



**HAL**  
open science

## **Input of DNA microarrays to identify novel mechanisms in multiple myeloma biology and therapeutic applications.**

Karène Mahtouk, Dirk Hose, John de Vos, Jérôme Moreaux, Michel Jourdan, Jean François Rossi, Thierry Rème, Harmut Goldschmidt, Bernard Klein

### **► To cite this version:**

Karène Mahtouk, Dirk Hose, John de Vos, Jérôme Moreaux, Michel Jourdan, et al.. Input of DNA microarrays to identify novel mechanisms in multiple myeloma biology and therapeutic applications.: DNA microarrays in multiple myeloma. *Clinical Cancer Research*, 2007, 13 (24), pp.7289-95. 10.1158/1078-0432.CCR-07-1758 . inserm-00270566

**HAL Id: inserm-00270566**

**<https://www.hal.inserm.fr/inserm-00270566>**

Submitted on 5 Jul 2010

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

**Input of DNA microarrays to identify novel mechanisms in multiple myeloma  
biology and therapeutic applications.**

Karène Mahtouk<sup>1,2</sup>, Dirk Hose<sup>3</sup>, John De Vos<sup>1,2,4</sup>, Jérôme Moreaux<sup>1,2</sup>, Michel Jourdan<sup>2</sup>,  
Jean François Rossi<sup>1,4,5</sup>, Thierry Rème<sup>1,2</sup>, Harmut Goldschmidt<sup>3</sup>, Bernard Klein<sup>1,2,4</sup>

1 CHU Montpellier, Institut de Recherche en Biothérapies, hôpital Saint-Eloi, F-34285 France ; 2 INSERM, U847, Montpellier, F-34197 France ; 3 Medizinische Klinik und Poliklinik V, Universitätsklinikum Heidelberg, INF410, Germany; 4 Université Montpellier1, UFR de médecine, F-34967 France ; 5 CHU Montpellier, Service d'Hématologie et d'Oncologie Médicale, F-34285 France

Running title: DNA microarrays in multiple myeloma

Key words: microarrays, myeloma, prognostic factors, microenvironment, therapeutic targets

Correspondance: Bernard Klein. Institut de Recherche en Biothérapies, CHU Montpellier, Hôpital Saint-Eloi, Av Augustin Fliche, Montpellier F-34285 France. Tel: +33 4 67 33 78 88. Fax: + 33 4 67 33 70 05. e-mail: klein@montp.inserm.fr

Acknowledgments. This work was supported by the Ligue nationale contre le cancer (équipe labellisée), Paris, France. It is a part of a national program called " carte d'identité des tumeurs" founded by the Ligue nationale contre le cancer (<http://www.ligue-cancer.net>). Karène Mahtouk has been financed by a grant of the association Guillaume Espoir (<http://www.gespoir.com>).

## **Abstract**

Multiple myeloma (MM) is a B cell neoplasia characterized by the proliferation of a clone of malignant plasma cells in the bone marrow. We review here the input of gene expression profiling (GEP) of myeloma cells and of their tumor microenvironment to develop new tumor classifiers, to better understand the biology of myeloma cells, to identify some mechanisms of drug sensitivity and resistance, to identify new myeloma growth factors, and to depict the complex interactions between tumor cells and their microenvironment. We discuss how these findings may improve the clinical outcome of this still incurable disease.

## Introduction

Multiple myeloma (MM) is a B cell neoplasia that affects 15 000 new patients per year in Europe and 15 000 in the United States. It is characterized by the accumulation of a clone of malignant plasma cells in the bone marrow. Myeloma cells are strongly dependant of their microenvironnement which produces cell communication signals, in particular myeloma growth factors. One key finding to understand the biology of myeloma has been the identification of interleukin-6 (IL-6) as a major survival and proliferation factor for myeloma cells in 1988 (1, 2). However, though IL-6 is essential, it is not sufficient and additional factors, produced by the microenvironment or the tumor cells themselves, are required together with IL-6 to promote tumor growth (3). We and others have identified such factors, as for example insulin-like growth factor-1 (IGF-1) (4, 5), interferon-alpha (6), interleukin-10 (7), hepatocyte growth factor (8), Wnt family (9), and more recently members of the epidermal growth factor family (10) and BAFF (B-cell activating factor)/APRIL (A proliferation-inducing ligand) (11). MM is still an incurable disease with an average 5-year survival after high dose chemotherapy and autologous stem cells transplantation (12). A recent study has pointed out that allografting with HLA-identical sibling stem cells and immune cells of patients with newly-diagnosed MM may considerably increase patients' outcome (13). New drugs, such as Thalidomide or Bortezomib, have also improved MM patient treatment (14). By understanding the biology of MM and defining important potential therapeutic targets, effective new treatments can be developed to improve patient outcome in MM. We review here the major inputs of DNA microarrays in the understanding of MM disease and their clinical applications.

## I – New molecular classifications of MM

### I-1 Genetic abnormalities and prognosis in MM

In MM, genetic abnormalities led to overexpression of (putative) oncogenes such as cyclin D1 - t(11;14) and trisomy 11-, cyclin D3 - t(6;14)-, MMSET/FGFR3 - t(4;14)- or c-MAF - t(14;16)-. In addition, monoallelic deletions of chromosome 13q14 (del(13)) involving the *retinoblastoma* gene, or chromosome 17p13 (del(17)) involving *P53* are found in 50% and 10-30% of patients with MM, respectively (15). The t(4;14) translocation confers a bad prognosis, even after high dose chemotherapy and autologous stem cell transplantation (16). Del(13) or del(17) have also been reported to be associated with poor prognosis (16, 17), however, the prognostic value of del(13) would be related to the t(4;14) and del(17) that are frequently associated with del(13) (18). Chromosome 1q21 amplification is found in 43% and 72% of patients with newly diagnosed MM or relapsed MM, respectively, and it is associated with a poor prognosis and a shortened post relapse survival (19). Thus, cytogenetics is a powerful technique to identify some major chromosome aberrations and their clinical implications in myeloma. In the following, we will review the input of DNA microarrays to further identify myeloma disease heterogeneity and the mechanisms targeted by these gene abnormalities

### I-2 Gene expression profile (GEP)-based classifications of newly-diagnosed patients with MM.

Microarrays profiling has helped to establish relevant MM subclassifications. For example, GEP analysis of primary myeloma cells from 92 patients was analysed according to their Ig types and light chain subtypes, revealing that several clusters of genes involved in various biologic functions could significantly discriminate those different types of myeloma

(20). Moreover, we and others have confirmed that patients whose myeloma cells have t(4;14) and t(11;14) translocations can be pointed out by DNA microarrays through an overexpression of FGFR3, cyclin D1 or MAF genes, respectively (21). Based on the hypothesis that the major genetic abnormalities can be picked up by DNA microarrays, Bergsagel et al. proposed a GEP-based molecular classification of MM taking into account the unifying expression of cyclins (22). They pointed out a high expression of one of the 3 cyclin D genes as a general feature of MM and proposed a classification of patients within 8 TC (translocation/cyclin D) groups (22). More recently, using Affymetrix GEP of 414 newly-diagnosed patients, Zhan and al. proposed a classification of MM in 7 groups which are driven by the presence of translocations or hyperdiploidy (23). These groups are characterized by: 1) An overexpression of genes involved in cell cycle and proliferation (PR group for proliferation). 2) A lower expression of genes involved in bone disease, like the wnt signalling antagonists Dickkopf 1 (DKK1) and Frizzled B, and a lower number of magnetic resonance imaging (MRI)-defined focal (LB group for "Low Bone disease"). 3) An overexpression of FGFR3 and MMSET genes (MS group for MMSET). 4) A hyperdiploid signature (HY group). 5-6) An overexpression of cyclin D1 and cyclin D3 genes (CD-1 and CD-2 groups). 7) An overexpression of MAF and MAFB genes (MF group). With a 36-month median follow-up on the training group, HY, CD-1, CD-2 and LB groups had a higher event free survival (EFS) and overall survival (OAS) compared to PR, MS and MF groups. On multivariate analysis, this 7 group GEP classification is a significant and independent predictor for survival (23). Using the same series of Affymetrix GEP data, the Shaughnessy's group identified 70 genes whose up-regulation or down-regulation were linked with a bad prognosis in a subset of 13% of newly-diagnosed patients (24). 17 out of the 70 genes are sufficient to predict for this bad prognosis subset. A high risk score based on the  $\text{Log}_2$  of average of up-regulated gene minus the  $\text{Log}_2$  of

average of down-regulated genes has a strong independent prognostic value, in particular eliminating the prognostic value of ISS staging. 30% out of the 70 genes are located on chromosome 1, mainly 1q. One major up-regulated gene is *CKSB1*. *CKSB1* is a member of the Cks/Suc1 family of small proteins that bind the catalytic subunit of cyclin-dependent protein kinases and modulate their function. In particular, *CKSB1* promote the ubiquitin-mediated P27<sub>Kip1</sub> degradation, resulting in cell cycle progression. Shaughnessy's group has shown that knockout of *CKSB1* in myeloma cells with a shRNA lentivirus result in P27 accumulation and apoptosis emphasizing the major role of *CKSB1* upregulation in MM disease aggressivity (25).

Combining analysis of expression changes by GEP and cytogenetic aberrations by iFISH in highly purified myeloma cells, we could classify 128 newly-diagnosed patients in four groups noted EC for "Expression-Cytogenetic" (26). EC1 groups are characterized by cyclin D1 overexpression and either additional copy of 11q13 (EC1-1) or t(11;14) translocations (EC1-2). EC2 groups are defined by cyclin D2 overexpression, either without 11q13+, t(11;14) and t(4;14) (EC2-1), or with t(4;14) and *FGFR3* upregulation (EC2-2). The EC2.1 group comprises several rare translocations that indirectly increase cyclin D2 expression, like the t(14;16), indicated by a spiked *MAF*-expression. Patients of the EC1.1 group are hyperdiploid while those in EC1.2 and EC2.2 groups are mostly non-hyperdiploid. Highly significant differences in EFS were found between the 4 groups of patients treated with high dose therapy and peripheral blood stem cell transplantation. In agreement with the published data for the t(4;14) translocation, patients of the EC2.2 group had the shortest median EFS. Patients of the EC1.2 group had the longest EFS, those of EC1.1 and EC2.1 groups represented an intermediate risk.

**Frequent NF- $\kappa$ B pathway activation through recurrent genetic abnormalities.**

Using GEP and aCGH arrays, two groups recently identified a promiscuous array of genetic alterations yielding to activation of NF- $\kappa$ B pathway in at approximately 20% of MM patients and 41% of HMCLs (27, 28) . They included overexpression and/or gain-of-functions of *NIK*, *NFKB2*, *NFKB1*, *CD40*, *LTBR* and *TACI*, all these genes coding for proteins activating the NF- $\kappa$ B pathway. They also included inactivating abnormalities of *TRAF3*, *cIAP1*, *cIAP2*, *CYLD* and *TRAF2* which are negative regulators of NF- $\kappa$ B. These two studies disagree whether the canonical or non canonical NF- $\kappa$ B pathways are mainly deregulated. Of note, Annunziata et al. clearly demonstrated that activation of NIK affected both canonical and non canonical pathways (28), emphasizing the interest of IKK2 inhibitors in patients (29) In addition, Keats et al reported that DEX was poorly efficient compared to Bortezomib in patients with NF- $\kappa$ B alteration whereas they were both efficient in other patients (27). In normal plasma cells, NK- $\kappa$ B pathway is highly activated, likely in part due to the activation of TACI and BCMA by BAFF/APRIL (30)that are produced by bone marrow cells, in particular osteoclasts (31).

## **II - Understanding of the biology of myeloma cells.**

II - 1 Comparison of the GEP of myeloma cells, of plasma cells from healthy individuals and of plasma cells from patients with MGUS.

Several analysis comparing the GEP of malignant plasma cells to that of their normal counterpart have provided new insights to understand the molecular mechanisms of the mutistep pathogenesis of MM. Comparing the GEP of purified myeloma cells and myeloma cell lines to that of B cells (32) or *in vitro*-generated plasmablastic cells (PPC) (33), we identified genes that were statistically significantly overexpressed in myeloma, including oncogenes and genes coding for tumor antigens or intercellular communication

signals (growth factors, chemokines, pro- and anti-apoptotic proteins) (34). A quarter of those genes were also identified by Zhan et al (35), like MYC and cyclin D1, using purified bone marrow plasma cells from healthy individuals as a normal counterpart of myeloma cells. The use of either mature plasma cells or immature plasmablasts may explain the partial overlap of the gene lists described in these two studies providing complementary information. In 2004, Munshi and al. analyzed the GEP of normal and malignant plasma cells purified from the bone marrow of genetically identical twins; *i.e* from an identical genetic background. Among genes overexpressed in myeloma cells vs. plasma cells of the healthy twin, they found oncogenes/transcriptional factors (FGFR3, Jun-D, v-fos) and genes involved in cell survival pathway (MCL-1) (36). Recently, using the U133 Affymetrix microarrays to analyse a larger cohort of patients, we found that *CD200* - a highly conserved type I transmembrane glycoprotein expressed by thymocytes, B and T cells, dendritic cells, endothelial cells and neurons (37) - was significantly overexpressed in myeloma cells compared with normal plasma cells, plasmablastic cells and B cells, and was a bad prognostic factor independent of ISS stage or  $\beta$ 2-microglobulin, for newly diagnosed patients (38). *CD200* is a potent immunosuppressive molecule, downregulating activation of T cells that express *CD200* receptor (39). Antibodies to *CD200* can block the immunosuppression induced by *CD200*<sup>+</sup> Burkitt cell lines and promote tumor rejection in SCID mice receiving the tumor cell lines and allogenic human T cells (40). This emphasizes that *CD200* may a potential therapeutic target in MM.

Concerning MGUS patients, it was first reported that whereas MGUS and myeloma could be distinguished from normal plasma cells, those two conditions were very difficult to differentiate from each other (41, 42). Using whole genome microarrays on larger cohorts of patients, Zhan et al. recently identified genes differentially expressed between plasma cells from normal donors, patients with MGUS and patients with MM (43). Unsupervised

hierarchical clustering of the genes differentially expressed between MGUS and MM, identified a subset of MGUS-like MM which had favourable clinical features and a longer survival. This MGUS-like signature was found in the majority of the patients surviving more than 10 years after initiation of total therapy (43).

## II - 2 Identification of myeloma growth factors.

Based on GEP data, we have identified new families of proteins which play a major role in the biology of the MM, and which are therefore promising therapeutic targets in this pathology.

EGF (Epidermal Growth Factor) family members and ErbB receptors.

The EGF/EGF-receptor family comprises 4 receptors (ErbB1-4) and 10 ligands (see Figure 1A) (44). Expression and/or activation of ErbB receptors are altered in many epithelial tumors but this family of protein had never been involved in hematological malignancies. Using Affymetrix microarrays, we have shown that five genes (*AREG*, *TGF- $\alpha$* , *NRG1*, *NRG2* and *NRG3*) are expressed by primary myeloma cells. Among them, two – *NRG2* and *NRG3* – are “myeloma genes”, *i.e* they are significantly overexpressed in myeloma cells compared to their normal counterpart, whereas other three genes – *AREG*, *TGF- $\alpha$*  and *NRG1* – are “plasma cell genes”, *i.e* they are expressed both in normal and malignant plasma cells but not in B cells and plasmablastic cells (10). We further demonstrated that ErbB receptor expression is induced during plasma cell differentiation (ErbB1-2) and oncogenesis (ErbB3-4) (Figure 1B) (45). The importance of ErbB receptor activation for myeloma cell survival has been demonstrated by the finding that a pan-ErbB kinase inhibitor (PD169540, Pfizer), either used alone or combined with other drugs, induced a dramatic apoptosis of primary myeloma cells cultured *in vitro* for 5 days with

their environment, in 10/14 patients (Figure 1C). The ErbB inhibitor did not affect the viability of other bone marrow cells present in the culture (Figure 1C) (46). More recently, we have demonstrated that syndecan-1, the main heparan sulphate proteoglycan present on plasma cells, plays a major role in the EGF/ErbB pathway, concentrating high levels of heparin-binding-EGF-ligands at the cell membrane which likely facilitates ErbB-activation (10). Altogether, these data indicate that ErbBs are excellent candidates for targeted therapy and that inhibition of the EGF-family pathway may be useful in the treatment of MM.

BAFF, APRIL, and their receptors.

BAFF is a Tumor Necrosis Factor family member involved in the survival of normal and malignant B cells. APRIL is highly expressed in several tumor tissues where it can stimulate tumor cell growth (47). Using Affymetrix microarrays, we found that TACI and BCMA, each gene coding for a receptor of BAFF and APRIL, were overexpressed in malignant plasma cells compared with their normal counterparts. We and others have demonstrated that BAFF and APRIL can support the growth of myeloma cell lines and conversely, an inhibitor of BAFF and APRIL can induce apoptosis of primary myeloma cells (11, 48). BAFF and APRIL serum levels are increased in patients with MM compared to age related healthy individuals (11). Using Affymetrix microarrays, real time RT-PCR and ELISA, we found that BAFF and APRIL are mainly produced by the tumor environment, in particular by monocytes and myeloid cells (31). Furthermore, GEP data of purified myeloma cells from 65 newly-diagnosed patients were analyzed by supervised clustering of groups with higher ( $TACI^{hi}$ ) vs. lower ( $TACI^{lo}$ ) *TACI* expression levels.  $TACI^{hi}$  group displayed a mature plasma cell gene signature, indicating dependence on the bone marrow environment. In contrast, the  $TACI^{lo}$  group had a plasmablastic gene signature,

suggesting a weaker dependence on the microenvironment (31). In 2005, a phase I-II trial using a receptor TACI coupled to Fc fragments of Ig (TACI-Ig, which blocks the effect of BAFF and APRIL) has been initiated at the university hospital of Montpellier and Heidelberg. This is the first clinical trial using this molecule (30). One can anticipate that GEP may help in identifying groups of patients who might benefit most from treatment with BAFF/APRIL inhibitors.

### II - 3 Understanding signalling pathways involved in myeloma cell growth.

Hallmarks of MM are the presence of genetic abnormalities, the requirement of the bone marrow environment for tumor cell survival/proliferation and the major role of IGF-1 and IL-6 as myeloma growth factors. In order to understand how different stimuli can affect myeloma cell growth, Cronquist and co-workers compared the GEP of the ANBL-6 human myeloma cell line, either treated with IL-6, or cocultured with bone marrow stromal cells (BMSC), or carrying N-Ras activating mutation, *i.e* that is independent on exogenous IL-6 (49). They demonstrated that the different activating signals share some genetic pathways, but they also affect myeloma cells distinctively through the regulation of specific gene signatures (49). It suggested that the GEP of myeloma cells is not only influenced by intrinsic oncogenic events but also by exogenous signals from the microenvironment. In another study, the anti-apoptotic activity of IL-6 was evaluated on the INA-6 myeloma cell line. The authors demonstrated that IL-6 dependant survival of INA-6 cells relies on an anti-apoptotic pathway in which STAT3 plays a central role (50). Chatterjee et al. have shown that in the presence of BMSCs, the concomitant inhibition of both IL-6/STAT3 and Ras/MEK/ERK pathways is required to induce apoptosis of IL-6-dependant myeloma cells (51). In order to understand how those two pathways mediate their effect, the same team compared the GEP of the INA-6 HMCL cocultured with BMSCs, and treated either with

Sant7 or with a MEK-inhibitor or with a combination of both inhibitors (52). They identified genes specifically down-regulated after the combined pathway blockade, among which heat shock proteins (HSP) were highly represented. The authors focused on the two HSP90 genes, HSP90 $\alpha$  and HSP90 $\beta$ , which were further demonstrated to be critical for myeloma cell survival within the context of their microenvironment (52).

### **III – Understanding mechanisms of drug-induced anti-tumor activity and drug resistance**

These studies were mainly conducted by K. Anderson's group comparing the GEP of myeloma cells treated or not with various inhibitors. They provided important insights to identify genes modulated in response to a given drug or genes that confer tumor chemoresistance. Chauhan et al. determined the GEP of myeloma cells treated with dexamethasone (dex) using oligonucleotide arrays (53). They reported that transient activation of genes involved in DNA repair is followed by downregulation of genes promoting growth and survival. In the same study, comparing the GEP of dex-sensitive (MM1S) and dex-resistant (MM1R) cells, they pinpointed genes differentially expressed between those cell lines which may confer dex-resistance to MM1R. GEP analysis of primary myeloma cells validated those findings and confirmed their relevance *in vivo* (53). In a second study, the authors compared the GEP of MM1R and cells treated with 2-methoxyestradiol (2ME2), an estrogenic derivative with anti-myeloma activity. They demonstrated that 2ME2 downregulates genes overexpressed in MM1R, suggesting mechanisms whereby 2ME2 overcomes dex-resistance (54). In further studies, oligonucleotide microarrays were used to characterize the transcriptional profile of myeloma cells treated with anti-tumor agents such as SAHA, an histone deacetylase

inhibitor (55), or Velcade, a proteasome inhibitor (56). One of the interests of studying the molecular profile of tumor cells treated with different drugs is to establish a framework for optimal design of combination therapies with conventional or targeted antitumor agents. Furthermore, a recent publication has provided the first description of the prospective development of a genomic classifier that predicts clinical response between myeloma patients treated with bortezomib or dexamethasone (57). Response and survival classifiers were developed and shown to be significantly associated with outcome via testing on independent data. Among gene sets significantly associated with response to bortezomib, the authors pointed out pathways that had already been described to be involved in Bortezomib activity *in vitro* such as NF- $\kappa$ B activity or cell adhesion (57).

#### **IV – Using microarrays to analyse the role of the microenvironment in MM**

IV - 1 Comparison of GEP of normal and MM bone marrow mesenchymal stem cells (BMMSCs).

In MM, it is now admitted that the bone marrow microenvironment plays a major role suggesting that the tumor may select a favourable microenvironment or that microenvironment cells could be abnormal. This hypothesis was recently demonstrated by our group comparing the GEP of BMMSCs from normal donors to that of BMMSCs from patients with MM (58). An unsupervised analysis delineated 2 distinct homogeneous clusters of genes: a myeloma BMMSC cluster and a normal one. Interestingly, genes identified by a supervised analysis to be differentially expressed between normal and myeloma BMMSC were mainly classified in the “tumor microenvironment” category, according to Gene ontology annotations. Genes overexpressed in myeloma BMMSCs included known myeloma growth factors (IL-6, amphiregulin, IL-1 $\beta$ ), angiogenic factors,

and proteins involved in bone disease (DKK1) (58). In agreement with GEP data, normal and myeloma BMMSCs had functional differences as well. Myeloma BMMSCs showed a reduced matrix mineralization capability, and they could support the growth of the stroma-dependant Molp-6 cell line better than normal BMMSCs. Those data suggest that BMMSCs likely create a very efficient niche to support the survival and proliferation of myeloma cells.

#### IV - 2 GEP analysis of bone marrow subpopulations of myeloma patients.

In order to analyse comprehensively the various intercellular communication pathways involved in MM, we analyzed for the first time the GEP of whole bone marrow cells of 39 patients (including the tumor cells and environment cells) and that of myeloma cells purified from the bone marrow of those 39 patients. This makes it possible to determine the relative contribution of the environment and the tumour itself for the expression of a given gene. Using this approach, we could identify various genes that are mainly expressed by cells of the environment, as illustrated for BAFF in Figure 2 (3<sup>rd</sup> panel). Another interesting example is provided by the gene encoding heparanase (HPSE), an enzyme that cleaves heparan sulphate chains and controls syndecan-1 gene expression and shedding in myeloma cells (59, 60). In MM, HPSE stimulates angiogenesis and promotes metastasis of myeloma cells to bone in a murine model (61). Using Affymetrix microarrays, we found a high *HPSE* expression in the whole bone marrow samples, which was 7.6-fold higher than that found in the corresponding myeloma cells. Combining GEP and clinical data, we demonstrated that *HPSE* expression in the whole bone marrow of patients with MM is an indicator of poor prognosis (59). Classifying patients according to *HPSE* expression in purified myeloma cells did not result in any significant differences in survival (59). This is of major interest as it is the first study showing that a gene's

expression mainly in the bone marrow environment, *i.e.* *HSPE*, has prognostic value in MM.

In order to dissect the role of each component of the bone marrow of patients with MM, we simultaneously purified four bone marrow subpopulations (polymorphonuclear cells, myeloma cells, T cells and monocytes) we analysed their GEP. Osteoclasts and BMMSC that cannot be harvested *in vivo* were generated *in vitro* from MM patients' cells. This approach makes it possible to identify at a glance which cell component express a gene of interest. Four representative genes are shown in Figure 2. We observed a high IL-6 expression in BMMSCs compared to other cell populations indicating that those cells are the main source of IL-6, in agreement with previous data (1). Neuregulin 3, an EGF-family member, was exclusively expressed by myeloma cells. As it was not expressed by subpopulations of the environment, the neuregulin 3 expression found in some whole bone marrow samples is the reflexion of its expression in myeloma cells. In contrast, BAFF was mainly expressed by cells from the environment, in particular by CD14+ and CD15+ cells. Osteoclasts are huge APRIL producer emphasizing the importance of osteoclasts in promoting myeloma stem cell growth (62).

IV – 3 GEP and prediction of bone disease.

Tian et al. compared the GEP of 137 myeloma patients with bone lesions to that of 36 patients in whom focal bone lesions could not be detected by magnetic resonance imaging. In this study, only 4 genes were shown to be significantly overexpressed by MMC from patients with focal bone lesions (63). One of these genes was DKK1, a soluble inhibitor of the Wnt pathway. Consistently, recombinant DKK1 or BM serum containing high levels of DKK1 inhibited osteoblastic differentiation from BMMSCs *in vitro* (63). Hence, DKK1 produced by MMC would shift the balance between bone formation by

osteoblasts and bone destruction by osteoclasts in favour of osteoclasts, thereby enhancing bone resorption.

## **V- Conclusion**

Measurement of the expression of thousands of genes in hundreds of patient samples using microarrays has revealed novel molecularly defined subclasses of tumor, some of them predicting clinical behaviour. Furthermore, DNA microarrays proved to be very useful to improve our understanding of the myeloma pathogenesis, first, through a comparative analysis of tumor cells and their normal counterpart, and secondly, through a comprehensive analysis of the complex interactions between tumor cells and the BM microenvironment. The challenge will be now to translate this fundamental knowledge into new prognostic, diagnostic and therapeutic tools that will improve treatment and outcome of patients with MM.

## References.

1. Klein B, Zhang XG, Jourdan M, et al. Paracrine rather than autocrine regulation of myeloma-cell growth and differentiation by interleukin-6. *Blood* 1989;73:517-26.
2. Kawano M, Hirano T, Matsuda T, et al. Autocrine generation and essential requirement of BSF-2/IL-6 for human multiple myeloma. 1988;332:83-5.
3. Klein B, Tarte K, Jourdan M, et al. Survival and proliferation factors of normal and malignant plasma cells. *Int J Hematol* 2003;78:106-13.
4. Ferlin M, Noraz N, Hertogh C, et al. Insulin-like growth factor induces the survival and proliferation of myeloma cells through an IL-6-independent transduction pathway. *British Journal of Haematology* 2000;111:626-34.
5. Georgii-Hemming P, Wiklund HJ, Ljunggren O, and Nilsson K Insulin-like growth factor I is a growth and survival factor in human multiple myeloma cell lines. *Blood* 1996;88:2250-8.
6. Ferlin-Bezombes M, Jourdan M, Liautard J, et al. IFN-alpha is a survival factor for human myeloma cells and reduces dexamethasone-induced apoptosis. *J.Immunol.* 1998;161:2692-9.
7. Lu ZY, Zhang XG, Rodriguez C, et al. Interleukin-10 is a proliferation factor but not a differentiation factor for human myeloma cells. *Blood* 1995;85:2521-7.
8. Derksen PW, Keehnen RM, Evers LM, et al. Cell surface proteoglycan syndecan-1 mediates hepatocyte growth factor binding and promotes Met signaling in multiple myeloma. *Blood* 2002;99:1405-10.
9. Derksen PW, Tjin E, Meijer HP, et al. Illegitimate WNT signaling promotes proliferation of multiple myeloma cells. *Proc Natl Acad Sci U S A* 2004;101:6122-7.
10. Mahtouk K, Cremer FW, Reme T, et al. Heparan sulphate proteoglycans are essential for the myeloma cell growth activity of EGF-family ligands in multiple myeloma. *Oncogene* 2006;25:7180-91.
11. Moreaux J, Legouffe E, Jourdan E, et al. BAFF and APRIL protect myeloma cells from apoptosis induced by interleukin 6 deprivation and dexamethasone. *Blood* 2004;103:3148-57.
12. Attal M, Harousseau JL, Facon T, et al. Single versus double autologous stem-cell transplantation for multiple myeloma. *N Engl J Med* 2003;349:2495-502.
13. Bruno B, Rotta M, Patriarca F, et al. A comparison of allografting with autografting for newly diagnosed myeloma. *N Engl J Med* 2007;356:1110-20.
14. Piazza FA, Gurrieri C, Trentin L, and Semenzato G Towards a new age in the treatment of multiple myeloma. *Ann Hematol* 2007;86:159-72.
15. Higgins MJ and Fonseca R Genetics of multiple myeloma. *Best Pract Res Clin Haematol* 2005;18:525-36.
16. Gertz MA, Lacy MQ, Dispenzieri A, et al. Clinical implications of t(11;14)(q13;q32), t(4;14)(p16.3;q32), and -17p13 in myeloma patients treated with high-dose therapy. *Blood* 2005;106:2837-40.
17. Kroger N, Schilling G, Einsele H, et al. Deletion of chromosome band 13q14 as detected by fluorescence in situ hybridization is a prognostic factor in patients with multiple myeloma who are receiving allogeneic dose-reduced stem cell transplantation. *Blood* 2004;103:4056-61.
18. Avet-Loiseau H, Attal M, Moreau P, et al. Genetic abnormalities and survival in multiple myeloma: the experience of the Intergroupe Francophone du Myelome. *Blood* 2007.
19. Hanamura I, Stewart JP, Huang Y, et al. Frequent gain of chromosome band 1q21 in plasma-cell dyscrasias detected by fluorescence in situ hybridization: incidence increases from MGUS to relapsed myeloma and is related to prognosis and disease progression following tandem stem-cell transplantation. *Blood* 2006;108:1724-32.
20. Magrangeas F, Nasser V, Avet-Loiseau H, et al. Gene expression profiling of multiple myeloma reveals molecular portraits in relation to the pathogenesis of the disease. *Blood* 2003;101:4998-5006.
21. De Vos J, Hose D, Reme T, et al. Microarray-based understanding of normal and malignant plasma cells. *Immunol Rev* 2006;210:86-104.
22. Bergsagel PL, Kuehl WM, Zhan F, et al. Cyclin D dysregulation: an early and unifying pathogenic event in multiple myeloma. *Blood* 2005;106:296-303.
23. Zhan F, Huang Y, Colla S, et al. The molecular classification of multiple myeloma. *Blood* 2006;108:2020-8.
24. Shaughnessy JD, Jr., Zhan F, Burington BE, et al. A validated gene expression model of high-risk multiple myeloma is defined by deregulated expression of genes mapping to chromosome 1. *Blood* 2007;109:2276-84.

25. Zhan F, Colla S, Wu X, et al. CKS1B, overexpressed in aggressive disease, regulates multiple myeloma growth and survival through SKP2- and p27Kip1-dependent and -independent mechanisms. *Blood* 2007; *109*:4995-5001.
26. Hose D, Rossi JF, Itrich C, et al. Molecular Classification of Multiple Myeloma (MM) Based on Gene Expression Profiling (GEP) and Fluorescence In Situ Hybridisation (FISH) Is an Independent Predictor for Event Free Survival (EFS). *Blood (ASH Annual Meeting Abstracts)* 2005; *106*.
27. Keats JJ, Fonseca R, Chesi M, et al. Promiscuous mutations activate the noncanonical NF-kappaB pathway in multiple myeloma. *Cancer Cell* 2007; *12*:131-44.
28. Annunziata CM, Davis RE, Demchenko Y, et al. Frequent engagement of the classical and alternative NF-kappaB pathways by diverse genetic abnormalities in multiple myeloma. *Cancer Cell* 2007; *12*:115-30.
29. Jourdan M, Moreaux J, Vos JD, et al. Targeting NF-kappaB pathway with an IKK2 inhibitor induces inhibition of multiple myeloma cell growth. *Br J Haematol* 2007; *138*:160-8.
30. Rossi J, Moreaux J, Rose M, et al. A Phase I/II Study of Atacicept (TACI-Ig) To Neutralize APRIL and BlyS in Patients with Refractory or Relapsed Multiple Myeloma (MM) or Active Previously Treated Waldenstrom's Macroglobulinemia (WM). *Blood (ASH Annual Meeting Abstracts)* 2006; *108*:3578.
31. Moreaux J, Cremer FW, Reme T, et al. The level of TACI gene expression in myeloma cells is associated with a signature of microenvironment dependence versus a plasmablastic signature. *Blood* 2005; *106*:1021-30.
32. De Vos J, Couderc G, Tarte K, et al. Identifying intercellular signaling genes expressed in malignant plasma cells by using complementary DNA arrays. *Blood* 2001; *98*:771-80.
33. Tarte K, De Vos J, Thykjaer T, et al. Generation of polyclonal plasmablasts from peripheral blood B cells: a normal counterpart of malignant plasmablasts. *Blood* 2002; *100*:1113-22.
34. De Vos J, Thykjaer T, Tarte K, et al. Comparison of gene expression profiling between malignant and normal plasma cells with oligonucleotide arrays. *Oncogene* 2002; *21*:6848-57.
35. Zhan F, Hardin J, Kordsmeier B, et al. Global gene expression profiling of multiple myeloma, monoclonal gammopathy of undetermined significance, and normal bone marrow plasma cells. *Blood* 2002; *99*:1745-57.
36. Munshi NC, Hideshima T, Carrasco D, et al. Identification of genes modulated in multiple myeloma using genetically identical twin samples. *Blood* 2004; *103*:1799-806.
37. Barclay AN, Wright GJ, Brooke G, and Brown MH CD200 and membrane protein interactions in the control of myeloid cells. *Trends Immunol* 2002; *23*:285-90.
38. Moreaux J, Hose D, Reme T, et al. CD200 is a new prognostic factor in multiple myeloma. *Blood* 2006; *108*:4194-7.
39. Gorczynski RM CD200 and its receptors as targets for immunoregulation. *Curr Opin Investig Drugs* 2005; *6*:483-8.
40. Kretz-Rommel A, Qin F, Dakappagari N, et al. CD200 expression on tumor cells suppresses antitumor immunity: new approaches to cancer immunotherapy. *J Immunol* 2007; *178*:5595-605.
41. Davies FE, Dring AM, Li C, et al. Insights into the multistep transformation of MGUS to myeloma using microarray expression analysis. *Blood* 2003; *102*:4504-11.
42. Shaughnessy J, Jr., Zhan F, Barlogie B, and Stewart AK Gene expression profiling and multiple myeloma. *Best Pract Res Clin Haematol* 2005; *18*:537-52.
43. Zhan F, Barlogie B, Arzoumanian V, et al. A gene expression signature of benign monoclonal gammopathy evident in multiple myeloma is linked to good prognosis. *Blood* 2006.
44. Hynes NE and Lane HA ERBB receptors and cancer: the complexity of targeted inhibitors. *Nat Rev Cancer* 2005; *5*:341-54.
45. Mahtouk K, Hose D, Reme T, et al. Expression of EGF-family receptors and amphiregulin in multiple myeloma. Amphiregulin is a growth factor for myeloma cells. *Oncogene* 2005; *24*:3512-24.
46. Mahtouk K, Jourdan M, De Vos J, et al. An inhibitor of the EGF receptor family blocks myeloma cell growth factor activity of HB-EGF and potentiates dexamethasone or anti-IL-6 antibody-induced apoptosis. *Blood* 2004; *103*:1829-37.
47. Mackay F and Ambrose C The TNF family members BAFF and APRIL: the growing complexity. *Cytokine Growth Factor Rev* 2003; *14*:311-24.
48. Novak AJ, Darce JR, Arendt BK, et al. Expression of BCMA, TACI, and BAFF-R in multiple myeloma: a mechanism for growth and survival. *Blood* 2004; *103*:689-94.
49. Croonquist PA, Linden MA, Zhao F, and Van Ness BG Gene profiling of a myeloma cell line reveals similarities and unique signatures among IL-6 response, N-ras-activating mutations, and coculture with bone marrow stromal cells. *Blood* 2003; *102*:2581-92.

50. Brocke-Heidrich K, Kretzschmar AK, Pfeifer G, et al. Interleukin-6-dependent gene expression profiles in multiple myeloma INA-6 cells reveal a Bcl-2 family-independent survival pathway closely associated with Stat3 activation. *Blood* 2004; *103*:242-51.
51. Chatterjee M, Stuhmer T, Herrmann P, et al. Combined disruption of both the MEK/ERK and the IL-6R/STAT3 pathways is required to induce apoptosis of multiple myeloma cells in the presence of bone marrow stromal cells. *Blood* 2004; *104*:3712-21.
52. Chatterjee M, Jain S, Stuhmer T, et al. STAT3 and MAPK signaling maintain overexpression of heat shock proteins 90alpha and beta in multiple myeloma cells, which critically contribute to tumor-cell survival. *Blood* 2007; *109*:720-8.
53. Chauhan D, Auclair D, Robinson EK, et al. Identification of genes regulated by dexamethasone in multiple myeloma cells using oligonucleotide arrays. *Oncogene* 2002; *21*:1346-58.
54. Chauhan D, Li G, Auclair D, et al. Identification of genes regulated by 2-methoxyestradiol (2ME2) in multiple myeloma cells using oligonucleotide arrays. *Blood* 2003; *101*:3606-14.
55. Mitsiades CS, Mitsiades NS, McMullan CJ, et al. Transcriptional signature of histone deacetylase inhibition in multiple myeloma: biological and clinical implications. *Proc Natl Acad Sci U S A* 2004; *101*:540-5.
56. Mitsiades N, Mitsiades CS, Poulaki V, et al. Molecular sequelae of proteasome inhibition in human multiple myeloma cells. *Proc Natl Acad Sci U S A* 2002; *99*:14374-9.
57. Mulligan G, Mitsiades C, Bryant B, et al. Gene expression profiling and correlation with outcome in clinical trials of the proteasome inhibitor bortezomib. *Blood* 2006.
58. Corre J, Mahtouk K, Attal M, et al. Bone marrow mesenchymal stem cells are abnormal in multiple myeloma. *Leukemia* 2007; *21*:1079-88.
59. Mahtouk K, Hose D, Raynaud P, et al. Heparanase influences expression and shedding of syndecan-1, and its expression by the bone marrow environment is a bad prognostic factor in multiple myeloma. *Blood* 2007; *109*:4914-23.
60. Yang Y, Macleod V, Miao HQ, et al. Heparanase enhances syndecan-1 shedding: a novel mechanism for stimulation of tumor growth and metastasis. *J Biol Chem* 2007; *282*:13326-33.
61. Sanderson RD, Yang Y, Suva LJ, and Kelly T Heparan sulfate proteoglycans and heparanase--partners in osteolytic tumor growth and metastasis. *Matrix Biol* 2004; *23*:341-52.
62. Yaccoby S, Wezeman MJ, Henderson A, et al. Cancer and the microenvironment: myeloma-osteoclast interactions as a model. *Cancer Res* 2004; *64*:2016-23.
63. Tian E, Zhan F, Walker R, et al. The role of the Wnt-signaling antagonist DKK1 in the development of osteolytic lesions in multiple myeloma. *N Engl J Med* 2003; *349*:2483-94.
64. Assou S, Lecarrou T, Tondeur S, et al. A meta-analysis of human embryonic stem cells transcriptome integrated into a web-based expression atlas. *Stem Cells* 2007.

## Legends to figures.

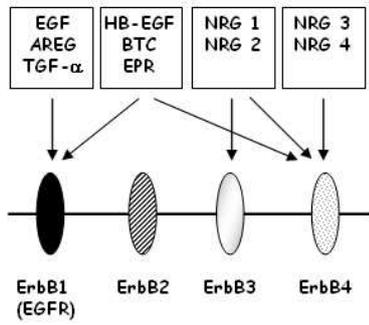
**Figure 1. Syndecan-1, ErbB receptors and EGF-ligands expression throughout plasma cell differentiation and malignant transformation.** A. The ten ligands can be subdivided in 4 groups according to their specificity (indicated with arrows) for ErbB receptors. B. Late plasma cell differentiation is associated with the development of a possible functional autocrine loop involving ErbB1 and ErbB2, their ligands, and their co-receptor syndecan-1. Malignant transformation is associated with the development of additional possible autocrine loop involving ErbB3 and ErbB4, neuregulins, and syndecan-1; as well as paracrine expression of EGF-ligands. TGF- $\alpha$  and epiregulin (represented in parentheses) do not have a HS-binding domain, *i.e* they are not able to promote myeloma cell growth. C. Total mononuclear cells from patients with MM were cultured for 5 days with either the PD169540 ErbB inhibitor or dexamethasone (Dex), or a combination of both. Cells were stained with an anti-CD138-PE antibody. Abbreviations: PPC; polyclonal plasmablastic cells. BMPC; bone marrow plasma cells. MMC; multiple myeloma cells. EGF; epidermal growth factor. AREG; amphiregulin. TGF- $\alpha$ ; tumor necrosis factor- $\alpha$ . HB-EGF; heparin-binding EGF-like growth factor. BTC; betacellulin. EPR; epiregulin. NRG; neuregulin.

**Figure 2. Expression of growth factors in myeloma cells and in cells from the environment.** Gene expression was measured by pan genomic U133 Plus2.0 Affymetrix oligonucleotides microarrays. The signal intensity for each gene is shown on the Y axis as arbitrary units determined by Affymetrix software. White boxes indicate that the gene is not detected, *i.e* it has an “absent” detection call according to the definition of Affymetrix. Histograms were generated with our “Amazonia!” web site (64). BMMSCs; bone marrow

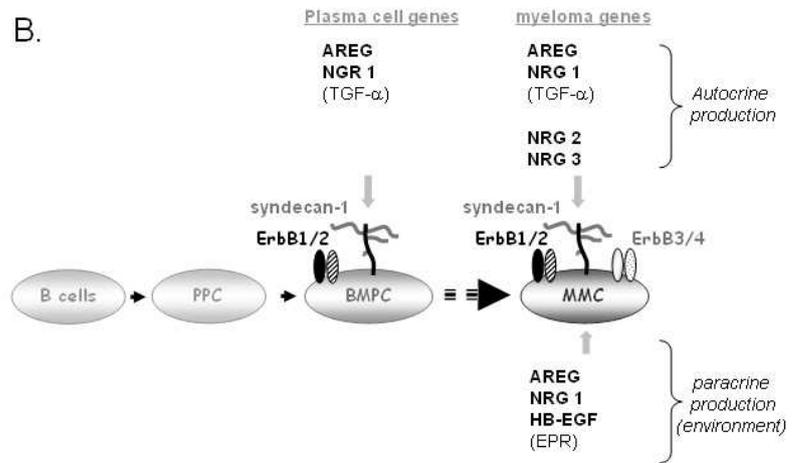
mesenchymal stem cells. CD15+; polynuclear cells. CD14+; monocytes. CD3+; T cells. MMC; multiple myeloma cells. The BM of patients with MM contains about 52% CD15<sup>+</sup> polynuclear cells, 14% CD3<sup>+</sup> T lymphocytes and 10% CD14<sup>+</sup> monocytes, other cells being mainly erythroid cells (the mean percentages were determined on 22 consecutive samples, unpublished data).

**Figure 1**

A.



B.



C.

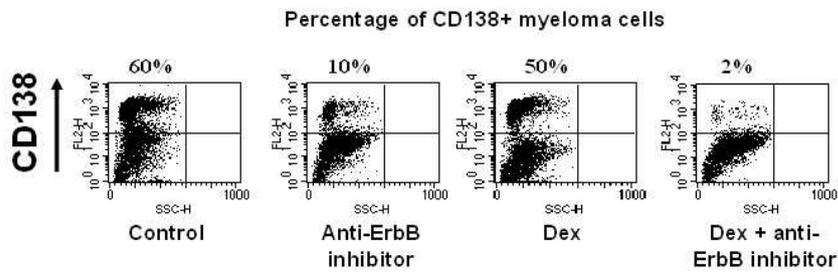


Figure 2

