REVIEW ARTICLE

Resistance to immunomodulatory drugs in multiple myeloma: the cereblon pathway and beyond

Phaik Ju Teoh,[1-5*] Mun Yee Koh,[1*] Constantine Mitsiades,[3-5] Sarah Gooding[6-8] and Wee Joo Chng[1,2,9]

[1]Cancer Science Institute of Singapore, National University of Singapore, Singapore; [2]Department of Medicine, Yong Loo Lin School of Medicine, National University of Singapore, Singapore; [3]Division of Molecular and Cellular Oncology (MCO), Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA, USA; [4]Department of Medicine, Harvard Medical School, Boston, MA, USA; [5]Broad Institute of Massachusetts Institute of Technology (MIT) and Harvard, Cambridge, MA, USA; [6]Department of Haematology, Oxford University Hospitals NHS Foundation Trust, Oxford, UK; [7]Oxford Centre for Translational Myeloma Research, University of Oxford, Oxford, UK; [8]MRC Molecular Haematology Unit, Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, UK and [9]Division of Haematology, Department of Haematology-Oncology, National University Cancer Institute, Singapore (NCIS), National University Health System, Singapore

**PJT and MYK contributed equally as first authors.*

Correspondence: P.J. Teoh
mdctpju@nus.edu.sg
W.J. Chng
mdccwj@nus.edu.sg

Received: April 19, 2024.
Accepted: November 8, 2024.
Early view: November 21, 2024.

https://doi.org/10.3324/haematol.2024.285636

Abstract

Acquired resistance to immunomodulatory drugs (IMiD) remains a significant unmet need in the treatment landscape of multiple myeloma (MM). The cereblon (CRBN) pathway-dependent mechanisms are known to be vital contributors to IMiD resistance; however, they may account for only a small proportion. Recent research has unveiled additional mechanisms of acquired IMiD resistance that are independent of the CRBN pathway. In this review, we provide a comprehensive overview of the existing work on IMiD resistance in MM, focusing specifically on the emerging evidence of CRBN pathway-independent mechanisms. Finally, we discuss the plausible actionable strategies and outlook for IMiD-based therapies moving forward.

Introduction

Immunomodulatory drugs (IMiD) are a major class of drugs that have changed the treatment paradigm for multiple myeloma (MM). Thalidomide, the first-in-class IMiD, was introduced in the late 1950s as a sedative and anti-emetic during pregnancy, but was soon withdrawn due to neuropathy and teratogenicity.[1] However, a breakthrough study in 1999 reported promising efficacy in MM patients, leading to accelerated approval for MM treatment in May 2006[2,3] (Figure 1). This success spurred the development of thalidomide analogs, namely, lenalidomide and pomalidomide, to enhance therapeutic effectiveness while reducing toxicities. Lenalidomide received US Food and Drug Administration (FDA) approval in June 2006 for use in MM patients who have had at least one prior line of therapy.[4] Pomalidomide was granted FDA approval seven years later for relapsed / refractory MM patients (RRMM) who had undergone at least two prior therapies, including lenalidomide and bortezomib.[3] While thalidomide is now less prescribed, lenalidomide is widely used as the backbone of numerous combination treatments for newly diagnosed MM (NDMM), as post-transplant maintenance, and in RRMM, whereas pomalidomide is commonly used for treatment of RRMM, especially for lenalidomide-refractory patients.[5]

The clinical benefit of IMiD in MM is well established; however, their therapeutic efficacy and durability are significantly limited by primary and acquired drug resistance. In MM, approximately 5% of patients demonstrate primary resistance to IMiD, while those who initially responded to IMiD-based regimens eventually acquire resistance over time.[6-9] Moreover, recent analyses suggest efficacy and key subset differences depend on age, particularly in older patients where clinical benefit is more limited. There is, therefore, a significant unmet need in understanding the underlying mechanisms of resistance to IMiD.

Landmark studies have revealed that IMiD exert their activity by binding to a specific tri-tryptophan pocket of cereblon (CRBN), a substrate adaptor protein of the $CRL4^{CRBN}$ E3 ubiquitin ligase complex, which consists of DNA damage-binding

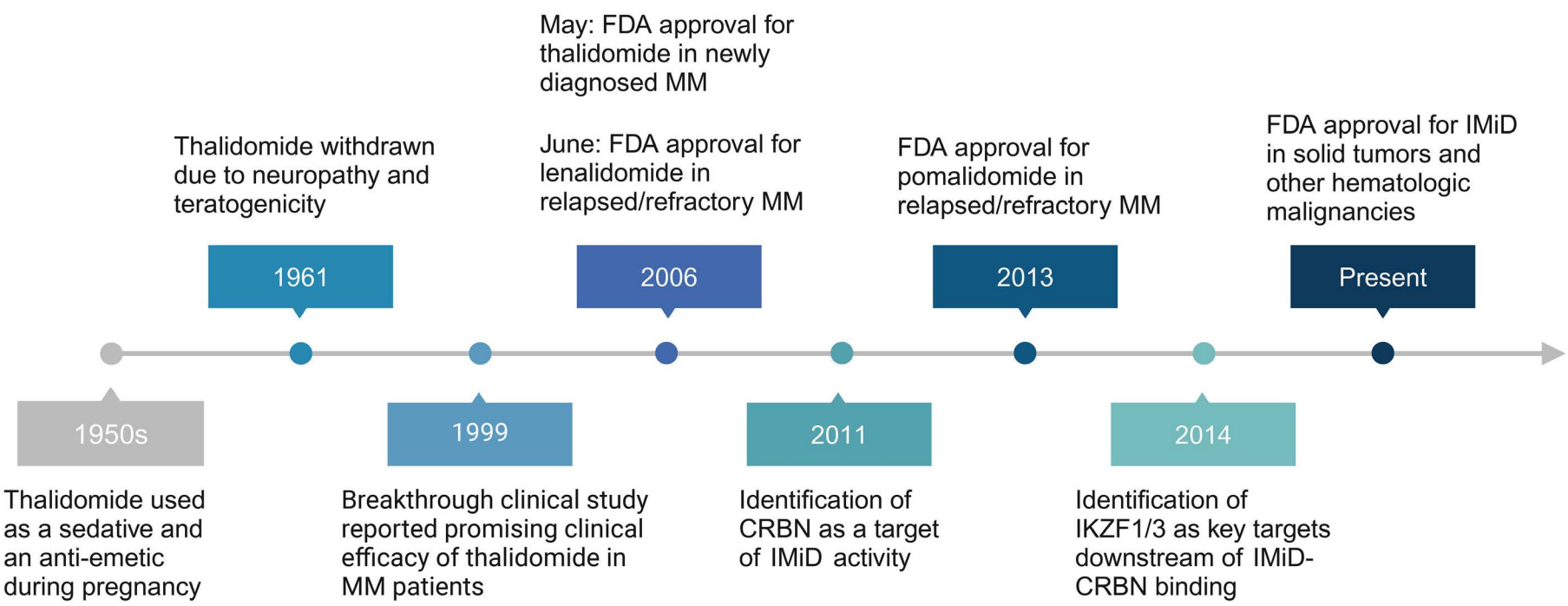

Figure 1. Timeline of the development of immunomodulatory drugs. The historical timeline of immunomodulatory drug (IMiD) development over the years from multiple myeloma (MM) to its therapeutic application in different malignancies. FDA: US Food and Drug Administration.

protein 1 (DDB1), cullin-4A/B (CUL4A/B), and regulator of cullins 1 (ROC1).[10,11]

Multiple studies on CRBN pathway abnormalities have facilitated our understanding of IMiD resistance.[12-14] Resistance mechanisms beyond the CRBN pathway, on the other hand, are gradually emerging, but their relative significance, and how one study relates to another, is not fully understood. In this current review, we explore in depth the evidence on CRBN pathway-independent mechanisms of IMiD resistance, dissect the details of the studies, and systematically describe the evidence based on how one may be supporting the findings of another. Additionally, we discuss areas of future research that may hold promise in advancing our understanding of IMiD resistance and propose plausible therapeutic strategies to overcome IMiD resistance in the clinic.

Mechanism of action of immunomodulatory drugs

By binding to CRBN, IMiD redirect the $CRL4^{CRBN}$ E3 ubiquitin ligase machinery to target and induce proteasomal degradation of a range of neo-substrates, including the transcription factors Ikaros and Aiolos, encoded by the *IKZF1* and *IKZF3* genes, respectively[10,11,15] (Figure 2A). Both *IKZF1* and *IKZF3* (*IKZF1*/3) are regulators of B-cell differentiation and were described to be essential genes in MM.[16,17] IMiD-induced-degradation of IKZF1/3 is, therefore, crucial for the anti-neoplastic effects in MM, mediating the cellular toxicity and/or induction of immunomodulatory responses (Figure 2B).

The direct anti-MM effect is mainly attributed to the downregulation of two MM essential genes, *IRF4* and *c-MYC*, causing disruption of their oncogenic drive and hence cytotoxicity.[18,19] At the microenvironment level, IMiD enhance immunomodulatory responses by: 1) promoting immune recognition through increased antigen presentation by dendritic cells; 2) increasing production of anti-tumor cytokines such as interferon-γ (IFN-γ) and interleukin-2 (IL-2) to drive T-cell expansion and natural killer (NK)-cell activation; 3) reducing adhesion molecules such as VCAM-1 and ICAM-1 on bone marrow stromal cells (BMSC) to impede tumor cell-BMSC interactions; 4) inhibiting immunosuppressive T-regulatory cells (Tregs); and 5) impairing signaling of angiogenic factors, vascular endothelial growth factors (VEGF) and fibroblast growth factors (FGF) in the BM niche.[20-22]

The distinct clinical efficacy of each thalidomide derivative reflects the differences in its CRBN binding affinity and the subtly different spectrum of neo-substrate degradation.[23] Thalidomide, lenalidomide, and pomalidomide share common phthalimide and glutarimide moieties, but all differ, respectively, in a carboxy and an amino group at the phthalimide ring (Figure 2C). These small but key structural variations lead to significant differences in clinical efficacy, with increased potency observed from thalidomide to lenalidomide and then to pomalidomide.[1] Compared with thalidomide, lenalidomide was documented to be 50-2,000 times more potent in inducing T-cell proliferation and 300-1,200 times more potent in augmenting T-cell activity due to increased IL-2 and IFNγ production. Pomalidomide is 10 times more efficient than its predecessor in stimulating T cell and inducing pro-inflammatory cytokines from Type 1 helper (Th1) cells, while reducing anti-inflammatory cytokines from Th2 cells.[24]

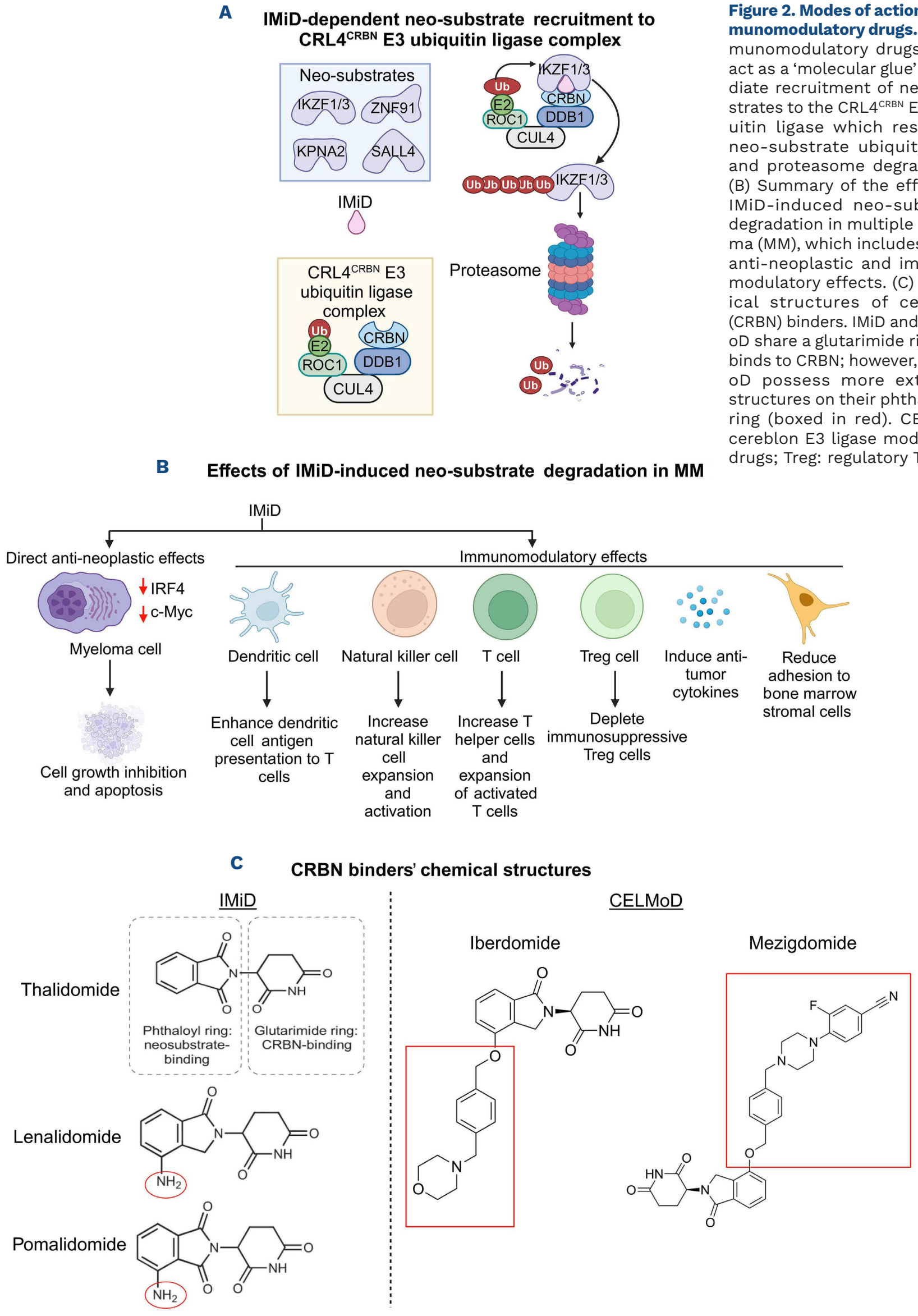

Figure 2. Modes of action of immunomodulatory drugs. (A) Immunomodulatory drugs (IMiD) act as a 'molecular glue' to mediate recruitment of neo-substrates to the CRL4^{CRBN} E3 ubiquitin ligase which results in neo-substrate ubiquitylation and proteasome degradation. (B) Summary of the effects of IMiD-induced neo-substrate degradation in multiple myeloma (MM), which includes direct anti-neoplastic and immunomodulatory effects. (C) Chemical structures of cereblon (CRBN) binders. IMiD and CELMoD share a glutarimide ring that binds to CRBN; however, CELMoD possess more extended structures on their phthalimide ring (boxed in red). CELMoD: cereblon E3 ligase modulatory drugs; Treg: regulatory T cell.

Immunomodulatory drug resistance associated with the cereblon pathway, and its paradox

Owing to the core function of CRBN in the activity of IMiD, disruption to the CRL4^{CRBN} E3 ubiquitin ligase components has been the most commonly reported mode of resistance to this group of drugs (Table 1). Here, we document the key findings from previous studies on the genomic and non-genomic abnormalities of CRBN and its pathway genes, and their association, or not, with patients' responses to IMiD.[25-28]

Genomic alterations in the CRBN gene that have been reported in MM patients include most commonly, single nucleotide variation (SNV) and copy number loss, while the non-genomic events involved epigenetic and transcriptomic aberrations that affect its stability and expression, including the abnormal exon 10 splicing.[25,26,29-31] Somatic SNV in *CRBN* are not frequent among newly diagnosed MM (<1%); however, their prevalence significantly increases to 9-12% of IMiD-refractory patients.[25,26] Genetic mapping identified that these SNV were predominantly located within the IMiD-binding domain,[26] and their ectopic introduction into MM cell lines

Table 1. Immunomodulatory drug resistance associated with cereblon pathway.

N	Study details	Key findings	Ref
1	Gene-expression profiling of MM patients (HOVON-65/GMMG-HD4 trial) on thalidomide maintenance (N=96)	Decreased *CRBN* gene expression was significantly associated with poorer PFS	37
2	qPCR of paired MM patients' samples at pre-treatment and at Len resistance (N=9)	20-90% reduction of CRBN expression in 8 patients and 2-fold increase in one patient	29
3	Targeted sequencing of paired MM patients' samples at pre- and post-Len treatment (N=25)	68% of post-Len patients showed reduced CRBN expression while 32% showed increased expression	39
4	Immunohistochemistry of paired MM patients' samples at diagnosis and at Len-refractory (N=55)	77% Len-refractory patients had reduced CRBN expression with a median decrease of 53.1% (range: 6.6-99.2%) whereas 23% patients had no decrease in CRBN expression IKZF1, IKZF3, and IRF4 protein expression: unchanged at Len-refractory c-Myc protein expression: slight increase at Len-refractory	31
5	WGS (N=455): newly diagnosed (N=198), Len-refractory (N=203), and Pom-refractory (N=54) cohorts RNA-seq: newly diagnosed (N=437), Len-refractory (N=176), and Pom-refractory (N=42)	Overall incidence of *CRBN* abnormalities: 20.7% of Len-refractory cases 29.6% of Pom-refractory cases Breakdown of *CRBN* abnormalities: *CRBN* mutations: newly diagnosed (0.5%), Len-refractory (2.2%), Pom-refractory (9%) *CRBN* gene copy loss: newly diagnosed (1.5%), Len-refractory (7.9%), Pom-refractory (24%) Exon 10 splicing: increased ratio of spliced transcript/full length from newly diagnosed to Len- and Pom-refractory patients	25
6	Targeted sequencing of IMiD-refractory patients (N=50)	Genomic mutations in *CRBN* (12%), *IKZF1* (2%), *IRF4* (4%), and *CUL4B* (6%)	26
7	WGS (N=522): newly diagnosed (N=198), Len-refractory (N=269), and Pom-refractory (N=55) cohorts	Mutation or copy loss in CSN members: *COPS3*: newly diagnosed (14%), Len-refractory (26%), Pom-refractory (22%) *COPS4*: newly diagnosed (10%), Len-refractory (14%), Pom-refractory (11%) *COPS5*: newly diagnosed (5%), Len-refractory (8%), Pom-refractory (3%) *COPS6*: newly diagnosed (1%), Len-refractory (2%), Pom-refractory (3%) *COPS7A*: newly diagnosed (17%), Len-refractory (20%), Pom-refractory (19%) *COPS7B*: newly diagnosed (6%), Len-refractory (12%), Pom-refractory (19%) *COPS8*: newly diagnosed (8%), Len-refractory (16%), Pom-refractory (22%) Mutation or copy loss in UBE2: *UBE2GD3*: newly diagnosed (5%), Len-refractory (9%) *UBE2G1*: newly diagnosed (14%), Len-refractory (28%), Pom-refractory (25%)	28

CRBN: cereblon; IMiD: immunomodulatory drugs; Len: lenalidomide; MM: multiple myeloma; N: number; PFS: progressionn-free survival; Pom: pomalidomide; qPCR: quantitative polymerase chain reaction; Ref: reference; WGS: whole-genome sequencing.

obliterated responses to lenalidomide.[26,32] There was also an increase in the frequency of CRBN copy number loss, from 1.5% in NDMM to 7.9% in lenalidomide (Len)-refractory and a significant 24% in pomalidomide (Pom)-refractory patients.[25] In addition, higher levels of alternative splicing of exon-10 in *CRBN*, which prevents IMiD-binding, has an incidence reaching up to 10% of Len-refractory patients, and has been consistent in predicting poor responses to IMiD.[25,33] These reports underscore the biological role of genomic and non-genomic lesions of *CRBN* in IMiD resistance. However, it is noteworthy that they represent only a small proportion of IMiD-refractory patients (up to 20% for lenalidomide and up to 30% for pomalidomide), suggesting that the majority of acquired IMiD resistance cases (i.e., >70-80%) are unaccounted for by *CRBN* abnormalities. Studies from smaller independent cohorts have not reported changes in the frequency of *CRBN* mutations in Len-refractory *versus* NDMM patients,[34-36] but are mainly small and/or limited to SNV detection.

Pre-clinical modeling of acquired IMiD-resistance demonstrated that resistant cell lines had depleted CRBN expression.[29] This is consistent with clinical observations, whereby high CRBN expression correlated with improved progression-free survival (PFS) in IMiD-treated-patients, while the IMiD-non-responders exhibited reduced CRBN expression.[30,37] However, it is also notable in other studies that CRBN levels were not predictive for IMiD responses.[34,38] Importantly, amongst all types of CRBN abnormalities described in IMiD-RRMM, there was no one mechanism that rendered a complete loss of CRBN expression.[25,29,31] For example, neither the cases with CRBN gene copy loss nor those with an aberrant exon 10 splicing demonstrated changes/reduction in CRBN expression compared to their counterparts without these aberrations.[25] More interestingly, a substantial proportion of the IMiD-RRMM (32%) paradoxically exhibited increased CRBN expression with no loss-of-function variant detected.[39] These data suggest that in the cases without genetic loss-of-function, the activity of the CRBN pathway was putatively retained in the IMiD-resistant MM cells. This supports the observation that len-refractory patients were responsive to subsequent pomalidomide treatment,[5,40,41] suggesting that low but intact CRBN expression does not abolish the functional $CRL4^{CRBN}$ E3 ubiquitin ligase activity and residual CRBN signaling may still mediate responsiveness to the more potent pomalidomide.

Investigations into CRBN axis genes have also yielded inconclusive results. For example, IMiD-refractory disease had an increased mutation frequency in *IKZF1*, *IRF4* and *CUL4B* compared to NDMM,[26] but another study showed no difference in the mutation status of *DDB1*, *CUL4A*, *CUL4B*, *IKZF1*, *IKZF2*, and *IKZF3*.[36] High IKZF1/3 expression has been associated with poorer PFS in lenalidomide-treated patients,[42] and, in contrasting data, with a favorable response to lenalidomide and better PFS.[43,44] In another patient cohort, IKZF1/3 protein levels were non-prognostic.[45] Further upstream in the CRBN pathway, COP9 signalosome (CSN) and E2 ubiquitin ligase proteins (UBE) are required for the maintenance of $CRL4^{CRBN}$ E3 ubiqitin ligase activity. Whole-genome sequencing (WGS) of MM patients (N=522) revealed increased incidence of copy number loss of chromosomal 2q37, the region containing CSN members (*COPS7B* and *COPS8*) in the lenalidomide-refractory, and lenalidomide-then-pomalidomide-refractory patients compared to the NDMM patients.[28] Importantly, however, the enrichment of this abnormality was again observed only in a small subset of the refractory patients (10-16%) and mutational analysis revealed low frequency for CSN and UBE members, implicating that once those with CRBN aberrations were also removed, >60% of IMiD-refractory patients are still unaccounted for by the aberrancy in these CRBN pathway proteins.[28]

In summary, CRBN pathway abnormalities are not a universal event in IMiD-refractory patients and the mechanism of resistance is likely to extend beyond this canonical IMiD-pathway.

Immunomodulatory drug resistance independent of the cereblon pathway

Supporting this notion, evidence on IMiD resistance mechanisms independent of the CRBN pathway are gradually emerging. These have been described to include myeloma cell-intrinsic factors and myeloma cell-extrinsic factors (Table 2).

Intrinsic mechanisms

The main myeloma cell-intrinsic mechanisms of IMiD resistance in MM involve the dysregulation of various oncogenic mediators, including known MM essential genes and other under-studied MM drivers. Some of these studies reported direct mechanistic evidence, while others showed clinical association without clear mechanisms (Figure 3).

Interleukin-6 (IL-6)/STAT3 signaling, a crucial MM driver, was found to be up-regulated in an acquired lenalidomide resistant MM cell line, XG1 (XG1-LenRes).[46,47] The authors identified autocrine production of IL6 in the XG1-LenRes, which was further enhanced in the presence of lenalidomide treatment. Stimulation of parental-XG1 cells with IL6 induced lenalidomide resistance, whereas inhibition of STAT3 with a selective STAT3 inhibitor (PB-1-102) re-sensitized its isogenic resistant counterpart to lenalidomide. Constitutive IL6/STAT3 activity in XG1-Len-resistant (LenRes) was associated with sustained expression of IRF4. Notably, XG1-LenRes did not have any accompanying abnormalities in CRBN and its downstream components. There was no change in CRBN expression, and neither were there any differential effects on lenalidomide-induced IKZF1/3 degradation. In agreement with this, introduction of exogenous CRBN also

Table 2. Cereblon pathway-independent evidence in Immunomodulatory drug resistance.

N	CRBN pathway-independent mechanisms/features	Type of IMiD	Study model/cohort	Key findings	Ref
Intrinsic mechanisms					
1	IL-6/STAT3 pathway	Len	aCGH, mRNA-seq, (XG1 parental *vs.* acquired-Len-resistance)	High IL-6/STAT3 signaling led to sustained expression of IRF4 in Len-resistant cells Introduction of exogenous CRBN failed to restore Len sensitivity	47
2	Wnt/β-catenin signaling	Len	Affymetrix microarray GEP	Increased Wnt/β-catenin activity: resulted in upregulation of Myc	48
			Len-refractory (N=26) *versus* IMiD-naïve (N=13)	Significant increase in CD44: (downstream target of Wnt/β-catenin) surface expression in Len-refractory patients	49
3	MEK/ERK pathway	Len; Pom	Mouse xenograft model	Len- or Pom-resistant plasmacytomas showed robust stabilization of pERK1/2 compared to responsive tumors	53
		Len; Pom	Genome-wide CRISPR-Cas9 KO screen	TNF-α and IL-6 in the BM milieu induced proteasome degradation of TRAF2 and activation of the MEK/ERK and NF-κB pathways *TRAF2* knockout showed no effect on CRBN expression and degradation of IKZF1/3 and IRF4	55
			IHC of newly diagnosed *versus* refractory to single-agent Len maintenance therapy (N=6)	Lower expression of TRAF2 protein at the time of relapse	
		Len	RNA-seq of MM patients (N=69) at first relapse	Nearly universal ERK pathway activation at relapse on Len maintenance therapy	
4	Epigenetic alterations	Len; Pom	Genome-wide methylation array; RNA-seq	Acquired Len- and Pom-resistant-OPM2 and -H929 displayed global increased DNA methylation, and reduced chromatin accessibility and gene expression with SMAD3 being commonly down-regulated IMiD-resistant cell lines No alteration in chromatin accessibility or DNA methylation profile of *CRBN*, *IKZF1*, *IKZF3*, and *IRF4*	57
		Len	ChIP-seq in 16 MM cell lines: Len-resistant (AMO1, JJN3, KMS-12-BM, SKMM2, XG1, XG5, XG7, XG12, XG20, and XG21) *versus* Len-sensitive (OPM2, RPMI8226, XG2, XG6, XG13, and XG19)	Len-resistant cells displayed mixture of enrichment/depletion of the active H3K4me3 marks Differential H3K4me3 sites enriched in Len-resistant HMCL were associated to interferon signaling and cytokine signaling *CUL4B* gene, but not other CRBN complex genes, in Len-sensitive cells was enriched with H3K4me3 mark	60
		Len; Pom	WES	Continuous IMiD treatment induced loss-of-function mutation and downregulation of NCOR2 leading to MYC upregulation, via increased CD180 expression, independent of CRBN mechanisms	61
5	IKZF1/3 transcription factor redundancy for maintenance of c-MYC and IRF4 expression	Len; Pom	RNA-seq, ATAC-Seq, ChIP-seq	92% of the Pom-treated MM cell lines remained highly viable despite significant downregulation of IKZF1/3 Several inherently IMiD-resistant cell lines expressed high levels of the AP-1 factor BATF BATF heterodimerization sustained IRF4 expression, compensating for IMiD-induced loss of IKZF1/3	62

Continued on following page.

N	CRBN pathway-independent mechanisms/features	Type of IMiD	Study model/cohort	Key findings	Ref
5	IKZF1/3 transcription factor redundancy for maintenance of c-MYC and IRF4 expression	Len; Pom	Paired newly diagnosed *versus* RRMM patients treated with IMiD (N=35)	Significant upregulation of BAFT upon relapse	62
			MMRF CoMMpass dataset (N=484)	BATF expression is associated with poorer survival outcomes	
		Len	ChIP-seq, ATAC-seq, RNA-seq	ETV4 bound to the same enhancers as IKZF1 ETV4 maintained BRD4 and P300 occupancy and oncogenic enhancer function to compensate for the IMiD-mediated loss of IKZF1/3 in IMiD-resistant cells to drive MYC overexpression	63
			MMRF CoMMpass (36 paired samples) and POLLUX (14 paired samples)	*ETV4* expression was associated with poorer PFS and overall survival *ETV4* was significantly up-regulated at the time of relapse No change in the expression of *IKZF1*, *IKZF3*, *IRF4*, or *MYC*	
6	High-risk MM markers: gain/amp(1q21) and del(17p)	Len; Pom	Longitudinal genomic analysis of relapsed MM patients (N=386)	Significant increasing trend of gain/amp(1q21) and del(17p) in IMiD-refractory MM cases	65, 66
			MMRF CoMMpass dataset (N=730)	High ADAR1 and hyperedited transcriptome in 1q21(gain/amp) is associated with reduced response to IMiD	67
7	Differential expression pattern of genome-wide circRNAs	Len; Pom	RNA-sequencing	More up-regulated than down-regulated circRNA in IMiD-resistant *versus* -sensitive cells Major overlap in the specific circRNA up-regulated upon acquired Len and Pom resistance	70
8	CDK6 activity	Len; Pom	Proteomics and RNA sequencing (pre-treatment and relapsed patient samples)	CDK6: Up-regulated in IMiD- based-RRMM patients CRBN: No change in RNA, protein or phosphorylation levels No genetic alterations in the $CRL4^{CRBN}$ E3 ligase complex	71
9	Modulation of cell surface expression	Len	RRMM (N=15) *versus* newly diagnosed (N=90)	Loss of CD138 surface expression: significant increase in CD138-negative cells in RRMM patients *ex vivo* cultured Len-RRMM cells from a single patient had low CD138 expression that was associated with an immature phenotype, downregulation of IRF4 and increased BCL6 expression	73
		Len	Glycoprotein CSC proteomics analysis	Acquired lenalidomide-resistant-OPM2 and -H929 showed increased CD33 and CD45/PTPRC expression	75
			CoMMpass dataset (IA14): paired diagnosis-first-relapse samples (Len-based induction regimens)	*CD33* and *PTPRC* mRNA expression was significantly increased at first relapse	
Extrinsic mechanisms					
1	Tumor-TME crosstalk	Len	WGS MANHATTAN clinical trial (N=49)	Failed MRD negativity was associated with shorter PFS, high APOBEC mutational activity, 1p22 (RPL5) deletions, *IKZF3* loss, low $CD14^{+}$ monocyte, T-cell exhaustion, persistence of IFNγ-expressing NK cells, and decreased TCR diversity	81
2	Exhausted T-cell phenotype	Len	Immune-profiling on Len-resistant MM patients	Significant expansion of exhausted effector T-cell populations expressing elevated levels of LAG3 and PD-1	83

Continued on following page.

N	CRBN pathway-independent mechanisms/features	Type of IMiD	Study model/cohort	Key findings	Ref
Extrinsic mechanisms					
3	Downregulation of chemokine, CCL20	Len	Illumina Gene Expression microarray	Downregulation of CCL20 in U266 Len-resistant cells and in Len relapsed/refractory MM cases (N=5). Addition of CCL20 increases MM cell sensitivity to Len	85
4	Elevated lactate level in the TME	Len	Metabolomics	High-risk MM t(4;14) demonstrated elevated plasma lactate levels Lactate is a differential metabolite associated with PKCα Knockdown of PKCα increases the sensitivity to Len, independent of the CRBN-IKZF1/3 axis	86
5	Increased EV secretion and enhanced adherence abilities	Len	RNA-seq	Increased expression of SORT1 and LAMP2, core regulatory genes governing EV secretion in Len-resistant cells Knockdown of SORT1 or LAMP2 reduced EV secretion, decreased cell adhesion and restored Len sensitivity in resistant cells without affecting CRBN expression	89
			Analysis of GSE19784 (N=300) and GSE136324 (N>200)	High SORT1 and LAMP2 expression are associated with poor survival in patients treated with Len	

aCGH: array comparative genome hybridization; ATAC-Seq: assay for transposase-accessible chromatin with sequencing; BM: bone marrow; ChIP-seq: chromatin immunoprecipitation sequencing; CircRNA: circular RNA; CRBN: cereblon; CSC: cell surface capture; EV: extracellular vesicle; GEP: gene expression profiling; IFN: interferon; IMiD: immunomodulatory drugs; Len: lenalidomide; MMRF: Multiple Myeloma Research Foundation; MRD: minimal residual disease; NK: natural killer; Pom: pomalidomide; PFS: progression-free survival; Ref: reference; RRMM: relapsed/refractory multiple myeloma; TME: tumor microenvironment; WGS: whole-genome sequencing.

failed to restore lenalidomide sensitivity in XG1-LenRes,[47] indicating the involvement of CRBN pathway-independent mechanisms.

Dysregulation of another oncogenic pathway, the Wnt/β-catenin, was also observed in LenRes MM cells. Through gene expression profiling of lenalidomide-resistant-U266, -ANBL-6, -KAS-6 and -MM1.S *versus* their parental cells, several Wnt/β-catenin intermediates (Wnt-3, Fzd-4, β-catenin) were found to be up-regulated. This increase in Wnt/β-catenin activity led to stabilization of cytoplasmic β-catenin and upregulation of MM drivers CyclinD1 and c-Myc. Knocking down β-catenin, in turn, restored MM cell sensitivity to lenalidomide.[48] Another report showed that CD44, a downstream transcriptional target of β-catenin, was also associated with IMiD resistance. The authors found that increased CD44 expression in the LenRes cells enhanced MM cell adhesion to BMSC to promote cell survival. Inhibition of β-catenin, and consequently CD44, with all-trans retinoic acid (ATRA) successfully re-sensitized resistant MM cells to lenalidomide.[49]

The main players in the oncogenic MAPK pathway such as *NRAS*, *KRAS* and, to a lesser extent *BRAF*, are the most frequently mutated genes in MM patients, with NDMM and RRMM cases bearing a high 20-50% and 45-80% frequency, respectively.[26,50-52] BRAF/KRAS/NRAS are upstream mediators of the mitogen-activated protein kinase (MEK)/extracellular signal-regulated kinase (ERK) kinases and activating mutations of *BRAF/KRAS/NRAS* genes trigger these kinases to up-regulate a series of proliferative and cell cycle signals.[51] A mouse xenograft study bearing MM1.S plasmacytomas with acquired IMiD resistance showed that resistance onset was accompanied by hyperactivity of the MEK1/ERK pathway (increased pMEK1/2 and pERK1/2). The addition of selumetinib, a small-molecule MEK inhibitor, effectively reinstated IMiD sensitivity, both *in vivo* and *ex vivo*,[53] hence suggesting the role of the BRAF/KRAS/NRAS /MEK/ERK signaling cascade in mediating IMiD resistance. Nevertheless, it should be noted that *BRAF/KRAS/NRAS* gene mutations are a general predictor of poor clinical outcome and are observed widely in all RRMM states.[50] There is a diverse range of SNV reported[54] and the functional impact of these different *BRAF/KRAS/NRAS* point mutations on the activation of MEK/ERK pathway leading specifically to IMiD resistance requires more study.

The biological role of MEK/ERK signaling was further demonstrated in a genome-wide CRISPR-Cas9 knockout screen in pomalidomide-treated MM.1s cells, in which TNF receptor-associated factor 2 (*TRAF2*) appeared as a modulator of resistance.[55] In cells co-cultured with BMSC or BMSC supernatants, the authors identified that IL-6 directly activates MEK/ERK signaling while triggering proteasomal degradation of *TRAF2* to stimulate nuclear factor κB (NF-

κB) and ERK signaling. MM1.S cells with *TRAF2* knockout exhibited significant resistance to lenalidomide and pomalidomide, alongside activation of NF-κB and MEK/ERK pathways, independently of the CRBN-IKZF1/3 axis. Consistent with the former study, inhibition of MEK with selumetinib effectively overcame IMiD resistance in *TRAF2* knockout MM cells; however, the authors also identified that *TRAF2* knockout conferred higher resistance to dexamethasone and melphalan treatments, indicating that *TRAF2* knockout-induced drug resistance may not be specific to IMiD.[55] Epigenetic alterations are widely implicated in cancer drug resistance. In the case of IMiD in MM: 1) a global increase in DNA methylation with 2) a reciprocal decrease in chromatin accessibility, and 3) a dominance of gene downregulation,

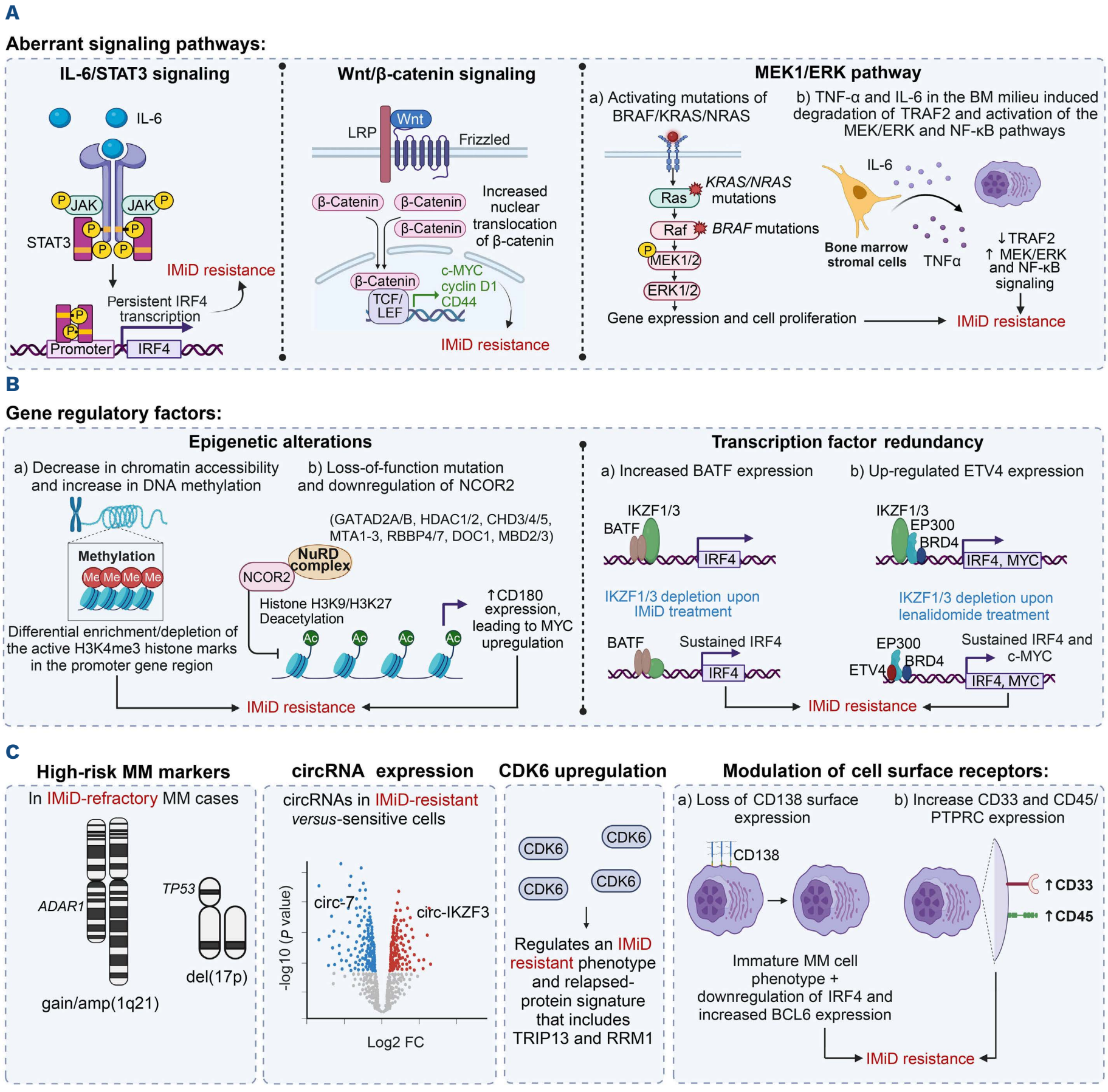

Figure 3. Intrinsic cereblon-independent immunomodulatory drug resistance mechanisms. Intrinsic factors that contribute to immunomodulatory drug (IMiD) resistance beyond the cereblon (CRBN) pathway include (A) aberrant activation of signaling pathways (such as IL-6/STAT3, Wnt/β-catenin and MEK/ERK), (B) epigenetic alterations and transcription factor redundancy, and (C) high-risk prognostic markers (such as gain/amp(1p21) and del(17p)), differential expression of circRNA, CDK6 overexpression, and dysregulated cell surface receptors. BM: bone marrow; MM: multiple myeloma.

were observed in acquired IMiD-resistant-OPM2 and -H929, with the main components of the CRBN pathway (*CRBN*, *IKZF1/3*, and *IRF4*) being unaffected. This is consistent with reports that promoter silencing of CRBN and its pathway genes were not associated with CRBN pathway deficiency and IMiD resistance.[39,56,57] In this study, the authors, instead, identified SMAD3 (a transcription factor and cell signaling regulator) as the novel gene commonly down-regulated in the resistant counterpart of both the cell lines. Treatment with a combination of 5-azacytidine (Aza) and the EZH2 inhibitor (EPZ-6438) reverted chromatin repression, increased SMAD3 expression, and ultimately re-sensitized the resistant cells to IMiD.[57] In support of this pre-clinical finding, Kalff *et al*. have reported some clinical efficacy in combining oral Aza with lenalidomide-dexamethasone (Rd) in heavily treated LEN-resistant RRMM patients (overall response rate [ORR] 37.5%, clinical benefit rate 50%).[58] Although Khouri *et al.*'s Rd-Aza (subcutaneous) treatment protocol in another patient cohort yielded a lower response rate (ORR 22%, clinical benefit rate 32%), the authors propose that Rd-Aza may overcome some IMiD refractoriness with careful regime optimization and correct patient selection.[59]
Further evidence of epigenetic involvement in IMiD resistance was demonstrated in a chromatin immunoprecipitation sequencing (ChIP-seq) study across 16 MM cell lines, comparing LenRes *versus* len-sensitive cells.[60] At the gene specific level, the authors reported that in the lenalidomide-resistant cells, the promoter regions of *ANKRD30B* and *SLAMF6* exhibited the highest occupancy of the active H3K4me3 mark, while the promoter regions of *GPR15* and *NKX6-1* demonstrated a marked depletion. Among the CRBN pathway genes, only *CUL4B* displayed enriched H3K4me3 at its promoter region in the len-sensitive cells. Nonetheless, the underlying mechanism by which IMiD induce epigenetic reprogramming, and the extent to which changes in epigenetics contribute to IMiD loss of function (LOF), was not described, and this represents an essential area of study as work moves forward.
Furthermore, NCOR2, an epigenetic remodeling gene, has been implicated in multi-drug resistance in MM, including to IMiD.[61] The authors identified that NCOR2 was interacting with nucleosome remodeling and deacetylase (NuRD) complex, to repress the expression of CD180 by directly binding to its promoter and that it resulted in the down-regulation of MYC. They showed in IMiD-resistant cells that low NCOR2 and CD180 expression was associated with increased MYC expression. There was no change in CRBN and IKZF1 expression, and thus they concluded that high MYC in IMiD-resistance was induced by deregulation of the NCOR2-CD180 pathway, independently of CRBN. It is noteworthy that, in this study, NCOR2 knockout also led to resistance to BET and HDAC inhibitors; thus, the therapeutic implication of loss of NCOR2 in MM was not specific to IMiD.
A more specific epigenetic dissection of IMiD resistance has been recently reported.[62] Out of 48 MM cell lines challenged with pomalidomide, 44 (92%) remained viable, despite significant depletion of IKZF1/3. These MM cells displayed high growth rate with most of the IMiD-resistant cell lines retaining high levels of MYC and IRF4 expression.[62] Further investigations using an assay for transposase-accessible chromatin with sequencing (ATAC-Seq) revealed reduced chromatin accessibility for IKZF1-binding in the pomalidomide- *versus* DMSO-treated cells. Notably, the sites that lost chromatin accessibility for IKZF1 were enriched for BATF, IRF4 and FOX bHLH binding motifs. RNA-sequencing of 66 MM cell lines revealed that the inherently IMiD-resistant cell lines (e.g., KMS-12BM, RPMI-8226) expressed high levels of BATF. They identified that BATF heterodimerization was compensating for the IMiD-induced loss of IKZF1/3 to sustain IRF4 overexpression, ultimately leading to IMiD resistance. In the analysis of paired NDMM and RRMM patients treated with IMiD (N=35), a significant upregulation of BATF upon relapse was observed. Cross-referencing these findings in the CoMMpass dataset, the authors identified that high BATF expression did indeed confer poorer survival outcome (N=484) in IMiD-treated patients.[62]
IKZF1/3 redundancy in IMiD responses was also highlighted in a complementary study whereby two other factors, EP300 and BRD4, compensated for the IMiD-mediated loss of IKZF1/3.[63] On ChIP-seq analysis, half the chromatin-bound IKZF1/3 sites overlapped with EP300 and BRD4 binding sites. While lenalidomide universally depleted chromatin-bound IKZF1 in both IMiD-sensitive MM1.S and IMiD-resistant RPMI-8226 cells, the IMiD-resistant MM cells maintained BRD4 and P300 super-enhancer occupancy. Further investigation revealed that this was acting through transcription factor *ETV4*, which co-binds the enhancers with IKZF1 to induce IMiD resistance. They also reported that *ETV4* expression was associated with poorer PFS and overall survival (OS) for CoMMpass patients treated with IMiD, and for POLLUX (NCT02076009) patients treated with Rd. Analysis of 36 paired CoMMpass patients and 14 paired POLLUX patients showed that *ETV4* was significantly up-regulated at relapse, whereas no change was observed in the expression of *IKZF1*, *IKZF3*, *IRF4* or *MYC*.[63] Alongside the immediate former publication,[62] this work identifies that CRBN-mediated-degradation of IKZF1/3 can be bypassed in sustaining the oncogenic IRF4-MYC axis to drive IMiD resistance.
High-risk MM markers, specifically t(4;14), t(14;16), del(17p), and gain/amp(1q21), have been associated with early relapses following IMiD-based therapy.[64] A longitudinal genomic analysis of RRMM patients (N=386) highlighted the enrichment of gain/amp(1q21) and del(17p) in IMiD-refractory cases, underscoring the potential impact of genes up-regulated in chr1q and deleted in chr17p on IMiD responses.[65] In agreement with this, the Myeloma XI trial (N=556) revealed in their multivariate analysis that isolated gain(1q21) and double-hit cases (defined as two concomitant high-risk

features) derived no survival benefit from lenalidomide maintenance.[66] We note, however, that 1q21(gain/amp) and 17p13(del) are poor predictive markers for a broad range of MM therapeutics, and, therefore, interactions between mechanisms specific to IMiD *versus* general drivers of resistance / early relapse need to be further interrogated. Notably, adenosine deaminase acting on RNA (ADAR1), encoding an RNA editing enzyme, is located in the amplified chr1q21 region. Our work has shown a close association between high ADAR1 expression and hyperedited MM transcriptome with reduced responsiveness to IMiD,[67] implicating the involvement of aberrant RNA editing in the mechanism of IMiD resistance. Another study has also shown that ADAR1-mediated editing of glioma-associated oncogene homolog 1 (GLI1), a Hedgehog pathway transcriptional activator and self-agonist, promotes malignant regeneration and IMiD resistance in MM.[68] In view of the growing interest and the biological relevance of RNA abnormalities in MM, our team is currently investigating the mechanism by which ADAR1 and its aberrant activity regulate IMiD responses in MM. We have identified a novel mechanism involving the ADAR1-regulated-dsRNA sensing pathway in modulating IMiD resistance.[69]

Further evidence of RNA-related aberrations in IMiD resistance was reported in a recent circular RNA (circRNA) profiling study.[70] Totals of 200 and 277 differentially expressed circRNA were observed, in H929-LenRes and H929-Pom-resistant cells, respectively, compared to their sensitive counterpart. The authors identified ciRS-7 to be consistently down-regulated, while circIKZF3 was commonly up-regulated in the lenalidomide- and pomalidomide-resistant cells. The depletion of ciRS-7 correlated with increased methylation levels of the promoter CpG island of its host gene, *LINC00632*. Combination treatment of an EZH2 inhibitor (EPZ-6438) and a DNA methyl transferase inhibitor (5-azacytidine) partially restored the expression of LINC00632 and ciRS-7 and the IMiD sensitivity of the cells. Nevertheless, silencing ciRS-7 in sensitive parental cells did not increase resistance to IMiD, potentially suggesting indirect modes of action. In the case of circIKZF3, its underlying mechanism remains elusive, due to the challenges faced by the authors in knocking down circIKZF3 in both LenRes and Pom-resistant cells.[70]

Investigation of the proteomics and RNA-sequencing analyses of RRMM patients treated with lenalidomide-based therapy has identified CDK6 upregulation as a driver of IMiD resistance.[71] Overexpression of CDK6 in IMiD-sensitive MM cell lines resulted in reduced IMiD sensitivity, while the inhibition of CDK6 through Palbociclib or CDK6-specific proteolysis-targeting chimeras (PROTAC) (BSJ-03-123 or CST528) demonstrated synergy with IMiD both *in vitro* and in MM1.S xenografts. In their patient cohort, they did not detect any CRBN pathway abnormalities (RNA, protein, or phosphoprotein), genetic alterations to the other genes in the $CRL4^{CRBN}$ E3 ligase complex, or association between CRBN and CDK6 protein expression. CRISPR / Cas9-knockout of CRBN in MM lines led to no change in the expression of cyclin dependent kinase 6 (CDK6). Although CDK6 inactivation in conjunction with IMiD resulted in significant inhibition of MYC, downstream functions of CDK6 in RRMM remain unclear.[71]

CD138 is a marker for terminally differentiated plasma cells during normal B-cell development and serves as a specific surface antigen for MM cells.[72] A significant increase in CD138-negative MM cells has been observed in relapsed or progressing patients (N=15) compared to NDMM patients (N=90).[73] Characterization of two MM cell lines (KYMM-1 and KYMM-2) established from a single patient showed that the cell population with decreased CD138 surface expression had higher lenalidomide resistance. The downregulation of IRF4 and upregulation of BCL6 was suggested as the mechanism for this resistance,[73] citing another study that documented high IRF4 expression was correlated with increased lenalidomide sensitivity.[74]

In another study on surfaceome, glycoprotein cell surface capture (CSC) proteomics on LenRes OPM2 and H929 showed a common signature of increased CD33 and CD45/PTPRC, when compared to their sensitive counterparts.[75] Analysis of the Multiple Myeloma Research Foundation (MMRF) CoMMpass dataset from paired diagnosis and first-relapse tumor cells (verIA14, N=50, where 94% of patients had received lenalidomide and dexamethasone with a proteasome inhibitor [PI] as part of their induction regimen) revealed that both *CD33* and *PTPRC* transcripts were significantly increased at first relapse.[75] The authors noted that plasma cell expression of either of these markers has been associated with poor prognosis for NDMM, who exhibited more aggressive disease upon lenalidomide resistance.[76,77] They, however, did not describe, nor further investigate, the basic or plausible mechanisms underlying CD33 and PTPRC associations with disease progression.

Lastly, various other genes have appeared in genome-wide CRISPR-screens in cell lines as regulators of IMiD sensitivity, for example, *TOP2B*, *EDC4*, *RARA*, *SNRNP25*, *OTUB1*, *PLAA*, *DEPDC5*, *SRP14*, *XRN1*, *EIF4A1*, *ARID2*, *MBTPS1/2*, and SCAP.[78-80] Whether they have any relevance to clinical IMID resistance, and if so how, remains a topic for future research.

Extrinsic mechanisms

The interaction of MM cells with BM components such as secreted growth factors/cytokines, BMSC, and immune cells can promote growth, survival, and drug resistance of MM cells (Figure 4).

Characterization of the MM tumor microenvironment (TME) landscape in patients from the MANHATTAN trial (clinicaltrials.gov 03290950; N=49 patients) who did not achieve minimal residual disease (MRD) negativity and have shorter PFS, demonstrated TME dysregulations including low population of $CD14^{+}$ monocytes, increased frequency of T-cell exhaustion, persistence of IFNγ-expressing NK cells, and

decreased T-cell receptor (TCR) diversity.[81] These were observed alongside genomic defects such as high *APOBEC* mutational activity, 1p22 (*RPL5*) deletions and *IKZF3* loss, suggesting an interplay between tumor genomic features with the composition of TME in driving drug resistance.

In a prospective observational clinical trial, immune-profiling of lenalidomide-RRMM, non-refractory MM and non-MM-healthy individuals revealed that the lenalidomide-RRMM patients had a significant expansion of effector T-cell populations that express elevated levels of checkpoint molecules, lymphocyte activating 3 (LAG3) and programmed cell death 1 (PD-1). Their frequency was positively correlated with increased serum inflammatory cytokines, IL6, IL17, and TNFα.[82] High levels of PD-1 and LAG3-positive T cells were predictive of inferior survival and clinical outcomes in these RRMM patients, as in previous studies.[83,84]

The chemokine (C-C motif) ligand 20 (CCL20) was found to be down-regulated in an acquired lenalidomide-resistant MM cell line (U266-LenRes), as well as in the MM cells and plasma of lenalidomide-treated RRMM patients (N=5). At both the *in vitro* and *in vivo* level, the addition of CCL20 was able to re-sensitize MM cells to lenalidomide.[85] The authors postulated that CCL20 plays a role in increasing lymphocyte chemotaxis to the tumor areas and in assisting the cell-mediated immunity.

Our group has recently reported metabolic reprogramming, with the release of metabolic waste product such as lactate into the TME, to be associated with IMiD resistance.[86] Elevated lactate secretion is known to promote acidosis in the TME, driving metastasis, angiogenesis, and drug resistance.[87] t(4;14) is a high-risk MM marker with a prevalence rate of 15-20% that drives overexpression of the histone methyltransferase, nuclear receptor binding SET domain protein 2 (NSD2).[88] NSD2 promotes plasma cell transformation by catalyzing the active histone mark H3K36me2. We identified that protein kinase C alpha (PKCα) is an epigenetic target of NSD2.[86] Through metabolomics analysis, we found that lactate was a differential metabolite associated with PKCα. High lactate levels were associated with reduced responsiveness to lenalidomide. Knockdown of PKCα resulted in reduced intracellular and extracellular lactate levels, consequently increasing cellular sensitivity to lenalidomide, independent of the CRBN-IKZF1/3 axis. Clinically, t(4;14) MM patients demonstrated elevated plasma lactate levels compared to non-t(4;14) patients and did not derive significant benefits from lenalidomide-based regimens.[86]

Multiple myeloma cells with an IMiD-resistance phenotype have also been associated with increased secretion of extracellular vesicles (EV) and enhanced adherence abil-

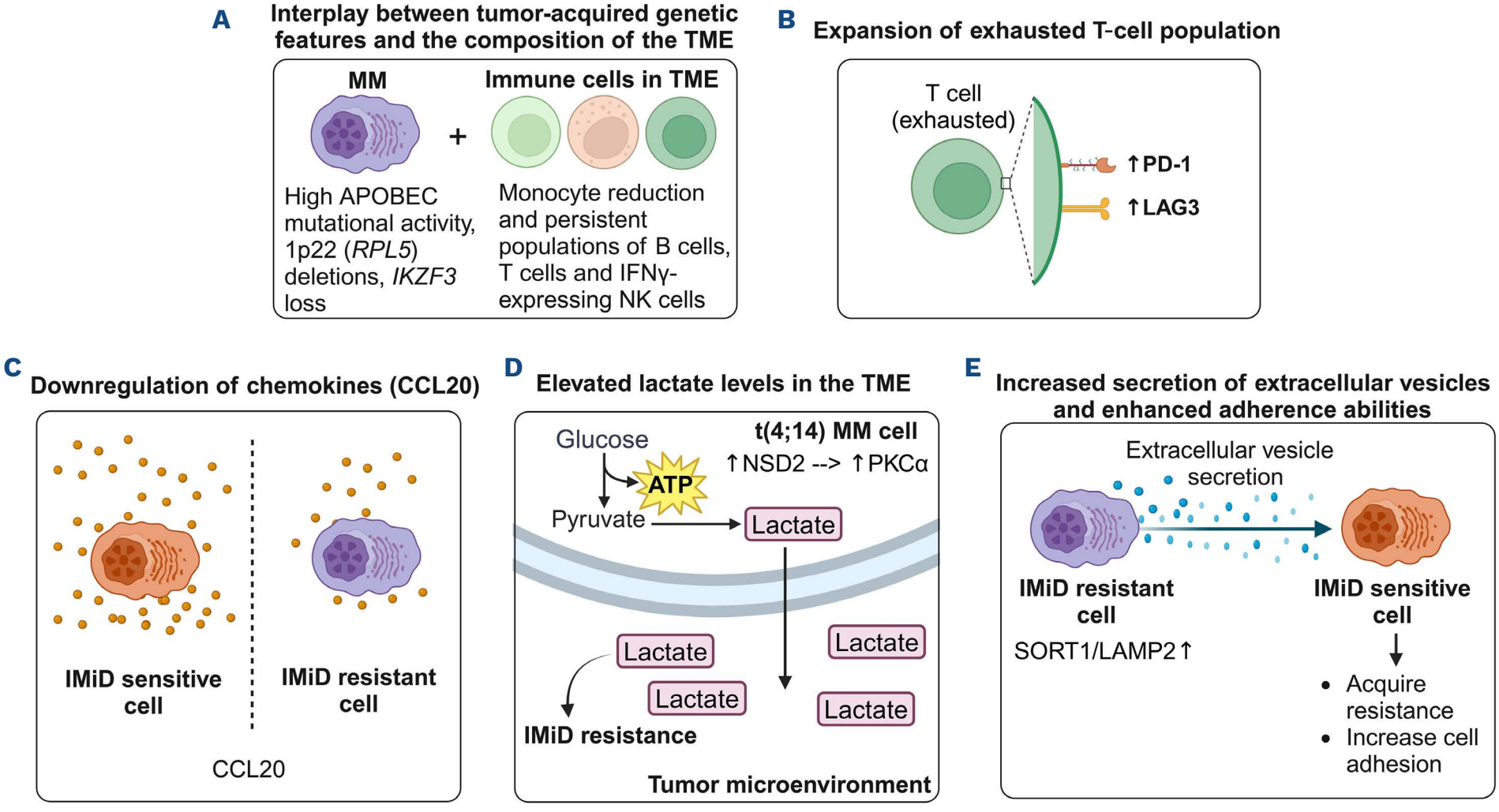

Figure 4. Extrinsic mechanisms independent of the cereblon pathway. Potential tumor microenvironment (TME) mechanisms of immunomodulatory drug (IMiD) resistance which include (A) composition of the immune cells in the TME with tumor-acquired genetic features, (B) expansion of exhausted T-cell population, (C) downregulation of CCL20, (D) elevated lactate levels in the TME, and (E) increased secretion of extracellular vesicle (EV)-mediated multiple myeloma (MM) cell adhesion and resistance.

ities.[89] Through a comprehensive transcriptomic analysis of acquired lenalidomide-resistant MM cell lines (KMS-21, KMS-27, KMS-34), core regulatory genes governing EV secretion, including sortilin 1 (SORT1) and lysosomal associated membrane protein 2 (LAMP2), were found to be significantly up-regulated compared to their sensitive counterparts. Knockdown of SORT1 or LAMP2 reduced EV secretion, decreased cell adhesion and restored lenalidomide sensitivity in lenalidomide-resistant cells without affecting CRBN expression. Further analysis of publicly available clinical data revealed that high SORT1 and LAMP2 expression were associated with poor survival in MM patients (GSE19784, N=300 patients) and in patients treated with lenalidomide (GSE136324, N >200 patients).[89]

Taken together, the above findings suggest that aberrations at the TME may impinge on the efficacy of IMiD in MM, further highlighting that IMiD resistance is likely to involve an interplay of many biological factors.

Future perspectives

The discovery of CRBN as a pivotal target of IMiD has been instrumental in advancing our understanding of the molecular mechanism of these therapeutic agents.[10,11] Given the inevitable occurrence of IMiD-resistance in MM, the identification of biomarkers that can accurately predict for IMiD responses is of paramount importance to increase the prospects of therapeutic efficacies.

At present, exploring CRBN status as a potential biomarker for predicting IMiD responses and resistance seems a conceivable strategy; however, as reviewed in the earlier sections, the results reported hitherto have been rather inconclusive. In light of this, several critical limitations should be addressed to ensure the reliability of using CRBN as a biomarker. Firstly, standardized assays for quantifying functional levels of CRBN expression in clinical samples are currently lacking.[90] The development of robust and reproducible measurement techniques is crucial if CRBN expression is to have any role as a biomarker in clinical practice. Next, determining the optimal approach for assessing functional CRBN levels, whether at the mRNA or protein level or both, and evaluating their genomic aberrations are equally important. Identifying the cancer clonal fraction (CCF) harboring these genomic events at relapse *versus* diagnosis will provide insights into the clonal selection of IMiD-resistant subclones and whether longitudinal exposure to the therapies could drive clonal selection. In addition, determining the threshold level at which the CCF is deemed prognostic for patients will be essential in guiding personalized treatment strategies. Considering that CRBN expression may be down-regulated but not completely abrogated in RRMM, it will also be paramount to consider whether a threshold of expression, and which and how different transcript splice variants should be measured to identify a non-functional CRBN activity, could predict treatment failure. It is likely that any such biomarker parameters would also vary between different IMiD and cereblon E3 ligase modulators (CELMoD).

Similarly, the approach to measure other CRBN pathway proteins, such as IKZF1/3, should be undertaken in greater depth. For example, the rate rather than the magnitude of IKZF1/3 degradation was found to be the more important determinant for modulating IRF4 expression, and, thus, the efficacy of IMiD.[91] This highlights the need to investigate the timepoint and protein level at which IKZF1/3 cease to be sufficient to support transcription of the downstream IRF4 oncogenic events, and whether any compensatory mechanisms such as BATF and ETV4 may assume the transcriptional regulation roles of IKZF1/3. Further, IMiD-bound CRBN has binding affinity for IKZF1/3 and other reported competing neo-substrates, which, at variable levels between different myelomas, may be differentially responsible for response and resistance. These are some of the interesting questions that could form the basis of future research.

High-risk copy number alterations in gain/amp(1q21) and del(17p) involve a large number of genes. It is plausible that some of them may play a role in driving IMiD resistance. For example, in chromosome 17p, a few IMiD-response pathway genes (*UBE2G1*, *NCOR1*, and *COPS3*) reside close to tumor suppressor *TP53*. The functional impact of the loss of these genes in del(17p) MM has not been investigated. Whether IMiD resistance might be driven by these genes independently or by their co-deletion with *TP53* awaits further investigation.

The growing body of evidence on the significance of non-canonical pathways and CRBN-independent mechanisms in regulating the efficacy of IMiD have been demonstrated mostly in cell line models (Table 2), which means that clinical evidence is not well-established. This highlights the need to determine how these alternative genes/modes of action are translationally relevant, given that many of the CRBN-independent abnormalities seem to be enriched also in patients resistant to non-IMiD treatment regimens. The proposed associations should, therefore, be validated in clinical samples, alongside the well-studied CRBN pathway genes, to determine the prognostic value of any associations found, and, if they prove worthwhile, to be further explored as a novel predictor and specific biomarker for IMiD treatment.

As described, there are also quite a number of studies on clinical samples that report associations with IMiD response but do not yet have direct mechanistic explanations. Greater effort into elucidating these mechanisms and how they modulate IMiD responses in MM is of paramount importance as we move forward in identifying information that can be used to aid clinical decision making.

Previous genome-wide CRISPR/Cas9-mediated studies were conducted with the typical aim of identifying genes that regulate IMiD sensitivity.[78-80,92] It is also worthwhile to

validate these findings in a converse manner, i.e., CRISPR studies on IMiD-resistant cells to decipher their novel dependencies and therapeutic vulnerabilities. This might identify genes that, when knocked out or activated, will result in the re-sensitization or killing of IMiD-resistant MM cells.

Tumor heterogeneity leads to the development of multiple mechanisms of resistance to IMiD.[93] Rapid advancements in single cell profiling technology have enabled us to dissect the heterogeneity of cells at both single and spatial resolution. Spatial single-cell transcriptomics is a burgeoning tool to decipher tumor architecture and TME,[94,95] and studies utilizing this cutting-edge technology in MM is still in its infancy. Given the current lack of knowledge surrounding the association between cell-intrinsic and cell-extrinsic mechanisms in IMiD resistance, it will be pertinent to adopt spatial single-cell technology in future investigations to enable detailed characterization of the MM cell-TME interaction.

It is becoming evident that the mechanisms underpinning IMiD resistance in MM involves a complex interplay of genomic, transcriptomic, and proteomic events both in tumor and immune cells. Future approaches to overcoming IMiD resistance may, therefore, call for targeting a combination of CRBN pathway-dependent and pathway-independent mechanisms. This would entail adopting personalized multi-drug strategies that concurrently target individually-relevant pathways. With the immense array of possible target combinations, predicting the optimal drug combination for an individual patient presents a future trajectory for optimal therapy. In this era of artificial intelligence, it is likely to require deep machine learning techniques to effectively map individualized drug-drug interactions, rank different drug combinations, and determine a more accurate drug dosage for optimal clinical efficacy. Such an approach has been successfully adopted in our previous proof-of-concept study in MM and lymphoma.[96,97]

Next-generation cereblon targeting agents, CELMoD (iberdomide and mezigdomide), are in ongoing clinical trials in MM as a means to overcome IMiD resistance. They promise higher potency, more robust degradation of known targets, and an expanded repertoire of neo-substrate targets.[98,99] Compared to IMiD, the binding affinity of iberdomide for CRBN was 20-fold higher (IC_{50} for CRBN binding was approx. 3 uM and approx. 0.15 uM, respectively) which leads to a more rapid IKZF1/3 degradation and, therefore, enhanced treatment efficacy.[100] Iberdomide and mezigdomide have both shown meaningful clinical outcome in heavily pre-treated IMiD-refractory MM patients with ORR 26% for the former and 40% for the latter.[101,102] However, this also implies that up to 60% of IMiD-refractoriness cannot yet be rescued by next-generation counterparts. Given that CELMoD share the same mechanisms of action as IMiD, it remains to be seen whether any particular CRBN-dependent or -independent mechanisms also contribute to the therapeutic insufficiency of CELMoD.[103-105] The comprehensive understanding of the range of biology of IMiD resistance, therefore, holds the key in facilitating the successful integration of these new agents into clinical practice.

Conclusions

Immunomodulatory drug resistance associated with the CRBN pathway has been extensively reviewed previously.[12-14] In this review, we extended our discussion into several other important aspects including potential CRBN pathway-independent IMiD resistance mechanisms, ranging from the cell intrinsic IMiD-resistance to the extrinsic components in the TME. The transformation of IMiD from a teratogenic 'dark remedy' to the pioneering standard-of-care treatment today in MM marks a significant shift in treatment paradigms, but lasting responses are inevitably hindered by acquired resistance, which represents an unmet therapeutic need. As we move forward, a clearer understanding of which resistance mechanisms are clinically relevant and why will lead us to new avenues for personalized and effective therapeutic interventions as we manoeuvre the challenging IMiD landscape, with IMiD resistance and the differential impact of new agents across age gaps[41] in particular, and so better translate these promising results from the clinical trial to real-world practice.[106]

Disclosures

No conflicts of interest to disclose.

Contributions

PJT and MYK were involved in the conception, literature searches, writing and revision of the manuscript through its entirety. MYK designed the figures and tables. SG, CM and WJC reviewed the manuscript and provided input. PJT and WJC supervised and finalized the manuscript.

Acknowledgments

Figures were created with BioRender.com.

Funding

This work was supported by the Cancer Science Institute of Singapore, National University of Singapore, through the National Research Foundation Singapore and the Singapore Ministry of Education under its Research Centres of Excellence initiative.

References

1. Gao S, Wang S, Song Y. Novel immunomodulatory drugs and neo-substrates. Biomarker Res. 2020;8:2.
2. Singhal S, Mehta J, Desikan R, et al. Antitumor activity of thalidomide in refractory multiple myeloma. N Engl J Med. 1999;341(21):1565-1571.
3. Holstein SA, McCarthy PL. Immunomodulatory drugs in multiple myeloma: mechanisms of action and clinical experience. Drugs. 2017;77(5):505-520.
4. Weber DM, Chen C, Niesvizky R, et al. Lenalidomide plus dexamethasone for relapsed multiple myeloma in North America. N Engl J Med. 2007;357(21):2133-2142.
5. van de Donk NWCJ, Pawlyn C, Yong KL. Multiple myeloma. Lancet. 2021;397(10272):410-427.
6. Pawlyn C, Davies FE, Kaiser MF, et al. Primary IMiD refractory myeloma; results from 3894 patients treated in the phase III Myeloma XI study. Blood. 2016;128(22):1144.
7. Lecat CSY, Taube JB, Wilson W, et al. Defining unmet need following lenalidomide refractoriness: real-world evidence of outcomes in patients with multiple myeloma. Front Oncol. 2021;11:703233.
8. Pawlyn C, Cairns D, Kaiser M, et al. The relative importance of factors predicting outcome for myeloma patients at different ages: results from 3894 patients in the Myeloma XI trial. Leukemia. 2020;34(2):604-612.
9. Pawlyn C, Schjesvold FH, Cairns DA, et al. Progression-free survival as a surrogate endpoint in myeloma clinical trials: an evolving paradigm. Blood Cancer J. 2024;14(1):134.
10. Krönke J, Udeshi ND, Narla A, et al. Lenalidomide causes selective degradation of IKZF1 and IKZF3 in multiple myeloma cells. Science. 2014;343(6168):301-305.
11. Lu G, Middleton RE, Sun H, et al. The myeloma drug lenalidomide promotes the cereblon-dependent destruction of Ikaros proteins. Science. 2014;343(6168):305-309.
12. Martinez-Høyer S, Karsan A. Mechanisms of lenalidomide sensitivity and resistance. Exp Hematol. 2020;91:22-31.
13. Wang S, Li Z, Gao S. Key regulators of sensitivity to immunomodulatory drugs in cancer treatment. Biomarker Res. 2021;9(1):43.
14. Chen LY, Gooding S. Tumor and microenvironmental mechanisms of resistance to immunomodulatory drugs in multiple myeloma. Front Oncol. 2022;12:1038329.
15. Gandhi AK, Kang J, Havens CG, et al. Immunomodulatory agents lenalidomide and pomalidomide co-stimulate T cells by inducing degradation of T cell repressors Ikaros and Aiolos via modulation of the E3 ubiquitin ligase complex CRL4(CRBN.). Br J Haematol. 2014;164(6):811-821.
16. John LB, Ward AC. The Ikaros gene family: transcriptional regulators of hematopoiesis and immunity. Mol Immunol. 2011;48(9-10):1272-1278.
17. Krönke J, Fink EC, Hollenbach PW, et al. Lenalidomide induces ubiquitination and degradation of CK1α in del(5q) MDS. Nature. 2015;523(7559):183-188.
18. Shaffer AL, Emre NC, Lamy L, et al. IRF4 addiction in multiple myeloma. Nature. 2008;454(7201):226-231.
19. Jovanović KK, Roche-Lestienne C, Ghobrial IM, Facon T, Quesnel B, Manier S. Targeting MYC in multiple myeloma. Leukemia. 2018;32(6):1295-1306.
20. D'Souza C, Prince HM, Neeson PJ. Understanding the role of T-cells in the antimyeloma effect of immunomodulatory drugs. Front Immunol. 2021;12:632399.
21. Noonan K, Rudraraju L, Ferguson A, et al. Lenalidomide-induced immunomodulation in multiple myeloma: impact on vaccines and antitumor responses. Clin Cancer Res. 2012;18(5):1426-1434.
22. Krämer I, Engelhardt M, Fichtner S, et al. Lenalidomide enhances myeloma-specific T-cell responses in vivo and in vitro. OncoImmunology. 2016;5(5):e1139662.
23. Thakurta A, Pierceall WE, Amatangelo MD, Flynt E, Agarwal A. Developing next generation immunomodulatory drugs and their combinations in multiple myeloma. Oncotarget. 2021;12(15):1555-1563.
24. Corral LG, Haslett PAJ, Muller GW, et al. Differential cytokine modulation and T cell activation by two distinct classes of thalidomide analogues that are potent inhibitors of TNF-α1. J Immunol. 1999;163(1):380-386.
25. Gooding S, Ansari-Pour N, Towfic F, et al. Multiple cereblon genetic changes are associated with acquired resistance to lenalidomide or pomalidomide in multiple myeloma. Blood. 2021;137(2):232-237.
26. Kortüm KM, Mai EK, Hanafiah NH, et al. Targeted sequencing of refractory myeloma reveals a high incidence of mutations in CRBN and Ras pathway genes. Blood. 2016;128(9):1226-1233.
27. Barrio S, Munawar U, Zhu YX, et al. IKZF1/3 and CRL4(CRBN) E3 ubiquitin ligase mutations and resistance to immunomodulatory drugs in multiple myeloma. Haematologica. 2020;105(5):e237-e241.
28. Gooding S, Ansari-Pour N, Kazeroun M, et al. Loss of COP9 signalosome genes at 2q37 is associated with IMiD resistance in multiple myeloma. Blood. 2022;140(16):1816-1821.
29. Zhu YX, Braggio E, Shi C-X, et al. Cereblon expression is required for the antimyeloma activity of lenalidomide and pomalidomide. Blood. 2011;118(18):4771-4779.
30. Heintel D, Rocci A, Ludwig H, et al. High expression of cereblon (CRBN) is associated with improved clinical response in patients with multiple myeloma treated with lenalidomide and dexamethasone. Br J Haematol. 2013;161(5):695-700.
31. Laurens EF, Inger SN, Suzana C, et al. Cereblon loss and up-regulation of c-Myc are associated with lenalidomide resistance in multiple myeloma patients. Haematologica. 2018;103(8):e368-e371.
32. Barrio S, Munawar U, Zhu YX, et al. IKZF1/3 and CRL4CRBN E3 ubiquitin ligase mutations and resistance to immunomodulatory drugs in multiple myeloma. Haematologica. 2020;105(5):e237-e241.
33. Borsi E, Mazzocchetti G, Dico AF, et al. High levels of CRBN isoform lacking IMiDs binding domain predicts for a worse response to IMiDs-based upfront therapy in newly diagnosed myeloma patients. Clin Exp Med. 2023;23(8):5227-5239.
34. Jones JR, Barber A, Le Bihan YV, et al. Mutations in CRBN and other cereblon pathway genes are infrequently associated with acquired resistance to immunomodulatory drugs. Leukemia. 2021;35(10):3017-3020.
35. Bohl SR, Schmalbrock LK, Bauhuf I, et al. Comprehensive CRISPR-Cas9 screens identify genetic determinants of drug responsiveness in multiple myeloma. Blood Adv. 2021;5(9):2391-2402.
36. Weinhold N, Ashby C, Rasche L, et al. Clonal selection and double-hit events involving tumor suppressor genes underlie relapse in myeloma. Blood. 2016;128(13):1735-1744.
37. Broyl A, Kuiper R, van Duin M, et al. High cereblon expression is associated with better survival in patients with newly diagnosed

multiple myeloma treated with thalidomide maintenance. Blood. 2013;121(4):624-627.
38. Dimopoulos K, Fibiger Munch-Petersen H, Winther Eskelund C, et al. Expression of CRBN, IKZF1, and IKZF3 does not predict lenalidomide sensitivity and mutations in the cereblon pathway are infrequent in multiple myeloma. Leuk Lymphoma. 2019;60(1):180-188.
39. Tachita T, Kinoshita S, Ri M, et al. Expression, mutation, and methylation of cereblon-pathway genes at pre- and post-lenalidomide treatment in multiple myeloma. Cancer Science. 2020;111(4):1333-1343.
40. Paludo J, Mikhael JR, LaPlant BR, et al. Pomalidomide, bortezomib, and dexamethasone for patients with relapsed lenalidomide-refractory multiple myeloma. Blood. 2017;130(10):1198-1204.
41. Richardson PG, Oriol A, Beksac M, et al. Pomalidomide, bortezomib, and dexamethasone for patients with relapsed or refractory multiple myeloma previously treated with lenalidomide (OPTIMISMM): a randomised, open-label, phase 3 trial. Lancet Oncol. 2019;20(6):781-794.
42. Krönke J, Kuchenbauer F, Kull M, et al. IKZF1 expression is a prognostic marker in newly diagnosed standard-risk multiple myeloma treated with lenalidomide and intensive chemotherapy: a study of the German Myeloma Study Group (DSMM). Leukemia. 2017;31(6):1363-1367.
43. Pourabdollah M, Bahmanyar M, Atenafu EG, Reece D, Hou J, Chang H. High IKZF1/3 protein expression is a favorable prognostic factor for survival of relapsed / refractory multiple myeloma patients treated with lenalidomide. J Hematol Oncol. 2016;9(1):123.
44. Zhu YX, Braggio E, Shi CX, et al. Identification of cereblon-binding proteins and relationship with response and survival after IMiDs in multiple myeloma. Blood. 2014;124(4):536-545.
45. Kalff A, Khong T, Ramachandran M, et al. Cereblon pathway biomarkers and immune profiles in patients with myeloma receiving post-ASCT lenalidomide maintenance (LEOPARD). Leuk Lymphoma. 2021;62(12):2981-2991.
46. de Matos Simoes R, Shirasaki R, Downey-Kopyscinski SL, et al. Genome-scale functional genomics identify genes preferentially essential for multiple myeloma cells compared to other neoplasias. Nat Cancer. 2023;4(5):754-773.
47. Zhu YX, Shi C-X, Bruins LA, 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.
48. Bjorklund CC, Ma W, Wang ZQ, 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):11009-11020.
49. Bjorklund CC, Baladandayuthapani V, Lin HY, et al. Evidence of a role for CD44 and cell adhesion in mediating resistance to lenalidomide in multiple myeloma: therapeutic implications. Leukemia. 2014;28(2):373-383.
50. Giesen N, Paramasivam N, Toprak UH, et al. Comprehensive genomic analysis of refractory multiple myeloma reveals a complex mutational landscape associated with drug resistance and novel therapeutic vulnerabilities. Haematologica. 2022;107(8):1891-1901.
51. Pasca S, Tomuleasa C, Teodorescu P, et al. KRAS/NRAS/BRAF mutations as potential targets in multiple myeloma. Front Oncol. 2019;9:1137.
52. Perroud C, Thurian D, Andres M, et al. Effect of MAPK activation via mutations in NRAS, KRAS and BRAF on clinical outcome in newly diagnosed multiple myeloma. Hematol Oncol. 2023;41(5):912-921.
53. Ocio EM, Fernández-Lázaro D, San-Segundo L, et al. In vivo murine model of acquired resistance in myeloma reveals differential mechanisms for lenalidomide and pomalidomide in combination with dexamethasone. Leukemia. 2015;29(3):705-714.
54. Lionetti M, Barbieri M, Todoerti K, et al. Molecular spectrum of BRAF, NRAS and KRAS gene mutations in plasma cell dyscrasias: implication for MEK-ERK pathway activation. Oncotarget. 2015;6(27):24205-24217.
55. Liu J, Hideshima T, Xing L, et al. ERK signaling mediates resistance to immunomodulatory drugs in the bone marrow microenvironment. Sci Adv. 2021;7(23):eabg2697.
56. Dimopoulos K, Gimsing P, Liang G, Grønbæk K, Helbo Søgaard A, Lakshminarasinham R. Cereblon is downregulated by promoter nucleosome occupancy in acquired IMiD resistance: the potential of IMiD resensitization by epigenetic therapy. Blood. 2016;128(22):3258-3258.
57. Dimopoulos K, Søgaard Helbo A, Fibiger Munch-Petersen H, et al. Dual inhibition of DNMTs and EZH2 can overcome both intrinsic and acquired resistance of myeloma cells to IMiDs in a cereblon-independent manner. Mol Oncol. 2018;12(2):180-195.
58. Kalff A, Khong T, Mithraprabhu S, et al. Oral azacitidine (CC-486) in combination with lenalidomide and dexamethasone in advanced, lenalidomide-refractory multiple myeloma (ROAR study). Leuk Lymphoma. 2019;60(9):2143-2151.
59. Khouri J, Faiman BM, Grabowski D, et al. DNA methylation inhibition in myeloma: experience from a phase 1b study of low-dose continuous azacitidine in combination with lenalidomide and low-dose dexamethasone in relapsed or refractory multiple myeloma. Semin Hematol. 2021;58(1):45-55.
60. Alaterre E, Ovejero S, Herviou L, et al. Comprehensive characterization of the epigenetic landscape in multiple myeloma. Theranostics. 2022;12(4):1715-1729.
61. Mori T, Verma R, Nakamoto-Matsubara R, et al. Low NCOR2 levels in multiple myeloma patients drive multidrug resistance via MYC upregulation. Blood Cancer J. 2021;11(12):194.
62. Welsh SJ, Barwick BG, Meermeier EW, et al. Transcriptional heterogeneity overcomes super-enhancer disrupting drug combinations in multiple myeloma. Blood Cancer Discov. 2023;5(1):34-55.
63. Neri P, Barwick BG, Jung D, et al. ETV4-dependent transcriptional plasticity maintains MYC expression and results in IMiD resistance in multiple myeloma. Blood Cancer Discov. 2024;5(1):56-73.
64. Hagen P, Zhang J, Barton K. High-risk disease in newly diagnosed multiple myeloma: beyond the R-ISS and IMWG definitions. Blood Cancer J. 2022;12(5):83.
65. Ansari-Pour N, Samur M, Flynt E, et al. Whole-genome analysis identifies novel drivers and high-risk double-hit events in relapsed / refractory myeloma. Blood. 2023;141(6):620-633.
66. Panopoulou A, Cairns DA, Holroyd A, et al. Optimizing the value of lenalidomide maintenance by extended genetic profiling: an analysis of 556 patients in the Myeloma XI trial. Blood. 2023;141(14):1666-1674.
67. Teoh PJ, An O, Chung T-H, et al. Aberrant hyperediting of the myeloma transcriptome by ADAR1 confers oncogenicity and is a marker of poor prognosis. Blood. 2018;132(12):1304-1317.
68. Lazzari E, Mondala PK, Santos ND, et al. Alu-dependent RNA editing of GLI1 promotes malignant regeneration in multiple

myeloma. Nat Commun. 2017;8(1):1922.
69. Koh MY, Chung T-H, Tang NXN, et al. ADAR1-regulated cytoplasmic dsRNA-sensing pathway is a novel mechanism of lenalidomide resistance in multiple myeloma. Blood. 2024 Dec 9. doi: 10.1182 [Epub ahead of print]
70. Jakobsen T, Dahl M, Dimopoulos K, Grønbæk K, Kjems J, Kristensen LS. Genome-wide circular RNA expression patterns reflect resistance to immunomodulatory drugs in multiple myeloma cells. Cancers. 2021;13(3):365.
71. Ng YLD, Ramberger E, Bohl SR, et al. Proteomic profiling reveals CDK6 upregulation as a targetable resistance mechanism for lenalidomide in multiple myeloma. Nat Commun. 2022;13(1):1009.
72. Zhan F, Huang Y, Colla S, et al. The molecular classification of multiple myeloma. Blood. 2006;108(6):2020-2028.
73. Kawano Y, Fujiwara S, Wada N, et al. Multiple myeloma cells expressing low levels of CD138 have an immature phenotype and reduced sensitivity to lenalidomide. Int J Oncol. 2012;41(3):876-884.
74. Lopez-Girona A, Heintel D, Zhang L-H, et al. Lenalidomide downregulates the cell survival factor, interferon regulatory factor-4, providing a potential mechanistic link for predicting response. Br J Haematol. 2011;154(3):325-336.
75. Ferguson ID, Patiño-Escobar B, Tuomivaara ST, et al. The surfaceome of multiple myeloma cells suggests potential immunotherapeutic strategies and protein markers of drug resistance. Nat Commun. 2022;13(1):4121.
76. Arana P, Paiva B, Cedena MT, et al. Prognostic value of antigen expression in multiple myeloma: a PETHEMA/GEM study on 1265 patients enrolled in four consecutive clinical trials. Leukemia. 2018;32(4):971-978.
77. Shim H, Ha JH, Lee H, et al. Expression of myeloid antigen in neoplastic plasma cells is related to adverse prognosis in patients with multiple myeloma. Biomed Res Int. 2014;2014:893243.
78. Sievers QL, Gasser JA, Cowley GS, Fischer ES, Ebert BL. Genome-wide screen identifies cullin-RING ligase machinery required for lenalidomide-dependent CRL4(CRBN) activity. Blood. 2018;132(12):1293-1303.
79. Liu J, Song T, Zhou W, et al. A genome-scale CRISPR-Cas9 screening in myeloma cells identifies regulators of immunomodulatory drug sensitivity. Leukemia. 2019;33(1):171-180.
80. Costacurta M, Vervoort SJ, Hogg SJ, Martin BP, Johnstone RW, Shortt J. Whole genome CRISPR screening identifies TOP2B as a potential target for IMiD sensitization in multiple myeloma. Haematologica. 2020;106(7):2013-2017.
81. Maura F, Boyle EM, Coffey D, et al. Genomic and immune signatures predict clinical outcome in newly diagnosed multiple myeloma treated with immunotherapy regimens. Nat Cancer. 2023;4(12):1660-1674.
82. Chen M, Zhu J, Yang X, Yao J, Liu Y, Liu Q. PD-1 and LAG-3-positive T cells are associated with clinical outcomes of relapsed / refractory multiple myeloma patients. Eur J Med Res. 2022;27(1):296.
83. Lucas F, Pennell M, Huang Y, et al. T cell transcriptional profiling and immunophenotyping uncover LAG3 as a potential significant target of immune modulation in multiple myeloma. Biol Blood Marrow Transplant. 2020;26(1):7-15.
84. Lee L, Alrasheed N, Khandelwal G, et al. Increased immune-regulatory receptor expression on effector T cells as early indicators of relapse following autologous stem cell transplantation for multiple myeloma. Front Immunol. 2021;12:618610.
85. Wang H, Shi H, He X, Liao A. Downregulation of chemokine CCL20 involved in myeloma cells resistant to elotuzumab and lenalidomide. Onco Targets Ther. 2021;14:2789-2795.
86. Chong PSY, Chooi J-Y, Lim JSL, et al. Histone methyltransferase NSD2 activates PKCα to drive metabolic reprogramming and lenalidomide resistance in multiple myeloma. Cancer Res. 2023;83(20):3414-3427.
87. Dhup S, Dadhich RK, Porporato PE, Sonveaux P. Multiple biological activities of lactic acid in cancer: influences on tumor growth, angiogenesis and metastasis. Curr Pharm Des. 2012;18(10):1319-1330.
88. Kuo AJ, Cheung P, Chen K, et al. NSD2 links dimethylation of histone H3 at lysine 36 to oncogenic programming. Mol Cell. 2011;44(4):609-620.
89. Yamamoto T, Nakayama J, Yamamoto Y, Kuroda M, Hattori Y, Ochiya T. SORT1/LAMP2-mediated extracellular vesicle secretion and cell adhesion are linked to lenalidomide resistance in multiple myeloma. Blood Adv. 2022;6(8):2480-2495.
90. Gandhi AK, Mendy D, Waldman M, et al. Measuring cereblon as a biomarker of response or resistance to lenalidomide and pomalidomide requires use of standardized reagents and understanding of gene complexity. Br J Haematol. 2014;164(2):233-244.
91. Bjorklund CC, Lu L, Kang J, 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.
92. Bohl SR, Schmalbrock LK, Bauhuf I, et al. Comprehensive CRISPR-Cas9 screens identify genetic determinants of drug responsiveness in multiple myeloma. Blood Adv. 2021;5(9):2391-2402.
93. Vasan N, Baselga J, Hyman DM. A view on drug resistance in cancer. Nature. 2019;575(7782):299-309.
94. Moffitt JR, Lundberg E, Heyn H. The emerging landscape of spatial profiling technologies. Nat Rev Genet. 2022;23(12):741-759.
95. Sorin M, Rezanejad M, Karimi E, et al. Single-cell spatial landscapes of the lung tumour immune microenvironment. Nature. 2023;614(7948):548-554.
96. Goh J, De Mel S, Hoppe MM, et al. An ex vivo platform to guide drug combination treatment in relapsed / refractory lymphoma. Sci Transl Med. 2022;14(667):eabn7824.
97. Rashid M, Toh TB, Hooi L, et al. Optimizing drug combinations against multiple myeloma using a quadratic phenotypic optimization platform (QPOP). Sci Transl Med. 2018;10(453):eaan0941.
98. Charliński G, Vesole DH, Jurczyszyn A. Rapid progress in the use of immunomodulatory drugs and cereblon E3 ligase modulators in the treatment of multiple myeloma. Cancers (Basel). 2021;13(18):4666.
99. Lonial S, Popat R, Hulin C, et al. Iberdomide (IBER) in combination with dexamethasone (DEX) in patients (pts) with relapsed / refractory multiple myeloma (RRMM): results from the dose-expansion phase of the CC-220-MM-001 trial. Blood. 2021;138(Suppl 1):162.
100. Matyskiela ME, Zhang W, Man HW, et al. A cereblon modulator (CC-220) with improved degradation of Ikaros and Aiolos. J Med Chem. 2018;61(2):535-542.
101. Richardson PG, Trudel S, Popat R, et al. Mezigdomide plus dexamethasone in relapsed and refractory multiple myeloma. N

Engl J Med. 2023;389(11):1009-1022.
102. Lonial S, Popat R, Hulin C, et al. Iberdomide plus dexamethasone in heavily pretreated late-line relapsed or refractory multiple myeloma (CC-220-MM-001): a multicentre, multicohort, open-label, phase 1/2 trial. Lancet Haematol. 2022;9(11):e822-e832.
103. Amatangelo M, Flynt E, Stong N, et al. Pharmacodynamic changes in tumor and immune cells drive iberdomide's clinical mechanisms of activity in relapsed and refractory multiple myeloma. Cell Rep Med. 2024;5(6):101571.
104. Bird SA, Barber A, Sialana FJ, et al. Multiomics analysis of IMiD/CELMoD resistant multiple myeloma models uncovers novel and targetable vulnerabilities in the SREBP lipid synthesis pathway. Blood. 2022;140(Suppl 1):600-601.
105. Chrisochoidou Y, LeBihan Y-V, Morales S, et al. Investigating the functional impact of CRBN mutations on response to IMiD/celmod agents in myeloma. Blood. 2023;142(Suppl 1):753.
106. Richardson PG, San Miguel JF, Moreau P, et al. Interpreting clinical trial data in multiple myeloma: translating findings to the real-world setting. Blood Cancer J. 2018;8(11):109.