Clinical Study Protocol

Study Title:

A prospective, multicentre European Registry for newly diagnosed patients with Myelodysplastic Syndromes (MDS), including acute myeloid leukaemia with 20-30 percent marrow blasts (former RAEB-t), and Chronic Myelomonocytic Leukaemia (CMML).

Short Title:

A prospective, multicentre European Registry for newly diagnosed patients with Myelodysplastic Syndromes (EUMDS).

Protocol Number:

5.1

Protocol Status:

Final *(modification of protocol V2.2)*

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Release Date:

18st of April 2016

Number of Pages:

41

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Study Title:

A prospective, multicentre European Registry for newly diagnosed patients with Myelodysplastic Syndromes (MDS), including acute myeloid leukaemia with 20-30 percent marrow blasts (former RAEB-t), and Chronic Myelomonocytic Leukaemia (CMML).

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Protocol Synopsis

Study Title:

A prospective, multicentre European Registry for newly diagnosed patients with Myelodysplastic Syndromes (MDS), including Acute Myeloid Leukaemia (AML) with 20-30 percent marrow blasts (former RAEB-t), and Chronic Myelomonocytic Leukaemia (CMML).

Study Objectives:

To collect and to describe the demographics, disease-management, and treatment outcomes of MDS[2] patients who are newly diagnosed and classified according to the WHO criteria[3], including therapy-related MDS and MDS-Fibrosis (MDS-F), AML with 20-30 percent marrow blasts (former RAEB-t), and CMML and other forms of mixed MDS/MPD.

To perform observational studies concerning relevant scientific research questions in MDS using clinical data and biological samples, and to present relevant research outcomes in the fields of diagnosis and prognostication, health related quality of life issues, health economics, and risk stratification for newly developed classes of drugs.

To disseminate the results of the studies to all stakeholders involved, including patients, health care givers, health care authorities, health insurance companies, pharmaceutical companies and health care professionals.

Methodology:

Data on patients with MDS will be collected prospectively at diagnosis and at 6-month intervals after diagnosis for all registered patients. The data will be collected by seventeen (or more)[1] countries that are represented within the LeukemiaNet MDS Working Party and will be combined in one central European Database. Data analyses will be conducted by the Data Management Centre at the University of York in various sub studies, after every 500 patients included in the European Registry and at the end of the follow-up period.

Number of Patients & Centres

Over 150 haematology centres in seventeen (or more)[1] different countries (Austria, Croatia, Czech Republic, Denmark, France, Germany, Greece, Israel, Italy, the Netherlands, Poland, Portugal, Republic of Serbia, Romania, Spain, Sweden, and United Kingdom) will participate in this Registry. The recruitment target is a minimum of 3000 lower-risk MDS and 1000 higher-risk cases.

Population:

The study population will consist of newly diagnosed patients with all subtypes of MDS classified according to the WHO criteria[3], including therapy-related MDS and MDS-F, AML with 20-30 percent marrow blasts (former RAEB-t), and CMML and other forms of mixed MDS/MPD.

Study Duration:

The enrolment time will continue at least until May 1st 2020 but extension of the recruitment period is possible. The follow-up period will be until termination of the EUMDS Registry (up to 12 years after enrolment or longer if the study is extended).

[2] The abbreviation of MDS will cover all subgroups described in the study population, if not mentioned otherwise

[3] Both the WHO-2008 and WHO-2016 classification will be recorded.

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List of abbreviations

AML	Acute Myeloid Leukaemia
AHCT	Allogeneic Haematopoietic Cell Transplantation
BM	Bone Marrow
BSC	Best Supportive Care
CCR	Conventional Care Regimen
CI	Confidence Interval
CMML	Chronic Myelomonocytic Leukaemia
CR	Complete Remission
CRF	Case Report/Record Form
CRP	C-Reactive Protein
DNA	Deoxyribonucleic Acid
EDTA	Ethylenediaminetetraacetic Acid
ELN-WP8	European LeukemiaNet MDS Working Party
EQ-5D	EuroQol 5D
ESA	Erythropoietin Stimulating Agent
EU	European Union
EUMDS ID	Unique identification number within the European MDS Registry
FAB	French-American-British Criteria
FCM	Flow cytometry
Hb	Haemoglobin
HDAC	Histone-Deacetylase
HMA	Hypomethylating Agent
ICH-GCP	International Conference on Harmonisation - Good Clinical Practice
IPSS	International Prognostic Scoring System
IPSS-R	Revised International Prognostic Scoring System
LDH	Lactate Dehydrogenase
LVEF	Left Ventricular Ejection Fraction
MCV	Mean Corpuscular Volume
MDS	Myelodysplastic Syndromes
MDS-F	Myelodysplastic Syndromes with Fibrosis
MPD	Myeloproliferative Disease
HRQoL	Health Related Quality of Life
PB	Peripheral Blood
PR	Partial Remission
PRO	Patient Reported Outcomes
RA	Refractory Anaemia
RARS	Refractory Anaemia with Ringed Sideroblasts
RAEB-1	Refractory Anaemia with Excess of Blasts 1
RAEB-2	Refractory Anaemia with Excess of Blasts 2
RAEB-t	Refractory Anaemia with Excess Of Blasts in transformation
RCMD	Refractory Cytopenia with Multilineage Dysplasia
RCMD-RS	Refractory Cytopenia with Multilineage Dysplasia and Ringed Sideroblasts
SUSAR	Suspected Unexpected Serious Adverse Reaction
tAML	Therapy-related Acute Myeloid Leukaemia
tMDS	Therapy-related Myelodysplastic Syndromes
VAS	Visual Analogue Scale
WHO	World Health Organization
WPSS	WHO Classification-Based Prognostic Scoring System

1. Introduction

Myelodysplastic syndromes (MDS)[2] are a heterogeneous group of haematopoietic stem cell disorders.[1] They are characterized by dysplasia in the myeloid, megakaryocytic and/or erythroid lineages. The abnormal cells belong to a (pre-)malignant clone, which usually represses progressively the remaining normal cells in the bone marrow. Patients with MDS suffer from peripheral blood cytopenias (anaemia, leukopenia and/or thrombocytopenia). The natural course of MDS ranges from an indolent disease that may span years, to a more acute manifestation with severe bone marrow failure resulting in life-threatening complications. About 30% of the patients show progression towards acute myeloid leukaemia (AML), but most patients eventually die from complications of bone marrow failure.

1.1 Incidence and diagnosis

The overall incidence of MDS is estimated to be 3-4 per 100,000 per year, but the incidence increases to 32.1%/100.000 per year among those aged 80 years.[2] The incidence is generally underestimated due to the complexity of diagnosing MDS, which accounts especially for the more indolent forms in elderly patients. In the last decades the incidence of MDS seems to have increased. In part this may be due to an increased readiness to perform bone marrow examinations in the increasing population of elderly persons, but there is also some evidence for a real increase due to occupational and environmental exposure to chemicals like benzene and other organic solvents. Furthermore, treatment with radiotherapy and / or certain chemotherapeutic agents promotes the development of therapy-related MDS and AML (tMDS/tAML).[3] Approximately 70% of the patients can be defined as low-risk (IPSS-R low & very low risk and intermediate risk) and 30% as high-risk disease (IPSS-R high & very high risk).[4]

In clinical practice today, cytomorphologic evaluation of the peripheral blood and bone marrow continues to be the basis of MDS diagnostics. The clonal haematopoietic cells show dysplastic features. However, there are a number of other conditions, such as infections or medication that can result in transient cytopenias and dysplastic cells, without clonal aberrations. In approximately 50% of patients chromosomal abnormalities are found using conventional cytogenetics, which can facilitate the diagnosis of MDS.

1.2 Classification

Classification systems have been developed to serve as a guide for the diagnosis, estimation of prognosis and management of patients with this disease. However, newly acquired knowledge about the pathogenesis of MDS and the development of novel forms of therapy require that classification systems are continuously open to changes. From 1982 up until 1997, the myelodysplastic syndromes have been classified according to the FAB (French-American-British)-criteria.[5, 6] In this classification five subcategories have been described, based on the percentage of blast cells in blood and bone marrow, the percentage of ring sideroblasts and the number of monocytes: refractory anaemia (RA), refractory anaemia with ring sideroblasts (RARS), refractory anaemia with excess blasts (RAEB), refractory anaemia with excess blasts in transformation (RAEB-t) and chronic myelomonocytic leukaemia (CMML). Advanced MDS is defined as RAEB with more than 10% blast cells and RAEB-t. Median survival of these patients is generally shorter than 12 months. Because of the limitations of the FAB classification, the World Health Organization (WHO) [7] has provided a new system that classifies patients according to the number of cell lineages affected, the number of blasts in peripheral blood and bone marrow, the presence of ringed sideroblasts and the result of cytogenetic analysis. Patients with RA (+/-RS), RCMD (+/-RS), or a solitary deletion of the long arm of chromosome 5, have a relatively good prognosis regarding survival and risk of developing AML. Prognosis is worse in the RAEB-1 subgroup. Patients with RAEB-2 in general have the highest risk of progression to AML and the lowest overall survival. One of the major changes in the WHO classification compared to the FAB classification is lowering the blast percentage for the diagnosis of AML

from 30% to 20%. Several studies have suggested that there is little difference between RAEB-t and AML in terms of prognosis and response to chemotherapy. As a consequence RAEB-t has been eliminated from the MDS classification and included in the AML diagnosis.[7]

CMML was eliminated from the MDS category because of features at the time of initial presentation of both a myelodysplastic disease as well as a myeloproliferative disease (MDS/MPD). The WHO-2001 criteria classified CMML into two prognostic subclasses, CMML-1 and CMML-2, based on the number of blast cells in the blood and bone marrow.[7] In 2008, the WHO classification was updated with several minor changes in comparison to the 2001 WHO classification scheme.[8] The WHO-2016 criteria (pre-published online April 2016), have minor changes in comparison to the WHO-2008.[9, 10]

The International Prognostic Scoring System (IPSS) was developed for assessment of prognosis of MDS.[11] The IPSS system comprises three parameters, bone marrow blast percentage, karyotype and number of cytopenias (appendix A2). Patients with a low or intermediate-1 score are more likely to have an indolent disease course (often defined as 'low-risk'). Patients in the intermediate-2 or high risk group are more likely to suffer from aggressive disease with a higher frequency of transformation to AML. In 2007 the WHO Classification-Based Prognostic Scoring System (WPSS) was developed, which can be used as a dynamic prognostic scoring system for predicting survival and leukemic evolution in patients with MDS.[12]

The IPSS has been revised in 2012.[4] Patients have been subdivided in 5 prognostic groups based on a more precise cytogenetic risk calculation, and more detailed subdivision of the cytopenias and percentage of marrow blasts. The IPSS-R has proven its value as a more refined risk stratification tool in our lower risk MDS registry by identifying a group of patients with a high/very high IPSS-R risk score (5%) within the IPSS low and intermediate-1 groups.[13] The EUMDS Registry has analysed the value of IPSS-R in the first 1,000 patients entered in the Registry. IPSS-R appeared to estimate prognosis more accurately especially in the intermediate-1 risk patients.[13] In the near future, it is expected that genetic markers will be increasingly incorporated in the classification of MDS.[14]

1.3 Treatment

Management decisions in MDS are partly based on the WHO classification and IPSS-R score.[15] Allogeneic haematopoietic cell transplantation (AHCT) remains the only potentially curative treatment. AHCT is recommended to patients with advanced disease stages. The intensity of this treatment and the average high age in MDS around 75 years precludes the general application of transplantation in this patient population although reduction of the conditioning regimens has allowed a wider application of transplantation.[16] Intensive anti-leukemic (anti-AML) chemotherapy in the treatment of higher risk MDS patients has not been proven as effective as in de novo AML due to a lower complete remission (CR) rate and remission duration.[17] In the last years a number of new drugs are under investigation such as the histone-deacetylase inhibitors (HDAC inhibitors) and hypomethylating agents (HMA). HMA or the DNA methyltransferase inhibitors are a relatively new class of drugs which have shown efficacy in the treatment of MDS. 5-Azacitidine (Vidaza®) and its deoxy derivative decitabine (Dacogen®) are pyrimidine nucleoside analogs of cytidine, which are thought to exert their anti-neoplastic activity by incorporating into the DNA of the cells and impairing methyltransferase, resulting in DNA hypomethylation and direct cytotoxicity to abnormal haematopoietic cells in the bone marrow.[18, 19] Also, genes that are critical for normal differentiation and proliferation may be restored by hypomethylation. 5-Azacitidine has been approved for treating intermediate-2 and high risk subtypes of MDS and decitabine only for patients with AML.[19] Data from an international phase III trial demonstrated an overall survival benefit in higher-risk MDS patients treated with 5-Azacitidine. This overall survival benefit extends to patients with WHO-defined AML.[18] Patients achieved significantly improved overall survival compared to those treated with a conventional care regimen (CCR). Therefore, these agents provide an alternative treatment for newly diagnosed patients with MDS of IPSS intermediate-2 and high-risk subtypes and patients with AML or CMML according to WHO definitions, who have significant co-morbidities that preclude stem cell transplantation or intensive chemotherapy.[19]

The care of patients with MDS has improved during the past decades. However, because treatment options are still limited, good supportive care remains a central aspect in the management of MDS patients with good prognosis or patients with poor prognosis who are not eligible for stem cell transplantation. For example, the implementation of growth factors, including erythropoietin stimulating agents (ESAs) [20], and immunomodulating agents thalidomide and more recently lenalidomide (for del5q) [21] has improved the management of patients with IPSS low and intermediate-1 risk MDS. Drugs have been developed to prevent and to treat the complications of MDS, such as infections or transfusion-induced iron overload. Although collaboration between centres has led to the development of national and international guidelines on the treatment of MDS, there is a large variation in clinical management.[16] Published data on the management of MDS are mainly based on local experience and expert opinions.[16, 22]

1.4 European MDS Registry (EUMDS)

The European MDS Registry (EUMDS) started as an observational pan-European study aiming to prospectively collect longitudinal data from a large number of lower-risk myelodysplastic syndromes (MDS) patients in April 2008. The registry has evolved into a valuable source containing data on diagnostics, demographics, clinical parameters, health-related quality of life (HRQoL), disease-management and outcome of over 2000 newly diagnosed lower-risk (IPSS low and intermediate-1) MDS patient across 142 centres in 17 countries. In a number of these countries, national MDS Registration projects are ongoing aiming at improving the knowledge of the local incidence and management of these patients. The EUMDS registry serves as a central international registry, using the national MDS Registries that are represented within the European LeukemiaNet MDS Working Party (ELN WP8) as the platform for registration, to study the demographics, disease-management and treatment outcomes in patients with newly diagnosed MDS more comprehensively. Recently, the demographics, treatment and prognostication of the first 1,000 patients in the EUMDS registry has been evaluated and published.[13]

The aim in the next phase of the EUMDS Registry is to extend the current registry to a general MDS registry including higher-risk (IPSS intermediate-2 and high-risk), therapy-related MDS and MDS-F, AML with 20-30 percent marrow blasts (former RAEB-t), CMML and other forms of mixed MDS/MPD.

2. Study objectives

2.1 Primary objective

To collect and to describe demographics, clinical and lab manifestations, epidemiological data, genetic characteristics, HRQoL, disease-management, and treatment outcomes of MDS patients who are newly diagnosed and classified according to the WHO-2008 and WHO-2016 criteria [8, 10], including therapy-related MDS and MDS-F, AML with 20-30 percent marrow blasts (former RAEB-t), CMML and other forms of mixed MDS/MPD.

Treatment outcomes are defined as: efficacy (including survival, CR, PR and haematological responses as defined in the revised Cheson criteria [23], safety, HRQoL, and Health Economics (see also: secondary objectives).

2.2 Secondary objectives

1. To investigate the relationship between:
   - Clinical characteristics (including WHO classification, genetic characteristics, and known prognostic factors) at inclusion and during follow-up
   - Treatments received, including transfusions,

   and
   - Responses to treatment as defined in the treatment section
   - Overall survival (censored at end of follow-up)
   - Time to progression to high risk MDS and to leukaemia
   - Karnofsky Performance Status (appendix A4), general and disease specific HRQoL
   - Health Economics

2. To derive and validate new prognostic scoring systems based on the data obtained

3. To perform observational studies concerning relevant scientific research questions in MDS using clinical data and biological samples and to present relevant research outcomes in the fields of diagnosis & prognostication, HRQoL issues, health economics, risk stratification for newly developed classes of drugs.

4. To disseminate the results of the studies to all stakeholders involved.

3. Investigational Plan

3.1 Overall Study Design

The registry is designed to collect information about a large cohort of newly diagnosed MDS patients from clinical centres within the participating European countries. Patients will be observed until death or until termination of the EUMDS Registry (up to 12 years after enrolment or longer if the study is extended).

- *Enrolment:* each centre should register all consecutive eligible patients who present during the enrolment period, or until the achievement of the study recruitment target. Patients can be included up to 100 days after diagnosis.
- *Follow-up:* follow-up visits will be scheduled according to the standard practice of the centre and to the treating physician's best judgment. Reports of the follow-up visits will be collected every six months. Clinical and laboratory evaluations for disease or treatment monitoring may be performed more often in dedicated studies running in the centres, but the EUMDS Registry will only collect data at 6 months intervals.

In this study, no clinical, instrumental, laboratory assessments, or therapeutic intervention will be performed other than those required for disease management according to local best practice. The only exceptions will be the Patient Reported Outcomes (PRO) questionnaires and blood sample collection for biological correlative studies, including molecular data. In selected countries and centres, ancillary HRQoL, cardiac function and pharmaco-economics sub-projects will be launched to collect information about the HRQoL of patients and cost implications of the therapeutic strategies (separate protocols).

3.2 Study Population

The European Registry will be limited to patients diagnosed with MDS, including therapy-related MDS and MDS-Fibrosis, patients with acute myeloid leukaemia (AML) with 20-30 percent marrow blasts (former RAEB-t), chronic myelomonocytic leukaemia patients (CMML) and other forms of mixed MDS/MPD. The abbreviation of MDS will cover all subgroups described in the study population, if not mentioned otherwise.

3.2.1 Study sample size

The recruitment target is a minimum of 3000 lower-risk MDS and 1000 higher-risk cases. All patients will have been diagnosed with MDS within 100 days of enrolment. This sample size is intended to be a broad representation of the European MDS patients and sufficiently large for meaningful analysis of MDS subgroups.

3.2.2 Inclusion Criteria

Patients must meet all of the following criteria to be included in the European MDS Registry:

- Age $\geq$ 18 years
- Newly diagnosed patient (within 100 days from the date of the diagnostic BM aspirate)
- MDS classified according to current WHO criteria (both 2008 [8] and 2016 [10] will be recorded)[3]
    - All sub groups of MDS

  - Therapy-related MDS
  - MDS with Fibrosis (MDS-F)
  - AML with 20-30 percent marrow blasts (former RAEB-t)
  - CMML and other forms of mixed MDS/MPD
- IPSS and IPSS-R Risk group classification *(mandatory)*[4]
- Able and willing to provide the written informed consent

3.2.3 Exclusion Criteria

- Age <18 years
- Patient unwilling or unable to give consent
- AML with ≥30 percent marrow blasts according to WHO
- Patients with inv(16), t(5;17) and t(8;21) are considered AML and therefore not eligible
- Patients with higher risk MDS progressed from a previously diagnosed lower risk MDS that was not registered within 100 days after first diagnosis of (lower risk) MDS

3.2.4 Follow-up & withdrawal from the Study

Patients will be followed until termination of follow-up (i.e. death, withdrawal, loss to follow-up, or termination of follow-up period). Patients will be withdrawn from the study in case of:

- Withdrawal of consent. A patient may withdraw consent at any time, without providing a reason.

In these cases, only data on survival will be collected.

3.3 Visits and Assessments

3.3.1 Visit Schedule and Assessments

3.3.1.1 At inclusion

The following data will be collected at inclusion of the patient:

- Inclusion and Exclusion Criteria
- Date of patient inclusion
- Demographic information: sex, date of birth
- Weight, height
- Karnofsky Performance Score (appendix A4), EQ-5D and Visual Analogue Score (appendix A5), MDS specific HRQoL (e.g. QUALMS-1*, to be implemented based on availability of language*)

[4] Cytogenetic data form the basis of MDS risk stratification and proper state of the art treatment of MDS-patients. As of February 2nd, 2015 cytogenetic assessment is mandatory for inclusion in the EUMDS Registry.

- History of MDS: date of MDS diagnosis, WHO-2008 and 2016 classification, IPSS and IPSS-R risk groups
- If secondary MDS: prior disease and type of treatment or prior and type of exposure to cytotoxics or radiation therapy
- Treatment for MDS:
  - Therapies for MDS:
    - if haematopoietic stem cell transplantation: date of transplantation; graft type and source donor cells/type of donor; response after transplantation: CR (yes/no), date of CR, date of relapse.
    - if (intensive) chemotherapy: start and stop date and type of chemotherapy (schedule), number of cycles; response: CR (yes/no), date of CR, date of relapse.
    - if use of hypomethylating agents (HMA): start date and type of agent; number of cycles, date of last HMA dose; response, according to revised Cheson criteria [23], date end of response according to Cheson revised criteria and/or physician.
    - Other therapies, including haematopoietic growth factors: start date and type of therapy; date of last dose; response, according to revised Cheson criteria [23], date end of response according to Cheson revised criteria and/or physician and date of first post-ESA transfusion due to MDS (excluding operations etc).
  - Best supportive care (BSC, also when concomitant to other therapies for MDS):
    - red cell transfusion: date of first transfusion, number of transfusions in the prior year, date of last transfusion and number of units transfused during the follow-up interval, pre-transfusion haemoglobin (Hb) value of last transfusion before visit, serum erythropoietin value with date if available.
    - if treatment with iron chelator is given: dose and schedule, start and stop date and type of therapy, duration, reason for discontinuation, ferritin values with date if available.
- Concomitant diseases, including but not limited to cardiac insufficiency, ophthalmic conditions including lens opacities and cataract, hearing impairment, diabetes mellitus, endocrine dysfunctions, renal or liver disease
- All concomitant medication
- Laboratory values:
  - Peripheral blood: Hb concentration, white cell count, neutrophil, lymphocyte, monocyte, eosinophil and basophil count, platelet count, MCV, CRP, reticulocytes, glucose, albumin, LDH, liver transaminases, ferritin, erythropoietin, transferrin saturation level, serum creatinine and calculated creatinine clearance
  - Bone marrow: date of BM aspirate and/or biopsy, percentage of blasts, percentage of ring sideroblasts, cytogenetics (karyotype)[4]
  - Urine: urinalysis for protein (by dipstick)
- Samples for biological correlative studies, including molecular studies:

  *For all **new included** patients*
  - 2 x EDTA-blood tubes (each 7 ml) for molecular analyses ***at screening*** (see appendix A7.A for handling of samples).
    - *If EDTA-blood is not feasible:* BM aspirate 3-5 ml, or Isolated DNA 2-5 µg of ***screening visit*** (preferred) or at least collected within +/- 3 months before or after diagnosis. Only in the cases that 'patients are not treated' or 'patients are only treated with EPO', samples within +/- 6 months before or after diagnosis (see appendix A7.B for specifications per sample type).

Samples will be labelled only with EUMDS ID and date and time of sampling. *Collected samples for molecular analyses will be stored in the central tissue bank of the EUMDS Registry or can be stored in a local or in a national biobank if available.*

- ***Optional:*** Extra serum sampling only at screening for future research (see appendix A7.C for handling of samples)[5]

*For **already included** patients (if (re-)consent is adequate):*

- Isolated DNA 2-5 µg, viable cells/cell pellets, cytogenetic pellets, or (1-)3 unstained (or stained) BM smears of ***screening visit*** (preferred) or at least collected within +/- 3 months before or after diagnosis. Only in the cases that 'patients are not treated' or 'patients are only treated with EPO', samples within +/- 6 months before or after diagnosis (see appendix A7.A/B for handling of samples).

- Flow cytometry (FCM): performed yes/no, if performed according to the ELN FCM WP8 platform: diagnosis (see appendix A6)

3.3.1.2 At each follow-up visit, including end of study:

Follow-up data will be reported at 6-monthly intervals for all registered patients.

- Date of last visit prior to report
- Weight
- Karnofsky Performance Score (appendix A4), EQ-5D and VAS (appendix A5), MDS specific HRQoL (e.g. QUALMS-1*, to be implemented based on availability of language*).
- Changes in concomitant medical conditions and medication since last visit.
- Changes in MDS specific treatment since last visit:
  - Therapies for MDS.
    - <u>if haematopoietic stem cell transplantation:</u> date of transplantation; graft type and source donor cells/type of donor; response after transplantation: CR (yes/no), date of CR, date of relapse.
    - <u>if (intensive) chemotherapy</u>: start and stop date and type of chemotherapy (schedule), number of cycles; response: CR (yes/no), date of CR, date of relapse.
    - <u>if use of hypomethylating agents (HMA)</u>: start date and type of agent; number of cycles, date of last HMA dose; response, according to revised Cheson criteria [23], date end of response according to Cheson revised criteria and/or physician.
    - <u>Other therapies, including haematopoietic growth factors</u>: start date and type of therapy; date of last dose; response, according to revised Cheson criteria [23], date end of response according to Cheson revised criteria and/or physician and date of first post-ESA transfusion due to MDS (excluding operations etc).
  - Best supportive care (BSC, also when concomitant to other therapies for MDS).
    - <u>red cell transfusion</u>: date of first transfusion, number of transfusions since last visit, date of last transfusion and number of units transfused since last visit, pre-transfusion Hb value of last transfusion before visit, serum erythropoietin value with date if available.

[5] Serum collection should be decided on at country or local site level. Logistics and storage of samples should also be arranged at country or local site level. This will no longer be coordinated centrally by the project management. The protocol for collection and processing of samples is provided in appendix A6.C.

  - if treatment with iron chelator is given: dose and schedule, start and stop date and type of therapy, duration, reason for discontinuation, ferritin values with date if available.

- Suspected Unexpected Serious Adverse Reaction (SUSAR) only in case if reported to local/national registries

- Laboratory values:
  - Peripheral blood: Hb concentration, white cell count, neutrophil, lymphocyte, monocyte, eosinophil and basophil count, platelet count, MCV, CRP, reticulocytes, glucose, albumin, LDH , liver transaminases, ferritin, erythropoietin, transferrin saturation level, serum creatinine and calculated creatinine clearance.
  - Bone marrow: date of BM aspirate and/or biopsy, percentage of blasts, percentage of ring sideroblasts, cytogenetics (karyotype).[4,6]

- Patient outcome:
  - number of transfusions (see above)
  - patients treated with interventional therapies, including haematopoietic growth factors (see above)
  - in case of MDS progression to a more advanced WHO-2008 / 2016 subtype / AML: provide the date of progression, WHO-2008 and 2016 classification.
  - in case of death: provide date and cause of death.

- ***Optional:*** Samples for biological correlative studies, including molecular studies:

  *For **all included** patients:*
  - 2 x EDTA-blood tubes (each 7 ml) for molecular analyses at ***follow-up visit*** (see appendix A7.A for handling of samples).

    Samples will be labelled only with EUMDS ID and date and time of sampling. *Collected samples for molecular analyses will be stored in the central tissue bank of the EUMDS Registry or can be stored in a local or in a national biobank if available.*

  *For **already included** patients with serum samples stored:*
  - ***Recommended:*** Continue extra serum sampling at each follow-up visit only for already included patients who have serum samples of (one or more) previous visits stored for future research (see appendix A7.C for handling of samples).[5]

3.3.2 Laboratory Tests

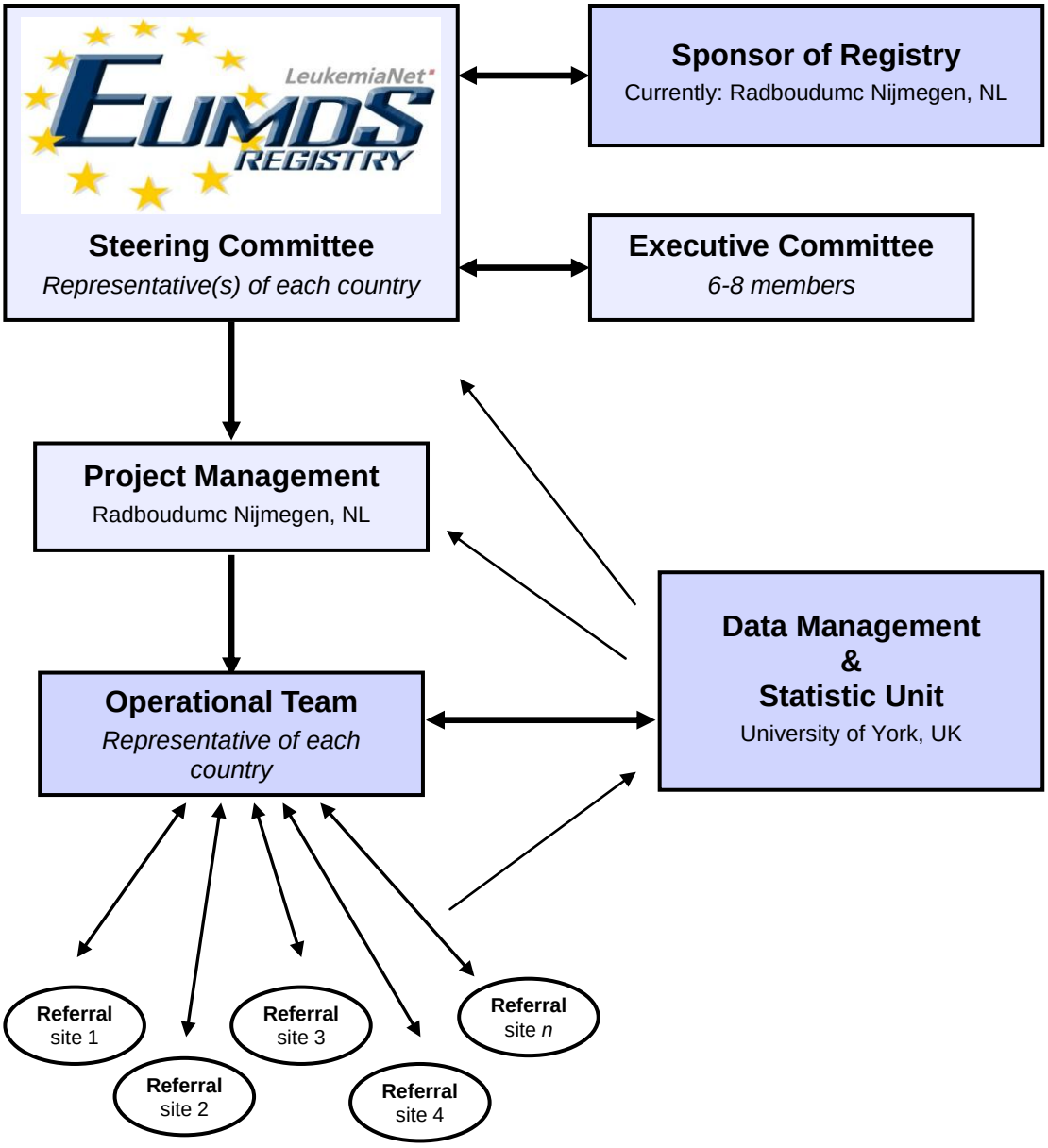
Laboratory tests will be performed as judged appropriate by the treating physician. This study does not require additional laboratory tests to be performed. The laboratory test results of interest will be registered if available.

[6] It is recommended to repeat bone marrow assessments (at the first follow-up visit) to confirm MDS diagnosis.

4. Organization and Responsibilities

4.1 Overall organization

The European MDS Registry is an initiative of the European LeukemiaNet MDS Work Package. The registry is built as a central international platform for registration of data collected by centres (referral sites) sometimes in the context of their local/national registries. The collection of data is coordinated by the operational team consisting of representatives from all countries involved. The central organization is responsible for the management of data and a central unit involved in the statistical analysis. An organisational structure, schematically represented below, has been designed for effective management of the EUMDS Registry.



4.2 Steering committee

The steering committee (SC) is the ultimate decision-making body of the registry and consists of (one or more) representatives from all participating countries, the Chief Investigator (voting members), the project manager, and a representative from the Central Data Management and Statistic Unit (non-voting members). The number of representatives per country will be based on the proportion of patients recruited in the Registry according to the Election policy as approved by the SC.

The SC is responsible for the general design (i.e. study protocol, CRFs), conduct, and overall progress of the Registry. The SC selects the sites for participation in the registry and monitors the availability of resources at all sites. The patient inclusion rate is monitored during the enrolment period of the study. Proposed research questions (sub studies) as well as statistical analysis plans have to be approved by the steering committee. Finally, the SC takes decisions concerning publication policy and authorship.
The SC is supported by **Project Management** and an **Executive Committee**. Project management is responsible for the general day-to-day coordination and execution of general tasks of the Registry (i.e. administrative, financial, contractual, newsletters). The Executive Committee (consisting of the Chief Investigator, 4-6 country representatives, the project manager, and a representative from the Central Data Management and Statistic Unit) is responsible for the day-to-day management of the Registry, and to advice / prepare proposals for the SC. Members of the Executive Committee are appointed according to the Election policy as approved by the SC.

During the inclusion period, the SC will meet every 6 months in a plenary session and by intercurrent TC if necessary. During the follow-up phase of the study, meetings will take place at least once a year in a plenary session. The secretary of the SC will be responsible for drafting the minutes of each meeting and circulating this document after approval.

4.3 Operational Team

The operational team (OT) is chaired by the project manager from the sponsor institution. The OT consists of the coordinators from the referral sites, representatives from the Central Data Management and Statistic Unit, an administrator for the regulatory issues and archive (non-voting member).

The OT is responsible for the overall coordination of the project in the participating countries. This includes the arrangement of support for the contract duties, the distribution of sites metrics, such as the number of patients included, coordination of the referral sites and the organization of Site Training. The project manager organizes all meetings, prepares and distributes the agenda and minutes. Finally, the project manager supports the preparation of publications.

The OT will meet every 12 months in a plenary session (during and after completion of recruitment) and TC will be scheduled on demand. The project manager is responsible for the preparation of the minutes of each meeting and circulation of this document. It is the duty of the project manager to report the important issues to the members of the SC.

4.4 Central Data Management and Statistic Centre

The Central Data Management and Statistics Centre is responsible for the design and maintenance of the core database, data transfer algorithms, and the EUMDS website (www.eumds.org), which will be part of the European MDS competence network website (www.mds-europe.eu). The data management centre prepares working instructions related to the data entry and cleaning, executes the data cleaning and provides a database lock. The statistics centre prepares and executes the statistical analysis. It provides statistical support during the preparation of publications and provides metrics by site.

5. Statistics

The Central Statistical Unit is responsible for the development of the details of the statistical analysis plan. The detailed statistical analysis plan has to be approved by the Steering Committee. This also applies whenever changes in the analysis plan are being considered.

5.1 Sample size

This study is exploratory in nature. Thus, the estimated sample size is not based on a statistical hypothesis, but on an estimation of the number of patients who are diagnosed with MDS per centre in an observation period and sufficiently large to perform some subgroup analyses.

5.2 Collection of clinical variables

All data collected for each patient are displayed in the patient data listings. Unless otherwise stated, *baseline* is defined as the first observation at the time of diagnosis. The tabulation of laboratory data, vital signs and LVEF indicate the normal ranges for each variable. Each value is classified as falling above, below or within normal limit. It is impossible to use a single central laboratory for all parameters and all patients. However, to avoid the issue of collecting hundreds of normal ranges, standard normal ranges will be defined and applied for the purpose of statistical analysis.

5.3 Demographics and disease management

Descriptive analyses will be undertaken at the end of the follow-up period using standard statistical methods to examine the subjects' demographics, disease characteristics and management of these disorders. Interim analyses are described in 5.5.

Time-to-event analyses, namely Kaplan-Meier and Cox proportional hazard regression will be used to estimate progression-free and overall survival:

- **The proportion (with 95% CI) of patients that has progressed to higher risk MDS and/or leukaemia.** The median, range and 95% CI for time to progression will be calculated. Time to progression is defined as the time from diagnosis to the date of the first objective progression to a higher stage of MDS or to leukaemia or the last date the patient was assessed and found to be progression-free. Patients who have been lost to follow-up or have died from any cause (including non disease-related deaths) without documentation of progression will be censored at the last date they were assessed and found to be progression-free. Patients who have not progressed or died will be censored at the last date they were found to be progression-free.

- **The proportion (with 95% CI) of patients that has died during follow-up**. The median, range and 95% CI for survival will be calculated. Overall survival is calculated for all patients from the date of MDS diagnosis to the date of death from any cause. Patients with no documented death are censored at the last date they were known to be alive.

- The proportion (with 95% CI) of patients that experiences an event (e.g. iron overload, cardiac failure, renal failure and/or other co-morbidities).

- The median, range and 95% CI for time to development of an event. Time to the initiation of treatment aimed at the event.

- The proportion (with 95% CI) of patients treated with any treatment for MDS recorded in the registry (including type, dose and schedule of treatment).

5.4 Correlation between patient characteristics and prognosis

Multivariate Cox proportional hazards regression models will be used to identify variables that are important in predicting variables that predict are applied to correlate survival and disease progression. These include clinical variables, such as the WHO classification at enrolment, but also the impact of various treatments received during the course of the disease.

Similar exploratory analyses are applied to investigate the relationship between these patient characteristics and development of co-morbidities (concerning cardiac and renal function) and PROs (including HRQoL assessments)

5.5 Interim analysis

Interim (descriptive) analyses will be conducted when requested for the various sub studies, and at specific time points as decided or requested by the SC, but at least once a year. These analyses will report the patient and disease characteristics, treatment pathways and examine recruitment level across the different centres and countries. These analyses will allow an accurate statistical analytical plan to be developed including formal power calculation to determine the sample size necessary to examine important secondary endpoints, including the impact of the various therapeutic interventions reported in the Registry study.

6. Data recording and data management

6.1 Data recording

Data are recorded and entered through the web-based e-CRF at each national registry site and at clinical sites within each country or uploaded from National Registries by means of tailor made data transfer algorithms *(if (re-)consent is adequate)*. A screening log is maintained at each site to ensure consecutive patient enrolment. Dedicated resources are available for collecting data by a specialized nurse, data manager or equivalent for each national registry site. This resource will be an employee of the Referral Site. This person co-ordinates data entry with the clinical sites and is responsible for validation of data from all clinical sites prior to upload into the central study Database. All data collected for each patient are displayed in the patient data listings. History and clinical conditions are assessed from routine documentation and clinical evaluation performed in the context of inclusion and follow-up visits. The data management centre is responsible for generation of queries.

6.2 Data Management

The Data Management Centre is responsible for the import of data from the national registry sites and for the merging of all data in a central database. Procedures concerning data export, cleaning and database merging will be described in the Data Management Manual. Training is provided for each site and a dedicated helpdesk is available.

The EU general Data Protection Regulation provides every EU citizen with the 'Right to be forgotten'[7]. This might have implications for the data management. Procedures concerning the 'Right to be forgotten' will be described in the Manual of Procedures.

[7] The proposed General Data Protection Regulation of the European Union provides any person with the 'Right to be forgotten'. In summary, this provides any person with the right - under certain conditions - to ask for personal data to be removed once the data is no longer necessary, inaccurate, inadequate, irrelevant, excessive to the purposes for which the data are collected.

7. Quality Control and Quality Assurance

The European Registry is a non-interventional study. Therefore, it is not considered necessary to conduct close monitoring activities with 100% source data verification for all patients. Instead, the quality of the data provided by the referral sites are evaluated on a sample of patients. This evaluation is conducted by a monitor independent from the clinical sites. The monitor reports the results directly to the Sponsor.

In order to ensure source data verification, the participating centres must provide access to all relevant clinical records. Information concerning the identity of the patient does not leave the premises of the centre.

8. Ethics and GCP Compliance

8.1 Subject identification and protection

Patients are cared for according to their treating physician's best judgement. They are not be subjected to any experimental treatment or examination for the purposes of this study. The only exceptions are the PRO questionnaires and blood and / or bone marrow sampling for biological correlative studies, including molecular studies. Patient identifiers will not be recorded in the Registry. An identification number will be allocated to each patient registered, including a code to indicate which local registry registered them.

This version of the protocol will be reviewed by the Local, Regional or National Ethics Committees.

8.2 Informed Consent

All patients who are eligible for inclusion are informed of the aims and nature of the study. They are informed that all their clinical data will be treated confidentially, but that their medical records may be reviewed by authorized persons other than their treating physician for study purposes.

All patients will be informed that participation is voluntary and that they can refuse participation at any time, without consequences for their further treatment. Documented informed consent will be obtained for all patients before they are registered. The informed consent procedure will be conform to the ICH guidelines on Good Clinical Practice (ICH-GCP) and will be in accordance with national and local regulatory requirements.

Human tissues collected in the context of this registration project will be used for scientific studies and genetic characteristics that play a role in MDS. Informed consent must be obtained for collection or 'further use' and storage of human tissues for ongoing or future research. The treating physician may inform the patient about new relevant information from this research which will affect their personal outcome in relation to their MDS. To ensure anonymity, all samples will be coded by the provider (or their staff) prior to transfer to the researcher.

Genetic or other types of research methods that might incur a risk of generating hitherto unknown congenital and clinically relevant findings about the current or future health of the patient can be considered. Efforts will be made to minimize the chance on these findings. An unsolicited finding policy will be implemented in compliance with Local, Regional or National regulatory requirements / ethical approval. Requirement will be established in a sample transfer agreement and registered in the database. Although policies might vary per country, in essence it should encompass informing the patient about the advantages and disadvantages of unsolicited findings, registration / policy for feedback of unsolicited findings to the patients (e.g. patients' preference). In the situation that a policy does not allow participation when the patient does not want to be informed, these samples will not be released for research using these research methods. In the situation that feedback is allowed / required, the researchers will inform the treating physician. The treating physician (with an ethicist/geneticist) will assess the importance of informing the patient. The patient's interest is paramount and overrides all other considerations.

For samples retrieved from biobanks, the policies of these biobanks will be adopted in line with requirements described above. For these samples approval by local or national research ethics committees will be required prior to release of samples.

8.3 Safety reporting

Monitoring of safety of the administered treatment takes place according to the local guidelines. If an adverse reaction has occurred and has been reported to the local or national authorities (according to institutional/regional/national guidelines) a copy of the report should be sent to the Sponsor of the study.

No reporting of adverse reactions is needed in case of:

- Progression to higher risk MDS
- Progression to AML
- Death because of other diseases
- Death because of MDS
- Cytopenia
- Disease related adverse reactions
- Expected side effects of treatment according to the guidelines
- Any event not reported to the local or national authorities, including events reported in local investigational studies

9. Financing and Insurance

The project is an investigator initiated study from the European LeukemiaNet. The study sponsor is the Radboud university medical centre, Nijmegen. Funding for the study is acquired from pharmaceutical companies (Patrons) and projects submitted to governmental and EU programmes, including Horizon 2020 program.

10. Publication Policy

Data and analyses will remain property of the sponsor. Patrons will have the right to use reported data according to the publication policy as defined by the Steering Committee.

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Appendices

A.1 WHO classification of MDS, incl. CMML, former RAEB-t

A1.1a WHO-2008 - MDS [8]

Type MDS	Blood findings	Bone marrow findings
Refractory cytopenias with unilineage dysplasia (RCUD) ▪ Refractory anaemia (RA) ▪ Refractory neutropenia (RN) ▪ Refractory thrombocytopenia (RT)	Unicytopenia or bicytopenia[#1] No or rare blasts (<1%)[#2]	Unilineage dysplasia: ≥10% of the cells in one myeloid lineage <5% blasts <15% of erythroid precursor are ring sideroblasts
Refractory anaemia with ringed sideroblasts (RARS)	Anaemia No blasts	≥15% of erythroid precursors are ring sideroblasts Erythroid dysplasia only <5% blasts
Refractory cytopenia with multilineage dysplasia (RCMD)	Cytopenia(s) No or rare blasts (<1%)[#2] No Auer rods <1x10^9/L monocytes	Dysplasia in ≥10% of the cells in ≥ two myeloid lineages *(neutrophil and/or erytroid precursor and/or megakaryocytes)* <5% blasts in marrow No Auer rods ±15% ring sideroblasts
Refractory anaemia with excess blasts-1 (RAEB-1)	Cytopenia(s) <5% blasts[#2] No Auer rods <1x10^9/L monocytes	Unilineage or multilineage dysplasia 5-9% blasts[#2] No Auer rods
Refractory anaemia with excess blasts-2 (RAEB-2)	Cytopenia(s) 5-19% blasts Auer rods ±[#3] <1x10^9/L monocytes	Unilineage or multilineage dysplasia 10%-19% blasts Auer rods ±[#3]
Myelodysplastic syndrome - unclassified (MDS-U)	Cytopenias ≤yt blasts	Unequivocal dysplasia in less than 10% of cells in one or more myeloid cell lines when accompanied by a cytogenetic abnormality considered as presumptive evidence for diagnosis of MDS (see table chromosomal abnormalities below) <5% blasts
MDS associated with isolated del(5q)	Anaemia Usually normal or increased platelet count No or rare blasts (<1%)	Normal to increased megakaryocytes with hypolobated nuclei <5% blasts Isolated del(5q) cytogenetic abnormality No Auer rods

[#1] Bicytopenia may occasionally be observed. Cases with pancytopenia should be classified as MDS-U.

[#2] If the marrow myeloblast percentage is <5% but there are 2-4% myeloblast in the blood, the diagnostic classification is RAEB-1. Cases of RCUD and RCMD with 1% myeloblasts in the blood should be classified as MDS-U

[#3] Cases with Auer rods and <5% myeloblasts in the blood and <10% in the marrow should be classified as RAEB-2.

Recurring chromosomal abnormalities (incl. frequency) in the MDS at diagnosis

Abnormality	MDS	t-MDS		MDS	t-MDS
Unbalanced			*Balanced*		
+8*	10%		t(11;16)(q23;p13.3)		3%
-7 or del(7q)	10%	50%	t(3;21)(q26.2;q22.1)		2%
-5 or del(5q)	10%	40%	t(1;3)(p36.3;q21.2)	1%	
del(20q)*	5-8%		t(2;11)(p21;q23)	1%	
-Y*	5%		inv(3)(q21;q26.2)	1%	
i(17q) or t(17p)	3-5%		t(6;9)(p23;q34)	1%	
-13 or del(13q)	3%				
del(11q)	3%				
del(12p) or t(12p)	3%				
del(9q)	1-2%				
idic(X)(q13)	1-2%				

* The presence of these abnormalities as the sole cytogenetic abnormality in the absence of morphological criteria is not considered definitive evidence for MDS. In the setting of persistent cytopenias of undetermined origin, the other abnormalities shown are considered presumptive evidence of MDS in the absence of definitive morphologic features

A1.1b WHO-2008 – CMML [8]

1. Persistent peripheral blood monocytosis >1x10^9/L
2. No Philadelphia chromosome or BCR-ABL1 fusion gene
3. No rearrangement of PDGFRA or PDGFRB *(should be specifically excluded in cases with eosinophilia)*
4. Fewer than 20% blasts^ in the blood or bone marrow. *(The finding of >20% blasts in the blood and/or bone marrow indicates AML rather than CMML)*

Type CMML	Blood findings	Bone marrow findings
CMML-1	<5% blasts *(including promonocytes)*	<10% blasts *(including promonocytes)*
CMML-2	5-19% blasts *(including promonocytes)* *Or* Auer rods + *(irrespective of the blast plus promonocyte count)*	10-19% blasts *(including promonocytes)* *Or* Auer rods + *(irrespective of the blast plus promonocyte count)*

5. Dysplasia in one or more myeloid lineages. If myelodysplasia is absent or minimal, the diagnosis of CMML may still be make if the other requirements are met, and:
   - an acquired, clonal cytogenetic or molecular genetic abnormality is present in the haematopoietic cells, *Or*
   - the monocytosis has persistent for at least 3 months, *and*
   - all other causes of monocytosis have been excluded.

^ Blast include myeloblasts, monoblasts and promonocytes. Promonocytes are monocytic precursors with abundant light gray or slightly basophilic cytoplasm with a few scattered, fine lilac-coloured granules, finely-distributed, stippled nuclear chromatin, variably prominent nucleoli, and delicate nuclear folding or creasing, and in this classification are equivalent to blasts. Abnormal monocytes which can be present both in the peripheral blood and bone marrow are excluded from the blast count.

A1.1c AML with 20-30% blasts (former RAEB-t - FAB classification) [5, 6]

Type MDS	Blood findings	Bone marrow findings
Refractory anaemia with excess blasts in transformation (RAEB-t)¥	Blasts ≥5% *Or* Auer rods	>20 and <30% blasts *Or* Auer rods

¥ Patients with inv(16), t(5;17) and t(8;21) are considered AML and therefore not eligible for the EUMDS Registry

A1.2a WHO-2016 - MDS [9, 10]

Type MDS	Dysplastic lineages	Cytopenias §1	Ring sideroblasts as % of marrow erythroid elements	BM and PB Blasts	Cytogenetics by conventional karyotype analysis
with single lineage dysplasia (MDS-SLD)	1	1 or 2	<15% / <5% §2	BM <5% / PB <1% No Auer rods	Any, unless fulfils all criteria for MDS with isolated del(5q)
with multiple lineage dysplasia (MDS-MLD)	2 or 3	1-3			
with ring sideroblasts *(MDS-RS)*			≥15% / ≥5% §2	BM <5% / PB <1% No Auer rods	Any, unless fulfils all criteria for MDS with isolated del(5q)
- with single lineage dysplasia **(MDS-RS-SLD)**	1	1 or 2			
- with multiple lineage dysplasia **(MDS-RS-MLD)**	2 or 3	1-3			
MDS associated with isolated del(5q)	1-3	1-2	None or any	BM <5% / PB <1% No Auer rods	del(5q) alone or with 1 additional abnormality except -7 or del(7q)
with excess blasts *(MDS-EB)*	0-3	1-3	None or any		Any
- type 1 **(MDS-EB-1)**				BM 5-9% or PB 2-4% No Auer rods	
- type 2 **(MDS-EB-2)**				BM 10-19% or PB 5-19% *Or* Auer rods	
unclassifiable (MDS-U)					
▪ with 1% blood blasts	1-3	1-3	None or any	BM <5% / PB <1% §3 No Auer rods	Any
▪ with single lineage dysplasia and pancytopenia	1	3	None or any	BM <5% / PB <1% No Auer rods	Any
▪ based on defining cytogenetic abnormality	0	1-3	<15% §4	BM <5% / PB <1% No Auer rods	MDS-defining abnormality

§1 Cytopenias defined as haemoglobin <10 g/dL, platelet count <100 x 10^9/L, and absolute neutrophil count <1.8 x 10^9/L; rarely, MDS may present with mild anaemia or thrombocytopenia above these levels. PB monocytes must be <1 x 10^9/L

[§2] If SF3B1 mutation is present.

[§3] 1% PB blasts must be recorded on at least two separate occasions.

[§4] Cases with ≥15% ring sideroblasts by definition have significant erythroid dysplasia, and are classified as MDS-RS-SLD

A1.2b WHO-2016 - CMML [9, 10]

1. Persistent peripheral blood monocytosis >1x10^9/L, with monocytes accounting for ≥10% of the white blood cell count
2. Not meeting WHO criteria for BCR-ABL1-positive CML, primary myelofibrosis, polycythaemia vera or essential thrombocythaemia[^1]
3. No evidence of PDGFRA, PDGFRB or FGFR1 rearrangement or PCM1-JAK2 *(should be specifically excluded in cases with eosinophilia)*
4. Fewer than 20% blasts[^2] in the blood or bone marrow

Type CMML	Blood findings	Bone marrow findings
CMML-0	<2% blasts *(including promonocytes)*	<5% blasts *(including promonocytes)*
CMML-1	2-4% blasts *(including promonocytes)*	5-9% blasts *(including promonocytes)*
CMML-2	5-19% blasts *(including promonocytes)* *And/Or* Auer rods + *(irrespective of the blast plus promonocyte count)*	10-19% blasts *(including promonocytes)* *And/Or* Auer rods + *(irrespective of the blast plus promonocyte count)*

5. Dysplasia in one or more myeloid lineages. If myelodysplasia is absent or minimal, the diagnosis of CMML may still be make if the other requirements are met, and:
   - an acquired, clonal cytogenetic or molecular genetic abnormality is present in the haematopoietic cells[^3], *Or*
   - the monocytosis (as previously defined) has persistent for at least 3 months, *and*
   - all other causes of monocytosis have been excluded.

[^1] Cases of MPN can be associated with monocytosis or they can develop it during the course of the disease. These cases may simulate CMML. In these rare instances, a previous documented history of MPN excludes CMML, while the presence of MPN features in the bone marrow and/or of MPNassociated mutations (JAK2, CALR or MPL) tend to support MPN with monocytosis rather than CMML.

[^2] Blasts and blast equivalents include myeloblasts, monoblasts and promonocytes. Promonocytes are monocytic precursors with abundant light grey or slightly basophilic cytoplasm with a few scattered, fine lilac-coloured granules, finely-distributed, stippled nuclear chromatin, variably prominent nucleoli, and delicate nuclear folding or creasing. Abnormal monocytes, which can be present both in the peripheral blood and bone marrow are excluded from the blast count.

[^3] The presence of mutations in genes often associated with CMML (e.g. TET2, SRSF2, ASXL1, SETBP1) in the proper clinical contest can be used to support a diagnosis. It should be noted however, that many of these mutations can be age-related or be present in subclones. Therefore caution would have to be used in the interpretation of these genetic results.

A1.2b AML with 20-30% blasts

See A1.1c

A.2 International Prognostic Scoring System [11]

IPSS scoring system:

Prognostic Variable	Score Value 0	0.5	1.0	1.5	2.0
BM blasts (%)	<5	5-10	-	11-20	21-30
Karyotype *	Good	Intermediate	Poor	-	-
Cytopenias	0/1	2/3	-	-	-

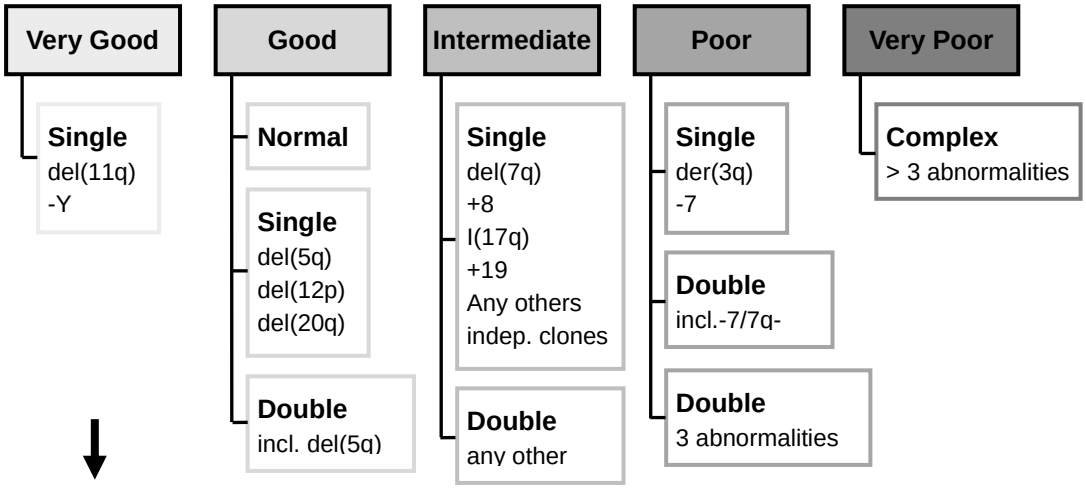
* ***Good:*** normal, -Y, del(5q), del(20q); ***Poor:*** complex (≥ 3 abnormalities) or chromosome 7 anomalies; ***Intermediate :*** all other abnormalities.

IPSS risk groups are classified according to the following sum scores:

IPSS risk group	Sum Scores
Low	0
Intermediate-1	0.5-1.0
Intermediate-2	1.5-2.0
High	≥ 2.5

A.3 Revised International Prognostic Scoring System [4]

Five prognostic cytogenetic groups used in IPSS-R:



IPSS-R scoring system:

Prognostic Variable	Score Value 0	0.5	1	1.5	2	3	4
Cytogenetics	Very good	-	Good	-	Inter-mediate	Poor	Very poor
Blasts	≤2%	-	>2 - <5%	-	5-10%	>10%	-
Hb	≥10	-	8 - <10	<8	-	-	-
Platelets	≥100	50 - <100	<50	-	-	-	-
ANC	≥0.8	<0.8	-	-	-	-	-

IPSS-R risk groups are classified according to the following sum scores:

IPSS-R risk group	Sum Scores
Very Low	≤ 1.5
Low	> 1.5-3
Intermediate	> 3-4.5
High	> 4.5-6
Very High	> 6

A.4 Karnofsky Performance Status

The Karnofsky Performance Scale Index allows patients to be classified as to their functional impairment. This can be used to compare effectiveness of different therapies and to assess the prognosis in individual patients. The lower the Karnofsky score, the worse the survival for most serious illnesses.

Able to carry on normal activity and to work; no special care needed.	100	Normal no complaints; no evidence of disease.
	90	Able to carry on normal activity; minor signs or symptoms of disease.
	80	Normal activity with effort; some signs or symptoms of disease.
Unable to work; able to live at home and care for most personal needs; varying amount of assistance needed.	70	Cares for self; unable to carry on normal activity or to do active work.
	60	Requires occasional assistance, but is able to care for most of his personal needs.
	50	Requires considerable assistance and frequent medical care.
Unable to care for self; requires equivalent of institutional or hospital care; disease may be progressing rapidly.	40	Disabled; requires special care and assistance.
	30	Severely disabled; hospital admission is indicated although death not imminent.
	20	Very sick; hospital admission necessary; active supportive treatment necessary.
	10	Moribund; fatal processes progressing rapidly.
	0	Dead

A.5 EQ-5D and VAS

Figure 1: EQ-5D (UK English version)

By placing a tick in one box in each group below, please indicate which statements best describe your own health state today.

Mobility

I have no problems in walking about	❑
I have some problems in walking about	❑
I am confined to bed	❑

Self-Care

I have no problems with self-care	❑
I have some problems washing or dressing myself	❑
I am unable to wash or dress myself	❑

Usual Activities (*e.g. work, study, housework, family or leisure activities)*

I have no problems with performing my usual activities	❑
I have some problems with performing my usual activities	❑
I am unable to perform my usual activities	❑

Pain/Discomfort

I have no pain or discomfort	❑
I have moderate pain or discomfort	❑
I have extreme pain or discomfort	❑

Anxiety/Depression

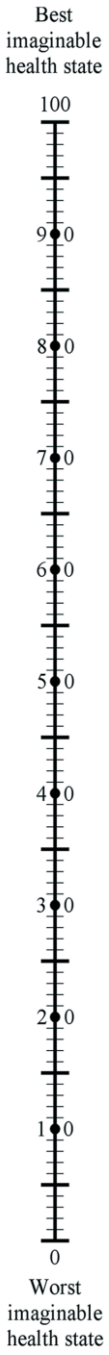
I am not anxious or depressed	❑
I am moderately anxious or depressed	❑
I am extremely anxious or depressed	❑

To help people say how good or bad a health state is, we have drawn a scale (rather like a thermometer) on which the best state you can imagine is marked 100 and the worst state you can imagine is marked 0.

We would like you to indicate on this scale how good or bad your own health is today, in your opinion.

Please do this by drawing a line from the box below to whichever point on the scale indicates how good or bad your health state is today.

YOUR OWN HEALTH STATE TODAY



A.6 Flow cytometric diagnostic algorithm (according to the ELN FCM WP8 platform) [24, 25]

Diagnostic score	<2	<2	<2	<2	<2	<2	<2	<2	≥2	≥2	≥2	≥2	≥2	≥2	≥2	≥2
Dysplasia by FC myeloid progenitor	-	-	-	-	+	+	+	+	-	-	-	-	+	+	+	+
Dysplasia by FC - Neutrophils - Monocytes	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+
Dysplasia by FC - Erythrocytes	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+
Conclusion (see below)	A	B	B	C	B	C	C	C	B	C	C	C	C	C	C	C

A = Results show no MDS-related features (As good as normal)
B = Results show limited number of changes associated with MDS (Borderline Benign)
C = Results are consistent with MDS (Consider MDS)

For more explanation or details see [24, 25]

A.7 Protocol for sample collection and handling

A7.a Whole blood:

Collection:

1. Collect 2 tubes of at least 7 ml EDTA-blood.
   - ⚠ Please collect the blood directly into the tubes.
   - ⚠ Record date and time of sample collection.
2. Write or label each tube with the patients EUMDS ID and sample date and time using a permanent marker pen or sticker.
   - ⚠ Make sure that the information is clearly readable using block capitals.

Handling:

Whole blood can be handled according to one of the following options. For each individual centre, Project Management will decide which of the 2 options will be used based on feasibility of sample transport.

Option 1: Immediate transport to Nijmegen without any handling:

1. Put the properly labelled samples and forms in appropriate packaging.
2. Send this package immediately to the Radboudumc, the Netherlands. Samples should arrive in Nijmegen within 36 - max. 72 hours. (Address will be provided).
3. Send an e-mail to the lab in Nijmegen when sample is sent. (E-mail address will be provided).

Option 2: Immediate storage at -20 ºC or -80 ºC:

1. Put the properly labelled samples without any handling in a freezer at -20 ºC or -80 ºC.
2. Transport of a batch of samples will be arranged with Project Management.

A7.b All other samples for molecular analyses:

If the preferred source for molecular research (EDTA-blood see section A7.A) is not feasible, one of the following sources (see table A7.b1 and A7.b2) are suitable for molecular research. For all these other samples for molecular analyses, collection and handling instructions will be provided and arrangement for transport will be made between the centre and Project Management if applicable.

- ⚠ Samples have to be collected have to be collected +/- 3 months before or after diagnosis
- ⚠ In two cases samples can be collected within +/- 6 months before or after diagnosis:
  - Patients are not treated
  - Patients are only treated with EPO

A7.b1: prospective collection of samples (newly included patients, only if EDTA blood is not feasible):

Type	Requirements		Quantity
1. BM aspirate			3-5ml
2. Isolated DNA	A. BM ***(preferred)***	A1. Mononuclear cells (***preferred***) A2. Whole BM	2-5 µg
	B. Blood	B1. Mononuclear cells (***preferred***) B2. Whole blood	

A7.b2: retrospective collection of samples (patients already included):

Type *Ranked by preference*	**Requirements**		**Quantity**
1. Isolated DNA	A. BM ***(preferred)***	A1. Mononuclear cells (***preferred***) A2. Whole BM	2-5 µg
	B. Blood	B1. Mononuclear cells (***preferred***) B2. Whole blood	
2. Viable cells / cell pellets	From **BM *preferred*** over blood		
3. Cytogenetic pellets	From **BM *preferred*** over blood		
4. BM smears	Preferences: - Uncoloured smears - Without cover slip If not available: coloured with cover slip		(1-)3 smears

A7.c Serum:

Collection:

1. Collect 20 mls of blood into a plain tube(s) containing silica activator. Record time of sample collection on the sample record form.
   - The anticoagulant used for serum should be plain clot activator tubes (silica activator only) *(These Tubes are typically red top (serum) when sourced from Greiner and Becton Dickinson for example).*
   - Please do not use tubes containing gel or separators.
   - Please collect the blood directly into the tubes, not via a syringe

Handling:

2. Serum should be allowed to clot for 1 hour before centrifugation.
3. Centrifuge the samples for 10 minutes, 20 ºC, 2,000 g (approximately 3,000 rpm in many bench top centrifuges - needs to be checked as varies with centrifuge type and size).
   - Any deviations from these times should be recorded on the sample record form.
4. Following centrifugation of the serum tube(s), remove as much of the serum as possible without disturbing the red cells using a fine point Pastette and place into a pooling tube (bijoux). Once serum collection is complete for a sample, use a second Pastette and divide the serum equally between 4 pre-labelled screw top or Eppendorf storage tubes.
   - For efficient storage, 2ml tubes of maximum 4.6 cm height and 1.2 cm diameter (example: see below) are recommended.
5. Write or label each tube with the patients EUMDS ID and sample date using a permanent marker pen or sticker.
   - Make sure that the information is clearly readable using block capitals.
6. Store the sample tubes at -70 ºC / -80 ºC. Record the time of freezing and any deviations from the above protocol.
   - Logistics and storage of samples should be arranged at country or local site level. This will no longer be coordinated centrally by the project management.

IF SAMPLES ARE TO BE SENT FOR PROCESSING TO A REGIONAL LABORATORY THEY **MUST BE PROCESSED ON THE DAY OF COLLECTION**. THE TIME OF COLLECTION AND TIME OF CENTRIFUGATION MUST BE RECORDED ON THE SAMPLE COLLECTION FORM.

A.8 Serum sample collection record for EUMDS Registry

Sample Record Form EUMDS Registry

Please complete in block capitals and using a black ball point pen

EUMDS ID (study no.)…….. Clinical centre ……

Visit number ……..……..

Date of Sample (DD/MM/YY) ☐☐/☐☐/☐☐

	24hr (hh/mm)			
Time of venepuncture				
Time of freezing - SERUM samples				

Number of tubes frozen

Serum ☐

Blood tubes used for venepuncture

Becton Dickinson ☐

Greiner ☐

Sarstedt ☐

Other (please specify) _____

Comments (please document any deviations from the protocol, for example sample processing delays, missing parts of samples, haemolysis etc)

_____

_____

_____

When this form has been completed please:

- Fax a copy to Leeds (F.A.O: UK EUMDS Coordinator, FAX no. ++44 113 2067468)
  - ⚠ Keep the original safely in the site file