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

A collaborative EWOG-MDS and ITCC study

Study ITCC-015/EWOG-MDS-Azacytidine-2010 or VZ-MDS-PI-0246)

Sponsor: Erasmus MC, Rotterdam

Trial Management and Data Center: ‘DCOG – Early Clinical Trial Consortium’

EUDRACT nr: 2010-022235-10

The Netherlands Trial Register: number 2578

Including amendment 2 and administrative changes
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  M.R. Rodriguez, Associate Manager Medical Affairs-IITs

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The Netherlands
APPROVAL OF A STUDY PROTOCOL

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

The following persons declare their consent with the study protocol:

Coordinating investigators:

MM van den Heuvel-Eibrink

(date, dd/MON/yy) (signature)

CM Zwaan

(date, dd/MON/yy) (signature)

Sponsor (on behalf of Erasmus MC):

CM Zwaan

(date, dd/MON/yy) (signature)

Herewith I confirm that I read the study protocol carefully and declare my consent with it. I will treat and examine the patients in accordance with the study protocol, the national applicable laws, the international guidelines on good clinical practice (ICH-GCP) and the most recent version of the Declaration of Helsinki.

Investigator:

Prof. Dr.____________________  ___________________ __________________

(name) (date, dd/MON/yy) (signature)

Site: _____________________

(name)
# LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>6-MP</td>
<td>6-Mercaptopurine</td>
</tr>
<tr>
<td>ALL</td>
<td>Acute lymphoblastic leukemia</td>
</tr>
<tr>
<td>AML</td>
<td>Acute myeloid leukemia</td>
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<tr>
<td>Ara-C</td>
<td>Cytarabine</td>
</tr>
<tr>
<td>ASR</td>
<td>Annual Safety Report</td>
</tr>
<tr>
<td>BLQ</td>
<td>Below the Limit of Quantification</td>
</tr>
<tr>
<td>BM</td>
<td>Bone marrow</td>
</tr>
<tr>
<td>BSA</td>
<td>Body surface area</td>
</tr>
<tr>
<td>BU</td>
<td>Busulfan</td>
</tr>
<tr>
<td>CBC</td>
<td>Complete blood count</td>
</tr>
<tr>
<td>CCR</td>
<td>Complete cytogenetic response</td>
</tr>
<tr>
<td>CMR</td>
<td>Complete molecular remission</td>
</tr>
<tr>
<td>CNS</td>
<td>Central nervous system</td>
</tr>
<tr>
<td>CR</td>
<td>Complete remission</td>
</tr>
<tr>
<td>CRF</td>
<td>Case report form</td>
</tr>
<tr>
<td>CY</td>
<td>Cyclophosphamide</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>DSMB</td>
<td>Data safety monitoring board</td>
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<tr>
<td>EBMT</td>
<td>European Blood and Marrow Transplantation</td>
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<tr>
<td>EFS</td>
<td>Event free survival</td>
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<tr>
<td>EMEA</td>
<td>European Medicines Agency</td>
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<tr>
<td>ESR</td>
<td>Expedited Safety Report</td>
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<tr>
<td>EWOG-MDS</td>
<td>European Working Group of MDS in childhood</td>
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<tr>
<td>Term</td>
<td>Definition</td>
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<td>--------------------</td>
<td>-------------------------------------------------</td>
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<tr>
<td>FTase</td>
<td>Farnesyl transferase</td>
</tr>
<tr>
<td>GCP</td>
<td>Good clinical practice</td>
</tr>
<tr>
<td>GFR</td>
<td>Glomerular Filtration Rate</td>
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<tr>
<td>GM-CSF</td>
<td>Granulocyte macrophage-colony stimulating factor</td>
</tr>
<tr>
<td>HbF</td>
<td>Fetal hemoglobin</td>
</tr>
<tr>
<td>HI</td>
<td>Hematological improvement</td>
</tr>
<tr>
<td>(H) SCT</td>
<td>(Hematopoietic) stem cell transplantation</td>
</tr>
<tr>
<td>IB</td>
<td>Investigator’s Brochure</td>
</tr>
<tr>
<td>IEC</td>
<td>Independent ethics committee</td>
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<tr>
<td>ICH</td>
<td>International Conference on Harmonisation</td>
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<tr>
<td>IRD</td>
<td>Institutional review board</td>
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<tr>
<td>IV</td>
<td>Intravenous</td>
</tr>
<tr>
<td>Kg</td>
<td>Kilogram</td>
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<tr>
<td>JMML</td>
<td>Juvenile myelomonocytic leukemia</td>
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<tr>
<td>LLQ</td>
<td>Lower Limit of Quantification</td>
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<tr>
<td>MAPK</td>
<td>Mitogen-activated protein kinase</td>
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<tr>
<td>MDR-AML</td>
<td>Myelodysplasia-related AML</td>
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<tr>
<td>MDS</td>
<td>Myelodysplastic syndrome</td>
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<tr>
<td>MFD</td>
<td>Matched family donor</td>
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<tr>
<td>NCI-CTCAE</td>
<td>National Cancer Institute- Common Toxicity Criteria for Adverse Events</td>
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<tr>
<td>NF1</td>
<td>Neurofibromatosis type 1</td>
</tr>
<tr>
<td>OS</td>
<td>Overall survival</td>
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<tr>
<td>PB</td>
<td>Peripheral blood</td>
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<tr>
<td>PCR</td>
<td>Partial cytogenetic response</td>
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<tr>
<td>PD</td>
<td>Pharmacodynamics</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<td>--------------</td>
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<tr>
<td>PoD</td>
<td>Progression of Disease</td>
</tr>
<tr>
<td>PK</td>
<td>Pharmacokinetics</td>
</tr>
<tr>
<td>PR</td>
<td>Partial response</td>
</tr>
<tr>
<td>RAEB</td>
<td>Refractory cytopenia with excess blasts</td>
</tr>
<tr>
<td>RAEB-t</td>
<td>Refractory cytopenia with excess blasts in transformation</td>
</tr>
<tr>
<td>RARS</td>
<td>Refractory anemia with ringed sideroblasts</td>
</tr>
<tr>
<td>RC</td>
<td>Refractory cytopenia</td>
</tr>
<tr>
<td>SC</td>
<td>Subcutaneous</td>
</tr>
<tr>
<td>SPC</td>
<td>Summary of Product Characteristics</td>
</tr>
<tr>
<td>UD</td>
<td>Unrelated donor</td>
</tr>
<tr>
<td>ULN</td>
<td>Upper Limit of Normal</td>
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<tr>
<td>WHO</td>
<td>World Health Organization</td>
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## SYNOPSIS

<table>
<thead>
<tr>
<th>Main Study Objective</th>
<th>To establish the recommended dose and preliminary efficacy of azacitidine in children with newly diagnosed and relapsed advanced MDS (either primary or secondary MDS) or JMML.</th>
</tr>
</thead>
</table>
| Additional Study Objectives | To determine the safety and tolerability of azacitidine in newly diagnosed and relapsed advanced MDS (either primary or secondary MDS) and JMML  
• To determine (preliminary) the hematological remission rate in these patients  
• To describe the durability of response and long-term follow-up, including that of patients undergoing stem-cell transplant after treatment with azacitidine  
• To determine the plasma pharmacokinetic parameters of azacitidine  
• To study the pharmacodynamic effects of azacitidine in 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 in 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, either at initial diagnosis or at relapse. A separate cohort of patients with secondary advanced MDS will be enrolled, excluding secondary MDS-cases that are associated with bone-marrow failure syndromes or familial MDS. 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 evidence 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. |
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)

<table>
<thead>
<tr>
<th>Study Population</th>
<th>Inclusion criteria:</th>
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<tbody>
<tr>
<td></td>
<td>In this study 5 subgroups of patients are eligible, which will be enrolled in 5 different strata:</td>
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<tr>
<td></td>
<td>• <strong>stratum 1</strong>: newly diagnosed patients with primary advanced MDS (RAEB or RAEB-t) in a ‘pre stem cell transplantation window’.</td>
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<td></td>
<td>• <strong>stratum 2</strong>: relapsed patients with advanced primary MDS in a ‘re-transplantation window’. At relapse azacitidine may also be continued when a 2\textsuperscript{nd} transplant is not feasible, as long as the patient benefits from treatment.</td>
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<tr>
<td></td>
<td>• <strong>stratum 3</strong>: newly diagnosed patients with JMML in a ‘pre-stem cell transplantation window’.</td>
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<tr>
<td></td>
<td>• <strong>stratum 4</strong>: relapsed patients with JMML in a ‘re-transplantation window’. Azacitidine may also be continued when a 2\textsuperscript{nd} transplant is not feasible and as long as the patient benefits from treatment.</td>
</tr>
<tr>
<td></td>
<td>• <strong>stratum 5</strong>: 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.</td>
</tr>
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</table>

**General conditions:**

• Advanced primary or secondary MDS or JMML confirmed by the diagnostic criteria as specified in the EWOG-MDS 2006 protocol (see appendix 1)
• 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\textsuperscript{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.
- For patients with childbearing potential, a negative test for pregnancy is to be considered before entry on study. If applicable, use of an effective contraceptive method.
- Written informed consent from patients or from parents or legal guardians for minor patients, according to local law and regulations.

**Exclusion criteria:**

*Prior or current history:*
- 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.

**Detailed Study Design**

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.

However, there are strict time restriction between dissolving azacitidine for and the end of the infusion (45 minutes), which is logistically difficult in most centers. Therefore, sites are allowed to choose between IV and SC administration, whichever suits best for them (see administration guidelines below). However, the route of administration should always be the same for all patients enrolled at one study-site, and should also be fixed per patient.

**SC administration and time-lines between dissolving and administration:**
- For immediate use of the SC administration the time between dissolving and administration is 45 minutes, when the
reconstituted product is kept at room temperature.

- For delayed administration: when the reconstituted product is refrigerated immediately, it may be held under refrigerated conditions (2°C - 8°C; 36°F - 46°F) for up to 8 hours. After removal from refrigerated conditions, the suspension may be allowed to equilibrate to room temperature for up to 30 minutes prior to administration.

- 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:**

In case of IV administration the total dose will be delivered in a period of 10 to 15 minutes on a syringe pump. The administration must be completed within 45 minutes of reconstitution of the azacitidine vial. 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 we will use a mg/kg dose. Two dose levels will be studied. One course is defined as 7 days of azacitidine, 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 of 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 clear progressive disease will be taken of study, and can either be transplanted directly in case a donor is available, or receive other chemotherapy.

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 perceive 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 Biopharmaceuticals.

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 duration and time schedule**

Initially, the aim is to recruit a total of 6 relapsed MDS or JMML patients (stratum 2, 4 or 5) at the starting dose-level. When 6 patients are treated in either of these strata, cohort 1 and 3 (including newly diagnosed patients) may start enrolling patients provided the drug is safe in the relapsed setting. An independent Data Safety Monitoring Board (DSMB) will be set up to determine this.

Based on the available data (safety, response, PK) following treatment of 6 patients in each of the strata, we will enrol another 6 patients per stratum at the proposed recommended dose, to expand the safety data. Therefore, we will recruit a maximum of 12 patients in each stratum, and hence 60 patients in total. Including screen failures or drop-outs we may need to recruit approximately 65 patients. The study will last approximately 4 years from first patient first visit (FPFV) to last patient last visit (LPLV).

Note: since the stem cell transplantation techniques continue to improve the number of relapses may drop, and we may not be able to recruit enough patients in stratum 2 or 4. Within EWOG-MDS approximately 8 patients with secondary MDS after chemo/radiotherapy are registered each year, hence, we feel that recruitment of 12 patients in stratum 5 should be feasible. This stratum consists of patients with very different...
pre-treatment, and prognosis is poor, and hence we decided to combine patients with newly diagnosed and relapsed secondary MDS in one stratum.

| Guidelines for Dose Escalation of Azacitidine | Intra-patient dose-escalation in newly diagnosed MDS patients enrolled in stratum 1 is not allowed, because of their anticipated slow response to azacitidine, and because they will likely be transplanted after the 3rd course of treatment.  
   
   Intra-patient dose-escalation in patients with relapsed MDS (stratum 2) 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). This is also allowed for stratum 5 patients.  
   
   In newly diagnosed JMML patients enrolled in stratum 3, intra-patient dose-escalation is allowed if the patient does not receive a PR after 1 course, or a CR after 2 courses (see section 6.7 for response criteria).  
   
   For relapsed JMML patients in stratum 4 this may take place after the 1st course similar to newly diagnosed patients.  
   
   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.  
   
   Inter-patient dose-escalation  
   Especially in JMML patients the required dose-level is unknown, as this disease does not occur in adults. For the cohorts in whom intra-patient dose-escalation (stratum 3 and 4) is allowed the number of patients requiring dose-escalation will be assessed as soon as the first cohort of 6 patients has been treated.  
   
   In case 3 or more patients required dose-escalation and there have been no major safety concerns with the higher dose, the higher dose-level and/or shorter interval between courses, will be considered to be used for the 2nd cohort of 6 patients. At this moment in time we will aim to have the clinical response available for these 6 patients. When we see recovery of disease activity during the interval between courses we may also consider shortening the interval to 21 rather than 28 days. We will seek advice of the Data Safety Monitoring Board (DSMB) before implementing the dose-level of the 2nd cohort of JMML (stratum 3 and 4) patients. |
Safety Assessment and Guidelines for Dose Reduction of Azacitidine

Toxicity monitoring will be done applying the most recent 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 not 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.

Hematologic DLTs:
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.

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:
<table>
<thead>
<tr>
<th><strong>Dose reduction for hematological toxicity</strong></th>
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<tbody>
<tr>
<td>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 4.4.2.</td>
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</table>

<table>
<thead>
<tr>
<th><strong>Dose reduction for non-hematological toxicity</strong></th>
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</thead>
<tbody>
<tr>
<td>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 ( \leq ) grade 1 (or ( \leq ) grade 2, if this is baseline). Subsequent courses may then be given at the next lower dose-level. Please see details in paragraph 4.4.2 in the protocol.</td>
</tr>
</tbody>
</table>

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.
STUDY FLOWCHART

ELIGIBILITY

MDS
- Stratum 1: Newly diagnosed MDS, pre HSCT
- Stratum 2: Relapsed advanced MDS, pre second HSCT

JMML
- Stratum 1: Newly JMML, pre HSCT window
- Stratum 2: Relapsed JMML, pre second HSCT window

Secondary MDS
- Stratum 1: Newly or relapsed sec. advanced MDS

SCREENING
- Age and body weight
- < 1 year of age or < 10 kg
  - Level 1: 2.5 mg/kg/day IV in 10-15 min or SC x 7 days with a 28-day interval
  - Level 2: 3.3 mg/kg/day IV in 10-15 min or SC x 7 days with a 28-day interval
- > 1 year of age and > 10 kg
  - Level 1: 75 mg/m²/day IV in 10-15 min or SC x 7 days with a 28-day interval
  - Level 2: 100 mg/m²/day IV in 10-15 min or SC x 7 days with a 28-day interval

STUDY FLOWCHART

TREATMENT

Cycle 1
- Adverse Events and Response Evaluation CRF to sponsor

Cycle 2
- Adverse Events and Response Evaluation CRF to sponsor

Cycle 3
- Adverse Events and Response Evaluation CRF to sponsor

Cycle 4
- Donor available and HSCT preparations finalized

Progression of Disease
- OFF STUDY

Dose adjustments:
- C, E escalation / reduction / continuation

Death
- Per IRB

A: Stratrum 1 and 3 will be opened by signal of the DSMB (after n=6 in stratum 2, 4 and or 5)
B: Written informed consent has to obtained before any study specific procedure;
C: Intra patient dose escalation rules
1) Intra-patient dose escalation in Stratrum 1 is not allowed;
2) Intra-patient dose escalation in patients with relapsed MDS or secondary MDS (Stratum 2 or 3) 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)
3) In newly diagnosed JMML patients enrolled in Stratrum 3, intra-patient dose escalation is allowed if the patient does not receive a PR after 1 course, or a CR after 2 courses.
4) For relapsed JMML patients in Stratrum 4 this may take place after the 1st course similar to newly diagnosed patients.
D: Until donor available, as long there is benefit and no major safety concerns, or until more curative options become available, or until clear Progressive disease
E: Inter-patient dose escalation rules: see section 4.4.1 of the protocol.
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1 BACKGROUND AND STUDY RATIONALE

Myelodysplastic and myeloproliferative disorders in childhood are heterogeneous diseases, and include both MDS and JMML (Hasle et al, 2003). 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 (Niemeyer et al, Hematology 2011). All other cases are referred to as primary MDS, although it is reasonable to assume that most of these disorders are secondary to some as-yet-unknown genetic predisposition. These presumed underlying genetic changes may also give rise to subtle phenotypic abnormalities observed in many children with primary MDS.

1.1.1 Primary childhood MDS

Myelodysplastic syndrome (MDS) is a clonal proliferative stem cell disease, leading to bone marrow insufficiency. MDS is morphologically characterized by dysplasia in at least two cell lines (erythrocytes, leukocytes, thrombocytes).

Childhood MDS differs in presentation from adult MDS, as a relatively large proportion of cases are hypoplastic in contrast to adults in which the majority is myeloproliferative. In children, there are no data to indicate whether a blast threshold of 20%, as proposed in the WHO classification issued in 2000, is superior to the traditional 30% to distinguish MDS from AML (Hasle et al, 2003). Moreover, there are very few cases with ring-sideroblasts, and the 5q- syndrome is exceedingly rare in children. Therefore a pediatric modification of the WHO-classification is being used.

<table>
<thead>
<tr>
<th>Type</th>
<th>Abbreviation</th>
<th>Bone marrow</th>
<th>Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>Refractory cytopenia</td>
<td>RC</td>
<td>&lt; 5 % blasts</td>
<td>&lt; 2 % blasts</td>
</tr>
<tr>
<td>Refractory anemia with excess blasts</td>
<td>RAEB</td>
<td>5 – 19 % blasts</td>
<td>2 – 19 % blasts</td>
</tr>
<tr>
<td>RAEB in transformation</td>
<td>RAEBt</td>
<td>20 – 29 % blasts</td>
<td>20 – 29 % blasts</td>
</tr>
<tr>
<td>Myelodysplasia-related AML</td>
<td>MDR-AML</td>
<td>&gt; 30 % blasts</td>
<td>Any percentage</td>
</tr>
</tbody>
</table>

Patients with non-random translocations, such as t(15;17)(q22;q12), inv16(p13q22) or t(8;21)(q22;q22), and patients with Down Syndrome (DS) and myelodysplasia/myeloid leukemia are not considered MDS patients, but are treated according to de novo AML protocols independent of blast percentage (Chan et al, 1997). Otherwise the separation between high-grade MDS and de novo AML should be done according to the European Working Group on MDS in Children (EWOG–MDS group) recommendations, which include parameters such as monosomy 7 and blast count <20% (more likely MDS) versus blasts count >30%, leucocytosis/organomegaly (more likely AML). A rise in blast percentage in a 2nd bone marrow taken after 2 weeks may also be indicative of AML rather than MDS.

About 500 newly diagnosed pediatric MDS patients have been registered in the EWOG central database in Freiburg. About 40% of the patients suffer from high-grade MDS (RAEB + RAEBt), whereas another 40% has refractory cytopenia (RC).
In contrast to adult MDS, the therapeutic aim in childhood MDS is cure, which implies disease eradication rather than palliation. Currently, in order to cure the children, hematopoietic stem cell transplantation is the only effective therapeutic option. Hence, most children with MDS will be transplanted and approximately half of these transplanted children will experience long-term survival (Stary et al, 2005).

**High-grade or advanced MDS**
A recent analysis of EWOG of 105 pediatric patients (median age of 10.6 years at diagnosis and range 1.0-18.2 years) with high-grade MDS (also referred to as advanced MDS) showed that the probability of event-free survival was 56% after stem-cell transplantation. Disease recurrence was reported in approximately a quarter of the transplanted cases, with transplant related mortality as competing event. Given this high number of relapses, it could be anticipated that better disease control pre-HSCT may lead to improved outcome. However, the advantage of intensive chemotherapy pre-HSCT has never been shown, and carries the risk of long-lasting aplasia with transfusion dependency and infections, including serious fungal infections. Therefore, less bone-marrow toxic induction therapy while waiting for a suitable donor could be of benefit to prevent disease progression and avoid serious side effects. Clearly, azacitidine would qualify for this, given the favorable safety profile reported in adults, especially as it does not increase the rate of infections or bleeding (Silverman et al, 2006). Reduction of disease levels pre-transplant may also result in less relapses post-HSCT, as has already been shown for various subtypes of leukemia (Bader et al, 2002, Bader et al, 2009). In fact, in current ALL protocols it is acceptable to use experimental treatment approaches to reduce minimal residual disease levels to less than 1x10-3, as higher levels are invariably associated with relapse post-HSCT.

**Current treatment of newly diagnosed and relapsed MDS**
Current EWOG-MDS therapy guidelines for newly diagnosed advanced MDS consist of allogeneic stem-cell transplantation. The current HSCT trial of EWOG-MDS utilizes a preparative regimen consisting of busulfan, cyclophosphamide and melphalan. Intensive chemotherapy prior to HSCT is not recommended. In case of relapse a 2nd transplant should be considered.

**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.(Hasle H et al, Br J Haematol, 2011) The most frequently involved drugs causing secondary MDS include alkylating agents, epipodophyllotoxins and anthracyclines. The majority of secondary MDS are morphologically characterized by multi-lineage myeloid dysplasia; the great majority have chromosome abnormalities, the most common being the loss of either part or all of chromosome 7 and/or 5 (7q/-7, /5q-, -5). Approximately 20-30% of children diagnosed with MDS in SCT-series have secondary MDS. (Madureira et al, 2011; Woodard, P et al, 2011). However, the real frequency is not known as many patients may not be offered SCT and may not be treated apart from palliative care. In the EWOG/MDS 2006 study, 42 patients with MDS secondary after chemotherapy or radiotherapy were registered, hence approximately 8 new patients per year.

In secondary MDS, cure can only be achieved with a stem cell transplantation. Whether this is feasible depends on the patients prior history, i.e. on the cumulative dosage of prior chemotherapy and/or radiotherapy, the interval between the prior treatment episode and the occurrence of secondary MDS, specific toxicities due to prior treatment, and/or prior stem-cell transplantation that a patient may have received. Children with MDS secondary to chemo- or radiation-therapy generally have a very poor survival. AML-type therapy may induce remission but very few patients remain in remission and even HSCT has been reported to offer cure to only 20–30% of patients. (Hasle et al, Br J Haematol 2011)
Given the poor outcome of these patients and the fact that some of these patients may not be fit to undergo transplant, we consider it reasonable to offer them treatment with azacitidine. MDS in childhood may be associated with Fanconi's anemia, Kostmann's syndrome, Schwachman-Diamond syndrome, and other inherited diseases characterized by chromosome breakage. Since these diseases are entirely different in origin, cases with secondary MDS after bone marrow failure syndromes are not included in this study and not discussed in further detail here.

1.1.2 JMML

Juvenile myelomonocytic leukemia (JMML) is a rare clonal myeloproliferative disorder, accounting for less than 3% of all hematologic malignancies in children (Locatelli et al., 2005, Niemeyer & Kratz, 2008). JMML predominates in young children (median age at diagnosis, 2 years). There is a male predominance with a male: female ratio of 2:1 (Castro-Malaspina et al., 1984, Niemeyer et al., 1997). The reason for this male predominance is unknown.

Pallor, fever, infection, skin infiltration and dry cough are the most commonly presenting symptoms. The disease is characterized by generally marked splenomegaly and hepatomegaly, thrombocytopenia, monocytosis and elevated HbF. Other typical presenting symptoms can be tachypnoe or failure to thrive. Infiltration of the monocytes into the lungs and gastrointestinal tract can respectively lead to respiratory problems and diarrhea. Anemia and thrombocytopenia are often present. Unlike acute monoblastic leukemia, JMML rarely involves the central nervous system (CNS).

Karyotype aberrations are mainly found in chromosome 7. Monosomy 7, deletion of 7q and other chromosome 7 abnormalities occur in approximately 25-30% of cases (Niemeyer et al., 1997), but are not associated with outcome. Besides chromosome 7 abnormalities, other karyotype changes are noted in 5-10%; predominately involving chromosome 3 and 8 (Emanuel, 1999).

JMML was diagnosed in the past by demonstrating GMCSF hypersensitivity, which is mediated by activation of the RAS-pathway. Molecular aberrations in this pathway are frequently found in JMML patients, and have recently been included in the diagnostic criteria for JMML, including mutations in NF1, PTPN11 and RAS itself. These mutations are present in a mutually exclusive manner. Taken together, 35% of the JMML patients show somatic mutations in PTPN11 (Tartaglia et al., 2003), 20-25% aberrations in the RAS gene (Miyauchi et al., 1994; Flotho et al., 1999), 11% of JMML were diagnosed with clinical NF1 (Niemeyer et al., 1997), whereas ~15% of the patients as described by Side et al. (1998) had mutations in the NF1 gene without showing a clinical diagnosis of NF1. Recently, CBL was found to be mutated in 15% of all JMML patients. This mutation was not detected in patients with a known RAS or PTPN11 mutation (Loh et al., 2009).

The diagnostic criteria were set up by the International JMML Working Group and include the features given in appendix 1 (Chan et al., 2009, de Vries 2010).

For most children, JMML is a rapidly fatal disorder if left untreated. Most patients die from respiratory failure due to pulmonary infiltration with monocytes. The median survival time without haematopoietic stem cell transplantation (HSCT) is approximately 1 year (Niemeyer et al., 1997). Despite this, some factors such as higher age at diagnosis, increased HbF, and lower platelets are predictors of poor outcome in this disease (Niemeyer et al., 1997; Locatelli et al., 2005).

Long-term survival has only been achieved with HSCT, and there are no confirmed drugs that are curative for JMML in the absence of HSCT (Locatelli et al., 2005). The analysis of the EWOG-MDS/EBMT trial of 100 patients with JMML transplanted with a preparative regimen of busulfan
Azacitidine in high grade MDS and JMML pediatric patients

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(BU), cyclophosphamide (CY) and melphalan showed a 5-year EFS of 52% (Locatelli et al, 2005). The EFS of patients transplanted from a matched family donor (MFD) and unrelated donor (UD) were not significantly different. Relapse is a major cause of treatment failure, which is observed in up to 50% patients, with toxicity as competing event. Re-transplantation is the only option for relapsed patients, but may sometimes not be achieved due to lack of disease control or morbidity/toxicity from the previous procedure.

In JMML, as advised by the EWOG-MDS group, the role of anti-leukemic therapy prior to transplantation is uncertain. 6-Mercaptopurine (6-MP) is probably the drug most commonly applied in JMML prior to HSCT to control the tumor burden, and may be administered either as single-agent or in combination with low-dose cytarabine. However, the response is transient, and there are no data indicating that it influences the duration of survival. Other investigators pointed out that intensive chemotherapy is unsuccessful, especially in patients with aggressive disease, and durable remission may not be achievable. In a recent prospective analysis of the EWOG-MDS/EBMT trial, neither EFS was improved, nor was relapse incidence reduced in patients who had received intensive chemotherapy before the allograft. Thus, in view of these results, intensive chemotherapy prior to HSCT cannot be recommended outside clinical trials.

The benefit of splenectomy for prevention of post-transplant relapse has never been proven. In the current HSCT study of the EWOG-MDS, splenectomy did not improve the survival of the patient after HSCT.

In the United States a trial with tipifarnib (Zarnestra), a farnesyl transferase inhibitor, has been performed. FTase cause a post-translational modification required for RAS activation. As described above, activation of the RAS pathway is one of the central factors in the pathogenesis of JMML, and therefore a FTase inhibitor could be an appropriate agent to treat MDS. In this study, promising CR/PR rates were found, but this clinical activity was not due to blocking RAS farnesylation and did not correlate with RAS mutational status (Castleberry et al, 2005). In addition no advantage regarding overall survival has been reported so far.

**Newly diagnosed and relapsed JMML**

Current treatment guidelines provided by EWOG-MDS suggest that all children with JMML should receive an allogeneic HSCT as soon as the diagnosis is established. There is currently no evidence that therapy prior to HSCT improves survival following HSCT. Oral 6-MP can be administrated in patients with very high leukocytes count, pulmonary problems and/or prominent organomegaly. More intensive chemotherapy is only administrated in severely ill children with rapidly progressive disease before HSCT. First, intravenous cytarabine (Ara-C) can be administrated and if this fails, high dose Ara-C in combination with fludarabine can be considered.

Clearly there is an unmet medical need for a non-toxic agent that may bridge time to HSCT, and reduce disease burden in the preparation time before HSCT. Moreover, effective targeted agents may contribute to decreasing the relapse risk post-HSCT.

### 1.2 EPIGENETICS

#### 1.2.1 Epigenetics in adult MDS

Based on several *in vitro* studies it has been shown that targeting the epigenome may be a useful future strategy. Aberrant methylation of CpG-islands of promoter regions of tumour suppressor genes in adult MDS has been described by several groups. Genes of interest that are frequently hypermethylated in adult MDS include *CDKN2B, CDKN2A, CALCA, CDH1, HIC1, p73, p14ARF, MGMT, APC, RARβ,*

The CDKN2B gene is frequently hypermethylated in adult MDS patients (Uchida et al, 1997), and hypermethylation of this gene might be related to disease progression. Uchida et al. (1997) reported that hypermethylation was significantly more frequent in high-risk MDS patients (78%) compared with low-risk MDS patients (8%; p=.002). This finding was also seen in a study by Aoki et al. (Aoki et al, 2003). Quesnel et al. (1998) reported that methylation of CDKN2B was correlated with blastic bone marrow involvement and disease evolution towards AML. All patients with myelodysplasia-related AML had CDKN2B hypermethylation (Quesnel et al, 1998). On interest, hypermethylation was also linked to clinical outcome. Patients with hypermethylated CDKN2B at diagnosis had a significantly shorter survival than those without hypermethylation of this gene (11 months vs 26 months; p<0.04) (Tien et al, 2001).

In a study by Figueroa et al. (2009) a genome wide approach was used to find promoter hypermethylation in adult MDS patients. They found that epigenetic deregulation was not restricted to cancer-associated genes but appeared to be a more widespread phenomenon. A difference between de novo AML was found in comparison with MDS; hypermethylation was more found in MDS (absolute fold change in log > 1.5; p<0.0005). This hyper-methylation was also more pervasive in the MDS group: 81.6% of genes of the genes differentially methylated between the de novo AML and MDS groups were hypermethylated in the MDS cohort. Aberrantly methylated genes in MDS were found in cancer-related genes such as CDKN2A, genes from the WNT-signaling pathways, MAPK-activity and synaptogenesis. Genes uniquely affected in MDS were those encoding certain transcription factors, genes involved in DNA damage repair and notch signaling genes. The gene PPARD was also hypermethylated; loss of expression of this gene is known to be associated with disease aggressiveness in colon cancer. Hypermethylated genes were re-expressed after the use of azacitidine or etinostat. Data generated at day +15 following the start of treatment still showed significant hypomethylation from baseline genes, which was evenly distributed across all chromosomal regions. This indicates that azacitidine and/or etinostat have a uniform demethylating effect across the genome (Figueroa et al, 2009).

### 1.2.2 Epigenetica in pediatric MDS

In childhood high risk MDS, it was shown that especially the CALCA and CDKN2B were frequently hypermethylated ((Hasegawa et al, 2005; Flotho, 2007; Vidal et al, 2007). Vidal et al. (2007) also found hypermethylation of the CDH1 gene, but this was also seen in normal bone marrow samples. The CDKN2A gene was also investigated but not aberrant (Hasegawa et al, 2005). In Table 2 a summary of the data is provided.

<table>
<thead>
<tr>
<th>Study</th>
<th>N of patients</th>
<th>Hypermethylated gene</th>
<th>% of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vidal et al. (2007)</td>
<td>21</td>
<td>CALCA</td>
<td>75%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CDH1</td>
<td>86%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CDKN2B</td>
<td>50%</td>
</tr>
<tr>
<td>Hasegawa et al. (2005)</td>
<td>9</td>
<td>CDKN2B</td>
<td>78%</td>
</tr>
</tbody>
</table>
The similarities in hypermethylated genes between adult and childhood MDS may indicate that epigenetic targeting is a reasonable strategic option in childhood MDS as well. Many studies have evaluated the use of demethylating agents in adult MDS (see 4.2.2) (Kaminskas et al., 2005; Wijermans et al., 2005; Silverman et al., 2006). In contrast, clinical studies using demethylating agents in pediatric patients are lacking so far.

1.2.3 Epigenetics in JMML

Olk-Batz et al. recently reported the first large study on methylation in JMML patients, analyzing 14 genes in 127 children with JMML. It concerned a candidate gene approach where genes were selected, based on previous reported hypermethylation in other types of cancer or leukemia, or on involvement in the RAS pathway. They found that 4 of the 14 investigated genes were aberrantly hypermethylated in JMML patients, and not in healthy controls (see Table 2). This aberrant methylation was clonal and therefore might be an initiating event in this rare disease (Olk-Batz et al., 2011).

In 2005, Hasegawa et al. investigated the methylation status of CDKN2B and CDKN2A, two cell cycle regulatory genes, in 18 JMML patients. Only in 17% of the JMML patients hypermethylation of CDKN2B was detected; aberrant methylation in CDKN2A was not found (see Table 3) (Hasegawa et al., 2005).

Johan et al. (2005) investigated the methylation status of RASSF1A, SHP-1 and SOCS-1 in 5 JMML patients. These genes are frequently methylated in other adult myeloid malignancies. In the 5 patients, only hypermethylation of the RASSF1A gene (20%) was found (see Table 3) (Johan et al., 2005).

PTEN hypermethylation was found in 23 of 30 JMML patients assessed by Liu et al. (2009) and not seen in healthy controls. This hypermethylation may be responsible for the low PTEN transcription that was in that study (Liu et al., 2009). However, Batz et al. (2009) reported on 90 JMML patients and did not detect hypemethylation of the PTEN gene found (Batz et al., 2009).

Considering the relationship between methylation status and clinical outcome, Olk-Batz et al. divided the patients in a group without hypermethylation (46%), intermediate hypermethylation (1-2 genes; 33.3%) and hypermethylation of 3 or more genes; 20.7%). The group with the highest methylation status had a significantly poorer prognosis (5-year overall survival without hypermethylation 63%, and with hypermethylation 24%, p<0.01) and represented the older patients with an increased level of hemoglobin F at diagnosis (p<0.05) and a lower platelet count (p=0.1) compared with the non methylated group (Olk-Batz et al., 2011). This was due to a higher relapse rate in the patients with the hypermethylation phenotype. This is in contrast with the study performed by Hasegawa et al. (2005), who did not find differences in clinical characteristics between patients with and without hypermethylation. Two patients with progressive JMML evolving into AML showed no hypermethylation in CDKN2B and CDKN2A, so no evidence for progression of disease due to hypermethylation was found. This study however was hampered by a limited number of patients (Hasegawa et al., 2005).

Of interest, the study by Olk-Batz et al also provided evidence of clonal evolution. Patients could show a more hypermethylated phenotype at time of relapse, when compared to their methylation status at diagnosis (Olk-Batz et al., 2011).
Table 3: Frequency of hypermethylated genes in several pediatric JMML studies

<table>
<thead>
<tr>
<th>Study</th>
<th>N of patients</th>
<th>Hypermethylated gene</th>
<th>% of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Olk-Batz et al. (2011)</td>
<td>127</td>
<td>BMP4</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CALCA</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CDKN2B</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RARB</td>
<td>13</td>
</tr>
<tr>
<td>Hasegawa et al. (2005)</td>
<td>18</td>
<td>CDKN2B</td>
<td>17</td>
</tr>
<tr>
<td>Johan et al. (2005)</td>
<td>5</td>
<td>RASSF1A</td>
<td>20</td>
</tr>
<tr>
<td>Lui et al. (2009)</td>
<td>34</td>
<td>PTEN</td>
<td>77</td>
</tr>
</tbody>
</table>

1.3 5-AZACITIDINE

Azacitidine (Vidaza®) is a pyrimidine nucleoside analogue of cytidine. The antineoplastic effect of azacitidine is mainly caused by hypomethylation of DNA, as well as by direct cytotoxicity on abnormal hematopoietic cells in the bone marrow. The predominant effect may very well be dose-dependent.

1.3.1 Mechanism of Action

Azacitidine was developed in 1964 as a classical cytostatic agent (Sorm et al, 1964). Later, it was shown that azacitidine inhibits DNA methylation in human cell lines (Jones et al, 1980).

When azacitidine is transported into the cell, intracellular azacitidine undergoes 3 sequential phosphorylation steps resulting in azacitidine triphosphate. The first phosphorylation reaction is mediated by the enzyme uridine-cytidine kinase. This reaction is subject to feedback inhibition by either uridine triphosphate (UTP) or cytidine triphosphate (CTP). Azacitidine triphosphate may then be incorporated into DNA resulting in inactivation of DNA methyltransferase, leading to hypomethylation of DNA. This DNA hypomethylation of for instance aberrantly methylated genes involved in normal cell cycle regulation, differentiation and cell-survival pathways may result in gene re-expression and restoration of tumor-suppression function in cancer cells.

1.3.2 Pharmacokinetics and Pharmacology

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 pharmacokinetics 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 4: PK variables for SC and IV administration of 75 mg/m\(^2\) azacitidine.**

<table>
<thead>
<tr>
<th>Route</th>
<th>(C_{\text{max}}) ng/mL</th>
<th>AUC(_{(0 \rightarrow \infty)}) ng·h/ml</th>
<th>AUC(_{(0 \rightarrow z)}) ng·h/ml</th>
<th>(T_{1/2}) h</th>
<th>CL L/h</th>
</tr>
</thead>
<tbody>
<tr>
<td>SC</td>
<td>750.0 (± 403.3)</td>
<td>960.53 (± 458.06)</td>
<td>923.88 (± 473.61)</td>
<td>0.69 (± 0.14)</td>
<td>167.48 (± 48.69)</td>
</tr>
<tr>
<td>IV</td>
<td>2750.0 (± 1069.0)</td>
<td>1044.26 (± 285.67)</td>
<td>1025.11 (± 298.06)</td>
<td>0.36 (± 0.02)</td>
<td>146.70 L/h (± 46.91)</td>
</tr>
</tbody>
</table>

\(C_{\text{max}}\): 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

**Pharmacokinetics of azacitidine in Pediatric Patients with MDS**

No pharmacokinetic studies on the administration of azacitidine in pediatric patients have been done. Pharmacokinetic parameters in pediatric patients cannot simply be extrapolated from adult data because of differences in growth and development and changes in kidney and liver functions (metabolizing and eliminating mechanism). Children often tolerate higher dosages than adult patients as a result of increased renal clearance (Kearns et al., 2003).

### 1.3.3 Clinical Experience with azacitidine

**Adult studies**

On May 19, 2004 the U.S. Food and Drug Administration approved azacitidine as injectable suspension for treatment of adult patients with several types of MDS. Hereby, azacitidine was the first agent approved for treatment of MDS. The Cancer and leukemia group B (CALGB) performed 3 studies (phase II and III studies) to reveal the role of azacitidine in MDS patients (Silverman et al., 1993; Silverman et al., 1994; Silverman et al., 2002).

The CALGB 9221 study was reported by Silverman et al. (2002) and concerned a phase III study with azacitidine. This was a controlled study and concerned a randomization to compare the safety and efficacy of SC administrated azacitidine with best supportive care in patients with MDS. Azacitidine (75 mg/m\(^2\)/day) was given for 7 consecutive days every 28 days. If a beneficial effect was not seen at day 57 (start of course 3) and no significant toxicity had occurred, the dose was increased by 33%. If benefit was seen, azacitidine was continued unless toxicity developed. Patients with no response after four azacitidine courses were considered as treatment failures and taken off the study (Silverman et al., 2002).

The group receiving best supportive care was allowed to cross over to the azacitidine arm after 4 months if their disease had worsened. Withdrawal before four months was only permitted when patients transformed to AML with less or equal to 40% of blasts.

In total 191 patients were randomized, the azacitidine group and best supportive care group consisted of respectively 99 and 92 patients. Patients had a median age of 68 years (range 31-92 years) and consisted of 132 male and 59 female subjects. The two groups were well balanced at study entry according to FAB classification, cytogenetic abnormalities, prognostic scoring rate and time from diagnosis to study entry.
Bone marrow samples were obtained before study entry and at day 57 (start of course 3) and day 113 (start of course 4). Response criteria were defined as follows:

- **trilineage response:** \( \geq 50\% \) restitution of the initial deficit from normal in all three peripheral-blood cell counts and elimination of all blood transfusion requirements.
- **improvement:** monolineage or bilineage response: \( \geq 50\% \) restitution of the initial deficit from normal in one or two peripheral blood cell counts.
- **complete response (CR):** complete normalization of the peripheral blood cell counts and bone marrow blast percentages (< 5% blasts) for at least four weeks.
- **partial response (PR):** \( \leq 50\% \) of the initial bone marrow blasts, absence of blasts in peripheral blood and a trilineage response in the peripheral blood.

This study showed that the best supportive care group showed an improvement in 5% of the patients but no PR or CR was achieved. In the azacitidine group a total of 60% of the patients showed a response \((p<.0001)\) (CR in 7% \((p=.01)\), PR in 16% or hematological improvement in 37%; CR and PR \((p<.0001)\)). This response was independent of MDS classification and the best responding patients demonstrated response at the beginning of the third or fourth month. In detail, the initial response occurred after 64 days whereas the best response was shown after 93 days. The median duration of the response was 15 months.

Forty-nine patients crossed over from supportive care to azacitidine treatment. CR was achieved in 10%, PR in 4% and 33% showed an improvement, which means that an overall response rate of 47% was found.

The median time to transformation to AML or death was 12 months in the best supportive care group and 21 months in the azacitidine group \((p=.007)\). For patients with MDS RAEB, RAEBT or CMMoL, the median time to AML or death for the supportive care group was 8 months compared with 19 months in the azacitidine group \((p=.004)\). They concluded that FAB subtype was a significant predictor of time to AML or death \((p=.0003)\).

Quality of life improvement over time was significantly greater in azacitidine treated patients than in the supportive care group. Even the group patients who crossed over from supportive care to azacitidine treatment showed improvement in quality of life.

The median survival rate was assessed in different groups after 6 months: patients in the best supportive care group without or with late (after 6 months) crossover had a median survival of 11 months, the best supportive care group with a crossover before 6 months had a median survival time of 14 months. The azacitidine group had a median survival of 18 months, this was significantly longer than the supportive care group without or late crossover \((p=.03)\).

The most common experienced toxicity was myelosuppression. This toxicity was transient and patients usually recovered in time for the next treatment course. Furthermore, infections related to treatment and nausea or vomiting were seen.

Another phase III study investigated the use of azacitidine in high grade MDS patients compared with conventional care \((\text{Fenaux et al., 2009})\). The study design comprised a conventional care group as a comparator, which contained patients treated with the most common treatments options for patients with high risk MDS, namely supportive care, low dose cytarabine and intensive chemotherapy. No crossover was allowed in this study. Azacitidine \((75 \text{ mg/m}^2/\text{day})\) was administrated SC for 7 days every 28 days and was given for at least 6 courses. Best supportive care consisted of blood transfusions and antibiotics with granulocyte-colony-stimulating factor for neutropenic infection. Low dose cytarabine
(20 mg/m²/day) was administrated SC for 14 days q 28 days for at least 4 courses. Intensive chemotherapy was based on an induction phase (cytarabine (100-200 mg/m²/day) per continuous intravenous infusion for 7 days plus 3 days of either intravenous daunorubicin (45-60 mg/m²/day), idarubicin (9-12 mg/m²/day) or mitoxantrone (8-12 mg/m²/day)). When achieving complete or partial remission after the induction phase, one or two consolidation courses were given followed by supportive care.

Investigators determined which of the 3 consolidations treatments was most appropriate for each patient. Therefore, 222 patients were selected to supportive care, 94 to low-dose cytarabine and 42 to intensive chemotherapy. These patients, in total 358, were randomly assigned one to one to either the best consolidation or the azacitidine group (n = 179). The median age in the azacitidine group was 69 years (range: 42-83) and in the conventional care group 70 (range: 38-88).

The primary efficacy endpoint was overall survival. The median overall survival showed a difference of 9.4 months comparing the two groups. In the azacitidine group median overall survival was 24.5 months (range: 9.9 – not reached) compared with 15 months (range: 5.6 – 24.1) in the conventional care group (p=.0001). This effect of azacitidine was consistent in the different subgroups of patients e.g. in cytogenetic differences.

The overall survival effect was also seen in the different conventional care groups compared with azacitidine. There were significant differences in overall survival favoring azacitidine between azacitidine and best supportive care (9.6 months; p=.0045) and azacitidine and cytarabine (9.2 months; p=.0006). The differences between azacitidine and intensive chemotherapy was also in favor of azacitidine but not statistically significant (9.3 months; p=.51), possible through the small number of patients receiving intensive chemotherapy.

Duration of the responses were significantly longer in the azacitidine group than in the conventional care group (n=13.6 vs. 5.2 months; p=.0002). Median time to transfer to AML was significantly longer for the azacitidine group (17.8 months) compared to the conventional care group (11.5 months; p<.0001).

After 2 years, 26.2% patients in the conventional care group were alive compared to 50.8% in the azacitidine group (p<.0001).

Azacitidine was well tolerated in most patients. The most common events were peripheral blood cytopenias for all treatment groups. Discontinuation of treatment before study completion was mostly related to hematological adverse events. Non-hematological treatment related adverse events were injection site reactions for azacitidine. For all treatment groups were seen nausea, vomiting, diarrhea and fatigue as non-hematological events.

After about 3 months after the first azacitidine course a survival advantage was seen in the azacitidine group compared with the conventional care group. This suggested that long term treatment gave the best survival benefit. This is confirmed by the median number of administrated azacitidine courses (median of 9 courses).

Field et al. (2009) investigated the effect of pre-transplant azacitidine treatment on post transplant outcomes in a retrospective study (Field et al, 2009). Patients with MDS or chronic myelomonocytic leukemia (CMML) who had a HSCT were retrospectively reviewed regarding exposure to azacitidine. In this cohort, in total 54 patients (age 25 – 69 years), 30 did receive azacitidine in the past and 24 did not. This study showed that treatment with azacitidine before HSCT did not significantly affect the outcomes, including rates of remission, relapse, acute and chronic GVHD and survival, compared with patients without azacitidine treatment. However, a trend towards decreased early relapse was seen in
patients using azacitidine (1 year post HSCT the cumulative incidence of relapse was 32 vs 20%, 2 year post HSCT cumulative incidence of relapse was 36 vs 31%). No adverse effects were seen.

**Pediatric studies**

The use of azacitidine in children is limited to multiple pediatric acute leukemia patients, but there is no experience in pediatric MDS or JMML apart from case reports.

A phase I study by Karon et al. (1973) treated 37 children from 2 to 17 year with azacitidine monotherapy to establish a maximum tolerated dose. Azacitidine was administrated for 5 days every 14 days as an IV bolus starting with 2mg/m². The maximum tolerated dose was measured between 150 to 200 mg/m².

More studies were done to evaluate azacitidine combination therapy in AML pediatric patients. Kalwinsky et al. reported in 1988 a complete remission rate of 85% in 68 previously untreated children with AML. In this study azacitidine was administered IV at a dosage of 300 mg/m² at day 4 and 5 in combination with etoposide, cytarabine, daunorubicin and thioguanine.

A phase II randomized study by Steuber et al. (1996) in 41 AML patients who had failed to respond to primary induction therapy with standard doses of daunorubicin, cytarabine and thioguanine, randomized patients to a treatment regime with amsacrine and etoposide with or without azacitidine (250 mg/m²/day on days 4 and 5 IV). The group with azacitidine (n=19) showed a significantly higher CR rate than the group without azacitidine (CR 53% vs 18% p=.03).

Furlan et al. (2009) reported the first case of the use of azacitidine in a JMML patient with monosomy 7 with complete hematologic and molecular response. IV azacitidine was initiated at a dosage of 100 mg/m² on 5 consecutive days repeated every 4 weeks. In this case of JMML the clinical and hematologic response was impressive. After 1 course, already a response was seen in spleen size and monocytes were decreasing (start=2260/µL, course 1=1270/µL). After the 6th course, monosomy 7 disappeared and the RAS mutation, present at diagnosis, was undetectable after 8 courses of azacitidine. After this last course a HSCT was performed and 4 months post-SCT the child remained disease free. Additional molecular studies on samples from this patient showed a reduction in aberrant DNA methylation which reflected the efficacy of treatment. Initially, 55.4% of the CALCA gene was methylated, before the 8th course of azacitidine this was decreased to 4.5%.

### 1.4 RATIONALE FOR THIS STUDY

There is clear medical need in advanced primary or secondary MDS and JMML to control disease pre-SCT 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. Based on adult data in MDS and the favorable safety profile we feel that a study in 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. 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).
2 ELIGIBILITY CRITERIA

2.1 Introduction

A diagnosis of advanced MDS (primary or secondary) or JMML should be established by using the diagnostic criteria as specified in the EWOG-MDS 2006 protocol. These criteria are also provided in appendix 1.

Note: this study does require centralized morphology review, cytogenetics and mutation analysis (the latter for JMML patients only), hence please consider this when planning these assessments.

In this study 5 subgroups of pediatric MDS and JMML patients are eligible, and will be enrolled in 5 different strata:

- **Stratum 1**: newly diagnosed patients with advanced MDS (RAEB or RAEB-t) in a ‘pre stem cell transplantation window’.
- **Stratum 2**: relapsed patients with advanced MDS in a ‘re-transplantation window’. At relapse azacitidine may also be continued when a 2nd transplant is not feasible, as long as the patient benefits from treatment.
- **Stratum 3**: newly diagnosed patients with JMML in a ‘pre stem cell transplantation window’.
- **Stratum 4**: relapsed patients with JMML in a ‘re-transplantation window’. Azacitidine may also be continued when a 2nd transplant is not feasible and as long as the patient benefits from treatment.
- **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. Note: secondary MDS cases after bone-marrow failures or familial cases are not eligible.

2.2 In- and exclusion criteria

**Inclusion criteria**

General conditions:
- Advanced primary or secondary MDS or JMML confirmed by the diagnostic criteria as specified in the EWOG-MDS 2006 protocol (see appendix 1)
- 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 > 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.
- For patients with childbearing potential, a negative test for pregnancy is to be considered before entry on study. If applicable, use of an effective contraceptive method.
• Written informed consent from patients or from parents or legal guardians for minor patients, according to local law and regulations.

**Exclusion criteria:**

Prior or current history:

• 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.
3 PRODUCT CHARACTERISTICS

Azacitidine is the investigational medicinal product in this study, and will be made available free of charge by Celgene. For the complete summary of product characteristics see the latest available approved SPC text in case of subcutaneous usage (current approved version is from 12/2011). For intravenous usage of azacitidine please refer to the latest investigators brochure. The sponsor will provide the English approved version as well as revised and updated versions.

3.1 Azacitidine drug information

3.1.1 Nomenclature

Azacitidine is also known as Vidaza®.

3.1.2 Molecular Structure

Figure: Molecular structure of azacitidine (C₈H₁₂N₄O₅).

3.1.3 Physical and Chemical Characteristics

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 with further dilution for intravenous (IV) infusion.

3.1.4 Storage and Handling

The first drug shipment to a site will be issued after release of an “Investigational Product Authority To Ship Form”, which authorizes Celgene to ship drug to the site once the sponsor (Erasmus MC) has agreed that all regulatory documents are in place. Further details of the drug ordering procedure are specified in the Procedure Manual.

3.1.5 Subcutaneous Administration

Reconstitution: Azacitidine degrades rapidly at room temperature following reconstitution; therefore, the azacitidine suspension should be prepared immediately before use or should be refrigerated immediately after reconstitution.

Azacitidine should be reconstituted aseptically with 4 mL sterile water for injection. The diluent should be injected slowly into the vial. Vigorously shake or roll the vial until a uniform suspension is achieved. The resulting suspension will be cloud. The resulting azacitidine concentration will be 25 mg/mL. Do not filter the suspension after reconstitution. Doing so could remove the active substance.
If a dose in excess of 100 mg (4 mL) is required, repeat the above step for preparation of the suspension. In such circumstances the dose should be equally divided into 2 syringes and injected into 2 separate sites. For example a dose of 150 mg (final suspension volume = 6 mL) should be equally divided into 2 syringes with 3 mL suspension in each. All syringes should be prepared prior to starting administration.

Azacitidine is supplied in single-use vials that cannot be used more than once.

**Subcutaneous Administration:** To provide a homogeneous suspension, the contents of the dosing syringe must be re-suspended immediately prior to administration. To re-suspend, vigorously roll the syringe between the palms until a uniform, cloudy suspension is achieved.

For SC dosing, rotate sites for each injection (thigh, abdomen, or upper arm). New injections should be given at least one inch (2.5 cm) from an old site and never into areas where the site is tender, bruised, red, or hard. Subcutaneous doses greater than 100 mg (4 mL) should be divided equally into 2 syringes and injected into 2 separate sites.

**Suspension Stability:** Vidaza® reconstituted for SC administration should be stored as directed (see Storage of the reconstituted product)

### 3.1.6 Storage of the reconstituted product for SC administration

**For immediate use:**
The Vidaza suspension may be prepared immediately before use and the reconstituted suspension should be administered within 45 minutes. If elapsed time is greater than 45 minutes, the reconstituted suspension should be discarded appropriately and a new dose prepared.

**For delayed use:**
When reconstituting using water for injections that has not been refrigerated, the reconstituted suspension must be placed in a refrigerator (2°C to 8°C) immediately after reconstitution, and kept in the refrigerator for a maximum of 8 hours. If the elapsed time in the refrigerator is greater than 8 hours, the suspension should be discarded appropriately and a new dose prepared.

When reconstituting using **refrigerated (2°C to 8°C) water** for injections, the reconstituted suspension must be placed in a refrigerator (2°C to 8°C) immediately after reconstitution, and kept in the refrigerator for a maximum of 22 hours. If the elapsed time in the refrigerator is greater than 22 hours, the suspension should be discarded appropriately and a new dose prepared.

The syringe filled with reconstituted suspension should be allowed up to 30 minutes prior to administration to reach a temperature of approximately 20°C-25°C. If the elapsed time is longer than 30 minutes, the suspension should be discarded appropriately and a new dose prepared.

### 3.1.7 Intravenous Administration

**Reconstitution:** Reconstitute the appropriate number of vials to achieve the desired dose. Reconstitute each vial with 10 mL of sterile water for injection. Vigorously shake or roll the vial until all solids are dissolved. The resulting solution will contain azacitidine 10 mg/mL. 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.
Withdraw the required amount of product solution to deliver the desired dose and inject into a 50-100 mL infusion bag of either 0.9% Sodium Chloride Injection or Lactated Ringer’s Injection.

**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.

### 3.1.8 Drug accountability

It is the responsibility of the Investigator to ensure that the investigational product (azacitidine) is only dispensed to study subjects. The investigational product must be dispensed only from official study sites by authorized personnel, according to local regulations.

The lot numbers, dosing start dates and the number of vials for each dosage strength must be recorded. The drug accountability must be up to date and available.

It is the responsibility of the Investigator to ensure that a current record of investigational product disposition is maintained at each study site where investigational product is inventoried and disposed. Records or logs must comply with applicable regulations and guidelines, and should include:

- Amount received and placed in storage area.
- Amount currently in storage area.
- Label ID number or batch number and use date or expiry date.
- Dates and initials of person responsible for each investigational product inventory entry/movement.
- Method of reconstitution
- Method of administration (IV/SC)
- Time interval between reconstitution and administration
- Amount dispensed to and returned from each subject, including unique subject identifiers.
- Amount transferred to another area/site for dispensing or storage.
- Non-study disposition (e.g., lost, wasted, broken).
- Amount destroyed at study site.

Used or partially used investigational products will be destroyed on site, as well as unused vials, according to hospital procedures. It is the Investigator’s responsibility to ensure that arrangements have been made for disposal, and that written authorization has been granted by the sponsor, and that procedures for proper disposal have been established according to applicable regulations, guidelines, and institutional procedures. Appropriate records of the disposal must be maintained.
3.1.8 Toxicity

Adult Patients

The most common adverse reactions of azacitidine in adult patients are thrombocytopenia, neutropenia, anemia, leucopenia, nausea, vomiting, pyrexia, diarrhea, injection site erythema, constipation, ecchymosis, petechiae, rigors, weakness and hypokalemia.

In case of SC administration the majority of skin and subcutaneous adverse reactions were associated with the injection site. None of these adverse reactions led to temporary or permanent discontinuation of azacitidine, or reduction of azacitidine dose in the pivotal study. The majority of adverse reactions occurred during the first 2 courses and tended to decrease with subsequent courses. Adverse reactions associated with SC use such as injection site rash/inflammation/pruritus, rash, erythema and skin lesion may require management with concomitant medicinal products, such as antihistamines, corticosteroids and non-steroidal anti-inflammatory drugs (NSAIDs).

Adverse reactions that result most frequently in clinical intervention (such as discontinuation of azacitidine) are leucopenia, thrombocytopenia or neutropenia. Dose interruptions can occur due to leucopenia, neutropenia, thrombocytopenia, pyrexia, pneumonia or febrile neutropenia.

For detailed information on azacitidine toxicology refer to the full prescribing information for azacitidine in the SPC.

Azacitidine should not be administered to subjects allergic to azacitidine or mannitol.

3.1.9 Interaction with other medicinal products and other forms of interaction

Based on in vitro data, azacitidine metabolism does not appear to be mediated by cytochrome P450 isoenzymes (CYPs), UDP-glucuronosyltransferases (UGTs), sulfotransferases (SULTs), and glutathione transferases (GSTs); interactions related to these metabolizing enzymes in vivo are therefore considered unlikely. Clinically significant inhibitory or inductive effects of azacitidine on cytochrome P450 enzymes are unlikely. No formal clinical drug interaction studies with azacitidine have been conducted.
4 STUDY DESIGN AND TREATMENT PLAN

4.1 Primary Objectives
The current study aims to establish the recommended dose, safety and preliminary efficacy of azacitidine administered IV or SC in children with newly diagnosed or relapsed/refractory advanced primary or secondary MDS or JMML, in 5 different subgroups (strata) of patients.

4.2 Secondary Objectives
Secondary objectives:
• To determine the safety and tolerability of azacitidine per stratum.
• To determine (preliminary) the hematological remission rate in these patients.
• To describe the durability of response and long-term follow-up, including that of patients undergoing stem-cell transplant after treatment with azacitidine.
• To determine the plasma pharmacokinetic parameters of azacitidine.
• To study the pharmacodynamic effects of azacitidine in pediatric MDS and JMML.

4.3 Administration
In the first adult studies azacitidine was administered using continuous IV infusion (Silverman et al., 1993). Later studies showed that SC administration was as effective (Kaminskas et al., 2005). The bioavailability 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). Both routes of administration are approved by the FDA, whereas in Europe only the SC administration is approved.

In this study both IV infusion or SC administration will be allowed. Therefore sites are allowed to choose between IV and SC administration. However, the route of administration should always be the same in a given patient, and should be fixed per site.

Dosing will be 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 azacitidine, 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

4.4 Dose Modifications

4.4.1 Dose escalation
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/m$^2$ 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 azacitidine is mainly eliminated by renal excretion, creatinine has to be carefully monitored.

As the preferred dose in adults (75 mg/m$^2$ 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.

**Intra-patient dose-escalation**

Stratum 1: Intra-patient dose-escalation in newly diagnosed MDS patients is not allowed, because of their anticipated slow response to azacitidine (based on experience in adults), and because these patients will likely be transplanted directly after the 3$^{rd}$ course of treatment.

Stratum 2: Intra-patient dose-escalation in patients with relapsed MDS who started at dose-level 1 may take place after the 3$^{rd}$ course, in patients not achieving CR but showing some response to azacitidine (hence a PR or stable disease). Patients are to be taken off study when they do not show any response after the 6$^{th}$ course of treatment, or when there is clear progressive disease (for instance development of MDS-related AML).

Stratum 3: In newly diagnosed JMML patients, intra-patient dose-escalation is allowed if the patient does not receive PR after 1 course or CR after 2 courses (see 6.7). Note: in case JMML patients develop need for supplemental oxygen therapy they should come off study and be treated with regular chemotherapeutic alternatives such as cytarabine or FLAG.

Stratum 4: For relapsed JMML patients dose-escalation may take place after the 1$^{st}$ course following similar criteria as patients in stratum 3. Note: in case JMML patients develop need for supplemental oxygen therapy they should come off study and be treated with regular chemotherapeutic alternatives such as cytarabine or FLAG.

Stratum 5: dose escalation guidelines for stratum 5 follow the rules given above for stratum 2.

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

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

Especially in JMML patients the required dose-level is unknown, as this disease does not occur in adults. For the cohorts in whom intra-patient dose-escalation (stratum 3 and 4) is allowed the number of patients requiring dose-escalation will be assessed as soon as the first cohort of 6 patients has been treated. In case 3 or more patients required dose-escalation and there have been no major safety concerns with the higher dose, the higher dose-level and/or shorter interval between courses, will be considered to be used for the 2nd cohort of 6 patients. At this moment in time we will aim to have the clinical response available for these 6 patients. When we see recovery of disease activity during the interval between courses we may also consider to shorten the interval to 21 rather than 28 days. We will seek that advice of the Data Safety Monitoring Board before implementing the dose-level of the 2nd cohort of JMML (stratum 3 and 4) patients.

4.4.2 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**: for patients with advanced MDS there are usually reduced counts at start of treatment. WBC counts may vary depending upon the number of circulating blasts. Given that we will provide 3 courses of azacitidine prior to SCT, we will not take hematological toxicity into account and administrate azacitidine courses every 28 days irrespective of counts, unless there is clear evidence of progressive disease towards MDS-related AML (defined as increase of blast percentage > 30% in the bone marrow in 2 consecutive examinations), which will lead to discontinuation of study drug.

- **Stratum 2**:
  - In case patients are to be re-transplanted the advice is similar to the stratum 1 patients.
  - 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 10^9/l and ANC > 1.5 x 10^9/l, and platelets > 75.0 x 10^9/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 1000x10^6 and/or platelets below 50X10^9/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 10^9/l or ANC < 1.5 x 10^9/l or platelets < 75.0 x 10^9/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 3:** 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.

- **Stratum 4:** This is handled in a similar fashion as stratum 3.

**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.

### 4.5 Anti-emetics

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

### 4.6 Administration related supportive care measures

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

### 4.7 Supportive Care

#### Blood Products

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

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

#### Infection Prophylaxis

The use of antibacterial or antifungal prophylaxis is left to the discretion of the investigator and will follow institutional guidelines.

#### Treatment of Fever and Neutropenia

Patients with neutropenia (ANC \( \leq 500 \times 10^6/\text{l} \) (or < 1,000 and falling) and fever should have empiric systemic antibiotics started immediately. Broad-spectrum antibiotics should be initiated according to institutional guidelines, and should cover 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 will only be administrated in case of serious or life-threatening febrile neutropenia.

#### Oral anticonceptives

If applicable, females should receive oral contraceptives during the entire course of this protocol. Suppression of menses should be continued at least until the platelet count is \( 50 \times 10^9/\text{l} \) without transfusion support.

#### Nutrition

Active measures should be used to prevent weight loss of greater than 10% of pre-illness body weight. If possible, enteral feedings are preferred to parenteral.
4.8 Concomitant Therapy

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

Hepatic and renal function should be assessed prior to and during treatment with azacitidine and it is recommended that the patient’s fluid status and hepatic and renal function be carefully monitored during the drug administration period.

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

4.9 Duration of Therapy

In adult MDS, the median time to respond to azacitidine (CR, PR or hematological improvement) was 3 courses (range 1-17 courses), with 75% of adults having responded by course 4, and 90% by course 6 (Silverman et al, 2006).

Therefore, in all patients, every effort should be made to transplant patients only after having received at least 3 courses of azacitidine prior to HSCT (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 stratum 2 and 4, following relapse, again every effort should be made to transplant patients after 3 courses of azacitidine only, similar to newly diagnosed patients. In case a 2nd HSCT is not possible or HSCT takes longer to prepare, patients may continue azacitidine if they benefit from treatment per investigator’s discretion.

It needs to be mentioned that the HSCT procedure itself is not part of this protocol, and should be performed according to EWOG-MDS or institutional guidelines at the discretion of the principle investigator. We will, however, capture data on the type and date of HSCT as well as outcome data (relapse/survival) to determine whether the prior azacitidine treatment influences the outcome of HSCT. Follow-up will be restricted to one year post-HSCT.

However, patients with clear progressive disease will be taken of study, and can either be transplanted directly in case a donor is available, or receive other chemotherapy.

4.10 Off study criteria

Patients with relapsed advanced MDS should preferably be taken off study only following the 6th course in case of lack of response given that in adults it may take up to 6 courses before a response becomes evident. JMML patients who are clear non-responders may stop therapy after the 2nd course following intra-patients dose-escalation. 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 will be offered to continue with azacitidine as long as demonstrable benefit or safety is allowing. In that case there is no maximum to the number of courses of study treatment, unless:

- general or specific changes in the patient’s condition render the patient unacceptable for further treatment per investigator’s judgment.
- Patients for whom other more curative treatment options (i.e. a stem-cell transplant) will become available, will be taken off study.
- no benefit / lack of efficacy (MDS after at least 6 courses; JMML after at least 2 courses) unless clear progressive disease
- the patient chooses to withdraw from the study.
- the patient becomes pregnant or fails to use adequate birth control if able to conceive.
- the patient is not able to comply with the protocol requirements.
- the sponsor decides to terminate the study for significant risk-benefit concerns.
- Celgene no longer makes study drug available for free for the purpose of this study
- the patient dies or is lost to follow-up.

4.11 Centralized morphology review

We will centrally assess bone marrow smears, trephines and peripheral blood morphology from patients included in this study. Please send peripheral blood and bone marrow slides and when applicable also the trephine biopsies to Freiburg according to the procedure manual.

4.12 Centralized cytogenetics and molecular genetics

Centralized cytogenetics

Centralized cytogenetics (karyotype and FISH for monosomy 7) for all patients (both MDS and JMML) included in this study will be performed by dr. Berna Beverloo at Erasmus MC in Rotterdam, and will include FISH for monosomy 7.

Cytogenetic follow up at end of treatment for MDS patients will be done in Rotterdam as well. Hence when a patient is transplanted after 3 courses centralized cytogenetics should be repeated after the last course of azacitidine. Please send both peripheral blood and bone marrow to Rotterdam as specified in the procedure manual.

In case a monosomy 7 is apparent in JMML patients regular follow up of monosomy 7 FISH will be done in Freiburg to determine the clone size (see the section on pharmacodynamic studies).

Centralized mutation analysis for JMML patients

Peripheral blood samples from JMML patients will be screened routinely for disease-specific mutations in the RAS-pathway (see figure below, taken from De Vries et al, Haematologica 2010). Material for this analysis should be sent to Freiburg according to the procedure manual. In most cases this will already have been done in the diagnostic work-up of JMML. In case a molecular abnormality is found this will be used for quantification of the disease clone over time, especially with regards to the planned methylation studies (see the section on pharmacodynamic studies).
4.13 Pharmacokinetic Sample Collection

The aim is to study the PK parameters of azacitidine derived in pediatric MDS and JMML patients and compare them with available data in adult studies.

Given the young median age of JMML patients versus the older median age of MDS patients we will probably need to do a stratified analysis, aiming at least 10 subjects per group to be included in the PK-analysis. Further age stratification may need to take place in patients below and over one year of age. This will be decided during an interim analysis based on the available data at that point in time. Since the half-life of azacitidine in adults is very short and there is no accumulation with repeated dosing, PK parameters will not be used for dose-(de-) escalation decisions.

From each subject participating in the PK evaluation, blood samples (2 mL) will be collected for the determination of plasma azacitidine concentrations by in-dwelling catheter or by venipuncture into sample collection tubes as described in the Investigator Manual. In this study, the same catheter will be used for IV dosing and pharmacokinetic blood sampling collection. The catheter will be flushed vigorously with saline for injection immediately following dosing.

Days and timing of pharmacokinetic blood sampling is only applicable in course 1 and will be as follows (see table 5):

- On Days 5 and 6 prior to dosing (within 1 hour prior to dosing);
- Day 7:
  - For SC use: prior to dosing (within 1 hour prior to dosing) and at the following post-dose times: 15 minutes, 30 minutes, 1, 2, 4 and 6 hours.
  - For IV use: prior to dosing (within 1 hour prior to dosing) and at the following post-dose times: 5 minutes, 30 minutes, 1, 2, 4 and 6 hours.
Table 5: PK sampling schedule in 1st course of treatment

<table>
<thead>
<tr>
<th>PK sampling schedule</th>
<th>Day +5</th>
<th>Day +6</th>
<th>Day +7 (Last day of infusion)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Prior to dosing</td>
<td>Prior to dosing</td>
<td>Prior to dosing (≤1 hour)</td>
</tr>
<tr>
<td></td>
<td>(≤1 hour)</td>
<td>(≤1 hour)</td>
<td>(≤1 hour)</td>
</tr>
<tr>
<td></td>
<td>Post-dose 5 minutes</td>
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<tr>
<td></td>
<td>(IV azacitidine treated patients only)</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Post-dose 15 minutes</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>(SC azacitidine treated patients only)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Post-dose 30 minutes</td>
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<tr>
<td></td>
<td>Post-dose 1 hour</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Post-dose 2 hours</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Post-dose 4 hours</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Post-dose 6 hours</td>
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<td></td>
</tr>
</tbody>
</table>

The label on each sample tube will be pre-printed with an internal Covance code. The subject number (patient number) should be completed on the label, using indelible ink. The exact date and time of each sample collection will be recorded in the source documentation. These details will be transferred on the Laboratory Requisition Forms (see Investigator Manual). Azacitidine is unstable in blood and plasma; all blood samples will be processed and plasma harvested immediately per the instructions in the Investigator Manual. In general, these procedures will involve collection of blood using pre-chilled vacutainers; gently mixing blood with a preservative and anticoagulant then immediately placing the vacutainer of freshly collected blood into an ice bath. The blood will then be centrifuged using a refrigerated (4°C) centrifuge for 10 minutes at 2,000 x g, followed by immediate harvest of the plasma and storage in an ultra-low temperature freezer at -70°C or colder. All plasma samples will be kept frozen at -70°C or colder and shipped in dry ice to Covance, according to instructions provided in the Investigator Manual. Covance will take care of shipment to the central PK-laboratory. Analysis of plasma samples for azacitidine will be performed using a high performance liquid chromatography/tandem mass spectrometry (LC-MS/MS) method specifically validated for the determination for azacitidine in plasma, as organized by Celgene. A maximum total of 9 samples (approximately 18 mL of blood) over the course of the study will be collected from each subject participating in the PK evaluation, for pharmacokinetic analysis. The pharmacokinetic population will consist of all subjects who had sufficient concentration time data to enable the calculation of pharmacokinetic parameters for azacitidine. For subjects that were
determined to be noncompliant with respect to administration of azacitidine, or for subjects with incomplete data, a decision as to their inclusion in the analysis will be made on a case-by-case basis.

4.14 Pharmacodynamic Sample Collection

**MDS**
Bone marrow and peripheral blood pharmacodynamic assessments will be done analyzing methylation changes of the CpG islands of genome-wide methylation patterns, as well as a using a candidate gene approach. In addition, gene expression levels of these candidate candidate genes will be analyzed. This requires the quantification of methylation in relation to the proportion of malignant cells in the bone marrow (advanced MDS patients), which will be done using morphology in advanced MDS patients. As quantification and correction for blast counts in MDS patients is difficult the main PD-endpoint is already at day 8 directly following the first treatment course, which will rule out large differences in clone size, as azacitidine is not expected to induce major differences in clone size in this time-interval. Another sample will be studies at day 28 prior to course 2.

**JMML**
In JMML, we aim at weekly assessments of the methylation status during the first course of treatment. We will study this by determining the methylation status of candidate genes. The first time-point is directly prior to starting treatment, hence at baseline, after which weekly follow-up during the first course is planned.

In order to quantify methylation in relation to the proportion of malignant cells in the peripheral blood of JMML patients (the clone size), the peripheral blood will be assessed either by FISH for monosomy 7 (when detected at screening) or with a quantative PCR for molecular aberrations in the RAS pathway.

Details regarding the work-up and shipment of the samples are given in the Procedure Manual.
Table 6: Pharmacodynamic sampling schedule in 1st course of treatment, including follow-up of clone size in JMML patients.

<table>
<thead>
<tr>
<th></th>
<th>Screening</th>
<th>Baseline</th>
<th>Day 8</th>
<th>Day 15</th>
<th>Day 22</th>
<th>Day 28</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MDS patients</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bone marrow</td>
<td>X</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Peripheral blood</td>
<td>X</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td><strong>JMML patients</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peripheral Blood for methylation status</td>
<td></td>
<td>X</td>
<td>X</td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Peripheral blood for monosomy 7 FISH</td>
<td></td>
<td>X</td>
<td>X</td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Molecular mutations in the RAS pathway to determine clone size</td>
<td></td>
<td>X</td>
<td>X</td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

1: Pharmacodynamic analysis of MDS patients will be done in Rotterdam, of JMML patients in Freiburg.
2: Only in case a monosomy 7 is present at disease diagnosis or in the screening sample.
3: Only in case a molecular abnormality is present at disease diagnosis or in the screening sample.
5 PROCEDURES

5.1 Pre-treatment Evaluations
To diagnose MDS or JMML we will apply the diagnostic criteria as given in the EWOG-MDS protocol (see appendix 1). Only patients with an established diagnosis of advanced MDS or JMML are eligible for this study. The initial diagnostic work-up is not part of this study; however, there will be centralized morphology review, as well as centralized cytogenetics and mutation analysis, which will need to be taken into account when planning these investigations. Please note that informed consent should be obtained before any study specific procedures are carried out.

5.2 Screening Evaluations/Procedures
These evaluations should be carefully reviewed to determine the patient’s eligibility for the study. Please note that some procedures are part of standard diagnostic work-up for these diseases despite the fact that we will capture the data for the study.

Stratum 1 patients (newly diagnosed primary advanced MDS) or Stratum 5 patients (newly diagnosed secondary MDS)

1. Signed, written informed consent.
2. Complete physical examination, including vital parameters (temperature, pulse, blood pressure).
3. Height, weight and body surface area.
4. Medical history: detailed documentation of disease (including family history, concomitant medication, and transfusions).
5. Lansky or Karnofsky performance status (given in appendix 2).
6. Hematology: CBC with differential and platelet count. Hematology should be repeated to have a baseline measurement within 2 days before starting therapy with azacitidine.
7. Serum chemistry: electrolytes (sodium, potassium, calcium, magnesium, phosphate, chloride, and bicarbonate), blood urea nitrogen (BUN), creatinine, uric acid, CRP, glucose, and liver function tests (aspartate aminotransferase (AST) and/or alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin, lactate dehydrogenase (LDH)). Serum chemistry should be repeated to have a baseline measurement within 2 days before starting therapy with azacitidine.
8. Peripheral blood and bone marrow aspirate (bilateral) for morphology (smears) and flowcytometry (immunological classification) according to standard procedures at the site within 2 weeks before starting treatment.
10. Send peripheral blood and bone marrow slides as well as trephines to Freiburg for central morphology review (see procedure manual).
11. Send bone marrow and peripheral blood for cytogenetic analysis within 2 weeks before start of treatment to Rotterdam. Cytogenetic analysis will include FISH for monosomy 7. Results will be reported to the site and to the PD-laboratories. Details will be provided in the procedure manual.
12. Send peripheral blood and bone marrow for pharmacodynamic studies to Rotterdam. Details will be provided in the procedure manual.
13. Lumbar puncture to assess potential CNS-involvement (optional, mainly when a differential diagnosis of AML is considered)
14. Chest X-ray, in case of suspected fungal infection on chest X-ray perform HR-CT scan of the chest.
15. Abdominal sonography (ultrasound: liver and spleen enlargement, renal abnormalities).
17. Pregnancy test in females with child bearing potential.

**Stratum 2 patients (relapsed primary advanced MDS) or Stratum 5 patients (relapsed secondary advanced MDS)**

1. Signed, written informed consent
2. High-grade relapsed MDS according to EWOG criteria (appendix 1).
3. Complete physical examination, including vital parameters (temperature, pulse, blood pressure).
4. Height, weight and body surface area.
5. Medical history: detailed documentation of disease, prior treatment, concomitant medication and transfusions. Carefully assess any remaining toxicities from a prior stem cell transplant procedure.
6. Lansky or Karnofsky performance status (given in appendix 2).
7. Hematology: CBC with differential and platelet count. Hematology should be repeated to have a baseline measurement within 2 days before starting therapy with azacitidine.
8. Serum chemistry: electrolytes (sodium, potassium, calcium, magnesium, phosphate, chloride, and bicarbonate), blood urea nitrogen (BUN), creatinine, uric acid, CRP, glucose, and liver function tests (aspartate aminotransferase (AST) and/or alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin, lactate dehydrogenase (LDH)). Serum chemistry should be repeated to have a baseline measurement within 2 days before starting therapy with azacitidine.
9. Peripheral blood and bone marrow aspirate (bilateral) for morphology (smears) and flowcytometry (immunological classification) according to standard procedures at the site within 2 weeks before starting treatment.
11. Send peripheral blood and bone marrow slides as well as trephines to Freiburg for central morphology review (see procedure manual).
12. Send bone marrow and peripheral blood for cytogenetic analysis within 2 weeks before start of treatment to Rotterdam. Cytogenetic analysis will include FISH for monosomy 7. Results will be reported to the site and the PD-laboratories. Details will be provided in the procedure manual.
13. Send peripheral blood and bone marrow for pharmacodynamic studies to Rotterdam. Details will be provided in the procedure manual.
14. Lumbar puncture to assess CNS-involvement (optional, mainly when a differential diagnosis of AML is considered)
15. Chest X-ray, in case of suspected fungal infection on chest X-ray perform HR-CT scan of the chest.
17. Echocardiography (shortening fraction).
18. Pregnancy test in females with child bearing potential.
Stratum 3 patients (newly diagnosed JMML patients)

1. Signed, written informed consent.
2. Diagnosis of JMML according to EWOG criteria (see appendix 1), including work-up for molecular abnormalities (see appendix 1).
3. Complete physical examination, including vital parameters (temperature, pulse, blood pressure).
4. Height, weight and body surface area.
5. Medical history: detailed documentation of disease and treatment history (including family history, concomitant medication and transfusions.).
6. Lansky or Karnofsky performance status (see appendix 2).
7. Document spleen size by physical examination within 1 day prior to start of treatment as baseline measurement (in centimeters in the midclavicular line below the costal margin at the end of inspiration with the child in supine position).
8. Abdominal sonography (ultrasound) for liver and spleen size within 7 days before starting treatment as baseline measurement (spleen size: optically maximum distance on the longitudinal coronal view between the most superomedial and the most inferolateral points), and other abnormalities.
9. Hematology: CBC with differential and platelet count. Hematology should be repeated to have a baseline measurement within 1 day prior to start treatment.
10. Absolute monocyte count in peripheral blood. Repeat to have a baseline measurement within 1 day prior to start treatment.
11. Serum chemistry: electrolytes (sodium, potassium, calcium, magnesium, phosphate, chloride, and bicarbonate), blood urea nitrogen (BUN), creatinine, uric acid, CRP, glucose, and liver function tests (aspartate aminotransferase (AST) and/or alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin, lactate dehydrogenase (LDH)). Serum chemistry should be repeated to have a baseline measurement within 2 days before treatment start.
12. Peripheral blood and bone marrow aspirate for morphology (smears) and flowcytometry (immunological classification) according to standard procedures at the site within 2 weeks before starting treatment.
13. Send peripheral blood slides to Freiburg for central morphology review (see procedure manual).
14. Send bone marrow and peripheral blood for cytogenetic analysis within 2 weeks before start of treatment to Rotterdam. Cytogenetic analysis will include FISH for monosomy 7. Results will be reported to the site and the PD laboratory. Details will be provided in the procedure manual.
15. Send peripheral blood for pharmacodynamic studies to Freiburg. Details will be provided in the procedure manual.
16. Send peripheral blood to Freiburg for mutation analysis involving the RAS-pathway (see procedure manual). 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.
18. Echocardiography (shortening fraction).
Stratum 4 patients (relapsed JMML patients)

1. Signed, written informed consent.
2. Diagnosis of JMML according to EWOG criteria (see appendix 1), including work-up for molecular abnormalities (see appendix 1).
3. Complete physical examination, including vital parameters (temperature, pulse, blood pressure). Carefully assess any remaining toxicities from a prior stem cell transplant procedure.
4. Height, weight and body surface area.
5. Medical history: detailed documentation of disease and treatment history (including concomitant medication and transfusions.).
6. Lansky or Karnofsky performance status (see appendix 2).
7. Document spleen size by physical examination within 1 day prior to start of treatment as baseline measurement (in centimeters in the midclavicular line below the costal margin at the end of inspiration with the child in supine position).
8. Abdominal sonography (ultrasound) for liver and spleen size within 7 days before start of treatment as baseline measurement (spleen size: optically maximum distance on the longitudinal coronal view between the most superomedial and the most inferolateral points), and other abnormalities.
9. Hematology: CBC with differential and platelet count within 1 day prior to start treatment.
10. Absolute monocyte count in peripheral blood. Repeat to have a baseline measurement within 1 day prior to start treatment.
11. Serum chemistry within 2 days prior to staring treatment: electrolytes (sodium, potassium, calcium, magnesium, phosphate, chloride, and bicarbonate), blood urea nitrogen (BUN), creatinine, uric acid, CRP, glucose, and liver function tests (aspartate aminotransferase (AST) and/or alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin, lactate dehydrogenase (LDH)). Serum chemistry should be repeated to have a baseline measurement within 2 days before treatment start.
12. Peripheral blood and bone marrow aspirate for morphology (smears) and flowcytometry (immunological classification) according to standard procedures at the site within 2 weeks before starting treatment.
13. Send peripheral blood slides to Freiburg for central morphology review (see procedure manual).
14. Send bone marrow and peripheral blood for cytogenetic analysis within 2 weeks before start of treatment to Rotterdam. Cytogenetic analysis will include FISH for monosomy 7. Results will be reported to the site. Details will be provided in the procedure manual.
15. Send peripheral blood for pharmacodynamic studies to Freiburg. Details will be provided in the procedure manual.
16. Send peripheral blood to Freiburg for mutation analysis involving the RAS-pathway (see procedure manual). 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.
17. Chest X-ray, in case of suspected fungal infection or pulmonary infiltration on chest X-ray perform HR-CT scan of the chest.
18. Echocardiography (shortening fraction).

Stratum 5 patients (newly secondary and relapsed advanced secondary MDS)

Follow guidelines for stratum 1 patients in case of newly diagnosed secondary MDS, for relapsed cases follow guidelines as in stratum 2.
5.3 On Study Evaluations during 1\textsuperscript{st} course of treatment for all advanced MDS patients (primary and secondary)

1. Hematology: CBC with differential and platelet count twice weekly during azacitidine administration, or more frequently when clinically indicated. After that at least once weekly, but may be needed more frequently depending on clinical circumstances.

2. Serum chemistries: electrolytes (sodium, potassium, calcium, phosphate, chloride, and bicarbonate), uric acid, BUN, creatinine, and liver function tests (AST, ALT, ALP, total bilirubin, LDH): every other day during azacitidine administration, or more frequently when clinically indicated. After that once weekly until the next course, but may be needed more frequently depending on clinical circumstances.

3. Assess Adverse Events according to the CTCAE, version 4.0.

4. Record concomitant medication and transfusions.

5. Perform PK sampling according to schedule (day 5, 6 and 7). The samples should be stored locally until collection by Covance (see Investigator Manual).

6. Send PD peripheral blood and bone marrow samples to Rotterdam directly after each sampling point (it should arrive within 24 hours, see Procedure Manual).

5.4 On Study Evaluations during 1\textsuperscript{st} course of treatment for all JMML patients

1. Hematology: CBC with differential and platelet count: twice weekly during azacitidine administration, or more frequently when clinically indicated. After that at least once weekly, but may be needed more frequently depending on clinical circumstances.

2. Absolute monocyte count in peripheral blood twice weekly during azacitidine administration and at least once weekly after that.

3. Serum chemistries: electrolytes (sodium, potassium, calcium, phosphate, chloride, and bicarbonate), uric acid, BUN, creatinine, and liver function tests (AST, ALT, ALP, total bilirubin, LDH): every other day during azacitidine administration, or more frequently when clinically indicated. After that once weekly until the next course, but may be needed more frequently depending on clinical circumstances.

4. Assess Adverse Events according to the CTCAE, version 4.0.

5. Record concomitant medication and transfusions.

6. Perform PK sampling according to schedule (day 5, 6 and 7). The samples should be stored locally until collection by Covance (see Investigator Manual).

7. Document spleen size at least once weekly.

8. Send peripheral blood samples to Freiburg for PD studies on a weekly basis (see section on PD and Procedure Manual), which will include the determination of monosomy 7 FISH and/or mutation analysis for clone size follow-up next to methylation studies.
5.5 **Response evaluation after 1\textsuperscript{st} course for MDS patients**

1. Response evaluation should consist of the following evaluations:
   a. Bone marrow (bilateral), trephine biopsy (bilateral) and peripheral blood morphology to determine response. Repeat lumbar puncture if abnormal at inclusion.
   b. Send peripheral blood and bone marrow slides for central morphology review to Freiburg as described in the Procedure Manual.
   c. In case of persisting aplasia with an empty bone marrow at day +28, the next bone marrow evaluation may be delayed to day +42, to neutrophil recovery (ANC at least 1000x10\(^6\)/l), or to progressive disease, whichever occurs first.

5.6 **Response evaluation after 1\textsuperscript{st} course for JMML patients**

1. Response evaluation should consist of the following evaluations:
   a. Peripheral blood examination for morphology.
   b. Determine the absolute monocyte count in peripheral blood.
   c. Send peripheral blood slides for central morphological review to Freiburg, as described in the procedure manual.
   d. Send peripheral blood for pharmacodynamic studies to Freiburg as described in the Procedure Manual. This will include FISH for monosomy 7 or molecular aberrations when present in the diagnostic sample.
   e. Determine the spleen size by physical examination (see work-up at screening).
   f. Perform abdominal sonography to document liver and spleen size (use same methodology as at screening).

5.7 **Criteria to start subsequent courses of treatment**

1. Must have recovered from the acute side-effects of previous courses according to the guidelines provided in section 4.4.
2. Consider intra-patient dose-escalation according to section 4.4.1
3. Patients with progressive disease are to be taken of study. For MDS patients this is defined as evolution to MDS-related AML (>30% bone marrow blasts). In JMML, progressive disease is defined as a >25% increase in WBC and an increase in spleen size of >25%.
4. Assess the performance status on a regular basis, but at least before each new treatment course will start. Performance status should be ≥60 (Lansky or Karnofsky).

5.8 **On study evaluation during subsequent courses of treatment**

These are essentially similar as given in paragraph 5.6 and 5.7.

For MDS patients who will be transplanted after the 3\textsuperscript{rd} course of azacitidine please refer to paragraph 5.9 for details of the required evaluation, as this concerns the end of treatment evaluation.

However, in case MDS patients are treated with > 3 courses of azacitidine, bone marrow evaluation will be repeated every 3\textsuperscript{rd} courses until the patient goes off study. This should include
threphines and cytogenetic evaluation, which need to be send to the central labs as mentioned in the Procedure Manual.

The evaluation of JMML patients will continue after each course as this is done on peripheral blood only. This will also include molecular analysis and/or FISH for monosomy 7 to determine clone size, for which samples need to be shipped to Freiburg.

5.9 **End of treatment Evaluation advanced MDS before SCT**

Planned last evaluation before proceeding to SCT
1. Complete physical examination, including vital parameters (temperature, pulse, blood pressure).
2. Lansky or Karnofsky performance status (see appendix 2).
3. Adverse event assessment, concomitant medication, and transfusions.
5. Bone marrow (bilateral) and peripheral blood morphology to determine response.
7. Lumbar puncture to assess CNS-involvement when present at inclusion.
8. Send peripheral blood and bone marrow slides for central morphology review to Freiburg as described in the Procedure Manual.
9. Send peripheral blood and bone marrow to Rotterdam for cytogenetic evaluation.
10. Serum chemistries: electrolytes (sodium, potassium, calcium, magnesium, phosphate, chloride, and bicarbonate), blood urea nitrogen (BUN), creatinine, CRP, glucose, and liver function tests (aspartate aminotransferase (AST) and/or alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin, lactate dehydrogenase (LDH), uric acid.

5.10 **End of treatment Evaluation JMML before SCT**

Planned last evaluation before proceeding to SCT
1. Complete physical examination, including vital parameters (temperature, pulse, blood pressure).
2. Lansky or Karnofsky performance status (see appendix 2).
3. Adverse event assessment, concomitant medication, and transfusions.
5. Absolute monocyte count in peripheral blood.
6. Send peripheral blood slides for central morphological review to Freiburg as described in the Procedure Manual.
7. Send peripheral blood to Freiburg for follow-up of clone size looking at molecular mutations in the RAS-pathway or monosomy 7.
8. Document spleen size (see screening).
9. Perform abdominal sonography to document liver and spleen size (use same methodology as at screening).
10. Serum chemistries: electrolytes (sodium, potassium, calcium, magnesium, phosphate, chloride, and bicarbonate), blood urea nitrogen (BUN), creatinine, CRP, glucose, and liver function tests (aspartate aminotransferase (AST) and/or alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin, lactate dehydrogenase (LDH), uric acid.
5.11 Follow up visits

All subjects will be followed (3-monthly) for 1 year after end-of-azacitidine treatment, or, in case patients are transplanted following azacitidine, for 1 year post stem cell transplantation for date of progression (only the first occurrence of disease progression after study entry) and date of death.

If drug-related AEs are present at end-of-treatment, early follow-up visits are required at a maximum interval of 4 weeks until all such AEs resolve to baseline or CTC Grade $\leq 1$, or are deemed irreversible.

Although the HSCT procedure is not part of this protocol we will capture data considering type and date of SCT and follow-up data post-HSCT, to preliminary assess whether azacitidine may affect the outcome or complications of HSCT or relapse post-SCT.

5.12 Study Calendars

See appendix 3.
6 DEFINITIONS AND CRITERIA FOR EVALUATION

6.1 Safety and toxicity

The safety profile of azacitidine will be evaluated. Clinical and laboratories toxicities symptoms will be graded according to the CTCAE v4.0 (http://ctep.cancer.gov/reporting/ctc.html).

Any adverse events, which are not reported in the NCI common toxicity criteria, will be graded as mild, moderate or severe.

Safety and tolerability of study treatment will be reported for all subjects who received at least one dose of azacitidine. Safety assessments will be performed after informed consent is obtained on a continuous basis until the end-of-treatment visit. If drug-related AEs are present at end-of-treatment, early follow-up visits are required at a maximum interval of 4 weeks until all such AEs resolve to baseline or CTC Grade ≤ 1, or are deemed irreversible.

Safety assessments will include physical examination, vital signs (systolic/diastolic blood pressure, pulse rate, and body temperature), clinical laboratory tests (hematology, serum chemistry), and reported or observed adverse events.

Treatment interruptions and discontinuations, and dose reductions for toxicity will be analyzed by dose level. Moreover, the frequency and severity of all laboratory abnormalities will be tabulated.

All laboratory test values captured as part of the study should be recorded on the appropriate laboratory test results pages of the case report form (CRF). In addition, in order for the principal investigators to collect additional information about clinically-important laboratory abnormalities, at a minimum, the following laboratory abnormalities recorded on an AE or SAE form as appropriate:

- Any laboratory test result that meets the criteria for a Serious Adverse Event.
- Any laboratory abnormality that requires the subject to have investigational product discontinued or interrupted.

It is expected that wherever possible, the clinical, rather than the laboratory term would be used by the reporting Investigator (e.g. fatigue rather than low hemoglobin).

6.2 Adverse events

Adverse events (AEs) are defined as any untoward medical occurrence in a patient or a clinical investigation subject administered a pharmaceutical product, and which does not necessarily have a causal relationship with the treatment. An AE can therefore be any unfavourable and unintended sign, symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related.

Any medical condition that was present prior to study treatment and that remains unchanged or improved should not be recorded as an AE. If there is a worsening of that medical condition, this should be considered an AE. A diagnosis or syndrome should be recorded on the AE page of the Case Report Form rather than the individual signs or symptoms of the diagnosis or syndrome.

AEs will be assessed continuously and graded according to NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 (a copy can be downloaded from the CTEP website (http://ctep.cancer.gov/reporting/ctc.html).
All AEs will be recorded by the Investigators from the time of signing the informed consent through to the end of the designated follow-up period.

The causality relationship of azacitidine to the AEs will be assessed by the investigator as either:
1. Certain: There is a reasonable causal relationship between the combined drug treatment and the AE. The event responds to withdrawal of study treatment (dechallenge), and recurs with rechallenge when clinically feasible.
2. Probable: There is a reasonable causal relationship between the treatment and the AE. The event responds to dechallenge. Rechallenge is not required.
3. Possible: There is reasonable causal relationship between the treatment and the AE. Dechallenge information is lacking or unclear.
4. Not likely: There is a temporal relationship to treatment administration, but there is not a reasonable causal relationship between the treatment and the AE.
5. Not related: There is not a temporal relationship to treatment administration (too early, or late, or study drug not taken), or there is a reasonable causal relationship between another drug, concurrent disease, or circumstance and the AE.

AEs should be followed to resolution or stabilization, and reported as SAEs as they become serious. AEs occurring in the first course (DLTs) will be tabulated separately from the 2nd or subsequent courses.

### 6.3 Serious adverse events

A serious adverse event (SAE) is any AE which:
- Results in death.
- Is life-threatening (i.e., in the opinion of the Investigator(s) the subject is at immediate risk of death from the AE).
- Requires inpatient hospitalization or prolongation of existing hospitalization.
- Results in persistent or significant disability/incapacity (a substantial disruption of the subject’s ability to conduct normal life functions).
- Is a congenital anomaly/birth defect.
- Constitutes an important medical event.

Important medical events are defined as those occurrences that may not be immediately life threatening or result in death, hospitalization, or disability, but may jeopardize the subject or require medical or surgical intervention to prevent one of the other outcomes listed above. Medical and scientific judgment should be exercised in deciding whether such an AE should be considered serious.

**NOTE 1:**
- Pregnancy: pregnancy is not considered as a SAE. Pregnancy must, however, be reported immediately by E-mail or by phone to the principal investigators of this study.
- Overdose: All cases of overdose must be reported immediately by E-mail or by phone to the principal investigators of this study.

**NOTE 2:**
Criteria for hospitalizations that should not be reported as SAEs include admissions for:
- Planned as per protocol medical/surgical procedure.
- Routine health assessment requiring admission for baseline/trending of health status documentation.
- Medical/surgical admission for purpose other than remedying ill health state (planned prior to entry into study trial; appropriate documentation required).
- Admission encountered for other life circumstance that carries no bearing on health status and requires no medical/surgical intervention (e.g. lack of housing, economic inadequacy, family circumstances, administrative).
- Admissions for protocol-scheduled procedures or blood product transfusions will not be considered SAEs.

An SAE report should be completed for any event where doubt exists regarding its status of seriousness.

For each SAE, the Investigator will provide information on severity, start and stop dates, relationship to study drug, action taken regarding study drug, and outcome.

6.4 Dose-limiting toxicities

DLTs are AEs considered at least possibly drug-related and will be limited to the first course of azacitidine.

Non-hematologic DLT will be defined as:

- Any ≥ grade 3 study treatment 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 not be considered DLT: grade 3 nausea and/or vomiting that can be subsequently controlled, including by pre-medication (uncontrollable conditions will be considered DLT), alopecia, drug fever, anorexia, and transient grade 3 transaminase elevations that return to ≤ grade 1 within 7 days.

For hematologic toxicity, it is anticipated that the disease itself as well as the study treatment regimen will result in severe myelosuppression and its associated complications. Therefore, myelosuppression/pancytopenia and grade 3 febrile neutropenia will not be considered DLTs. However, prolonged myelosuppression will be considered a DLT, and 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% without evidence of persisting or recurrent leukemia). DLTs may result in delay of subsequent treatment courses, or dose adjustments.

6.5 SUSARs

The coordinating investigator will have the responsibility to monitor all SAE’s and to decide whether they should be labeled as SUSARs. A suspected unexpected serious adverse reaction (SUSAR) indicates a serious adverse reaction related to the study drug (a side-effect), which was not anticipated, and has not yet been reported in the Summary of Product Characteristics and the Investigator’s Brochure.
6.6 Data safety monitoring board (DSMB)

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 at least make recommendations on 2 major issues regarding study conduct. This concerns mainly whether the observed safety and efficacy data will allow to:

1. open the 2 cohorts of newly diagnosed patients with advanced MDS and JMML after an initial phase for relapsed patients only

2. decide which dose-level should be used in JMML patients. This concerns using dose-level 1 or dose-level 2 and/or the dosing interval (21 or 28 days) in the expanded cohort of patients after the initial cohort is treated.

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).

6.7 Response definitions

6.7.1 Response definitions for advanced MDS (adapted from Cheson et al, 2006)

Complete response (CR): disappearance of blasts (<5% blasts in the bone marrow aspirate and absence of peripheral blood leukemic blasts, or leukemic cells at any other localization), with recovery of trilineage hematopoiesis without signs of dysplasia (ANC >1000/µl and platelets >100x10^9/l.)

Partial response (PR): a >50% relative reduction (with a minimum of 10% absolute reduction) of blast % in the bone marrow aspirate, irrespective of recovery of the peripheral blood counts and no transfusion requirements.

Stable disease (SD): This includes all patients who do not qualify as CR, PR or PD.

Progressive disease (PD): evolution to MDS-related AML (>30% bone marrow blasts).

Complete cytogenetic response (CCyR): disappearance of a chromosomal abnormality which was present at inclusion.

Partial cytogenetic response (PCyR): at least a 50% reduction of a chromosomal abnormality which was present at inclusion.

The overall morphological response rate is the number of patients with either CR or PR.

Treatment failure includes those patients for whom treatment has failed to achieve less than a PR.
6.7.2 Response definitions for JMML (adapted from Chan et al, 2009)

Chan et al redefined the response criteria using WBC<20x10^9/l as cut-off for clinical response. However, approximately one-third of JMML patients present with a WBC <20x10^9/l, and hence would need to be excluded using this definition. We therefore decided to use absolute monocyte count, and use greater than 1x10^9/l as the cut-off value, which is in line with the diagnostic criteria for JMML.

*Complete response* (CR): normalization of spleen size, and peripheral blood absolute monocyte count ≤1x10^9/l

*Partial response* (PR): at least 25% decrease from initial spleen size, and absolute monocyte count <50%, but still greater than1x10^9/l

*Stable disease* (SD). This includes all patients who do not qualify as CR, PR or PD.

*Progressive disease* (PD). Progressive disease is a clinical diagnosis as there are no clear cut laboratory criteria to establish progressive disease. Clinical criteria 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.

*Complete cytogenetic response* (CCyR): disappearance of a chromosomal abnormality which was present at inclusion.

*Partial cytogenetic response* (PCyR): at least a 50% reduction of a chromosomal abnormality which was present at inclusion.

*Complete molecular remission* (CMolR): negative molecular markers after previous positivity for mutations of genes involved in the RAS pathway.

The overall morphological response rate is the number of patients with either CR or PR.

*Treatment failure* includes those patients for whom treatment has failed to achieve less than a PR.

6.8 Other Definitions

**Early Death**
Death during the first 3 weeks of treatment (i.e., before the time complete remission could have been documented). The cause of death (disease, therapy or both) should be recorded as well as the last known percentage of blasts in the BM.

**Event-free survival**
Event-free survival (EFS) will be defined as the time between diagnosis and first event (relapse, death of any cause, failure to achieve remission or second malignancy) or date of last follow-up. Patients who were classified as treatment failures will be considered as failures at time zero. Probabilities of event-free survival will be estimated by the method of Kaplan and Meier.
Overall Survival
Overall survival (OS) is defined as time from first dose of study treatment to date of death. All subjects will be followed until death or (if study drug is stopped) until 1 year after end-of-treatment. Subjects lost to follow-up will be censored on the last date the subject was known to be alive. Probabilities of survival will be estimated by the method of Kaplan and Meier.

Relapse definition MDS
After a documented CR or PR, this designation is defined as a reappearance of blasts in the peripheral blood, or \( \geq 5\% \) blasts in the bone marrow not attributable to any other cause (eg. bone marrow regeneration after consolidation therapy), and confirmed with flow cytometry.

Relapse definition JMML
After a documented CR or PR reappearance of organomegaly in combination with elevated WBC with peripheral blood monocytosis (greater than \( 1 \times 10^9/l \)) 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.
7 REGISTRATION OF PATIENTS

Patients will be recruited from a population of children and adolescents treated at or referred to one of the participating study sites.

A study site can only start enrolling patients once the appropriate ethical committee and other relevant authorities have agreed with the study, if the contract with the sponsor (and co-sponsor if applicable) has been signed, and if the study drug (azacitidine) is available at the study site.

The study site will be able to enroll patients into the study by procedure of registration available via the website [http://www.skion.nl/dcog-ectc/studies](http://www.skion.nl/dcog-ectc/studies).

This method of registration will be done utilizing a web-based service called TenALEA. This web-based registration program will provide continuous service during 24 hours 7 days per week. Notification of a registration will be sent automatically to all involved parties. A subject number will be assigned at this time. Each study site will request a login for the local person responsible for the registration of the patients. Detailed instructions will be available in the Procedure Manual.

Alternatively, patient registration may be done by faxing the signed Randomization Form to:

DCOG – ECTC Trial Office
Fax: +31 (0) 10 703 6681 (in case of dysfunction, fax +31 (0) 10 703 1134; in case assistance is needed: T+31-10-7036325)

The signed Registration Form may also be sent by e-mail to: research-kocr@erasmusmc.nl

The following information will be required to register a patient:

1. Date of birth
2. Stratum
3. All eligibility criteria

In case any enrollment issues need to be discussed prior to faxing the enrollment forms, please send an e-mail to both Coordination Investigators simultaneously for discussion: c.m.zwaan@erasmusmc.nl and/or m.vandenheuvel@erasmusmc.nl, and send a copy to research-kocr@erasmusmc.nl

It is the responsibility of the Principle Investigator to ensure that the subject is eligible for the study before enrolling the subject.

The first dose of study therapy should be administered within 5 days.
8 REGULATORY AND REPORTING REQUIREMENTS

8.1 Adverse Event Monitoring and Reporting

The Principal Investigator is responsible for monitoring the safety of patients who enroll in the study. All AEs occurring after any administration of azacitidine will be followed until End of Treatment or resolution or to return to base-line values. If drug-related AEs are present at End of Treatment, early follow-up visits are required at a maximum interval of 4 weeks until all such AEs resolve to baseline or CTC Grade $\leq 1$, or are deemed irreversible. 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 website (http://ctep.cancer.gov/reporting/ctc.html).

Adverse events, as well as serious adverse events (see section 6), need to be reported until 30 days after the last administration of study medication, or until another treatment regimen is started, whichever occurs first. However, in the case of AEs occurring later than this deadline but which are considered related to the study medication by the principle investigator, such AEs still need to be reported.

8.2 Reporting Serious Adverse Events

All SAEs occurring during the study or within 30 days of the last administration of azacitidine treatment must be reported to the coordinating investigators / sponsor within 24 hours of occurrence. Adverse events classified as "serious" must be recorded on the SERIOUS AE (SAE) page of the CRF and require expeditious handling and reporting (within 24 hours after occurrence) to the DCOG-ECTC Safety Desk to comply with regulatory requirements.

SAE reporting by TELEPHONE, FAX and E-MAIL:
DCOG-Early Clinical Trial Consortium Safety Desk
Tel: +31 (0)10 703 6568
Fax: +31-(0)8486 70 480
E-mail: c.m.zwaan@erasmusmc.nl and m.vandenheuvel@erasmusmc.nl; and copy e-mail to: research-kocr@erasmusmc.nl.

Collection of complete information concerning SAEs is extremely important. If only limited information is initially available, follow-up reports are required. Thus, follow-up information which becomes available as the SAE evolves, as well as supporting documentation (e.g., hospital discharge summaries and autopsy reports), should be collected subsequently, if not available at the time of the initial report, and immediately sent using the same procedure as the initial SAE report. For ongoing SAEs a follow-up report should be sent at least once monthly. The investigator is responsible for submitting these follow-up reports for all SAEs, until the SAE has resolved or until the patient’s condition stabilizes (in the case of persistent impairment), or the patient dies.

The sponsor (and national co-sponsors) is responsible for reporting SAEs/SUSARS to the Institutional Review Board (IRB) or other applicable regulatory authority.

In accordance with local regulations, the DCOG-ECTC Safety Desk will notify the Principle Investigators of all AEs that are serious, unexpected, and certainly, probably, or possibly related to the investigational product. This notification will be in the form of a SUSAR report. Upon receiving such notices, the Principle Investigator must review and retain the SUSAR reports in the trial file.
Where required by local regulations or when there is a central Institutional Review Board (IRB)/Independent Ethics Committee (IEC) for the study, the sponsor will submit the SUSAR report to the appropriate IRB/IEC. The sponsor, together with the principal investigators, will determine if the informed consent requires revision.

The sponsor will inform relevant IRB/IEC:

- of all relevant information about serious unexpected adverse events suspected to be related to the study medication that are fatal or life threatening as soon as possible, and in any case no later than seven days after knowledge of such a case. Relevant follow-up information for these cases will subsequently be submitted within an additional eight days.

- of all other serious unexpected events suspected to be related to the study medication as soon as possible, but within a maximum of fifteen days of first knowledge by the investigator.

The Principle Investigator will inform the sponsor of all SAEs within 24 hours in order that the sponsor can fulfill his regulatory reporting obligations within the required timeframes.

The sponsor will supply Celgene with a copy of all SAEs within 24 hours of being made aware of the event regardless of whether or not the event is listed in the reference document (e.g IB, SPC).

The sponsor will provide Celgene with a copy of the Annual Safety Report (ASR) at the time of the submission to the regulatory authority and the Ethics Committee.

8.3 Compliance with the protocol

The study should be conducted as described in this approved protocol. All revisions to the protocol must be discussed with, and be prepared by the sponsor. The Principle Investigator should not implement any deviation or change to the protocol without prior review and documented approval/favorable opinion from the IRB/IEC of an Amendment, except where necessary to eliminate an immediate hazard(s) to study subjects.

If a deviation or change to a protocol is implemented to eliminate an immediate hazard(s) prior to obtaining IRB/IEC approval/favorable opinion, as soon as possible the deviation or change will be submitted to:

- IRB/IEC for review and approval/favorable opinion;
- the sponsor;
- Regulatory Authority(ies), if required by local regulations.

Documentation of approval signed by the chairperson or designee of the IRB(s)/IEC(s) must be sent to the sponsor.

If the revision is a non-substantial amendment, the principle investigators must inform their IRB(s)/IEC(s).

If an amendment substantially alters the study design or increases the potential risk to the subject:

1. the consent form must be revised and submitted to the IRB(s)/IEC(s) for review and approval/favorable opinion;
2. the revised form must be used to obtain consent from subjects currently enrolled in the study if they are affected by the amendment; and
3. the new form must be used to obtain consent from new subjects prior to enrollment.
8.4 CRFs and Monitoring

The Principle Investigator will have access to web based case report forms (CRF) for documentation. All relevant data collected during the study for all of the patients enrolled into the study have to be entered into these CRF’s by the responsible investigator or someone authorized by the Principle Investigator in a timely manner. The Principal Investigator will review all the CRF’s of each patient and confirm the completeness, medical correctness and plausibility of the documented data by his signature on a special CRF page.

Additions and corrections in the CRF will be dated and signed by the responsible investigator or an authorized person. Reasons must be given for corrections that are not self-explanatory. Queries will be raised by the Data Center and are part of the CRF.

Monitors will ensure that the clinical trial is conducted, recorded, and reported in accordance with the protocol, ICH-GCP, and the applicable regulatory requirement(s). The sponsors may delegate monitoring activities to national co-sponsors in each of the participating countries, provided they are appropriately qualified and approved by the sponsor.

Representatives of the sponsor and/or co-sponsor must be allowed to visit all study site locations periodically to assess the data, quality and study integrity. On site they will review study records and directly compare them with source documents, and discuss the conduct of the study with the Principle Investigator, and verify that the facilities remain acceptable. Source documents are defined as: patient files, letters, laboratory / histology records. The type and scope of monitoring will be defined in the Monitoring Manual and documented on study specific Source Data Verification Forms / Monitoring report forms.

With his participation in the study the Principle Investigator is obligated to support the activities of the monitors, provide them with direct access to the files and give them the opportunity to inspect the laboratory facilities, storage of the investigational product, etc.

8.5 Quality assurance

Apart from the monitoring process described above, the sponsor may implement procedures to assure the quality of every aspect of the study. During the course of the study, external auditors contracted by the sponsor may conduct an onsite audit visit.

In addition, the study may be evaluated by government inspectors who must be allowed access to CRFs, source documents and other study files. The Principle Investigator must notify the sponsor promptly of any inspections scheduled by regulatory authorities, and promptly forward copies of inspection reports to the sponsor.

8.6 Delegation of authority

The Principle Investigator will maintain a Delegation of Authority Form in the Investigator File to document signatures and initials of all persons to provide a legal delegation of study specific principal responsibilities. Each member of study staff should sign to agree the acceptance of these, and each delegation should be signed by the Investigator.
8.7 Records retention

The confidentiality of records that could identify subjects must be protected, respecting the privacy and confidentiality rules in accordance with the applicable regulatory requirement(s).

The investigator must retain investigational product disposition records, copies of CRFs and source documents for the maximum period required by applicable regulations and guidelines, or institution procedures, or for the period specified by the sponsor, whichever is longer. The investigator must contact the sponsor prior to destroying any records associated with the study.

8.8 Institutional Review Board/Independent Ethics Committee (IRB/IEC)

Before study initiation, the Principle Investigator must have written and dated approval/favorable opinion from the local IRB/IEC for the protocol, consent form, subject recruitment materials/process (e.g. advertisements), and any other written information to be provided to subjects. The investigator or sponsor should also provide the IRB/IEC with a copy of the Investigator Brochure or product labeling, and of information to be provided to subjects.

The Principle Investigator or sponsor should provide the IRB/IEC with reports, updates and other information (e.g. ASR, Amendments, Administrative Letters) according to regulatory requirements or Institution procedures.

Freely given written informed consent must be 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.

For minors, according to local legislation, one or both parents or legally acceptable representative must be informed of the study procedures and must sign the informed consent form approved for the study prior to clinical trial participation. The explicit wish of a minor who is capable of forming an opinion and assessing this information to refuse participation in, or to be withdrawn from, the clinical trial at any time should be considered by the investigator.

8.9 Informed Consent Procedures

Preparation of the consent form is the responsibility of the investigator and must include all elements required by ICH, GCP and applicable regulatory requirements, and must adhere to GCP and to the ethical principles that have their origin in the Declaration of Helsinki. The consent form must also include a statement that the sponsor and regulatory authorities have direct access to subject records.

Prior to the beginning of the study, the investigator must have the IRB/IEC’s written approval/favorable opinion of the written informed consent form and any other information to be provided to the subjects.

The investigator must provide the subject or legally acceptable representative with a copy of the consent form and written information about the study in the language in which the subject is most proficient. The language must be non-technical and easily understood. The investigator should allow time necessary for subject or subject's legally acceptable representative to inquire about the details of the study, then informed consent must be signed and personally dated by the subject or the subject's legally acceptable representative and by the person who conducted the informed consent discussion. The subject or legally acceptable representative should receive a copy of the signed informed consent and any other written information provided to study subjects prior to subject's participation in the trial.
8.10 Update of Informed Consent

The informed consent and any other information provided to subjects or the subject's legally acceptable representative, should be revised whenever important new information becomes available that is relevant to the subject's consent, and should receive IRB/IEC approval/favorable opinion prior to use. The investigator, or a person designated by the investigator should fully inform the subject or the subject's legally acceptable representative of all pertinent aspects of the study and of any new information relevant to the subject's willingness to continue participation in the study. This communication should be documented. During a subject's participation in the trial, any updates to the consent form and any updates to the written information will be provided to the subject.
9 STATISTICAL CONSIDERATIONS

The primary objective of this study is to establish a recommended dose and preliminary efficacy of azacitidine in children with newly diagnosed or relapsed advanced MDS or JMML.

Other objectives concern:

• To determine the safety and tolerability of azacitidine in newly diagnosed and relapsed primary or secondary MDS and/or JMML.
• To determine (preliminary) the hematological remission rate in these patients.
• To describe the durability of response and long-term follow-up (at least 1 year after end of treatment), including that of patients undergoing stem-cell transplant after treatment with azacitidine.
• To determine the plasma pharmacokinetic parameters of azacitidine.
• To study the pharmacodynamic effects of azacitidine in pediatric MDS or JMML.

9.1 Data set description

The following data sets will be used in this study:

• Enrolled subjects: All subjects who signed the informed consent form. This dataset will be used for eligibility tabulation.
• Treated subjects: All subjects who received at least one dose of study treatment. Demographic and baseline characteristics and safety analyses will be performed on all treated subjects.
• Evaluable subjects: All subjects who received at least one course of study treatment and who have had at least one on-treatment efficacy evaluation. Analyses of dosing and efficacy will be performed on the dataset of all efficacy-evaluable subjects.

9.2 Number of patients

The patients in the various strata need to be analyzed separately as there may be marked differences in tolerability and response.

Initially, the aim is to recruit a total of 6 relapsed patients into stratum 2, 4 or 5 (combined) at the starting dose-level. In order to avoid any safety issues in the newly diagnosed patients, stratum 1 and 3 will only open in the absence of dose limiting toxicities (DLTs) in 6 patients treated either in stratum 2, 4 or 5. We will set up an independent Data Safety Monitoring Board (DSMB) to advise on the safety aspects and the opening of the newly diagnosed cohort.

Based on the available data (safety, response, PK) following treatment of 6 patients in each of the strata, we will enrol another 6 patients per stratum to further expand the safety data and to gather preliminary efficacy data. Therefore, we will recruit a maximum of 12 evaluable patients in each stratum, and hence 60 patients in total in this study. However, we will replace screen-failures and non-evaluable patients; hence the total number of included patients may be higher and is anticipated to approximately 65 patients. As DLTs are defined as occurring in the 1st course of treatment, patients need to have received the full first course of treatment in order to be evaluable for DLTs (referred to as ‘treated subjects’ in the statistical analysis plan).
9.3 Safety
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.

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).

9.4 Preliminary Efficacy
The primary efficacy variable is the overall response rate to azacitidine in the various strata. The number of patients with cytogenetic responses for advanced MDS patients and cytogenetic and/or molecular response for JMML patients will be reported separately. The second efficacy variable is the duration of the responses, the time to progression and survival, as well as the number of patients undergoing SCT post-induction with azacitidine.

9.5 Pharmacokinetics
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:

- $C_{\text{max}}$: observed maximum plasma concentration
- $T_{\text{max}}$: observed time to maximum plasma concentration
- $AUC_{0-t}$: area under the plasma concentration time curve from time zero to the last quantifiable time point, calculated by the linear trapezoidal rule
- $AUC_{0-\infty}$: 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:
  $$AUC_{0-\infty} = AUC_{0-t} + \left(\frac{C_t}{\lambda z}\right)$$
  where $C_t$ is the last quantifiable concentration
- $\lambda z$: terminal phase rate constant, determined by linear regression of the terminal points of the log-linear plasma-concentration-time curve
- $t/2$: terminal phase half-life, will be calculated according to the following equation:
  $$t/2 = \frac{0.693}{\lambda z}$$
- $CL$: total clearance, calculated as Dose/AUC$_{0-\infty}$
- $Vd$: volume of distribution will be calculated according to the equation: $Vd = (CL)/\lambda z$

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.

Pharmacokinetic parameters will be derived using with WinNonlin® Professional Version 5.2, or higher, (Pharsight Corp., Mountain View, California). All PK computations and graphics will be performed using WinNonlin Professional Version 5.2, or higher; Excel 2002, or higher (Microsoft Corp., Seattle, Washington); or SAS® Version 9.1, or higher (SAS Institute, Inc., Cary, North Carolina).

A stratified analysis will be performed for patients with JMML with a young median age versus patients with advanced MDS who have a higher median age. Other age cut-off points maybe studies as well. PK data will be collected for at least 10 subjects per stratum.

For sample processing, PK-kits and shipment of PK-samples: see the Procedure Manual.

The PK analysis will be done under supervision of Eric Laille, manager clinical pharmacology at Celgene. Details are provided in the procedure manual.

9.6 Duration of the study

The study will last approximately 4 years from first patient first visit (FPFV) to last patient last visit (LPLV). Therefore we expect to provide the final study report 5 years after enrolment of the first subject into the study.

Within the EWOG-MDS group (Nordic countries, Germany, Netherlands, Italy, Belgium, Switzerland, Austria and Poland) a total number of 60 pediatric patients with high-grade MDS are expected to be diagnosed every 4 years (hence ~15/year). Approximately 20-25% of patients with high-grade MDS will suffer from relapse following HSCT, indicating that per year ~5-7 patients could be included (Kardos et al, 2003; Niemeyer & Kratz, 2008). Therefore it is anticipated that we will be able to recruit the required number of patients in stratum 1 and 2 (n=24) in approximately 3-4 years (Kardos et al, 2003, Niemeyer & Kratz, 2008, Stary et al, 2008).

The number of patients with secondary MDS after prior treatment for chemotherapy and radiotherapy, excluding patients after prior aplastic anemia, is approximately 8 patients per year. Pre-treatment will be very heterogenous. Therefore this stratum is explorative and we aim to recruit 12 patients with either ‘newly diagnosed or relapsed’ secondary MDS in one stratum, and provide descriptive results.

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 35%. This means that 10-15 newly diagnosed patients per year are eligible for this study, and approximately 5 relapses/year. Therefore we anticipate that a sufficient number of patients can be enrolled in a 3-4-year time period.
10 ETHICS

This study will be conducted in accordance with the ethical principles that have their origin in the current Declaration of Helsinki, and will be consistent with International Conference on Harmonization Good Clinical Practice (ICH GCP) and applicable regulatory requirements.

The study should be conducted in compliance with the protocol. The protocol and any substantial amendment and the subject informed consent forms need to be approved by the Institutional Review Board (IRB)/Independent Ethics Committee (IEC) prior to initiation of the study.

The rights, safety and well being of the trial subjects are the most important considerations and should prevail over interests of science and society.

Study personnel involved in conducting this trial will be qualified by education, training, and experience to perform their respective task(s), and will be authorized to perform study related procedures as laid down in the delegation of authority log.

Systems with procedures that assure the quality of every aspect of the study will be implemented.

Left over cells from extra blood and bone-marrow samples taken for central laboratory diagnostic or PK/PD procedures will be stored for a period of at least 15 years for future studies on JMML and/or MDS. Patients/parents will be asked for permission during the informed consent process. No additional informed consent will be asked for using the left-over cells. Use of left-over cells for additional studies not currently described in this protocol or the procedure manual will be decided upon by the principal investigators involved in this study.

11 END OF STUDY REPORT

The sponsor will notify the accredited IRB/IEC and the competent authority of the end of the study within a period of 90 days. The end of the study is defined as the last patient’s last visit. In case the study is ended prematurely, the sponsor will notify the IRD/IEC and the competent authority within 15 days, including the reasons for the premature termination.

Within one year after the end of the study, the Coordinating Investigator/sponsor will submit a final study report with the results of the study, including any publications / abstracts of the study, to the accredited IRB/IEC and the Competent Authority.

12 PUBLICATION/PRESENTATION OF RESULTS

The results of the clinical trial will be published after complete data collection and evaluation. Partial or preliminary results will not be published beforehand. Publications and/or presentations are to be initiated and/or authorized by the Coordinating Investigators, and will be prepared by the Coordinating Investigators as first and last author of the paper.

The following persons will be considered as co-authors:

- principle investigators who have recruited at least 1 patient into the trial. In case the journal we want to publish the paper in does not allow a large number of co-authors this may have to be restricted to investigators enrolling at least 2 or more patients.
• the national principle investigators representing their country/national group as co-sponsor.

The final publication manuscript should be prepared within six months after last patient last visit. The co-authors must notify the main author in writing concerning their approval or proposed changes to the manuscript within four weeks after receiving the publication draft. Failing to do this, their approval will be assumed.

Any publication in the form of a lecture, poster or publication of data must be approved by the coordinating investigator. Such publication should generally not occur before the joint publication of the study group. Enquiries from the press and general public concerning study results may only be answered by the investigator after consulting the sponsor.

13 INVESTIGATOR SPONSOR AND CO-SPONSOR RESPONSIBILITIES AND PATIENT INSURANCE

This study is an investigator-initiated study, which will be a collaborative effort between the Erasmus MC and the Academic Medical Center in Amsterdam, working together in the Dutch Childhood Oncology Group - Early Clinical Trial Consortium (DCOG-ECTC). Erasmus MC will act as the sponsor of the study.

This study will be performed in collaboration with the EWOG MDS Group and the ITCC consortium.

Erasmus MC is the coordinating sponsor of the study. There is a co-sponsor for each country involved in the study. The (co)-sponsor of each country will provide insurance or indemnity in accordance with the applicable regulatory requirements for all patients within that country.

Any investigator or co-investigator who signed this protocol agrees to carry out this research in accordance with the protocol approved by the ethic committee, GCP and regulatory requirements.

Study personnel involved in conducting this trial should be qualified by education, training, and experience to perform their respective task(s).

The Principle Investigator should provide the sponsor with his/her signed and dated CV.

The sponsor has the right to prematurely discontinue the study for significant efficacy or safety problems and will notify the investigator in writing, as well as the IRB/ethic committees and the competent authorities according to local law and regulations.
APPENDIX 1. Diagnostic criteria for MDS and JMML

Part A: 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

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

**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
- a first degree relative (parent or sibling) with NF1.
Part B: advanced 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 time, the subtype of RAEB was redefined, now accommodating all cases with up to 20% blasts in PB. Six MDS subtypes are described by the WHO.

- 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%)

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/RARA), 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 $\geq 30\%$ 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.
**APPENDIX 2: PERFORMANCE STATUS CRITERIA**

Please use the score which is most applicable for this particular child.

<table>
<thead>
<tr>
<th>Lansky score: for younger children</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fully active</td>
<td>100</td>
</tr>
<tr>
<td>Minor restriction in normal physical activity</td>
<td>90</td>
</tr>
<tr>
<td>Active, but tires more quickly</td>
<td>80</td>
</tr>
<tr>
<td>Both greater restriction and less time spent in active play</td>
<td>70</td>
</tr>
<tr>
<td>Minimal active play, busy with quieter activities</td>
<td>60</td>
</tr>
<tr>
<td>Gets dressed, but no active play, able to participate in all quiet play and activities</td>
<td>50</td>
</tr>
<tr>
<td>Mostly in bed, participates in quiet activities</td>
<td>40</td>
</tr>
<tr>
<td>In bed, needs assistance even for quiet play</td>
<td>30</td>
</tr>
<tr>
<td>Often sleeping, play limited to passive activity</td>
<td>20</td>
</tr>
<tr>
<td>No play, does not get out of bed</td>
<td>10</td>
</tr>
<tr>
<td>Unresponsive</td>
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</table>

<table>
<thead>
<tr>
<th>Karnofsky score: for older children</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
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<tr>
<td>Able to carry on normal activities</td>
<td>90</td>
</tr>
<tr>
<td>Normal activity with effort</td>
<td>80</td>
</tr>
<tr>
<td>Cares for self, unable to carry on normal activity or to do active work</td>
<td>70</td>
</tr>
<tr>
<td>Requires occasional assistance, is able to care for most of own needs</td>
<td>60</td>
</tr>
<tr>
<td>Requires considerable assistance, frequent medical care</td>
<td>50</td>
</tr>
<tr>
<td>Disabled, requires special care/assistance</td>
<td>40</td>
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<tr>
<td>Severely disabled, hospitalization</td>
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<td>Hospitalization, very sick, active treatment</td>
<td>20</td>
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<td>Moribund, fatal processes in progression</td>
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<tr>
<td>Dead</td>
<td>0</td>
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<tr>
<td>Assessment</td>
<td>Screening</td>
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<tr>
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<td>--------------------</td>
</tr>
<tr>
<td>Signed written Informed Consent</td>
<td>X</td>
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<tr>
<td>Physical examination/Vital Signs</td>
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<tr>
<td>Height, Weight, BSA</td>
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<td>Medical History/Concurrent Conditions</td>
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<tr>
<td>Performance Status</td>
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<tr>
<td>Hematology (CBC with differential; including platelets)</td>
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</tr>
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<td>Serum Chemistry</td>
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<tr>
<td>BM aspirate, bilateral</td>
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</tr>
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<td>BM trephine biopsy, bilateral</td>
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<td>BM and PB for central cytogenetics and FISH in Rotterdam</td>
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<td>Study Treatment Administration</td>
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<td>Concomitant Medications/Transfusions</td>
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<tr>
<td>Adverse Event Assessment</td>
<td>X</td>
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<tr>
<td>Follow up</td>
<td>X</td>
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</table>
Hematology frequency: twice weekly during Azacitidine administration (or more frequently when clinically indicated). After that at least once weekly until the next treatment course.

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.

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.

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

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

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

Lumbar puncture in case CNS-involvement at inclusion.

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

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.

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

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.

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

If end of a course coincides with end of treatment, end of treatment examinations should be done.
<table>
<thead>
<tr>
<th>Assessment</th>
<th>Screening</th>
<th>Course 1</th>
<th>Course 2</th>
<th>Course 3</th>
<th>Additional Courses</th>
<th>End l, m of Treatment</th>
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<tbody>
<tr>
<td><strong>Assessment</strong></td>
<td>Baseline</td>
<td>During</td>
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<td>Baseline</td>
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<td>Signed written Informed Consent</td>
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<td>Physical examination/Vital Signs</td>
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<td>Height, Weight, BSA</td>
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<td>Medical History/Concurrent Conditions</td>
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<td>Spleen size (physical examination)</td>
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<td>Abdominal sonography a (ultrasound spleen size)</td>
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<td>Hematology b (CBC with differential: including platelets)</td>
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<td>PB and BM aspirate for morphology and flow cytometry at site</td>
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<td>PB slides for central review morphology (send to Freiburg)</td>
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<td>BM and PB for central cytogenetics and FISH in Rotterdam</td>
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<td>PB for Pharmacodynamics f, g (send to Freiburg)</td>
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<td>PB for Molecular diagnostics (send to Freiburg)</td>
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<td>Pharmacokinetic sampling (PK)</td>
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Abdominal ultrasound: at end of each treatment course.

Hematology frequency: twice weekly during Azacitidine administration (or more frequently when clinically indicated). After that at least once weekly until the next treatment course. Absolute Monocyte Count: twice weekly during each course of azacitidine administration and at least once weekly after that.

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

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.

Pharmacodynamic studies: clone-size and methylation status.

Clone-size follow up.

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

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.

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.

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 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 CT grade ≤1, or are deemed irreversible.

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

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


