Cereblon-Targeting Ligase Degraders in Myeloma

Mechanisms of Action and Resistance

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KEYWORDS

• IMiDs • CELMoDs • Thalidomide • Lenalidomide • Pomalidomide • Iberdomide • Mezigdomide

KEY POINTS

- Cereblon ligase degraders bind the adaptor cereblon (CRBN) of the CUL4-RING E3 ubiquitin ligase (CRL4) neomorphing its substrate bindings.
- Immunomodulatory imide drugs (IMiDs) and cereblon E3 ligase modulators (CELMoDs) promote the proteasomal degradation of two plasma cells essential transcription factors Ikaros and Aiolos, decommissioning myeloma active promoters and oncogenic enhancers they regulate and their targets (*MYC, IRF4, and so forth*).
- Resistance to IMiDs and CeLMoDs is largely mediated by mutations in CRBN and CRL4 subunits as well as transcriptional plasticity at the myeloma oncogenic enhancers.

INTRODUCTION

Cereblon-targeting degraders, such as immunomodulatory imide drugs (IMiDs) lenalidomide and pomalidomide and their derivative cereblon E3 ligase modulators (CELMoDs) iberdomide and mezigdomide, have exhibited clear anti-myeloma activity. The integration of this class of drugs into various therapeutic regimens and disease stages in multiple myeloma (MM) underscores their pivotal therapeutic role. Despite their pronounced anti-MM activity, it is worth noting that ∼ 5% to 10% of patients demonstrate primary refractoriness to lenalidomide and patients invariably develop resistance to this class of drugs. Consequently, an improved understanding of both the mechanisms of action and the mechanisms underlying their resistance is critical for the refining and development of novel therapeutic combinations with this class of drugs.

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MECHANISMS OF ACTION

Structural Basis of Immunomodulatory Imide Drugs and Cereblon E3 Ligase Modulators

Thalidomide, α-*N*-phthalimido-glutarimide, consists of two moieties, phthalimide and glutarimide, and serves as the foundational structure from which IMiDs as well as CELMoDs are derived. Although these molecules share the glutarimide moiety which is essential for binding to the adaptor protein cereblon (CRBN), each is distinguished by their phthaloyl ring variations which define their unique pharmacokinetic and pharmacodynamic properties, as summarized in **Fig. 1** and **Table 1**.

CUL4-RING E3 Ubiquitin Ligase Complex Assembly and Immunomodulatory Imide Drug/Cereblon E3 Ligase Modulator Binding

CRBN, in conjunction with the DNA damage-binding protein 1 (DDB1), cullin 4 (CUL4), and RING-box protein 1 (Roc1) assemble to form the CUL4-RING E3 ubiquitin ligase (CRL4) complex ($Cul4A^{CRBN}$), as depicted in **Figs. 2** and **3**. IMiDs and CELMoDs execute their cellular functions through engagement with CRBN[1] and neomorphing substrates binding to the CRL4 E3 ligase, rendering the substrates for polyubiquitination and degradation by the 26S proteasome.

The glutarimide moiety of IMiDs and CELMoDs engages into a shallow hydrophobic pocket of CRBN formed by three tryptophan residues (Trp380, Trp386, and Trp400) located within the thalidomide binding domain (TBD) of CRBNs C terminus (exons 10–11)[2] (**Fig. 4**). Both thalidomide and pomalidomide's glutarimide rings establish two hydrogen bonds with the peptide backbone of His378 and Trp380 of CRBN.

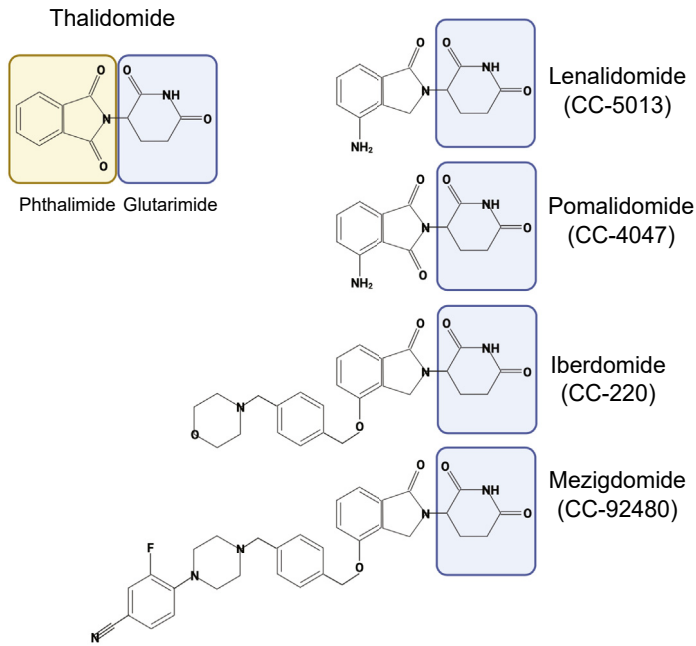

Fig. 1. Chemical structures of immunomodulatory drugs (IMiDs) and cereblon E3 ligase modulators (CELMoDs). The glutarimide ring (in *blue*) is present in all IMiDs and CELMoDs, whereas differences in the phthaloyl ring (in *yellow*) distinguish the distinctive pharmacokinetic and pharmacodynamic properties of each molecule.

Table 1
IMiD and CELMoD chemical structure and pharmacokinetics

	Lenalidomide (CC-5013)	Pomalidomide (CC-4047)	Iberdomide (CC-220)	Mezigdomide (CC-92480)
Molecular formula	$C_{13}H_{13}N_3O_3$	$C_{13}H_{11}N_3O_4$	$C_{25}H_{27}N_3O_5$	$C_{32}H_{30}FN_5O_4$
Molecular weight (g/mol)	259.3	273.2	449.5	567.6
Half-life (hours)	3–5	7.5–9.5	9–13	16–19
Renal dosing	Adjustments for CrCl <60 mL/min	Adjustments for severe renal impairment requiring dialysis	No available data	No available data
Liver metabolism	Minimal	Partially metabolized by CYP1A2, CYP3A4, substrate for p-glycoprotein CYP2C19, CYP2D6, CYP3A4	Primarily metabolized by CYP3A	Primarily liver metabolized

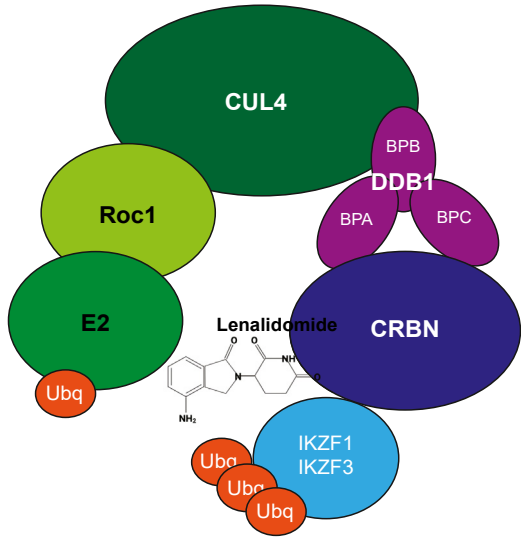

Fig. 2. CRBN E3 ligase complex ($Cul4A^{CRBN}$). The $Cul4A^{CRBN}$ complex is formed by CRBN, DNA damage-binding protein 1 (DDB1), cullin 4 (CUL4), and RING-box protein 1 (Roc1). Roc1 recruits E2 ubiquitin conjugating enzyme (E2). Lenalidomide (or other IMiDs/CELMoDs) binds CRBN and induces the recruitment of substrates (such as IKZF1 or IKZF3) which undergo ubiquitination (Ubq).

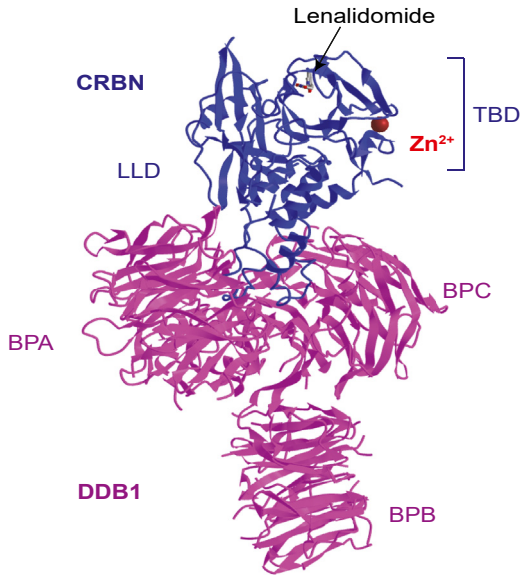

Fig. 3. Crystal structure of human Cereblon, CRBN, (in *blue*) in complex with DNA damage-binding protein 1, DDB1 (in *pink*), and lenalidomide. CRBN contains thalidomide binding domain (TBD) and LON-like domain (LLD). DDB1 consists of three domains including β-propeller A (BPA), β-propeller B (BPB), and β-propeller C (BPC). (Figure was adapted from crystal structure deposited in Protein Data Bank (PDB ID: 4TZ4) by Chamberlain et al. 2014 (Reference 12: Hansen JD, Correa M, Nagy MA, et al. Discovery of CRBN E3 Ligase Modulator CC-92480 for the Treatment of Relapsed and Refractory Multiple Myeloma. J Med Chem 2020;63(13):6648-6676).)

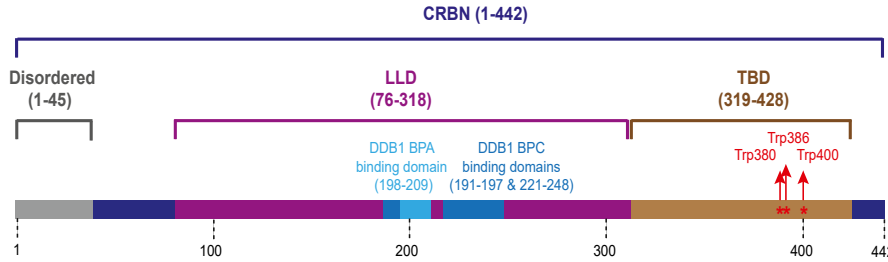

Fig. 4. Cereblon (CRBN) interaction domains. Depicted in this figure are the disordered domain, LON-like domain (LLD), and thalidomide binding domain (TBD). LLD contains DDB1 BPA and BPC binding domains. TBD domain contains three tryptophan residues (Trp 380, 386, and 400), which form the shallow hydrophobic pocket of CRBN.

Lenalidomide forms an additional third hydrogen bond with the side chain of His378 of CRBN.[2] On the docking of the glutarimide moiety to CRBN, the isoindolinone ring (of lenalidomide) or phthalimide ring (of pomalidomide) remains exposed on the surface of the CRBN complex. These structures retain the C4 amine with unquenched hydrogen molecules. This configuration contributes to defining target substrate specificity, influencing interactions with various substrates at the neomorphic interface.[2]

A cornerstone of IMIDs anti-MM activity hinges on CRBN E3 ligase dependent modulation of various transcriptional networks, effectively accomplished by the degradation of neo-substrates, including zinc finger (ZF) proteins IKZF1 (Ikaros) and IKZF3 (Aiolos), two canonical plasma cell transcription factors[3–10] with MYC and IRF4 as their downstream transcriptional targets.

Cereblon E3 Ligase Modulators Offer Enhanced Interaction, Binding Affinity, and Potency

CELMoDs, including iberdomide (CC-220) and mezigdomide (CC-92480), exhibit substantially greater potency by eliciting modulated CRBN substrate specificity compared with IMiDs. Of note, they have higher binding affinities to CRBN, as highlighted in **Table 2**, compared with those of lenalidomide and pomalidomide.[11,12]

Iberdomide, by its common glutarimide ring shared with IMIDs, interacts with CRBN. The molecule further integrates extensive modifications involving phenyl and morpholino moieties to the isoindolinone ring. These adaptations foster additional interactions with CRBN and/or substrates.[11] Indeed, iberdomide exhibits approximately 20-fold stronger affinity for CRBN compared with lenalidomide or pomalidomide, resulting in more efficient and rapid degradation of IKZF1 and IKZF3, as outlined in **Table 2**.[11] Iberdomide and mezigdomide exhibited varying degrees of IMiD resistance

Table 2
IMiD and CELMoD half-maximal inhibitory and effective concentrations

	Lenalidomide (CC-5013)	Pomalidomide (CC-4047)	Iberdomide (CC-220)	Mezigdomide (CC-92480)
CRBN binding (C50	1.5 μM	1.2 μM	0.06 μM	0.03 μM
CRBN closed conformation	–	20%	50%	100%
IKZF1 degradation EC50	67 nM	24 nM	1 nM	–
IKZF3 degradation EC50	87 nM	22 nM	0.5 nM	–

reversal in MM cell lines with reduced CRBN levels.[12,13] This CELMoDs improved binding affinity to CRBN stems from an extended area of contact between CRBN and the extended chemical moieties present in CELMoDs.[13] Cryo-electron microscopy-based structural analyses have characterized the allosteric modulation of CRBNs conformation, transitioning between open and closed states on IMiD or CELMoD binding.[14] This analysis revealed that mezigdomide extends its interaction to the TBD and the Lon domain of CRBN, leading to CRBNs closed conformation in 100% of the time, whereas pomalidomide primarily induces closure by binding to the TBD with only 20% of CRBN in closed confirmation. Mezigdomide and iberdomide's ability to stabilize the closed CRBN conformation without relying on the N-terminal belt contributes to their superior enhanced neosubstrate recruitment and therapeutic efficacy.[14]

Diverse Neo-Substrates Targeted by Immunomodulatory Imide Drug and Cereblon E3 Ligase Modulators

Recently, C-terminal cyclic imides, emerging from posttranslational modifications via intramolecular cyclization of glutamine or asparagine residues, were characterized to be the endogenous CRBN substrates.[15] IMiD or CELMoD-bound CRBN extends these substrates to IKZF1 and IKZF3 and differentially influences a broad spectrum of Cys_2-His_2 (C2H2) ZF proteins[16] such as ZFP91 that is uniquely targeted by pomalidomide, iberdomide, and mezigdomide, but not thalidomide or lenalidomide.[17,18] In addition to targeting shared neo-substrates, each IMiD and CELMoD confer distinct neo-substrate specificities as recently summarized in a review.[19] The spectrum of neo-substrates expands further to include substrates that lack ZF domains, such as casein kinase 1 alpha (CSNK1A1),[20,21] GSPT1,[22] and DTWD1.[18] CSNK1A1 degradation forms the basis of lenalidomide specific action in myelodysplastic syndrome with deletion of chromosome 5q.[20,21] The extent to which these substrates, beyond IKZF1 and IKZF3, contribute to IMiD and CELMoD anti-myeloma activity remains to be defined.

Altogether, the anti-MM effects of IMiDs and CelMoDs are largely linked to the integrity and expression levels of CRBN and the CRL4 E3 ligase subunits. Hence, resistance to these drugs in MM has been well documented in both primary plasma cells and cell lines that exhibit reduced or mutated CRBN.[7,23]

Additional Mechanisms of Action: Chaperon-Like Function, H_2O_2 Decomposition

Apart from its role within the E3 ligase complex, CRBN is reported to exhibit a chaperone-like function promoting the maturation and stabilization of CD147 and MCT1 proteins, which together form CD147-MCT1 transmembrane complex that supports malignant cells proliferation.[24] It has been proposed that IMiDs competitively interact with CRBN, disrupting and countering the CD147-MCT1 complex function, contributing to an additional anti-MM mechanism of IMiDs.[24] In a separate study, however, knockdown of CD147 and subsequent MCT1 downregulation did not correlate with MM cell viability or lenalidomide sensitivity.[25]

IMiDs also affect their anti-MM activity by inhibiting intracellular H_2O_2 decomposition within MM cells, with pomalidomide as the most potent agent.[26] MM cells with lower capacity for H_2O_2 decomposition were more susceptible to lenalidomide-induced cytotoxicity, regardless of CRBN protein expression levels. Lenalidomide was shown to reduce intracellular H_2O_2 decomposition, increasing intracellular oxidative stress. Elevated H_2O_2 contributed to the downregulation of IKZF1 and IKZF3 by directly attenuating their expression as well as precipitated and endoplasmic reticulum stress response with BH3 protein Bim activation and apoptotic cell death.

Immunomodulatory Effects of Immunomodulatory Imide Drugs and Cereblon E3 Ligase Modulators

IMiDs also exhibit a broad range of effects beyond their direct antitumor actions, influencing various aspects of the adaptive and innate immune system and the tumor microenvironment. IMiDs exert immunomodulatory activities by facilitating co-stimulation of CD4+ and CD8+ T cells through activation of CD28 axis, enabling them to bypass co-stimulation from antigen-presenting cell interactions. Furthermore, they enhance interleukin-2 (IL-2) and interferon gamma (IFNγ) production, while also boosting AP-1 transcriptional activity.[27–30] In T cells, IKZF1 and IKZF3 bind the promoter of IL-2 gene and repress gene transcription,[4,28,31–33] and therefore, the degradation of IKZF1 and IKZF3 by IMiDs further supports IL-2 expression.[4,34] Of note, pomalidomide and CELMoDs exhibit a more potent T-cell stimulatory effect compared with lenalidomide or thalidomide.[35]

Beyond T-cell activation, CRBN-dependent degraders contribute to the expansion and activation of NK and NK T cells, resulting in increased IFNγ production and enhanced antibody-dependent cellular cytotoxicity.[35–39] This activation of NK cells creates a positive feedback loop that promotes immune responses and the secretion of cytokines that further attract T cells and dendritic cells, ultimately enhancing local cellular immunity.[40] In addition, IMiDs have also been shown to restrain regulatory T-cell activity, further strengthening their immunomodulatory effects.[41]

Apart from the direct immunomodulation of T- and NK-cell functions, this class of drugs also modulate the bone marrow environment through reported anti-inflammatory properties[35] as well as antiangiogenic effects by inhibiting among others basic fibroblast growth factor and vascular endothelial growth factors.[42–45] In addition, IMiDs modulate the interaction between bone marrow stromal cells and plasma cells by downregulating adhesion molecules receptors and ligands reducing adhesion-mediated drug resistance. This attenuated MM cell–stromal interactions also result in reduced secretion of pro-survival cytokines, counteraction of osteoclastogenesis, and inhibition of IL-6 secretion from bone marrow stromal cells.[35,44,46–48]

MECHANISMS OF RESISTANCE TO IMMUNOMODULATORY IMIDE DRUGS AND CEREBLON E3 LIGASE MODULATORS

Resistance to CRBN-dependent degraders can be generally classified as CRBN or non-CRBN mediated. A summary of some of these reported mechanisms of resistance is depicted in **Fig. 5**.

Cereblon-Dependent Resistance Mechanisms

Cereblon, encoded by the CRBN gene on chromosome 3p26.2, is a key target of acquired resistance to IMiDs and CELMoDs in MM. Of note, genetic alterations within CRBN are detected in approximately 30% of patients on progression from IMiDs.[49] The frequency of missense or truncating mutations increases as patients progress from newly diagnosed-to lenalidomide- and pomalidomide-refractory MM with the incidence rates rising from 0.5% in newly diagnosed cases to 2.2% and 9% in lenalidomide and pomalidomide refractory patients, respectively. Furthermore, structural variations, encompassing copy number loss, inversions, or translocations, follow a similar pattern of escalation from newly diagnosed to refractory cases, with incidences of 1.5%, 7.9%, and 24%, respectively.[49] CRBN mutations often emerge as subclonal mutations and are observed more frequently with extended duration of IMiD therapy in the relapsed refractory setting.[50]

CRBN modifications can also result from alteration of CRBN transcripts through exon 10 splicing, which generates a stable protein that retains its interaction capacity

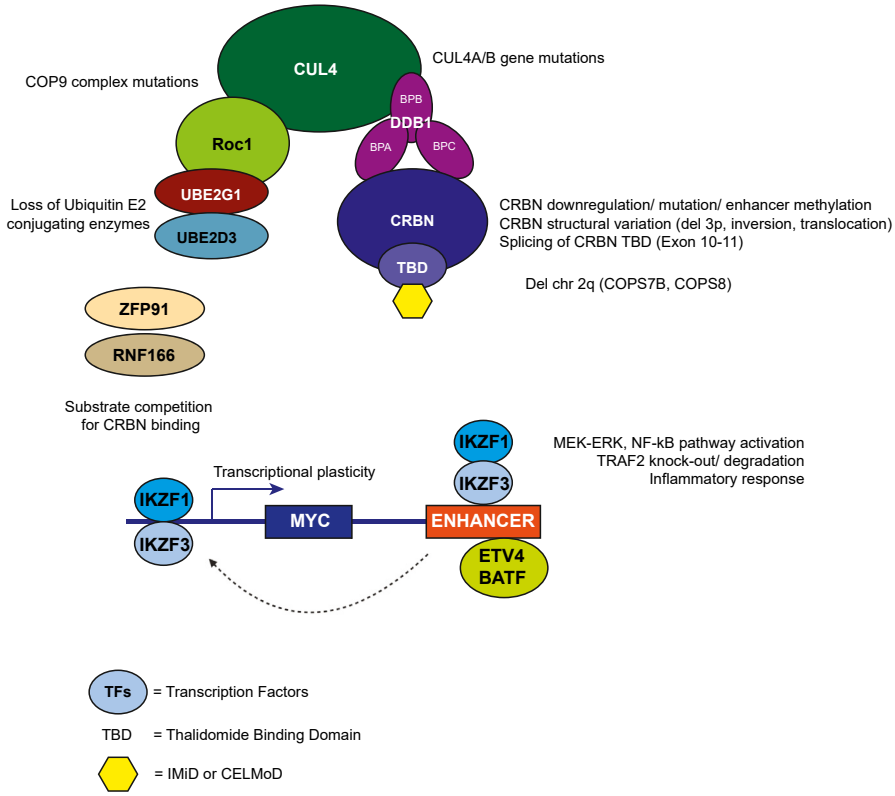

Fig. 5. Summary of mechanisms of resistance to CRBN-dependent degraders.

with DDB1 and CUL4A, but loses its ability to bind the glutarimide moiety of lenalidomide and pomalidomide.[51] The ratio between the exon 10 splice variant and the full-length transcript has been shown to correlate with the clinical response to IMiDs.[49,51] Tumors harboring CRBN exon-10 splice variants exhibit elevated TNF alpha signaling via NF-κB pathway activation, accompanied by a heightened inflammatory response characterized by elevated IL-1 and IL-10 signaling, and activation of transcriptional programs, including EZH2.[52] In this context, a potential for therapeutically leveraging molecular vulnerability arises, wherein EZH2 inhibitors could potentially address IMiD-resistant MM driven by the exon-10 splice variant of CRBN.[53]

Epigenetic regulation of CRBN was also reported.[54] DNA hypermethylation in an active intronic CRBN enhancer was observed in IMiD-refractory patients and correlated with decreased CRBN levels and in vitro DNA methyltransferase inhibitor sensitized myeloma cells to lenalidomide.

Posttranscriptional resistance mechanisms of resistance also reported competitive binding to CRBN or its substrates by other molecules hindering degradation of Ikaros and Aiolos.[55,56]

CRBN-INDEPENDENT RESISTANCE MECHANISMS

Transcriptional Plasticity

Oncogenic enhancers drive the aberrant expression of key drivers of myeloma cells survival and proliferation such as MYC and IRF4. Translocations involving the activation of

potent myeloma superenhancers such as IGLL5 on chr.22 were associated with decreased response to IMiDs.[57] Chromatin immunoprecipitation sequencing studies of IKZF1 revealed the mapping of this plasma cells essential transcription factors to the promoters and oncogenic enhancers and superenhancers of transcribed myeloma genes.[58,59] Although IMiDs efficiently deplete Ikaros and Aiolos from oncogenic myeloma enhancers in both IMiDs sensitive and resistant cell lines, they only displace of the acetyltransferase (EP300) and the bromodomain acetyl mark reader (BRD4) at these enhancers in sensitive cell lines. This retention of EP300/BRD4 at these enhancers was recently revealed to result from the co-occupancy of these myeloma enhancers by other transcription factors along IKZF1 and IKZF3. Such factors included, among others, the ETS family member ETV4[58] and the bZIP family member BATF.[59] Silencing of these factors indeed sensitized IMiDs resistant myeloma cell lines to lenalidomide or pomalidomide. Therapeutically, direct targeting of the acetyl transferase EP300 with small molecule inhibitors exhibited cytotoxicity toward myeloma cell lines and synergized with IMiDs.[59,60]

Mutations in COP9 Signasolome Complex

A CRISPR-based genome wide screens have demonstrated the importance of $Cul4A^{CRBN}$ complex regulators, including components of the COP9 signalosome, in driving IMIDs' anti-MM effectiveness.[61–64] UBE2M and COP9 signalosome complex constituents regulate $Cul4A^{CRBN}$ complex neddylation—the covalent addition of the ubiquitin-like activator NEDD8 protein—and deneddylation of the cullin backbone. This modulation dictates the ligase's active and inactivate states.[61,65] Altered neddylation of the CUL4A complex was shown to attenuate IMiD-induced $Cul4A^{CRBN}$ activity.[61] E2 ubiquitin-conjugating enzymes, UBE2D3, and UBE2G1 prime the neo-substrates of $Cul4A^{CRBN}$ by monoubiquitination, with subsequent polyubiquitination formed by UBE2G1 through lysine 48-linked ubiquitin chains.[61,66] Inactivation of the cullin-RING ligase regulators by targeting of COPS5, UBE2D3, and UBE2G1 led to IMiD resistance.[45] Of note, although UBE2G1 deficiency rendered MM cells resistant to lenalidomide and pomalidomide, iberdomide retained its activity.[66]

Copy number loss in chromosome 2q37, which carries vital COP9 signalosome constituents *COPS7B* and *COPS8*, is reported to be frequently observed as patients progress from initial MM diagnosis to the more refractory stages following lenalidomide and pomalidomide treatment.[67] The incidence rates were 5.5%, 10%, and 16.5%, respectively, underscoring the potential of this alteration as a predictive marker for clinical response to IMiDs.[67]

MEK-ERK Pathway Activation

An alternative facet of IMiD resistance, distinct from the CUL4A-CRBN-IKZF1/3 axis, involves TRAF2 downregulation,[68] which leads to the activation of the noncanonical NF-kB and ERK pathways, and culminates in the elevation of phosphorylated mitogen-activated protein kinase 1 or 2 (ERK1-2) levels and upstream mitogen-activated protein kinase kinase 1 (MEK1) activity. Of note, engagement of the ERK pathway is evident in nearly all (97%) cases of relapse post-lenalidomide maintenance.[68] TNF alpha, secreted by bone marrow stromal cells, lowers TRAF2 levels and attenuates the efficacy of IMiD-induced anti-MM cytotoxicity. TNF alpha mediates this effect, independent of CRBN expression, by the activation of both the ERK and noncanonical NF-kB pathways. Importantly, the bone marrow microenvironment contributes to IMiD resistance by secreting TNF alpha and IL-6, which directly fuels ERK signaling.[68] Collectively, this demonstrates that the presence of soluble factors, by orchestrating MEK-ERK activation, shields MM cells from IMiD effect within the bone marrow.[68]

Resistance within this context was overcome by MEK inhibitor, AZD6244, suggesting the potential use of MEK/ERK inhibitors for restoration of IMiD sensitivity.[68,69]

Other Reported CRBN-Independent Mechanisms of Resistance

In a genome wide screening using short hairpin RNA (shRNA) libraries, KPNB1 was identified as a critical factor for nuclear import of CRBN, and its deficiency led to resistance to pomalidomide by inhibiting nuclear import of CRBN and reducing pomalidomide-dependent degradation of IKZF3.[63] Disturbance in the subcellular localization of CRBN had significant impact on the effectiveness of CRBN modulators, underscoring the significance of "spatial overlap" between CRBN and its targets to ensure effective ubiquitination.[63]

Epigenetically an increase in genome-wide DNA methylation and reduction in chromatin accessibility were observed in MM cells lines derived to acquire resistance to lenalidomide and pomalidomide with SMAD3 downregulation. In these cells, simultaneous inhibition of DNA methyl transferases and EZH2 restored myeloma cells to sensitivity to IMiDs in a CRBN-independent mechanism.[70]

IL6 and STAT3 activation has also been implicated in IMiD resistance both in in vitro generated lenalidomide-resistant cell lines and validated in a comprehensive genomic data set of newly diagnosed MM patients, revealing a notable correlation between heightened IL6 and STAT3 expression and shorter treatment response.[25]

SUMMARY

CRBN-dependent degraders represent a remarkable class of drugs with a pleotropic mechanisms of action with direct anti-MM as well as immunomodulatory effects. Defining the mechanisms of action and resistance to these molecules allowed the development of next-generation CRBN-dependent degraders (iberdomide, mezigdomide, CFT7455) with enhanced activity and potency compared with first-generation IMiDs. Ongoing explorations of these molecules and the combination of CRBN-dependent degraders with other novel anti-myeloma therapeutics with further unravel the pandora box effects of this class of drugs.

CLINICS CARE POINTS

- Cereblon-targeting ligase degraders neomorphe the CRL4 E3 ubiquitin ligase substrates binding and degradation with two myeloma essential transcription factors IKZF1 and IKZF3. In addition, these molecules are potent inducers of T- and NK-cell activation and expansion.
- Mutations in *CRBN,* CRL4 E3 ligase, or the COP9 signalosome subunits as well as transcriptional plasticity are the major mediators of resistance to cereblon-targeting ligase degraders
- Therapeutically, among cereblon-targeting ligase degraders, novel molecules with enhanced binding affinity for CRBN and ability to retain it in a close confirmation, exhibit more potent anti-myeloma activity and in some instances overcome resistance to earlier generation of CRBN binders. Furthermore, epigenetic modifiers such as the acetyltransferase EP300 or BRD4 inhibitors display potent preclinical synergy with cereblon-targeting ligase degraders.
- Cereblon-degraders are potent activators of innate and adaptive immunity and hence represent ideal partners to T cells redirecting antibodies and chimeric antigen receptor engineered T cells in multiple myeloma.

DISCLOSURE

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