umcg

Protocol NL43844.042.13

Bone marrow evaluation in patients with leukemia and myelodysplasia

Protocol Number: NL43844.042.13 Final

Protocol: 06-JAN-2014

Version: 1.3

PROTOCOL TITLE Bone marrow evaluation in patients with leukemia and myelodysplasia

Protocol ID	**Beenmerg evaluatie bij patiënten met leukemie en myelodysplasie** **Bone marrow evaluation in patients with leukemia and myelodysplasia**
Short title	
EudraCT number	***Not applicable***
Version	**1.3**
Date	**06 January 2014**
Coordinating investigator/project leader	***Prof.dr.E.Vellenga*** ***University Medical Center Groningen*** ***Department of Hematology, DA 21*** ***P.O. Box 30001*** ***9700 RB Groningen, The Netherlands*** ***Tel. +31-(0)50-361 2354*** ***Fax +31-(0)50-361 5960*** ***Email: e.vellenga@umcg.nl***
Principal investigators	***Prof. dr. E. Vellenga*** ***University Medical Center Groningen*** ***Dr. R. Komdeur*** ***Martini Ziekenhuis Groningen***
Sponsor	***Prof. dr. E. Vellenga*** ***University Medical Center Groningen*** ***Department of Hematology, DA 21*** ***P.O. Box 30001*** ***9700 RB Groningen, The Netherlands*** ***Tel. +31-(0)50-361 2354*** ***Fax +31-(0)50-361 5960*** ***Email: e.vellenga@umcg.nl***

Subsidising party	***Not applicable***
Independent expert	***Prof. dr. J.A. Gietema*** ***University Medical Center Groningen*** ***Department of Medical Oncology, DA 11*** ***P.O. Box 30001*** ***9700 RB Groningen, The Netherlands*** ***Tel. +31-(0)50-361 1334*** ***Fax +31-(0)50-361 4862*** ***Email: j.a.gietema@umcg.nl***
Laboratory sites	***Not applicable***
Pharmacy	***Not applicable***

PROTOCOL SIGNATURE SHEET

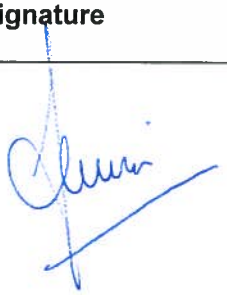
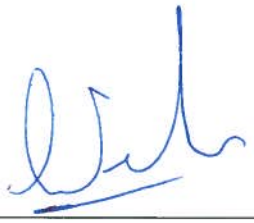
Name	Signature	Date
Head of Department: Prof. dr. J.C.Kluin-Nelemans Head of the Department of Hematology		13/1/14
Coordinating Investigator/ Project leader: Prof. dr. E. Vellenga Hematologist		7/1/14

TABLE OF CONTENTS

1. INTRODUCTION AND RATIONALE 8
2. OBJECTIVES 9
3. STUDY DESIGN 9
4. STUDY POPULATION 9
4.1 Population (base) 9
4.2 Inclusion criteria 9
4.3 Exclusion criteria 10
4.4 Sample size calculation 10
5. METHODS 10
5.1 Study parameters/endpoints 10
5.1.1 Main study parameter/endpoint 11
5.2 Study procedures 11
5.3 Withdrawal of individual subjects 11
5.3.1 Specific criteria for withdrawal (if applicable) 11
6. STATISTICAL ANALYSIS 12
6.1 Primary study parameter(s) 12
7. ETHICAL CONSIDERATIONS 12
7.1 Regulation statement 12
7.2 Recruitment and consent 12
7.3 Benefits and risks assessment, group relatedness 13
7.4 Compensation for injury 13
7.5 Handling and storage of data and documents 13
7.6 Amendments 13
7.7 Annual progress report 13
7.8 End of study report 13
7.9 Public disclosure and publication policy 14
8. REFERENCES 14

SUMMARY

Rationale:

Rationale: Patients with AML or MDS frequently demonstrate relapsing disease following intensive chemotherapy. This likely relates to a small population of malignant cells that have an intrinsic resistance to chemotherapy. By studying the properties of these malignant cells, in particular the role of polycomb proteins in conjunction with the surrounding microenvironment, new insights might be obtained for the differences in chemotherapy susceptibility of the AML cells compared to normal hematopoietic cells. In addition this protocol will provide the opportunity that all patients presenting with AML/MDS can be included in the ongoing side studies linked to treatment protocols. In patients participating in the ongoing AML/MDS HOVON studies additional bone marrow samples are already collected. However, AML/MDS patients not included in these HOVON studies but treated according to the standard arms of the protocols cannot be included in these type of studies since no additional informed consent is given for bone marrow collection. The present protocol will make it feasible that these patients can also participate in the ongoing scientific studies.

Study design:

Observational study.

Study population:

Subjects with AML and MDS at the age of 18 years or more.

Main study parameters/endpoints:

Proliferation and differentiation markers, gene profiling, new molecular markers of AML cells. In addition, bone marrow mesenchymal stem cells (MSC) of the patient will be collected and the interaction with the malignant cells will be analyzed in *in vitro* culture assays in conjunction with gene profiling.

Nature and extent of the burden and risks associated with participation, benefit and group relatedness:

The sample for additional bone marrow will be collected during the standard diagnostic bone marrow test. In total 20 ml bone marrow cells will be collected. The collection of the sample will give a short stab during the suction procedure of the material from the bone marrow.

1. INTRODUCTION AND RATIONALE

Patients treated for leukemia and myelodysplasia still have an unfavorable prognosis despite treatment with intensive chemotherapy and allogenic stem cell transplantation[1-3]. These results are in general not related to the incapability to treat the patients with chemotherapy but are due to the fact that the malignant cells re-emerge within 6 – 12 months after cessation of therapy[1-3]. These findings indicate that the malignant cells, at least a small subpopulation of them, are intrinsically resistant to the therapeutic modalities applied. More knowledge regarding the underlying survival mechanisms might be highly relevant for further improvements in treatment results for these patients. Especially additional information of the cellular and molecular mechanisms that operate in the AML cells and the interaction with the microenvironment that provides protective signals to these cells, might be highly informative. During the last years we have demonstrated that in particular epigenetic changes occurs in AML cells by an altered function of BMI1 and additional polycomb proteins[4-7]. In addition the micro-environment is affected reflected by enhanced micro-vessel density and neo-angiogenesis[4-8]. Both components will give the leukemic cells a growth advantage and future studies will focus on how both partners interact with each other.

A second reason for this protocol is that AML/MDS patients participate in general in ongoing HOVON studies. To these treatment protocols side studies are linked which include molecular and cellular characterization of the malignant cells. For these studies additional bone marrow cells are collected and used for the defined side studies according to the protocols. However, a subgroup of the MDS/AML patients is not eligible for the HOVON study or is not willing to participate in the HOVON treatment protocols. In that case, patients are treated according to the standard arm of the ongoing HOVON protocol. To make it feasible that still these type of studies can be performed an additional informed consent procedure is required for collecting the additional bone marrow cells. The present protocol will make it feasible that these patients can be included in the on-going scientific studies.

2. OBJECTIVES

The present study will be focused on defining the underlying differences between malignant vs. normal cells by analyzing the process of proliferation and differentiation of the AML cells in conjunction with gene profiling or DNA signature. Special attention will be given to the role of polycomb proteins and the resulting epigenetic alterations. Standard assays present at our lab will be used for culturing these cells on MS5 stromal layer under different conditions. In addition, it will be defined in which degree the own microenvironment is supportive and behaves differently, and what processes are triggered by the altered micro-environment. MSC will be isolated from the CD34- cell fraction and after 2-3 passages MSCs can be used for studying the interaction with AML stem cells.

3. STUDY DESIGN

In subjects diagnosed with AML or MDS additional bone marrow cells (20 ml) will be collected during the standard diagnostic bone marrow test.

4. STUDY POPULATION

4.1 Population (base)

Patients with newly diagnosed AML or MDS

4.2 Inclusion criteria

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

- newly diagnosed AML or MDS according to WHO guidelines

- ≥18 years
- not eligible for participation in HOVON-trials

4.3 Exclusion criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study:

- Age < 18 years

4.4 Sample size calculation

At the moment different subgroups can be recognized within AML (n=6) and MDS (n=4). To obtain adequate information per subgroup, at least 6 samples from each subgroup have to be studied. In addition, it is assumed that a bone sample can be used for 2 different experiments. Based on these assumptions 120 subjects will be included.

5. METHODS

5.1 Study parameters/endpoints

Study parameters analyzed in collected bone marrow samples:

To obtain information regarding the disturbed cellular processes in the malignant cells, the *in vitro behavior* of the cells will be studied in the absence and presence of their own microenvironment. Subsequently it will be determined, how these changes translate in altered gene expression by performing gene profiling studies and how this is correlated with the genetic background of the cells. In particular attention will be given to the role of polycomb genes and the epigenetic alterations generated by an altered expression of these proteins. Relevant target genes will be identified and functionally inactivated by RNAi methods. In addition leukemic cells will be exposed *in vitro* to relevant drugs that affect the disturbed cellular pathways in the leukemic

cells and the responses between different subgroups will be studied and defined in which degree protective signals are provided by the microenvironment.

5.1.1 Main study parameter/endpoint

The AML and MDS cells will be cultured in *in vitro* culture assays and analyzed with regard to cell proliferation, differentiation and cell survival according to ongoing protocols. In addition patients' MSC's will be collected from the same sample and co-culture experiments will be performed. Moreover, additional information will be obtained with regard to the cellular make-up of the cells by performing gene profiling studies, drug susceptibility assays, and DNA methylation alterations against their own genetic background. This will be compared to normal hematopoietic cells and will provide information which pathways are altered in the leukemic compartments.

5.2 Study procedures

During the standard diagnostic bone marrow test an additional 20 ml bone marrow sample will be collected. No additional puncture is required. The patient will experience a short stab during the suction procedure of the material from the bone marrow. In the lab the AML cell fraction will be isolated following ficoll separation and the $CD34^-$ cell fraction will be used for isolation of the mesenchymal stem cells and frozen. At a later time point the *in vitro* experiments will be performed separately. In addition RNA, DNA will be isolated for defining the molecular markers.

5.3 Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so, without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons.

5.3.1 Specific criteria for withdrawal (if applicable)

Subjects can leave the study at any time for any reason if they wish to do so without any consequences.

6. STATISTICAL ANALYSIS

The proposed *in vitro* experiments are explorative investigations and will generate new hypotheses. In line with the approach, the statistical analysis will be describing.

6.1 Primary study parameter(s)

Different cellular parameters will be defined within defined subgroups of AML (n=7) or MDS (n=4) including cellular proliferation, cell survival, interaction with MSC's, gene profiling, alterations in methylation and changes following overexpression or knocking down target genes by RNAi approach. These findings will be compared with results of similar experiments with normal cells.

7. ETHICAL CONSIDERATIONS

7.1 Regulation statement

This study will be conducted in compliance with the International Conference on Harmonization (ICH) E6 Good Clinical Practice (GCP) guidance, and the most recent version of the Declaration of Helsinki as amended in 2013. In addition, all applicable local laws and regulatory requirements will be adhered to.

7.2 Recruitment and consent

The subjects will be informed and asked for participation by one of the staff members of the department of Hematology. Time to consider participation will be 7 days. The patient information letter and informed consent form are enclosed.

7.3 Benefits and risks assessment, group relatedness

The subject will not experience benefit from the tests performed. Hopefully, the treatment results of this patient group might improve as a result of these investigations in future.

7.4 Compensation for injury

The investigator has a liability insurance which is in accordance with the WMO regulation. No additional insurance is required since the investigation is not a threat for the subjects' health.

7.5 Handling and storage of data and documents

The material will be collected and frozen and stored with an anonymous code based on year data-sample number. In publications patients will be reported anonymously.

7.6 Amendments

Amendments are changes made to the research after a favorable opinion by the accredited METC has been given. All amendments will be notified to the METC that gave a favorable opinion.

7.7 Annual progress report

The sponsor/investigator will submit a summary of the progress of the investigations to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject and the total numbers of subjects that have been included. In addition a short summary will be given regarding the *in vitro* studies that have been performed.

7.8 End of study report

The investigator will notify the accredited METC of the end of the study within a period of 8 weeks. Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study.

7.9 Public disclosure and publication policy

The results of the scientific studies will be submitted to scientific journals

8. REFERENCES

1. Pabst T, Vellenga E, van Putten W, Schouten HC, Graux C, Vekemans MC, Biemond B, Sonneveld P, Passweg J, Verdonck L, Legdeur MC, Theobald M, Jacky E, Bargetzi M, Maertens J, Ossenkoppele GJ, Löwenberg B; Dutch-Belgian Hemato-Oncology Cooperative Group (HOVON); German AML Study Group (AMLSG); Swiss Collaborative Group for Clinical Cancer Research (SAKK). Favorable effect of priming with granulocyte colony-stimulating factor in remission induction of acute myeloid leukemia restricted to dose escalation of cytarabine. Blood. 2012;119:5367-73.

2. Vellenga E, van Putten W, Ossenkoppele GJ, Verdonck LF, Theobald M, Cornelissen JJ, Huijgens PC, Maertens J, Gratwohl A, Schaafsma R, Schanz U, Graux C, Schouten HC, Ferrant A, Bargetzi M, Fey MF, Löwenberg B; Dutch-Belgian Hemato-Oncology Cooperative Group (HOVON); Swiss Group for Clinical Cancer Research Collaborative Group (SAKK). Autologous peripheral blood stem cell transplantation for acute myeloid leukemia. Blood. 2011;118:6037-42.

3. Löwenberg B, Pabst T, Vellenga E, van Putten W, Schouten HC, Graux C, Ferrant A, Sonneveld P, Biemond BJ, Gratwohl A, de Greef GE, Verdonck LF, Schaafsma MR, Gregor M, Theobald M, Schanz U, Maertens J, Ossenkoppele GJ; Dutch-Belgian Cooperative Trial Group for Hemato-Oncology (HOVON) and Swiss Group for Clinical Cancer Research (SAKK) Collaborative Group. Cytarabine dose for acute myeloid leukemia. N Engl J Med. 2011;364:1027-36.

4. Rizo A, Olthof S, Han L, Vellenga E, de Haan G, Schuringa JJ. Repression of BMI1 in normal and leukemic human CD34(+) cells impairs self-renewal and induces apoptosis. Blood. 2009;114:1498-505.

5. Schuringa JJ, Vellenga E. Role of the polycomb group gene BMI1 in normal and leukemic hematopoietic stem and progenitor cells. Curr Opin Hematol. 2010;17:294-9.

6. van den Boom V, Rozenveld-Geugien M, Bonardi F, Malanga D, van Gosliga D, Heijink AM, Viglietto G, Morrone G, Fusetti F, Vellenga E, Schuringa JJ. Nonredundant and locus-specific gene repression functions of PRC1 paralog family members in human hematopoietic stem/progenitor cells. Blood. 2013;121:2452-61.

7. Rizo A, Horton SJ, Olthof S, Dontje B, Ausema A, van Os R, van den Boom V, Vellenga E, de Haan G, Schuringa JJ. BMI1 collaborates with BCR-ABL in leukemic transformation of human CD34+ cells. Blood. 2010;116:4621-30.

8. Horton SJ, Jaques J, Woolthuis C, van Dijk J, Mesuraca M, Huls G, Morrone G, Vellenga E, Schuringa JJ. MLL-AF9-mediated immortalization of human hematopoietic cells along different lineages changes during ontogeny. Leukemia. 2013;27:1116-26.