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Can we change the disease biology of multiple myeloma?

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Abstract

Despite improvements in disease management, multiple myeloma (MM) remains incurable. Conventional treatment methods are unsatisfactory, leading to a pattern of regression and remission, and ultimately failure. This pattern suggests that one of the possible strategies for improving outcomes is continuous therapy to maintain suppression of the surviving tumor cells. Optimal management of MM requires potent agents and modalities with direct tumoricidal activity, which can also provide continuous suppression of the residual tumor to prevent disease relapse. Immunomodulatory agents exert immunomodulatory and tumoricidal effects, and cause disruption of stromal cell support from the bone marrow microenvironment. Therefore continuous therapy with immumomodulatory agents may be able to provide both tumor reduction and tumor suppression, enabling physicians to consider the possibility of incorporating continuous therapy into the treatment paradigm of patients with MM.

Keywords

Multiple myeloma; Cell biology; Bone marrow microenvironment; Mode of action; Immunomodulatory drugs; Proteosome inhibitors

1. Introduction

Multiple myeloma (MM) is characterized by the accumulation of clonal plasma cells in the bone marrow, the presence of monoclonal immunoglobulin (Ig) in the serum or urine, osteolytic bone lesions, renal disease, and immunodeficiency. It is principally a disease of older patients, with a median age at diagnosis of 65–70 years. The first stage in the development of MM is the emergence of asymptomatic monoclonal gammopathy of undetermined significance (MGUS). In some of these patients, this progresses to smoldering MM and ultimately to symptomatic MM, with an annual risk of around 1% for patients with MGUS [1]. The reasons why MGUS progresses to MM in only a small proportion of patients are unclear, and both genetic and environmental factors have been implicated [2]. Progression to MM is associated with a series of complex genetic events in MM cells, as well as changes in the bone marrow microenvironment, including increased angiogenesis, suppression of the immune response, increased bone resorption, and the establishment of aberrant signaling-loops involving cytokines and growth factors associated with the clinical features of MM and its resistance to treatment [3].

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Despite the improvements in overall survival associated with the use of conventional high-dose chemotherapy and autologous stem-cell transplantation (HDT-ASCT), median overall survival remained at around 33 months until the introduction of the novel anti-myeloma agents, thalidomide, lenalidomide, and bortezomib [4]. For patients diagnosed since 2000, the use of the novel agents has improved survival times significantly, particularly for younger patients [5,6]. The median survival time for patients under 65 years of age treated with novel agents is 56 months [6]. The course of MM treated with conventional chemotherapy, such as single-agent alkylating drugs, corticosteroids or combination chemotherapy involving novel anti-myeloma agents is characterized by a pattern of remission and relapse, with a decreasing duration of response and increasing number of salvage regimens (Fig. 1). This reflects the development of drug resistance, which eventually results in refractory disease (Fig. 1) [7]. This pattern suggests the presence of residual disease after treatment, even following an apparently complete response. Therefore, the incurable nature of MM necessitates treatment with agents and modalities that not only provide direct tumoricidal effects to reduce tumor burden, but also suppress residual disease with continuous use.

The development of novel anti-MM agents relies on an understanding of the biology of MM and the multiple factors involved in its pathogenesis and response to treatment. As well as genetic aberrations in essential growth- and tumor-suppressor genes, there is increasing evidence that interactions between tumor cells and their bone marrow microenvironment play a pivotal role in the development, maintenance, and progression of MM, and thus, in the development of drug resistance. This knowledge has improved treatment options leading to the approval of drugs such as thalidomide, bortezomib, and lenalidomide, which not only target malignant cells directly, but also their supporting bone marrow microenvironment. In addition to their tumoricidal effects, immunomodulatory agents also act on the immune system, potentially helping to overcome MM-associated immunodeficiency and enhancing anti-MM immune activity.

This article aims to give an overview of the biology of MM, focusing on the pivotal role of the bone marrow microenvironment and its relevance to tumor survival and proliferation. It will also discuss how new agents have the potential to modify MM biology, offering the prospect of a shift in treatment paradigm to a focus on sustaining disease control with long-term treatment, which may transform myeloma into a chronic disease.

2. The biology of MM

The bone marrow of patients with MM contains malignant cells that have the morphology of mature plasma cells or plasmablasts. However, the origin of MM cells and their developmental relationship to non-malignant counterpart cells remains obscure. The vast majority of MM cells appear to be mature, quiescent, and terminally differentiated; therefore, they do not have long-term proliferative potential. This raises questions about which cells in MM patients are clonogenic and capable of proliferation, and at what stage these cells develop. It is possible that clonogenic growth may be restricted to a minor, specialized population of cells that is distinct from the differentiated/mature cells that comprise the bulk of disease, such as post-germinal center memory B cells [8], and that these cells may be similar to the cancer stem cells seen in other malignancies [9].

Identification of the stage at which tumor growth develops in MM has been an important research aim. During normal differentiation from stem cells to plasma cells, immature B lymphocytes first differentiate in the bone marrow, where they undergo immunoglobulin heavy-chain (IgH) VDJ gene rearrangement, resulting in the expression of surface IgM (Fig. 2). The B cells then migrate as naïve B lymphocytes to the secondary lymphoid tissue where

antigen stimulation leads to their proliferation. During this period, somatic hypermutation in the IgH and light-chain genes gives rise to the selection of B-cell clones expressing high-affinity Igs. At this stage the cells may either leave the secondary lymphoid organs and circulate as memory B cells or differentiate into post-follicular plasmablasts following a switch in Ig class from IgM to IgG, IgA, IgD, or IgE. Plasmablasts migrate back to the bone marrow to undergo terminal differentiation into plasma cells. During this process, B cells express a range of surface markers used to assess their developmental stage (Fig. 2). Cell-surface markers and IgH chain gene sequences in MM cells define the nature of the malignant cells, and their analysis has revealed both similarities and differences between normal plasma cells and MM cells. Both cell types typically express CD138 (syndecan-1), which is considered to be a universal marker of both cell types; they also express CD38, although expression levels have been found to vary between cell types [10,11]. MM cells also show extensive somatic hypermutations of rearranged Ig genes and almost exclusively express Ig isotypes other than IgM, indicating a mature, post-follicular B-cell origin.

MM cells express not only markers associated with plasma cells, but also markers associated with natural killer (NK) cells (CD56/NCAM), T cells (CD28); and occasionally also the pan-B-cell marker, CD20 [11]. Interestingly, cell-surface markers usually found on B-cells during the early stages of differentiation, such as CD10 and CD19, have been observed on subpopulations of MM cells [12–14]. This provides evidence for the existence of an early-lineage precursor for MM cells, which is further supported by the identification of unique MM idiotypic determinants in pre-B-cell populations [15] and mRNA expression of cell-surface proteins characteristic of myeloid, erythroid, and platelet lineages in MM cells [16]. Several studies have described varying frequencies of clonal cells expressing B-cell characteristics rather than plasma cell characteristics in the bone marrow and peripheral blood of patients with MM [17,18]. Such studies have suggested the presence of clonogenic stem cells in MM arising from a post-germinal center compartment [18,19], possibly equivalent to memory B cells [9]. The exact phenotype of the clonogenic cells in MM remains to be definitively established, and their role in the pathogenesis of disease is controversial [9]. The results of a recent study revealed the presence of a stem cell phenotype [20], and those of other studies have suggested that such clonogenic cells may be resistant to chemotherapy and that they may persist following treatment [14,21,22], making them a particularly interesting target for MM therapy.

Whatever the cell of origin, the majority of MM cases are characterized by complex chromosomal abnormalities (Table 1) [23–30]. Karyotype analysis has demonstrated the presence of two major cytogenetic categories: hyperdiploid MM, which includes numerous chromosomal trisomies and is associated with a low prevalence of IgH translocations; and non-hyperdiploid MM, which encompasses hypodiploid, pseudodiploid, and near-tetraploid MM and is associated with a high level of IgH rearrangements [23]. Some of the most frequent and early genetic events involve the IgH gene locus on 14q32, which is commonly part of a translocation [24]. Interphase fluorescence *in situ* hybridization (FISH) analysis has shown that these translocations are present in about half of patients with MGUS and up to 75% of patients with MM [24,29,31]. The most common of these translocations leads to dysregulation of oncogenes at translocation partner regions (Table 1) [23,24]. Frequent translocations involving the IgH gene locus and 14q32 are t(11;14)(q13;q32), t(4;14)(p16;q32), and t(14;16)(q32;q23) [23–27, 30], some of which have been associated with poor survival [28,32]. In terms of prognosis, deletion of 17p13, involving the tumor suppressor gene p53, is the most important cytogenetic factor; it is associated with worse treatment outcomes (Table 1) [23,24]. However, 13q deletion, which has been traditionally considered an adverse prognostic factor, is associated with poor prognosis only if other cytogenetic abnormalities, such as t(4;14) and deletion of 17p13, are present [28]. Although several genetic mutations seen in MM patients have been linked to disease progression,

clinical findings, and response to therapy, it is important to note that the behavior of MM cells at the biological and clinical level is also crucially influenced by interactions between tumor cells and the bone marrow microenvironment [33].

3. The role of the bone marrow microenvironment in MM pathogenesis

MM has become the prototypical tumor model for characterizing the interaction between tumor cells and their local milieu [34,35]. The bone marrow microenvironment refers not only to bone marrow stromal cells (BMSCs), but also to the non-cellular component composed of extracellular matrix (ECM) proteins such as collagen, fibronectin and laminin, and the extracellular fluid containing cytokines and growth factors. The bone marrow microenvironment supports normal hematopoiesis and these support mechanisms are harnessed by MM cells. The provision of various cytokines, growth factors, and receptors to MM cells increases their replicative capacity and confers resistance to pro-apoptotic signals, including those induced by conventional chemotherapy drugs [33]. The key processes include: direct MM cell–BMSC interactions and MM cell interactions with other components of the bone marrow microenvironment; indirect effects of cytokines produced by BMSCs or MM cells following such interactions; and the resulting activation of proliferative and anti-apoptotic signaling pathways (Fig. 3) [36]. The list of adhesion molecules and cytokines implicated in the pathophysiology of MM is extensive and expanding. The signaling cascades induced by these factors affect not only the proliferation and survival of tumor cells, but also other key aspects of MM pathology, including the development of osteolytic lesions and angiogenesis. A full discussion of the molecules and signaling pathways implicated in MM pathophysiology is beyond the scope of this article; however, some of the most important points are discussed below.

Cell adhesion molecules (CAMs) such as CD44 (H-CAM), CD56 (N-CAM), members of the CD49 integrin family (including very-late antigen VLA-4 and VLA-5), lymphocyte function-associated antigen-1, syndecan-1, and selectin have been shown to mediate the interaction of tumor cells with, and their adherence to, ECM proteins and BMSCs [37]. These molecules play crucial roles in MM pathogenesis, including in the homing and localization of MM cells to the bone marrow [37]. Binding of MM cells to BMSCs induces the activation of p42/44 mitogen-activated protein kinase, and nuclear factor κB (NF-κB), resulting in increased expression of adhesion molecules on both MM cells and BMSCs, which in turn leads to increased production of cytokines, in particular interleukin (IL)-6 [38–40]. Adhesion molecules may also play a direct role in the response of MM to therapy; for example, MM cells resistant to melphalan and doxorubicin typically overexpress VLA-4. Adherence of VLA-4 to ECM proteins, such as fibronectin, induces CAM-mediated drug resistance, including up-regulation of p27 in tumor cells [41]. Concomitant exposure of MM cells to IL-6 and adhesion to fibronectin has been shown to result in an increase in STAT3 phosphorylation, nuclear translocation, and DNA binding, leading to transactivation of genes involved in proliferation, differentiation, and survival [42]. Another adhesion molecule, selectin, has been shown to modulate the interactions between MM cells and surrounding stromal cells; indeed, selectin inhibitors and the proteasome inhibitor bortezomib have been shown to reduce tumor burden [43].

Of the cytokines involved in MM pathogenesis, IL-6 is a major growth and survival factor for MM cells that is predominantly produced by BMSCs. Adhesion of MM cells to BMSCs up-regulates IL-6 secretion via NF-κB-dependent transcription [38,39]. IL-6 acts via its receptor (CD126) to activate signal transduction pathways (JAK/STAT3 and PI3K/Akt), inducing proliferation and preventing apoptosis, and thereby contributing to drug resistance [24,33]. IL-6 is known to mediate both MM cell proliferation and inhibition of Fas-induced

apoptosis [44,45]. Additionally, it enhances production of vascular endothelial growth factor (VEGF) [44–46] and it may also play a role in the differentiation of osteoclasts [47].

Tumor necrosis factor (TNF)-α is a potent mediator of inflammation and bone resorption [48]. It has been shown to enhance MM-cell survival, trigger proliferation, and promote cell migration [49,50]. Levels of the pro-inflammatory chemokines mediated by TNF-α are higher in patients with MM than in patients with MGUS, suggesting that TNF-α levels increase with disease severity [51]. In MM, TNF-α also mediates the up-regulation of adhesion molecules on MM cells and BMSCs, which in turn results in activation of the NF-κB pathway [48]. High serum levels of TNF-α in MM patients are associated with osteolytic lesions, because TNF-α induces osteoclast differentiation [51]. B-cell activating factor (BAFF), a member of the TNF superfamily of proteins, is crucial for the maintenance and homeostasis of normal B-cell development, and has been shown to confer a survival advantage on MM cells [52–54]. MM cell adhesion to BMSCs augments BAFF production via NF-κB activation, and BAFF itself strengthens this adhesion [52].

Other relevant cytokines include insulin-like growth factor-1, a growth and survival factor in MM cells [55] that induces sustained activation of proliferative/anti-apoptotic signaling cascades (e.g., PI3K/Akt; IKK/NF-κB) [56]. VEGF is a pro-angiogenic molecule expression which is up-regulated by MM cell–BMSC adhesion, and is associated with tumor cell migration, growth, and survival [44,45]. VEGF also stimulates microvascular endothelial cells and BMSCs to increase IL-6 secretion, thus contributing further to MM cell proliferation and survival. IL-17 is a cytokine that, in addition to exerting an effect on cell survival [57], has also recently been identified as a key mediator of bone disease in myeloma [58]. Interestingly, the extent of lytic bone disease appears to be largely mediated by IL-17 produced by T cells, independent of the tumor burden, underscoring the crucial interplay of the immune system with the tumor microenvironment in the pathogenesis of MM [58].

It is clear that there is a complex web of autocrine and paracrine interactions between components of the bone marrow microenvironment and MM cells. However, other factors also contribute to the many aspects of MM pathophysiology, including the effects of the malignant cells on the immune system.

4. Immunodeficiency in MM

Immune dysfunction is an important feature of MM and is associated with an increased incidence of infections, which are a major cause of morbidity and mortality in myeloma patients. Importantly, immunodeficiency impacts disease progression and resistance to chemotherapy. Several factors produced as a result of MM cell–BMSC interactions also alter the functions of the host immune effector cells, thus interfering with immune surveillance and preventing immune-mediated tumor rejection [59].

MM patients show a pattern of global immunosuppression, with significantly reduced numbers of NK, B, and memory T cells, and low levels of non-myeloma Igs [60]. CD4+ and CD8+ T cells from MM patients show multiple abnormalities, particularly in more advanced stages, including reduced expression of cell-surface markers associated with T-cell signaling (such as CD28 and CD152), aberrant signal transduction, and impaired activation-induced cytokine production [61]. Defects have also been demonstrated in NK T cells [62] and antigen-presenting cells [63], as well as during B-cell differentiation and antibody responses [64,65]. Cytokine production resulting from MM cell–BMSC interactions is thought to play a role in immune dysfunction; for example, transforming growth factor-β has several effects in MM, including suppression of normal B-cell function [66].

Increasing knowledge about the interactions among cells, signaling proteins, and the bone marrow microenvironment has been important in providing potential targets for new therapies. Therapies that affect both the tumor cells and their bone marrow microenvironment, and that enable the patient's own immune system to mount a more effective anti-MM response, are required.

5. Mode of action of immunomodulatory agents

Thalidomide, a synthetic derivative of glutamic acid, has been found to have a range of properties including anti-inflammatory effects via inhibition of TNF-α [67], inhibition of angiogenesis [68], and immunomodulatory properties, including enhancement of T cell- and NK cell-mediated immunity [69,70]. These properties stimulated interest in thalidomide as an anti-cancer drug, particularly for the treatment of MM. Thalidomide has been relatively successful in improving survival in patients with MM, initially as monotherapy and later in combination with dexa-methasone. However, it is associated with a range of toxicities in addition to its known teratogenic effect, including polyneuropathy, somnolence and, particularly when administered in combination with dexamethasone or chemotherapy, venous thromboembolism.

Immunomodulatory agents, synthetic analogs of thalidomide, were developed by Celgene Corporation to have increased potency with less toxicity compared with the parent compound [71]. Lenalidomide and pomalidomide are more potent inhibitors of TNF-α *in vivo* than thalidomide [72], and are more potent T-cell co-stimulators [73]. To date, clinical data on this class of compounds have come predominantly from studies on lenalidomide (phases I-IV), with pomalidomide currently in phase II–III development [73–78]. Lenalidomide is administered orally, has a favorable safety profile and has been shown to be highly effective in treating MM [79]. It is currently approved for use with dexamethasone in patients with MM who have received at least one prior therapy. Although their exact mode of action in MM remains unknown, studies suggest that immunomodulatory agents have a combination of anti-myeloma actions including direct tumoricidal effects, disruption of stromal cell support from the bone marrow microenvironment, and a number of immunomodulatory effects including anti-proliferative, apoptotic, anti-inflammatory, and anti-angiogenic effects. Recently, expression of cereblon (CRBN), a thalidomide-binding protein and teratogenic target [80] has been shown to be an essential requirement for immunomodulatory activity [81], and to be an important molecular target of lenalidomide and pomalidomide [82]. A positive association has been identified between high levels of CRBN expression and a good clinical response to treatment with lenalidomide and dexamethasone [83]. The multiple effects of immunomodulatory agents have been cited as the likely reason for the breadth of activity of this class of drugs [84].

6. Direct tumoricidal effects and modulation of the tumor microenvironment

Immunomodulatory agents have been shown to have several direct and indirect effects on MM cells, via both direct tumoricidal effects and modulation of the bone marrow microenvironment, including the prevention of angiogenesis and osteoclastogenesis.

Lenalidomide down-regulates expression of the MM cell survival factor interferon regulatory factor-4 [85–87]. Conversely, it induces the expression of cyclin-dependent kinase inhibitors, including p21, p27, and p15, and the early response transcription factors Egr1, Egr2, and Egr3, which are implicated in the regulation of tumor suppressor and cell-cycle regulatory genes [85,88,89]. Lenalidomide has also been shown to activate caspases, directly triggering tumor cell death [89,90], with the activation of caspases 3, 8, and 9 by lenalidomide being synergistically enhanced by dexamethasone [89,90].

Lenalidomide and pomalidomide also act by disrupting the stromal support within the bone marrow that is needed for the production of a range of cytokines including VEGF, IL-6, and TNF-α [72,91,92]. By inhibiting TNF-α expression, and thereby, reducing the expression of adhesion molecules on both MM cells and BMSCs [93], immunomodulatory agents have been shown to reduce levels of IL-6 induced by MM–BMSC interactions [44,91]. The down-regulation of adhesion molecules also has implications for signaling pathways. Indeed, lenalidomide has been shown to down-regulate NF-κB *in vitro* [92], resulting in reduced expression of anti-apoptotic proteins [93].

Angiogenesis in MM has been associated with active disease and the adhesion-molecule-mediated interactions between MM cells and the microvasculature have been implicated in the ability of a tumor to disseminate [94,95]. The anti-angiogenic effects of immunomodulatory agents are likely to be due to anti-migratory mechanisms mediated via modulation of chemotactic factors such as TNFα, VEGF, and basic fibroblast growth factor rather than direct inhibition of endothelial cell proliferation [92,96,97]. Lenalidomide has also been shown to inhibit growth factor-induced phosphorylation of Akt, a key signaling step in the Akt pathway involved in malignant transformation, chemoresistance, and invasiveness, by inducing cell survival, growth, migration, and angiogenesis [97,98]. Immunomodulatory agents have also been shown to directly inhibit osteoclast maturation, associated with a reduction in osteoclast expression of cathespin K, markers of osteoclast differentiation [99], and markers of bone metabolism [100].

7. Immunomodulatory properties

In vitro, immunomodulatory agents have been shown to augment both the adaptive and innate immune systems via enhancement of T-cell and NK-cell immune responses, both of which are reduced in MM patients [69,89,101–103]. Immunomodulatory agents induce cell-surface expression of positive co-stimulatory molecules on T cells, including CD28, which is down-regulated in MM. T-cell co-stimulation by immunomodulatory agents via the B7-CD28 pathway is associated with up-regulation of cytokines, such as IL-2 and interferon (IFN)-γ, which mediate T-cell activation, proliferation, and anti-tumor immune responses [73].

Treatment with immunomodulatory agents has been shown to diminish the expression of suppressor of cytokine signaling (SOCS)1, a negative regulator of IL-2 and IFN-γ signaling, in immune effector cells (CD4+, CD8+ cells, NKT cells, and NK cells) from both the peripheral blood and bone marrow of MM patients [104]. SOCS1 also negatively regulates IL-6 signaling, and is silenced by hypermethylation in approximately 75% of MM patients [105]. Interestingly, immunomodulatory agents were found to demethylate the SOCS1 gene; this not only abrogated IL-6 expression, but also enhanced the susceptibility of MM cell lines to cytotoxic T-lymphocyte killing [104].

The increase in production of immunostimulatory cytokines by immunomodulatory agents also results in augmentation of other aspects of the immune response, including NK-cell function and dendritic cell activity [69,103,106,107]. For example, immunomodulatory agents have been shown to augment NK-cell proliferation and activity in the presence of IL-2, resulting in NK cell-mediated lysis of MM cells [69]. The use of lenalidomide as salvage therapy after ASCT for MM resulted in increased levels of activated NK cells [108]. Lenalidomide has also been shown to enhance anti-tumor antibody-dependent cellular cytotoxicity as a result of increased IL-2 production by T cells [107,109]. This effect is reflected in patients responding to treatment with thalidomide, who showed increases in both the numbers of NK cells, and in IL-2 and IFN-γ secretion [69]. Taken together, these data

suggest that immunomodulatory compounds exert a positive regulatory function, enhancing the anti-MM immune response [93,104].

There is also evidence that immunomodulatory agents regulate humoral immune responses in MM. The positive co-stimulatory surface marker ICOS and its ligand, which are up-regulated by immunomodulatory agents [104], regulate T-cell-mediated immune responses by controlling T-cell/B-cell interactions. This is achieved by stimulating the production of cytokines, such as IL-4 and IL-10, which play crucial roles in B-cell growth, maturation, and isotype switching [110]. Lenalidomide-based therapy was found to improve the humoral immune response in a significant proportion of responding patients [111]. In addition, MM patients treated with the polyvalent pneumococcal vaccine, in combination with lenalidomide, showed significantly greater B-cell and T-cell responses to the vaccine, underscoring its potential role as an immunostimulatory vaccine adjuvant [112].

Given the complexity of the immune response *in vivo*, the ultimate immunomodulatory effects of immunomodulatory agents in patients are probably considerably more complex and dynamic than current evidence indicates, and are also dependent on the individual's immune status and cytokine profile in the bone marrow.

8. Mode of action of proteasome inhibitors

Proteasome inhibitors act by targeting intracellular protein turnover via inhibition of the ubiquitin–proteasome pathway [113]. The proteasome inhibitor bortezomib, a specific inhibitor of the 26S proteasome, was the first of its class to enter clinical trials for the treatment of MM. Bortezomib is administered by injection and is indicated for the treatment of patients who have already received at least one prior therapy and undergone, or are unsuitable for, bone marrow transplantation, or those patients with previously untreated MM who are ineligible for high-dose chemotherapy with bone marrow transplantation. The second generation proteasome inhibitor carfilzomib is currently undergoing phase III trials in patients with relapsed/refractory MM in the ASPIRE and FOCUS trials, as well as in newly diagnosed (CYCLONE) and elderly (NCT01279694) MM patients. Carfilzomib is selective and structurally distinct from bortezomib, and shows more sustained proteasome inhibition as its effect is mechanistically irreversible [114] it is being assessed in a head-to-head phase III trial versus bortezomib (ENDEAVOR study). As such, the majority of data on proteasome inhibitors in MM have been derived from studies on bortezomib.

9. Direct tumoricidal effects and modulation of the tumor microenvironment

The therapeutic effects of bortezomib probably result from a combination of direct toxicity and its effects on the bone marrow microenvironment [3,115]. Proteasome inhibition results in cytoplasmic accumulation of IκB, which blocks the nuclear translocation and transcriptional activity of NF-κB. As discussed previously, inhibition of NF-κB results in a decrease in expression of a range of adhesion molecules and cytokines such as IL-6, which is involved in the growth and survival of MM cells. *In vitro*, proteasome inhibitors have been shown to inhibit growth, induce apoptosis, and overcome drug resistance in MM cells [116,117]. The exact means by which "non-specific" proteasome inhibition results in selective killing of tumor cells is unclear [118]. Inhibition of the NF-κB pathway and associated down-regulation of inhibitors of apoptosis such as Bcl-2, A1, cIAP-2, and XIAP, promotion of endoplasmic reticulum stress-induced apoptosis, induction of p53-dependent apoptosis, and cell cycle disruption, have all been implicated in the selective killing of tumor cells by bortezomib [115,116,118,119]. In keeping with this, bortezomib has been shown to directly inhibit proliferation and induce apoptosis in MM cell lines and tumor cells from MM patients [36]. Bortezomib has also been shown to overcome chemoresistance following conventional chemotherapy *in vitro* via inhibition of NF-κB [117,120], and by lowering the

apoptotic threshold of resistant cells via down-regulation of effectors involved in the cellular response to genotoxic stress induced by DNA-damaging chemotoxic agents [120].

Bortezomib has both direct and indirect effects on endothelial cells, inhibiting the proliferation of MM patient-derived endothelial cells and human umbilical vein endothelial cells, and inhibiting angiogenesis *in vitro*. It inhibits VEGF and IL-6 production dose-dependently, and down-regulates expression of angiopoietin-1 and -2 [121]. In addition, bortezomib has been shown to inhibit osteoclast differentiation and bone resorption activity *in vitro* via inhibition of p38, AP-1, and NF-κB [122], although clinical observations suggest that response to treatment is associated with osteoblast activation in patients with MM [123,124]. Inhibition of the ubiquitin–proteasome pathway also reportedly modulates osteoblast differentiation through up-regulation of the expression of bone morphogenetic protein-2 [125].

10. Effects on the immune system

Proteasome inhibition sensitizes tumor cells to NK cell-mediated lysis through TNF-related apoptosis-inducing ligand and/or Fas/Fas ligand pathways [126,127]. However, bortezomib has also been shown to disrupt NK-mediated immunity via induction of NK-cell apoptosis and suppression of NKp46 receptor-mediated cytotoxicity [128,129]. In addition, proteasome inhibitors induce apoptosis in activated and proliferating human T cells [130], interfere with the dendritic cell function [131] and antigen presentation [132], and suppress essential immune functions of CD4+ T cells [133], all of which contribute to an overall immunosuppressive action. Clinically, the immunomodulatory effects of bortezomib result in an increased incidence of herpes zoster reactivation during treatment [134]. The long-term effects of this action in patients with MM-induced immunosuppression are unclear.

11. Summary

In summary, MM is an incurable disease in which relapse is characterized by re-growth of residual tumor and immune suppression with a complex biology that affects many aspects of the disease and its response to treatment. As such, this disease requires effective long-term treatment strategies [135,136]. Conventional treatment, even with the addition of novel anti-myeloma agents, remains unsatisfactory. The disease is characterized by a pattern of regression and remission, and ultimately failure, indicating the presence of residual disease or resistant cells, even in patients who initially show a complete clinical response to treatment. This pattern suggests that continuous therapy may be required to maintain suppression of surviving tumor cells, which confound conventional therapy (Fig. 4), although unanswered questions remain regarding the long-term safety of continuous treatment with these agents.

Studies of the biology of MM have highlighted the need for agents to target not only the tumor cells themselves, but also to disrupt their supportive microenvironment in the bone marrow. Immunomodulatory agents and proteasome inhibitors act directly on malignant clones and also on their environment through inhibition of stromal cell support. This may explain the high clinical response rates associated with treatment regimens involving these agents. In addition to their ability to directly induce tumor cell death and to interfere with tumor cell–microenvironment interactions, immunomodulatory agents also offer the potential to enhance anti-tumor immune responses in MM as a consequence of the immunostimulatory effects they exert on both cellular and humoral immunity. The ability to modify the biology of MM using such new therapies raises the question of whether a change in treatment paradigm towards continuous therapy, providing both tumor reduction and tumor suppression, is warranted. Future studies are needed to test such paradigms.

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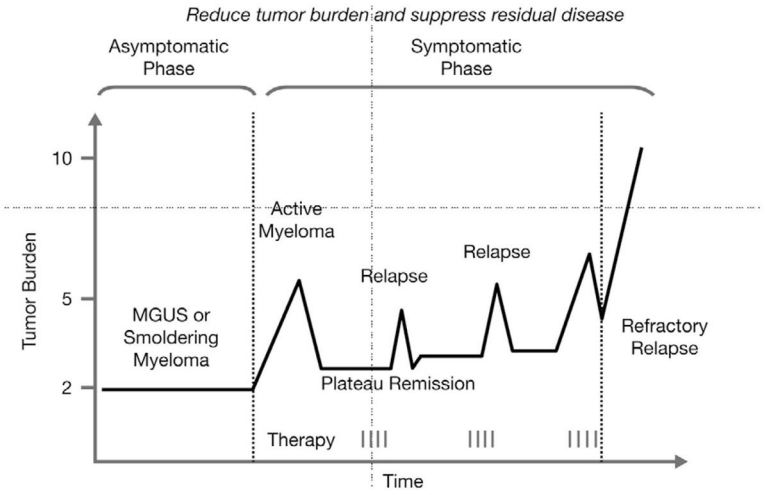

Fig. 1.

Characteristic pattern of remission and relapse following conventional chemotherapy in multiple myeloma. MGUS, monoclonal gammopathy of undetermined significance.

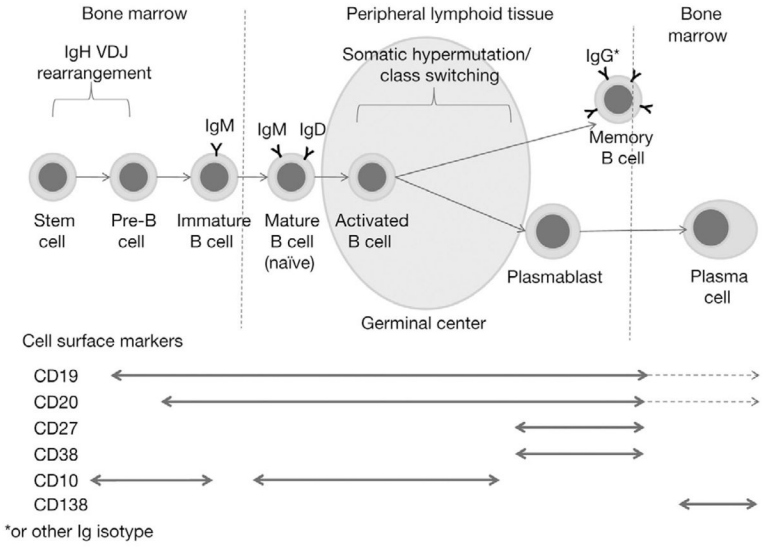

Fig. 2.

B-cell maturation and cell-surface marker expression. * Or other Ig isotype. Ig, immunoglobulin; VDJ, variable diversity, and joining.

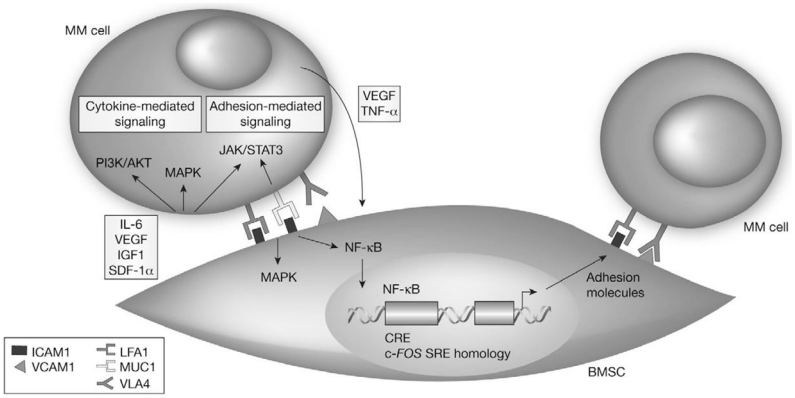

Fig. 3.

Multiple myeloma (MM) cell–bone marrow stromal cell (BMSC) interaction. Reproduced with permission from [36] © 2002, Rights Managed by Nature Publishing Group. AKT, protein kinase B; CRE, cyclic AMP-responsive element; ICAM1; intercellular adhesion molecule 1; IGF1, insulin-like growth factor-1; IL-6, interleukin-6; JAK, janus kinase; LFA1, lymphocyte function-associated antigen 1; MAPK, mitogen-activated protein kinase; muc1, mucin 1; NF-κB, nuclear factor κB; PI3K, phosphatidylinositol 3-kinase; SDF-1α, stromal-cell-derived factor-1α; SRE, serum response element; STAT3, signal transducer and activator of transcription 3; TNF-α, tumor necrosis factor-α; VCAM1, vascular cell adhesion molecule 1; VEGF, vascular endothelial growth factor; VLA4, very-late antigen 4.

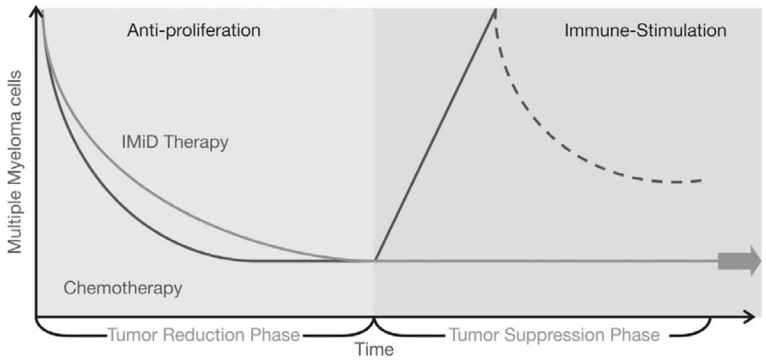

Fig. 4.
Continuous therapy with IMiD® immunomodulatory agents versus conventional chemotherapy.

Table 1

Chromosomal aberrations in multiple myeloma (MM) [23–30].

Chromosomal aberrations	Protein affected	Biological consequence	Frequency	Effect on treatment efficacy
t(11;14)(q13;q32) [23,24]	Cyclin D1	Over-expression; cell cycle dysregulation	15%	
t(4;14)(p16;q32) [24–27]	FGFR3 [23] or MMSET	Over-expression and activation; MM cell proliferation/apoptosis prevention MMSET probably linked to crucial transforming event	25%	Poor survival [28,29]
t(14;16)(q32;q23) [30]	*c-MAF*	Over-expression; involvement in IL-4 regulation	5%	Poor survival [28,29]
del 17p13 [23]	p53	Cell-cycle dysregulation/apoptosis	Rare in newly diagnosed MM; frequency increasing in relapsed patients; not present in MGUS	Short survival, aggressive disease, short response duration following ASCT [23]

ASCT, autologous stem-cell transplantation; del, deletion; FGFR3, fibroblast growth factor receptor 3; IL, interleukin; MGUS, monoclonal gammopathy of undetermined significance; MM, multiple myeloma; MMSET, multiple myeloma SET; t, translocation.