Resistance to Targeted Anti-Cancer Therapeutics 22
Series Editor: Benjamin Bonavida

Silvia CW Ling
Steven Trieu *Editors*

Resistance to Targeted Therapies in Multiple Myeloma

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Resistance to Targeted Anti-Cancer Therapeutics

Volume 22

Series Editor

Benjamin Bonavida
Los Angeles, CA, USA

For several decades, treatment of cancer consisted of chemotherapeutic drugs, radiation, and hormonal therapies. Those were not tumor specific and exhibited several toxicities. During the last several years, targeted cancer therapies (molecularly targeted drugs) have been developed and consisting of immunotherapies (cell mediated and antibody) drugs or biologicals that can block the growth and spread of cancer by interfering with surface receptors and with specific dysregulated gene products that control tumor cell growth and progression. These include several FDA-approved drugs/antibodies/inhibitors that interfere with cell growth signaling or tumor blood vessel development, promote the cell death of cancer cells, stimulate the immune system to destroy specific cancer cells and deliver toxic drugs to cancer cells. Targeted cancer therapies are being used alone or in combination with conventional drugs and other targeted therapies.

One of the major problems that arise following treatment with both conventional therapies and targeted cancer therapies is the development of resistance, preexisting in a subset of cancer cells or cancer stem cells and/or induced by the treatments. Tumor cell resistance to targeted therapies remains a major hurdle and, therefore, several strategies are being considered in delineating the underlining molecular mechanisms of resistance and the development of novel drugs to reverse both the innate and acquired resistance to various targeted therapeutic regimens.

The new Series *"Resistance of Targeted Anti-Cancer Therapeutics"* was inaugurated and focuses on the clinical application of targeted cancer therapies (either approved by the FDA or in clinical trials) and the resistance observed by these therapies. Each book will consist of updated reviews on a specific target therapeutic and strategies to overcome resistance at the biochemical, molecular and both genetic and epigenetic levels. This new Series is timely and should be of significant interest to clinicians, scientists, trainees, students, and pharmaceutical companies.

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Editors

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Aims and Scope

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One of the major problems that arise following treatment with both conventional therapies and targeted cancer therapies is the development of resistance, preexisting in a subset of cancer cells or cancer stem cells and/or induced by the treatments. Tumor cell resistance to targeted therapies remains a major hurdle, and, therefore, several strategies are being considered in delineating the underlining molecular mechanisms of resistance and the development of novel drugs to reverse both the innate and acquired resistance to various targeted therapeutic regimens.

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Benjamin Bonavida

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Objective

Multiple myeloma remains an incurable malignancy with a 5-year survival rate of about 51% despite a plethora of advances in therapy. It is genetically and clonally heterogeneous with no single genetic target. It is usually sensitive to first-line treatment. However, as disease progresses, it invariably becomes resistant to treatment and almost all patients develop refractory disease.

There are multiple different types of targeted therapies and many of them are used in combination at different stages of disease. Targeted therapies that are approved to be used include proteasome inhibitors, immunomodulatory drugs, and monoclonal antibodies. Second and third generations of these drugs have been developed to overcome resistance and have unique mechanism of actions. Targeted therapies that are undergoing clinical trials include CAR-T cells, bi-specific antibodies, vaccines, ubiquitin ligase inhibitors, and BCL-2 inhibitors.

The purpose of this book is to develop an understanding of targeted therapies in multiple myeloma. Its goal is to provide a unique review of the mechanism of action and resistance of the many targeted therapies in multiple myeloma. The targeted audience includes students in medical science, clinicians, health professionals, scientists, pharmaceutical industry, drug developers, and policy makers.

This book will provide an insightful knowledge of the biology of multiple myeloma, the mechanism of action and resistance of targeted therapies, interactions of complex pathways, potential novel molecular markers, targets and strategies in overcoming resistance.

Preface

Multiple myeloma is an incurable malignancy of the plasma cells. The treatment of multiple myeloma has evolved from palliative, traditional chemotherapy 70 years ago to the current multi-targeted therapies which have significantly improved the survival and lives of multiple myeloma patients. This advancement is a credit to many clinicians and scientists who work on the biology of this disease.

I thank Dr. Ben Bonavida for the opportunity to be the author and editor of this volume. The goal is to review the science that underpins targeted therapies in multiple myeloma. My coauthors, Dr. Alice Kwok, Dr. Adrian Yeung, Dr. Opelo Sefhore, and Dr. Ashley McEwan decipher and discuss the evolution of therapies in myeloma, the mechanism of action and resistance of immunomodulatory drugs, HDAC inhibitors, and targeted bone therapies. Dr. Craig T. Wallington-Beddoe, Dr. Melissa K. Bennett, and Dr. Stuart M. Pitson evaluate the clinical impact, mechanism of action, and resistance of proteasome inhibitors. Dr. Minh Hua reviews the latest evidence of daratumumab, a monoclonal antibody which targets CD38, a high expressed antigen on myeloma cells. Dr. Adam Bryant describes the unique target of elotuzumab, SLAMF7 which underscores the importance of this drug and possible future development. Dr. Christian Bryant and Dr. Caroline Dix covers a wide array of novel targeted agents undergoing clinical development. These include the excitement and promises of CAR-T cell therapies, bi-specific antibodies, and small molecular inhibitors.

I sincerely thank my coeditor, Steven Trieu, for his generous and timely contribution and Dr. Bonavida for his unwavering encouragement. I thank the many reviewers of the manuscripts.

Liverpool Hospital, NSW, Australia — Silvia CW Ling
Liverpool Hospital, NSW, Australia — Steven Trieu

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Series Editor Biography

About the Series Editor

Benjamin Bonavida, PhD (Series Editor) is currently Distinguished Research Professor at the University of California, Los Angeles (UCLA). His research career, thus far, has focused on basic immunochemistry and cancer immunobiology. His research investigations have ranged from the mechanisms of cell-mediated killing, sensitization of resistant tumor cells to chemo-/immunotherapy, characterization of resistant factors in cancer cells, cell-signaling pathways mediated by therapeutic anticancer antibodies, and characterization of a dysregulated NF-κB/Snail/YY1/RKIP/PTEN loop in many cancers that regulates cell survival, proliferation, invasion, metastasis, and resistance. He has also investigated the role of nitric oxide in cancer and its potential antitumor activity. Many of the above studies are centered on the clinical challenging features of cancer patients' failure to respond to both conventional and targeted therapies. The development and activity of various targeting agents, their modes of action, and resistance are highlighted in many refereed publications.

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Dr. Ling received the degrees of MBBS and PhD from the University of Sydney. She is a fellow of the Royal Australasian College of Physicians and the Royal College of Pathologists of Australasia. She is a clinician scientist, with a focus on translational research in the mechanism of drug resistance in multiple myeloma. Her original research has been published in peer-reviewed journals.

She organizes the teaching program of biomedical science of the South Western Sydney Clinical School of UNSW Sydney. She is a research supervisor of undergraduate and postgraduate students of UNSW Sydney and Western Sydney University. She is an invited reviewer of manuscripts, grants, and examiner of masters and PhD thesis.

Steven Trieu is a PhD candidate at the University of New South Wales, focused on drug resistance in multiple myeloma. He has received a Bachelor of Advanced Science with First-Class Honors from the University of New South Wales and is currently a recipient of an Australian Government Research Training Program Scholarship.

Chapter 1
The Role of Targeted Therapy in Multiple Myeloma

Alice C. Y. Kwok and Silvia CW Ling

Abstract Targeted therapies are the cornerstone of the treatment of all stages of multiple myeloma (MM), from newly diagnosed to relapsed/refractory myeloma (RRMM). The advent of targeted therapies has improved the overall survival for MM patients compared to conventional cytotoxic therapies alone. Despite increasing response rates, deeper depths of response, and prolonged survival, MM remains incurable. Almost all patients with MM eventually relapse, suggesting the presence of residual clones that are resistant to therapy. This review series explores the available evidence and literature on previous, current, and emerging therapies in MM, as well as the mechanisms of resistance to these therapies. A deeper understanding of these resistance mechanisms will be necessary for the development of improved treatments, potentially reaching a cure for MM.

Targeted therapies are widely incorporated into treatment strategies across the scope of MM including incorporation into induction regimens for transplant-eligible patients. For transplant-ineligible patients, combination therapies including targeted agents are used with the aim to prolong disease free progression. In the setting of RRMM, targeted therapies have become the backbone of treatment in combination with chemotherapy. This chapter will review the history and classes of targeted therapies as well as recent therapies still under trial.

Keywords Multiple myeloma · Targeted therapy · Treatment resistance · Immunomodulatory imide drugs · Proteasome inhibitors · Monoclonal antibody treatment · Histone deacetylase inhibitors

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Abbreviations

BCMA	B-cell maturation antigen
CAR	Chimeric antigen receptor
ECOG	Eastern Cooperative Oncology Group
Fc	Fragment crystallizable
FDA	US Food and Drug Administration
FISH	Fluorescence in situ hybridization
HDAC	Histone deacetylase
HDACi	Histone deacetylase inhibitor
IgG1	Immunoglobulin G1
IL-6	Interleukin-6
IMiD	Immunomodulatory imide drug
IMWG	International Myeloma Working Group
MGUS	Monoclonal gammopathy of undetermined significance
MM	Multiple myeloma
MoAb	Monoclonal antibody
NK	Natural killer
ORR	Overall response rate
OS	Overall survival
PFS	Progression free survival
PI	Proteasome inhibitor
RANK	Receptor activator of nuclear factor kappa-B
RANKL	Receptor activator of nuclear factor kappa-B ligand
RRMM	Relapsed/refractory multiple myeloma
SLAMF7	Signaling lymphocytic activation molecule F7
SWOG	Southwest Oncology Group
VAD	Vincristine, doxorubicin, and dexamethasone
VEGF	Vascular endothelial growth factor
XPO1	Exportin 1

1.1 Multiple Myeloma Overview

Multiple myeloma is the malignant proliferation of plasma cells derived from a single clone. It accounts for approximately 1% of neoplastic disease and 13% of all hematologic cancers [1]. Multiple myeloma usually evolves from an asymptomatic premalignant stage of clonal plasma cell proliferation—known as monoclonal gammopathy of undetermined significance (MGUS) that progresses to smoldering (asymptomatic) MM and finally to symptomatic MM [1]. Commonly, MGUS may progress to MM or related malignancy at a rate of 1% per year [2]. The annual incidence of MM in the US is 4–5 per 100,000. With advancements in treatment options, the 5-year survival has steadily increased in the last decade and is currently 49%.

In MM, there are important prognostic factors which stratify patients into high and standard risks. These factors include cytogenetic abnormalities deletion 17p or immunoglobulin heavy chain translocations t(4;14) or t(14;16) detected by interphase FISH (fluorescence in situ hybridization). The median overall survival (OS) of patients with high-risk disease is only 2–3 years even with tandem stem cell transplantation compared to >7–10 years in those with standard risk disease [3].

Multiple myeloma displays a complicated karyotype and high levels of genomic instability associated with various gene mutations and chromosomal translocations [4]. Elevated aberrant homologous recombination in myeloma cells is one of the main contributing mechanisms to this instability, resulting in loss of cell cycle control and apoptosis and thus increased disease aggressiveness and treatment resistance [4]. Many studies over the years have demonstrated that MM has complex genetic features. At the chromosome level, MM can be classified into hyperdiploid (48–74 chromosomes) and non-hyperdiploid (common in MGUS) although currently there are no known triggers leading to change in ploidy [5]. There are also various chromosomal gains and losses with no known triggers for chromosomal changes. Furthermore, translocation rearrangements can occur within the immunoglobulin heavy chain gene which can be seen in MGUS and acts as a potential trigger for transformation to MM. Genetic mutations can also affect cell signaling pathways such as RAS, which is one of the most commonly mutated pathways in MM. Due to the capability of various and complex genetic and molecular abnormalities, MM is a very genetically heterogeneous disease.

Currently, there is no strong evidence that early treatment of patients with smoldering MM prolongs survival. However, there are ongoing clinical trials to determine whether targeted agents can delay progression and improve survival in smoldering MM. In transplant-eligible patients with standard risk disease, the current recommendation is induction with triple therapy, with one or two targeted agents followed by stem cell transplant. In patients with high-risk disease, the recommendation is initial treatment with proteasome inhibitor-containing therapy with consideration of clinical trials given that high-risk disease does not respond well to conventional therapy.

The introduction of novel agents has dramatically improved outcomes for MM patients. However, there is no established curative therapy and consequently patients will relapse and eventually develop refractory disease with a limited duration of response to subsequent lines of treatment. The choice of salvage therapy is affected by several considerations including initial therapy, degree, and duration of response to primary therapy, age, performance status, and previous toxicities. Novel agents including proteasome inhibitors (PI) and immunomodulatory imide drugs (IMiD) are currently part of the treatment paradigm.

1.2 Historical Treatment of Multiple Myeloma Until Present

There have been substantial changes in drug design and treatment regimens which have transformed MM from an acute to a chronic condition. With the advent of novel agents introduced as first-line therapy, the 5-year survival rate has been reported to be as high as 80% [6]. A review of the historical treatments for MM demonstrates both the significant changes over the last century and continuing development of new agents.

The first documented case of MM was in 1844 with the first treatment consisting of rhubarb and orange peel mixture with the application of leeches as "maintenance therapy" [2]. In 1947, Alwall reported that urethane reduced serum globulin and decreased bone marrow plasma cells [2]. This was proven to be ineffective as demonstrated in a small trial by Holland [7] in 1966 whereby 83 patients were randomized to either urethane or placebo and no differences in objective improvement or survival were observed.

From 1958 to 1962, Blokhin reported significant improvement in MM patients treated with melphalan [8]. This was followed by Hoogstraten [2] who found that a melphalan loading dose followed by maintenance therapy achieved a response in approximately 78% of patients with either newly diagnosed or previously treated MM [9].

In 1962, Maas was the first to test corticosteroids in MM. At the time, prednisone was trialed as a single agent; however, no difference in survival was observed. The first regimen of melphalan and prednisone was established after Alexanian et al. completed a randomized trial with 183 multiple myeloma patients [10]. This study observed that patients receiving combination regimen had a 6 months longer survival compared to melphalan alone [10]. Pulsed corticosteroids have been an important backbone of myeloma therapy until now [11]. The combination of multiple alkylating agents is efficacious in MM. For example, the M-2 protocol, consisting of carmustine, melphalan, cyclophosphamide, and prednisone resulted in response rates of up to 87% were observed in some groups of patients [12]. Triple therapy of vincristine, doxorubicin, and dexamethasone (VAD) was the main treatment for about 2 decades as induction therapy in newly diagnosed and RRMM [13, 14].

Autologous stem cell transplantation has been a standard first-line therapy for patients who can tolerate the intensity of high dose chemotherapy, due to its impact on survival advantage [15]. This procedure consists of induction chemotherapy, followed by autologous stem cell harvest, high dose melphalan chemotherapy, and reinfusion of harvested stem cells. Prior to the advent of target therapy, induction therapy/chemotherapies included dexamethasone [16], VAD and VAD with dexamethasone, cyclophosphamide, etoposide, and cisplatin [14, 17, 18]. However, the incorporation of novel targeted agents into induction regimens was proven to be superior to conventional cytotoxic chemotherapy like VAD [19]. Hence, the current standard of care involves targeted therapy in the induction phase followed by autologous stem cell transplant.

Maintenance therapy post autologous stem cell transplantation or other induction therapy is important in prolonging the duration of remission and possibly overall survival. Maintenance therapy has evolved over the years from corticosteroids and interferon to novel targeted therapies such as thalidomide, lenalidomide, and bortezomib.

The first targeted agent used in myeloma therapy was thalidomide, an IMiD. An Eastern Cooperative Oncology Group (ECOG) randomized trial demonstrated that the thalidomide-dexamethasone combination was superior to dexamethasone alone as an induction regimen for newly diagnosed MM [20]. This led to the accelerated approval for thalidomide-dexamethasone in 2006 by the US Food and Drug Administration (FDA) for the treatment of newly diagnosed MM. For transplant-ineligible patients, melphalan, prednisone, and thalidomide demonstrated higher response and progression free survival (PFS) when compared with standard melphalan and prednisone [21].

The next targeted therapy utilized in MM was bortezomib, the first PI with significant survival benefit in both transplant-eligible and ineligible MM patients [19, 22]. The combination of melphalan, prednisone, and bortezomib in patients older than 65 years of age has achieved an associated response rate of 89% and increased PFS [23]. Thereafter, there has been ongoing development in subsequent generations of IMiDs and PIs with the aim to further improve overall survival and side effect profiles.

Despite the ongoing development of IMiDs and PIs, resistance to these agents does occur in RRMM. This has led to the development of other agents including monoclonal antibodies (MoAb) such as daratumumab, which have demonstrated rapid and deep responses when used as monotherapy [24] and in combination with bortezomib, lenalidomide, and pomalidomide [25–29].

Histone deacetylase inhibitors (HDACi) target the deacetylation of histones and nonhistone proteins and have synergistic activity with other target therapies. Panobinostat is a pan-HDACi which targets the protein degradation pathway, aggrephagy, and has synergistic activity with bortezomib in MM.

Immunotherapy including chimeric antigen receptor (CAR) T cell therapies and bispecific T cell and natural killer (NK) antibodies are currently being tested in clinical trials. In CAR T cell therapy, T cells are engineered to target specific antigens on myeloma cells such as CD38 or B cell maturation antigen (BCMA). Bispecific antibodies have two targets, engaging T cells or NK cells and specific antigens on the myeloma cells, bringing the myeloma cells in close proximity to activated T cells or NK cells which cause the demise of the myeloma cells.

The outcomes of MM have improved substantially over the past 20 years with the introduction of PIs and IMiDs. These agents, either in triplets or doublets, form the backbone of therapy for MM.

1.3 Immunomodulatory Imide Drugs

Immunomodulatory imide drugs were the first targeted therapy in MM. As a class, IMiDs have a wide spectrum of mechanisms of action including augmentation of NK cells, alterations in cytokine production and T cell activity, and decreasing vascular endothelial growth factor (VEGF) and interleukin-6 (IL-6) expression which inhibits angiogenesis. The introduction of IMiDs into induction regimens has been observed to increase rates of complete response.

Thalidomide was the first IMiD introduced in 1957. Although initially introduced as a sedative and treatment for morning sickness in pregnancy, severe teratogenic malformations were associated with thalidomide use and was subsequently removed from most markets globally by the end of 1961. In certain conditions, thalidomide continued to be used as a therapeutic agent and was approved for the treatment of erythema nodosum leprosum in 1998 [30]. In 1994, thalidomide was found to have significant antiangiogenic properties [31]. Subsequently, in 1997 Barlogie et al. initiated a compassionate-use trial of thalidomide as antiangiogenic therapy [32]. At the time, 84 patients were enrolled with 32% responding to single agent thalidomide [32]. Response rates in newly diagnosed and RRMM were 63% and 50%, respectively when combined with dexamethasone [20, 33]. The response rate of the three-drug combination of thalidomide, steroids, and cyclophosphamide ranged from 60% in RRMM to 80% in newly diagnosed MM [34–36].

Due to the significant neurotoxicity of thalidomide that commonly results in therapy cessation, the second- and third-generation IMiDs, lenalidomide, and pomalidomide, respectively, were developed. Lenalidomide is a derivative of thalidomide, interfering with multiple signaling and survival pathways within myeloma cells and the bone marrow microenvironment. It is more potent than thalidomide but has significantly less neurotoxicity. The FIRST trial demonstrated better median PFS and trends towards better OS when lenalidomide and dexamethasone were used in an upfront setting for transplant-ineligible patients with PFS of 22.5 months, compared to 21.2 months in the melphalan, prednisone, and thalidomide group [37]. This led to its approval in 2015 by the FDA for use as first-line therapy in transplant-ineligible patients. Lenalidomide was also studied in RRMM, with the MM-009 trial demonstrating an improvement in median time of progression (11.1 months in the lenalidomide group compared to 4.7 months in the placebo group) with complete, near-complete, or partial response rates of 60.2% in the lenalidomide group compared to 24% in the placebo group [38].

Pomalidomide is the third-generation IMiD that is chemically related to both thalidomide and lenalidomide but is more active and potent. Currently, pomalidomide is approved by the FDA for third-line treatment in patients with relapsed or progressive MM who have received at least two prior therapies, including lenalidomide and bortezomib. This is based on its significant improvement in PFS and OS in this group of MM patients.

1.4 Proteasome Inhibitors

Proteasome inhibitors were developed following an increased understanding of the role of the ubiquitin-proteasome pathway in MM. This pathway is responsible for the degradation of misfolded and unfolded intracellular proteins. Proteasome inhibition leads to the accumulation of these unfolded or misfolded proteins that induce stress in the endoplasmic reticulum and ultimately apoptosis [39, 40]. Bortezomib is the first in-class PI developed and used for the treatment of MM and acts through multiple mechanisms to suppress tumor survival pathways and arrest tumor growth, spread, and angiogenesis [40]. Preclinical studies demonstrated that bortezomib had potent cytotoxic and growth inhibitory effects on myeloma cells [41]. An open-label phase II study of bortezomib in 202 patients with RRMM and who had failed two prior lines of therapy observed an overall response rate (ORR) of 28% [42]. This led to FDA approval in 2004 for bortezomib to be used as a single agent for the treatment of RRMM. Many randomized studies including the MM5 German study demonstrated the efficacy of bortezomib combined with cyclophosphamide and dexamethasone in untreated MM patients, resulting in lower rates of disease progression and high response rates. Bortezomib has also been used as induction therapy for both transplant-eligible and transplant-ineligible patients [43, 44]. The Southwest Oncology Group (SWOG) S0777 trial demonstrated that combining a PI with an IMiD (bortezomib combined with lenalidomide and dexamethasone) improves OS and PFS compared to the conventional regimen of lenalidomide and dexamethasone [45]. Bortezomib has also been investigated as a potential posttransplantation maintenance therapy. There is evidence that bortezomib-based maintenance may increase response rates and prolong PFS [46, 47].

Despite its efficacy, resistance to bortezomib in MM is inevitable. The mechanism of resistance is heterogeneous and is difficult to predict. The potential resistance mechanisms studied so far include mutations in the β5-subunit of the proteasome, derangement of stress responses, increased proteasomes and survival, and anti-apoptotic pathways [48–52]. In response to developing resistance, the second-generation PI carfilzomib was developed and approved for the treatment of RRMM.

Carfilzomib is indicated for RRMM after at least one previous therapy. It is an epoxyketone-based, irreversible PI that binds the chymotrypsin catalytic site within the β5-subunit of the 20S proteasome. Carfilzomib is active against bortezomib-resistant myeloma cells. It induces extrinsic and intrinsic apoptosis and activates stress response pathways in human MM [53]. In the ASPIRE trial, carfilzomib, lenalidomide, and dexamethasone were compared with lenalidomide and dexamethasone. A longer PFS was observed in the carfilzomib group [54]. Carfilzomib is also superior to bortezomib in RRMM with improved response rates and PFS [55].

Ixazomib is an oral, selective, and reversible PI. It preferentially binds and inhibits the chymotrypsin-like activity of the β5-subunit of the 20S proteasome [56]. Ixazomib demonstrated in vitro cytotoxicity against primary myeloma cells from patients who had relapsed after multiple prior therapies including bortezomib,

lenalidomide, and dexamethasone. Ixazomib in combination with a regimen of lenalidomide and dexamethasone was shown to result in a significantly longer median PFS of 20.6 months when compared to 14.7 months with lenalidomide and dexamethasone, with a hazard ratio of 0.74 [57].

1.5 Monoclonal Antibodies

Monoclonal antibodies were developed to target specific antigens and pathways driving MM. Current MoAbs available for treatment are daratumumab and elotuzumab, both of which are approved for RRMM.

Daratumumab is a human MoAb that targets the highly expressed CD38 glycoprotein on MM cells [58, 59]. It is generated by immunized transgenic mice. Daratumumab is approved for use in combination with lenalidomide and dexamethasone, or with bortezomib and dexamethasone, for the treatment of patients with MM and who have received at least one prior therapy [60]. Approval for the use of daratumumab was based on two randomized clinical trials where the addition of daratumumab to lenalidomide and dexamethasone (POLLUX), and to bortezomib and dexamethasone (CASTOR) improved the 12-month PFS significantly; 83.2% in the daratumumab group versus 60.1% in the control group in the POLLUX trial, and 60.7% in the daratumumab group versus 29% in the control group in the CASTOR trial [29, 61]. Daratumumab has also been found to improve PFS when combined with carfilzomib and dexamethasone in RRMM (CANDOR study).

Isatuximab is a chimeric immunoglobulin G1 (IgG1) kappa anti-CD38 MoAb which is generated by immunized wild type mice [59]. ICARIA-MM43, a multicenter, multinational, randomized, open-label phase III study comparing isatuximab, pomalidomide, and low-dose dexamethasone against pomalidomide and low-dose dexamethasone showed a 40% reduction in risk of disease progression or death with the addition of isatuximab [62]. This led to the FDA approval of isatuximab for use in RRMM.

Elotuzumab is a humanized IgG1 immunostimulatory MoAb targeted against the signaling lymphocytic activation molecule F7 (SLAMF7), a glycoprotein expressed on myeloma cells and NK cells [63]. Expression of SLAMF7 is nearly universal in MM irrespective of cytogenetic abnormalities and disease progression. Elotuzumab exerts a dual effect by directly activating NK cells and mediating antibody-dependent, cell-mediated cytotoxicity through the CD16 pathway [64]. As a single agent, elotuzumab has little clinical activity; however, when combined with lenalidomide and dexamethasone, it reduced the risk of progression or death by 30% with a median PFS of 19.4 months [63, 65]. Elotuzumab attained FDA approval in 2015 for use in the treatment of RRMM.

1.6 Histone Deacetylase Inhibitors

Histone deacetylase inhibitors (HDACi) acetylate histones and nonhistone proteins. The hyperacetylation of histones can reverse the silencing of specific genes. Moreover, hyperacetylation of nonhistone proteins affects their cellular function. Currently, panobinostat is the only HDACi approved for the treatment of relapsed myeloma. Panobinostat, bortezomib, and dexamethasone have been reevaluated as a third-line therapy in MM patients with improvement in PFS when compared to bortezomib and dexamethasone (11.99 months versus 8.08 months), leading to accelerated FDA approval in 2015 [66].

Despite these survival benefits, panobinostat has significant toxicity including diarrhea and cardiac events such as arrhythmias [66]. As such, selective HDACIs are being developed. Ricolinostat is a selective HDAC6 inhibitor which inhibits autophagic protein degradation. In a phase I/II trial, promising results were shown in RRMM when ricolinostat was combined with bortezomib and dexamethasone, with ongoing phase I/II trials further investigating other combinations including ricolinostat with lenalidomide and pomalidomide [67].

1.7 Bone Targeted Therapy

Bone targeted therapy is important in MM as it reduces skeletal lesions and has antitumor effects. Multiple myeloma is characterized by osteolytic lesions, osteopenia, tumor-induced hypercalcemia, and skeletal complications such as pathologic fractures in the long bones or vertebral collapses. Skeletal complications are a major cause of morbidity and mortality.

Targeted agents such as IMiDs and PIs have some direct effects on bone remodeling. Immunomodulatory imide drugs inhibit osteoclasts in vitro and in vivo and also reduces bone resorption in some MM patients. Bortezomib inhibits osteoclasts and activates osteoblast differentiation, reducing bone resorption in MM patients and increasing the receptor activator of nuclear factor kappa-B ligand (RANKL) to osteoprotegerin ratio [68].

Bisphosphonates are the main agent for bone directed therapy in MM. Zoledronic acid, the nitrogen-containing bisphosphonate is likely to have anticancer activity as it improves the PFS and OS of newly diagnosed MM when compared with clodronate [69]. The International Myeloma Working Group recommends that intravenous bisphosphonates be initiated in all patients with active MM and administered in 3–4 weekly intervals to reduce skeletal complications [70].

Denosumab is a fully humanized MoAb that binds to RANKL, inhibiting the interaction with the receptor activator of nuclear factor kappa-B (RANK) receptor and leading to the inhibition of osteoclasts [71]. A phase III study demonstrated the non-inferiority of denosumab to zoledronic acid at delaying time to the first skeletal-related events in MM patients and prolonged PFS [72]. In 2018, the FDA

approved denosumab for use in the prevention of skeletal-related events in MM patients.

1.8 New Agents on the Horizon

Despite the advent of a wide variety of novel agents, MM remains an incurable hematological malignancy with drug resistance an ongoing issue. Research is focused on developing new generations of targeted therapies, finding new targets, and discovering novel targeted therapies with unique mechanisms of action. New drugs currently in phase I and II trials include bispecific T cell or NK cell engager antibodies, CAR T cell therapy, Ulocuplumab (an anti-CXCR4 MoAb), Pembrolizumab (an anti-PD1 MoAb) in combination with radiation therapy, Nivolumab, and various other targeted therapies.

Bispecific antibodies are immunoglobulins that lack fragment crystallizable (Fc) regions and can simultaneously bind to two different epitopes—CD3 molecules on T cells and a specific antigen on myeloma cells—resulting in the destruction of myeloma cells. Currently, there are ongoing early phase clinical trials in RRMM.

Chimeric antigen receptor T cell therapy refers to the adoptive transfer of effector immune cells, either T cells or NK cells, which are engineered to recognize tumor-specific antigens such as the BCMA on myeloma cells and consists of a co-stimulatory molecule [73]. A number of early phase clinical trials of anti-BCMA CAR T cell therapy have reported the safety and toxicity profile in RRMM [74–76].

Ulocuplumab is a CXCR4 chemokine receptor MoAb that induces apoptosis in myeloma cells. In a phase Ib trial, Ulocuplumab, lenalidomide, and dexamethasone showed a high response rate of over 50% in patients with RRMM and who had been previously treated with two lines of therapy including lenalidomide and bortezomib [77].

Several targets have been investigated following the development of pathway receptor inhibitors. These drugs include Vemurafenib (BRAF inhibitor), Dovitinib (FGFR3 inhibitor), Alvocidib and Dinaciclib (targeted kinase inhibitors), Selumetinib (MEK inhibitors), Selinexor (Exportin-inhibitor), and Venetoclax (BCL-2 inhibitor).

Early clinical trials suggest that MM carrying t(11;14) translocations is sensitive to Venetoclax [78]. Currently, Venetoclax, Bortezomib, and dexamethasone are being tested in t [11, 14] RRMM in an ongoing clinical trial.

Selinexor is a selective inhibitor of exportin 1 (XPO1) which blocks export nuclear proteins such as tumor suppressor proteins. It inhibits nuclear factor kappa-B and reduces oncoprotein messenger RNA translation. In an early-phase clinical trial, Selinexor showed a tolerable safety profile and an ORR of 26% [79].

Dinaciclib (cyclin-dependent kinase inhibitor) and Filanesib (kinesin spindle protein inhibitor) have been tested in early phase clinical trials [80–83]. Filanesib was tested in RRMM as monotherapy and in combination with bortezomib and carfilzomib [81–83].

1.9 Conclusion

Although MM remains incurable, the advent of new and novel agents in recent times has transformed it from an acute disease into a chronic condition. However, the increasing availability of therapeutic options is leading to a developing era of drug resistance in MM. To overcome drug resistance and improve patient outcomes, the research and development of improved targeted agents continue. These novel agents and current therapies outlined in this chapter will be further explored in this series.

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References

1. Palumbo A, Anderson K. Multiple myeloma. N Engl J Med. 2011;364(11):1046–60.
2. Kyle RA, Rajkumar SV. Multiple myeloma. Blood. 2008;111(6):2962–72.
3. Rajkumar SV. Multiple myeloma: 2018 update on diagnosis, risk-stratification, and management. Am J Hematol. 2018;93(8):981–1114.
4. Abdi J, Chen G, Chang H. Drug resistance in multiple myeloma: latest findings and new concepts on molecular mechanisms. Oncotarget. 2013;4(12):2186–207.
5. de Mel S, Lim SH, Tung ML, Chng WJ. Implications of heterogeneity in multiple myeloma. Biomed Res Int. 2014;2014:232,546.
6. Naymagon L, Abdul-Hay M. Novel agents in the treatment of multiple myeloma: a review about the future. J Hematol Oncol. 2016;9(1):52.
7. Holland JR, Hosley H, Scharlau C, Carbone PP, Frei E 3rd, Brindley CO, et al. A controlled trial of urethane treatment in multiple myeloma. Blood. 1966;27(3):328–42.
8. Blokhin N, Larionov L, Perevodchikova N, Chebotareva L, Merkulova N. Clinical experiences with sarcolysin in neoplastic diseases. Ann N Y Acad Sci. 1958;68(3):1128–32.
9. Hoogstraten B, Sheehe PR, Cuttner J, Cooper T, Kyle RA, Oberfield RA, et al. Melphalan in multiple myeloma. Blood. 1967;30(1):74–83.
10. Alexanian R, Haut A, Khan AU, Lane M, McKelvey EM, Migliore PJ, et al. Treatment for multiple myeloma. Combination chemotherapy with different melphalan dose regimens. JAMA. 1969;208(9):1680–5.
11. Alexanian R, Yap BS, Bodey GP. Prednisone pulse therapy for refractory myeloma. Blood. 1983;62(3):572–7.
12. Tirelli U, Crivellari D, Carbone A, Veronesi A, Galligioni E, Trovò MG, et al. Combination chemotherapy for multiple myeloma with melphalan, prednisone, cyclophosphamide, vincristine, and carmustine (BCNU) (M-2 protocol). Cancer Treat Rep. 1982;66(11):1971–3.
13. Barlogie B, Smith L, Alexanian R. Effective treatment of advanced multiple myeloma refractory to alkylating agents. N Engl J Med. 1984;310(21):1353–6.
14. Anderson H, Scarffe JH, Ranson M, Young R, Wieringa GS, Morgenstern GR, et al. VAD chemotherapy as remission induction for multiple myeloma. Br J Cancer. 1995;71(2):326–30.
15. Attal M, Harousseau JL, Stoppa AM, Sotto JJ, Fuzibet JG, Rossi JF, et al. A prospective, randomized trial of autologous bone marrow transplantation and chemotherapy in multiple myeloma. Intergroupe Français du Myélome. N Engl J Med. 1996;335(2):91–7.

16. Kumar S, Lacy MQ, Dispenzieri A, Rajkumar SV, Fonseca R, Geyer S, et al. Single agent dexamethasone for pre-stem cell transplant induction therapy for multiple myeloma. Bone Marrow Transplant. 2004;34(6):485–90.
17. Corso A, Barbarano L, Zappasodi P, Cairoli R, Alessandrino EP, Mangiacavalli S, et al. The VAD-DCEP sequence is an effective pre-transplant therapy in untreated multiple myeloma. Haematologica. 2004;89(9):1124–7.
18. Barlogie B, Jagannath S, Desikan KR, Mattox S, Vesole D, Siegel D, et al. Total therapy with tandem transplants for newly diagnosed multiple myeloma. Blood. 1999;93(1):55–65.
19. Harousseau JL, Attal M, Leleu X, Troncy J, Pegourie B, Stoppa AM, et al. Bortezomib plus dexamethasone as induction treatment prior to autologous stem cell transplantation in patients with newly diagnosed multiple myeloma: results of an IFM phase II study. Haematologica. 2006;91(11):1498–505.
20. Rajkumar SV, Blood E, Vesole D, Fonseca R, Greipp PR. Phase III clinical trial of thalidomide plus dexamethasone compared with dexamethasone alone in newly diagnosed multiple myeloma: a clinical trial coordinated by the eastern cooperative oncology group. J Clin Oncol. 2006;24(3):431–6.
21. Rajkumar SV, Blood E, Vesole D, Fonseca R, Greipp PR, Eastern Cooperative Oncology Group. Phase III clinical trial of thalidomide plus dexamethasone compared with dexamethasone alone in newly diagnosed multiple myeloma: a clinical trial coordinated by the Eastern Cooperative Oncology Group. J Clin Oncol. 2006;24(3):431–6.
22. Mateos MV, Richardson PG, Schlag R, Khuageva NK, Dimopoulos MA, Shpilberg O, et al. Bortezomib plus melphalan and prednisone compared with melphalan and prednisone in previously untreated multiple myeloma: updated follow-up and impact of subsequent therapy in the phase III VISTA trial. J Clin Oncol. 2010;28(13):2259–66.
23. Mateos MV, Hernandez JM, Hernandez MT, Gutierrez NC, Palomera L, Fuertes M, et al. Bortezomib plus melphalan and prednisone in elderly untreated patients with multiple myeloma: results of a multicenter phase 1/2 study. Blood. 2006;108(7):2165–72.
24. Usmani SZ, Weiss BM, Plesner T, Bahlis NJ, Belch A, Lonial S, et al. Clinical efficacy of daratumumab monotherapy in patients with heavily pretreated relapsed or refractory multiple myeloma. Blood. 2016;128(1):37–44.
25. Dimopoulos MA, San-Miguel J, Belch A, White D, Benboubker L, Cook G, et al. Daratumumab plus lenalidomide and dexamethasone versus lenalidomide and dexamethasone in relapsed or refractory multiple myeloma: updated analysis of POLLUX. Haematologica. 2018;103(12):2088–96.
26. Mateos MV, Cavo M, Blade J, Dimopoulos MA, Suzuki K, Jakubowiak A, et al. Overall survival with daratumumab, bortezomib, melphalan, and prednisone in newly diagnosed multiple myeloma (ALCYONE): a randomised, open-label, phase 3 trial. Lancet (London, England). 2020;395(10,218):132–41.
27. Mateos MV, Dimopoulos MA, Cavo M, Suzuki K, Jakubowiak A, Knop S, et al. Daratumumab plus Bortezomib, Melphalan, and prednisone for untreated myeloma. N Engl J Med. 2018;378(6):518–28.
28. Nooka AK, Joseph NS, Kaufman JL, Heffner LT, Gupta VA, Gleason C, et al. Clinical efficacy of daratumumab, pomalidomide, and dexamethasone in patients with relapsed or refractory myeloma: utility of re-treatment with daratumumab among refractory patients. Cancer. 2019;125(17):2991–3000.
29. Palumbo A, Chanan-Khan A, Weisel K, Nooka AK, Masszi T, Beksac M, et al. Daratumumab, bortezomib, and dexamethasone for multiple myeloma. N Engl J Med. 2016;375(8):754–66.
30. Rehman W, Arfons LM, Lazarus HM. The rise, fall and subsequent triumph of thalidomide: lessons learned in drug development. Ther Adv Hematol. 2011;2(5):291–308.
31. D'Amato RJ, Loughnan MS, Flynn E, Folkman J. Thalidomide is an inhibitor of angiogenesis. Proc Natl Acad Sci U S A. 1994;91(9):4082–5.

32. Barlogie B, Desikan R, Eddlemon P, Spencer T, Zeldis J, Munshi N, et al. Extended survival in advanced and refractory multiple myeloma after single-agent thalidomide: identification of prognostic factors in a phase 2 study of 169 patients. Blood. 2001;98(2):492–4.
33. Dimopoulos MA, Zervas K, Kouvatseas G, Galani E, Grigoraki V, Kiamouris C, et al. Thalidomide and dexamethasone combination for refractory multiple myeloma. Ann Oncol. 2001;12(7):991–5.
34. Sidra G, Williams CD, Russell NH, Zaman S, Myers B, Byrne JL. Combination chemotherapy with cyclophosphamide, thalidomide and dexamethasone for patients with refractory, newly diagnosed or relapsed myeloma. Haematologica. 2006;91(6):862–3.
35. Dimopoulos MA, Hamilos G, Zomas A, Gika D, Efstathiou E, Grigoraki V, et al. Pulsed cyclophosphamide, thalidomide and dexamethasone: an oral regimen for previously treated patients with multiple myeloma. Hematol J. 2004;5(2):112–7.
36. Morgan GJ, Davies FE, Gregory WM, Bell SE, Szubert AJ, Navarro Coy N, et al. Cyclophosphamide, thalidomide, and dexamethasone as induction therapy for newly diagnosed multiple myeloma patients destined for autologous stem-cell transplantation: MRC myeloma IX randomized trial results. Haematologica. 2012;97(3):442–50.
37. Benboubker L, Dimopoulos MA, Dispenzieri A, Catalano J, Belch AR, Cavo M, et al. Lenalidomide and dexamethasone in transplant-ineligible patients with myeloma. N Engl J Med. 2014;371(10):906–17.
38. Weber DM, Chen C, Niesvizky R, Wang M, Belch A, Stadtmauer EA, et al. Lenalidomide plus dexamethasone for relapsed multiple myeloma in North America. N Engl J Med. 2007;357(21):2133–42.
39. Lee AH, Iwakoshi NN, Anderson KC, Glimcher LH. Proteasome inhibitors disrupt the unfolded protein response in myeloma cells. Proc Natl Acad Sci U S A. 2003;100(17):9946–51.
40. Adams J. Development of the proteasome inhibitor PS-341. Oncologist. 2002;7(1):9–16.
41. Boccadoro M, Morgan G, Cavenagh J. Preclinical evaluation of the proteasome inhibitor bortezomib in cancer therapy. Cancer Cell Int. 2005;5(1):18.
42. Bross PF, Kane R, Farrell AT, Abraham S, Benson K, Brower ME, et al. Approval summary for bortezomib for injection in the treatment of multiple myeloma. Clin Cancer Res. 2004;10(12 Pt 1):3954–64.
43. Mai EK, Bertsch U, Durig J, Kunz C, Haenel M, Blau IW, et al. Phase III trial of bortezomib, cyclophosphamide and dexamethasone (VCD) versus bortezomib, doxorubicin and dexamethasone (PAd) in newly diagnosed myeloma. Leukemia. 2015;29(8):1721–9.
44. Reeder CB, Reece DE, Kukreti V, Chen C, Trudel S, Hentz J, et al. Cyclophosphamide, bortezomib and dexamethasone induction for newly diagnosed multiple myeloma: high response rates in a phase II clinical trial. Leukemia. 2009;23(7):1337–41.
45. Durie BGM, Hoering A, Abidi MH, Rajkumar SV, Epstein J, Kahanic SP, et al. Bortezomib with lenalidomide and dexamethasone versus lenalidomide and dexamethasone alone in patients with newly diagnosed myeloma without intent for immediate autologous stem-cell transplant (SWOG S0777): a randomised, open-label, phase 3 trial. Lancet (London, England). 2017;389(10,068):519–27.
46. Sonneveld P, Schmidt-Wolf IG, van der Holt B, El Jarari L, Bertsch U, Salwender H, et al. Bortezomib induction and maintenance treatment in patients with newly diagnosed multiple myeloma: results of the randomized phase III HOVON-65/ GMMG-HD4 trial. J Clin Oncol. 2012;30(24):2946–55.
47. Mateos MV, Oriol A, Martinez-Lopez J, Gutierrez N, Teruel AI, de la Guia AL, et al. Maintenance therapy with bortezomib plus thalidomide or bortezomib plus prednisone in elderly multiple myeloma patients included in the GEM2005MAS65 trial. Blood. 2012;120(13):2581–8.
48. Oerlemans R, Franke NE, Assaraf YG, Cloos J, van Zantwijk I, Berkers CR, et al. Molecular basis of bortezomib resistance: proteasome subunit beta5 (PSMB5) gene mutation and overexpression of PSMB5 protein. Blood. 2008;112(6):2489–99.

49. Franke NE, Niewerth D, Assaraf YG, van Meerloo J, Vojtekova K, van Zantwijk CH, et al. Impaired bortezomib binding to mutant beta5 subunit of the proteasome is the underlying basis for bortezomib resistance in leukemia cells. Leukemia. 2012;26(4):757–68.
50. Wu YX, Yang JH, Saitsu H. Bortezomib-resistance is associated with increased levels of proteasome subunits and apoptosis-avoidance. Oncotarget. 2016;7(47):77,622–34.
51. Hamouda MA, Belhacene N, Puissant A, Colosetti P, Robert G, Jacquel A, et al. The small heat shock protein B8 (HSPB8) confers resistance to bortezomib by promoting autophagic removal of misfolded proteins in multiple myeloma cells. Oncotarget. 2014;5(15):6252–66.
52. Markovina S, Callander NS, O'Connor SL, Kim J, Werndli JE, Raschko M, et al. Bortezomib-resistant nuclear factor-kappaB activity in multiple myeloma cells. Mol Cancer Res. 2008;6(8):1356–64.
53. Kuhn DJ, Chen Q, Voorhees PM, Strader JS, Shenk KD, Sun CM, et al. Potent activity of carfilzomib, a novel, irreversible inhibitor of the ubiquitin-proteasome pathway, against preclinical models of multiple myeloma. Blood. 2007;110(9):3281–90.
54. Stewart AK, Rajkumar SV, Dimopoulos MA, Masszi T, Spicka I, Oriol A, et al. Carfilzomib, lenalidomide, and dexamethasone for relapsed multiple myeloma. N Engl J Med. 2015;372(2):142–52.
55. Dimopoulos MA, Moreau P, Palumbo A, Joshua D, Pour L, Hajek R, et al. Carfilzomib and dexamethasone versus bortezomib and dexamethasone for patients with relapsed or refractory multiple myeloma (ENDEAVOR): a randomised, phase 3, open-label, multicentre study. Lancet Oncol. 2016;17(1):27–38.
56. Shirley M. Ixazomib: first global approval. Drugs. 2016;76(3):405–11.
57. Moreau P, Masszi T, Grzasko N, Bahlis NJ, Hansson M, Pour L, et al. Oral ixazomib, lenalidomide, and dexamethasone for multiple myeloma. N Engl J Med. 2016;374(17):1621–34.
58. de Weers M, Tai YT, van der Veer MS, Bakker JM, Vink T, Jacobs DC, et al. Daratumumab, a novel therapeutic human CD38 monoclonal antibody, induces killing of multiple myeloma and other hematological tumors. J Immunol (Baltimore, MD:1950). 2011;186(3):1840–8.
59. Bannas P, Koch-Nolte F. Perspectives for the development of CD38-specific heavy chain antibodies as therapeutics for multiple myeloma. Front Immunol. 2018;9:2559.
60. Bhatnagar V, Gormley NJ, Luo L, Shen YL, Sridhara R, Subramaniam S, et al. FDA approval summary: daratumumab for treatment of multiple myeloma after one prior therapy. Oncologist. 2017;22(11):1347–53.
61. Dimopoulos MA, Oriol A, Nahi H, San-Miguel J, Bahlis NJ, Usmani SZ, et al. Daratumumab, lenalidomide, and dexamethasone for multiple myeloma. N Engl J Med. 2016;375(14):1319–31.
62. Attal M, Richardson PG, Rajkumar SV, San-Miguel J, Beksac M, Spicka I, et al. Isatuximab plus pomalidomide and low-dose dexamethasone versus pomalidomide and low-dose dexamethasone in patients with relapsed and refractory multiple myeloma (ICARIA-MM): a randomised, multicentre, open-label, phase 3 study. Lancet (London, England). 2019;394(10,214):2096–107.
63. Lonial S, Dimopoulos M, Palumbo A, White D, Grosicki S, Spicka I, et al. Elotuzumab therapy for relapsed or refractory multiple myeloma. N Engl J Med. 2015;373(7):621–31.
64. Collins SM, Bakan CE, Swartzel GD, Hofmeister CC, Efebera YA, Kwon H, et al. Elotuzumab directly enhances NK cell cytotoxicity against myeloma via CS1 ligation: evidence for augmented NK cell function complementing ADCC. Cancer Immunol Immunother. 2013;62(12):1841–9.
65. Dimopoulos MA, Lonial S, White D, Moreau P, Palumbo A, San-Miguel J, et al. Elotuzumab plus lenalidomide/dexamethasone for relapsed or refractory multiple myeloma: ELOQUENT-2 follow-up and post-hoc analyses on progression-free survival and tumour growth. Br J Haematol. 2017;178(6):896–905.
66. San-Miguel JF, Hungria VT, Yoon SS, Beksac M, Dimopoulos MA, Elghandour A, et al. Panobinostat plus bortezomib and dexamethasone versus placebo plus bortezomib and dexamethasone in patients with relapsed or relapsed and refractory multiple myeloma: a multicentre, randomised, double-blind phase 3 trial. Lancet Oncol. 2014;15(11):1195–206.

67. Vogl DT, Raje N, Jagannath S, Richardson P, Hari P, Orlowski R, et al. Ricolinostat, the first selective histone deacetylase 6 inhibitor, in combination with bortezomib and dexamethasone for relapsed or refractory multiple myeloma. Clin Cancer Res. 2017;23(13):3307–15.
68. Terpos E, Dimopoulos MA, Sezer O. The effect of novel anti-myeloma agents on bone metabolism of patients with multiple myeloma. Leukemia. 2007;21(9):1875–84.
69. Morgan GJ, Davies FE, Gregory WM, Cocks K, Bell SE, Szubert AJ, et al. First-line treatment with zoledronic acid as compared with clodronic acid in multiple myeloma (MRC Myeloma IX): a randomised controlled trial. Lancet (London, England). 2010;376(9757):1989–99.
70. Terpos E, Morgan G, Dimopoulos MA, Drake MT, Lentzsch S, Raje N, et al. International myeloma working group recommendations for the treatment of multiple myeloma-related bone disease. J Clin Oncol. 2013;31(18):2347–57.
71. Rizzoli R, Yasothan U, Kirkpatrick P. Denosumab. Nat Rev Drug Discov. 2010;9(8):591–2.
72. Raje N, Terpos E, Willenbacher W, Shimizu K, García-Sanz R, Durie B, et al. Denosumab versus zoledronic acid in bone disease treatment of newly diagnosed multiple myeloma: an international, double-blind, double-dummy, randomised, controlled, phase 3 study. Lancet Oncol. 2018;19(3):370–81.
73. Feinberg D, Paul B, Kang Y. The promise of chimeric antigen receptor (CAR) T cell therapy in multiple myeloma. Cell Immunol. 2019;345:103964.
74. Cohen AD, Garfall AL, Stadtmauer EA, Melenhorst JJ, Lacey SF, Lancaster E, et al. B cell maturation antigen-specific CAR T cells are clinically active in multiple myeloma. J Clin Investig. 2019;129(6):2210–21.
75. Raje N, Berdeja J, Lin Y, Siegel D, Jagannath S, Madduri D, et al. Anti-BCMA CAR T-cell therapy bb2121 in relapsed or refractory multiple myeloma. N Engl J Med. 2019;380(18):1726–37.
76. Xu J, Chen LJ, Yang SS, Sun Y, Wu W, Liu YF, et al. Exploratory trial of a biepitopic CAR T-targeting B cell maturation antigen in relapsed/refractory multiple myeloma. Proc Natl Acad Sci U S A. 2019;116(19):9543–51.
77. Ghobrial IM, Liu CJ, Redd RA, Perez RP, Baz R, Zavidij O, et al. A phase Ib/II trial of the first-in-class anti-CXCR4 antibody ulocuplumab in combination with lenalidomide or bortezomib plus dexamethasone in relapsed multiple myeloma. Clin Cancer Res. 2020;26(2):344–53.
78. Kumar S, Kaufman JL, Gasparetto C, Mikhael J, Vij R, Pegourie B, et al. Efficacy of venetoclax as targeted therapy for relapsed/refractory t(11;14) multiple myeloma. Blood. 2017;130(22):2401–9.
79. Chari A, Vogl DT, Gavriatopoulou M, Nooka AK, Yee AJ, Huff CA, et al. Oral selinexor-dexamethasone for triple-class refractory multiple myeloma. N Engl J Med. 2019;381(8):727–38.
80. Kumar SK, LaPlant B, Chng WJ, Zonder J, Callander N, Fonseca R, et al. Dinaciclib, a novel CDK inhibitor, demonstrates encouraging single-agent activity in patients with relapsed multiple myeloma. Blood. 2015;125(3):443–8.
81. Chari A, Htut M, Zonder JA, Fay JW, Jakubowiak AJ, Levy JB, et al. A phase 1 dose-escalation study of filanesib plus bortezomib and dexamethasone in patients with recurrent/refractory multiple myeloma. Cancer. 2016;122(21):3327–35.
82. Lee HC, Shah JJ, Feng L, Manasanch EE, Lu R, Morphey A, et al. A phase 1 study of filanesib, carfilzomib, and dexamethasone in patients with relapsed and/or refractory multiple myeloma. Blood Cancer J. 2019;9(10):80.
83. Shah JJ, Kaufman JL, Zonder JA, Cohen AD, Bensinger WI, Hilder BW, et al. A phase 1 and 2 study of Filanesib alone and in combination with low-dose dexamethasone in relapsed/refractory multiple myeloma. Cancer. 2017;123(23):4617–30.

Chapter 2
Lenalidomide

Adrian Jun-Ting Yeung and Silvia CW Ling

Abstract Lenalidomide is the second-generation immunomodulatory imide drug (IMiD) derived from thalidomide, approved for the treatment of multiple myeloma (MM). Lenalidomide exerts its anti-myeloma action through multiple effects, including effects on the cereblon pathway, cytokine production, immune cells, and angiogenesis. Despite high overall response rates (ORR) to lenalidomide, resistance to lenalidomide can be seen in the proportion of MM patients that fail to respond. This chapter will review data from notable clinical trials of lenalidomide, the mechanisms of action, and explore the potential mechanisms of lenalidomide resistance in MM. Previous attempts to manage patients with lenalidomide resistance/refractory disease will also be reviewed.

Keywords Multiple myeloma · Lenalidomide · Immunomodulatory drug · Cereblon pathway · Lenalidomide resistance

Abbreviations

BMI	Body mass index
CDK	Cyclin-dependent kinase
CR	Complete response
CTLA-4	Cytotoxic T lymphocyte-associated protein 4
EMA	European Medicines Agency

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FDA	US Food and Drug Administration
IL-1	Interleukin-1
IL-10	Interleukin-10
IL-12	Interleukin-12
IL-2	Interleukin-2
IL-6	Interleukin-6
IMiD	Immunomodulatory imide drug
IRF4	Interferon regulatory factor 4
MM	Multiple myeloma
MoAb	Monoclonal antibody
MRD	Minimal residual disease
mRNA	Messenger RNA
NF-κβ	Nuclear factor-kappa B
NK	Natural killer
ORR	Overall response rate
OS	Overall survival
PFS	Progression-free survival
PI	Proteasome inhibitor
PR	Partial response
RRMM	Relapsed/refractory multiple myeloma
shRNA	Short hairpin RNA
TGA	Therapeutic Goods Administration
TNF-α	Tumor necrosis factor alpha
TRAIL	TNF-related apoptosis inducing ligand
TRAP	Tartrate-resistant acid phosphatase
VEGF	Vascular endothelial growth factor
VGPR	Very good partial response

2.1 Introduction

Lenalidomide is an immunomodulatory imide drug (IMiD) and derivative of thalidomide, an earlier IMiD. Thalidomide was originally synthesized in the early 1950s as a remedy for morning sickness but was withdrawn from the market after it was found to be teratogenic. Further research revealed the anti-angiogenic properties of thalidomide, leading to interest in its potential as a cancer treatment. However, the ongoing concerns with its side effects led to the development of thalidomide analogs such as lenalidomide. Lenalidomide shares the same glutarimide portion with thalidomide but has a modified phthalimide portion. This modification resulted in increased potency and less severe adverse side effects [1].

2.2 Indications

Within the realm of multiple myeloma (MM), lenalidomide is approved for use by the Therapeutic Goods Administration (TGA) in Australia for (1) treatment of MM whose disease has progressed after one therapy, (2) newly diagnosed MM, and (3) maintenance therapy post autologous stem cell transplantation. Lenalidomide is approved by the US Food and Drug Administration (FDA) for the treatment of MM and as maintenance therapy post autologous stem cell transplantation. Likewise, the European Medicines Agency (EMA) has also approved lenalidomide for use in maintenance therapy following autologous stem cell transplant, as well as the common indications of relapsed/refractory multiple myeloma (RRMM), and newly diagnosed MM in patients not eligible for stem cell transplantation (European Medicines Agency, 2019).

2.3 Efficacy of Lenalidomide

2.3.1 Efficacy in Relapsed or Refractory Multiple Myeloma

2.3.1.1 Lenalidomide and Dexamethasone

Initial data regarding the efficacy of lenalidomide was produced in the context of RRMM. Pivotal studies demonstrating the efficacy of lenalidomide include the MM-009 [2] and MM-010 studies [3], where patients treated with lenalidomide and dexamethasone were compared in a randomized control trial to dexamethasone alone. Both treatment arms consisted of 28-day cycles, with lenalidomide dosed at 25 mg daily on days 1–21, and dexamethasone dosed at 40 mg once daily on days 1–4, 9–12, and 17–20 for the first 4 cycles, then subsequently reduced to 40 mg once daily on days 1–4. In a pooled follow-up analysis of the two studies combined [3], the addition of lenalidomide to dexamethasone demonstrated a significantly longer time to progression (48.3 weeks compared to 20.1 weeks), and progression-free survival (PFS) (median time 47.3 weeks compared to 20.1 weeks). Complete response (CR) and overall response rates (ORR) were also higher in the lenalidomide treated patient groups. Overall survival (OS) at 1 year was 82% in patients treated with lenalidomide and dexamethasone compared to 75% in the placebo and dexamethasone group. A meta-analysis of seven randomized controlled trials investigating lenalidomide by Qiao et al. (2015) further confirmed the efficacy of lenalidomide compared to control groups, with improved ORR, PFS, and OS [4].

2.3.1.2 Bortezomib, Lenalidomide, and Dexamethasone

Along with IMiDs, proteasome inhibitors (PI) such as bortezomib have changed the landscape of MM treatment, demonstrating superior results compared to dexamethasone. The combination therapy of bortezomib, lenalidomide, and dexamethasone was investigated in a phase II study [5] in patients with RRMM who had received one to three prior regimes but were naïve to combination bortezomib and lenalidomide therapy. Bortezomib was dosed at 1 mg/m^2 intravenously on days 1, 4, 8, and 11 in a 21-day cycle. Lenalidomide was dosed at 15 mg/day on days 1–14, in combination with dexamethasone 40 mg for the first 4 cycles, then decreased to 20 mg in cycles 5–8 on the day of and day after bortezomib dosing. This regime of bortezomib, lenalidomide, and dexamethasone demonstrated a median OS of 30 months, with median PFS of 9.5 months. The 6-month progression-free survival rate was 75% and the ORR was 64%. The median treatment duration was 8 months, suggesting that despite toxicities including myelosuppression and peripheral neuropathy, the combination regime was generally well tolerated.

2.3.1.3 Daratumumab, Lenalidomide, and Dexamethasone

Since its introduction into the MM sphere, daratumumab has shown promising efficacy. Triple therapy with daratumumab, lenalidomide, and dexamethasone was compared to lenalidomide and dexamethasone alone in a randomized control trial [6]. The enrolled patients had RRMM and had previously received and responded to one or more lines of therapy. Of note is that patients who had lenalidomide refractory disease were excluded. Daratumumab was dosed at 16 mg/kg and administered weekly for the first 8 weeks, followed by every 2 weeks for the following 16 weeks, and then every 4 weeks thereafter. Lenalidomide was given at a standard dose of 25 mg/day daily from days 1 to 21 of each cycle (or reduced to 10 mg daily if creatinine clearance was 30–60 mL/min), while dexamethasone was dosed at 40 mg weekly in the lenalidomide and dexamethasone group. In the triple therapy group, dexamethasone was split in 20 mg prior to daratumumab infusion for infusion-related reaction prophylaxis, and 20 mg the next day. Patients older than 75 years of age or with body mass index (BMI) <18.5 could have their dexamethasone dose reduced to 20 mg weekly instead of the standard 40 mg dose. The results demonstrated a benefit for daratumumab combination therapy with lenalidomide with PFS at 12 months of 83.2% compared to 60.1% in the lenalidomide and dexamethasone group. Overall response rates were also higher in the daratumumab group (92.9% compared with 76.4%), with higher rates of deeper responses (evidenced by higher rates of very good partial responses [VGPR] or better) in the daratumumab combination arm (75.8% vs 44.2%), as well as higher rates of CR or better (43.1% vs 19.2%). In a subgroup analysis, the benefits of daratumumab addition were irrespective of previous treatment, as well as the number of lines of previous treatment. As expected, the higher efficacy with the addition of daratumumab was associated with increased rates of serious adverse effects (48.8% vs 42%). Despite this, the

percentage of patients with adverse events leading to treatment discontinuation was similar in both groups (6.7% vs 7.8%).

2.3.1.4 Carfilzomib, Lenalidomide, and Dexamethasone

Carfilzomib has been studied in combination with lenalidomide and dexamethasone in patients with relapsed multiple myeloma. In a phase III study [7], carfilzomib in combination with lenalidomide and dexamethasone was compared with lenalidomide and dexamethasone. The addition of carfilzomib demonstrated reduced risk of disease progression or death, where the median PFS was improved by 12 months after the first relapse and by 9 months in patients with >2 previous lines of therapy. Subgroup analysis showed that the addition of carfilzomib to lenalidomide and dexamethasone consistently improved PFS, even in patients previously exposed to bortezomib as well as lenalidomide [8].

2.3.2 Efficacy in Newly Diagnosed Multiple Myeloma

2.3.2.1 Transplant Ineligible Patients

Lenalidomide and Dexamethasone

The combination of lenalidomide and dexamethasone first demonstrated efficacy in newly diagnosed MM in 2014 when it was compared in a randomized control trial [9] against the standard combination therapy at the time; melphalan, prednisone, and thalidomide. In this trial, the lenalidomide-dexamethasone regime consisted of lenalidomide 25 mg administered on days 1–21 of each 28 day cycle and dexamethasone 40 mg administered on days 1, 8, 15, and 22. Two durations of treatment were studied, one being a continuous lenalidomide-dexamethasone arm where lenalidomide and dexamethasone were given in 28-day cycles until disease progression and a limited treatment arm where 18 cycles (or 72 weeks) of treatment were given. The comparison group received melphalan, prednisone, and thalidomide, with standard doses of melphalan (0.25 mg/kg of body weight per day on days 1–4), prednisone (2 mg per kg on days 1–4), and thalidomide (200 mg per day) in 42 day cycles for 12 cycles (or 72 weeks). The results showed a benefit with continuous lenalidomide-dexamethasone treatment, with patients responding 13% more frequently compared to the comparison group, as well as a significantly improved PFS of 25.5 months compared to the 21.2 months obtained with melphalan, prednisone, and thalidomide. The shorter course of 18 cycles of lenalidomide and dexamethasone had a similar outcome to melphalan, prednisolone, and thalidomide with a PFS of 20.7 months. Continuous lenalidomide and dexamethasone significantly reduced the risk of progression or death by 28%, and the risk of death by 22% compared to melphalan, thalidomide, and prednisone. The duration of response

was also significantly improved with continuous lenalidomide-dexamethasone compared to both 18 cycle lenalidomide-dexamethasone as well as melphalan, prednisolone, and thalidomide. Notably, the PFS benefit is also sustained through further lines of therapy in patients who relapse, suggesting that lenalidomide does not negatively affect second-line treatment options. Overall survival also favored the addition of lenalidomide, with 4-year survival of 59% for continuous lenalidomide-dexamethasone, and 56% for 18 cycles of lenalidomide-dexamethasone. Although continuous lenalidomide and dexamethasone had a significantly better OS compared to melphalan, prednisone, and thalidomide (which had a 4-year survival of 51%), there was no significant difference in OS between continuous and 18 cycles of lenalidomide-dexamethasone.

The benefit of lenalidomide maintenance has also been demonstrated with comparisons between melphalan, prednisone, and lenalidomide induction with lenalidomide maintenance, melphalan, prednisone, and lenalidomide induction alone, and melphalan with prednisone [10]. Progression-free survival was prolonged in the lenalidomide maintenance arm with a median PFS of 31 months compared with 14 months with melphalan, prednisone, and lenalidomide induction alone, and 13 months with melphalan and prednisone. Upon specific analysis of maintenance therapy, lenalidomide significantly extended progression-free survival from the start of maintenance therapy by a median of 26 months, compared to 7 months with placebo following the same induction therapy. This benefit was consistently observed across all subgroup analyses except for elderly patients >75 years of age.

Cyclophosphamide, Lenalidomide, and Dexamethasone

Cyclophosphamide in addition to lenalidomide and dexamethasone demonstrated a median PFS of 28 months, with OS at 2 years of 87% in phase II trials studying the combination of alkylating agents with standard lenalidomide and dexamethasone therapy [11]. Cyclophosphamide was dosed at 300 mg/m^2 weekly for 3 weeks in a 4-week cycle. 85% of patients achieved a partial response (PR) or better, and 47% of patients achieved a VGPR or better. Although not directly compared, the addition of cyclophosphamide seemingly results in higher response rates compared to studies of lenalidomide and dexamethasone.

Bortezomib, Lenalidomide, and Dexamethasone

The combination of bortezomib, lenalidomide, and dexamethasone has been explored in patients with newly diagnosed MM without intention for transplantation [12]. When compared with lenalidomide and dexamethasone, the addition of bortezomib appeared to improve outcomes, with improvement in PFS of 43 months in the bortezomib arm compared to 31 months in lenalidomide and dexamethasone alone. Overall survival was also improved with a hazard ratio of 0.666.

2.3.2.2 Transplant Eligible Patients

Bortezomib, Lenalidomide, and Dexamethasone

Combination therapy with bortezomib, lenalidomide, and dexamethasone has not only been studied in RRMM but has also been studied in the transplant setting as both induction and maintenance therapy. Patients eligible for autologous stem cell transplant in newly diagnosed MM were studied [13] with 3-week cycles of therapy consisting of intravenous bortezomib (1.3 mg/m^2 on days 1, 4, 8, and 11), oral lenalidomide (25 mg on days 1–14), and oral dexamethasone 40 mg (days 1, 8, and 15) as induction therapy. Patients subsequently proceeded to transplantation with melphalan conditioning. Patients continued to receive two further cycles of bortezomib, lenalidomide, and dexamethasone posttransplant, followed by lenalidomide maintenance therapy. After consolidation therapy, 97% of patients achieved a PR or better, with 50% achieving CR or better. The estimated 3-year PFS and OS rates were 77% and 100%, respectively. Bortezomib, lenalidomide, and dexamethasone did not appear to adversely affect mobilization and stem cell collection as only 1 of the 31 patients studied in the trial experienced stem cell collection failure. Lenalidomide maintenance posttransplantation demonstrated a benefit and improved response in 27% of patients, with 13% changing minimal residual disease (MRD) status from positive to negative with lenalidomide maintenance.

2.4 Mechanisms of Action

2.4.1 Cereblon Pathway

Immunomodulatory imide drugs such as lenalidomide exert their effects via their interactions with cereblon, an important protein within the E3 ubiquitin ligase complex. Binding to cereblon results in the ubiquitination and proteasomal degradation of substrate proteins Ikaros (encoded by the IKZF1 gene) and Aiolos (encoded by the IKZF3 gene), which in turn results in the downregulation of the proteins interferon regulatory factor 4 (IRF-4) and c-Myc and subsequently growth inhibition and apoptosis of myeloma cells [14, 15].

2.4.2 Effect on Cytokines

Lenalidomide also affects cytokine production, resulting in a net effect of downregulation of pro-inflammatory cytokines tumor necrosis factor-alpha (TNF-α), interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-12 (IL-12) as well as upregulation of the anti-inflammatory cytokine interleukin-10 (IL-10) [1]. Lenalidomide

has a particularly potent effect on TNF-α downregulation, 50,000 times more potent than thalidomide. The exact mechanism of action of lenalidomide on TNF-α is yet to be defined. However, it is known that thalidomide has an effect on the degradation of TNF-α messenger RNA (mRNA) thus lenalidomide may act via similar mechanisms. TNF-α plays multiple roles within the body but has been suggested to be involved in the pathogenesis of various hematological malignancies. IL-6 downregulation is beneficial as IL-6 inhibits the apoptosis of malignant myeloma cells and increases their proliferation. In addition to directly downregulating IL-6 production, Lenalidomide inhibits the interaction between myeloma cells and IL-6 generating bone marrow stromal cells.

2.4.3 T Cell Activation

Lenalidomide affects the B7-CD28 costimulatory signaling pathways, allowing antigen presenting cells to activate T cells and augment their response and proliferation. Lenalidomide induces tyrosine phosphorylation of CD28 on T cells, resulting in the activation of several downstream targets (including phosphoinositide 3-kinase (PI3K) and nuclear factor-kappa B [NF-κβ]) and a Th1-type cytokine response that further stimulates clonal T cell proliferation and natural killer (NK) cell activity, bolstering the body's antitumor immune response to myeloma cells [1]. Importantly, lenalidomide is capable of bypassing the CD28 blockade by cytotoxic T lymphocyte-associated protein 4 (CTLA-4) present on tumor cells, to exert its T cell activating effects.

2.4.4 Effect on Natural Killer Cells

Immunomodulatory imide drugs appear to improve the potency of NK cells, although the exact mechanism is unknown [1]. Treatment with IMiDs including lenalidomide appears to increase NK cells as well as enhance antibody cell-mediated cytotoxicity [1]. The interaction of immunoglobulin G (IgG) with FC-gamma receptors in the presence of interleukin-2 (IL-2) or IL-12 appears to be induced by lenalidomide, and it may be the enhancement of this interaction that leads to lenalidomide's effect on augmenting the potency of NK cells [2].

2.4.5 Anti-Angiogenic Activity

Lenalidomide, along with the other IMiDs, exhibits an inhibitory effect on the expression of the angiogenic factors vascular endothelial growth factor (VEGF) and IL-6, resulting in reduced angiogenesis (the formation of new blood vessels) [1]. Angiogenesis is required to support the dysregulated growth of tumor cells; thus, the inhibition of angiogenesis may contribute to the anti-myeloma effect of IMiDs. The newer IMiDs, lenalidomide and pomalidomide, have 2–3 times more potent anti-angiogenic activity compared to thalidomide [16]. The anti-angiogenic activity of lenalidomide is not only brought about by the direct reduction in expression of VEGF and IL-6 alone, as lenalidomide also disrupts VEGF-mediated angiogenesis indirectly by partially inhibiting Akt phosphorylation after VEGF stimulation, as well as inhibiting the phosphorylation of Gab1, a protein upstream of Akt1 [17, 18].

2.4.6 Direct Antitumor Activity

Lenalidomide has anti-proliferative activity against myeloma cells. Lenalidomide upregulates the cyclin-dependent kinase (CDK) inhibitor p21 (WAF1/CIP1), modulating the activity of CDKs and leading to IMiD induced growth arrest [19]. Lenalidomide also results in apoptosis via multiple mechanisms, including TNF-related apoptosis-inducing ligand (TRAIL), increased sensitivity to Fas-mediated cell death, caspase-8 activation, downregulation of caspase-8 inhibitors FLIP and cIAP2, downregulation of NF-κβ, and inhibition of the pro-survival effects of insulin-like growth factor 1 (IGF-1) [20].

2.4.7 Myeloma Microenvironment

Both osteoclasts and bone marrow stromal cells promote MM survival and growth via a number of pathways. Bone marrow stromal cells interact with myeloma cells which prompts the secretion of IL-6 and other growth factors, leading to the growth of both myeloma cells and osteoclasts. In turn, osteoclasts lead to bone resorption and produce factors which increase myeloma cell survival. Lenalidomide disrupts this synergistic relationship by reducing tartrate-resistant acid phosphatase (TRAP) positive osteoclast-precursor cells and downregulating important mediators in osteoclastogenesis including the transcription factor PU.1 and MAP kinase pERK [21]. Lenalidomide also disrupts bone resorption by decreasing the adhesion molecule αVβ3-integrin which is required for osteoclast activation, reducing cathepsin K which limits the osteoclasts bone resorptive ability, and reducing the receptor activator of nuclear factor-kappa B ligand which plays a role in bone remodeling factor receptor activation [21]. The interaction between myeloma cells and bone marrow

stromal cells is also disrupted by lenalidomide's effects on surface adhesion molecules including ICAM-1, VCAM-1, and E-selectin [22].

2.5 Lenalidomide Resistance

Lenalidomide resistance in MM can be seen in the proportion of patients failing to respond to therapy in previous clinical trials. The ORR to lenalidomide and dexamethasone in a cohort of newly diagnosed, transplant ineligible MM patients was approximately 73–75% [9]. Therefore, approximately 25% of patients did not respond to the treatment and may have possessed primary resistance to lenalidomide and dexamethasone. In RRMM patients [2], the overall response rate to IMiD therapy was 61%, with a CR rate of 14.1%, near CR rate of 10.2%, and a median duration of response of 15.8 months. In this cohort of patients, about 41.8% of patients received thalidomide. It is unclear how many of these patients responded to lenalidomide. Overall 39% of RRMM patients failed to respond to lenalidomide. In a phase III study of the combination carfilzomib, lenalidomide, and dexamethasone in RRMM patients [7], the ORR was 87% in the carfilzomib, lenalidomide, and dexamethasone drug group, and 67% in the lenalidomide and dexamethasone group. As patients that had previously received lenalidomide (approximately 20%) and bortezomib (approximately 66%) were included in the study, it would be difficult to assess the proportion of patients possessing primary resistance to lenalidomide not resulting from prior therapy.

2.5.1 Potential Mechanisms of Lenalidomide Resistance

2.5.1.1 Decreased Cereblon Expression and Downstream Factors

Given the importance of cereblon as a target protein for lenalidomide, decreased expression of cereblon is postulated as a mechanism of lenalidomide resistance [14]. Although not completely understood, decreased cereblon levels via short hairpin RNA (shRNA) mediated depletion, epigenetic modification of the cereblon promoter region, gene mutations, or chromosomal deletions appear to result in IMiD resistance but does not affect the sensitivity of myeloma cells to other anti-myeloma agents. Decreased cereblon expression is associated with lenalidomide resistance in patients, and studies have demonstrated restoration of IMiD sensitivity following the introduction of wild-type cereblon back into myeloma cells, further supporting the link between cereblon expression and resistance [14, 23, 24]. However, cereblon alone does not appear to be the only factor contributing to IMiD resistance as there are populations of patients possessing both lenalidomide resistance and high levels of cereblon. Another hypothesis is that the downstream substrates or factors of celebron are key in the development of lenalidomide resistance. Although further

research is required, various indirect downstream factors of celebron such as IRF4 and beta-catenin appear to be overexpressed in lenalidomide-resistant cases [15, 25].

2.5.1.2 Increase in c-Myc

In lenalidomide refractory patients, c-Myc expression is noted to have increased, compared to the time of diagnosis [14]. Although requiring further investigation, c-Myc is noted to be increased during the progression from monoclonal gammopathy of undetermined significance (a precursor stage) to MM and is also linked to adverse outcomes and poorer survival. Inducing resistance to lenalidomide may be another mechanism by which c-Myc affects myeloma cells.

The IGL/MYC translocation was associated with poor prognosis and no response to IMiDs based on the whole genome sequencing in the Clinical Outcomes in Multiple Myeloma to Personal Assessment (CoMMpass) study. The IGL locus is a super-enhancer of myeloma cells and is bound by high levels of IKZF1 which may be a contributor of resistance to lenalidomide [3].

Efforts to target increased c-Myc include using BET domain inhibitors in combination with lenalidomide and dexamethasone [26]. This results in the synergistic downregulation of MYC and IKZF1 in vitro and in vivo [26].

2.5.2 Management of Lenalidomide-Resistant Disease

There have been very few studies investigating the best therapy for lenalidomide-resistant/refractory disease. Randomized controlled trials in this area have since explored pomalidomide, proteasome inhibitors, and daratumumab as potential therapies.

2.5.2.1 Pomalidomide-Based Regimes

The most commonly investigated regimes in lenalidomide-resistant disease were based around pomalidomide. Pomalidomide dosed at 4 mg daily from days 1 to 21 in 28-day cycles in combination with dexamethasone 40 mg weekly demonstrated increased efficacy compared to both dexamethasone and pomalidomide monotherapy [4]. However, there was no statistically significant difference to continuous regimes of pomalidomide administered without a week off the drug [5], nor was there any statistically significant benefit with the addition of cyclophosphamide 400 mg on days 1, 8, and 15 other than improved ORR of 65% [6]. This improvement in ORR did not translate to a survival benefit, suggesting the lack of deep responses and a short-lived response. Overall response rates in regimens containing standard doses of pomalidomide in addition to at least dexamethasone appear to demonstrate a disease response in at least 30% of patients with lenalidomide

refractory disease. Given that both lenalidomide and pomalidomide belong to IMiD class, this suggests that pomalidomide is either much more potent or has effects on other cellular targets aside from cereblon, the shared target between the two IMiDs. This is supported by subgroup analysis in Richardson et al.'s study which demonstrated no significant difference in the efficacy of Pomalidomide when comparing the study's general patient cohort to the lenalidomide refractory subgroup (overall response rates of 33% in the general population versus 30% in the lenalidomide refractory population), although notably 79% of the general cohort were lenalidomide refractory [7]. Other than evaluating the efficacy of pomalidomide, Sehgal et al.'s study also demonstrated increases in T cells, NK cells, the production of cytokines associated with these cells including interferon-γ, TNF-α, IL-2, and interleukin-4 (IL-4), increases in CD8 T cells and increased NK cell expression of granzyme B and perforin [8]. The authors suggest that the anti-myeloma effect of pomalidomide, even in lenalidomide refractory settings, may be due to its ability to alter both immune cells and the tumor microenvironment.

2.5.2.2 Proteasome Inhibitor and Daratumumab-Based Regimes

Proteasome inhibitor-based treatment regimes containing bortezomib or carfilzomib have been tested in lenalidomide refractory disease. In the ENDEAVOR study, patients who were refractory to lenalidomide achieved a median PFS of 8.6 and 6.6 months with carfilzomib and bortezomib, respectively [9]. In a subgroup analysis, there was a statistically significant difference in the median PFS of patients with previous lenalidomide exposure treated with carfilzomib (12.9 months) compared to bortezomib (7.3 months) [9]. Carfilzomib differs from bortezomib as it is the second-generation PI with differing structure and activity on the 20S proteasome through which it exerts its anti-myeloma activity. Contrasting to bortezomib, carfilzomib binds irreversibly and has increased selectivity for chymotrypsin-like activity in the 20S proteasome which may explain its greater efficacy. There is no direct comparative clinical study between pomalidomide and proteasome inhibitors in lenalidomide refractory disease.

Spencer et al. performed a subgroup analysis of lenalidomide refractory disease in the CASTOR trial (a phase III clinical trial comparing daratumumab, bortezomib, and dexamethasone with bortezomib, dexamethasone in RRMM). The addition of daratumumab as a third agent in this study improved the median PFS from 4.4 months to 9.3 months and the ORR from 50% to 81% in the lenalidomide refractory subgroup [10]. Response rates were similar to other daratumumab studies which did not specifically analyze the lenalidomide refractory population. Daratumumab is a MoAb that exerts its anti-myeloma effect by targeting CD38 which is expressed on plasma cells. This unique mechanism of action compared to other anti-myeloma treatments may explain its ability to overcome lenalidomide refractory disease.

2.6 Conclusion

The mechanism of lenalidomide resistance remains an important area of research. Currently, switching therapeutic class is a common strategy to overcome resistance. Future drug development could aim at targeting mechanisms of resistance such as MYC deregulation.

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References

1. Chang X, Zhu Y, Shi C, Stewart AK. Mechanism of immunomodulatory drugs' action in the treatment of multiple myeloma. Acta Biochimt Biophys Sin. 2014;46(3):240–53.
2. Weber DM, Chen C, Niesvizky R, Wang M, Belch A, Stadtmauer EA, et al. Lenalidomide plus dexamethasone for relapsed multiple myeloma in North America. N Engl J Med. 2007;357(21):2133–42.
3. Dimopoulos MA, Chen C, Spencer A, Niesvizky R, Attal M, Stadtmauer EA, et al. Long-term follow-up on overall survival from the MM-009 and MM-010 phase III trials of lenalidomide plus dexamethasone in patients with relapsed or refractory multiple myeloma. Leukemia. 2009;23(11):2147–52.
4. Qiao SK, Guo XN, Ren JH, Ren HY. Efficacy and safety of lenalidomide in the treatment of multiple myeloma: a systematic review and meta-analysis of randomized controlled trials. Chin Med J (Engl). 2015;128(9):1215–22.
5. Richardson PG, Xie W, Jagannath S, Jakubowiak A, Lonial S, Raje NS, et al. A phase 2 trial of lenalidomide, bortezomib, and dexamethasone in patients with relapsed and relapsed/refractory myeloma. Blood. 2014;123(10):1461–9.
6. Dimopoulos MA, Oriol A, Nahi H, San-Miguel J, Bahlis NJ, Usmani SZ, et al. Daratumumab, lenalidomide, and dexamethasone for multiple myeloma. N Engl J Med. 2016;375(14):1319–31.
7. Stewart AK, Rajkumar SV, Dimopoulos MA, Masszi T, Spicka I, Oriol A, et al. Carfilzomib, lenalidomide, and dexamethasone for relapsed multiple myeloma. N Engl J Med. 2015;372(2):142–52.
8. Dimopoulos MA, Stewart AK, Masszi T, Spicka I, Oriol A, Hajek R, et al. Carfilzomib-lenalidomide-dexamethasone vs lenalidomide-dexamethasone in relapsed multiple myeloma by previous treatment. Blood Cancer J. 2017;7(4):e554.
9. Benboubker L, Dimopoulos MA, Dispenzieri A, Catalano J, Belch AR, Cavo M, et al. Lenalidomide and dexamethasone in transplant-ineligible patients with myeloma. N Engl J Med. 2014;371(10):906–17.
10. Palumbo A, Blade J, Boccadoro M, Palladino C, Davies F, Dimopoulos M, et al. How to manage neutropenia in multiple myeloma. Clin Lymphoma Myeloma Leuk. 2012;12(1):5–11.
11. Kumar SK, Lacy MQ, Hayman SR, Stewart K, Buadi FK, Allred J, et al. Lenalidomide, cyclophosphamide and dexamethasone (CRd) for newly diagnosed multiple myeloma: results from a phase 2 trial. Am J Hematol. 2011;86(8):640–5.
12. Durie BGM, Hoering A, Abidi MH, Rajkumar SV, Epstein J, Kahanic SP, et al. Bortezomib with lenalidomide and dexamethasone versus lenalidomide and dexamethasone alone in patients with newly diagnosed myeloma without intent for immediate autologous stem-cell transplant (SWOG S0777): a randomised, open-label, phase 3 trial. Lancet (London, England). 2017;389(10,068):519–27.

13. Roussel M, Lauwers-Cances V, Robillard N, Hulin C, Leleu X, Benboubker L, et al. Front-line transplantation program with lenalidomide, bortezomib, and dexamethasone combination as induction and consolidation followed by lenalidomide maintenance in patients with multiple myeloma: a phase II study by the Intergroupe Francophone du Myelome. J Clin Oncol. 2014;32(25):2712–7.
14. Franssen LE, Nijhof IS, Couto S, Levin MD, Bos GMJ, Broijl A, et al. Cereblon loss and up-regulation of c-Myc are associated with lenalidomide resistance in multiple myeloma patients. Haematologica. 2018;103(8):e368–e71.
15. Bjorklund CC, Lu L, Kang J, Hagner PR, Havens CG, Amatangelo M, et al. Rate of CRL4(CRBN) substrate Ikaros and Aiolos degradation underlies differential activity of lenalidomide and pomalidomide in multiple myeloma cells by regulation of c-Myc and IRF4. Blood Cancer J. 2015;5(10):e354.
16. Kotla V, Goel S, Nischal S, Heuck C, Vivek K, Das B, et al. Mechanism of action of lenalidomide in hematological malignancies. J Hematol Oncol. 2009;2:36.
17. Gandhi AK, Kang J, Naziruddin S, Parton A, Schafer PH, Stirling DI. Lenalidomide inhibits proliferation of Namalwa CSN.70 cells and interferes with Gab1 phosphorylation and adaptor protein complex assembly. Leuk Res. 2006;30(7):849–58.
18. Dredge K, Horsfall R, Robinson SP, Zhang LH, Lu L, Tang Y, et al. Orally administered lenalidomide (CC-5013) is anti-angiogenic in vivo and inhibits endothelial cell migration and Akt phosphorylation in vitro. Microvasc Res. 2005;69(1–2):56–63.
19. Escoubet-Lozach L, Lin IL, Jensen-Pergakes K, Brady HA, Gandhi AK, Schafer PH, et al. Pomalidomide and lenalidomide induce p21 WAF-1 expression in both lymphoma and multiple myeloma through a LSD1-mediated epigenetic mechanism. Cancer Res. 2009;69(18):7347–56.
20. Mitsiades N, Mitsiades CS, Poulaki V, Chauhan D, Richardson PG, Hideshima T, et al. Apoptotic signaling induced by immunomodulatory thalidomide analogs in human multiple myeloma cells: therapeutic implications. Blood. 2002;99(12):4525–30.
21. Breitkreutz I, Raab MS, Vallet S, Hideshima T, Raje N, Mitsiades C, et al. Lenalidomide inhibits osteoclastogenesis, survival factors and bone-remodeling markers in multiple myeloma. Leukemia. 2008;22(10):1925–32.
22. Terpos E, Migkou M, Christoulas D, Gavriatopoulou M, Eleutherakis-Papaiakovou E, Kanellias N, et al. Increased circulating VCAM-1 correlates with advanced disease and poor survival in patients with multiple myeloma: reduction by post-bortezomib and lenalidomide treatment. Blood Cancer J. 2016;6(5):e428.
23. Zhu YX, Shi CX, Bruins LA, Wang X, Riggs DL, Porter B, et al. Identification of lenalidomide resistance pathways in myeloma and targeted resensitization using cereblon replacement, inhibition of STAT3 or targeting of IRF4. Blood Cancer J. 2019;9(2):19.
24. Lopez-Girona A, Mendy D, Ito T, Miller K, Gandhi AK, Kang J, et al. Cereblon is a direct protein target for immunomodulatory and antiproliferative activities of lenalidomide and pomalidomide. Leukemia. 2012;26(11):2326–35.
25. Bjorklund CC, Ma W, Wang ZQ, Davis RE, Kuhn DJ, Kornblau SM, et al. Evidence of a role for activation of Wnt/beta-catenin signaling in the resistance of plasma cells to lenalidomide. J Biol Chem. 2011;286(13):11009–20.
26. Díaz T, Rodríguez V, Lozano E, Mena MP, Calderón M, Rosiñol L, et al. The BET bromodomain inhibitor CPI203 improves lenalidomide and dexamethasone activity in in vitro and in vivo models of multiple myeloma by blockade of Ikaros and MYC signaling. Haematologica. 2017;102(10):1776–84.

Chapter 3
Pomalidomide

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Abstract Pomalidomide is the third-generation immunomodulatory imide drug (IMiD) derived from thalidomide, approved for the treatment of multiple myeloma (MM). The exact mechanisms of action of pomalidomide are unclear; however, given the structural similarities between pomalidomide and the second-generation IMiD lenalidomide, it is postulated that the two IMiDs share common effects. Pomalidomide is more potent than lenalidomide and is efficacious in lenalidomide-resistant cases. However, pomalidomide-resistant cases have been observed. This chapter will review data from notable clinical trials of pomalidomide and explore the potential mechanisms of pomalidomide action and resistance.

Keywords Multiple myeloma · Pomalidomide · Immunomodulatory imide drug · Cereblon pathway · Pomalidomide resistance

Abbreviations

EMA	European Medicines Agency
FDA	US Food and Drug Administration
IMiD	Immunomodulatory imide drug

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mRNA	Messenger RNA
NF-κβ	Nuclear factor-kappa B
ORR	Overall response rate
OS	Overall survival
PFS	Progression-free survival
PI	Proteasome inhibitor
RRMM	Relapsed/refractory multiple myeloma
TGA	Therapeutic Goods Administration

3.1 Introduction

Pomalidomide is an analog of thalidomide and the third drug to be developed belonging to the immunomodulatory imide drug (IMiD) class. It shares common phthalimide and glutarimide moieties as thalidomide but differs in that it has a substituted amino acid at position 4 on the isoindole ring system [1].

3.2 Clinical Indication of Pomalidomide

In Australia pomalidomide, in combination with dexamethasone, is indicated in relapsed/refractory multiple myeloma (RRMM) patients who have undergone at least two prior lines of therapy, which must include bortezomib and lenalidomide based regimes, and with demonstrated disease progression on their last line of therapy (Therapeutic Goods Administration, 2014). It is licensed for the same indication by the US Food and Drug Administration (FDA) (Food and Drug Administration, 2013) and the European Medicines Agency (EMA).

The combination of pomalidomide and bortezomib is approved by the Therapeutic Goods Administration (TGA) of Australia and the European Medicines Agency (EMA) for the treatment of RRMM patients who have undergone at least one prior line of therapy, including lenalidomide.

The combination of daratumumab, pomalidomide, and dexamethasone has been licensed by the FDA since 2017 for the treatment of RRMM patients who have received at least two prior therapies, including lenalidomide and a proteasome inhibitor (PI). Isatuximab and pomalidomide was approved by the FDA in 2020 for the same indication.

3.3 Efficacy

3.3.1 Efficacy in Relapsed and Refractory Multiple Myeloma

3.3.1.1 Pomalidomide and Dexamethasone

A multicenter, open-label, randomized phase III trial by Miguel et al. set the foundation for TGA and FDA approval of pomalidomide and dexamethasone [2]. In this study patients with RRMM who had failed at least two previous treatment lines including bortezomib and lenalidomide were randomized to either pomalidomide and dexamethasone or high dose dexamethasone without pomalidomide. Pomalidomide was dosed at 4 mg daily on days 1–21 of 28-day cycles, with weekly doses of dexamethasone 40 mg orally. The high dose dexamethasone arm was dosed at 40 mg daily orally on days 1–4, 9–12, and 17–20. For patients older than 75 years of age, dexamethasone was reduced to 20 mg at the same dosing frequency. Four hundred and fifty five patients were enrolled in the study with 302 in the pomalidomide and dexamethasone arm and 153 in the high dose dexamethasone arm. Overall response rates (ORR) in the pomalidomide arm were reported at 31%, with median progression-free survival (PFS) in the intention to treat a population of 16 weeks in the pomalidomide and dexamethasone arm, compared to 8.1 weeks in the high dose dexamethasone arm. Subgroup analysis including age stratified (65 years and younger compared to above 65 years old), lenalidomide refractory, bortezomib intolerant, refractory to both lenalidomide and bortezomib, lenalidomide as last treatment, and bortezomib as last treatment groups demonstrated similar results in favor of the pomalidomide arm. Median overall survival (OS) also favored the pomalidomide arm at 55.4 weeks compared to 35.1 weeks for high dose dexamethasone.

Pomalidomide as a single agent has also been compared to pomalidomide and dexamethasone but was observed to result in both a lower median PFS and median OS [3]. Other variations of pomalidomide and dexamethasone dosing strategies have also been studied, including continuous pomalidomide at 4 mg daily on days 1–28, with weekly dexamethasone [4], and continuous low dose pomalidomide at 2 mg daily on days 1–28 in combination with weekly dexamethasone [5]. These strategies were compared to the standard dosing of pomalidomide 4 mg on days 1–21 and weekly dexamethasone and demonstrated comparable PFS and OS benefits. However, continuous pomalidomide dosing slightly increased the incidence of grade 3 and 4 adverse effects.

3.3.1.2 Pomalidomide + Dexamethasone + Cyclophosphamide

A phase I/II randomized controlled trial compared pomalidomide and dexamethasone in combination with cyclophosphamide to pomalidomide and dexamethasone alone [6]. Cyclophosphamide was dosed at 400 mg on days 1, 8, and 15. Thirty-four

patients were enrolled in the triple therapy arm and 36 patients were enrolled in the pomalidomide and dexamethasone arm. Although an increased ORR was observed in the triple therapy arm (65% vs 39%), this did not translate to a significant improvement in PFS or OS.

3.3.1.3 Pomalidomide, Bortezomib, and Dexamethasone

A randomized, open-label phase III trial by Richardson et al. compared pomalidomide, bortezomib, and dexamethasone to bortezomib and dexamethasone in patients with RRMM who had undergone one to three previous regimens, one of which must have been a lenalidomide-containing regimen for at least two consecutive cycles [7]. Bortezomib was dosed at 1.3 mg/m^2, given either intravenously or subcutaneously on days 1, 4, 8, and 11 for the first eight cycles, and then on days 1 and 8 of subsequent cycles. Each cycle was 21 days in length. Dexamethasone was dosed at 20 mg on the day of and the day after bortezomib administration. The dexamethasone dose was reduced to 10 mg for patients older than 75 years of age. Patients allocated to the pomalidomide arm were given 4 mg pomalidomide orally on days 1–14. In total, 559 patients were enrolled with 281 in the pomalidomide, bortezomib, and dexamethasone arm, and 278 in the bortezomib and dexamethasone arm. An improvement in median PFS was observed with the addition of pomalidomide (11.2 months vs 7.1 months [p value <0.0001]).

3.3.1.4 Pomalidomide, Daratumumab, and Dexamethasone

The addition of daratumumab to the standard dosing of pomalidomide and dexamethasone was evaluated in an open-label, nonrandomized phase Ib trial [8]. The standard dosing of pomalidomide 4 mg daily days 1–21 with weekly dexamethasone was evaluated with the addition of daratumumab at 16 mg/kg intravenously weekly for the first two 28-day cycles, every 2 weeks from cycles 3 to 6, and every 4 weeks in each subsequent cycle. Eligible patients must have received at least two prior lines of therapy which must have included lenalidomide and bortezomib but must also be naïve to daratumumab and pomalidomide. One hundred and three patients were enrolled, with an ORR of 60% and median PFS of 8.8 months. The estimated survival rates at 3, 6, and 12 months were 89%, 79%, and 66%, respectively. These results appear to be improved compared to pomalidomide and dexamethasone alone; however, there is a paucity of phase III trials comparing these regimes.

3.3.1.5 Pembrolizumab, Pomalidomide, and Dexamethasone

A randomized phase trial investigating pembrolizumab combined with pomalidomide and dexamethasone compared to pomalidomide and dexamethasone alone was halted due to risks in the triple therapy arm outweighing benefits [9]. A total of 125 patients were randomized to the pembrolizumab combined with pomalidomide and dexamethasone arm, compared to 124 in the pomalidomide and dexamethasone alone arm. The median PFS was 5.6 months in the triple therapy arm compared to 8.4 months in the pomalidomide and dexamethasone arm, with serious adverse events occurring in 63% of patients in the triple therapy arm compared to 46% in the pomalidomide and dexamethasone arm. Of these serious adverse events, 3% were considered treatment-related in the triple therapy arm, with none in the pomalidomide and dexamethasone arm considered treatment-related. From these early results, there is no current data to support the addition of pembrolizumab with standard dosing of pomalidomide and dexamethasone.

3.4 Mechanisms of Pomalidomide Action

Belonging to the same IMiD class, it is postulated that pomalidomide shares a similar mechanism of action to the other second-generation IMiD lenalidomide. Pomalidomide and lenalidomide have multiple anti-myeloma effects, including induction of cell cycle arrest and apoptosis, inactivation of nuclear factor-kappa β (NF-κβ), downregulation of C/Ebβ, activation of caspase-8, disruption of the interaction between myeloma cells and the bone marrow microenvironment, enhancement of T cell proliferation and modulation of regulatory T cells, and effects on proinflammatory cytokines [10–12]. The same cereblon pathway which lenalidomide affects also seems to be an important factor in the efficacy of pomalidomide. Although the exact molecular mechanisms behind this myriad of changes are not yet known, pomalidomide does appear to be more potent than both thalidomide and lenalidomide with regards to its effect on cereblon [13].

3.5 Potential Mechanism of Pomalidomide Resistance and Overcoming Resistance

The specific mechanism for pomalidomide resistance remains unknown. Given the similar effects of lenalidomide and pomalidomide, it can be assumed that lenalidomide-resistant cases would also be pomalidomide resistant. However, lenalidomide resistance does not translate to pomalidomide resistance, as pomalidomide has clearly been proven to be efficacious in lenalidomide-resistant

populations. Whether this is solely due to its more potent nature compared to lenalidomide or an undescribed effect of pomalidomide is yet to be understood.

Cereblon is the key binding protein of IMiDs. The expression of cereblon protein and its messenger RNA (mRNA) had been shown to correlate with clinical response to pomalidomide. Higher cereblon protein expression was associated with increased depth of response and improved PFS and OS [14].

The mechanism of acquired resistance to lenalidomide and pomalidomide was studied in a xenograft plasmacytoma model. It appeared that there was a differential mechanism of resistance between the two drugs. This was supported by the lack of cross-resistance in vivo and differences in gene expression levels and cereblon expression levels. Cereblon expression was significantly downregulated in pomalidomide-resistant cases but not in lenalidomide-resistant ones. The gene expression profile was also significantly different between cases of lenalidomide resistance and pomalidomide resistance. However, in both situations there was upregulation of the MEK/ERK pathway and MEK inhibition by selumetinib could overcome both lenalidomide resistance and pomalidomide resistance in the animal model. It appears that pomalidomide action is more dependent on cereblon than lenalidomide, whereas lenalidomide may rely more on non-cereblon pathways for its anti-myeloma effect.

3.6 Conclusion

There is little data on pomalidomide-resistant cases given pomalidomide itself is reserved for relapsed/refractory cases. As described previously, the combination of pomalidomide with anti-myeloma agents from different classes such as cyclophosphamide, bortezomib, and daratumumab seem to have benefits, regardless of how small. Unfortunately, this does not appear to be the case with pembrolizumab which appeared to result in detrimental outcomes. Further studies of pomalidomide combinations with current drugs, as well as newly developed drugs will be required to determine the optimum approach to pomalidomide-resistant myeloma.

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References

1. PubChem Compound Summary for CID 134780, Pomalidomide; [cited 2020 Sept. 24]. [Internet]. PubChem [Internet]. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information. 2004. Retrieved from https://pubchem.ncbi.nlm.nih.gov/compound/Pomalidomide.
2. Miguel JS, Weisel K, Moreau P, Lacy M, Song K, Delforge M, et al. Pomalidomide plus low-dose dexamethasone versus high-dose dexamethasone alone for patients with relapsed

and refractory multiple myeloma (MM-003): a randomised, open-label, phase 3 trial. Lancet Oncol. 2013;14(11):1055–66.
3. Richardson PG, Siegel DS, Vij R, Hofmeister CC, Baz R, Jagannath S, et al. Pomalidomide alone or in combination with low-dose dexamethasone in relapsed and refractory multiple myeloma: a randomized phase 2 study. Blood. 2014;123(12):1826–32.
4. Leleu X, Attal M, Arnulf B, Moreau P, Traulle C, Marit G, et al. Pomalidomide plus low-dose dexamethasone is active and well tolerated in bortezomib and lenalidomide-refractory multiple myeloma: Intergroupe Francophone du Myelome 2009-02. Blood. 2013;121(11):1968–75.
5. Sehgal K, Das R, Zhang L, Verma R, Deng Y, Kocoglu M, et al. Clinical and pharmacodynamic analysis of pomalidomide dosing strategies in myeloma: impact of immune activation and cereblon targets. Blood. 2015;125(26):4042–51.
6. Baz RC, Martin TG 3rd, Lin HY, Zhao X, Shain KH, Cho HJ, et al. Randomized multicenter phase 2 study of pomalidomide, cyclophosphamide, and dexamethasone in relapsed refractory myeloma. Blood. 2016;127(21):2561–8.
7. Richardson PG, Hofmeister CC, Raje NS, Siegel DS, Lonial S, Laubach J, et al. Correction: Pomalidomide, bortezomib, and low-dose dexamethasone in lenalidomide-refractory and proteasome inhibitor-exposed myeloma. Leukemia. 2018;32(10):2305.
8. Chari A, Suvannasankha A, Fay JW, Arnulf B, Kaufman JL, Ifthikharuddin JJ, et al. Daratumumab plus pomalidomide and dexamethasone in relapsed and/or refractory multiple myeloma. Blood. 2017;130(8):974–81.
9. Mateos MV, Blacklock H, Schjesvold F, Oriol A, Simpson D, George A, et al. Pembrolizumab plus pomalidomide and dexamethasone for patients with relapsed or refractory multiple myeloma (KEYNOTE-183): a randomised, open-label, phase 3 trial. Lancet Haematol. 2019;6(9):e459–e69.
10. Chang X, Zhu Y, Shi C, Stewart AK. Mechanism of immunomodulatory drugs' action in the treatment of multiple myeloma. Acta Biochim Biophys Sin. 2014;46(3):240–53.
11. Li S, Pal R, Monaghan SA, Schafer P, Ouyang H, Mapara M, et al. IMiD immunomodulatory compounds block C/EBP{beta} translation through eIF4E down-regulation resulting in inhibition of MM. Blood. 2011;117(19):5157–65.
12. Galustian C, Meyer B, Labarthe MC, Dredge K, Klaschka D, Henry J, et al. The anti-cancer agents lenalidomide and pomalidomide inhibit the proliferation and function of T regulatory cells. Cancer Immunol Immunother. 2009;58(7):1033–45.
13. Lopez-Girona A, Mendy D, Ito T, Miller K, Gandhi AK, Kang J, et al. Cereblon is a direct protein target for immunomodulatory and antiproliferative activities of lenalidomide and pomalidomide. Leukemia. 2012;26(11):2326–35.
14. Steven R, Schuster KMK, Zhu YX, Braggio E, Shi C-X, Bruins L, Schmidt J, Ahmann G, Kumar SK, Rajkumar SV, Mikhael JR, Roy V, LaPlant BR, Laumann K, Barlogie B, Shaughnessy JD, Fonseca R, Bergsagel L, Lacy MQ, Stewart K. Cereblon expression predicts response, progression free and overall survival after pomalidomide and dexamethasone therapy in multiple myeloma. Blood. 2012;120(21)

Chapter 4
Mechanisms Driving Resistance to Proteasome Inhibitors Bortezomib, Carfilzomib, and Ixazomib in Multiple Myeloma

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Abstract The first clinically available proteasome inhibitor (PI) bortezomib was trialed in multiple myeloma (MM) approximately two decades ago and has since become a mainstay of myeloma therapy, significantly enhancing the overall survival of patients. However, bortezomib resistance continues to be a significant hurdle in the treatment of MM, despite the introduction of next-generation PIs such as carfilzomib and ixazomib. Unlike resistance to some other targeted therapies such as tyrosine kinase inhibitors, bortezomib resistance is highly complex and is able to arise through multiple mechanisms. This chapter discusses the current known mechanisms underlying bortezomib resistance, as well as resistance to the next-generation proteasome inhibitors carfilzomib and ixazomib.

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Keywords Multiple myeloma · Proteasome inhibitors · Bortezomib · Carfilzomib · Ixazomib · Proteasome inhibitor resistance · Autophagy · Unfolded protein response

Abbreviations

ABC	ATP-binding cassette
ATF4	Activating transcription factor 4
ATF6	Activating transcription factor 6
BiP	Binding immunoglobulin protein
BMSC	Bone marrow stromal cell
eIF2α	Eukaryotic initiation factor 2 alpha
ER	Endoplasmic reticulum
ERAD	ER-associated decay of proteins
FDA	US Food and Drug Administration
HDAC6	Histone deacetylase 6
IGF-1	Insulin-like growth factor 1
IL	Interleukin
IRE1	Inositol-requiring enzyme 1
JNK	c-Jun N-terminal kinase
MHC-1	Major histocompatibility complex class I
MM	Multiple myeloma
MSC	Mesenchymal stem cells
NF-κB	Nuclear factor kappa-B
p38MAPK	p38 mitogen-activated protein kinase
PERK	PKR-like ER kinase
PFS	Progression-free survival
PI	Proteasome inhibitor
PI3K	Phosphotidylinositol 3-kinase
RIDD	Regulated IRE1-dependent decay
RRMM	Relapsed/refractory multiple myeloma
TNF-α	Tumor necrosis factor-alpha
UPR	Unfolded protein response
XBP1	X-box binding protein 1

4.1 Introduction

Multiple myeloma (MM) is a hematological malignancy arising from plasma cells [1]. This plasma cell origin means myeloma cells often produce and secrete very high levels of nonfunctional, monoclonal immunoglobulin termed paraprotein [2].

This paraprotein production, and the subsequent endoplasmic reticulum stress and unfolded protein response activation, has been dubbed an "Achilles heel" of myeloma, which proteasome inhibitors (PI) such as bortezomib, carfilzomib, and ixazomib are able to exploit to induce myeloma cell death [2]. Although PIs have rapidly become a foundation of myeloma therapy, resistance is still a major hurdle in the treatment of patients with myeloma.

4.2 The Proteasome

The proteasome is responsible for the degradation of 70–90% of proteins that are unfolded, misfolded, or otherwise marked for degradation [3]. In eukaryotes, the 26S proteasome consists of two major subunits, a barrel-shaped core 20S subunit, and two regulatory 19S subunits bound to either end [4]. The 20S subunit is where proteolysis occurs and it contains six proteolytic centers composed of three different β subunits, β1, β2, and β5 [5]. These different subunits each have different activities; a caspase-like activity which cleaves after acidic amino acids (β1), a trypsin-like activity which cleaves after basic amino acids (β2), and a chymotrypsin-like activity which cleaves after neutral amino acids (β5) [3, 5]. Some mammalian cells also possess an immunoproteasome, where β1, β2, and β5 are replaced with β1i, β2i, and β5i, respectively [3]. The immunoproteasome is generally stimulated by γ-interferon, but can also be induced by other factors such as tumor necrosis factor α (TNF-α), and has altered substrate specificity to produce peptides optimized in size and composition for presentation to the major histocompatibility complex class I (MHC-I) on the cell surface during the immune response [6].

4.3 Endoplasmic Reticulum Stress

The extensive production of paraprotein in myeloma cells results in an increase in unfolded protein levels within the endoplasmic reticulum (ER), which in turns causes ER stress [2]. As a result of this, a cellular cascade known as the unfolded protein response (UPR) is triggered [2]. The UPR is activated by three ER transmembrane stress sensing proteins, inositol-requiring enzyme 1 (IRE1), PKR-like ER kinase (PERK), and activating transcription factor 6 (ATF6) [7]. Under homeostatic conditions, these ER stress sensors are kept inactive by the binding of the ER-specific chaperone binding immunoglobulin protein (BiP, also known as GRP78) to their luminal domain [8]. However, if unfolded protein levels increase, BiP is titrated away from these ER stress sensors due to its high affinity for unfolded proteins [8]. For IRE1 and PERK, the loss of BiP, as well as the direct binding of unfolded proteins to the luminal domains of IRE1 and potentially PERK, leads to their oligomerization and autophosphorylation, resulting in activation [7, 9, 10]. Meanwhile, the loss of BiP from ATF6 results in the exposure of Golgi localization

signal sequences, which results in the relocation of ATF6 to the Golgi, where it is cleaved by site-1 and site-2 proteases into its active form as a transcription factor [11, 12].

Activation of the UPR results in a complex signaling cascade, the main components of which are summarized in Fig. 4.1. This activation initially elicits a pro-survival response, aimed at restoring ER homeostasis via several mechanisms [2, 13]. However, if ER homeostasis is unable to be restored, then the UPR switches from pro-survival to pro-apoptotic signaling and induces cell death. Exactly how the

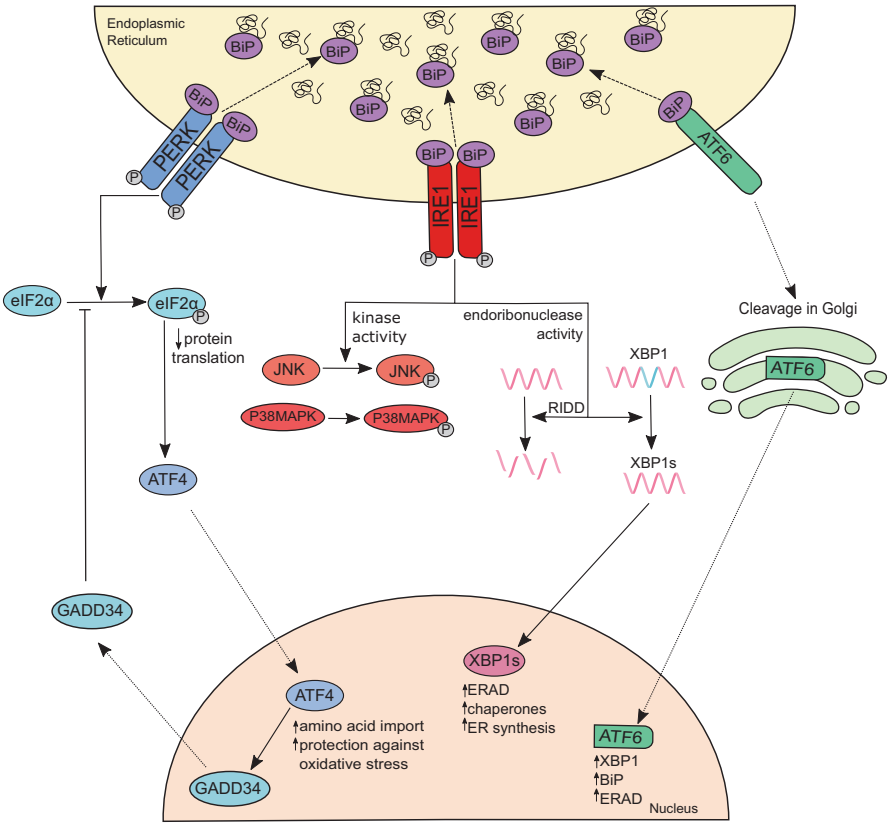

Fig. 4.1 A summary of the unfolded protein response. As unfolded protein levels increase, BiP dissociates from the luminal domain of ER stress sensors PERK, IRE1, and ATF6, resulting in their activation. IRE1 oligomerizes and autophosphorylates, activating its endoribonuclease and protein kinase activity. The endoribonuclease activity of IRE1 results in the production of the transcription factor XBP1, as well as IRE1-dependent decay (RIDD) of certain RNAs. Activation of the protein kinase activity of IRE1 results in the recruitment of binding partners and the phosphorylation of multiple targets, including p38MAPK and JNK. PERK also activates via oligomerization and autophosphorylation, resulting in reduced cap-dependent translation, and the production of transcription factor ATF4. When released by BiP, ATF6 translocates to the Golgi, where it is cleaved to form active ATF6, a transcription factor capable of upregulating several key UPR genes, including XBP1 and BiP

cell makes this decision however is still not fully understood [8, 14]. For a comprehensive review on the UPR and its role in cell fate, see Hetz and Papa (2018) [11].

Each ER stress sensor is able to activate separate but overlapping pathways [11]. Activated IRE1 is able to act as both an endoribonuclease and a protein kinase [11]. By far the most important target of the endoribonuclease activity of IRE1 is x-box binding protein 1 (XBP1), the splicing of which allows for the translation of XBP1s, a transcription factor which is important for both plasma cell differentiation and pro-survival UPR signaling [2]. XBP1s is able to upregulate several pathways, including ER membrane synthesis, ER chaperones, and ER-associated decay of proteins (ERAD) [2]. The endoribonuclease activity of IRE1 is also able to induce the degradation of certain RNAs via regulated IRE1-dependent decay, or RIDD, which is more closely associated with apoptosis [15, 16]. The protein kinase activity of IRE1 is also more closely associated with apoptosis, leading to the phosphorylation of stress-activated protein kinases such as c-Jun N-terminal kinase (JNK) and p38 mitogen-activated protein kinase (p38MAPK) through association with binding partners such as TRAF2 and ASK1 [7].

The protein kinase activity of activated PERK results in the phosphorylation of eukaryotic initiation factor 2 alpha (eIF2α), which suppresses cap-dependent translation, reducing the protein burden on the ER [17]. This leads to upregulation of activating transcription factor 4 (ATF4), which contributes to both cell-survival and cell-death pathways depending on how long it is present in the cell [11]. The extended presence of ATF4 results in the production of the pro-apoptotic transcription factor CHOP, as well as GADD34, which blocks eIF2α phosphorylation and thus restarts cap-dependent translation [18]. Cleavage of ATF6 into an active transcription factor also results in the upregulation of a number of UPR associated genes, including BiP and XBP1 [11].

4.4 Proteasome Inhibitors in Multiple Myeloma

Due to their high paraprotein production, myeloma cells have relatively high intrinsic levels of ER stress, and UPR is often already active in these cells as a pro-survival mechanism [2]. Proteasome inhibitors are able to take advantage of this by blocking proteasomal degradation, and therefore ERAD, further increasing unfolded protein levels and thus eliciting an apoptotic UPR [2]. The first PI bortezomib (Velcade) was approved by the US Food and Drug Administration (FDA) in 2003 for patients with relapsed/refractory multiple myeloma (RRMM) [19]. Since then, bortezomib has become one of the central drugs in myeloma treatment [20]. It is a reversible PI which acts on the 20S subunit of the proteasome, inhibiting primarily the β5 subunit (chymotrypsin-like activity), although inhibition of the β2 subunit (trypsin-like activity) and β1 subunit (caspase-like activity) also occurs, albeit with a lower affinity [21]. Proteasome inhibitors are thought to cause the death of myeloma cells through several mechanisms. One of the first proposed mechanisms was through inhibition of nuclear factor kappa-B (NF-κB), which in itself is an

inhibitor of apoptosis, although it has since been suggested that this is unlikely to be the main mechanism [21, 22]. It is now known that PIs also induce a pro-apoptotic UPR and cause changes in the bone marrow microenvironment that make it less hospitable to myeloma cells [21, 23].

Since the development of bortezomib, next-generation PIs have been developed. Of these, carfilzomib and ixazomib have both been FDA approved for the treatment of RRMM, in 2012 and 2015, respectively [24, 25]. Carfilzomib has a different active moiety to bortezomib (epoxyketone as opposed to the boronate of bortezomib) and is more specific for the chymotrypsin-like activity of the proteasome than bortezomib, which it inhibits in an irreversible manner [26]. Ixazomib, on the other hand, is based on the same structural moiety as bortezomib (boronate), and, thus, unsurprisingly is a reversible inhibitor of primarily chymotrypsin-like proteasome activity, but also trypsin- and caspase-like activity [21]. However, unlike bortezomib and carfilzomib, ixazomib is orally bioavailable, and has a better pharmacokinetic profile than bortezomib [21]. The structures of these inhibitors and their similar mode of binding to the β5 subunit of the proteasome are shown in Fig. 4.2. Both carfilzomib and ixazomib have been shown to be effective in bortezomib-resistant patients, though some cross-resistance between PIs has been observed [27–29].

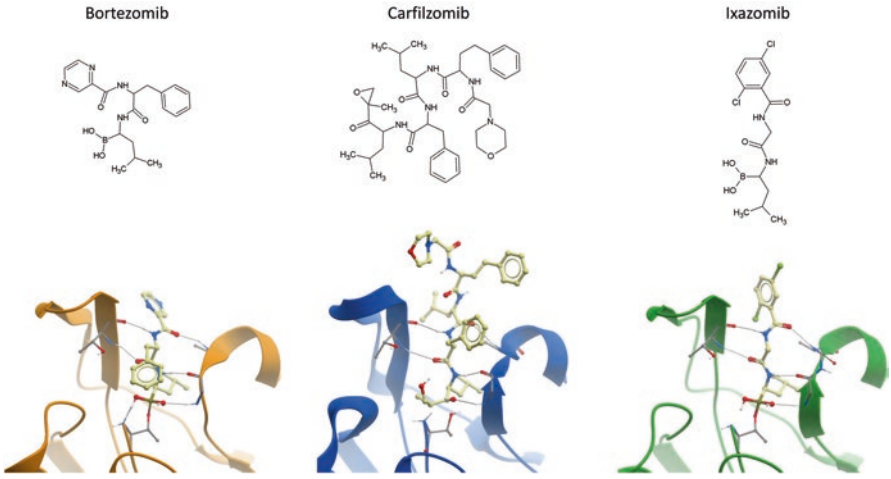

Fig. 4.2 Proteasome inhibitors and their interactions with the proteasomal subunit PSMB5. The structures of bortezomib, carfilzomib, and ixazomib are shown, along with how these drugs interact with the proteasomal subunit PSMB5. The PSMB5 protein is shown in ribbon format, and the atoms within the inhibitors are represented with different colors; red is oxygen, blue is nitrogen, white is hydrogen, green is chlorine, and brown is boron. Black dots represent hydrogen bonds between the protein and the inhibitor. Modeling was performed in Molsoft's ICM-Pro, and structures were obtained from the protein database (code 5LF3 for structure with bortezomib, 4R67 for structure with carfilzomib, and 5LF7 for structure with ixazomib)

4.5 Bortezomib Resistance Mechanisms

Almost as soon as bortezomib was FDA approved, research into bortezomib resistance and how it may potentially be overcome was already underway [30]. In the last two decades, a significant amount of research concerning the mechanisms of bortezomib resistance has been conducted.

4.5.1 Proteasome Mutation and Overexpression

One of the first proposed mechanisms of bortezomib resistance was mutation and/or overexpression of the proteasome, especially the β5 subunit (PSMB5, encoded by the *PSMB5* gene) to which bortezomib primarily binds [31]. In bortezomib-resistant cell lines, generated by exposing cells in vitro to escalating doses of bortezomib, both mutations in the bortezomib binding pocket of PSMB5 (Ala49→Thr), as well as up to 60-fold upregulation of PSMB5 protein expression, were observed [31]. The Ala49Thr mutation has since been found in independently generated bortezomib-resistant myeloma cell lines [32], as well as other bortezomib-resistant cell lines from different hematological lineages generated in a similar way [33, 34]. Furthermore, bortezomib-resistant cell lines which do not possess any PSMB5 mutations have been shown to have upregulated PSMB5 expression, although this has not always appeared to be the main mechanism of resistance [35, 36].

However, until recently, these observations from in vitro studies had not been seen in patients with myeloma. Several studies that sequenced patient samples, largely at diagnosis but also at relapse, showed no correlation between PSMB5 single nucleotide polymorphisms (SNPs) and bortezomib resistance, and no mutations within the bortezomib binding pocket [37–40]. Therefore, for a time, the idea of proteasome mutation and upregulation playing an important role in bortezomib-resistant patients fell out of favor among researchers. However, a recent study which conducted deep sequencing on a patient with relapsed myeloma found low-frequency PSMB5 mutations which correlated with resistance, and that have been confirmed to confer resistance in vitro [41]. Furthermore, overexpression of PSMB5 that correlated with bortezomib resistance has been detected in one patient [42]. Thus, these mechanisms of resistance may play an important role for some patients resistant to bortezomib.

4.5.2 Drug Efflux

The ATP-binding cassette (ABC) transporters are a superfamily of membrane transport proteins that play a well-established role in the efflux of drugs, and thus the development of drug resistance, so much so that some of them were originally

discovered and named as multidrug resistance proteins [43]. Though bortezomib efflux by multidrug resistance proteins MRP1, MRP2, MRP3 (ABCC1, ABCC2, and ABCC3), and breast cancer resistance protein (ABCG2) has been tested, only the multidrug resistance protein MDR1 (also known as ABCB1 or p-glycoprotein) has been associated with bortezomib efflux and bortezomib resistance in in vitro settings [44–46]. Although this has been largely demonstrated in overexpression systems, a recent study has shown that hypoxia increased both MDR1 and proteasome inhibitor resistance, and that this resistance could be reversed using a MDR1 inhibitor [47]. However, analysis of parental myeloma cell lines and clinical samples has found little to no association between MDR1 and bortezomib resistance, suggesting that bortezomib may be a poor substrate for MDR1, and that MDR1 is unlikely to play a significant role in bortezomib-resistant myeloma [44, 48–50].

4.5.3 Plasma Cell Differentiation

Expression of the UPR-activated transcription factor XBP1s is required for B-cells to differentiate into plasma cells and produce immunoglobulin [51]. Leung-Hagesteijn et al. found that loss of XBP1s, and thus de-commitment to plasma cell differentiation, is able to confer bortezomib resistance in myeloma [52]. Suppression of XBP1s in myeloma cell lines induced a switch from a mature plasma cell phenotype to a pre-plasmablast phenotype, including a decrease in immunoglobulin production [52]. With a lower protein production load, the pre-plasmablast-like cells showed lower basal UPR activation, and thus increased resistance to proteasome inhibitors [52]. The reverse also holds true; myeloma cells with a more mature phenotype express higher levels of XBP1 have higher immunoglobulin production, and are more sensitive to bortezomib [53]. Furthermore, loss of plasma cell maturation has also been associated with bortezomib resistance in animal models [54]. Both innate and acquired bortezomib resistance in a plasma cell malignancy in Bcl-xl/Myc transgenic mice was found to correlate with loss of plasma cell maturation markers, and induction of plasma cell maturation was able to render these cells sensitive to bortezomib [54].

Changes to XBP1s and plasma cell maturation with bortezomib resistance have also been observed in patient samples. It was found that, at diagnosis, the majority of myeloma cells were XBP1s positive plasma cells or plasmablasts [52]. However, some patients whose disease progressed on bortezomib had a large subpopulation of XBP1 negative, less differentiated myeloma cells [52]. Furthermore, it was cells of this phenotype which went on to survive bortezomib-based therapies as a minimal residual disease [52]. Other studies have also found that patients with myeloma that was sensitive to bortezomib therapy had higher paraprotein expression and higher levels of XBP1s [53]. Other studies have also identified XBP1/XBP1s levels to be a marker of bortezomib response, and overexpression of XBP1s was able to increase

the bortezomib sensitivity of a bortezomib-resistant myeloma cell line, although notably XBP1 knockdown was unable to induce bortezomib resistance in bortezomib sensitive cells [55]. Thus, there is solid evidence to suggest that plasma cell dedifferentiation contributes to bortezomib resistance, though this is unlikely to be the case for all patients.

4.5.4 Upregulation of Heat Shock Proteins

Heat shock proteins are a large family of molecular chaperones which play a key role in protein folding and trafficking, as well as degradation of unfolded proteins [56]. Thus, heat shock proteins are upregulated by the UPR as a cytoprotective mechanism and have been found to be upregulated in myeloma cells exposed to bortezomib [56]. It is therefore unsurprising that heat shock proteins may play a role in bortezomib resistance.

BiP is a member of the heat shock protein family and plays a critical role in activation of the UPR [2]. Some studies have reported that BiP expression increases with disease progression, although other studies suggest that this is not always the case [57–59]. Despite this, upregulation of BiP has been found to correlate with bortezomib resistance, and inhibition of BiP via multiple mechanisms was able to enhance cell death caused by bortezomib exposure [57, 60, 61]. To this end, an anti-BiP monoclonal antibody has been engineered, as BiP has also been observed on the surface of myeloma cells, but to date it has only been tested in one relapsed refractory patient, who achieved a partial remission before relapse [59].

HSP90 is another heat shock protein involved in the regulation of unfolded proteins in the ER and has been found to be upregulated by bortezomib treatment [62, 63]. The combination of bortezomib and HSP90 inhibition causes synergistic cell death in both myeloma cell lines and primary samples [64, 65], and has also been tested in phase I/II clinical trials, although these are yet to progress further [63, 66, 67]. Given its role in protein homeostasis, it is plausible that HSP90 not only synergizes with bortezomib but may contribute to bortezomib resistance. Although this has been shown in other hematological cancers, there is yet to be an in-depth study examining the role HSP90 plays in the development of bortezomib resistance [68, 69]. However, heat shock protein HSPB8 has been shown to play a role in bortezomib resistance, as least in vitro [70]. A myeloma cell line made resistant to bortezomib was found to have increased levels of HSPB8, and overexpression of HSBP8 in wildtype cells to a similar level to that found in their resistant counterparts was able to confer bortezomib resistance by increasing the clearance of protein aggregates [70].

4.5.5 Autophagy

Activation of the UPR has been shown to upregulate autophagy, a process by which cytosolic contents are surrounded by a double membrane to form a vesicle called an autophagosome, which then fuses with the lysosome in order to degrade its contents [71]. Autophagy has been shown to be critical for plasma cell survival, especially of long-lived plasma cells [72]. Furthermore, autophagic degradation of proteins marked for degradation can promote cell survival during proteasomal inhibition, and thus bortezomib treatment often results in upregulation of autophagy-related proteins [71, 72]. Thus, it is not surprising that autophagy has been implicated in bortezomib resistance. Indeed, the ability of both BiP and HSPB8 to confer bortezomib resistance has, in some cases, been tied to the development of autophagy [57, 61, 70].

The ability of a myeloma cell to increase autophagy has been correlated with sensitivity to bortezomib, with cells that are unable to increase their autophagic capacity having greater sensitivity to proteasome inhibition [73]. Furthermore, overexpression of autophagy-inducing proteins has been shown to cause bortezomib resistance, while inhibition of these same proteins enhances bortezomib-induced cell death [73–75]. Comparing the differential expression of microRNAs in bortezomib sensitive and resistant myeloma cells, Jagannathan et al. found that miR-29b is downregulated in bortezomib-resistant cells, and its replacement with a synthetic mimetic increased bortezomib-induced cell death through both reduction in proteasome activity and inhibition of autophagosome formation [76]. Application of an anti-β_2-microglobin (β_2M) monoclonal antibody to bortezomib-resistant myeloma cell lines and patient samples enhances bortezomib-induced cell death, which was in part due to inhibition of autophagy [77], while a phase I clinical trial of the autophagy-inducing drug hydroxychloroquine in combination with bortezomib in patients with relapsed/refractory myeloma has also been conducted, though results were modest at best [78].

Combined, the above findings would suggest a key role for autophagy in bortezomib resistance. However, as is often the case in cancer, the situation is complex [72]. Kawaguchi et al. found that inhibition of the later stages of autophagy enhanced bortezomib-induced cell death, but inhibition of early autophagy actually attenuated it [79]. Furthermore, although autophagy was upregulated in bortezomib-resistant cells, knockdown of ATG5, required for autophagosome formation, inhibited bortezomib-induced cell death of myeloma cells [80]. It has been suggested that these divergent responses may be due to what stage of autophagy is inhibited, with inhibition of late autophagy, where cellular contents have already been sequestered but are unable to be recycled, being more likely to cause cytotoxic effects [72].

4.5.6 The Bone Marrow Microenvironment

It is becoming increasingly clear that the tumor microenvironment plays a key role in resistance to therapy [81]. The bone marrow microenvironment is complex, consisting of several types of cells, including bone marrow stromal cells (BMSCs), endothelial cells, osteoblasts, osteoclasts, and many types of immune cells, as well as extracellular matrix, chemokines, and growth factors [82]. Bortezomib resistance conferred by the bone marrow microenvironment can be generally classified into two main categories, resistance generated by adhesion to various components of the microenvironment, and resistance mediated by soluble factors secreted by the microenvironment.

There are several different physical interactions between myeloma cells and their microenvironment which are able to confer drug resistance [83]. Integrin-β7 expression in myeloma cells correlates with poor patient survival and assists in myeloma cell adhesion to bone marrow stromal cells and fibronectin, the latter of which is able to convey bortezomib resistance [84]. Coculture with BMSCs also confers bortezomib resistance in myeloma cell lines, which can be prevented by inhibition of the chemokine receptor CXCR4, which blocks adhesion [85]. Furthermore, myeloma cells are able to induce a microenvironment more permissive to bortezomib resistance, for example, through inducing BMSCs to become more like cancer-associated fibroblasts [86]. Direct contact with these cells has been shown to induce bortezomib resistance in myeloma cell lines via β-catenin upregulation [86].

As well as physical contact, the bone marrow microenvironment secretes a number of soluble factors which are able to contribute to bortezomib resistance in myeloma cells [83]. For example, multiple members of the interleukin family have been found to play a role in bortezomib resistance. Interleukin (IL)-6 is very important for myeloma survival and proliferation, and BMSCs from myeloma patients have been shown to produce more IL-6 than normal BMSC [83, 87]. Furthermore, IL-6 can induce bortezomib resistance via upregulation of JunB, a transcription factor which appears to promote cell proliferation and regulate apoptosis in myeloma [88]. IL-8 is also produced at higher levels by BMSCs from myeloma patients compared to healthy controls, and this can confer bortezomib resistance via NF-κB activation [89]. Similarly, exposure to IL-10, produced by BMSCs upon exposure to the chemokine CCL27, confers bortezomib resistance, which can be reversed by an IL-10 blocking antibody [90].

In myeloma cells, there can exist cross-activation between IL-6 and insulin-like growth factor-1 (IGF-1) [91]. IGF-1 promotes myeloma proliferation through activation of pathways such as Ras and Akt, and the IGF-1 receptor has been shown to be upregulated in bortezomib-resistant cells, with inhibition restoring bortezomib sensitivity [35, 91]. Both IGF-1 and IL-6 have also been shown to activate the phosphotidylinositol 3-kinase (PI3K) pathway, and inhibition of PI3K activity reduced bortezomib resistance induced by coculturing myeloma cells with BMSCs [92]. Other factors able to influence levels of ERK1/2, Akt and/or NF-κB signaling, such as B-cell activating factor, macrophage inflammatory protein-1α, and exosomes

from BMSCs, have also been shown to play a role in bortezomib resistance, suggesting that these pathways may be common resistance mechanisms [93]. Recently, it has also been found that mesenchymal stem cells (MSCs) from bortezomib-resistant patients, but not sensitive patients, produce exosomes which can induce bortezomib resistance via increasing levels of the proteasome subunit PSMA3, which contributes to the chymotrypsin-like activity of the proteasome [94].

4.6 Resistance Mechanisms to Second Generation Proteasome Inhibitors

Second-generation PIs, including carfilzomib and ixazomib, have been clinically available for a significantly shorter period of time than bortezomib, and as such there has been less research into potential resistance mechanisms. However, it is interesting to note that many bortezomib resistance mechanisms, such as heat shock protein regulation, autophagy, and plasma cell dedifferentiation, provide the cell with ways to counteract proteasome inhibition, instead of preventing inhibition from occurring. As such, one might anticipate that these resistance mechanisms result in resistance to any proteasome inhibitor, as they are not dependent on the structure of bortezomib in the way proteasome mutations may be. Indeed, while second-generation PIs have been shown to be effective in the bortezomib-resistant setting, it has already been noted that a degree of cross-resistance does occur, with bortezomib-naïve patients more likely to respond than those who have developed bortezomib resistance [28, 29, 95].

4.6.1 Carfilzomib Resistance Mechanisms

4.6.1.1 Proteasome Mutations

Carfilzomib is based on a different active moiety, and thus interacts with slightly different residues within the binding pocket on the proteasome, as seen in Fig. 4.2 [21, 41]. A number of residues within the binding pocket of PSMB5 do however interact with both bortezomib and carfilzomib, meaning there are mutations in PSMB5 which can confer both bortezomib and carfilzomib resistance [41]. However, other unique interactions between carfilzomib and PSMB5, along with the fact that carfilzomib binds irreversibly, where bortezomib does not, means that often PSMB5 subunits bearing these mutations are less resistant to carfilzomib than they are to bortezomib [41].

4.6.1.2 Drug Efflux

Unlike bortezomib, evidence suggests that carfilzomib is much more likely to be a true MDR1/p-glycoprotein substrate [34, 50, 96, 97]. MDR1 overexpression is seen in both carfilzomib-resistant cell lines generated by long-term exposure to carfilzomib and in carfilzomib-resistant patients, and engineering myeloma cell lines to overexpress MDR1 is sufficient to convey carfilzomib resistance [34, 50, 96, 97]. Upregulation of another ABC transporter, ABCG2 (alternatively referred to as breast cancer resistance protein) has also been seen in carfilzomib-resistant patients but not carfilzomib-resistant cell lines; the significance of this is yet to be investigated [98]. Furthermore, it has been found that pharmacological inhibition of MDR1 is able to significantly increase carfilzomib-induced cell death in carfilzomib-resistant myeloma cell lines [98]. A similar result has also been seen using MDR1 peptide inhibitors in carfilzomib-resistant adenocarcinoma cell lines [96].

4.6.1.3 Autophagy

Carfilzomib is also able to upregulate autophagy in myeloma cells, and the autophagy-linked miR-29b, found downregulated in bortezomib-resistant myeloma cells, was also found to be downregulated in carfilzomib-resistant cells [76, 99]. Furthermore, myeloma cells made resistant to carfilzomib have shown an upregulation of SQSTM1, an autophagy receptor that gathers misfolded proteins into aggregates and links them to autophagic membranes [100]. Notably, overexpression of SQSTM1 is enough to convey resistance to carfilzomib [100, 101]. Inhibition of the autophagic system, both directly by chloroquine and indirectly via histone deacetylase 6 (HDAC6) inhibition (which stops unfolded proteins forming aggregates called aggresomes that can be degraded by autophagy) potentiates carfilzomib-induced cell death [99, 101, 102]. Interestingly, the combination of chloroquine and bortezomib has little to no effect in vitro, suggesting this may be a mechanism more specific to carfilzomib [99, 101].

4.6.1.4 Bone Marrow Microenvironment

As well as conferring bortezomib resistance, exposure to CCL27, which is produced by BMSCs, also confers resistance to carfilzomib [90]. Culturing myeloma cells with BMSCs is able to confer carfilzomib resistance as well as bortezomib resistance [85, 103, 104], and Azab et al. found that inhibition of PI3K was able to prevent this resistance [92]. Like bortezomib resistance, it has also been found that carfilzomib resistance can be induced by incubating cells with exosomes from bortezomib-resistant patient MSCs and that this is due to increases in PSMA3 [94]. The fact that bortezomib-resistant patient MSCs are able to directly generate carfilzomib resistance highlights the potential similarities between carfilzomib and bortezomib resistance generated by the bone marrow microenvironment.

4.6.2 Ixazomib Resistance Mechanisms

Ixazomib is the newest PI to be approved by the FDA [25]. Ixazomib has been found to increase progression free survival (PFS) of RRMM patients and is highly efficacious even in patients with high cytogenetic risk or patients who have previously been treated with a PI [105, 106]. This may be due at least partially to a more favorable pharmacokinetic profile, resulting in a higher plasma concentration and a greater distribution of ixazomib from the blood into tissue compared to bortezomib [107]. Given how new it is to the clinic, relatively little research has been conducted regarding potential resistance mechanisms to ixazomib. However, given its structural similarities to bortezomib, it is likely that there will be overlap in resistance mechanisms, despite the effectiveness of ixazomib in relapsed/refractory myeloma.

This has already been seen with proteasome mutants found during deep sequencing analysis, where mutations in PSMB5 which conferred bortezomib resistance also conferred resistance to ixazomib [41]. Given that ixazomib is much closer in structure to bortezomib than carfilzomib, as it is based on the same boronate backbone (Fig. 4.2), it is more likely that proteasome mutations which convey bortezomib resistance will also convey ixazomib resistance [21].

While looking at resistance to more commonly used PI, it was found that the autophagy-linked miR-29b, downregulated in both bortezomib and carfilzomib-resistant cells, was also found to be downregulated in myeloma cells that had been made resistant to ixazomib [76]. Using similar cell lines which had been made resistant to either bortezomib, carfilzomib, or ixazomib, Malek et al. found a high degree of cross-resistance between proteasome inhibitors, and that expression of certain long noncoding RNAs (lncRNAs) were dysregulated in all resistant cells compared to the parental cell lines [108]. These same lncRNAs were found to be dysregulated in myeloma cells from patients compared to healthy plasma cells [108]. The lncRNA which stabilizes PSMA3, along with PSMA3 itself, which increases the chymotrypsin-like activity of the proteasome, has also been found to be upregulated in ixazomib-resistant cell lines, as it is in bortezomib and carfilzomib-resistant lines, further highlighting potential similarities between bortezomib, carfilzomib, and ixazomib resistance [94].

4.7 Conclusion

Within the last two decades, PIs have become a standard-of-care in myeloma treatment. However, myeloma cells inevitably become resistant to PIs, posing a significant hurdle to the treatment of patients. Research reaching back almost as long as bortezomib has been in the clinic has demonstrated that bortezomib resistance is highly complex, and can include a variety of mechanisms such as proteasome mutations, upregulation of cellular pathways including heat shock proteins and

autophagy, plasma cell dedifferentiation, and interactions with the bone marrow microenvironment [109].

While newer generations of PIs, including carfilzomib and ixazomib, have proven to be effective in the bortezomib-resistant setting, cross-resistance is already being recognized as an issue [34, 50, 96, 97]. This is likely due to the fact that many bortezomib resistance mechanisms assist the cell in surviving proteasome inhibition, instead of preventing it, and are thus able to promote survival regardless of the structure of the proteasome inhibitor used. Thus, although proteasome inhibitors have been an important advance in myeloma pharmacotherapy, resistance to these agents represents a serious clinical problem that often requires combining more than one novel agent to target non-overlapping aspects of myeloma biology.

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References

1. Bianchi G, Anderson KC. Understanding biology to tackle the disease: multiple myeloma from bench to bedside, and back. CA Cancer J Clin. 2014;64(6):422–44.
2. Vincenz L, Jager R, O'Dwyer M, Samali A. Endoplasmic reticulum stress and the unfolded protein response: targeting the Achilles heel of multiple myeloma. Mol Cancer Ther. 2013;12(6):831–43.
3. Jung T, Grune T. Structure of the proteasome. Prog Mol Biol Transl Sci. 2012;109:1–39.
4. Forster F, Unverdorben P, Sledz P, Baumeister W. Unveiling the long-held secrets of the 26S proteasome. Structure. 2013;21(9):1551–62.
5. Livneh I, Cohen-Kaplan V, Cohen-Rosenzweig C, Avni N, Ciechanover A. The life cycle of the 26S proteasome: from birth, through regulation and function, and onto its death. Cell Res. 2016;26(8):869–85.
6. Murata S, Takahama Y, Kasahara M, Tanaka K. The immunoproteasome and thymoproteasome: functions, evolution and human disease. Nat Immunol. 2018;19(9):923–31.
7. Schroder M. Endoplasmic reticulum stress responses. Cell Mol Life Sci. 2008;65(6):862–94.
8. Szegezdi E, Logue SE, Gorman AM, Samali A. Mediators of endoplasmic reticulum stress-induced apoptosis. EMBO Rep. 2006;7(9):880–5.
9. Gardner BM, Walter P. Unfolded proteins are Ire1-activating ligands that directly induce the unfolded protein response. Science. 2011;333(6051):1891–4.
10. Karagöz GE, Acosta-Alvear D, Nguyen LCP, Chu F, Walter P. An unfolded protein-induced conformational switch activates mammalian IRE1. Elife. 2017;6:e30700.
11. Hetz C, Papa FR. The unfolded protein response and cell fate control. Mol Cell. 2018;69(2):169–81.
12. Walter P, Ron D. The unfolded protein response: from stress pathway to homeostatic regulation. Science. 2011;334(6059):1081–6.
13. Hetz C. The unfolded protein response: controlling cell fate decisions under ER stress and beyond. Nat Rev Mol Cell Biol. 2012;13(2):89–102.

14. Urra H, Dufey E, Lisbona F, Rojas-Rivera D, Hetz C. When ER stress reaches a dead end. BBA. 2013;1833(12):3507–17.
15. Hollien J, Lin JH, Li H, Stevens N, Walter P, Weissman JS. Regulated Ire1-dependent decay of messenger RNAs in mammalian cells. J Cell Biol. 2009;186(3):323–31.
16. Maurel M, Chevet E, Tavernier J, Gerlo S. Getting RIDD of RNA: IRE1 in cell fate regulation. Trends Biochem Sci. 2014;39(5):245–54.
17. Harding HP, Zhang Y, Bertolotti A, Zeng H, Ron D. PERK is essential for translational regulation and cell survival during the unfolded protein response. Mol Cell. 2000;5:897–904.
18. Han J, Back SH, Hur J, Lin YH, Gildersleeve R, Shan J, et al. ER-stress-induced transcriptional regulation increases protein synthesis leading to cell death. Nat Cell Biol. 2013;15(5):481–90.
19. Kane RC, Bross PF, Farrell AT, Pazdur R. Velcade: U.S. FDA approval for the treatment of multiple myeloma progressing on prior therapy. Oncologist. 2003;8(6):508–13.
20. Merin NM, Kelly KR. Clinical use of proteasome inhibitors in the treatment of multiple myeloma. Pharmaceuticals (Basel). 2014;8(1):1–20.
21. Kubiczkova L, Pour L, Sedlarikova L, Hajek R, Sevcikova S. Proteasome inhibitors—molecular basis and current perspectives in multiple myeloma. J Cell Mol Med. 2014;18(6):947–61.
22. Hideshima T, Ikeda H, Chauhan D, Okawa Y, Raje N, Podar K, et al. Bortezomib induces canonical nuclear factor-kappaB activation in multiple myeloma cells. Blood. 2009;114(5):1046–52.
23. Obeng E, Carlson L, Gutman D, Harrington W Jr, Lee K, Boise L. Proteasome inhibitors induce a terminal unfolded protein response in multiple myeloma cells. Blood. 2006;107:4907–16.
24. Raedler LA. Kyprolis (Carfilzomib) received new indications as combination therapy for use in relapsed and/or refractory multiple myeloma. Am Health Drug Benefits. 2016;9:93–6.
25. Raedler LA. Ninlaro (Ixazomib): first oral proteasome inhibitor approved for the treatment of patients with relapsed or refractory multiple myeloma. Am Health Drug Benefits. 2016;9:102–5.
26. Gandolfi S, Laubach JP, Hideshima T, Chauhan D, Anderson KC, Richardson PG. The proteasome and proteasome inhibitors in multiple myeloma. Cancer Metastasis Rev. 2017;36(4):561–84.
27. Kuhn DJ, Chen Q, Voorhees PM, Strader JS, Shenk KD, Sun CM, et al. Potent activity of carfilzomib, a novel, irreversible inhibitor of the ubiquitin-proteasome pathway, against preclinical models of multiple myeloma. Blood. 2007;110(9):3281–90.
28. Dimopoulos MA, Moreau P, Palumbo A, Joshua D, Pour L, Hájek R, et al. Carfilzomib and dexamethasone versus bortezomib and dexamethasone for patients with relapsed or refractory multiple myeloma (ENDEAVOR): a randomised, phase 3, open-label, multicentre study. Lancet Oncol. 2016;17(1):27–38.
29. Khan ML, Stewart AK. Carfilzomib: a novel second-generation proteasome inhibitor. Future Oncol. 2011;7(5):607–12.
30. Chauhan D, Li G, Podar K, Hideshima T, Mitsiades C, Schlossman R, et al. Targeting mitochondria to overcome conventional and bortezomib/proteasome inhibitor PS-341 resistance in multiple myeloma (MM) cells. Blood. 2004;104(8):2458–66.
31. Oerlemans R, Franke NE, Assaraf YG, Cloos J, van Zantwijk I, Berkers CR, et al. Molecular basis of bortezomib resistance: proteasome subunit beta5 (PSMB5) gene mutation and overexpression of PSMB5 protein. Blood. 2008;112(6):2489–99.
32. Ri M, Iida S, Nakashima T, Miyazaki H, Mori F, Ito A, et al. Bortezomib-resistant myeloma cell lines: a role for mutated PSMB5 in preventing the accumulation of unfolded proteins and fatal ER stress. Leukemia. 2010;24(8):1506–12.
33. Lu S, Yang J, Song X, Gong S, Zhou H, Guo L, et al. Point mutation of the proteasome beta5 subunit gene is an important mechanism of bortezomib resistance in bortezomib-selected variants of Jurkat T cell lymphoblastic lymphoma/leukemia line. J Pharmacol Exp Ther. 2008;326(2):423–31.

34. Verbrugge SE, Assaraf YG, Dijkmans BA, Scheffer GL, Al M, den Uyl D, et al. Inactivating PSMB5 mutations and P-glycoprotein (multidrug resistance-associated protein/ATP-binding cassette B1) mediate resistance to proteasome inhibitors: ex vivo efficacy of (immuno)proteasome inhibitors in mononuclear blood cells from patients with rheumatoid arthritis. J Pharmacol Exp Ther. 2012;341(1):174–82.
35. Kuhn DJ, Berkova Z, Jones RJ, Woessner R, Bjorklund CC, Ma W, et al. Targeting the insulin-like growth factor-1 receptor to overcome bortezomib resistance in preclinical models of multiple myeloma. Blood. 2012;120(16):3260–70.
36. Balsas P, Galan-Malo P, Marzo I, Naval J. Bortezomib resistance in a myeloma cell line is associated to PSMB5 overexpression and polyploidy. Leuk Res. 2012;36(2):212–8.
37. Lichter DI, Danaee H, Pickard MD, Tayber O, Sintchak M, Shi H, et al. Sequence analysis of B-subunit genes of the 20S proteasome in patients with relapsed multiple myeloma treated with bortezomib or dexamethasone. Blood. 2012;120(23):4513–6.
38. Kortuem KM, Braggio E, Bruins L, Barrio S, Shi CS, Zhu YX, et al. Panel sequencing for clinically oriented variant screening and copy number detection in 142 untreated multiple myeloma patients. Blood Cancer J. 2016;6:e397.
39. Walker BA, Boyle EM, Wardell CP, Murison A, Begum DB, Dahir NM, et al. Mutational spectrum, copy number changes, and outcome: results of a sequencing study of patients with newly diagnosed myeloma. J Clin Oncol. 2015;33(33):3911–20.
40. Egan JB, Shi CX, Tembe W, Christoforides A, Kurdoglu A, Sinari S, et al. Whole-genome sequencing of multiple myeloma from diagnosis to plasma cell leukemia reveals genomic initiating events, evolution, and clonal tides. Blood. 2012;120(5):1060–6.
41. Barrio S, Stuhmer T, Da-Via M, Barrio-Garcia C, Lehners N, Besse A, et al. Spectrum and functional validation of PSMB5 mutations in multiple myeloma. Leukemia. 2019;33:447–56.
42. Shuqing L, Jianmin Y, Chongmei H, Hui C, Wang J. Upregulated expression of the PSMB5 gene may contribute to drug resistance in patient with multiple myeloma when treated with bortezomib-based regimen. Exp Hematol. 2011;39(12):1117–8.
43. Chen Z, Shi T, Zhang L, Zhu P, Deng M, Huang C, et al. Mammalian drug efflux transporters of the ATP binding cassette (ABC) family in multidrug resistance: a review of the past decade. Cancer Lett. 2016;370(1):153–64.
44. Clemens J, Seckinger A, Hose D, Theile D, Longo M, Haefeli WE, et al. Cellular uptake kinetics of bortezomib in relation to efficacy in myeloma cells and the influence of drug transporters. Cancer Chemother Pharmacol. 2015;75(2):281–91.
45. O'Connor R, Ooi MG, Meiller J, Jakubikova J, Klippel S, Delmore J, et al. The interaction of bortezomib with multidrug transporters: implications for therapeutic applications in advanced multiple myeloma and other neoplasias. Cancer Chemother Pharmacol. 2013;71(5):1357–68.
46. Rumpold H, Salvador C, Wolf AM, Tilg H, Gastl G, Wolf D. Knockdown of PgP resensitizes leukemic cells to proteasome inhibitors. Biochem Biophys Res Commun. 2007;361(2):549–54.
47. Muz B, Kusdono HD, Azab F, de la Puente P, Federico C, Fiala M, et al. Tariquidar sensitizes multiple myeloma cells to proteasome inhibitors via reduction of hypoxia-induced P-gp-mediated drug resistance. Leuk Lymphoma. 2017;58(12):2916–25.
48. Wiberg K, Carlson K, Aleskog A, Larsson R, Nygren P, Lindhagen E. In vitro activity of bortezomib in cultures of patient tumour cells—potential utility in haematological malignancies. Med Oncol. 2009;26(2):193–201.
49. Buda G, Ricci D, Huang CC, Favis R, Cohen N, Zhuang SH, et al. Polymorphisms in the multiple drug resistance protein 1 and in P-glycoprotein 1 are associated with time to event outcomes in patients with advanced multiple myeloma treated with bortezomib and pegylated liposomal doxorubicin. Ann Hematol. 2010;89(11):1133–40.
50. Lu S, Wang J. The resistance mechanisms of proteasome inhibitor bortezomib. Biomarker Res. 2013;1:13.

51. Iwakoshi NN, Lee AH, Vallabhajosyula P, Otipoby KL, Rajewsky K, Glimcher LH. Plasma cell differentiation and the unfolded protein response intersect at the transcription factor XBP-1. Nat Immunol. 2003;4(4):321–9.
52. Leung-Hagesteijn C, Erdmann N, Cheung G, Keats JJ, Stewart AK, Reece DE, et al. Xbp1s-negative tumor B cells and pre-plasmablasts mediate therapeutic proteasome inhibitor resistance in multiple myeloma. Cancer Cell. 2013;24(3):289–304.
53. Gu JL, Li J, Zhou ZH, Liu JR, Huang BH, Zheng D, et al. Differentiation induction enhances bortezomib efficacy and overcomes drug resistance in multiple myeloma. Biochem Biophys Res Commun. 2012;420(3):644–50.
54. Stessman HA, Mansoor A, Zhan F, Linden MA, Van Ness B, Baughn LB. Bortezomib resistance can be reversed by induced expression of plasma cell maturation markers in a mouse in vitro model of multiple myeloma. PLoS One. 2013;8(10):e77608.
55. Ling SC, Lau EK, Al-Shabeeb A, Nikolic A, Catalano A, Iland H, et al. Response of myeloma to the proteasome inhibitor bortezomib is correlated with the unfolded protein response regulator XBP-1. Haematologica. 2012;97(1):64–72.
56. Shah SP, Lonial S, Boise LH. When cancer fights back: multiple myeloma, proteasome inhibition, and the heat-shock response. Mol Cancer Res. 2015;13(8):1163–73.
57. Abdel Malek MA, Jagannathan S, Malek E, Sayed DM, Elgammal SA, Abd El-Azeem HG, et al. Molecular chaperone GRP78 enhances aggresome delivery to autophagosomes to promote drug resistance in multiple myeloma. Oncotarget. 2015;6(5):3098–110.
58. Steiner N, Borjan B, Hajek R, Johrer K, Gobel G, Willenbacher W, et al. Expression and release of glucose-regulated protein-78 (GRP78) in multiple myeloma. Oncotarget. 2017;8(34):56,243–54.
59. Rasche L, Menoret E, Dubljevic V, Menu E, Vanderkerken K, Lapa C, et al. A GRP78-directed monoclonal antibody recaptures response in refractory multiple myeloma with extramedullary involvement. Clin Cancer Res. 2016;22(17):4341–9.
60. Adomako A, Calvo V, Biran N, Osman K, Chari A, Paton JC, et al. Identification of markers that functionally define a quiescent multiple myeloma cell sub-population surviving bortezomib treatment. BMC Cancer. 2015;15:444.
61. Jagannathan S, Abdel-Malek MA, Malek E, Vad N, Latif T, Anderson KC, et al. Pharmacologic screens reveal metformin that suppresses GRP78-dependent autophagy to enhance the anti-myeloma effect of bortezomib. Leukemia. 2015;29(11):2184–91.
62. Mitsiades N, Mitsiades C, Poulaki V, Chauhan D, Fanourakis G, Gu X, et al. Molecular sequelae of proteasome inhibition in human multiple myeloma cells. Proc Natl Acad Sci U S A. 2002;99(22):14,374–9.
63. Zhang L, Fok JHL, Davies F. Heat shock proteins in multiple myeloma. Oncotarget. 2014;5(5):1132–48.
64. Khong T, Spencer A. Targeting HSP 90 induces apoptosis and inhibits critical survival and proliferation pathways in multiple myeloma. Mol Cancer Ther. 2011;10(10):1909–17.
65. Ishii T, Seike T, Nakashima T, Juliger S, Maharaj L, Soga S, et al. Anti-tumor activity against multiple myeloma by combination of KW-2478, an Hsp90 inhibitor, with bortezomib. Blood Cancer J. 2012;2(4):e68.
66. Cavenagh J, Oakervee H, Baetiong-Caguioa P, Davies F, Gharibo M, Rabin N, et al. A phase I/II study of KW-2478, an Hsp90 inhibitor, in combination with bortezomib in patients with relapsed/refractory multiple myeloma. Br J Cancer. 2017;117(9):1295–302.
67. Usmani SZ, Chiosis G. HSP90 inhibitors as therapy for multiple myeloma. Clin Lymphoma Myeloma Leuk. 2011;11(Suppl 1):S77–81.
68. Shringarpure R, Catley L, Bhole D, Burger R, Podar K, Tai YT, et al. Gene expression analysis of B-lymphoma cells resistant and sensitive to bortezomib. Br J Haematol. 2006;134(2):145–56.
69. Roue G, Perez-Galan P, Mozos A, Lopez-Guerra M, Xargay-Torrent S, Rosich L, et al. The Hsp90 inhibitor IPI-504 overcomes bortezomib resistance in mantle cell lymphoma

in vitro and in vivo by down-regulation of the prosurvival ER chaperone BiP/Grp78. Blood. 2011;117(4):1270–9.
70. Hamouda MA, Belhacene N, Puissant A, Colosetti P, Robert G, Jacquel A, et al. The small heat shock protein B8 (HSPB8) confers resistance to bortezomib by promoting autophagic removal of misfolded proteins in multiple myeloma cells. Oncotarget. 2014;5(15):6252–66.
71. Benbrook DM, Long A. Integration of autophagy, proteasomal degradation, unfolded protein response and apoptosis. Exp Oncol. 2012;34(3):286–97.
72. Yun Z, Zhichao J, Hao Y, Ou J, Ran Y, Wen D, et al. Targeting autophagy in multiple myeloma. Leuk Res. 2017;59:97–104.
73. Milan E, Perini T, Resnati M, Orfanelli U, Oliva L, Raimondi A, et al. A plastic SQSTM1/p62-dependent autophagic reserve maintains proteostasis and determines proteasome inhibitor susceptibility in multiple myeloma cells. Autophagy. 2015;11(7):1161–78.
74. Zhang H, Pang Y, Ma C, Li J, Wang H, Shao Z. ClC5 decreases the sensitivity of multiple myeloma cells to bortezomib via promoting prosurvival autophagy. Oncol Res. 2018;26(3):421–9.
75. Lu Y, Wang Y, Xu H, Shi C, Jin F, Li W. Profilin 1 induces drug resistance through Beclin1 complex-mediated autophagy in multiple myeloma. Cancer Sci. 2018;109(9):2706–16.
76. Jagannathan S, Vad N, Vallabhapurapu S, Vallabhapurapu S, Anderson KC, Driscoll JJ. MiR-29b replacement inhibits proteasomes and disrupts aggresome+autophagosome formation to enhance the antimyeloma benefit of bortezomib. Leukemia. 2015;29(3):727–38.
77. Zhang M, He J, Liu Z, Lu Y, Zheng Y, Li H, et al. Anti-β2-microglobulin monoclonal antibody overcomes bortezomib resistance in multiple myeloma by inhibiting autophagy. Oncotarget. 2015;6(11):8567–78.
78. Vogl DT, Stadtmauer EA, Tan KS, Heitjan DF, Davis LE, Pontiggia L, et al. Combined autophagy and proteasome inhibition: a phase 1 trial of hydroxychloroquine and bortezomib in patients with relapsed/refractory myeloma. Autophagy. 2014;10(8):1380–90.
79. Kawaguchi T, Miyazawa K, Moriya S, Ohtomo T, Che XF, Naito M, et al. Combined treatment with bortezomib plus bafilomycin A1 enhances the cytocidal effect and induces endoplasmic reticulum stress in U266 myeloma cells: crosstalk among proteasome, autophagy-lysosome and ER stress. Int J Oncol. 2011;38(3):643–54.
80. Jaganathan S, Malek E, Vallabhapurapu S, Vallabhapurapu S, Driscoll JJ. Bortezomib induces AMPK-dependent autophagosome formation uncoupled from apoptosis in drug resistant cells. Oncotarget. 2014;5(23):12,358–70.
81. Murray MY, Auger MJ, Bowles KM. Overcoming bortezomib resistance in multiple myeloma. Biochem Soc Trans. 2014;42(4):804–8.
82. Kawano Y, Moschetta M, Manier S, Glavey S, Gorgun G, Roccaro AM, et al. Targeting the bone marrow microenvironment in multiple myeloma. Immunol Rev. 2015;263(1):160–72.
83. Di Marzo L, Desantis V, Solimando AG, Ruggieri S, Annese T, Nico B, et al. Microenvironment drug resistance in multiple myeloma: emerging new players. Oncotarget. 2016;7(37):60,698–711.
84. Neri P, Ren L, Azab AK, Brentnall M, Gratton K, Klimowicz AC, et al. Integrin beta7-mediated regulation of multiple myeloma cell adhesion, migration, and invasion. Blood. 2011;117(23):6202–13.
85. Azab AK, Runnels JM, Pitsillides C, Moreau AS, Azab F, Leleu X, et al. CXCR4 inhibitor AMD3100 disrupts the interaction of multiple myeloma cells with the bone marrow microenvironment and enhances their sensitivity to therapy. Blood. 2009;113(18):4341–51.
86. Zi FM, He JS, Li Y, Wu C, Wu WJ, Yang Y, et al. Fibroblast activation protein protects bortezomib-induced apoptosis in multiple myeloma cells through beta-catenin signaling pathway. Cancer Biol Ther. 2014;15(10):1413–22.
87. Hao M, Zhang L, An G, Sui W, Yu Z, Zou D, et al. Suppressing miRNA-15a/-16 expression by interleukin-6 enhances drug-resistance in myeloma cells. J Hematol Oncol. 2011;4:37.

88. Fan F, Bashari MH, Morelli E, Tonon G, Malvestiti S, Vallet S, et al. The AP-1 transcription factor JunB is essential for multiple myeloma cell proliferation and drug resistance in the bone marrow microenvironment. Leukemia. 2017;31(7):1570–81.
89. Markovina S, Callander NS, O'Connor SL, Xu G, Shi Y, Leith CP, et al. Bone marrow stromal cells from multiple myeloma patients uniquely induce bortezomib resistant NF-kappaB activity in myeloma cells. Mol Cancer. 2010;9:176.
90. Thangavadivel S, Zelle-Rieser C, Olivier A, Postert B, Untergasser G, Kern J, et al. CCR10/CCL27 crosstalk contributes to failure of proteasome-inhibitors in multiple myeloma. Oncotarget. 2016;7(48):78,605–18.
91. Podar K, Chauhan D, Anderson KC. Bone marrow microenvironment and the identification of new targets for myeloma therapy. Leukemia. 2009;23(1):10–24.
92. Azab F, Vali S, Abraham J, Potter N, Muz B, de la Puente P, et al. PI3KCA plays a major role in multiple myeloma and its inhibition with BYL719 decreases proliferation, synergizes with other therapies and overcomes stroma-induced resistance. Br J Haematol. 2014;165(1):89–101.
93. Farrell ML, Reagan MR. Soluble and cell-cell-mediated drivers of proteasome inhibitor resistance in multiple myeloma. Front Endocrinol (Lausanne). 2018;9:218.
94. Xu H, Han H, Song S, Yi N, Qian C, Qiu Y, et al. Exosome-transmitted PSMA3 and PSMA3-AS1 promote proteasome inhibitor resistance in multiple myeloma. Clin Cancer Res. 2019; https://doi.org/10.1158/1078-0432.CCR-18-2363.
95. Stessman HA, Baughn LB, Sarver A, Xia T, Deshpande R, Mansoor A, et al. Profiling bortezomib resistance identifies secondary therapies in a mouse myeloma model. Mol Cancer Ther. 2013;12(6):1140–50.
96. Ao L, Wu Y, Kim D, Jang ER, Kim K, Lee DM, et al. Development of peptide-based reversing agents for p-glycoprotein-mediated resistance to carfilzomib. Mol Pharm. 2012;9(8):2197–205.
97. Hawley TS, Riz I, Yang W, Wakabayashi Y, Depalma L, Chang YT, et al. Identification of an ABCB1 (P-glycoprotein)-positive carfilzomib-resistant myeloma subpopulation by the pluripotent stem cell fluorescent dye CDy1. Am J Hematol. 2013;88(4):265–72.
98. Besse A, Stolze SC, Rasche L, Weinhold N, Morgan GJ, Kraus M, et al. Carfilzomib resistance due to ABCB1/MDR1 overexpression is overcome by nelfinavir and lopinavir in multiple myeloma. Leukemia. 2017;32(2):391–401.
99. Jarauta V, Jaime P, Gonzalo O, de Miguel D, Ramirez-Labrada A, Martinez-Lostao L, et al. Inhibition of autophagy with chloroquine potentiates carfilzomib-induced apoptosis in myeloma cells in vitro and in vivo. Cancer Lett. 2016;382(1):1–10.
100. Riz I, Hawley TS, Hawley RG. KLF4-SQSTM1/p62-associated prosurvival autophagy contributes to carfilzomib resistance in multiple myeloma models. Oncotarget. 2015;6(17):14,814–31.
101. Baranowska K, Misund K, Starheim KK, Holien T, Johansson I, Darvekar S, et al. Hydroxychloroquine potentiates carfilzomib toxicity towards myeloma cells. Oncotarget. 2016;7(43):70,845–56.
102. Mishima Y, Santo L, Eda H, Cirstea D, Nemani N, Yee AJ, et al. Ricolinostat (ACY-1215) induced inhibition of aggresome formation accelerates carfilzomib-induced multiple myeloma cell death. Br J Haematol. 2015;169(3):423–34.
103. Bustany S, Bourgeais J, Tchakarsha G, Body S, Herault O, Gouilleux F, et al. Cyclin D1 unbalances the redox status controlling cell adhesion, migration, and drug resistance in myeloma cells. Oncotarget. 2016;7(29):45,214–24.
104. Waldschmidt JM, Simon A, Wider D, Muller SJ, Follo M, Ihorst G, et al. CXCL12 and CXCR7 are relevant targets to reverse cell adhesion-mediated drug resistance in multiple myeloma. Br J Haematol. 2017;179(1):36–49.
105. Moreau P, Masszi T, Grzasko N, Bahlis NJ, Hansson M, Pour L, et al. Oral ixazomib, lenalidomide, and dexamethasone for multiple myeloma. N Engl J Med. 2016;374(17):1621–34.

106. Mateos MV, Masszi T, Grzasko N, Hansson M, Sandhu I, Pour L, et al. Impact of prior therapy on the efficacy and safety of oral ixazomib-lenalidomide-dexamethasone vs. placebo-lenalidomide-dexamethasone in patients with relapsed/refractory multiple myeloma in TOURMALINE-MM1. Haematologica. 2017;102(10):1767–75.
107. Bonnet A, Moreau P. Safety of ixazomib for the treatment of multiple myeloma. Expert Opin Drug Saf. 2017;16(8):973–80.
108. Malek E, Kim BG, Driscoll JJ. Identification of long non-coding RNAs deregulated in multiple myeloma cells resistant to proteasome inhibitors. Genes (Basel). 2016;7(10)
109. Wallington-Beddoe CT, Sobieraj-Teague M, Kuss BJ, Pitson SM. Resistance to proteasome inhibitors and other targeted therapies in myeloma. Br J Haematol. 2018;182(1):11–28.

Chapter 5
Daratumumab

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Abstract Daratumumab is a monoclonal antibody approved for the treatment of multiple myeloma (MM). Daratumumab exerts its anti-myeloma effects by targeting the CD38 (a transmembrane glycoprotein highly expressed on myeloma cells) and inducing antibody-dependent cytotoxicity, complement-dependent cytotoxicity, and antibody-dependent cellular phagocytosis. Despite well-established efficacy in both newly diagnosed and relapsed/refractory MM patient populations, a large proportion of patients fail to respond to daratumumab and thus may be daratumumab-resistant. This chapter will review the efficacy of daratumumab in notable clinical trials and discuss its mechanisms of action and the potential mechanisms behind daratumumab-resistance.

Keywords Multiple myeloma · Daratumumab · Anti-CD38 antibody · Monoclonal antibody treatment · Daratumumab-resistance

Abbreviations

ADCC	Antibody-dependent cellular toxicity
ADCP	Antibody-dependent cellular phagocytosis
ATRA	All-trans retinoic acid
CDC	Complement dependent cytotoxicity
CR	Complete response
D-VMP	Daratumumab, bortezomib, melphalan, and dexamethasone
IgG1k	Immunoglobulin G1 kappa
IMiD	Immunomodulatory imide drug
MM	Multiple myeloma
MoAb	Monoclonal antibody
MRD	Minimal residual disease
NK	Natural killer
ORR	Overall response rate

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OS	Overall survival
PFS	Progression free survival
PI	Proteasome inhibitor
PR	Partial response
RRMM	Relapsed/refractory multiple myeloma
SIRPa	Signal regulatory protein-alpha
TAMS	Tumor associated macrophages
VGPR	Very good partial response
VMP	Bortezomib, melphalan, and dexamethasone

5.1 Introduction

Daratumumab was first introduced into the clinical setting in 2008. This was driven by the poor prognosis of multiple myeloma (MM) patients who were double refractory to immunomodulatory imide drugs (IMiDs) and proteasome inhibitors (PI) triggering a demand for new treatment options with unique mechanisms of action.

Daratumumab is a high-affinity monoclonal antibody (MoAb) targeting CD38 with unique cytotoxic abilities, shown to effectively kill myeloma cells from patients by antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC). It targets a unique epitope on CD38, a transmembrane glycoprotein with differential high expression on malignant myeloma cells [1]. It was developed by Genmab, a Danish-Dutch biotech company in collaboration with the scientists at the University of Utrecht [2].

Emerging clinical trials have demonstrated the efficacy and tolerability of daratumumab when used alone and in combination with standard anti-myeloma therapies in both the newly diagnosed and relapsed and refractory setting.

This chapter will focus on daratumumab's mechanism of action, mechanisms behind drug resistance, and the efficacy data from emerging clinical trials in both the newly diagnosed and relapsed refractory setting for myeloma.

5.2 Mechanism of Action

Daratumumab is an immunoglobulin G1 kappa (IgG1k) human MoAb binding to a unique CD38 epitope on CD38 expressing cells with high affinity and was developed by immunization of human immunoglobulin transgenic mice with recombinant CD38 protein [1]. CD38 is a 46-kDa type II transmembrane glycoprotein with physiological roles in receptor-mediated adhesion, signaling events and has a bifunctional ecto-enzymatic activity that contributes to intracellular calcium mobilization [3]. CD38 is highly expressed in myeloma cells and represents a promising target for MoAb-based immunotherapy. It is also expressed in relatively lower levels on lymphoid, myeloid, and non-hematopoietic tissue [4].

Daratumumab targets CD38-positive myeloma cells via several mechanisms. The immune-mediated mechanisms include CDC, ADCC, and antibody-dependent cellular phagocytosis (ADCP). It also exerts its effect via apoptosis and crosslinking [2, 5]. The unique epitope of daratumumab on CD38 clusters and positions the Fc region of the antibody in a way that facilitates optimal binding and activation of complement proteins [6].

In vitro experiments have shown that daratumumab induces ADCC in many different tumor cell lines with varying CD38 expression. The ADCC activity was preserved despite testing on myeloma patients who had undergone a variety of previous chemotherapeutic schedules [7]. This demonstrates the ability of daratumumab to circumvent the observation that Fc gamma receptor polymorphisms in cancer patients may have a negative impact on the therapeutic responses to antibodies [8].

Yu, Qiao et al demonstrated that daratumumab induced effective lysis via ADCC and CDC in the presence of both peripheral immune effector cells and bone marrow stem cells. This observation is suggestive of daratumumab activity in the bone marrow microenvironment, an advantage from a drug resistance perspective [1]. Daratumumab has also demonstrated high efficacy in interrupting tumor growth in mouse xenograft models [1]. Nijhof et al. showed that there was no difference in daratumumab induced ADCC or CDC between newly diagnosed, relapsed/refractory, or IMID refractory myeloma patients, suggesting that resistance to prior therapies does not affect the efficacy of daratumumab [9, 10].

The ADCC mechanism of daratumumab by natural killer (NK) cells has been shown to be enhanced by drugs that increase NK cell activity such as lenalidomide. Van de Veer et al. demonstrated in vitro that the pretreatment of peripheral blood mononuclear cells with lenalidomide enhanced daratumumab-induced ADCC against myeloma cell lines derived from patient bone marrow myeloma cells. The combination of daratumumab and lenalidomide was synergistic, increasing tumor lysis by 20% [11]. Other studies support the notion that it is the NK cell activation of lenalidomide that contributed to the synergistic effect of daratumumab [9, 10, 12].

A significant association was observed between CD38 expression and daratumumab-induced ADCC and CDC. They observed all-trans retinoic acid (ATRA) induced upregulation of CD38 expression and reduced expression of complement inhibitory proteins CD55 and CD59 in myeloma cells. This resulted in a significant increase in daratumumab activity in vitro, and enhanced activity in mouse models, providing rationale for further evaluation of daratumumab in combination with ATRA [9, 10].

In addition to ADCC and CDC, ADCP was another mechanism induced by daratumumab. Overijk and colleagues demonstrated daratumumab-induced ADCP in vitro and in vivo in leukemic xenograft mouse models. It also triggered macrophage-mediated phagocytosis ex vivo in patient-derived MM cell samples [13]. ADCP may have an important function in the bone marrow microenvironment, as tumor-associated macrophages in the marrow have been shown to have a Fc-dependent antitumor function [14].

The off-target immunomodulatory effects of daratumumab were studied in two earlier daratumumab monotherapy trials analyzing peripheral blood and bone

marrow samples before, during, and at relapse. These studies found that depletion of CD38 immunosuppressive cells was associated with an increase in T helper cells, cytotoxic T cells, and improvement in T cell functionality [5]. These findings may explain the significant prolongation in overall survival (OS) in these early clinical trials conducted with daratumumab monotherapy [15].

Elimination of immunosuppressive cells belonging to T cell, B cell, and monocyte-macrophage system expressing CD38 are observed with daratumumab. These immunosuppressive cells inhibit cytotoxic T cells from exerting antitumor control on myeloma patients. In addition, antibody-mediated inhibition of the enzymatic activity of CD38 on cytotoxic T cells may directly boost the antitumor activity of these cells [16].

Preclinical studies have demonstrated significant additive and synergistic effects of daratumumab in combination with other anti-myeloma therapies. This has been confirmed in multiple clinical trials, highlighting daratumumab's unique mechanism of action without overlapping toxicity.

5.3 Mechanisms Behind Daratumumab-Resistance

Despite the well-established efficacy of daratumumab, 60% of patients do not achieve partial response (PR) and the majority who initially respond will eventually experience disease progression [17]. Insights into the mechanisms of daratumumab-resistance have been highlighted in several studies.

It has been shown that the CD38 expression on myeloma cells correlates with in vitro daratumumab-mediated ADCC and CDC [9, 10]. However, it has also been shown that the variability in daratumumab-mediated killing in vitro is not completely explained by CD38 expression alone and that there are CD38 independent mechanisms at play. The overexpression of complement inhibitory proteins is known to play a role in tumor immune evasion and resistance against therapeutic antibodies. Resistance towards daratumumab was associated with the upregulation of CD55 and CD59 on myeloma cells. A reduced expression of CD38 on myeloma cells was also found to confer protection against daratumumab. All-trans retinoic acid was found to increase CD38 expression and reduce CD55 and CD59 expression, increasing CDC against myeloma cells [18].

The mechanisms of resistance to daratumumab can be summarized into the following categories:

5.3.1 Reduced Cell Surface Expression of Target Antigen CD38

A reduction in myeloma surface CD38 expression is a mechanism involved in primary and/or acquired resistance [18]. CD38 reduction was postulated to occur via clonal selection [19]. Another mechanism is the downstream effects of

daratumumab, triggering CD38 internalization leading to cytoskeletal reorganization and redistribution of CD38 into polar aggregates in myeloma cells. These are then released into the bone marrow microenvironment as microvesicles, leading to the modulation of inflammatory cytokines and the abrogation of anti-myeloma immune responses [20].

The IMiDs lenalidomide and pomalidomide can increase the expression of CD38 on myeloma cells and synergize its activity with daratumumab in vitro and in vivo [21, 22].

5.3.2 Antibody-Dependent Cell Cytotoxicity Resistance

Daratumumab induces fratricide of NK cells via its CD38 expression which can then in turn affect NK mediated ADCC, influencing its own efficacy [23]. Interestingly, ex vivo experiments have shown an enhanced proliferative and anti-myeloma activity in the remaining NK cells with low CD38 expression, lending to the hypothesis that daratumumab-resistance may be overcome by infusion of ex vivo expanded autologous NK cells [24].

The concept of bone marrow stromal cells conferring resistance to daratumumab mediated ADCC was demonstrated by de Haart et al. showing overexpression of the anti-apoptotic protein *survivin* in myeloma cells upon its interaction with BMSCs [25].

5.3.3 Antibody-Dependent Cellular Phagocytosis Resistance

The overexpression of CD47 on myeloma cells aids its immune escape from ADCP via its binding to signal regulatory protein-alpha (SIRPa) and tumor-associated macrophages (TAMS), effectively inhibiting phagocytosis [26].

5.3.4 Complement-Dependent Cytotoxicity Resistance

Overexpression of complement inhibitory proteins is known to play a role in tumor immune evasion and resistance against therapeutic antibodies. Cells are protected from complement activation by fluid phase regulators and by membrane-associated inhibitory proteins such as CD46 and glycosyl-phosphatidyl-inositol anchored proteins such as CD55 and CD59 [27].

Samples in the GEN501 study showed increased expression of CD55 and CD59 in myeloma cells during disease progression, confirming that overexpression of these complementary inhibitory proteins can be postulated to be a mechanism of resistance for daratumumab.

5.3.5 *Immune Modulated Resistance*

There is the intriguing hypothesis that the immune system itself is contributory to the resistance to daratumumab. This is postulated to be via several mechanisms, including the downregulation of intracellular pathways in the bone marrow stromal cells, a decrease in effector memory T cells and M1 macrophages, and the CD28 expression in T cells [28, 29].

5.4 Clinical Efficacy of Daratumumab

The initial clinical testing of daratumumab in the GEN501 phase I/II clinical trials enrolled 23 patients over three and a half years due to limited preclinical data, resulting from the lack of cross reactivity of daratumumab with the CD38 molecule in other species. The tested doses of antibody were extremely low, starting at 0.005 mg/kg to a maximum of 24 mg/kg [30]. Clinical efficacy was observed when the dosage was between 2 and 4 mg/kg, translating to a decrease in M protein with no major side effects observed. Target saturation was seen at doses of 16 mg/kg with eight weekly dosing, followed by eight bi-weekly dosing then dosing every 4 weeks, with a maximum tolerated dose not reached at even 24 mg/kg. Another clinical trial has observed superior efficacy at 16 mg/kg over 8 mg/kg [31].

Preclinical studies demonstrate significant synergistic and additive effects in combination with other anti-myeloma therapies. This has been confirmed in multiple clinical trials, supporting the unique mechanism of action of daratumumab without overlapping toxicity.

5.4.1 *Daratumumab in the Relapsed and Refractory Setting*

The GEN501 and SIRIUS study led to US Food and Drug Administration (FDA) approval of daratumumab for the treatment of multiple myeloma patients who had received at least three prior lines of therapy including a proteasome inhibitor (PI) and an IMiD or who are double refractory to a PI and an IMiD [31, 32].

Daratumumab monotherapy demonstrated approximately 30% response in patients with relapsed refractory multiple myeloma (RRMM) [15, 31]. Half of the patients in the trials demonstrated a significant prolongation of survival due to the immunomodulatory effect of daratumumab [5].

The enhanced efficacy and tolerability of several daratumumab-based combinations in both transplant ineligible and eligible patients have been demonstrated without compromising transplant ability [33].

A deeper response and increase in progression free survival (PFS) has been seen with the addition of daratumumab to a PI and an IMiD. Phase III studies (POLLUX and CASTOR) have demonstrated a higher response rate, depth of response, and

PFS in MM patients who have received more than one line of therapy. As a result, daratumumab is now placed in second- and first-line treatment in MM [34–38].

5.4.2 *Daratumumab in Newly Diagnosed, Transplant Ineligible Patients*

The phase III ALCYONE trial evaluated the efficacy of daratumumab in combination with bortezomib, melphalan, and dexamethasone (D-VMP) compared with bortezomib, melphalan, and dexamethasone alone (VMP). The addition of daratumumab demonstrated significant improvement in PFS among 706 transplant ineligible, newly diagnosed myeloma patients. The benefit in overall responses in the daratumumab group was also translated to other patient groups including older age (>75 years), higher ISS stage, and poorer performance status with impaired organ function. As expected, there was less benefit in the high-risk cytogenetic groups compared to the standard risk group [36].

The phase III MAIA trial demonstrated the benefit of daratumumab in addition to lenalidomide and dexamethasone in newly diagnosed, transplant ineligible myeloma patients. A superior PFS was demonstrated in interim analysis in the older age group of greater than 75, but not in the high-risk cytogenetic subgroup [39]. Interestingly, the POLLUX study evaluating the addition of daratumumab to lenalidomide and dexamethasone in RRMM patients demonstrated a longer PFS in the high-risk cytogenetics subgroup compared to the standard risk group [40].

Due to these promising results, to date daratumumab in combination with either VMP or lenalidomide and dexamethasone is being approved in Europe and the USA. Maturation of data will hopefully result in widespread global approval.

There is currently little evidence to guide treatment choice between the various standard regimens. Cao et al. recently published a meta-analysis comparing the efficacy of currently used regimens compared to lenalidomide and dexamethasone. In general, three drug combinations with daratumumab (with either lenalidomide and dexamethasone or VMP) showed superiority to two-drug combinations (lenalidomide and dexamethasone) [41]. Proteasome inhibitor-based doublet regimens in combination with daratumumab are being evaluated. The phase II HOVON 143 study demonstrated manageable side effects in its first planned safety analysis with promising overall response rates (ORR) [42]. Ongoing trials of daratumumab-based combinations in transplant ineligible, newly diagnosed myeloma patients are currently being conducted.

5.4.3 *Daratumumab in Newly Diagnosed, Transplant Eligible Patients*

The promising results of daratumumab in combination with transplant ineligible myeloma patients led to its evaluation in the transplant eligible group. There are several studies being conducted to evaluate its efficacy. The phase III CASSIOPEIA

trial evaluated the efficacy of daratumumab combined with bortezomib, thalidomide, and dexamethasone during induction and consolidation. The daratumumab treatment group showed favorable results with increased rates of stringent complete response (CR) at 100 days posttransplant and higher ORR including CR, very good partial response (VGPR) and minimal residual disease (MRD) negativity (64% versus 44%, $p < 0.0001$). This benefit was demonstrated in many patient groups including those over the age of 50, those with poorer performance status, and those with renal or hepatic dysfunction. However, a benefit was not seen in the ISS stage 3 subgroup. Less benefit was seen in the higher cytogenetic risk group. There was a higher incidence of grade 3 and 4 cytopenia and a lower yield of stem cells requiring plerixafor during mobilization in the daratumumab group. These results led to the approval of daratumumab in transplant eligible myeloma patients [43]. The phase II GRIFFIN study where patients received daratumumab with bortezomib, lenalidomide, and dexamethasone induction and posttransplant consolidation, followed by maintenance with daratumumab and lenalidomide, demonstrated promising results in safety profile and response rates after consolidation [44]. The subcutaneous formulation of daratumumab is currently being utilized to minimize toxicity in the phase III PERSUES trial comparing the efficacy of bortezomib, lenalidomide, and dexamethasone with or without daratumumab in transplant eligible populations.

5.5 Toxicity Profile

The most important side effect to note in daratumumab is the infusion-related reactions that may occur in the first infusion in approximately half of the patients with incidences subsiding thereafter. These reactions are managed with the premedications with glucocorticoids, antihistamines, montelukast, and paracetamol prior to the infusions. Patients with chronic obstructive pulmonary disease may require a prolonged course of glucocorticoids.

The expression of CD38 on erythrocytes complicates the antibody identification work-up in transfusion medicine [45]. Pan-agglutination caused by daratumumab and other anti-CD38 antibodies may mask the presence of a clinically significant RBC alloantibody in the patient's plasma during an antibody screen and identification process, consequently putting a patient at risk of an acute or delayed transfusion reaction [46]. Methods for circumventing this include group and screening for all potential baseline alloantibodies at baseline and extended red cell phenotyping and genotyping [47].

5.6 Conclusion

Daratumumab is a high-affinity monoclonal antibody targeting a unique epitope on CD38 and exerts its therapeutic effects via CDC, ADCC, ADCP, and off-target immunomodulatory effects with overall improvement in T cell functionality

observed in preclinical studies. Its unique mechanisms of action have led to its favorable tolerability profile with nonoverlapping toxicity when used in combination therapy.

Insights into Daratumumab's evolving mechanisms of resistance provide avenues for further drug and synergy development. These include upregulation of complement inhibitory proteins, reduced CD38 expression, inhibition of NK mediated ADCC, and escape from ADCP by overexpression CD47 among other immune modulatory effects.

Daratumumab has shown promising efficacy in both the newly diagnosed and relapsed refractory setting in emerging clinical trials. Its favorable tolerability profile has extended its benefit in OS even in the older and frail population. It serves as an important armamentarium in the treatment of multiple myeloma.

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References

1. de Weers M, Tai YT, van der Veer MS, Bakker JM, Vink T, Jacobs DC, et al. Daratumumab, a novel therapeutic human CD38 monoclonal antibody, induces killing of multiple myeloma and other hematological tumors. J Immunol (Baltimore, MD: 1950). 2011;186(3):1840–8.
2. Plesner T, Krejcik J. Daratumumab for the treatment of multiple myeloma. Front Immunol. 2018;9:1228.
3. Mehta K, Shahid U, Malavasi F. Human CD38, a cell-surface protein with multiple functions. FASEB J. 1996;10(12):1408–17.
4. Malavasi F, Deaglio S, Funaro A, Ferrero E, Horenstein AL, Ortolan E, et al. Evolution and function of the ADP ribosyl cyclase/CD38 gene family in physiology and pathology. Physiol Rev. 2008;88(3):841–86.
5. Krejcik J, Casneuf T, Nijhof IS, Verbist B, Bald J, Plesner T, et al. Daratumumab depletes CD38+ immune regulatory cells, promotes T-cell expansion, and skews T-cell repertoire in multiple myeloma. Blood. 2016;128(3):384–94.
6. Yu T, Qiao C, Lv M, Tang L. Novel anti-CD38 humanized mAb SG003 possessed enhanced cytotoxicity in lymphoma than Daratumumab via antibody-dependent cell-mediated cytotoxicity. BMC Biotechnol. 2019;19(1):28.
7. Stevenson FK, Bell AJ, Cusack R, Hamblin TJ, Slade CJ, Spellerberg MB, et al. Preliminary studies for an immunotherapeutic approach to the treatment of human myeloma using chimeric anti-CD38 antibody. Blood. 1991;77(5):1071–9.
8. Cartron G, Dacheux L, Salles G, Solal-Celigny P, Bardos P, Colombat P, et al. Therapeutic activity of humanized anti-CD20 monoclonal antibody and polymorphism in IgG fc receptor FcgammaRIIIa gene. Blood. 2002;99(3):754–8.
9. Nijhof IS, Groen RW, Lokhorst HM, van Kessel B, Bloem AC, van Velzen J, et al. Upregulation of CD38 expression on multiple myeloma cells by all-trans retinoic acid improves the efficacy of daratumumab. Leukemia. 2015a;29(10):2039–49.
10. Nijhof IS, Groen RWJ, Noort WA, van Kessel B, de Jong-Korlaar R, Bakker J, et al. Preclinical evidence for the therapeutic potential of CD38-targeted immuno-chemotherapy in multiple myeloma patients refractory to Lenalidomide and Bortezomib. Clin Cancer Res. 2015b;21(12):2802–10.

11. van der Veer MS, de Weers M, van Kessel B, Bakker JM, Wittebol S, Parren PW, et al. Towards effective immunotherapy of myeloma: enhanced elimination of myeloma cells by combination of lenalidomide with the human CD38 monoclonal antibody daratumumab. Haematologica. 2011;96(2):284–90.
12. Nijhof IS, van Bueren JJL, van Kessel B, Andre P, Morel Y, Lokhorst HM, et al. Daratumumab-mediated lysis of primary multiple myeloma cells is enhanced in combination with the human anti-KIR antibody IPH2102 and lenalidomide. Haematologica. 2015c;100(2):263–8.
13. Overdijk MB, Verploegen S, Bogels M, van Egmond M, van Bueren JJL, Mutis T, et al. Antibody-mediated phagocytosis contributes to the anti-tumor activity of the therapeutic antibody daratumumab in lymphoma and multiple myeloma. MAbs. 2015;7(2):311–21.
14. Grugan KD, McCabe FL, Kinder M, Greenplate AR, Harman BC, Ekert JE, et al. Tumor-associated macrophages promote invasion while retaining Fc-dependent anti-tumor function. J Immunol (Baltimore, MD: 1950). 2012;189(11):5457–66.
15. Usmani SZ, Weiss BM, Plesner T, Bahlis NJ, Belch A, Lonial S, et al. Clinical efficacy of daratumumab monotherapy in patients with heavily pretreated relapsed or refractory multiple myeloma. Blood. 2016;128(1):37–44.
16. Chatterjee S, Daenthanasanmak A, Chakraborty P, Wyatt MW, Dhar P, Selvam SP, et al. CD38-NAD(+)axis regulates immunotherapeutic anti-tumor T cell response. Cell Metab. 2018;27(1):85–100.e8.
17. Nooka AK, Joseph NS, Kaufman JL, Heffner LT, Gupta VA, Gleason C, et al. Clinical efficacy of daratumumab, pomalidomide, and dexamethasone in patients with relapsed or refractory myeloma: utility of re-treatment with daratumumab among refractory patients. Cancer. 2019;125(17):2991–3000.
18. Nijhof IS, Casneuf T, van Velzen J, van Kessel B, Axel AE, Syed K, et al. CD38 expression and complement inhibitors affect response and resistance to daratumumab therapy in myeloma. Blood. 2016;128(7):959–70.
19. van de Donk NWCJ, Usmani SZ. CD38 antibodies in multiple myeloma: mechanisms of action and modes of resistance. Front Immunol. 2018;9:2134.
20. Saltarella I, Desantis V, Melaccio A, Solimando AG, Lamanuzzi A, Ria R, et al. Mechanisms of resistance to anti-CD38 daratumumab in multiple myeloma. Cell. 2020;9(1):167.
21. Boxhammer R, Steidl S, Endell J. Effect of IMiD compounds on CD38 expression on multiple myeloma cells: MOR202, a human CD38 antibody in combination with pomalidomide. J Clin Oncol. 2015;33(15_suppl):8588.
22. Endell J, Boxhammer R, Wurzenberger C, Ness D, Steidl S. The activity of MOR202, a fully human anti-CD38 antibody, is complemented by ADCP and is synergistically enhanced by lenalidomide in vitro and in vivo. Blood. 2012;120(21):4018.
23. Casneuf T, Xu XS, Adams HC III, Axel AE, Chiu C, Khan I, et al. Effects of daratumumab on natural killer cells and impact on clinical outcomes in relapsed or refractory multiple myeloma. Blood Adv. 2017;1(23):2105–14.
24. Wang Y, Zhang Y, Hughes T, Zhang J, Caligiuri MA, Benson DM, et al. Fratricide of NK cells in daratumumab therapy for multiple myeloma overcome by ex vivo−expanded autologous NK cells. Clin Cancer Res. 2018;24(16):4006–17.
25. de Haart SJ, Holthof L, Noort WA, Minnema MC, Emmelot ME, Aarts-Riemens T, et al. Sepantronium bromide (YM155) improves daratumumab-mediated cellular lysis of multiple myeloma cells by abrogation of bone marrow stromal cell-induced resistance. Haematologica. 2016;101(8):e339–e42.
26. Matozaki T, Murata Y, Okazawa H, Ohnishi H. Functions and molecular mechanisms of the CD47–SIRPα signalling pathway. Trends Cell Biol. 2009;19(2):72–80.
27. Zipfel PF, Skerka C. Complement regulators and inhibitory proteins. Nat Rev Immunol. 2009;9(10):729–40.
28. Neri P, Maity R, Tagoug I, Duggan P, McCulloch S, Jimenez-Zepeda V, et al. Single cell resolution profiling defines the innate and adaptive immune repertoires modulated by daratumumab and IMiDs treatment in multiple myeloma (MM). Blood. 2017;130(Supplement 1):123.

29. Viola D, Dona A, Gunes EG, Troadec E, Wu X, Branciamore S, et al. Immune mediated mechanisms of resistance to daratumumab. Blood. 2018;132(Supplement 1):3201.
30. Lokhorst HM, Plesner T, Laubach JP, Nahi H, Gimsing P, Hansson M, et al. Targeting CD38 with daratumumab monotherapy in multiple myeloma. N Engl J Med. 2015;373(13):1207–19.
31. Lonial S, Weiss BM, Usmani SZ, Singhal S, Chari A, Bahlis NJ, et al. Daratumumab monotherapy in patients with treatment-refractory multiple myeloma (SIRIUS): an open-label, randomised, phase 2 trial. Lancet (London, England). 2016;387(10,027):1551–60.
32. Lokhorst HM, Laubach J, Nahi H, Plesner T, Gimsing P, Hansson M, et al. Dose-dependent efficacy of daratumumab (DARA) as monotherapy in patients with relapsed or refractory multiple myeloma (RR MM). J Clin Oncol. 2014;32(15_suppl):8513.
33. Syed YY. Daratumumab: a review in combination therapy for transplant-ineligible newly diagnosed multiple myeloma. Drugs. 2019;79(4):447–54.
34. Dimopoulos MA, Oriol A, Nahi H, San-Miguel J, Bahlis NJ, Usmani SZ, et al. Daratumumab, lenalidomide, and dexamethasone for multiple myeloma. N Engl J Med. 2016;375(14):1319–31.
35. Palumbo A, Chanan-Khan A, Weisel K, Nooka AK, Masszi T, Beksac M, et al. Daratumumab, bortezomib, and dexamethasone for multiple myeloma. N Engl J Med. 2016;375(8):754–66.
36. Mateos MV, Dimopoulos MA, Cavo M, Suzuki K, Jakubowiak A, Knop S, et al. Daratumumab plus bortezomib, melphalan, and prednisone for untreated myeloma. N Engl J Med. 2018;378(6):518–28.
37. Harousseau JL, Attal M. How I treat first relapse of myeloma. Blood. 2017;130(8):963–73.
38. Plesner T, Arkenau HT, Gimsing P, Krejcik J, Lemech C, Minnema MC, et al. Phase 1/2 study of daratumumab, lenalidomide, and dexamethasone for relapsed multiple myeloma. Blood. 2016;128(14):1821–8.
39. Facon T, Kumar S, Plesner T, Orlowski RZ, Moreau P, Bahlis N, et al. Daratumumab plus Lenalidomide and dexamethasone for untreated myeloma. N Engl J Med. 2019;380(22):2104–15.
40. Dimopoulos MA, San-Miguel J, Belch A, White D, Benboubker L, Cook G, et al. Daratumumab plus lenalidomide and dexamethasone versus lenalidomide and dexamethasone in relapsed or refractory multiple myeloma: updated analysis of POLLUX. Haematologica. 2018;103(12):2088–96.
41. Cao Y, Wan N, Liang Z, Xie J, Wang S, Lin T, et al. Treatment outcomes in patients with newly diagnosed multiple myeloma who are ineligible for stem-cell transplantation: systematic review and network meta-analysis. Clin Lymphoma Myeloma Leuk. 2019;19(8):e478–e88.
42. Stege CAM, Nasserinejad K, Levin M-D, Thielen N, Klein SK, Ludwig I, et al. Efficacy and tolerability of ixazomib, daratumumab and low dose dexamethasone (IDd) in unfit and frail newly diagnosed multiple myeloma (NDMM) patients; First Interim Safety Analysis of the Phase II HOVON 143 Study. Blood. 2018;132(Supplement 1):596.
43. Moreau P, Attal M, Hulin C, Arnulf B, Belhadj K, Benboubker L, et al. Bortezomib, thalidomide, and dexamethasone with or without daratumumab before and after autologous stem-cell transplantation for newly diagnosed multiple myeloma (CASSIOPEIA): a randomised, open-label, phase 3 study. Lancet (London, England). 2019;394(10,192):29–38.
44. Abdallah N, Kumar SK. Daratumumab in untreated newly diagnosed multiple myeloma. Ther Adv Hematol. 2019;10:2040620719894871.
45. Dizon MF. The challenges of daratumumab in transfusion medicine. Lab Med. 2017;48(1):6–9.
46. Quach H, Benson S, Haysom H, Wilkes AM, Zacher N, Cole-Sinclair M, et al. Considerations for pre-transfusion immunohaematology testing in patients receiving the anti-CD38 monoclonal antibody daratumumab for the treatment of multiple myeloma. Intern Med J. 2018;48(2):210–20.
47. Lancman G, Arinsburg S, Jhang J, Cho HJ, Jagannath S, Madduri D, et al. Blood transfusion management for patients treated with anti-CD38 monoclonal antibodies. Front Immunol. 2018;9:2616.

Chapter 6
Elotuzumab

Adam Bryant

Abstract Elotuzumab is approved for therapy in combination with dexamethasone and an immunomodulatory agent in relapsed and refractory myeloma patients. Given its relative recency of use in clinical practice, mechanisms of resistance are poorly understood and will require further study for full elucidation. Nevertheless, this chapter will examine what is known, extrapolate from concepts established in other monoclonal antibodies, and anticipate avenues for future research.

Keywords Multiple myeloma · Elotuzumab · Signaling lymphocytic activation molecule family member 7 · Anti-SLAMF7 antibody · Monoclonal antibody treatment · Elotuzumab resistance

Abbreviations

ADA	Anti-drug antibodies
ADCC	Antibody-dependent cellular cytotoxicity
EMA	European Medicines Agency
FDA	US Food and Drug Administration
IgG1	Immunoglobulin G1
IMiD	Immunomodulatory imide drug
MM	Multiple myeloma
MoAb	Monoclonal antibody
NK	Natural killer
ORR	Overall response rate
OS	Overall survival
PFS	Progression free survival

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RRMM	Relapsed/refractory multiple myeloma
siRNA	Small interfering RNA
SLAMF7	Signaling lymphocytic activation molecule family member 7

6.1 Introduction

Monoclonal antibodies (MoAb) approved by the US Food and Drug Administration (FDA) for administration in multiple myeloma (MM) include daratumumab (targeting CD38), isatuximab (targeting CD38), and elotuzumab (targeting SLAMF7). Elotuzumab has a unique mode of action among the approved antibodies through its targeting of signaling lymphocytic activation molecule family member 7 (SLAMF7) protein. Engagement of this protein results in anti-myeloma tumor activity both by direct activation of natural killer (NK) cells, as well as by activating antibody-dependant cellular cytotoxicity (ADCC) [1].

The clinical approval of elotuzumab by the FDA and the European Medicines Agency (EMA) is for administration in combination with an immunomodulatory imide drug (IMiD) and dexamethasone in relapsed/refractory multiple myeloma (RRMM). This is based on results of the ELOQUENT-2 study which combined elotuzumab with lenalidomide and dexamethasone [2] and the ELOQUENT-3 study which combined it with pomalidomide and dexamethasone [3]. While heavily pretreated myeloma patients administered elotuzumab monotherapy tolerated the agent well, objective responses to monotherapy were not seen [1]. As a consequence, higher phase clinical trials focussed on assessing its activity in combination with other anti-myeloma agents. Although it is conceivable that responses could be seen with monotherapy in patients earlier in their disease course, this is not where the development of this agent is headed.

This chapter will address the mechanisms of action, mechanisms of resistance, and clinical outcomes of elotuzumab therapy.

6.2 Mechanisms of Action

6.2.1 Preclinical Studies

SLAMF7, or the signaling lymphocytic activation molecule family member 7 protein, is a novel target in MM [4]. This protein is also variably known as CS1 (cell-surface glycoprotein CD2 subset 1), CD2 subset-1, CRACC, and CD319 and is involved in the regulation of natural killer (NK) cell function [5].

The utility of SLAMF7 as a potential target for anti-myeloma therapy lies in its high expression in both normal and malignant plasma cells, with minimal expression in other tissues. This high expression was first identified through gene

expression profiling in normal plasma cells, myeloma cell lines, and primary myeloma cells [4]. Some expression was also seen in other leukocytes (NK cells, activated monocytes, activated dendritic cells, and some T cell subsets) but other tissues had limited to no gene expression (lung, uterus, kidney, stomach, brain, breast, spleen, prostate, skeletal muscle, testis, thymus, liver, ovary, heart, and small intestine).

These gene expression results were confirmed with Western Blotting assays for SLAMF7, showing these tissues expressed levels of protein in keeping with their SLAMF7 gene profiles [4]. There was also minimal SLAMF7 in other hematological malignancies (B cell lymphoma, Hodgkin Lymphoma, acute myeloid leukemia, and acute lymphoblastic leukemia), though some expression was seen in a subset of cases peripheral T cell lymphoma and interestingly in significant proportion of lymphoplasmacytic lymphoma.

Hsi and colleagues confirmed these findings and further explored this target with in vitro assays of HuLuc63 (analogous to elotuzumab), a humanized immunoglobulin G1 (IgG1) antibody targeting SLAMF7 [4]. This antibody has been demonstrated to have an anti-myeloma action resulting from NK cell-mediated ADCC, confirmed by the finding that NK cell depletion ablates the anti-myeloma effects. Other researchers have also shown that HuLuc63 acts to enhance NK function, beyond its effect on ADCC alone [6, 7]. Furthermore, HuLuc63 leads to decreased adhesion of myeloma cells to bone marrow stroma [8].

This in vitro action has been correlated by other researchers in vivo using a CS1+ xenograft mouse model [8]. Treatment of CS1+ (SLAMF7) tumor inoculated mice with HuLuc63 resulted in tumor eradication in 9 of 27 subjects, whereas there was no eradication in CS1 negative tumors, or with humanized IgG1 control antibody. In a separate experiment, HuLuc63 was demonstrated to be dose dependant.

The utility of HuLuc63 in combination with established anti-myeloma agents (including dexamethasone, bortezomib, and lenalidomide) was also evaluated in vitro [8]. Myeloma cell lines were pretreated with these agents prior to exposure to HuLuc63, resulting in increased MM cell lysis. In vivo synergy with bortezomib has also been confirmed in a mouse model [9]. This synergistic effect suggested a possible utility of HuLuc63 as a component of combination therapy in humans, as well as in patients with disease resistant to these agents.

6.3 Pharmacological Characteristics of Elotuzumab

Pharmacological properties of elotuzumab were evaluated in a phase one study of 35 patients with advanced myeloma enrolled into six dosing cohorts [1]. There was a disproportionate increase in AUC across the dosing range suggesting nonlinear pharmacokinetics. The volume of distribution approximated the serum volume. SLAMF7 receptors were consistently saturated between 10 and 20 mg/kg of elotuzumab. On immunogenicity testing, 39% developed anti-drug antibodies (ADA),

though ADA responses were minimized in the 10 and 20 mg/kg range. Sustained lymphocyte depletion was neither seen on elotuzumab administration, nor was neutropenia or thrombocytopenia.

6.4 Mechanisms of Resistance to Elotuzumab

Due to the relatively new introduction of elotuzumab into clinical practice, studies of the mechanisms and pathways imparting resistance to this specific MoAb are sparse. This section will address mechanisms of resistance in several categories as follows and is largely speculative.

6.4.1 Expression of the Antigen Target of the Monoclonal Antibody

As a MoAb targeting surface proteins, it is reasonable to speculate that response to elotuzumab may correlate with expression of the target antigen. This has been demonstrated to be the case for daratumumab [10, 11] and isatuximab [12] with respect to CD38 expression. There is a paucity of data regarding the elotuzumab response to differential SLAMF7 expression. This may well be difficult to demonstrate given the near universal expression of SLAMF7 in primary myeloma cells.

While mostly SLAMF7 expression is retained at relapse [4], it is of interest that in one small study of 33 patients, 3 patients who had suffered an extramedullary relapse after exposure to elotuzumab had reduced SLAMF7 expression on biopsy [13]. Therefore, SLAMF7 downregulation by tumor cells may be one mechanism of acquired resistance to elotuzumab. NK cells also express SLAMF7 to a lesser extent [4], so it is possible that downregulated expression on these cells could affect NK activation by elotuzumab, though this remains to be shown.

6.4.2 CD16a Expression on NK Cells and Associated Polymorphisms

Elotuzumab cross-links SLAMF7 on plasma cells to FcγRIIIa (also called CD16a) on NK cells [14]. CD16a is encoded by the FCGR3A gene on the long arm of chromosome 1 [15]. The NK cell uses this receptor to phagocytose antibody-coated tumor cells. In this context, myeloma cells are coated by elotuzumab. It has been demonstrated that polymorphisms exist in the extracellular domain of the CD16a which impart differential affinity to immunoglobulin, with those homozygous for

FCGR3A 158 V (V/V) binding more IgG1 compared with those homozygous for FCGR3A 158 F (F/F) [16].

In their phase II study (discussed in more detail in a subsequent section of this chapter), Jakubowiak and colleagues were able to demonstrate a markedly improved survival in patients homozygous for the high-affinity V allele (22.3 months) versus those homozygous for low-affinity F allele (9.8 months) when elotuzumab was combined with bortezomib [14]. However, this was not seen in the ELOQUENT-2 study which employed elotuzumab in combination with lenalidomide [17]. It is conceivable that significant differential expression of the F allele could play a role in basal elotuzumab resistance, depending on the coadministered agent, though again this remains to be demonstrated.

6.4.3 Interactions with the Microenvironment

It has been demonstrated that SLAMF7 protein localizes to the uropod membrane domains in polarized myeloma cell lines and primary myeloma cells [8]. The finding that knockdown of SLAMF7 protein expression by small interfering RNA (siRNA) resulted in failure of a myeloma cell lines cell to bind to bone marrow mesenchymal cells suggested that SLAMF7 inhibition by elotuzumab may ablate essential survival interactions with the microenvironment. Further experiments confirmed that antibody inhibition of SLAMF7 reduced MM cell adhesion. It was furthermore shown that in myeloma cells cultured with mesenchymal stem cells, elotuzumab exposure inhibited myeloma cell viability. Given how important this mechanism is, it is feasible that myeloma cell and microenvironment adaptations could overcome the inhibition by elotuzumab and form a mechanism of resistance.

6.4.4 Development of Neutralizing Antibodies

While not necessarily a form of intrinsic myeloma cell or associated microenvironment resistance, it is certainly possible that the development of neutralizing antibodies against therapeutic MoAb could be a means for loss of response to elotuzumab. In the initial phase I human study [1], 12/31 (29%) of subjects had developed detectable anti-drug antibodies, of which 11/12 had neutralizing activity. This was seen to a lesser extent in the ELOQUENT-2 study, in which there was neutralizing antibody generation in 15% of subjects [2]. At this stage, the clinical significance of this observation is not clear and needs further evaluation to determine the extent to which this may mitigate the effect of elotuzumab.

6.5 Clinical Trials

Objective clinical responses to elotuzumab monotherapy were not seen in the first in human phase I study [1]. Furthermore, the synergistic action of elotuzumab when combined with other anti-myeloma therapies has been noted [8]. Therefore, the focus of the clinical development of elotuzumab has been as a component of combination therapy.

6.5.1 Relapsed and/or Refractory Myeloma

Immunomodulatory imide drugs (IMiD) are particularly attractive candidates as combination partners of elotuzumab, given their previously demonstrated ability to enhance the ADCC and immune function of MoAbs [18]. This prospect was supported by the findings of phase I clinical trial in which elotuzumab was added to a standard dosing schedule of lenalidomide and dexamethasone [19]. Objective responses were seen in 82% of a cohort of 29 RRMM patients (median of 3 prior lines of therapy). Hematological toxicities were the main adverse effects with neutropenia (36%) and thrombocytopenia (21%) being the commonest grade 3–4 toxicities. Infusion reactions were not a significant problem, having only been seen in 2/29 patients.

The results of the major higher level phase II and III studies are presented in Table 6.1. The largest study was a phase III comparison of lenalidomide and dexamethasone with elotuzumab versus lenalidomide and dexamethasone in 646 RRMM patients having received 1–3 prior lines of therapy [2]. The addition of elotuzumab resulted in a meaningful improvement in the primary endpoint of median progression free survival (PFS) at 19.4 months versus 14.9 months (hazard ratio, 0.70; 95% confidence interval, 0.57–0.85; $P < 0.001$). Favorable response rates were also noted with an overall response rate (ORR) of 79% in the elotuzumab arm versus 66% in the control arm ($P < 0.001$).

Elotuzumab has also been successfully combined with pomalidomide. ELOQUENT-3 was a phase II study in 117 patients with greater than two prior lines of therapy. Median PFS was 10.3 months in the elotuzumab group and 4.7 months in the control group (HR 0.54 (95% CI, 0.34–0.86; $P = 0.008$)). The ORR was 53% in the elotuzumab group versus 26% in the control arm (odds ratio, 3.25; 95% CI, 1.49–7.11).

As a consequence of these major two studies elotuzumab has been successively approved for administration in combination with lenalidomide (November 30, 2015) and pomalidomide (November 6,2018), with similar approvals having been made by the EMA (May 11, 2016 and July 25, 2019, respectively).

Elotuzumab has also been successfully combined with bortezomib and dexamethasone in myeloma patients who have had 1–3 lines of prior therapy. After the encouraging phase I results [20], this bortezomib and dexamethasone with or without elotuzumab combination was compared in a phase II study with 152 participants [14]. The primary endpoint of this study was met with a 24% reduction in the risk

Table 6.1 Major clinical trials utilizing elotuzumab

Patient population	Combination	Numbers	ORR (%)	>VGPR (%)	>CR (%)	Median PFS (mths)	Median OS (mths)	Reference
Phase 1b/2 Relapsed or refractory to 1–3 prior lines of therapy No prior lenalidomide exposure	Lenalidomide and dexamethasone with Elotuzumab	73[#] [#]phase 2 portion of study	83	56	14	28.6	Not reported	Richardson 2015 Study (1703) NCT00742560
Phase 2 (randomized) Received >2 prior lines of therapy	Pomalidomide dexamethasone with Elotuzumab	60	53	20	8	10.3	Not reported	Dimopoulos 2018 Eloquent-3 study NCT02654132
	Pomalidomide and dexamethasone	57	26	9	2	4.7		
Phase 3 Relapsed or refractory to 1–3 prior lines of therapy	Lenalidomide and dexamethasone with Elotuzumab	321	79	32	4	19.4	48	Lonial 2015 Eloquent-2 study NCT01239797
	Lenalidomide and dexamethasone	325	66	28	7	14.9	40	
Phase 2 (randomized) Relapsed or refractory to 1–3 prior lines of therapy	Bortezomib and dexamethasone with Elotuzumab (EBd)	77	66	37	4	9.7	Not reported but 2-year OS was 73% EBd versus 66% Bd	Jakubowiak 2016 NCT01478048
	Bortezomib and dexamethasone (Bd)	75	63	27	4	6.9		

ORR Overall response rate, *VGPR* Very good response rate, *CR* Complete response rate (including stringent complete response), *PFS* Progression free survival, *OS* Overall survival, *EBd* Bortezomib, dexamethasone, and elotuzumab, *Bd* Bortezomib and dexamethasone

of disease progression or death. Two-year overall survival (OS) analysis trended towards favoring the elotuzumab arm 73% (95% CI, 61%–82%) to 66% (95% CI, 54%–76%) but OS data was not mature by the time of publication.

Lenalidomide and dexamethasone with elotuzumab versus lenalidomide and dexamethasone has also been studied in newly diagnosed but transplant ineligible patients in the ELOQUENT-1 study [21]. However, it has been announced that this study failed to meet the primary endpoint measure of improved PFS (BMS Press Release March 9, 2020). Therefore, unless new further studies show otherwise, the main current utility of elotuzumab is for administration as part of a combination triplet with either and IMiD or bortezomib in RRMM patients. This combination is effective and is able to be administered with manageable toxicities in this patient population (Table 6.1).

6.6 Toxicities of Elotuzumab

Elotuzumab was able to be administered in the aforementioned studies with minimal additional toxicities over the control arms. As expected with MoAb therapy, modest infusional reactions have been seen. In the ELOQUENT-2 study utilizing lenalidomide containing triplet therapy [2], 10% of patients receiving elotuzumab experienced mild infusional reactions (fevers, chills, hypertension with 29/33 of these being grade 1 or 2). Only two subjects (1%) required discontinuation of these agents due to infusional reactions.

In the ELOQUENT-2 study, the major differential toxicity seen in the elotuzumab arm including a higher rate of grade 3 or 4 lymphopenia (77% versus 49%) as well as a higher rate of herpes zoster infection (4.1 versus 2.2 events per 100 patient years). Forty-five patients (15%) developed ADA though the significance of these were not clear. There was also no difference in pain or quality of life measures between the arms of the study.

6.7 Conclusion

Elotuzumab is an established, if not widely adopted therapeutic option, that is approved for therapy combination with dexamethasone and an IMiD in RRMM patients. By its action in promoting ADCC and enhancing NK cell function through the binding of SLAMF7, the addition to elotuzumab has improved PFS when added to dexamethasone with either lenalidomide, pomalidomide, or bortezomib. Given its relative recency of use in clinical practice, mechanisms of resistance are poorly understood and will require further study for full elucidation.

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References

1. Zonder JA, Mohrbacher AF, Singhal S, van Rhee F, Bensinger WI, Ding H, et al. A phase 1, multicenter, open-label, dose escalation study of elotuzumab in patients with advanced multiple myeloma. Blood. 2012;120(3):552–9.
2. Lonial S, Dimopoulos M, Palumbo A, White D, Grosicki S, Spicka I, et al. Elotuzumab therapy for relapsed or refractory multiple myeloma. N Engl J Med. 2015;373(7):621–31.
3. Dimopoulos MA, Dytfeld D, Grosicki S, Moreau P, Takezako N, Hori M, et al. Elotuzumab plus pomalidomide and dexamethasone for multiple myeloma. N Engl J Med. 2018;379(19):1811–22.
4. Hsi ED, Steinle R, Balasa B, Szmania S, Draksharapu A, Shum BP, et al. CS1, a potential new therapeutic antibody target for the treatment of multiple myeloma. Clin Cancer Res. 2008;14(9):2775–84.
5. Kumaresan PR, Lai WC, Chuang SS, Bennett M, Mathew PA. CS1, a novel member of the CD2 family, is homophilic and regulates NK cell function. Mol Immunol. 2002;39(1–2):1–8.
6. Collins SM, Bakan CE, Swartzel GD, Hofmeister CC, Efebera YA, Kwon H, et al. Elotuzumab directly enhances NK cell cytotoxicity against myeloma via CS1 ligation: evidence for augmented NK cell function complementing ADCC. Cancer Immunol Immunother. 2013;62(12):1841–9.
7. Balasa B, Yun R, Belmar NA, Fox M, Chao DT, Robbins MD, et al. Elotuzumab enhances natural killer cell activation and myeloma cell killing through interleukin-2 and TNF-alpha pathways. Cancer Immunol Immunother. 2015;64(1):61–73.
8. Tai YT, Dillon M, Song W, Leiba M, Li XF, Burger P, et al. Anti-CS1 humanized monoclonal antibody HuLuc63 inhibits myeloma cell adhesion and induces antibody-dependent cellular cytotoxicity in the bone marrow milieu. Blood. 2008;112(4):1329–37.
9. van Rhee F, Szmania SM, Dillon M, van Abbema AM, Li X, Stone MK, et al. Combinatorial efficacy of anti-CS1 monoclonal antibody elotuzumab (HuLuc63) and bortezomib against multiple myeloma. Mol Cancer Therap. 2009;8(9):2616–24.
10. Nijhof IS, Casneuf T, van Velzen J, van Kessel B, Axel AE, Syed K, et al. CD38 expression and complement inhibitors affect response and resistance to daratumumab therapy in myeloma. Blood. 2016;128(7):959–70.
11. Nijhof IS, Groen RW, Lokhorst HM, van Kessel B, Bloem AC, van Velzen J, et al. Upregulation of CD38 expression on multiple myeloma cells by all-trans retinoic acid improves the efficacy of daratumumab. Leukemia. 2015;29(10):2039–49.
12. Moreno L, Perez C, Zabaleta A, Manrique I, Alignani D, Ajona D, et al. The mechanism of action of the anti-CD38 monoclonal antibody isatuximab in multiple myeloma. Clin Cancer Res. 2019;25(10):3176–87.
13. Danhof S, Strifler S, Hose D, Kortum M, Bittrich M, Hefner J, et al. Clinical and biological characteristics of myeloma patients influence response to elotuzumab combination therapy. J Cancer Res Clin Oncol. 2019;145(3):561–71.
14. Jakubowiak A, Offidani M, Pegourie B, De La Rubia J, Garderet L, Laribi K, et al. Randomized phase 2 study: elotuzumab plus bortezomib/dexamethasone vs bortezomib/dexamethasone for relapsed/refractory MM. Blood. 2016;127(23):2833–40.
15. Ravetch JV, Perussia B. Alternative membrane forms of Fc gamma RIII(CD16) on human natural killer cells and neutrophils. Cell type-specific expression of two genes that differ in single nucleotide substitutions. J Exp Med. 1989;170(2):481–97.
16. Koene HR, Kleijer M, Algra J, Roos D, von dem Borne AE, de Haas M. Fc gammaRIIIa-158V/F polymorphism influences the binding of IgG by natural killer cell fc gammaRIIIa, independently of the fc gammaRIIIa-48L/R/H phenotype. Blood. 1997;90(3):1109–14.
17. Poulart V, Jou YM, Delmonte T, Robbins M. Fc gamma receptor polymorphisms and progression free survival-analysis of three clinical trials of elotuzumab in multiple myeloma. Copenhagen, Denmark: EHA 2018; 2018.

18. Hernandez-Ilizaliturri FJ, Reddy N, Holkova B, Ottman E, Czuczman MS. Immunomodulatory drug CC-5013 or CC-4047 and rituximab enhance antitumor activity in a severe combined immunodeficient mouse lymphoma model. Clin Cancer Res. 2005;11(16):5984–92.
19. Lonial S, Vij R, Harousseau JL, Facon T, Moreau P, Mazumder A, et al. Elotuzumab in combination with lenalidomide and low-dose dexamethasone in relapsed or refractory multiple myeloma. J Clin Oncol. 2012;30(16):1953–9.
20. Jakubowiak AJ, Benson DM, Bensinger W, Siegel DS, Zimmerman TM, Mohrbacher A, et al. Phase I trial of anti-CS1 monoclonal antibody elotuzumab in combination with bortezomib in the treatment of relapsed/refractory multiple myeloma. J Clin Oncol. 2012;30(16):1960–5.
21. Dimopoulos M, Facon T, Richardson P, Orlowski R, San Miguel J, Lonial S, et al. ELOQUENT-1: a phase III, randomized, open-label trial of lenalidomide/dexamethasone with or without elotuzumab in subjects with previously untreated multiple myeloma (CA204-006). J Clin Oncol. 2017;30

Chapter 7
Histone Deacetylase Inhibitors

Opelo Sefhore and Silvia CW Ling

Abstract Histone Deacetylase Inhibitors (HDACi) inhibits deacetylases of histones and nonhistones. As such it has potential widespread biological effects. However, cancer cells are preferentially affected than normal cells making it a useful targeted therapy in cancer. HDACi has therapeutic effects in hematological malignancies like acute myeloid leukemia and multiple myeloma. The molecular effect is dependent on the cancer type, the specific class of histone deacetylase, and the chemical structure of the HDACi. This chapter focuses on the basics of HDAC classification and the specific molecular effects in multiple myeloma.

Keywords Multiple myeloma · Histone deacetylase · Histone deacetylase inhibitor · Pan-HDAC inhibitor · Histone deacetylase inhibitor resistance

Abbreviations

ABC	ATP binding cassette
ATF6	Activating transcription factor 6
CCND1	Cyclin D1
CDK	Cyclin-dependent kinase
CR	Complete response
CRBN	Celebron
DNMT1	DNA-methyltransferase 1
HDAC	Histone deacetylase

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HDACi	Histone deacetylase
HSP	Heat shock protein
IGF-1	Insulin-like growth factor 1
IL	Interleukin
IMiD	Immunomodulatory imide drug
IRF4	Interferon 4
MAPK	Mitogen-activated protein kinase
MEF2	Myocyte enhancer factor 2
MM	Multiple myeloma
N-CoR	Nuclear receptor corepressor
PAI-1	Plasminogen activator-inhibitor 1
PERK	Protein kinase R-like ER kinase
PI	Proteasome inhibitor
RRMM	Relapsed/refractory multiple myeloma
SAHA	Suberoylanilide hydroxamic acid
siRNA	Small interfering RNA
SMRT	Silencing mediator for retinoic acid for thyroid hormone receptors
STAT	Signal transducer and activator of transcription pathway
TAFI68	TATA-box binding protein associated factor 1
TIF	Transcription initiation factor
TRAIL	Tumor necrosis factor-related apoptosis-inducing ligand
TSA	Trichostatin A

7.1 Introduction

The introduction of new therapeutic agents such as proteasome inhibitors (PI) and immunomodulatory imide drugs (IMiD) in the last 1–2 decades has dramatically improved the outcome of multiple myeloma (MM) patients. Multiple myeloma is characterized by heterogeneous and complex genetic alterations such as structural chromosomal abnormalities, point mutations, and epigenetic alterations. Epigenetic alterations refer to changes in gene expression without changes in the DNA code. Examples of epigenetic alterations include DNA methylation, acetylation, phosphorylation, ubiquitination, and sumoylation which modify the posttranslational structure of histone. In cancer, dysregulation in epigenetics affects the expression of proteins involved in tumor suppression, cell cycling, DNA repair, apoptosis, protein homeostasis, and tumor immunity.

Histone Deacetylase inhibitors (HDACi) is a promising new group of anti-myeloma therapy that acetylates histone and nonhistone proteins. There have been encouraging clinical responses observed with HDACi used in combination with other targeted therapies such as proteasome inhibitors in MM. To further understand the efficacy of HDACi, it is important to explore the biology of histone deacetylases and their roles in MM.

7.2 Histone Deacetylases

Human DNA is organized into basic structural units called nucleosomes which are packed and wound around two copies each of four different histone proteins (H2A, H2B, H3, and H4). The accessibility of the DNA to regulatory proteins is dependent on the covalent modifications of these histones and the position of the nucleosomes [1].

Histone deacetylases (HDAC) belong to a class of enzymes that removes acetyl groups from lysine within the tail of histones. This process, known as deacetylation, allows DNA to tightly coil around histones to form chromatin. The reverse process, termed acetylation, is mediated by histone acetyltransferases and leads to the uncoiling of DNA, exposing promoter genes to transcription factors. The balance between acetylation and deacetylation determines the degree of gene accessibility to transcription factors [2]. There is growing evidence that deacetylation plays an important role in silencing tumor suppressor genes, dysregulating cellular function, and contributing to cancer development and resistance to chemotherapy [2]. Hence, research has been invested to develop drugs targeting epigenetic regulation.

There are 18 HDACs in humans, which are divided into four classes (I–IV) based on their homology to the yeast enzyme Rpd3, their intracellular localization, and organization with the DNA-binding complexes. Class I, II, and IV HDACs require zinc as a cofactor for their deacetylase activity [3–6]. Class III HDACs, also referred to as sirtuins, are homologs of the yeast enzyme Sir2 and are dependent on NAD+ for their activity rather than zinc [3, 7–16].

7.2.1 Class I Histone Deacetylases

Class I HDACs are homologous to the yeast enzyme Rpd3 and consist of four subtypes (HDAC1, 2, 3, and 8). They are predominantly found in the nucleus, ubiquitously expressed in all tissues, with the main function of histone deacetylation. HDAC1 and HDAC2 are similar with 86% homology and require multiple protein cofactors for enzyme activity [1]. For example, HDAC1 and HDAC2 are part of the multiple protein complexes with Sin3, Rb-associated protein 48, and RbAp46 which function as transcription repressors [17–19]. HDAC1 and HDAC2 also bind directly to DNA-binding proteins, such as YY1, retinoblastoma protein (pRb), pRb-binding protein 1, Sp1, and breast cancer-associated susceptibility protein 1 [19–23]. HDAC1 and HDAC2 activity can be modulated by phosphorylation, increasing enzyme activity but mediating dissociation from multiprotein complexes [24]. HDAC3 is evolutionarily most closely related to HDAC8, with 34% overall sequence identity. SMRT (silencing mediator for retinoic acid and thyroid hormone receptors) and N-CoR (nuclear receptor corepressor) are necessary factors for HDAC3 activity [25, 26]. Besides histone deacetylation, HDAC1, HDAC2, and HDAC3 localize in the endoplasmic reticulum where they deacetylase nonhistone proteins,

such as GRP78, the major molecular chaperone. Inhibition of HDAC1, HDAC2, and HDAC3 leads to the acetylation of the GRP78 and activation of the protein kinase R-like ER kinase (PERK) and activating transcription factor 6 (ATF6) arm of the unfolded protein response [27].

Class I HDAC overexpression has been studied in both solid and hematological malignancies, with prognostic implications, discussed later in this chapter [2]. HDAC1 overexpression has been observed in renal cell carcinoma and ovarian cancers [3, 4]. Another main function of Class I and II HDACs is regulation of tissue regeneration [5–8].

7.2.2 Class II Histone Deacetylases

Class II HDACs are homologous to the yeast enzyme Hda1, which consists of a N-terminal deacetylase domain and a long C-terminal extension. This class is subdivided into IIa (HDACs 4, 5, 7, and 9) and IIb (HDAC6 and 10). Class IIa HDACs possess a conserved N-terminal extension that binds myocyte enhancer factor 2 (MEF2) and 14–3-3 proteins. Class IIa HDACs can shuttle between the nucleus and the cytosol in response to different stimuli. HDAC4, 5, and 9 are expressed predominantly in heart, cardiac, and skeletal muscle, whereas HDAC7 is found in CD4/CD8 double-positive thymocytes [28], endothelial and smooth muscle cells [29]. Class IIa HDACs do not bind chromatin directly. Their activity is dependent on their association with other multiprotein complexes and other HDACs.

HDAC6 is a class IIb HDAC which contains tandem deacetylase domains and a C-terminal zinc finger which is homologous to the non-catalytic domain of ubiquitin-specific proteases (USPs). HDAC6 is localized in the microtubular network of the cytoplasm and acts as a deacetylase of tubulin. It binds ubiquitin via its zinc finger with high affinity. HDAC6 is important in multiple myeloma as it is necessary for the clearance of misfolded proteins via aggresomes and aggrephagy.

The other class IIb HDAC member is HDAC10, which has an N-terminal half similar to the first deacetylase domain of HDAC6, but its C-terminal half is leucine-rich. HDAC10 deacetylases polyamines, such as spermidine and spermine, which are critical in the regulation of the function of biological macromolecules [30]. In addition, HDAC10 overexpression is a poor prognostic marker in neuroblastoma, as it mediates lysosomal exocytosis of doxorubicin in neuroblastoma cells causing resistance to doxorubicin [31].

7.2.3 Class III Histone Deacetylases (Sirtuins)

Class III HDACs (sirtuins) consist of seven subclasses (SIRT 1–7) [32]. This class has sequence homology with the yeast gene silent information regulator, Sir2 [33, 34]. Its enzyme activity is NAD^+ dependent whereas other HDACs require Zn^{2+} as a

cofactor [35, 36]. Sirtuins are deacetylases and mono-ADP-ribosyl transferases [32]. Sirtuins are insensitive to inhibitors of "classical" (Class I, II, and IV) HDACs.

SIRT1 targets both histone and nonhistone proteins. SIRT1 deacetylases the lysine residues at positions 9 and 26 of histone H1, position 14 of H3, and position 16 of H4. Nonhistone targets of SIRT1 include p53 and TAF_I68 [TBP (TATA-box binding protein)-associated factor I] [68]. Deacetylation of p53 leads to its suppression and hence inactivation of apoptosis in response to DNA damage and oxidative stress [37]. TAF_I68 is a subunit of TIF (transcription initiation factor)-IB/SL, which regulates transcription of RNA polymerase I [38]. Deacetylation of TAF_I68 leads to the repression of RNA polymerase I. SIRT1 has been shown to act as an oncogene or a tumor suppressor gene in vitro and in vivo, depending on the specific cancer type. SRT1720 is a novel first-in-class SIRT1 activator which triggers apoptosis in MM cell lines via the activation of the DNA repair pathway, ATM-CHK2 [39].

SIRT6 is highly expressed in human multiple myeloma and is virtually absent in normal human mononuclear cells. High SIRT6 levels are associated with an adverse prognosis in MM. SIRT6 downregulates the expression of mitogen-activated protein kinase (MAPK) pathway genes. It inactivates ERK2/p90RSK signaling, allowing DNA repair via Chk1 hence conferring resistance to DNA damage treatment [40].

7.2.4 Class IV Histone Deacetylases

HDAC11 is the sole class IV HDAC. It is isolated in tissues such as the heart, muscle, and kidney but there is limited knowledge about its function. HDAC11 is important in the development of plasma cells. HDAC11 knockout mice exhibited an 88% decrease in bone marrow plasma cells compared to wild-type mice. Selective inhibition of HDAC11 pharmacologically and by small interfering RNA (siRNA) reduced the viability of MM cell lines [41]. *Mithraprabhu* et al. demonstrated variable expression of HDAC11 in primary MM cells and human MM cell lines. Overexpression of HDAC11 along with HDAC1, HDAC2, HDAC4, HDAC6, was associated with poor prognosis in human MM patients [42].

Dysregulation of HDAC expression has been implicated in the pathogenesis of many cancers. Specific to hematological malignancies, HDAC dysregulation has been reported in peripheral T cell lymphomas, cutaneous T cell lymphomas, diffuse large B cell lymphomas, pediatric acute lymphoblastic leukemia, myeloproliferative neoplasms, and MM.

7.3 Histone Deacetylases in Multiple Myeloma

HDACs repress gene transcription by deacetylation of histones and regulate multiple cellular pathways by deacetylation of nonhistone proteins. These pathways include cell cycling, apoptosis, DNA repair, oxidative stress response, unfolded

protein response, autophagy, and angiogenesis. In MM, inhibition of HDAC has significant synergistic therapeutic effects with proteasome inhibitors. The combination of the pan-HDAC inhibitor, panobinostat with the proteasome inhibitor bortezomib is an approved therapy in relapsed/refractory multiple myeloma (RRMM). This has led to further research and understanding of the role of HDAC in the pathogenesis and the mechanism of drug resistance in MM and other cancers. Despite the therapeutic effects of HDAC inhibition, the specific roles of HDAC in MM are still unclear.

7.3.1 Histone Deacetylases and Protein Clearance

Multiple myeloma is highly dependent on the ubiquitin proteasome pathway for the disposal of misfolded and unfolded proteins. Proteasome inhibitors are the backbone of anti-myeloma therapy. The aggresome/aggrephagy pathway is an alternate pathway for protein degradation when the ubiquitin proteasome pathway is overwhelmed or inhibited by drugs and is dependent on HDAC6. HDAC6 localizes in and deacetylates microtubules, binding ubiquitin which tags onto misfolded protein aggregates [43–45]. It mediates the transport of protein aggregates along the microtubules to the microtubule organization center, where aggresomes and autophagosomes are formed. Autophagosomes fuse with lysosomes where proteins and polymers are degraded by hydrolases [46]. In addition, HDAC6 dissociates from heat shock protein (HSP)-90 in the presence of protein aggregates leading to the activation of HSF1 [47, 48]. HSF1 activation leads to further activation of heat shock proteins/chaperones. Therefore, HDAC6 plays a crucial role in the clearance of misfolded proteins when the proteasomes are inhibited. This underscores the synergism between HDAC and proteasome inhibition in MM and the development and approval of panobinostat, in the treatment of multiple myeloma [49–51].

7.3.2 Histone Deacetylase Overexpression and Increased Activity in Multiple Myeloma

Overexpression of Class I HDACs (HDAC1, 2, 3, and 8) and Class II HDACs (HDAC5 and 10) has been observed in human MM cell lines compared with normal plasma cells [42]. In primary human MM samples, overexpression of HDAC1, 2, 4, 6, and 11 were shown to be associated with poor prognosis [42]. Elevated HDAC1 protein expression by immunohistochemistry was associated with inferior overall survival in MM [42]. It is clear that HDACs are dysregulated in MM however the role of each HDAC in the pathogenesis of MM remains unclear.

Increased HDAC activity plays a role in the growth of MM cells. In the MM cell line MOLP8, acetylation of H3K9 is markedly reduced in the c-myc proto-oncogene coding regions and the MCL1 coding regions and promoter. When treated with

vorinostat, an inhibitor of Class I, II, and IV HDACs, these regions became acetylated [52]. In addition, it has been shown that melphalan and gamma radiation-induced apoptosis was associated with hyperacetylation of MYC and cyclin D1 (CCND1) oncoprotein [53]. This suggests that the growth of MM is associated with deacetylation and cytotoxicity is associated with hyperacetylation.

Further research of the specific function of HDACs in MM is important to develop and improve the therapeutic role of HDACi in MM.

7.4 Histone Deacetylase Inhibitors

7.4.1 Types of Histone Deacetylase Inhibitors

There are five broad categories of HDACi based on their chemical structure: aliphatic fatty acids, hydroxamic acid, benzamides, cyclic peptides, and mercaptoketone (Table 7.1).

7.4.2 Mechanisms of Action

Histone deacetylase inhibitors induce growth arrest, differentiation, and apoptosis in cancer cells in vitro and in vivo. In most cancers including MM, HDACi are used in combination with other anticancer drugs. It increases the acetylation of histone

Table 7.1 HDAC inhibitors

Name	Target HDAC Class	Examples
Aliphatic fatty acids	I and IIa	Butyrate Valporic acid
Hydroxamic acid	All classes All classes All classes Class I and II Class IIb HDAC6 Class IIb HDAC6	SAHA (Vorinostat) Belinostat (PXD101) Panobinostat (LBH-589) Givinostat (ITF2357) Resminostat (4SC-201 Abexinostat (PCI-24781) Tubacin Ricolinostat (ACY-1215)
Benzamides	Class I Class I Class I Class I and IV	Entinostat (MS-275) Mocetinostat (MGCD0103) Tacedinaline (CI-994) MGCD-0103
Cyclic peptides	Class I Class I Class I	Depsipeptide (FK228) Romidepsin Apicidin
Mercaptoketone	Class I and II	KD5170

and nonhistone proteins and affects the transcriptional modulation of 7%–10% of the genes in MM and lymphoma cell lines [15]. HDAC inhibitor effects on nonhistone proteins are even more extensive with at least 50 candidate nonhistone proteins including transcription factors, transcription regulators, signal transduction mediators, DNA repair enzymes, nuclear import regulators, chaperone proteins, structural proteins, inflammation mediators, and viral proteins. The efficacy of HDACi is dependent on cell type, context, dose, and chemical structure of the inhibitor. Cancer cells are more susceptible to the effects of HDACi than normal cells, supporting its development as an anticancer drug.

7.4.2.1 Altered Gene Expression

Histone deacetylase inhibitors acetylate the histones of specific genes. They are able to induce the expression of cyclin-dependent kinase (CDK) inhibitor p21 (WAF1/CIP1) by increasing the acetylation of histones H3 and H4 associated with the p21 promoter region [54, 55]. This enables cell cycle arrest, repair, terminal differentiation, and prevention of DNA replication in response to DNA damage.

7.4.2.2 Induction of Apoptosis

Tumor death mainly occurs via apoptosis through mitochondrial (intrinsic) and death receptor (extrinsic) pathways. These pathways converge to activate caspases and trigger cell death. HDACi is able to induce both intrinsic and extrinsic apoptosis. Apicidin, a cyclic tetrapeptide HDACi induces extrinsic apoptosis of HL60 cell lines by induction of Fas/Fas ligand and induces intrinsic apoptosis, evidenced by the translocation of Bax from the cytosol to the mitochondria and the release of cytochrome c [56]. Suberoylanilide hydroxamic acid (SAHA) and trichostatin A (TSA) induce the expression of DR3 and DR4, mediating tumor necrosis family-related apoptosis-inducing ligand (TRAIL) induced extrinsic apoptosis.

HDAC inhibitors also induce intrinsic apoptosis by the upregulation of the pro-apoptotic factors (Bax, Bak, Bid, and Bim) of the Bcl-2 related proteins, relative to the pro-survival factors (Bcl-2, Bcl-xL, Bcl-w, Mcl-1, and A1). Cancer cells are more susceptible to HDACi induced apoptosis than their normal counterparts, partly because cancer is dependent on the upregulation of the pro-survival factors. In MM there is overexpression of pro-survival factors Bcl-2 or Mcl-1 and downregulation of the pro-apoptotic protein Bax [57, 58]. SAHA and TSA upregulate the pro-apoptotic factors Bim, Bak, Noxa, PUMA β/δ, and Bax and downregulate the pro-survival factors Bcl-2 and Bcl-X_L [59].

The mechanism by which HDACi interferes with the balance of pro-apoptotic and pro-survival factors is heterogeneous. HDAC3 inhibition increases the acetylation and ubiquitination of DNA-methyltransferase 1 (DNMT1), leading to reduced DNMT1 expression and downregulation of the members of the XIAP family (apoptosis inhibitors) and Bcl-2 [60]. Panobinostat, a pan-HDACi, induces apoptosis

through caspase 3 mediated interferon regulatory factor 4 (IRF4) and MYC degradation [61]. Pharmacologic and genetic inhibition of HDAC4 (Class IIa) was shown to induce ATF4 and CHOP expression and upregulate intrinsic pro-apoptotic factors Bim, Puma, and Bax [62].

7.4.2.3 Cell Cycle Arrest

Most, if not all, HDACi induce G0/S/G1 arrest. This is mediated by p53-dependent and independent upregulation of cyclin-dependent kinase inhibitor proteins. SAHA and TSA induce G1 growth arrest by upregulation of p21WAF1, p27Kip1, and p53 in myeloma cell lines [55, 59, 63]. Resminostat, a potent inhibitor of HDAC1, 3, and 6, induces G0/G1 cell cycle arrest in MM cell lines by decreased levels of Cyclin D1, Cdc25a, Cdk4, pRb, and upregulation of p21 [64].

7.4.2.4 Inhibition of Angiogenesis

Histone deacetylases play a significant role in angiogenesis during embryogenesis, tissue repair, and cancer growth. HDAC7, a class IIa HDAC is an essential regulator of embryonic blood vessel development [9]. HDAC7 controls endothelial angiogenic functions, such as tube formation, migration, and proliferation [10–12]. Conversely, HDAC5 represses angiogenic gene expression in endothelial cells and angiogenesis [13]. HDAC5 and HDAC7 are controlled by protein kinase D-dependent phosphorylation which mediates their nuclear export [14, 15].

HDAC6 is important in endothelial cell sprouting, tube formation, and perfusion of blood vessels. It is transcriptionally activated by hypoxia and deacetylates cortactin independently of deacetylation of alpha-tubulin in the cytoplasm. Cortactin is essential for endothelial cell migration and blood vessel formation. However, HDAC6 deficiency is not embryonically lethal as HDAC10 compensates its angiogenic function [65, 66].

Class I and II HDACi inhibit angiogenesis in vitro and in animal models [16]. The pan-HDAC inhibitor AR-42 inhibits angiogenesis by hyperacetylation of histones H3 and H4, upregulation of miR-9-5p, and downregulation of CD44 [67]. CD44 is a receptor for hyaluronic acid which induces the expression of plasminogen activator-inhibitor-1 (PAI-1) [68]. PAI-1 overexpression is associated with an unfavorable prognosis in many cancers. It stimulates the endothelial cell migration from vitronectin to fibronectin, promoting vascularization and tumor invasion from the vitronectin rich perivascular space into the fibronectin rich, poorly vascularized tumor stroma as well as promoting fibrosis [69].

R306465, a hydroxamate-based, potent inhibitor of HDAC1 and HDAC8 (class I HDACs) inhibits angiogenesis in vivo and induces G1 cell cycle arrest and apoptosis in solid and hematological malignancies including MM [70]. Panobinostat, a hydroxamic, pan-HDAC inhibitor has anti-angiogenic activity in prostate cancer xenografts. It acetylates histone H3 and alpha-tubulin in human umbilical vein

endothelial cells [71]. Panobinostat also inhibits angiogenesis in Hodgkin Lymphoma cell lines by inhibition of HIF-1α expression [72]. TSA is a HDACi that induces the ubiquitination of histone acetylases, leading to the reduction of NOX4 expression and inhibition of angiogenesis [73, 74].

7.4.2.5 Regulation of Cytokines

HDAC inhibitors downregulate the expression of genes involved in cytokine signaling in MM. These include insulin-like growth factor 1 (IGF-1), IGF-1 receptor, and interleukin (IL)-6 receptor, important components in the interaction between MM cells and the bone marrow microenvironment.

IL-6 is a major growth factor for myeloma cells which is produced by the tumor microenvironment [75]. IL-6 binds to soluble IL-6 Receptor to form the IL-6/IL-6R complex, which binds to CD130 (gp130) on MM cells to activate downstream STAT3 signaling and ultimately MM cell survival and proliferation. This process is called IL-6 trans-signaling [76]. The natural inhibitor of IL-6 trans-signaling is the soluble gp130 (sgp130) which is dependent on HDAC3 mediated secretion by the bone marrow stromal cells [77].

Vorinostat (SAHA) had been shown to suppress the expression of IGF-1 and its receptor IGF-1R, IL-6R receptor and its key signal transducer gp130, TNF-receptor-1 (TNF-R1), BCMA, and paracrine IL-6 secretion by BMSC [78].

7.4.2.6 Suppressed DNA Damage Repair

HDAC inhibitors affect the function of certain DNA repair proteins resulting in double-stranded breaks in DNA. KD5170, a novel mercaptoketone-based HDACi induced oxidative stress and oxidative DNA damage in myeloma cells as evidenced by the upregulation of heme oxygenase-1 and H2A.X phosphorylation [79].

SAHA selectively suppressed DNA repair proteins in cancer cells but not in normal cells, and therefore preferentially causing cell death in cancer cells [80].

7.4.2.7 Ubiquitin Proteasome System

Multiple myeloma is highly dependent on the ubiquitin proteasome system. Misfolded proteins are often refolded with the help of chaperones. If this process fails, misfolded proteins are ubiquitinated and are predominantly degraded by the 26S proteasome. Failure of this degradation process results in the accumulation of these proteins which are toxic to the cells. HR23B (also known as UV excision repair protein RAD23 homolog B, XP-C repair complementing complex 58 kDa protein and p58) is a protein which is situated on the proteasome and shuttles ubiquitinated proteins into the proteasome for degradation [81]. HDAC inhibitors can result in the hyperacetylation of HR23B, aberrant proteasomal activity, and cell death. HR23B is essential to the action of HDACi (TSA, SAHA, and BELINOSTAT).

In cutaneous T cell lymphoma, HR23B is a biomarker that predicts sensitivity to HDACi [82].

Panobinostat decreases DNMT1 by hyperacetylation of HSP90, disruption of the HSP90 and DNMT1 complex, and mediating proteasomal degradation of DNMT1. This contributes to the anticancer activity of panobinostat in breast cancer as DNMT1 is an essential breast cancer stem cell survival factor [83, 84].

7.4.2.8 Aggresome Pathway

The aggresome pathway is an alternate protein degradation pathway to the proteasome pathway. Unfolded or misfolded proteins can form protein aggregates, which are not degradable by the proteasomes and are toxic to cells. To avoid cell death, protein aggregates are ubiquitinated and are taken up by aggresomes. Autophagosomes then engulf the aggresomes and fuse with lysosomes where their contents are degraded by lysosomal hydrolases [19].

HDAC6 is essential for the formation of aggresomes. HDAC6 localizes in the microtubule and regulates the acetylation of microtubules. HDAC6 binds to polyubiquitinated misfolded proteins and dynein motors, which transport the polyubiquitinated misfolded proteins to aggresomes at the microtubule-organizing center [46]. Histone deacetylase inhibitors induce proteotoxic stress and cell death by blocking HDAC6.

Catley et al. has shown that the proteasome inhibitor bortezomib and the pan-HDACi panobinostat synergistically induced apoptosis of myeloma cells by hyperacetylation of tubulin and accumulation of small aggresomes [49]. This finding laid the foundation to the clinical development and subsequent approval of panobinostat in combination with bortezomib in the treatment of RRMM.

7.5 Approved Histone Deacetylase Inhibitors

The US Food and Drug Administration (FDA) has approved four HDACi in hematological malignancies: vorinostat (Zolinza) and romidepsin (Istodax) for the treatment of cutaneous T cell lymphoma; belinostat (Beleodaq) and panobinostat (Farydak) for the treatment of peripheral T cell lymphoma and panobinostat for MM [21].

7.5.1 Panobinostat

Histone deacetylase inhibitors and PIs have been shown to act synergistically to induce cell death in MM [25]. Proteasome inhibition results in the accumulation of misfolded proteins that are prone to aggregation. The presence of HDACi prevents

the removal of these protein aggregates by inhibiting the aggresome pathway, resulting in cytotoxic stress and downstream activation of cell apoptosis.

In February 2015, FDA approved panobinostat (Farydak; Novartis Pharmaceuticals), an orally administered, pan-HDACi, in combination with bortezomib for the treatment of patients with MM who have received at least two prior lines of therapy including bortezomib and IMiDs.

Panobinostat has a stronger inhibitory effect against HDAC classes I, II, and IV compared to Vorinostat [25]. At the molecular level panobinostat affects cell cycle progression and apoptosis. Ninety percent of the drug is bound to plasma protein and the peak concentration is reached within 2 h. Metabolism of panobinostat occurs via reduction, hydrolysis, oxidation, and glucuronidation. Approximately 40% of panobinostat is eliminated via CYP3A and approximately another 40% is eliminated by CYP2D6. Panobinostat is excreted from the body via the urine (29–51%) and via the feces (44–77%) [26].

Panobinostat was shown to improve survival in the PANORAMA1 trial, a multicenter, double-blinded phase III clinical trial of bortezomib, panobinostat, and dexamethasone compared with placebo, bortezomib, and dexamethasone in RRMM patients who had received one to three previous treatment regimens. Approximately 768 eligible patients were randomized. The study observed increased rates of complete response (CR) or near CR with panobinostat compared to the placebo group (27.6% vs 16.7%) [85]. It also showed a prolonged median duration of response (13.14 vs 10.87 months), median PFS (11·99 vs 8·08 months), and median overall survival (OS) (33.6 vs 30.4 months) favoring the panobinostat group [85]. A subgroup analysis showed that panobinostat was associated with an improved PFS of 12.5 vs 4.7 months (HR 0.47; 95% CI 0.31–0.72) in patients who had received two or more prior regimens including bortezomib and an IMiD [86].

Panobinostat is the first HDACi used in MM. It is not widely used as other targeted therapy probably due to its relatively small benefit compared with its added toxicities including mainly gastrointestinal effects and cytopenia.

7.5.2 Vorinostat

Vorinostat was the first HDACi approved for the treatment of cancer. It was approved in October 2006 for the treatment of progressive, persistent, or recurrent cutaneous T cell lymphoma [28]. It is a SAHA and an oral nonselective inhibitor of class I and II HDACs. When combined with bortezomib, vorinostat showed mild increases in efficacy in RRMM in multiple phase I, II, and III clinical trials compared with bortezomib alone [28]. However, vorinostat is currently not approved for use in MM.

7.5.3 *Ricolinostat*

Ricolinostat is a selective inhibitor of HDAC6 and has been tested as monotherapy and in combination with bortezomib and dexamethasone in a phase I/II study [87]. The combination therapy was well tolerated at ricolinostat doses of up to 160 mg/day. The overall response rate (ORR) was 29% with a clinical benefit rate of 39%. The most common treatment emergent adverse events were thrombocytopenia (71%), diarrhea (67%), anemia (42%), fatigue (42%), nausea (38%), hypokalemia (33%), vomiting (29%), peripheral neuropathy (29%), hyperglycemia (25%), and renal insufficiency (21%) [87].

The combination of ricolinostat, lenalidomide, and dexamethasone was tested in an early phase clinical trial with 38 patients. Two dose-limiting toxicities were observed with ricolinostat 160 mg twice daily [88]. More clinical trials are required to further establish the safety and efficacy of this combination.

A meta-analysis has shown a weaker anti-MM effect with ricolinostat compared to vorinostat and panobinostat [89]. The highest ORR of panobinostat in RRMM was 64% versus 51% and 38% in those treated with vorinostat and ricolinostat, respectively. The main adverse events were pancytopenia, fatigue, diarrhea, and nausea which was more pronounced in patients treated with ricolinostat [89].

7.6 Immunomodulatory Imide Drugs and Histone Deacetylase Inhibitors

Immunomodulatory imide drugs are a class of novel targeted therapy in MM consisting of thalidomide, lenalidomide, and pomalidomide. The effects of IMiDs on MM are mediated by the protein cereblon (CRBN) through ubiquitin-dependent and ubiquitin-independent pathways [90]. Studies have shown that the knockdown of CRBN results in resistance to treatment with IMiDs. The combination of IMiDs and HDACi has been shown to have synergistic activity in MM cell lines [91]. This combination induces caspase 8 and caspase 9 cleavage, activating the intrinsic and extrinsic apoptotic pathway and downregulating the anti-apoptotic XIAP protein [91].

Vorinostat (Class I and II HDACi) and Entinostat (Class I HDACi) have synergistic effects with lenalidomide despite the downregulation of CRBN. Their cytotoxic effect is due to the downregulation of c-Myc and is independent of CRBN [91]. On the contrary, ricolinostat, a selective HDAC6 inhibitor, does not affect CRBN activity. It downregulates IKZF1 which in turn decreases IRF4 and c-Myc, inhibiting MM growth [91].

7.7 Potential Mechanisms of Resistance to Histone Deacetylase Inhibitors

The basis of HDACi resistance is largely unknown and complex. This is due to the ability of HDACi to cause alterations at various cellular levels. Many molecular mechanisms of resistance have been demonstrated in vitro in cutaneous T cell lymphoma and solid cancers with limited evidence in humans.

7.7.1 Drug Transporters

ATP binding cassette transporter expression has been implicated in HDACi resistance. ATP binding cassette transporters (ABC transporters) are essential for many processes in the cell and are often overexpressed in cancer cells. Overexpression of these transporters results in increased drug expulsion from cells. The ABC transporter proteins that have been implicated in resistance to HDACi include ABCB1 and ABCC1. Romidepsin is a substrate for ABCB1 and ABCC1 [92, 93].

7.7.2 Cell Signaling

Genome wide gene expression studies of MM cells with different sensitivities to HDACi suggest that HDACi resistance is associated with a 35-gene signature. This signature primarily involves two pathways: the regulation of the actin cytoskeleton and protein processing in the endoplasmic reticulum. Synergism between HDACi and drugs that target the regulation of the actin cytoskeleton has been observed. These drugs include MEK/ERK, PI3K, and FAK inhibitors [94].

In cutaneous T cell lymphoma, the activation of the signal transducer and activator of transcription (STAT) pathway is associated with resistance to vorinostat. STAT1, STAT3, and STAT5 are highly expressed in lymphoma cell lines that are resistant to vorinostat [95].

7.7.3 Antioxidant Pathway

HDACi induced apoptosis is associated with the generation of reactive oxygen species [96]. The activation of an antioxidant signature was shown to be associated with resistance to vorinostat in acute myeloid leukemia and myelodysplasia [97]. Thioredoxin, a major reducing protein, is protective against HDACi induced cell

death. In malignant cells thioredoxin levels are relatively reduced compared with normal cells, and hence they are more sensitive to HDACi induced cell death compared with normal cells. However, when malignant cells are transfected with thioredoxin siRNA, they become more sensitive to HDACi. Therefore, antioxidant mechanisms may mediate resistance to HDACi [98].

7.7.4 Cell Cycle Proteins

HDAC inhibition is associated with the induction of $p21^{CIP1}$. Leukemic cell lines transfected with antisense $p21^{CIP1}$ have increased sensitivity to HDACi, suggesting that induction of $p21^{CIP1}$ could be a potential mechanism of resistance to HDACi [99].

7.7.5 Nuclear Factor-Kappa B

Resistance to panobinostat in cutaneous T cell lymphoma is associated with constitutive activation of NF-kB which activates pro-survival factors, including inhibitors of apoptotic proteins and Bcl-2 family proteins. Inhibition of Bcl-2 by ABT-737 overcomes resistance to panobinostat [100].

7.7.6 Anti-Apoptotic Proteins

Overexpression of anti-apoptotic proteins is another potential mechanism of resistance to HDACi. Valproate and ITF2357 are HDACi with cytotoxic activity in hepatoma cell lines. They inhibit Bcl-xL expression and induce apoptosis. Overexpression of Bcl-xL can induce resistance to Valproate and ITF 2357 on hepatoma cell lines [101].

7.7.7 Altered Histone Deacetylases

Altered expression or structures of HDAC proteins may confer HDACi resistance. Cells of the HL-60 leukemic cell line that were selected for HDACi resistance were observed to express higher levels of HDAC1, HDCA2, and HDAC4 [102]. In melanoma cell lines, overexpression of HDAC1 confers resistance to sodium butyrate [103]. In breast cancer patients, HDAC2 expression level has been correlated with vorinostat response [104].

7.7.8 Autophagy

Autophagy is a cellular process that removes damaged cellular components and misfolded proteins. Aggrephagy is a type of autophagy that targets and removes protein aggregates. HDAC6 is necessary for this process, recognizing protein aggregates through its ubiquitin-binding domain. HDAC6 binds to dynein which is a motor protein mediating the retrograde transport of the protein aggregates on the microtubules to the perinuclear region at the microtubule-organizing center. The protein aggregate is then enclosed by a vimentin cage, becoming an aggresome, which further develops into an autophagosome. The autophagosome then fuses with a lysosome which contains acid hydrolases for the degradation of the enclosed protein aggregates.

The induction of autophagy has been shown to be a mechanism of resistance to other anticancer therapy. Inhibitors of autophagy such as chloroquine and hydroxychloroquine have been shown to overcome resistance to chemotherapy in vitro and in vivo. The combination of chloroquine or hydroxychloroquine and other anticancer drugs are being investigated in ongoing clinical trials for the treatment of a range of cancer types. In a clinical trial comparing gemcitabine hydrochloride and nab-paclitaxel with and without hydroxychloroquine in patients with advanced pancreatic cancer, OS was similar in both groups. However, an improved ORR was observed in the hydroxychloroquine cohort [105]. In an early-phase clinical trial of vorinostat and hydroxychloroquine in metastatic colon cancer, there was preliminary evidence of autophagy inhibition, seen in the accumulation of lysosomal protease cathepsin D and p62 in biopsies [106].

The activation of autophagy could be a potential mechanism of resistance to HDAC6 inhibition. Autophagy activation in MM can be achieved by short hairpin RNA knockdown of HDAC1 or by treatment with SAHA, which upregulates the transcription of LC3, activating the ULK1 Complex and suppressing mTOR [107].

It can be seen that there are multiple potential mechanisms of resistance to HDACi, many of which are not yet fully understood. Further deciphering the mechanisms of HDACi resistance is important as it may enable the discovery of more synergistic combination therapies, the development of novel HDACi, and the discovery of biomarkers that could predict resistance.

7.8 Conclusion

Multiple myeloma remains incurable with most patients either relapsing or becoming refractory to treatments. The incorporation of various novel therapies has resulted in significant survival benefits not only in newly diagnosed MM patients but also in those with RRMM disease. Despite these advances, resistance to therapy leads to eventual relapse and fatal outcomes in the vast majority of patients. There remains an unmet need for novel drugs and efficacious therapies for continued

improvement in outcomes. The incorporation of HDACi with current MM therapies may improve long-term outcomes. The use of these drugs is however limited by unfavorable side effects and drug resistance. Further studies to address this, particularly focusing on combining selective and better tolerated HDACi with PIs and IMiDs offer the possibility of improving outcomes in MM.

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References

1. Cutter AR, Hayes JJ. A brief review of nucleosome structure. FEBS Lett. 2015;589(20 Pt A):2914–22.
2. Struhl K. Histone acetylation and transcriptional regulatory mechanisms. Genes Dev. 1998;12(5):599–606.
3. Hu E, Chen Z, Fredrickson T, Zhu Y, Kirkpatrick R, Zhang GF, et al. Cloning and characterization of a novel human class I histone deacetylase that functions as a transcription repressor. J Biol Chem. 2000;275(20):15,254–64.
4. Schuetz A, Min J, Allali-Hassani A, Schapira M, Shuen M, Loppnau P, et al. Human HDAC7 harbors a class IIa histone deacetylase-specific zinc binding motif and cryptic deacetylase activity. J Biol Chem. 2008;283(17):11,355–63.
5. Finnin MS, Donigian JR, Cohen A, Richon VM, Rifkind RA, Marks PA, et al. Structures of a histone deacetylase homologue bound to the TSA and SAHA inhibitors. Nature. 1999;401(6749):188–93.
6. Hassig CA, Tong JK, Fleischer TC, Owa T, Grable PG, Ayer DE, et al. A role for histone deacetylase activity in HDAC1-mediated transcriptional repression. Proc Natl Acad Sci U S A. 1998;95(7):3519–24.
7. Grozinger CM, Hassig CA, Schreiber SL. Three proteins define a class of human histone deacetylases related to yeast Hda1p. Proc Natl Acad Sci U S A. 1999;96(9):4868–73.
8. Taunton J, Hassig CA, Schreiber SL. A mammalian histone deacetylase related to the yeast transcriptional regulator Rpd3p. Science (New York, NY). 1996;272(5260):408–11.
9. Yang WM, Yao YL, Sun JM, Davie JR, Seto E. Isolation and characterization of cDNAs corresponding to an additional member of the human histone deacetylase gene family. J Biol Chem. 1997;272(44):28,001–7.
10. Emiliani S, Fischle W, Van Lint C, Al-Abed Y, Verdin E. Characterization of a human RPD3 ortholog, HDAC3. Proc Natl Acad Sci U S A. 1998;95(6):2795–800.
11. Dangond F, Hafler DA, Tong JK, Randall J, Kojima R, Utku N, et al. Differential display cloning of a novel human histone deacetylase (HDAC3) cDNA from PHA-activated immune cells. Biochem Biophys Res Commun. 1998;242(3):648–52.
12. Fischer DD, Cai R, Bhatia U, Asselbergs FA, Song C, Terry R, et al. Isolation and characterization of a novel class II histone deacetylase, HDAC10. J Biol Chem. 2002;277(8):6656–66.
13. Gao L, Cueto MA, Asselbergs F, Atadja P. Cloning and functional characterization of HDAC11, a novel member of the human histone deacetylase family. J Biol Chem. 2002;277(28):25,748–55.
14. Kao HY, Lee CH, Komarov A, Han CC, Evans RM. Isolation and characterization of mammalian HDAC10, a novel histone deacetylase. J Biol Chem. 2002;277(1):187–93.
15. Guardiola AR, Yao TP. Molecular cloning and characterization of a novel histone deacetylase HDAC10. J Biol Chem. 2002;277(5):3350–6.

16. Buggy JJ, Sideris ML, Mak P, Lorimer DD, McIntosh B, Clark JM. Cloning and characterization of a novel human histone deacetylase, HDAC8. Biochem J. 2000;350(Pt 1):199–205.
17. Lai A, Lee JM, Yang WM, DeCaprio JA, Kaelin WG Jr, Seto E, et al. RBP1 recruits both histone deacetylase-dependent and -independent repression activities to retinoblastoma family proteins. Mol Cell Biol. 1999;19(10):6632–41.
18. Heinzel T, Lavinsky RM, Mullen TM, Söderstrom M, Laherty CD, Torchia J, et al. A complex containing N-CoR, mSin3 and histone deacetylase mediates transcriptional repression. Nature. 1997;387(6628):43–8.
19. Brehm A, Miska EA, McCance DJ, Reid JL, Bannister AJ, Kouzarides T. Retinoblastoma protein recruits histone deacetylase to repress transcription. Nature. 1998;391(6667):597–601.
20. Doetzlhofer A, Rotheneder H, Lagger G, Koranda M, Kurtev V, Brosch G, et al. Histone deacetylase 1 can repress transcription by binding to Sp1. Mol Cell Biol. 1999;19(8):5504–11.
21. Yarden RI, Brody LC. BRCA1 interacts with components of the histone deacetylase complex. Proc Natl Acad Sci U S A. 1999;96(9):4983–8.
22. Yang WM, Inouye C, Zeng Y, Bearss D, Seto E. Transcriptional repression by YY1 is mediated by interaction with a mammalian homolog of the yeast global regulator RPD3. Proc Natl Acad Sci U S A. 1996;93(23):12845–50.
23. Takaki T, Fukasawa K, Suzuki-Takahashi I, Hirai H. Cdk-mediated phosphorylation of pRB regulates HDAC binding in vitro. Biochem Biophys Res Commun. 2004;316(1):252–5.
24. Galasinski SC, Resing KA, Goodrich JA, Ahn NG. Phosphatase inhibition leads to histone deacetylases 1 and 2 phosphorylation and disruption of corepressor interactions. J Biol Chem. 2002;277(22):19618–26.
25. Guenther MG, Barak O, Lazar MA. The SMRT and N-CoR corepressors are activating cofactors for histone deacetylase 3. Mol Cell Biol. 2001;21(18):6091–101.
26. Wen YD, Perissi V, Staszewski LM, Yang WM, Krones A, Glass CK, et al. The histone deacetylase-3 complex contains nuclear receptor corepressors. Proc Natl Acad Sci U S A. 2000;97(13):7202–7.
27. Kahali S, Sarcar B, Prabhu A, Seto E, Chinnaiyan P. Class I histone deacetylases localize to the endoplasmic reticulum and modulate the unfolded protein response. FASEB J. 2012;26(6):2437–45.
28. Kasler HG, Young BD, Mottet D, Lim HW, Collins AM, Olson EN, et al. Histone deacetylase 7 regulates cell survival and TCR signaling in CD4/CD8 double-positive thymocytes. J Immunol (Baltimore, MD: 1950). 2011;186(8):4782–93.
29. Chang S, Young BD, Li S, Qi X, Richardson JA, Olson EN. Histone deacetylase 7 maintains vascular integrity by repressing matrix metalloproteinase 10. Cell. 2006;126(2):321–34.
30. Hai Y, Shinsky SA, Porter NJ, Christianson DW. Histone deacetylase 10 structure and molecular function as a polyamine deacetylase. Nat Commun. 2017;8:15368.
31. Ridinger J, Koeneke E, Kolbinger FR, Koerholz K, Mahboobi S, Hellweg L, et al. Dual role of HDAC10 in lysosomal exocytosis and DNA repair promotes neuroblastoma chemoresistance. Sci Rep. 2018;8(1):10,039.
32. Frye RA. Characterization of five human cDNAs with homology to the yeast SIR2 gene: Sir2-like proteins (sirtuins) metabolize NAD and may have protein ADP-ribosyltransferase activity. Biochem Biophys Res Commun. 1999;260(1):273–9.
33. Brachmann CB, Sherman JM, Devine SE, Cameron EE, Pillus L, Boeke JD. The SIR2 gene family, conserved from bacteria to humans, functions in silencing, cell cycle progression, and chromosome stability. Genes Dev. 1995;9(23):2888–902.
34. Afshar G, Murnane JP. Characterization of a human gene with sequence homology to Saccharomyces cerevisiae SIR2. Gene. 1999;234(1):161–8.
35. Imai S, Armstrong CM, Kaeberlein M, Guarente L. Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. Nature. 2000;403(6771):795–800.
36. Landry J, Sutton A, Tafrov ST, Heller RC, Stebbins J, Pillus L, et al. The silencing protein SIR2 and its homologs are NAD-dependent protein deacetylases. Proc Natl Acad Sci U S A. 2000;97(11):5807–11.

37. Luo J, Nikolaev AY, Imai S, Chen D, Su F, Shiloh A, et al. Negative control of p53 by Sir2alpha promotes cell survival under stress. Cell. 2001;107(2):137–48.
38. Muth V, Nadaud S, Grummt I, Voit R. Acetylation of TAF(I)68, a subunit of TIF-IB/SL1, activates RNA polymerase I transcription. EMBO J. 2001;20(6):1353–62.
39. Chauhan D, Bandi M, Singh AV, Ray A, Raje N, Richardson P, et al. Preclinical evaluation of a novel SIRT1 modulator SRT1720 in multiple myeloma cells. Br J Haematol. 2011;155(5):588–98.
40. Cea M, Cagnetta A, Adamia S, Acharya C, Tai YT, Fulciniti M, et al. Evidence for a role of the histone deacetylase SIRT6 in DNA damage response of multiple myeloma cells. Blood. 2016;127(9):1138–50.
41. Brayer JB, Distler A, Meads MB, Sahakian E, Powers JJ, Nguyen T, et al. HDAC11 is a candidate therapeutic target in multiple myeloma. Blood. 2017;130(supplement 1):1800.
42. Mithraprabhu S, Kalff A, Chow A, Khong T, Spencer A. Dysregulated class I histone deacetylases are indicators of poor prognosis in multiple myeloma. Epigenetics. 2014;9(11):1511–20.
43. Hubbert C, Guardiola A, Shao R, Kawaguchi Y, Ito A, Nixon A, et al. HDAC6 is a microtubule-associated deacetylase. Nature. 2002;417(6887):455–8.
44. Seigneurin-Berny D, Verdel A, Curtet S, Lemercier C, Garin J, Rousseaux S, et al. Identification of components of the murine histone deacetylase 6 complex: link between acetylation and ubiquitination signaling pathways. Mol Cell Biol. 2001;21(23):8035–44.
45. Boyault C, Gilquin B, Zhang Y, Rybin V, Garman E, Meyer-Klaucke W, et al. HDAC6-p97/VCP controlled polyubiquitin chain turnover. EMBO J. 2006;25(14):3357–66.
46. Kawaguchi Y, Kovacs JJ, McLaurin A, Vance JM, Ito A, Yao TP. The deacetylase HDAC6 regulates aggresome formation and cell viability in response to misfolded protein stress. Cell. 2003;115(6):727–38.
47. Boyault C, Zhang Y, Fritah S, Caron C, Gilquin B, Kwon SH, et al. HDAC6 controls major cell response pathways to cytotoxic accumulation of protein aggregates. Genes Dev. 2007;21(17):2172–81.
48. Hook SS, Orian A, Cowley SM, Eisenman RN. Histone deacetylase 6 binds polyubiquitin through its zinc finger (PAZ domain) and copurifies with deubiquitinating enzymes. Proc Natl Acad Sci U S A. 2002;99(21):13425–30.
49. Catley L, Weisberg E, Kiziltepe T, Tai YT, Hideshima T, Neri P, et al. Aggresome induction by proteasome inhibitor bortezomib and alpha-tubulin hyperacetylation by tubulin deacetylase (TDAC) inhibitor LBH589 are synergistic in myeloma cells. Blood. 2006;108(10):3441–9.
50. Hideshima T, Bradner JE, Wong J, Chauhan D, Richardson P, Schreiber SL, et al. Small-molecule inhibition of proteasome and aggresome function induces synergistic antitumor activity in multiple myeloma. Proc Natl Acad Sci U S A. 2005;102(24):8567–72.
51. Redic KA, Hough SM, Price EM. Clinical developments in the treatment of relapsed or relapsed and refractory multiple myeloma: impact of panobinostat, the first-in-class histone deacetylase inhibitor. Oncol Targets Therapy. 2016;9:2783–93.
52. Foltankova V, Legartova S, Kozubek S, Bartova E. Tumor-specific histone signature and DNA methylation in multiple myeloma and leukemia cells. Neoplasma. 2012;59(4):450–62.
53. Krejcí J, Harnicarová A, Streitová D, Hájek R, Pour L, Kozubek S, et al. Epigenetics of multiple myeloma after treatment with cytostatics and gamma radiation. Leuk Res. 2009;33(11):1490–8.
54. Richon VM, Sandhoff TW, Rifkind RA, Marks PA. Histone deacetylase inhibitor selectively induces p21WAF1 expression and gene-associated histone acetylation. Proc Natl Acad Sci U S A. 2000;97(18):10014–9.
55. Gui CY, Ngo L, Xu WS, Richon VM, Marks PA. Histone deacetylase (HDAC) inhibitor activation of p21WAF1 involves changes in promoter-associated proteins, including HDAC1. Proc Natl Acad Sci U S A. 2004;101(5):1241–6.
56. Kwon SH, Ahn SH, Kim YK, Bae GU, Yoon JW, Hong S, et al. Apicidin, a histone deacetylase inhibitor, induces apoptosis and Fas/Fas ligand expression in human acute promyelocytic leukemia cells. J Biol Chem. 2002;277(3):2073–80.

57. Jourdan M, De Vos J, Mechti N, Klein B. Regulation of Bcl-2-family proteins in myeloma cells by three myeloma survival factors: interleukin-6, interferon-alpha and insulin-like growth factor 1. Cell Death Differ. 2000;7(12):1244–52.
58. Slomp A, Peperzak V. Role and regulation of pro-survival BCL-2 proteins in multiple myeloma. Front Oncol. 2018;8:533.
59. Fandy TE, Shankar S, Ross DD, Sausville E, Srivastava RK. Interactive effects of HDAC inhibitors and TRAIL on apoptosis are associated with changes in mitochondrial functions and expressions of cell cycle regulatory genes in multiple myeloma. Neoplasia (New York, NY). 2005;7(7):646–57.
60. Harada T, Ohguchi H, Grondin Y, Kikuchi S, Sagawa M, Tai YT, et al. HDAC3 regulates DNMT1 expression in multiple myeloma: therapeutic implications. Leukemia. 2017;31:2670–7.
61. Tang S, Ma D, Cheng B, Fang Q, Kuang X, Yu K, et al. Crucial role of HO-1/IRF4-dependent apoptosis induced by panobinostat and lenalidomide in multiple myeloma. Exp Cell Res. 2018;363(2):196–207.
62. Kikuchi S, Suzuki R, Ohguchi H, Yoshida Y, Lu D, Cottini F, et al. Class IIa HDAC inhibition enhances ER stress-mediated cell death in multiple myeloma. Leukemia. 2015;29(9):1918–27.
63. Yuan XG, Huang YR, Yu T, Jiang HW, Xu Y, Zhao XY. Chidamide, a histone deacetylase inhibitor, induces growth arrest and apoptosis in multiple myeloma cells in a caspase-dependent manner. Oncol Lett. 2019;18(1):411–9.
64. Mandl-Weber S, Meinel FG, Jankowsky R, Oduncu F, Schmidmaier R, Baumann P. The novel inhibitor of histone deacetylase resminostat (RAS2410) inhibits proliferation and induces apoptosis in multiple myeloma (MM) cells. Br J Haematol. 2010;149(4):518–28.
65. Kaluza D, Kroll J, Gesierich S, Yao TP, Boon RA, Hergenreider E, et al. Class IIb HDAC6 regulates endothelial cell migration and angiogenesis by deacetylation of cortactin. EMBO J. 2011;30(20):4142–56.
66. Kaluza D, Kroll J, Gesierich S, Yao T-P, Boon RA, Hergenreider E, et al.
67. Canella A, Cordero Nieves H, Sborov DW, Cascione L, Radomska HS, Smith E, et al. HDAC inhibitor AR-42 decreases CD44 expression and sensitizes myeloma cells to lenalidomide. Oncotarget. 2015;6(31):31,134–50.
68. Park D, Kim Y, Kim H, Kim K, Lee YS, Choe J, et al. Hyaluronic acid promotes angiogenesis by inducing RHAMM-TGFβ receptor interaction via CD44-PKCδ. Mol Cells. 2012;33(6):563–74.
69. Isogai C, Laug WE, Shimada H, Declerck PJ, Stins MF, Durden DL, et al. Plasminogen activator inhibitor-1 promotes angiogenesis by stimulating endothelial cell migration toward fibronectin. Cancer Res. 2001;61(14):5587–94.
70. Arts J, Angibaud P, Mariën A, Floren W, Janssens B, King P, et al. R306465 is a novel potent inhibitor of class I histone deacetylases with broad-spectrum antitumoral activity against solid and haematological malignancies. Br J Cancer. 2007;97:1344–53.
71. Qian DZ, Kato Y, Shabbeer S, Wei Y, Verheul HM, Salumbides B, et al. Targeting tumor angiogenesis with histone deacetylase inhibitors: the hydroxamic acid derivative LBH589. Clin Cancer Res. 2006;12(2):634–42.
72. Lemoine M, Derenzini E, Buglio D, Medeiros LJ, Davis RE, Zhang J, et al. The pan-deacetylase inhibitor panobinostat induces cell death and synergizes with everolimus in Hodgkin lymphoma cell lines. Blood. 2012;119(17):4017–25.
73. Hakami NY, Dusting GJ, Peshavariya HM. Trichostatin a, a histone deacetylase inhibitor suppresses NADPH oxidase 4-derived redox Signalling and angiogenesis. J Cell Mol Med. 2016;20(10):1932–44.
74. Hakami NY, Dusting GJ, Peshavariya HM.
75. Klein B, Zhang XG, Jourdan M, Boiron JM, Portier M, Lu ZY, et al. Interleukin-6 is the central tumor growth factor in vitro and in vivo in multiple myeloma. Eur Cytokine Netw. 1990;1(4):193–201.

76. Jones SA, Rose-John S. The role of soluble receptors in cytokine biology: the agonistic properties of the sIL-6R/IL-6 complex. Biochim Biophys Acta. 2002;1592(3):251–63.
77. Ho M, Chen T, Liu J, Dowling P, Hideshima T, Zhang L, et al. Targeting histone deacetylase 3 (HDAC3) in the bone marrow microenvironment inhibits multiple myeloma proliferation by modulating exosomes and IL-6 trans-signaling. Leukemia. 2020;34(1):196–209.
78. Mitsiades CS, Mitsiades NS, McMullan CJ, Poulaki V, Shringarpure R, Hideshima T, et al. Transcriptional signature of histone deacetylase inhibition in multiple myeloma: biological and clinical implications. Proc Natl Acad Sci U S A. 2004;101(2):540–5.
79. Feng R, Ma H, Hassig CA, Payne JE, Smith ND, Mapara MY, et al. KD5170, a novel mercaptoketone-based histone deacetylase inhibitor, exerts antimyeloma effects by DNA damage and mitochondrial signaling. Mol Cancer Ther. 2008;7(6):1494–505.
80. Lee JH, Choy ML, Ngo L, Foster SS, Marks PA. Histone deacetylase inhibitor induces DNA damage, which normal but not transformed cells can repair. Proc Natl Acad Sci U S A. 2010;107(33):14,639–44.
81. Elsasser S, Chandler-Militello D, Müller B, Hanna J, Finley D. Rad23 and Rpn10 serve as alternative ubiquitin receptors for the proteasome. J Biol Chem. 2004;279(26):26817–22.
82. Fotheringham S, Epping MT, Stimson L, Khan O, Wood V, Pezzella F, et al. Genome-wide loss-of-function screen reveals an important role for the proteasome in HDAC inhibitor-induced apoptosis. Cancer Cell. 2009;15(1):57–66.
83. Zhou Q, Agoston AT, Atadja P, Nelson WG, Davidson NE. Inhibition of histone deacetylases promotes ubiquitin-dependent proteasomal degradation of DNA methyltransferase 1 in human breast cancer cells. Mol Cancer Res. 2008;6(5):873–83.
84. Pathania R, Ramachandran S, Elangovan S, Padia R, Yang P, Cinghu S, et al. DNMT1 is essential for mammary and cancer stem cell maintenance and tumorigenesis. Nat Commun. 2015;6:6910.
85. San-Miguel JF, Hungria VT, Yoon SS, Beksac M, Dimopoulos MA, Elghandour A, et al. Panobinostat plus bortezomib and dexamethasone versus placebo plus bortezomib and dexamethasone in patients with relapsed or relapsed and refractory multiple myeloma: a multicentre, randomised, double-blind phase 3 trial. Lancet Oncol. 2014;15(11):1195–206.
86. Richardson PG, Hungria VT, Yoon SS, Beksac M, Dimopoulos MA, Elghandour A, et al. Panobinostat plus bortezomib and dexamethasone in previously treated multiple myeloma: outcomes by prior treatment. Blood. 2016;127(6):713–21.
87. Vogl DT, Raje N, Jagannath S, Richardson P, Hari P, Orlowski R, et al. Ricolinostat, the first selective histone deacetylase 6 inhibitor, in combination with Bortezomib and dexamethasone for relapsed or refractory multiple myeloma. Clin Cancer Res. 2017;23(13):3307–15.
88. Yee AJ, Bensinger WI, Supko JG, Voorhees PM, Berdeja JG, Richardson PG, et al. Ricolinostat plus lenalidomide, and dexamethasone in relapsed or refractory multiple myeloma: a multicentre phase 1b trial. Lancet Oncol. 2016;17(11):1569–78.
89. Gao X, Shen L, Li X, Liu J. Efficacy and toxicity of histone deacetylase inhibitors in relapsed/refractory multiple myeloma: systematic review and meta-analysis of clinical trials. Exp Ther Med. 2019;18(2):1057–68.
90. Shi Q, Chen L. Cereblon: a protein crucial to the multiple functions of immunomodulatory drugs as well as cell metabolism and disease generation. J Immunol Res. 2017;2017:9130608.
91. Hideshima T, Cottini F, Ohguchi H, Jakubikova J, Gorgun G, Mimura N, et al. Rational combination treatment with histone deacetylase inhibitors and immunomodulatory drugs in multiple myeloma. Blood Cancer J. 2015;5(5):e312.
92. Xiao JJ, Foraker AB, Swaan PW, Liu S, Huang Y, Dai Z, et al. Efflux of depsipeptide FK228 (FR901228, NSC-630176) is mediated by P-glycoprotein and multidrug resistance-associated protein 1. J Pharmacol Exp Ther. 2005;313(1):268–76.
93. Ruefli AA, Bernhard D, Tainton KM, Kofler R, Smyth MJ, Johnstone RW. Suberoylanilide hydroxamic acid (SAHA) overcomes multidrug resistance and induces cell death in P-glycoprotein-expressing cells. Int J Cancer. 2002;99(2):292–8.

94. Mithraprabhu S, Khong T, Spencer A. Overcoming inherent resistance to histone deacetylase inhibitors in multiple myeloma cells by targeting pathways integral to the actin cytoskeleton. Cell Death Dis. 2014;5(3):e1134.
95. Fantin VR, Loboda A, Paweletz CP, Hendrickson RC, Pierce JW, Roth JA, et al. Constitutive activation of signal transducers and activators of transcription predicts vorinostat resistance in cutaneous T-cell lymphoma. Cancer Res. 2008;68(10):3785–94.
96. Ruefli AA, Ausserlechner MJ, Bernhard D, Sutton VR, Tainton KM, Kofler R, et al. The histone deacetylase inhibitor and chemotherapeutic agent suberoylanilide hydroxamic acid (SAHA) induces a cell-death pathway characterized by cleavage of bid and production of reactive oxygen species. Proc Natl Acad Sci U S A. 2001;98(19):10833–8.
97. Garcia-Manero G, Yang H, Bueso-Ramos C, Ferrajoli A, Cortes J, Wierda WG, et al. Phase 1 study of the histone deacetylase inhibitor vorinostat (suberoylanilide hydroxamic acid [SAHA]) in patients with advanced leukemias and myelodysplastic syndromes. Blood. 2008;111(3):1060–6.
98. Ungerstedt JS, Sowa Y, Xu WS, Shao Y, Dokmanovic M, Perez G, et al. Role of thioredoxin in the response of normal and transformed cells to histone deacetylase inhibitors. Proc Natl Acad Sci U S A. 2005;102(3):673–8.
99. Vrana JA, Decker RH, Johnson CR, Wang Z, Jarvis WD, Richon VM, et al. Induction of apoptosis in U937 human leukemia cells by suberoylanilide hydroxamic acid (SAHA) proceeds through pathways that are regulated by Bcl-2/Bcl-XL, c-Jun, and p21CIP1, but independent of p53. Oncogene. 1999;18(50):7016–25.
100. Shao W, Growney JD, Feng Y, O'Connor G, Pu M, Zhu W, et al. Activity of deacetylase inhibitor panobinostat (LBH589) in cutaneous T-cell lymphoma models: defining molecular mechanisms of resistance. Int J Cancer. 2010;127(9):2199–208.
101. Armeanu S, Pathil A, Venturelli S, Mascagni P, Weiss TS, Göttlicher M, et al. Apoptosis on hepatoma cells but not on primary hepatocytes by histone deacetylase inhibitors valproate and ITF2357. J Hepatol. 2005;42(2):210–7.
102. Fiskus W, Rao R, Fernandez P, Herger B, Yang Y, Chen J, et al. Molecular and biologic characterization and drug sensitivity of pan-histone deacetylase inhibitor-resistant acute myeloid leukemia cells. Blood. 2008;112(7):2896–905.
103. Bandyopadhyay D, Mishra A, Medrano EE. Overexpression of histone deacetylase 1 confers resistance to sodium butyrate-mediated apoptosis in melanoma cells through a p53-mediated pathway. Cancer Res. 2004;64(21):7706–10.
104. Munster PN, Thurn KT, Thomas S, Raha P, Lacevic M, Miller A, et al. A phase II study of the histone deacetylase inhibitor vorinostat combined with tamoxifen for the treatment of patients with hormone therapy-resistant breast cancer. Br J Cancer. 2011;104(12):1828–35.
105. Karasic TB, O'Hara MH, Loaiza-Bonilla A, Reiss KA, Teitelbaum UR, Borazanci E, et al. Effect of gemcitabine and nab-paclitaxel with or without hydroxychloroquine on patients with advanced pancreatic cancer: a phase 2 randomized clinical trial. JAMA Oncol. 2019;5(7):993–8.
106. Patel S, Hurez V, Nawrocki ST, Goros M, Michalek J, Sarantopoulos J, et al. Vorinostat and hydroxychloroquine improve immunity and inhibit autophagy in metastatic colorectal cancer. Oncotarget. 2016;7(37):59,087–97.
107. Gammoh N, Lam D, Puente C, Ganley I, Marks PA, Jiang X. Role of autophagy in histone deacetylase inhibitor-induced apoptotic and nonapoptotic cell death. Proc Natl Acad Sci U S A. 2012;109(17):6561–5.

Chapter 8
Bone Targeted Therapies

Ashley McEwan and Silvia CW Ling

Abstract Myeloma bone disease (MBD) is present in up to 90% of multiple myeloma (MM) patients and is a product of osteolytic lesions due to dysregulated osteoblast and osteoclast function. Myeloma bone disease is a cause of significant morbidity and decreased quality of life in MM patients as it leads to several skeletal-related events including pathologic fractures, severe bone pain, and spinal cord compression. Bisphosphonate drugs and the monoclonal antibody denosumab are currently the only approved treatments for MBD, despite their potential severe adverse events such as osteonecrosis of the jaw. Further studies and the continued development of novel treatments for MBD are needed to better combat MBD. This chapter will review the available efficacy data of current bisphosphonate drugs in use and denosumab and their mechanisms of action, explore the pathways and potential targets involved in MBD, and review the current progress in the developments of a number of potential novel treatments for MBD.

Keywords Multiple myeloma · Myeloma bone disease · Bisphosphonate drugs · Pamidronate · Zoledronic acid · Denosumab · Myeloma bone disease treatment

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Abbreviations

ASCO	American Society of Clinical Oncology
BAFF	B cell activating factor
bALP	Bone-specific alkaline phosphatase
BMSC	Bone marrow stem cell
BTK	Bruton's tyrosine kinase
CCL	Chemokine (C-C motif) ligand
CCR	Chemokine (C-C motif) receptor
DKK1	Dickkopf-1
FDA	US Food and Drug Administration
HGF	Hepatocyte growth factor
IL	Interleukin
IMiD	Immunomodulatory imide drug
IMWG	International Myeloma Working Group
MBD	Myeloma bone disease
MGUS	Monoclonal gammopathy of uncertain significance
MIP-1α	Macrophage-inhibitory protein 1 alpha
MM	Multiple myeloma
NF-κB	Nuclear factor kappa-B
OAF	Osteoclast-activating factor
OPG	Osteoprotegerin
OS	Overall survival
PI	Proteasome inhibitor
RANK	Receptor activator of nuclear factor kappa-B
sFRP3	Soluble frizzled-related protein 3
SRE	Skeletal related event
TGF-β	Transforming growth factor-beta
TNF	Tumor necrosis factor
TNF-α	Tumor necrosis factor-alpha
TSP1	Thrombospondin 1
uNTX	Urinary N-telopeptide of collagen type 1
VEGF	Vascular endothelial growth factor
β-CTX	Beta-isomerized C-terminal telopeptide of collagen type 1

8.1 Myeloma Bone Disease

Multiple myeloma (MM) is a cancer of differentiated B lymphocytes (plasma cells) and is characterized by clonal proliferation of these plasma cells in the bone marrow, the secretion of a monoclonal protein, and osteolytic bone disease [1]. Myeloma bone disease (MBD) is present in up to 90% of patients and is a result of plasma cell proliferation, characterized by osteolytic lesions and the suppression of osteoblast

differentiation and function [2]. Current International Myeloma Working Group (IMWG) guidelines require the presence of at least one osteolytic lesion to meet the criteria for MBD [3]. Myeloma bone disease leads to several skeletal-related events (SREs) including pathologic fractures, severe bone pain, and spinal cord compression which can result in the need for radiotherapy or surgical fixation [4]. In MM patients, pathologic fractures increase the risk of death by more than 20% compared to patients without fractures [5]. Hence, despite increasing overall survival (OS) for patients with multiple myeloma, MBD and secondary SREs can result in significant morbidity and reduced quality of life, highlighting the need for advancements in the current standard of care [1].

8.1.1 Diagnosis

Myeloma bone disease is diagnosed through the use of plain radiograph, whole body low dose computed tomography, or whole body magnetic resonance imaging skeletal surveys to detect the presence of osteolytic bone lesions [6]. Plain radiograph is the least sensitive available type of imaging, as a bone lesion needs to be at least 1 cm in size and associated with at least 30% loss of bone mineral content before it can be detected [7]. Despite this, most current guidelines recommend plain radiograph skeletal survey as the primary method for the detection of MBD, followed by the utilization of other modalities if there is a suspicion of MBD and conventional radiography is negative [3, 8]. Imaging findings such as osteoporosis, osteopenia, or compression fractures without the presence of osteolytic lesions is insufficient to meet the current criteria for MBD [6]. The IMWG has also noted that increased fluorodeoxyglucose uptake on positron emission tomography (PET) scan without an associated destructive bone lesion does not meet the criteria for MBD [3].

8.1.2 Pathogenesis

Myeloma bone disease occurs as a result of numerous interactions between plasma cells and various pathways that affect osteoclasts and osteoblasts, leading to overall bone loss and the development of lytic bone lesions [1]. While augmented osteoclast function is a key pathogenic mechanism in the development of MBD, the reduction in trabecular thickness, calcification rate, and osteoblast numbers in bone specimens from multiple myeloma patients suggest that dysfunctional osteoblast activity is a significant contributor [9].

Osteoblasts and osteoclasts are the major cells involved in bone remodeling, with other factors including osteocytes, cytokines, and hormones also contributing to the process [10]. Osteoclasts are large multinucleated cells derived from monocyte-macrophage lineage which generate enzymes that breakdown the bone mineral matrix [11]. Osteoblasts are mononuclear cells originating from mesenchymal stem

cells which contain the enzyme alkaline phosphatase [12]. Immature osteoblasts secrete interleukin (IL)-6 that upregulates osteoclasts, while mature osteoblasts secrete osteoprotegerin (OPG) which inhibits the activation of osteoclasts [1]. Osteoblasts create the bone mineral matrix through the secretion of collagen and eventually become trapped as part of the mineralized matrix before differentiating into osteocytes [11]. Osteocytes communicate with surrounding cells in the bone surface and bone marrow via cytoplasmic projections and contribute factors such as receptor activator of nuclear factor kappa-B ligand (RANKL) and sclerostin that affect both osteoblastic and osteoclastic activity [13].

The normal signaling pathways are disrupted by interactions between malignant plasma cells (myeloma cells) and cells of the bone marrow microenvironment [14]. A group of mediators known as osteoclast-activating factors (OAFs) have been identified which include IL-6, interleukin-1 (IL-1), interleukin-3 (IL-3), macrophage-inhibitory protein 1 alpha (MIP-1α), tumor necrosis factor-alpha (TNF-α), hepatocyte growth factor (HGF), and vascular endothelial growth factor (VEGF) [14]. These OAFs affect various components of other pathways including the receptor activator of nuclear factor kappa-B (RANK), RANKL, and OPG. Additionally, certain molecules have been shown to inhibit osteoblast differentiation, including transforming growth factor-beta (TGF-β), HGF and Wnt-signaling inhibitors dickkopf-1 (DKK1), soluble frizzled-related protein-3 (sFRP3), and sclerostin [14].

In the early stages of MBD, myeloma cells secrete IL-1 and tumor necrosis factor (TNF) which stimulate osteoblast differentiation and recruitment to affected sites. These recruited osteoblasts in turn secrete IL-6, which recruits osteoclasts and is a myeloma growth factor [7]. Later in MBD, osteoblast numbers decrease secondary to unclear mechanisms; postulated to be related to osteoblast inhibitory factors or decorin, a proteoglycan produced by osteoblasts which causes an anti-myeloma effect by inhibiting TGF-β [1]. Myeloma also affects osteoprogenitor cells, disrupting the normal production of osteoblasts, resulting in a net effect of reduced osteoblast levels and over activation of osteoclasts and leading to the lytic bone lesions found on imaging in MBD [15]. Hence, any coupled bone remodeling between osteoclasts and osteoblasts is disordered in MM. The numerous factors that affect osteoclasts and osteoblasts and contribute to the pathogenesis of MBD and may be potential foci for bone targeted therapies and are explored in further detail below [4].

8.1.3 Osteoclastic Activation

8.1.3.1 The RANK/RANKL Pathway

The RANK/RANKL pathway plays a major role in osteoclast function and bone remodeling [4]. RANK is a transmembrane receptor that is expressed on the surface of osteoclast precursors, and RANKL is a membrane-bound protein on stromal cells of the osteoblast line and activated T lymphocytes [11]. The binding of RANKL to

RANK triggers the maturation of osteoclast precursors into osteoclasts that bind to the bone surface and initiate bone resorption [14]. OPG is a cytokine secreted by osteoblasts and stromal cells that is a soluble decoy receptor for RANKL that inhibits osteoclast development; in MM patients OPG levels are reduced while RANKL levels are increased [4]. RANKL is produced by myeloma cells and increased RANKL expression by osteoblasts and bone marrow stromal cells can result from stimulation by other contributory cytokines such as increased levels of parathyroid hormone-related peptide [11]. This imbalanced ratio of RANKL to OPG results in a net increase in osteoclast stimulation and bone resorption. Treatments including thalidomide and autologous stem cell transplant can normalize the RANKL to OPG ratio, reducing bone resorption. Denosumab, a human monoclonal antibody (MoAb) targeting RANKL, prevents the activation of this signaling pathway and reduces the burden of MBD as well as progression of disease [16].

8.1.3.2 Interleukins

IL-3 stimulates osteoclast formation and inhibits osteoblast differentiation. IL-3 exerts its osteoclastogenic effect by inducing activin A production by macrophages, a factor involved in promoting osteoclast differentiation [11]. IL-3 also acts in conjunction with RANKL and MIP-1α to increase osteoclastogenesis [14]. Increased levels of IL-3 have been detected in studies of myeloma patient's bone marrow plasma [17]. IL-6 augments osteoclast differentiation by simulating myeloma cells to secrete VEGF which activates osteoclasts via surface receptor binding [11]. IL-6 levels are lowered with DKK1 protein inhibition [14]. IL-17 promotes osteoclast activation and can result in osteolytic lesion formation, although this has been seen mainly in preclinical models [18].

8.1.3.3 Hepatocyte Growth Factor

HGF is produced by bone marrow stem cells (BMSCs) and myeloma cells. The binding of HGF to MET receptor on the surface of myeloma cells triggers downstream signaling via the RAS pathway, causing the growth of myeloma plasma cells and inhibiting apoptosis [11]. HGF also acts as a coupling factor between osteoclasts and osteoblasts and mediates autocrine regulation of osteoclasts and paracrine regulation of osteoblasts.

8.1.3.4 Notch Pathway

There are four Notch transmembrane receptors on myeloma cells that can bind to their ligands on the same cell or on adjacent BMSCs which results in the production of RANKL by the myeloma cells [11]. This generates a feedback loop which stimulates Notch2. The resulting Notch2 signaling cascade further stimulates osteoclast

differentiation and proliferation. Inhibition of this pathway results in apoptosis of myeloma cells and inhibits osteoclastogenesis, representing a potential therapeutic target [11].

8.1.3.5 Chemokines

Certain chemokines are involved in osteoclastogenesis. Chemokine (C-C motif) ligand 3 (CCL-3) is secreted by myeloma cells. Chemokine (C-C motif) ligand 20 (CCL-20) is overexpressed in the multiple myeloma bone marrow. These chemokines induce osteoclastogenesis, and higher levels are detected in myeloma patients, correlating with the extent of bone disease [11]. Chemokine receptors including the CCL-3 receptor, CCR1 receptor, and CCR5 receptor are expressed on BMSCs, osteoclasts, osteoblast, and MM cells. The activation of these receptors attracts immature osteoclasts, promoting differentiation and stimulating RANKL and IL-6 [11]. Inhibitors of CCL-3 and its receptors are promising in preclinical studies for MBD therapy [16].

8.1.3.6 Activin A

When activin A binds to activin type 2A receptor, subsequent signaling results in increased bone resorption and reduced bone formation [16]. Activin A is elevated in myeloma patients, correlating with the extent MBD [11]. Activin A is further upregulated by IL-3, a cytokine released from the BMSCs of myeloma patients [16]. There is a synergistic effect between Activin A and RANKL, resulting in more potent stimulation of osteoclastogenesis. Preclinical studies have demonstrated that osteoclast formation is blocked following treatment with soluble activin receptor type 2A [11].

8.1.3.7 The TNF Superfamily

Key members of the TNF superfamily involved in MBD include TNF-α and B-cell activating factor (BAFF). TNF-α acts together with RANKL to induce osteoclast differentiation and growth and is elevated in MBD patients [11]. BAFF is secreted by myeloma cells, osteoclasts, and BMSCs, causing the activation of nuclear factor kappa-B (NF-κB), resulting in osteoclastogenesis and myeloma cell survival [14].

8.1.3.8 BTK and SDF-1α

Bruton's tyrosine kinase (BTK) regulates osteoclast differentiation and is expressed in osteoclasts. Higher levels of BTK expression have been observed in myeloma patients [16]. BTK is linked to CXCR4 expression. The CXCR4SDF-1α pathway

induces BTK activation, promoting osteoclastogenesis [11]. Inhibition of osteoclastic activity through agents like ibrutinib is being investigated in current clinical trials [19]. This could potentially be an added therapy in patients with severe myeloma bone disease.

8.1.4 Osteoblastic Suppression

8.1.4.1 The WNT Pathway

The Wnt-signaling pathway cascade ultimately results in gene expression favoring bone formation and imminent bone resorption [11]. It is activated via Wnt ligands and parathyroid hormone binding to receptors in the Wnt pathway. Preclinical models demonstrate that increased Wnt signaling inhibits the development of MBD [20]. Conversely, aberrant Wnt signaling can result in the proliferation of myeloma cells and the subsequent development of MBD [11]. This dysregulated Wnt pathway signaling contributes to the invasion of myeloma cells and is linked to their adhesion-mediated drug resistance [21]. The canonical Wnt pathway is inhibited by sclerostin, DKK1, and sFRP2. These proteins have been observed to be elevated in cases of MBD and may be potential therapeutic targets [11].

8.1.4.2 Sclerostin

Sclerostin is a protein encoded by the SOST gene and is produced by osteocytes. Sclerostin induces the apoptosis of mature osteoblasts and reduces osteoblast-driven bone formation [14]. It is an inhibitor of the canonical Wnt pathway through binding to LRP5/6 transmembrane receptors on osteoblasts, blocking the Wnt pathway cascade [11]. Sclerostin is secreted by myeloma cells and suppresses bone formation by inhibiting osteoblastogenesis while stimulating osteoclastogenesis by increasing the ratio of RANKL to OPG ratio [22]. High levels of sclerostin have been observed in patients with more severe disease and pathologic fractures at diagnosis [11]. Monoclonal antibodies against sclerostin are under investigation as a sole therapy for MBD and in conjunction with proteasome inhibitors (PI) [23].

8.1.4.3 DKK1

DKK1 inhibits the Wnt-signaling pathway by competitively binding LRP5/6 receptors and removing transmembrane proteins [14]. DKK1 inhibits osteoblastogenesis by blocking osteoblast differentiation and acts together with sclerostin resulting in osteoblast dysfunction [11]. DKK1 also indirectly increases osteoclastogenesis, by blocking the osteoclastogenesis inhibitor OPG and increasing the osteoclastogenesis activator RANKL [14]. Higher DKK1 levels have been observed in patients with

more extensive MBD [24]. DKK1 expression can be utilized to predict early SREs, and reduced levels can be observed after myeloma treatment has commenced [11].

8.1.4.4 Periostin

Periostin is a protein produced by BMSCs that activates the integrin-AKT-FAK-β-catenin pathway and is implicated in the Wnt-signaling pathway [11]. High periostin levels in myeloma patients are associated with SREs, lytic bone lesions, and more advanced disease [25].

8.1.4.5 RUNX2/CBFA1 and IL-7

IL-7 reduces osteoblast differentiation and stimulates T lymphocytes to secrete RANKL. IL-7 reduces transcriptional levels of runt-related transcription factor 2 (RUNX2) via the noncanonical Wnt-signaling pathway [11]. RUNX2 is required for osteoblastogenesis, and both reduced levels of RUNX2 [12] and increased levels of IL-7 [14] have been observed in myeloma patients with MBD.

8.2 Indications for Bone Targeted Therapies

Bisphosphonate drugs are currently the only therapy approved in Australia for the treatment of MBD. The IMWG recommends considering bisphosphonate therapy [23], including patients with no visible lesions on conventional radiology [31]. It is difficult to accurately determine whether MM patients without radiological evidence of bone disease would benefit from bisphosphonates as most clinical trials did not stratify patients according to the presence of lytic lesions prior to treatment. Certainly, there is no indication that bisphosphonate therapy in the setting of smoldering myeloma or monoclonal gammopathy of uncertain significance (MGUS) reduces the time or likelihood of progression to MM [31].

8.3 Utility of Bone Resorption Markers to Guide Therapy

Currently, the use of bone turnover markers to guide therapy and predict response or disease progression in myeloma is controversial, with mixed results across studies and a lack of consensus regarding appropriate markers. International guidelines do not currently support the utilization of bone turnover or resorption markers to guide therapy given the lack of convincing evidence [31]. A recent study examining 123 patients with newly diagnosed multiple myeloma measured β-isomerized C-terminal telopeptide of collagen type I (β-CTX), which reflects the resorptive

osteoclast activity, and bone-specific alkaline phosphatase (bALP) over a 12-month period [26]. This study found that bALP levels did not have a clear relationship with the degree of underlying bone disease; however, β-CTX levels were increased in those patients with underlying bone disease, with a correlation between the degree of bone lesions and β-CTX levels [26]. Hence, changes in β-CTX levels could potentially reflect the degree of MBD burden and may be a clinically useful marker. A small retrospective study of patients receiving bisphosphonate therapy found that increasing levels of β-CTX were linked to increased likelihoods of disease progression [27]. Several other studies have utilized urinary N-telopeptide of type 1 collagen (uNTX) as well as ALP to predict fracture risk in MM patients on bisphosphonates and concluded that these biomarkers did not correlate to fracture risk [28, 31]. Until further studies provide stronger evidence for the utility of bone turnover markers in guiding therapy for MBD, there is currently no support for their use.

8.4 Current Treatments for Myeloma Bone Disease

8.4.1 Bisphosphonates

8.4.1.1 Mechanism of Action

Bisphosphonates are the mainstay of MBD prevention and treatment. They are pyrophosphate analogs which bind with varying affinity to hydroxyapatite and become integrated into the bone matrix [31]. Bisphosphonates are then released from hydroxyapatite secondary to bone resorption and are potent inhibitors of osteoclast activity and signaling [4]. Bisphosphonates are absorbed by macrophages and mature osteoclasts and induce apoptosis via ATP metabolites [14]. The potency of different bisphosphonates is dependent on their binding affinity for hydroxyapatite; the nitrogen group in the phosphate-carbon-phosphate core of pamidronate and zoledronic acid renders them 100–10,000 times more potent than etidronate and clodronate, as seen in Table 8.1 [29].

Table 8.1 Comparative potencies of bisphosphonates

Bisphosphonate	Potency	IC50[a] (nmol/L)
Etidronate	~1×	–
Clodronate	~10×	–
Pamidronate	~100×	200
Alendronate	>100 to <1000×	50
Ibandronate	>1000 to <10,000×	20
Risedronate	>1000 to <10,000×	10
Zoledronate	>10,000×	3

[a]IC50 half-maximal inhibitory concentration

8.4.1.2 Evidence in MBD

Treatment with pamidronate or zoledronic acid has been proven to improve symptoms related to MBD and prevent SREs. A 1996 study compared pamidronate with placebo in 392 MM patients demonstrated significant protection against SREs [30]. A non-inferiority trial comparing pamidronate with zoledronic acid for MBD demonstrated equivalence between the two therapies [4]. As a result, pamidronate and zoledronic acid have become the standard of care for MBD. There is currently some evidence for the use of oral clodronate, which has been shown to lower the incidence of SREs compared with placebo [31].

8.4.1.3 Comparison Between Bisphosphonates

Pamidronate and zoledronic acid are the two most commonly utilized bisphosphonates for MBD. They are nitrogen-containing bisphosphonates, which were proven to be superior at reducing SREs compared with non-nitrogen containing bisphosphonates in the MRC Myeloma IX trial [32]. A review of 20 studies found that zoledronic acid improved overall survival compared to placebo and etidronate, but not compared with other bisphosphonates [31]. An observational study comparing zoledronic acid with pamidronate found that patients treated with zoledronic acid had significantly less mortality and SREs compared with patients treated with pamidronate [33]. However, a randomized controlled trial comparing zoledronic acid with pamidronate found that they had similar efficacies in MBD [34]. Hence, current recommendations suggest the use of zoledronic acid or pamidronate as primary treatment for MBD.

8.4.1.4 Adverse Events

Bisphosphonates can cause several rare but serious side effects including osteonecrosis of the jaw, atypical femoral fractures, and renal impairment. More common side effects include acute phase reactions, injection site reactions, transient fevers, myalgias and flu-like symptoms, hypocalcemia, and hypophosphatemia [31]. Bisphosphonates also cause ocular side effects, which typically have a rapid onset ranging from within days to hours, such as conjunctivitis, uveitis, episcleritis, scleritis, and keratitis [35]. The more severe side effects are discussed in greater detail below.

8.4.1.5 Renal Impairment

The kidneys are responsible for 40% of bisphosphonate excretion via glomerular filtration and active tubular excretion, and as such, are extremely sensitive to bisphosphonates [4]. Both acute and chronic renal impairment can result from

bisphosphonate therapy, but the level of kidney damage is related to blood drug levels [31]. Nephrotoxicity is directly linked to the maximum plasma concentration, bisphosphonate dose, and infusion time [4]. Renal impairment is seen equally with zoledronic acid and pamidronate however zoledronic acid has more potential for accumulation in the kidneys due to its prolonged half-life [4]. Kidney injury related to zoledronic acid is most often caused by tubular toxicity, and hence acute tubular necrosis. Comparatively, pamidronate more commonly causes acute kidney injury and nephrotic range proteinuria [31]. Patients with elevated creatinine levels at baseline are at higher risk of developing acute renal injury related to bisphosphonate treatment [36]. Dosing variations are required in renal impairment, as described in Table 8.2.

8.4.1.6 Osteonecrosis of the Jaw

Osteonecrosis of the jaw is a rare but serious side effect of bisphosphonate therapy. Characteristic features include exposed bone in the oral cavity, severe pain, necrosis, and increased risk of secondary infections at the site of osteonecrosis [4]. The symptoms preceding an osteonecrotic lesion include mucosal swelling, ulceration, loose dentition, pain, or a nonhealing socket after tooth extraction [4]. The pathogenic etiology of osteonecrosis of the jaw is not clear but thought to be related to reduced vascularity of the bones of the maxilla and mandible, risk of dental infections, and bone turnover suppression [31]. The anti-angiogenic properties of bisphosphonates also contribute to osteonecrosis risk, due to an interruption of blood supply [37]. The suppression of bone remodeling appears to play a major role, evidenced by the higher rates of osteonecrosis of the jaw associated with treatment with higher potency bisphosphonates like zoledronic acid [31]. Excessive inhibition of bone remodeling can occur secondary to the long half-life of bisphosphonates and potential accumulation if administered monthly [31]. Osteonecrosis may not only be due to bisphosphonate therapy but may be an outcome of bone

Table 8.2 Renal dosing adjustments in bisphosphonate therapy [6]

Creatinine clearance	Sodium clodronate	Pamidronate	Zoledronic acid
>60	1600 mg	90 mg 2–4 h	4 mg over 15 min
50–60	1600 mg	Reduce dose or infuse over 4–6 h	3.5 mg over 15–30 min
30–50	1200 mg	Reduce dose or infuse over 4–6 h	40–49 mL/min: 3.3 mg 30–39 mL/min: 3 mg Over 15–30 min
10–30	800 mg	30 mg to be given over 2–4 h	Not recommended
<10	Not recommended	30 mg to be given over 2–4 h	Not recommended
Haemodialysis	Not recommended	On renal advice only 30 mg to be given over 2–4 h	On renal advice only

modeling suppression, supported by animal models and given that osteonecrosis of the jaw can occur with denosumab therapy [38].

The risk factors for osteonecrosis of the jaw include potency, dosage, and exposure duration to bisphosphonate therapy [31]. Several studies have shown that the incidence of osteonecrosis of the jaw increases with longer exposure to bisphosphonate therapy. The median time to development of osteonecrosis of the jaw was shorter for intravenous administration compared to oral administration in a single center study; 34–54 months for intravenous versus 16 months for oral bisphosphonates [39]. Patients with preexisting dental disease or concomitant dental procedures had significant increases in the risk of osteonecrosis of the jaw. Dental extraction caused an approximately nine times higher risk of osteonecrosis of the jaw development [38]. Alterations within the genes that affect bone turnover and collagen formation, and metabolic bone diseases may also predispose patients to osteonecrosis of the jaw [40].

As such, dental assessment prior to initiation of bisphosphonate is recommended in order to minimize the risk of developing osteonecrosis of the jaw. Any existing lesions should be addressed prior to bisphosphonate therapy, and bisphosphonates should be withheld for 90 days before or after any dental extraction [31]. In the event of osteonecrosis of the jaw developing, bisphosphonate therapy should be discontinued until the lesion is healed and treatment has been given as required [23].

8.4.1.7 Subtrochanteric and Other Atypical Femoral Fractures

The symptoms of atypical femoral fractures can be subtle and preceded by thigh or groin pain prior to diagnosis [31]. Long-term bisphosphonate use is linked to the development of atypical femoral fractures in approximately 93.9% of cases [41]. The majority of atypical femoral fractures occur at the proximal femur, with a minority occurring at the subtrochanteric region along the femoral shaft [42].

8.4.1.8 Duration, Frequency, and Monitoring of Therapy

Doses for bisphosphonate therapy are adjusted for renal function as described in Table 8.2. Zoledronic acid is not recommended in creatinine clearance rates <30 mL/min given to the lack of available evidence. Pamidronate can be utilized in patients with significant renal disease, defined as having an estimated creatinine clearance of <30 mL/min. While expert guidelines do not currently include dosing guidelines in patients with a creatinine clearance of <30 mL/min, expert panels recommend that a reduced dose is used [43].

The American Society of Clinical Oncology (ASCO) guidelines suggest that bone targeted therapy continues for a period of at least 2 years, and if ceased, should resume upon multiple myeloma relapse with any new SREs [43]. The frequency of dosing is recommended to be around every 3–4 weeks with intravenous zoledronic acid or pamidronate, or daily for oral clodronate therapy [31]. Less frequent dosing

may be considered in patients with responsive or stable disease. Three-month intervals of therapy may be considered in patients with inactive myeloma or on maintenance therapy [3].

Renal function should be monitored before each dose of pamidronate or zoledronic acid, and in the event of an acute rise in creatinine, therapy should be withheld until renal function normalizes within 10% of baseline [31]. Serum calcium and vitamin D levels should be reviewed intermittently during therapy and replaced as required. Intermittent review of albuminuria on a spot urine sample is recommended every 3–6 months, and if present should lead to further evaluation with a 24-h urine collection [43].

8.4.1.9 Future of Bisphosphonate Therapy

Bisphosphonates are the cornerstone of bone targeted therapy in MM. Despite adverse effects and the development of new therapies, bisphosphonates remain the standard of care for MBD in all major guidelines [44]. There is ongoing research evaluating different dosing strategies and administration schedules of bisphosphonates as sole therapy and in conjunction with new bone therapies. Denosumab, a human MoAb against RANKL, has recently been approved by the US Food and Drug Administration (FDA) for the treatment of MBD and was shown to be non-inferior to zoledronic acid in phase III randomized controlled trial [45]. Further agents under investigation include anti-DKK1 MoAbs, and therapies targeting activin A and CCR1 [16].

8.4.2 Denosumab

8.4.2.1 Mechanism of Action

Denosumab is a fully humanized MoAb that binds RANKL, preventing RANKL from activating RANK on the surface of osteoclasts. This inhibits osteoclast function and osteoclastogenesis by preventing the RANK–RANKL interaction [14]. Denosumab mimics the endogenous effects of OPG by lowering the amount of RANKL and thus decreasing the osteoclastogenesis [1]. Unlike bisphosphonates, denosumab does not become embedded in the bone mineral matrix but binds RANKL in the extracellular fluid and circulation to inhibit osteoclasts formation [10]. Denosumab is cleared from the circulation by the reticuloendothelial system and has a half-life of approximately 26 days [10].

8.4.2.2 Evidence in MBD

Denosumab has been approved by the FDA for the treatment of bone disease in solid organ cancers since 2010 but was not been approved for use in MBD until 2018. The approval of denosumab for use in MBD was largely based upon the results of phase III randomized, controlled trial of denosumab versus zoledronic acid in patients with newly diagnosed MM [45]. The study recruited 1718 patients, with 859 patients assigned to each arm of the study. In the denosumab treatment arm, subcutaneous denosumab 120 mg was given along with intravenous placebo every 4 weeks. In the zoledronic acid treatment arm, intravenous zoledronic acid 5 mg was given along with subcutaneous placebo every 4 weeks. The study showed that the median time to the first on-study SRE was similar between the denosumab and zoledronic acid arms (22.83 months with denosumab versus 23.98 months with zoledronic acid). Median progression free survival (PFS) was prolonged in the denosumab arm by 10.7 months compared to the zoledronic acid arm ($P = 0.036$). Prior to this landmark study, denosumab had been successfully studied in phase II and III trials in patients with solid organ cancer bone disease and those at high risk of developing bony metastatic disease, with results reporting that denosumab was equal to zoledronic acid in preventing or delaying SREs [16].

8.4.2.3 Adverse Events

The adverse events seen most commonly with denosumab therapy include neutropenia, thrombocytopenia, and anemia [45]. Renal toxicity is significantly lower in denosumab therapy compared with bisphosphonate therapy, and denosumab can be utilized in patients with poor baseline renal function without dose adjustment [46]. In a study of single-dose denosumab in patients with varying degrees of renal impairment, 15% of patients developed hypocalcemia. The severity of hypocalcemia appeared to relate to the severity of renal impairment, and two patients required hospitalization for treatment [46]. The serum nadir of hypocalcemia tends to appear approximately 10 days after administration of denosumab. Other associated adverse events include osteonecrosis of the jaw, nausea and vomiting, pneumonia, back pain, headache, arthralgia, and injection site reactions. Interestingly, in the phase III trial of denosumab versus zoledronic acid, the incidence of osteonecrosis of the jaw was not significantly different between denosumab and zoledronic acid arms [45].

8.4.2.4 Duration and Frequency of Therapy

Denosumab therapy is typically administered at 120 mg doses every 4 weeks subcutaneously with no dose adjustments required for renal impairment [45]. The length of therapy in MBD is not yet established given the recency of approval for denosumab therapy in this area. However, given the findings of the phase III trial of denosumab versus zoledronic acid, it can be extrapolated that denosumab may be

dosed at the same length and frequency as current bisphosphonate guidelines. This is the area that currently needs further development as denosumab is incorporated into current treatment guidelines.

8.4.3 Novel Therapies

8.4.3.1 Anti-Sclerostin Antibodies

Sclerostin is a soluble Wnt antagonist produced by osteocytes, which binds to Wnt coreceptors LRP5/6 to inhibit the Wnt-signaling pathway during bone formation, leading to an increase in osteoclastogenesis via RANKL production and OPG inhibition [1]. Multiple myeloma results in elevated sclerostin expression, and a reciprocal decrease in osteoblast markers. Sclerostin expression has been observed to be restricted to osteocytes, presenting a potential therapeutic target [47]. Anti-sclerostin therapies such as romosozumab, a humanized anti-sclerostin MoAb, have shown improvements in bone formation and bone mineral density in osteoporosis [1]. The effects of anti-sclerostin treatment on MBD currently remain unknown.

Anti-sclerostin MoAbs have been effective in mouse models and humans in promoting bone formation in the context of osteoporosis [16]. Anti-sclerostin treatment in mice with multiple myeloma has resulted in the normalization of bone volumes by increasing trabecular bone volume and thickness, increased osteoblastogenesis, reduced osteolytic lesions, and reduced bone loss [1, 47]. These mouse models also demonstrated a potential link between DKK1 and sclerostin; as DKK1 appeared to control increases in sclerostin by inducing its release from osteoblasts [48]. Romosozumab has been shown to increase bone mineral density and bone formation in numerous studies, mainly in the setting of postmenopausal osteoporosis [48]. However, romosozumab has been shown to increase the risk of cardiovascular events by 2.5% [1].

Anti-sclerostin antibodies have also been shown to reduce bone marrow adipose tissue, which has a downstream effect of reducing signaling molecules such as adipokines and fatty acids that normally promote the growth of myeloma plasma cells and osteolytic lesions [49]. Overall, sclerostin inhibition has been demonstrated to be beneficial in postmenopausal osteoporosis but there is currently a lack of evidence to support its use in MM [50].

8.4.3.2 Anti-DKK1 Neutralizing Antibodies

The DKK1 protein negatively regulates the Wnt/β-catenin signaling pathway. DKK1 binds to LRP 5/6 receptors, causing β-catenin breakdown by the proteasome and reducing osteoblast differentiation [51]. DKK1 prevents the differentiation of stem cells into mature osteoblasts by downregulating the Wnt signaling required for osteoblast differentiation [11]. The undifferentiated stem cells secrete IL-6,

promoting the expansion of myeloma cells which further secrete DKK1 [52]. DKK1 also causes an increase in the ratio of RANKL to OPG, increasing osteoclastogenesis and leading to the development of MBD [53].

DKK1 inhibition via a neutralizing antibody has been studied in murine and humanized MM models. Antibodies such as BHQ880 and DKN-01 aim to increase osteoblast differentiation and activity by DKK1 blockade and Wnt-signaling pathway modulation [54]. In vitro studies have observed that DKK1 inhibition promotes osteoblast differentiation and activity and inverts the negative effects of myeloma cells on osteoblast differentiation. These findings were demonstrated by the increased trabecular thickness of bone [55]. In vivo studies have demonstrated that DKK1 antibodies can improve bone formation, osteoblast numbers, and decrease lytic lesions [4].

A phase IB trial has evaluated the combination of DKK1 antibodies and bisphosphonate therapy in MM patients [56]. The study demonstrated that the combination therapy resulted in a delay in SREs and increased bone density. The contribution of DKK1 inhibition to these results is unclear.

Overall, DKK1 inhibition has been shown to positively affect osteoblastogenesis but the effects on osteoclastogenesis are currently unclear. The utility of DKK1 inhibition in MM at present may be limited as some myeloma patients do not show increased DKK1 levels, and DKK1 levels have been observed to decrease in later stages of the disease [57].

8.4.3.3 Activin Receptor Ligand Traps

Activin A is a cytokine that is upregulated in MM patients, especially in the setting of MBD. Activin A levels correlate with the severity of bone disease and disease stage [58]. Activin A inhibits bone mineralization by binding to the activin type 2A receptor, resulting in increased bone resorption and reduced bone formation [59]. Activin receptor ligand traps have been shown to increase markers of bone formation and decrease bone pain in MM patients [14].

A murine analog of sotatercept, a recombinant activin type 2A receptor ligand trap, has demonstrated dual anabolic and anti-bone resorptive effects in preclinical trials [60]. The safety and tolerability of sotatercept in combination with melphalan, prednisolone, and thalidomide have been evaluated in a phase IIA trial [60]. A total of 24 patients received sotatercept during the study, with three patients experiencing adverse events secondary to therapy. These three patients each experienced significant hypertension, which was resolved following antihypertensive therapy or interruption of sotatercept therapy. One patient had a grade five adverse event of sudden death following a second dose of sotatercept. The efficacy and safety of activin A receptor ligand traps for the treatment of MBD currently remains unclear. Further studies are required to the utility of this treatment in MBD.

8.4.3.4 Bruton's Tyrosine Kinase (BTK) Inhibitors

BTK is expressed in many hematopoietic lineages and affects the development and function of B cells via B cell antigen receptor signaling pathways [16]. BTK inhibitors have proven efficacy in the setting of chronic lymphocytic leukemia. BTK is highly expressed in patients with MM and is involved in the promotion of osteoclastic bone resorption [61].

PCI-32765 (ibrutinib), a potent BTK inhibitor, has been shown to reduce osteoclast differentiation and bone resorption [19]. Ibrutinib has also resulted in a reduction of chemokine and cytokine secretion from osteoclasts.

Overall, BTK activation in MM facilitates osteoclast differentiation and osteoclastic bone resorption. BTK inhibitors such as ibrutinib appear to reduce this effect, and further investigations into this potential therapy for the management of MBD are warranted.

8.4.3.5 B Cell Activating Factor (BAFF) Neutralizing Antibodies

BAFF is a TNF superfamily member that promotes normal B cell development [62]. It is expressed by myeloma cells, osteoclasts, and bone marrow stromal cells, and is increased in MM patients and mediates survival of myeloma plasma cells in the bone marrow [16]. BAFF neutralizing antibodies have been tested in a mouse MM model [62]. Anti-BAFF treated animals showed decreased IL-6 receptor levels, suggesting anti-myeloma activity. Additionally, a survival advantage and reduction in radiologically evident lytic lesions were observed.

A phase I study examined tabalumab, a human MoAb against BAFF, in combination with the PI bortezomib in 48 patients [28]. Twenty of 46 evaluable patients achieved a partial response or better following the combination treatment, showing some promise for BAFF neutralizing antibodies.

Although early studies have so far promising results of BAFF neutralizing antibodies in both animals and humans, further studies are still required to fully assess the utility and impact of this therapy in MBD.

8.4.3.6 Transforming Growth Factor-β (TGF-β) Inhibitors

The TGF-β protein has been observed to result in increased tumor-induced bone disease, although the exact mechanism is unclear. Increased levels of TGF-β are released by osteoclasts in MBD [1]. The use of TGF-β inhibitor neutralizing antibody (1D11) in mice has been shown to improve the trabecular architecture and increase osteoblast differentiation in mouse MM models [63]. 1D11 in combination with bortezomib was shown to reduce tumor burden and bone disease, but 1D11 alone did not reduce tumor burden.

Thrombospondin 1 (TSP1) binds to and activates TGF-β. SRI31277, a TSP1 antagonist that acts by reducing TGF- β activation, has been tested in mice with

MBD [64]. SRI31277 treatment resulted in a decrease in osteoclasts and an increase in osteoblastogenesis. It is unclear if these benefits would be seen in humans as only mice with highly osteolytic lesions and the human CAG-HPSE myeloma cell line were studied.

8.4.3.7 Parathyroid Hormone

Parathyroid hormone has been shown to be beneficial in osteoporotic bone disease via anabolic pathways at intermittent lower doses. The mechanism of action is thought to be due to the inhibition of sclerostin which normally promotes osteoclastogenesis, as well as direct activity on osteoblasts to promote osteoblastogenesis [65].

Treatment with teriparatide therapy has been linked to the development of MM in several case reports [66]. Additionally, it has been shown that high parathyroid hormone levels could facilitate the development of myeloma cells and have been correlated with a reduced progression free survival [67]. Conversely, a study of parathyroid hormone administration in murine MM models found an increase in bone mineral density via upregulation of osteoblasts [68].

Overall, there is minimal evidence to support the role for, and investigation of, parathyroid hormone therapy in the setting of MBD.

8.4.3.8 Proteasome Inhibitors

Bortezomib is a PI which impairs osteoclastogenesis and stimulates osteoblast differentiation, and hence actively modulating bone remodeling in MM [4]. Proteasome inhibitors produce an anabolic effect through the stimulation of osteoblast differentiation via the reduction of sclerostin levels, and the upregulation of BMP-2 and transcription factor RUNX2 via inhibition of proteasomal degradation [1].

It has been demonstrated that bortezomib therapy results in a reduction of sclerostin levels [58]. Patients with active MM and pathologic fractures at diagnosis possessed very high levels of sclerostin compared to other patient groups (relapsed myeloma, MGUS, and a control group). Higher sclerostin levels were associated with reduced survival with a median survival of 27 months for those with higher sclerostin levels versus 98 months for other patient groups [58]. Bortezomib monotherapy resulted in a significant reduction of sclerostin levels by almost 50%. Bortezomib has been shown to have anabolic activity leading to increased bone formation through promoting osteoblastogenesis and increasing bone mineral density.

Other PIs such as carfilzomib and ixazomib have also been shown to have bone anabolic effects similar to bortezomib [1]. Overall, PIs have been shown to improve MBD, disease control, and progression. They are a promising therapeutic in the setting of MBD.

8.4.3.9 Immunomodulatory Imide Drugs

Immunomodulatory imide drugs (IMiD) such as thalidomide, lenalidomide, and pomalidomide have a direct inhibitory effect on MM growth, as well as exerting an immunomodulatory effect and inhibiting angiogenesis [4]. Immunomodulatory imide drugs have been shown to reduce both osteoclastogenesis and growth factors associated with bone destruction [16]. Lenalidomide decreases RANKL secretion and increases OPG in MM patients, restoring the balance of RANKL and OPG and resulting in reduced osteoclast differentiation and activation. By decreasing RANKL secretion in MM patients, lenalidomide also causes OPG to increase, restoring the RANKL-OPG balance and resulting in reduced osteoclast formation and activation.

It has been demonstrated that thalidomide downregulates transcriptional factor PU.1 [69]. Lenalidomide and pomalidomide also both downregulate PU.1 expression in osteoclast precursors, causing a net reduction in osteoclast differentiation. Additionally, lenalidomide causes inhibition of the osteoclast-activating factors APRIL and BAFF [14].

Overall, it is clear that IMiDs have numerous effects on tumor growth, growth factors, and signaling proteins that result in a reduction in osteoclastogenesis. Further studies are warranted to further examine and understand the direct impacts of IMiDs on MBD.

8.5 Conclusion

Myeloma bone disease can result in significant associated morbidity and mortality. Effective therapies to combat the development and progression of MBD are crucial in maintaining quality of life, reducing cost, and improving overall survival. The current treatments available to combat MBD are limited to bisphosphonates and denosumab. Given the plethora of pathways and proteins involved in MBD, the potential targets for therapy are numerous. Further studies of novel agents, as well as studies of new combinations of existing and novel therapies, are needed to better manage MBD, reduce morbidity and mortality, and increase the quality of life and survival in patients with MM.

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References

1. Ring ES, Lawson MA, Snowden JA, Jolley I, Chantry AD. New agents in the treatment of myeloma Bone disease. Calcif Tissue Int. 2018;102(2):196–209.

2. Kyle RA, Gertz MA, Witzig TE, Lust JA, Lacy MQ, Dispenzieri A, et al. Review of 1027 patients with newly diagnosed multiple myeloma. Mayo Clin Proc. 2003;78(1):21–33.
3. Rajkumar SV, Dimopoulos MA, Palumbo A, Blade J, Merlini G, Mateos MV, et al. International myeloma working group updated criteria for the diagnosis of multiple myeloma. Lancet Oncol. 2014;15(12):e538–48.
4. Raje N, Roodman GD. Advances in the biology and treatment of bone disease in multiple myeloma. Clin Cancer Res. 2011;17(6):1278–86.
5. Saad F, Lipton A, Cook R, Chen YM, Smith M, Coleman R. Pathologic fractures correlate with reduced survival in patients with malignant bone disease. Cancer. 2007;110(8):1860–7.
6. Lee OL, Horvath N, Lee C, Joshua D, Ho J, Szer J, et al. Bisphosphonate guidelines for treatment and prevention of myeloma bone disease. Intern Med J. 2017;47(8):938–51.
7. Edelstyn GA, Gillespie PJ, Grebbell FS. The radiological demonstration of osseous metastases. Experimental observations. Clin Radiol. 1967;18(2):158–62.
8. Bird JM, Owen RG, D'Sa S, Snowden JA, Pratt G, Ashcroft J, et al. Guidelines for the diagnosis and management of multiple myeloma 2011. Br J Haematol. 2011;154(1):32–75.
9. Bataille R, Chappard D, Marcelli C, Dessauw P, Sany J, Baldet P, et al. Mechanisms of bone destruction in multiple myeloma: the importance of an unbalanced process in determining the severity of lytic bone disease. J Clin Oncol. 1989;7(12):1909–14.
10. Hanley DA, Adachi JD, Bell A, Brown V. Denosumab: mechanism of action and clinical outcomes. Int J Clin Pract. 2012;66(12):1139–46.
11. Terpos E, Ntanasis-Stathopoulos I, Gavriatopoulou M, Dimopoulos MA. Pathogenesis of bone disease in multiple myeloma: from bench to bedside. Blood Cancer J. 2018;8(1):7.
12. Hameed A, Brady JJ, Dowling P, Clynes M, O'Gorman P. Bone disease in multiple myeloma: pathophysiology and management. Cancer Growth Metastasis. 2014;7:33–42.
13. Nakashima T, Hayashi M, Fukunaga T, Kurata K, Oh-Hora M, Feng JQ, et al. Evidence for osteocyte regulation of bone homeostasis through RANKL expression. Nat Med. 2011;17(10):1231–4.
14. Walker RE, Lawson MA, Buckle CH, Snowden JA, Chantry AD. Myeloma bone disease: pathogenesis, current treatments and future targets. Br Med Bull. 2014;111(1):117–38.
15. Stewart JP, Shaughnessy JD Jr. Role of osteoblast suppression in multiple myeloma. J Cell Biochem. 2006;98(1):1–13.
16. Webb SL, Edwards CM. Novel therapeutic targets in myeloma bone disease. Br J Pharmacol. 2014;171(16):3765–76.
17. Lee JW, Chung HY, Ehrlich LA, Jelinek DF, Callander NS, Roodman GD, et al. IL-3 expression by myeloma cells increases both osteoclast formation and growth of myeloma cells. Blood. 2004;103(6):2308–15.
18. Noonan K, Marchionni L, Anderson J, Pardoll D, Roodman GD, Borrello I. A novel role of IL-17-producing lymphocytes in mediating lytic bone disease in multiple myeloma. Blood. 2010;116(18):3554–63.
19. Tai YT, Chang BY, Kong SY, Fulciniti M, Yang G, Calle Y, et al. Bruton tyrosine kinase inhibition is a novel therapeutic strategy targeting tumor in the bone marrow microenvironment in multiple myeloma. Blood. 2012;120(9):1877–87.
20. Qiang YW, Shaughnessy JD Jr, Yaccoby S. Wnt3a signaling within bone inhibits multiple myeloma bone disease and tumor growth. Blood. 2008;112(2):374–82.
21. Kobune M, Chiba H, Kato J, Kato K, Nakamura K, Kawano Y, et al. Wnt3/RhoA/ROCK signaling pathway is involved in adhesion-mediated drug resistance of multiple myeloma in an autocrine mechanism. Mol Cancer Ther. 2007;6(6):1774–84.
22. Colucci S, Brunetti G, Oranger A, Mori G, Sardone F, Specchia G, et al. Myeloma cells suppress osteoblasts through sclerostin secretion. Blood Cancer J. 2011;1(6):e27.
23. Terpos E, Morgan G, Dimopoulos MA, Drake MT, Lentzsch S, Raje N, et al. International myeloma working group recommendations for the treatment of multiple myeloma-related bone disease. J Clin Oncol. 2013;31(18):2347–57.

24. Kaiser M, Mieth M, Liebisch P, Oberlander R, Rademacher J, Jakob C, et al. Serum concentrations of DKK-1 correlate with the extent of bone disease in patients with multiple myeloma. Eur J Haematol. 2008;80(6):490–4.
25. Terpos E, Christoulas D, Kastritis E, Bagratuni T, Gavriatopoulou M, Roussou M, et al. High levels of periostin correlate with increased fracture rate, diffuse MRI pattern, abnormal bone remodeling and advanced disease stage in patients with newly diagnosed symptomatic multiple myeloma. Blood Cancer J. 2016;6(10):e482.
26. Auzina D, Erts R, Lejniece S. Prognostic value of the bone turnover markers in multiple myeloma. Exp Oncol. 2017;39(1):53–6.
27. Pochintesta L, Mangiacavalli S, Cocito F, Pompa A, Albertini R, Pascutto C, et al. Serum C terminal telopeptide maintains its correlation with bone disease in patients with myeloma even under treatment with bisphosphonates. Leuk Lymphoma. 2014;55(6):1397–8.
28. Raje N, Vescio R, Montgomery CW, Badros A, Munshi N, Orlowski R, et al. Bone marker-directed dosing of zoledronic acid for the prevention of skeletal complications in patients with multiple myeloma: results of the Z-MARK study. Clin Cancer Res. 2016;22(6):1378–84.
29. Dunford JE, Thompson K, Coxon FP, Luckman SP, Hahn FM, Poulter CD, et al. Structure-activity relationships for inhibition of farnesyl diphosphate synthase in vitro and inhibition of bone resorption in vivo by nitrogen-containing bisphosphonates. J Pharmacol Exp Ther. 2001;296(2):235–42.
30. Berenson JR, Lichtenstein A, Porter L, Dimopoulos MA, Bordoni R, George S, et al. Efficacy of pamidronate in reducing skeletal events in patients with advanced multiple myeloma. Myeloma Aredia study group. N Engl J Med. 1996;334(8):488–93.
31. McCloskey EV, Dunn JA, Kanis JA, MacLennan IC, Drayson MT. Long-term follow-up of a prospective, double-blind, placebo-controlled randomized trial of clodronate in multiple myeloma. Br J Haematol. 2001;113(4):1035–43.
32. Morgan GJ, Davies FE, Gregory WM, Bell SE, Szubert AJ, Cook G, et al. Long-term follow-up of MRC myeloma IX trial: survival outcomes with bisphosphonate and thalidomide treatment. Clin Cancer Res. 2013;19(21):6030–8.
33. Sanfilippo KM, Gage B, Luo S, Weilbaecher K, Tomasson M, Vij R, et al. Comparative effectiveness on survival of zoledronic acid versus pamidronate in multiple myeloma. Leuk Lymphoma. 2015;56(3):615–21.
34. Rosen LS, Gordon D, Kaminski M, Howell A, Belch A, Mackey J, et al. Long-term efficacy and safety of zoledronic acid compared with pamidronate disodium in the treatment of skeletal complications in patients with advanced multiple myeloma or breast carcinoma: a randomized, double-blind, multicenter, comparative trial. Cancer. 2003;98(8):1735–44.
35. Fraunfelder FW, Fraunfelder FT. Bisphosphonates and ocular inflammation. N Engl J Med. 2003;348(12):1187–8.
36. Berenson JR, Yellin O, Crowley J, Makary A, Gravenor DS, Yang HH, et al. Prognostic factors and jaw and renal complications among multiple myeloma patients treated with zoledronic acid. Am J Hematol. 2011;86(1):25–30.
37. Santini D, Vincenzi B, Dicuonzo G, Avvisati G, Massacesi C, Battistoni F, et al. Zoledronic acid induces significant and long-lasting modifications of circulating angiogenic factors in cancer patients. Clin Cancer Res. 2003;9(8):2893–7.
38. Badros A, Weikel D, Salama A, Goloubeva O, Schneider A, Rapoport A, et al. Osteonecrosis of the jaw in multiple myeloma patients: clinical features and risk factors. J Clin Oncol. 2006;24(6):945–52.
39. Mehrotra B, Ruggiero S. Bisphosphonate complications including osteonecrosis of the jaw. Hematology Am Soc Hematol Educ Program. 2006;356–60:515.
40. Katz J, Gong Y, Salmasinia D, Hou W, Burkley B, Ferreira P, et al. Genetic polymorphisms and other risk factors associated with bisphosphonate induced osteonecrosis of the jaw. Int J Oral Maxillofac Surg. 2011;40(6):605–11.

41. Shane E, Burr D, Ebeling PR, Abrahamsen B, Adler RA, Brown TD, et al. Atypical subtrochanteric and diaphyseal femoral fractures: report of a task force of the American Society for Bone and Mineral Research. J Bone Min Res. 2010;25(11):2267–94.
42. Nieves JW, Bilezikian JP, Lane JM, Einhorn TA, Wang Y, Steinbuch M, et al. Fragility fractures of the hip and femur: incidence and patient characteristics. Osteoporos Int. 2010;21(3):399–408.
43. Anderson K, Ismaila N, Flynn PJ, Halabi S, Jagannath S, Ogaily MS, et al. Role of bone-modifying agents in multiple myeloma: American Society of Clinical Oncology clinical practice guideline update. J Clin Oncol. 2018;36(8):812–8.
44. Pozzi S, Raje N. The role of bisphosphonates in multiple myeloma: mechanisms, side effects, and the future. Oncologist. 2011;16(5):651–62.
45. Raje N, Terpos E, Willenbacher W, Shimizu K, Garcia-Sanz R, Durie B, et al. Denosumab versus zoledronic acid in bone disease treatment of newly diagnosed multiple myeloma: an international, double-blind, double-dummy, randomised, controlled, phase 3 study. Lancet Oncol. 2018;19(3):370–81.
46. Block GA, Bone HG, Fang L, Lee E, Padhi D. A single-dose study of denosumab in patients with various degrees of renal impairment. J Bone Min Res. 2012;27(7):1471–9.
47. Reagan MR, McDonald M, Terry R, Pettitt J, Le L, Mohanty S, et al. Anti-sclerostin treatment prevents multiple myeloma induced bone loss and reduces tumor burden. ASH Publication Blood. 2015;126(23):119.
48. Eda H, Santo L, Wein MN, Hu DZ, Cirstea DD, Nemani N, et al. Regulation of sclerostin expression in multiple myeloma by Dkk-1: a potential therapeutic strategy for myeloma bone disease. J Bone Min Res. 2016;31(6):1225–34.
49. Falank C, Fairfield H, Reagan MR. Signaling interplay between bone marrow adipose tissue and multiple myeloma cells. Front Endocrinol (Lausanne). 2016;7:67.
50. McClung MR, Grauer A, Boonen S, Bolognese MA, Brown JP, Diez-Perez A, et al. Romosozumab in postmenopausal women with low bone mineral density. N Engl J Med. 2014;370(5):412–20.
51. Pozzi S, Fulciniti M, Yan H, Vallet S, Eda H, Patel K, et al. In vivo and in vitro effects of a novel anti-Dkk1 neutralizing antibody in multiple myeloma. Bone. 2013;53(2):487–96.
52. Gunn WG, Conley A, Deininger L, Olson SD, Prockop DJ, Gregory CA. 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. 2006;24(4):986–91.
53. Qiang YW, Chen Y, Stephens O, Brown N, Chen B, Epstein J, et al. Myeloma-derived Dickkopf-1 disrupts Wnt-regulated osteoprotegerin and RANKL production by osteoblasts: a potential mechanism underlying osteolytic bone lesions in multiple myeloma. Blood. 2008;112(1):196–207.
54. Heusschen R, Muller J, Duray E, Withofs N, Bolomsky A, Baron F, et al. Molecular mechanisms, current management and next generation therapy in myeloma bone disease. Leuk Lymphoma. 2018;59(1):14–28.
55. Fulciniti M, Tassone P, Hideshima T, Vallet S, Nanjappa P, Ettenberg SA, et al. Anti-DKK1 mAb (BHQ880) as a potential therapeutic agent for multiple myeloma. Blood. 2009;114(2):371–9.
56. Iyer SP, Beck JT, Stewart AK, Shah J, Kelly KR, Isaacs R, et al. A phase IB multicentre dose-determination study of BHQ880 in combination with anti-myeloma therapy and zoledronic acid in patients with relapsed or refractory multiple myeloma and prior skeletal-related events. Br J Haematol. 2014;167(3):366–75.
57. Tian E, Zhan F, Walker R, Rasmussen E, Ma Y, Barlogie B, et al. The role of the Wnt-signaling antagonist DKK1 in the development of osteolytic lesions in multiple myeloma. N Engl J Med. 2003;349(26):2483–94.
58. Terpos E, Kastritis E, Christoulas D, Gkotzamanidou M, Eleutherakis-Papaiakovou E, Kanellias N, et al. Circulating activin-A is elevated in patients with advanced multiple myeloma and correlates with extensive bone involvement and inferior survival; no alterations post-lenalidomide and dexamethasone therapy. Ann Oncol. 2012;23(10):2681–6.

59. Roodman GD. Osteoblast function in myeloma. Bone. 2011;48(1):135–40.
60. Abdulkadyrov KM, Salogub GN, Khuazheva NK, Sherman ML, Laadem A, Barger R, et al. Sotatercept in patients with osteolytic lesions of multiple myeloma. Br J Haematol. 2014;165(6):814–23.
61. Lee SH, Kim T, Jeong D, Kim N, Choi Y. The tec family tyrosine kinase Btk regulates RANKL-induced osteoclast maturation. J Biol Chem. 2008;283(17):11526–34.
62. Neri P, Kumar S, Fulciniti MT, Vallet S, Chhetri S, Mukherjee S, et al. Neutralizing B-cell activating factor antibody improves survival and inhibits osteoclastogenesis in a severe combined immunodeficient human multiple myeloma model. Clin Cancer Res. 2007;13(19):5903–9.
63. Nyman JS, Merkel AR, Uppuganti S, Nayak B, Rowland B, Makowski AJ, et al. Combined treatment with a transforming growth factor beta inhibitor (1D11) and bortezomib improves bone architecture in a mouse model of myeloma-induced bone disease. Bone. 2016;91:81–91.
64. Lu A, Pallero MA, Lei W, Hong H, Yang Y, Suto MJ, et al. Inhibition of transforming growth factor-beta activation diminishes tumor progression and osteolytic bone disease in mouse models of multiple myeloma. Am J Pathol. 2016;186(3):678–90.
65. Lombardi G, Di Somma C, Rubino M, Faggiano A, Vuolo L, Guerra E, et al. The roles of parathyroid hormone in bone remodeling: prospects for novel therapeutics. J Endocrinol Invest. 2011;34(7 Suppl):18–22.
66. Koski AM, Sikio A, Forslund T. Teriparatide treatment complicated by malignant myeloma. BMJ Case Rep. 2010;2010
67. Kang MG, Won EJ, Choi HW, Kim HR, Choi HJ, Park HR, et al. Serum parathyroid hormone is a new potential risk factor in multiple myeloma. Biomed Res Int. 2014;2014:804182.
68. Pennisi A, Ling W, Li X, Khan S, Wang Y, Barlogie B, et al. Consequences of daily administered parathyroid hormone on myeloma growth, bone disease, and molecular profiling of whole myelomatous bone. PLoS One. 2010;5(12):e15233.
69. Anderson G, Gries M, Kurihara N, Honjo T, Anderson J, Donnenberg V, et al. Thalidomide derivative CC-4047 inhibits osteoclast formation by down-regulation of PU.1. Blood. 2006;107(8):3098–105.

Chapter 9
New Targeted Therapies for Multiple Myeloma Under Clinical Investigation

Caroline Dix and Christian Bryant

Abstract The current landscape of multiple myeloma treatment has greatly improved outcomes for the majority of patients and includes proteasome inhibitors, immunomodulatory imide drugs, and more recently, a number of monoclonal antibodies. However, there remains a proportion of patients that possess inherent resistance and fail to achieve optimal responses to these treatments. To overcome this problem, the continued research into and development of novel therapies is needed with the hope of further improving outcomes for all MM patients. This chapter will discuss novel targeted therapies currently being evaluated for the treatment of MM including antibody therapies, cellular therapies, and small-molecule inhibitors.

Keywords Multiple myeloma · Novel targeted therapies · Antibody therapies · Checkpoint inhibitors · Small-molecule inhibitors · Cellular therapies

Abbreviations

BCMA	B cell maturation antigen
BET	Bromodomain and extraterminal
BiTe	Bispecific T cell engager
CAR	Chimeric antigen receptor
CR	Complete response
CRS	Cytokine release syndrome
CTCAE	Common terminology criteria for adverse events
DC	Dendritic cell
FDA	US Food and Drug Administration
FGFR3	Fibroblast growth receptor 3
IgG	Immunoglobulin G
IgG1	Immunoglobulin G1

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IMiD	Immunomodulatory imide drug
KSP	Kinesin spindle protein
MAPK	Mitogen-activated protein kinases
MM	Multiple myeloma
MoAb	Monoclonal antibody
MR	Minimal response
mRNA	Messenger RNA
NK	Natural killer
ORR	Overall response rate
PFS	Progression free survival
PI	Proteasome inhibitor
PR	Partial response
RRMM	Relapsed/refractory multiple myeloma
SINE	Selective inhibitor of nuclear export
SLAMF7	Signaling lymphocytic activation molecule family member 7
TNF	Tumor necrosis factor
VGPR	Very good partial response
XPO1	Exportin 1

9.1 Introduction

Despite major advances in pharmacotherapy for multiple myeloma (MM), it remains incurable. There is a complex clonal molecular architecture present at diagnosis, and while current therapies control sensitive cells, there is an inevitable escape of cells with inherent resistance. Targeted therapies hold the promise of overcoming this problem and inducing deeper, more long-lasting remissions. Targeted therapies can be broadly divided into (1) antibody therapies, (2) cellular therapies, and (3) small-molecule inhibitors. While clinical testing of antibody therapies is the most advanced, there is considerable activity in MM in all three groups. In this chapter, the most promising targeted therapies for myeloma will be discussed.

9.2 Antibodies

9.2.1 Monoclonal Antibodies Directed at Plasma Cells

Monoclonal antibodies (MoAb) have revolutionized cancer therapy. While they have been used for a number of years in both solid organ and hematological malignancies, it was not until 2015 that the first monoclonal antibodies directed at myeloma cells were approved by the FDA—daratumumab and elotuzumab—signaling a paradigm shift in the way MM is treated. As discussed in previous chapters,

daratumumab, a CD38 specific MoAb, has single agent efficacy [1] and promotes deep remission when combined with immunomodulatory imide drugs (IMiDs) [2]. In addition, CD38 expression on nonmalignant immune cells such as T regulatory cells means daratumumab has immunoregulatory effects as well as direct antitumor effects [3, 4]. This is associated with increases in clonally expanded T cells, and absolute numbers of helper and cytotoxic T cells, suggesting daratumumab drives antigen-specific immunity [4]. Isatuximab is another humanized immunoglobulin G (IgG) antibody directed against CD38, although it targets a different epitope to daratumumab [5]. It is currently being evaluated in phase I and II dose-escalation studies. A phase 1b study recently confirmed that isatuximab is active in combination with lenalidomide and dexamethasone in heavily pretreated relapsed/refractory multiple myeloma (RRMM) patients, with an overall response rate (ORR) of 52% and progression free survival (PFS) of 8.5 months [6].

There are other MM antigens that have been targeted with MoAbs. Elotuzumab is a humanized IgG MoAb targeting Signaling Lymphocytic Activation Molecular family member 7 (SLAMF7), a surface glycoprotein that is expressed on both normal plasma cells and myeloma cells. CD138 is another interesting target as it is specific to plasma cells [7]. Indatuximab ravtansine is an antibody-drug conjugate comprising a CD138 chimerized antibody and the maytansinoid DM4 as the cytotoxic agent. It binds to the CD138 on cancer cells and releases the DM4 after internalization, resulting in cell death [8]. Indatuximab ravtansine has been evaluated as both single agent and in combination with lenalidomide or pomalidomide and dexamethasone for heavily pretreated RRMM. In a study reported in 2016, with a patient group having received between one and six prior therapies, the ORR of those treated with the lenalidomide/indatuximab combination was 77% and the median duration of response 21 months, compared to an ORR of 79% in the pomalidomide/indatuximab combination group where the duration of response was not reached [9]. Treatment was well tolerated with >90% of adverse events being Common Terminology Criteria for Adverse Events (CTCAE) grade 1 or 2.

Other antibody-drug conjugates have also been developed and are currently in preclinical or early clinical trial stages. By targeting specific molecules on the surface of the neoplastic cells, the cytotoxic agent is delivered locally and at higher levels than could be achieved otherwise, without the systemic toxicity associated with traditional chemotherapy. A study evaluating amanitin as the drug (an RNA polymerase II and III inhibitor) and B cell maturation antigen (BCMA) as the antibody target ("Hdp-101") found it to have cytotoxic effects ex vivo and was safe in monkey models [10]. The first human trials of this antibody-drug conjugate are expected in 2018. BCMA is a member of the Tumor Necrosis Factor (TNF) receptor superfamily and is expressed on B cells predominantly in the interfollicular region of germinal centers and on differentiated plasma cells and plasmablasts [7]. High levels of messenger RNA (mRNA) have been found in the plasma cells of all MM patients [11].

A phase I study of a BCMA-monomethyl auristatin-F conjugate ("GSK2857916") in 38 patients with heavily pretreated RRMM and with limited therapeutic options demonstrated an ORR of 60%, with 51% obtaining greater than very good partial

response (VGPR) [12]. The safety profile was manageable. The results of this study are remarkable given that more than half the recruited patients had received at least 5 or more prior lines of therapy, 97% were refractory to proteasome inhibitors (PI), 91% were refractory to IMiDs and almost 40% were refractory to both PIs and IMiDs [12].

9.2.2 Bispecific Antibodies

Bispecific T cell engager (BiTe) antibodies direct a specific autologous immune response to MM. They are formed from single fragment chain variable components (i.e., the antigen-binding domain) with specificity for two antigens, joined by a linker domain [13]. They generally bind the target antigen on the tumor cell to CD3 in the T cell receptor complex, leading to activation of T cells in a tumor-specific fashion [14, 15]. This has proven highly effective in acute lymphoblastic leukemia when directed at the pan-B cell antigen CD19, with Blinotumumab inducing 43% complete response (CR) rates in poor prognosis relapsed patients [16].

Preclinical data supports the potential utility of bispecific antibodies in MM. BCMA is particularly appealing as it is highly restricted to, and is expressed by all malignant plasma cells [17]. BCMA and CD3-specific bispecific antibodies kill MM cell lines and increase survival in xenograft models [18]. Antibody therapeutics have the great advantage of being highly modifiable, for example, using a high Fc affinity immunoglobulin G1 (IgG1) region induced greater activation of natural killer (NK) and T cells, and enhanced MM cell killing [19].

BiTe antibodies for MM are currently in clinical development, with some having reached phase I dose-escalation studies. A BiTe targeting BCMA and CD3 (BI 836909) has been shown to induce selective lysis of BCMA-positive myeloma cells in both ex vivo assays, mouse and primate studies [20]. EM801, another such BiTe, has been shown to induce myeloma cell death by autologous T cells in ex vivo bone marrow samples, including in high-risk patients with multiple lines of previous treatment [21]. BiTes targeting other surface antigens, including Fc receptor-like protein 5 (FcRH5, a B cell lineage marker overexpressed on myeloma cells), and CD38 have undergone preclinical testing and show great promise [21, 22]. Currently, numerous Phase I clinical trials are underway or are being set up (NCT03269136, NCT02514239).

9.2.3 Checkpoint Inhibitor

Antibodies blocking immune checkpoints have demonstrated marked efficacy in malignancies such as melanoma [23] and Hodgkin's lymphoma [24]. They work by blocking signaling molecules (e.g., CTLA4, PD1, 41BB) which act as brakes on existing natural autologous antitumor immunity [25]. The PD-1/PD-L1 axis is of

particular interest as the expression of PD-1 is high in MM, T, and NK cells [26, 27], and the expression of PD-L1 is high on MM cells [26]. Furthermore, PD-1 blockade improved anti-MM T cell responses in vitro to a DC-tumor fusion vaccine [27].

The PD-1 inhibitor Nivolumab had little single agent efficacy in heavily treated MM patients [28, 29], with the best result being a 63% rate of stable disease with a median PFS of only 10 weeks [28]. However, the combination of pembrolizumab, another PD-1 inhibitor, with IMiDs has produced more impressive results [30–32]. Pembrolizumab combined with lenalidomide resulted in an overall response rate of 76% in heavily pretreated patients, 75% of which were refractory to lenalidomide [32]. Pembrolizumab combed with pomalidomide is also potent, with an ORR of 60% in patients with a median of three prior lines of therapy, with 40% of these patients having high-risk MM factors [31]. It is difficult to assess the additive effect of the checkpoint inhibitor to pomalidomide in this phase II study; however, the response rate is higher than the response rate to pomalidomide and low dose dexamethasone in patients with a median of five prior lines of therapy (35% ORR in IFM2009-02) [33], and in patients with a median of three prior lines (34% ORR in MM-03) [34]. More impressively, the PFS with pembrolizumab and pomalidomide was 17.4 months at a median follow-up of 15.6 months. There were ongoing decreases in patients' paraproteins after many months of treatment and patients who did not have deep responses did not progress for long periods. This is strikingly different from the experience with pomalidomide and dexamethasone where progression is more likely when only a partial response (PR) is achieved [35]. This indicates that the link between the kinetics and depth and durability of responses of PD-1 inhibition are fundamentally different when combined with an immunotherapeutic. However, the FDA placed a clinical hold on all IMiD and pembrolizumab combination trials in July 2017 stating that it had "determined that the data available … indicate that the risks of Keytruda plus pomalidomide or lenalidomide outweigh any potential benefit for patients with multiple myeloma." There has been no further detailed public explanation of the safety concerns, but it would seem for now that this approach will not be pursued.

9.2.4 Cellular Therapies

9.2.4.1 Chimeric Antigen Receptor T Cells

The ability of the cytotoxic T cell to eliminate tumor cells has been recently showcased by the remarkable success of chimeric antigen receptor (CAR) T cells [36]. CAR T cells are autologous T cells, modified with retroviruses to express constructs, which have antigen-specific binding domains of antibodies in their extracellular domains, and signaling components of the T cell receptor and costimulatory receptors in their intracellular domains.

This renders these T cells specific to the antigen of choice and avoids the need for interactions with antigen presenting cells and the normal limitations of affinity

maturation. Infusion of these cells induces the recognition and killing of MM cells by T cells.

CAR T cells have been produced against a number of antigens for MM. Although CD19 is known to be largely negative on MM cells, a CD19 CAR T cell product generated a clear response in one patient with MM following autologous stem cell transplantation who was previously refractory to nine lines of therapy [37]. This success stimulated considerable interest and was attributed to a potential CD19 positive MM precursor cell [38]. However, when this was tested in a larger cohort there were no striking responses [39].

CAR T cells recognizing the more myeloma-restricted antigen BCMA have also shown activity in preclinical testing [17]. Subsequently, numerous early phase clinical trials have been conducted. The most impressive results have been seen in a 2017 study of 19 patients with RRMM [40]. The median follow-up was 208 days. Of 7 patients followed up for longer than 6 months, 6 were in minimal residual disease negative CR, and of 12 patients followed up for less than 6 months, all 12 were in near CR or VGPR with progressively dropping paraproteins, suggesting that they would reach CR [40]. These promising results came at the cost of prevalent cytokine release syndrome (CRS), with 10 of 19 patients having CTCAE Grade 1–3 CRS, and one death resulting from CRS. These results were so impressive that the word "cure" has been used, but clearly clinical assessment is required, and a multinational study is planned.

A separate phase I study of BCMA CAR T cells in MM has also been conducted [41]. The study treated a total of 21 RRMM patients who were very heavily pretreated with a median of seven prior lines of therapy, in three separate cohorts. Although follow-up was short, there appeared to be more limited efficacy. The first cohort utilized a higher CAR T cell dose ($1\text{-}5 \times 10^8$ cells) but no lymphodepleting chemotherapy (1.5 g/m^2 cyclophosphamide given to other cohorts). In this cohort, 6 of 9 patients responded with 1 stringent CR ongoing at 21 months, 2 VGPR, 1 PR, and 2 minimal response (MR). Only 2 of 5 patients responded in the second cohort with lymphodepletion but a lower CAR T cell dose ($1\text{-}5 \times 10^7$ cells). The third cohort combined the higher cell dose with lymphodepletion and had too little follow-up to make comment. Notably, toxicity was high in the first cohort, with CTCAE Grade 3–4 CRS in 4 of 9 patients and fatal neurotoxicity in 2 of 9 patients.

A third study has also presented early data of BMCA CAR T cells in MM [42]. Very heavily pretreated patients who had received a median of seven prior lines were treated BMCA CAR T cells in a dose-escalation design. While CTCAE Grade 1–2 CRS was prevalent (71%), there was little grade 3 CRS (2 of 18 patients). The ORR was 89% and responses were seen in 100% of patients that received the highest CAR T cell dose (1.5×10^8 cells) [42]. At presentation, 10 of 18 patients achieved CR, and at 52 weeks, five patients had remained progression free for 1 year, with the longest duration of response at 58 weeks.

9.2.4.2 DC Vaccination

It is difficult to assess the place of dendritic cell (DC) vaccination in MM given the early successes seen with CAR T cells. However, it is still worth noting the considerable amount of work that has gone into the development of DC vaccination for MM. Dendritic cells are professional antigen presenting cells with the ability to generate new immune responses, in particular T cell responses [43]. Their use as a therapeutic requires extracting them from the patient (as monocytes or preformed blood DCs), activating them in vitro, exposing them to some form of tumor antigen, then readministering them to traffic to lymph nodes to stimulate T cells. There is a sound rationale for performing this process ex vivo, as this avoids the tumor microenvironment which renders the DC nonfunctional [44–47].

Dendritic cell vaccination has been assessed by multiple groups in MM, and definitive clinical efficacy has so far been difficult to demonstrate. The first substantial trial suggested a survival advantage with a blood-derived DC vaccine of 5.3 years versus 3.4 years seen with a historical control cohort; however, this may not be a fair comparator [48]. A monocyte-derived DC vaccine produced by fusing cells with tumor cells using polyethylene glycol was tested as a consolidation strategy after autologous transplantation [49]. The study reported that paraprotein responses deepened in 24% of patients, with conversion of some PRs to CRs during the vaccination schedule [49]. However, paraprotein levels continue to drop in 39% of patients in the 3 months after autograft [50], so the improvement in responses may not be completely attributable to the vaccine. It is worth remembering that with Sipeleucel T, the only DC vaccine to receive FDA approval [51], 512 patients with castrate-resistant prostate cancer were enrolled in a randomized trial design, and while there was no change in PFS, there was a significant increase in OS (2–3 months). Therefore, immunological control may still significantly prolong survival despite difficulties in detecting it by conventional measures of disease burden. This puts extra emphasis on immunological biomarkers to detect the vaccine's effect and reinforces the need to perform randomized studies with OS as a primary endpoint. In fact, numerous clinical trials of DC vaccination in MM have demonstrated disease stability along with convincing T cell responses [52–54], potentially representing a meaningful clinical effect. At present, a combination of checkpoint inhibitors and DC vaccination is being assessed in a clinical trial (NCT01067287). This is a rational way forward, as the specificity of a DC vaccine may pair well with checkpoint inhibitors, avoiding the autoimmunity seen with checkpoint inhibitors combined with IMiDs.

9.2.5 Small-Molecule Inhibitors

9.2.5.1 Targeting Specific Subsets of Patients

Venetoclax for Patients with Chromosomal Translocation t(11;14)

Venetoclax is an oral small-molecule inhibitor of BCL-2 that has shown some promising results both as monotherapy and in combination with conventional anti-myeloma therapy in RRMM. BCL-2 is a protein expressed in many malignant cells; it has the ability to block apoptosis, in particular when cells are exposed to chemotherapeutic agents, and has been shown to mediate the survival of myeloma cells [55, 56]. Specific genetic subtypes of MM cells are particularly sensitive to venetoclax, including those with the t(11;14) chromosomal translocation, which have a high ratio of BCL-2 to MCL1 [56].

A phase I study of venetoclax monotherapy for 36 patients with RRMM, 26 of whom had had prior autologous stem cell transplant, revealed a tolerable safety profile, with better responses and a longer time on venetoclax for t(11;14) patients [56]. A 2017 study of venetoclax monotherapy again confirmed anti-myeloma activity and improved responses in those with the t(11;14) abnormality [57]. This study treated 66 patients who had received a median of five prior therapies, 61% of whom were bortezomib and lenalidomide double refractory, and 46% of whom possessed the t(11;14) abnormality. The ORR was 21% and 15% of patients achieved a VGPR or better. In those with t(11;14), the ORR was 40% and 27% of these patients achieved VGPR or better [57].

The combination of venetoclax, bortezomib, and dexamethasone has greater potency, with bortezomib inhibiting MCL-1 indirectly through the stabilization of MCL-1 neutralizing protein NOXA [58], while dexamethasone increases the expression of the pro-apoptotic molecule BIM which binds to BCL-2 [59]. Subsequently, a Phase Ib study has been completed in patients with a median of 3 prior lines of therapy, demonstrating acceptable toxicity (29% of patients developing grade 3 or 4 thrombocytopenia) and an ORR of 67% with a median time to progression of 9.5 months. Notably, 73% of patients were not refractory to bortezomib, and this subgroup achieved at least a VGPR [60].

Patients with BRAF V600E Mutation

Targeting intracellular proteins that influence the regulation of the cell cycle has been an area of interest in cancer therapy, particularly in the solid malignancy setting. The MAPK (mitogen-activated protein kinases) pathway, also known as the Ras-Raf-Mek-Erk pathway, regulates diverse cellular programs by relaying extracellular signals to intracellular processes [61]. One of the major programs it regulates is cellular proliferation, and mutations in any of the steps of the pathway can result in malignancy. The role of this pathway in the pathogenesis of MM is

fundamental to its clinical targeting. Both BRAF and MEK have been examined in the MM setting.

BRAF V600E mutations in MM have been investigated, but no clinical implications of the mutation have been found. This mutation activates the MAPK signaling pathway, resulting in the growth and survival of tumor cells. The incidence of the mutation in MM has been reported to be between 4 and 10% [62]. A case report, consisting of only seven patients, found the BRAF V600E mutation conferred shorter OS and higher prevalence of extramedullary disease [63]. However, a study in 2014 found the mutation in 6.2% of 209 patient biopsies and reported discordant results—with no evidence of a prognostic role or clinical phenotype for this mutation in early MM [64]. A small trial enrolling patients with the BRAF V600E mutation using vemurafenib in RRMM found a clinical benefit in 71%, with one patient having a PR and four having stable disease [65]. More work is clearly needed in this area to establish firstly whether the BRAF mutation has any clinical implications in MM and secondly whether targeting it will produce any definite clinical benefit. A well-known issue with BRAF inhibitors, identified in the melanoma setting, is that they can result in paradoxical activation of the MAPK pathway, requiring MAPK (MEK) inhibitors (see next section below) [66].

Potential MEK Inhibition

MEK1 and MEK2, similar to BRAF, are involved in the MAPK pathway, and play similar roles in cellular proliferation and apoptosis [67]. Although MEK1/2 have rarely mutated themselves, upstream mutations may result in MEK being constitutively activated, which in turn activates downstream signals, leading to altered transcription and cellular proliferation; thus MEK inhibitors can halt this cascade and lead to cell cycle arrest [68].

Approximately 50% of multiple myeloma patients have NRAS and KRAS mutations leading to the activation of the MAPK pathway via MEK [69]. MEK inhibitors such as trametinib have been used in RRMM, with response rates between 30 and 50%, albeit not in a formal clinical trial setting [66]. An assessment of the importance of RAS mutation status in sensitivity to MEK inhibitors found that those with the RAS mutation were sensitive to MEK inhibitors, while those with RAS wild type were resistant [69]. Furthermore, if there is coexpression of the t(14;16) chromosomal translocation which leads to MAF overexpression, patients are resistant to MEK inhibitors. In a very small study evaluating a combined RAF/MEK inhibitor as monotherapy in a range of patients with KRAS, NRAS, or BRAF mutated tumors, one included patient with MM achieved a PR [70].

9.2.5.2 Patients with Overexpressed FGFR3

FGFR3 (fibroblast growth factor receptor 3) is a glycoprotein belonging to the tyrosine kinase receptor family, and its activation leads to the activation of several key pathways implicated in neoplastic signaling, including the MAPK pathway [71]. Germline mutations in FGFR3 have long been known to lead to congenital anomalies such as achondroplasia [72]. It has only been recently that somatic FGFR3 mutations have been discovered, particularly in bladder cancer and endometrial cancer, and the discovery of its oncogenic role [71].

Approximately 10–20% of MM patients have the t(4;14)(p16.3;q32.2) translocation, which results in overexpression of FGFR3 and has an adverse prognosis [73]. Preclinical studies suggest FGFR3 inhibitors can induce cytotoxic responses in cells harboring a t(4;14) translocation, as well as a synergistic response with dexamethasone and additive response with melphalan or bortezomib [74]. Unfortunately, the Phase I/II clinical trial (NCT00304590) with the FGFR3 inhibitor XL999 has been discontinued because of unexpected cardiac toxicity.

9.2.5.3 Targeting Inherent Weaknesses in MM

SINE Compounds

Selective inhibitor of Nuclear Export (SINE) compounds are a family of small-molecule drugs that inhibit exportin 1 (XPO1)-mediated nuclear export, resulting in the retention of important tumor suppressor proteins in the nucleus, such as p53, FOXO, pRB, and IκB and ultimately leading to cancer cell death [75]. XPO1 is overexpressed in a wide variety of cancers including multiple myeloma and often correlates with a poor prognosis [76].

Selinexor is an oral SINE compound currently undergoing clinical trials in patients with both hematological and solid malignancies. In a study of 28 heavily pretreated RRMM patients, the combination of selinexor and dexamethasone resulted in an ORR of 60%, with 10% of patients achieving CR and 50% achieving PR. Patients given selinexor without dexamethasone had significantly worse outcomes [76]. These trials were initially halted due to incomplete safety documentation but have now resumed.

Selinexor has been evaluated in combination with a variety of IMiDs, PIs, MoAbs, and traditional chemotherapeutic agents, and there have been attempts to exploit synergism between agents with different mechanisms of action. An early-phase trial of selinexor with lenalidomide and low dose dexamethasone in 18 patients, found better results in lenalidomide naïve patients, with an ORR of 91%. Thrombocytopenia was the most common side effect without major organ toxicity [77]. Pomalidomide and dexamethasone alone in the R/R myeloma setting had an ORR of 30% and PFS of 3.6 months; when combined with selinexor the ORR improved to 54% without any additive toxicity [78].

Selinexor has also now been combined with bortezomib and dexamethasone in patients with RRMM. Selinexor was able to restore sensitivity to bortezomib in those that were previously PI-resistant [79]. Selinexor has demonstrated excellent activity when combined with bortezomib and dexamethasone in RRMM, with an ORR of 83% in PI-naïve patients, and an ORR of 42% in PI-refractory patients, both with lower rates of peripheral neuropathy than bortezomib alone [79]. A phase Ib study has assessed the combination of selinexor and daratumumab in patients previously exposed to PIs and IMiDs and reported responses without significant safety issues [80]. Combining selinexor with liposomal doxorubicin was shown to have no clinical benefit over selinexor alone [81].

Second-generation SINE compounds have also been developed, including eltanexor which, in phase I studies, has been shown to be safe and has anti-myeloma activity [82].

Bromodomain Inhibitors

The bromodomain and extraterminal (BET) family regulate and activate gene transcription by binding to acetylated lysine residues on histones, and thus play a key role as epigenetic regulators [83]. Bromodomain proteins are involved in tumorigenesis, regulating the expression of certain oncogenes, including those involved in cellular proliferation and apoptosis [84]. Small-molecule inhibitors of BET proteins have been investigated in both solid organ and hematologic malignancies and have shown some promise. They have been shown to have potent anti-myeloma activity in preclinical studies [85].

A next-generation bromodomain inhibitor, which causes degradation rather than just inhibition of BET bromodomains, has shown promising results ex vivo, encouraging its development as a myeloma therapy [86]. BET inhibitors have also been shown in myeloma cell lines to improve response when combined with lenalidomide and dexamethasone, even in cases with previous suboptimal prior response to IMiDs [87]. Phase Ib trials of BET inhibitors are currently underway.

Kinesin Spindle Protein Inhibitors

The kinesin spindle protein (KSP) is a member of the kinesin superfamily of microtubule-based motors and plays a crucial role in mediating centrosome separation and assembling bipolar spindles during mitosis [88]. Inhibition of the KSP leads to cell cycle arrest and reduction in the MCL-1 protein [89], which is an anti-apoptotic factor in MM cells.

Filanesib, a KSP inhibitor, has demonstrated promising clinical activity both as a single agent and when combined with dexamethasone in RRMM, with an ORR of 16% response rate (greater than partial response) in heavily pretreated patients who had no other therapeutic options [90]. A Phase II study of filanesib plus carfilzomib, in carfilzomib-naïve patients who had received at least two prior lines of therapy and

were refractory to their last therapy, showed that the addition of filanesib increased the median PFS to 8.5 months versus 3.7 months for carfilzomib alone. The ORR of filanesib and carfilzomib was 30% versus 10% for carfilzomib alone [91]. Clinical trials are continuing to establish the efficacy of filanesib in combination with other standard-of-care anti-myeloma agents, including pomalidomide, in heavily pre-treated RRMM patients.

9.3 Conclusion and Perspectives

While the armamentarium of targeted agents is growing, none are likely to be highly effective as single agents (outside of perhaps CAR T cells). This poses challenges around the clinical testing of optimal combinations. Adding new agents to current standard-of-care regimens is feasible, for example, the addition of daratumumab to a novel agent backbone, as clinical trials with randomization to the additional agents are ethical and practical. However, it becomes more difficult to assess the efficacy of multiple new agents in combination. The "pick a winner design" used in acute myeloid leukemia may accelerate this process [92].

There are also challenges in assessing combinations of antibodies, cellular therapies, and small-molecule inhibitors, all with differing response kinetics and mechanisms of disease control. It is possible to assess the effects of small-molecule inhibitor in a subset of "sensitive" patients with a defined molecular abnormality, but it is not as simple as immune therapies. Responses may not only be determined by tumor characteristics therefore conventional pre-therapy prognostic factors (including tumor karyotype, gene expression, clinical prognostic factors, etc.) may no longer predict responses, requiring an appreciation of complex immunological factors. Incorporating thorough assessments of immune biomarkers into clinical trials will be essential to direct these therapies to those who are most likely to respond.

While we may demonstrate that a new agent provides a progression free survival benefit of a few months in heavily pretreated patients, the cost and toxicity of these agents are significant, and funding bodies will increasingly ask whether the efforts put into these new agents are justifiable. Specific combinations of effective agents, directed to those likely to respond, at suitable points in their disease, may provide more meaningful benefits to patients.

References

1. Lokhorst HM, et al. Targeting CD38 with daratumumab monotherapy in multiple myeloma. N Engl J Med. 2015;373(13):1207–19.
2. Palumbo A, et al. Daratumumab, bortezomib, and dexamethasone for multiple myeloma. N Engl J Med. 2016;375(8):754–66.
3. Feng X, et al. Targeting CD38 suppresses induction and function of T regulatory cells to mitigate immunosuppression in multiple myeloma. Clin Cancer Res. 2017;23(15):4290–300.

4. Krejcik J, et al. Daratumumab depletes CD38+ immune regulatory cells, promotes T-cell expansion, and skews T-cell repertoire in multiple myeloma. Blood. 2016;128(3):384–94.
5. Deckert J, et al. SAR650984, a novel humanized CD38-targeting antibody, demonstrates potent antitumor activity in models of multiple myeloma and other CD38+ hematologic malignancies. Clin Cancer Res. 2014;20(17):4574–83.
6. Martin T, et al. A phase 1b study of isatuximab plus lenalidomide and dexamethasone for relapsed/refractory multiple myeloma. Blood. 2017;129(25):3294–303.
7. O'Connell FP, Pinkus JL, Pinkus GS. CD138 (syndecan-1), a plasma cell marker immunohistochemical profile in hematopoietic and nonhematopoietic neoplasms. Am J Clin Pathol. 2004;121(2):254–63.
8. Heffner LT, et al. BT062, an antibody-drug conjugate directed against CD138, given weekly for 3 weeks in each 4 week cycle: Safety and further evidence of clinical activity. Blood. 2012;120(21):4042.
9. Kelly KR, et al. Indatuximab Ravtansine (BT062) in combination with low-dose dexamethasone and Lenalidomide or Pomalidomide: Clinical activity in patients with relapsed/refractory multiple myeloma. Blood. 2016;128(22):4486.
10. Ko, J., et al., Preclinical evaluation of Hdp-101, a novel anti-BCMA antibody-drug conjugate, in multiple myeloma. 2017.
11. Sanchez E, et al. Serum B-cell maturation antigen is elevated in multiple myeloma and correlates with disease status and survival. Br J Haematol. 2012;158(6):727–38.
12. Trudel S, Lendvai N, Popat R, Voorhees PM, Reeves B, Libby EN, Richardson PG, Anderson L, Sutherland H, Yong K, Hoos A, Gorczyca M, Lahiri S, He Z, Jewell RC, Opalinska JB, Cohen AD. Deep and durable responses in patients (pts) with relapsed/refractory multiple myeloma (MM) treated with monotherapy GSK2857916, an antibody drug conjugate against B-cell maturation antigen (BCMA): Preliminary results from part 2 of study BMA117159. The American Society of Hematology. Atlanta; 2017.
13. Huehls AM, Coupet TA, Sentman CL. Bispecific T-cell engagers for cancer immunotherapy. Immunol Cell Biol. 2015;93(3):290–6.
14. Brischwein K, et al. Strictly target cell-dependent activation of T cells by bispecific single-chain antibody constructs of the BiTE class. J Immunother. 2007;30(8):798–807.
15. Offner S, et al. Induction of regular cytolytic T cell synapses by bispecific single-chain antibody constructs on MHC class I-negative tumor cells. Mol Immunol. 2006;43(6):763–71.
16. Topp MS, et al. Safety and activity of blinatumomab for adult patients with relapsed or refractory B-precursor acute lymphoblastic leukaemia: A multicentre, single-arm, phase 2 study. Lancet Oncol. 2015;16(1):57–66.
17. Carpenter RO, et al. B-cell maturation antigen is a promising target for adoptive T-cell therapy of multiple myeloma. Clin Cancer Res. 2013;19(8):2048–60.
18. Hipp S, et al. BI 836909, a novel Bispecific T cell engager for the treatment of multiple myeloma induces highly specific and efficacious Lysis of multiple myeloma cells in vitro and shows anti-tumor activity in vivo. Blood. 2015;126(23):2999.
19. Zou J, et al. Immunotherapy based on bispecific T-cell engager with hIgG1 fc sequence as a new therapeutic strategy in multiple myeloma. Cancer Sci. 2015;106(5):512–21.
20. Hipp S, et al. A novel BCMA/CD3 bispecific T-cell engager for the treatment of multiple myeloma induces selective lysis in vitro and in vivo. Leukemia. 2017;31(10):2278.
21. Seckinger A, et al. Target expression, generation, preclinical activity, and pharmacokinetics of the BCMA-T cell Bispecific antibody EM801 for multiple myeloma treatment. Cancer Cell. 2017;31(3):396–410.
22. Li J, et al. Anti-FcRH5/CD3 T cell dependent Bispecific antibody (TDB) for the treatment of multiple myeloma. Blood. 2016;128(22):4475.
23. Topalian SL, et al. Survival, durable tumor remission, and long-term safety in patients with advanced melanoma receiving nivolumab. J Clin Oncol. 2014;32(10):1020–30.
24. Ansell SM, et al. PD-1 blockade with nivolumab in relapsed or refractory Hodgkin's lymphoma. N Engl J Med. 2015;372(4):311–9.

25. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer. 2012;12(4):252–64.
26. Gorgun G, et al. Lenalidomide enhances immune checkpoint blockade-induced immune response in multiple myeloma. Clin Cancer Res. 2015;21(20):4607–18.
27. Rosenblatt J, et al. PD-1 blockade by CT-011, anti-PD-1 antibody, enhances ex vivo T-cell responses to autologous dendritic cell/myeloma fusion vaccine. J Immunother. 2011;34(5):409–18.
28. Lesokhin AM, et al. Nivolumab in patients with relapsed or refractory hematologic malignancy: Preliminary results of a phase Ib study. J Clin Oncol. 2016;34(23):2698–704.
29. Suen H, et al. The failure of immune checkpoint blockade in multiple myeloma with PD-1 inhibitors in a phase 1 study. Leukemia. 2015;29(7):1621–2.
30. Görgün G, et al. Lenalidomide enhances immune checkpoint blockade-induced immune response in multiple myeloma. Clin Cancer Res. 2015;21(20):4607–18.
31. Badros AZ, et al. A phase II study of anti PD-1 antibody pembrolizumab, pomalidomide and dexamethasone in patients with relapsed/refractory multiple myeloma (RRMM). Blood. 2015;126(23):506.
32. San Miguel J, et al. Pembrolizumab in combination with lenalidomide and low-dose dexamethasone for relapsed/refractory multiple myeloma (RRMM): Keynote-023. Blood. 2015;126(23):505.
33. Leleu X, et al. High response rates to pomalidomide and dexamethasone in patients with refractory myeloma, final analysis of IFM 2009-02. Blood. 2011;118(21):812.
34. Lacy MQ, et al. Pomalidomide plus low-dose dexamethasone (Pom/Dex) in relapsed myeloma: Long term follow up and factors Predicing outcome in 345 patients. Blood. 2012;120(21):201.
35. San Miguel JF, et al. Impact of prior treatment and depth of response on survival in MM-003, a randomized phase 3 study comparing pomalidomide plus low-dose dexamethasone versus high-dose dexamethasone in relapsed/refractory multiple myeloma. Haematologica. 2015;100(10):1334–9.
36. Ikeda H. T-cell adoptive immunotherapy using tumor-infiltrating T cells and genetically engineered TCR-T cells. Int Immunol. 2016;28(7):349–53.
37. Garfall AL, et al. Chimeric antigen receptor T cells against CD19 for multiple myeloma. N Engl J Med. 2015;373(11):1040–7.
38. Garfall AL, Stadtmauer EA, June CH. Chimeric antigen receptor T cells in myeloma. N Engl J Med. 2016;374(2):194.
39. Garfall AL, et al. Pilot study of anti-CD19 chimeric antigen receptor T cells (CTL019) in conjunction with salvage autologous stem cell transplantation for advanced multiple myeloma. Blood. 2016;128(22):974.
40. Fan F, et al. Durable remissions with BCMA-specific chimeric antigen receptor (CAR)-modified T cells in patients with refractory/relapsed multiple myeloma. J Clin Oncol. 2017;35(18_suppl):LBA3001-LBA3001.
41. Cohen AD, Garfall AL, Stadtmauer EA, Lacey SF, Lancaster E, Vogl DT, Weiss BM, Ambrose DE, Nelson AM, Chen F, Plesa G. Safety and efficacy of B-cell maturation antigen (BCMA)-specific chimeric antigen receptor T cells (CART-BCMA) with cyclophosphamide conditioning for refractory multiple myeloma (MM). ASH. Atlanta; 2017.
42. Berdeja L, Lin Y, Raje N, Munshi N, Siegel D, Liedtke M, Jagannath S, Maus MV, Turka A, Lam LP, Hege K, Morgan RA, Quigley MT, Kochenderfer JN. Durable clinical responses in heavily pretreated patients with relapsed/refractory multiple myeloma: Updated results from a multicenter study of bb2121 anti-Bcma CAR T cell therapy. The American Society of Hematology. Atlanta; 2017.
43. Anguille S, et al. Dendritic cells as pharmacological tools for cancer immunotherapy. Pharmacol Rev. 2015;67(4):731–53.
44. Brown R, et al. Dendritic cells from patients with myeloma are numerically normal but functionally defective as they fail to up-regulate CD80 (B7-1) expression after huCD40LT

stimulation because of inhibition by transforming growth factor-beta1 and interleukin-10. Blood. 2001;98:2992–8.
45. Ratta M, et al. Dendritic cells are functionally defective in multiple myeloma: The role of interleukin-6. Blood. 2002;100(1):230–7.
46. Raje N, et al. Bone marrow and peripheral blood dendritic cells from patients with multiple myeloma are phenotypically and functionally normal despite the detection of Kaposi's sarcoma herpesvirus gene sequences. Blood. 1999;93(5):1487–95.
47. Pfeiffer S, et al. Dendritic cells generated from the blood of patients with multiple myeloma are phenotypically and functionally identical to those similarly produced from healthy donors. Br J Haematol. 1997;98(4):973–82.
48. Lacy MQ, et al. Idiotype-pulsed antigen-presenting cells following autologous transplantation for multiple myeloma may be associated with prolonged survival. Am J Hematol. 2009;84(12):799–802.
49. Rosenblatt J, et al. Vaccination with dendritic cell/tumor fusions following autologous stem cell transplant induces immunologic and clinical responses in multiple myeloma patients. Clin Cancer Res. 2013;19(13):3640–8.
50. Gonsalves WI, et al. Implications of continued response after autologous stem cell transplantation for multiple myeloma. Blood. 2013;122(10):1746–9.
51. Kantoff PW, et al. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. N Engl J Med. 2010;363(5):411–22.
52. Curti A, et al. Phase I/II clinical trial of sequential subcutaneous and intravenous delivery of dendritic cell vaccination for refractory multiple myeloma using patient-specific tumour idiotype protein or idiotype (VDJ)-derived class I-restricted peptides. Br J Haematol. 2007;139(3):415–24.
53. Rosenblatt J, et al. Vaccination with dendritic cell/tumor fusion cells results in cellular and humoral antitumor immune responses in patients with multiple myeloma. Blood. 2011;117(2):393–402.
54. Yi Q, et al. 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. 2010;150(5):554–64.
55. Tu Y, et al. Upregulated expression of BCL-2 in multiple myeloma cells induced by exposure to doxorubicin, etoposide, and hydrogen peroxide. Blood. 1996;88(5):1805–12.
56. Kumar SK, et al. Safety and efficacy of Venetoclax (ABT-199/GDC-0199) monotherapy for relapsed/refractory multiple myeloma: Phase 1 preliminary results. Blood. 2015;126(23):4219.
57. Kumar S, et al. Efficacy of venetoclax as targeted therapy for relapsed/refractory t(11;14) multiple myeloma. Blood. 2017;130(22):2401–9.
58. Qin J-Z, et al. Proteasome inhibitors trigger NOXA-mediated apoptosis in melanoma and myeloma cells. Cancer Res. 2005;65(14):6282–93.
59. Matulis SM, et al. Dexamethasone treatment promotes Bcl-2 dependence in multiple myeloma resulting in sensitivity to venetoclax. Leukemia. 2016;30(5):1086–93.
60. Moreau P, et al. Promising efficacy and acceptable safety of venetoclax plus bortezomib and dexamethasone in relapsed/refractory MM. Blood. 2017;130(22):2392–400.
61. Cargnello M, Roux PP. Activation and function of the MAPKs and their substrates, the MAPK-activated protein kinases. Microbiol Mol Biol Rev. 2011;75(1):50–83.
62. Danu A, et al. BRAF V600E targetable mutation in relapsed/refractory multiple myeloma (R/R MM) patients: A high incidence in R/R MM detected using cell sorting screening. Blood. 2016;128(22):5638.
63. Andrulis M, et al. Targeting the BRAF V600E mutation in multiple myeloma. Cancer Discov. 2013;3(8):862–9.
64. Rustad EH, et al. BRAF V600E mutation in early-stage multiple myeloma: Good response to broad acting drugs and no relation to prognosis. Blood Cancer J. 2015;5(3):e299.
65. Raje N, Chau I, Hyman D, Ribrag V, Blay J-Y, Tabernero J, Elez-Fernandez M, Wolf J, Sirzen F, Yee A, Faris J, Kaiser M, Landau H, Michot J-M, Veronese L, Makrutzki M, Lasserre F,

Puzanov I, Baselga J. Vemurafenib (VEM) in relapsed refractory multiple myeloma harbouring BRAF V600E mutations (V600m): a cohort of the histology-independent VE-basket study. The American Society of Hematology; 2015.
66. Heuck CJ, et al. Inhibiting MEK in MAPK pathway-activated myeloma. Leukemia. 2016;30(4):976–80.
67. Zhao Y, Adjei AA. The clinical development of MEK inhibitors. Nat Rev Clin Oncol. 2014;11(7):385–400.
68. Luke JJ, Ott PA, Shapiro GI. The biology and clinical development of MEK inhibitors for cancer. Drugs. 2014;74(18):2111–28.
69. Qiang Y-W, et al. The co-occurrence of MAF translocations in RAS mutated multiple myeloma confers resistance to MEK inhibition. Blood. 2016;128(22):1138.
70. Chenard-Poirier M, et al. Results from the biomarker-driven basket trial of RO5126766 (CH5127566), a potent RAF/MEK inhibitor, in RAS- or RAF-mutated malignancies including multiple myeloma. J Clin Oncol. 2017;35(15_suppl):2506.
71. Kalff A, Spencer A. The t(4;14) translocation and FGFR3 overexpression in multiple myeloma: Prognostic implications and current clinical strategies. Blood Cancer J. 2012;2:e89.
72. Naski MC, et al. Graded activation of fibroblast growth factor receptor 3 by mutations causing achondroplasia and thanatophoric dysplasia. Nat Genet. 1996;13(2):233–7.
73. Benard B, et al. FGFR3 mutations are an adverse prognostic factor in patients with t(4;14)(p16;q32) multiple myeloma: An Mmrf Compass analysis. Blood. 2017;130(Suppl 1):3027.
74. Trudel S, et al. Evaluation of XL999, a potent inhibitor of FGFR3, for the potential treatment of t(4;14) positive multiple myeloma. Blood. 2007;110(11):2515.
75. Kashyap T, et al. Selinexor, a selective inhibitor of nuclear export (SINE) compound, shows synergistic anti-tumor activity in combination with dexamethasone characterized by specific pattern of gene expression in multiple myeloma (MM). Blood. 2015;126(23):3683.
76. Chen CI, et al. Selinexor demonstrates marked synergy with dexamethasone (Sel-Dex) in preclinical models and in patients with heavily pretreated refractory multiple myeloma (MM). Blood. 2014;124(21):4773.
77. White D, Bahlis N, Venner C, Schiller G, Gasparetto C, Sutherland H, Sebag M, Lentzsch S, Koth R, Bensinger W, Lipe B, Chen C, Del Col A, Kauffman M, Shacham S, Jeha J, Saint-Martin J-R, Shah J, Leblanc R. A phase Ib/II trial of selinexor combined with lenalidomide and low dose dexamethasone in patients with relapsed/refractory multiple myeloma. The American Society of Hematology. Atlanta; 2017.
78. Chen C, Sutherland H, Koth R, Sebag M, White D, Bensinger W, Gasparetto C, Leblanc R, Venner C, Lentzsch S, Schiller G, Lipe B, Del Col A, Kauffman M, Shacham S, Jeha J, Saint-Martin J-R, Shah S, Bahlis N. Selinexor in combination with pomalidomide and low dose dexamethasone in a relapsed/refractory multiple myeloma patient population with prior proteasome inhibitor and lenalidomide exposure. The American Society of Hematology. Atlanta; 2017.
79. Bahlis NJ, Sutherland H, White D, Sebag M, Lentzsch S, Koth R, Venner CP, Gasparetto C, Del Col A, Neri P, Reece D, Kauffman M, Shacham S, Unger TJ, Jeha J, Saint-Martin J-R, Shah J, Chen C. Selinexor plus low-dose bortezomib and dexamethasone for patients with relapsed or refractory multiple myeloma. Blood 2018; 132(24):2546–2554.
80. Gasparetto C, Lentzsch S, Schiller G, Bensinger W, Bahlis N, Sutherland H, White D, Sebag M, Koth R, Venner C, Lebland R, Chen C, Del Col A, Kauffman M, Shacham S, Jeha J, Saint-Martin J-R, Shah J, Lipe B. A phase Ib study to assess the combination of selinexor and daratumumab in patients with relapsed/refractory multiple myeloma previously exposed to proteasome inhibitors (PI) and immunomodulatory drugs (IMiDs). The American Society of Hematology. Atlanta; 2017.
81. Baz R, Zonder J, Shain K, Alsina M, Brayer J, Melody M, Turner J, Dawson J, Kim J, Sullivan D. Phase I/II study of liposomal doxorubicin (DOX) in combination with selinexor (SEL) and dexamethasone (Dex) for relapsed and refractory multiple myeloma (RRMM). The American Society of Hematology. Atlanta; 2017.

82. Cornell R, Rossi A, Baz R, Hofmeister C, Shustik C, Richter J, Chen C, Vogl D, Baloglu E, Senapedis W, Ellis J, Williams T, Shacham S, Kauffman M. Eltanexor (KPT-8602), a second-generation selective inhibitor of nuclear export (SINE) compound, in patients with refractory multiple myeloma. The American Society of Hematology. Atlanta; 2017.
83. Perez-Salvia M, Esteller M. Bromodomain inhibitors and cancer therapy: From structures to applications. Epigenetics. 2017;12(5):323–39.
84. Wadhwa E, Nicolaides T. Bromodomain inhibitor review: bromodomain and extra-terminal family protein inhibitors as a potential new therapy in central nervous system tumors. Cureus. 2016;8(5):e620.
85. Chaidos A, et al. Potent antimyeloma activity of the novel bromodomain inhibitors I-BET151 and I-BET762. Blood. 2014;123(5):697–705.
86. Matthews GM, et al. BET Bromodomain degradation as a therapeutic strategy in multiple myeloma. Blood. 2016;128(22):1062.
87. Diaz T, et al. The BET bromodomain inhibitor CPI203 improves lenalidomide and dexamethasone activity in in vitro and in vivo models of multiple myeloma by blockade of Ikaros and MYC signaling. Haematologica. 2017;102(10):1776–84.
88. Marra E, et al. Kinesin spindle protein SiRNA slows tumor progression. J Cell Physiol. 2013;228(1):58–64.
89. Hernandez-Garcia S, et al. The kinesin spindle protein inhibitor filanesib enhances the activity of pomalidomide and dexamethasone in multiple myeloma. Haematologica. 2017;102(12):2113–24.
90. Shah JJ, et al. Prolonged survival and improved response rates with ARRY-520 in relapsed/refractory multiple myeloma (RRMM) patients with low α-1 acid glycoprotein (AAG) levels: Results from a phase 2 study. Blood. 2013;122(21):285.
91. Zonder JA, et al. Phase 2 study of Carfilzomib (CFZ) with or without Filanesib (FIL) in patients with advanced multiple myeloma (MM). Blood. 2015;126(23):728.
92. Hills RK, Burnett AK. Applicability of a "pick a winner" trial design to acute myeloid leukemia. Blood. 2011;118(9):2389–94.

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