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Mechanism of action of immunomodulatory agents in multiple myeloma

Thomas Reske,

VA Boston Healthcare System, Boston, MA, USA

Boston University Medical Center, Boston, MA, USA

Mariateresa Fulciniti, and

Dana-Farber Cancer Institute, Harvard Medical School, 44 Binney Street, Boston, MA 02115, USA

Nikhil C. Munshi

VA Boston Healthcare System, Boston, MA, USA

Dana-Farber Cancer Institute, Harvard Medical School, 44 Binney Street, Boston, MA 02115, USA, nikhil_munshi@dfci.harvard.edu

Abstract

Immunomodulatory agents (IMiD's) have become an important drug category in the treatment of multiple myeloma. The agents have a complex mechanism of action that influence the microenvironment in the bone marrow. The microenvironment is an essential promotor of disease progression and therefore important in targeting the disease. The article reviews mechanism of action and essential pathways of IMiD's that are important in disease treatment.

Keywords

Immunomodulatory agents; Bone marrow microenvironment; Multiple myeloma

Introduction

The increased understanding of the interactions between multiple myeloma (MM) cells and the extracellular matrix proteins as well as the bone marrow stromal cells (BMSC) in the bone marrow milieu has spurred the development of novel agents targeting these interactions, besides direct effects on MM cells. The BM microenvironment promotes MM cell growth, survival, migration, and drug resistance. Different cellular components of the BM microenvironment participate in these effects. Besides BMSC, the cells in BM milieu that affect MM cell growth and survival include immune cells, endothelial cells, osteoclasts, and osteoblasts. These broad arrays of cells not only interact with MM cells but also secrete growth or anti-apoptotic factors that affect MM cell growth and survival and may promote development of drug resistance. Therefore, it is important that the therapeutic interventions not only target MM cells, but also their supporting environment.

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Correspondence to: Nikhil C. Munshi.

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The immunomodulatory agents (IMiD), a group of orally bioavailable agents, derived from thalidomide are one of the important novel agents available for the treatment of MM. Currently, thalidomide and lenalidomide are commercially available, and pomalidomide has demonstrated early encouraging clinical activity. These agents share a similar chemical structure and are characterized by a wide range of similar biologic and molecular activity with effects on adhesion molecules, MM–BMSC interactions, cytokines, and growth factors supporting MM growth, angiogenesis, and immune cells (Fig. 1) [1]. Their mechanism of action and effect on the microenvironment as a single agent and in combination with other conventional and novel agents will be reviewed here.

Thalidomide, the first in this group, was introduced as anti-emetic and sedative but was revisited in the 1990s for the treatment of erythema nodosum. The reports of increased bone marrow angiogenesis in multiple myeloma coupled with known anti-angiogenic properties of thalidomide provided the initial rationale for its use to treat MM. Given the broad spectrum of activity, the IMiDs appear to have anti-MM effects in several ways.

Apoptosis and cell cycle arrest

Certain IMiD's may have a direct effect on the MM cells and/or BMSCs to inhibit their growth and survival. Mitsiades et al. [2] demonstrated that immunomodulators induce apoptosis in multiple myeloma cells with activation of caspase-8 and enhanced sensitivity to Fas-mediated apoptosis. Cell culture and in vivo studies revealed a down-regulation of NF κ B activity as well as cellular inhibition of apoptosis protein 2 (cIAP-2) and FLICE inhibitory protein (FLIP) expression; inhibition of the pro-survival effects of IGF-1, restoration of the anti-MM activity of TNF-related apoptosis-inducing ligand (TRAIL/Apo2L). Thalidomide analogs appear more potent than Thalidomide in inducing these pathways. Gandhi et al. [3] showed in vitro that lenalidomide induced G0/G1 cell arrest and apoptosis. Direct cytotoxic activity of lenalidomide has been observed via a mechanism involving modulation of p21 expression, sub-G1 cell cycle arrest, and apoptosis [4]. Lenalidomide has also been shown to disrupt the phosphatidylinositol 3 (PI3) kinase/Akt signaling pathway which plays a critical role in cell survival. Adaptor proteins that assemble into scaffolding complexes and act as signaling intermediates between growth factor and cytokine receptors with PI3 have been identified. Grb2-associated binding protein Gab1 is an adaptor protein that contains a pleckstrin-homology domain for membrane targeting, multiple tyrosine phosphorylation sites that can recruit SH2 domain containing molecules such as PI3 kinase to the cell membrane for activation and a proline-rich domain that can interact with SH3 domain containing proteins. Gab1 was first identified as a docking protein downstream of epidermal growth factor and insulin receptor signaling. The Gab1 pathway has been shown to be disrupted by Lenalidomide [3]. In the cell culture model, it inhibited the phosphorylation of the adaptor protein Gab1 upon erythropoietin (Epo) stimulation and disrupted assembly of the Gab1 scaffolding complex with the Epo receptor.

Inhibition of MM–BMSC interaction

Adhesion of MM cells to BMSC has been shown to trigger adhesion-mediated cell signaling as well as secretion of cytokines that supports MM cell growth, survival, and development of drug resistance. IMiDs have its significant effect on both inhibiting the MM–BMSC interaction and production of cytokines. The effect on the MM–BMSC interaction is mediated by inhibition of expression of cell surface adhesion molecules [5]. The vascular cell adhesion molecule (VCAM-1), intercellular adhesion molecule 1 (ICAM-1), and VLA-4 have been shown to play a significant role in MM–BMSC adhesion within the bone marrow microenvironment [6]. MM–BMSC adhesion leads to adhesion-mediated activation of signaling pathways including the Ras/Raf MAPK cascade and PKC-dependent pathway as

well as secretion of growth and survival-related cytokines. Thalidomide and its analogs downregulate VCAM-1 and ICAM-1 expression along with consequent inhibition of adhesion-mediated cell signaling as well as cytokine production in the BM milieu [7].

Inhibition of cytokine circuits

Growth factors production triggered by MM–BMSC interaction includes interleukin (IL)-6, vascular endothelial growth factor (VEGF), tumor necrosis factor (TNF)- α , stromal cell derived factor (SDF) 1 α , and B cell-activating factor BAFF. IL-6 secretion by BMSC seems to play a significant role in cell adhesion. Although IL-6 is involved in normal B cell development, overproduction of this cytokine is considered to be an important component of the MM pathogenesis. The most common source of IL-6 is BMSC with its paracrine rather than autocrine effects. Chauhan et al. [8] showed in vitro that adhesion of MM cells to BMSCs and BMSC lines triggered 5–15-fold and 2–4-fold increases in IL-6 secretion, respectively. Gene expression studies by Mitsiades et al. [9] have also confirmed the induction of IL-6 gene expression by BMSC during MM–BMSC adhesion [9]. NF- κ B is considered an essential regulatory element for adhesion-induced IL-6 transcription by BMSCs. IMiDs have demonstrated inhibition of IL-6 secretion by inhibiting NF κ B as well as by direct effects on IL-6 production. The inhibition of NF κ B is triggered by down-regulated TNF- α that leads to a down-regulated IL-6 transcription [10].

IGF-1 is a potent growth and survival factor for MM cells [16–18] that activates NF- κ B [15] associated with up-regulation of the expression of several caspase inhibitors, including the caspase-8 inhibitors cIAP-2 and FLIP. IGF-1 regulates cell proliferation, differentiation, and apoptosis. It has been shown to augment the proliferative and anti-apoptotic effects of IL-6 [11]. Lenalidomide is able to overcome the induction of these anti-apoptotic proteins by IGF-1. Other cytokines secreted into the BM microenvironment include TNF- α . Thalidomide has been shown to selectively inhibit TNF- α production by human monocytes in vivo and in vitro [12, 13]. In comparison with thalidomide, lenalidomide is 50,000 fold more potent on a molar basis in inhibiting TNF- α production in vitro. Other anti-inflammatory cytokines include IL-10 and IL-4. IL-10 has been shown to be stimulated by IMiD's.

Anti-angiogenic activity

VEGF and basic fibroblast growth factor 2 (bFGF-2) secreted by MM cells or BMSC may play a role in tumor cell growth and survival, as well as the bone marrow angiogenesis. Thalidomide and its analogs inhibit angiogenesis by inhibiting VEGF, as well as bFGF-2. Thalidomide has been shown to inhibit angiogenesis in experimental models. For example, angiogenesis induced by bFGF in a rabbit cornea micro pocket assay was inhibited by Thalidomide [14, 15]. Anti-angiogenic activity for IMiD's has also been confirmed using an in vitro multicellular human assay system and the established rat aorta assay [16]. IMiDs are able to significantly inhibit angiogenesis even at low concentrations [17]. The anti-angiogenic potency is not related to inhibition of endothelial cell proliferation, nor their TNF- α /PDE type inhibitory properties. Lentzsch et al. [18] characterized the effect of IMiDs on growth of B cell malignancies and angiogenesis. Using a beige-nude-xid (BNX) mouse model to simultaneously measure in vivo anti-tumor and anti-angiogenic effects of thalidomide and its analogs, the anti-angiogenic effects were confirmed by a decrease in micro-vessel density.

Immune modulation: T cell and NK cell effects

IMiDs significantly modulate immune responses by altering cytokine production, regulating T cell co-stimulation and augmenting NK cell number and cytotoxicity, providing the

cellular and molecular basis for use of IMiDs as an adjuvant in immunotherapeutic treatment strategies in MM. Lenalidomide has been shown to inhibit production of pro inflammatory cytokines TNF- α , IL-1, IL-6, IL-12 and elevate the production of anti-inflammatory cytokine IL-10 from human PBMCs [19]. Lenalidomide stimulates T cell proliferation, increases IL-2 and IFN- γ production following CD3 ligation, and enhances T cell function by providing T cell co-stimulatory signals through B7-CD28 pathway [20, 21]. Although thalidomide and its analogs do not directly induce T cell activation, they increase proliferation of T cells following CD 3 activation. T cell proliferation triggered by dendritic cells (DC) is abrogated by cytotoxic T lymphocyte antigen (CTLA-4) [21, 22]. However, thalidomide and its analogs are able to overcome this inhibitory effect and induce co-stimulation via CD28-B7 pathway. Cell culture studies did not show up-regulation of CD28 expression on T cells or CD80-CD86 expression on DCs. The IMiDs, however, triggered tyrosine phosphorylation of CD28 on T cells followed by activation of NF κ B, a known downstream target of CD28 signaling. T cell co-stimulation by lenalidomide leads to an increased Th1 type cytokine response resulting in increased secretion of IFN- γ and IL-2 that in turn stimulate clonal T cell proliferation and NK cell activity. Lenalidomide is 50–2,000 times more potent than thalidomide in stimulating T cell proliferation triggered via the T cell receptor and 50–100 times more potent than thalidomide in augmenting IL-2 and IFN- γ production. Moreover, IMiDs induce NK cell cytotoxicity. NK cell proliferation and ADCC activity are enhanced by IL-2 production from T cells. IMiD effects the IL-2 and IFN- γ cytokine production with direct and indirect anti-MM effects [23]. This increased NK cell activity has also been demonstrated in patients receiving IMiDs.

Recently, it has also been shown that lenalidomide augments the Th1 polarization by CD1d-restricted invariant natural killer T (iNKT) cells. These cells play an important immunoregulatory role in anti-tumor immune responses, via increased Th1 cytokine production and reduced Th2 cytokine production [24].

Molecular basis for combination containing IMiDs

As single agents, the IMiDs achieve response in 30% of relapsed/refractory patients [25]. Additionally, those who respond eventually relapse suggesting the need to combine these agents with other novel or conventional agents to improve outcome. Molecularly based rational strategies have been used to combine other agents with IMiDs to achieve additive or synergistic activity. By combining agents with different effects on apoptotic or signaling pathways, this approach may overcome some drug resistance mechanisms. The IMiDs ability to enhance host immune mechanism has also been exploited by combining it with immunotherapeutic approaches.

Combining agents with complementary apoptotic pathways

The apoptotic signaling cascades triggered in MM cells by both conventional and novel agents have been characterized [26–29] (Fig. 2). Lenalidomide and other IMiDs-induced apoptosis in MM cells is associated with several outcomes: activation of the intrinsic apoptotic pathway, through caspase-8 cleavage; enhanced sensitivity to Fas-mediated apoptosis; down-regulation of NF- κ B activity as well as cIAP-2 and FLIP expression; and inhibition of the prosurvival effects of IGF-1¹⁰.

Lenalidomide-induced caspase-8 activation suggests that it may synergize with other activators of the caspase-8-dependent apoptotic pathway or more importantly activators of alternate apoptotic pathways. The death receptor Fas triggers apoptosis via caspase-8 [30] and Lenalidomide enhances Fas-mediated apoptosis in MM cells [2]. Importantly, as already discussed above, by reducing NF- κ B activity, lenalidomide also downregulates the expression of cIAP-2 and FLIP proteins, which inhibit apoptosis triggered by TRAIL/

Apo2L in MM cells [31], thus providing the rationale for combination strategies with TRAIL-activators. In vitro, pretreatment with IMiDs sensitizes MM cells to TRAIL/Apo2L-induced apoptosis providing dual triggering of caspase 8 death signaling [2].

The combination of dexamethasone with IMiDs provides synergistic cytotoxicity. A high level of NF- κ B activity in MM cells is associated with dexamethasone resistance [32]. The combination of dexamethasone with lenalidomide results in complete abrogation of NF- κ B activity [4]. This combination is associated with activation of dual apoptotic signaling cascades: dexamethasone-induced apoptosis is mediated by caspase 9 activation [33, 34] while IMiDs triggers caspase-8 activation [2, 35]. Moreover, lenalidomide impairs IL-6 secretion by BMSC induced by MM cell adhesion, overcoming the protective effect of IL-6 against Dex-induced apoptosis.

Lenalidomide is also able to overcome the protective effect of IGF-1 against Dex-induced apoptosis in MM cells in vitro. As described above, Lenalidomide is able to overcome the IGF-1-mediated induction of caspase-8 inhibitors cIAP-2 and FLIP anti-apoptotic proteins providing additional rational for its combination with dexamethasone.

The combination of lenalidomide and bortezomib enhances tumor cytotoxicity in vitro [2]. Preclinical studies show IMiDs trigger caspase 8 pathway and related genes while bortezomib triggers both caspase 8- and caspase 9-mediated MM cell death providing potential for synergistic activity. Although both agents suppress the transcriptional activity of NF κ B in MM cells, they act at different checkpoints to regulate NF κ B activity. Bortezomib, in addition to its inhibition of NF- κ B, also blocks MAPK, but not STAT3, activation by IL-6 [33].

Lenalidomide has been studied in vitro also for its effects on osteoclastogenesis. IMiDs, specifically lenalidomide, is an effective anti-osteoclastogenic drug, inhibiting both osteoclast formation and activity [36], through the down-regulation of PU.1 and cathepsin K gene expression, and the reduction of RANKL secretion by BMSC derived from patients with MM and of the serum level of sRANKL/ OPG ratio in patients with MM [37]. On the other hand, bortezomib stimulates osteoblast generation by activating the RUNX-2 pathway in mesenchymal cells, the osteoblast precursors [38]. Combining these two agents therefore should restore the impaired bone remodeling balance of MM in vivo and prevent osteolytic lesions, in addition to reducing the tumor burden.

Combining agents with complementary signaling pathways

Lenalidomide and mTOR inhibitors, such as rapamycin or its analogs CCI-779 and RAD001, have also been successfully combined in vitro [39] as they act by interrupting different signaling cascades, further supporting the role of combining these agents in clinical practice. mTOR is a downstream target in the AKT signaling pathway that modulates cell growth and rapamycin acts downstream of the PI3K pathway, as demonstrated by the inhibition of p70S kinase and pSTAT3. In contrast, STAT3 phosphorylation is not affected by lenalidomide, however, it inhibits the activation of the MAPK pathway signaling [4], whereas rapamycin either does not affect or at higher doses up-regulates this pathway. The combination of lenalidomide and an mTOR inhibitor enhances anti-tumor response by inhibiting MAPK signaling, blocking STAT3 phosphorylation, and overcoming the protective effects of IL-6, IGF-1 and BMSCs from rapamycin-induced cytotoxicity [39] providing the preclinical rationale for ongoing clinical study.

Combining agents to overcome drug resistance

Despite advances, MM remains incurable due to intrinsic or acquired chemotherapeutic resistance. Multiple myeloma cells manifest intrinsic genetic mechanisms of drug resistance

(owing, for example, to p53 mutations) or can acquire resistance following exposure to conventional chemotherapeutic treatment (for example, through over-expression of the P-glycoprotein that can confer multidrug resistance following exposure to alkylating agents or anthracyclines). In addition, the binding of MM cells to extracellular matrix components, such as fibronectin, as well as BMSC confers cell adhesion-mediated drug resistance (CAMDR) to conventional chemotherapy by NF- κ B activation [40, 41]. BMSC–MM cell interaction further induces transcription and secretion of cytokines, which in turn confer drug resistance (for example, interleukin 6 secretion by BMSC, osteoclasts, and endothelial cells abrogates the apoptosis triggered by dexamethasone).

Thalidomide and lenalidomide can overcome CAMDR by downregulating the expression of adhesion molecules, like ICAM-1, via NF- κ B inhibition [2, 42], inducing apoptosis or growth arrest in MM cells resistant to conventional therapeutic agents [4]. Preclinical studies have shown that IMiDs inhibit the proliferation of Doxorubicine- and Melphalan-resistant MM cells by 20–35% and of dexamethasone-resistant MM cells by 50% [4]. Lenalidomide, for example, is highly effective in combination with melphalan, mainly based on the inhibition of the supportive role of BMSC [43, 44]. Indeed, clinical trials combining lenalidomide with melphalan induce high remission rates. The combination strategies with other alkylating agents, such as cyclophosphamide, also show promising results in terms of overall response rate and toxicity in heavily pretreated patients [45].

Combining agents to enhance host anti-MM immunity

IMiDs significantly modulate T, NK, and DC-mediated immune responses, as described above. In vitro combination studies have shown that lenalidomide markedly augments ADCC induced by SGN-40, an anti-CD40 monoclonal antibody, in MM, both by directly affecting MM cells and indirectly increasing NK cell number, providing the rationale for ongoing combination clinical trial [24]. Combined lenalidomide and SGN-40 significantly induced MM apoptosis, evidenced by enhanced cleavage of caspase-3/8/poly (ADP-ribose) polymerase and increased sub-G₀ cells, compared with either single agents at the same doses. Pretreatment of effector cells with lenalidomide augmented SGN-40–induced MM cell lysis, associated with an increased number of CD56⁺CD3[−] NK cells expressing CD16 and LFA-1. Importantly, pretreatment with thalidomide or lenalidomide and SGN-40 markedly enhanced NK-cell–mediated lysis of autologous patient MM cells triggered by SGN-40. Lenalidomide also up-regulated CD40L on CD56⁺CD3[−] NK cells, facilitating IL-2–mediated activation of NK cells. In addition, lenalidomide induced the CD56^{dim} NK subset, which is more potent mediators of ADCC against target MM cells than the CD56^{bright} NK subset. Finally, pretreatment of both effectors and target MM cells with lenalidomide markedly enhanced SGN-40–mediated ADCC against CD40-expressing MM cells. Similarly, recent studies have shown that a novel humanized mAb against CS1, a cell surface glycoprotein highly expressed by myeloma cells with a role in cell adhesion-mediated drug resistance in the BM milieu (HuLuc63), induces significant ADCC against MM cells including drug-resistant cells, which can be augmented by lenalidomide. The effects of IMiDs on NKT cells also lend itself for use in combination with NKT cells in multiple myeloma [24].

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Fig. 1.
IMiD mechanism of action—IMiDs Target MM cells in its microenvironment

Fig. 2.
Activation of caspase pathways by IMiDs and other agents active in myeloma