5

Targeted Inhibition of Multiple Proinflammatory Signalling Pathways for the Prevention and Treatment of Multiple Myeloma

Radhamani Kannaiyan[1], Rohit Surana[1,2], Eun Myoung Shin[2], Lalitha Ramachandran[1], Gautam Sethi[1,2,*] and Alan Prem Kumar[1,2,3,*]

[1]Department of Pharmacology, Yong Loo Lin School of Medicine, National University of Singapore,
[2]Cancer Science Institute of Singapore, National University of Singapore,
[3]School of Anatomy and Human Biology, The University of Western Australia, Crawley, Perth, Western Australia
[1,2]Singapore
[3]Australia

1. Introduction

Multiple myeloma (MM) is a B cell malignancy involving the post germinal centre B cells. The disease is characterized by the presence of blood and urinary monoclonal proteins, osteolytic bone lesions and infiltration of bone marrow with malignant plasma cells of low proliferative index. Multiple myeloma is mainly a disease of elderly males, but, there is evidence to support that there is increasing incidence in younger individuals as well. American blacks are more prone than American whites. MM is the most common non Hodgkin's haematological malignancy, contributing 13% of all malignancies and 1% of all neoplasias. The median survival is 3-4 years, but with autologous stem cell transplantation and high dose chemotherapy, the median survival has increased to 5-7 years [1].

Most, if not all, multiple myeloma evolve from a premalignant condition known as 'Multiple Gammapathy of Undetermined Significance (MGUS)'. It then progresses via a 'smouldering multiple myeloma' stage, to a full blown disease and finally to an 'extramedullary MM' condition, where the malignant cells are no longer dependent on the bone marrow microenvironment for their proliferation. On a cellular scale, the origin of MM is thought to be post germinal centre B cell or memory B cell, indicated by the presence of hypermutated immunoglobulin gene. Evidence also supports the stem cell origin of the disease, as indicated by activated Wnt and Hedgehog signalling in the subset of cells in MM primary samples [2].

Cornelius Celsus, a Roman physician, first described the features of inflammation (inflammation - to set on fire) with the following signs: heat (calor), pain (dolour), redness (rubor) and swelling (tumour). The main purpose of inflammation is to protect the host

* Corresponding Authors

organism from the microbes and other noxious stimuli. However, when the infection cannot be controlled or when there is a constant presence of the damaging stimuli, inflammatory process gets deregulated, resulting in a condition called chronic inflammation, which is destructive to the host organism. Thus inflammation is aptly termed as a 'double edged sword'. The link between inflammation and cancer was first established in 1897, by a German pathologist named Dr. Rudolf Virchow. He found that leukocytes infiltrate tumour tissue and therefore, termed tumours as 'wounds that do not heal'. Since then there has been much evidence to link inflammation and cancer, so as to be able to add inflammation as one of the hallmarks of cancer [3-5].

In the linking of inflammation and cancer, two pathways are said to exist - extrinsic and intrinsic. In the extrinsic pathway, chronic inflammation leads to autoimmune diseases, which eventually culminate in cancer. For example, H.pylori infection in the stomach, Hepatitis B and Hepatitis C infections in the liver, inflammatory bowel diseases and inflammation of the prostate gland (prostatitis); lead to incidences of gastric cancer, hepatocarcinoma, colon cancer and prostate cancer, respectively. In fact, about 20% of all cancers are said to arise in an inflammatory environment. In the intrinsic pathway, activation of oncogenes or inactivation of the tumour suppressor genes, causing both cancer and inflammation, which complement each other [6, 7]. Irrespective of the pathways involved, the perpetrators of the cancer related inflammation are inflammatory cells and inflammatory mediators, such as cytokines, chemokines, growth factors, all of which finally converge on a few transcription factors [8]. Not surprisingly, agents modulating cancer-related inflammation have been tried in cancer therapeutics [9].

MM cells depend largely on a bone marrow microenvironment for their growth and survival, until the last stage of the disease, where they invade other areas to be termed as extramedullary MM. The bone marrow microenvironment can be broadly divided into cellular and non-cellular components. Cellular components include myeloma cells, bone marrow stromal cells or bone marrow fibroblasts, haematopoietic precursor cells, osteoclasts, osteoblasts, endothelial cells and immune cells. Of these, the supportive role of stromal cells in MM has been studied extensively. The interactions between myeloma cells and osteoclasts have also been studied to an extent. The bone marrow stromal cells and osteoclasts provide the myeloma cells with the ability to grow and survive, either by direct adhesion and/or by secreting growth and survival cytokines.

The non-cellular compartment is comprised of the extracellular matrix and the soluble factors. Extracellular matrix consists of various proteins like collagen, fibronectin and laminin. The extracellular matrix not only acts as depots for the growth factors, but also provides the myeloma cells with the ability to resist cell death induced by chemotherapeutic agents. The survival advantage offered by the bone marrow microenvironment to the MM cells is achieved by 1. the soluble growth factors which are secreted by various cellular components, 2. insoluble growth factors that are bound to the extracellular matrix component and 3. adhesion molecules that help MM cells adhere to the extracellular matrix and the cellular compartment. In fact, in a recent study, 22 out of the 51 multiple myeloma growth factor genes that could be interrogated by affymetrix were found to be significantly overexpressed by at least one bone marrow environment population compared to others [10].

The stromal derived factor (SDF/CXCL12), secreted by the bone marrow stromal cells, plays an important role in the homing of MM cells to the bone marrow, which expresses receptor CXCR4. Moreover, adhesion of MM cells to stromal cells or fibronectin, induces chemoresistance in MM cells, mediated by integrins [11]. The adhesion molecules namely, very late antigen (VLA-4), vascular cell adhesion molecule (VCAM-1) and lymphocyte function–associated antigen 1 (LFA-1), intercellular adhesion molecule (ICAM–1), mediate integrin induced chemoresistance [12]. The resistance is mediated partly due to the activation of NF-κB, which upregulates anti-apoptotic gene products. MM samples are found to have various mutations activating both classical and alternative NF-κB. Apart from the mutations, the NF-κB pathway can also be stimulated by B cell growth factors like BAF and APRIL, which are secreted by the bone marrow microenvironment [13].

Adhesion of MM cells to the stromal cells, induces the latter to secrete IL-6. IL-6 is the main growth factor for the MM cells. IL-6 then induces JAK/STAT 3, PI3/AKT and MAPK survival pathways. STAT 3 transcription factor upregulates its targets, namely, cyclin D1 and Mcl-1, which promote cell proliferation and antiapoptosis respectively. In addition to the IL-6 induced activation of STAT 3, DNA methylation is found to silence the negative regulators of STAT 3. On the other hand, IGF secreted by bone marrow stromal cells induces PI3/AKT pathways [14]. AKT promotes cell proliferation by phosphorilating GSK3β, which regulates cyclin D1 proteolysis. Activated MAPK pathway leads to the activation of ERK, promoting MM growth and survival [15]. The following section will elaborate on the very common and important inflammatory player, involved in the progression of MM.

2. Role of proinflammatory cytokines and growth factors

2.1 Interleukin - 6

Interleukin-6, a pleotropic cytokine, is involved in processes such as haematopoiesis, immunity and inflammation. It was discovered as a factor secreted from mitogen stimulated T cells, which helps mature B cells transform into antibody producing plasma cells [16]. Because of its pleotropic nature, various laboratories were working with its different functions, giving it different names: B cell stimulating factor II (BSF II) as it stimulated B cells to turn into plasma cells and secrete antibodies, interferon-ß2 [17] as it was thought to have the properties of interferon but later it was proven that IL-6 does not have properties of interferon, 26 kDa protein - named after its molecular weight, a hybridoma/plasmacytoma growth factor as it induced plasmacytoma in balb/c mice injected with mineral oil [18] and a hepatocyte-stimulating factor as it stimulated hepatocytes to produce acute phase proteins [19].

IL-6 binds to its receptor, which is either membrane bound or in soluble form. It then activates ubiquitously expressed receptor gp130 [20]. Once gp130 gets activated, IL-6 acts by three of the following signalling pathways: JAK-STAT pathway, MAPK-ERK and PI3-AKT pathway. Most of the actions of IL-6 are executed by JAK-STAT pathway [21]. IL-6 is found to be involved in the growth of many solid tumours like prostate cancer and renal cancer. Pathogenesis of Kaposi sarcoma has been proven to be due to the secretion of IL-6 [22-24]. IL-6 is also involved in the growth of many haematological malignancies.

IL-6 is one of the main growth factors in multiple myeloma [25]. In fact, IL-6 knock out mice failed to develop MM [26]. Moreover, the serum level of IL-6 and soluble IL-6 receptor has been proven to be a prognostic marker for tumour load, disease progression and survival [27-31]. Moreover, serum levels of IL-6 in patients with smouldering MM and monoclonal gammapathy of undetermined significance are comparable with healthy individuals, indicating the important role of IL-6 in the disease progression [32].

Initially, it was thought based on the following findings that myeloma cells secrete and respond to IL-6 in an autocrine manner. Firstly, IL-6 induces in vitro growth of freshly isolated MM cells. Secondly, MM cells express the IL-6 receptor (IL-6R). Thirdly, purified MM cells produce IL-6 and lastly, in vitro growth of MM cells is inhibited by anti-IL-6 antibodies [33]. But, again controversies prevailed among the research laboratories on the autocrine secretion of IL-6 by myeloma cells. Because, though all myeloma derived cell lines and patients cells express IL-6 receptor, only subsets of cell lines express IL-6 mRNA [34]. It was also found that bone marrow stromal cells are the main source of IL-6 [35-37]. Interestingly, when myeloma cells were co-cultured with bone marrow stromal cells, they tend to adhere to each other tightly and the IL-6 secretion by these cells reaches the peak. But, when the bone marrow stromal cells were fixed by paraformaldehyde, there was no increase in the level of IL- 6, confirming that the source of IL-6 was bone marrow stromal cells and not myeloma cells. Moreover, it was found that the stromal cells secrete IL-6 when stimulated by the adhesion of myeloma cells to the stromal cells. This is evident from experimental setup where these cells were cultured in transwell chambers without any physical contact with the myeloma cells. As a result, the bone marrow cells failed to secrete IL-6, emphasising the importance of adhesion molecules in the cross talk between the group of cells and pathophysiology of myeloma [38]. The adhesion mediated secretion of IL-6 was found to be NF-κB dependent [39].

In addition to bone marrow stromal cells, adhesion of myeloma cells to the peripheral blood derived osteoclastic cells protected myeloma cells from serum deprivation induced apoptosis and doxorubicin induced apoptosis. Osteoclasts produced osteopontin (OPN) and IL-6, and adhesion of MM cells to osteocleasts increased IL-6 production from osteoclasts. In addition, IL-6 and osteopontin in combination, enhanced MM cell growth and survival. However, the effects of osteoclasts on MM cell growth and survival were only partially suppressed by a simultaneous addition of anti–IL-6 and anti-osteopontin antibodies and were completely abrogated by inhibition of cellular contact between MM cells and osteoclasts. Osteoclasts enhance MM cell growth and survival through a cell-cell contact-mediated mechanism that is partially dependent on IL-6 and osteopontin [40].

The IL-6 induced survival of myeloma cells is mediated by STAT3, which upregulates anti-apoptotic proteins Bcl-XL and Mcl-1 and cell cycle proteins like cyclin D1, c-Myc and Pim. The IL-6 induced proliferation is mediated by MAPK-ERK pathway [41]. A PI3-AKT pathway mediates proliferation and induces survival by phosphorilating Bad and activating cell cycle proteins and NF-κB. Gene expression profiling studies demonstrated that out of 138 genes shown to be regulated by IL-6 in myeloma cells, 54% regulated cell cycle progression. This finding emphasises the role of IL-6 in myeloma cell proliferation [42]. IL-6 was shown to inhibit Fos induced apoptosis [43]. IL-6 can inhibit dexamethasone induced apoptosis of myeloma cells by gp130 induced activation of SHP2, which deactivates related adhesion focal tyrosine kinase (RAFTK) [44, 45] and activates the PI3/AKT pathway [46]. Partial reduction in the levels of IL-6 can sensitise the myeloma cells to chemotherapeutic agents [47, 48].

Various strategies, including IL-6 antagonist, IL-6 receptor inhibitor (CNTO 328), antisense oligonucleotides against IL-6 and IL-6 super antagonist (SANT7), have been tried for MM, but even after effectively blocking IL-6 receptor by the monoclonal antibody, the results were disappointing in clinical trials [49]. Accordingly, in the presence of bone marrow stromal cells, IL-6 receptor inhibition did not induce apoptosis, indicating the significance of the pleotropism offered by other growth and survival factors present in the bone marrow microenvironment [50, 51].

2.2 TNFα

In 1894, William Coley noticed that an injection of bacterial extracts into the tumour, could induce necrosis of tumours [52]. O'Malley et al. demonstrated that serum from mice injected with bacterial endotoxin can induce tumour regression [53]. The factor that can induce anti-cancer activity in vivo and in vitro, present in the sera of mice treated with endotoxin or LPS, was identified as Tumour Necrosis Factor α [54, 55]. The gene expressing human TNFα was cloned in 1984 [56]. Thereafter, the recombinant TNFα was used for experimental and therapeutic purposes. The therapeutic dose of TNFα induced serious hemodynamic instability and septic shock-like symptoms preclinically. TNFα can induce necrosis of the tumour by selective destruction of the blood vessels, only when injected at higher concentrations loco-regionally [57]. Its induction of apoptosis is highly context dependent. Physiologically, TNFα is an important cytokine regulating inflammation, immunity and haematopoiesis. Its deregulation is involved in lots of inflammatory and autoimmune conditions like rheumatoid arthritis and Crohn's disease. Recent research has realised the potent protumerigenic effect of TNFα [58]. TNFα KO and TNFα-R1 KO mice do not develop chemical carcinogen induced skin cancers [59, 60]. TNFα-R1 KO mice do not develop chemical carcinogen induced liver cancer [61]. TNFα antagonists are in various stages of clinical trials for a variety of cancers.

In MM, TNFα is not a strong growth factor, but it is an important factor secreted from myeloma cells to act on BMSCs to stimulate the secretion of IL-6. TNFα induces the expression of adhesion molecules on both myeloma cells and BMSCs. TNFα secreted by myeloma cells acts both directly and by increasing the adhesion between myeloma cells and the bone marrow stromal cells to secrete IL-6 by an NF-κB mediated mechanism in bone marrow stromal cells. TNFα is very potent when compared to other growth factors [62]. TNFα also participates in transendothelial migration of myeloma cells by acting via TNF-R2 and upregulating the secretion of MCP-1 in myeloma cells [63]. Clinically, the agents which are known to inhibit TNFα; namely, thalidomide and its derivates and bortezomib, have significant anti-myeloma activity.

2.3 BAFF and APRIL

BAFF and APRIL also belong to the TNF family of cytokines. They act by binding to receptors TACI (transmembrane activator and calcium modulator, and cyclophilin ligand interactor), BCMA (B-cell maturation antigen) and BAFF-R (BAFF Receptor) which is specific for BAFF. Myeloma cells express these receptors in a heterogeneous manner [64]. In fact, patient groups whose myeloma cells had low expression of TACI receptor were less differentiated and showed attenuated dependence on the bone marrow and portending poor prognosis; whereas patients whose myeloma cells express high levels of TACI receptor showed mature plasma cell signature exhibiting good prognosis [65]. There is evidence for these cytokines being secreted from myeloma cells [64, 66], bone marrow cells [67] and osteoclastic cells [65]. BAFF and APRIL seem to induce myeloma cell growth and inhibit dexamethasone induced apoptosis. BAFF and APRIL activate NF-κB, PI3kinase/AKT, and MAPK pathways in myeloma cells and induce a strong upregulation of the Mcl-1 and Bcl-2 anti-apoptotic proteins [68, 69]. Cell adhesion induced bone marrow cells secrete BAFF, which acts on myeloma cells to regulate their growth and survival [67]. Interestingly, bortezomib has been found to inhibit BAFF and APRIL induce proliferation of myeloma cells [66].

2.4 Insulin-like Growth Factor 1 (IGF-1)

Recent studies have delineated the role of IGF-1 in MM. IGF-1 was shown to be a strong indicator of prognosis in MM patients [70]. In the bone marrow milieu, IGF-1 is mainly produced and secreted from bone marrow stromal cells and mediates cell growth and survival in MM cells both in vitro [71, 72] and in vivo [73-75]. IGF-1 and its receptor were shown to be acting as growth factors [76] and preferentially expressed in MM cells [77] as compared to B-Lymphoblastoid cell lines.

IGF-1 inhibits Dexametasone-induced apoptosis in MM cell lines [78]. IGF-1 augments the proliferative and anti-apoptotic effects of IL-6 [71, 79] . Although IL-6 has mostly been described as a proliferation factor for MM, it has become clear that IGF-1 has an equally important proliferative and anti-apoptotic effect [80-82]. It could be that IGF-1 plays an even more pivotal role in the survival of MM cells, as IL-6 independent lines still respond to IGF-1 [80, 82]. Another group demonstrates that IGF-1 serves as a chemoattractant for MM cells [73]. In vivo induction of the receptor IGF-1R helps murine multiple myeloma cells in their homing and growth in the bone marrow [83].

IGF-1 transduces its signal by receptor phosphorylation of the insulin response substrate 1 and its activation of PI-3K and subsequently Akt kinase (PI-3K pathway). In fact, IGF-I increases adhesion of MM cell lines to fibronectin (FN) in a time and dose-dependent manner, as a consequence of IGF-1R activation and subsequent activation of β1- integrin and PI3-kinase/AKT signalling [84]. Several important biological characteristics have been associated with this segment of the PI-3K pathway [85]. Akt subsequently phosphorylates Bad, a member of the Bcl-2 family, producing an anti-apoptotic effect. The second pathway associated with IGF-I stimulation signals through the Shc, Grb-2, Sos complex, resulting in activation of Ras and subsequently the mitogen-activated protein kinase (MAPK) signalling cascade.

IGF-1 is also shown to mediate the activation of NF-κB [86], induce the phosphorylation of FKHR (forkhead) transcription factor, upregulate a series of intracellular anti-apoptotic proteins (including FLIP, survivin, cIAP-2, A1/Bfl-1 and XIAP) and decrease drug sensitivity of MM cells [75]. Caveolin-1, which is usually absent in blood cells, is expressed in MM cells and plays a crucial role in IGF-1-mediated signalling cascades [87]. Specifically, IGF-1 induces HIF-1α, which triggers VEGF expression [88, 89]; consequently, inhibition of IGFR-1 activity markedly decreases VEGF secretion in MM/BMSC co-cultures [75].

Therapies targeting IGF-1, such as inhibitors of IGF-1 receptor, have already shown preclinical anti-MM activity and will soon undergo clinical evaluation [75]. IGF-1R inhibition with neutralizing antibody, antagonistic peptide, or the selective kinase inhibitor NVP-ADW742 has in vitro activity against MM cell types and in orthotopic xenograft MM model had synergistic anti-tumour activity in combination with conventional chemotherapy. Another study [90] reports that IGF-1R inhibition blunts tumour cell response to other growth factors, overcomes the drug resistance phenotype conferred by the bone microenvironment and abrogates the production of proangiogenic cytokines. These sets of studies provide in vivo proof of the principle for therapeutic use of selective IGF-1R inhibitors in cancer.

2.5 Fibroblast Growth Factor (FGF)

Besides bone marrow microvessel density (MVD), serum levels of FGF, along with VEGF, are predicted to be prognostic markers of MM disease activity [91, 92]. Expression of bFGF correlates with clinical characteristics of MM and its high level also indicates poor prognosis [93]. However, the levels of bFGF may serve as a predictor for good response to the treatment of MM with Thalidomide [94]. Patients responsive to Thalidomide may have significantly higher concentrations of bFGF than non-responsive patients, but this observation is not consistent even between the same authors [95, 96]. Stimulation of BMSCs with FGF-2 induced a time and dose-dependent increase in IL-6 secretion, a well studied cytokine, which was completely abrogated by anti-bFGF antibodies. Conversely, stimulation with IL-6 enhanced bFGF expression and secretion by myeloma cell lines as well as MM patient cells, suggested a paracrine interaction between the myeloma and the stromal cells with respect to the above cytokines [97].

The FGF receptor 3 (FGFR3) is now recognized as a potential oncogene. Ectopic expression of FGFR3 originates from the translocation t(4;14) occurring in 10-25% of MM patients [98, 99]. Gain of function mutations in FGF receptors, especially FGFR3, have been widely implicated and studied in MM pathogenesis [98]. Suppression of FGFR3 using short hairpin RNAs (shRNAs), lead to apoptosis and anti-tumour effects in MM [100, 101].

FGF binding to the FGFR, results in dimerization of the receptor and autophosphorylation of the FGFR dimer at intracellular tyrosine residues. The activated receptor either binds directly to signalling molecules or recruits adapter molecules to link the activated receptor to downstream targets at the cell membrane.

Three FGF signalling downstream pathways have been identified in MM [102]: the Ras mitogen-activated protein kinase (MAPK) pathway, the phosphoinositol pathway and the signal transducer and activator of transcription (STAT) pathway.

2.6 Transforming Growth Factor (TGF-β)

Transforming Growth Factor beta (TGF-β) is a growth factor that controls proliferation, cellular growth and differentiation [103], and embryonic development [104]. During tumourigenesis, the TGF-β signalling pathway becomes mutated and TGF-β no longer controls the cell cycle [105, 106]. The cancer cells along with the surrounding stromal cells (fibroblasts) proliferate unchecked. Both these cells increase their production of TGF-β, which acts on the surrounding stromal cells, immune cells, endothelial and smooth-muscle cells, causing immunosuppression [106, 107] and tumour angiogenesis, and increasing the invasiveness [108, 109] and motility [110] of cancer.

TGF-β also plays a role in the suppression of bone formation in MM bone lesions [111]. Overproduction of TGF-beta 1 in MM patients was reported by Kroning et al. [112]. TGF-β is mainly produced by BMSCs, but is also secreted by malignant plasma cells and can regulate interleukin-6 (IL-6) secretion [113]. According to Cook et al., TGF-β produced by MM cells plays a significant role in suppressing host T cells and immune responses [114, 115]. TGF-β inhibition was able to suppress MM cell growth within the bone marrow while preventing bone destruction in MM-bearing animal models [116].

3. Role of chemokines

In MM, chemokines mainly help homing the myeloma cells to the bone marrow microenvironment. Their role in proliferation and survival of myeloma cells is only moderate. This effect can be either direct or mediated indirectly by inducing the secretion of IL-6, VEGF, or any other growth factor involved in the growth and survival of myeloma cells. The role of chemokines, especially that of MIPs, in osteolytic bone lesions is well established. Homing is defined by transendothelial migration of cells from the blood stream towards the chemokine gradient. This involves adhesion of cells to the endothelial layer, transendothelial migration and eventually residing in the microenvironment. So, it is apparent that bone marrow endothelial cells play an active role in the migration of plasma cells. They do so by secreting various chemokines and expressing adhesion molecules; thereby helping myeloma cells to migrate towards them. Upon adherence, MM cells will extravasate using their MMP arsenal to move through the basal lamina of bone marrow sinusoids. This process is also aided by the chemokine gradient in the bone marrow microenvironment because certain chemokine are said to be present in higher concentrations in the bone marrow microenvironment than in bone marrow endothelial cells which make sure that the cells are confined to the bone marrow microenvironment.

3.1 Macrophage Inflammatory protein: (MIP-1, CCL3)

MIP1 belongs to the CC family of chemokine and mainly acts via CCR1, CCR5 and CCR9 receptors. Myeloma cells have been shown to express both the receptors (CCR1, CCR5) and the chemokine [117, 118]. Controversial findings on the effect of growth and survival of myeloma cells could be due to usage of different experimental models and design [118, 119], but its role in migration and homing of myeloma cells, and in the progression of the myeloma bone disease, are clearly demonstrated. SCID mice injected with stable MIP1 knock-down clones of ARH cell line showed comparably less adhesion to the bone marrow, reduced survival and less bone pathology when compared to wild type ARH cell line injected group [117]. Suzanne Lentzsch et al. showed in vitro evidence that MIP1α can induce myeloma cell migration. Interestingly, they also showed that MIP1α can induce proliferation and survival of myeloma cells by inducing MAPK/ERK pathway, PI3/AKT pathway [118]. There is a study in which the various effects of MIP1α on 5TMM has been dissected. MIP1α induced migration has been attributed to the CCR5 and CCR1 receptor mediated signalling. Both the receptors mediate the MIP1α induced bone marrow angiogenesis and at least CCR1 mediates this effect directly [119].

3.2 MCP1 (or monocyte chemoattractant protein - CCL2)

As mentioned earlier, endothelial cells play an active role in the extravasation of myeloma cells and eventually their homing to the microenvironment. Murine endothelial cells are shown to secrete CCL2 and murine myeloma cells express the cognate receptor CCR2. Myeloma cells migrated towards the endothelial cell conditioned medium and this migration was inhibited by using antibodies against MCP1 [120]. Human bone marrow stromal cells also secrete MCP1, MCP2 and MCP 3, and myeloma cells migrate towards a stromal cell conditioned medium. This effect was inhibited by using antibodies against the MCPs and maximal inhibition was observed when all the three MCPs were blocked together, suggesting the role of various MCPs in myeloma homing [121].

3.3 CXC chemokines and CXCR3 receptor involvement in MM

CXCR3 receptor is expressed by activated T cells. It binds to CXC chemokines namely,: CXCL11 or Interferon-inducible T-cell Alpha Chemoattractant (I-TAC), Mig (Monocyte/macrophage-activating IFNϒ-inducible protein)/CXCL9 and IP10 (IFNϒ - inducible 10 kDa protein)/CXCL10. Myeloma cells derived from patients with myeloma, as well as myeloma derived cell lines, express CXCR3 receptor and they respond to their ligands by inducing tyrosine kinase phosphorylation and secreting MMP2 and MMP9 [122]. Bone marrow endothelial cells also secrete CXC chemokines and certain myeloma cells expressing their cognate receptors migrate in response to these chemokines [123].

3.4 Stromal Derived Factor (SDF-1α/CXCL12)

Stromal derived factor is a member of CXC family of cytokines and its cognate receptor is CXCR4. CXC12/CXCR4 is the most extensively studied chemokine/receptor system with respect to cancer. It has been implicated in progression, migration, invasion and metastasis of various cancers. The role of CXC12/CXCR4 has been well established in the homing of haematopoietic progenitor cells. Bone marrow plasma and bone marrow stromal cells secrete this chemokine, with the myeloma cells from the patient sample and myeloma derived cell lines expressing the cognate receptors. The chemokine mediates the secretion of IL-6 and VEGF, and induces proliferation, migration and inhibits dexamethasone induced cell death [124]. In the 5TMM model, bone marrow stromal cells and endothelial cells secrete SDF-1α and myeloma cells express the receptor. In vitro, SDF-1α induces moderate proliferation of myeloma cells, which was abrogated by blocking antibodies. 5T myeloma cells migrated towards a stromal cells conditioned medium which was partially inhibited by CXCR4 inhibitor. SDF also stimulated myeloma cells to secrete MMP9, demonstrated by zymography. Accordingly, SDF induces invasion and the CXCR4 inhibitor inhibits SDF induced invasion. In vivo, CXCR4 inhibitor inhibited the tumour burden and the immediate homing to about 40% [125].

When the myeloma cells were mobilized, the CXCL12/CXCR4 axis is downregulated. There is a downregulation of very late antigen (VLA4) in the peripheral blood myeloma cells after mobilization. This results in a suppression of the adhesion of myeloma cells to the bone marrow stromal cells, which can be rescued by induction with IL-6 [126]. Moreover, bone marrow endothelial cells are also shown to secrete CXCL12 and induce migration of myeloma cells towards the bone marrow endothelial cells. Thus, angiogenesis induced migration of myeloma cells is also mediated by CXCL12 chemokine [123]. The expression of CXCR4 was higher in bone marrow plasma cells of patients with myeloma than patients with MGUS. Moreover, the bone marrow plasma of myeloma patients has higher SDF-1α levels than that of peripheral blood of myeloma cells and bone marrow plasma of healthy individuals [127]. Consistent with its effect on migration, invasion, homing, proliferation and survival, CXCL12/CXCR4 axis induced MAPK/ERK, AKT, PKC and NF-kB pathways [124, 127].

4. Role of proinflammatory transcription factors

4.1 STAT3

STAT3 is a member of the STAT family of transcription factors. STAT family proteins were first discovered in the context of the specificity of the IFN signalling [128]. STAT3 was first described as a DNA-binding factor, in IL-6 stimulated hepatocytes, capable of selectively interacting with an enhancer element in the promoter region of acute-phase genes [129].

STAT3 is constitutively phosphorylated in v-Src–transformed cells and has been found to be necessary for the v-Src induced carcinogenesis. Expression of a constitutively active version of STAT3 on its own can lead to fibroblast transformation, showing that STAT3 is an oncogene [130]. Consistent with its role in various cancers, STAT3 regulates various genes involved in different aspects of cancer progression. Genes regulated by STAT3 that are involved in proliferation and growth include c-myc, cyclinD3, cyclin A, cdc25a, p21, cyclinD1, Pim-1 and Pim-2. Genes regulated by STAT3 that are involved in survival include proteins belonging to the family of Bcl-2 and IAPs, namely, Bcl-2, Bcl-xL, Mcl-1 and survivin. STAT3 has also been shown to downregulate the Fas cytokine. STAT3 mediated angiogenesis is mediated by VEGF; STAT3 also regulates MMP family members MMP2 and MMP9 [131]. STAT3 is vital for development, seen from STAT3 knock out mice which succumb to embryonic lethality [132]. However, disruption of STAT3 function either by deleting the gene or by introducing the dominant negative form of STAT3, leads to only a few phenotypical changes [133]. These findings are critical for the development of therapeutic strategies with high therapeutic index. In MM, STAT3 plays an important role in survival. It upregulates anti-apoptotic proteins like Bcl2, Bcl-XL and Mcl-1 [134-136]. Constitutive expression of STAT3 confers myeloma cells resistance to apoptosis [137]. Out of all the anti-apoptotic proteins regulated by STAT3, Mcl-1 seems to be more important. While antisense inhibition of Bcl-xL did not inhibit survival, knock down of Mcl-1 was sufficient to inhibit survival in myeloma cells. Overexpression of Mcl-1 was able to promote proliferation of multiple myeloma cells lines, even in the absence of IL-6 [138].

Knock down of Bcl-2 can augment dexamethasone induced apoptosis [139], but again, the importance of STAT3 in regulating the anti-apoptotic proteins and thereby the survival of myeloma cells remains controversial in the light of a lack of correlation between the constitutive expression of STAT3 and the anti-apoptotic proteins [140]. However, it is clear that STAT3 is not the only factor which regulates the survival of myeloma cells because myeloma cells become independent of a IL-6-gp130-STAT3 pathway in the presence of bone marrow stromal cells [51]. Almost 48% of MM patients have constitutively activated STAT3 [140]. There has been no activating mutations of STAT3 detected in MM. But, there has been epigenetic silencing of negative regulators of STAT3, namely, SHP1 and SOCS in MM. 27 of

34 (79.4%) myeloma samples showed SHP1 hypermethylation. At least in U266 MM cells, methylation of SHP1 may be responsible for constitutive STAT3 activation, because treatment with 5-azacytidine, a DNA demethylator, led to a progressive demethylation of SHP1 and a parallel downregulation of phosphorylated STAT3 [15]. SOCS-1 is hypermethylated in 23 out of 35 (62.9%) MM patient samples and consistently expression of this protein is upregulated after treatment with demethylators. So, it can be concluded that suppression of the expression of negative regulators of IL6-JAK-S TAT pathway by epigenetic silencing increases the sensitivity of myeloma cells to IL-6 induced proliferation and survival [141]. Moreover, overexpression of SOCS using adenoviral vector inhibited the IL-6 induced proliferation in IL-6 dependent multiple myeloma cells, hinting at another strategy to inhibit IL-6 induced downstream signal transduction pathways [142].

There are lots of therapeutic strategies that are being developed to target JAK-STAT3 pathway in MM. In fact, the novel agents that are being used nowadays namely, thalidomide and its derivatives and bortezomib, act partially to disrupt the NF-κB induced activation of IL-6 and thereby STAT3 activation. Numerous drugs that inhibit IL-6-JAK-STAT3 pathway at various levels induce apoptosis, both in vitro and in vivo [143-175].

4.2 NF-κB pathway

NF-κB is a Rel family of transcription factors consisting of p50, p52, c-Rel, p65/RelA and RelB subunits [176, 177]. It was discovered by Dr. Baltimore and colleagues in 1986 as a DNA binding protein, recognising specific sequences in the immunoglobulin kappa light chain joining (J) segment gene region in B cells [178].

Various inflammatory stimuli activate the NF-κB pathway. There are two pathways involved in the activation of the NF-κB pathway: the classical pathway and the alternative pathway.

NF-κB is a main transcription factor regulating various genes involved in inflammation. NF-κB has been casually implicated in various types of tumours [179]. Selective deletion of NF-κB in hepatocytes or inhibition of TNF-α production by neighbouring parenchymal cells, induced programmed cell death of transformed hepatocytes and reduced the incidence of liver tumours. Paracrine activation of NF-κB in initiated cells was not important in the early stages of liver tumour development, but it was crucial for malignant conversion [180]. In colitis associated cancer model of mice, selective deletion of IKK-β in inflammatory cells that are surrounding the enterocytes reduced the mRNA of inflammatory cytokine levels and subsequently decreased the tumour formation. However, selective deletion of IKK-β in enterocytes did not reduce inflammatory features, but it induced enhanced cell death in enterocytes leading to a decrease in the incidence of colon cancer [181]. It is quite obvious from these experiments that NF-κB affects both tumour cells and inflammatory stromal cells to induce and promote cancer. NF-κB acts on enteroctyes to inhibit apoptosis and also acts on inflammatory cells to stimulate the secretion of various mediators of inflammation which inturn acts on the enteroctyes to induce cancer. However, in some tissues, NF-κB acts to prevent cancer. For example, inhibition of NF-κB in keratinocytes leads to squamous cell carcinoma of skin [182]. In MM, patient samples show a constitutive activation of NF-κB to a variable degree [183]. How these cells activate NF-κB in a constitutive manner is still under investigation. Soluble cytokines belonging to TNFα super family including TNF-α, BAFF, APRIL, lymphotoxin b, are known to activate NF-κB and are present in the bone marrow microenvironment. Adhesion of myeloma cells to the bone marrow stromal cells and osteoclasts also activates the NF-κB pathway in both myeloma cells and osteoclasts, and bone marrow stromal cells.

Moreover, around 15-20% of myeloma samples and 40% of the cell lines show activating mutations in the NF-κB pathway [13, 184, 185]. There could be some unidentified genetic mutations or epigenetic modifications that might explain the constitutive activation in the remaining tumours. Gain of function mutations include ones encoding receptors known to activate NF-κB namely, CD40, LTβR, TAC1, NIK (NF-κB-inducing kinase), and direct mutations involving NF-κB1 p50/p105 and NF-κB2 p52/ p100. Loss of function mutations include those that involve negative regulators of NF-κB activation namely, TRAF3, TRAF2, CYLD and cIAP1/cIAP2, inactivation of TRAF3 being the most common. These mutations activate both classical and alternative pathways of NF-κB. CD40, LTβR, TAC1 and receptor overexpression may be sufficient to activate the NF-κB pathway or might enhance the sensitivity of MM cells to factors in the tumour microenvironment. Overexpression of NIK or NF-κB1 p105 directly leads to constitutive activation of NF-κB. Deletion of sequences in the p100 IκB-like domain of NF-KB2 promotes processing of p100 to p52 and activation of the alternative NF-κB pathway [184, 185]. Activating mutations of the NF-κB pathway helps the myeloma cells become independent of the bone marrow, as they overcome the need for external cytokines activating the pathway [13].

Activation of NF-κB in myeloma cells induces proliferation, survival and chemoresistance. When compared to chemosensitive myeloma cell lines, chemoresistant myeloma cells express higher levels of NF-κB, suggesting a link between NF-κB and development of chemoresistance [186, 187]. Moreover, dexamethasone induced apoptosis is associated with a decrease in the NF-κB DNA binding activity. Interestingly, NF-κB can also serve as a prognostic indicator for response to dexamethasone. Only patients who responded to dexamethasone, demonstrated decreased NF-κB DNA binding activity in their samples. Enforced ectopic expression of Bcl-2 in myeloma cells conferred resistance to dexamethasone induced apoptosis, and this was also associated with enhanced NF-κB DNA binding [187]. Inhibition of NF-κB by IKK inhibitor abrogates the protective effect of IL-6 on dexamethesone induced apoptosis. It also potentiated TNFα induced apoptosis in myeloma cells. NF-κB inhibition abrogated the TNFα induced upregulation of ICAM-1, both in myeloma cells and in bone marrow stromal cells. It also inhibited the myeloma cell adhesion induced IL-6 secretion by bone marrow stromal cells and resulting proliferation of myeloma cells. These findings indicate that pro-survival functions of the bone marrow microenvironment are abrogated upon NF-κB inhibition. The novel therapeutic agents namely, bortezomib and thalidomide and its derivatives, act at least partially by inhibiting NF-κB [188].

5. Role of matrix proteinases, angiogneic and adhesion molecules

5.1 Matrix metalloproteinase

Matrix metalloproteinase belong to a family of proteases, capable of degrading all kinds of extracellular matrix proteins. In 1962, Gross et al. discovered MMP, when they found collagenase activity in the tail of a tadpole during metamorphogenesis [189]. These proteins function not only to remodel the extracellular matrix, but also are involved in the cleavage and thereby activation and inactivation of various biologically significant proteins like chemokines and growth factors. In the context of cancer, both the cancer cells and stromal cells secrete MMPs. Their involvement in invasion and metastasis was examined in various clinical models. Recent evidence suggests the role of MMPs in various hallmarks of cancer progression [190]. Culture supernatants of bone marrow derived stromal cells from multiple myeloma patients were found to have higher levels of MMP-1 and MMP-2 than control samples [191]. Moreover, endothelial cells secrete hepatocyte growth factor, which acts on myeloma cells to stimulate the secretion of MMP-9 [192]. 5T MM bone marrow expresses various MMPs, such as MMP2, MMP8, MMP9 and MMP13. Adequate inhibition of these MMPs by a broad spectrum MMP inhibitor SC-964 suppresses angiogenesis, reduces tumour load and osteolytic lesions [193].

5.2 Vascular Endothelial Growth Factor (VEGF)

VEGF is a signal protein that stimulates formation of new blood vessels, through vasculogenesis and angiogenesis. The activity of VEGF is mediated through three receptor tyrosine kinases: VEGFR-1 (Flt-1), VEGFR-2 (KDR/Flk-1) and VEGFR-3 [194]. Dysregulation of VEGF has been shown to be a major contributor to tumour angiogenesis as well, promoting tumour growth, invasion and metastasis [195]. Upon stimulation by VEGF, bovine capillary endothelial cells were shown to proliferate and show signs of capillary-like tube structures [196]. Significantly elevated levels of VEGF are observed in a variety of haematologic malignancies [197-201]. Several studies link VEGF inactivation to anti-tumour effects [202]. Angiogenesis appears to play a role in haematological malignancies [203]. There is growing evidence that increased bone marrow angiogenesis occurs in myeloma [204, 205] and is related to disease activity [206, 207]. Angiogenesis in myeloma also appears to be correlated with the Plasma Cell Labelling Index, PCLI [206]. Micro vessel density (MVD) increases five-to-six fold in magnitude with progression from gammopathy of undetermined significance (MGUS) or non-active MM to the active MM [93, 208]. Moreover, after chemotherapy, MVD decreases significantly in patients in complete or partial remission [209]. MM cells release angiogenic factors, such as FGF and VEGF [93, 210], and are shown to induce angiogenesis in vivo in the Chick Chorioallantoic Membrane assay [93]. They secrete matrix metalloproteinase-2 and -9 (MMP-2 and MMP-9) and urokinase-type plasminogen activator [93] and cytokines recruiting inflammatory cells, such as mast cells, that then induce angiogenesis through secretion of angiogenic factors in their granules [211]. A better understanding of some of the above angiogenic factors would help in developing novel therapeutic targets against MM. A few of the widely prominent angiogenic factors are reviewed in detail in the following section.

A number of studies implicate dysregulation of VEGF in MM pathogenesis and associated clinical features, including lytic lesions of the bone and immune deficiency. VEGF protein was found in malignant cells from 75% of MM patients studied [212]. Increased serum levels of VEGF have been correlated with a poor prognosis in patients with advanced stages of MM [213]. In fact, Iwasaki T et al. report predicting treatment responses and disease progression in myeloma using serum vascular endothelial growth factor [214]. Another patient study claims that the levels of VEGF, along with FGF, parallel disease activity [210]. VEGF may also affect the immune response in MM patients. Sera from MM patients' bone marrow inhibits antigen presentation by dendritic cells (DCs); conversely, anti-VEGF antibodies neutralized this inhibitory effect, confirming that VEGF mediates immunosuppression in MM patients [215]. The cytokine is probably involved in the progression of MM to plasma cell leukaemia (PCL) [216]. Not just the ligand, its receptor VEGFR-1 is also widely expressed on both MM cell lines and patient MM cells, confirmed both by reverse-transcriptase polymerase chain reaction (RT-PCR) analyses and immunoprecipitation [217-219]. VEGF is generally present in the bone marrow (BM) microenvironment of patients with MM and associated with neovascularization at sites of MM cell infiltration [220]. The induction of VEGF enhances the microvascular density of bone marrow and accounts for the abnormal structure of myeloma tumour vessels [221]http://www.nejm.org.libproxy1.nus.edu.sg/doi/full/10.1056/NEJMra1011442 - ref12. VEGF increases both osteoclastic bone-resorbing activity [222] and osteoclast chemotaxis [223], and inhibits maturation of dendritic cells [224]. As marrow neovascularization parallels disease activity in MM, it is reasonable to postulate that the vascular growth factor is acting in an autocrine fashion. However, MM cells express VEGF receptors only weakly, if at all. Therefore, the mechanism may be paracrine and result from a VEGF-induced time and dose-dependent increase in stromal cell secretion of interleukin-6 (IL-6), a known MM growth factor [225]. Another cytokine, TNFα, has been reported to be involved in the control of VEGF production by myeloma cells [226]. Moreover, VEGF directly, or indirectly through its stimulatory activity on TNF-α and IL-β1, stimulates the activation of osteoclasts and thus contributes to the lytic lesions in MM [222].

Other factors modulating VEGF secretion include Interleukins: IL-1β [227], IL-10 and IL-13 [228]; secretion of IL-6 [218, 225, 229] or VEGF by both BMSCs and tumour cells (paracrine/autocrine loop); hypoxia and the presence of mutant oncogenes (i.e., mutant Ras [mutRas] or Bcr-Abl, which up-regulate VEGF expression via HIF-1α protein); secretion of growth factors, such as insulin-like growth factor-1 (IGF-1) [88, 230], fibroblast growth factor- 4 (FGF-4) [231], platelet-derived growth factor (PDGF) [232], TGF-β [233], TNF-α [234] and gonadotropins [235]; c-maf–driven expression of tumour integrin β7 [236]; tumour cell expression of ICAM1 and LFA1 modulating adhesion to ECM and BMSCs, thereby increasing VEGF production and secretion; and CD40 activation, which induces p53-dependent VEGF secretion. Binding of VEGF to MM cells triggers VEGFR tyrosine phosphorylation, activating several downstream signalling pathways, particularly involving phosphatidyl-inositol-3 kinase [237, 238]. PI3-kinase- dependent cascade mediates MM cell migration on fibronectin, evidenced by using the PI3-kinase inhibitor bis-indolylmaleimide I and LY294002 [237]. This signal transduction pathway is mediated by focal adhesion proteins [239], such as FAK, paxillin and cortactin, which are responsible for the stabilization of focal adhesion plaques and the reorganization of actin fibres [240]. VEGF also regulates MM cell survival by modulating the expression of Mcl-1 and survivin [241].

MAP kinases (MAPK) are the final effectors of the signal to the nucleus, thereby activating genes for proliferation, migration and survival [242]. This increased migration and cell proliferation is because of the activation of VEGFR-2, since it is totally inhibited by a VEGFR-2 blocking antibody [243]. In fact, MEK-extracellular signal-regulated protein kinase (ERK) pathway is shown to mediate MM cell proliferation, evidenced by use of anti-VEGF antibody and PD098059 [217]. Approaches to disrupt the VEGF/VEGF receptor signalling pathways range from small molecule VEGF/VEGFR inhibitors, anti-VEGF and anti-VEGF receptor antibodies, such as bevacizumab [244, 245], and VEGF transcription inhibitors. Of interest are various kinase inhibitors that block the signal transduction mediated by VEGF. The VEGF receptor tyrosine kinase inhibitor PTK787 is active preclinically and undergoing clinical protocol testing in MM [246, 247]. It acts directly on MM cells to inhibit VEGF-induced MM cell growth and migration, and inhibits paracrine IL-6–mediated MM cell growth in the BM milieu. Pazopanib [248], another VEGF receptor tyrosine kinase inhibitor, has been studied for cancer therapy.

5.3 Adhesion molecules

Cell adhesion is a key physiological event involved in morphogenesis and histogenesis. Adhesion molecules mediate cell-cell and cell-ECM interactions [249], and are also involved in intracellular signalling after engagement with their receptors. Broadly, there are five groups of adhesion molecules. They are 1) the integrins-mediating cell-ECM and cell-cell adhesion 2) the cadherin family-mediating homotypic cell-cell adhesion 3) the selectin family-mediating heterotypic cell-cell adhesion 4) the immunoglobulin superfamily-mediating cell-cell adhesion and 5) other transmembrane proteoglycans, such as CD44 adhesion molecules and syndecan that mediate cell-extracellular matrix adhesion [12]. Dysregulated expression or function of adhesion molecules are involved in various steps of cancer progression.

In MM, there is evidence that adhesion molecules mediate homing of MM cells to the bone marrow, secretion of cytokines and growth factors, and development of chemoresistance. Out of all the adhesion molecules, VLA-4 and VLA-5 expressed by the myeloma cells play a crucial role in the myeloma pathogenesis [250]. VCAM-1 and fibronectin are the receptors for VLA. VLA adheres to the bone marrow stromal cells by binding to VCAM, CS-1 fragment and H1 region of fibronectin [251]. Inhibition of VLA using blocking antibodies inhibit the adhesion of myeloma cells to the bone marrow stromal cells and fibronectin [252]. VLA dependent adhesion to the bone marrow is regulated by the CXCL12/CXCR4 axis [253]. This is further supported by the finding that disruption of CXCL12/CXCR4 axis results in downregulation of VLA-4 and decreased adhesive capacity in the mobilised myeloma cells when compared to premobilisation bone marrow myeloma cells [126].

VLA dependent adhesion of MM cells to the bone marrow stromal cells induces secretion of IL-6 by an NF-κB mediated mechanism [38, 39]. Drug-sensitive 8226 human myeloma cells, expressing both VLA-4 and VLA-5 receptors, are relatively resistant to the apoptotic effects of doxorubicin and melphalan, when pre-adhered to FN and compared with cells grown in suspension. Upon exposure to chemotherapeutic agents, myeloma cells expressing high levels of VLA-4 have survival advantage over those that express them at low levels. When the cells were removed from a chronic drug exposure, the VLA-4 expression decreased. However, there was no upregulation of common mediators of drug resistance like anti-apoptotic proteins and drug exporting glycoproteins in the cells. It was concluded that though the survival advantage offered by VLA-4 induced adhesion to fibronectin is less, it is significant in helping them survive the acute drug exposure and gives them adequate time to employ the classic mechanisms of drug resistance [254]. How adhesion of cells to fibronecin is rendering the cells resistance to chemotherapy, is still not completely understood. It was shown that adhesion of myeloma cells to fibronectin activates NF-κB and its regulated gene products, leading to drug resistance [255]. Moreover, it seems that IL-6 and fibronectin collaborate to stimulate STAT3 and fibronectin augments IL-6 induced STAT3 activation [256].

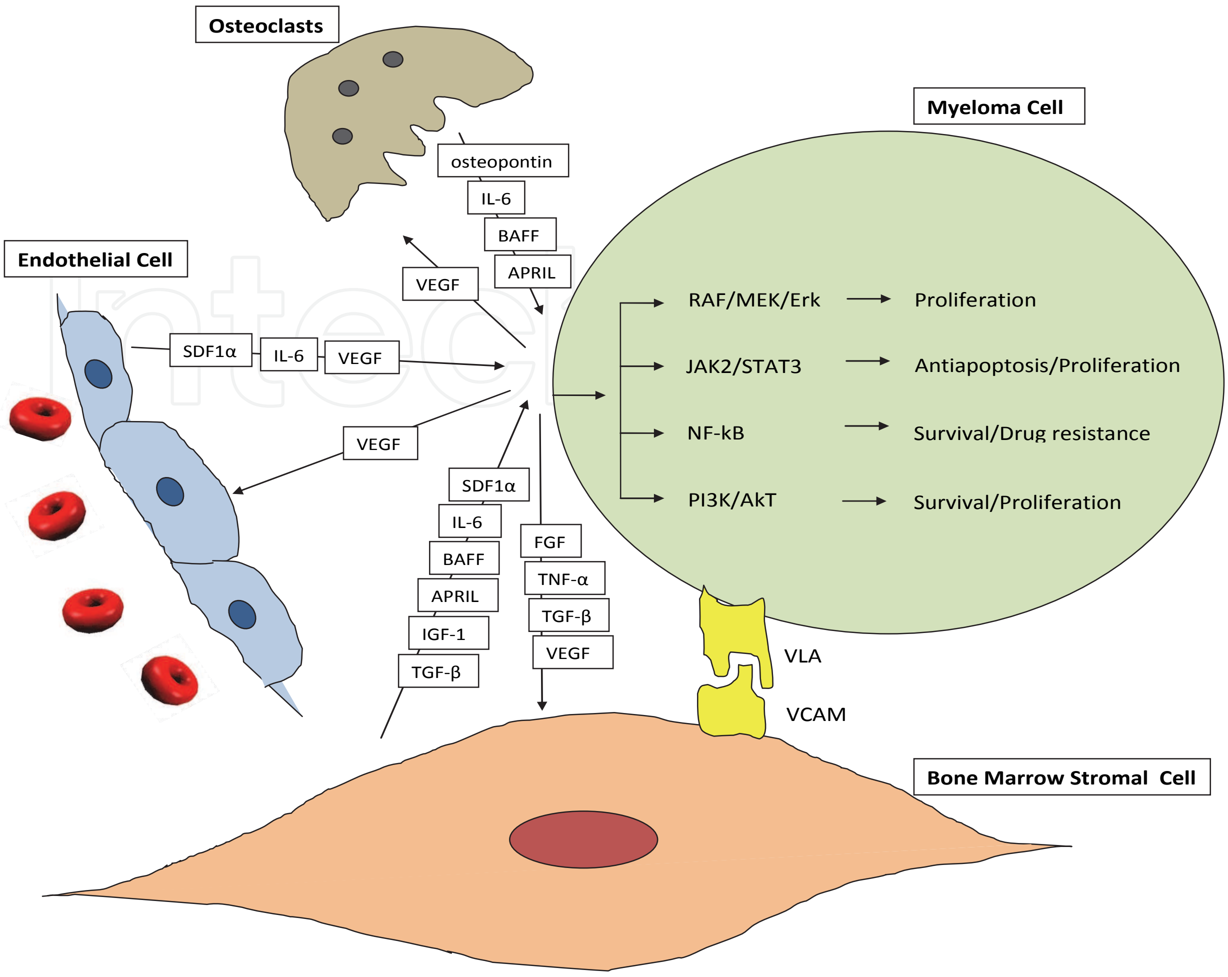


Fig. 1. Comprehensive representation of the role(s) of various inflammatory mediators in MM

	Pharmacological/ Biological Blockers	***Mechanism(s) of Action***	**References**
IL-6	1339	high-affinity fully humanized anti-IL-6 mAb	[257]
	6-amino-4-quinazoline	inhibits IL-6 signalling	[166]
	Bortezomib		
	CHIR-12.12 (Human	downregulates gp130	[258]
	anti-CD40 antagonist antibody)	inhibits CD-40 enhanced IL-6 secretion	[259]
	CNTO 328 (siltuximab)	IL-6 neutralizing monoclonal antibody	[260-262]
	ITF2357 histone deacetylase inhibitor	down-modulates the interleukin-6 receptor α (CD126)	[263]
	Novel indolinone BIBF 1000	abrogates stroma-derived IL-6 secretion	[264]
	Sant7	IL-6 receptor superantagonist	[265]

	Pharmacological/ Biological Blockers	*Mechanism(s) of Action*	**References**
TNFα	Rituximab	monoclonal antibody	[266]
	Thalidomide and its analogues	suppresses the expression of TNFα	[267]
BAFF & APRIL	Atacicept	blocks the binding of BAFF and APRIL	[268]
VEGF	CHIR-12.12 (Human anti-CD40 antagonist antibody)	inhibits CD-40 enhanced VEGF secretion	[259]
	Bevacizumab	humanized murine anti--VEGF monoclonal antibody	[269]
	PTK787/ZK222584, SU6668, SU5416	VEGF receptor tyrosine kinase inhibitors	[203, 270-272] [273]
	Sorafenib	dual Raf kinase/VEGF-R inhibitor	
IGF	α-IR3	neutralizing monoclonal antibody to IGF-1R	[274]
	JB-1	IGF-1 like competitive peptide antagonist	[275, 276]
	NVP-ADW742	IGF-1R tyrosine kinase inhibitor	[277]
TGF-β	SD-208	TGF-β receptor I kinase inhibitor	[278]
CXCL12	4F-benzoyl-TN14003 AMD3100	CXCR4 antoganist	[279]
	Thalidomide	CXCR4 inhibitor	[267, 280]
		immnunomodulator- downregulates CXCL12 and CXCR4	[281]
STAT3	AR-42	downregulates gp130	[175]
	Atiprimod	inhibits STAT3 activation	[143]
	Auranofin	inhibits activation of JAK2	[162]
	Avicin D	activates protein phosphatase-1	[282]
	Azaspirane	inhibits STAT3	[283]
	AZD1480	JAK-2 inhibitor	[174]
	Baicalein	Inhibits IL-6-mediated phosphorylation of signalling proteins	[157]
	Betulinic acid	inhibits the activation of Src kinase, JAK1 and JAK2	[165]
	Butein	inhibits the activation of Src kinase, JAK1 and JAK2; and upregulates SHP-1	[164]
	Cantharidin	inhibits phosphorylation of STAT3	[172]
	Capsaicin	inhibits the activation of Src kinase, JAK1	[146]
	Celastrol	inhibits JAK2 and Src kinase phosphorylation upregulates SHP-1	[284]

	Pharmacological/ Biological Blockers	*Mechanism(s) of Action*	**References**
	Compound K	inhibits constitutive and IL-6-inducible	[167]
	Curcumin	STAT3 phosphorylation	[145]
		inhibits activation of JAK2	
	Decursin	upregulates PTEN	[150]
	Embelin	inhibitor of JAK-2	[149]
	Emodin	inhibits the activation of c-src	[161]
	Genipin	induces protein tyrosine phosphatase	[152]
	Guggulsterone	SHP-1	[172]
		inhibits the activation of c-src and	
	Icariside II	JAK-2 and upregulates the expression	[151]
		of SHP-1 and PTEN	
		JAK1/2 selective inhibitor	
	INCB16562	Janus kinase inhibitor	[155]
	INCB20	forced overexpression of SOCS	[285]
	Infectivity-enhanced adenoviral vector of		[142]
	SOCS	inhibits Aurora kinase A, Aurora	
	Multitargeted kinase	kinase B, and Janus kinase 2/3	[173]
	inhibitor, AT9283	reduces Jak kinase auto-	
	Nifuroxazide	phosphorylation	[163]
		induces the expression of the protein	
	Plumbagin	tyrosine phosphatase, SHP-1	[286]
		pan-Janus-activated kinase inhibitor	
	Pyridone 6	inhibits both constitutive and IL-6	[169]
	Resveratrol	induced activation of STAT3	[287]
		inhibits activation JAK2	
	TG101209	inhibits of c-Src and JAK2 activation	[171]
	Thymoquinone	JAK2 tyrosine kinase inhibitor	[154]
	Tyrphostin AG490	inhibits the activation of Src kinase,	[148]
	Ursolic acid	JAK1 and JAK2, and upregulates SHP-1	[168]
NF-κB	Azacitidine	inhibits both NF-κB nuclear translocation and DNA binding	[288]
	Azaspirane	inhibits IκBα NFκB- p65	[283]
		phosphorylation TNF-α	
	Bay 11-7082	pharmacological NF-κB inhibitors	[289]
	Celastrol	inhibits JAK2 and Src kinase phosphorylation	[284]
	Curcumin	suppresses NF-κB activation	[183]
	Genistein	suppresses constitutively active NF-κB	[290]
		IκB kinase β inhibitor	
	MLN120B	suppresses NF-κB activation	[291]
	Parthenolide	suppresses constitutively active NF-κB	[292]
	Resveratrol	through inhibition of IκBα kinase and	[287]

	Pharmacological/ Biological Blockers	*Mechanism(s) of Action*	**References**
		the phosphorylation of IκBα and of p65	
MMPs	Chitosan	a marine phospholipid that inhibits the activity of MMP-2 and MMP-9	[293]
	SST0001	a chemically modified heparin with antiheparanase activity	[294]
Integrins	Anti-alpha4 Ab	monoclonal antibody to alpha4 integrin	[295, 296]
	QLT0267	integrin-linked kinase inhibitor	[297297]

Table 1. List of various pharmacological/biological agents modulating inflammatory mediators in MM

6. Conclusions

Understanding the various growth and survival pathways activated in both myeloma cells and various components of the bone marrow microenvironment is of paramount importance, not only to the basic understanding of the biology of MM, but also to effectively produce efficacious and safer anti-myeloma agents. In essence, myeloma is initiated by the primary genetic abnormalities and supported by the bone marrow microenvironment induced growth and survival. The secondary genetic mutations and epigenetic abnormalities emancipate myeloma cells of their dependence on the bone marrow microenvironment, which is when they progress to extramedullary MM. There are multiple signalling pathways activated, which serve overlapping functions. Combined inhibition of multiple signalling pathways offers better effects.

7. Acknowledgements

This work was supported by grants from the National Medical Research Council of Singapore [Grant R-713-000-124-213] and Cancer Science Institute of Singapore, Experimental Therapeutics I Program [Grant R-713-001-011-271] to APK; NUS Academic Research Fund [Grants R-184-000-170-112 and R-184-000-177-112], National kidney Foundation [Grant R-184-000-196-592] and National Medical Research Council of Singapore [Grant R-184-000-201-275] to GS.

8. References

[1] Raab, M.S., et al., *Multiple myeloma.* The Lancet, 2009. 374(9686): p. 324-339.
[2] Agarwal, J.R. and W. Matsui, *Multiple myeloma: A paradigm for translation of the cancer stem cell hypothesis.* Anti-Cancer Agents in Medicinal Chemistry, 2010. 10(2): p. 116-120.
[3] Colotta, F., et al., *Cancer-related inflammation, the seventh hallmark of cancer: Links to genetic instability.* Carcinogenesis, 2009. 30(7): p. 1073-1081.
[4] Hanahan, D. and R.A. Weinberg, *Hallmarks of cancer: The next generation.* Cell, 2011. 144(5): p. 646-674.

[5] Balkwill, F. and A. Mantovani, *Inflammation and cancer: Back to Virchow?* Lancet, 2001. 357(9255): p. 539-545.

[6] Mantovani, A., *Molecular pathways linking inflammation and cancer.* Current Molecular Medicine, 2010. 10(4): p. 369-373.

[7] Porta, C., et al., *Cellular and molecular pathways linking inflammation and cancer.* Immunobiology, 2009. 214(9-10): p. 761-777.

[8] Mantovani, A., et al., *Cancer-related inflammation.* Nature, 2008. 454(7203): p. 436-444.

[9] Balkwill, F. and A. Mantovani, *Cancer and inflammation: Implications for pharmacology and therapeutics.* Clinical Pharmacology and Therapeutics, 2010. 87(4): p. 401-406.

[10] Mahtouk, K., et al., *Growth factors in multiple myeloma: A comprehensive analysis of their expression in tumor cells and bone marrow environment using Affymetrix microarrays.* BMC Cancer, 2010. 10.

[11] Katz, B.Z., *Adhesion molecules-The lifelines of multiple myeloma cells.* Seminars in Cancer Biology, 2010. 20(3): p. 186-195.

[12] Bewick, M.A. and R.M. Lafrenie, *Adhesion dependent signalling in the tumour microenvironment: The future of drug targeting.* Current Pharmaceutical Design, 2006. 12(22): p. 2833-2848.

[13] Demchenko, Y.N., et al., *Classical and/or alternative NF-κB pathway activation in multiple myeloma.* Blood, 2010. 115(17): p. 3541-3552.

[14] Kawauchi, K., et al., *The PI3K/Akt pathway as a target in the treatment of hematologic malignancies.* Anti-Cancer Agents in Medicinal Chemistry, 2009. 9(5): p. 550-559.

[15] Chim, C.S., et al., *SOCS1 and SHP1 hypermethylation in multiple myeloma: Implications for epigenetic activation of the Jak/STAT pathway.* Blood, 2004. 103(12): p. 4630-4635.

[16] Hirano, T., T. Taga, and N. Nakano, *Purification to homogeneity and characterization of human B-cell differentiation factor (BCDF or BSFp-2).* Proceedings of the National Academy of Sciences of the United States of America, 1985. 82(16): p. 5490-5494.

[17] Van Damme, J., G. Opdenakker, and R.J. Simpson, *Identification of the human 26-kD protein, interferon β2 (IFN-β2), as a B cell hybridoma/plasmacytoma growth factor induced by interleukin 1 and tumor necrosis factor.* Journal of Experimental Medicine, 1987. 165(3): p. 914-919.

[18] Aarden, L.A., *Hybridoma growth factor.* Annals of the New York Academy of Sciences, 1989. 557: p. 192-199.

[19] Van Damme, J. and J. Van Snick, *Induction of hybridoma growth factor (HGF) identical to IL-6, in human fibroblasts by IL-1: Use of HGF activity in specific and sensitive biological assays for IL-1 and IL-6.* Developments in Biological Standardization, 1988. 69: p. 31-38.

[20] Nishimoto, N., et al., *Oncostatin M, leukemia inhibitory factor, and interleukin 6 induce the proliferation of human plasmacytoma cells via the common signal transducer, GP130.* Journal of Experimental Medicine, 1994. 179(4): p. 1343-1347.

[21] Hirano, T., K. Ishihara, and M. Hibi, *Roles of STAT3 in mediating the cell growth, differentiation and survival signals relayed through the IL-6 family of cytokine receptors.* Oncogene, 2000. 19(21): p. 2548-2556.

[22] Nishimoto, N. and T. Kishimoto, *Interleukin 6: From bench to bedside.* Nature Clinical Practice Rheumatology, 2006. 2(11): p. 619-626.

[23] Barton, B.E., *Interleukin-6 and new strategies for the treatment of cancer, hyperproliferative diseases and paraneoplastic syndromes.* Expert Opinion on Therapeutic Targets, 2005. 9(4): p. 737-752.

[24] Adachi, Y., N. Yoshio-Hoshino, and N. Nishimoto, *The blockade of IL-6 signaling in rational drug design.* Current Pharmaceutical Design, 2008. 14(12): p. 1217-1224.
[25] Barut, B.A., et al., *Role of interleukin 6 in the growth of myeloma-derived cell lines.* Leukemia Research, 1992. 16(10): p. 951-959.
[26] Hilbert, D.M., et al., *Interleukin 6 is essential for in vivo development of B lineage neoplasms.* Journal of Experimental Medicine, 1995. 182(1): p. 243-248.
[27] Pulkki, K., et al., *Soluble interleukin-6 receptor as a prognostic factor in multiple myeloma.* British Journal of Haematology, 1996. 92(2): p. 370-374.
[28] Kyrtsonis, M.C., et al., *Soluble interleukin-6 receptor (sIL-6R), a new prognostic factor in multiple myeloma.* British Journal of Haematology, 1996. 93(2): p. 398-400.
[29] Stasi, R., et al., *The prognostic value of soluble interleukin-6 receptor in patients with multiple myeloma.* Cancer, 1998. 82(10): p. 1860-1866.
[30] Pelliniemi, T.T., et al., *Immunoreactive interleukin-6 and acute phase proteins as prognostic factors in multiple myeloma.* Blood, 1995. 85(3): p. 765-771.
[31] Bataille, R., et al., *Serum levels of interleukin 6, a potent myeloma cell growth factor, as a reflection of disease severity in plasma cell dyscrasias.* Journal of Clinical Investigation, 1989. 84(6): p. 2008-2011.
[32] Bataille, R., et al., *Serum levels of interleukin 6, a potent myeloma cell growth factor, as a reflection of disease severity in plasma cell dyscrasias.* The Journal of Clinical Investigation, 1989. 84(6): p. 2008-2011.
[33] Kawano, M., et al., *Autocrine generation and requirement of BSF-2/IL-6 for human multiple myelomas.* Nature, 1988. 332(6159): p. 83-84.
[34] Anderson, K.C., et al., *Response patterns of purified myeloma cells to hematopoietic growth factors.* Blood, 1989. 73(7): p. 1915-1924.
[35] Caligaris-Cappio, F., et al., *'Role of bone marrow stromal cells in the growth of human multiple myeloma.* Blood, 1991. 77(12): p. 2688-2693.
[36] Klein, B., et al., *Paracrine rather than autocrine regulation of myeloma-cell growth and differentiation by interleukin-6.* Blood, 1989. 73(2): p. 517-526.
[37] Lichtenstein, A., et al., *Production of cytokines by bone marrow cells obtained from patients with multiple myeloma.* Blood, 1989. 74(4): p. 1266-1273.
[38] Uchiyama, H., et al., *Adhesion of human myeloma-derived cell lines to bone marrow stromal cells stimulates interleukin-6 secretion.* Blood, 1993. 82(12): p. 3712-3720.
[39] Chauhan, D., et al., *Multiple myeloma cell adhesion-induced interleukin-6 expression in bone marrow stromal cells involves activation of NF-κB.* Blood, 1996. 87(3): p. 1104-1112.
[40] Abe, M., et al., *Osteoclasts enhance myeloma cell growth and survival via cell-cell contact: A vicious cycle between bone destruction and myeloma expansion.* Blood, 2004. 104(8): p. 2484-2491.
[41] Ogata, A., et al., *IL-6 triggers cell growth via the ras-dependent mitogen-activated protein kinase cascade.* Journal of Immunology, 1997. 159(5): p. 2212-2221.
[42] Croonquist, P.A., et al., *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(7): p. 2581-2592.
[43] Chauhan, D., et al., *Interleukin-6 inhibits Fas-induced apoptosis and stress-activated protein kinase activation in multiple myeloma cells.* Blood, 1997. 89(1): p. 227-234.
[44] Chauhan, D., et al., *RAFTK/PYK2-dependent and -independent apoptosis in multiple myeloma cells.* Oncogene, 1999. 18(48): p. 6733-6740.

[45] Chauhan, D., et al., *SHP2 mediates the protective effect of interleukin-6 against dexamethasone-induced apoptosis in multiple myeloma cells.* Journal of Biological Chemistry, 2000. 275(36): p. 27845-27850.

[46] Hideshima, T., et al., *Biologic sequelae of interleukin-6 induced PI3-K/Akt signaling in multiple myeloma.* Oncogene, 2001. 20(42): p. 5991-6000.

[47] Salem, M., et al., *Identification of predictors of disease status and progression in patients with myeloma (MM).* Hematology, 2000. 5(1): p. 41-45.

[48] Thavasu, P.W., et al., *Multiple myeloma: An immunoclinical study of disease and response to treatment.* Hematological Oncology, 1995. 13(2): p. 69-82.

[49] Trikha, M., et al., *Targeted Anti-Interleukin-6 Monoclonal Antibody Therapy for Cancer: A Review of the Rationale and Clinical Evidence.* Clinical Cancer Research, 2003. 9(13): p. 4653-4665.

[50] Chatterjee, M., 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(12): p. 3712-3721.

[51] Chatterjee, M., et al., *In the presence of bone marrow stromal cells human multiple myeloma cells become independent of the IL-6/gp130/STAT3 pathway.* Blood, 2002. 100(9): p. 3311-3318.

[52] Wiemann, B. and C.O. Starnes, *Coley's toxins, tumor necrosis factor and cancer research: A historical perspective.* Pharmacology and Therapeutics, 1994. 64(3): p. 529-564.

[53] O'Malley, W.E., B. Achinstein, and M.J. Shear, *ACTION OF BACTERIAL POLYSACCHARIDE ON TUMORS. III. REPEATED RESPONSE OF.* Cancer research, 1963. 23: p. 890-895.

[54] Carswell, E.A., L.J. Old, and R.L. Kassel, *An endotoxin induced serum factor that causes necrosis of tumors.* Proceedings of the National Academy of Sciences of the United States of America, 1975. 72(9): p. 3666-3670.

[55] Oettgen, H.F., et al., *Endotoxin-induced tumor necrosis factor.* Recent results in cancer research. Fortschritte der Krebsforschung. Progres dans les recherches sur le cancer, 1980. 75: p. 207-212.

[56] Pennica, D., G.E. Nedwin, and J.S. Hayflick, *Human tumour necrosis factor: Precursor structure, expression and homology to lymphotoxin.* Nature, 1984. 312(5996): p. 724-729.

[57] Lejeune, F.J., *Clinical use of TNF revisited: Improving penetration of anti-cancer agents by increasing vascular permeability.* Journal of Clinical Investigation, 2002. 110(4): p. 433-435.

[58] Balkwill, F., *TNF-a in promotion and progression of cancer.* Cancer and Metastasis Reviews, 2006. 25(3): p. 409-416.

[59] Moore, R.J., et al., *Mice deficient in tumor necrosis factor-a are resistant to skin carcinogenesis.* Nature Medicine, 1999. 5(7): p. 828-831.

[60] Arnott, C.H., et al., *Expression of both TNF-a receptor subtypes is essential for optimal skin tumour development.* Oncogene, 2004. 23(10): p. 1902-1910.

[61] Knight, B., et al., *Impaired preneoplastic changes and liver tumor formation in tumor necrosis factor receptor type 1 knockout mice.* Journal of Experimental Medicine, 2000. 192(12): p. 1809-1818.

[62] Hideshima, T., et al., *The role of tumor necrosis factors in the pathophysiology of human multiple myeloma: Therapeutic applications.* Oncogene, 2001. 20(33): p. 4519-4527.

[63] Johrer, K., et al., *Transendothelial Migration of Myeloma Cells Is Increased by Tumor Necrosis Factor (TNF)-a via TNF Receptor 2 and Autocrine Up-Regulation of MCP-1.* Clinical Cancer Research, 2004. 10(6): p. 1901-1910.

[64] Novak, A.J., et al., *Expression of BCMA, TACI, and BAFF-R in multiple myeloma: A mechanism for growth and survival.* Blood, 2004. 103(2): p. 689-694.

[65] Moreaux, J., 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(3): p. 1021-1030.

[66] Li, W., et al., *New targets of PS-341: BAFF and APRIL.* Medical Oncology, 2010. 27(2): p. 439-445.

[67] Tai, Y.T., et al., *Role of B-cell-activating factor in adhesion and growth of human multiple myeloma cells in the bone marrow microenvironment.* Cancer research, 2006. 66(13): p. 6675-6682.

[68] Moreaux, J., et al., *BAFF and APRIL protect myeloma cells from apoptosis induced by interleukin 6 deprivation and dexamethasone.* Blood, 2004. 103(8): p. 3148-3157.

[69] Quinn, J., et al., *APRIL promotes cell-cycle progression in primary multiple myeloma cells: Influence of D-type cyclin group and translocation status.* Blood, 2011. 117(3): p. 890-901.

[70] Standal, T., et al., *Serum insulin-like growth factor is not elevated in patients with multiple myeloma but is still a prognostic factor.* Blood, 2002. 100(12): p. 3925-3929.

[71] Jelinek, D.F., T.E. Witzig, and B.K. Arendt, *A Role for Insulin-Like Growth Factor in the Regulation of IL-6-Responsive Human Myeloma Cell Line Growth.* Journal of Immunology, 1997. 159(1): p. 487-496.

[72] Ogawa, M., et al., *Cytokines prevent dexamethasone-induced apoptosis via the activation of mitogen-activated protein kinase and phosphatidylinositol 3-kinase pathways in a new multiple myeloma cell line1.* Cancer Research, 2000. 60(15): p. 4262-4269.

[73] Vanderkerken, K., et al., *Insulin-Like Growth Factor-1 Acts as a Chemoattractant Factor for 5T2 Multiple Myeloma Cells.* Blood, 1999. 93(1): p. 235-241.

[74] Ge, N.-L. and S. Rudikoff, *Insulin-like growth factor I is a dual effector of multiple myeloma cell growth.* Blood, 2000. 96(8): p. 2856-2861.

[75] Mitsiades, C.S., et 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(3): p. 221-230.

[76] Georgii-Hemming, P., et al., *Insulin-like growth factor I is a growth and survival factor in human multiple myeloma cell lines.* Blood, 1996. 88(6): p. 2250-2258.

[77] Freund, G.G., et al., *Functional Insulin and Insulin-like Growth Factor-1 Receptors Are Preferentially Expressed in Multiple Myeloma Cell Lines as Compared to B-Lymphoblastoid Cell Lines.* Cancer Research, 1994. 54(12): p. 3179-3185.

[78] Xu, F., et al., *Multiple myeloma cells are protected against dexamethasone-induced apoptosis by insulin-like growth factors.* British Journal of Haematology, 1997. 97(2): p. 429-440.

[79] Abroun, S., et al., *Receptor synergy of interleukin-6 (IL-6) and insulin-like growth factor-I that highly express IL-6 receptor a myeloma cells.* Blood, 2004. 103(6): p. 2291-2298.

[80] Ferlin, M., et al., *Insulin-like growth factor induces the survival and proliferation of myeloma cells through an interleukin-6-independent transduction pathway.* British Journal of Haematology, 2000. 111(2): p. 626-634.

[81] Tu, Y., A. Gardner, and A. Lichtenstein, *The Phosphatidylinositol 3-Kinase/AKT Kinase Pathway in Multiple Myeloma Plasma Cells: Roles in Cytokine-dependent Survival and Proliferative Responses.* Cancer Research, 2000. 60(23): p. 6763-6770.

[82] Qiang, Y.-W., E. Kopantzev, and S. Rudikoff, *Insulin-like growth factor–I signaling in multiple myeloma: downstream elements, functional correlates, and pathway cross-talk.* Blood, 2002. 99(11): p. 4138-4146.

[83] Asosingh, K., et al., *In Vivo Induction of Insulin-like Growth Factor-I Receptor and CD44v6 Confers Homing and Adhesion to Murine Multiple Myeloma Cells.* Cancer Research, 2000. 60(11): p. 3096-3104.

[84] Tai, Y.T., et al., *Insulin-like growth factor-1 induces adhesion and migration in human multiple myeloma cells via activation of β1-integrin and phosphatidylinositol 3'-kinase/AKT signaling.* Cancer Research, 2003. 63(18): p. 5850-5858.

[85] Ge, N.L. and S. Rudikoff, *Insulin-like growth factor I is a dual effector of multiple myeloma cell growth.* Blood, 2000. 96(8): p. 2856-2861.

[86] Akiyama, M., et al., *Cytokines modulate telomerase activity in a human multiple myeloma cell line.* Cancer Research, 2002. 62(13): p. 3876-3882.

[87] Podar, K., et al., *Essential role of caveolae in interleukin-6- and insulin-like growth factor I-triggered Akt-1-mediated survival of multiple myeloma cells.* Journal of Biological Chemistry, 2003. 278(8): p. 5794-5801.

[88] Fukuda, R., et al., *Insulin-like Growth Factor 1 Induces Hypoxia-inducible Factor 1-mediated Vascular Endothelial Growth Factor Expression, Which is Dependent on MAP Kinase and Phosphatidylinositol 3-Kinase Signaling in Colon Cancer Cells.* Journal of Biological Chemistry, 2002. 277(41): p. 38205-38211.

[89] Miele, C., et al., *Insulin and Insulin-like Growth Factor-I Induce Vascular Endothelial Growth Factor mRNA Expression via Different Signaling Pathways.* Journal of Biological Chemistry, 2000. 275(28): p. 21695-21702.

[90] Mitsiades, C.S., et 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(3): p. 221-230.

[91] Sucak, G.T., et al., *Prognostic value of bone marrow microvessel density and angiogenic cytokines in patients with multiple myeloma undergoing autologous stem cell transplant.* Leukemia & Lymphoma, 2011. 52(7): p. 1281-1289.

[92] Sezer, O., et al., *Serum levels of the angiogenic cytokines basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF) in multiple myeloma.* European Journal of Haematology, 2001. 66(2): p. 83-88.

[93] Vacca, A., et al., *Bone Marrow Neovascularization, Plasma Cell Angiogenic Potential, and Matrix Metalloproteinase-2 Secretion Parallel Progression of Human Multiple Myeloma.* Blood, 1999. 93(9): p. 3064-3073.

[94] Bertolini, F., et al., *Thalidomide in multiple myeloma, myelodysplastic syndromes and histiocytosis. Analysis of clinical results and of surrogate angiogenesis markers.* Annals of Oncology, 2001. 12(7): p. 987-990.

[95] Neben, K., et al., *High Plasma Basic Fibroblast Growth Factor Concentration Is Associated with Response to Thalidomide in Progressive Multiple Myeloma.* Clinical Cancer Research, 2001. 7(9): p. 2675-2681.

[96] Neben, K., et al., *Response to thalidomide in progressive multiple myeloma is not mediated by inhibition of angiogenic cytokine secretion.* British Journal of Haematology, 2001. 115(3): p. 605-608.

[97] Bisping, G., et al., *Paracrine interactions of basic fibroblast growth factor and interleukin-6 in multiple myeloma.* Blood, 2003. 101(7): p. 2775-2783.

[98] Chesi, M., et al., *Frequent translocation t(4;14)(p16.3;q32.3) in multiple myeloma is associated with increased expression and activating mutations of fibroblast growth factor receptor 3.* Nat Genet, 1997. 16(3): p. 260-264.

[99] Richelda, R., et al., *A Novel Chromosomal Translocation t(4; 14)(p16.3; q32) in Multiple Myeloma Involves the Fibroblast Growth-Factor Receptor 3 Gene.* Blood, 1997. 90(10): p. 4062-4070.

[100] Zhu, L., et al., *Fibroblast growth factor receptor 3 inhibition by short hairpin RNAs leads to apoptosis in multiple myeloma.* Molecular Cancer Therapeutics, 2005. 4(5): p. 787-798.

[101] Hadari, Y. and J. Schlessinger, *FGFR3-targeted mAb therapy for bladder cancer and multiple myeloma.* The Journal of Clinical Investigation, 2009. 119(5): p. 1077-1079.

[102] Eswarakumar, V.P., I. Lax, and J. Schlessinger, *Cellular signaling by fibroblast growth factor receptors.* Cytokine & Growth Factor Reviews, 2005. 16(2): p. 139-149.

[103] Alexandrow, M.G. and H.L. Moses, *Transforming Growth Factor β and Cell Cycle Regulation.* Cancer Research, 1995. 55(7): p. 1452-1457.

[104] Pepper, M.S., *Transforming growth factor-beta: Vasculogenesis, angiogenesis, and vessel wall integrity.* Cytokine & Growth Factor Reviews, 1997. 8(1): p. 21-43.

[105] Taipale, J., J. Saharinen, and J. Keski-Oja, *Extracellular Matrix-Associated Transforming Growth Factor-[beta]: Role in Cancer Cell Growth and Invasion*, in *Advances in Cancer Research*, F.V.W. George and K. George, Editors. 1998, Academic Press. p. 87-134.

[106] Norgaard, P., et al., *Transforming growth factor β and cancer.* Cancer treatment reviews, 1995. 21(4): p. 367-403.

[107] Letterio, J.J. and A.B. Roberts, *Regulation of immune responses by TGF-beta.* Annual Review of Immunology, 1998. 16: p. 137-161.

[108] Maehara, Y., et al., *Role of Transforming Growth Factor-ß1 in Invasion and Metastasis in Gastric Carcinoma.* Journal of Clinical Oncology, 1999. 17(2): p. 607.

[109] Picon, A., et al., *A subset of metastatic human colon cancers expresses elevated levels of transforming growth factor beta1.* Cancer Epidemiology Biomarkers & Prevention, 1998. 7(6): p. 497-504.

[110] Hojo, M., et al., *Cyclosporine induces cancer progression by a cell-autonomous mechanism.* Nature, 1999. 397(6719): p. 530-534.

[111] Matsumoto, T. and M. Abe, *TGF-β-related mechanisms of bone destruction in multiple myeloma.* Bone, 2011. 48(1): p. 129-134.

[112] Kroning, H., et al., *Overproduction of IL-7, IL-10 and TGF-beta 1 in multiple myeloma.* Acta Haematologica, 1997. 98(2): p. 116-118.

[113] Urashima, M., et al., *Transforming growth factor-beta1: differential effects on multiple myeloma versus normal B cells.* Blood, 1996. 87(5): p. 1928-1938.

[114] Cook, G., et al., *Transforming growth factor beta from multiple myeloma cells inhibits proliferation and IL-2 responsiveness in T lymphocytes.* Journal of Leukocyte Biology, 1999. 66(6): p. 981-988.

[115] Cook, G. and J.D.M. Campbell, *Immune regulation in multiple myeloma: the host–tumour conflict.* Blood reviews, 1999. 13(3): p. 151-162.

[116] Takeuchi, K., et al., *TGF-β Inhibition Restores Terminal Osteoblast Differentiation to Suppress Myeloma Growth.* PLoS ONE, 2010. 5(3): p. e9870.

[117] Choi, S.J., et al., *Antisense inhibition of macrophage inflammatory protein 1-a blocks bone destruction in a model of myeloma bone disease.* Journal of Clinical Investigation, 2001. 108(12): p. 1833-1841.

[118] Lentzsch, S., et al., *Macrophage inflammatory protein 1-alpha (MIP-1a) triggers migration and signaling cascades mediating survival and proliferation in multiple myeloma (MM) cells.* Blood, 2003. 101(9): p. 3568-3573.

[119] Menu, E., et al., *Role of CCR1 and CCR5 in homing and growth of multiple myeloma and in the development of osteolytic lesions: A study in the 5TMM model.* Clinical and Experimental Metastasis, 2006. 23(5-6): p. 291-300.

[120] Vanderkerken, K., et al., *Monocyte chemoattractant protein-1 (MCP-1), secreted by bone marrow endothelial cells, induces chemoattraction of 5T multiple myeloma cells.* Clinical and Experimental Metastasis, 2002. 19(1): p. 87-90.

[121] Vande Broek, I., et al., *Chemokine receptor CCR2 is expressed by human multiple myeloma cells and mediates migration to bone marrow stromal cell-produced monocyte chemotactic proteins MCP-1, -2 and -3.* British Journal of Cancer, 2003. 88(6): p. 855-862.

[122] Pellegrino, A., et al., *CXCR3-binding chemokines in multiple myeloma.* Cancer Letters, 2004. 207(2): p. 221-227.

[123] Pellegrino, A., et al., *Bone marrow endothelial cells in multiple myeloma secrete CXC-chemokines that mediate interactions with plasma cells.* British Journal of Haematology, 2005. 129(2): p. 248-256.

[124] Hideshima, T., et al., *The biological sequelae of stromal cell-derived factor-1alpha in multiple myeloma.* Molecular cancer therapeutics, 2002. 1(7): p. 539-544.

[125] Menu, E., et al., *The involvement of stromal derived factor 1a in homing and progression of multiple myeloma in the 5TMM model.* Haematologica, 2006. 91(5): p. 605-612.

[126] Gazitt, Y. and C. Akay, *Mobilization of Myeloma Cells Involves SDF-1/CXCR4 Signaling and Downregulation of VLA-4.* Stem Cells, 2004. 22(1): p. 65-73.

[127] Alsayed, Y., et al., *Mechanisms of regulation of CXCR4/SDF-1 (CXCL12)-dependent migration and homing in multiple myeloma.* Blood, 2007. 109(7): p. 2708-2717.

[128] Darnell Jr, J.E., I.M. Kerr, and G.R. Stark, *Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins.* Science, 1994. 264(5164): p. 1415-1421.

[129] Akira, S., et al., *Molecular cloning of APRF, a novel IFN-stimulated gene factor 3 p91-related transcription factor involved in the gp130-mediated signaling pathway.* Cell, 1994. 77(1): p. 63-71.

[130] Bromberg, J., *Stat proteins and oncogenesis.* Journal of Clinical Investigation, 2002. 109(9): p. 1139-1142.

[131] Alvarez, J.V. and D.A. Frank, *Genome-wide analysis of STAT target genes: Elucidating the mechanism of STAT-mediated oncogenesis.* Cancer Biology and Therapy, 2004. 3(11): p. 1045-1050.

[132] Takeda, K., et al., *Targeted disruption of the mouse Stat3 gene leads to early embryonic lethality.* Proceedings of the National Academy of Sciences of the United States of America, 1997. 94(8): p. 3801-3804.

[133] Akira, S., *Roles of STAT3 defined by tissue-specific gene targeting.* Oncogene, 2000. 19(21): p. 2607-2611.

[134] Puthier, D., R. Bataille, and M. Amiot, *IL-6 up-regulates Mcl-1 in human myeloma cells through JAK/STAT rather than Ras/MAP kinase pathway.* European Journal of Immunology, 1999. 29(12): p. 3945-3950.
[135] Puthier, D., et al., *Mcl-1 and Bcl-X(L) are co-regulated by IL-6 in human myeloma cells.* British Journal of Haematology, 1999. 107(2): p. 392-395.
[136] Spets, H., et al., *Expression of the bcl-2 family of pro- and anti-apoptotic genes in multiple myeloma and normal plasma cells: Regulation during interleukin-6 (IL-6)-induced growth and survival.* European Journal of Haematology, 2002. 69(2): p. 76-89.
[137] Catlett-Falcone, R., et al., *Constitutive activation of Stat3 signaling confers resistance to apoptosis in human U266 myeloma cells.* Immunity, 1999. 10(1): p. 105-115.
[138] Song, L., et al., *Mcl-1 mediates cytokine deprivation induced apoptosis of human myeloma cell line XG-7.* Chinese Medical Journal, 2002. 115(8): p. 1241-1243.
[139] Chanan-Khan, A.A., *Bcl-2 antisense therapy in multiple myeloma.* Oncology (Williston Park, N.Y.), 2004. 18(13 Suppl 10): p. 21-24.
[140] Quintanilla-Martinez, L., et al., *Analysis of signal transducer and activator of transcription 3 (Stat 3) pathway in multiple myeloma: Stat 3 activation and cyclin D1 dysregulation are mutually exclusive events.* American Journal of Pathology, 2003. 162(5): p. 1449-1461.
[141] Galm, O., et al., *SOCS-1, a negative regulator of cytokine signaling, is frequently silenced by methylation in multiple myeloma.* Blood, 2003. 101(7): p. 2784-2788.
[142] Yamamoto, M., et al., *Suppressor of cytokine signaling-1 expression by infectivity-enhanced adenoviral vector inhibits IL-6-dependent proliferation of multiple myeloma cells.* Cancer Gene Therapy, 2006. 13(2): p. 194-202.
[143] Amit-Vazina, M., et al., *Atiprimod blocks STAT3 phosphorylation and induces apoptosis in multiple myeloma cells.* British Journal of Cancer, 2005. 93(1): p. 70-80.
[144] Bai, L.Y., et al., *OSU-03012 sensitizes TIB-196 myeloma cells to imatinib mesylate via AMP-activated protein kinase and STAT3 pathways.* Leukemia Research, 2010. 34(6): p. 816-820.
[145] Bharti, A.C., N. Donato, and B.B. Aggarwal, *Curcumin (diferuloylmethane) inhibits constitutive and IL-6-inducible STAT3 phosphorylation in human multiple myeloma cells.* Journal of Immunology, 2003. 171(7): p. 3863-3871.
[146] Bhutani, M., et al., *Capsaicin is a novel blocker of constitutive and interleukin-6 - Inducible STAT3 activation.* Clinical Cancer Research, 2007. 13(10): p. 3024-3032.
[147] Che, Y., et al., *Serenoa repens induces growth arrest and apoptosis of human multiple myeloma cells via inactivation of STAT 3 signaling.* Oncology Reports, 2009. 22(2): p. 377-383.
[148] De Vos, J., et al., *JAK2 tyrosine kinase inhibitor tyrphostin AG490 downregulates the mitogen-activated protein kinase (MAPK) and signal transducer and activator of transcription (STAT) pathways and induces apoptosis in myeloma cells.* British Journal of Haematology, 2000. 109(4): p. 823-828.
[149] Heo, J.Y., et al., *Embelin suppresses STAT3 signaling, proliferation, and survival of multiple myeloma via the protein tyrosine phosphatase PTEN.* Cancer Letters, 2011. 308(1): p. 71-80.
[150] Kim, H.J., et al., *Decursin chemosensitizes human multiple myeloma cells through inhibition of STAT3 signaling pathway.* Cancer Letters, 2011. 301(1): p. 29-37.
[151] Kim, S.H., et al., *Janus activated kinase 2/signal transducer and activator of transcription 3 pathway mediates icariside II-induced apoptosis in U266 multiple myeloma cells.* European Journal of Pharmacology, 2011. 654(1): p. 10-16.

[152] Kim, S.H., et al., *Signal transducer and activator of transcription 3 pathway mediates genipin-induced apoptosis in U266 multiple myeloma cells.* Journal of Cellular Biochemistry, 2011. 112(6): p. 1552-1562.

[153] Kunnumakkara, A.B., et al., *Boswellic acid blocks signal transducers and activators of transcription 3 signaling, proliferation, and survival of multiple myeloma via the protein tyrosine phosphatase SHP-1.* Molecular Cancer Research, 2009. 7(1): p. 118-128.

[154] Li, F., P. Rajendran, and G. Sethi, *Thymoquinone inhibits proliferation, induces apoptosis and chemosensitizes human multiple myeloma cells through suppression of signal transducer and activator of transcription 3 activation pathway.* British Journal of Pharmacology, 2010. 161(3): p. 541-554.

[155] Li, J., et al., *INCB16562, a JAK1/2 selective inhibitor, is efficacious against multiple myeloma cells and reverses the protective effects of cytokine and stromal cell support.* Neoplasia, 2010. 12(1): p. 28-38.

[156] Lin, L., et al., *A novel small molecule inhibits STAT3 phosphorylation and DNA binding activity and exhibits potent growth suppressive activity in human cancer cells.* Molecular Cancer, 2010. 9.

[157] Liu, S., et al., *Inhibitory effect of baicalein on IL-6-mediated signaling cascades in human myeloma cells.* European Journal of Haematology, 2010. 84(2): p. 137-144.

[158] Ma, J., et al., *Therapeutic potential of cladribine in combination with STAT3 inhibitor against multiple myeloma.* BMC Cancer, 2011: p. 255.

[159] Ma, J., et al., *Mechanism of MS-275 blocking STAT3 and NF-κB signaling, inducing apoptosis in U266 cells.* Chinese Journal of Cancer Prevention and Treatment, 2009. 16(16): p. 1234-1237.

[160] Malara, N., et al., *Simultaneous inhibition of the constitutively activated nuclear factor κB and of the Interleukin-6 pathways is necessary and sufficient to completely overcome apoptosis resistance of human U266 myeloma cells.* Cell Cycle, 2008. 7(20): p. 3235-3245.

[161] Muto, A., et al., *Emodin has a cytotoxic activity against human multiple myeloma as a Janus-activated kinase 2 inhibitor.* Molecular cancer therapeutics, 2007. 6(3): p. 987-994.

[162] Nakaya, A., et al., *The gold compound auranofin induces apoptosis of human multiple myeloma cells through both down-regulation of STAT3 and inhibition of NF-κB activity.* Leukemia Research, 2011. 35(2): p. 243-249.

[163] Nelson, E.A., et al., *Nifuroxazide inhibits survival of multiple myeloma cells by directly inhibiting STAT3.* Blood, 2008. 112(13): p. 5095-5102.

[164] Pandey, M.K., et al., *Butein suppresses constitutive and inducible signal transducer and activator of transcription (stat) 3 activation and stat3-regulated gene products through the induction of a protein tyrosine phosphatase SHP-1.* Molecular Pharmacology, 2009. 75(3): p. 525-533.

[165] Pandey, M.K., B. Sung, and B.B. Aggarwal, *Betulinic acid suppresses STAT3 activation pathway through induction of protein tyrosine phosphatase SHP-1 in human multiple myeloma cells.* International Journal of Cancer, 2010. 127(2): p. 282-292.

[166] Park, J., et al., *Blockage of interleukin-6 signaling with 6-amino-4-quinazoline synergistically induces the inhibitory effect of bortezomib in human U266 cells.* Anti-Cancer Drugs, 2008. 19(8): p. 777-782.

[167] Park, S., et al., *Inhibition of JAK1/STAT3 signaling mediates compound K-induced apoptosis in human multiple myeloma U266 cells.* Food and Chemical Toxicology, 2011. 49(6): p. 1367-1372.

[168] Pathak, A.K., et al., *Ursolic acid inhibits STAT3 activation pathway leading to suppression of proliferation and chemosensitization of human multiple myeloma cells.* Molecular Cancer Research, 2007. 5(9): p. 943-955.
[169] Pedranzini, L., et al., *Pyridone 6, a Pan-Janus-activated kinase inhibitor, induces growth inhibition of multiple myeloma cells.* Cancer research, 2006. 66(19): p. 9714-9721.
[170] Peng, J., et al., *Patrinia scabiosaefolia extract suppresses proliferation and promotes apoptosis by inhibiting the STAT3 pathway in human multiple myeloma cells.* Molecular Medicine Reports, 2011. 4(2): p. 313-318.
[171] Ramakrishnan, V., et al., *TG101209, a novel JAK2 inhibitor, has significant in vitro activity in multiple myeloma and displays preferential cytotoxicity for CD45+ myeloma cells.* American Journal of Hematology, 2010. 85(9): p. 675-686.
[172] Sagawa, M., et al., *Cantharidin induces apoptosis of human multiple myeloma cells via inhibition of the JAK/STAT pathway.* Cancer Science, 2008. 99(9): p. 1820-1826.
[173] Santo, L., et al., *Antimyeloma activity of a multitargeted kinase inhibitor, AT9283, via potent Aurora kinase and STAT3 inhibition either alone or in combination with lenalidomide.* Clinical Cancer Research, 2011. 17(10): p. 3259-3271.
[174] Scuto, A., et al., *The novel JAK inhibitor AZD1480 blocks STAT3 and FGFR3 signaling, resulting in suppression of human myeloma cell growth and survival.* Leukemia, 2011. 25(3): p. 538-550.
[175] Zhang, S., et al., *The novel histone deacetylase inhibitor, AR-42, inhibits gp130/Stat3 pathway and induces apoptosis and cell cycle arrest in multiple myeloma cells.* International Journal of Cancer, 2011. 129(1): p. 204-213.
[176] Sethi, G., B. Sung, and B.B. Aggarwal, *Nuclear factor-κB activation: From bench to bedside.* Experimental Biology and Medicine, 2008. 233(1): p. 21-31.
[177] Sethi, G. and V. Tergaonkar, *Potential pharmacological control of the NF-κB pathway.* Trends in Pharmacological Sciences, 2009. 30(6): p. 313-321.
[178] Weaver, D. and D. Baltimore, *B lymphocyte-specific protein binding near an immunoglobulin κ-chain gene J segment (DNA binding protein/B cell-specific protein/DNA rearranggement).* Proceedings of the National Academy of Sciences of the United States of America, 1987. 84(6): p. 1516-1520.
[179] Aggarwal, B.B., *Nuclear factor-κB: The enemy within.* Cancer Cell, 2004. 6(3): p. 203-208.
[180] Pikarsky, E., et al., *NF-κB functions as a tumour promoter in inflammation-associated cancer.* Nature, 2004. 431(7007): p. 461-466.
[181] Greten, F.R., et al., *IKKβ links inflammation and tumorigenesis in a mouse model of colitis-associated cancer.* Cell, 2004. 118(3): p. 285-296.
[182] Seitz, C.S., et al., *Alterations in NF-κB function in transgenic epithelial tissue demonstrate a growth inhibitory role for NF-κB.* Proceedings of the National Academy of Sciences of the United States of America, 1998. 95(5): p. 2307-2312.
[183] Bharti, A.C., et al., *Nuclear factor-κB and STAT3 are constitutively active in CD138 + cells derived from multiple myeloma patients, and suppression of these transcription factors leads to apoptosis.* Blood, 2004. 103(8): p. 3175-3184.
[184] Keats, J.J., et al., *Promiscuous Mutations Activate the Noncanonical NF-κB Pathway in Multiple Myeloma.* Cancer Cell, 2007. 12(2): p. 131-144.
[185] Annunziata, C.M., et al., *Frequent Engagement of the Classical and Alternative NF-κB Pathways by Diverse Genetic Abnormalities in Multiple Myeloma.* Cancer Cell, 2007. 12(2): p. 115-130.

[186] Berenson, J.R., H.M. Ma, and R. Vescio, *The role of nuclear factor-κB in the biology and treatment of multiple myeloma.* Seminars in Oncology, 2001. 28(6): p. 626-633.

[187] Feinman, R., et al., *Role of NF-κB in the rescue of multiple myeloma cells from glucocorticoid-induced apoptosis by bcl-2.* Blood, 1999. 93(9): p. 3044-3052.

[188] Hideshima, T., et al., *NF-κB as a therapeutic target in multiple myeloma.* Journal of Biological Chemistry, 2002. 277(19): p. 16639-16647.

[189] Brinckerhoff, C.E. and L.M. Matrisian, *Matrix metalloproteinases: A tail of a frog that became a prince.* Nature Reviews Molecular Cell Biology, 2002. 3(3): p. 207-214.

[190] Egeblad, M. and Z. Werb, *New functions for the matrix metalloproteinases in cancer progression.* Nature Reviews Cancer, 2002. 2(3): p. 161-174.

[191] Zdzisińska, B., et al., *Matrix metalloproteinase and cytokine production by bone marrow adherent cells from multiple myeloma patients.* Archivum Immunologiae et Therapiae Experimentalis, 2006. 54(4): p. 289-296.

[192] Vande Broek, I., et al., *Bone marrow endothelial cells increase the invasiveness of human multiple myeloma cells through upregulation of MMP-9: Evidence for a role of hepatocyte growth factor.* Leukemia, 2004. 18(5): p. 976-982.

[193] Van Valckenborgh, E., et al., *Multifunctional role of matrix metalloproteinases in multiple myeloma: A study in the 5T2MM mouse model.* American Journal of Pathology, 2004. 165(3): p. 869-878.

[194] Klagsbrun, M. and P. A. D'Amore, *Vascular endothelial growth factor and its receptors.* Cytokine & Growth Factor Reviews, 1996. 7(3): p. 259-270.

[195] Dvorak, H.F., *Vascular Permeability Factor/Vascular Endothelial Growth Factor: A Critical Cytokine in Tumor Angiogenesis and a Potential Target for Diagnosis and Therapy.* Journal of Clinical Oncology, 2002. 20(21): p. 4368-4380.

[196] Asahara, T., et al., *Synergistic Effect of Vascular Endothelial Growth Factor and Basic Fibroblast Growth Factor on Angiogenesis In Vivo.* Circulation, 1995. 92(9): p. 365-371.

[197] Aguayo, A., et al., *Angiogenesis in acute and chronic leukemias and myelodysplastic syndromes.* Blood, 2000. 96(6): p. 2240-2245.

[198] Salven, P., et al., *Simultaneous elevation in the serum concentrations of the angiogenic growth factors VEGF and bFGF is an independent predictor of poor prognosis in non-Hodgkin lymphoma: a single-institution study of 200 patients.* Blood, 2000. 96(12): p. 3712-3718.

[199] Molica, S., et al., *Increased serum levels of vascular endothelial growth factor predict risk of progression in early B-cell chronic lymphocytic leukaemia.* British Journal of Haematology, 1999. 107(3): p. 605-610.

[200] Aguayo, A., et al., *Clinical relevance of intracellular vascular endothelial growth factor levels in B-cell chronic lymphocytic leukemia.* Blood, 2000. 96(2): p. 768-770.

[201] *A Predictive Model for Aggressive Non-Hodgkin's Lymphoma.* New England Journal of Medicine, 1993. 329(14): p. 987-994.

[202] Inoue, M., et al., *VEGF-A has a critical, nonredundant role in angiogenic switching and pancreatic ² cell carcinogenesis.* Cancer cell, 2002. 1(2): p. 193-202.

[203] Moehler, T.M., et al., *Angiogenesis in hematologic malignancies.* Critical reviews in oncology/hematology, 2003. 45(3): p. 227-244.

[204] Rajkumar, S.V. and R.A. Kyle, *Angiogenesis in multiple myeloma.* Seminars in oncology, 2001. 28(6): p. 560-564.

[205] Jakob, C., et al., *Angiogenesis in multiple myeloma.* European journal of cancer (Oxford, England : 1990), 2006. 42(11): p. 1581-1590.

[206] Vacca, A., et al., *Bone marrow angiogenesis and progression in multiple myeloma.* British Journal of Haematology, 1994. 87(3): p. 503-508.
[207] Vacca, A., et al., *Angiogenesis in B Cell Lymphoproliferative Diseases. Biological and Clinical Studies.* Leukemia & Lymphoma, 1995. 20(1-2): p. 27-38.
[208] Rajkumar, S.V., et al., *Prognostic Value of Bone Marrow Angiogenesis in Multiple Myeloma.* Clinical Cancer Research, 2000. 6(8): p. 3111-3116.
[209] Sezer, et al., *Relationship between bone marrow angiogenesis and plasma cell infiltration and serum ß2-microglobulin levels in patients with multiple myeloma.* Annals of Hematology, 2001. 80(10): p. 598-601.
[210] Di Raimondo, F., et al., *Angiogenic factors in multiple myeloma: higher levels in bone marrow than in peripheral blood.* Haematologica, 2000. 85(8): p. 800-805.
[211] Ribatti, D., et al., *Bone marrow angiogenesis and mast cell density increase simultaneously with progression of human multiple myeloma.* Br J Cancer, 1999. 79(3-4): p. 451-455.
[212] Bellamy, W.T., *Expression of vascular endothelial growth factor and its receptors in multiple myeloma and other hematopoietic malignancies.* Seminars in oncology, 2001. 28(6): p. 551-559.
[213] Ugurel, S., et al., *Increased Serum Concentration of Angiogenic Factors in Malignant Melanoma Patients Correlates With Tumor Progression and Survival.* Journal of Clinical Oncology, 2001. 19(2): p. 577-583.
[214] Iwasaki, T. and H. Sano, *Predicting Treatment Responses and Disease Progression in Myeloma using Serum Vascular Endothelial Growth Factor and Hepatocyte Growth Factor Levels.* Leukemia & Lymphoma, 2003. 44(8): p. 1347-1351.
[215] Hayashi, T., et al., *Ex vivo induction of multiple myeloma-specific cytotoxic T lymphocytes.* Blood, 2003. 102(4): p. 1435-1442.
[216] Hideshima, T., et al., *Novel therapies targeting the myeloma cell and its bone marrow microenvironment.* Seminars in oncology, 2001. 28(6): p. 607-612.
[217] Podar, K., et al., *Vascular endothelial growth factor triggers signaling cascades mediating multiple myeloma cell growth and migration.* Blood, 2001. 98(2): p. 428-435.
[218] Bellamy, W.T., et al., *Expression of Vascular Endothelial Growth Factor and Its Receptors in Hematopoietic Malignancies.* Cancer Research, 1999. 59(3): p. 728-733.
[219] Kumar, S., et al., *Expression of VEGF and its receptors by myeloma cells.* Leukemia, 0000. 17(10): p. 2025-2031.
[220] Yaccoby, S., B. Barlogie, and J. Epstein, *Primary Myeloma Cells Growing in SCID-hu Mice: A Model for Studying the Biology and Treatment of Myeloma and Its Manifestations.* Blood, 1998. 92(8): p. 2908-2913.
[221] Hideshima, T., et al., *Understanding multiple myeloma pathogenesis in the bone marrow to identify new therapeutic targets.* Nature Reviews Cancer, 2007. 7(8): p. 585-598.
[222] Nakagawa, M., et al., *Vascular endothelial growth factor (VEGF) directly enhances osteoclastic bone resorption and survival of mature osteoclasts.* Febs Letters, 2000. 473(2): p. 161-164.
[223] Henriksen, K., et al., *RANKL and Vascular Endothelial Growth Factor (VEGF) Induce Osteoclast Chemotaxis through an ERK1/2-dependent Mechanism.* Journal of Biological Chemistry, 2003. 278(49): p. 48745-48753.
[224] Gabrilovich, D.I., et al., *Production of vascular endothelial growth factor by human tumors inhibits the functional maturation of dendritic cells (vol 2, pg 1096, 1996).* Nature Medicine, 1996. 2(11): p. 1267-1267.

[225] Dankbar, B., et al., *Vascular endothelial growth factor and interleukin-6 in paracrine tumor-stromal cell interactions in multiple myeloma.* Blood, 2000. 95(8): p. 2630-2636.
[226] Neufeld, G., et al., *Vascular endothelial growth factor (VEGF) and its receptors.* The FASEB Journal, 1999. 13(1): p. 9-22.
[227] Li, J., et al., *Induction of Vascular Endothelial Growth Factor Gene Expression by Interleukin-1 in Rat Aortic Smooth Muscle Cells.* Journal of Biological Chemistry, 1995. 270(1): p. 308-312.
[228] Matsumoto, K., H. Ohi, and K. Kanmatsuse, *Interleukin 10 and interleukin 13 synergize to inhibit vascular permeability factor release by peripheral blood mononuclear cells from patients with lipoid nephrosis.* Nephron, 1997. 77(2): p. 212-218.
[229] Gupta, D., et al., *Adherence of multiple myeloma cells to bone marrow stromal cells upregulates vascular endothelial growth factor secretion: therapeutic applications.* Leukemia, 2001. 15(12): p. 1950-1961.
[230] Goad, D.L., et al., *Enhanced expression of vascular endothelial growth factor in human SaOS-2 osteoblast-like cells and murine osteoblasts induced by insulin-like growth factor I.* Endocrinology, 1996. 137(6): p. 2262-8.
[231] Deroanne, C.F., et al., *Angiogenesis by Fibroblast Growth Factor 4 Is Mediated through an Autocrine Up-Regulation of Vascular Endothelial Growth Factor Expression.* Cancer Research, 1997. 57(24): p. 5590-5597.
[232] Finkenzeller, G., et al., *Sp1 recognition sites in the proximal promoter of the human vascular endothelial growth factor gene are essential for platelet-derived growth factor-induced gene expression.* Oncogene, 1997. 15(6): p. 669-76.
[233] Pertovaara, L., et al., *Vascular endothelial growth factor is induced in response to transforming growth factor-beta in fibroblastic and epithelial cells.* Journal of Biological Chemistry, 1994. 269(9): p. 6271-6274.
[234] Ryuto, M., et al., *Induction of Vascular Endothelial Growth Factor by Tumor Necrosis Factor a in Human Glioma Cells.* Journal of Biological Chemistry, 1996. 271(45): p. 28220-28228.
[235] Wang, T.-H., et al., *Human Chorionic Gonadotropin-Induced Ovarian Hyperstimulation Syndrome Is Associated with Up-Regulation of Vascular Endothelial Growth Factor.* Journal of Clinical Endocrinology & Metabolism, 2002. 87(7): p. 3300-3308.
[236] Hurt, E.M., et al., *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(2): p. 191-199.
[237] Gerber, H.P., et al., *Vascular endothelial growth factor regulates endothelial cell survival through the phosphatidylinositol 3'-kinase/Akt signal transduction pathway: Requirement for Flk-1/KDR activation.* Journal of Biological Chemistry, 1998. 273(46): p. 30336-30343.
[238] Podar, K., et al., *Vascular Endothelial Growth Factor-induced Migration of Multiple Myeloma Cells Is Associated with β1 Integrin- and Phosphatidylinositol 3-Kinase-dependent PKCa Activation.* Journal of Biological Chemistry, 2002. 277(10): p. 7875-7881.
[239] Qi, J.H. and L. Claesson-Welsh, *VEGF-induced activation of phosphoinositide 3-kinase is dependent on focal adhesion kinase.* Experimental Cell Research, 2001. 263(1): p. 173-182.

[240] Waltenberger, J., et al., *DIFFERENT SIGNAL-TRANSDUCTION PROPERTIES OF KDR AND FLT1, 2 RECEPTORS FOR VASCULAR ENDOTHELIAL GROWTH-FACTOR.* Journal of Biological Chemistry, 1994. 269(43): p. 26988-26995.
[241] Le Gouill, S., et al., *VEGF induces Mcl-1 up-regulation and protects multiple myeloma cells against apoptosis.* Blood, 2004. 104(9): p. 2886-2892.
[242] Meyer, R.D., et al., *The presence of a single tyrosine residue at the carboxyl domain of vascular endothelial growth factor receptor-2/FLK-1 regulates its autophosphorylation and activation of signaling molecules.* Journal of Biological Chemistry, 2002. 277(30): p. 27081-27087.
[243] Rousseau, S., et al., *Vascular Endothelial Growth Factor (VEGF)-driven Actin-based Motility Is Mediated by VEGFR2 and Requires Concerted Activation of Stress-activated Protein Kinase 2 (SAPK2/p38) and Geldanamycin-sensitive Phosphorylation of Focal Adhesion Kinase.* Journal of Biological Chemistry, 2000. 275(14): p. 10661-10672.
[244] Kim, K.J., et al., *Inhibition of vascular endothelial growth factor-induced angiogenesis suppresses tumour growth in vivo.* Nature, 1993. 362(6423): p. 841-844.
[245] Ferrara, N., et al., *Discovery and development of bevacizumab, an anti-VEGF antibody for treating cancer.* Nat Rev Drug Discov, 2004. 3(5): p. 391-400.
[246] Lin, B., et al., *The Vascular Endothelial Growth Factor Receptor Tyrosine Kinase Inhibitor PTK787/ZK222584 Inhibits Growth and Migration of Multiple Myeloma Cells in the Bone Marrow Microenvironment.* Cancer Research, 2002. 62(17): p. 5019-5026.
[247] Wood, J.M., et al., *PTK787/ZK 222584, a Novel and Potent Inhibitor of Vascular Endothelial Growth Factor Receptor Tyrosine Kinases, Impairs Vascular Endothelial Growth Factor-induced Responses and Tumor Growth after Oral Administration.* Cancer Research, 2000. 60(8): p. 2178-2189.
[248] Sloan, B. and N.S. Scheinfeld, *Pazopanib, a VEGF receptor tyrosine kinase inhibitor for cancer therapy.* Current Opinion in Investigational Drugs, 2008. 9(12): p. 1324-1335.
[249] Thiery, J.P., *Cell adhesion in cancer.* Comptes Rendus Physique, 2003. 4(2): p. 289-304.
[250] Sanz-Rodríguez, F. and J. Teixidó, *VLA-4-dependent myeloma cell adhesion.* Leukemia and Lymphoma, 2001. 41(3-4): p. 239-245.
[251] Sanz-Rodríguez, F., et al., *Characterization of VLA-4-dependent myeloma cell adhesion to fibronectin and VCAM-1.* British Journal of Haematology, 1999. 107(4): p. 825-834.
[252] Uchiyama, H., et al., *Characterization of adhesion molecules on human myeloma cell lines.* Blood, 1992. 80(9): p. 2306-2314.
[253] Sanz-Rodríguez, F., A. Hidalgo, and J. Teixidó, *Chemokine stromal cell-derived factor-1a modulates VLA-4 integrin-mediated multiple myeloma cell adhesion to CS-1/fibronectin and VCAM-1.* Blood, 2001. 97(2): p. 346-351.
[254] Damiano, J.S., et al., *Cell adhesion mediated drug resistance (CAM-DR): Role of integrins and resistance to apoptosis in human myeloma cell lines.* Blood, 1999. 93(5): p. 1658-1667.
[255] Landowski, T.H., et al., *Cell adhesion-mediated drug resistance (CAM-DR) is associated with activation of NF-κB (RelB/p50) in myeloma cells.* Oncogene, 2003. 22(16): p. 2417-2421.
[256] Shain, K.H., et al., *β1 integrin adhesion enhances IL-6-mediated STAT3 signaling in myeloma cells: Implications for microenvironment influence on tumor survival and proliferation.* Cancer research, 2009. 69(3): p. 1009-1015.
[257] Fulciniti, M., et al., *A high-affinity fully human anti-IL-6 mAb, 1339, for the treatment of multiple myeloma.* Clinical Cancer Research, 2009. 15(23): p. 7144-7152.
[258] Hideshima, T., et al., *Proteasome inhibitor PS-341 abrogates IL-6 triggered signaling cascades via caspase-dependent downregulation of gp130 in multiple myeloma.* Oncogene, 2003. 22(52): p. 8386-8393.

[259] Tai, Y.T., et al., *Human anti-CD40 antagonist antibody triggers significant antitumor activity against human multiple myeloma.* Cancer research, 2005. 65(13): p. 5898-5906.

[260] Voorhees, P.M., et al., *Inhibition of interleukin-6 signaling with CNTO 328 enhances the activity of bortezomib in preclinical models of multiple myeloma.* Clinical Cancer Research, 2007. 13(21): p. 6469-6478.

[261] Hunsucker, S.A., et al., *Blockade of interleukin-6 signalling with siltuximab enhances melphalan cytotoxicity in preclinical models of multiple myeloma.* British Journal of Haematology, 2011. 152(5): p. 579-592.

[262] Voorhees, P.M., et al., *Targeted inhibition of interleukin-6 with CNTO 328 sensitizes pre-clinical models of multiple myeloma to dexamethasone-mediated cell death.* British Journal of Haematology, 2009. 145(4): p. 481-490.

[263] Todoerti, K., et al., *Pleiotropic anti-myeloma activity of ITF2357: Inhibition of interleukin-6 receptor signaling and repression of miR-19a and miR-19b.* Haematologica, 2010. 95(2): p. 260-269.

[264] Bisping, G., et al., *Targeting receptor kinases by a novel indolinone derivative in multiple myeloma: Abrogation of stroma-derived interleukin-6 secretion and induction of apoptosis in cytogenetically defined subgroups.* Blood, 2006. 107(5): p. 2079-2089.

[265] Tassone, P., et al., *Combination therapy with interleukin-6 receptor superantagonist Sant7 and dexamethasone induces antitumor effects in a novel SCID-hu in vivo model of human multiple myeloma.* Clinical Cancer Research, 2005. 11(11): p. 4251-4258.

[266] Lamm, W., et al., *Bortezomib combined with rituximab and dexamethasone is an active regimen for patients with relapsed and chemotherapy-refractory mantle cell lymphoma.* Haematologica, 2011. 96(7): p. 1008-1014.

[267] Wemeau, M., S. Balkaran, and X. Leleu, *Increased sensitivity to bortezomib after mobilization of multiple myeloma cells with the CXCR4 antagonist AMD3100.* Majoration de la sensibilité au bortézomib dans le myélome multiple en favorisant la migration des cellules tumorales dans le sang par un inhibiteur de CXCR4, l'AMD3100, 2009. 15(3): p. 194-196.

[268] Rossi, J.F., et al., *Atacicept in relapsed/refractory multiple myeloma or active Waldenstrom's macroglobulinemia: a phase I study.* Br J Cancer, 0000. 101(7): p. 1051-1058.

[269] Presta, L.G., et al., *Humanization of an Anti-Vascular Endothelial Growth Factor Monoclonal Antibody for the Therapy of Solid Tumors and Other Disorders.* Cancer Research, 1997. 57(20): p. 4593-4599.

[270] Lin, B., et al., *The vascular endothelial growth factor receptor tyrosine kinase inhibitor PTK787/ZK222584 inhibits growth and migration of multiple myeloma cells in the bone marrow microenvironment.* Cancer research, 2002. 62(17): p. 5019-5026.

[271] Hagedorn, M. and A. Bikfalvi, *Target molecules for anti-angiogenic therapy: from basic research to clinical trials.* Critical reviews in oncology/hematology, 2000. 34(2): p. 89-110.

[272] *Development of SU5416, a selective small molecule inhibitor of VEGF receptor tyrosine kinase activity, as an anti-angiogenesis agent.* Anti-Cancer Drug Design, 2000. 15: p. 29-41.

[273] Ramakrishnan, V., et al., *Sorafenib, a dual Raf kinase/vascular endothelial growth factor receptor inhibitor has significant anti-myeloma activity and synergizes with common anti-myeloma drugs.* Oncogene, 2010. 29(8): p. 1190-1202.

[274] Flier, J.S., P. Usher, and A.C. Moses, *Monoclonal antibody to the type I insulin-like growth factor (IGF-I) receptor blocks IGF-I receptor-mediated DNA synthesis: clarification of the*

mitogenic mechanisms of IGF-I and insulin in human skin fibroblasts. Proceedings of the National Academy of Sciences, 1986. 83(3): p. 664-668.

[275] Hayry, P., et al., *Stabile D-peptide analog of insulin-like growth factor-1 inhibits smooth muscle cell proliferation after carotid ballooning injury in the rat.* The FASEB Journal, 1995. 9(13): p. 1336-1344.

[276] Pietrzkowski, Z., et al., *Inhibition of Growth of Prostatic Cancer Cell Lines by Peptide Analogues of Insulin-like Growth Factor 1.* Cancer Research, 1993. 53(5): p. 1102-1106.

[277] *Insulin-Like Growth Factor 1 Receptor Targeted Therapeutics: Novel Compounds and Novel Treatment Strategies for Cancer Medicine.* Recent Patents on Anti-Cancer Drug Discovery, 2009. 4: p. 54-72.

[278] Hayashi, T., et al., *Transforming growth factor β receptor I kinase inhibitor down-regulates cytokine secretion and multiple myeloma cell growth in the bone marrow microenvironment.* Clinical Cancer Research, 2004. 10(22): p. 7540-7546.

[279] Beider, K., et al., *CXCR4 antagonist 4F-benzoyl-TN14003 inhibits leukemia and multiple myeloma tumor growth.* Experimental Hematology, 2011. 39(3): p. 282-292.

[280] Azab, A.K., et al., *CXCR4 inhibitor AMD3100 disrupts the interaction of multiple myeloma cells with the bone marrow microenvironment and enhances their sensitivity to therapy.* Blood, 2009. 113(18): p. 4341-4351.

[281] Oliveira, A.M., et al., *Thalidomide treatment down-regulates SDF-1a and CXCR4 expression in multiple myeloma patients.* Leukemia Research, 2009. 33(7): p. 970-973.

[282] Haridas, V., et al., *Avicin D: A protein reactive plant isoprenoid dephosphorylates Stat3 by regulating both kinase and phosphatase activities.* PLoS ONE, 2009. 4(5).

[283] Hamasaki, M., et al., *Azaspirane (N-N-diethyl-8,8-dipropyl-2-azaspiro [4.5] decane-2-propanamine) inhibits human multiple myeloma cell growth in the bone marrow milieu in vitro and in vivo.* Blood, 2005. 105(11): p. 4470-4476.

[284] Kannaiyan, R., et al., *Celastrol Inhibits Proliferation and Induces Chemosensitization through downregulation of NF-kappaB and STAT3 Regulated Gene Products in Multiple Myeloma Cells.* Br J Pharmacol, 2011.

[285] Burger, R., et al., *Janus kinase inhibitor INCB20 has antiproliferative and apoptotic effects on human myeloma cells in vitro and in vivo.* Molecular cancer therapeutics, 2009. 8(1): p. 26-35.

[286] Sandur, S.K., et al., *5-Hydroxy-2-methyl-1,4-naphthoquinone, a vitamin K3 analogue, suppresses STAT3 activation pathway through induction of protein tyrosine phosphatase, SHP-1: Potential role in chemosensitization.* Molecular Cancer Research, 2010. 8(1): p. 107-118.

[287] Bhardwaj, A., et al., *Resveratrol inhibits proliferation, induces apoptosis, and overcomes chemoresistance through down-regulation of STAT3 and nuclear factor-κB-regulated antiapoptotic and cell survival gene products in human multiple myeloma cells.* Blood, 2007. 109(6): p. 2293-2302.

[288] Khong, T., J. Sharkey, and A. Spencer, *The effect of azacitidine on interleukin-6 signaling and nuclear factor-κB activation and its in vitro and in vivo activity against multiple myeloma.* Haematologica, 2008. 93(6): p. 860-869.

[289] Dai, Y., et al., *Interruption of the NF-κB pathway by Bay 11-7082 promotes UCN-01-mediated mitochondrial dysfunction and apoptosis in human multiple myeloma cells.* Blood, 2004. 103(7): p. 2761-2770.

[290] He, H., et al., *Genistein down-regulates the constitutive activation of nuclear factor-κB of bone marrow stromal cells in multiple myeloma, leading to suppression of gene expression and proliferation.* Drug Development Research, 2008. 69(4): p. 219-225.

[291] Hideshima, T., et al., *MLN120B, a novel IκB kinase β inhibitor, blocks multiple myeloma cell growth in vitro and in vivo.* Clinical Cancer Research, 2006. 12(19): p. 5887-5894.

[292] Kong, F., et al., *Inhibitory effects of parthenolide on the angiogenesis induced by human multiple myeloma cells and the mechanism.* Journal of Huazhong University of Science and Technology - Medical Science, 2008. 28(5): p. 525-530.

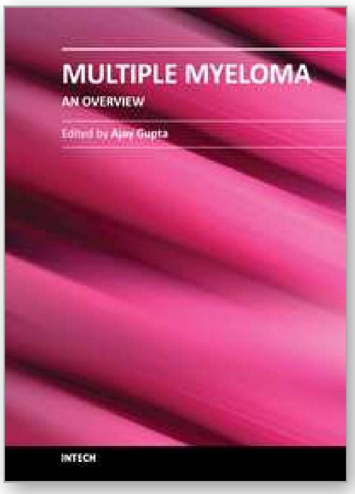
[293] Hossain, Z., et al., *Chitosan and marine phospholipids reduce matrix metalloproteinase activity in myeloma SP2 tumor-bearing mice.* European Journal of Lipid Science and Technology, 2009. 111(9): p. 877-883.

[294] Ritchie, J.P., et al., *SST0001, a chemically modified heparin, inhibits myeloma growth and angiogenesis via disruption of the heparanase/syndecan-1 axis.* Clinical Cancer Research, 2011. 17(6): p. 1382-1393.

[295] Mori, Y., et al., *Anti-a4 integrin antibody suppresses the development of multiple myeloma and associated osteoclastic osteolysis.* Blood, 2004. 104(7): p. 2149-2154.

[296] Olson, D.L., et al., *Anti-a4 integrin monoclonal antibody inhibits multiple myeloma growth in a murine model.* Molecular cancer therapeutics, 2005. 4(1): p. 91-99.

[297] Wang, X., Z. Zhang, and C. Yao, *Targeting integrin-linked kinase increases apoptosis and decreases invasion of myeloma cell lines and inhibits IL-6 and VEGF secretion from BMSCs.* Medical Oncology, 2010: p. 1-5.



Multiple Myeloma - An Overview

Edited by Dr. Ajay Gupta

ISBN 978-953-307-768-0
Hard cover, 274 pages
Publisher InTech
Published online 20, January, 2012
Published in print edition January, 2012

Multiple myeloma is a malignant disorder characterized by the proliferation of plasma cells. Much insight has been gained into the molecular pathways that lead to myeloma and indeed much more remains to be done. The understanding of these pathways is closely linked to their therapeutic implications and is stressed upon in the initial chapters. Recently, the introduction of newer agents such as bortezomib, lenalidomide, thalidomide, liposomal doxorubicin, etc. has led to a flurry of trials aimed at testing various combinations in order to improve survival. Higher response rates observed with these agents have led to their integration into induction therapies. The role of various new therapies vis a vis transplantation has also been examined. Recent advances in the management of plasmacytomas , renal dysfunction, dentistry as well as mobilization of stem cells in the context of myeloma have also found exclusive mention. Since brevity is the soul of wit our attempt has been to present before the reader a comprehensive yet brief text on this important subject.

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821