Open Journal of Hematology

ROSS Science Publishers

Review Article

OPEN ACCESS

Cell Trafficking in Multiple Myeloma

Giada Bianchi[1,*], *Shaji Kumar*[1], *Irene M. Ghobrial*[2], *Aldo M. Roccaro*[2,*]

[1] Mayo Clinic, Rochester, MN, USA

[2] Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA, USA

Corresponding Authors & Addresses:

Giada Bianchi[*]

Mayo Clinic, Rochester, MN, USA; Email: bianchi.giada@mayo.edu

Aldo M. Roccaro[*]

Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA, USA;

Email: Aldo_roccaro@dfci.harvard.edu

Published: 21st February, 2012 Accepted: 21st February, 2012

Received: 19th October, 2011

Open Journal of Hematology, 2012, **3(S1)**-4

ABSTRACT

Multiple myeloma (MM) is an incurable cancer of terminally differentiated plasma cells (PC) and represents the second most frequent hematologic malignancy in the western world. MM cells localize preferentially to the bone marrow where they interact closely with bone marrow stroma cells (BMSC) and extracellular matrix (ECM) proteins in a reciprocal pro-survival loop. Such a bone marrow niche guarantees a survival advantage for MM cells and has a crucial role in mediating drug resistance to chemotherapy agents. As the name suggests, hallmark characteristic of MM is the ability to localize in multiple, distant bone sites causing disruption of the normal bone architecture and impairment of normal hematopoiesis. The pathogenic mechanisms of MM rely then not only on proliferation of cancerous cells, but also on the ability of myeloma cells to traffic between sites and home to appropriate survival niches.

Identifying the mechanisms that regulate the homing of MM cells to the bone marrow, the MM-BMSC interaction and the trafficking of MM cells from the bloodstream to distant bone locations is therefore crucial to design new, more effective therapies capable of overcoming the maladaptive interaction between BMSCs and MM and help in finding a cure for MM.

INTRODUCTION

MM is a cancer of terminally differentiated B lymphocytes or PC [1, 2]. It accounts for over 10% of all hematologic malignancy and 2% of annual cancer-related deaths [3, 4]. The American Cancer Society estimates that almost 21,000 new cases of MM will be diagnosed in the year 2011 and 10,600 MM-related deaths are expected in the same year. Despite significant progresses in the treatment of MM over the last 10 years, MM remains incurable and the current 5-year relative survival rate has been estimated around 40%.

MM cells are the malignant counterpart of

terminally differentiated long lived PC.

As the latter, MM cells home to the bone marrow (BM) where they abnormally survive and proliferate and generally secrete a monoclonal immunoglobulin (M spike or M component). Together with the typical punch out lytic lesions, the M spike, clearly visible on serum protein electrophoresis, is the historical hallmark of MM [4, 5].

The abnormal proliferation of MM cells within the BM with disruption of normal bone architecture and impairment of hematopoiesis is the main pathogenetic mechanism of MM and accounts for the majority of MM patients' presenting symptoms and signs (i.e.: back or bone pain, constitutional symptoms and, if severe, altered mental status secondary to hypercalcemia, or fatigue secondary to anemia). However, the M component can be pathogenetic per se as in the case of some forms of glomerular and tubular renal damage; peripheral neuropathy; cardiac and hepatic toxicity due to amyloidogenic monoclonal light chain deposition and hyperviscosity syndrome in extremely high M spikes [6].

While MM can present as an isolated site of soft tissue or bone disease (plasmacytoma), the overall majority of patients present with multiple sites of bone disease, hence the name "multiple" myeloma [5]. The homing process, characterized by adhesion of circulating cells to vascular endothelium, transendothelial migration, penetration of the basement membrane and ECM and, finally, localization and retention into a niche on the base of a chemotactic gradient, is therefore crucial in mediating MM pathogenicity [7].

The CRAB criteria for diagnosis of symptomatic MM (hyperCalcemia, Renal impairment, Anemia and Bone disease) as well as the common finding of hypogammaglobulinemia and subsequent predisposition to recurrent infections can all be ascribed to proliferation of the malignant clone in the BM, recirculation of MM cells with homing to distant bony site and their maladaptive interaction with cellular elements present in the BM, including osteoclasts [6].

The interaction of MM cells with BMSC and ECM components in the BM microenvironment has been proven to sustain MM cell proliferation, provide MM cells with a survival advantage and be responsible for drug resistance to chemotherapeutic agents [7].

In this perspective, understanding the mechanisms of MM cell trafficking is crucial to further understand the pathophysiology of MM in order to design novel therapies capable of successfully targeting MM cells in their environment, disrupt the vicious MM-BMSC loop and finally help curing MM patients.

In this review, we will discuss the role of chemokines in homing of MM cells to BM; the molecules involved in adhesion and survival within the BM; proteases responsible for ECM invasion and seeding to distant bone sites; the available therapeutic armamentarium and future treatment perspective.

HOMING TO THE BONE MARROW: THE SDF-1/CXCR4 AXIS IN HEALTH AND DISEASE

Ontogenesis of long lived plasma cells

Long lived PC are a key element of immunological memory, being responsible for the maintenance of sustained, low titer, high affinity antibody production [8, 9]. Upon encounter with an appropriate T cell-dependent antigen, B cells form germinal centers (GC) in secondary lymphoid tissues, supporting the formation of plasmablasts [10, 11]. These enter circulation and home to different sites in a process strictly regulated by chemokines and adhesion molecules [12]. The vast majority of post-germinal PC secrete high titer, high affinity antibody over a short period of time, being doomed to die after 3-7 days upon antigen encounter [12]. The tightly regulated apoptosis of short lived PC is an effective mechanism to ensure suppression of antibody production and avoid side effect from prolonged humoral response, including autoimmunity [13-15].

On the contrary, a subpopulation of GC derived plasmablasts homes to the BM where they survive for years and ensure long term specific antibody production, key to maintain an adequate antigenic memory. These cells are named long lived PC. They are typically non-recirculating, IgG secreting PC [8, 12, 16].

It has been observed that while these cells are characterized by sustained survival *in vivo*,

apoptosis ensues quickly *ex vivo* [9, 17]. However, long lived PCs can be rescued *ex vivo* by supplementation of media with stromal cell-derived factor 1 (SDF-1) and interleukin 6 (IL-6).

Along the process of B cell differentiation to plasma cell, B lymphocytes undergo rearrangement of their pool of chemokine receptors, downregulating CXCR5 and CCR7, while upregulating CXCR4 and maintaining functional CCR3, CXCR6 and CCR 10. This modified asset of receptors results in decreased responsiveness to B and T-cell chemokines such as CCXCL13, CCL12 and CCL19 and increased sensitivity to SDF-1 gradient [18-20].

Initially identified as a growth factor for pre-B cells, SDF-1 has been subsequently shown to have a key role in multiple steps of B cell-poiesis [21-23]. Also know as CXCL-12, SDF-1 is a chemokine constitutively expressed by BMSC and exerts its function as chemoattractant and growth factor by binding its cognate receptor CXCR4 [20, 24]. The latter belongs to the family of G protein coupled receptors, also known as 7 transmembrane domain proteins, and is expressed by B cells throughout different stages of their ontogenesis: in pre-B cells, it mediates localization to dedicated BM niches; in mature B cells, it is crucial for appropriate organization of germinal centers; in PC it is a fundamental factor to guide homing to the BM [20, 22, 25]. Once in the BM, long lived PC localize in close proximity with a subset of BMSC secreting high levels of SDF-1 [22]. Recruitment and accumulation of PC in the BM has been shown to be significantly delayed in mice genetically engineered to lack expression of CXCR-4 in the B cell lineage [26]. However, a consistent number of long-lived PCs was still present in the BM of such mice, therefore suggesting redundant mechanisms for homing and retention of PC beyond the SDF-1/CXCR4 axis. The authors suggested the existence of two different ontogenic processes for long-lived PC: the classic post-GC differentiation into plasma blasts expressing high level of CXCR-4 and being dependent on SDF-1 gradient for homing and retention into the BM; and a second population of plasma-blasts capable of homing to the BM in a CXCR4/SDF-1 independent mechanisms and undergoing terminal differentiation to long-lived PC *in situ*.

The reproduction of an identical, lethal phenotype in both Cxcr4 and Cxcl12 gene-deleted mice is a further confirmation of the reciprocal, univocal relationship between CXCR4 and its ligand SDF-1. Knockout mice for either one of these genes presented with impaired myelopoiesis, especially involving the B-cell lineage, and defective cardiovascular and neuronal development [27-29].

THE SDF-1/CXCR4 AXIS IN MULTIPLE MYELOMA

Nowakowski et al. showed that circulating PC are present in more than 70% of newly diagnosed MM patients and represent an independent predictor of survival [30, 31]. In order to reach the favorable environment of the BM niche, circulating MM cells need to adhere to endothelial cells, cross the endothelial lining and migrate to the BM, a process collectively known as homing [7].

Flow cytometry studies on primary cells of MM patients obtained from BM, peripheral blood and extra medullary sites showed CXCR4 to be present in 70% of BM MM cells and 100% MM from peripheral blood or extramedullary sites [32]. In this particular study, a subset of malignant cells was capable of spontaneous migration, independently from chemotactic gradient, and such capacity correlated with more aggressive disease, advanced stages and tendency to extra-medullary localization and leukemic phase.

Multiple myeloma cells have been shown to be dependent on the SDF-1/CXCR4 axis for BM homing, ECM and transendothelial migration and survival [33, 34]. As their normal counterpart, MM cells express CXCR4 and its interaction with SDF-1 is responsible for adhesion to vascular cell adhesion protein 1 (VCAM-1), ECM components including fibronectin, and endothelial cells via expression of α4β1 [35, 36]. Interestingly, SDF-1 level was significantly higher in the BM milieu of patients with MM compared to healthy donors [35].

As the interaction of MM with the BM microenvironment has been shown to be crucial for pathogenicity, MM cell survival and drug-resistance, recent years have witness a growing interest in understanding the mechanisms underlying SDF-1-mediated recruitment of MM cells to the BM.

In multiple myeloma cells, SDF-1 induces

relocalization and polarization of CXCR4, followed by pseudopodia formation, leading to chemotaxis of MM cells toward the source of SDF-1 [35]. *In vitro*, the dose response to SDF-1 was distributed according to a bell-shaped curve, with peaked effect around 30nM and decreased chemotaxis for higher concentration of SDF-1. This mechanism was thought to be responsible for retention of MM in the BM where high concentrations of SDF-1 are achieved. SDF-1 causes internalization of CXCR4 *in vitro* and primary cells from MM patients showed higher surface expression of CXCR4 while circulating in the peripheral blood, when compared to BM resident MM cells, suggesting a potential negative feedback loop between SDF-1 and CXCR4.

CXCR-4 was proved necessary for SDF-1 induced migration of MM cells as pharmacologic inhibition of CXCR4/SDF-1 binding, via anti CXCR-4 monoclonal antibody (mAb) or CXCR4 knockdown, abolished SDF-1 induced migration [35]. Differently from hematopoietic stem cells (HSC), the phosphoinositide 3-kinase inhibitor (PI3K) and extracellular signal-regulated kinase/mitogen-activated protein kinase (ERK/MAPK) pathways were shown to be activated downstream CXCR4/SDF-1 interaction. Inhibition of CXCR4 signaling via the Gi protein inhibitor PTX abolished phosphorylation of ERK and AKT. The PI3K inhibitor LY294002 reproduced a similar effect with inhibition of ERK and AKT phosphorylation while the MAP/ERK kinase (MEK) inhibitor PD098059 only affected phosphorylation of ERK 1/2, therefore suggesting a hierarchy with PI3K upstream of ERK/MAPK.

De Gorter et al. showed that SDF-1 stimulation of MM cells results in activation of the small GTPase RalB which in turn is necessary, but not sufficient to drive SDF-1 induced chemotaxis [37]. The authors showed that activation of RalB is independent of cytosolic kinases Lyn/Syk, phospholipase C (PLC), Bruton's tyrosine kinase (Btk), PI3K and Ras and postulated β-Arrestin and p38 pathway to be responsible for SDF-1-mediated RalB activation [38, 39].

RhoA and Rac1, members of the Rho guanosine triphosphatase(GTPase) family, are involved in mediating MM adhesion and SDF-1-induced chemotaxis, as well [40]. Gene expression profiling and flow cytometry in MM cell lines and primary myeloma cells showed RhoA and Rac1 to be expressed at a significantly higher level in MM when compared to bone marrow plasma cells from control individuals [41]. Rho-associated protein kinase (ROCK) is a major effector molecule along the RhoA signaling pathway [42-44]. ROCK and Rac1 are involved in MM cell adhesion to endothelium and fibronectin and in triggering MM-BMSC binding via a very late antigen 4/vascular cell adhesion molecule 1 (VLA 4/VCAM-1)-dependent and leukocyte function-associated antigen 1/intracellular adhesion molecule 1 (LFA-1)/ICAM-1-independent mechanism [41].

Chemotaxis along SDF-1 gradient was dependent upon RhoA, but not Rac1. Confocal microscopy studies showed that SDF-1-induced actin polymerization and polarization were substantially reduced by the ROCK inhibitor Y27632, while Rac1 blockade via its inhibitor NSC23766 had a less prominent effect on the former and did not affect the latter. Moreover, ROCK, but not Rac1 inhibition, resulted in increased dephosphorylation of myosin light chain (MLC), a key molecule in the regulation of contraction and relaxation of actin [45].

Pharmacologic inhibition of either ROCK or Rac1 in a murine model resulted in delayed MM cell exit from circulation and bone marrow engraftment, thus further confirming the role of both molecules in MM cell homing to the BM niche [41].

In a similar mouse model, significantly reduced homing of intravenously injected MM cells to bone marrow niches was obtained by pharmacological inhibition of SDF-1/CXCR4 interaction by AMD3100 (plerixafor, AnorMED, Toronto, ON, Canada), a selective, reversible bicyclam antagonist of SDF-1 [35].

THE BONE MARROW MICROENVIRONMENT: A SURVIVAL NICHE FOR MM

The bone marrow microenvironment is composed of a cellular and a non-cellular component. The former includes stroma cells, endothelial cells, hematopoietic stem cells and pluripotent precursors, adipocytes, osteoblasts and osteoclasts; the latter is the pool of ECM proteins such as laminin, fibronectin and collagen [46, 47]. The binding of MM cells to BM cells has been

reported to increase autocrine and paracrine production and secretion of molecules involved in survival, proliferation and neoangiogenesis such as IL-6, SDF-1, vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), insulin-like growth factor 1 (IGF-1), tumor necrosis factor α (TNF- α) and transforming growth factor β (TGF- β) [48-50]. This bidirectional positive feedback loop between MM cells and BMSC is sustained by the induction of several pro-survival signaling pathways, including nuclear factor kB (NF-kB), MEK/ERK pathway, Janus kinase/signal transducers and activator of transcription 3 (JNK/STAT3) pathway, PI3K/AKT pathway [17, 36, 51]. The downstream effects of these signaling pathways include upregulation of cyclin D1; induction of antiapoptotic, multi dimers Bcl proteins; stimulation of osteoclastogenesis and inhibition of osteoblast differentiation; induction of adhesion molecules (i.e., ICAM-1), pro-survival cytokines (i.e., IL-6), neo-angiogenesis factors (i.e., VEGF) and matrix metalloproteinases (i.e., MMP-9 and MMP-1) [7, 52-55].

Importantly, the adhesion of MM cells to BMSCs mediates cell-adhesion mediated drug resistance (CAM-DR) while some of the chemokines, including IL-6, have been shown to cause drug resistance by mechanisms independent from cell-cell contact [49, 51, 55-58].

MM-BMSCs interaction occurs via a plethora of adhesions molecules as CD44, VLA 4 and 5, LFA-1, ICAM-1, VCAM-1 and syndecan-1 or CD138. The latter is a transmembrane proteoglycan strongly expressed by terminally differentiated plasma cells and MM cells that mediates adherence to ECM via binding of type I collagen. The CD138-type I collagen interaction triggers expression of matrix metalloproteinase 1 (MMP-1) which has been shown to be crucial for ECM reabsorption and tumor invasion [52, 59, 60].

Disruption of the normal balance between osteoclast and osteoblast activity in the MM niche is fundamental to perseverate MM survival and proliferation and is a major determinant of MM-related morbidity including pathologic fractures and hypercalcemia [61, 62]. MM is characterized by increased number and activity of osteoclasts and impairment of new bone deposition due to suppression of osteoblast function [63, 64]. Several humoral factors are involved in mediating the maladaptive MM-osteoclast loop, including IL-6, receptor activator of NF-κB ligand (RANKL), B-cell activating factor (BAFF), VEGF and dickkopf (DKK)–1 [65, 66].

A relative excess of the receptor activator of NF-κB ligand (RANKL) in comparison to the decoy receptor osteoprotegerin (OPG) was demonstrated to play a major role in determining an unbalance between bone reabsorption and deposition and to be a prognostic factor for bone disease in MM [63, 67, 68].

Given the hypoxemic nature of the bone marrow, there is growing interest in the role of HIF-1 in MM progression, neoangiogenesis and trafficking [69-73]. Interestingly, HIF-1 and 2 induces expression of CXCL-12 with a tentative teleological goal of attracting circulating stem cells to ischemic areas in order to guarantee tissue repair [74]. HIF-1 is also responsible for inducing CXCR4 in normal and malignant cells, potentially contributing to bone marrow metastatic spread [75, 76]. Immunohistochemistry studies found HIF-1α and HIF-2α to be frequently overexpressed in primary MM cells and their expression to correlate with VEGF production and neoangiogenesis [77]. Interestingly, HIF-2α was found to be a positive regulator of aberrant expression of CXCL12 in MM cell lines [78].

RECIRCULATION TO DISTANT SITE: THE ROLE OF METALLOPROTEINASES AND INTEGRINS

MM cell homing is a complex multistep process that sequentially requires tenacious adhesion of circulating cells to the endothelial surface; migration through endothelium, basement membrane, subendothelial space and extracellular matrix; and, finally, localization and retention into the bone marrow niche. MMPs are a family of multidomain, zinc-dependent, endopeptidases which have been implicated in both physiological (leukocyte extravasation, tissue remodeling) and pathological processes (metastasis, tissue invasion) [79-81]. MMPs exist in two forms: as zymogenic enzymes that are secreted in an inactive form and undergo activation by cleavage, or as integral transmembrane proteins, namely membrane-type MMPs (MT-MMP) [82-84]. The activity of MMPs is modulated in a sophisticated manner at different

levels: beyond transcriptional and translational control, MMP function is affected by stereotactic interaction with other ECM proteins, including tissue inhibitors of MMPs (TIMP), a family of small molecular weight proteins whose function is reversible inhibition of MMPs [85]. The fine equilibrium between MMPs activity and their counter-regulatory mechanisms defines the outcome [86-89].

In vitro, primary MM cells and BMSC from MM patients produce an excess of several MMPs, including MMP-1, MMP-2, MMP-8, MMP-9, MMP-13 and MTI-MMP when compared to their normal tissue counterparts [52, 60]. *In vitro* experiments with primary MM cells and MM cell lines using Matrigel, an extracellular matrix extract that mimics *in vitro* the composition of basement membrane, showed that migration was dependent on CXCL12 and substantially inhibited by anti-CXCR4 mAb, CXCR4-CXCL12 binding inhibitors and broad metalloproteinase inhibitor GM6001 [90]. Further studies showed that exposure to CXCL-12 resulted in increased secretion of MMP-9 by MM cells and that specific blocking antibodies against MMP-9 or the use of TIMP-1, a naturally produced inhibitor of MMP-9, significantly abrogate CXCL-12 induced MM migration. Exposure of MM cells to CXCL-12 also resulted in increased expression of MT1-MMP and its discrete polarization along a unique site on the cell membrane. Inhibition studies with blocking mAb and specific pharmacologic inhibitors, showed MT1-MMP to also play a role in mediating migration of MM cells through Matrigel. Further *in vitro* experiments proved transendothelial migration of MM cells to be dependent upon an intact CXCL-12/CXCR4 axis and to be mediated by MMP-9. A second mechanism by which MMP-9 is thought to contribute to metastatic spread and MM progression, is its ability to release matrix-bond VEGF-A, resulting in VEGF-A binding to its receptor and initiation of neoangiogenesis cascade [91].

Mouse models have been used to further investigate the putative role of MMPs in MM trafficking and disease progression. In 5T2MM bearing mice, treatment with SC-964, a broad MMP inhibitor, resulted in a significant decrease in burden of disease with reduction in osteolytic lesions and decreased neoangiogenesis when compared to untreated mice, suggesting a pivotal role for MMP in MM pathogenesis [92].

MM cells express a wide array of integrins, including α4, α5, αv, β1, β2, β3 and β7 whose activity is regulated both by binding with ECM and by inside-out signalling [54, 93]. The expression of several integrins in MM cells has been shown to be regulated by cytokines, chemokines and oncogenes [94, 95].

Integrins VLA-4 (α4 β1), VLA-5 (α5 β1) and αvβ3 have been long known to mediate MM homing to the BM; malignant cell trafficking and drug resistance [56, 96-99]. More recently, a role for integrin β7 as a negative prognostic factor for survival and predictor of poor response to standard and novel therapy has been established in MM [100]. Integrin β7 was identified as a downstream target of the oncogene C-MAF, which is overexpressed in almost half of MM patients either via genetic rearrangement (translocation t(14;16)) or due to paracrine stimulation along the BAFF/APRIl/TACI (B-cell activating factor / a proliferation-inducing ligand / transmembrane activator and calcium modulator and cyclophilin ligand interactor) axis. *In vitro* studies showed that knock down or mAb-mediated blockade of integrin β7 resulted in diminished MM cell line adhesion to fibronectin, E-cadherin and BMSCs; reduced capacity of invasion of Matrigel and of migration along an SDF-1 gradient and increased sensitivity to standard and novel chemotherapy agents such as melphalan and bortezomib. Moreover, integrin β7 was implicated in inducing VEGF, macrophage inflammatory protein 1β (MIP-1β) and interleukin 1β (IL-1β) secretion by MM and BMSC, suggesting a role in mediating neo-angiogenesis and osteoclast activity.

Consistent with *in vitro* studies, knock down of integrin β7 resulted in delayed homing and tumor engraftment in a murine model as well as decreased MM-related neoangiogenesis.

ANIMAL MODELS IN MM TRAFFICKING

In order to better understand the pathophysiologic mechanisms behind MM homing to the BM and cell trafficking, animal models have been used as a tool to recapitulate the natural history and mechanicistic evolution of MM. Nevertheless, the technological difficulties related to following real time the circulation of cancer cell as well as the intrinsic differences between the murine and human bone marrow

microenvironment had made it difficult to satisfactory reproduce MM in animals [101].

The 5T2MM is a well characterized myeloma cell lines obtained by C57Bl/KaLwRij mice. When elderly, these mice spontaneously develope a population of cells whose behavior recapitulate human MM. When injected intravenously in younger syngeneic mice, these cells are capable of homing to the BM, producing osteolytic lesions, secreting a monoclonal serum Ig and inducing neoangiogenesis [102-104].

The 5T2MM mouse model has been used to evaluate the effect of MMP inhibition in MM cell homing, survival and proliferation. Although the authors were able to assess BM homing, neo-angiogenesis and disease burden, no real time monitoring of distribution of MM cells could be achieved with this model [92].

The plasmacytoma mouse model, obtained by subcutaneous injection of human MM cells in immunodeficient mice, was the first animal model to utilize human MM cells [105]. Unfortunately, no data about the interaction of MM cells with their natural environment, the bone marrow, could be obtained by this model. Moreover, by definition, homing and trafficking could not be evaluated in a plasmacytoma model [106]. Intravenous injection of MM cells in severe combined immunodeficient/non obese diabetic (SCID/NOD) mice provided a considerable improvement in the validity of animal model for assessment of trafficking as it would recapitulate the processes of multi-site homing of MM cells. However, rapid, accurate and real time evaluation of those processes could not be obtained [101].

The implementation of novel optic techniques based on luminescence and fluorescence provided the unique opportunity to visualize real time and study the kinetics of homing and re-trafficking of MM cells [107].

Irradiated, SCID/NOD mice injected intravenously with luciferase positive (Luc+) or lentivirally transfected GFP-luciferin-Neo MM.1S cells (a widely used, MM cell line) were used to study the effect of the SDF-1 inhibitor AMD3100 on MM cell trafficking and homing to the BM [35, 108].

The kinetic of homing of malignant cells was followed at close interval by means of whole body, real time bioluminescence [109, 110]. In mice treated with combination of bortezomib and AMD3100, *in vivo* flow cytometry provided real time detection of apoptosis in circulating MM cells without need for blood draws [108].

Alsayed et al. further confirmed their findings by using Balb/c mice injected with fluorescently labeled MM.1S cells. *In vivo* flow cytometry allowed live monitoring of myeloma cells as they exit the circulation and localize to the bone marrow, while fluorescence confocal microscopy provided high resolution images throughout the intact animal skull and direct visualization of the bone marrow niche [111].

This same mouse model was used by Neri et al. to evaluate the role of integrin β7 in BM homing [100]. SCID mice subcutaneously injected with wild type and ITGB7^{silenced} MM.1S cells provided the authors the opportunity to evaluate the effects of integrin β7 silencing on proliferation of MM cells *in vivo*.

In vivo flow cytometry and fluorescence confocal microscopy was also used in SCID mice intravenously injected with fluorescent MM.1S cells to address the impact of the ROCK inhibitor Y27632 and the Rac1 inhibitor NSC23766 [41].

THE CORNER OF THE PHARMACOLOGIST: HOW TO DISRUPT MM TRAFFICKING

The interaction between MM cells and BMSCs mediates not only MM cells proliferation and survival, but also drug resistance. Recent efforts have been invested in pharmacologically targeting the MM-BMSC interaction to circumvent the pro-survival and growth advantage conferred by the BM microenvironment, overcome standard chemotherapy resistance and to improve therapy against MM.

Given its key role in mediating homing and retention of MM cells in the bone marrow, the CXCR4-SDF-1 axis was the natural target of such therapies.

In vitro studies showed the SDF-1 antagonist AMD3100 to cause sensitization of MM cells to novel and standard chemotherapy agents including bortezomib, melphalan, doxorubicin and dexamethasone [108]. In the presence of BMSC,

the co-treatment with AMD3100 resulted in significant inhibition of phosphorylation of AKT in MM cells treated with bortezomib, providing a molecular mechanism for the improved bortezomib effect in the presence of AMD3100. Azeb et al. showed that in a murine model, treatment with AMD3100 caused mobilization of MM cells to the peripheral blood with delayed kinetics when compared to mobilization of hematopoietic stem cell, suggesting that a timely planned administration of chemotherapy agents after AMD3100 could spare HSC and be an effective anti-MM therapy. Mice treated with AMD3100 showed neither a more aggressive course of disease nor an increase in extramedullary engraftment of MM cells. Moreover, combinatory treatment with bortezomib and AMD3100 resulted in increased overall effectiveness of bortezomib with a significant percentage of circulating MM cells undergoing apoptosis and with no evidence of increased toxicity against HSC [108].

Currently AMD3100 has been successfully used in phase I, II and III clinical studies to aid mobilization of CD34+ positive cells in patient undergoing harvesting for autologous bone marrow transplantation [112, 113]. No significant early or delayed toxicity or inadequate HSC engraftment were noticed with the use of AMD3100 alone or in combination with G-CSF [114-118]. Indeed, the addition of AMD3100 to G-CSF was proved to successfully mobilize CD34+ cells in patients with hematologic malignancies, including MM, who had previously failed mobilization with cytokines alone [119].

Noticeably, the use of AMD3100 was not associated with significantly increased mobilization of MM cells to the peripheral blood or with tumor contamination of the CD34 apheresis products [120]. In the context of the data reported by Azeb et al, this observation could be the result of delayed mobilization of MM cells when compared to HSC after administration of AMD3100.

A phase I/II clinical trial of combination of plerixafor and bortezomib is currently undergoing in relapsed/refractory MM patients and several clinical trials of AMD3100 in combination with standard and novel anti-MM agents are currently enrolling.

T140 is a 14 amino acid, CXCR4 antagonist derived from a naturally occurring horseshoe crab produced molecule. Comparative studies showed AMD3100 to have weak partial agonist activity upon binding of CXCR4 while T140 functions as an inverse agonist and produces no signalling upon binding to the receptor [121, 122]. Head to head comparison of the two molecules in MM cell lines showed a transient pro-proliferative and pro-survival effect caused by AMD3100, but not T140, followed by a sustained anti-proliferative action of both [123]. In this perspective, T140 could have an advantage when compared to AMD3100 as no paradoxical signaling through CXCR4 would be elicited. Clinical studies are currently undergoing in solid tumors, while T140 has not been currently tested further in hematologic malignancies.

Given their role in promoting tissue invasion and cancer metastasization in preclinical studies, MMPs have also been at the centre of pharmacologic research as potential oncologic drugs [124, 125]. Unfortunately, multiple clinical trials with MMP inhibitors have been largely unsatisfactory, prompting further bench research to better understand the role of specific MMP in cancer progression and metastatic seeding, in order to design more effective treatments [126].

CONCLUSIONS AND FUTURE PERSPECTIVES

MM typically present with multiple distant sites of bone disease and the presence of circulating malignant cells at the time of diagnosis appears to be a common phenomenon. The process by which MM cells exit the bone marrow, enter venous circulation and home to a distant bone marrow site in accordance to a chemotactic gradient is collectively known as trafficking. Given the protective environment provided by the bone marrow niche, disrupting trafficking and homing is a promising therapeutic intervention to improve sensitivity to standard and novel chemotherapy drugs.

The SDF-1 antagonist AMD3100 has been proven safe in clinical trials as a tool to help mobilization of HSC in patients with hematologic malignancies; including MM. Preclinical studies *in vitro* and *in vivo* suggest this molecule to be effective in mobilizing MM cells and in increasing sensitivity to several anti-MM agents, including bortezomib.

The fact that the extent of circulating malignant cells in newly diagnosed MM patients was found to be a poor prognostic factor and a marker of advanced, aggressive disease raises concerns that the mobilization of MM cells outside the bone marrow by AMD3100 could potentially be detrimental. As the CXCR4-SDF-1 axis is also implicated in retention of HSC to the bone marrow, combined treatment with AMD3100 and chemotherapy agents could theoretically results in more severe bone marrow suppression.

Data from mouse models showed that AMD3100 treatment did not cause more aggressive disease or increased extramedullary engraftment of MM cells. Moreover, combined treatment with bortezomib resulted in a significant percentage of mobilized MM cells to undergo apoptosis in the peripheral blood and no significant additional toxicity on HSC was noticed. Phase I/II clinical trials of AMD3100 and bortezomib in relapsed refractory MM patients are currently enrolling and results will help clarify these questions.

It is evident that additional knowledge of the mechanisms underlying homing and trafficking of MM cells is of pivotal importance in order to identify novel targets, design more effective therapies and aid in the quest for the cure of MM.

CONFLICT OF INTEREST

The authors have no conflict of interest.

REFERENCES

[1] Kyle RA, Rajkumar SV. Multiple myeloma. *N Engl J Med.* **2004;** 351: 1860-73.

[2] Anderson KC, Carrasco RD. Pathogenesis of myeloma. *Annu Rev Pathol.* **2011;** 6: 249-74.

[3] Kyle RA, Rajkumar SV. Epidemiology of the plasma-cell disorders. *Best Pract Res Clin Haematol.* **2007;** 20: 637-64.

[4] Kyle RA, Rajkumar SV. Multiple myeloma. *Blood.* **2008;** 111: 2962-72.

[5] Kyle RA. Multiple myeloma: an odyssey of discovery. *Br J Haematol.* **2000;** 111: 1035-44.

[6] Munshi NC LD, Anderson KC. Multiple Myeloma. In: Longo DL KD, Jameson JL, Fauci AJ, Hauser SH, Loscalzo J ed. Harrison's Principles of Internal Medicine, 18th e McGraw Hill; **2011**.

[7] Hideshima T, Mitsiades C, Tonon G, Richardson PG, Anderson KC. Understanding multiple myeloma pathogenesis in the bone marrow to identify new therapeutic targets. *Nat Rev Cancer.* **2007;** 7: 585-98.

[8] Moser K, Tokoyoda K, Radbruch A, MacLennan I, Manz RA. Stromal niches, plasma cell differentiation and survival. *Curr Opin Immunol.* **2006;** 18: 265-70.

[9] Minges Wols HA, Underhill GH, Kansas GS, Witte PL. The role of bone marrow-derived stromal cells in the maintenance of plasma cell longevity. *J Immunol.* **2002;** 169: 4213-21.

[10] Calame KL. Plasma cells: finding new light at the end of B cell development. *Nat Immunol.* **2001;** 2: 1103-8.

[11] Calame KL, Lin KI, Tunyaplin C. Regulatory mechanisms that determine the development and function of plasma cells. *Annu Rev Immunol.* **2003;** 21: 205-30.

[12] Shapiro-Shelef M, Calame K. Regulation of plasma-cell development. *Nat Rev Immunol.* **2005;** 5: 230-42.

[13] Cascio P, Oliva L, Cerruti F, Mariani E, Pasqualetto E, Cenci S, Sitia R. Dampening Ab responses using proteasome inhibitors following *in vivo* B cell activation. *Eur J Immunol.* **2008;** 38: 658-67.

[14] Cenci S, Mezghrani A, Cascio P, Bianchi G, Cerruti F, Fra A, Lelouard H, Masciarelli S, Mattioli L, Oliva L, Orsi A, Pasqualetto E, Pierre P, Ruffato E, Tagliavacca L, Sitia R. Progressively impaired proteasomal capacity during terminal plasma cell differentiation. *EMBO J.* **2006;** 25: 1104-13.

[15] Neubert K, Meister S, Moser K, Weisel F, Maseda D, Amann K, Wiethe C, Winkler TH, Kalden JR, Manz RA, Voll RE. The proteasome inhibitor bortezomib depletes plasma cells and protects mice with lupus-like disease from nephritis. *Nat Med.* **2008;** 14: 748-55.

[16] Manz RA, Arce S, Cassese G, Hauser AE, Hiepe F, Radbruch A. Humoral immunity and long-lived plasma cells. *Curr Opin Immunol.* **2002;** 14: 517-21.

[17] Cassese G, Arce S, Hauser AE, Lehnert K, Moewes B, Mostarac M, Muehlinghaus G, Szyska M, Radbruch A, Manz RA. Plasma cell survival is mediated by synergistic effects of cytokines and adhesion-dependent signals. *J Immunol.* **2003;** 171: 1684-90.

[18] Hargreaves DC, Hyman PL, Lu TT, Ngo VN, Bidgol A, Suzuki G, Zou YR, Littman DR, Cyster JG. A coordinated change in chemokine responsiveness guides plasma cell movements. *J Exp Med.* **2001;** 194: 45-56.

[19] Hauser AE, Debes GF, Arce S, Cassese G, Hamann A, Radbruch A, Manz RA. Chemotactic responsiveness toward ligands for CXCR3 and CXCR4 is regulated on plasma blasts during the time course of a memory immune response. *J Immunol.* **2002;** 169: 1277-82.

[20] Nakayama T, Hieshima K, Izawa D, Tatsumi Y, Kanamaru A, Yoshie O. Cutting edge: profile of chemokine receptor expression on human plasma cells accounts for their efficient recruitment to target tissues. *J Immunol.* **2003;** 170: 1136-40.

[21] Campbell DJ, Kim CH, Butcher EC. Chemokines in the systemic organization of immunity. *Immunol Rev.* **2003;** 195: 58-71.

[22] Tokoyoda K, Egawa T, Sugiyama T, Choi BI, Nagasawa T. Cellular niches controlling B lymphocyte behavior within bone marrow during development. *Immunity.* **2004;** 20: 707-18.

[23] Ansel KM, Cyster JG. Chemokines in lymphopoiesis and lymphoid organ development. *Curr Opin Immunol.* **2001;** 13: 172-9.

[24] Bleul CC, Fuhlbrigge RC, Casasnovas JM, Aiuti A, Springer TA. A highly efficacious lymphocyte chemoattractant, stromal cell-derived factor 1 (SDF-1). *J Exp Med.* **1996;** 184: 1101-9.

[25] Allen CD, Ansel KM, Low C, Lesley R, Tamamura H, Fujii N, Cyster JG. Germinal center dark and light zone organization is mediated by CXCR4 and CXCR5. *Nat Immunol.* **2004;** 5: 943-52.

[26] Nie Y, Waite J, Brewer F, Sunshine MJ, Littman DR, Zou YR. The role of CXCR4 in maintaining peripheral B cell compartments and humoral immunity. *J Exp Med.* **2004;** 200: 1145-56.

[27] Nagasawa T, Hirota S, Tachibana K, Takakura N, Nishikawa S, Kitamura Y, Yoshida N, Kikutani H, Kishimoto T. Defects of B-cell lymphopoiesis and bone-marrow myelopoiesis in mice lacking the CXC chemokine PBSF/SDF-1. *Nature.* **1996;** 382: 635-8.

[28] Ma Q, Jones D, Borghesani PR, Segal RA, Nagasawa T, Kishimoto T, Bronson RT, Springer TA. Impaired B-lymphopoiesis, myelopoiesis, and derailed cerebellar neuron migration in CXCR4- and SDF-1-deficient mice. *Proc Natl Acad Sci U S A.* **1998;** 95: 9448-53.

[29] Zou YR, Kottmann AH, Kuroda M, Taniuchi I, Littman DR. Function of the chemokine receptor CXCR4 in haematopoiesis and in cerebellar development. *Nature.* **1998;** 393: 595-9.

[30] Nowakowski GS, Witzig TE, Dingli D, Tracz MJ, Gertz MA, Lacy MQ, Lust JA, Dispenzieri A, Greipp PR, Kyle RA, Rajkumar SV. Circulating plasma cells detected by flow cytometry as a predictor of survival in 302 patients with newly diagnosed multiple myeloma. *Blood.* **2005;** 106: 2276-9.

[31] Billadeau D, Van Ness B, Kimlinger T, Kyle RA, Therneau TM, Greipp PR, Witzig TE. Clonal circulating cells are common in plasma cell proliferative disorders: a comparison of monoclonal gammopathy of undetermined significance, smoldering multiple myeloma, and active myeloma. *Blood.* **1996;** 88: 289-96.

[32] Trentin L, Miorin M, Facco M, Baesso I, Carraro S, Cabrelle A, Maschio N, Bortoli M, Binotto G, Piazza F, Adami F, Zambello R, Agostini C, Semenzato G. Multiple myeloma plasma cells show different chemokine receptor profiles at sites of disease activity. *Br J Haematol.* **2007;** 138: 594-602.

[33] Möller C, Strömberg T, Juremalm M, Nilsson K, Nilsson G. Expression and function of chemokine receptors in human multiple myeloma. *Leukemia.* **2003;** 17: 203-10.

[34] Aggarwal R, Ghobrial IM, Roodman GD. Chemokines in multiple myeloma. *Exp Hematol.* **2006;** 34: 1289-95.

[35] Alsayed Y, Ngo H, Runnels J, Leleu X, Singha UK, Pitsillides CM, Spencer JA, Kimlinger T, Ghobrial JM, Jia X, Lu G, Timm M, Kumar A, Côté D, Veilleux I, Hedin KE, Roodman GD, Witzig TE, Kung AL, Hideshima T, Anderson KC, Lin CP, Ghobrial IM. Mechanisms of regulation of CXCR4/SDF-1 (CXCL12)-dependent migration and homing in multiple myeloma. *Blood.* **2007;** 109: 2708-17.

[36] Hideshima T, Chauhan D, Hayashi T, Podar K, Akiyama M, Gupta D, Richardson P, Munshi N, Anderson KC. The biological sequelae of stromal cell-derived factor-1alpha in multiple myeloma. *Mol Cancer Ther.* **2002;** 1: 539-44.

[37] de Gorter DJ, Reijmers RM, Beuling EA, Naber HP, Kuil A, Kersten MJ, Pals ST, Spaargaren M. The small GTPase Ral mediates SDF-1-induced migration of B cells and multiple myeloma cells. *Blood.* **2008;** 111: 3364-72.

[38] Fong AM, Premont RT, Richardson RM, Yu YR, Lefkowitz RJ, Patel DD. Defective lymphocyte chemotaxis in beta-arrestin2- and GRK6-deficient mice. *Proc Natl Acad Sci U S A.* **2002;** 99: 7478-83.

[39] Sun Y, Cheng Z, Ma L, Pei G. Beta-arrestin2 is critically involved in CXCR4-mediated chemotaxis, and this is mediated by its enhancement of p38 MAPK activation. *J Biol Chem.* **2002;** 277: 49212-9.

[40] Etienne-Manneville S, Hall A. Rho GTPases in cell biology. *Nature.* **2002;** 420: 629-35.

[41] Azab AK, Azab F, Blotta S, Pitsillides CM, Thompson B, Runnels JM, Roccaro AM, Ngo HT, Melhem MR, Sacco A, Jia X, Anderson KC, Lin CP,

Rollins BJ, Ghobrial IM. RhoA and Rac1 GTPases play major and differential roles in stromal cell-derived factor-1-induced cell adhesion and chemotaxis in multiple myeloma. *Blood.* **2009;** 114: 619-29.

[42] Leung T, Chen XQ, Manser E, Lim L. The p160 RhoA-binding kinase ROK alpha is a member of a kinase family and is involved in the reorganization of the cytoskeleton. *Mol Cell Biol.* **1996;** 16: 5313-27.

[43] Matsui T, Amano M, Yamamoto T, Chihara K, Nakafuku M, Ito M, Nakano T, Okawa K, Iwamatsu A, Kaibuchi K. Rho-associated kinase, a novel serine/threonine kinase, as a putative target for small GTP binding protein Rho. *EMBO J.* **1996;** 15: 2208-16.

[44] Amano M, Fukata Y, Kaibuchi K. Regulation and functions of Rho-associated kinase. *Exp Cell Res.* **2000;** 261: 44-51.

[45] Kamm KE, Stull JT. Signaling to myosin regulatory light chain in sarcomeres. *J Biol Chem.* **2011;** 286: 9941-7.

[46] Basak GW, Srivastava AS, Malhotra R, Carrier E. Multiple myeloma bone marrow niche. *Curr Pharm Biotechnol.* **2009;** 10:345-6.

[47] Mitsiades CS, McMillin DW, Klippel S, Hideshima T, Chauhan D, Richardson PG, Munshi NC, Anderson KC. The role of the bone marrow microenvironment in the pathophysiology of myeloma and its significance in the development of more effective therapies. *Hematol Oncol Clin North Am.* **2007;** 21: 1007-34, vii-viii.

[48] Caligaris-Cappio F, Gregoretti MG, Merico F, Gottardi D, Ghia P, Parvis G, Bergui L. Bone marrow microenvironment and the progression of multiple myeloma. *Leuk Lymphoma.* **1992;** 8: 15-22.

[49] Hayashi T, Hideshima T, Nguyen AN, Munoz O, Podar K, Hamasaki M, Ishitsuka K, Yasui H, Richardson P, Chakravarty S, Murphy A, Chauhan D, Higgins LS, Anderson KC. Transforming growth factor beta receptor I kinase inhibitor down-regulates cytokine secretion and multiple myeloma cell growth in the bone marrow microenvironment. *Clin Cancer Res.* **2004;** 10: 7540-6.

[50] Podar K, Chauhan D, Anderson KC. Bone marrow microenvironment and the identification of new targets for myeloma therapy. *Leukemia.* **2009;** 23: 10-24.

[51] Chauhan D, Uchiyama H, Akbarali Y, Urashima M, Yamamoto K, Libermann TA, Anderson KC. Multiple myeloma cell adhesion-induced interleukin-6 expression in bone marrow stromal cells involves activation of NF-kappa B. *Blood.* **1996;** 87: 1104-12.

[52] Barillé S, Akhoundi C, Collette M, Mellerin MP, Rapp MJ, Harousseau JL, Bataille R, Amiot M. Metalloproteinases in multiple myeloma: production of matrix metalloproteinase-9 (MMP-9), activation of proMMP-2, and induction of MMP-1 by myeloma cells. *Blood.* **1997;** 90: 1649-55.

[53] Dankbar B, Padró T, Leo R, Feldmann B, Kropff M, Mesters RM, Serve H, Berdel WE, Kienast J. Vascular endothelial growth factor and interleukin-6 in paracrine tumor-stromal cell interactions in multiple myeloma. *Blood.* **2000;** 95: 2630-6.

[54] Uchiyama H, Barut BA, Mohrbacher AF, Chauhan D, Anderson KC. Adhesion of human myeloma-derived cell lines to bone marrow stromal cells stimulates interleukin-6 secretion. *Blood.* **1993;** 82: 3712-20.

[55] Gupta D, Treon SP, Shima Y, Hideshima T, Podar K, Tai YT, Lin B, Lentzsch S, Davies FE, Chauhan D, Schlossman RL, Richardson P, Ralph P, Wu L, Payvandi F, Muller G, Stirling DI, Anderson KC. Adherence of multiple myeloma cells to bone marrow stromal cells upregulates vascular endothelial growth factor secretion: therapeutic applications. *Leukemia.* **2001;** 15: 1950-61.

[56] Damiano JS, Cress AE, Hazlehurst LA, Shtil AA, Dalton WS. Cell adhesion mediated drug resistance (CAM-DR): role of integrins and resistance to apoptosis in human myeloma cell lines. *Blood.* **1999;** 93: 1658-67.

[57] Damiano JS, Dalton WS. Integrin-mediated drug resistance in multiple myeloma. *Leuk Lymphoma.* **2000;** 38: 71-81.

[58] Vincent T, Mechti N. Extracellular matrix in bone marrow can mediate drug resistance in myeloma. *Leuk Lymphoma.* **2005;** 46: 803-11.

[59] Zdzisińska B, Walter-Croneck A, Kandefer-Szerszeń M. Matrix metalloproteinases-1 and -2, and tissue inhibitor of metalloproteinase-2 production is abnormal in bone marrow stromal cells of multiple myeloma patients. *Leuk Res.* **2008;** 32: 1763-9.

[60] Kelly T, Børset M, Abe E, Gaddy-Kurten D, Sanderson RD. Matrix metalloproteinases in multiple myeloma. *Leuk Lymphoma.* **2000;** 37: 273-81.

[61] Abe M, Hiura K, Wilde J, Shioyasono A, Moriyama K, Hashimoto T, Kido S, Oshima T, Shibata H, Ozaki S, Inoue D, Matsumoto T. Osteoclasts enhance myeloma cell growth and survival via cell-cell contact: a vicious cycle between bone destruction and myeloma expansion. *Blood.* **2004;** 104: 2484-91.

[62] Bataille R, Chappard D, Marcelli C, Dessauw P, Sany J, Baldet P, Alexandre C. Mechanisms of bone destruction in multiple myeloma: the

importance of an unbalanced process in determining the severity of lytic bone disease. *J Clin Oncol.* **1989;** 7: 1909-14.

[63] Giuliani N, Bataille R, Mancini C, Lazzaretti M, Barillé S. Myeloma cells induce imbalance in the osteoprotegerin/osteoprotegerin ligand system in the human bone marrow environment. *Blood.* **2001;** 98: 3527-33.

[64] Ashcroft AJ, Davies FE, Morgan GJ. Aetiology of bone disease and the role of bisphosphonates in multiple myeloma. *Lancet Oncol.* **2003;** 4: 284-92.

[65] Tian E, Zhan F, Walker R, Rasmussen E, Ma Y, Barlogie B, Shaughnessy JD Jr. 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.

[66] Raje N, Roodman GD. Advances in the biology and treatment of bone disease in multiple myeloma. *Clin Cancer Res.* **2011;** 17: 1278-86.

[67] Seidel C, Hjertner Ø, Abildgaard N, Heickendorff L, Hjorth M, Westin J, Nielsen JL, Hjorth-Hansen H, Waage A, Sundan A, Børset M; Nordic Myeloma Study Group. Serum osteoprotegerin levels are reduced in patients with multiple myeloma with lytic bone disease. *Blood.* **2001;** 98: 2269-71.

[68] Roux S, Meignin V, Quillard J, Meduri G, Guiochon-Mantel A, Fermand JP, Milgrom E, Mariette X. RANK (receptor activator of nuclear factor-kappaB) and RANKL expression in multiple myeloma. *Br J Haematol.* **2002;** 117: 86-92.

[69] Martin SK, Diamond P, Gronthos S, Peet DJ, Zannettino AC. The emerging role of hypoxia, HIF-1 and HIF-2 in multiple myeloma. *Leukemia.* **2011;** 25: 1533-42.

[70] Asosingh K, De Raeve H, de Ridder M, Storme GA, Willems A, Van Riet I, Van Camp B, Vanderkerken K. Role of the hypoxic bone marrow microenvironment in 5T2MM murine myeloma tumor progression. *Haematologica.* **2005;** 90: 810-7.

[71] Colla S, Storti P, Donofrio G, Todoerti K, Bolzoni M, Lazzaretti M, Abeltino M, Ippolito L, Neri A, Ribatti D, Rizzoli V, Martella E, Giuliani N. Low bone marrow oxygen tension and hypoxia-inducible factor-1alpha overexpression characterize patients with multiple myeloma: role on the transcriptional and proangiogenic profiles of CD138(+) cells. *Leukemia.* **2010;** 24: 1967-70.

[72] Danet GH, Pan Y, Luongo JL, Bonnet DA, Simon MC. Expansion of human SCID-repopulating cells under hypoxic conditions. *J Clin Invest.* **2003;** 112: 126-35.

[73] Hu J, Handisides DR, Van Valckenborgh E, De Raeve H, Menu E, Vande Broek I, Liu Q, Sun JD, Van Camp B, Hart CP, Vanderkerken K. Targeting the multiple myeloma hypoxic niche with TH-302, a hypoxia-activated prodrug. *Blood.* **2010;** 116: 1524-7.

[74] Ceradini DJ, Kulkarni AR, Callaghan MJ, Tepper OM, Bastidas N, Kleinman ME, Capla JM, Galiano RD, Levine JP, Gurtner GC. Progenitor cell trafficking is regulated by hypoxic gradients through HIF-1 induction of SDF-1. *Nat Med.* **2004;** 10: 858-64.

[75] Staller P, Sulitkova J, Lisztwan J, Moch H, Oakeley EJ, Krek W. Chemokine receptor CXCR4 downregulated by von Hippel-Lindau tumour suppressor pVHL. *Nature.* **2003;** 425: 307-11.

[76] Schioppa T, Uranchimeg B, Saccani A, Biswas SK, Doni A, Rapisarda A, Bernasconi S, Saccani S, Nebuloni M, Vago L, Mantovani A, Melillo G, Sica A. Regulation of the chemokine receptor CXCR4 by hypoxia. *J Exp Med.* **2003;** 198: 1391-402.

[77] Giatromanolaki A, Bai M, Margaritis D, Bourantas KL, Koukourakis MI, Sivridis E, Gatter KC. Hypoxia and activated VEGF/receptor pathway in multiple myeloma. *Anticancer Res.* **2010;** 30: 2831-6.

[78] Martin SK, Diamond P, Williams SA, To LB, Peet DJ, Fujii N, Gronthos S, Harris AL, Zannettino AC.Hypoxia-inducible factor-2 is a novel regulator of aberrant CXCL12 expression in multiple myeloma plasma cells. *Haematologica.* **2010;** 95: 776-84.

[79] Stefanidakis M, Koivunen E. Cell-surface association between matrix metalloproteinases and integrins: role of the complexes in leukocyte migration and cancer progression. *Blood.* **2006;** 108: 1441-50.

[80] Gialeli C, Theocharis AD, Karamanos NK. Roles of matrix metalloproteinases in cancer progression and their pharmacological targeting. *FEBS J.* **2011;** 278: 16-27.

[81] Egeblad M, Werb Z. New functions for the matrix metalloproteinases in cancer progression. *Nat Rev Cancer.* **2002;** 2: 161-74.

[82] Nagase H, Woessner JF Jr. Matrix metalloproteinases. *J Biol Chem.* **1999;** 274: 21491-4.

[83] McCawley LJ, Matrisian LM. Matrix metalloproteinases: they're not just for matrix anymore! *Curr Opin Cell Biol.* **2001;** 13: 534-40.

[84] Stamenkovic I. Extracellular matrix remodelling: the role of matrix metalloproteinases. *J Pathol.* **2003;** 200: 448-64.

[85] Bourboulia D, Stetler-Stevenson WG. Matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs): Positive and negative regulators in tumor cell adhesion. *Semin Cancer Biol.* **2010;** 20: 161-8.

[86] Visse R, Nagase H. Matrix metalloproteinases and tissue inhibitors of metalloproteinases:

structure, function, and biochemistry. *Circ Res.* **2003;** 92: 827-39.

[87] Hojilla CV, Mohammed FF, Khokha R. Matrix metalloproteinases and their tissue inhibitors direct cell fate during cancer development. *Br J Cancer.* **2003;** 89: 1817-21.

[88] Cruz-Munoz W, Khokha R. The role of tissue inhibitors of metalloproteinases in tumorigenesis and metastasis. *Crit Rev Clin Lab Sci.* **2008;** 45: 291-338.

[89] Sternlicht MD, Werb Z. How matrix metalloproteinases regulate cell behavior. *Annu Rev Cell Dev Biol.* **2001;** 17: 463-516.

[90] Parmo-Cabañas M, Molina-Ortiz I, Matías-Román S, García-Bernal D, Carvajal-Vergara X, Valle I, Pandiella A, Arroyo AG, Teixidó J. Role of metalloproteinases MMP-9 and MT1-MMP in CXCL12-promoted myeloma cell invasion across basement membranes. *J Pathol.* **2006;** 208: 108-18.

[91] Bergers G, Brekken R, McMahon G, Vu TH, Itoh T, Tamaki K, Tanzawa K, Thorpe P, Itohara S, Werb Z, Hanahan D. Matrix metalloproteinase-9 triggers the angiogenic switch during carcinogenesis. *Nat Cell Biol.* **2000;** 2: 737-44.

[92] Van Valckenborgh E, Croucher PI, De Raeve H, Carron C, De Leenheer E, Blacher S, Devy L, Noël A, De Bruyne E, Asosingh K, Van Riet I, Van Camp B, Vanderkerken K. Multifunctional role of matrix metalloproteinases in multiple myeloma: a study in the 5T2MM mouse model. *Am J Pathol.* **2004;** 165: 869-78.

[93] Podar K, Tai YT, Lin BK, Narsimhan RP, Sattler M, Kijima T, Salgia R, Gupta D, Chauhan D, Anderson KC. Vascular endothelial growth factor-induced migration of multiple myeloma cells is associated with beta 1 integrin- and phosphatidylinositol 3-kinase-dependent PKC alpha activation. *J Biol Chem.* **2002;** 277: 7875-81.

[94] Hurt EM, Wiestner A, Rosenwald A, Shaffer AL, Campo E, Grogan T, Bergsagel PL, Kuehl WM, Staudt LM. Overexpression of c-maf is a frequent oncogenic event in multiple myeloma that promotes proliferation and pathological interactions with bone marrow stroma. *Cancer Cell.* **2004;** 5: 191-9.

[95] Holt RU, Baykov V, Rø TB, Brabrand S, Waage A, Sundan A, Børset M. Human myeloma cells adhere to fibronectin in response to hepatocyte growth factor. *Haematologica.* **2005;** 90: 479-88.

[96] Pellat-Deceunynck C, Barillé S, Puthier D, Rapp MJ, Harousseau JL, Bataille R, Amiot M. Adhesion molecules on human myeloma cells: significant changes in expression related to malignancy, tumor spreading, and immortalization. *Cancer Res.* **1995;** 55: 3647-53.

[97] Sanz-Rodríguez F, Ruiz-Velasco N, Pascual-Salcedo D, Teixidó J. Characterization of VLA-4-dependent myeloma cell adhesion to fibronectin and VCAM-1. *Br J Haematol.* **1999;** 107: 825-34.

[98] Van Riet I, De Waele M, Remels L, Lacor P, Schots R, Van Camp B. Expression of cytoadhesion molecules (CD56, CD54, CD18 and CD29) by myeloma plasma cells. *Br J Haematol.* **1991;** 79: 421-7.

[99] Uchiyama H, Barut BA, Chauhan D, Cannistra SA, Anderson KC. Characterization of adhesion molecules on human myeloma cell lines. *Blood.* **1992;** 80: 2306-14.

[100] Neri P, Ren L, Azab AK, Brentnall M, Gratton K, Klimowicz AC, Lin C, Duggan P, Tassone P, Mansoor A, Stewart DA, Boise LH, Ghobrial IM, Bahlis NJ. Integrin {beta}7-mediated regulation of multiple myeloma cell adhesion, migration, and invasion. *Blood.* **2011;** 117: 6202-13.

[101] Mitsiades CS, Anderson KC, Carrasco DR. Mouse models of human myeloma. *Hematol Oncol Clin North Am.* **2007;** 21: 1051-69, viii.

[102] Vanderkerken K, De Raeve H, Goes E, Van Meirvenne S, Radl J, Van Riet I, Thielemans K, Van Camp B. Organ involvement and phenotypic adhesion profile of 5T2 and 5T33 myeloma cells in the C57BL/KaLwRij mouse. *Br J Cancer.* **1997;** 76: 451-60.

[103] Asosingh K, Radl J, Van Riet I, Van Camp B, Vanderkerken K. The 5TMM series: a useful *in vivo* mouse model of human multiple myeloma. *Hematol J.* **2000;** 1: 351-6.

[104] Vanderkerken K, Asosingh K, Croucher P, Van Camp B. Multiple myeloma biology: lessons from the 5TMM models. *Immunol Rev.* **2003;** 194: 196-206.

[105] LeBlanc R, Catley LP, Hideshima T, Lentzsch S, Mitsiades CS, Mitsiades N, Neuberg D, Goloubeva O, Pien CS, Adams J, Gupta D, Richardson PG, Munshi NC, Anderson KC. Proteasome inhibitor PS-341 inhibits human myeloma cell growth *in vivo* and prolongs survival in a murine model. *Cancer Res.* **2002;** 62: 4996-5000.

[106] Pilarski LM, Hipperson G, Seeberger K, Pruski E, Coupland RW, Belch AR. Myeloma progenitors in the blood of patients with aggressive or minimal disease: engraftment and self-renewal of primary human myeloma in the bone marrow of NOD SCID mice. *Blood.* **2000;** 95: 1056-65.

[107] Novak J, Georgakoudi I, Wei X, Prossin A, Lin CP. *In vivo* flow cytometer for real-time detection and quantification of circulating cells. *Opt Lett.* **2004;** 29: 77-9.

[108] Azab AK, Runnels JM, Pitsillides C, Moreau AS, Azab F, Leleu X, Jia X, Wright R, Ospina B, Carlson AL, Alt C, Burwick N, Roccaro AM, Ngo HT, Farag

M, Melhem MR, Sacco A, Munshi NC, Hideshima T, Rollins BJ, Anderson KC, Kung AL, Lin CP, Ghobrial IM. CXCR4 inhibitor AMD3100 disrupts the interaction of multiple myeloma cells with the bone marrow microenvironment and enhances their sensitivity to therapy. *Blood.* **2009;** 113: 4341-51.

[109] Mitsiades CS, Mitsiades NS, McMullan CJ, Poulaki V, Shringarpure R, Akiyama M, Hideshima T, Chauhan D, Joseph M, Libermann TA, García-Echeverría C, Pearson MA, Hofmann F, Anderson KC, Kung AL. Inhibition of the insulin-like growth factor receptor-1 tyrosine kinase activity as a therapeutic strategy for multiple myeloma, other hematologic malignancies, and solid tumors. *Cancer Cell.* **2004;** 5: 221-30.

[110] Mitsiades CS, Mitsiades NS, Bronson RT, Chauhan D, Munshi N, Treon SP, Maxwell CA, Pilarski L, Hideshima T, Hoffman RM, Anderson KC. Fluorescence imaging of multiple myeloma cells in a clinically relevant SCID/NOD *in vivo* model: biologic and clinical implications. *Cancer Res.* **2003;** 63: 6689-96.

[111] Sipkins DA, Wei X, Wu JW, Runnels JM, Côté D, Means TK, Luster AD, Scadden DT, Lin CP. *In vivo* imaging of specialized bone marrow endothelial microdomains for tumour engraftment. *Nature.* **2005;** 435: 969-73.

[112] Pelus LM, Horowitz D, Cooper SC, King AG. Peripheral blood stem cell mobilization. A role for CXC chemokines. *Crit Rev Oncol Hematol.* **2002;** 43: 257-75.

[113] Lapidot T, Petit I. Current understanding of stem cell mobilization: the roles of chemokines, proteolytic enzymes, adhesion molecules, cytokines, and stromal cells. *Exp Hematol.* **2002;** 30: 973-81.

[114] Devine SM, Vij R, Rettig M, Todt L, McGlauchlen K, Fisher N, Devine H, Link DC, Calandra G, Bridger G, Westervelt P, Dipersio JF. Rapid mobilization of functional donor hematopoietic cells without G-CSF using AMD3100, an antagonist of the CXCR4/SDF-1 interaction. *Blood.* **2008;** 112: 990-8.

[115] Devine SM, Flomenberg N, Vesole DH, Liesveld J, Weisdorf D, Badel K, Calandra G, DiPersio JF. Rapid mobilization of CD34+ cells following administration of the CXCR4 antagonist AMD3100 to patients with multiple myeloma and non-Hodgkin's lymphoma. *J Clin Oncol.* **2004;** 22: 1095-102.

[116] Liles WC, Rodger E, Broxmeyer HE, Dehner C, Badel K, Calandra G, Christensen J, Wood B, Price TH, Dale DC. Augmented mobilization and collection of CD34+ hematopoietic cells from normal human volunteers stimulated with granulocyte-colony-stimulating factor by single-dose administration of AMD3100, a CXCR4 antagonist. *Transfusion.* **2005;** 45: 295-300.

[117] Broxmeyer HE, Orschell CM, Clapp DW, Hangoc G, Cooper S, Plett PA, Liles WC, Li X, Graham-Evans B, Campbell TB, Calandra G, Bridger G, Dale DC, Srour EF. Rapid mobilization of murine and human hematopoietic stem and progenitor cells with AMD3100, a CXCR4 antagonist. *J Exp Med.* **2005;** 201: 1307-18.

[118] Flomenberg N, Devine SM, Dipersio JF, Liesveld JL, McCarty JM, Rowley SD, Vesole DH, Badel K, Calandra G. The use of AMD3100 plus G-CSF for autologous hematopoietic progenitor cell mobilization is superior to G-CSF alone. *Blood.* **2005;** 106: 1867-74.

[119] Calandra G, McCarty J, McGuirk J, Tricot G, Crocker SA, Badel K, Grove B, Dye A, Bridger G. AMD3100 plus G-CSF can successfully mobilize CD34+ cells from non-Hodgkin's lymphoma, Hodgkin's disease and multiple myeloma patients previously failing mobilization with chemotherapy and/or cytokine treatment: compassionate use data. *Bone Marrow Transplant.* **2008;** 41: 331-8.

[120] Fruehauf S, Ehninger G, Hübel K, Topaly J, Goldschmidt H, Ho AD, Müller S, Moos M, Badel K, Calandra G. Mobilization of peripheral blood stem cells for autologous transplant in non-Hodgkin's lymphoma and multiple myeloma patients by plerixafor and G-CSF and detection of tumor cell mobilization by PCR in multiple myeloma patients. *Bone Marrow Transplant.* **2010;** 45: 269-75.

[121] Zhang WB, Navenot JM, Haribabu B, Tamamura H, Hiramatu K, Omagari A, Pei G, Manfredi JP, Fujii N, Broach JR, Peiper SC. A point mutation that confers constitutive activity to CXCR4 reveals that T140 is an inverse agonist and that AMD3100 and ALX40-4C are weak partial agonists. *J Biol Chem.* **2002;** 277: 24515-21.

[122] Trent JO, Wang ZX, Murray JL, Shao W, Tamamura H, Fujii N, Peiper SC. Lipid bilayer simulations of CXCR4 with inverse agonists and weak partial agonists. *J Biol Chem.* **2003;** 278: 47136-44.

[123] Kim HY, Hwang JY, Kim SW, Lee HJ, Yun HJ, Kim S, Jo DY. The CXCR4 Antagonist AMD3100 Has Dual Effects on Survival and Proliferation of Myeloma Cells In Vitro. *Cancer Res Treat.* **2010;** 42: 225-34.

[124] Folgueras AR, Pendás AM, Sánchez LM, López-Otín C. Matrix metalloproteinases in cancer: from new functions to improved inhibition strategies. *Int J Dev Biol.* **2004;** 48: 411-24.

[125] López-Otín C, Palavalli LH, Samuels Y. Protective roles of matrix metalloproteinases: from mouse models to human cancer. *Cell Cycle.* **2009;** 8: 3657-62.

[126] Overall CM, Kleifeld O. Tumour microenvironment - opinion: validating matrix metalloproteinases as drug targets and anti-targets for cancer therapy. *Nat Rev Cancer.* **2006;** 6: 227-39.