SHORT COMMUNICATION

O-9. DETECTION AND CHARACTERIZATION OF CIRCULATING ENDOTHELIAL CELLS IN MULTIPLE MYELOMA VERSUS MONOCLONAL GAMMAPATHY OF INDETERMINATED SIGNIFIANCE

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Introduction
Cancer, a proliferative disease hallmarked by abnormal cell growth and spread, is largely dependent on abnormal angiogenesis, whereby new vessel formation ensures an adequate supply of nutrients, oxygen and growth factors to the growing tumor. Novel ways to assess vascular function in cancer include measuring levels of circulating endothelial cells (CEC). Rare in healthy individuals, increased CEC in peripheral blood reflects vascular damage and dysfunction. A related circulating cell population are endothelial progenitor cells (EPC), which originate from the bone marrow, rather than from vessel walls. Seen in small numbers in healthy individuals, their number tends to increase following vascular injury. Recent evidence has suggested the involvement of EPC in tumor vasculogenesis (1).

Multiple myeloma (MM) is characterized by the infiltration of malignant plasma cells in the bone marrow. It can occur de novo or evolve from benign monoclonal gammapathy of unknown significance (MGUS). Approximately 1% of individuals with MGUS evolve to MM per year. It accounts for approximately 13% of hematologic cancers. This disease remains incurable, despite major treatment improvements. One of the objectives of the present study was to identify whether the measurement of CECs and EPCs has potential as a surrogate marker for monitoring the evolution of the disease.

Materials and Methods
Study participants, collection of blood and bone marrow :
Blood samples and bone marrow samples are collected in compliance with French legislation at the time of diagnosis. An informed consent was obtained from all participants. To date, nineteen patients have been included. The median age for the MGUS patients was 69 years and 65 years for the myeloma patients.

Enumeration of CECs and EPCs by Flow Cytometry :
Circulating mononuclear cells (MNCs) were obtained by density-gradient centrifugation within 2 hours of blood collection. Freshly isolated peripheral blood mononuclear cells (PBMNCs) were analyzed by 6-color flow cytometry using combinations of monoclonal antibodies (mAbs) obtained from Beckman Coulter: anti-CD38 (FITC), anti-CD 31 (PE), anti-CD45 (ECD), anti-CD146 (PC5), anti-CD34 (PC7); bone marrow mononuclear cells (BMMNCs) were analyzed by 8-color flow cytometry using the following monoclonal antibody-combinations: anti-CD31 (FITC), anti-CD146 (PE), anti-CD34 (ECD), anti-CD38 (PC5.5), anti-CD309 (PC7), anti-CD45 (APC). DAPI was added to all samples in order to exclude apoptotic cells. Isotypic controls were done to measure background noise and to adjust the gates precisely. Cells were analyzed on a Cyan TM analyzer (Beckman Coulter). To ensure detection of low levels of CECs and EPCs, at least 1 x 106 events from each sample was acquired. Data were analysed with

the use of Kaluza Software® (Beckman Coulter). To determine the number of CECs and EPCs, we developed a gating strategy adapted from the standardised so-called ISHAGE (International Society of Hematotherapy and Graft Engineering) sequential strategy (2). Data are expressed as number of events and a relative percentage was calculated on PBMNC for the blood sample and CD34+ cells for the bone marrow.

<u>Statistical analysis :</u>
Statistical analysis was perfomed by Graph-Pad Software® (Graph-Pad Software Inc., San Diego, USA). Groups were compared with the use of a nonparametric Mann-Whitney test. A P value ≤0.05 was considered as statistically significant.

Results
At this state of the study, statistical analysis of the bone marrow samples showed that CEC and CEP numbers are not significantly increased in the group of patients with myeloma when compared to the group of patients with MGUS (Figure 1 and 2). Interestingly, the number of EPCs exceeded the number of CECs in the MM group. Blood sample analysis is still going on.
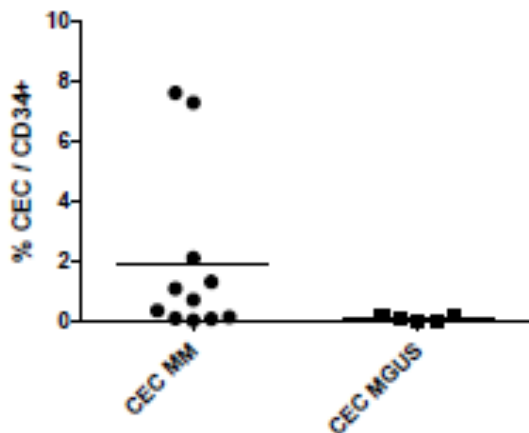

Fig.1: Proportion of CECs in the bone marrow of patients with MM and MGUS. The y-axis represents the number of CECs per CD34+ BMMNC. CEC MM CEC MGUS 02468 10 % CEC / CD34+
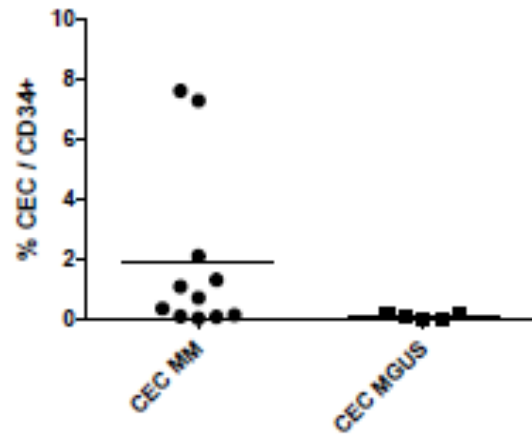CEP MM CEP MGUS 05 10 15 20 25 % CEP / CD34+

Fig. 2: Proportion of EPCs in the bone marrow of patients with MM and MGUS. The y-axis represents the number of EPCs per CD34+ BMMNC.

Discussion
In this study we identified CECs and EPCs in patients with MM and MGUS. Mature CECs have no proliferative potential and have been found to be a marker for cellular damage. In contrast to CECs, EPCs are bone-marrow derived cells with a high proliferation potential and have been found to be a potential marker for neovascularisation. Mancuso et al.(4) found a fivefold increase of CECs in PBMNCs in breast cancer and lymphoma patients compared to healthy donors. Zhang et al. (5) showed an increase of CECs in myeloma versus healthy controls. A recent study (6) found elevated levels of EPCs in patients with breast cancer before therapy with a rapid decline after tumour excision (6). In our study we could not demonstrate a statistically significant difference in CEC and CEP numbers between MM and benign MGUS. However, our actual results only evaluated the number of CECs and EPCs in the bone marrow. It has to be pointed out that MM patients with a low level of CECs and EPCs are patients who just developed the disease or presenting an indolent myeloma, whereas patients presenting high levels of CECs/EPCs are at an advanced stage of the disease.
Furthermore, from the 19 patients included, only 16 could be analyzed. With only 16 patients available, a valid comparison between the 2 groups is not possible for the moment.

Conclusion
Preliminary results obtained in the bone marrow samples show no significant difference in CEC and EPC numbers. Considering the low number of inclusions, the study has to go on,

to draw a valid conclusion.

References

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