Evaluation of VEGF expression with $^{89}\text{Zr}$-bevacizumab PET scan in patients with relapsing multiple myeloma; a feasibility study

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1. Background and introduction

1.1 Multiple myeloma and imaging techniques
Multiple Myeloma (MM) is a clonal B cell disorder characterised by a monoclonal plasma cell population in bone marrow, with bone pain, anaemia, hypercalcaemia, and kidney dysfunction as clinically presenting symptoms. Osseous involvement is one of the most predominant features of patients with MM; 90% of the patients develop lytic bone lesions. Lytic bone lesions are the result of increased bone resorption and reduced bone formation. The regular method to detect bone lesions is skeletal survey. This technique can only detect lesions that have lost 30% or more of the trabecular bone. Another weakness is the fact that lesions persist after treatment with chemotherapy or radiotherapy and no clear distinction can be made whether vital tumour cells persist in these lesions. So unless progressive defects are observed, skeletal survey has limited value in relapsing multiple myeloma. As mentioned before, 90% of the myeloma patients will develop bone lesions. New bone lesions are a sign of disease progression. Furthermore they give clinical signs as bone pain and in the worse case scenario pathological fractures. Alternative scanning methods have been developed to visualize the emergence of new malignant plasma cells that make use of tracers that identify tumour-specific receptors or identify enhanced metabolic activity of the malignant plasma cells. Recently it was shown that \(^{111}\text{In}\)-labeled somatostatin receptor scintigraphy (SRS) can visualize disease activity in a better way than the conventional used X-ray examination. A positive SRS scan was observed in 83% of the relapsing patients compared to 40% of the patients with new defects on X-ray examination. To define whether these results could further be improved by making use of enhanced metabolic activity of the plasma cells defined by the uptake of \(^{18}\text{F}\)-fluorodeoxyglucose -Positron Emission tomography (FDG-PET), we compared SRS scans with the FDG-PET scans in relapsing myeloma patients. The results of this study demonstrate that more abnormal lesions could be detected in relapsing MM with FDG-PET vs. SRS. The use of FDG-PET in newly diagnosed MM patients is well studied. A number of studies demonstrate that FDG-PET has a higher probability to show affected areas both medullar and extramedullar than whole body X-ray. Some studies even demonstrate that a positive FDG-PET scan following treatment is an independent prognostic parameter for overall survival.

1.2 Multiple myeloma and angiogenesis.
The increased FDG-uptake by the tumour is related to a high metabolic activity. This might be a consequence of tumour hypoxia causing new vessel formation. There seems to be a relationship between MM and angiogenesis, the formation of new blood vessels from exciting blood vessels. There is an increased microvessel density (MVD) of the affected bone marrow in patients with active MM. Bone marrow angiogenesis was studied in 400 patients with a plasma cell neoplasma. The MVD was determined in patients having monoclonal gammapathy of undetermined significance (MGUS), smouldering myeloma and active myeloma. The MVD was significantly
increased, showing progressively increased bone marrow angiogenesis, during disease progression\(^9\). Vascular endothelial growth factor (VEGF) is an important mediator of angiogenesis. MM cell lines were found to express VEGF mRNA and secrete the protein in the extracellular environment thereby stimulating angiogenesis. Furthermore plasma cells in the bone marrow of MM patients expressed VEGF as well as VEGF receptor 1 and 2\(^{10,11,12}\). Thus, also direct stimulation of MM growth and survival might be mediated by VEGF, through an autocrine pathway\(^{11,12}\). Therefore, inhibition of the process of angiogenesis but also blocking VEGF itself, is of interest in the treatment of MM.

Inhibition of the process of angiogenesis is used in the treatment of MM, for instance by means of thalidomide and lenalidomide\(^{13}\). The anti-angiogenic proportion of thalidomide led to the first clinical use in MM. Immunomodulatory drugs, like thalidomide and lenalidomide, induce its anti-myeloma effect through several pathways including angiogenesis. During treatment with thalidomide VEGF levels in bone marrow and plasma and MVD decreases\(^{14,15}\). Blocking VEGF itself can be obtained by means of bevacizumab, a recombinant, humanised monoclonal antibody that binds to all isoforms of human VEGF with high affinity. Treatment with bevacizumab is well established in solid tumours, like colon cancers and renal cell carcinomas and is currently tested in acute myeloid leukaemia and MM\(^8\). Thus, the direct or indirect VEGF mediated growth and survival in MM, underline the importance of VEGF in MM.

1.3 Bevacizumab scanning

At the University Medical Center Groningen, non-invasive in vivo VEGF imaging with radiolabeled bevacizumab has been developed. Systemic VEGF levels only consist of splice variants VEGF\(_{121}\) and a small proportion of VEGF\(_{165}\), whereas VEGF\(_{189,206}\) and a large proportion of VEGF\(_{165}\) are mainly located in the extracellular matrix, resulting in high concentrations in the tumor micro-environment. Bevacizumab binds to all VEGF splice variants and can be labeled with the single \(\gamma\)-emitting isotope Indium-111 (\(^{111}\)In) and with the PET isotope Zirconium-89 (\(^{89}\)Zr) while preserving VEGF binding properties. In a human ovarian tumor xenograft, PET imaging 24 hours after injection of \(^{89}\)Zr-bevacizumab showed high uptake in well perfused organs and in the tumor, with an increase in tumor to background ratio in time, resulting in clear tumor localization after 72 hours\(^{16}\). A feasibility study in our institution, with \(^{89}\)Zr-bevacizumab PET imaging in renal cell carcinoma patients, showed a superior diagnostic yield compared to other imaging techniques. In breast cancer patients, \(^{89}\)Zr-bevacizumab PET was used for evaluation of therapy induced decline of VEGF in tumor lesions\(^{17}\).

The high VEGF production by myeloma cells makes VEGF a very interesting target for tumor visualization. \(^{89}\)Zr-bevacizumab PET imaging could be more sensitive for myeloma lesions. In addition, \(^{89}\)Zr-bevacizumab PET imaging might give information about treatment options, in other
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words quantification of the VEGF excretion by tumor cells by measuring standard uptake values (SUV) may provide in the future an additional parameter for selecting patients for anti-angiogenic treatment\(^{13}\).

So, in conclusion, VEGF is expressed by MM plasma cells, thereby providing a rationale that the assessment of VEGF-levels in the micro-environment of MM tumors could potentially be used as a diagnostic tool to see if there is disease activity. Especially in the relapsed setting this is of invaluable importance, since conventional skeletal survey has limitations in this setting. Furthermore, \(^{89}\text{Zr}\)-bevacizumab PET imaging could provide information about treatment options and treatment response.

2. Objective of the study

In the present study we will perform a feasibility study to demonstrate that \(^{89}\text{Zirconium-bevacizumab PET scanning can visualize multiple myeloma lesions. Data from the present study may be used to design further studies with regard to the expression of VEGF and the selection of patients for anti-angiogenic therapy. It might predict which patient will benefit from anti-angiogenetic treatment and for future studies to see which patient might benefit from adding bevacizumab to the treatment regime. Furthermore, \(^{89}\text{Zirconium-bevacizumab PET scanning can be used for monitoring therapy effect and the degree of uptake defined by SUV might also provide prognostic information. In addition, in vitro staining of bone marrow material will be performed to demonstrate whether VEGF is up regulated by the malignant plasma cells or surrounding cells. Furthermore, the MVD will be defined. These results will be combined with the results of the \(^{89}\text{Zr}\)-bevacizumab PET imaging to see if there is a correlation between positivity of the \(^{89}\text{Zr}\)-bevacizumab PET imaging and up regulation of angiogenesis parameters.\)

2.1 Primary objective

Feasibility study to demonstrate that VEGF PET imaging by using \(^{89}\text{Zr}\)-bevacizumab as tracer can be used to detect multiple myeloma lesions.

2.2 Secondary Objectives

The secondary objective will be the comparison of \(^{89}\text{Zr}\)-bevacizumab PET imaging with the FDG-PET scan to demonstrate whether increased angiogenesis is associated with enhanced FDG uptake. Furthermore bone marrow material will be stained to for MVD and VEGF levels. In
addition we will see if there is a correlation between lesion found on the $^{89}$Zr-bevacizumab PET scan and expression of VEGF and MVD.

2.3 End-points

- **The primary endpoint** will be $^{89}$Zr-bevacizumab tracer uptake in multiple myeloma lesions.

- **The secondary endpoints** will be:
  - The correlation between positive lesions found on the FDG-PET and lesions found on $^{89}$Zr-bevacizumab PET.
  - The correlation between VEGF levels and MVD in bone marrow samples of MM patients and lesions found on $^{89}$Zr-bevacizumab PET.

3. Patient selection criteria

3.1 Inclusion criteria

Patients with relapsing MM according to international defined guidelines:

Relapse after having achieved CR:
1. Reappearance of paraprotein
2. More than 5% plasma cells in bone marrow.
3. New lytic lesions or progression of old lesions.

Relapse after having achieved PR
1. Increases of paraprotein with more than 25%
2. Increase of urine paraprotein with more than 25%
3. Increase of plasma cells in bone marrow with 10%
4. New lytic lesions or progression of old lesions
5. New hypercalcaemia

3.2 Exclusion criteria

- Radiotherapy in the last 3 months.
- Ineligible to lay supine during the PET scan.
- Age ≤18 years.
- Pregnancy, documentation of a negative pregnancy test must be available for pre-menopausal women with intact reproductive organs and for women less than two years after menopause
- Claustrophobia
- Severe kidney dysfunction; serum-creatinine ≥250 µM.

4. Trial design
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This is a pilot-study, thus no formal group size calculation can be given. For the purpose of this study, 20 patients will be included. Patients must have a relapsing multiple myeloma according to international guidelines. A bone marrow biopsy and a FDG-PET scan will be performed. It is expected that 20 patients will cover the variability of the $^{89}$Zr-bevacizumab uptake of MM patients. Currently, the total number of patients fulfilling the eligibility criteria of this study is 20-30 on a yearly basis.

4.1 Timetable

<table>
<thead>
<tr>
<th>Evaluation in-exclusion criteria</th>
<th>baseline</th>
<th>T=0</th>
<th>T=1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Informed consent</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>FDG-PET scan</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bone marrow biopsy</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$^{89}$Zr bevacizumab injection</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$^{89}$Zr bevacizumab imaging</td>
<td>X</td>
<td></td>
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</tbody>
</table>

T=1: 4 days after tracer injection

5. $^{89}$Zr-bevacizumab PET imaging

5.1.1 Radiolabelling of bevacizumab

Conjugation and radiolabelling of the monoclonal antibody is essentially performed as has been described by Verel et al.\(^{19}\), with only minor adjustments with regard to purification. In short: FTP-N-sucDf-Fe (kindly provided by the VU University Medical Center, Amsterdam, The Netherlands) was conjugated to the mAb at a 10:1 molar ratio. Fe(III) is subsequently removed by an excess of EDTA (Calbiochem, San Diego, California, USA) for 30 min at 35°C and the antibody is purified by ultrafiltration (Vivaspin-2 Centrifugal Concentrator, filter 30 kDa, Sartorius, Göttingen, Germany). This purified antibody is either stored at -80°C or immediately used for radiolabelling. The conjugated antibody is added to $^{89}$Zr-oxalate (100-1000 MBq/mg mAb, kindly provided by the VU University Medical Center, Amsterdam, The Netherlands), 0.9% NaCl (Braun Melsungen AG, Oss, The Netherlands), 2M Na$_2$CO$_3$ (OPG Farma, Utrecht, The Netherlands) and 0.5M HEPES buffer (Sigma-Aldrich, Zwijndrecht, The Netherlands). After 60 minutes, the radiolabeled antibody is purified by ultrafiltration Vivaspin-2 Centrifugal Concentrator, filter 30 kDa, Sartorius, Göttingen, Germany) and diluted in 0.9% NaCl/gentisic acid (5 mg/ml, Merck Schuchardt OHG, Hohenbrunn, Germany). The $^{89}$Zr isotope has a physical t½ of 78 hours. $^{89}$Zr-bevacizumab has radiochemical
purity >95% and excellent long term stability in human serum (> 1 week). Conjugation and radiolabeling is performed in the radiopharmacy facilities of the department of Nuclear Medicine and Molecular Imaging under GMP-conditions, under responsibility of a hospital pharmacist. More detailed information regarding $^{89}$Zr-bevacizumab is found in the investigational medicinal product dossier (IMPD).

5.1.2 PET imaging
The $^{89}$Zr-bevacizumab PET scan must be performed within three weeks after the FDG-PET scan. Patients will be injected intravenously with 37 MBq $^{89}$Zr-bevacizumab (protein dose 5 mg) at day 0. Subsequently, images will be made 4 days after the injection of $^{89}$Zr-bevacizumab. For imaging a Siemens PET/CT (Biograph mCT with 64 slice CT) camera will be used. Total PET imaging will be performed in 3D mode from head to feet, with total scanning time 30-40 minutes, depending on the length of the patient. A low dose CT will also be performed to serve as attenuation correction scan and anatomical co-registration. The Department of Nuclear Medicine and Molecular Imaging is certified according to the ISO-NEN-9001 and EFQM standard. Actual operation of the PET camera and scan acquisition will be performed by a nuclear medicine technologist. Final image analysis will be performed by a nuclear medicine specialist.

5.2 Data Analysis and statistics
Data will be assessed both visually and semi-quantitatively, using the SUVmax within a threshold isocontour of 50%. For SUV calculation the standard routine available in the software will be used in order to eliminate inter-observer variability. Lesions found on the FDG-PET or the $^{89}$Zr-bevacizumab scan can be focal in the bone or extramedullary due to increases amount of tracer uptake. The low-dose CT with the scans gives information about the localisation. FDG-PET gives information about high metabolic activity so when there is for example an infection higher uptake will be seen. By means of the low dose CT and clinical information differentiation will be made between MM lesions and for example infection. The number, localisation and SUV of focal lesions will be recorded of both scans and placed in a table. Bone marrow uptake will be described as negative or diffuse according to the degree of uptake. Since this is a feasibility study no power calculation can be made. When FDG-PET lesions are driven by a high metabolic activity due to angiogenesis we expect to see the same amount of lesions on the FDG-PET as on the VEGF PET imaging using $^{89}$Zr-bevacizumab scan. We also expect to see patients with lesions found on the $^{89}$Zr-bevacizumab PET scan to have high expression of VEGF and MVD in the immunohistochemistry on bone marrow material.

5.3 Radiation dose
The radiation dose of a single $^{89}$Zr-bevacizumab PET scan, with administered activity of 37 MBq, has been calculated by our clinical physicist (dr J.R. de Jong) at 18 mSv. This effective dose
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equivalent is comparable to the dose of a full body CT. The radiation dose of the low dose CT for attenuation correction is 1.5 mSv.

6. Immunohistochemical staining of the bone marrow

Bone marrow material obtained during the diagnostic procedure will be used. Bone marrow biopsy will be done from the crista iliaca, as described by international guidelines for diagnosis of multiple myeloma. Bone marrow biopsy will take place before $^{89}$Zr-bevacizumab PET scan is performed. The bone marrow biopsies will be fixed in phosphate buffered formaldehyde and decalcified. After standard processing the bone marrow sample will be stained for angiogenesis factor vascular endothelial growth factor (VEGF) and MVD. The immunohistochemistry will be done with commercial available antibodies. The intensity of VEGF staining will be analysed semi quantitatively: 0 = no staining, 1 = slight staining, 2 = moderate staining and 3 = maximal staining. The vessel count will be measured by FVIIIRA using light microscopy in areas of the slide containing highest numbers of blood vessels per selected area at a x 400 magnification.

7. Ethical considerations

The study will be conducted according to the principles of the Declaration of Helsinki 1975, Amended by the 59th WMA General Assembly, Seoul, October 2008, and in accordance with the principles of ‘Good Clinical Practice’ and the Medical Research involving Human Subjects acts (see appendix 5, the Declaration of Helsinki 1975, Amended by the 59th WMA General Assembly, Seoul, October 2008).

8. Patient information

Eligible patients will be fully informed about the study and asked to participate. The patient will receive a patient information sheet (see Appendix 1) and will have the opportunity to ask remaining questions. The patient will have 2 weeks time to consider the implications of the study before deciding to participate. Patients consent will be noted on an informed consent form
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compliant with the local and ethical regulations. If during the study the patient for any reason no longer wishes to participate, the subject can withdraw at any time.

Prior to the start of the study, the protocol has to be approved by the medical ethics review committee of the UMCG. Approval will be indicated in writing with reference to the final protocol number and date.

9. Patients records.

Eligible patients will be given a number under which their records will be stored. Only the principal investigator will have access to this storage file. The result of the FDG-PET scan will be visible in the clinical database to which the medical doctor will have access. The data of VEGF PET imaging using $^{89}$Zr-bevacizumab scan will only be visible for the investigators. To keep track of all the investigations for which radiation was used, patients' names will be mentioned on the scans. Data of the bone marrow immunohistochemically staining will only be visible to the investigators. Finally, the data will be combined and published.

10. SAE and SUSAR.

Suspected Unexpected Serious Adverse Reactions (SUSAR's) and Serious Adverse Events (S.A.E.'s) will be reported to the Medical Ethical Commission of the UMCG. SUSAR's and SAE will also be reported to toetsingonline (www.ccmo.nl).

Adverse event that are related to a relapse multiple myeloma are:
- infections
- pathologic fractures
- kidney dysfunction
- anaemia, due to disease activity or treatment
- hypercalcaemia.
- spinal cord injury

Adverse event that are related to $^{89}$Zr-bevacizumab PET imaging:
- Hypersensitivity reactions to bevacizumab within a short term after administration (within 3 hours).
- Hypertension can occur after administration of bevacizumab. However, the risk is
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considered minimal due to the low tracer dose used in this study (see IMPD).
So far no side effects have been seen using $^{89}$Zr-bevacizumab$^{17}$.

11. Responsibilities

The responsible physician for the correct carrying out of this protocol is drs E.G.M. de Waal, who is the principle investigator. Dr.R.H.J.A. Slart, MD, PhD, specialist in nuclear medicine, is responsible for the PET-aspects of the protocol. Prof. Dr. Ph.M. Kluin is responsible for the immunohistochemical staining at the department of pathology. Independent information regarding the protocol can be obtained by Prof.dr. J.A. Gietema (phone 050 – 3612821).

12. Trial insurance

In accordance with article 7, subsection 6 “Wet medisch wetenschappelijk onderzoek met mensen” (Staatsblad 1998, 161), the investigator of this study, the University Medical Center Groningen, took out an liability insurance policy in order to cover any loss by death or injury from participating patients caused by this study (see Appendix 1). The insurance policy is taken out by insurance company Onderlinge Waarborgmaatschappij Centramed, Postbus 191, 2270 AD Voorburg. The assurer and the insurance policy comply with the requirements laid down by order of “Besluit verplichte verzekering bij medisch-wetenschappelijk onderzoek met mensen” (Staatsblad 2003, 266).
13. References


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