The Immune Microenvironment of Myeloma

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Abstract The bone marrow (BM) is the site of disease in myeloma and possesses unique immune characteristics involved in the pathobiology of the disease. Interactions of plasma cells with stromal cells, osteoclasts, osteoblasts, myeloid and lymphoid cells make up the unique bone marrow milieu that mediates myeloma disease progression. Independently or through a complex network of interactions these cells impart immune changes leading to immune evasion and disease progression. The critical role of these factors in disease progression has led to the intense development of therapeutic strategies aimed at either disrupting the immune mechanisms mediating disease progression or augmenting those with anti-tumor benefits. This review discusses the major contributors of immunity in the bone marrow microenvironment, their interactions, and mechanisms whereby immune modulation can be translated into therapies with anti-myeloma efficacy.

Keywords Bone marrow microenvironment · Immunology · T cells · Osteoclasts

Introduction

Plasma cells secrete antibodies in response to antigenic stimulus and mediate the humoral response. In multiple myeloma (MM), plasma cells show features of post-germinal center modifications with somatic hypermutation and features of long-lived plasma cells. Multiple myeloma

is a clonal plasma cell malignancy that is diagnosed in approximately 20,000 new patients each year with 11,000 patient deaths reported annually. While significant progress has been made in treating this disease, median survival remains 5 years from diagnosis [1].

Myeloma falls into a category of plasma cell dyscrasias. The earliest stage of these disorders is a monoclonal gammopathy of unknown significance (MGUS). This is a premalignant state in which the risk of progression of MM averages 1% per year and is categorized only by an abnormal monoclonal spike without end organ dysfunction. Smoldering MM shows a higher disease, between 10% and 30% plasma cell bone marrow involvement and a 10–20% per year conversion to active disease [2–4]. Increasing evidence demonstrates that the endogenous immune response towards the plasma cells has a critical role in preventing progression to active MM. Specifically, SOX-2 specific T cells are present in MGUS and undetectable in patients with MM [5]. Considering that SOX-2 is a putative stem cell antigen, this data suggests that immune surveillance plays a critical role in preventing disease progression to active MM.

The bone marrow plays a major role in normal immune function and contains multiple cell types that are both influenced by the MM microenvironment and likely contribute to disease advancement. Decreases of regulatory T cells (Tregs) [6–8], natural killer (NK) cell dysfunction [9, 10], and dendritic cell impairment [11] have all been described in myeloma and likely play a role in disease progression.

In recent years there has been an increased understanding of the biology of the disease and the development of several novel treatment options [12–14]. Immunomodulatory drugs alone or in combination are increasingly becoming standard therapy for patients with myeloma [15–18]. Furthermore

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immunotherapeutic vaccine strategies [19, 20] and adoptive T cell therapies [21–23] represent exciting new arenas of exploration in myeloma therapy.

This review will elucidate the role of the immune system in myeloma and highlight how these cell interactions lead to disease progression and immune evasion. Finally, it will provide an overview of immunotherapeutic strategies currently available and in development for the treatment of multiple myeloma.

The Bone Marrow and the Immune Cells Involved in Myeloma

The complex nature of the bone marrow (BM) microenvironment involves the interaction of multiple cell types. Mature B cells and/or potentially myeloma stem cells undergo post-germinal modifications and develop into clonal plasma cells which migrate to the BM where they interact with stromal and endothelial cells. These cells, as well as myeloma cells themselves, may be influenced by the T cells and NK cells in the BM microenvironment. Changes in dendritic cells, myeloid derived suppressor cells and eosinophils likely influence myeloma and lytic bone disease progression. Finally, adipocytes impact the immune system and may play an important immune role in the genesis and evolution of myeloma. Here we describe the relationship of these cell populations with myeloma growth and advancement.

Myeloma Stem Cells: Progenitor Cells

Plasma cells are terminally differentiated cells of the B cell lineage with limited proliferative potential [24–26]. As such, a hypothesis has recently been generated that this cell lacks the suitable properties to ensure self-propagation considered the *sine qua non* of a malignant clone. In myeloma, the post-germinal memory B cell possesses many of the required properties capable of self-renewal and differentiation into a mature plasma cell with the clonally restricted light chains [27, 28].

Myeloma progenitor stem cells have been described experimentally by depleting bone marrow of mature CD138⁺ plasma cells, CD3⁺, CD34⁺ hematopoietic stem cells and reproducibly generating mature plasma cells with the same light chain restriction as the parental malignant, mature plasma cells [25]. These clonotypic B cells show specific immunoglobulin gene rearrangements expressed in human MM with extensive somatic hyper-mutation in addition to recombination of the variable (V), diversity (D), and joining (J) genes within the immunoglobulin locus suggesting that MM arises from a germinal center or post-germinal center B cell [29]. These gene sequences were unaltered during the

disease course further implicating memory B cells or plasmablasts. Developmental pathways such as Hedgehog (Hh), Wnt and Notch signaling are generally silenced in adult tissues. However these pathways can be seen in several human cancers [30] including myeloma [31, 32], indicating the involvement of a less mature progenitor cell.

Therapies targeting CD138⁺ plasma cells are not curative. One possible explanation has been the inability to effectively eliminate MM stem cells with currently available therapies. A better understanding of the biology of myeloma stem cells may lead to critical advances in treating subsets of patients with early stage disease such as MGUS who are at greater risk of progressing to multiple myeloma [33]. Therapies targeting both mature plasma cells as well as myeloma progenitor cells may profoundly impact the disease [21].

Plasma Cells and the Bone Marrow Stroma

B cell development ends with the production of plasma cells (PCs), terminally differentiated, non-dividing cells whose function is to secrete antigen-specific antibodies [34]. Disease progression in myeloma is described by an increase in clonal plasma cells that secrete a monoclonal immunoglobulin. These cells home to the bone marrow through CXCR4/CXCL-12 (stromal derived factor 1/ SDF1) interactions [34, 35].

Adhesion molecules such as CD44, LFA-1, CD56, syndecan-1, and MPC-1 mediate their attachment to either extracellular matrix (ECM) proteins or bone marrow stromal cells (BMSC) and play a vital role in the pathogenesis of disease progression [36, 37]. MM cells bind to BMSCs and to the ECM mediated by VLA-4 to VCAM-1 and by syndecan-1 (CD138) to type I collagen plus VLA-4 to fibronectin. This binding causes homing of the tumor cells to the BM microenvironment and stimulates interleukin 6 (IL-6) transcription and secretion from BMSCs which aids the paracrine growth of MM cells [38]. CD138 is probably the most important adhesion molecule associated with MM. This protein is a regulator of tumor cell growth and survival and is involved in bone cell differentiation. Syndecan-1 is shed from the surface of most MM cells and has multiple roles in the pathogenesis of MM [39]. Acquisition of other adhesion molecules on plasma cells, such as CD11b, CD44, and RHAMM, occur during disease progression [40]. Targeting adhesion molecules may play a direct role in the response of MM to therapy.

The BMSCs in myeloma act to produce numerous cytokines and chemokines responsible for plasma cell migration and survival. The interaction of myeloma PC with its BM microenvironment is important for the homing pattern, survival, and proliferation of malignant plasma cells [41]. Stromal production of CXCL-12 facilitates migration of

mature PC to the BM [34] through binding to CXCR4 on plasma cells.

IL-6 is a key cytokine in the pathogenesis and disease progression of myeloma and is produced by both the BMSC and MM cells [42]. IL-6 effects on plasma cells occurs through an autocrine mechanism whereas a paracrine mechanism produces IL-6 by bone marrow stromal cells through an interaction between adhesion molecules present on myeloma plasma cells and their respective receptors present on BMSCs and osteoblasts [37]. Serum levels of IL-6 and the soluble IL-6 receptor (sIL-6R) inversely correlate with disease-free survival. Furthermore, high sIL-6R serum levels correlate with poor response to chemotherapy [43]. A recent murine study showed that an IL-6R knockdown-DC vaccine enhanced tumor-specific CD8⁺ CTLs and generated more CD8⁺ memory T cells, leading to prolonged survival of tumor-bearing mice [44].

Tumor necrosis factor alpha (TNF- α) acts as a proinflammatory immunoregulatory cytokine and is a prominent mediator of immune regulation [45]. TNF- α stimulates IL-6 secretion in bone-marrow stromal-cells and induces the expression of adhesion molecules [46] therefore aiding in the genesis of malignant cells [47]. TNF- α may play a role in the development of osteolytic bone disease [48]. A recent study has shown that the TNF- α -238 polymorphism is associated with a favorable clinical outcome in MM patients receiving thalidomide plus dexamethasone regimen while TNF- α -308 polymorphisms may protect against developing myeloma [49].

Osteoclasts and Osteoblasts

Lytic bone disease is a hallmark of myeloma and is caused by an increased formation of osteoclasts and decrease in osteoblasts [50]. Myeloma plasma cells produce molecules implicated in both the extensive bone destruction and impaired new bone formation. Receptor activator of NF- κ B ligand (RANKL), macrophage inflammatory protein-1 α (MIP-1 α) and IL-6 [51–53] are all produced by MM cells and play a role to increase osteoclast activity.

RANKL is an essential growth factor for osteoclasts (OC). Myeloma PCs binding to stromal cells within the bone marrow regulates RANKL expression on the surface of the BMSCs. Subsequently, this enhances OC activity through binding of RANKL to its receptor on OC cells promoting their differentiation [54], and possibly inhibiting OC apoptosis [55]. The soluble decoy for RANKL, osteoprotegerin (OPG), is also produced by BMSC and can inhibit the actions of RANKL on OC activation. The RANKL to OPG ratio determines the level of OC formation and activity with a higher ratio favoring OC formation. Interactions between MM cells and bone marrow stromal cells decrease production of OPG resulting in further OC

activation and enhanced bone destruction [55]. It has also been shown in myeloma patients that an imbalance exists between OPG and RANKL levels in the bone marrow microenvironment, favoring OC formation as MM cells do not express RANKL and produce low amounts of OPG [53]. Denosumab (AMG-162) is a fully human monoclonal antibody that binds RANKL and inhibits the RANKL–RANK interaction thus mimicking the endogenous effect of OPG. In a recent phase III trial, denosumab showed good efficacy and little toxicity with similar efficacy to zoledronic acid [56].

IL-6 is not only important for myeloma tumor cell development but also plays a critical role in OC development. Osteoclasts produce high levels of IL-6 when grown in co-culture with MM cells which further enhances MM cell growth and inhibits apoptosis [57, 58]. Although its precise role is still under debate, IL-6 production by OCs may increase MM tumor burden, and lead to enhanced bone destruction. Insight into the exact nature of this relationship was recently clarified through the link connecting OC bone disease and IL-17. Specifically, the elevated IL-6 levels in myeloma bone marrow skews T cells towards an IL-17 producing phenotype [8]. Increased IL-17 secretion by these BM T cells results in up-regulation of RANKL and increased OC formation. To underscore the importance of the immune response in MM-induced bone disease, IL-17 concentrations (and its associated pathway: IL-1 β and IL-23) in the BM plasma of MM patients statistically correlated to the extent of bone disease in these patients. Interestingly, no correlation was observed with total plasma cell numbers and bone disease. Further evidence shows that expression of the IL17 receptor on MM plasma cells leads to IL-17-mediated growth of plasma cells [59] and that MM antigen presenting cells (APCs) also produce IL-17 [6]. Taken together, targeting IL-17 production in MM could have a significant therapeutic benefit for bone disease, immune function as well as anti-tumor control.

Interferon gamma (IFN γ) can overcome RANKL-induced OC formation in in vitro studies [60]. It has been previously shown that activation of T cells or marrow infiltrating lymphocytes (MILs) with anti-CD3/CD28 beads augments anti-myeloma specificity by inducing a potent Th1 phenotype that produces high levels of IFN γ [21]. In fact, activated MILs (aMILs) co-cultured in an osteoclast outgrowth assay significantly blunted OC outgrowth [8]. The role of aMILs on control of OC is being examined in a current clinical trial of adoptive immunotherapy with activated MILs in MM.

IL-3 levels in BM plasma from patients with MM are increased in approximately 70% of patients compared with healthy controls. IL-3 increased OC formation and MM cell growth in vitro [61]. IL-3 acts as a bi-functional mediator of

MM bone disease, increasing osteoclasts and simultaneously suppressing osteoblast formation. Unlike DKK-1, which directly suppresses osteoblast formation, IL-3 works indirectly to blunt the growth of osteoblasts and is mediated by CD45⁺/CD11b⁺ monocytes/macrophages in both human and mouse primary culture systems [62].

T Cells

T cells play a central role in cell-mediated immunity. In recent years it has been shown that the bone marrow is a reservoir of effector memory T cells (T_{EM}). In melanoma it has been shown that the BM may be an important compartment for tumor surveillance harboring a tumor-specific memory T-cell pool in addition to effector T cells [63]. Similarly, the identification and characterization of a functionally enhanced T_{EM} in the BM of patients undergoing total joint replacement for osteoarthritis has been described [64]. Another example in patients with thymoma-associated pure red cell aplasia reports the expansion of CD8⁺/perforin⁺ T_{EM} cells in the bone marrow of these patients [65]. A dendritic cell based vaccine trial in myeloma showed that T cells from the tumor microenvironment of patients with progressive myeloma generate strong, tumor-specific cytolytic responses to autologous, tumor-loaded dendritic cells more so than cells from the peripheral blood (PBL) of these patients [66]. Marrow infiltrating lymphocytes (MILs) from MM patients show a significant tumor specificity as compared to the PBL of the same patient upon activation with anti-CD3/anti-CD28 beads [21]. However, the profound global immune-suppression associated with myeloma prevents the generation of an effective, endogenous, anti-myeloma effect of the MILs.

The bone marrow is a reservoir of regulatory T cells (T_{regs}) in *normal* donors [67]. However, their role in MM remains less clear. While some groups have reported an overabundance of T_{regs} [68, 69] many groups are beginning to report a paucity of functional T_{regs} in myeloma patients [7, 8]. Part of these differences reported in MM lie in the compartment examined (blood vs. BM) as well as the criteria used to define these cells (phenotype vs. functional suppression assays). However, one important emerging characteristic is that profound differences exist between the BM microenvironments of normal as compared to MM patients. As discussed above, IL-6 plays a critical role in determining the fate of CD4 toward Th17 or Treg differentiation [70, 71]. IL-6 and IL-23 in combination with TGF- β activate retinoic acid-related orphan receptor- γ t (ROR γ t) to generate the Th-17 phenotype via MM dendritic cells in a STAT3 dependent manner [6]. IL-17 induces myeloma tumor cell growth and inhibits immune function in myeloma patients [59]. Furthermore, it plays a critical role

in osteolytic bone disease in MM [8]. In summary, MM induces profound alterations in the immune function of the BM microenvironment leading to a dramatic reduction in Tregs with a reciprocal increase in IL-17 producing T cells. These data should lead to a re-examination of the role of Tregs in disease progression in MM the results of which may have significant therapeutic implications for future immune-based therapies.

NK and NKT Cells

Natural killer cells (NK) are cytotoxic lymphocytes involved in the innate immune system and implicated in anti-tumor immunity. Natural killer T (NKT) cells are a heterogeneous group of T cells that share properties of both T cells and NK cells and recognize the non-polymorphic CD1d molecule that binds self- and foreign lipids and glycolipids [72]. NKT cells have been shown to be part of a broader repertoire of lipid-specific T cells [73].

Recently, NK cells have been shown to mediate Ag-specific recall responses in several different model systems [74]. Impaired differentiation and function of NK and NKT cells have been identified in MM. Clinical progression in patients with MGUS is associated with the loss of ligand-dependent IFN- γ production by invariant NK and NKT cells. A critical step in the transition to progressive tumors may be an acquired capacity of the myeloma plasma cells to disable the NKT arm of host resistance [75]. The functional deficit of NK and NKT cells can be overcome using dendritic cells pulsed with the NKT ligand, α -GalCer. Injection of α -GalCer-pulsed DCs led to greater than 100-fold expansion of several subsets of NKT cells in all patients that could be detected for up to 6 months after vaccination. NKT activation was associated with an increase in serum levels of IL-12 and IFN- γ -inducible protein-10. There was also an increase in tumor specific T cells as well as cytomegalovirus specific memory CD8⁺ T cells after injection of α -GalCer-loaded DCs [76]. Lenalidomide and pomalidomide also increase NK function in myeloma [77] and may be critical in augmenting anti-tumor immunity in myeloma.

Dendritic Cells

Dendritic cells (DC) process and present antigen to T cells and serve as a critical link between the innate and adaptive arms of the immune system. Two distinct subsets of DCs have been defined, namely, the myeloid DC (CD11c⁺) (mDC) and the plasmacytoid DC (CD11c⁻ CD123⁺) (pDC) [78]. Dendritic cell function is impaired in patients with myeloma [11]. The absolute number of circulating myeloid and plasmacytoid DC precursors was significantly lower in myeloma patients than in healthy matched controls. Upon

maturation, DCs from MM patients showed significantly lower expression of HLA-DR, CD40, and CD80 antigens and impaired induction of allogeneic T-cell proliferation compared with controls. Remarkably, these DCs were incapable of presenting the patient-specific tumor idiotype to autologous T cells. A major contributing factor to this immune dysfunction is believed to be IL-6 mediated.

To address the role of IL-6 induced immune dysfunction, a recent murine study silenced the IL-6 receptor alpha chain (IL-6R α) in DCs in an attempt to restore the functional competence of DC vaccines in mice with an IL-6-producing tumor and give rise to protective immunity. The IL-6R α knockdown-DC vaccine significantly enhanced the frequency of tumor-specific CD8 $^{+}$ CTLs producing effector molecules such as IFN- γ , TNF- α , FasL, perforin, and granzyme B, increased CD8 $^{+}$ memory T cells and led to substantially prolonged tumor-free survival [44].

Several clinical studies have attempted to overcome the intrinsic deficits of antigen presentation through vaccination with ex vivo expanded autologous monocyte-derived DCs. A small study of patients with stage-I myeloma described some success with monocyte derived DCs pulsed with autologous patient idiotype (Id) and keyhole limpet hemocyanin (KLH). Five out of 9 patients (56%) developed Id-specific T cells and 8 out of 9 patients (89%) showed specific T-cell-mediated cytokine release after Id stimulation with minimal clinical responses [19]. Another vaccine strategy combines patient-derived myeloma cells chemically fused with autologous DC's. This affords a broad spectrum of myeloma-associated antigens that are presented in the context of DC-mediated co-stimulation. Seventeen out of the 18 patients in the study responded to the vaccine with antitumor responses and stabilization of disease [79].

Myeloid Derived Suppressor Cells

Myeloid-derived suppressor cells (MDSCs) are a heterogeneous population of cells that expands during cancer, inflammation and infection, and that have a remarkable ability to suppress T-cell responses [80]. In mice, MDSCs express a CD11b $^{+}$ /GR1 $^{+}$ /IL4R α [81]. The human MDSC phenotype is CD14 $^{+}$ /CD15 $^{+}$ /HLA-DR low /IL4R α $^{+}$ [82]. Their suppressive function is largely mediated through up-regulation of Arginase-1 (Arg-1) which depletes the extracellular matrix of the essential amino acid, arginine, and iNOS [83]. ARG directly promotes tumor progression [84] and iNOS blocks T cell activity through nitration of tyrosine residues inhibiting their phosphorylation and thus T cell function [85].

The role of MDSCs in solid tumors such as head and neck carcinoma [18], melanoma [86], colon [87], renal

[88], breast [89], and prostate cancer [90] has been well described. The role of MDSC in hematologic malignancies such as lymphoma [91] and multiple myeloma [18, 68] have also been described although they are less well understood. A recent study showed a 5-fold increase in CD14 $^{+}$ /HLA-DR low MDSCs in newly diagnosed MM patients compared to healthy controls [68].

Phosphodiesterase-5(PDE-5) inhibitors such as sildenafil and tadalafil can eliminate the suppressive function of MDSC [18]. PDE5 inhibition reverses tumor-induced immunosuppressive mechanisms and enables a measurable antitumor immune response to be generated that substantially delays tumor progression. Sildenafil down-regulates Arg-1 and iNOS expression which reduces the suppressive machinery of MDSC recruited by growing tumors and ultimately removing the tumor escape mechanisms imparted by these cells. PDE-5 inhibitors enhance intra-tumoral T cell infiltration and activation, reduce tumor outgrowth, and may improve the antitumor efficacy of adoptive T cell therapy. Sildenafil also restores in vitro T cell proliferation of peripheral blood mononuclear cells from multiple myeloma and may therefore be a beneficial treatment for patients with myeloma.

Adipocytes

Adipocytes primarily compose adipose tissue, specialized in storing energy as fat. Age related accumulation of bone marrow adipocytes occurs and is accompanied by a loss of osteoblasts [92]. In addition to their role in fat metabolism, increasing data also suggests a critical role of adipocytes in mediating immune function. A major physiologic process associated with aging is the gradual replacement of the BM mesenchymal stromal elements with adipocytes. These cells release leptin and increase the serum levels of IL-6 [93]. Leptin has been strongly implicated in supporting the growth of hematopoietic malignancies such as myeloma [94]. Leptin activated DCs improve their activity and generate a Th1 response [95]. Leptin also increases IL12 production upon CD40 activation to increase CD8 responses [96]. This data would hypothesize a role of leptin in maintaining hematopoiesis, immune response and possible tumor growth within the BM microenvironment in MM.

Immuno-therapeutic Strategies in Myeloma

Evidence clearly points to an altered intrinsic immune response within the BM of MM patients. Strategies aimed at correcting these defects may have significant therapeutic implications. Several of these approaches are outlined below.

Monoclonal Antibody (mAb) Therapies

CD20 is expressed in a fraction of mature plasma cells in patients with multiple myeloma [97], but is constitutively expressed on the clonogenic precursor memory B cells [25]. The human–mouse chimeric monoclonal antibody against the CD20 antigen, rituximab, has been used in a number of clinical settings in patients with B-cell tumors and has been shown to prolong the progression free survival of indolent B cell lymphomas [98]. Most recently a trial examining the role of rituximab in MM showed the ability of the antibody to bind to the clonogenic MM precursors but did not kill the cells. Interestingly, while this initial approach failed to achieve clinically meaningful outcomes, there was a clinical correlation observed between the reappearance of the clonogenic memory B cell population and subsequent serologic relapse in MM patients (Huff, CA personal communication). However, targeting the clonotypic B-cell fraction or myeloma stem cell fraction, especially in the minimum residual disease stage, theoretically has the potential to prevent relapse although this remains to be proven.

A recent report shows that 20-C2-2b, a bi-specific monoclonal antibody which comprises two copies of IFN α 2b and a stabilized F-ab humanized anti-HLA-DR site-specifically linked to velutuzumab (humanized anti-CD20) may be more effective than anti-CD20 therapy alone. In vitro, 20-C2-2b inhibited the growth of eight myeloma cell lines, and was more effective than monospecific CD20-targeted MAb-IFN α or a mixture comprising the parental antibodies in the treatment of MM [99].

Daratumumab is a novel, high-affinity, therapeutic human mAb against CD38 (an epitope expressed on myeloma plasma cells.) In vitro, daratumumab induced potent antibody dependent cellular cytotoxicity in CD38-expressing myeloma derived cell lines as well as in primary myeloma patient cells. It induces complement-dependent cytotoxicity in patient myeloma cells. Interestingly, daratumumab-induced antibody dependent cellular cytotoxicity and complement dependent cytotoxicity which was not affected by the presence of bone marrow stromal cells implies the ability of this antibody to effectively kill myeloma tumor cells in a tumor-preserving bone marrow microenvironment. Low dose daratumumab was also highly effective in a murine model in vivo. The data generated may support the development of this drug clinically [100].

Denosumab (AMG 162) is a fully humanized monoclonal antibody that targets RANKL and has been developed and used for the treatment of osteoporosis, rheumatoid arthritis, bone metastases and the lytic bone disease associated with multiple myeloma [101–103]. Because it targets RANKL, denosumab inhibits the formation, function, and survival of osteoclasts. Several ongoing clinical

studies using denosumab alone or in the context of bisphosphonate therapies have shown that targeting RANK/RANKL signaling may prevent skeletal complications in patients with myeloma [104, 105].

Immunomodulatory Drugs

Immunomodulatory drugs or IMiDs are structural and functional analogues of thalidomide that work as immunomodulators for the treatment of a variety of inflammatory, autoimmune, and neoplastic diseases including multiple myeloma [106].

Lenalidomide, is a second generation IMiD and analogue of thalidomide. It possesses immunomodulatory and antiangiogenic properties as well as direct apoptotic properties which culminate in cancer cell death either through direct interference with key functions of tumor cells or indirectly through modulation of signaling pathways that regulate their interaction to bone marrow immune cells. However, the exact clinical immunomodulatory effects of lenalidomide remain to be elucidated [107]. Dexamethasone is often used in conjunction with lenalidomide. However a recent study has shown that dexamethasone synergizes with lenalidomide to inhibit multiple myeloma tumor growth, but reduces the capacity of lenalidomide-induced immunomodulation of T and NK cell function [108]. In vitro studies have shown enhancement of T cell cytokine generation, chemotaxis, proliferation and survival [109] as well as decreases in the proliferation and function of Tregs with lenalidomide [110]. Clinical data in a small trial showed the ability of lenalidomide to augment both humoral and cellular vaccine specific responses to the polyvalent pneumococcal vaccine, Prevanr, in patients with relapsed myeloma [111]. Further studies will be needed to fully elucidate the immune potentiating clinical effects of lenalidomide.

Pomalidomide, another thalidomide analogue, with potent anti-myeloma activity, also possesses immunomodulatory properties. It has T cell costimulatory activity that enhances durable, antigen-specific Th1 type response in vivo [112] and decreases the proliferation and function of Tregs [110]. Furthermore, pomalidomide directly impacts myeloma associated lytic bone disease by inhibiting osteoclast production and function [113].

Adoptive T Cell Therapy

Adoptive T cell therapy (ACT) offers the possibility to deliver a greater percentage of antigen specific T cells that potentially impart durable responses and overcome endogenous immunosuppressive mechanisms. In a recent phase 1/2 two-arm trial, 54 patients with myeloma received autografts followed by ex vivo anti-CD3/anti-CD28 cos-

stimulated autologous T cells at day 2 after transplantation. Arm A of the study received activated peripheral blood lymphocytes (aPBL) that had previously been exposed to a vaccine of h-TERT, Survivin, CMV and Pevnar. Arm B was only pre-vaccinated with the Pevnar vaccine. The anticipated result was that patients positive for HLA-A2 who received the hTERT/survivin vaccine (arm A) might show better myeloma control. However, patients in arm A exhibited an inferior event free survival (EFS) compared with patients in arm B with no difference in overall survival (OS.) [114]. Previous trials in myeloma infusing autologous activated peripheral blood T cells in the context of an autologous transplant imparted an accelerated and profound in vivo expansion of T cells. However there was no myeloma specific response found in these patients [115].

In solid tumors, such as melanoma, tumor infiltrating lymphocytes (TILs) are most often used for expansion in adoptive T cell therapy [116]. Although these T cell expansions are lengthy and expensive, when clinical results are produced in patients they are profound. The T cells used in these expansions have been both previously exposed to tumor antigen but also tolerized by their immune microenvironment. However, removing the T cells from this suppressive environment, and activating and expanding them with clonal restriction restores the previously tolerized function of these cells [117].

Coupling this experience with the current literature that the bone marrow is a privileged site for effector memory T cells [63] with the bone marrow being the site of tumor in myeloma, supports the preferential use of marrow infiltrating lymphocytes (MILs) as opposed to peripheral blood lymphocytes (PBL) in adoptive immunotherapy for myeloma patients. T cells from myeloma bone marrow, upon activation with anti-CD3/anti-CD28 beads, show a significant tumor specificity as compared to the PBL of the same patient [21]. The specificity of these cells was not only to mature plasma cells but also their clonogenic precursors. Furthermore evidence of both IFN γ production and cytotoxicity towards tumor cells was greater in the MILs than the PBLs from the same patient. MILs, compared to PBLs, had increased expression of CXCR-4 which favored their ability to traffic towards an SDF-1 gradient which might impart their ability to traffic back to the bone marrow, the site of tumor in myeloma. These data suggest that the use of MILs over PBLs may be a superior approach for adoptive T cell therapy in myeloma.

Myeloma Vaccines

Vaccines designed for use in cancer immunotherapy differ greatly from infectious vaccines. Cancer immunotherapy vaccines are attempting to mount an immune response against a previously established tumor burden rather than to

prime immune responses in a prophylactic manner as with infectious vaccines.

Wilms Tumor1 (WT1) and Proteinase-3 (Pr3) are presented in the context of major histocompatibility complex (MHC) class I epitopes on leukemic blasts, and are potentially immunogenic. Another candidate tumor antigen is the mucin 1 protein (MUC1) [118]. Many studies have investigated the use of these target antigens as possible vaccine strategies in other hematologic malignancies [119, 120]. However, a recent study showed that these vaccine strategies employed on advanced stage cancer patients could not produce T cells that proliferated nor secreted cytokines upon peptide stimulation and in fact T cells were depleted rather than rescued after vaccination [118].

It bears mentioning that overcoming profound immunologic deficits, especially in patients with large tumor burdens, may be overwhelming for the current vaccine strategies. More effective use of a vaccine for myeloma may be to integrate it in the setting of minimal residual disease. With the current treatment paradigms and successes being seen with them, the two obvious settings would be either in the post-transplant setting where the possibility to skew the developing immune response towards greater tumor recognition during the period of immune reconstitution offers significant theoretical and practical advantages [121]. However, this must be weighed with the intrinsic immunosuppression accompanying transplantation. One approach that has been utilized to overcome this has been the integration of myeloma vaccines with adoptive T cell therapy strategies. In a recently published report of activated peripheral blood T cells administered in combination with HLA-A2-restricted hTERT and survivin, no significant improvement in overall survival was observed in MM patients undergoing an autologous peripheral stem cell transplant [114] despite the fact that it had previously been shown that pre-transplant vaccinations can be effective adoptively transferred when the T cells are infused early in the post-transplant period [122]. An alternative approach is the integration of a vaccine strategy with an immunomodulatory agent. To this effect, we currently have a clinical study in which patients on lenalidomide with an undetectable monoclonal protein spike but still showing evidence of immunofixation positivity of their plasma cell clone will be maintained on lenalidomide and will be vaccinated with a GM-CSF cell based allogeneic myeloma vaccine. This strategy aims to integrate the immunomodulatory aspects of lenalidomide with a minimal residual disease state with a MM-specific vaccine.

PDE-5 Inhibitors

PDE-5 inhibitors, such as sildenafil and tadalafil, abrogate the suppressive function of MDSC [18]. PDE5 inhibition

reverses tumor-induced immunosuppressive mechanisms and enables a measurable antitumor immune response to be generated that substantially delays tumor progression. Sildenafil down-regulates Arg-1 and iNOS expression through downregulation of IL4R α and destabilization of the mRNA encoding for iNOS, respectively. Furthermore, PDE-5 mediated inhibition of MDSCs also reduces the generation of Tregs as an additional mechanism resulting in enhanced immune responsiveness [91]. Clinical studies are currently planned to add PDE-5 inhibitors to standard MM therapy in an effort to augment anti-tumor efficacy.

Conclusion

Increasing evidence exists pointing to the role of the immune microenvironment of the BM that significantly contributes to disease progression in MM. The interaction of the myeloma tumor cells with the bone marrow stroma, osteoclast, osteoblasts, T cells, dendritic cells, MDSCs and adipocytes enables the tumor to survive and evade immune recognition. Our increasing understanding of these mechanisms has also led to the development of numerous therapeutic strategies targeting many of these pathways. These advances coupled to our ever-increasing understanding of these tumor-associated immunosuppressive mechanisms will eventually result in the development of either combinatorial immune based therapies or integration of immunotherapeutic approaches with conventional cytotoxic approaches. While not likely to show considerable anti-tumor efficacy alone, the field is quickly approaching a point in which these approaches will enter into the standard armamentarium of treatment options.

References

- Mahindra A, Hideshima T, Anderson KC (2010) Multiple myeloma: biology of the disease. *Blood Rev* 24(Suppl 1):S5–11
- Kyle RA, Therneau TM, Rajkumar SV, Offord JR, Larson DR, Plevak MF et al (2002) A long-term study of prognosis in monoclonal gammopathy of undetermined significance. *N Engl J Med* 346(8):564–9
- Kyle RA, Rajkumar SV (2007) Monoclonal gammopathy of undetermined significance and smoldering multiple myeloma. *Hematol Oncol Clin North Am* 21(6):1093–113, ix
- Kyle RA, Rajkumar SV (2007) Monoclonal gammopathy of undetermined significance and smoldering multiple myeloma: emphasis on risk factors for progression. *Br J Haematol* 139(5):730–43
- Spisek R, Kukreja A, Chen LC, Matthews P, Mazumder A, Vesole D et al (2007) Frequent and specific immunity to the embryonal stem cell-associated antigen SOX2 in patients with monoclonal gammopathy. *J Exp Med* 204(4):831–40, PMID: 2118551
- Dhodapkar KM, Barbuto S, Matthews P, Kukreja A, Mazumder A, Vesole D et al (2008) Dendritic cells mediate the induction of polyfunctional human IL17-producing cells (Th17-1 cells) enriched in the bone marrow of patients with myeloma. *Blood* 112(7):2878–85, PMID: 2556623
- Gupta R, Ganeshan P, Hakim M, Verma R, Sharma A (2010) Kumar L. Significantly reduced regulatory T cell population in patients with untreated multiple myeloma. *Leuk Res*
- Noonan K, Marchionni L, Anderson J, Pardoll D, Roodman GD, Borrello I (2010) A novel role of IL-17 producing lymphocytes in mediating lytic bone disease in multiple myeloma. *Blood*
- von Lilienfeld-Toal M, Frank S, Leyendecker C, Feyler S, Jarmin S, Morgan R et al (2010) Reduced immune effector cell NKG2D expression and increased levels of soluble NKG2D ligands in multiple myeloma may not be causally linked. *Cancer Immunol Immunother* 59(6):829–39
- Dhodapkar MV (2011) Richter J. Progress and challenges. *Clin Immunol*, Harnessing natural killer T (NKT) cells in human myeloma
- Ratta M, Fagnoni F, Curti A, Vescovini R, Sansoni P, Oliviero B et al (2002) Dendritic cells are functionally defective in multiple myeloma: the role of interleukin-6. *Blood* 100(1):230–7
- Shah N, Lonial S (2010) Evidence-based mini-review: treatment options for patients with relapsed/refractory myeloma previously treated with novel agents and high-dose chemotherapy and autologous stem-cell transplantation. *Hematology Am Soc Hematol Educ Program* 2010:310–3
- Harousseau JL (2010) Multiple myeloma in the elderly: when to treat, when to go to transplant. *Oncology (Williston Park)* 24(11):992–8
- Kumar A, Galeb S, Djulbegovic B (2011) Treatment of patients with multiple myeloma: an overview of systematic reviews. *Acta Haematol* 125(1–2):8–22
- Lacy MQ (2011) New immunomodulatory drugs in myeloma. *Curr Hematol Malig Rep*
- Harousseau JL (2010) Ten years of improvement in the management of multiple myeloma: 2000–2010. *Clin Lymphoma Myeloma Leuk* 10(6):424–42
- van de Donk NW, Lokhorst HM, Dimopoulos M, Cavo M, Morgan G, Einsele H et al (2011) Treatment of relapsed and refractory multiple myeloma in the era of novel agents. *Cancer Treat Rev* 37(4):266–83
- Serafini P, Meckel K, Kelso M, Noonan K, Califano J, Koch W et al (2006) Phosphodiesterase-5 inhibition augments endogenous antitumor immunity by reducing myeloid-derived suppressor cell function. *J Exp Med* 203(12):2691–702, PMID: 2118163
- Rollig C, Schmidt C, Bornhauser M, Ehninger G, Schmitz M, Auffermann-Gretzinger S (2011) Induction of cellular immune responses in patients with stage-I multiple myeloma after vaccination with autologous idiotype-pulsed dendritic cells. *J Immunother* 34(1):100–6
- Yi Q, Szmania S, Freeman J, Qian J, Rosen NA, Viswamitra S et al (2010) Optimizing dendritic cell-based immunotherapy in multiple myeloma: intranodal injections of idiotype-pulsed CD40 ligand-matured vaccines led to induction of type-1 and cytotoxic T-cell immune responses in patients. *Br J Haematol* 150(5):554–64, PMID: 2924470
- Noonan K, Matsui W, Serafini P, Carbley R, Tan G, Khalili J et al (2005) Activated marrow-infiltrating lymphocytes effectively target plasma cells and their clonogenic precursors. *Cancer Res* 65(5):2026–34
- Barber A, Meehan KR, Sentman CL (2011) Treatment of multiple myeloma with adoptively transferred chimeric NKG2D receptor-expressing T cells. *Gene Ther*
- Ocadlikova D, Kryukov F, Mollova K, Kovarova L, Buresdova I, Matejkova E et al (2010) Generation of myeloma-specific T cells using dendritic cells loaded with MUC1- and hTERT- driven

- nonapeptides or myeloma cell apoptotic bodies. *Neoplasma* 57 (5):455–64
24. Pilarski LM, Hipperson G, Seeberger K, Pruski E, Coupland RW, Belch AR (2000) Myeloma progenitors in the blood of patients with aggressive or minimal disease: engraftment and self-renewal of primary human myeloma in the bone marrow of NOD SCID mice. *Blood* 95(3):1056–65
 25. Matsui W, Huff CA, Wang Q, Malehorn MT, Barber J, Tanheco Y et al (2004) Characterization of clonogenic multiple myeloma cells. *Blood* 103(6):2332–6
 26. Matsui W, Wang Q, Barber JP, Brennan S, Smith BD, Borrello I et al (2008) Clonogenic multiple myeloma progenitors, stem cell properties, and drug resistance. *Cancer Res* 68(1):190–7
 27. Yaccoby S, Barlogie B, Epstein J (1998) Primary myeloma cells growing in SCID-hu mice: a model for studying the biology and treatment of myeloma and its manifestations. *Blood* 92(8):2908–13
 28. Yaccoby S, Epstein J (1999) The proliferative potential of myeloma plasma cells manifest in the SCID-hu host. *Blood* 94 (10):3576–82
 29. Bakkus MH, Heirman C, Van Riet I, Van Camp B, Thielemans K (1992) Evidence that multiple myeloma Ig heavy chain VDJ genes contain somatic mutations but show no intracлонаl variation. *Blood* 80(9):2326–35
 30. Taipale J, Beachy PA (2001) The Hedgehog and Wnt signalling pathways in cancer. *Nature* 411(6835):349–54
 31. Peacock CD, Wang Q, Gesell GS, Corcoran-Schwartz IM, Jones E, Kim J et al (2007) Hedgehog signaling maintains a tumor stem cell compartment in multiple myeloma. *Proc Natl Acad Sci USA* 104(10):4048–53, PMID: 1805487
 32. Agarwal JR, Matsui W (2010) Multiple myeloma: a paradigm for translation of the cancer stem cell hypothesis. *Anticancer Agents Med Chem* 10(2):116–20, PMID: 3033115
 33. Landgren O, Kyle RA, Rajkumar SV (2011) From myeloma precursor disease to multiple myeloma: new diagnostic concepts and opportunities for early intervention. *Clin Cancer Res* 17 (6):1243–52
 34. Shapiro-Shelef M, Calame K (2005) Regulation of plasma-cell development. *Nat Rev Immunol* 5(3):230–42
 35. Radbruch A, Muehlinghaus G, Luger EO, Inamine A, Smith KG, Dorner T et al (2006) Competence and competition: the challenge of becoming a long-lived plasma cell. *Nat Rev Immunol* 6(10):741–50
 36. Bergsagel PL, Kuehl WM (2005) Molecular pathogenesis and a consequent classification of multiple myeloma. *J Clin Oncol* 23 (26):6333–8
 37. Teoh G, Anderson KC (1997) Interaction of tumor and host cells with adhesion and extracellular matrix molecules in the development of multiple myeloma. *Hematol Oncol Clin North Am* 11(1):27–42
 38. Uchiyama H, Barut BA, Mohrbacher AF, Chauhan D, Anderson KC (1993) Adhesion of human myeloma-derived cell lines to bone marrow stromal cells stimulates interleukin-6 secretion. *Blood* 82(12):3712–20
 39. Dhodapkar MV, Abe E, Theus A, Lacy M, Langford JK, Barlogie B et al (1998) Syndecan-1 is a multifunctional regulator of myeloma pathobiology: control of tumor cell survival, growth, and bone cell differentiation. *Blood* 91(8):2679–88
 40. Damiano JS, Cress AE, Hazlehurst LA, Shtil AA, Dalton WS (1999) Cell adhesion mediated drug resistance (CAM-DR): role of integrins and resistance to apoptosis in human myeloma cell lines. *Blood* 93(5):1658–67
 41. Cao Y, Luetkens T, Kobold S, Hildebrandt Y, Gordic M, Lajmi N et al (2010) The cytokine/chemokine pattern in the bone marrow environment of multiple myeloma patients. *Exp Hematol* 38 (10):860–7
 42. Medinger M, Fischer N, Tzankov A (2010) Vascular endothelial growth factor-related pathways in hemato-lymphoid malignancies. *J Oncol* 2010:729725, PMID: 2875768
 43. Lauta VM (2003) A review of the cytokine network in multiple myeloma: diagnostic, prognostic, and therapeutic implications. *Cancer* 97(10):2440–52
 44. Hwang W, Jung K, Jeon Y, Yun S, Kim TW, Choi I (2010) Knockdown of the interleukin-6 receptor alpha chain of dendritic cell vaccines enhances the therapeutic potential against IL-6 producing tumors. *Vaccine* 29(1):34–44
 45. Chan KF, Siegel MR, Lenardo JM (2000) Signaling by the TNF receptor superfamily and T cell homeostasis. *Immunity* 13(4):419–22
 46. Richardson P, Hideshima T, Anderson K (2002) Thalidomide: emerging role in cancer medicine. *Annu Rev Med* 53:629–57
 47. Sawamura M, Murakami H, Tsuchiya J (1996) Tumor necrosis factor-alpha and interleukin 4 in myeloma cell precursor differentiation. *Leuk Lymphoma* 21(1–2):31–6
 48. Abildgaard N, Glerup H, Rungby J, Bendix-Hansen K, Kassem M, Brixen K et al (2000) Biochemical markers of bone metabolism reflect osteoclastic and osteoblastic activity in multiple myeloma. *Eur J Haematol* 64(2):121–9
 49. Du J, Yuan Z, Zhang C, Fu W, Jiang H, Chen B et al (2010) Role of the TNF-alpha promoter polymorphisms for development of multiple myeloma and clinical outcome in thalidomide plus dexamethasone. *Leuk Res* 34(11):1453–8
 50. Roodman GD (2010) Targeting the bone microenvironment in multiple myeloma. *J Bone Miner Metab* 28(3):244–50
 51. Gunn WG, Conley A, Deininger L, Olson SD, Prockop DJ, Gregory CA (2006) A crosstalk between myeloma cells and marrow stromal cells stimulates production of DKK1 and interleukin-6: a potential role in the development of lytic bone disease and tumor progression in multiple myeloma. *Stem Cells* 24(4):986–91
 52. Choi SJ, Cruz JC, Craig F, Chung H, Devlin RD, Roodman GD et al (2000) Macrophage inflammatory protein 1-alpha is a potential osteoclast stimulatory factor in multiple myeloma. *Blood* 96(2):671–5
 53. Giuliani N, Colla S, Rizzoli V (2004) New insight in the mechanism of osteoclast activation and formation in multiple myeloma: focus on the receptor activator of NF-kappaB ligand (RANKL). *Exp Hematol* 32(8):685–91
 54. Ehrlich LA, Roodman GD (2005) The role of immune cells and inflammatory cytokines in Paget's disease and multiple myeloma. *Immunol Rev* 208:252–66
 55. Sezer O, Heider U, Jakob C, Zavrski I, Eucker J, Possinger K et al (2002) Immunocytochemistry reveals RANKL expression of myeloma cells. *Blood* 99(12):4646–7, author reply 7
 56. Henry DH, Costa L, Goldwasser F, Hirsh V, Hungria V, Prausova J et al (2011) Randomized, double-blind study of denosumab versus zoledronic Acid in the treatment of bone metastases in patients with advanced cancer (excluding breast and prostate cancer) or multiple myeloma. *J Clin Oncol* 29(9):1125–32
 57. Abe M, Hiura K, Wilde J, Shioyasono A, Moriyama K, Hashimoto T et al (2004) Osteoclasts enhance myeloma cell growth and survival via cell-cell contact: a vicious cycle between bone destruction and myeloma expansion. *Blood* 104 (8):2484–91
 58. Anderson KC, Jones RM, Morimoto C, Leavitt P, Barut BA (1989) Response patterns of purified myeloma cells to hemato-poietic growth factors. *Blood* 73(7):1915–24
 59. Prabhala RH, Pelluru D, Fulciniti M, Prabhala HK, Nanjappa P, Song W et al (2010) Elevated IL-17 produced by TH17 cells promotes myeloma cell growth and inhibits immune function in multiple myeloma. *Blood* 115(26):5385–92, PMID: 2902136

60. Takahashi N, Mundy GR, Roodman GD (1986) Recombinant human interferon-gamma inhibits formation of human osteoclast-like cells. *J Immunol* 137(11):3544–9
61. Abstracts of the American Society of Hematology 46th Annual Meeting. December 4–7, 2004, San Diego, California, USA. *Blood*. 2004;104(11 Pt 1):1a-1093a
62. Ehrlich LA, Chung HY, Ghobrial I, Choi SJ, Morandi F, Colla S et al (2005) IL-3 is a potential inhibitor of osteoblast differentiation in multiple myeloma. *Blood* 106(4):1407–14
63. Letsch A, Keilholz U, Assfalg G, Mailander V, Thiel E, Scheibenbogen C (2003) Bone marrow contains melanoma-reactive CD8+ effector T cells and, compared with peripheral blood, enriched numbers of melanoma-reactive CD8+ memory T cells. *Cancer Res* 63(17):5582–6
64. Zhang X, Dong H, Lin W, Voss S, Hinkley L, Westergren M et al (2006) Human bone marrow: a reservoir for “enhanced effector memory” CD8+ T cells with potent recall function. *J Immunol* 177(10):6730–7
65. Nitta H, Mihara K, Sakai A, Kimura A (2010) Expansion of CD8+/perforin+ effector memory T cells in the bone marrow of patients with thymoma-associated pure red cell aplasia. *Br J Haematol* 150(6):712–5
66. Dhodapkar MV, Krasovsky J, Olson K (2002) T cells from the tumor microenvironment of patients with progressive myeloma can generate strong, tumor-specific cytolytic responses to autologous, tumor-loaded dendritic cells. *Proc Natl Acad Sci USA* 99(20):13009–13, PMID: 130577
67. Zou L, Barnett B, Safah H, Larussa VF, Evdemon-Hogan M, Mottram P et al (2004) Bone marrow is a reservoir for CD4+CD25+ regulatory T cells that traffic through CXCL12/CXCR4 signals. *Cancer Res* 64(22):8451–5
68. Brimnes MK, Vangstedt AJ, Knudsen LM, Gimsing P, Gang AO, Johnsen HE et al (2010) Increased level of both CD4+FOXP3+ regulatory T cells and CD14+HLA-DR/low myeloid-derived suppressor cells and decreased level of dendritic cells in patients with multiple myeloma. *Scand J Immunol* 72(6):540–7
69. Feyler S, von Lilienfeld-Toal M, Jarmin S, Marles L, Rawstron A, Ashcroft AJ et al (2009) CD4(+)CD25(+)FoxP3(+) regulatory T cells are increased whilst CD3(+)CD4(–)CD8(–)alpha-betaTCR(+) Double Negative T cells are decreased in the peripheral blood of patients with multiple myeloma which correlates with disease burden. *Br J Haematol* 144(5):686–95
70. Bettelli E, Carrier Y, Gao W, Korn T, Strom TB, Oukka M et al (2006) Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. *Nature* 441(7090):235–8
71. Korn T, Mitsdoerffer M, Croxford AL, Awasthi A, Dardalhon VA, Galileos G et al (2008) IL-6 controls Th17 immunity in vivo by inhibiting the conversion of conventional T cells into Foxp3+ regulatory T cells. *Proc Natl Acad Sci USA* 105(47):18460–5, PMID: 2587589
72. Jerud ES, Bricard G, Porcelli SA (2006) CD1d-restricted natural killer T cells: roles in tumor immunosurveillance and tolerance. *Transf Med Hemother* 33(1):18–36
73. Godfrey DI, MacDonald HR, Kronenberg M, Smyth MJ, Van Kaer L (2004) NKT cells: what's in a name? *Nat Rev Immunol* 4(3):231–7
74. Sun JC, Lopez-Verges S, Kim CC, DeRisi JL, Lanier LL (2011) NK cells and immune “memory”. *J Immunol* 186(4):1891–7
75. Dhodapkar MV, Krasovsky J, Osman K, Geller MD (2003) Vigorous premalignancy-specific effector T cell response in the bone marrow of patients with monoclonal gammopathy. *J Exp Med* 198(11):1753–7
76. Chang DH, Osman K, Connolly J, Kukreja A, Krasovsky J, Pack M et al (2005) Sustained expansion of NKT cells and antigen-specific T cells after injection of alpha-galactosyl-ceramide loaded mature dendritic cells in cancer patients. *J Exp Med* 201(9):1503–17, PMID: 1389847
77. Gorgun G, Calabrese E, Soydan E, Hideshima T, Perrone G, Bandi M et al (2010) Immunomodulatory effects of lenalidomide and pomalidomide on interaction of tumor and bone marrow accessory cells in multiple myeloma. *Blood* 116(17):3227–37, PMID: 2995353
78. Brown R, Murray A, Pope B, Sze DM, Gibson J, Ho PJ et al (2004) Either interleukin-12 or interferon-gamma can correct the dendritic cell defect induced by transforming growth factor beta in patients with myeloma. *Br J Haematol* 125(6):743–8
79. Rosenblatt J, Vasir B, Uhl L, Blotta S, Macnamara C, Somaiya P et al (2011) Vaccination with dendritic cell/tumor fusion cells results in cellular and humoral antitumor immune responses in patients with multiple myeloma. *Blood* 117(2):393–402, PMID: 3031474
80. Gabrilovich DI, Nagaraj S (2009) Myeloid-derived suppressor cells as regulators of the immune system. *Nat Rev Immunol* 9(3):162–74, PMID: 2828349
81. Gallina G, Dolcetti L, Serafini P, De Santo C, Marigo I, Colombo MP et al (2006) Tumors induce a subset of inflammatory monocytes with immunosuppressive activity on CD8+ T cells. *J Clin Invest* 116(10):2777–90, PMID: 1578632
82. Mandruzzato S, Solito S, Falisi E, Francescato S, Chiarion-Sileni V, Mocellin S et al (2009) IL4Ralpha+ myeloid-derived suppressor cell expansion in cancer patients. *J Immunol* 182(10):6562–8
83. Serafini P, Borrello I, Bronte V (2006) Myeloid suppressor cells in cancer: recruitment, phenotype, properties, and mechanisms of immune suppression. *Semin Cancer Biol* 16(1):53–65
84. Medina MA, Quesada AR, Nunez de Castro I, Sanchez-Jimenez F (1999) Histamine, polyamines, and cancer. *Biochem Pharmacol* 57(12):1341–4
85. Brito C, Naviliat M, Tiscornia AC, Vuillier F, Gualco G, Dighiero G et al (1999) Peroxynitrite inhibits T lymphocyte activation and proliferation by promoting impairment of tyrosine phosphorylation and peroxynitrite-driven apoptotic death. *J Immunol* 162(6):3356–66
86. Poschke I, Mougiakakos D, Hansson J, Masucci GV, Kiessling R (2010) Immature immunosuppressive CD14+HLA-DR-/low cells in melanoma patients are Stat3hi and overexpress CD80, CD83, and DC-sign. *Cancer Res* 70(11):4335–45
87. Schmielau J, Finn OJ (2001) Activated granulocytes and granulocyte-derived hydrogen peroxide are the underlying mechanism of suppression of t-cell function in advanced cancer patients. *Cancer Res* 61(12):4756–60
88. Zea AH, Rodriguez PC, Atkins MB, Hernandez C, Signoretti S, Zabaleta J et al (2005) Arginase-producing myeloid suppressor cells in renal cell carcinoma patients: a mechanism of tumor evasion. *Cancer Res* 65(8):3044–8
89. Diaz-Montero CM, Salem ML, Nishimura MI, Garrett-Mayer E, Cole DJ, Montero AJ (2009) Increased circulating myeloid-derived suppressor cells correlate with clinical cancer stage, metastatic tumor burden, and doxorubicin-cyclophosphamide chemotherapy. *Cancer Immunol Immunother* 58(1):49–59
90. Vuk-Pavlovic S, Bulur PA, Lin Y, Qin R, Szumlanski CL, Zhao X et al (2010) Immunosuppressive CD14+HLA-DRlow/- monocytes in prostate cancer. *Prostate* 70(4):443–455, PMID: 2935631
91. Serafini P, Mgebroff S, Noonan K, Borrello I (2008) Myeloid-derived suppressor cells promote cross-tolerance in B-cell lymphoma by expanding regulatory T cells. *Cancer Res* 68(13):5439–5449, PMID: 2887390
92. Schilling T, Noth U, Klein-Hitpass L, Jakob F, Schutze N (2007) Plasticity in adipogenesis and osteogenesis of human mesenchymal stem cells. *Mol Cell Endocrinol* 271(1–2):1–17

93. Tang CH, Lu DY, Yang RS, Tsai HY, Kao MC, Fu WM et al (2007) Leptin-induced IL-6 production is mediated by leptin receptor, insulin receptor substrate-1, phosphatidylinositol 3-kinase, Akt, NF-kappaB, and p300 pathway in microglia. *J Immunol* 179(2):1292–1302
94. Mouzaki A, Panagoulas I, Dervilli Z, Zolota V, Spadidea P, Rodi M et al (2009) Expression patterns of leptin receptor (OB-R) isoforms and direct in vitro effects of recombinant leptin on OB-R, leptin expression and cytokine secretion by human hematopoietic malignant cells. *Cytokine* 48(3):203–211
95. Mattioli B, Straface E, Quaranta MG, Giordani L, Viora M (2005) Leptin promotes differentiation and survival of human dendritic cells and licenses them for Th1 priming. *J Immunol* 174(11):6820–6828
96. Mattioli B, Straface E, Matarrese P, Quaranta MG, Giordani L, Malorni W et al (2008) Leptin as an immunological adjuvant: enhanced migratory and CD8⁺ T cell stimulatory capacity of human dendritic cells exposed to leptin. *FASEB J* 22(6):2012–2022
97. Robillard N, Avet-Loiseau H, Garand R, Moreau P, Pineau D, Rapp MJ et al (2003) CD20 is associated with a small mature plasma cell morphology and t(11;14) in multiple myeloma. *Blood* 102(3):1070–1071
98. Vidal L, Gafter-Gvili A, Leibovici L, Dreyling M, Ghilmini M, Hsu Schmitz SF et al (2009) Rituximab maintenance for the treatment of patients with follicular lymphoma: systematic review and meta-analysis of randomized trials. *J Natl Cancer Inst* 101(4):248–255
99. Rossi EA, Rossi DL, Stein R, Goldenberg DM, Chang CH (2010) A bispecific antibody-IFNalpha2b immunocytokine targeting CD20 and HLA-DR is highly toxic to human lymphoma and multiple myeloma cells. *Cancer Res* 70(19):7600–7609
100. de Weers M, Tai YT, van der Veer MS, Bakker JM, Vink T, Jacobs DC et al (2011) Daratumumab, a novel therapeutic human CD38 monoclonal antibody, induces killing of multiple myeloma and other hematological tumors. *J Immunol* 186(3):1840–1848
101. Boyle WJ, Simonet WS, Lacey DL (2003) Osteoclast differentiation and activation. *Nature* 423(6937):337–342
102. McClung MR, Lewiecki EM, Cohen SB, Bolognese MA, Woodson GC, Moffett AH et al (2006) Denosumab in postmenopausal women with low bone mineral density. *N Engl J Med* 354(8):821–831
103. Ellis GK, Bone HG, Chlebowski R, Paul D, Spadafora S, Smith J et al (2008) Randomized trial of denosumab in patients receiving adjuvant aromatase inhibitors for nonmetastatic breast cancer. *J Clin Oncol* 26(30):4875–4882
104. Terpos E, Efstathiou E, Christoulas D, Roussou M, Katodritou E, Dimopoulos MA (2009) RANKL inhibition: clinical implications for the management of patients with multiple myeloma and solid tumors with bone metastases. *Expert Opin Biol Ther* 9(4):465–479
105. Body JJ, Facon T, Coleman RE, Lipton A, Geurs F, Fan M et al (2006) A study of the biological receptor activator of nuclear factor-kappaB ligand inhibitor, denosumab, in patients with multiple myeloma or bone metastases from breast cancer. *Clin Cancer Res* 12(4):1221–1228
106. Knight R (2005) IMiDs: a novel class of immunomodulators. *Semin Oncol* 32(4 Suppl 5):S24–S30
107. Tajeja N (2011) Lenalidomide—current understanding of mechanistic properties. *Anticancer Agents Med Chem* 11(3):315–326
108. Gandhi AK, Kang J, Capone L, Parton A, Wu L, Zhang LH et al (2010) Dexamethasone synergizes with lenalidomide to inhibit multiple myeloma tumor growth, but reduces lenalidomide-induced immunomodulation of T and NK cell function. *Curr Cancer Drug Targets* 10(2):155–167
109. Huang MC, Greig NH, Luo W, Tweedie D, Schwartz JB, Longo DL et al (2011) Preferential enhancement of older human T cell cytokine generation, chemotaxis, proliferation and survival by lenalidomide. *Clin Immunol* 138(2):201–211
110. Galustian C, Meyer B, Labarthe MC, Dredge K, Klaschka D, Henry J et al (2009) The anti-cancer agents lenalidomide and pomalidomide inhibit the proliferation and function of T regulatory cells. *Cancer Immunol Immunother* 58(7):1033–1045
111. Noonan K, Ferguson A, Huff CA, Emerling A, Mgebroff S, Wilson R et al (2008) The immunomodulatory role of lenalidomide on Prevnar[®] responses in patients with relapsed multiple myeloma: a comprehensive analysis of the immune response. *Blood (ASH Annual Meeting Abstracts)* 112:2772
112. Dredge K, Marriott JB, Todryk SM, Muller GW, Chen R, Stirling DI et al (2002) Protective antitumor immunity induced by a costimulatory thalidomide analog in conjunction with whole tumor cell vaccination is mediated by increased Th1-type immunity. *J Immunol* 168(10):4914–4919
113. Anderson G, Gries M, Kurihara N, Honjo T, Anderson J, Donnenberg V et al (2006) Thalidomide derivative CC-4047 inhibits osteoclast formation by down-regulation of PU.1. *Blood* 107(8):3098–3105
114. Rapoport AP, Aqui NA, Stadtmauer EA, Vogl DT, Fang HB, Cai L et al (2011) Combination immunotherapy using adoptive T-cell transfer and tumor antigen vaccination on the basis of hTERT and survivin after ASCT for myeloma. *Blood* 117(3):788–797, PMID: 3035073
115. Siegel DS, Vij R, Vescio R, Borrello I, Martin TG, Berenson JR et al (2004) A phase I/II study of Xcellerated T cells after autologous peripheral blood stem cell transplantation in patients with multiple myeloma. *Blood (ASH Annual Meeting Abstracts)* 104:925
116. Zhang L, Kerkar SP, Yu Z, Zheng Z, Yang S, Restifo NP et al (2011) Improving Adoptive T Cell Therapy by Targeting and Controlling IL-12 Expression to the Tumor Environment. *Mol Ther* 19(4):751–759
117. Prieto PA, Durflinger KH, Wunderlich JR, Rosenberg SA, Dudley ME (2010) Enrichment of CD8⁺ cells from melanoma tumor-infiltrating lymphocyte cultures reveals tumor reactivity for use in adoptive cell therapy. *J Immunother* 33(5):547–556
118. Kuball J, de Boer K, Wagner E, Wattad M, Antunes E, Weeratna RD et al (2011) Pitfalls of vaccinations with WT1-, Proteinase3- and MUC1-derived peptides in combination with MontanideISA51 and CpG7909. *Cancer Immunol Immunother* 60(2):161–171, PMID: 3024516
119. Maslak PG, Dao T, Krug LM, Chaneil S, Korontsvit T, Zakhaleva V et al (2010) Vaccination with synthetic analog peptides derived from WT1 oncoprotein induces T-cell responses in patients with complete remission from acute myeloid leukemia. *Blood* 116(2):171–179, PMID: 2910606
120. Van Tendeloo VF, Van de Velde A, Van Driessche A, Cools N, Anguille S, Ladell K et al (2010) Induction of complete and molecular remissions in acute myeloid leukemia by Wilms' tumor 1 antigen-targeted dendritic cell vaccination. *Proc Natl Acad Sci USA* 107(31):13824–13829, PMID: 2922237
121. Borrello I, Sotomayor EM, Rattis FM, Cooke SK, Gu L, Levitsky HI (2000) Sustaining the graft-versus-tumor effect through post-transplant immunization with granulocyte-macrophage colony-stimulating factor (GM-CSF)-producing tumor vaccines. *Blood* 95(10):3011–3019
122. Rapoport AP, Stadtmauer EA, Aqui N, Badros A, Cotte J, Chrisley L et al (2005) Restoration of immunity in lymphopenic individuals with cancer by vaccination and adoptive T-cell transfer. *Nat Med* 11(11):1230–1237