve from time zero to the last quantifiable time point; Cmax = observed maximum plasma concentration; CLss = total clearance; t½ = terminal phase half-life; tmax = observed time to maximum plasma concentration; Vz = of distribution.

*Subject 6 had samples analyzed ~30 days outside of stability (Note: stability period = 980 days)

**Infusion duration was 48 minutes relative to 15 min per protocol

Table 2. SC PK Parameters by Individual

Subject	AUCt (hr*ng/ml)	AUC∞ (hr*ng/ml)	Cmax (ng/ml)	T1/2 (hr)	Tmax (hr)	CLss/F (L/hr)	Vz/F (L)
2	910.53	913.31	1430.00	0.77	0.08	45.44	50.35
3	1040.47	1041.60	1630.00	0.42	0.08	50.85	30.96
8	698.75	700.02	729.00	0.42	0.08	191.20	116.83

AUC∞ = area under the plasma concentration time curve from time zero to infinity; AUCt = area under the concentration time curve from time zero to the last quantifiable time point; Cmax = observed maximum plasma concentration; CL/F = apparent total clearance; t½ = terminal phase half-life; tmax = observed time to maximum plasma concentration; Vz /F = apparent volume of distribution.

Figure 1. Linear scale Concentration-time plots by individual and administration route

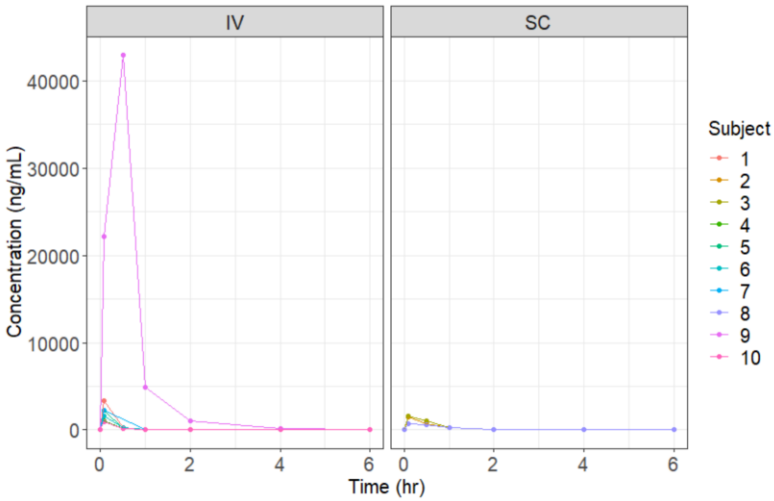


Figure 2. Log scale Concentration-time plots by individual and administration route

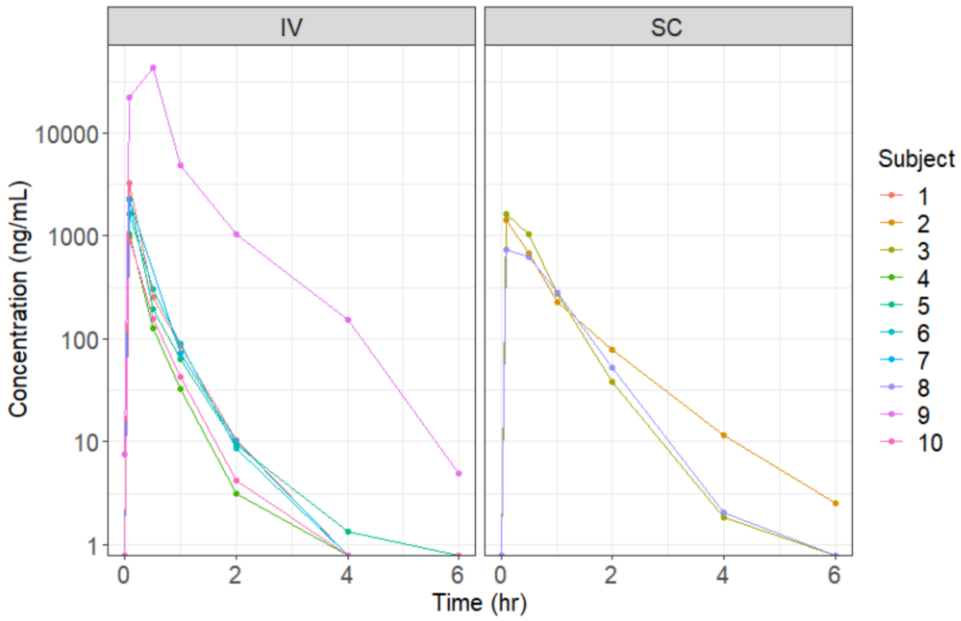


Table 3. Summary of IV Subjects (excluding subject 9)

Variable	N	Mean	SD	CV%	Min	Median	Max	Geometric Mean	Geometric CV%
AUC∞ (hr*ng/ml)	5	475.76	198.67	41.76	273.55	521.99	749.79	441.83	45.82
AUCt (hr*ng/ml)	6	512.51	201.07	39.23	272.27	531.08	744.95	475.97	45.81
CLss (L/hr)	5	179.11	47.23	26.37	147.10	165.10	261.04	174.88	23.86
Cmax (ng/mL)	6	1920.33	888.21	46.25	972.00	1945.00	3310.00	1748.47	51.13
T1/2 (hr)	5	0.34	0.09	27.09	0.28	0.29	0.50	0.33	24.13
Tmax (hr)	6	0.08	0.00	0.00	0.08	0.08	0.08	0.08	0.00
Vz (L)	5	86.09	25.80	29.97	62.41	71.21	118.81	83.15	29.83

AUC∞ = area under the plasma concentration time curve from time zero to infinity; AUCt = area under the concentration time curve from time zero to the last quantifiable time point; Cmax = observed maximum plasma concentration; CV% = percentage coefficient of variation; CL = total clearance; Max = maximum; Min = minimum; NA = not applicable; SD = standard deviation; t½ = terminal phase half-life; tmax = observed time to maximum plasma concentration; Vz = volume of distribution.

Table 4. Summary Statistics for SC administration

Variable	N	Mean	SD	CV%	Min	Median	Max	Geometric Mean	Geometric CV%
AUC∞ (hr*ng/ml)	3	884.98	172.55	19.50	700.02	913.31	1041.60	873.26	20.46
AUCt (hr*ng/ml)	3	883.25	172.49	19.53	698.75	910.53	1040.47	871.53	20.47
CLss/F (L/hr)	3	95.83	82.64	86.24	45.44	50.85	191.20	76.16	94.55
Cmax (ng/mL)	3	1263.00	473.15	37.46	729.00	1430.00	1630.00	1193.30	45.27
T1/2 (hr)	3	0.54	0.20	37.05	0.42	0.42	0.77	0.52	35.51
Tmax (hr)	3	0.08	0.00	0.00	0.08	0.08	0.08	0.08	0.00
Vz/F (L)	3	66.05	45.03	68.19	30.96	50.35	116.83	56.68	75.53

AUC∞ = area under the plasma concentration time curve from time zero to infinity; AUCt = area under the concentration time curve from time zero to the last quantifiable time point; Cmax = observed maximum plasma concentration; CV% = percentage coefficient of variation; CL/F = apparent total clearance; Max = maximum; Min = minimum; NA = not applicable; SD = standard deviation; t½ = terminal phase half-life; tmax = observed time to maximum plasma concentration; Vz/F = apparent volume of distribution.

Figure 3. Linear IV Mean (± SD) Concentration vs Time Profile (excluding Subject 9)

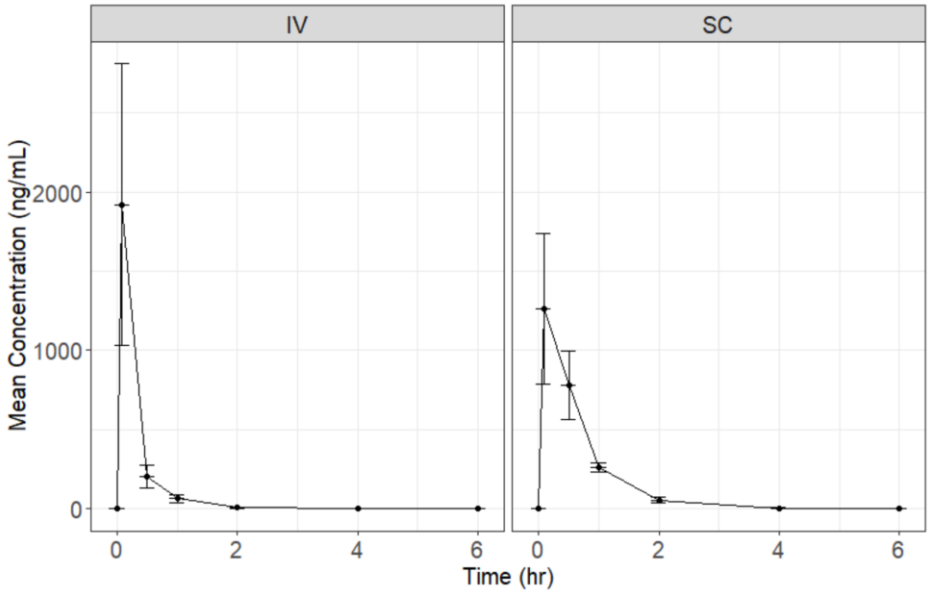
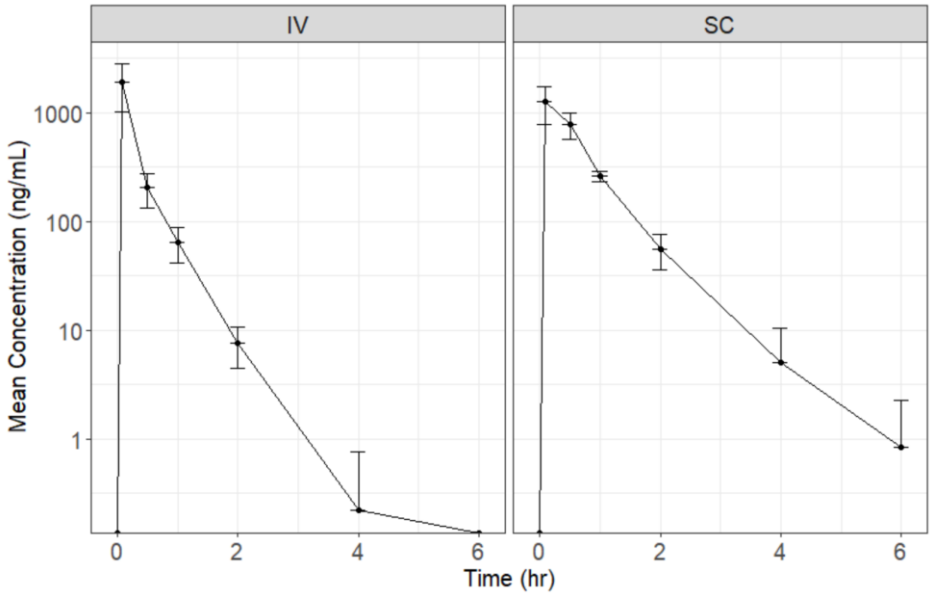


Figure 4. Log IV Mean (± SD) Concentration vs Time Profile (excluding Subject 9)



Pharmacokinetics of azacitidine in Adult Patients with MDS

In the first adult studies azacitidine was administered using continuous intravenous (IV) infusion (Silverman *et al*, 1993). Later studies showed that subcutaneous (SC) administration was as effective (Kaminskas *et al*, 2005). The bio-availability of azacitidine was somewhat lower after SC administration when compared with a 10-minute duration IV administration (89%, range 70-112%) (Marcucci *et al*, 2005).

Vidaza pharmakokinetics was assessed in a study with 6 adult MDS patients with normal renal function. The maximum plasma azacitidine concentration after SC administration (mean peak plasma was 750.0 ng/mL (± 403.3)) was observed in 0.5 hour. After IV infusion mean maximum plasma concentrations were approximately 4-fold higher (2750.0 ng/mL (± 1069.0)). Apparent clearance after SC dosing was 167.48 (± 48.69) L/h compared with a clearance after IV dosing of 146.70 L/h (± 46.91). These clearance rates far exceed the glomerular filtration rate, which suggests that non-renal elimination plays a role in the elimination of azacitidine.

Mean SC half-life time was 0.69 ± 0.14 hours, which is approximately 41 minutes, compared to approximately 22 (± 1) minutes for half-life time after IV administration (0.36 ± 0.02 hours). SC half-life times were approximately 2-fold greater than IV half-life times.

$AUC_{(0 \rightarrow \infty)}$ values were measured for both SC and IV administration, and were respectively 960.53 ng·h/ml (± 458.06) and 1044.26 ng·h/ml (± 285.67) (Marcucci *et al*, 2005).

Table 5. Adult PK variables for SC and IV administration of 75 mg/m2 azacitidine.

Route	C_{max} ng/mL	$AUC_{(0 \rightarrow \infty)}$ ng·h/ml	$AUC_{(0 \rightarrow z)}$ ng·h/ml	$T_{1/2}$ h	CL L/h
SC	750.0 (± 403.3)	960.53 (± 458.06)	923.88 (± 473.61)	0.69 (± 0.14)	167.48 (± 48.69)
IV	2750.0 (± 1069.0)	1044.26 (± 285.67)	1025.11 (± 298.06)	0.36 (± 0.02)	146.70 L/h (± 46.91)

C_{max}: maximum concentration, $AUC_{(0 \rightarrow \infty)}$: area under the plasma concentration time curve from 0 to infinity, $AUC_{(0 \rightarrow z)}$: area under the plasma concentration time curve from 0 to the last measurable time point, $T_{1/2}$: half-time, CL: apparent clearance

The PK behavior of azacitidine in pediatric patients was similar to adult PK.

9.6 Statistical Methods Planned in the Protocol and Determination of Sample Size

9.7.1 Statistical and analytical plans

The primary statistical analysis (SAP) plan can be found in Annex IV

9.6.2 Determination of sample size

The study has incorporated a two-stage design per disease stratum. The JMML arm will had an option safety run-in cohort consisting of 3 patients. Per stratum, stage one and stage two will each consist of a cohort of at most 6 patients. This means a total of up to 12 patients should have been enrolled to the JMML disease indication and up to 12 patients in the MDS disease indication, i.e. a maximum of 24 patients in total. Considering that some patients may not have been available for evaluation, a maximum of 28 patients was anticipated.

9.7 Changes in the Conduct of the Study or Planned Analyses

Changes to the conduct of the study were made in three separate study amendments. Details per amendment are provided in the table below.

Table 6. Study amendments

Amendment no	Date	Summary of amendment
1.1	07-03-2012	Major changes: • Due to the limited stability of azacitidine when reconstituted for intraveneous administration the principle investigator may choose to administer azacitidine intravenously or subcutaneously. Method of administration should be fixed per patient and per partcipating site. • New version of the SPC allows the reconstituted azacitidine for SC adminstration to be kept refrigerated up till 22 hrs at 2-8 centigrade Celsius when reconstituted with refrigerated water. • Additionally, for MDS patients an extra BM and PB sample will be taken at day 28 of the first course to be able to evaluate the pharmacodynamic analysis. This time point was previously not incorporated in the protocol by mistake.
1.2	12-09-2012	Major changes involving: • Additional definitions of MDS (primary and secondary). Addition of stratum 5: newly diagnosed or relapsed patients with secondary advanced MDS, occurring after chemotherapy, radiotherapy and or stem-cell transplantation, or secondary cases after prior treatment for aplastic anemia. • Additional text on a 1 week wash-out period for 6-MP or low-dose cytarabine in JMML patients. • Addition of two exclusion criteria: JMML patients in whom a diagnosis of Noonan syndrome is suspected based on clinical history and/or presenting symptoms and patients with secondary MDS with underlying bone-marrow failure syndromes or with familial MDS. • Changes in the SC and IV administration and time-lines between dissolving and administration. • Adding a statement that stability for azacitidine has not been confirmed for longer storage times, and hence no delayed administration is allowed. • Dose level and interval was changed and specified. One course is defined as 7 days of azacitdine, which is repeated once every 28 days. <u>For children ≥1 year of age and ≥10 kg body weight:</u> Addition of level -1 (dose reduction): 50 mg/m^2/day IV/SC x 7 days, one course every 28 days <u>For children<1 year of age or <10 kg body weight:</u>

		Addition of level -1 (dose reduction): 1,7 mg/kg/day IV/SC x 7 days, one course every 28 days
1.3	13-02-2017	Major changes involving: • IV administration and additional clarification on time-lines between dissolving and administration • Removal of newly diagnosed and secondary MDS stratum as included in company sponsored studies by Celgene • Revision of trial methodology (adding a formal design), and sample size calculation • Adding language for highly effective methods of contraception. Male and female patients of child-bearing potential must agree to use an *highly* effective method of contraception approved by the investigator during the study and for 90 days after the last dose of azacitidine. • Administrative changes, including adding a new statistician and institute change of one of the Coordinating Investigators • Implementing information from recent IB releases • Adapting study time-lines • Relapse definitions for JMML and MDS were added • Updated literature review
1.4	27-7-2018	• Clarification on reconstitution of vidaza for IV administration • Administrative changes

Additionally, during the whole study period the inclusion rate was very low and in recent years the inclusion rate dropped even further. Especially for JMML patients a strong decline of inclusion was noticed, with a 5 year time period between the last two included JMML patients.

Possible explanations for the lack of inclusion of JMML patients are that the outcomes after transplantation for primary JMML patients have improved. As a result, fewer patients were available than anticipated when setting up the study. In addition, azacitidine is also freely available and commonly used for patients with JMML. Patients will therefore be less likely to travel to a study center to participate in the study.

As the results of the AZA-JMML-001 study presented at the ASCO 2019 showed that monotherapy with azacitidine was well tolerated in newly diagnosed JMML patients we found that the clinical equipoise is decreased to a level that the investment (time and costs) to include the remaining JMML patients is too large.

It was therefore decided, in consultation with the PIs, to stop the study prematurely on the 12th of February 2020. This had consequences for the analyses which will now be mostly descriptive.

10. STUDY PATIENTS

10.1 Disposition of Patients

A total of 10 patients (6 MDS/ 4 JMML) signed informed consent forms and were included in the study

Table 7: Basic characteristics of patients

Patient ID	Stratum	first diagnosis	Sex	Age at Reg (Years)	Weight(kg)	Height(cm)	Spleen Size (cm)	Prior SCT	date of relapse	Type of sct
1	MDS	2011-11-17	Female	14.2	48.1	159.0	0	Yes	2013-05-06	Matched Unrelated Donor
2	JMML	2011-08-03	Male	3.1	12.7	87.0	11	Yes	2013-07-07	Other
3	JMML	2012-12-27	Male	4.8	16.8	108.0	0	Yes	2013-11-20	Matched Unrelated Donor
4	JMML	2012-11-17	Male	3.2	15.9	96.0	4	Yes	2014-03-05	Matched Unrelated Donor
5	MDS	2013-08-12	Male	14.4	34.0	147.0	0	Yes	2014-08-28	Matched Unrelated Donor
6	MDS	2015-04-17	Female	9.7	28.8	133.5	0	Yes	2015-11-17	Other
7	MDS	2014-01-15	Female	18.2	58.0	161.8	2	Yes	2016-02-09	Matched Unrelated Donor
8	MDS	2015-08-15	Male	15.2	64.0	181.0	0	Yes	2016-05-19	Matched Unrelated Donor
9	MDS	2016-08-19	Male	11.5	77.7	165.6	0	Yes	2018-03-09	Haplo-identical donor
10	JMML	2014-09-05	Male	7.3	28.8	129.0	0	Yes	2019-03-08	Matched Unrelated Donor

10.2 Protocol Deviations

There were 2 patients with protocol deviations: For one patient there was a fault in the formula for the dose on the label due to which half of the dose was noted on the label (i.e. 70 mg instead of 140 mg). The patient was however dosed correctly (14 ml azacitidine 10 mg/ml) had been added to the infusion bag. One patient did not meet all the exclusion criteria. However, due to the rarity of the disease and several clinical features it was concluded that the patients had an early stage of relapsed JMML. It was therefore decided to include the patient in the trial.

Both were considered a minor protocol deviation, and patients were not excluded from the analyses.

11. EFFICACY EVALUATION

11.1 Data Sets Analysed

Full Analysis Set (FAS)

The Full Analysis Set (FAS) consists of all enrolled patients, regardless of whether they received study therapy. Tabulations of subject disposition, analysis subsets, demographic and baseline characteristics, medical history, concomitant medication and protocol deviations will be based on this analysis set.

Efficacy-Evaluable Analysis Set (EEAS)
The Efficacy Evaluable Analysis Set (EEAS) consists of all patients in the FAS receiving at least one dose of study therapy and having at least one post-baseline assessment. Assessment of all efficacy-based endpoints in both phase I and phase II will be performed on the EEAS.

Safety Analysis Set
The Safety Analysis Set will include all subjects in the FAS who received at least one dose of study medication and had any safety data collected after enrollment. This set will be used for the analysis of safety endpoints.

11.2 Demographic and Other Baseline Characteristics

Among all 10 patients recruited for this study by 16NOV2020, 6 belong to the MDS stratum (patient IDs 1, 5, 6, 7, 8 and 9) and 4 patients belong to the JMML stratum (patient IDs 2, 3, 4, 10). All patients are included in the FAS, EEAS and Safety Set, since they all received at least one dose of study medication and had at least one post-baseline assessment.

Patient baseline characteristics
Median follow-up is 8.76 months (interquartile range 4.09 - 9.57) in the MDS stratum, and 14.7 months (interquartile range 6.59 - 14.42) in the JMML stratum. Further details are provided in paragraph 14.1

11.3 Measurements of Treatment Compliance

All patients received the prescribed dosage per course as the drug was given either IV or SC.

As in adult MDS patients the median time to respond to azacitidine was 3 months, and because this complies with the usual preparation time for HSCT the protocol prescribes that In all patients, every effort should be made to transplant patients only after having received at least 3 courses of azacitidine (hence ~3 months of pre-HSCT treatment). Two patients (Patient ID 5 and 6) received only 2 courses (Table 8) due to complications. However, both patients are evaluable as they received at least one course of study treatment and had at least one on-treatment efficacy evaluation performed.

11.4 Efficacy Results and Tabulations of Individual Patient Data

11.4.1 Analysis of efficacy

Of the 6 MDS patients 5 had stable disease and 1 progressive disease. Of the 4 JMML patients 1 had stable disease, 1 patient a partial response and 2 patients a complete response. In some cases there was a discrepancy between the reported responses based on lab values from the central lab (Table 9 and 12) and responses based on lab values reported within the participating centers (Table 10 and 13). As lab values from the participating center were used for guidance in the treatment of these patients (Due to the delay of several weeks in the reporting of the central lab values) we chose to use the response data from Table 10 and 13 for evaluation purposes. Final conclusions are presented in Table 11 and 14

|For further details see paragraph 14.2

11.4.2 Statistical/analytical issues

11.4.2.1 Adjustments for Covariates

Not applicable

11.4.2.2 Handling of Dropouts or Missing Data
No imputation technique, such as last observation carried forward (LOCF), was used. As this report is primarily descriptive. Data is reported as available.

11.4.2.3 Interim Analyses and Data Monitoring
Data was fully monitored. No formal interim analysis was performed

11.4.2.4 Multicentre Studies
Due to the small sample size used in this study, results by center are not reported.

11.4.2.5 Multiple Comparisons/Multiplicity
Not applicable

11.4.2.6 Use of an "Efficacy Subset" of Patients
Not applicable

11.4.2.7 Active-Control Studies Intended to Show Equivalence
Not applicable

11.4.2.8 Examination of Subgroups
Patients were only stratified according to their primary pathology, i.e., MDS or JMML

11.4.3 Tabulation of individual response data
See paragraph 14.2

11.4.4 Drug dose, drug concentration, and relationships to response
Not applicable

11.4.5 Drug-drug and drug-disease interactions
Not applicable

11.4.6 By-patient displays
See paragraph 14.2

11.4.7 Efficacy conclusions
In general, azacitidine was well tolerated in pediatric patients. The ORR in the MDS patients was not satisfactory.

Looking at the whole study period the inclusion rate was very low. In recent years the inclusion rate dropped even further. Especially for JMML patients a strong decline of inclusion was noticed, with a 5 year time period between the last two included JMML patients. Possible explanations for the lack of inclusion of JMML patients are that the outcomes after transplantation for primary JMML patients have improved. As a result, fewer patients are available than anticipated when setting up the study. In addition, azacitidine is also freely available and commonly used for patients with JMML. Patients will therefore be less likely to travel to a study center to participate in the study.

As the results of the AZA-JMML-001 (NCT02447666) study presented at the ASCO 2019 showed that monotherapy with azacitidine was well tolerated in newly diagnosed JMML patients we found that the clinical equipoise was decreased to a level that the investment (time and costs) to include the remaining JMML patients was too large. It was therefore decided, in consultation with the PIs, to stop the study prematurely on the 12th of February 2020. However, for the 4 included JMML patients we see signs of activity. The PK behavior of azacitidine was similar to adult PK.

12. SAFETY EVALUATION

12.1 Extent of Exposure

Table 8. dosing details per patient

Patient ID	Stratum	Planned daily dose per course	Cumulative dose	Daily administered dose per course
1	MDS	1= 75 mg/m2/day or 2.5 mg/kg/day mg/day, IV	C1 : 777.7 mg IV / C2 : 763 mg IV / C3 : 766.5 mg IV	C1: 111.1mg / C2: 109mg / C3: 109.5mg
2	JMML	1= 75 mg/m2/day or 2.5 mg/kg/day mg/day, SC	C1 : 290.5 mg SC / C2 : 378 mg SC / C3 : 378 mg SC	C1: 41.5mg / C2: 54mg / C3: 54mg
3	JMML	1= 75 mg/m2/day or 2.5 mg/kg/day mg/day, SC	C1 : 371 mg SC / C2 : 364 mg SC / C3 : 364 mg SC / C4 : 364 mg SC / C5 : 371 mg SC	C1: 53mg / C2: 52mg / C3: 52mg / C4: 52mg / C5: 53mg
4	JMML	1= 75 mg/m2/day or 2.5 mg/kg/day mg/day, IV	C1 : 336 mg IV / C2 : 336 mg IV / C3 : 350 mg IV / C4 : 350 mg IV / C5 : 350 mg IV / C6 : 350 mg IV	C1: 48mg / C2: 48mg / C3: 50mg / C4: 50mg / C5: 50mg / C6: 50mg
5	MDS	1= 75 mg/m2/day or 2.5 mg/kg/day mg/day, IV	C1 : 630 mg IV / C2 : 630 mg IV	C1: 90mg / C2: 90mg
6	MDS	1= 75 mg/m2/day or 2.5 mg/kg/day mg/day, IV	C1 : 546 mg IV / C2 : 546 mg IV	C1: 78mg / C2: 78mg
7	MDS	1= 75 mg/m2/day or 2.5 mg/kg/day mg/day, IV	C1 : 840 mg IV / C2 : 875 mg IV / C3 : 875 mg IV	C1: 120mg / C2: 125mg / C3: 125mg
8	MDS	1= 75 mg/m2/day or 2.5 mg/kg/day mg/day, SC	C1 : 938 mg SC / C2 : 902 mg SC / C3 : 882 mg SC	C1: 134mg / C2: 134mg / C3: 126mg
9	MDS	1= 75 mg/m2/day or 2.5 mg/kg/day mg/day, IV	C1 : 980 mg IV / C2 : 980 mg IV / C3 : 980 mg IV / EOS : 980 mg IV	C1: 140mg / C2: 140mg / C3: 140mg / EOS: 140mg
10	JMML	1= 75 mg/m2/day or 2.5 mg/kg/day mg/day, IV	C1 : 532 mg IV / C2 : 532 mg IV / C3 : 518 mg IV / C4 : 518 mg IV / C5 : 518 mg IV	C1: 76mg / C2: 76mg / C3: 74mg / C4: 74mg / C5: 74mg

12.2 Adverse Events (AEs)

12.2.1 Brief summary of adverse events

See paragraph 14.3.2.1

12.2.2 Display of adverse events

See paragraph 14.3.2.1

12.2.3 Analysis of adverse events

See paragraph 14.3.2.1

12.2.4 Listing of adverse events by patient

See paragraph 14.3.2.1

12.3 Deaths, Other Serious Adverse Events, and Other Significant Adverse Events

12.3.1 Listing of deaths, other serious adverse events, and other significant adverse events

12.3.1.1 Deaths

See paragraph 14.3.2.2

12.3.1.2 Other Serious Adverse Events

See paragraph 14.3.2.1

12.3.1.3 Other Significant Adverse Events

See paragraph 14.3.2.1

12.3.2 Narratives of deaths, other serious adverse events, and certain other significant adverse events

See paragraph 14.3.2

12.3.3 Analysis and discussion of deaths, other serious adverse events, and other significant adverse events

Not applicable

12.4 Clinical Laboratory Evaluation

12.4.1 Listing of individual laboratory measurements by patient (16.2.8) and each abnormal laboratory value (14.3.4)

Not applicable

12.4.2 Evaluation of each laboratory parameter

Not applicable

12.4.2.1 Laboratory Values Over Time

Not applicable

12.4.2.2 Individual Patient Changes

Not applicable

12.4.2.3 Individual Clinically Significant Abnormalities

Not applicable

12.5 Vital Signs, Physical Findings, and Other Observations Related to Safety

Not applicable

12.6 Safety Conclusions

In general, azacitidine was well tolerated in pediatric patients.

13. DISCUSSION AND OVERALL CONCLUSIONS

In general, azacitidine was well tolerated in pediatric patients. Unfortunately we found no efficacy for MDS patients. However, there are signs of activity in JMML patients. Due to the low number of inclusion we cannot conclude the efficacy, however the signs of activity are in line with the results of the AZA-JMML-001 (NCT02447666) study in newly diagnosed patients. As SCT efficiency has gone up over the years the number of available patients in this study was low and the recruitment below expectations, which led to termination of the study.

14. TABLES, FIGURES AND GRAPHS REFERRED TO BUT NOT INCLUDED IN THE TEXT

14.1 Demographic Data

See table 7.

14.2 Efficacy Data

Table 9: Response listing per course (Central Lab form)

Patient ID	Time evaluation	Overall morph resp	Cytogen resp
1	C2D1	3=SD (Stable Disease)	4=not evaluable
1	EOS	3=SD (Stable Disease)	3=no cytogenetic response
6	C2D1		1= complete cytogenetic response (CCyR)
6	EOS		1= complete cytogenetic response (CCyR)
7	C2D1	3=SD (Stable Disease)	4=not evaluable
7	EOS	3=SD (Stable Disease)	3=no cytogenetic response
8	C2D1	3=SD (Stable Disease)	3=no cytogenetic response
8	EOS	3=SD (Stable Disease)	3=no cytogenetic response
9	C2D1	3=SD (Stable Disease)	4=not evaluable
9	EOS	3=SD (Stable Disease)	4=not evaluable

Table 10: MDS - Response listing per course (Response form)

Patient ID	Time evaluation	Overall resp
1	C2D1	3=SD (Stable Disease)
1	C3D1	3=SD (Stable Disease)
1	EOS	3=SD (Stable Disease)
5	C2D1	4=PD (Progressive disease)
5	EOS	4=PD (Progressive disease)
6	C2D1	3=SD (Stable Disease)
6	EOS	3=SD (Stable Disease)
7	C2D1	3=SD (Stable Disease)
7	C3D1	3=SD (Stable Disease)
7	EOS	3=SD (Stable Disease)
8	C2D1	3=SD (Stable Disease)
8	C3D1	3=SD (Stable Disease)
8	EOS	3=SD (Stable Disease)
9	C2D1	3=SD (Stable Disease)
9	C3D1	
9	EOS	3=SD (Stable Disease)

Table 11: MDS – Best response listing (End of Study form)

Patient ID	Best response on protocol
1	3=SD (Stable Disease)
5	4=PD (Progressive disease)
6	3=SD (Stable Disease)
7	3=SD (Stable Disease)
8	3=SD (Stable Disease)
9	3=SD (Stable Disease)

Table 12: JMML - Response listing per course (Central Lab form)

Patient ID	Time evaluation	Overall morph resp
2	C1D8	1=CR (Complete Response)
2	C2D1	1=CR (Complete Response)
2	C3D1	1=CR (Complete Response)
2	EOS	1=CR (Complete Response)
3	C2D1	2=PR (Partial Response)
3	C3D1	1=CR (Complete Response)
3	C4D1	1=CR (Complete Response)
3	C5D1	1=CR (Complete Response)
3	EOS	1=CR (Complete Response)
4	C4D1	1=CR (Complete Response)
4	C5D1	1=CR (Complete Response)
4	C6D1	1=CR (Complete Response)
4	EOS	1=CR (Complete Response)
10	C2D1	5=Relapse (after an initial response)
10	C3D1	4=PD (Progressive disease)
10	C4D1	2=PR (Partial Response)
10	EOS	2=PR (Partial Response)

Table 13: JMML - Response listing per course (Response form)

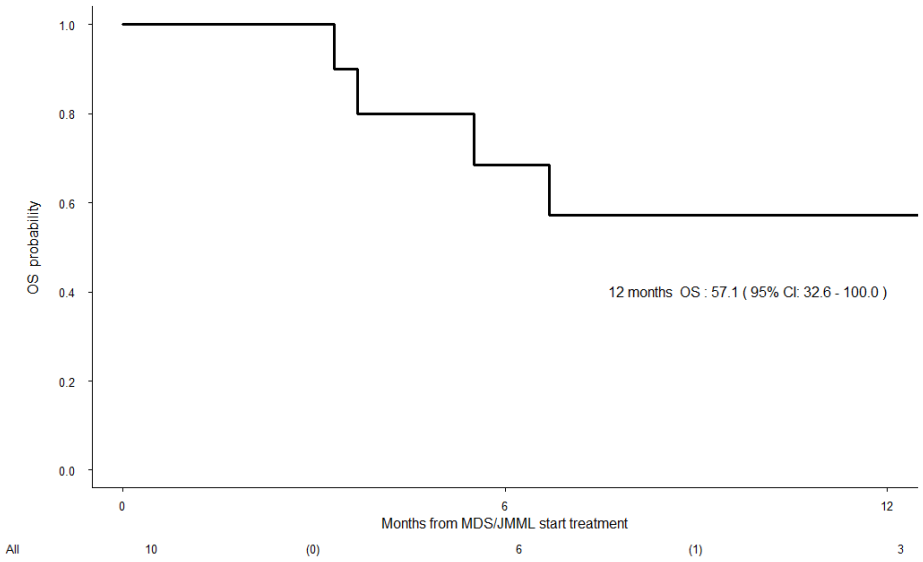
Patient ID	Time evaluation	Overall resp
2	C2D1	3=SD (Stable Disease)
2	C3D1	3=SD (Stable Disease)
2	EOS	3=SD (Stable Disease)
3	C2D1	2=PR (Partial Response)
3	C3D1	2=PR (Partial Response)
3	C4D1	2=PR (Partial Response)
3	C5D1	2=PR (Partial Response)
3	EOS	2=PR (Partial Response)
4	C2D1	1=CR (Complete Response)
4	C3D1	1=CR (Complete Response)
4	C4D1	1=CR (Complete Response)
4	C5D1	1=CR (Complete Response)
4	C6D1	1=CR (Complete Response)
4	EOS	1=CR (Complete Response)
10	C2D1	3=SD (Stable Disease)
10	C3D1	2=PR (Partial Response)
10	C4D1	2=PR (Partial Response)
10	C5D1	1=CR (Complete Response)
10	EOS	1=CR (Complete Response)

Table 14: JMML – Best response listing (End of Study form)

Patient ID	Best response on protocol
2	3=SD (Stable Disease)
3	2=PR (Partial Response)
4	1=CR (Complete Response)
10	1=CR (Complete Response)

Figure 5 (A) Overall Survival (OS) from start of treatment for JMML and MDS patients (B) OS from start of treatment for JMML and MDS patients differentiated to transplantation status.

A



B

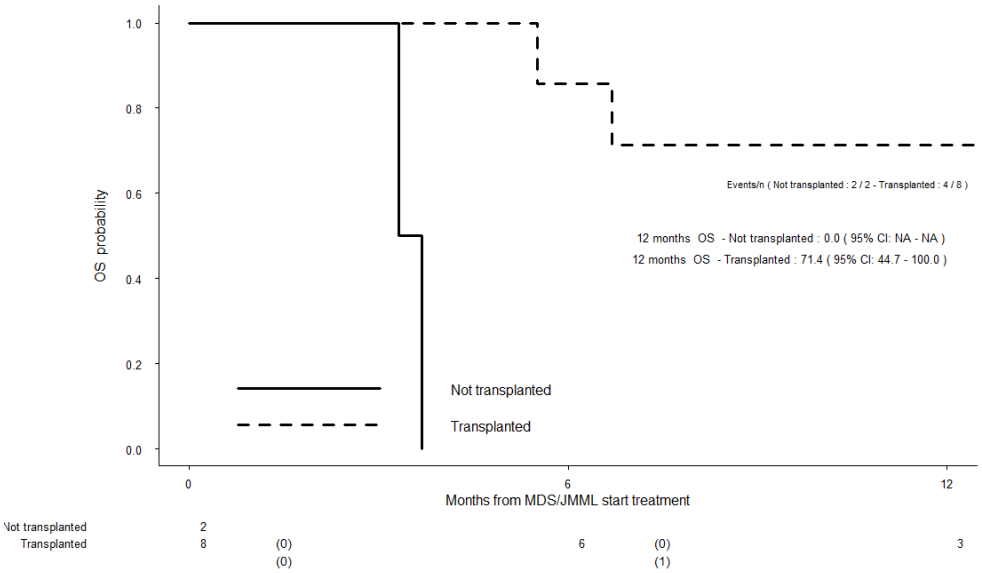
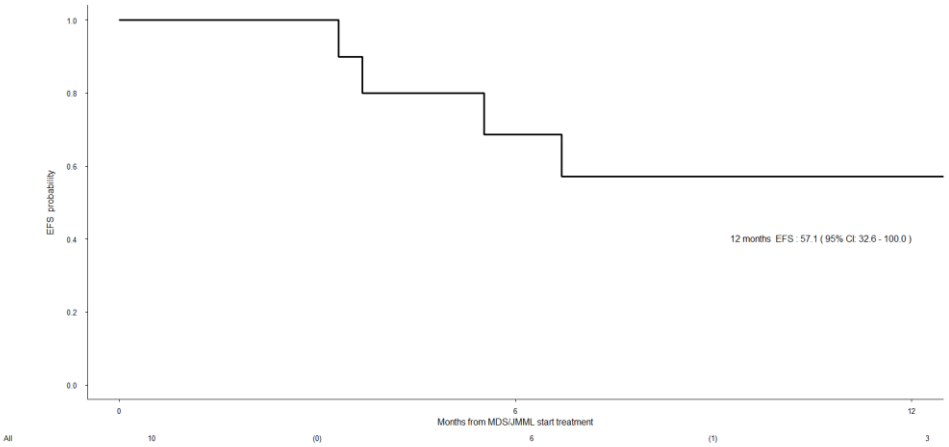
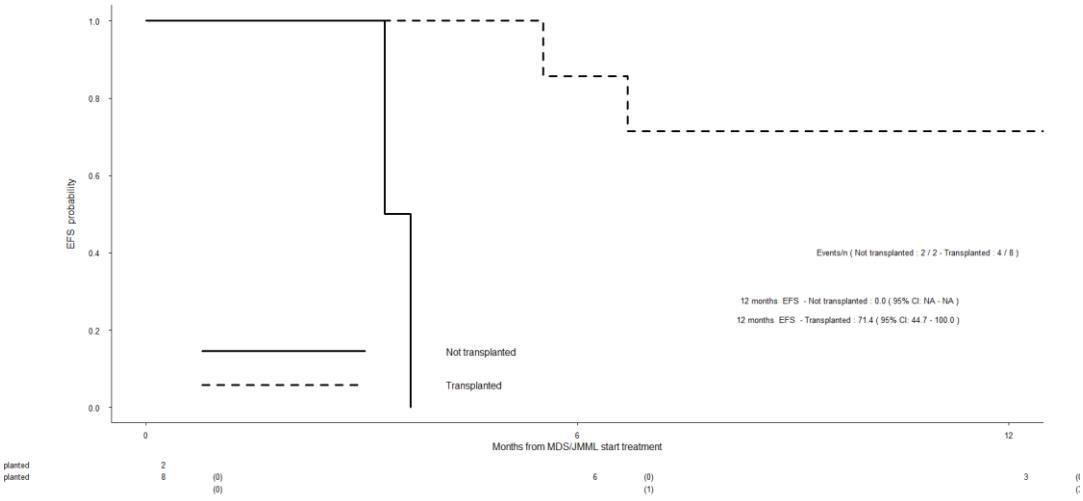


Figure 6 (A) Event Free Survival (EFS) from start of treatment for JMML and MDS patients (B) EFS from start of treatment for JMML and MDS patients differentiated to transplantation status.

A



B



14.3 Safety Data

14.3.1 Displays of adverse events

Table 15: DLT listing (DLT form)

Patient ID	Stratum	DLT occurred?
1	MDS	0=no
2	JMML	0=no
3	JMML	0=no
4	JMML	0=no
5	MDS	0=no
6	MDS	1=yes
7	MDS	0=no
8	MDS	0=no
9	MDS	0=no
10	JMML	0=no

Table 16: DLT listing (AE forms) - only seen in MDS stratum

Patient ID	DLT occurred?	Relation to medic	Outcome	SAE	Treatment change	AE term	SOC	AE onset date	AE end date	Grade
6	1=yes	3=Possible	4=ongoing	1=yes	0=no	Splenomegaly and extramedullary hematopoiesis	Blood and lymphatic system disorders - Other, specify	29-12-2015		3
6	1=yes	2=Probable	6=ongoing at death	0=no	0=no	Erythrocytosis	Blood and lymphatic system disorders - Other, specify	29-12-2015		3
6	1=yes	3=Possible	1=resolved completely	1=yes	1=yes	Pulmonary hemosiderosis	Immune system disorders - Other, specify	5-1-2016	18-1-2016	3

Table 17: Worst Grade AE per SOC within each patient

AE	3	4	Total
Grade			
Alanine aminotransferase increased	2	0	2
Anemia	4	0	4
Anorexia	1	0	1
Blood and lymphatic system disorders - Other, specify:Splenomegaly and extramedullary hematopoiesis	1	0	1
Blood bilirubin increased	1	0	1
Blood bilirubin increased:Direct bilirubin	0	1	1
Bronchial infection	1	0	1
Febrile neutropenia	4	0	4
Flu like symptoms	1	0	1
Headache	1	0	1
Immune system disorders - Other, specify:Pulmonary hemosiderosis	1	0	1
Infections and infestations - Other, specify:Lunginfection	1	0	1
Infections and infestations - Other, specify:Parotitis/Zellulitis	1	0	1
Investigations - Other, specify:CRP	1	0	1
Lymphocyte count decreased	1	0	1
Nervous system disorders - Other, specify:multifokal Leukencephalopathie	1	0	1
Neutrophil count decreased	0	7	7
Platelet count decreased	0	5	5
Respiratory, thoracic and mediastinal disorders - Other, specify:Idiopathic pulmonary hemosiderosis	1	0	1
Sepsis:streptococcus pneumonae	1	0	1
Skin and subcutaneous tissue disorders - Other, specify:Erythema nodosum	1	0	1

Table 18: SAE listing (SAE form)

Patient ID	Stratum	AE term	Severity	SAE category	Onset date	Date AE serious	Outcome	Resolved	Dose level	Protocol phase
1	MDS	Increased CRP level	Grade 3	3=(Prolongation of) hospitalization	15-7-2013	15-7-2013	1=Resolved	25-7-2013	1=dose level 1	Course 1
1	MDS	Increased CRP level	Grade 3	3=(Prolongation of) hospitalization	2-8-2013	19-8-2013	1=Resolved	20-8-2013	1=dose level 1	Course 2
2	JMML	Fever	Grade 1	3=(Prolongation of) hospitalization	22-8-2013	22-8-2013	1=Resolved	28-8-2013	1=dose level 1	Course 1
2	JMML	Febrile Neutropenia	Grade 3	3=(Prolongation of) hospitalization	11-10-2013	11-10-2013	1=Resolved	15-10-2013	2=escalation	Course 2
3	JMML	Jaundice	Grade 3	3=(Prolongation of) hospitalization	14-2-2014	14-2-2014	1=Resolved	19-2-2014	1=dose level 1	Course 2
6	MDS	Idiopathic pulmonary hemosiderosis	Grade 3	3=(Prolongation of) hospitalization	5-1-2016	5-1-2016	1=Resolved	18-1-2016	1=dose level 1	Course 1
6	MDS	Hemosiderosis	Grade 3	3=(Prolongation of) hospitalization	4-2-2016	4-2-2016	1=Resolved	5-2-2016	1=dose level 1	Course 2
6	MDS	Febrile Neutropenia	Grade 3	3=(Prolongation of) hospitalization	10-2-2016	10-2-2016	1=Resolved	15-2-2016	1=dose level 1	Course 2
6	MDS	Erythema nodosum	Grade 3	3=(Prolongation of) hospitalization	1-1-2016	17-2-2016	1=Resolved	19-2-2016	1=dose level 1	Course 2
6	MDS	Splenomegaly with extramedullary hematopoiesis	Grade 3	3=(Prolongation of) hospitalization	29-12-2015	1-1-2016	3=Ongoing		1=dose level 1	Course 1
6	MDS	Erythema nodosum	Grade 2	3=(Prolongation of) hospitalization	1-1-2016	1-1-2016	1=Resolved	18-1-2016	1=dose level 1	Course 1
9	MDS	Febrile Neutropenia	Grade 3	3=(Prolongation of) hospitalization	20-6-2018	20-6-2018	1=Resolved	21-6-2018	1=dose level 1	Follow-up

Table 19: SUSAR evaluation (SAE form)

Patient ID	Stratum	AE SOC	AE term	Suspected causal relationshiop	Unexpected	SUSAR	Consequences*
1	MDS	Increased CRP level		1=yes	0=no	0=No SUSAR	
1	MDS	Increased CRP level		1=yes	0=no	0=No SUSAR	
2	JMML	Fever cause unknown		0=no	0=no	0=No SUSAR	
3	JMML	Jaundice		0=no	0=no	0=No SUSAR	0=no
6	MDS	Pulmonary hemosiderosis		1=yes	1=yes	1=SUSAR	0=no
6	MDS	hemosiderosis		1=yes	1=yes	1=SUSAR	
6	MDS	Febrile neutropenia		1=yes	0=no	0=No SUSAR	
6	MDS	Erythema Nodosum		1=yes	1=yes	1=SUSAR	0=no
6	MDS	Splenomegaly with extramedullary hematopoiesis		1=yes	1=yes	1=SUSAR	0=no
6	MDS	Erythema Nodosum		1=yes	1=yes	1=SUSAR	0=no
9	MDS	Febrile neutropenia	10016288	1=yes	0=no	0=No SUSAR	

** Consequences of this SUSAR for the patients or for the continuation of the trial according to the Principal Investigator*

Table 20: GvHD and HSCT listing (follow-up form)

Patient ID	Stratum	Manifestation of a GvHD	Date onset	Type GvHD	Staging acute GvHD	HSCT	Date HSCT	Type donor specify
1	MDS	0=no				1=yes	25-10-2013	
2	JMML	0=no				1=yes	26-11-2013	
3	JMML	1=yes	2-7-2014	1=acute	2=stage II	1=yes	18-6-2014	
4	JMML	0=no				1=yes	11-9-2014	
5	MDS					0=no		
6	MDS					0=no		
7	MDS	1=yes	27-10-2016	2=chronic		1=yes	2-6-2016	
7	MDS	1=yes	4-1-2017	2=chronic		1=yes	2-6-2016	
8	MDS	1=yes	2-11-2016	1=acute	1=stage I	1=yes	13-10-2016	
9	MDS	0=no				1=yes	3-7-2018	Allogeneic cord blood stemcell transplantation

14.3.2 Listings of deaths, other serious and significant adverse events

Table 21: Listing of deaths

Patient ID	Stratum	Date initial diagnosis	Date registration	Date of death	Specification	Cause of death
2	JMML	3-8-2011	21-8-2013	6-2-2014	graft failure	3=complications
3	JMML	27-12-2012	30-12-2013	11-3-2015	Relapse 02.02.2015	1=disease progression
5	MDS	12-8-2013	2-9-2014	25-12-2014		1=disease progression
6	MDS	17-4-2015	15-12-2015	25-3-2016		3=complications
8	MDS	15-8-2015	23-6-2016	30-8-2017		1=disease progression
10	JMML	5-9-2014	22-3-2019	19-10-2019	Veno-occlusive disease. Macrophage activation syndrome.	3=complications

14.3.3 Narratives of deaths, other serious and certain other significant adverse events

After transplant one patient died after graft failure (pt. 2), one after disease progression despite HSCT (pt. 8) and one because of Veno-occlusive disease and macrophage activation syndrome (pt10).

In 3 cases the patients deceased before being transplanted: the one patient (pt. 6) with splenomegaly was treated with splenectomy prior to HSCT, and died following surgery due to respiratory insufficiency and multi-organ failure; one other patient died after relapse (pt. 3) and one patient died after progression of disease (pt. 5).

Patient 6 with relapsed MDS experienced DLTs before the second stem-cell transplantation with several Serious Unexpected Adverse Events, including pulmonary hemosiderosis, erythema nodosum, and splenomegaly with extramedullary hematopoiesis). These findings are not undoubtedly attributable to azacitidine, but may be related to an auto-immune phenomenon that can be observed in MDS, however we could also not rule out the possibility that treatment with azacitidine provoked the side-effects, as they occurred after drug exposure in both course 1 and course 2 this patient received. These events were all clustered in one patient, and were not reported before in the Celgene safety database (date 06-08-2015). The events were therefore reported as SUSARs (Table 19).

14.3.4 Abnormal laboratory value listing (each patient)

Abnormal lab values are presented for ALAT, ASAT, bilirubin, creatinine. Five times the ULN was determined as abnormal for ALAT/ASAT (grade 3), and three times the ULN as normal for bilirubin and creatinine (grade 3). Data is presented in table 22

Table 22 abnormal laboratory values.

Patient ID	Sex	Stratum	Time evaluation	Abn Gr3 Bilirubin	Abn Gr3 ASAT	Abn Gr3 ALAT
3	0=male	JMML	C2D15	127		
6	1=female	MDS	C1D15		151	
9	0=male	MDS	C3D4			340

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16. APPENDICES

16.1 Study Information

16.1.1 Protocol and protocol amendments

16.1.2 Sample case report form (unique pages only)

16.1.3 List of IECs or IRBs (plus the name of the committee Chair if required by the regulatory authority) - representative written information for patient and sample consent forms

Country	Coordinating site (Co-Sponsor)	Competent authority (CA):	Coordinating ethic committee (CEC):
Germany	**Freiburg**	Bundesinstitut für Arzneimittel und Medizinprodukte Kurt-Georg-Kiesinger-Allee 3 D-53175 Bonn	Ethik-Kommission der Albert-Ludwigs-Universität Freiburg Mark Schmidt Engelberger Straße 21 79106 Freiburg
Austria	**Vienna**	Bundesambt fur Sicherheit im Gesundheitsesen Schirchgasse 9 A-1030 Wien	Ethik-Komission der Medizinischen Universitat Wien und des Allgemeinen Krankenhauses der Stadt Wien AKH Borschkegasse 8b/6 - A-1-90 Wien Austria T:+43-1-404 00-2147 , 2244 F: +43-1-404 00-1690 E: ethik-kom@meduniwien.ac.at
Netherlands	**Rotterdam** International Sponsor	Centrale Commissie Mensgebonden Onderzoek (CCMO) Postbus 16302 2500 BH Den Haag T: 31-70-340 6700 E: bi@ccmo.nl	METC Erasmus MC Dr. Molewaterplein 50 3015 GE Rotterdam Kamer Fd 209 T: +31-10-70 34428; +31-10-70 33625 E: metc@erasmusmc.nl
Italy	**IRCCS, San Matteo**	Associazione Italiana del Farmaco (AIFA) Via del Tritone 181 00187 Roma T: 06-5978401 E: aifa@aifa.mailcert.it	Comitato Bioetica Fondazione Policlinico San Matteo Viale Golgi 19 27100 Pavia T: +39-0382-0382.503408 E: comitato.bioetica@smatteo.pv.it

Czech Republic	**Prague**	SUKL Srobarova 48 100 41 Praha 10 T:+420-272 185 111 E: posta@sukl.cz Statni Ustav Pro Kontrolu Leciv Srobarova 48 100 41 Praha 10 T: +420-272-185 111 E: posta@sukl.cz	Eticka Komise pro Multicentricke Klinicke Hodnoceni Fakultni Nemocnice v Motole V uvalu 84 150 06 Praha 5 T: +224-431 195 E:etickakomise@fnmotol.cz
Denmark	**Aarhus**	Sundhedsstyrelsen Axel Heides Grade 1 2300 København S E: kf@sst.dk	Videnskabsetisk komite Region Midt Skottenborg 26 8800 Viborg E: komite@rm.dk

16.1.4 List and description of national coordinating investigators and other important participants in the study, including brief (1 page) CVs or equivalent summaries of training and experience relevant to the performance of the clinical study

Site no.	Country	City	Hospital name	Local PI
03	GERMANY	Freiburg	University Medical Center Freiburg	Prof. C. Niemeyer, MD PhD
04	AUSTRIA	Vienna	St. Anna Children's Hospital	Prof. M. Dworzak, MD PhD
05	NETHERLANDS	Rotterdam	Erasmus MC - Sophia	Prof. C.M. Zwaanl, MD PhD
12	ITALY	Pavia	Ospedale San Matteo	Prof. M. Zecca, MD PhD
13	CZECH REPUBLIC	Prague	University Hospital Motol	Prof. J. Stary, MD PhD
15	DENMARK	Aarhus	Aarhus University Hospital Skejby	Prof. H. Hasle, MD PhD
19	NETHERLANDS	Utrecht	Princess Maxima Center	Dr. I.M. van der Sluis, MD PhD

16.1.5 Signatures of principal or coordinating investigator(s) or sponsor's responsible medical officer, depending on the regulatory authority's requirement

Attached annex II

16.1.6 Listing of patients receiving test drug(s)/investigational product(s) from specific batches, where more than one batch was used

Not applicable.

16.1.7 Randomisation scheme and codes (patient identification and treatment assigned)

Not applicable

16.1.8 Audit certificates (if available)

No applicable

16.1.9 Documentation of statistical methods

Statistical methods are described in Annex iV

16.1.10 Documentation of inter-laboratory standardisation methods and quality assurance procedures if used

Not applicable

16.1.11 Publications based on the study

First manuscript is in preparation.

16.1.12 Important publications referenced in the report

A full reference list is provided under 15. Reference list

16.2. Patient Data Listings

16.2.1 Discontinued patients

Patient 6

16.2.2 Protocol deviations

Patient ID	Center	Stratum	Reason
09	Erasmus MC, Rotterdam, Netherlands	MDS	
09	Erasmus MC, Rotterdam, Netherlands	MDS	Wrong dose on label of infusion bag. For the patient 09 study medication was prepared by the Pharmacy on Wednesday 28th March 29, 2018. There was a fault in the formula for the dose on the label due to which half of the dose was

	noted on the label (i.e. 70 mg instead of 140 mg). The preparation was correct (14 ml azacitidine 10 mg/ml) had been added to the infusion bag. The pharmacist on call was called by the nurses on the ward after seeing the wrong dose (dose on label did not match with the dose of the prescription in the electronic prescribing module, Practocol Planner) and questioned. He confirmed that the preparation was correct, and as such the infusion could be started. Due to the short expiration time and the delay which had followed given the query regarding the dose the infusion extended a couple of minutes beyond the expiration time. He corrected the dose on the label by hand. For the second infusion on thursday march 29th the label had been corrected. Explanation. The protocol had been authorized by in the past by another pharmacist and already previously used for other patients (last time in December 2015). However never in a dose higher than 100 mg; the formula for the dose on the label had been copied from the sc protocol in which doses > 100mg were divided in 2 syringees and as such the dose on the label halve that of the prescription. All test patients had been for a dose < 100 mg

10	Fondazione IRCCS Policlinico San Matteo, Pavia, Italy	JMML	Patient number 10 included in Fondazione IRCCS Policlinico San Matteo, Pavia, Italy didn't fulfil all the inclusion criteria. However, due to the rarity of the disease and several clinical features it was concluded that the patients had an early stage of relapsed JMML. It was therefore decided to include the patient in the ITCC-015 trial. The patient didn't fulfil the following inclusion criteria: peripheral blood monocyte count >= 1.0x109/L reappearance of organomegaly The decision to include the patient was made on the following clinical features. 13% Blasts in the Bone Marrow (sample 27-MAR-2019) c.226G>A mutation of PTPN11 Myeloid precursors on PB smears Unfortunately, the patient couldn't be registered without checking the following box on the registration form.: peripheral blood monocyte count >= 1.0x109/L. this was noted by the SPONSOR and this Note to File was prepared to clarify this discrepancy. The inclusion was discussed with the SPONSOR and it was decided to include the patient.

16.2.3 Patients excluded from the efficacy analysis
Not applicable

16.2.4 Demographic data
Details are provided in paragraph 14.1

16.2.5 Compliance and/or Drug Concentration Data (if available)
Details on drug concentration are provided in paragraph 9.5.4

16.2.6 Individual Efficacy Response data
Details are provided in paragraph 14.2

16.2.7 Adverse event listings (each patient)
Details are provided in paragraph 14.3

16.2.8. Listing of individual laboratory measurements by patient, when required by regulatory authorities
Not applicable.

16.3 Case Report Forms

16.3.1 CRFs of deaths, other serious adverse events and withdrawals for AE

16.3.2 Other CRFs submitted

ANNEX I

Diagnostic criteria for MDS and JMML

JMML

JMML diagnostic procedures

- Bone marrow aspirate
- Cytogenetics
- Mutational analysis (PTPN11 and other RAS-patway players), which can be send Freiburg. Note: When mutation analysis for JMML patients has already been performed in Freiburg in the diagnostic evaluation process there is no need to repeat it at entry into the study. In case it has been done elsewhere please provide another sample to the central lab in Freiburg plus information on the mutation that was detected elsewhere.
- GM-CSF hypersensitivity in vitro (optional)

Diagnostic criteria according to the EWOG-MDS protocol:

I. Clinical and hematological features (all three features mandatory)

- Peripheral blood monocyte count > $1x10^9$/L
- Blast percentage in PB and BM < 20%
- Splenomegaly

II. Oncogenetic studies (1 parameter sufficient)

- Mutations in the RAS pathway
- *NF1* mutation or clinical diagnosis of NF1
- Monosomy 7

III. In the absence of one parameter listed under II, the following criteria have to be fulfilled:

- Absence of Philadelphia chromosome (*BCR/ABL* rearrangement) (mandatory)
- And at least two of the following criteria
    - Spontaneous growth or GM-CSF hypersensitivity in colony assay
    - Hemoglobin F increased for age
    - Myeloid precursors on peripheral blood smear
    - White blood count > $10x10^9$/L
    - Clonal abnormality besides monosomy 7

Proposed revised JMML diagnostic criteria by Chan et al, Leukemia Research 2009

Category 1	Category 2	Category 3
All of the following:	*At least 1 of the following:*	*At least 2 of the following:*
Splenomegaly	Somatic mutation in RAS or PTPN11	Circulating myeloid precursors
Absolute monocyte count > 1000/µL	Clinical diagnosis of NF1 or NF1 gene mutation	WBC > 10,000/µL
Blasts in PB/BM < 20%	Monosomy 7	Increased fetal hemoglobin (HgF) for age
Absence of the t(9;22) BCR/ABL fusion gene		Clonal cytogenetic abnormality excluding monosomy 7
Age less than 13 years		

Note regarding NF1 and JMML

In patients with a confirmed diagnosis of JMML a clinical diagnosis of NF1 can be made in the presence of

- ≥ 6 café-au-lait macules greater than 5 mm in diameter

or

- a first degree relative (parent or sibling) with NF1.

MDS

In 2000, the World Health Organization (WHO) classification of neoplastic diseases of the hematopoietic and lymphoid tissues incorporating both morphology and genetic changes was introduced. For the definition of MDS, the WHO classification eliminated RAEB-T by reducing the threshold of blasts required to make the diagnosis of AML to 20%. At the same the subtype of RAEB was redefined, now accommodating all cases with up to 20% blasts in PB. The 6 MDS subtypes are described by the WHO for adults therefore are as follows:

- Refractory anemia
- Refractory anemia with ringed sideroblasts
- Refractory cytopenia with multi-lineage dysplasia
- Refractory anemia with excess blasts
- Myelodysplastic syndrome, unclassifiable
- Myelodysplastic syndrome associated with isolated del(5q) chromosome abnormality.

In children, there are no data to indicate whether a blast threshold of 20% is better than the traditional 30% to distinguish MDS from *de novo* AML. In addition, the subdivision of MDS does not reflect the hematological and clinical picture of MDS in childhood: ringed sideroblasts are very infrequently seen in children, the importance of multi-lineage dysplasia is unknown and the unique 5q- syndrome has not been described. The last category, "MDS not otherwise categorized" may not be very useful. Therefore, a pediatric modification of the WHO classification MDS and myelo-dysplastic/myeloproliferative diseases was developed.

Myelodysplastic Syndrome (MDS) pediatric classification:

- Refractory cytopenia (RC) (PB blasts <2% and BM blasts <5%)
- Refractory anemia with excess blasts (RAEB) (PB blasts 2-19% or BM blasts 5-19%)
- RAEB in transformation (RAEB-T) (PB or BM blasts 20-29%)

Advanced MDS with increased blast count comprises the MDS-subtypes RAEB and RAEB-T.

Separation from AML

The separation of MDS with increased blast count from *de novo* AML remains challenging and thresholds of blast counts, whether set at 20% or 30%, are arbitrary. Assuming that the underlying genetic changes between MDS and *de novo* AML are different and therapy approaches will differ, the distinction between these entities becomes important. *De novo* AML is a chemo-sensitive disease characterized by balanced translocations, while the typical genetic changes in MDS, typically resistant to chemotherapy, are numerical aberrations. Patients with recurrent cytogenetic abnormalities typically associated with AML, e.g. t(15;17) (PML/RARα), t(8;21) (AML1/ETO), inv(16)(CBFβ/MYH11), t(9;11) (MLL/AF9), should be diagnosed and treated as *de novo* AML regardless of the blast count. The only chromosomal abnormality which may be regarded as marker of MDS-like biology is monosomy 7.

MDS progressing to disease with BM blast counts > 30% is referred to as myelodysplasia-related AML (MDR-AML). For monosomy 7, it is unknown, whether cases evolving from MDS to MDRAML have the same biology than cases diagnosed as AML with monosomy 7. In AML studies, patients diagnosed as AML with monosomy 7 have a lower response rate to chemotherapy and a higher relapse rate compared with AML without -7.

It should be emphasized, however, that most MDS patients have a blast percentage < 20% at

diagnosis, while the vast majority of children with *de novo* AML present with a frank leukemic BM. For patients with an ambiguous blast count, organomegaly, CNS infiltration or chloroma are indicative of de *novo* AML. In patients presenting with a BM blast percentage > 20% and no clinical or cytogenetic changes characteristic of MDS or *de novo* AML, it is recommended to repeat the BM examination after 2 weeks. If the blast count has increased to ≥ 30% the patient most likely has *de novo*-AML. If the blast count is stable over an arbitrary period of 4 weeks the diagnosis of RAEB-T can be made.

ANNEX II Principal or Coordinating Investigator(s) Signature(s)

Prof dr. C.M. Zwaan _____

Date _____

Prof dr. M.M. van den Heuvel-Eibrink _____

Date _____

ANNEX III a schedule of Assessments MDS

Assessment	Screening	Course 1			Course 2			Course 3			Additional Courses	End [l, m] of Treatment
		Baseline	During	End [l]	Baseline	During	End [l]	Baseline	During	End [l, m]		
Signed written Informed Consent	X											
Physical examination/Vital Signs	X	X		X	X		X	X		X	X	X
Height, Weight, BSA	X	X			X			X		X	X	X
Medical History/Concurrent Conditions	X											
Performance Status	X	X		X	X		X	X		X	X	X
Hematology [a] (CBC with differential: including platelets)	X	X	X [a]	X	X	X [a]	X	X	X [a]	X	X [a]	X
Serum Chemistry [b, c]	X [b]	X [b]	X [b, c]	X [b]	X [b]	X [b, c]	X [b]	X [b]	X [b, c]	X [b]	X [b, c]	X [b]
BM aspirate, bilateral [d, e]	X			X						X	X (every 3 courses)	X
BM trephine biopsy, bilateral [d, e]	X			X						X	X (every 3 courses)	X
BM, PB and trephines for central review morphology (Freiburg)	X			X						X	X (every 3 courses)	X
BM and PB for central cytogenetics and FISH in Rotterdam	X									X		X
BM and PB for pharmacodynamics [f] (Rotterdam)	X		X (day 8)	X (day 28)								
Lumbar puncture [g]	X [g] (optional)			X [g]						X [g]	X [g]	X [g]
Chest X-ray [h]	X											
Ultrasound abdomen	X											
Echocardiography (shortening fraction)	X											

Pregnancy test and highly-effectice contraception measures (if applicable)	X	X			X			X			X (at baseline)	X
Study Treatment Administration [i]			X			X			X		X	
Pharmacokinetic sampling (PK)			X (day 5, 6, 7)									
Concomitant Medications/Transfusions	X	X	X	X	X	X	X	X	X	X	X	X
Adverse Event Assessment [j]	X	X	X	X	X	X	X	X	X	X	X	X [j, k]
Follow up [k]												X [j]

a Hematology frequency: twice weekly during Azacitidine administration (or more frequently when clinically indicated). After that at least once weekly until the next treatment course.

b Serum chemistry examinations: electrolytes (sodium, potassium, chloride, calcium, magnesium, phosphate, chloride and bicarbonate), BUN, creatinine, uric acid, CRP, glucose, AST, ALT, alkaline phosphatase, total bilirubin, and LDH.

c Serum chemistry frequency: every other day during Azacitidine administration (or more frequently when clinically indicated). After that at least once weekly until the next treatment course.

d BM aspirate for morphology, flow-cytometry, and cytogenetics including FISH. Cytogenetics (including FISH) is done in Rotterdam at the central cytogenetic laboratory.

e Progressive Disease may be diagnosed on PB when morphology is confirmed by flow-cytometry.

f Extra pharmacodynamics samples (BM and PB) limited to first course of azacitidine course (baseline, day 8 and day 28 first course).

g Lumbar puncture in case CNS-involvement at inclusion (optional, mainly when a differential diagnosis of AML is considered).

h Chest X-ray: In case of suspected fungal infection: high-resolution CT-scan.

i Study treatment: at least for 3 Courses or progression of disease if earlier; In case donor search and HSCT preparations have not been finalized or a HSCT is not possible. Every attempt should be made to administer 3 Courses.

j Adverse Events should be followed up 4-weekly until resolved to baseline or CTCAE v4.0 grade ≤1, or deemed irreversible.

k Follow up should Include safety (limited to severe infections (≥ grade 3 CTCAE v4.0, Graft versus Host), response, survival, and new therapy (3-monthly) until 1 year after HSCT or until 1 year after last azacitidine administration when HSCT did not take place. Early follow-up visits are required at a maximum interval of 4 weeks until all drug-related AEs resolve to baseline or CTC grade ≤1, or are deemed irreversible.

l End of a course can coincide with baseline of next course. If applicable examinations need not to be repeated at both time points.

m If end of a course coincides with end of treatment, end of treatment examinations should be done.

ANNEX III b Schedule of Assessments JMML

Assessment	Screening	Course 1			Course 2			Course 3			Additional Courses	End [l, m] of Treatment
		Baseline	During	End [l]	Baseline	During	End [l]	Baseline	During	End [l, m]		
Signed written Informed Consent	X											
Physical examination/Vital Signs	X	X		X	X		X	X		X	X	X
Height, Weight, BSA	X	X			X			X			X	
Medical History/Concurrent Conditions	X											
Performance Status	X	X		X	X		X	X		X	X	X
Spleen size (physical examination)	X	X		X	X		X	X		X	X	X
Abdominal sonography [a] (ultrasound spleen size)	X	X		X [a]			X [a]			X [a]	X [a]	X
Hematology [b] (CBC with differential: including platelets)	X	X	X [b]	X	X	X [b]	X	X	X [b]	X	X [b]	X
Absolute Monocyte count [c]	X	X	X [c]	X	X	X [c]	X	X	X [c]	X	X [c]	X
Serum Chemistry [d, e]	X [d]	X [d]	X [d, e]	X [d]	X [d]	X [d, e]	X [d]	X [d]	X [d, e]	X [d]	X [d, e]	X [d]
PB and BM aspirate for morphology and flow cytometry *at site*	X											
PB slides for central review morphology (send to Freiburg)	X			X			X			X	X	X
BM and PB for central cytogenetics and FISH in Rotterdam	X											
PB for Pharmacodynamics [f, g] (send to Freiburg)		X [f]	X [f] (day 8, 15, 22)	X [f] (day 28)			X [g]			X [g]		X [g]
PB for Molecular diagnostics (send to Freiburg)	X											
Chest X-ray [h]	X [h]											
Echocardiography (shortening fraction)	X											

Pregnancy test and highly-effectice contraception measures (if applicable)	X	X			X			X			X (at baseline)	X
Study Treatment Administration [i]			X [i]			X [i]			X [i]		X [i]	
Pharmacokinetic sampling (PK)			X (day 5, 6, 7)									
Concomitant Medications/Transfusions	X	X	X	X	X	X	X	X	X	X	X	X
Adverse Event Assessment [j]	X	X	X	X	X	X	X	X	X	X	X	X [j, k]
Follow up [k]												X [k]

[a] Abdominal ultrasound: at end of each treatment course.

[b] Hematology frequency: twice weekly during Azacitidine administration (or more frequently when clinically indicated). After that at least once weekly until the next treatment course.

[c] Absolute Monocyte Count: twice weekly during each course of azacitidine administration and at least once weekly after that.

[d] Chemistry examinations: electrolytes (sodium, potassium, chloride, calcium, magnesium, phosphate, chloride, bicarbonate), BUN, creatinine, uric acid, CRP, glucose, AST, ALT, alkaline phosphatase, total bilirubin, LDH.

[e] Chemistry frequency: every other day during Azacitidine administration (or more frequently when clinically indicated). After that at least once weekly until the next treatment course.

[f] Pharmacodynamic studies: clone-size and methylation status.

[g] Clone-size follow up.

[h] Chest X-ray: In case of suspected fungal infection a high-resolution CT-scan should be performed.

[i] Study treatment: at least for 3 Courses or progression of disease if earlier; In case donor search and HSCT preparations have not been finalized or a HSCT is not possible. very attempt should be made to administer 3 Courses.

[j] Adverse Event assessment: drug related AE's should be followed up 4-weekly until resolved to baseline or CTCAE v4.0 grade ≤1, or deemed irreversible.

[k] Follow up: including safety (limited to severe infections (≥ grade 3 CTCAE v4.0)), Graft versus Host , response, survival, and new therapy (3-monthly) until 1 year after HSCT or until 1 year after last vidaza administration when HSCT did not take place. Early follow-up visits are required at a maximum interval of 4 weeks until all drug-related AEs resolve to baseline or CTC grade ≤1, or are deemed irreversible.

[l] End of a course can coincide with baseline of next course. If applicable, examinations need not to be repeated at both time points.

[m] If end of a course coincides with end of treatment, end of treatment examinations should be done.

ANNEX IV Statistical Analysis Plan

1 Introduction

1.1 Background and rationale

This Statistical Analysis Plan (SAP) provides the detailed methodology for summary and statistical analyses of the data collected in study ITCC-015/EWOG-MDS-Azacitidine-2010 (VZ-MDS-PI-0246): A Phase I/II study of Azacitidine (Vidaza®) in pediatric patients with relapsed high-grade pediatric MDS or JMML, protocol version 1.4, dated 27-Jul-2018.

This document may modify the plans outlined in the protocol; however, any major modifications of the primary endpoint definition and/or its analysis will also be reflected in a protocol amendment.

Myelodysplastic syndromes (MDS) and juvenile myelomonocytic leukemia (JMML) are rare malignant diseases of childhood. MDS in children can be divided in primary and secondary MDS. MDS after prior chemotherapy or radiation therapy, after prior acquired aplastic anemia, or in bone marrow failure disorders and familial diseases is generally classified as secondary MDS. So far, stem cell transplantation is the only curative treatment option for both MDS and JMML. No other agents are available to treat these diseases successfully, and HSCT results in approximately 50% survival only; hence there is clear unmet medical need. Over the past few years, we have increasing evidence that aberrant methylation contributes to the malignant phenotype of JMML and childhood advanced MDS. The demethylating agent azacitidine has been shown to improve survival and is authorized for adults with MDS, but so far no studies are available in children with MDS or JMML.

In the current study we want to establish the recommended dose and preliminary efficacy of azacitidine, in children with primary advanced MDS or JMML in a pre-transplantation window at relapse. This study will provide a preliminary proof of concept whether a demethylating agent is able to induce responses in these diseases, and whether this agent indeed results in hypomethylation. Pharmacodynamic studies should provide further support for this proof of concept.

1.2 Study Design

This is an international, collaborative, prospective, open label, phase I/II trial. The study will be conducted as an investigator-initiated study in a European network (EWOG-MDS and ITCC) with Erasmus MC acting as international sponsor, and with free drug provided by Celgene, who are also responsible for the PK-studies. Financial support is provided by the Go4Children foundation and Celgene.

It needs to be mentioned that the HSCT procedure itself is not part of this protocol and should be performed under EWOG or institutional guidelines at the discretion of the principle investigator. We will however capture data to assess whether azacitidine influences outcome Azacitidine in high grade MDS and JMML pediatric patients post-HSCT (follow-up for relapse/survival one year post-HSCT).

This phase I/II study looks at two different disease indications (relapsed MDS and JMML). Each disease indication will be considered in independent treatment arms which will run in parallel with each other. One dose escalation will be allowed for each arm.

Due to the rarity of the two diseases being enrolled, this study will combine dose escalation in the phase I setting with efficacy based rules for discontinuing a study arm early or not, and declaring the study treatment in a given arm positive or not, in a phase II setting with regards to the primary endpoint, which is response rate. As such, moving from stage 1 to stage 2 in this two stage design takes into account study treatment tolerability and response data.

Each stage (stage one and stage two) will consist of a minimum of 3 patients per disease indication. Both study arms will follow a 2-stage design, with the option of a safety run-in in the case of the JMML arm. For the MDS arm, if there is at least one patient achieving response (defined as CR or PR) and there are no patients experiencing a dose-limiting toxicity among the three first patients in stage one,

another three patients will be treated at the next higher dose level, if applicable. In case of one dose-limiting toxicity among the first three patients, the cohort will be expanded to 6 patients at the starting dose-level. If there is at least one out of six patients achieving response and no more than one patient experiences a dose-limiting toxicity in stage one, stage two shall open for enrolment. In case of no responses the arm shall be closed to enrollment. In case there is more than one dose-limiting toxicity in stage one, the dose is set to the previous level (if applicable), and stage 2 shall open for enrolment if at least one patient responded at that dose-level. Otherwise, the arm shall be closed to enrolment.

The dose will be increased only if <2 of the 6 evaluable patients (30%, across stage-one and stage-two) achieve a response, and/or there are ≤ two dose-limiting toxicities; otherwise the therapy will be deemed unpromising for further consideration.

For the JMML arm, the safety run-in will include 3 patients and the tolerability of the therapy will be considered using a classic 3+3 design. Should the therapy be considered tolerable, stage one shall enroll patients to a higher dose, or otherwise the patients in the safety run-in will be considered part of stage one. During stage-one, if ≥ 1 of the 3 evaluable patients for the primary endpoint achieve a response then stage two shall open to enrolment, or otherwise that arm shall be closed to enrolment. At the end of stage two, the therapy will be considered positive for possible further investigation if ≥ 2 of the 6 evaluable patients (30%, across stage-one and stage-two) achieve a response; or otherwise considered unpromising for further consideration.

1.3 Study Objectives

1.3.1 Primary Objectives

To establish the recommended dose and preliminary efficacy of azacitidine in children with relapsed advanced MDS or JMML.

1.3.2 Secondary Objectives

- To determine the safety and tolerability of azacitidine in relapsed advanced MDS and JMML
- To determine (preliminary) the haematological remission rate in these patients
- To describe the durability of response, disease free and overall survival, including the number of patients undergoing stem-cell transplant after treatment with azacitidine
- To describe the number of patients transforming into AML
- To determine the plasma pharmacokinetic parameters of azacitidine
- To study the pharmacodynamic effects of azacitidine in relapsed pediatric advanced MDS or JMML

2 Interim Analyses, Final Analyses and Unblinding

An independent DSMB will be established to perform ongoing safety and efficacy surveillance, and to perform interim analyses on the safety data as mentioned in the protocol.

the DSMB will be involved in the decision regarding the RP2D before the expansion cohort opens.

The DSMB will be composed of a statistician and a pediatric oncologist with specific expertise in JMML and MDS. These members should not have conflicts of interest with the sponsor, company or the involved study. Since this study will be running in Europe only, it is likely that the DSMB members will be recruited from a US early clinical trial pediatric oncology network (TACL consortium).

This is a single-arm, open-label study, so unblinding is not required.

3 Sample Size and Power Considerations

The study will incorporate a two-stage design per disease stratum. The JMML arm will have an option

safety run-in cohort consisting of 3 patients. Per stratum, stage one and stage two will each consist of a cohort of at most 6 patients. This means a total of up to 12 patients being enrolled to the JMML disease indication and up to 12 patients in the MDS disease indication, i.e. a maximum of 24 patients in total. Considering that some patients may not be available for evaluation, a maximum of 28 patients is anticipated.

4 Data Source

The data will be exported from Open Clinica using validated SAS Exports.

5 Hypotheses and Decision Rules

5.1 Statistical Hypotheses

Not applicable.

5.2 Statistical Decision Rules

Not applicable.

6 Analysis sets and protocol deviations

6.1 Analysis Sets

6.1.1 Full Analysis Set (FAS)

The Full Analysis Set (FAS) consists of all enrolled patients, regardless of whether they received study therapy. Tabulations of subject disposition, analysis subsets, demographic and baseline characteristics, medical history, concomitant medication and protocol deviations will be based on this analysis set.

6.1.1 Efficacy-Evaluable Analysis Set (EEAS)

The Efficacy Evaluable Analysis Set (EEAS) consists of all patients in the FAS receiving at least one dose of study therapy and having at least one post-baseline assessment. Assessment of all efficacy-based endpoints in both phase I and phase II will be performed on the EEAS.

6.1.2 Per Protocol Set

The Per Protocol Analysis Set (PPS) includes all subjects in the EEAS who did not have any major protocol deviation after enrollment, which can be:

- Patient did not meet entry criteria
- Patient received forbidden medication.

The PPS will be used to perform sensitivity analyses on all efficacy-based endpoints.

6.1.1 Safety Analysis Set

The Safety Analysis Set will include all subjects in the FAS who received at least one dose of study medication and had any safety data collected after enrollment. This set will be used for the analysis of safety endpoints.

6.2 Protocol Deviations

6.2.1 Treatment Misallocations

Not applicable.

6.2.2 Protocol Deviations

The full list of protocol deviations for the study report will be compiled prior to database lock.

7 Endpoints and covariates

Demographic, medical history and baseline variables

7.1 Clinical and Laboratory Parameters

Clinical and laboratory parameters, including:

- Physical examination/vital signs

- Height, weight, BSA
- Medical history/concurrent conditions
- Performance status
- Hematology (CBC with differential, including platelets)
- Serum chemistry
- BM aspirate, bilateral
- BM trephine biopsy, bilateral
- Chest X-ray
- Ultrasound abdomen
- Echocardiography
- Pregnancy test
- Concomitant medications/transfusions
- Adverse Event assessment

7.2 Primary endpoint

- To establish the recommended dose and preliminary efficacy of azacitidine in children with relapsed advanced MDS or JMML.

Response data combined with safety data will be used to evaluate efficacy and establish the recommended dose. Depending on the disease indication, variables used for response evaluation will include, among others, bone marrow and peripheral blood morphology response. See Section 5 of the study protocol for details.

Dose-limiting toxicities (DLTs) will be reported and considered in the dose-escalation phase of the study. DLTs are adverse events (AEs) considered at least possibly drug-related, and will be limited to the first course of azacitidine. A definition of DLTs for this study can be found in Appendix 1.

The primary efficacy variable is the overall response rate in the 2 strata, defined as the number of patients with either a CR or PR, as defined in Appendix 2, over the total number of patients evaluable for the analysis. Patient discontinuation from study treatment without a disease assessment but with the cause for treatment discontinuation being disease progression will be considered as having a disease progression.

Cytogenetic/Molecular Response Rate is defined as the proportion of patients achieving a cytogenetic (MDS) / molecular (JMML) response, i.e. number of responders over the number of patients included in the EEAS.

7.3 Secondary endpoints

- To determine the safety and tolerability of azacitidine in relapsed advanced MDS and JMML

See primary endpoint and references to the study protocol therein.

- To determine (preliminary) the haematological remission rate in these patients

Haematological remission status (CR, PR, SD or progression/relapse) assessed during follow-up.

- To describe the durability of response, progression-free and overall survival, including the number of patients undergoing stem-cell transplant after treatment with azacitidine

Progression-free survival is defined as time from first study dose administration to disease progression or death from any cause, whichever happens first. Overall survival is defined as time from first study dose administration to death from any cause. Patients without an event are censored at the last known alive date.

Duration of Response (DoR) is defined as the time from first observed response until first observed disease progression thereafter. Only patients observed with a response will be included in the analysis of DoR.

Stem Cell Transplantation (HSCT) rate is calculated as the number of patients undergoing a HSCT post study drug administration over the number of subjects in the 1) the EEAS, 2) the safety set.

- To describe the number of patients transforming into AML

Blast crisis/transformation to AML as measured in the response form.

- To determine the plasma pharmacokinetic parameters of azacitidine

Pharmacokinetic parameters of azacitidine will be calculated from plasma concentration-time profiles using non-compartmental methods, though compartmental analysis may be employed if appropriate. Plasma PK parameters will include, but not be limited to:

o Cmax: observed maximum plasma concentration

o Tmax: observed time to maximum plasma concentration

o AUC0-t: area under the plasma concentration time curve from time zero to the last quantifiable time point, calculated by the linear trapezoidal rule

o AUC0-∞: area under the plasma concentration time curve from time zero to infinity, calculated by the linear trapezoidal rule and extrapolated to infinity will be calculated according to the following equation: AUC0-∞ = AUC0 t + (Ct/λz), where Ct is the last quantifiable concentration

o λz: terminal phase rate constant, determined by linear regression of the terminal points of the log-linear plasma-concentration-time curve

o t½: terminal phase half-life, will be calculated according to the following equation: t½ = 0.693/λz

o CL: total clearance, calculated as Dose/AUC0-∞

o Vd: volume of distribution will be calculated according to the equation: Vd = (CL)/λz

- To study the pharmacodynamic effects of azacitidine in relapsed pediatric advanced MDS or JMML

7.4 Details on safety endpoints

7.4.1 Exposure

Exposure and compliance data will be collected during this study. The primary exposure endpoints while the patient is on treatment are duration of treatment, dose received, percent of planned dose received by treatment course, reasons for dose modifications (reduction, delay, or escalation), and reasons for treatment discontinuation. Duration of treatment is the time from first non-zero dose to last non-zero dose.

7.4.2 Safety Assessments

Safety assessments will include collection of AEs, SAEs, vital signs and physical examination, echocardiography, laboratory assessments including pregnancy tests, and verification of concomitant treatments as described in the sections below.

7.4.2.1 Adverse Events

Adverse events should be documented and recorded at each visit using the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) version 4.0. AEs and SAEs should be recorded on the eCRF from the time the patient provided informed consent. AEs should be followed to resolution or stabilization, and reported as SAEs as they become serious. AEs occurring in the first course will be tabulated separately from the 2nd or subsequent courses.

Details about AEs and SAEs/SUSARs reporting are provided in section 6 of the study protocol.

7.4.2.2 Laboratory Safety Assessments

Blood tests include:

- Hematology: CBC with differential, including platelets.
- Serum chemistry: electrolytes (sodium, potassium, chloride, calcium, magnesium, phosphate, chloride and bicarbonate), BUN, creatinine, uric acid, CRP, glucose, AST, ALT, alkaline phosphatase, total bilirubin, and LDH.

Investigators may order additional blood tests for planning treatment administration, dose modification, or further evaluation of adverse events.

7.4.2.3 Vital signs and Physical Examination

Patients will have a physical exam to include height, weight, body surface area, vital signs (temperature, pulse, blood pressure), assessment of Lansky or Karnofsky performance status (Appendix 2 of the study protocol) at the scheduled time points.

7.4.2.4 Echocardiography

An echocardiography will be performed at screening.

7.4.3 Pregnancy Testing and Contraception Check

For all female patients with childbearing potential, a serum or urine pregnancy test will be performed prior to starting enrollment. Following a negative pregnancy result at screening, appropriate contraception for patients of childbearing potential must be commenced. Pregnancy tests will also be routinely repeated as per the schedule of events.

7.4.4 Disease Response Assessments

Response evaluation will consist of the following evaluations (depending on study group): bone marrow (bilateral), trephine biopsy (bilateral) and peripheral blood morphology; determination of spleen size by physical examination; abdominal sonography to document liver and spleen size.

Disease response assessments will be based upon disease specific response criteria (see Section 5 of the study protocol), and will in part be analyzed at a central laboratory.

7.4.5 Death

Date and cause of death will be collected during this study.

8 Handling of missing values and other data conventions

The baseline value will be defined as the last non-missing value prior to first dose, unless specifically stated otherwise.

Partial dates are handled in the following way: if the month and year are present but day is missing, date is set to 15th of the month. If month is missing, it is set to June.

No other imputation of missing data will be performed.

9 Statistical procedures

9.1 Statistical Methods and Properties

9.1.1 Analysis of Continuous Data

Descriptive statistics for continuous variables will include total counts, mean, median, standard deviation (SD), range and interquartile range (IQR).

9.1.2 Analysis of Categorical Data

Categorical variables will be presented as frequencies and percentages.

9.1.3 Analysis of Binary Endpoints

The binary endpoints (response rates) will be presented as a proportion with exact 2-sided 95% confidence intervals.

9.1.1 Analysis of Time-To-Event Endpoints

Time-to-event endpoints (PFS, OS, DoR) will be analyzed using the Kaplan Meier method.

When applying the Kaplan Meier method, median times and quartiles with associated 2-sided 95% confidence intervals (CIs) based on the Brookmeyer-Crowley linear transformation method (Brookmeyer & Crowley, J., 1982) will be provided, assuming no ties among observed survival times. If median times will not be reached at the time of the analysis, survival rates at the specific time point will be provided together with the associated 2-sided 95% CI based on Greenwood's formula.

9.1.2 Patient Accrual and Study Duration

The study will last approximately 8 years from first patient first visit (FPFV) to last patient last visit (LPLV). Therefore we expect to provide the final study report 9 years after enrolment of the first subject into the study. The total number of patients for this study is at maximum 28 subjects, as detailed in Section 3.

Approximately 120 pediatric patients with high-grade MDS are expected to be diagnosed every 8 years (hence ~15/year). Approximately 20-25% of patients with high-grade MDS will suffer from relapse following HSCT, indicating that per year ~3-4 patients could be included (Kardos et al, 2003; Niemeyer & Kratz, 2008). Considering JMML, a recent report describes the outcome of 100 patients transplanted over a 10-year period within the EWOG group (Locatelli et al, 2005). The risk of leukemia relapse was ~25%. Taken together recruitment should be feasible in this time-frame when sufficient sites are enrolling and patients are being referred.

9.2 Statistical Analyses

9.2.1 Analysis of the primary endpoint

Each stage (stage one and stage two) will consist of a minimum of 3 patients per disease indication. Both study arms will follow a 2-stage design, with the option of a safety run-in in the case of the JMML arm.

For the MDS arm, if there is at least one patient achieving response (defined as CR or PR) and there are no patients experiencing a dose-limiting toxicity among the three first patients in stage one, another three patients will be treated at the next higher dose level, if applicable. In case of 1 dose-limiting toxicity among the first three patients, the cohort will be expanded to 6 patients at the starting dose-level. If there is at least one out of six patients achieving response and no more than one patient experiences a dose-limiting toxicity in stage one, stage two shall open for enrolment. In case of no responses the arm shall be closed to enrolment. In case there is more than one dose-limiting toxicity in stage one, the dose is set to the previous level (if applicable), and stage 2 shall open for enrolment if at least one patient responded at that dose-level. Otherwise, the arm shall be closed to enrolment.

The dose will be increased only if <2 of the 6 evaluable patients (30%, across stage-one and stage-two) achieve a response, and/or there are ≤ two dose-limiting toxicities; otherwise the therapy will be deemed unpromising for further consideration.

For the JMML arm, the safety run-in will include 3 patients and the tolerability of the therapy will be considered using a classic 3+3 design. Should the therapy be considered tolerable, stage one shall

enrol patients to a higher dose, or otherwise the patients in the safety run-in will be considered part of stage one. During stage-one, if ≥ 1 of the 3 evaluable patients for the primary endpoint achieve a response then stage two shall open to enrolment, or otherwise that arm shall be closed to enrolment. At the end of stage two, the therapy will be considered positive for possible further investigation if ≥ 2 of the 6 evaluable patients (30%, across stage-one and stage-two) achieve a response; or otherwise considered unpromising for further consideration.

Overall response rate in the 2 strata will be estimated as the number of patients with either a CR or PR over the total number of patients evaluable for the analysis. Patient discontinuation from study treatment without a disease assessment but with the cause for treatment discontinuation being disease progression will be considered as having a disease progression for this analysis. Point estimates and exact 2-sided 95% confidence intervals will be reported.

Cytogenetic/Molecular Response Rate and the corresponding Clopper-Pearson 95% confidence interval shall be presented per disease strata and the per disease phase.

9.2.2 Analysis of secondary endpoints

The median PFS and OS time shall be calculated using the Kaplan-Meier method. Median, minimum and maximum values will be reported as well as the corresponding 95% confidence interval of the median per disease strata and per treatment phase.

Stem Cell Transplantation (HSCT) rate and the corresponding Clopper-Pearson 95% confidence interval shall be presented per disease strata and per disease phase.

In the analysis of Duration of Response (DoR) only patients observed with a response will be included. Due to the anticipated low number of patients expected to be observed as having a response, the median DoR time shall be calculated by the median time per patient who is responding, and also by means of the Kaplan-Meier method if more than 5 patients respond in any stratum. Median, minimum and maximum values will be reported as well as the corresponding 95% confidence interval of the median per disease strata and per treatment phase.

By-subject listing of pharmacokinetic blood sample collection times, derived sampling time deviations, and PK parameters will be provided. Azacitidine plasma concentrations and resulting PK parameters will be summarized using descriptive statistics (N, arithmetic mean, standard deviation, minimum, median, maximum, percent coefficient of variation, and geometric mean) for each treatment. Concentrations that are below the limit of quantitation (BLQ) will be treated as zero for the computation of descriptive statistics and listed with the lower limit of quantitation (LLQ) indicated. Missing concentrations will be omitted from the calculation of descriptive statistics.

Figures of mean azacitidine concentration-time data will also be illustrated for each treatment. Individual azacitidine subject concentration-time data for each treatment will be graphically presented on linear and semi-logarithmic scales.

9.2.2.1 Safety Data

Safety analysis includes frequency, severity, and relatedness of all AEs, frequency and severity of all laboratory abnormalities, frequency of dose interruptions, dose reductions and treatment discontinuation for toxicity, and use of concomitant medications. Individual safety data, as well as the absolute and relative incidence of toxicities, will be presented in tables. The worst grades of these toxicities will be given for all patients who were evaluable for toxicity measurements. These toxicities will be scored from the moment the patients received their first study drug until end of treatment. The toxicities might be classified and presented in different tables, based on the nature of the toxicities (hematological or non-hematological) or the causality of the toxicities (treatment-related or all observed toxicities).

All AEs occurring after any administration of the study drug will be followed until resolution or return to baseline values. The descriptions and grading scales found in the revised NCI CTCAE version 4.0 will be used for adverse event reporting. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov/reporting/ctc.html).

9.2.2.1.1 Laboratory Test Abnormalities

The number and percentage of patients who experience laboratory test abnormalities will be summarized according to worst toxicity grade observed for each laboratory test. The analyses will summarize laboratory abnormalities both during the entire study period and by specific time periods if applicable.

9.2.3 Serious Adverse Events (SAE)

The SAEs, grade (according to CTC-AE v.4.0), and possible relatedness to the study drug, will be presented in a table.

10 Sensitivity analysis

The PPS will be used to perform sensitivity analyses on all efficacy-based endpoints.

11 Rationale for any deviation from pre-specified analysis plan

Not applicable.

12 QC plans

Prior to the study start the electronic case report forms (eCRFs) will be reviewed by a statistician to check if all the endpoints can be reliably built based on the collected data. A sample of test patients will be entered in OpenClinica to verify proper data structure.

13 Programming plans

If applicable, validation of data and primary objectives results will be performed by double programming.