A Phase II randomized multicenter study to assess the efficacy of lenalidomide with or without erythropoietin and granulocyte-colony stimulating factor in patients with low and intermediate-1 risk myelodysplastic syndrome

PROTOCOL

Principal Investigator: A.A. van de Loosdrecht

Study Coordinator : P. Muus
G.E. de Greef
P.W. Wijermans

Sponsor : HOVON
VU University Medical Center
P.O.Box 7057
1007 MB Amsterdam

Statistician : C.A.G. van Montfort

Datamanagement : HOVON Data Center

Registration : HOVON Data Center
Erasmus MC - Daniel den Hoed
P.O.Box 5201
3008 AE ROTTERDAM
The Netherlands
tel. +31.10.7041560
fax +31.10.7041028
https://www.hdc.hovon.nl/top

EudraCT number : 2008-002195-10

First version : February 11, 2008

Final version : April 15, 2009

Date of activation : May 27, 2009

Approved : May 06, 2009

Amendment sites-1 : September 21, 2009

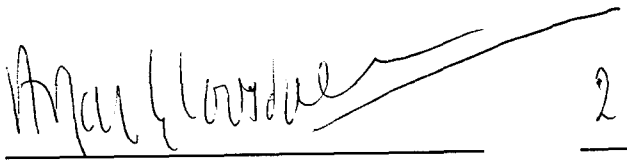
Amendment sites-2 : August 11, 2011

Amendment sites-3 : September 20, 2011

Amendment 4 : December 14, 2011

PRINCIPAL INVESTIGATOR SIGNATURE PAGE

Principal Investigator:



Signature of Investigator

2 | 2 | 2012

Date

A.A. Vande Loosdrecht

Printed Name of Investigator

LOCAL INVESTIGATOR SIGNATURE PAGE

Local site name: _____

Local Investigator:

_____ _____

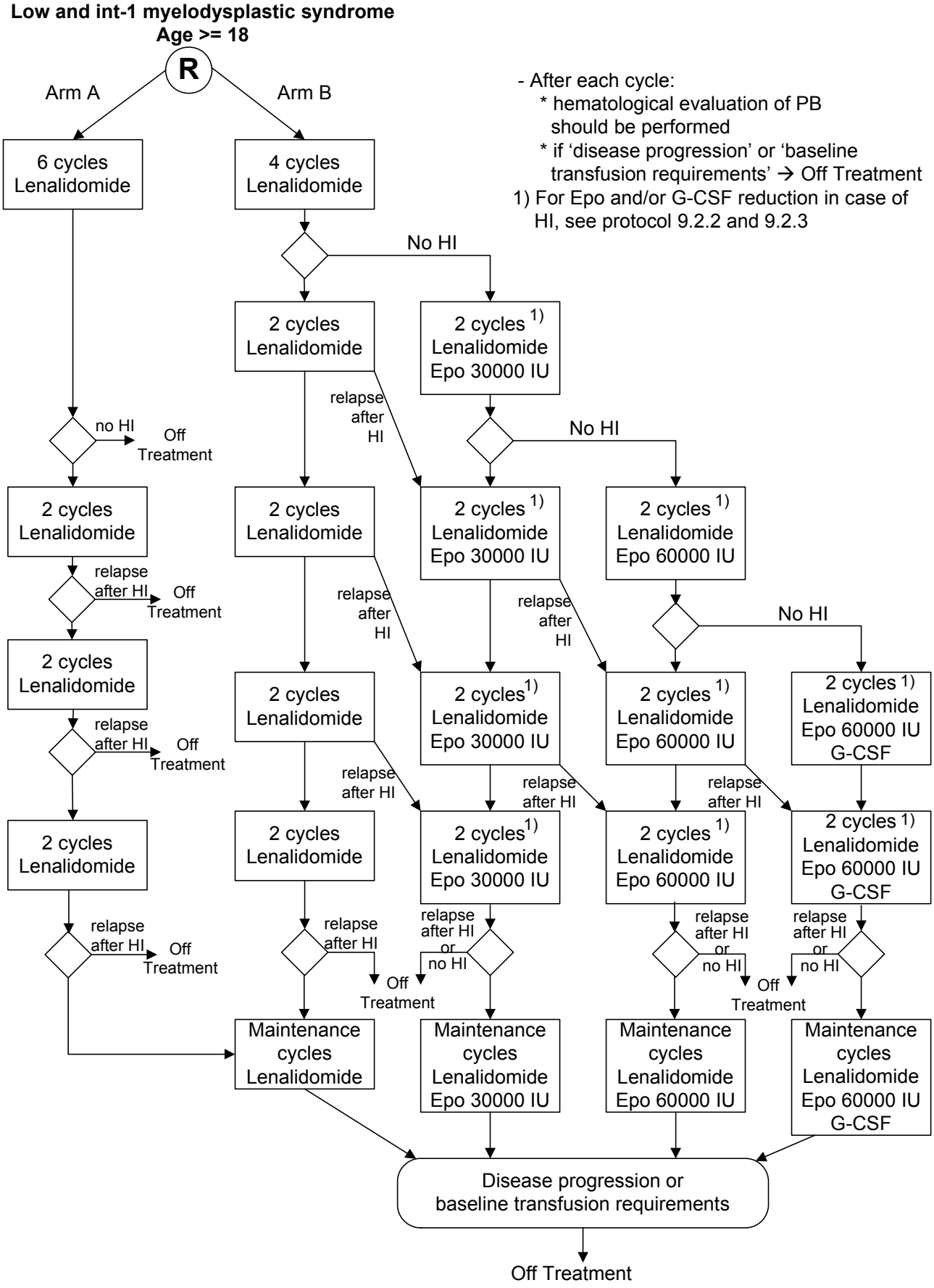
Signature of Investigator Date

_____

Printed Name of Investigator

By my signature, I agree to personally supervise the conduct of this study and to ensure its conduct in compliance with the protocol, informed consent, IRB/EC procedures, the Declaration of Helsinki, ICH Good Clinical Practices guideline, the EU directive Good Clinical Practice (2001-20-EG), and local regulations governing the conduct of clinical studies.

1 Scheme of study



2 Table of contents

Page

3 Synopsis

Study phase	Phase II
Study objectives	To evaluate the efficacy of lenalidomide (Revlimid™) in low/intermediate-1 risk MDS with or without treatment with Epo (NeoRecormon™)/G-CSF (Neupogen™) To evaluate the safety and tolerability of lenalidomide (Revlimid™) in low/intermediate-1 risk MDS with or without Epo (NeoRecormon™)/G-CSF (Neupogen™)
Patient population	Patients with low/intermediate-1 risk myelodysplastic syndrome
Study design	Prospective, phase II, multicenter, open label, with randomization between lenalidomide (Revlimid™) with or without a regimen with Epo (NeoRecormon™)/ G-CSF (Neupogen™)
Duration of treatment	Minimum of 6 months for arm A and 12 months for arm B or until relapse or disease progression; continuation thereafter if responsive. All patients will be followed until 5 years after registration.
Number of patients	200 patients
Adverse events	Adverse events will be documented if observed, mentioned during open questioning or when spontaneously reported
Planned start and end of recruitment	Start of recruitment: I 2009 End of recruitment: II 2013

4 Investigators and study administrative structure

4.1 Responsibilities

Responsibility	Name	Affiliation/Address
Study Coordinators	A.A. van de Loosdrecht, PI	VU University Medical Center Amsterdam
	P. Muus	University Medical Center st.Radboud Nijmegen
	G.E. de Greef	Erasmus MC Rotterdam
	P.W. Wijermans	Hagaziekenhuis Den Haag
Representative of Sponsor (HOVON)	P.C. Huijgens, chairman	VU University Medical Center Amsterdam
Registration	HOVON Data Center	Erasmus MC - Daniel den Hoed P.O.Box 5201 3008 AE ROTTERDAM, The Netherlands tel. +31.10.7041560 fax +31.10.7041028 https://www.hdc.hovon.nl/top
Monitoring	HOVON Data Center	Erasmus MC Rotterdam
Writing Committee	D. Breems	Hospital Stuivenberg Antwerp (B)
	A. Ferrant	Clinique Universitaires St. Luc Bruxelles (B)
	T. Fischer	Johannes Gutenberg-Universität Mainz (D)
	B. Gjertsen	University of Bergen (NO)
	A. Gratwohl	University Hospital Basel (CH)
	P. Huijgens	VU University Medical Center Amsterdam (NL)
	B. Löwenberg	Erasmus MC Rotterdam (NL)
	G.J. Ossenkoppele	VU University Medical Center Amsterdam (NL)
	Th. Pabst	University Hospital Bern (CH)
	W. van Putten	Erasmus MC Rotterdam (NL)
	R. Schaafsma	Medisch Spectrum Twente Enschede (NL)
	H.C. Schouten	University Hospital Maastricht (NL)
	P. Sonneveld	Erasmus MC Rotterdam (NL)
	E. Vellenga	University Medical Center Groningen (NL)
	G. Verhoef	University Hospital Leuven (B)
	Th. De Witte	University Medical Center st.Radboud Nijmegen(NL)
	S. Wittebol	Meander Hospital Amersfoort (NL)
Cytological review	M. Jongen	Erasmus MC Rotterdam (NL)
Cytogenetic review	M. Stevens Kroef	Radboud Nijmegen (NL)
	D. Olde Weghuis	MS Twente; Enschede (NL)
Statistician	C.A.G. van Montfort	HOVON Data Center, Rotterdam
Datamanagement	HOVON Data Center	Erasmus MC Rotterdam
SAEs notification	HOVON Data Center	Erasmus MC Rotterdam fax +31.10.423 9084

4.2 Cytological review

Cytological review has to be performed at diagnosis. Four unstained blood and 6 unstained bone marrow smears should be sent - together with a filled out cytology form - to dr. Mojca Jongen-Lavrencic, Hematocytology Review Committee (HRC), Erasmus MC - Daniel den Hoed, Groene Hilledijk 301, 3075 EA Rotterdam, the Netherlands. Confirmation of diagnosis is not necessary for randomization and start of treatment but sending in of smears for review is required.

4.3 Cytogenetic review

Each cytogeneticist, responsible for the cytogenetic analysis of the MDS patients will be notified automatically by email of the registration of a patient in the study. Review has to be performed for cytogenetic investigation at diagnosis. A filled out cytogenetic form (including FISH results if appropriate) together with 2 representative karyotypes and a copy of the original cytogenetic reports is requested to be sent within 1 month to the HOVON Data Center for central review.
Furthermore, cytogenetic investigation should be performed for safety reasons on a regular basis (after 3, 6, 9 and 12 months and every 6 months thereafter and at disease progression), also in cases of normal cytogenetics at study entry. Reports must be sent to the HOVON Data Center.

5 Introduction

5.1 Myelodysplastic syndromes

The myelodysplastic syndrome (MDS) is a clonal hematopoietic disorder characterized by ineffective erythropoiesis associated with morphological evidence of marrow cell dysplasia resulting in refractory pancytopenia despite normal or hypercellular bone marrow [1]. Various pathogenetic mechanisms have been defined, whereby an augmented apoptosis in MDS patients is a prominent phenomenon [1-3]. Augmented apoptotic cell death of the erythroid lineage is restricted to early MDS subtypes (RA/RARS) in contrast to more advanced stages of MDS (RAEB/RAEB-t) [2-6]. Apoptosis was shown to be largely restricted to the CD34+- compartment reflecting the more primitive progenitor cells [7-10]. Additional evidence that apoptosis plays a role in MDS is provided by studies showing that in approximately 50% of the patients with MDS, increased Fas and Fas-L expression on erythroblasts were found as compared to erythroblasts in normal individuals [11-14]. In normal hematopoiesis, Fas is rapidly upregulated in early erythroblasts and expressed at high levels through terminal differentiation. In contrast, Fas-L is selectively induced in early differentiating Fas-insensitive erythroblasts. Fas-L bearing mature erythroblasts displayed a Fas-based cytotoxicity against immature erythroblasts which is abrogated by high levels of erythropoietin (Epo). These findings suggest a negative feedback apoptotic control mechanism between mature and immature erythroblasts dependent on Epo and thereby regulating erythrocyte production in physiologic

erythropoiesis. The differential expression of Fas/Fas-L in MDS might be related to a differentiation defect and may result in a dysfunctional negative feedback control mechanism and an increased tendency to apoptosis. Finally, Fas-L expression in bone marrow cells at diagnosis in MDS patients who were more anemic correlated directly with red cell transfusion requirements over the subsequent course of the disease and was predictive of survival [14]. In addition, TNF-related family members such as the expression of TRAIL and TRAIL-decoy receptors may influence susceptibility to receptor mediated induction of apoptosis of MDS progenitor cells [15,16].

5.2 Apoptosis in myelodysplastic syndromes: implications for intervention

The data reviewed on the pathogenesis of ineffective hematopoiesis in MDS support the hypothesis that cell death that might be caspase dependent and independent might play an essential role in (pan)cytopenias, particulary in early stages of disease e.g. in RA, RARS and possibly in RAEB with less than 10% blasts. Anti-cytokine based therapies e.g. anti-TNFα may inhibit apoptosis by binding TNFα and/or by downregulating Fas receptors (CD95) [17]. More downstream in the signalling cascade of apoptosis, caspase inhibitors as well as blocking other pro-apoptotic proteins by anti-sense strategies may interfere with ineffective erythropoiesis in early stages of MDS [18]. Treatment of patients with MDS with colony-stimulating growth factors (CSF) such as interleukin-3 (IL-3), granulocyte-macrophage-CSF (GM-CSF), granulocyte-CSF (G-CSF), erythropoietin (Epo) and combinations have shown to ameliorate transfusion requirements and infection rates [19-22]. Treatment with Epo alone is insufficient in the majority of patients as is the use of GM-CSF and G-CSF as monotherapy. In several studies, the combined treatment of G-CSF with Epo has shown a response with respect to erythropoiesis in approximately 45% (up to 57%) of the patients. The predictive response to Epo was shown to be dependent on the MDS-subgroups e.g. RA > RAEB (less than 10% blasts) > RARS, transfusion independency, normal cytogenetics and Epo levels < 200 U/l [23]. Since high levels of TNFα may suppress endogenous Epo production, combination therapies with anti-cytokine based strategies e.g. anti-TNFα, PTX or combination with ciprofloxacin and dexamethason with Epo and G-CSF may further circumvent ineffective erythropoiesis in MDS. Moreover, Epo and G-CSF interfere with apoptosis of erythroid progenitor cells in MDS via blocking the release of cytochrome-c from the mitochondrion [24,25]. Treatment of specific subgroups of patients with MDS with Epo and G-CSF is currently seen as a standard treatment of low/int-1 risk MDS in the USA as well as in some European countries following the recently published guidelines of the treatment of MDS (www.leukemia-net.org) [26,27]. In addition, Epo/G-CSF treatment may not only have positive impact on the quality of live but also shows an increase in overall survival as compared to WHO and IPSS matched control patients [28]. Indeed, evidence is provided that Epo/G-CSF may interfere with immune surveillance mechanisms in MDS by enhancing effective control of potential leukemic outgrowth of the MDS clones [29]. A study comparing all available studies using growth factor treatment versus other treatment approaches e.g. ATG, cyclosporine

(and others) shows a significant superior outcome in terms of overall survival (OS) in patients treated with growth factors [30,31]. In these studies, no significant increase in leukemic evolution could be noted in these growth factor treated patients [28,30,31].

5.3 Immuno modulatory agents (IMiDs) in myeloid neoplasia

IMiDs are structural and functional analogues of thalidomide that represent a new class of immunomodulators for treatment of a variety of inflammatory, autoimmune and neoplastic diseases. The discovery of the antiangiogenic and T-cell co-stimulatory functions of IMiD compounds has led to the investigation of these agents for treatment of hematologic neoplasms such as multiple myeloma and myelodysplastic syndromes [32-33]. The second-generation IMiDs, such as lenalidomide, exhibited a greatly enhanced potency for immunomodulation and antiangiogenesis as compared to thalidomide. IMiDs appear to have reduced sedative and neurotoxicity effects, which are often associated with the use of thalidomide [34]. The precise mechanism of action of IMiDs e.g lenalidomide in the treatment of specific diseases is not entirely clear and may differ for various diseases depending on their underlying pathobiologies. The proposed mechanisms of action of lenalidomide are the inhibition of TNFα (anti-cytokine approach; including IL-1β; IL-6; IL-12), enhancement of co-stimulation of T-cells which might improve the generation of leukemia specific cytotoxic T-lymphocytes (CTLs), an improvement of heterotypic adhesion of hematopoietic progenitors to stroma, the inhibition of angiogenic factors (VEGFs), an improvement of erythroid response of Epo and inhibition of proliferation of chromosome 5 deleted hematopoietic tumour cells (cell lines; in vitro) [35,36]. Finally a recent study suggests epigenetic modulation of erytroid specific genes. Complex interactions between the affected clone and the bone marrow microenvironment drive the pathogenesis and progression of MDS, resulting in ineffective hematopoiesis, blast accumulation, and a variable predisposition for progression to acute leukemia.
Lenalidomide is an orally bioavailable analogue of thalidomide. In in-vitro studies, the effects of lenalidomide include potentiation of the response to erythropoietin via activation of integrin-mediated adhesion, cell cycle arrest, sensitization to apoptotic signals and abrogation of cellular response to receptor-initiated trophic signals [32-36]. Therefore, it is reasonable to combine lenalidomide with a standardized regimen of Epo/G-CSF. These effects have the potential to impact survival and apoptosis of erythropoietic progenitor cells and their progeny. Three major clinical trials of lenalidomide in MDS have shown erythropoietic- and cytogenetic-remitting activities that frequently result in transfusion independence, particularly in patients with 5q deletion and lower risk MDS [37-39].

5.4 Lenalidomide and MDS: the MDS-001, -002 and -003 trials

The MDS-001 phase I-II open label single center clinical trial evaluated safety and efficacy of lenalidomide in 43 patients with symptomatic anemia with either normal, abnormal and sole del(5)(q31.1) cytogenetic abnormalities. Oral lenalidomide dose was 25 mg daily, 10 mg daily, and 10 mg daily for 21 days of a 28-day cycle. The overall erythroid response was 56% in transfusion dependent and/or low probability to respond to EPO low/int-1 risk MDS patients. Although only 12 patients show a del(5q) abnormality, 83% of these patients has an erythroid response. The median time to response was 9 weeks. The cytogenetic response was rapid and was reached at a median of 8 weeks. From in-vitro studies it appears that lenalidomide was cytotoxic leading to sustained suppression and/or apoptosis of the MDS clone in del(5q) patients. In the other patients, lenalidomide promotes the arrest of proliferating cells to restore effective hematopoiesis.
The pivotal MDS-003 multicenter phase II study evaluated oral lenalidomide (10 mg daily or 10 mg daily for 21 days of a 28-day cycle) in 148 patients with del(5q) with or without additional chromosome abnormalities in low/int-1 risk MDS patients, all transfusion dependent. The overall erythroid response was 76%; 67% of the patients became transfusion independent of whom 75% showed an isolated del(5q). The median time to respond was 4.6 weeks (range: 1-49 weeks). The overall cytogenetic response was 73% whereas 45% of the patients were complete cytogenetic responders. 50% of the patients with a complex karyotype showed cytogenetic response, 67% with a del(5q) and 1 additional abnormality, whereas an increase to 77% was shown in patients with only del(5q). The erythroid response was durable. With a median follow-up of 24 months, the median duration of transfusion independency was not reached.
Finally, in the MDS-002 multicenter phase II trial using the treatment schedule of lenalidomide from the MDS-003 trial, the use of oral lenalidomide was investigated in transfusion-dependent patients with low/int-1 risk MDS without del(5q) abnormalities. 44% of the patients achieved a 50% or greater reduction in transfusion requirements. 33% achieved transfusion-independency by week 24 with an overall response rate of 51%. These responses were observed after a median of 4.5 weeks (r: 0.3 - 39.1 weeks). The median response duration was 43 weeks after a median follow-up of 58 weeks.
Currently, a multicenter randomized double-blind placebo-controlled phase-III clinical study (MDS-004) is ongoing investigating two dosages of oral lenalidomide vs placebo in patients with del(5q) MDS. In one arm, 5 mg daily of lenalidomide is given; in the other arm 10 mg daily during 21 days in a 28-daily cycle will be investigated. Since this study is ongoing, no data are available yet.

5.5 Lenalidomide in low/int-1 risk MDS: toxicity and safety issues

In all three MDS studies as outlined above, myelosuppression was moderate to severe and dose-dependent making treatment interruption and dose-reduction necessary. Especially in patients with del(5q) serious neutropenic and trombocytic events occured in 62% of the patients at a starting dose

of 10 mg once daily, orally. In the non-del(5q) patients less adverse events were seen with limiting neutropenia or thrombocytopenia in only 24% and 19% of patients, respectively. Other adverse events were infrequently and mild, including urticaria, diarrhea and fatigue. No adverse events were reported with respect to deep venous thrombosis and/or pulmonary embolism.
The major concern raising from the European Medicines Agency (EMEA)[doc EMEA/CHMP/ 271288/2008] on the use of lenalidomide in patients with 5q abnormalities in MDS, which hampered the registration of lenalidomide for MDS in Europe, is the observation that their might be an increased risk of leukemic evolution. The decision of the refusal of authorisation of lenalidomide in Europe for MDS is primarily based on the way safety issues have been recorded in the MDS-003 international study of lenalidomide in MDS in one European Center. Although it is not likely that lenalidomide might increase the risk of leukemic evolution in low-intermediate-I risk MDS based on the immunemodulatory effects of this new drug as discussed above, the proposed study of HOVON89 will specifically address safety issues on leukemic transformation. This will be done by both extensively monitoring the potential leukemic evolution by BM examination as well as by planned interim analysis by an independent data and safety monitoring board (DSMB).

5.6 Rationale of the study

In low/int-1 risk MDS no current standard treatment programs are available except for Epo/G-CSF in selected cases based on the predictive model of response to Epo/G-CSF (E. Helstrom-Lindberg; ELN 2008 recommendations; www.leukemia-net.org), in accordance with guidelines of several MDS working parties [23,26,27]. Based on new insights in the pathobiology of low/int-1 risk MDS new targets for therapy are emerging interfering with apoptosis of hematopoietic progenitors and interfering with the complex interactions of the microenvironment, the immune system and (leukemic)- progenitor cells. In this respect, lenalidomide with the pleiotropic effects including erythropoietic remitting activity in low/int-1 risk MDS is of particular interest. Epo/G-CSF might further potentiate the effects of lenalidomide by interfering with apoptotic signalling of hematopoietic precursor cells as well as with the optimisation of immune effector cell function. This might have impact on the survival of erythropoietic progenitor cells and their progeny with clinical and hematological improvement of patients with MDS. With respect to safety concerns, it is not likely that the addition of Epo/G-CSF to lenalidomide may induce an increased risk in hematological and/or non-hematological toxicities.

6 Study objectives

6.1 Primary objective

- To evaluate the efficacy of lenalidomide (Revlimid™) in low/int-1 risk MDS with or without a treatment with Epo (NeoRecormon™)/G-CSF (Neupogen™) in terms of hematological improvement (HI) as defined by the modified response criteria of the IWG for MDS [40], see appendix C.

6.2 Secondary objectives

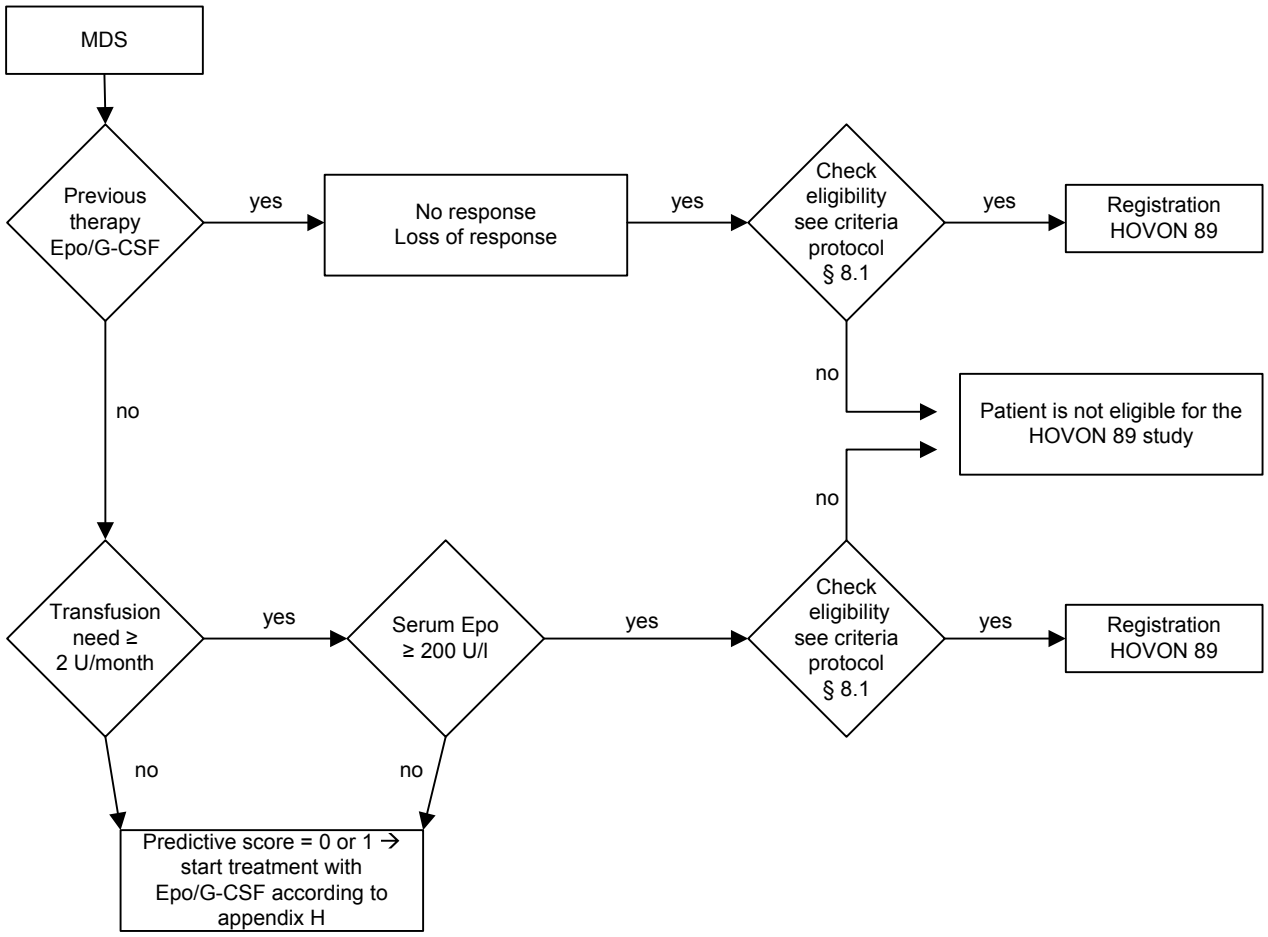
- To evaluate the safety and tolerability of lenalidomide (Revlimid™) in low/int-1 risk MDS with or without Epo (NeoRecormon™)/G-CSF (Neupogen™)
- Time-to-HI and duration-of-HI
- The number of given treatment cycles per patient and for arm B the number of patients receiving Epo and/or G-CSF
- The response rate (in terms of CR, PR, including cytogenetic response according to the modified response criteria of the IWG for MDS [40], see appendix C)
- Progression-Free-Survival (i.e. time from registration to disease progression, including progression to leukemia, or death from any cause)
- Transfusion requirements of red blood cells

7 Study design

This is a prospective, phase II, multi-center, open label, randomized trial within HOVON/SAKK. Randomization will be performed between lenalidomide (Revlimid™) with or without a standardized regimen with Epo (NeoRecormon™)/G-CSF (Neupogen™)

8 Study population

8.1 Eligibility for randomization



FLOW DIAGRAM FOR ELIGIBILITY

8.1.1 Inclusion criteria

- Patients with MDS classified as
  - RA, RARS and RAEB (with <10% myeloid blasts), CMML (with <10% myeloid blasts), according to FAB (see appendix A3) or
  - RA, RARS, RCMD, RCMD-RS, RAEB-1, MDS-U according to WHO (see appendix A1) or
  - patients with MPD/MDS (CMML-1 according to WHO) with a WBC ≤ $12x10^9$/l (see appendix A2)

  with an IPSS ≤ 1.0 (see appendix B1)
- Hb ≤ 6.2 mmol/l (10.0 g/dl)

  or Hb ≤ 7.2 mmol/l and ANC ≤ $1.0x10^9$/l

  or red blood cell transfusion dependent (≥ 2 units RBC during at least 8 weeks; units must be given for a Hb ≤ 5.6 mmol/l)

- Age ≥ 18 years
- WHO performance status 0-2 (see appendix D)
- Patient not previously treated with Epo/G-CSF, or
  failure of response or relapse after hematological improvement or disease progression to maximal RAEB-1 after previous therapy with Epo/G-CSF
- Serum creatinin < 150 µmol/l
- Serum billirubin < 25 µmol/l and ASAT, ALAT and Alkaline phosphatase < 2.5 times the upper limit of normal, except if related to disease
- The patient must give written informed consent
- Negative pregnancy test within 7 days prior to start of study drug, if applicable.
- Patient (all men, pre-menopausal women) agrees to use adequate contraceptive methods.
- Serum erythropoietin level
  - > 200 U/l or
  - ≤ 200 U/l if failure of response or loss of hematological improvement or disease progression to maximal RAEB-1 after prior standard therapy with Epo/G-CSF; Epo/G-CSF should be stopped at least 1 month before randomization.

Note: any cytogenetic karyotype can be included (normal and abnormal (including del(5q) abnormalities) as long as IPSS score ≤ 1.0 (see appendix B1).

8.1.2 Exclusion criteria

- Severe cardiac, pulmonary, neurologic, metabolic or psychiatric diseases or active malignancies.
- Anemia due to other causes than MDS including iron, B12 and folate deficiencies, auto-immune hemolysis and/or paroxysmal noctural hemoglobinuria (PNH)
- Hypoplastic MDS
- High predictive score (score 0 or 1) to respond on standard treatment with Epo/G-CSF according to guidelines; see appendix H
- Active uncontrolled infection
- Absolute neutrophil count (ANC) < $0.5x10^9$/l
- Patients dependent on platelet transfusions or with platelet counts < $25x10^9$/l or patients with active bleeding
- Patients treated with biological response modifiers (i.e. growth factors, immunosuppressive agents and/or chemotherapy) within 1 month prior to randomization
- Lactating women
- Prior treatment with lenalidomide
- Prior CTCAE ≥ grade 3 allergic reaction/hypersensitivity to thalidomide

- Prior CTCAE ≥ grade 3 rash/blistering while taking thalidomide
- Prior CTCAE ≥ grade 3 allergic/hypersensitivity to Epo and/or G-CSF

9 Treatment

For a detailed scheme of study see appendix I.

For arm A and B at any time (also during maintenance): in case of disease progression or when transfusion requirement returns to baseline, the patient will go off protocol treatment.
Baseline transfusion requirements are defined as the amounts of units RBC/8 weeks. HI is defined for each parameter e.g. Hb, if responses are last for at least 8 weeks. Transfusion requirements will be evaluated accordingly over a period of at least 8 weeks. According to the guidelines a reduction of at least 4 units of RBC over 8 weeks is considered relevant.

9.1 Arm A: treatment with lenalidomide monotherapy

The dosing regimen for lenalidomide (Revlimid™) is 10 mg once daily, orally on days 1-21 every 28 days. Dosing will be in the morning at approximately the same time each day. Lenalidomide can be taken with food. Subjects experiencing adverse events may need study treatment modifications (see section 9.3). When tolerated, this dosing regimen is to be continued for at least 6 cycles. If no HI according to the modified IWG response criteria for MDS (see appendix C) is obtained after 6 cycles, the patient will go off protocol treatment. If HI is reached after 6 cycles, the patient will receive another two cycles with lenalidomide and thereafter HI will be evaluated. If relapse after HI, the patient will go off protocol treatment, if no relapse after 8 cycles, the patient will continue with another two cycles with lenalidomide and thereafter HI will be evaluated. If relapse after HI, the patient will go off protocol treatment. If no relapse after 10 cycles, the patient will continue with another two cycles with lenalidomide and thereafter HI will be evaluated. If relapse after HI, the patient will go off protocol treatment. If no relapse after 12 cycles, the patient will continue with maintenance cycles of lenalidomide on the same dose as the last cycle until disease progression or transfusion requirement returns to baseline.

9.1.1 Treatment schedule Arm A

Agent	Dose	Route	Days (28d cycle)	Cycle / Maintenance
Lenalidomide (Revlimid™)	10 mg/day[1]	p.o.	1-21	1-12 / 13-last maintenance cycle

1) For dose reduction see 9.3

9.2 Arm B: treatment with lenalidomide and Epo/G-CSF

The dosing regimen for lenalidomide (Revlimid™) is 10 mg once daily, orally on days 1-21 every 28 days for the first 4 cycles. Dosing will be in the morning at approximately the same time each day. Lenalidomide can be taken with food. Subjects experiencing adverse events may need study treatment modifications (see section 9.3).

If after cycle 4 HI according to the modified IWG response criteria for MDS (see appendix C) has been reached, the patient will continue for cycles with lenalidomide monotherapy. Epo/G-CSF (NeoRecormon™ and Neupogen™ must be used) according to the scheme described below (see also detailed Scheme of study in appendix I) will be added if no HI or relapse after HI is observed after the first 4 cycles or in the following cycles.

If no HI is obtained after 4 cycles of lenalidomide, Epo (NeoRecormon™) will be started by s.c. administration at a dose of 30000 IU weekly for 2 cycles (5th and 6th cycle). The dose of Epo (NeoRecormon™) will be increased to 60000 IU weekly if no HI is obtained after 2 cycles with Epo (7th and 8th cycle). If no HI has been reached after 2 cycles of 60000 IU Epo weekly, G-CSF (Neupogen™) will be administrated at cycle 9-12. G-CSF will be administrated 3 times weekly (3x300 µg/week s.c. for patients ≤ 75kg; 3x480 µg/week s.c. for patients > 75 kg). Treatment will continue for at least 12 cycles for arm B. If no HI after 12 cycles is obtained, the patient will go off protocol treatment. Otherwise, the patient will continue with maintenance cycles of lenalidomide with or without Epo/G-CSF at the dose level reached after 12 cycles. During G-CSF administration the leucocytes should remain ≤ $30x10^9$/l (for dose adjustments see 9.3.3). See 9.2.2 and 9.2.3 for dose modifications for Epo and/or G-CSF in case of HI.

9.2.1 Treatment schedule Arm B

Agent	**Dose**[2]	**Route**	**Days (28d cycle)**	**Cycle / Maintenance**
Lenalidomide (Revlimid™)	10 mg/day	p.o.	1-21	1-12 / 13-last maintenance cycle
Epo (NeoRecormon™)	30000 IU/day[1]	s.c.	1x/wk	5-6
Epo (NeoRecormon™)	30000-60000 IU/day[1]	s.c.	1x/wk	7-12 / 13-last maintenance cycle
G-CSF (≤ 75kg) (Neupogen™)	300 µg/day[1]	s.c.	3x/wk	9-12 / 13-last maintenance cycle
G-CSF (> 75kg) (Neupogen™)	480 µg/day[1]	s.c.	3x/wk	9-12 / 13-last maintenance cycle

1) Epo and G-CSF should be given depending on achievement of HI. The Epo dose is also dependent on the achievement of HI (see detailed Scheme of Study in appendix I; for HI criteria see appendix C; for Epo/G-CSF dose modification in case of HI see 9.2.2 and 9.2.3

2) For other dose modification and interruption see 9.3

9.2.2 Dose modification for Epo in case of HI

In cases of HI the Epo dose will be reduced with 10000 IU/week, each reduction lasting for 4 weeks at a minimum maintenance dose of 20000 IU/week. See also 9.3 for interruption of Epo.

9.2.3 Dose modification for G-CSF in case of HI

In case of HI the G-CSF dose will be reduced to 2x or 1x 300µg/week (≤ 75kg) or 480µg/week (> 75kg); each dose reduction lasts for 4 weeks. See also 9.3 and 9.3.3 for interruption of G-CSF.

9.3 Dose modification and treatment interruption

If hematocrit increases to >50% during treatment, lenalidomide and/or Epo/G-CSF is to be stopped. After normalization of hematocrit to less than 45%, a stepping-up treatment program will be started dependent on the previous clinical course. If rapid cytoreduction is warranted, phlebotomy is considered to the discretion of the physician.

9.3.1 Dose modification or interruption for lenalidomide

If treatment-related thrombocytopenia and/or neutropenia occurs, the dose of lenalidomide has to be modified according to tables 1 and 2. If an adverse event mentioned in table 4 occurs, dose reduction steps have to be performed according to table 3 and dose modification according to table 4. See also 9.3 and 9.3.2 for interruption for lenalidomide.

Table 1: Recommended lenalidomide dose adjustment in patients with treatment-related thrombocytopenia

Time to Thrombocyopenia	Baseline value	When platelets	Recommended course
During cycle 1 with 10 mg daily	≥100 x10^9/l	• Fall to <50 x10^9/l	• Interrupt lenalidomide therapy
		• Return to ≥50 x10^9/l	• Resume lenalidomide at 5mg daily
	<100 x10^9/l	• Fall to 50% of baseline	• Interrupt lenalidomide therapy
		• If baseline was ≥60 x10^9/l and returns to ≥50 x10^9/l	• Resume lenalidomide at 5mg daily
		• If baseline was <60 x10^9/l and returns to ≥30 x10^9/l	• Resume lenalidomide at 5mg daily
After cycle 1 with 10 mg daily		• <30 x10^9/l or <50 x10^9/l and platelet transfusions (i.e. hemostatic failure)	• Interrupt lenalidomide therapy
		• Return to ≥30 x10^9/l (without hemostatic failure)	• Resume lenalidomide at 5mg daily
After cycle 1 with 5 mg daily		• <30 x10^9/l or <50 x10^9/l and platelet transfusions (i.e. hemostatic failure)	• Interrupt lenalidomide therapy
		• Return to ≥30 x10^9/l (without hemostatic failure)	• Resume lenalidomide at 5mg every other day

Table 2: Recommended lenalidomide dose adjustment in patients with treatment-related neutropenia

Time to neutropenia	Baseline ANC	• When neutrophils	• Recommended course
During cycle 1 with 10mg daily	≥1.0 x10^9/l	• Fall to <0.75 x10^9/l	• Interrupt lenalidomide therapy
		• Return to ≥1.0 x10^9/l	• Resume lenalidomide at 5mg daily
	<1.0 x10^9/l	• Fall to <0.5 x10^9/l	• Interrupt lenalidomide therapy
		• Return to ≥0.5 x10^9/l	• Resume lenalidomide at 5mg daily
After cycle 1 with 10mg daily		• <0.5 x10^9/l for ≥ 7 days or <0.5 x10^9/l associated with fever (38.5°C)	• Interrupt lenalidomide therapy
		• Return to ≥0.5 x10^9/l	• Resume lenalidomide at 5mg daily
After cycle 1 with 5mg daily		• <0.5 x10^9/l for ≥ 7 days or <0.5 x10^9/l associated with fever (38.5°C)	• Interrupt lenalidomide therapy
		• Return to ≥0.5 x10^9/l	• Resume lenalidomide at 5mg every other day

Table 3: Dose reduction steps for all other adverse events

Starting dose level	10mg once daily 1-21 every 28 days
Dose reduction #1	5mg once daily on days 1-21
Dose reduction #2	5mg every other day on days 1-21
Dose reduction #3	5mg on Monday, Wednesday, and Friday on days 1-21
Dose reduction #4	5mg on Monday and Friday on days 1-21
Dose reduction #5	5mg once a week on days 1-21

Table 4: Lenalidomide dose modification for adverse events possibly, probably or definite related to lenalidomide

CTC AE Grade	Day 2-14 of Cycle	≥ Day 15 of Cycle
Non-blistering rash **Grade 3**	• Hold (interrupt) dose. Follow weekly. • If the toxicity resolves to ≤ grade 2 prior to Day 21 restart at next lower dose level and continue the cycle until Day 21.	• Omit lenalomide for remainder of cycle.
Grade 4	• Discontinue lenalidomide study drug.	• Discontinue lenalidomide study drug.
Desquamating (blistering) rash- any Grade	• Discontinue lenalidomide study drug.	• Discontinue lenalidomide study drug.
Erythema multiforme **≥ Grade 3**	• Discontinue lenalidomide study drug.	• Discontinue lenalidomide study drug.
Neuropathy **Grade 3**	• Hold (interrupt) dose. Follow weekly. • If the toxicity resolves to ≤ grade 2 prior to Day 21 restart at next lower dose level and continue the cycle until Day 21.	• Omit lenalidomide for remainder of cycle.
Grade 4	• Discontinue lenalidomide study drug.	• Discontinue lenalidomide study drug.
Sinus bradycardia/ other cardiac arrhythmia **Grade 2**	• Hold (interrupt) dose. Follow at least weekly. • If the toxicity resolves to ≤ grade 1 prior to Day 21, restart at next lower dose level and continue the cycle until Day 21.	• Omit lenalidomide for remainder of cycle.
≥ Grade 3	• Discontinue lenalidomide study drug.	• Discontinue lenalidomide study drug.
Allergic reaction or hypersensitivity **Grade 2-3**	• Hold (interrupt) dose. Follow at least weekly. • If the toxicity resolves to ≤ grade 1 prior to Day 21 restart at next lower dose level and continue the cycle until Day 21.	• Omit lenalidomide for remainder of cycle.
Grade 4	• Discontinue lenalidomide study drug.	• Discontinue lenalidomide study drug
Constipation **Grade 1-2**	• Initiate bowel regimen and maintain dose level.	• Initiate bowel regimen and maintain dose level.
≥ Grade 3	• Hold (interrupt) dose. If the toxicity resolves to ≤ grade 2 prior to Day 21 restart at next lower dose level and continue the cycle until Day 21	• Omit lenalidomide for remainder of cycle.
Venous thrombosis/embolism **≥ Grade 3**	• Hold (interrupt) dose and start anticoagulation; restart at investigator's discretion (maintain dose level).	• Omit lenalidomide for remainder of cycle and start anticoagulation.
Other non-hematologic toxicity assessed as lenalidomide-related **≥ Grade 3**	• Hold (interrupt) dose. Follow at least weekly. • If the toxicity resolves to ≤ grade 2 prior to Day 21 restart at next lower dose level and continue the cycle until Day 21.	• Omit lenalidomide for remainder of cycle.
Hyperthyroidism or hypothyroidism	• Omit lenalidomide for remainder of cycle, evaluate etiology, and initiate appropriate therapy. Restart lenalidomide next cycle (decrease dose by one dose level).	• Omit lenalidomide for remainder of cycle, evaluate etiology, and initiate appropriate therapy. Restart lenalidomide next cycle (decrease dose by one dose level).

9.3.2 Procedures for subjects with falling Hb after achieving RBC transfusion independency

Chronic lenalidomide therapy has been associated with bone marrow suppression. Subjects who achieved RBC independency and whose Hb level persistently decreases during lenalidomide therapy should undergo (renewed) BM analysis to exclude disease progression. Lenalidomide should be interrupted.

9.3.3 Dose interruption and modification for G-CSF in case of AEs

G-CSF will be interrupted if the WBC >30x10^9/l and restarted after normalization of the WBC. G-CSF will be re-started at a dose of 300 or 480µg (dependant on weight) once a week for two weeks and will be escalated to 2x300 µg/week thereafter. See also 9.3 for interruption of G-CSF.

9.4 Concomitant therapy and supportive care

Patients should receive full supportive care, including antibiotics and anti-emetics when appropriate. Ciprofloxacin (500 mg twice daily) and fluconazol (50 mg daily) is recommended if ANC < 0.5x10^9/l. Subjects who have sustained CTCAE ≥ grade 3 neutropenia or develop fever associated with CTCAE ≥ grade 3 neutropenia may receive G-CSF at the discretion of the treating physician.

Individual requirements for blood transfusion products may vary. The thresholds and guidelines according to the Dutch CBO consensus are recommended. Platelets should be administered if life threatening thrombocytopenia occurs or with signs of hemostatic failure (i.e. mucosal bleeding or petechiae).

If platelet counts increases to > 600x10^9/l acetylsalicylic acid (100 mg once daily) is recommended under strict platelet control during treatment.

If corticosteroids (prednisone or dexamethasone) are indicated during therapy with lenalidomide for any reason, e.g. vasculitis, Ascal is recommended as prohylaxis for deep venous thromboembolism if no contraindication, e.g. platelet counts below 80x10^9/l, exists.

Patients with previous VTE low-molecular weight heparins should be used to prevent thrombosis. Dose interruptions should be monitored by careful platelet count control.

9.4.1 Prohibited concomitant therapy

Concomitant use of hematopoietic growth factors, with the exception of G-CSF and erythropoietin, other anti-cancer therapies, including radiation, thalidomide, or other investigational agents is not permitted while subjects are receiving study drug during the treatment phase of the study.

9.4.2 Obligatory contraception

Effective contraception must be used by patients for at least 4 weeks before beginning lenalidomide therapy, during lenalidomide therapy, during dose interruptions and for 4 weeks following discontinuation of lenalidomide therapy. Reliable contraception is indicated even where there has been a history of infertility, unless due to hysterectomy or because the patient has been postmenopausal naturally for at least 24 consecutive months. One reliable form of contraception must be used unless continuous abstinence from heterosexual sexual contact is the chosen method. Females of childbearing potential should be referred to a qualified provider of contraceptive methods, if needed. Sexually mature females who have not undergone a hysterectomy or who have not been postmenopausal naturally for at least 24 consecutive months (i.e., who have had menses at some time in the preceding 24 consecutive months) are considered to be females of childbearing potential.
Male patients: it is not known whether lenalidomide is present in the semen of patients receiving the drug. Therefore, males receiving lenalidomide must always use a latex condom during any sexual contact with females of childbearing potential even if they have undergone a successful vasectomy.

9.5 Lenalidomide

9.5.1 Supplier(s)

Celgene Corporation will supply Revlimid®, lenalidomide (CC-5013).

9.5.2 Dosage form

Lenalidomide will be supplied as 5 mg capsules for oral administration.

9.5.3 Packaging

Drug will be shipped to the pharmacy at the study site in individual wallets with tear-off labels. Two wallets will contain a sufficient number of capsules to last for 21 days of dosing.

9.5.4 Special Handling Instructions

Women of childbearing potential should not handle or administer the clinical dosage forms unless they are wearing gloves.

9.5.5 Labeling

Lenalidomide investigational supplies are dispensed to the patient in wallets of capsules that are labeled in accordance with the appropriate regulatory requirements. Each wallet will identify the contents as study medication and bear the patient number and protocol number. In addition, the label will bear the name, address and telephone number of the sponsor, quantity contained (21 capsules of 5 mg) and message use according to instructions of treating physician

9.5.6 Storage

At the study site, all investigational study drugs will be stored in a double locked, safe area to prevent unauthorized access.
The study drug should be stored at room temperature away from direct sunlight and protected from excessive heat and cold.

9.5.7 Unused study drug supplies

Celgene will instruct the Investigator on the return or destruction of unused study drug. If any study drug is lost or damaged, its disposition should be documented in the source documents. Study drug supplies will be retained at the clinical site pending instructions for disposition by Celgene. Patients will be instructed to return empty wallets or unused capsules in wallets.

9.5.8 Record of administration

Accurate records will be kept and stored of all study drug administration (including dispensing and dosing) .

9.6 Treatment compliance

At all times, when dispensing study drug, research center personnel will review the instructions, printed on the packaging, with subjects. Subjects will be asked to bring any unused study drug to the research center at their next visit. Research personnel will count and record the number of used and unused study drug capsules at each visit.

10 End of protocol treatment

A patient may discontinue participation in the study for any one of the following reasons categorized on the Off Treatment Form of the CRF as one of the following:

- No HI after 6 completed cycles for arm A or after 12 completed cycles for arm B
- Relapse (after HI) after 8, 10 or 12 cycles for arm A or after 12 cycles for arm B
- Disease progression or when transfusion requirement returns to baseline
- Excessive non-hematological and hematological drug toxicity preventing continuation of treatment
- Hypoplastic bone marrow abnormalities preventing continuation of treatment
- Major violation of the study protocol
- No compliance of the patient
- Death
- Suspected pregnancy

All relevant information related to the reason for treatment discontinuation, including contributory factors, must be included in the Off Treatment Form of the CRF and recorded in the patient medical records.

11 Required clinical evaluations

11.1 Time of clinical evaluations

- At entry: before start of treatment
- During treatment: visits including laboratory monitoring to assess hematological parameters will occur every cycle. Visits will occur:
  - for cycles 1-2 on day 1, 8, 15, and 22
  - for cycles 3-6 on days 1 and 15
  - for cycles 7-12 and during maintenance every 28 days on day 1 of each cycle until off treatment
  - To meet safety concerns bone marrow aspirate will be taken at 3,6,9 and 12 months and every 6 months thereafter
- Follow up: patients who are off protocol treatment for any reason should be followed until 5 years after registration. Follow up visits should occur every 3 months for the first three years and thereafter every 6 months or when signs and symptoms of disease progression are present (if disease progression was not the reason for going off protocol treatment).
  To meet safety concerns bone marrow aspirate will be taken at 3,6,9 and 12 months (from registration) and every 6 months thereafter, or when signs and symptoms of disease progression are present.

11.2 Required investigations

Required investigations at entry, during treatment, maintenance and follow-up (if applicable)

Procedure	At entry	Cycle 1-2		Cycle 3-6		Cycle 7-12 and (cycles) maintenance	Off treatment	Follow-up
		Day 1	Day 8, 15, 22	Day 1	Day 15	Day 1		
Medical history/anamnesis	X	X	X	X	X	X	X	X
Physical examination	X	X	X	X	X	X	X	X
Hematology	X	X	X	X	X	X	X	X
Add-on study 1-3 (PB)	X					X[10]	X	X[6]
Blood chemistry	X	X	X	X	X	X	X	X[7]
Additional blood chemistry	X			X[2]		X[2]		X[7]
Coagulation	X			X[2]		X[2]		
Thyroid function	X			X[2]		X[2]		
Urine analysis	X	X		X		X		
Serum or urine pregnancy test	X	X[3]		X		X		
Serum erythropoietin level	X							
Bone marrow aspirate								
Morphology	X[1]			X[4]		X[4]	X	X[4]
Cytogenetics/ FISH	X[1]			X[4]		X[8]	X	X[8]
Add-on study 1-3	X[1]					X[10]	X	X[6]
Bone marrow biopsy								
Histopathology	X[1]							
Specific investigations								
IPSS	X							
WPSS	X							
X-thorax	X							
ECG	X							
HI assessment		X[3,5]		X[5]		X[5]	X[5]	X[7]
Record adverse events		X[3]	X	X	X	X	X	X
Cytological review (see 4.2)	X							
Cytogenetic review (see 4.3)	X							

[1]: within 4 weeks prior to study entry
[2]: once every 3 months
[3]: not at cycle 1
[4]: at start cycle 4; 7; 10 and after 12 months and every 6 months thereafter and at disease progression
[5]: see 11.3 for consequences on protocol treatment when relapse after HI
[6]: only when progressive disease
[7]: until progressive disease
[8]: every 6 months
[9]: only when HI relapse
[10] only at 6 and 12 months

Remark A total of 40 ml peripheral blood is sufficient for the proposed investigations at diagnosis, month 6,12, when off protocol treatment or disease progression during follow-up. A total of 20 ml of bone marrow aspirate is sufficient for the proposed investigations at diagnosis, month 6, 12, when off protocol treatment or disease progression during follow-up.

11.2.1 Medical history/anamnesis

- Infections
- Bleeding tendency
- Obstipation
- Polyneuropathy

Only at entry:

- Standard medical history, with special attention for previous treatment and transfusion history
- Occupational history
- Prior and present other diseases
- Antecedent hematological or oncological diseases
- Previous chemotherapy or radiotherapy

11.2.2 Physical examination

- Standard physical examination including WHO performance status, body weight and height, with special attention for:
- Orthostatic hypotension
- Polyneuropathy or other neurologic symptoms
- Edema
- Infections
- Bleeding tendency

11.2.3 Hematology

- Hemoglobin
- Hematocrit
- Reticulocytes
- Leukocyte count, differential count
- Platelets

11.2.4 Coagulation

- APTT
- PT
- Fibrinogen

11.2.5 Blood chemistry

- Creatinin
- Liver enzymes
- Total bilirubin
- Alkaline phosphatase
- Total proteins
- Albumin
- LDH
- Calcium

11.2.6 Additional blood chemistry

- CRP
- Iron
- TIBC
- Ferritine

11.2.7 Thyroid function

- TSH
- T4

11.2.8 Urine analysis

- Routine screen strip

11.2.9 Pregnancy test

Before prescribing lenalidomide, females of childbearing potential should have 2 negative pregnancy tests (sensitivity of at least 50 mIU/mL). The first test should be performed within 10 – 14 days, and the second test within 24 hours prior to prescribing lenalidomide. A prescription for lenalidomide for a female of childbearing potential must not be issued by the prescriber until negative pregnancy tests have been verified by the prescriber.
Once treatment has started and during dose interruptions, pregnancy testing for females of childbearing potential should occur once every 4 weeks in females with regular menstrual cycles. A similar schedule of one pregnancy test every 4 weeks should be implemented if menstrual cycles are irregular. Pregnancy testing and counseling should be performed if a patient misses her period or if there is any abnormality in her pregnancy test or in her menstrual bleeding. Lenalidomide treatment must be discontinued during this evaluation.

11.2.10 Bone marrow aspirate

- Morphology (with special attention for iron staining)
- Cytogenetic analysis: conventional karyotyping and recording according to ISCN with at least 20-25 bone marrow metaphases. In those cases where no metaphases could be analyzed additional fluorescence in situ hybridization (FISH) should be performed according to recommendations. Such FISH investigations included 5q31, CEP7, 7q31, CEP8, 20q, CEPY and p53. [42,45]

11.2.11 Bone marrow biopsy

- Histopathology

11.2.12 Specific investigations

- X-Thorax
- ECG

11.2.13 Add-on studies

For the Add-on studies blood and bone marrow samples should be sent to a central lab. See Appendix G for (logistic) details. The add-on studies include assessments of specific immunologic, cytogenetic/molecular biology parameters for which bone marrow aspirates are needed.

11.3 Response evaluation/clinical criteria for evaluation

All responses will be assessed according to the modified IWG response criteria [40] (see appendix C). Hematological improvement (HI) will be assessed on day 1 of every cycle starting form day 1 of cycle 2.
HI will be assessed for each cell line. Dependent on the relevant pre-treatment parameter e.g. Hb, platelet count or ANC, HI will be assessed and responses must last at least for 8 weeks.
For arm A relapse after HI has only consequences for the protocol treatment after the first 6 cycles and thereafter every 2 cycles
For arm B relapse after HI has consequences for the protocol treatment after the first 4 cycles and thereafter every 2 cycles. See also scheme of study in appendix I.
Disease and cytogenetic response should be assessed at month 6 and 12 or at any indication (e.g. off treatment or (signs of) progressive disease).

12 Toxicity assessment

Toxicities will be scored according to the NCI Common Terminology Criteria for Adverse Events, version 3.0 (see appendix E).

Lenalidomide

Most frequently reported adverse events during clinical studies with lenalidomide in oncologic and non-oncologic indications, regardless of presumed relationship to study medication include: anemia, neutropenia, thrombocytopenia and pancytopenia, abdominal pain, nausea, vomiting and diarrhea, dehydration, rash, itching, infections, sepsis, pneumonia, urinary tract infection (UTI), upper respiratory infection, cellulitis, atrial fibrillation, congestive heart failure, myocardial infarction, chest pain, weakness, hypotension, hypercalcemia, hyperglycemia, back pain, bone pain, generalized pain, dizziness, mental status changes, syncope, renal failure, dyspnea, pleural effusion, pulmonary embolism, deep vein thrombosis, CVA, convulsions, dizziness, spinal cord compression, disease progression, death not specified and fractures.

Complete and updated adverse events are available in the Investigational Drug Brochure and the IND Safety Letters.

G-CSF (granulocyte-colony stimulating factor)

Fever, diarrhoea, abdominal pain, vomiting, skin rash, headaches, bone pain and injection site reactions have been reported following the use of G-CSF.

Erythropoietin

The most frequently reported adverse events for treatment of anemia in oncology is hypertension, thrombo-embolitic complication and headache.

13 Reporting serious adverse events and SUSARS

13.1 Definitions

Adverse event (AE)

An adverse event (AE) is any untoward medical occurrence in a patient or clinical study subject during protocol treatment. An AE does not necessarily have a causal relationship with the treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a (investigational) medicinal product, whether or not related to the (investigational) medicinal product.

Adverse reaction (AR)

Adverse reactions (AR) are those AEs of which a reasonable causal relationship to any dose administered of the investigational medicinal product and the event is suspected.

Serious adverse event (SAE)

A serious adverse event is defined as any untoward medical occurrence that at any dose results in:

- death
- a life-threatening event (i.e. the patient was at immediate risk of death at the time the reaction was observed)
- hospitalization or prolongation of hospitalization
- significant / persistent disability
- a congenital anomaly / birth defect
- Second Primary Malignancy
- any other medically important condition (i.e. important adverse reactions that are not immediately life threatening or do not result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed above)

Note that ANY death, whether due to side effects of the treatment or due to progressive disease or due to other causes is considered as a serious adverse event.

Unexpected SAE

Unexpected Serious Adverse Events are those SAE's of which the nature or severity is not consistent with information in the relevant source documents. For a medicinal product not yet approved for marketing, the Investigator's Brochure will serve as a source document.

Suspected unexpected serious adverse reaction (SUSAR)

All suspected ARs which occur in the trial and that are both unexpected and serious.

Protocol treatment period

The protocol treatment period is defined as the period from the first study-related procedure until 30 days following the last dose of protocol treatment or until the start of another systemic anti-cancer treatment off protocol, if earlier.

13.2 Reporting of (serious) adverse events

Adverse event

All AEs of CTCAE grade 2 or higher, with the exception of alopecia, nausea/vomiting and progression of the disease under study, have to be reported on the Adverse Events CRF.

Adverse events will be reported from the first study-related procedure until 30 days following the last protocol treatment or until the start of subsequent systemic therapy for the disease under study, if earlier.
Hematological toxicity grade 2 or higher should be reported when it occurs during an uninterrupted period of 8 weeks. Since lenalidomide is associated with myelosuppresion as early sign of response it should be emphasized that drug toxicity is only of relevance if long lasting, defined as at least during an 8-week period.
Adverse events occurring after 30 days should also be reported if considered related to study drug.
Grade 3 or 4 adverse events considered related to study drug must be followed until recovery or until 6 months after the last protocol treatment, whichever comes first.
All other adverse events must be followed until recovery or until 30 days after the last protocol treatment, whichever comes first.

Serious Adverse Events
Serious Adverse Events (SAEs) will be reported from the first study-related procedure until 30 days following the last protocol treatment or until the start of subsequent systemic therapy for the disease under study, if earlier.
Adverse events occurring after 30 days should also be reported if considered to be at least suspected to be related to the study drug.

All SAEs must be reported to the HOVON Data Center by fax **within 24 hours of the initial observation of the event**, except hospitalizations for:

- a standard procedure for protocol therapy administration. Hospitalization or prolonged hospitalization for a complication of therapy administration will be reported as a Serious Adverse Event.
- the administration of blood or platelet transfusion. Hospitalization or prolonged hospitalization for a complication of such transfusion remains a reportable serious adverse event.
- a procedure for protocol/disease-related investigations (e.g., surgery, scans, endoscopy, sampling for laboratory tests, bone marrow sampling). Hospitalization or prolonged hospitalization for a complication of such procedures remains a reportable serious adverse event.
- prolonged hospitalization for technical, practical, or social reasons, in absence of an adverse event.
- a procedure that is planned (i.e., planned prior to starting of treatment on study; must be documented in the source document and the CRF). Prolonged hospitalization for a

complication considered to be at least possibly related to the study drug remains a reportable serious adverse event.

All details should be documented on the Serious Adverse Event Report. In circumstances where it is not possible to submit a complete report an initial report may be made giving only the mandatory information. Initial reports must be followed-up by a complete report within a further 2 working days and sent to the HOVON Data Center. All SAE Reports must be dated and signed by the responsible investigator or one of his/her authorized staff members.

The investigator will decide whether the serious adverse event is related to the treatment (i.e. unrelated, unlikely, possible, probable, definitely and not assessable) and the decision will be recorded on the serious adverse event form. The assessment of causality is made by the investigator using the following:

RELATIONSHIP	DESCRIPTION
UNRELATED	There is no evidence of any causal relationship
UNLIKELY	There is little evidence to suggest there is a causal relationship (e.g. the event did not occur within a reasonable time after administration of the trial medication). There is another reasonable explanation for the event (e.g. the patient's clinical condition, other concomitant treatments).
POSSIBLE	There is some evidence to suggest a causal relationship (e.g. because the event occurs within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (e.g. the patient's clinical condition, other concomitant treatments).
PROBABLE	There is evidence to suggest a causal relationship and the influence of other factors is unlikely.
DEFINITELY	There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.
NOT ASSESSABLE	There is insufficient or incomplete evidence to make a clinical judgement of the causal relationship.

13.3 Processing of serious adverse event reports

The HOVON Data Center will forward all SAE reports within 24 hours of receipt to the prinicipal investigator, the study central datamanager and Celgene. Any suspected unexpected serious adverse reactions (SUSARs) arising from this trial will be reported expedited by HOVON to the investigators, and to all applicable Ethics Committees and Health Authorities within the timelines required by the EU Clinical Trial Directive.

13.4 Pregnancies

Pregnancies occurring while subjects are on study drug or within 4 weeks after a subject's last dose of study drug are considered events to be reported immediately to Celgene. If the subject is on study drug the study drug is to be discontinued immediately and the subject is to be instructed to return any unused portion of the study drug to the Investigator. The pregnancy must be reported to Celgene within 24 hours of the Investigator's knowledge of the pregnancy by phone and facsimile using the SAE Form. The pregnancy must also be reported to the sponsor.
The Investigator will follow the subject until completion of the pregnancy, and must notify the sponsor and Celgene of the outcome within 5 days or as specified below. The Investigator will provide this information as a follow-up to the initial pregnancy report.
If the outcome of the pregnancy meets the criteria for immediate classification as a SAE (i.e., spontaneous abortion [any congenital anomaly detected in an aborted foetus is to be documented], stillbirth, neonatal death, or congenital anomaly), the Investigator should follow the procedures for reporting SAEs. All neonatal deaths that occur within 30 days of birth should be reported, without regard to causality, as SAEs. In addition, any infant death after 30 days that the Investigator suspects is related to the in utero exposure to the study drug should also be reported.
In the case of a live "normal" birth, Celgene should be informed as soon as the information is available.

Any suspected foetal exposure to lenalidomide must be reported to Celgene within 24 hours of being made aware of the event. The patient should be referred to an obstetrician/gynaecologist experienced in reproductive toxicity for further evaluation and counselling.

Contact details for Celgene Europe Drug Safety:
Celgene International
Fax: +31 (0)30 28 44 511
Celgene B.V. (The Netherlands)
P.O. Box 2507
3500 GM Utrecht
The Netherlands
Tel : +31 (0)30 28 44 525
Email : DrugSafety-netherlands@celgene.com

14 Endpoints

All response endpoints are based on the recent updated Cheson criteria for response in MDS [40].

14.1 Primary endpoint

- Hematological improvement (HI) according to IWG 2006 criteria

14.2 Secondary endpoints

- Adverse events of CTCAE ≥ grade 2 (see appendix E)
- Time-to-HI and duration-of-HI (i.e. time from HI to relapse after HI or death from any cause)
- Number of given treatment cycles per patient, and especially for arm B the number of patients receiving Epo and/or G-CSF
- Response rate (in terms of CR, PR, including cytogenetic response according to the modified response criteria of the IWG for MDS [40], appendix C)
- Progression-free-survival, i.e. time from registration to relapse, disease progression (as defined in appendix C) or death from any cause
- Leukemic evolution. The risk of leukemic evolution will be calculated with competing risk death without previous evolution
- Number of transfusions of red blood cells and duration of RBC transfusion independence

Add/on studies (experimental design; see appendix G): exploratory analysis of identification of flowcytometric, molecular and cellbiological features of MDS at diagnosis, during treatment and follow-up

- response on MDS-flow dysplasia score (add-on study 1)
- prognostic impact of SNP array (add-on study 2)
- response on apoptosis/autophagy in MDS progenitor cells (add-on study 3)

15 Randomization

15.1 Regulatory Documentation

The following documents must be provided to the HOVON Data Center before shipment of study drug to the investigational site and before enrollment of the first patient.
By the principal investigator or study coordinator for all sites within their country:

- name and address of the (central) Ethical Committee including a current list of the members and their function;
- any other documentation required by local regulations.

By the local investigator for each investigational site:

- HDC Hospital Registration Form, signed and dated by the local investigator;
- a copy of the dated and signed (central) Ethical Committee approval of the protocol, any amendments and informed consent form for the investigational site. This approval must clearly identify the specific protocol by title, number and version date and must be signed by the chairman or authorized designee. The approval must also clearly identify the site(s) the approval applies to;
- a copy of the approved local version of the Patient Information and Informed Consent form;
- approval of participation by site's Board of Directors, if required by local regulations;
- CV of local investigator (dated and signed; if not recently provided);
- signed local investigator signature page ;
- local lab accreditation and list of local lab normal values (if not recently provided);
- any other documentation required by local regulations.

15.2 Randomization

Eligible patients should be randomized before start of treatment. Patients need to be registered at the HOVON Data Center of the Erasmus MC Rotterdam – location Daniel den Hoed, via the Internet via TOP (Trial Online Process; https://www.hdc.hovon.nl/top) or by phone call: +31.10.7041560 or fax +31.10.7041028, Monday through Friday, from 09:00 to 17:00 CET. A logon to TOP can be requested at the HOVON Data Center for participants.

The following information will be requested at registration:

- Protocol number
- Institution name
- Name of caller/responsible investigator
- Sex
- Date of birth
- Date written informed consent
- 'Risk Management Program' is discussed with patient
- Approval for central tissue review
- Approval for blood and/or bone marrow storage for scientific research
- Presence of cytogenetic abnormalities
- MDS diagnosis (FAB and/or WHO)
- Eligibility criteria

All eligibility criteria will be checked with a checklist. Patients will be randomized, stratified by center with a minimization procedure, ensuring balance within each stratum and overall balance
Each patient will be given a unique patient study number. Patient study number and result of randomization will be given immediately by TOP or phone and confirmed by fax or email.

16 Data collection

16.1 Reporting of leukemic evolution

Since leukemic evolution is of major concern, leukemic evolution of any patient at any time during treatment or follow up must be reported within 24 hours of determination to the HOVON Data Center.
In order to closely monitor the occurrence of leukemic evolution it is important that the Leukemic Evolution Report form is sent immediately by fax if leukemic evolution is established. Please FAX the form first and then send it by regular mail.

16.2 Reporting of Second Primary Malignancies

Second Primary Malignancies (SPM) should be reported as SAE during treatment and during the Follow Up period. SPM is always considered to be at least possibly related to the investigational medicinal product, making it a reportable SAE at all times including Follow Up. The SAE form together with the Second Primary Malignancy CRF must be reported to the HOVON Data Center by fax **within 24 hours of the initial observation of the Second Primary Malignancy.**
For each case of SPM occurring during treatment, contact the Principal Investigator to discuss if treatment needs to be discontinued.

16.3 CRF's

Data will be collected on Case Report Forms (CRF) to document eligibility, safety and efficacy parameters, compliance to treatment schedules and parameters necessary to evaluate the study endpoints. Data collected on the CRF are derived from the protocol and will include at least:

- inclusion and exclusion criteria;
- baseline status of patient including medical history and stage of disease;
- timing and dosage of protocol treatment;
- adverse events;
- parameters for response evaluation;
- any other parameters necessary to evaluate the study endpoints;
- survival status of patient;

- reason for end of protocol treatment;
- reporting of Second Primary Malignancies;
- reporting of leukemic evolution.

Each CRF page will be identified by a pre-printed trial number, and a unique combination of patient study number (assigned at registration), hospital and patient name code (as documented at registration) to be filled out before completing the form.
The CRF will be completed on site by the local investigator or an authorized staff member. Each page must be dated and signed by the local investigator upon completion. All CRF entries must be based on source documents. The CRF and written instructions for completing the CRF will be provided by the HOVON Data Center.
Copies of the CRF will be kept on site. The original CRF pages must be sent to the HOVON Data Center at the requested time points. How and when to send in forms is described in detail in the CRF header and the CRF instructions.
All data from the CRF will be entered into the study database by the HOVON Data Center.

17 Statistical considerations

The aim of this study is to decide whether the (response dependent) addition of EPO/G-CSF to lenalidomide increases the effectiveness of the treatment. An increase of 20% in hematological improvement is considered as clinically relevant and as an indication to continue in a Phase III study. The target number of patients for this Phase II study is 200 (100 patients in each arm).

All main analyses will be done according to the intention to treat principle.

17.1 Statistical analysis

Primary endpoint for this study is response rate, where response is defined as hematologic improvement (HI). Two interim analyses are planned, one regarding safety when 40 patients (20 in each arm) are off protocol or evaluable for response for at least 10 cycles and one interim analysis regarding efficacy when 100 patients (50 in each arm) are off protocol or evaluable for response for at least 10 cycles.

17.1.1 Safety interim analysis

The first interim analysis is planned for safety reasons. Results of this interim analysis will be presented confidentially to an independent data and safety monitoring board (DSMB). Only if the DSMB recommends that the study should be stopped or modified, the results will be made public to

the principal investigators for further discussion. The interim analysis is planned when 40 patients (20 patients in each arm) are off protocol or evaluable for response for at least 10 cycles.
At this interim analysis a detailed report will be generated and presented to the DSMB. The report includes by treatment arm the number of entered patients and at that time evaluable patients, treatment given, response (HI) rate and the incidence of SAEs and other adverse events and infections. Also the actuarial competing risk of (a) leukemic evolution [appendix C] and (b) death without previous leukemic evolution will be presented by treatment arm and split by cytogenetic subgroups, especially patients with and without 5q-. It is anticipated that within the low-Intermediate-1 risk MDS group 15% of the patients will evolve to leukemia within 2 years without any treatment. An increase to more than 20% of patients with leukemic evolution in either arm A and/or B during treatment will be considered as a signifcicant increase and reported directly to the DSMB.
The DSMB is free in her public recommendations to the principal investigator and the confidential recommendations to the statistician, but the following guidelines apply:
More than 25% of the patients (of both arms together) experience hematological toxicity of CTCAE grade 3 or more is a good reason to recommend stopping of the trial or recommendations for modifications

The study will be closely and sequentially monitored before the first interim analysis. Monitoring will be based on the reported SAEs which are not subject to data delay. Since leukemic evolution is of major concern, leukemic evolution of any patient should be reported immediately to the HOVON Data center. The actuarial risk of leukemic evolution will be monitored and calculated weekly on the basis of the reports received. As soon as there are at least 2 patients with leukemic evaluation and the estimated projected risk at 2 years is more than 20%, this will be reported to the DSMB for consideration.

17.1.2 Efficacy

The efficacy interim analysis is planned when 100 patients (50 patients in each arm) are off protocol or evaluable for response for at least 10 cycles. The following decision rules will be used regarding efficacy.

Let LLCI(nn) and ULCI(nn) be the lower and upper limit of the nominal nn% twosided confidence interval for the estimates of the true (unobserved) difference in response rates of between both arms.

Decisions	**Conditions**
At interim analysis	
Stop because of inefficacy	ULCI(90)<20%
Continue in a Phase III	LLCI(95)>0
Continue as Phase II	Otherwise (also if both above mentioned conditions are fullfilled)
At final analysis (if not stopped before)	
Stop because of inefficacy	Dr<=0 orULCI(80)<20%
Continue in a Phase III	Otherwise

Assuming that the true response rate in the lenalidomide arm is around 50%, and that a total of 200 patients (100 each arm) will be registered in the trial the decision rules lead to the following characteristics.

True difference Dr in response rate arm B minus arm A	Probability to continue in a Phase III	Probability to continue in a Phase III at interim analysis	Probability to stop at interim analysis
-10%	0.3%	0%	82%
0%	7%	3%	46%
10%	44%	18%	13%
20%	90%	54%	2%

Thus if the lenalidomide+Epo/G-CSF arm would in truth be as effective as the lenalidomide arm (true Dr=0%), there is still a probability of 7 % that the trial will continue in a Phase III (type I error). If the lenalidomide +Epo/G-CSF arm would be 20% more effective (true Dr=20%) the probability to continue in a Phase III (power) is 90%.

At this interim analysis a detailed report will be generated and presented to the DSMB. The report includes by treatment arm the number of entered patients and at that time evaluable patients, treatment given, response rates (HI as well as CR/PR) and the incidence of SAEs and other adverse events and infections. Note that the above considerations apply if the decisions would be purely based on the primary endpoint only. In reality actual decisions at the interim/final analysis will also take into account outcomes with respect to secondary endpoints and information from outside this trial.

17.2 Data and Safety monitoring board

A data and safety monitoring board will be installed before start of the study.

18 Ethics

18.1 Accredited ethics committee or Institutional review board

The study protocol and any substantial amendment will be approved by an accredited Ethics Committee or Institutional Review Board. The principal investigator will inform the subjects and the reviewing accredited METC if anything occurs, on the basis of which it appears that the disadvantages of participation may be significantly greater than was foreseen in the research proposal. The study will be suspended pending further review by the accredited METC, except insofar as suspension would jeopardize the subject's health. The investigator will take care that all subjects are kept informed.

18.2 Ethical conduct of the study

The study will be conducted in accordance with the ethical principles of the Declaration of Helsinki, the ICH-GCP Guidelines, the EU directive for Good Clinical Practice (2001/20/EG), and applicable regulatory requirements. The local investigator is responsible for the proper conduct of the study at the study site.

18.3 Investigator responsibilities

Investigator responsibilities are set out in the ICH guideline for Good Clinical Practice (GCP). Investigators must enter study data onto CRFs or other data collection system.
The Investigator, or a designated member of the Investigator's staff, must be available at some time during monitoring visits to review data and resolve any queries and to allow direct access to the subject's records (e.g., medical records, office charts, hospital charts, and study related charts) for source data verification.

18.4 Patient information and consent

Written Informed consent of patients is required before randomization. The procedure and the risks and the opinions for therapy will be explained to the patient.

18.5 Ethical and regulatory considerations

18.5.1 Subject confidentiality

HOVON affirms the subject's right to protection against invasion of privacy. HOVON requires the Investigator to permit HOVON's representatives and, when necessary, representatives of the regulatory authorities to review and/or copy any medical records relevant to the study in accordance with local laws.
Should direct access to medical records require a waiver or authorization separate from the subject.s statement of informed consent, it is the responsibility of the Investigator to obtain such permission in writing from the appropriate individual.

18.5.2 Study records requirements

The Investigator must ensure that the records and documents pertaining to the conduct of the study and the distribution of the study drug, that is copies of CRFs and source documents (original documents, data, and records [e.g., hospital records; clinical and office charts; laboratory notes; memoranda; subjects diaries or evaluation checklists; pharmacy dispensing records; recorded data from automated instruments; copies or transcriptions certified after verification as being accurate copies; microfiches; photographic negatives, microfilm, or magnetic media; x-rays; subject files; and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical study; documents regarding subject treatment and study drug accountability; original signed informed consents, etc.) be retained by the Investigator for as long as needed to comply with national and international regulations (generally 2 years after discontinuing clinical development or after the last marketing approval). The Investigator agrees to adhere to the document/records retention procedures by signing the protocol.

18.5.3 Premature discontinuation of study

Single center

The responsible local clinical Investigator as well as HOVON have the right to discontinue this study at any time for reasonable medical or administrative reasons in any single center. Possible reasons for termination of the study could be but are not limited to:

- Unsatisfactory enrollment with respect to quantity or quality.
- Inaccurate or incomplete data collection.
- Falsification of records.
- Failure to adhere to the study protocol.

Study as a whole

HOVON reserves the right to terminate this clinical study at any time for reasonable medical or ethical reasons.
Any possible premature discontinuation would be documented adequately with reasons being stated, and information would have to be issued according to local requirements (e.g., IRB/EC, regulatory authorities, etc.).

19 Trial insurance

The HOVON insurance program covers all patients from participating centers in the Netherlands according to Dutch law (WMO). The WMO insurance statement can be viewed on the HOVON Web site www.hovon.nl.

20 Publication policy

The final publication of the trial results will be written by the Principal Investigator and Study Coordinator(s) on the basis of the statistical analysis performed at the HOVON Data Center. A draft manuscript will be submitted to the Data Center and all co-authors for review. After revision by the other co-authors the manuscript will be sent to a peer reviewed scientific journal.

Authors of the manuscript will include the study coordinator(s), investigators who have included more than 5% of the evaluable patients in the trial (by order of inclusion), the statistician(s) and the HDC datamanager in charge of the trial, and others who have made significant scientific contributions.

Interim publications or presentations of the study may include demographic data, overall results and prognostic factor analyses, but no comparisons between randomized treatment arms may be made publicly available before the recruitment is discontinued.

Any publication, abstract or presentation based on patients included in this study must be approved by the Principal Investigator and Study Coordinator(s). This is applicable to any individual patient or any subgroup of the trial patients. Such a publication cannot include any comparisons between randomized treatment arms nor an analysis of any of the study end-points unless the final results of the trial have already been published.

Glossary of abbreviations
(in alphabetical order)

AE	Adverse Event
ANC	Absolute Neutrophil Count
AR	Adverse Reaction
ATG	Antithymocyte Globulin
BM	Bone Marrow
CBO	Centraal Begeleidings Orgaan
CKTO	Commissie voor Klinisch Toegepast Onderzoek'
CMML	Chronic Myelomonocytic Leukemie
CR	Complete Remission
CRF	Case Report Form
CRP	C-Reactive Protein
CTC	Common Toxicity Criteria
CTL	Cytotoxic T Lymfocyte
CV	Curriculum Vitae
CVA	Cerebral Vascular Accident
DSMB	Data and Safety Monitoring Board
ECG	Electrocardiogram
ECOG	Eastern Cancer Oncology Group
ELN	European Leukemia Net
Epo	Erythropoietine
FISH	Fluorescence In Situ Hybridisation
GCP	Good Clinical Practice
G-CSF	Granulocyte-Colony Stimulating Factor
Hb	Hemoglobin
HI	Hematological Improvement
HOVON	Dutch-Belgian Hematology-Oncology Cooperative Group
ICH	International Conference on Harmonization of technical requirements for registration of pharmaceuticals for human use
IMiDs	Immuno Modulatory Drugs
IND	Investigational New Drug
IPSS	International Prognostic Scoring System
IRB/EC	Institutional Review Board / Ethics Committee
ISCN	International society for Cytogenetic Nomenclature
IU	International Units

IWG	International Working Group
LDH	Lactate Dehydrogenase
MDS	Myelodysplastic Syndrome
MDS-U	Myelodysplastic Syndrome-Unclassified
METC	Medical Ethical Review Committee
MPD	Myeloproliferative Disease
NCI	National Cancer Institute
OS	Overall Survival
PB	Peripheral Blood
PD	Progressive Disease
PFS	Progression Free Survival
PI	Principal Investigator
PNH	Paroxysmal Noctural Hemoglobinuria
PO	Per Os
PR	Partial Response
PTX	Pentoxifylline
RA	Refractory Anemia
RAEB (t)	Refractory Anemia with Excess of Blasts (in transformation)
RARS	Refractory Anemia with Ringsideroblasts
RBC	Red Blood Cell
RCMD	Refractory Cytopenia with Multilineage Dysplasia
RCMD-RS	Refractory Cytopenia with Multilineage Dysplasia and Ringed Sideroblasts
SAE	Serious Adverse Event
SAKK	Swiss Group for Clinical Cancer Research
SC	Subcutaneous
SD	Stable Disease
SNP	Single Nucleotide Polymorphism
SPM	Second Primary Malignancy
SUSAR	Suspected Unexpected Serious Adverse Reaction
TIBC	Total Iron Binding Capacity
TNF	Tumor Necrosis Factor
TOP	Trial Online Process
TRAIL	TNF related Apoptosis Inducing Ligand
TSH	Thyroid Stimulating Hormon
ULN	Upper Limit of Normal
UTI	Urinary Tract Infection
VEGF	Vascular Endothelial Growth Factor

VTE	Venous Trombotic Embolism
WBC	White Blood Cell Count
WHO	World Health Organization
WMO	Wet Medisch-Wetenschappelijk Onderzoek met mensen
WPSS	WHO Prognostic Scoring System

21 References

1. Corey SJ, Minden MD, Barber DL, Kantarijan H, Wang JCY, Schimmer AD. Myelodysplastic syndromes: The complexity of stem-cell diseases. Nat Rev Cancer 2007;7:118-129.
2. Loosdrecht AA van de, et al. Myelodysplasia and apoptosis. New insights into ineffective erythropoiesis. Med Oncol 2000:17:16.
3. Parker JE, Mufti GJ. Ineffective haemopoiesis and apoptosis in myelodysplastic syndromes. Br J Haematol 1998;101:220
4. Raza A, Gezer S, Mundle S, Gao XZ.i S, Borok R, Rifkin S, Iftikhar A, Shetty V, Parcharidou A, Loew J, Marcus B, Khan Z, Chaney C, Showel J, Gregory S, Preisler H. Apoptosis in bone marrow biopsy samples involving stromal and hematopoietic cells in 50 patients with myelodysplastic syndromes. Blood 1995;86:268
5. Bogdanovic AD, Jankovic GM, Colovic MD. Apoptosis in bone marrow of myelodysplastic syndrome patients. Blood 1996;87:3064
6. Loosdrecht AA van de, Brada SJL, Blom NR, et al. Mitochondrial disruption and limited apoptosis is associated with high risk myelodysplasia: an ultrastructural analysis. Leuk Res 2001;25:385.
7. Parker JE, Fishlock KL, Mijovic A, Czepulkowski B, Pagliuca A, Mufti GJ. Low-risk myelodysplastic syndrome is associated with excessive apoptosis and an increased ratio of pro- versus anti-apoptotic bcl-2-related proteins. Br J Haematol 1998;105:1075.
8. Rajapaksa R, Ginzton N, Rott LS, Greenberg PL. Altered oncoprotein expression and apoptosis in myelodysplastic syndrome marrow cells. Blood 1996;88:4275.
9. Mundle S, Venugopal P, Shetty V. The relative extent and propensity of CD34+ versus CD34_ cells to undergo apoptosis in myelodysplastic marrows. Int J Hematol 1999;69:152.
10. Span LFR, Vierwinden G, Pennings AH et al., Programmed cell death is an intrinsic feature of MDS progenotors, predominantly found in the cluster-forming cells. Exp Hematol 2005;33:435-422.
11. Kitagawa M, Yamaguchi S, Takahashi M, Tanizawa T, Hirokawa K, Kamiyama R. Localozation of Fas and Fas ligand in bone marrow cells demonstrating myelodysplasia. Leukemia 1998;12:486
12. Gersuk G, Beckham, C, Loken MR, Kiener P, Anderson JE, Farrand A, Troutt A, Ledbetter JA, Deeg HJ. A role for tumour necrosis factor-α, Fas and Fas-ligand in marrow failure associated with myelodysplastic syndrome. Br J Haematol 1998;103:176
13. De Maria R, Testa U, Luchetti L, Zeuner A, Stassi G, Pelosi E, Riccioni R, Felli N, Samoggia P, Peschle C. Apoptotic role of Fas/Fas-L ligand system in the regulation of erythropoiesis. Blood 1999;93:796

14. Gupta P, Niejans GA, LeRoy SC. Fas ligand expression in the bone marrow in myelodysplastic syndromes correlates with FAB subtype and anemia and predicts survival. Leukemia 1999;13:44
15. Plasilova M, Zivny J, Jelinek J et al., TRAIL (Apo2L) suppresses growth of primary human leukemia and myelodysplasia progenitors. Leukemia 2002;16:67-73.
16. Young Zang D, Goodwin RG, Loken MR, Bryant E, Deeg HJ. Expression of TRAIL, Apo2L, and its receptors in myelodysplastic syndrome: effects on in-vitro hemopoiesis. Blood 2001;98:3058-65.
17. Reza S, Shetty V, Dar S et al. Tumor necrosis factor-α levels decrease with anticytokine therapy in patients with myelodysplastic syndromes. J Interf Cyt Res 1998;18:871.
18. Graaf AO de, Witte T de, Jansen JH. Inhibitor of apoptosis proteins: new therapeutic targets in hematological cancer? Leukemia 2004;18:1751-59.
19. Economopoulos T, Mellou S, Papageorgiou E et al. Treatment of anemia in low risk myelodysplastic syndromes with granulocyte-macrophage colony-stimulating factor plus recombinant human erythropoietin. Leukemia 1999;13:1009.
20. Hellström-Lindberg E, Ahlgren T, Beguin Y et al. Treatment of anemia in myelodysplastic syndromes with granulocyte colony-stumulating factor plus erythropoietin: results from a randomized phase II study and long term follow-up of 71 patients. Blood 1998;92:68.
21. Hellström-Lindberg E, Kanter-Lewensohn L, Öst Å. Morphological changes and apoptosis in bone marrow from patients with myelodysplastic syndromes treated with granulocyte-CSF and erythropoietin. Leuk Res 1997;21:415.
22. Stasi R Pagano A, Terzoli E, Amadori S. Recombinant human granulocyte macrophage colony-stimulating factor plus erythropoietin for the treatment of cytopenias in patients with myelodysplastic syndromes. Br J Haematol 1999;105:141.
23. Hellström-Lindberg E, Gulbrandsen N, Lindberg G et al. A validated decision model for treating the anemia of MDS with erythopoietin and G-CSF: significant effects on quality of life. Br J Haematol 2003;120:1037-46.
24. Tehranchi R, Fadeel B, Forsblom AM et al. Granulocyte colony-stimulating factor inhibits spontaneous cytochrome c release and mitochondria-dependent apoptosis of MDS hematopoietic progenitors. Blood 2003;101:1080-86.
25. Tehranchi R, Fadeel B, Schmidt-Mende J et al. Antiapoptotic role of growth factors in teh MDS; concordance between in vitro and in vivo observations. Clin Cancer Res 2005:11:6292-99.
26. Hellström-Lindberg E. Update on supportive care and new therapies: immunomodulatory drugs, growth factors and epigenetic-acting agents. Hematology ASH Educational Program 2005:161-166.

27. Bowen D, Culligan D, Jowitt S et al. Guidelines for the diagnosis and therapy of adult MDS. Br J haematol 2003;120:187-200.
28. Jadersten M, Montgomery SM, Dybedal I et al. Long-term outcome of treatment of anemia in MDS with Epo and G-CSF. Blood 2005;106:803-11.
29. Chamuleau MED, Zevenbergen A, Dreunen L van, Westers TM, Ossenkoppele GJ, Loosdrecht AA van de. Treatment with Epo/G-CSF of patients with low/int-i risk MDS diminishes relative CLIP (Class-II associated invariant chain peptide) amount on hematopoietic precursor cells. Leuk Res 2007;31(suppl 1)S61.
30. Golshayan D, Jin T, Maciejewski J et al. Efficacy of growth factors compared to other therapies for low-risk MDS. Br J Haematol 2007;137:125-32.
31. Sekeres MA. Fu AZ, Maciejewski JP et al. A decision analysis to determine the appropriate treatment for low-risk MDS. Cancer 2007;109:1125-32.
32. Sekeres MA, List A. Immunomodulation in MDS. Best Practice Res Clin Haematol 2006;19:757-67.
33. Estey EH. Modulation of angiogenesis in patients with MDS. Best Practice Res Clin Haematol 2004;17:623-639.
34. Kale V, List A. Immunomodulatory drugs (IMiDs): a new treatment option for MDS. Curr Pharm Biotechnol 2006;7:339-42.
35. Nilsson L, Eden P, Olssen E et al. The molecular signature of MDS stem cells supports a stem cell origin of 5q- MDS. Blood 2007;110:3005-3014.
36. Pellagatti A, Jadersten M, Forsblom AM et al. Lenalidomide inhibits the malignant clone and upregulates the SPARC gene mapping to the commonly deleted region in 5q- syndrome patients. Proc Natl Acad Sci USA 2007;104:11406-11.
37. List AF, Kurtin S, Roe DJ et al., Efficacy of lenalidomide in MDS. N Engl J Med 2005;352:549-557.
38. List AF, Dewald G, Bennett J et al. Resulst of the MDS-002 and -003 international phaser II studies evaluating lenalidomide (CC-5013); Revlimid) in the treatment of MDS. Haematologica 2005;90(suppl 2):307a.
39. List AF, Dewald G, Bennett J et al. Lenalidomide in the MDS with chromosome 5q deletion. N Engl J Med 2006;355:1456-1465.
40. Cheson BD, Greenberg PL, Bennett JM et al. Clinical application and proposal for modification of the international working group (IWG) response criteria in MDS. Blood 2006;108:419-25.
41. List AF, Baker AF, Green S, Bellamy W. Lenalidomide; targeted anemia therapy for MDS. Cancer Control 2006;13:4-11.
42. Mitelman F. ed. ISCN: An international system for human cytogenetic nomenclature. 1995. Basel. Karger. Switzerland.

43. Greenberg P, Cox C, LeBeau MM et al. International Scoring System for evaluating prognosis in MDS. Blood 1997;89:2079-2088.
44. Malcovati L, Germing U, Kuendgen A et al. Time-Dependent Prognostic Scoring System for Predicting Survival and Leukemic Evolution in Myelodysplastic Syndromes. J Clin Oncol 2007; 25: 3503-3510.
45. Valent P, Horny HP, Bennett JM, Fonatsch C, Germing U, Greenberg P et al. Definitions and standards in the diagnosis and treatment of the myelodysplastic syndromes: Consensus statements and report from a working conference. Leuk Res 2007;31:727-36.
46. Gattermann N, Porter J, Lopes LF, Seymour J. Iron overload in MDS. Hematology/Oncology Clinics 2005;19(suppl 1):5-25.
47. Gattermann N. Guidelines on iron chelation therapy in patients with myelodysplastic syndromes and transfusional iron overload. Leuk Res 2007;31S3:S10-S15.
48. Valent P, Horny HP, Bennett JM, Fonatsch C, Germing U, Greenberg P et al. Definitions and standards in the diagnosis and treatment of the myelodysplastic syndromes: Consensus statements and report from a working conference. Leuk Res 2007;31:727-36.
49. Loken MR, Loosdrecht AA van de, Ogata K, Orfao A, Wells DA. Flow cytometry in myelodysplastic syndromes: Report from a working conference. Leuk Res 2008:32:5-17.
50. Wells DA, Benesch M, Loken MR, Vallejo C, Myerson D, Leisenring WM et al. Myeloid and monocytic dyspoiesis as determined by flow cytometric scoring in myelodysplastic syndrome correlates with the IPSS and with outcome after hematopoietic stem cell transplantation. Blood 2003;102:394-403.
51. Lochem EG Van, Velden HHJ van der, Wind HK, Marevelde JG te, Westerdaal NAC, Dongen JJM van. Immunophenotypic differentiation patterns of normal hematopoiesis in human bone marrow: reference patterns for age-related changes and disease-induced shifts. Cytometry B Clin Cytom 2004;60:1-13.
52. Loosdrecht AA van de, Westers TM, Westra G, Drager A, Van der Velden VHJ, Ossenkoppele GJ. Identification of distinct prognostic subgroups in low and intermediate-I risk myelodysplastic syndromes by flowcytometry. Blood 2008;111:1067-1077.
53. Mohamedali A, Gäken J, Twine NA, Ingram W, Westwood N, Lea NC, Hayden J, Donaldson N, Aul C, Gattermann N, Giagounidis A, Germing U, List AF, Mufti GJ. Prevalence and prognostic significance of allelic imbalance by single-nucleotide polymorphism analysis in low-risk myelodysplastic syndromes. Blood 2007;110:3365-73.
54. Gondek LP, Tiu R, O'Keefe CL, Sekeres MA, Theil KS, Maciejewski JP. Chromosomal lesions and uniparental disomy detected by SNP arrays in MDS, MDS/MPD, and MDS-derived AML. Blood. 2008;111:1534-42.
55. Langemeijer S, Kuiper R, Vandenberghe P, Verburgh E, Boezeman J, Geurts van Kessel A, van der Reijden BA, de Witte T, Jansen JH. Identification of Novel Genetic Lesions in

Myelodysplastic Syndromes Using High Density SNP-Arrays. Blood 2007;110;11 [Meeting abstract].

56. Houwerzijl EJ, Blom NR, van der Want JJ, et al. Increased peripheral platelet destruction and caspase-3-independent programmed cell death of bone marrow megakaryocytes in myelodysplastic patients. Blood. 2005;105:3472-3479.
57. Houwerzijl EJ, Blom NR, van der Want JJ, et al. Megakaryocytic dysfunction in myelodysplastic syndromes and idiopathic thrombocytopenic purpura is in part due to different forms of cell death. Leukemia. 2006;11:1937-1942.
58. Braun T, Carvalho G, Grosjean J, et al. Differentiating megakaryocytes in myelodysplastic syndromes succumb to mitochondrial derangement without caspase activation. Apoptosis. 2007;12:1101-1108.
59. Leist M, Jäättelä M. Four deaths and a funeral: from caspases to alternative mechanisms. Nat Rev Mol Cell Biol. 2001;2:589-598.
60. Blazsek I, Chagraoui J, Peault B. Ontogenic emergence of the hematon, a morphogenetic stromal unit that supports multipotential hematopoietic progenitors in mouse bone marrow. Blood. 2000;96:3763-3771.
61. Houwerzijl EJ, Pol HWD, Blom NR, et al. Erythroid precursors from patients with low-risk myelodysplasia demonstrate ultrastructural features of enhanced autophagy. (submitted)

A. Classification of Myelodysplastic Syndromes

A1 WHO classification for myelodysplastic syndromes

MDS Subtype	Blood Findings	Bone Marrow Findings
Refractory anemia (RA)	• Anemia • No or rare blasts	• Erythroid dysplasia only • < 5% blasts • < 15% ringed sideroblasts
Refractory anemia with ringed sideroblasts (RARS)	• Anemia • No blasts	• Erythroid dysplasia only • ≥ 15% ringed sideroblasts • < 5% blasts
Refractory cytopenia with multilineage dysplasia (RCMD)	• Cytopenias (bi- or pancytopenia) • No or rare blasts • No Auer rods • < 1 x 10^9/L monocytes	• Dysplasia in ≥ 10% of cells in 2 or more myeloid cell lines • < 5% blasts • No Auer rods • < 15% ringed sideroblasts
Refractory cytopenia with multilineage dysplasia and ringed sideroblasts (RCMD-RS)	• Cytopenias (bi- or pancytopenia) • No or rare blasts • No Auer rods • < 1 x 10^9/L monocytes	• Dysplasia in ≥ 10% of cells in 2 or more myeloid cell lines • ≥ 15% ringed sideroblasts • < 5% blasts • No Auer rods
Refractory anemia with excess blasts-1 (RAEB-1)	• Cytopenias • < 5% blasts • No Auer rods • < 1 x 10^9/L monocytes	• Unilineage or multilineage dysplasia • 5% to 9% blasts • No Auer rods
Refractory anemia with excess blasts-2 (RAEB-2)	• Cytopenias • < 5% to 19% blasts • Auer rods +/- • < 1 x 10^9/L monocytes	• Unilineage or multilineage dysplasia • 10%-19% blasts • Auer rods +/-
Myelodysplastic syndrome, unclassified (MDS-U)	• Cytopenias • No or rare blasts • No Auer rods	• Unilineage dysplasia in granulocytes or megakaryocytes • < 5% blasts • No Auer rods
MDS associated with isolated del(5q)	• Anemia • < 5% blasts • Platelets normal or increased	• Normal to increased megakarocytes with hypolobulated nuclei • < 5% blasts • No Auer rods • Isolated del(5q)

A2 WHO diagnostic criteria for chronic myelomonocytic leukemia (CMML)

- Persistent peripheral blood monocytosis > $1x10^9$/l
- No Philadelphia chromosome or *BCR/ABL* fusion gene
- < 20% blasts* in the blood or bone marrow
- Dysplasia in one or more myeloid lineages. If myelodysplasia is absent or minimal, the diagnosis of CMML may still be made if the other requirements are present and:
  - o an acquired, clonal cytogenetic abnormality is present in the marrow cells, or
  - o the monocytosis has been persistent for at least 3 months and all other causes of monocytosis have been excluded

Diagnose CMML-1 when blasts < 5% in blood and < 10% in bone marrow
Diagnose CMML-2 when blasts are 5-19% in blood, or 10-19% in marrow, or if Auer rods are present and blasts are < 20% in blood or marrow
Diagnose CMML-1 or CMML-2 with eosinophilia when the criteria above are present and when the eosinophil count in the peripheral blood > $1.5x10^9$/l

*In this classification of CMML, blasts include myeloblasts, monoblasts, and promonocytes.

A3 FAB classification for myelodysplastic syndromes

FAB type	Blood Findings	Bone Marrow Findings
RA	• Anemia • < 1% blasts	• < 5% blasts • < 15% ringed sideroblasts
RARS	• Anemia • < 1% blasts	• < 5% blasts • ≥15% ringed sideroblasts
RAEB	• Anemia • < 1% blasts • +/- leucocytopenia and/or thrombocytopenia	• 5-20% blasts
RAEB/T	• Anemia • < 5% blasts • +/- leucocytopenia and/or thrombocytopenia	• 20-30% blasts
CMML	• Anemia • ≥ 1 x 10^9/L monocytes • +/- leucocytopenia and/or thrombocytopenia	• < 20% blasts • increase monocytic cells

B Prognostic Scoring Systems for Myelodysplastic Syndromes

B1 International Prognostic Score System (IPSS) for MDS [43]

	Score value				
Prognostic Variable	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			

The IPSS score is calculated by summation of the score values for categories of the prognostic variables for a patient. Risk groups are defined on the basis of this sumscore as:

Low: 0; Int-1: 0.5-1.0; Int-2: 1.5-2.0; High: ≥ 2.5

* Karyotype

Good : normal, -Y, del(5q), del(20q)

Poor : complex (≥ 3 abnormalities in the same clone)
or chromosome 7 abnormalities

Intermediate : all other chromosomal abnormalities

**Cytopenias

Hb < 6.2 mmol/l

ANC < $1.8x10^9$/l

Platelets < $100x10^9$/l

B2 WHO Classification-based Prognostic Scoring System (WPSS) [44]

Score (points)				
Prognostic Variable	0	1	2	3
WHO category	RA, RARS, 5q-	RCMD, RCMD-RS	RAEB-1	RAEB-2
IPSS karyotype class	Good	Intermediate	Poor	-
Transfusion requirements[1]	No	Yes	-	-

1) defined as ≥ 1 unit of packed red blood cells per 8 wks

Risk groups were defined as follows: very low (score = 0), low (score = 1), intermediate (score = 2), high (score = 3 to 4), and very high (score = 5 to 6)

C. Response criteria

Modified International Working Group response criteria for altering natural history of MDS [40]

Category	Response criteria according to Cheson and applied for HOVON 89 (responses must last at least 4 wk)
Complete remission (CR)	**BM:** < 5% blasts < 10% dysplasia in each cell line **Blood:** Haematological improvement AND: Hb ≥ 6.8 mmol/l (11 g/dl) Platelets ≥ 100 x 10^9/l Neutrophils ≥ 1.0 x 10^9/l Blasts 0%
CRi	**BM:** < 5% blasts < 10% dysplasia in each cell line **Blood:** Haematological improvement No normalization of cell counts as defined by CR Blasts 0%
CRd[1]	**BM:** < 5% blasts Persistent dysplasia (≥ 10%) in one or more cell lines **Blood:** Haematological improvement Blasts 0%
Marrow CR	• This response is applicable in case of the following 'on study' condition: % bone marrow blasts is between 5 – 10% • Bone marrow: ≤ 5% myeloblasts and decrease by ≥ 50% over pretreatment
Stable disease (SD)	None of the criteria above, but no evidence of progression

Relapse after CR[2]	BM: If <5% blasts on study: blasts >5% If 5-9% blasts on study: return to pre-treatment level OR blood: Blasts ≥2% (in case of CMML: >5%) OR one of the following: ≥ 50% decrease of maximum response levels of platelets or neutrophils ≥ 0.9 mmol/l (1.5 g/dl) reduction in Hb level Transfusion dependence
Progressive disease[3]	BM: If <5% blasts on study: blasts >5% and increase ≥ 50% If 5-9% blasts on study: blasts >10% and increase ≥ 50% OR blood: Blasts ≥2% (in case of CMML: >5%) AND one of the following (only in case of previous HI): ≥ 50% decrease of maximum response levels of platelets or neutrophils ≥ 1.2 mmol/l (2 g/dl) reduction in Hb level Transfusion dependence
Leukemic evolution	An increase in BM blasts to ≥ 20% OR Blood ≥ 20% (According to the definition of AML in the WHO classification)

[1] In case of re-occurrence of dysplasia while peripheral blood cell counts are still normal, a CR to CRd transition is possible, without relapse.

[2] Relapse is only possible after CR, CRi, CRd and marrow CR.

[3] Progressive disease is only possible after SD or relapse, or when previous responses were unknown. Normally BM blasts at diagnosis will be the reference value for determiniation of disease progression. However if during treatment a lower % of BM blasts is observed than at diagnosis, the lowest value will be the reference value.

Cytogenetic response criteria

Complete response	Disappearance of the chromosomal abnormality without appearance of new ones
Partial response	At least 50% reduction of the chromosomal abnormality

Proposed modified International Working Group response criteria for hematologic improvement (HI) [40]

Hematologic improvement*	**Response criteria (responses must last at least 8 wk)**
Erythroid response (pretreatment, < 6.8 mmol/l (11 g/dL))	• Hb increase by ≥ 0.9 mmol/l (1.5 g/dl) OR • Relevant reduction of units of RBC transfusions by an absolute number of at least 4 RBC transfusions/8 wk compared with the pretreatment transfusion number in the previous 8 wk. Only RBC transfusions given for a Hb of ≤ 5.6 mmol/l (9.0 g/dl) pretreatment will count in the RBC transfusion response evaluation
Platelet response (pretreatment, < 100 x 10^9/L)	• Absolute increase of ≥ 30 x 10^9/l for patients starting with > 20 x 10^9/l platelets • Increase from < 20x 10^9/l to > 20 x 10^9/l and by at least 100%
Neutrophil response (pretreatment, < 1.0 x 10^9/L)	At least 100% increase and an absolute increase > 0.5 x 10^9/l
Progression or relapse after HI	At least 1 of the following: • At least 50% decrement from maximum response levels in granulocytes or platelets • Reduction in Hb by ≥ 0.9 mmol/l (1.5 g/dl) • Transfusion dependence

*** Pretreatment counts averages of at least 2 measurements (not influenced by transfusions) ≥ 1 week apart**

D. ZUBROD-ECOG-WHO Performance Status Scale

0 Normal activity

1 Symptoms, but nearly ambulatory

2 Some bed time, but to be in bed less than 50% of normal daytime

3 Needs to be in bed more than 50% of normal daytime

4 Unable to get out of bed

E. Common Terminology Criteria for Adverse Events

The grading of toxicity and adverse events will be done using the NCI Common Terminology Criteria for adverse events, CTCAE version 3.0, published Dec 12, 2003. A complete document (72 pages) may be downloaded from the following sites:

http://ctep.info.nih.gov/reporting/ctc.html
http://www.hovon.nl (under Studies > Documents)

F. Guideline for the use of iron chelation therapy

According to the Nagasaki 2005 and Florence 2007 criteria [46,47]

Patients with low or intermediate-1 risk MDS according to IPSS, who are transfusion dependent with serum ferritine levels of 1000-2000 µg/L or other evidence of significant tissue-iron overload should be considered for iron chelation therapy if a life expectancy of 5 years is likely. Treatment with iron chelators should be monitored every 3 months during treatment.

G. Add-on study protocols

Add-on study 1

Validation of flow cytometric parameters in myelodysplastic syndromes [48-52]

Primary investigators: A.A. van de Loosdrecht, T.M. Westers, VU University Medical Center Amsterdam, Netherlands

Background:

The myelodysplastic syndrome (MDS) is a clonal hematopoietic disorder characterized by ineffective hematopoiesis. The WHO classification of myeloid disorders contributes to a more refined classification and prognostication of MDS. Substantial differences in clinical behavior of pure refractory anemia (RA) versus refractory cytopenia with multilineage dysplasia (RCMD) stress the need for additional methods to identify dysplasia of haematopoietic bone marrow cells in MDS. Characterization of aberrant expression of differentiation antigens can be assessed by multi-color flow cytometry and may detect dysplasia where routine diagnostics fail. The amount and type of flow cytometric aberrancies may have impact on prognostication and justify different treatment modalities in low and intermediate-I risk MDS vs. high risk MDS in the current era of emerging new drugs for MDS.

Methods:

Recent studies showed that flow cytometric methods can identify distinct subgroups of low and intermediate-1 risk MDS. The current proposal aims are: 1. To validate the role of flow cytometry in the diagnosis and prognostication of MDS in a large patient cohort of 200 patients with de novo low-intermediate-I risk MDS; 2. To perform a multiparameter analysis on all current validated variables in risk assessment of patients with low and intermediate-I risk MDS prospectively within HOVON 89 including the newly designed flow cytometric approach; 3. To assess the role of flow cytometry in MDS as monitoring of disease during treatment with lenalidomide with or without Epo/G-CSF within the HOVON 89 study. Multicolor flow cytometric technology will be used enabling analysis of distinct well characterized subpopulations within MDS bone marrow on very small amount of cells. The panel of reagents [antibodies] is based on a recently defined protocol which has been discussed with the ELN working party on MDS and mainly based on current protocols within the Dutch Society for Cytometry. It is anticipated that the current proposal may add significantly to the diagnosis, prognostication and pathobiology of MDS which may have implications on clinical decision making in MDS.

Briefly, flow cytometric analysis of bone marrow samples will be performed using 4-color flow cytometry. Analysis will be performed on total nucleated bone marrow cells after NH_4Cl lysis of erythrocytes. All samples will be processed and analyzed within 24 hours. Samples will be analyzed using a FACS Calibur (BD); per sample a minimum of 105 white blood cells will be

collected. Data will be analyzed using Cell Quest Software (BD). The different cell compartments (progenitor cells, granulocytes and monocytes) will be identified using CD45 expression and sideward light scatter (SSC) and defining antigens. Within each cell compartment expression of several antigens and phenotypic patterns of maturation will be analyzed; results will be compared to normal bone marrow samples. Aberrant expression of certain antigens will be defined as >0.5 log different from normal expression of that specific antigen. Aberrancies in the progenitor cells (blasts), granulocytes and monocytes will be evaluated per subpopulation. All flow cytometry data will be evaluated based on a scoring system defined by Wells et al. (a guideline for scoring dysplasia in the myelomonocytic lineage).
A data base will be conducted containing all flow cytometric data and other parameters such as Hb levels, platelet counts; leucocyte counts, differential, LDH, iron status, bone marrow fibrosis (immunohistological examination) and clinical parameters such as transfusion dependency and leukemic evolution. Univariate analysis and multivariate analysis will be performed with assistance of biostaticians of the Vumc to detect independent predictors of response. Upon this effort a new prognostic scoring system is anticipated to predict more accurately clinical outcome of patients with low and intermediate-I risk MDS.
The role of flow cytometry in the diagnosis and prognostication in MDS as described above will also be evaluated dynamically during treatment with novel agents such as lenalidomide within HOVON 89. Clinical evaluation will be analyzed according to the recent updated Cheson criteria for disease monitoring in MDS as described in detail in HOVON 89.

Practical considerations:

A total of 5 ml PB and 7-10 ml (one-heparinized tube) of bone marrow aspirate will be needed for all experiments as described above at time of diagnosis and after 6 and 12 month or at any time for going of study (no HI; or progressive disease). All samples will be send to the VU University Medical Center. Within the Dutch Society for Cytometry extensive collaborations already exist for managing bone marrow samples for flow cytometric assessments in MDS. After validation of each center during the study period, the study coordinator will inform each participating center within HOVON89 how to manage new samples. Since additional studies which are described below (add-on study 2-3) wil be included within HOVON89, the Vumc will manage and process all samples.

Add-on study 2

Identification of Genetic Aberrations in MDS Using High resolution Single Nulcleotide Polymorphism (SNP) Arrays [53-55]

Primary investigators: J.H. Jansen and T. de Witte, UMCN Nijmegen, Netherlands

Background:

Although gross cytogenetic aberrations can be detected in approximately 50% of MDS patients, the crucially affected genes that are causally involved in the pathogenesis of this disease remain largely unknown. Using the recently developed high-resolution single nucleotide polymorphism-(SNP) array technology, genetic amplification and loss of heterozygosity caused by deletions as well as by mitotic recombination can be detected in the majority of MDS patients. As all SNPs that are analysed on these arrays are exacly mapped with respect to the genomic localization, this technology allows the rapid and exact mapping of deletions and amplifications, and allow the definition of critically affected genomic regions and genes when multiple patients are analyzed. Until now, prospective studies including this novel technology are lacking, and the prognostic value of the newly identified affected loci remains mostly uncertain.

In conjunction to the flowcytometric studies (see add-on study 1), and in addition to standard cytogenetics and FISH, all patients will be screened at diagnosis for genetic aberrations using single nucleotide polymorphism-arrays (SNP- arrays). For this, Affymetrix technology will be employed.

For the SNP-analysis, DNA from cells that belong to the clonally expanded hematological cells are required. As granulocytes are part of the clonally expanded population in MDS (whereas lymphocytes are usually not) granulocytes purified from the peripheral blood can be used.

Methods:

Drawing and shipment of peripheral blood: For the isolation of DNA, one tube (7-10 ml) of peripheral blood will be drawn using EDTA, citrate or heparin as anticoagulant. Samples will be shipped at room temperature together with the samples for flow cytometry to the VU University Medical Center in Amsterdam. As soon as possible, but maximally 24 hours after drawing of the blood, standard Ficoll 1.077 density gradient centrifugation will be performed to isolate the interphase cells and granulocytes separately.

Isolation and storage of interphase cells: Interphase cells containing mononuclear cells including lymphocytes will be isolated sterilely and will be viably frozen for later isolation of T cells. DNA from the T cells may be used to investigate whether aberrations in the DNA isolated from the granulocytes are germ-line or acquired according to standard methods.

Isolation and storage of granulocytes: Granulocytes (belonging to the clonally expanded cells in MDS) will be recovered from the pellet after standard lysis of the erythrocytes in hypotonic NH_4Cl

solution. After erythrocyte lysis, the granulocytes will be counted and pelleted by centrifugation (1400-1600rpm for 5-10 minutes). After removal of the supernatant, the pellet will be resuspended in 200µl physiological NaCl solution or culture medium, transferred to a 1 or 2 ml cryotube and frozen at –20^{o}C (do not add DMSO). For DNA isolation and SNP arrays the frozen samples will be shipped to the central SNP-array laboratory (UMC-St-Radboud Nijmegen, The Netherlands) by the VU University Medical Center.

Practical considerations:

If the sample cannot be shipped and handled within 24 hours to the VUmc in Amsterdam, centers that routinely perform Ficoll separation and erythrocyte lysis may choose to isolate the interphase cells and granulocytes locally, and send the frozen samples to the UMC St Radboud in Nijmegen on dry ice.

Add-on study 3

The predictive value of mitochondrial dysfunction in erythroblasts of low-risk MDS patients for response on therapy [56-61]

Primary investigator: E. Vellenga, UMCG Groningen, Netherlands

Background:

Myelodysplastic syndromes (MDS) are a heterogeneous group of clonal stem cell disorders. Especially in low-risk MDS, such as refractory anemia (RA) and refractory anemia with ringed sideroblasts (RARS), increased programmed cell death (PCD) of bone marrow hematopoietic cells has been described. This might be an important mechanism to explain the typical clinical findings of a hypercellular bone marrow and peripheral blood cytopenias. In particular, enhanced apoptosis has been reported in MDS. Recent studies have indicated that besides apoptosis other types of PCD can be distinguished such as autophagic cell death and certain types of necrosis. In addition, cells are capable to switch between the different types of PCD dependent on their cellular context. Loss of mitochondrial transmembrane potential plays an important role in these events, and the intensity of the stimulus and the cellular context often determine which type of cell death develops. Previous studies have shown that hematons can be isolated from the bone marrow light density fraction. These hematons are compact hematopoietic complexes containing several cell lineages, including mesenchymal cells, endothelial cells, and hematopoietic progenitor cells. In these hematons a high number of erythroblasts are located within their own microenvironment. To investigate whether the type of PCD of MDS erythroid precursors is dependent on their cellular context, ultrastructural, cytomorphometric and immunohistochemical studies were performed on MDS erythroid precursors from the mononuclear cell fraction versus the hematon fraction. The results demonstrate that immunohistochemistry of bone marrow MDS erythroblasts demonstrated no positively for active caspase -3 and -8. Ultra structurally, abnormal and iron-laden mitochondria were abundant, but apoptosis was found in only small number of cells. However, a high number of immature and mature MDS erythroblasts contained cytoplasmic vacuoles, partly double-membrane and positive for lysosomal marker LAMP-2 and mitochondrial markers, compatible with autophagic removal of dysfunctional mitochondria. In healthy controls only mature erythroblasts comprised these vacuoles. These findings were confirmed morphometrically showing an increased vacuolar surface in MDS erythroblasts compared to controls. In summary, these data indicate that MDS erythroblasts show primarily features of enhanced autophagy, which is probably initiated to remove defective iron-containing mitochondria, and may switch to apoptosis when lacking an appropriate microenvironment.

Methods:

Based on these data the following experiments will be performed to study whether mitochondrial dysfunction due to iron accumulation in erythroid precursors and degree of autophagy in these cells is an important determinant for the response to therapy. Therefore, the following experiments will be performed:

Firstly, bone marrow aspirate material including hematons will be studied by electron microscopy. Especially attention will be given to the degree of autophagy, the morphological abnormalities of the mitochondria, and the degree of iron accumulation in these organelles. Functional assays will be performed for studying the mitochondrial functional activity including cytochrome c expression and localization and ROS production by erythroid precursors according to standardized and validated protocols.

Secondly, in case of responsive disease to lenalidomide (Revlimid) with or without Epo/G-CSF bone marrow examinations will be repeated to determine in which way the remaining cells are distinct from the erythroid cells upfront treatment, not only by functional studies but also by EM. Since these studies are labour intensive, 20 evaluable patients with MDS-RA and 20 patients with MDS-RARS will be analyzed. Dependent on the results this number might be extended.

Practical considerations:

These analyses have to be performed on fresh isolated bone marrow aspirate material in view of the fragility of the studied cell population. In according to all add-on studies, samples will be shipped at room temperature together with the samples for flow cytometry [add-on study 1], SNP arrays [add-on study 2] to the VU University Medical Center in Amsterdam. As soon as possible, but maximally 24 hours after drawing of the bone marrow, material selected for this study will be send for [5 ml bone marrow suspension] to Research Lab Hematology, UMCG Groningen, Mol de Vitrine, phone +31 50 3613257.

Logistic details for Add-on study 1, 2 and 3.

Peripheral blood and Bone marrow samples for the three Add-on studies should be taken at time of diagnosis, after 6 and 12 months, when going off protocol treatment, and if progressive disease in follow up.
A total of 40 ml PB and 20 ml BM at each timepoint will be sufficient to perform all experiments.

All samples should be send to:

Dr. A.A. van de Loosdrecht or
Dr. T.M. Westers
VU University medical center
Dept. of Hematology
Laboratory hematilogy/V-ICI/CCA-building
De Boelelaan 1117
1081 HV Amsterdam
The Netherlands
Phone +31-20-4442604 or +31-20-4447280 or +31-20-4447349

H. Guideline for extraprotocol treatment with Epo/G-CSF in low/int-I risk MDS

According to international guidelines and Vumc approach (www.hematologie.nl)

Table Decision-model for treating the anemia of MDS with Epo/G-CSF

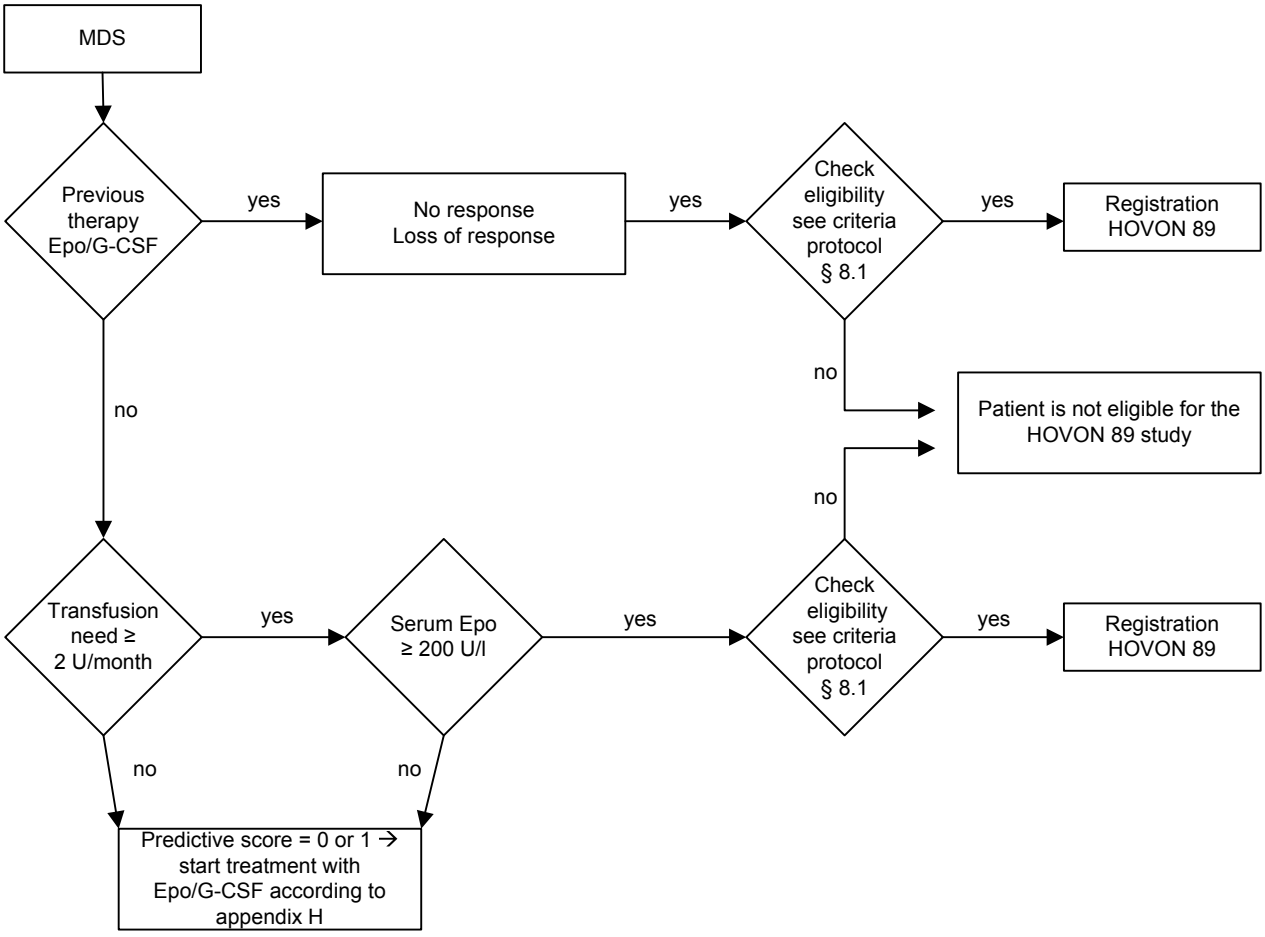
Variable	Value	Score	Value	Score
Transfusion need*	< 2 U/month	0	≥ 2 U/month	1
Serum-Epo*	< 200 U/liter**	0	≥ 200 U/liter**	1

Predicted response rate: total score 0=74%, 1=23%, 2=7%

* Pre-treatment evaluation

** serum-Epo level modified according to the British guidelines (see D. Boven et al..[27])

In patients with low or intermediate MDS with a predictive score of 0 or 1 according to a validated decision model, treatment with Epo/G-CSF is recommended according to international consensus. Patients with score 2 do not benefit from treatment with Epo/G-CSF and should be included in the HOVON 89 study.



FLOW DIAGRAM FOR ELIGIBILITY

Treatment with Epo/G-CSF according to international consensus.

Epoëtine bèta (Epo; NeoRecormon) will be administrated s.c. with a starting dose of 30000 IU weekly for 3 months. Dose escalation of 30000 IU is implemented after 6 weeks of treatment if no increase of Hb of at least 0.6 mmol/l has been reached. In case of complete remission the Epo dose will be reduced with 10000 IU/week, each reduction lasting for 6 weeks at a minimum maintenance dose of 20000 IU/week . After 12 weeks (3 months), G-CSF (filgrastim; Neupogen) will be administrated in combination with Epo if no increase is obtained in Hb of at least 0.6 mmol/l. G-CSF will be administrated 3 times weekly (3x300 µg/week s.c. for patients < 75kg; 3x480 µg/week s.c. > 75 kg). G-CSF will be interrupted if the WBC > $30x10^9$/l and restarted after normalization of the WBC. G-CSF will be re-started at a dose of 300 µg once a week and escalated to 2x300 µg/week in 6 weeks.