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REVIEW ARTICLE

Mechanisms of Action of Lenalidomide in B-Cell Non-Hodgkin Lymphoma

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ABSTRACT

Lenalidomide is an orally active immunomodulatory drug that has direct antineoplastic activity and indirect effects mediated through multiple types of immune cells found in the tumor microenvironment, including B, T, natural killer (NK), and dendritic cells. Recently, the E3 ubiquitin ligase cereblon was identified as a molecular target that may underlie the effects of lenalidomide on tumor cells, as well as on cells in the tumor microenvironment. Decreases in cereblon attenuate these effects and also confer resistance to lenalidomide. Tumoricidal effects of lenalidomide are associated with reduced interferon regulatory factor 4, a downstream target of cereblon. Lenalidomide stimulates proliferation and activation of NK cells, thereby enhancing NK cell–mediated cytotoxicity and antibody-dependent cellular cytotoxicity. These effects appear to be secondary to cytokine production from T cells. Lenalidomide has been shown to produce synergistic effects in experimental models when evaluated in combination with rituximab, dexamethasone, bortezomib, and B-cell receptor signaling inhibitors, consistent with mechanisms complementary to these agents. These experimental findings have translated to the clinic, where single-agent use displays durable responses in relapsed/refractory non-Hodgkin lymphoma, and combination with rituximab and other agents leads to improved responses at first line and in relapsed/refractory disease. The activity of lenalidomide is evident across multiple lymphoma subtypes, including indolent and aggressive forms. The interaction among cell types in the immune microenvironment is increasingly recognized as important to tumor cell recognition and destruction, as well as to protection of normal immune cells, as reflected by lenalidomide studies across multiple types of B-cell lymphomas.

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INTRODUCTION

B-cell non-Hodgkin lymphoma (NHL) comprises multiple clinico-pathologic subtypes, most commonly diffuse large B-cell lymphoma (DLBCL) and follicular lymphoma (FL).[1,2] First-line treatment typically consists of immunochemotherapy, which may be followed by rituximab-based maintenance therapy for FL, or consolidation with autologous stem-cell transplantation for mantle-cell lymphoma (MCL).[3] For patients with relapsed or refractory NHL, a wide range of treatment options is available, although consensus on the best approach and sequence remains to be determined.

Chemotherapy has a broad impact on both malignant and healthy cells. Advances in delineating pathways involved in cell signaling and tumor growth have led to novel, molecularly-based treatments.[4] The advent of rituximab provided proof-of-concept for targeted therapy in B-cell NHL. Since then, numerous novel agents have been evaluated, with favorable clinical activity portending improvements in patient outcome.[5] One such agent is lenalidomide, an oral, immune modulator. Its antineoplastic effects include direct antineoplastic activity, immunologic effects mediated by inhibition of tumor cell proliferation and angiogenesis, and stimulation of cytotoxicity mediated by T cells and NK cells.[6-13] Herein, we provide a comprehensive review of known mechanisms of action (MOAs) of lenalidomide in B-cell NHL. Lenalidomide was first approved for treatment of multiple myeloma, and much work has focused on its activity in this disease. Another immunomodulatory derivative of thalidomide family member, pomalidomide, has been approved for use in multiple myeloma, but it is not being explored in preclinical or clinical studies in lymphoma, and therefore this review focuses on lenalidomide only.

CEREBLON AS A DIRECT TARGET FOR LENALIDOMIDE

Cereblon is a ubiquitously expressed E3 ubiquitin ligase protein identified as the primary teratogenic target of thalidomide,[14] and cereblon is also a direct

and therapeutically important molecular target for lenalidomide. Direct binding of lenalidomide to endogenous cereblon isolated from cell line extracts and to recombinant cereblon–DNA damage-binding protein-1 complexes has been demonstrated in vitro.[15] Ikaros and Aiolos, zinc finger–containing transcription regulators of B- and T-cell development, are selectively bound by cereblon.[16-18] After direct binding, lenalidomide activates cereblon's E3 ligase activity, resulting in the rapid ubiquitination and degradation of Ikaros and Aiolos. Lenalidomide inhibits autoubiquitination of wild-type, but not mutant, cereblon protein. Zhu et al[19] found that transfection of myeloma cell lines with lentiviral constructs targeting cereblon was cytotoxic, and surviving cells with stable cereblon depletion became lenalidomide resistant. Cereblon silencing in myeloma cells attenuated the antiproliferative effect of lenalidomide, induction of tumor suppressor $p21^{WAF-1}$ expression, and decrease in interferon regulatory factor 4 (IRF4), and silencing in T cells decreased lenalidomide-induced interleukin (IL)-2 and tumor necrosis factor α (TNF-α) production.

Reduced or undetectable levels of cereblon were found in lenalidomide-resistant H929 and DF15R myeloma cells selected for incubation with increasing lenalidomide concentrations over extended periods,[15] and in patients with myeloma, lower cereblon levels were associated with lenalidomide resistance.[19] Translation of these findings to lymphoma remains to be shown.

EFFECT OF LENALIDOMIDE ON MALIGNANT B CELLS

Lenalidomide exhibits in vitro and in vivo activity against malignant lymphoma B cells,[6,11,12,20,21] and in specific tumor types, including DLBCL, FL, and MCL.[10,13,22-24] Early preclinical evaluation showed antineoplastic and antiproliferative effects on malignant B-cell lines while sparing $CD34^+$ progenitor and normal B cells (Fig 1).[11] Lenalidomide increased the percentage of cells arrested in the G_0-G_1 phase, and there was a corresponding decrease in the S and G_2-M phases. Lenalidomide upregulated protein and mRNA levels of $p21^{WAF-1}$, a regulator of cyclin-dependent kinases (CDKs) important for G_1-S progression, and promoted binding of $p21^{WAF-1}$ to CDK2, CDK4, and CDK6 in malignant, but not normal, B cells. Upregulation of $p21^{WAF-1}$ correlated with CDK inhibition, leading to hypophosphorylation of retinoblastoma protein, subsequent G_1 cell-cycle arrest, and decreased cell proliferation. Lenalidomide inhibited protein kinase B (also known as Akt) and GRB2-associated binding protein 1 phosphorylation and enhanced activator protein-1 expression, suggesting that it, in part, exerts its antineoplastic and antiproliferative effects through kinase signaling pathways.[7] Lenalidomide downregulates expression of checkpoint inhibitors, including programmed death-ligand 1 (PD-L1, CD274) on the surface of lymphoma cells.[29] Lenalidomide upregulates expression of several genes involved in immune responses in MCL cells, including CD86, CD40, CD58, and CD1c.[22]

Lenalidomide produces higher response rates in the activated B cell–like (ABC) subtype of DLBCL.[30,31] Lenalidomide preferentially suppressed ABC DLBCL cell proliferation and delayed malignant growth in a human tumor xenograft model, while minimally affecting non-ABC DLBCL cells.[24] The antineoplastic effects of lenalidomide in ABC DLBCL cells were associated with downregulation of IRF4 and, subsequently, B-cell receptor–dependent nuclear factor-κB (NF-κB) activity. Conversely, IRF4 overexpression led to enhanced NF-κB activation and a subsequent resistance to lenalidomide. Notably, cereblon expression was required for lenalidomide-induced downregulation of IRF4 and inhibition of B-cell receptor–mediated NF-κB signaling in ABC-type DLBCL cells.

A gain-of-function mutation of MYD88, an adaptor protein mediating Toll-like and IL-1 receptor signaling,[32] is commonly observed in ABC DLBCL. MYD88 mutation promotes NF-κB and Janus kinase/signal transducer and activator of transcription (STAT) 3 signaling pathways to sustain ABC DLBCL viability, while also inducing interferon beta (IFN-β) production and autocrine signaling, paradoxically promoting cell-cycle arrest and apoptosis.[33] On treatment of

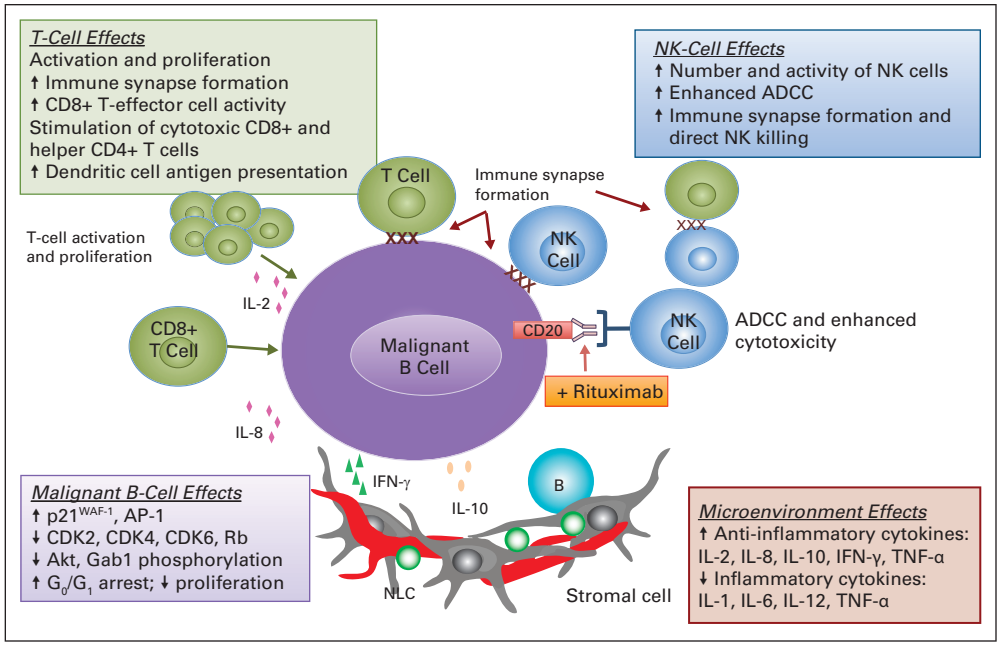

Fig 1. Mechanisms of action of lenalidomide in lymphoma cells and the nodal microenvironment.[6,9-13,25-28] ADCC, antibody-dependent cellular cytotoxicity; Akt, protein kinase B; AP-1, activator protein 1; CDK, cyclin-dependent kinase; Gab1, GRB2-associated binding protein 1; IFN, interferon; IL, interleukin; NK, natural killer; NLC, nurse-like cell; Rb, retinoblastoma; TNF, tumor necrosis factor.

ABC DLBCL cells with lenalidomide, mRNA and protein levels of IRF4 and SPi-B (an Ets family transcription factor) were reduced in a cereblon-dependent manner. SPi-B acted together with IRF4 to prevent IFN-β production, allowing survival and proliferation of ABC DLBCL cells with MYD88 mutations. By blocking these transcription factors, lenalidomide augmented IFN-β production and promoted cytotoxicity against ABC DLBCL cells. The mRNA levels of CARD11, a transcription factor regulating the activity of IκB kinase in the NF-κB pathway, were reduced alongside IRF4 and SPi-B. Further examination of the pathways involved in lenalidomide's cytotoxic activity in ABC DLBCL cells showed decreased CARD11 and IκB kinase activity (and, thus, reduced NF-κB activity) with accompanying IRF4 and SPi-B downregulation.

MOAs OF LENALIDOMIDE IN THE LYMPH NODE MICROENVIRONMENT

Recent studies have emphasized the importance of crosstalk between malignant and surrounding nonmalignant cells within localized tumor niches and the bone marrow.[34] Cells in the tumor microenvironment include macrophages, T cells, NK cells, dendritic cells, other myeloid-derived cells, and stromal cells. These cells not only provide a supportive network for tumor growth and progression but also can promote antitumor immune responses. Gene expression profiling (GEP) of 191 biopsy specimens from patients with FL who were treatment naive identified two immune response signature patterns (IR1 and IR2) predictive of survival.[35] These signatures reflected the biologic characteristics of nonmalignant immune cells rather than the tumor cell of origin, and were independent of clinically prognostic variables. IR1 comprised genes generally highly expressed in T cells, whereas IR2 encompassed genes highly expressed in monocytes. The two signatures ranked patients by survival-predictor scores with clearly differentiated quartiles ranging from 13.6 to 3.9 years of survival time, illustrating unique biologic characteristics of the host immune system microenvironment, their influence on tumors, and their association with survival time.

Colocalization of FL cells with $CD4^+$ T cells and follicular dendritic cells within follicular structures is necessary to support tumor cell proliferation.[36] FL cells demonstrated reduced proliferative activity in interfollicular regions.[37] Rather, FL cell proliferation depends on the surrounding immune system to support growth.[38,39] FL cells adapt to a germinal center B-cell (GCB) –like phenotype, including their dependence on immune cell interactions within the follicular microenvironment. Immune cells are influenced by both positive and negative regulatory molecules, governing whether antitumor responses or supportive signals are available for tumor cell growth and proliferation.

EFFECT OF LENALIDOMIDE ON T CELLS

T cells in the lymph node are influenced by the presence of lymphoma and display altered GEP and decreased immune synapse (IS) formation and effector function. GEP analysis of highly purified CD4 and CD8 tumor-infiltrating lymphocytes from baseline lymph node biopsies in 172 patients with FL who were treatment naive was altered compared with healthy donor reactive tonsils and peripheral blood.[40] Microarray analysis demonstrated multiple dysregulated genes in both CD4 and CD8. Multivariable analysis revealed that levels of expression of altered proteins on T cells were significantly prognostic for overall survival time and time to transformation in FL, further highlighting the role that lymphoma cells play in influencing the immune microenvironment and how this can affect outcome.

Tumor-infiltrating $CD4^+$ and $CD8^+$ T cells from lymphoma exhibit defective IS formation with antigen-presenting cells (APCs) compared with age-matched healthy donors,[10] resulting in impaired antigen presentation.[25,41] Ex vivo lenalidomide treatment of FL and autologous T cells repaired the F-actin IS activity and recruited tyrosine-phosphorylated protein independent of added antigen and irrespective of the patient's level of disease.[10]

When MCL and $\gamma\delta$ T cells were cocultured, lenalidomide induced reorganization of the actin cytoskeleton and cell surface markers and enhanced $\gamma\delta$ T-MCL cell IS formation, $\gamma\delta$ T-cell expansion, and cytotoxicity against MCL cells. These findings suggest that lenalidomide may have multiple mechanisms against MCL cells, including increased CD1c expression and enhancement of $\gamma\delta$ T cell–mediated cytotoxicity.

Although there is a considerable literature on the effects of lenalidomide on T-regulatory cells (Tregs), little has been published on lymphoma. In a murine model, lenalidomide was associated with reduced numbers of systemic Tregs, as well as myeloid-derived suppressor cells in tumor-bearing, but not naive, mice.[42] In a phase II study, Tregs were increased in the peripheral blood of patients with MCL compared with that of healthy volunteers, and they rose more after lenalidomide treatment.[43]

EFFECT OF LENALIDOMIDE ON NK CELLS

NK cells are important contributors to the innate immune response, with vital roles in clearing viruses, regulating dendritic cells, and rejecting malignant cells.[25] Lenalidomide treatment increased NK cell number, enhanced NK cell–induced cytotoxicity against cell lines,[26] and enhanced antibody-dependent cellular cytotoxicity (ADCC). The effects of lenalidomide-induced NK cell cytotoxicity and ADCC may be mediated indirectly via IL-2 production by T cells, as shown via the abrogation of NK cytotoxicity when IL-2 was inhibited with IL-2 antibody.[26]

Lenalidomide enhanced NK cell–mediated ADCC in several rituximab-treated NHL cell lines; the effects were dependent on rituximab binding to Fc-γ receptors and either IL-2 or IL-12 production.[12] Lenalidomide may stimulate NK cells by enhancing Fc-γ receptor signaling, which, in turn, elevates phosphorylated extracellular signal-regulated kinase and enhanced granzyme B and Fas ligand expression, contributing to enhanced ADCC.[12]

EFFECT OF LENALIDOMIDE ON DENDRITIC CELLS

Dendritic cells are APCs that are key messengers between the innate and adaptive immune systems, and function by processing and presenting antigens on their surface for recognition by T cells. Lenalidomide enhances expression of major histocompatibility complex class I and CD86 on bone marrow–derived murine dendritic cells, promotes uptake of tumor antigens by these APCs, and increases the efficiency of

antigen presentation to naive CD8$^+$ T cells.[44] The enhancement of dendritic cell function by lenalidomide may be important during immunosurveillance of cancer cells. Moreover, these findings suggest that lenalidomide may be useful in dendritic cell–based vaccines. The impact of lenalidomide on stromal cells, angiogenesis, and myeloid-derived suppressor cells, which have all been studied in myeloma, has yet to be fully addressed in studies in lymphoma.

EFFECT OF LENALIDOMIDE ON NORMAL HEMATOPOIESIS

Lenalidomide spares CD34$^+$ hematopoietic progenitor cells; indeed, lenalidomide has been shown to increase expansion of leukapheresis-derived CD34$^+$ cells.[11,45] The mechanism of lenalidomide-induced neutropenia has been associated with loss of PU.1, a key transcription factor involved in granulopoiesis.[45] Downregulation of PU.1 resulted in transient arrest of neutrophil maturation alongside accumulation of immature myeloid precursors and subsequent neutropenia.

EFFECT OF LENALIDOMIDE ON INFLAMMATORY CYTOKINES

Cytokines secreted by hematopoietic and nonhematopoietic cells are important factors for mediating innate and adaptive immune responses. Lenalidomide decreases the production of several proinflammatory cytokines (eg, TNF-α, IL-1, IL-6, and IL-12) and increases production of anti-inflammatory cytokine IL-10.[46,47] Modulation of these cytokines within the nodal microenvironment likely influences inflammatory responses, supports tumor growth and metastasis, and contributes to chemoresistance. The role of IL-6 was examined in preclinical studies of human MCL cells cocultured with peripheral blood mononuclear cells (PBMCs) or bone marrow–derived mononuclear cells.[48] IL-6 receptor ligation initiates a downstream kinase signaling cascade (eg, STAT3, Ras, phosphoinositide 3-kinase [PI3K]/Akt) to promote tumorigenesis. In some MCL cells, IL-6 secretion provides an autocrine growth signal. Bone marrow stromal cells secrete high levels of IL-6, and PBMCs secrete both IL-6 and the soluble gp80 IL-6 receptor subunit.[48] Because both stromal cells and PBMCs may be found in the MCL microenvironment, they may provide a paracrine source of IL-6 for supporting MCL growth. Consistent with this hypothesis, IL-6/gp80 knockdown effectively allows chemotherapy-induced apoptosis to occur on exogenous addition of IL-6 or gp80, rather than supporting tumor growth and proliferation. In line with IL-6 signaling, STAT3 phosphorylation and constitutive activation are dependent on autocrine and paracrine feedback loops for IL-6. The ability of lenalidomide to reduce IL-6 and STAT3 activity may provide mechanisms for reducing signaling within the MCL microenvironment, thereby inhibiting MCL cell growth and resistance to chemotherapy and promoting apoptosis.

Lenalidomide also stimulates production of IL-2 and other cytokines, including IFN-γ and TNF-α, and induces T-cell proliferation in the absence of CD28 stimulation.[25,46,49] Because T-cell receptor and costimulatory signals are required for IL-2 production, these observations suggest that lenalidomide may activate costimulatory-dependent signaling normally triggered by CD28. Consistent with this hypothesis, lenalidomide increases tyrosine phosphorylation of CD28 in the intracellular domain of T cells in the absence of costimulatory molecules, and stimulates NF-κB activation downstream from CD28.[50] Moreover, lenalidomide promotes nuclear translocation and binding of nuclear factor of activated T cells 2 and activator protein-1 to the IL-2 promoter, a process dependent on PI3K signaling, leading to enhanced IL-2 production.[26]

Although IL-2 and IL-12 are not required for monocyte-mediated cell lysis and ADCC for synergistic activity between lenalidomide and rituximab, enhancement of ADCC by lenalidomide is associated with increased cytokines on NK cells, including IL-8, monocyte chemotactic protein-1, RANTES (regulated on activation, normal T cell expressed and secreted), inducible protein-10, granulocyte-macrophage colony-stimulating factor, and with decreased IL-6.

COMBINATIONS OF LENALIDOMIDE WITH OTHER TREATMENTS

Lenalidomide may enhance or act synergistically with other treatments with complementary MOAs. Lenalidomide with dexamethasone and rituximab has been shown to synergistically inhibit growth and induce apoptosis of established MCL cell lines and ex vivo MCL cells from patients with relapsed or refractory MCL.[13,23] Mechanistically, lenalidomide enhanced dexamethasone-induced G_0-G_1 cell-cycle arrest through an intrinsic mitochondrial pathway of apoptosis, evidenced by increased Bcl-2 phosphorylation; upregulation of the proapoptotic proteins Bax, BAD, and Bim; activation of caspase-3 and -9; and cleavage of poly(ADP-ribose) polymerase (Table 1). Lenalidomide enhanced rituximab-induced apoptosis by upregulating c-Jun N-terminal protein kinase phosphorylation and activating the mitochondrial-derived apoptotic pathway.[13] In addition, lenalidomide increased the number of NK cells 10-fold and augmented rituximab-dependent NK cell–mediated cytotoxicity by increasing CD16 expression on a subset of NK cells considered key effector cells for ADCC in PBMCs. This increase was positively associated with elevated IFN-γ, TNF-α, and perforin expression. These preclinical findings translated into prolonged survival for severe combined immunodeficient mice inoculated with Mino MCL cells and treated with combined lenalidomide and rituximab; overall tumor burden was decreased two-fold ($P < .01$), and survival time significantly improved versus control or treatment with either single agent ($P < .05$). Further examination of tumor growth mechanisms demonstrated that after 21 days of treatment, lenalidomide increased the number of splenic NK cells 10-fold.

The synergistic effects of lenalidomide with anti-CD20 monoclonal antibodies appear to be independent of CD20 expression and density on the surface of different types of lymphoma cells.[27,51] In a severe combined immunodeficient mouse xenograft model bearing a disseminated Raji lymphoma, administration of lenalidomide significantly increased the number of circulating CD49b$^+$ NK cells from day 5 to day 10, whereas depleting NK cells with anti–IL-2 receptor monoclonal antibody before inoculation with lymphoma cells abrogated the antitumor effects of lenalidomide with or without rituximab. In a subsequent study, lenalidomide increased the infiltration of NK cells into tumor sites compared with vehicle-treated animals. Notably, infiltration was directed into the central part of the tumor in lenalidomide-treated animals but confined to the tumor periphery in control animals.[51] In an effort to explore other cellular effects in the

Table 1. Preclinical Mechanistic Rationale for Combinations With Lenalidomide

Combination Treatment	Known MOAs
Lenalidomide + rituximab[13,23,51]	NK cell ↑ NK-cell number and activity ↑ CD16 expression Malignant B cell G_0-G_1 cell cycle arrest ↑ *p*-Bcl-2, Bax, BAD, Bim Caspase-3 and -9 activation PARP cleavage *p*-JNK Antiangiogenic activity Microenvironment ↑ IFN-γ, MCP-1, TNF-α, and perforin
Lenalidomide + XmAb5574 (MOR208)[52]	NK cell Enhanced ADCC
Lenalidomide + SGN-40[53]	NK cell Enhanced ADCC Malignant B cell ↑ CD40 expression ↑ cytotoxicity
Lenalidomide + bortezomib[54]	Malignant B cell ↑ cytotoxicity
Lenalidomide + ibrutinib[33]	Malignant B cell ↓ IRF4 levels ↓ tumor growth
Lenalidomide + anti–PD-1 or anti–PD-L1[29]	Proposed preclinical rationale for enhanced checkpoint control inhibition

Abbreviations: ADCC, antibody-dependent cellular cytotoxicity; IFN-γ, interferon-gamma; IRF4, interferon regulatory factor 4; JNK, c-Jun N-terminal protein kinase; MCP-1, monocyte chemotactic protein-1; MOAs, mechanisms of action; NK, natural killer; PARP, poly(ADP-ribose) polymerase; PD-1, programmed death 1; PD-L1, programmed death ligand 1; TNF-α, tumor necrosis factor-alpha.

immune microenvironment, NK cell activity was associated with dendritic cell stimulation and alterations in the dendritic cell cytokine milieu, as shown by increased monocyte chemotactic protein-1, TNF-α, and IFN-γ, collectively augmenting rituximab-mediated ADCC. Lenalidomide also exhibited antiangiogenic activity in the Raji xenograft model, as shown by significantly decreased tumor microvessel density compared with vehicle-treated animals (50 *v* 109 vessels/5 low-power fields; $P = .009$).

The ability to augment NK cells and enhance rituximab-mediated cytotoxic mechanisms suggests that lenalidomide may also work cooperatively in combination with monoclonal antibodies for other surface antigens. Targeting other cell surface antigens provides alternative pathways to engage, as well as strategies for overcoming potential adaptive or acquired resistance to rituximab (Table 1).[55] In preclinical studies, ex vivo chronic lymphocytic leukemia (CLL) cells enhanced NK-mediated ADCC when lenalidomide was combined with XmAb5574 (MOR208), a humanized monoclonal antibody targeting CD19 found on the surface of normal and transformed B cells and involved in B-cell receptor signaling.[52] Another example is CD40, a member of the TNF receptor superfamily mainly expressed on B cells and other APCs (eg, dendritic cells and macrophages).[55] Lenalidomide increased CD40 expression and enhanced the direct cytotoxicity of anti-CD40 monoclonal antibody SGN-40 in CLL cells.[53] Moreover, lenalidomide enhanced anti–CD40-mediated ADCC after treatment with NK cells or PBMCs isolated from patients with CLL.

Given its unique MOAs, lenalidomide is expected to provide complementary effects with treatments other than monoclonal antibodies. As mentioned previously, lenalidomide acted synergistically with dexamethasone in promoting growth inhibition and apoptosis in MCL cells.[23] Similarly, lenalidomide synergistically enhanced bortezomib-induced cytotoxicity and apoptosis in FL and MCL cells.[54] Lenalidomide also displayed synergistic activity in combination with ibrutinib, a Bruton's tyrosine kinase inhibitor, which blocks B-cell receptor signaling.[33] Ibrutinib reduced IRF4 levels in ABC DLBCL cells, but when evaluated in combination with lenalidomide, IRF4 was decreased to undetectable levels. Lenalidomide and ibrutinib acted synergistically in inducing ABC DLCBL cell cytotoxicity in vitro, and the combination was effective in arresting tumor growth of OCI-Ly10 ABC DLBCL xenografts. These findings underscore the feasibility of lenalidomide combinations with other B-cell receptor pathway inhibitors, including the PI3Kδ inhibitor idelalisib and the spleen tyrosine kinase inhibitor entospletinib (GS-9973). Because checkpoint control inhibitors show activity in lymphoma, and because lenalidomide downregulates expression of PD-L1 on the surface of lymphoma cells,[29] there is a rationale for exploration of combining lenalidomide with anti–PD-1 or anti–PD-L1 antibodies to attempt to fully block the pathway.

TRANSLATION OF PRECLINICAL DATA TO CLINICAL STUDIES

The MOAs of lenalidomide identified in experimental studies appear to translate into therapeutic relevance in the clinical setting, both as monotherapy and in combination with other agents. Single-agent lenalidomide produced durable responses in patients with relapsed/refractory indolent or aggressive NHL in several phase II trials.[56-58] Subset analyses demonstrated that lenalidomide was active across multiple NHL subtypes; lenalidomide exhibited higher responses in non-GCB DLBCL compared with GCB[30] and showed particularly promising activity in MCL.[59,60] These latter findings led to a prospective international phase II trial known as MCL-001 (EMERGE [A Phase 2, Multicenter, Single-Arm, Open-Label Study to Determine the Efficacy and Safety of Single-Agent Lenalidomide (Revlimid) in Patients With Mantle Cell NHL Who Have Relapsed or Progressed After Treatment With Bortezomib or Are Refractory to Bortezomib]), which enrolled 134 patients with relapsed/refractory MCL.[56] Lenalidomide produced a 28% overall response rate (8% complete response) in patients. The duration of response lasted for a median of 16.6 months, notable given that patients were heavily pretreated and 60% refractory to bortezomib. Pooled data analyses for patients with MCL from MCL-001 and earlier phase II studies (NHL-002 and NHL-003) confirmed the clinical activity of single-agent lenalidomide and supported its approval by the US Food and Drug Administration for relapsed or refractory MCL after two earlier therapies, one of which included bortezomib.[56,59-61]

Recent reports of an increased risk of second primary malignancies (SPMs) in patients with multiple myeloma after lenalidomide maintenance have piqued interest in understanding the underlying mechanism that contributes to the emergence of SPMs.[62,63] Little has been reported in studies of lenalidomide in lymphoma because their follow-up times are shorter than those for multiple myeloma. The

Table 2. Ongoing Clinical Studies of Lenalidomide Combinations in Lymphoma

Clinical Study	Type of NHL	Phase	Treatment	Primary End Point
Previously untreated patients				
R2-ibrutinib (NCT01829568)	FL	I	R2-ibrutinib	MTD
MCL4 (LENA-BERIT; NCT00963534)	MCL (age > 65 years)	I/II	R2-B	Ph I: MTD Ph II: PFS
Lenalidomide + DA-EPOCH-R (NCT02213913)	MYC-associated B-cell lymphoma	I/II	Lenalidomide + DA-EPOCH-R	Ph I: MTD Ph II: PFS
SAKK 35/10 (NCT01307605)	FL	II	R ± lenalidomide	CR
ECOG E1412 (NCT01856192)	DLBCL	II	R2CHOP *v* R-CHOP	PFS
ROBUST (DLC-002) (NCT02285062)	ABC-type DLBCL	III	R2CHOP *v* R-CHOP	PFS
LYSA SENIOR (NCT02128061)	CD20+ DLBCL, age ≥ 80 years	III	R2miniCHOP *v* R-miniCHOP (subcutaneous R)	OS
Maintenance				
Lenalidomide maintenance post-chemotherapy (NCT01035463)	Chemotherapy-resistant or high-risk NHL	I/II	Lenalidomide maintenance post-BEAM ± rituximab and ASCT	Ph I: MTD Ph II: EFS, ORR, OS
FIL R2-B (NCT01737177)	MCL	II	Induction: R2-B Maintenance: lenalidomide	CR, PFS
ECOG E1411 (NCT01415752)	MCL	II	Induction: RB *v* RBV Maintenance: R2 *v* R	PFS
3-Arm randomized ECOG E2408 (NCT01216683)	High-risk stage II-IV FL	II	Arm I: BR to R Arm II: BVR to R Arm III: BR to lenalidomide to R	CR, DFS
RELEVANCE (NCT01650701)	FL	III	Induction: R2 *v* R-chemotherapy Maintenance: R2 (post-R2) *v* R (post-R-chemotherapy)	CR/CRu at 30 months, PFS
MCL R2 Elderly (NCT01865110)	Older MCL	III	Induction: R-CHOP + R-HAD *v* R-CHOP Maintenance: R2 *v* R	PFS
FIL MCL-0208 (NCT02354313)	Advanced MCL	III	Induction: R-high-dose chemotherapy and ASCT Maintenance: lenalidomide *v* observation	PFS
MAGNIFY (NHL-008) (NCT01996865)	FL, MZL, MCL	IIIb	Induction: R2 Maintenance: lenalidomide *v* R	PFS
Relapsed/refractory patients				
Lenalidomide + brentuximab vedotin (NCT02086604)	DLBCL	I	Lenalidomide + brentuximab vedotin	MTD
Lenalidomide + temsirolimus (NCT01076543)	NHL and HL	I/II	Lenalidomide + temsirolimus	Ph I: MTD Ph II: ORR, CR
R2 + chemotherapy (NCT01788189)	$CD20^+$ NHL (not MCL)	I/II	R2 + methotrexate, leucovorin, and cytarabine	Ph I: MTD Ph II: ORR
R2 + carfilzomib (NCT01729104)	MCL	I/II	R2 + carfilzomib	Ph I: MTD Ph II: ORR
Lenalidomide ± idelalisib (NCT01838434)	MCL	I/II	R2 with idelalisib; amended to lenalidomide ± idelalisib	Ph I: MTD Ph II: PFS
LR-ESHAP (NCT02340936)	DLBCL	I/II	Salvage LR-ESHAP in candidates for HDT and ASCT	Ph I: MTD Ph II: ORR
Dose-finding lenalidomide + obinutuzuab (NCT01995669)	iNHL	I/II	Lenalidomide + obinutuzumab	Ph I: MTD Ph II: ORR
GALEN (NCT01582776)	DLBCL, MCL	Ib/II	Lenalidomide + obinutuzumab	Ph I: MTD Ph II: ORR
Lenalidomide + ibrutinib (NCT01955499)	NHL	I	Lenalidomide + ibrutinib	MTD
Lenalidomide + ibrutinib ± R (NCT02077166)	DLBCL	Ib/II	Lenalidomide + ibrutinib ± rituximab	Ph I: MTD Ph II: ORR
Lenalidomide + ibrutinib + DA-EPOCH-R (NCT02142049)	DLBCL	Ib/II	Lenalidomide + ibrutinib + DA-EPOCH-R	Ph I: MTD Ph II: ORR
Lenalidomide + romidepsin (NCT01755975)	Lymphoma and myeloma	Ib/IIa	Lenalidomide + romidepsin	MTD, safety
R2 + carfilzomib + romidepsin (NCT02341014)	B- and T-cell lymphoma	Ib/IIa	R2 + carfilzomib + romidepsin	Ph I: MTD Ph II: ORR
LEGEND (NCT02060656)	DLBCL	II	LR-GEM *v* R-GEM-P	CR
AUGMENT (NHL-007) (NCT01938001)	FL and MZL	III	R2 *v* R	PFS

Abbreviations: ABC, activated B cell; ASCT, autologous stem-cell transplantation; B, bendamustine; BEAM, carmustine, etoposide, cytarabine, melphalan; CHOP, cyclophosphamide, doxorubicin, vincristine, prednisone; CR, complete response; CRu, CR unconfirmed; DA, dose adjusted; DFS, disease-free survival; DLBCL, diffuse large B-cell lymphoma; ECOG, Eastern Cooperative Oncology Group; EFS, event-free survival; EPOCH, etoposide, prednisone, doxorubicin, cyclophosphamide, vincristine; ESHAP, etoposide, methylprednisolone, cisplatin, and cytarabine; FIL, Fondazione Italiana Linfomi; FL, follicular lymphoma; GEM(-P), gemcitabine, methylprednisolone (cisplatin); HAD, high-dose cytarabine and dexamethasone; HDT, high-dose therapy; iNHL, indolent non-Hodgkin lymphoma; LR, lenalidomide + rituximab; LYSA, Lymphoma Study Association; MCL, mantle-cell lymphoma; MTD, maximum tolerated dose; MZL, marginal zone lymphoma; NHL, non-Hodgkin lymphoma; ORR, overall response rate; OS, overall survival; PFS, progression-free survival; Ph, phase; R, rituximab; R2, lenalidomide + rituximab; V, bortezomib.

MCL-001 study of single-agent lenalidomide identified invasive SPM rates consistent with the expected background occurrences reported by the SEER program for individuals 65 years of age and older.[56] Clear elucidation of the mechanisms involved in SPMs appears to be confounded by patients' prior exposure to multiple lines of therapy, making insights into the mechanisms involved speculative. Studies in multiple myeloma suggest that prior or concurrent exposure to the alkylating agent melphalan may increase the risk of developing SPMs through its DNA-damaging properties and potential synergy with lenalidomide's inhibition of DNA repair mechanisms (possibly via cereblon inhibition).[62,63] An alternate potential mechanism might include disruption of viral latency, as has been suggested for Epstein-Barr virus in preclinical studies of B cells.[64] For patients receiving lenalidomide for a long period of time, continued study is needed for better insight into the mechanisms involved in the development of SPMs. Clinically in lymphoma, a disease plagued by probable relapse, it is important to consider lenalidomide maintenance in the context of the risk to benefit ratio to the patient, as the risk of progressive disease or death is much greater than that of developing an SPM.

The single-agent activity of lenalidomide, combined with preclinical evidence of its ability to enhance the antitumor activity of rituximab, led to early trials of combination rituximab and lenalidomide (R2) therapy in first-line and relapsed settings. Enhanced activity has been observed with R2 in MCL,[28,65] DLBCL,[66-68] FL,[69,70] and indolent NHL.[71,72] A recently published study of R2 shows evidence of overcoming rituximab-resistance in indolent NHL and MCL.[73] The feasibility of administering lenalidomide or R2 in combination with either dexamethasone or bortezomib in patients with MCL was also demonstrated.[74-77] First-line R2 plus cyclophosphamide, doxorubicin, vincristine, prednisone (R2CHOP) produced encouraging response rates and progression-free survival times in patients with DLBCL and FL in several clinical trials, particularly when compared with historical data for R-CHOP alone.[78-80] Notably, patients with GCB and non-GCB DLBCL phenotypes achieved similar objective response rates with R2CHOP.[79] The combination of R2 with bendamustine is being explored as a first-line option in elderly patients with MCL (Table 2).[81] Studies of R2 with multiple combination partners are ongoing in phase I and II trials. Recent findings on the combination of R2 with idelalisib in relapsed/refractory NHL (A051201; NCT01838434) indicate that combined mechanisms of action may not always be complementary.[82] This triple combination led to unexpected toxicity suggestive of cytokine release syndrome (a rare event associated with rituximab), and the dosing regimen has been modified to include lenalidomide plus idelalisib without rituximab.

Numerous clinical trials are currently underway to further elucidate how to best exploit lenalidomide pathways in NHL treatment (Table 2). RELEVANCE (NCT01650701) is a phase III open-label study comparing R2 with rituximab-based immunochemotherapy followed by rituximab or R2 maintenance in 1,000 previously untreated FLs. The primary outcomes are complete response rate at 30 months and progression-free survival time. GALEN (NCT01582776) is a phase IB/II study evaluating the combination of lenalidomide with obinutuzumab in relapsed or refractory FL, DLBCL, and MCL.[83] The phase IB component recommended a dosage of 20 mg/d lenalidomide in combination with fixed-dose obinutuzumab in FLs; the ongoing phase II study will evaluate efficacy and safety in relapsed or refractory FLs and aggressive NHLs.

In conclusion, lenalidomide is an orally active immunomodulatory drug that has direct antineoplastic activity and indirect effects mediated through multiple types of immune cells found in the tumor microenvironment, including B, T, NK, and dendritic cells (Fig 1).[6,9-13,25-28] Recently, the E3 ubiquitin ligase cereblon was identified as a molecular target that likely underlies the effects of lenalidomide on tumor cells as well as on cells in the tumor microenvironment. On the basis of its overall profile, lenalidomide was evaluated initially as monotherapy in patients with relapsed or refractory NHL and exhibited activity across multiple lymphoma subtypes. The observation of durable responses in patients with MCL provided a focus for clinical development and led to approval of lenalidomide for relapsed/refractory MCL. Preclinical studies have shown that lenalidomide has enhanced or synergistic activity with other agents, including rituximab, dexamethasone, bortezomib, and B-cell receptor pathway inhibitors, reflecting its unique mechanisms of action. These experimental observations, combined with the single-agent activity observed clinically, provided the basis for evaluation of R2 and other combination regimens across a variety of treatment phases for both indolent and aggressive NHL types. Clinical results highlight the potential activity for lenalidomide-based combinations. Continued understanding of the mechanisms of lenalidomide against tumor cells and cells in the tumor microenvironment will help optimize lenalidomide's therapeutic effects for patients with NHL overall and on an individual basis.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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AUTHOR CONTRIBUTIONS

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Mechanisms of Action of Lenalidomide in B-Cell Non-Hodgkin Lymphoma

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