

Targeting Protein Synthesis and Degradation in Multiple Myeloma: A Look at What's on the Horizon

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Abstract

The natural history of multiple myeloma (MM) has profoundly changed over the last 20 years, largely due to the introduction of novel agents targeting MM in the context of the bone marrow microenvironment. Once a rapidly fatal cancer, MM can now be effectively treated in most patients, with median overall survival approaching 10 years. The landscape of MM therapy is rapidly changing, with 9 drugs approved by the Food and Drug Administration (FDA) for MM in the past 14 years, including 4 in 2015. In this on-the-horizon review, we will discuss the molecular mechanisms and scientific rationale of FDA-approved and investigational agents targeting protein synthesis, folding, and degradation in MM. In fact, a pathognomonic feature of MM is the abundant synthesis and secretion of a monoclonal immunoglobulin, or free light chain, underscoring the importance of an intact protein synthesis-folding-degradation axis in this cancer. Clinical use of proteasome inhibitors has proved successful in MM, but acquired resistance remains a major concern, prompting further research into targeting alternative proteolytic pathways for synergistic MM killing with the goal of improving outcome in patients with multiple myeloma.

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curable and resistance to novel agents represents a major clinical problem. This review will focus on the molecular mechanisms underlying protein handling in MM and on bench-to-bedside translation of therapies targeting protein synthesis, folding, and degradation in MM. Rational combination of these agents holds promise to help overcome proteasome inhibitor (PI) resistance in MM, with the goal of achieving prolonged remission, if not cure, for most patients with multiple myeloma.

Scientific Rationale for Targeting Protein-Handling Pathways in Multiple Myeloma

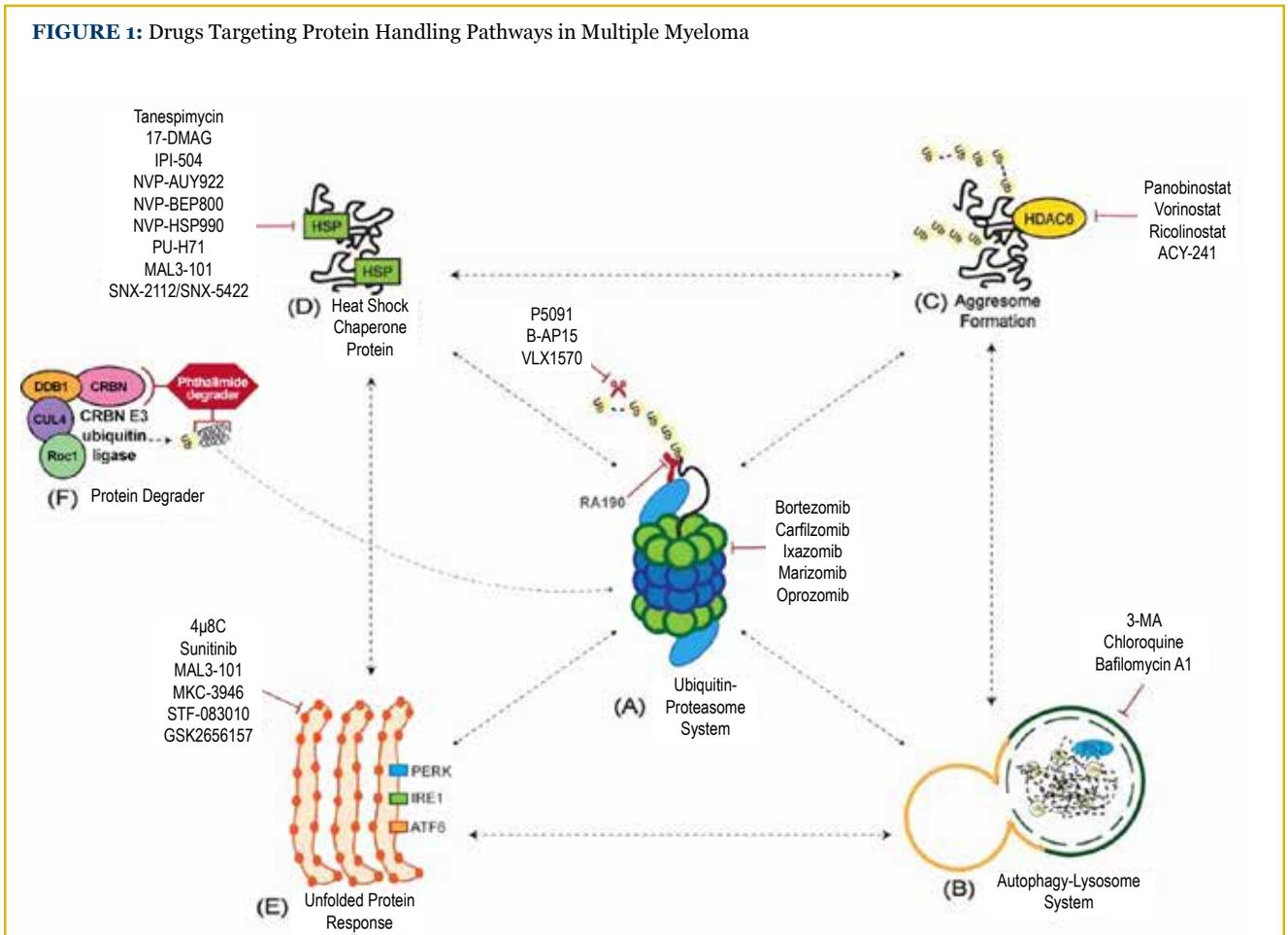
The process of protein synthesis and folding is intrinsically prone to errors, and eukaryotic cells are equipped with quality control mechanisms to ensure that native proteins adopt proper tertiary and quaternary conformations. The cytotoxic accumulation of misfolded proteins causes endoplasmic reticulum (ER) stress and activates the unfolded protein response (UPR), which, together with autophagy, aggresome, and the ubiquitin-proteasome system (UPS), has the goal of maintaining protein homeostasis.^{2,3} It is estimated that one-third of newly synthesized proteins are degraded via the proteasome within minutes of their synthesis due to an intrinsic inability to achieve stably folded conformations.⁴ These rapidly degraded proteins are termed “defective ribosomal products” (DRiPs). Due to high protein turnover, cancer cells typically produce an even higher percentage of DRiPs, making them reliant on an intact UPS for survival.^{5,8} This is especially true for MM, a cancer characterized by a high synthesis rate of immunoglobulins. In fact, MM cells exhibit stigmata of ongoing proteotoxic stress with baseline induction of UPR and accumulation of polyubiquitinated proteins, providing a substrate for proteasome-mediated degradation.^{9,11}

Studies have shown that an imbalance between the cargo for proteasomal degradation (polyubiquitinated proteins) and the activity of the proteasome is a key determinant of PI sensitivity in MM.¹² Drugs that increase proteasome workload (eg, heat shock protein [HSP] inhibitors, ER stressors) synergize with drugs that decrease proteasome activity (eg, PIs) in MM. The results of *in vitro* studies have shown that proteasome inhibition, perhaps even UPR induction, results in the compensatory activation of aggresome, autophagy, and heat shock response pathways in an

Introduction

Multiple myeloma (MM) is a cancer of plasma cells characterized by increased survival and proliferation of terminally differentiated plasma cells in the bone marrow.¹ Clinically, MM diagnosis is prompted by the detection of monoclonal intact immunoglobulin (M spike), also known as free light chains (not in association with heavy chains), in the serum and urine of patients presenting with 1 or more of hypercalcemia, renal failure, anemia, and bone disease (the CRAB criteria). Major progress in our understanding of MM biology over the past 4 decades has led to significant improvements in how we treat MM, reflected by a 3- to 4-fold increase in patient median survival. Although MM is now better controlled over longer periods for many patients, it remains in-

FIGURE 1: Drugs Targeting Protein Handling Pathways in Multiple Myeloma



effort to protect MM cells from proteotoxicity (Figure 1).^{2,13-15}

Further work assessing the combinatorial effects of blocking 2 or more of these pathways in MM are currently ongoing, some of which are highlighted below.

Ubiquitin-Proteasome System

At the core of protein homeostasis in eukaryotes is the UPS (Figure 1A). Proteins targeted for proteasome degradation are polyubiquitinated via a 3-enzyme cascade involving E1 (activating), E2 (conjugating), and E3 (ligase) enzymes, while deubiquitinating enzymes (DUBs) act in opposition to E3 ligases to remove ubiquitin.¹⁶⁻¹⁹ The 26S proteasome is an ATP-dependent, multicatalytic complex comprising a 20S catalytic core flanked on either side by 19S regulatory caps.²⁰ Polyubiquitinated substrates are recognized by the 19S regulatory subunit that, in concert with DUBs, remove ubiquitin and facilitate engagement with the 20S core that contains the catalytically active $\beta 1$ (caspase-like activity), $\beta 2$ (trypsin-like activity), and $\beta 5$ (chymotrypsin-like activity) subunits.^{19,21,22} The PIs bortezomib, carfilzomib, ixazomib, and oprozomib primarily target the $\beta 5$ subunit, while marizomib appears to have activity against all 3 β -subunits.²³⁻²⁶

Prior to degradation, proteasome-associated DUBs (eg, RPH11, UCH37, and USP14) remove ubiquitin chains, which would otherwise sterically hinder the translocation of target proteins to the 20S core.²⁷ Similar to PIs, DUB inhibitors trigger polyubiquitinated protein accumulation and apoptosis in MM, but without inhibiting the catalytic subunits of the proteasome.²⁸⁻³⁰ Thus, DUB inhibitors could theoretically overcome resistance to proteasome inhibition when this is mediated by mutations in the catalytic subunits of the proteasome. Furthermore, DUB inhibition offers the opportunity to promote the degradation of proteins that are preferential clients of specific DUBs.

Autophagy

Autophagy, a conserved process of autophagy that plays a key role in maintaining protein homeostasis (Figure 1B), participates in the quality control of protein synthesis/degradation by sequestering misfolded/aggregated proteins in autophagosomal vesicles for subsequent lysosome degradation.³¹ Studies have shown that crosstalk exists between UPS, ER stress, and autophagy.³²⁻³⁴ Although elevated basal autophagic activity in primary MM cells is associated with shorter overall survival (OS) and progression-free

survival (PFS), autophagy's role in MM is controversial given that it can be pro-survival and pro-apoptotic depending on factors we have yet to fully understand.³² The current consensus is that a basal level of autophagy is essential for MM survival as an alternative proteolytic pathway in the face of decreased proteasome activity/increased proteotoxic stress, thus providing a rationale for the combination of autophagy inhibitors with PI in MM. However, persistent, sustained, and uncontrolled autophagy is likely to result in cell death, outlining the difficulties in therapeutically targeting autophagy.³⁵

Aggresome Pathway

In vitro, the aggresome pathway is activated when proteasomes are blocked. Polyubiquitinated protein aggregates are transported along the microtubule to the microtubule-organizing center in a histone deacetylase 6 (HDAC6)-dependent manner to form aggresomes that target proteins for refolding or degradation by autophagy (**Figure 1C**).³⁶⁻³⁹ The results of in vitro studies show that combined inhibition of the proteasome and aggresome leads to synergistic cell death in MM, providing strong rationale for combining PI with HDAC6 selective inhibitors.⁴⁰

Heat Shock Chaperone Proteins

Heat shock chaperone proteins (HSPs) are a class of enzymes that chaperone the proper folding and function of proteins, and that direct misfolded proteins to degradation; therefore, they participate in protein quality control (**Figure 1D**).⁴¹⁻⁴⁴ In MM, HSPs support proliferation and survival by 1) facilitating proper folding of newly synthesized proteins to prevent proteotoxic stress and 2) preferentially supporting the folding and expression of several oncogenes.⁴⁵ Two main families of HSPs are being targeted therapeutically in MM: HSP90 and HSP70. Interestingly, HSP70 overexpression in neurons results in inhibition of caspase-dependent and -independent apoptosis, suggesting a third pro-survival function.^{46,47} Inhibition of HSP90 or the proteasome results in compensatory upregulation of HSP70, thereby making the latter an attractive target in combinatory anti-MM therapy. Recently, there has been growing interest in developing inhibitors against heat shock factor 1 (HSF1), the "master regulator" of heat shock response, in an attempt to avoid compensatory upregulation of individual chaperones.⁴⁵

Endoplasmic Reticulum Stress and Unfolded Protein Response

The UPR is a tripartite response triggered by the accumulation of unfolded/misfolded proteins in the ER (**Figure 1E**).^{48,49} The UPR functions to restore equilibrium in the ER; however, prolonged/persistent activation of UPR results in apoptosis.⁵⁰ The 3 distinct UPR branches are regulated by 3 kinases: IRE1, PERK, and ATF6. Activation of IRE1 results in the splicing of XBP1 mRNA which, together with activated ATF6, regulates ER expansion, increases expression of chaperone proteins, and initiates ER-as-

sociated de-gradation to reduce ER stress.⁵¹ Depending on the magnitude and duration of stress, IRE1 can either activate antiapoptotic signaling through protein kinase B or trigger apoptosis through c-Jun N-terminal kinases (JNK) activation.⁵²⁻⁵⁴ Furthermore, JNK activation can initiate autophagy, thereby serving as a link between ER stress and autophagy.⁵⁴ Finally, protein kinase R-like ER kinase (PERK) activation inhibits eIF2 α , leading to a repression of global protein synthesis while selectively inducing the translation of ATF4.⁵⁵ ATF4 activates cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP), and together they upregulate protective autophagy in the face of transient proteotoxic stress.⁵⁶ However, if the stress is prolonged, CHOP can trigger apoptosis, outlining the double-edged nature of this stress response pathway.⁵⁷

Forced expression of spliced X-box binding protein 1 (XBP1) in B cells induces an MM-like phenotype in mice, and high XBP1 expression in primary MM cells correlates with poor OS, suggesting that chronic IRE1-XBP1 activation may be important for MM survival.⁵⁸ However, it was recently demonstrated that decreased XBP1 splicing confers bortezomib resistance in MM.⁵⁹ By suppressing XBP1s, MM cells decommit to plasma cell maturation and decrease immunoglobulin production, proteasome load, and ER stress, resulting in acquired resistance to PI.^{12,59}

Clinical Translation of Therapies Targeting Protein-Handling Pathways in Multiple Myeloma

Drugs Targeting the Ubiquitin-Proteasome Pathway

Apart from FDA-approved bortezomib, carfilzomib, and ixazomib, there are 2 novel PIs in advanced clinical development, oprozomib and marizomib. Oprozomib (ONX 0912), an oral analogue of carfilzomib, is an irreversible epoxyketone PI. In preclinical studies, oprozomib demonstrated cytotoxicity in MM in combination with lenalidomide and/or HDAC inhibitor molecules, as well as bone anabolic effects.^{60,61} A phase Ib/II trial of single-agent oprozomib showed an overall response rate (ORR) of 22% to 34% in relapsed/refractory MM (R/R MM), including bortezomib- and carfilzomib-refractory MM (NCT01416428).⁶² In the R/R MM setting, the combination of oprozomib with dexamethasone (NCT01832727) or with pomalidomide and dexamethasone (NCT01993335) has resulted in ORRs of 42% and 50% to 59%, respectively.^{63,64}

Attempts at overcoming PI resistance by blocking all proteasome catalytic subunits prompted the development of marizomib, a pan-proteasome inhibitor.^{65,66} A phase I study of marizomib (NCT00461045) reported an ORR of 7.4% in bortezomib-, lenalidomide-, and/or thalidomide-refractory patients.⁶⁷ Based on encouraging preclinical data supporting the combination of marizomib and pomalidomide/dexamethasone, clinical trials evaluating this combination are now underway (NCT02103335).⁶⁸

TABLE. Investigational Drugs Targeting Protein Synthesis/Degradation Pathways in Multiple Myeloma*

Drug Name/ Sponsor	Mechanism of Action	Study Design	Status/ Clinical Trials ID/PMID
Drugs that target protein handling pathways			
Proteasome inhibitors			
Marizomib (NPI-0052) Triphase Research and Development I Corporation (USA)	Proteasome inhibition; caspase 8- and 9-mediated apoptosis	Marizomib alone	Phase I/II NCT00461045
		Marizomib + POM + low-dose dexamethasone in R/R MM	Phase I NCT02103335
Oprozomib (ONX 0912, PR-047) Onyx Therapeutics (USA)	Proteasome inhibition; caspase 8- and 9-mediated apoptosis; p53 and p21 upregulation terminal UPR induction; miR33b upregulation, PIM1 downregulation	Oprozomib alone	Phase I/II NCT01416428
		Oprozomib + DEX + LEN/ cyclophosphamide in ND MM	Phase I/II NCT01881789
		Oprozomib + DEX in R/R MM	Phase IB/II NCT01832727
		Oprozomib + POM + DEX in R/R MM	Phase I/II NCT01993335
		Oprozomib + melphalan + prednisone in ND MM (transplant-ineligible)	Phase I/II NCT02072863
Inhibitors of deubiquitinating enzymes (DUBs)			
VLX1570 Vivolux AB (Sweden)	Inhibits proteasome DUB (USP14) activity	VLX1570 + DEX in R/R MM	Phase I/II NCT02372240
P5091 NA	Inhibits DUB USP7 to induce apoptosis in MM cells	N/A	Preclinical PMID: 22975377 ⁹⁵
B-AP15 NA	Blocks USP14 and UCHL5: growth arrest via downregulation of CDC25C, CDC2, and cyclin B1; induction of caspase-dependent apoptosis; activation of UPR	N/A	Preclinical PMID: 24319254 ⁶⁹
RA 190 NA	Inhibits 19S proteasome-associated ubiquitin receptor Rpn13 to inhibit proteasome function without blocking proteasome activity or the 19S deubiquitylating activity	N/A	Preclinical PMID: 27118409 ⁹⁶
Histone deacetylase inhibitors			
Vorinostat Acetylon (USA)	Caspase 8/9-mediated apoptosis; terminal UPR induction; polyubiquitinated protein accumulation; aggresome disruption	Ricolinostat + LEN + DEX in R/R MM	Phase I/II NCT00773747
Ricolinostat Acetylon (USA)	Caspase 8/9-mediated apoptosis; terminal UPR induction; polyubiquitinated protein accumulation; aggresome disruption	Ricolinostat + LEN + DEX in R/R MM	Phase I/II NCT01583283
ACY-241 Acetylon (USA)	Downregulation of MYC and IRF4 proteins; inhibits aggresome formation	ACY-241 + POM + DEX vs ACY-241 alone in R/R MM	Phase Ia/Ib NCT02400242
Autophagy inhibitors			
Chloroquine New York University School of Medicine (USA)	Inhibits autophagy by inhibiting autophagosome and lysosome fusion	Chloroquine in combination with BTZ and cyclophosphamide in R/R MM	Phase I/II NCT01438177
3-MA NA	Inhibits autophagy at the level of PI3K class III	N/A	Preclinical PMID: 19648108 ⁹⁷
Bafilomycin A1 NA	Inhibits autophagy by inhibiting autophagosome and lysosome fusion	N/A	Preclinical PMID: 21174067 ³³

CDC25C indicates cell division cycle 25 homolog; DEX, dexamethasone; DUB, deubiquitinating enzyme, LEN, lenalidomide; MM, multiple myeloma; ND, newly diagnosed; PMID, PubMed; POM, pomalidomide; R/R, relapsed/refractory; UCHL5, ubiquitin C-terminal hydrolase L5; UPR, unfolded protein response; USP14, ubiquitin specific peptidase 14; CDC 25C, cell division cycle 25 homolog. *Table adapted from Bianchi et al (2015).

TABLE. Investigational Drugs Targeting Protein Synthesis/Degradation Pathways in Multiple Myeloma^a (continued)

Drug Name/Sponsor	Mechanism of Action	Study Design	Status Clinical Trials ID/PMID
Unfolded protein response			
Sunitinib (SU11248) National Cancer Institute (USA)	Inhibition of IRE1 kinase activity	Sunitinib in R/R MM	Phase II NCT00514137
Nelfinavir Swiss Group for Clinical Cancer Research (CHE)	Triggers UPR, inhibition of proteasome activity and p-AKT, synergizes with PI in both PI-sensitive and -resistant MM	Nelfinavir + LEN/DEX in progressive MM	Phase I/II NCT01555281
Lovastatin, zoledronic acid, digeranyl bisphosphonate NA	Inhibition of the isoprenoid biosynthetic pathway and Rab geranylgeranylation disrupts light chain trafficking, resulting in accumulations of light chain in the endoplasmic reticulum and activation of UPR	Nelfinavir + BTZ/DEX in resistant MM	Phase II NCT02188537
4μ8C NA	Inhibition of XBP1 mRNA splicing	N/A	Preclinical PMID: 22315414 ⁹⁹
MAL3-101 NA	Induction of XBP1 mRNA splicing	N/A	Preclinical PMID: 22750096 ¹⁰⁰
MKC-3946 NA	Inhibition of XBP1 mRNA splicing	N/A	Preclinical PMID: 14559994 ⁵¹
STF-083010 NA	Inhibition of XBP1 mRNA splicing	N/A	Preclinical PMID: 21081713 ⁸²
GSK2656157 NA	Inhibition of PERK and eIF2-phosphorylation, ATF4 translation and	N/A	Preclinical PMID: 23333938 ¹⁰¹
Heat shock protein inhibitors			
KW-2478 Kyowa Hakkō Kirin Pharma (Japan)	HSP90 inhibitor; apoptosis	KW-2478 in combination with BTZ in R/R MM	Phase II NCT01063907
NVP-AUY922 Novartis (Switzerland)	HSP90 inhibitor; apoptosis; downregulation of survival pathways	AUY922 +/- BTZ +/- DEX in R/R MM	Phase I/II NCT00708292
IPI-504 Infinity Pharmaceuticals (USA)	HSP90 inhibitor; inhibition of UPR	IPI-504 in R/R MM	Phase I NCT00113204
Tanespimycin (17-AAG) Bristol-Myers Squibb (USA)	HSP90 inhibitor; inhibition of downstream signaling pathways; induces UPR	Tanespimycin + BTZ in relapsed MM	Phase I NCT00546780
SNX-5422 Esanex (USA)	HSP90 inhibitor; apoptosis	SNX-5422 in refractory MM	Phase I NCT00595686
NVP-HSP990 NA	HSP90 inhibitor; induces apoptosis and cell cycle arrest	N/A	Preclinical PMID: 22309072 ¹⁰²
NVP-BEP800 NA	HSP90 inhibitor; apoptosis; inhibition of STAT3, ERK, and AKT pathways	N/A	Preclinical PMID: 21174067 ³³
SNX-2112 NA	HSP90 inhibitor; cell cycle arrest and cytotoxicity in MM; downregulation of AKT and ERK; inhibits angiogenesis and osteoclastogenesis	N/A	Preclinical PMID: 18948577 ¹⁰⁴
MAL3-101 NA	HSP70 inhibitor; induces apoptosis and cell cycle arrest in MM	N/A	Preclinical PMID: 21977030 ¹⁰⁵
PU-H71 NA	HSP90 inhibitor; induces cell cycle arrest, apoptosis, and UPR	N/A	Preclinical PMID: 20977755 ¹⁰⁶

BTZ indicates bortezomib; CHOP, cyclophosphamide, doxorubicin, vincristine, and prednisone; DEX, dexamethasone; HSP, heat shock protein; MM, multiple myeloma; PERK, protein kinase R-like ER kinase; R/R, relapsed/refractory; UPR, unfolded protein response; XBP1, X-box binding protein 1. ^aTable adapted from Bianchi et al (2015).

TABLE. Investigational Drugs Targeting Protein Synthesis/Degradation Pathways in Multiple Myeloma^a (continued)

Drug Name Sponsor	Mechanism of Action	Study Design	Status Clinical Trials ID/PMID
Protein degraders			
Phthalimide-conjugated degrader NA	Can be engineered to facilitate selective degradation of specific proteins by using phthalimide moiety to hijack the cereblon E3 ubiquitin ligase complex	N/A	Preclinical PMID: 25999370 ⁹⁴

MM indicates multiple myeloma; UPR, unfolded protein response. ^aTable adapted from Bianchi et al (2015).

Deubiquitinating Enzyme Inhibitors

The small-molecule DUB inhibitors RA190, P5091, and B-AP15 target RPN13, USP7, and USP14/UCHL5, respectively. In vitro, they induce proteotoxicity and apoptosis in MM without direct inhibition of catalytic subunits of the proteasome.⁶⁹⁻⁷¹ The USP14 inhibitor VLX1570 also demonstrated promising preclinical activity and is currently being evaluated in an early-phase clinical trial in MM.²⁸

Heat Shock Chaperone Protein Inhibitors

HSP90 is the most well-studied chaperone protein in MM. Several HSP90 inhibitors have completed phase I clinical studies (Table). Notably, among patients who were evaluable (n = 67), the combination of tanespimycin with bortezomib in R/R MM was associated with an ORR of 15%.⁷² However, modest activity and/or significant toxicity hampered clinical development of next-generation HSP90 inhibitors.⁷³

Histone Deacetylase Inhibitors

Panobinostat, in combination with bortezomib and dexamethasone, was recently approved as a third-line therapy in patients with MM with prior bortezomib and immunomodulatory drug (IMiD) exposure. Vorinostat is a class I and II HDAC inhibitor currently undergoing clinical trials. The phase III Vantage 008 trial reported improvements in ORR (54% vs 41%; $P < .0001$) and PFS (7.6 vs 6.8 months; $P = .10$) when vorinostat was added to bortezomib.⁷⁴ Although nonselective HDAC inhibitors show promising clinical activity, they are associated with significant adverse effects (particularly of a gastrointestinal and hematologic nature) due to the indiscriminate targeting of multiple HDACs, leading to extensive modulation of downstream histone and nonhistone protein functions.⁷⁵ Isoform-specific HDAC inhibitors, focusing on inhibition of HDAC6 and the aggresome pathway, have been developed in an effort to maintain efficacy and limit toxicities. Two promising HDAC6-selective inhibitors (ACY-241 and ACY-1215) are currently undergoing clinical trials. A phase Ib study of ricolinostat (ACY-1215) in combination with bortezomib/dexamethasone reported 45% and 25% ORRs in R/R MM and bortezomib-refractory MM, respectively, while ricolinostat in combination with lenalidomide/

dexamethasone had an ORR of 55% in R/R MM.^{76,77} BG45, an HDAC3-selective inhibitor, has also shown promising preclinical results, demonstrating anti-myeloma activity alone and in combination with bortezomib; translation to an early phase clinical trial is anticipated soon.⁷⁸

Unfolded Protein Response Modulators

Pharmacological induction of UPR via ER stressors, such as tunicamycin, thapsigargin, and brefeldin A, has proven to potentially synergize with a PI in vitro; however, clinical translation is limited by anticipated toxicities based on animal models.^{11,79-81} A more elegant way to exploit the UPR against MM hinges on identifying which of the tripartite signaling pathways and their downstream effectors are necessary for MM survival and proliferation. Such identification efforts were recently undertaken, revealing that single knockdown of each branch of the UPR has modest effects on MM viability at baseline; however, an intact IRE1-XBP1 pathway is required for bortezomib-mediated cytotoxicity.⁵⁹ Inhibition of either the endoribonuclease or kinase domains of IRE1 was found to have an anti-MM effect, especially combined with PI.^{82,83}

PERK inhibition has also recently emerged as a potential therapeutic option in MM. In preclinical studies, GSK2606414, a selective PERK inhibitor, was shown to synergistically enhance the apoptotic effect of bortezomib in MM.⁸⁴ Nelfinavir is an HIV-protease inhibitor that has demonstrated anti-MM activity through UPR induction, CHOP upregulation, poly (ADP-ribose) polymerase cleavage, and proteasome inhibition in preclinical studies.⁸⁵ Nelfinavir could also re-sensitize bortezomib-refractory primary MM cells towards bortezomib treatment.⁸⁵ A phase II trial of nelfinavir and bortezomib reported an ORR of 30% in the dose escalation cohort and an ORR of 50% in an exploratory extension cohort comprising patients with both bortezomib-refractory and lenalidomide-resistant MM.⁸⁶ No clinical-grade inhibitors of ATF6 have been reported at the time of writing.

Immunomodulatory Drugs

The clinical synergism between proteasome inhibitors and the IMiDs thalidomide, lenalidomide, and pomalidomide is well established.^{87,88} The molecular mechanisms of activity of these

compounds have long been obscure, and the anti-angiogenic effect was initially thought to be primarily responsible for their anti-MM activity.⁸⁹ However, the degradation of the transcription factors Ikaros (IKZF1) and Aiolos (IKZF3) was recently shown to be the base of the anti-MM effect of lenalidomide.^{90,91} In an unexpected twist, lenalidomide was shown to bind to the E3 ubiquitin-ligase complex made up of the damage-specific DNA-binding protein 1 (DDB1) and cereblon, enhancing its activity and facilitating ubiquitination and proteasome-mediated degradation of IKZF1 and IKZF3. IMiD-mediated stimulation of thymus and natural killer (NK) immunity similarly depends on the degradation of IKZF1 and IKZF3, resulting in IL-2 production in T lymphocytes. Based on these findings, the clinical synergism between bortezomib, a PI, and lenalidomide, a facilitator of proteasome-mediated IKZF1 and IKZF3 degradation, appears paradoxical and remains to be clarified at the cellular and molecular levels.

Conclusions and Future Directions

Although disrupting proteostasis via a PI has been successful in MM, innate or acquired resistance remains a major clinical challenge. Combination treatments have only partially overcome these issues, and progressive acquisition of resistance to multiple agents with each disease relapse is a well-known phenomenon in MM. Recent research efforts have focused on modulating other facets of protein homeostasis pathways (ie, aggresome, autophagy, UPR, DUB, HSP), with the goal of exacerbating proteotoxicity and overcoming MM drug resistance (**Figure 1**).

Recent insight into the mechanism of IMiDs has led to a novel therapeutic strategy (degronomid) that exploits the ability of IMiDs to redirect the cereblon E3 ubiquitin ligase complex toward specific proteins, thus targeting them for degradation.^{92,93} As a proof-of-concept, the phthalimide conjugate d-bromodomain and extra-terminal 1 was able to selectively induce cereblon-dependent BET protein degradation both in vitro and in mice (**Figure 1F**).⁹⁴ This ability to hijack the UPS to selectively degrade proteins that are otherwise considered undruggable (eg, MYC, β -catenin, and myeloid cell leukemia 1) could be a powerful tool in the treatment of MM and other malignancies.

In conclusion, the understanding of MM reliance on protein-handling pathways paved the way to therapeutically target this Achilles' heel by exacerbating baseline proteotoxic stress. In combination with IMiDs and immunotherapies, drugs targeting the protein synthesis/degradation machinery hold the key to achieving sustained remission, if not cure, in most MM patients.

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