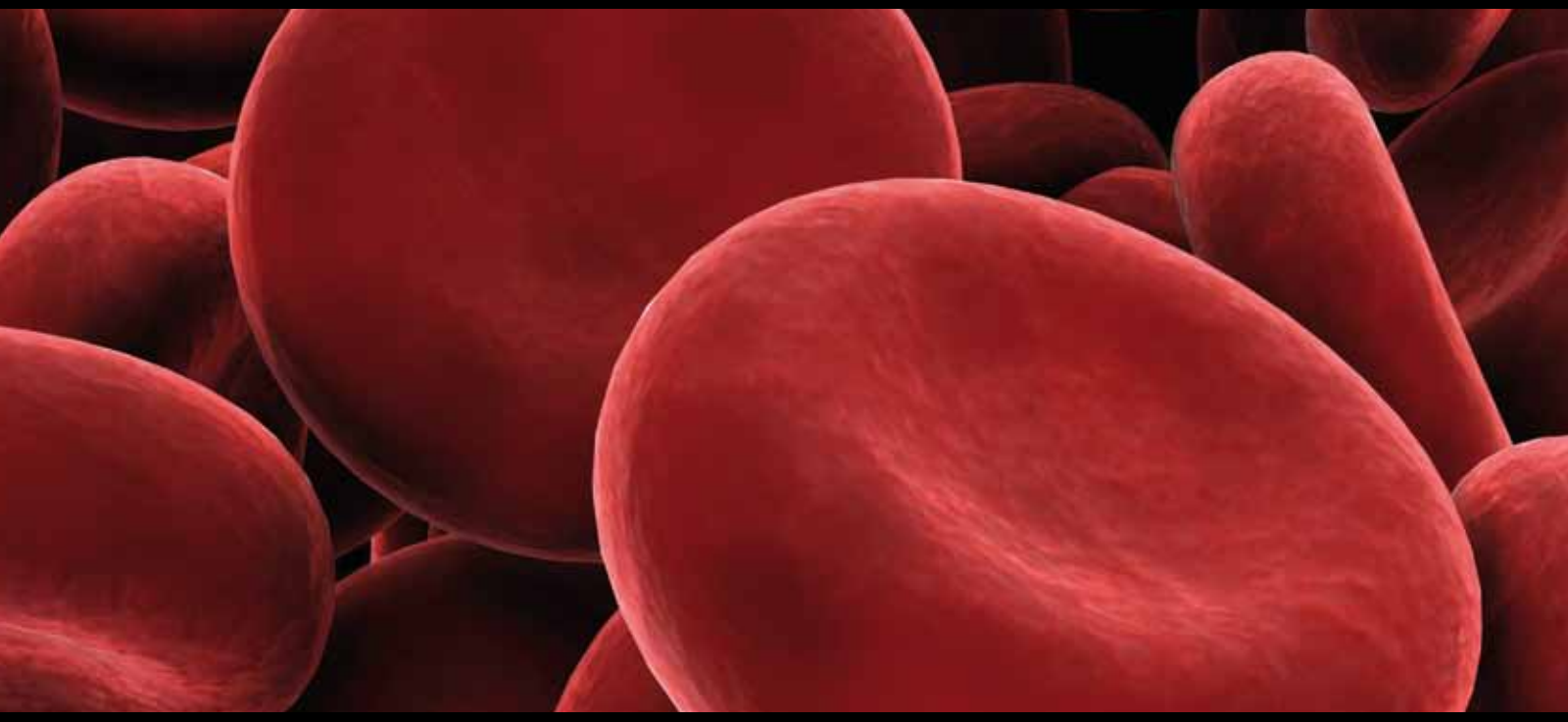
Advances in Hematology

Lenalidomide in the Treatment of Lymphoproliferative Disorders and Multiple Myeloma

Guest Editors: Anna Marina liberati, Umberto Vitolo, Antonio P. Palumbo, and Agostino Cortelezzi



Lenalidomide in the Treatment of Lymphoproliferative Disorders and Multiple Myeloma

Advances in Hematology

Lenalidomide in the Treatment of Lymphoproliferative Disorders and Multiple Myeloma

Guest Editors: Anna liberati, Umberto Vitolo, Antonio Palumbo, and Agostino Cortelezzi

Editorial Board

Contents

Hindawi Publishing Corporation
Advances in Hematology
Volume 2013, Article ID 812605, 2 pages
http://dx.doi.org/10.1155/2013/812605

Editorial

Lenalidomide in the Treatment of Lymphoproliferative Disorders and Multiple Myeloma

Anna Marina Liberati,[1] Umberto Vitolo,[2] Antonio Palumbo,[2] and Agostino Cortelezzi[3]

[1] *Medical Genetics Unit, University of Perugia, S. Andrea delle Fratte, Perugia, Italy*
[2] *Dipartimento di Ematologia, Azienda Ospedaliero-Universitaria San Giovanni Battista di Torino, Torino, Italy*
[3] *Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Pad. Marcora, Via F. Sforza 35, 20122 Milano, Italy*

Correspondence should be addressed to Anna Marina Liberati; marinal@unipg.it

Received 27 December 2012; Accepted 27 December 2012

Immunomodulating agents (ImiDs) are a novel class of anticancer drugs that have demonstrated impressive antitumor activity in various malignant disorders. Of this class, most recent research has been focused on the remarkably active agent, lenalidomide. Lenalidomide was designed to enhance immunologic and anticancer properties while potentially decreasing neurotoxic and teratogenic adverse effects of the parent compound thalidomide. The introduction of this novel agent has broadened the therapeutic landscape of hematologic malignant disorders including multiple myeloma (MM) and, more recently, other B-cell neoplasms. In this issue, we focused on mechanisms of action and results from clinical investigation that report the relevance of lenalidomide for the treatment of B-cell disorders including MM, chronic lymphocytic leukaemia (CLL), and non-Hodgkin's lymphomas by offering new mechanisms for targeting these diseases. In particular, papers defining the role of lenalidomide as part of the induction therapy before hematopoietic stem cells transplantation (HSCT) as well as its use as extended maintenance therapy post-HSTC in patients with MM will be of great interest.

In this special issue, we invited papers on potential topics including, but are not limited to, lenalidomide: mechanism of action, lenalidomide as part of the induction therapy before HSCT and its use as consolidation and maintenance therapy of multiple myeloma, lenalidomide in chronic lymphocytic leukaemia, lenalidomide in diffuse large B-cell lymphoma, and lenalidomide in mantle cell and low-grade non-Hodgkin's lymphomas. Ten articles discuss the role of lenalidomide in the treatment of malignant disorders.

The paper entitled "*Lenalidomide in diffuse large B-cell lymphoma*" by C. Thieblemont et al. presents the biological rational for the use of lenalidomide in DLBCL in light of recent advances in the pathophysiology of the disease and the therapeutic results of the most recent trials published in literature or reported in meetings in relapsed/refractory situations as well as in first-line treatment. The paper entitled "*Practical approaches to the use of lenalidomide in multiple myeloma: a Canadian consensus*" by D. Reece et al. discuss the use of lenalidomide in the management of RRMM in the Canadian environment. The Chair (DR) invited panelists to research and write individual sections of the paper. The various sections were collected, compiled, and distributed to the group, which discussed the paper via web conference. Panelists subsequently generated a revised draft in which all sections included specific clinical guidance (i.e., practice considerations). The revised paper was discussed at a final web conference, where all practice recommendations were considered, revised as appropriate, and ultimately adopted by the full panel; any areas of disagreement are noted. Celgene Canada provided the impetus for the panel to pursue this project freely and independently. Celgene Canada supported the process throughout, including support for the participation of a medical writer (JA) in preparing this paper. The opinions represented here are solely those of the physician-panelists.

The paper entitled "*Biological activity of lenalidomide and its underlying therapeutic effects in multiple myeloma*" by R. Martiniani et al. is about the direct and indirect antitumor effects of lenalidomide on malignant plasmacells, bone marrow microenvironment, bone resorption, and host's immune response. The molecular mechanisms and targets of lenalidomide remain largely unknown, but recent evidence shows cereblon (CRBN) as a possible mediator of its therapeutical effects.

The paper entitled "*Molecular action of lenalidomide in lymphocytes and hematologic malignancies*" by J. M. McDaniel et al. summarizes the current information about lenalidomide in proliferative neoplasms and describes our understanding of the molecular mechanism of action in lymphocytes. Based on the overwhelming success of lenalidomide for the treatment of several hematologic malignancies, there is potential for therapies that augment host immune responses to be extended from the relapsed and refractory setting, to primary therapy.

The paper entitled "*Secondary primary malignancies in multiple myeloma: an old nemesis revisited*" by J. Yang et al. reviews the developmental history of myeloma therapy, with particular emphasis on the risk of secondary cancers, and examine the available data with regard to the risk of SPMs seen with lenalidomide. We also speculate about the mechanism(s) by which lenalidomide could increase the risk of second cancers. To conclude, we make some recommendations about how our current understanding affects our treatment decisions and suggest directions for future research. As new data emerge about lenalidomide and the risk of SPMs, it is our hope that this paper will help to put that information in proper perspective.

The paper entitled "*Lenalidomide in the treatment of young patients with multiple myeloma: from induction to consolidation/maintenance therapy*" by B. Lupo et al. presents an overview of the results achieved with lenalidomide-containing combinations in patients eligible for high-dose therapies, namely, young patients. The advantages obtained should always be outweighed with the toxicity profile associated with the regimen used. Therefore, here, we will also provide a description of the main adverse events associated with lenalidomide and its combination.

The paper entitled "*Lenalidomide in the treatment of chronic lymphocytic leukemia*" by A. Cortelezzi et al. provides a comprehensive summary regarding mechanism of action, efficacy, and safety of lenalidomide in CLL patients. Relevant clinical trials using lenalidomide alone or in combination are discussed. Lenalidomide shows good activity also in relapsed/refractory or treatment-naive CLL patients. Definitive data from ongoing studies are needed to validate overall and progression-free survival. The toxicity profile might limit lenalidomide use because it can result in serious side effects, but largely controlled by gradual dose escalation. Further understanding of the exact mechanism of action in CLL will allow more efficacious use of lenalidomide alone or in combination regimens.

The paper entitled "*Lenalidomide in diffuse large B-cell lymphomas*" by A. Chiappella et al. reports the most relevant clinical trials for the use of lenalidomide in DLBCL. Monotherapy with lenalidomide showed an activity in terms of overall response rate, with acceptable hematological and extrahematological toxicities in relapsed/refractory aggressive NHL. The role of lenalidomide as salvage therapy in both cell of origin patterns in DLBCL (germinal center B cell/activated B cell) was reported in preliminary data. Preliminary data regarding the role of lenalidomide in addition to chemoimmunotherapy (R-CHOP) in first-line clinical trials were discussed; data of safety, feasibility, and efficacy were promising.

The paper entitled "*Therapeutic activity of lenalidomide in mantle cell lymphoma and indolent non-Hodgkin's lymphomas*" by M. Gunnellini et al. discusses the role of lenalidomide in the therapeutic armamentarium of patients with indolent NHL or MCL.

The tenth paper entitled "*Lenalidomide before and after autologous hematopoietic stem cell transplantation in multiple myeloma*" by S. A. Tuchman et al. summarizes existing data that pertains to lenalidomide in the specific context of ASCT, and we share our thoughts on how our own group applies these data to approach this complex issue clinically.

Anna Marina Liberati
Umberto Vitolo
Antonio Palumbo
Agostino Cortelezzi

Hindawi Publishing Corporation
Advances in Hematology
Volume 2012, Article ID 861060, 8 pages
doi:10.1155/2012/861060

Review Article

Lenalidomide in Diffuse Large B-Cell Lymphoma

Catherine Thieblemont,[1] Marie-Hélène Delfau-Larue,[2] and Bertrand Coiffier[3]

[1] *Hematology and Oncology Department, Hôpital Saint-Louis, AP-HP and IUH, INSERM U728, University Paris VII, 75010 Paris, France*
[2] *Immunbiology Department, Groupe Hospitalier Henri Mondor-Albert Chenevier, AP-HP, 94000 Créteil, France*
[3] *Hematology, Centre Hospitalier Lyon Sud, 69230 Pierre-Bénite, France*

Correspondence should be addressed to Catherine Thieblemont, catherine.thieblemont@sls.aphp.fr

Received 1 March 2012; Accepted 22 August 2012

Academic Editor: Umberto Vitolo

Diffuse large B-cell lymphoma (DLBCL) is the most common form of non-Hodgkin's lymphoma (NHL) in adults. Even if the natural history of DLBCL has been improved with the advent of immunochemotherapy, the survival results obtained with current treatment options clearly indicate that new agents or novel approaches are needed. Lenalidomide (Revlimid, Celgene Corporation, Summit, NJ, USA), an analogue of thalidomide, is an immunomodulatory drug with pleiotropic mechanisms of action potentially adding to immunochemotherapy. We present here the biological rational for the use of lenalidomide in DLBCL in light of recent advances in the pathophysiology of the disease and the therapeutic results of the most recent trials published in literature or reported in meetings in relapsed/refractory situations as well as in first-line treatment.

1. Introduction

The incidence of non-Hodgkin's lymphoma (NHL) has been increasing worldwide during the last 40 years and accounts for 4% of all cancer diagnoses. Among the NHL, diffuse large B-cell lymphoma (DLBCL) is the most common form in adults, accounting for 25–30% of NHL cases [1] and is recognized as an entity since the first classification of NHL [2]. However, complexity and heterogeneity of the disease have been demonstrated over the past ten years, first by the most recent WHO classification including not less than 13 different subentities [3], and second by the biological analyses, particularly the gene expression profiling analyses dividing the disease in at least two molecular subgroups, that is, germinal center B-cell-like (GBC)- and activated B-cell-like (ABC)-DLBCL [4]. These biological analyses have been able not only to capture the molecular heterogeneity of tumor cells [4], but also to demonstrate the existence of a complex interaction between the tumor and its microenvironnement involving multiple signaling pathways and regulatory mechanisms [5].

Standard first-line treatment for DLBCL patients is based since 2002 on the association of rituximab and CHOP (cyclophosphamide, vincristine, doxorubicin, and prednisone) [6]. Even if the natural history of DLBCL has been improved with treatments based on this association, there is clearly a need of improvement of long-term results. With R-CHOP, the expected 5-year and 10-year OS rates are, respectively, 58% and 43.5% [7, 8]. To improve these results, several changes to conventional R-CHOP have emerged either in shortening intervals between cycles [9] or giving alternative regimens with intensified doses of chemotherapy [10]. R-EPOCH (etoposide doxorubicin, vincristine associated with bolus cyclophosphamide, prednisone) has demonstrated to give an OS rate of 73% [11]. In patients <60 years old, GELA has developed R-ACVBP (doxorubicin, cyclophosphamide, vindesine, bleomycin, prednisone) given every 14 days [10] and subsequently demonstrated a superiority of R-ACVBP compared to R-CHOP in several additional randomized studies [12, 13]. However none of these intensified regimens are appropriate for patients with comorbidities or with older age, and the survival results obtained with these current treatment options for patients with DLBCL indicate that new treatment modalities are needed.

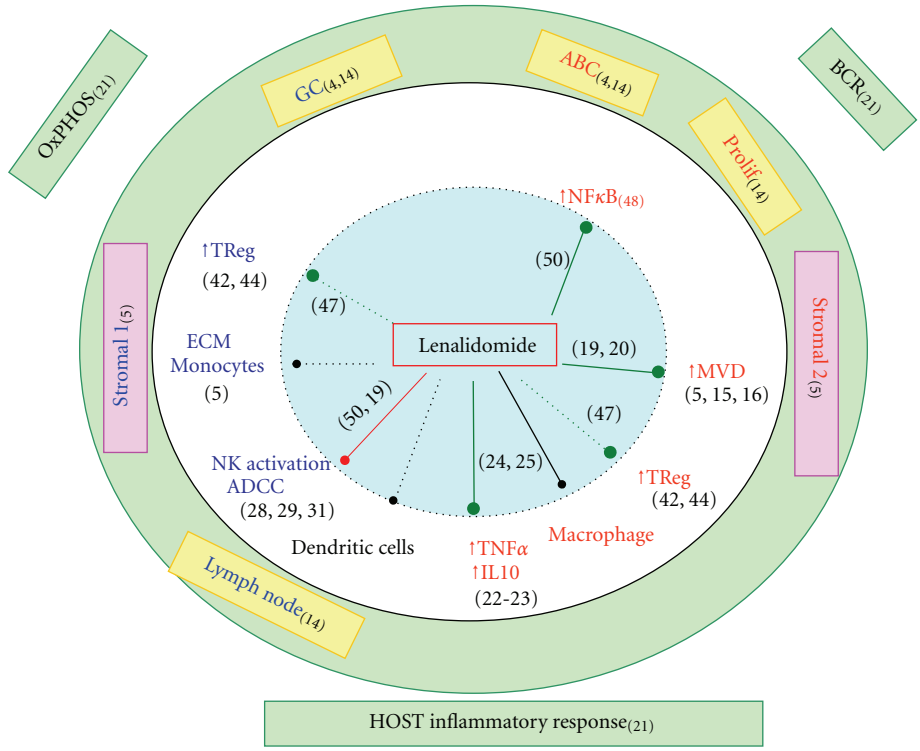


Figure 1: Biological effects of lenalidomide. Colored insets show the main transcriptomic signatures described in DLBCL. Just outside the circle are the signatures with prognostic impact. Inside the circle are indicated the factors studied in DLBCL, either with bad (red characters), good (blue), or undetermined (black) prognostic impact. Arrows indicate a negative (green), positive (red), or undetermined (black) regulation of lenalidomide on those factors in DLBCL. ECM: extracellular matrix components. MVD: microvascular density.

2. Part I: Biological Relevance of Lenalidomide for the Treatment of DLBCL

The antitumoral properties of lenalidomide in hematologic area (review in [14]) have been first studied in myeloma, and more recently in myelodysplastic syndromes and lymphomas, and can be grouped in 3 categories: (i) anti-angiogenesis, (ii) immune modulation, and (iii) direct tumor cell toxicities. Some progress on the understanding of DLBCL physiopathology enables us to speculate on biological pathways that could be targeted by lenalidomide (Figure 1).

2.1. Antiangiogenic Effects. Beside the two biologically and clinically distinct GC and ABC molecular subtypes of DLBCL defined by a tumoral cell signature [4, 15], different stromal gene signatures have been linked to prognosis [5, 15]. One was associated with reduced survival, includes markers of endothelial cells, regulators of angiogenesis, and was shown to correlate with a quantitative measure of blood-vessel density (MVD) in tumor [5]. Unfavorable prognostic of high MVD has been confirmed on tissue microarray (TMA) in CHOP [16], and R-CHOP [17] treated DLBCL patients. Vascular endothelial growth factor (VEGF)-A is the most prominent proangiogenic factor and value of serum VEGF has prognosis impact in lymphomas (review in [18]). However, the pathogenic association of MVDs and VEGF expression by tumor cell in DLBCL remain controversial [19]. On the basis of these results and on results on *in vivo* model [20], it can be hypothesized that patients with DLBCL characterized by increased tumor MVD may benefit from antiangiogenic effect of lenalidomide [21].

2.2. Effects on the Immune Microenvironment

2.2.1. Action on Proinflammatory Cytokines and T-cell Activation. Using whole genome arrays, and multiple clustering methods, Monti and colleagues have identified 3 discrete subsets of DLBCL [22], including one characterized by increased expression of T-natural killer cell receptor and activation pathway components, complement cascade members, macrophage/dendritic cell markers, and inflammatory mediators which has been referred as "host response" (HR) signature. Consistent with the signature of an ongoing inflammatory/immune response, HR tumors had increased expression of interferon-induced genes, tumor necrosis family (TNF) ligands and receptors, cytokine receptors, adhesion molecules, and extracellular matrix components. The role of microenvironment associated cytokine in DLBCL physiopathology has been approached in another way. Host immune gene polymorphisms including TNFα and IL10 predict late survival in DLBCL patients in the prerituximab era [23]. In accordance, the combined elevation of both TNFα and IL-10 in sera of DLBCL patients at diagnosis has been shown to negatively impact on prognosis [24]. The effect

of lenalidomide on TNFα production depends on immunological context. Lenalidomide has been shown to inhibit the production of proinflammatory cytokines including TNFα and to elevate the production of anti-inflammatory cytokine IL-10 from human PBMCs stimulated by LPS. In contrast, a strongly elevated production of TNFα [25] by CD3 stimulated T-cell during costimulation by lenalidomide [26] has been reported. In this model, the augmentation of TNFα is due to production by both CD4+ and CD8+ T cells, and is dependent on IL2-mediated signalling. Thus, depending on immune environment, lenalidomide could have differential impact on DLBCL tumors.

2.2.2. Action on NK Cells. Under normal circumstances of immune surveillance, human NK cells have inhibitory receptors that recognize MHC class I molecules as their cognate ligands on virtually every cell in the body [27] and activating receptors that sense stressed cells, that is, transformed or infected cells. Thus, NK cells spare healthy cells that express self-MHC class I molecules and low amounts of stress-induced self-molecules, whereas they selectively kill stressed target cells that downregulate MHC class I molecules and/or upregulate stress-induced self-molecules [28]. Investigating spontaneous B-cell lymphoma development in aging β2m-deficient mice, Street et al. [29] have shown that NK cells are critical in innate immune surveillance of B-cell lymphomas. In human, alteration of β2m expression leading to an altered HLA-I staining patterns has been report in 40/53 (75%) of DLBCL [30], a situation prone to activate NK cells. However, 75% of such HLA-I deficient tumors have concomitant alterations of CD58 (LFA3) expression leading to a potential defect in adhesion and activation of NK cells as it has been shown for LFA1/ICAM1 [31]. The frequency of β2m and CD58 expression defect was comparable in GCB and ABC DLBCL subgroups, but the correlation with the HR "Monti classification" has not been reported. We have previously shown that peripheral blood NK cell count deficiency was associated with lower response rate to CHOP like induction regiment [32] in DLBCL suggesting a cooperative contribution of the immune system to the chemotherapeutic response, a feature demonstrated in several mouse model [33]. *In vitro* addition of lenalidomide to PBMC of healthy individuals significantly increased their NK cell natural cytotoxicity (Davies et al., 2001 [34]) in a CD4+T cell and IL-2 dependent manner [35, 36]. The *in vivo* effect of lenalidomide on NK cell of patients with DLBCL treated with lenalidomide is under study.

The introduction of rituximab in therapeutic arsenal has greatly changed the clinical course of DLBCL [6, 37, 38]. Beside natural cytotoxicity mentioned above, NK cell may be involved in Rituximab mediated antidody dependant cytotoxicity (ADCC) by the engagement of the Fc portion of the antibody on their FcγRIII (CD16) receptors. It has been shown that FcγRIII polymorphisms impact on antibody binding, resulting in more effective antibody-dependent cellular cytotoxicity *in vitro* [39]. The association with FcγRIII polymorphisms and clinical outcome has been used to argue for an ADCC mechanism of action of rituximab *in vivo*, but the association is less significant in DLBCL [40] than initially report in follicular lymphoma [41].

Lenalidomide has been shown to enhance the NK-cell-mediated ADCC and NK cell IFN-γ production in a series of functional *in vitro* experiments using rituximab coated NHL cell lines, including one cell line derived from a DLBCL patient [42].

2.2.3. Action on Regulatory T Cells. Regulatory T cells (T_{REG}), defined as $CD4^+CD25^+$ T cells, play an important role in the immune system, not only by inhibiting autoimmunity, but also by hampering the antitumour response [43]. In human NHL (including 6 DLBCL) it has been shown *in vitro*, that intratumoral $CD4^+CD25^+$ cells can inhibit the proliferation of activated anti-tumour CTLs, and can inhibit the proliferation and the secretion of IFNγ and IL-4 by infiltrating CD4+CD25-T cells [44]. The expression of FOXP3 has been evaluated by immunohistochemical study on paraffin-embedded DLBCL tumor specimens and the number of FOXP3+ regulatory T cells has been first shown to be not predictive of clinical outcome [45]. However, the correlation between FOXP3+ infiltrating T cells and prognosis has been subsequently evaluated independently in GC and non-GC DLBCL subgroups [46], as defined by Hans algorithm [47]. Despite the fact that the absolute FOXP3+ cell numbers were similar in GC and non-GC DLBCL, a high amount of tumor-infiltrating FOXP3+ cells was of good prognostic value (DFS) in GC but was associated with an adverse clinical outcome in non-GC subgroup. In this study, localization of FOXP3+ cells within tumor, a feature that has been shown to impact their clinical value in solid tumor [48], has not been evaluated.

Lenalidomide can inhibit the proliferation of FOXP3+ CTLA4+CD4+$CD25^{high}$ T_{REG} cells in healthy donor PBMCs cultured for 7 days with IL-2 [49]. Moreover, lenalidomide inhibit the suppressor function of the T_{REG} cells against autologous responder cells *in vitro*. This inhibitory activity is associated with reduction of FOXP3 and OX40 expression. However, to our knowledge, nothing has been report on the effect of lenalidomide on T_{REG} extracted from DLBCL tumor samples.

2.3. Direct Effect on Tumor Cells. A hallmark of ABC DLBCL is the constitutive activation of the NFκB pathway, on which they rely for survival and proliferation [34]. NFκB activation in mediated through oncogenic driver mutations affecting B-cell receptor-NFκB signaling (review in [50]). Beside effects on microenvironment, lenalidomide has been shown to have a direct effect on DLBCL cell lines, with a decrease in NFκB activity and an arrest in DNA synthesis [51].

3. Part II: Lenalidomide and Treatment of DLBCL

3.1. Response to Lenalidomide in Relapsed/Refractory DLBCL. Data emerging from early clinical trials demonstrated that lenalidomide has a significant activity against relapsed/refractory DLBCL either as monotherapy or as an association with rituximab. Published results are shown in Table 1.

Table 1: Response to Lenalidomide in relapsed/refractory diffuse large B-cell lymphomas.

Lenalidomide	Monotherapy		Association	
Name of the protocol	NHL002 [52]	NHL003 [53]	Lenalidomide and rituximab [54]	
Year of publication	2008	2011	2011	
Type of study	Multicentric	International	Multicentric	
Phase	Phase II	Phase II	Phase II	
Treatment	Lenalidomide	Lenalidomide	Lenalidomide and rituximab	
Dose of lenalidomide:	25 mg/d, D1–21 every 28 days	25 mg/d, D1–21 every 28 days	20 mg/d, D1–21 every 28 days	
Duration or treatment or No. of cycles	52 weeks	—	4 cycles + maintenance ($n = 10$ pts)	
No. of patients	49	267	23	
No. of DLBCL	26	108	23	
Response*			Induction	Complete therapy
ORR *n*, (%)	5 (19)	30 (28)	8 (35)	8 (35)
CR *n*, (%)	1 (3)	8 (7)	7 (31)	8 (35)
CRU *n*, (%)	2 (8)	—	—	
PR *n*, (%)	2 (8)	22 (20)	1 (4)	0 (0)
Stable disease *n*, (%)	7 (27)	23 (21)	2 (8)	2 (8)
Progression *n*, (%)	14 (54)	40 (37)	13 (57)	
Followup		9.2	16	
Median time to response (month)	PR: 1.9 (1.2–3.7) CR: 4.3 (1.9–10.5)	1.9 (1.4–11.5)	—	
Median response duration (month)	6.2 (0–12.8)	1.6	—	
PFS, Median (month)	4	2.7	1-year DFS 34.8%	

ORR: overall response rate, CR: complete response, CRU: complete response unconfirmed, PR: partial response, PFS: progression free survival.
NHL002: the results of response are specifically reported for DLBCL.
NHL003: the results of response are specifically reported for DLBCL.

The first phase II trial was a single-arm, multicenter trial (NHL002) that evaluates the safety and efficacy of lenalidomide oral monotherapy (25 mg/day during 21 days every 28 days) in 49 patients with relapsed or refractory aggressive NHL [52]. Among them, 26 patients presented a relapsed/refractory DLBCL. The median age was 65 years. All these patients were heavily pretreated with a median of four prior treatment regimens. Overall response rate (ORR) was 19% ($n = 5/26$), including 3 complete responses (CRU + CR) and 2 partial responses (PR).

An international phase II study (NHL003) was then conducted enrolling 218 patients with refractory/relapsed B-cell aggressive lymphoma, and confirmed the efficacy of lenalidomide in this category of patients [53]. One hundred and eight patients with diffuse large B-cell lymphoma were included. As the previous study, the treatment consisted in lenalidomide 25 mg orally once daily on days 1–21 of every 28 day cycle. Thirty patients (28%) exhibited an objective response (8 CR, and 22 PR). Interestingly, response to lenalidomide therapy was independent of the tumor burden, and of the number and the type of prior treatment. Compared to other type of lymphomas included (mantle cell lymphoma, transformed large B-cell lymphoma, follicular lymphoma, grade III), progression-free survival (PFS) of the patients with DLBCL was the shortest.

In contrast, patients with large cell NHL of the transformed type ($n = 33$) had substantially better results [53]. Median PFS was of 5.1 months and median response duration of 12.8 months. These results were further explored in a study analysing 33 patients with transformed follicular lymphoma (tFL), transformed chronic lymphocytic leukaemia/small lymphocytic lymphoma (tCLL/SLL) [55]. Lenalidomide was administered at the same dosage. Among patients with tFL, ORR was 57%, with a median response duration of 12.8 months. None of the patients with tCLL/SLL responded to lenalidomide monotherapy.

These encouraging results are confirmed in a retrospective study (REVEAL study) showing an objective response rate after 3 cycles at 69.2% in heavily pretreated patients with relapsed LNH [59].

Table 2: Grade III-IV toxicities with lenalidomide as monotherapy in relapsed/refractory DLBCL.

	NHL002 [52]	NHL003 [53]
	%	%
Neutropenia	33	41
Febrile neutropenia	6.1	2.3
Thrombocytopenia	20.4	18.4
Anemia	6.1	9.2
Fatigue	6.1	4.6
Deep vein thrombosis	2	2.3
Neuropathy	0	0

Table 3: Response to lenalidomide in patients with diffuse large B-cell lymphoma in first-line treatment.

Name of the protocol	R2-CHOP [56]	LR-CHOP21 [57]	R2-CHOP [58]	
Year of publication	2011	2010	2011	
Type of study	Monocentric	Multicentric-IIL	Multicentric	
Phase	Phase I	Phase I-II	Phase I-II	
Treatment	Lenalidomide and R-CHOP21	Lenalidomide and R-CHOP21	Lenalidomideand R-CHOP21	
Dose of lenalidomide:	15 to 25 mg/d, D1–10 every 21 days	5 to 20 mg/d, D1–14 every 21 days	5 to 25 mg/d, D1–14 every 21 days	
No. of cycles	6	6	6	
No. of patients with DLBCL	24	21	27	
Recommended dose in function of DLT	25 mg	15 mg	25 mg	
Toxicity				
Hematologic	Grade III-IV	Grade III-IV	Grade III-IV	
Anemia	21%	4%	—	
Neutropenia	88%	28%	59%	
Thrombocytopenia	29%	10%	30%	
	Grade III	Grade III	Grade I-II	Grade III
Peripheral neurotoxicity	8%	14%	48%	0%
Vascular thrombosis	8%		7%	
Response				
ORR *n*, (%)	22 (87.5)	16 (72)	27 (100)	
CR *n*, (%)	18 (77)	15 (71)	20 (74)	
PR *n*, (%)		1 (5)	7 (26)	
Stable disease *n*, (%)			—	
Progression *n*, (%)	5 (21)	5 (16)	—	

R-CHOP: rituximab 375 mg/m^2 D1, cyclophosphamide 750 mg/m^2 D1, doxorubicin 50 mg/m^2 D1, vincristine 1.4 mg/m^2 D1 (capped at 2.0 mg) prednisone 50 mg/m^2 D1–5.
DTL: dose limiting toxicity.

When associated to rituximab, results of ORR seems equivalent to lenalidomide alone, with an ORR of 35% [54]. However number of CR seems higher as almost all but one responding patients were in CR. In this trial, the treatment plan comprised an induction phase with lenalidomide (20 mg/day, D1-D21 of a 28-day cycle for 4 cycles) and rituximab (375 mg/m2 on day 1 and day 21 of each cycle—total of 4 cycles) and maintenance therapy proposed to the responders (CR, PR SD) with lenalidomide. Interestingly one patient in PR after induction converted to CR during the maintenance.

Interestingly maintenance with lenalidomide is currently tested in patients with relapsed DLBCL who achieved at least a partial response to second-line chemotherapy (ICE, DHAP/DHAOx, or MINE regimen) and rituximab at the dose of 25 mg once daily for 21 days out of 28 until progression (NCT00799513).

3.2. Toxicity of Lenalidomide Alone in DLBCL. When lenalidomide is used in monotherapy at the "standard" dose of 25 mg/d D1–D21 cycling at 28 days, the most common grade 3 and 4 adverse events are neutropenia occurring in 33% to 41% of the patients, and thrombocytopenia in 20% of the patients. The neutropenia was rarely complicated with a febrile neutropenia, reported in 2–6% of the patients. No neuropathy was reported. Deep vein thrombosis was described in 2% of the patients. Other grade III-IV toxicities were anemia (<10%) and asthenia in 5% of the patients. These toxicities required a dose reduction in one third of the patients in both trials [52, 53] (Table 2). The median time to first dose reduction or interruption of treatment was 33 days [53]. The most common reasons for dose reduction were neutropenia (56%) and thrombocytopenia (31%) [53].

3.3. Lenalidomide in First-Line Treatment in DLBCL. Combination of lenalidomide and standard R-CHOP21 have been recently published or reported in meetings by several groups in phase I-II [56]. This strategy of "R2-CHOP" was proposed to patients in first-line treatment (Table 3). The lenalidomide dose levels tested were between 5 mg up to 25 mg/day. Duration of treatment by cycle was between 10 days to 14 days. Dose limiting toxicity principally occurred because of haematological toxicity described as the most frequent adverse event. Grade III-IV neutropenia occurred in 28% to 88% of the patients, and Grade III-IV thrombocytopenia in 10 to 30% of the patients. Grade I-II neuropathy was

observed in half of the patients, and grade III-IV in around 10% of the patients.

Beside concomitant association of lenalidomide and R-chemotherapy, alternative way to administer lenalidomide in front-line is a strategy of maintenance therapy after an induction of R-CHOP. Lenalidomide seems attractive to test in maintenance with several positive arguments. It is an oral drug, easy to administer. The early antitumoral efficacy and immunomodulatory effect have been shown, and finally tolerance is acceptable. This strategy is currently investigated in an international trial conducted by the LYSA (EUDRACT Number: 2008-008202-52), where lenalidomide is proposed in maintenance after R-CHOP21 or R-CHOP14 in responding patients (CR + PR) [60] aged from 60 to 80 years old with at least one adverse IPI prognostic factors.

3.4. Cell of Origin and Response to Lenalidomide. Based on the biological rational of Lenalidomide and the new categorization of DLBCL [4], Hernandez-Ilizaliturri et al., recently reported the response to lenalidomide in relapsed/refractory DLBCL in analyzing them within their subgroups: germinal center B-cell (GCB-)-like- or nongerminal center B-cell (non-GCB-)like- DLBCL [61]. Forty patients were retrospectively analyzed using the Hans's algorithm based on the expression of CD10, BCL6, and IRF4/MUM1 by immunohistochemistry [47]. Twenty-three were classified as GCB-like DLBCL and 17 as non-GCB-like DLBCL. Differences were observed in responses rates, PFS and OS. ORR rate was significantly higher in patients with non-GCB-like DLBCL compared to patients with GCB-like DLBCL (OOR rates, 53% versus 9%, $P = .006$). Complete response rate was 23.5 versus 4.3%. Median progression-free survival was 6.2 months versus 1.7 months. No difference in OS was yet observed.

4. Conclusion

Lenalidomide is a promising drug in DLBCL in relapse as well as in front-line therapy. Several trials have reported interesting results in monotherapy as well as in association with rituximab alone or with immunochemotherapy. Tolerance seems acceptable and long term results of the recently published trials should in the future help to define the place of this drug in the therapeutic strategy of patients with DLBCL. Numerous new therapeutic molecules are under development or in phase I/II evaluation and some additional biological works are necessary to decipher the precise mechanism of action of lenalidomide in DLBCL subgroups in order to develop rational combinations [62].

Authors' Contribution

C. Thieblemont and M.-H. Delfau-Larue have contributed equally to this paper.

References

[1] The Non-Hodgkin's Lymphoma Classification Project, "A clinical evaluation of the International Lymphoma Study Group classification of non-Hodgkin's lymphoma," *Blood*, vol. 89, no. 11, pp. 3909–3918, 1997.

[2] C. Percy, G. O'Conor, L. G. Ries, and E. S. Jaffe, "Non-Hodgkin's lymphomas. Application of the international classification of diseases for oncology (ICD-O) to the working formulation," *Cancer*, vol. 54, no. 7, pp. 1435–1438, 1984.

[3] S. H. Swerdlow, E. Campo, N. L. Harris et al., *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*, IARC Press, Lyon, France, 2008.

[4] A. A. Alizadeh, M. B. Elsen, R. E. Davis et al., "Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling," *Nature*, vol. 403, no. 6769, pp. 503–511, 2000.

[5] G. Lenz, G. Wright, S. S. Dave et al., "Stromal gene signatures in large-B-cell lymphomas," *New England Journal of Medicine*, vol. 359, no. 22, pp. 2313–2323, 2008.

[6] B. Coiffier, E. Lepage, J. Brière et al., "Chop chemotherapy plus rituximab compared with chop alone in elderly patients with diffuse large-B-cell lymphoma," *New England Journal of Medicine*, vol. 346, no. 4, pp. 235–242, 2002.

[7] P. Feugier, A. van Hoof, C. Sebban et al., "Long-term results of the R-CHOP study in the treatment of elderly patients with diffuse large B-cell lymphoma: a study by the groupe d'etude des lymphomes de l'adulte," *Journal of Clinical Oncology*, vol. 23, no. 18, pp. 4117–4126, 2005.

[8] B. Coiffier, C. Thieblemont, E. van den Neste et al., "Long-term outcome of patients in the LNH-98.5 trial, the first randomized study comparing rituximab-CHOP to standard CHOP chemotherapy in DLBCL patients: a study by the Groupe d'Etudes des Lymphomes de l'Adulte," *Blood*, vol. 116, no. 12, pp. 2040–2045, 2010.

[9] W. Klapper, H. Stoecklein, S. Zeynalova et al., "Structural aberrations affecting the MYC locus indicate a poor prognosis independent of clinical risk factors in diffuse large B-cell lymphomas treated within randomized trials of the German High-Grade Non-Hodgkin's Lymphoma Study Group (DSHNHL)," *Leukemia*, vol. 22, no. 12, pp. 2226–2229, 2008.

[10] H. Tilly, E. Lepage, B. Coiffier et al., "Intensive conventional chemotherapy (ACVBP regimen) compared with standard CHOP for poor-prognosis aggressive non-Hodgkin lymphoma," *Blood*, vol. 102, no. 13, pp. 4284–4289, 2003.

[11] W. H. Wilson, K. Dunleavy, S. Pittaluga et al., "Phase II study of dose-adjusted EPOCH and rituximab in untreated diffuse large B-cell lymphoma with analysis of germinal center and post-germinal center biomarkers," *Journal of Clinical Oncology*, vol. 26, no. 16, pp. 2717–2724, 2008.

[12] C. Récher, B. Coiffier, C. Haioun et al., "Intensified chemotherapy with ACVBP plus rituximab versus standard CHOP plus rituximab for the treatment of diffuse large B-cell lymphoma (LNH03-2B): an open-label randomised phase 3 trial," *The Lancet*, vol. 378, no. 9806, pp. 1858–1867, 2011.

[13] F. Reyes, E. Lepage, G. Ganem et al., "ACVBP versus CHOP plus radiotherapy for localized aggressive lymphoma," *New England Journal of Medicine*, vol. 352, no. 12, pp. 1197–1205, 2005.

[14] J. B. Bartlett, K. Dredge, and A. G. Dalgleish, "The evolution of thalidomide and its IMiD derivatives as anticancer agents," *Nature Reviews Cancer*, vol. 4, no. 4, pp. 314–322, 2004.

[15] A. Rosenwald, G. Wright, W. C. Chan et al., "The use of molecular profiling to predict survival after chemotherapy for diffuse large-B-cell lymphoma," *New England Journal of Medicine*, vol. 346, no. 25, pp. 1937–1947, 2002.

[16] D. Gratzinger, S. Zhao, R. J. Tibshirani et al., "Prognostic significance of VEGF, VEGF receptors, and microvessel density

in diffuse large B cell lymphoma treated with anthracycline-based chemotherapy," *Laboratory Investigation*, vol. 88, no. 1, pp. 38–47, 2008.

[17] T. M. Cardesa-Salzmann, L. Colomo, G. Gutierrez et al., "High microvessel density determines a poor outcome in patients with diffuse large B-cell lymphoma treated with rituximab plus chemotherapy," *Haematologica*, vol. 96, no. 7, pp. 996–1001, 2011.

[18] A. Koster and J. M. M. Raemaekers, "Angiogenesis in malignant lymphoma," *Current Opinion in Oncology*, vol. 17, no. 6, pp. 611–616, 2005.

[19] J. Ruan, K. Hajjar, S. Rafii, and J. P. Leonard, "Angiogenesis and antiangiogenic therapy in non-Hodgkin's lymphoma," *Annals of Oncology*, vol. 20, no. 3, pp. 413–424, 2009.

[20] N. Reddy, F. J. Hernandez-Ilizaliturri, G. Deeb et al., "Immunomodulatory drugs stimulate natural killer-cell function, alter cytokine production by dendritic cells, and inhibit angiogenesis enhancing the anti-tumour activity of rituximab in vivo," *British Journal of Haematology*, vol. 140, no. 1, pp. 36–45, 2008.

[21] L. Lu, F. Payvandi, L. Wu et al., "The anti-cancer drug lenalidomide inhibits angiogenesis and metastasis via multiple inhibitory effects on endothelial cell function in normoxic and hypoxic conditions," *Microvascular Research*, vol. 77, no. 2, pp. 78–86, 2009.

[22] S. Monti, K. J. Savage, J. L. Kutok et al., "Molecular profiling of diffuse large B-cell lymphoma identifies robust subtypes including one characterized by host inflammatory response," *Blood*, vol. 105, no. 5, pp. 1851–1861, 2005.

[23] T. M. Habermann, S. S. Wang, M. J. Maurer et al., "Host immune gene polymorphisms in combination with clinical and demographic factors predict late survival in diffuse large B-cell lymphoma patients in the pre-rituximab era," *Blood*, vol. 112, no. 7, pp. 2694–2702, 2008.

[24] E. Lech-Maranda, J. Bienvenu, F. Broussais-Guillaumot et al., "Plasma TNF-α and IL-10 level-based prognostic model predicts outcome of patients with diffuse large B-cell lymphoma in different risk groups defined by the international prognostic index," *Archivum Immunologiae et Therapiae Experimentalis*, vol. 58, no. 2, pp. 131–141, 2010.

[25] L. G. Corral, P. A. J. Haslett, G. W. Muller et al., "Differential cytokine modulation and T cell activation by two distinct classes of thalidomide analogues that are potent inhibitors of TNF-α," *Journal of Immunology*, vol. 163, no. 1, pp. 380–386, 1999.

[26] J. B. Marriott, I. A. Clarke, K. Dredge, G. Muller, D. Stirling, and A. G. Dalgleish, "Thalidomide and its analogues have distinct and opposing effects on TNF-α and TNFR2 during co-stimulation of both $CD4^+$ and $CD8^+$ T cells," *Clinical and Experimental Immunology*, vol. 130, no. 1, pp. 75–84, 2002.

[27] M. A. Caligiuri, "Human natural killer cells," *Blood*, vol. 112, no. 3, pp. 461–469, 2008.

[28] E. Vivier, E. Tomasello, M. Baratin, T. Walzer, and S. Ugolini, "Functions of natural killer cells," *Nature Immunology*, vol. 9, no. 5, pp. 503–510, 2008.

[29] S. E. A. Street, Y. Hayakawa, Y. Zhan et al., "Innate immune surveillance of spontaneous B cell lymphomas by natural killer cells and $\gamma\delta$ T cells," *Journal of Experimental Medicine*, vol. 199, no. 6, pp. 879–884, 2004.

[30] M. Challa-Malladi, Y. K. Lieu, O. Califano et al., "Combined genetic inactivation of β2-microglobulin and CD58 reveals frequent escape from immune recognition in diffuse large B cell lymphoma," *Cancer Cell*, vol. 20, no. 6, pp. 728–740, 2011.

[31] D. F. Barber, M. Faure, and E. O. Long, "LFA-1 contributes an early signal for NK cell cytotoxicity," *Journal of Immunology*, vol. 173, no. 6, pp. 3653–3659, 2004.

[32] A. Plonquet, C. Haioun, J. P. Jais et al., "Peripheral blood natural killer cell count is associated with clinical outcome in patients with aaIPI 2-3 diffuse large B-cell lymphoma," *Annals of Oncology*, vol. 18, no. 7, pp. 1209–1215, 2007.

[33] L. Zitvogel, O. Kepp, and G. Kroemer, "Immune parameters affecting the efficacy of chemotherapeutic regimens," *Nature Reviews Clinical Oncology*, vol. 8, no. 3, pp. 151–160, 2011.

[34] R. E. Davis, K. D. Brown, U. Siebenlist, and L. M. Staudt, "Constitutive nuclear factor κB activity is required for survival of activated B cell-like diffuse large B cell lymphoma cells," *Journal of Experimental Medicine*, vol. 194, no. 12, pp. 1861–1874, 2001.

[35] T. Hayashi, T. Hideshima, M. Akiyama et al., "Molecular mechanisms whereby immunomodulatory drugs activate natural killer cells: clinical application," *British Journal of Haematology*, vol. 128, no. 2, pp. 192–203, 2005.

[36] A. K. Hsu, H. Quach, T. Tai et al., "The immunostimulatory effect of lenalidomide on NK-cell function is profoundly inhibited by concurrent dexamethasone therapy," *Blood*, vol. 117, no. 5, pp. 1605–1613, 2011.

[37] T. M. Habermann, E. A. Weller, V. A. Morrison et al., "Rituximab-CHOP versus CHOP alone or with maintenance rituximab in older patients with diffuse large B-cell lymphoma," *Journal of Clinical Oncology*, vol. 24, no. 19, pp. 3121–3127, 2006.

[38] M. Pfreundschuh, L. Trümper, A. Österborg et al., "CHOP-like chemotherapy plus rituximab versus CHOP-like chemotherapy alone in young patients with good-prognosis diffuse large-B-cell lymphoma: a randomised controlled trial by the MabThera International Trial (MInT) Group," *Lancet Oncology*, vol. 7, no. 5, pp. 379–391, 2006.

[39] S. Dall'Ozzo, S. Tartas, G. Paintaud et al., "Rituximab-dependent cytotoxicity by natural killer cells: influence of FCGR3A polymorphism on the concentration-effect relationship," *Cancer Research*, vol. 64, no. 13, pp. 4664–4669, 2004.

[40] M. Ahlgrimm, M. Pfreundschuh, M. Kreuz, E. Regitz, K.-D. Preuss, and J. Bittenbring, "The impact of Fc-γ receptor polymorphisms in elderly patients with diffuse large B-cell lymphoma treated with CHOP with or without rituximab," *Blood*, vol. 118, no. 17, pp. 4657–4662, 2011.

[41] G. Cartron, L. Dacheux, G. Salles et al., "Therapeutic activity of humanized anti-CD20 monoclonal antibody and polymorphism in IgG Fc receptor FcγrIIIa gene," *Blood*, vol. 99, no. 3, pp. 754–758, 2002.

[42] L. Wu, M. Adams, T. Carter et al., "Lenalidomide enhances natural killer cell and monocyte-mediated antibody-dependent cellular cytotoxicity of rituximab-treated $CD20^+$ tumor cells," *Clinical Cancer Research*, vol. 14, no. 14, pp. 4650–4657, 2008.

[43] T. Yamaguchi and S. Sakaguchi, "Regulatory T cells in immune surveillance and treatment of cancer," *Seminars in Cancer Biology*, vol. 16, no. 2, pp. 115–123, 2006.

[44] Z. Z. Yang, A. J. Novak, M. J. Stenson, T. E. Witzig, and S. M. Ansell, "Intratumoral $CD4^+CD25^+$ regulatory T-cell-mediated suppression of infiltrating $CD4^+$ T cells in B-cell non-Hodgkin lymphoma," *Blood*, vol. 107, no. 9, pp. 3639–3646, 2006.

[45] S. Hasselblom, M. Sigurdadottir, U. Hansson, H. Nilsson-Ehle, B. Ridell, and P. O. Andersson, "The number of tumour-infiltrating TIA-1^+ cytotoxic T cells but not $FOXP3^+$

regulatory T cells predicts outcome in diffuse large B-cell lymphoma," *British Journal of Haematology*, vol. 137, no. 4, pp. 364–373, 2007.

[46] A. Tzankov, C. Meier, P. Hirschmann, P. Went, S. A. Pileri, and S. Dirnhofer, "Correlation of high numbers of intratumoral FOXP3$^+$ regulatory T cells with improved survival in germinal center-like diffuse large B-cell lymphoma, follicular lymphoma and classical Hodgkin's lymphoma," *Haematologica*, vol. 93, no. 2, pp. 193–200, 2008.

[47] C. P. Hans, D. D. Weisenburger, T. C. Greiner et al., "Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray," *Blood*, vol. 103, no. 1, pp. 275–282, 2004.

[48] C. Ménétrier-Caux, M. Gobert, and C. Caux, "Differences in tumor regulatory T-cell localization and activation status impact patient outcome," *Cancer Research*, vol. 69, no. 20, pp. 7895–7898, 2009.

[49] C. Galustian, B. Meyer, M. C. Labarthe et al., "The anticancer agents lenalidomide and pomalidomide inhibit the proliferation and function of T regulatory cells," *Cancer Immunology, Immunotherapy*, vol. 58, no. 7, pp. 1033–1045, 2009.

[50] A. L. Shaffer III, R. M. Young, and L. M. Staudt, "Pathogenesis of human B cell lymphomas," *Annual Review of Immunology*, vol. 30, pp. 565–610, 2012.

[51] F. J. Hernandez-Ilizaliturri, N. Reddy, B. Holkova, E. Ottman, and M. S. Czuczman, "Immunomodulatory drug CC-5013 or CC-4047 and rituximab enhance antitumor activity in a severe combined immunodeficient mouse lymphoma model," *Clinical Cancer Research*, vol. 11, no. 16, pp. 5984–5992, 2005.

[52] P. H. Wiernik, I. S. Lossos, J. M. Tuscano et al., "Lenalidomide monotherapy in relapsed or refractory aggressive non-Hodgkin's lymphoma," *Journal of Clinical Oncology*, vol. 26, no. 30, pp. 4952–4957, 2008.

[53] T. E. Witzig, J. M. Vose, P. L. Zinzani et al., "An international phase II trial of single-agent lenalidomide for relapsed or refractory aggressive B-cell non-Hodgkin's lymphoma," *Annals of Oncology*, vol. 22, no. 7, pp. 1622–1627, 2011.

[54] P. L. Zinzani, C. Pellegrini, L. Gandolfi et al., "Combination of lenalidomide and rituximab in elderly patients with relapsed or refractory diffuse large B-cell lymphoma: a phase 2 trial," *Clinical Lymphoma, Myeloma and Leukemia*, vol. 11, no. 6, pp. 462–466, 2011.

[55] M. S. Czuczman, J. M. Vose, T. E. Witzig et al., "The differential effect of lenalidomide monotherapy in patients with relapsed or refractory transformed non-Hodgkin lymphoma of distinct histological origin," *British Journal of Haematology*, vol. 154, no. 4, pp. 477–481, 2011.

[56] G. S. Nowakowski, B. LaPlant, T. M. Habermann et al., "Lenalidomide can be safely combined with R-CHOP (R2CHOP) in the initial chemotherapy for aggressive B-cell lymphomas: phase I study," *Leukemia*, vol. 25, pp. 1877–1881, 2011.

[57] U. Vitolo, A. Chiappella, A. Carella et al., "Prospective, multicenter phase I–II pilot trial to evaluate efficacy and safety of lenalidomide plus rituximab-CHOP21 (LR-CHOP21) for elderly patients with untreated diffuse large B-Cell lymphoma (DLBCL): interim analysis of the intergruppo italiano linfomi (IIL) REAL07 study," *Blood*, vol. 116, p. 2871, 2010, ASH Annual Meeting Abstracts.

[58] H. Tilly, F. Morschhauser, G. Salles et al., "Phase I study of escalating doses of lenalidomide combined with R-CHOP (R2-CHOP) for front-line treatment of B-Cell lymphomas," *Blood*, vol. 118, p. 1632, 2011, ASH Annual Meeting Abstracts.

[59] L. Rigacci, F. Zaja, A. Fabbri et al., "Lenalidomide administration in heavily pretreated patients with non hodgkin lymphoma—first report of the REVEAL study (REVlimid effectiveness of administration in patients with lymphoma)," *Blood*, vol. 118, 2011, ASH Annual Meeting Abstracts, Abstract 4979.

[60] B. D. Cheson, B. Pfistner, M. E. Juweid et al., "Revised response criteria for malignant lymphoma," *Journal of Clinical Oncology*, vol. 25, no. 5, pp. 579–586, 2007.

[61] F. J. Hernandez-Ilizaliturri, G. Deeb, P. L. Zinzani et al., "Higher response to lenalidomide in relapsed/refractory diffuse large b-cell lymphoma in nongerminal center b-cell-like than in germinal center b-cell-like phenotype," *Cancer*, vol. 117, no. 22, pp. 5058–5066, 2011.

[62] C. B. Reeder and S. M. Ansell, "Novel therapeutic agents for B-cell lymphoma: developing rational combinations," *Blood*, vol. 117, no. 5, pp. 1453–1462, 2011.

Hindawi Publishing Corporation
Advances in Hematology
Volume 2012, Article ID 621958, 14 pages
doi:10.1155/2012/621958

Review Article

Practical Approaches to the Use of Lenalidomide in Multiple Myeloma: A Canadian Consensus

Donna Reece,[1] C. Tom Kouroukis,[2] Richard LeBlanc,[3] Michael Sebag,[4] Kevin Song,[5] and John Ashkenas[6]

[1] *Princess Margaret Hospital, University Health Network, 610 University Avenue, Toronto, ON, Canada M5G 2M9*
[2] *Department of Oncology, Juravinski Cancer Centre, 699 Concession Street, Hamilton, ON, Canada L8V 5C2*
[3] *Hôpital Maisonneuve-Rosemont, University of Montreal, Montreal, QC, Canada H1T 2M4*
[4] *McGill University Health Centre, McGill University, Montreal, QC, Canada H3A 1A1*
[5] *Leukemia/BMT Program of British Columbia, Vancouver General Hospital, Vancouver, BC, Canada V5Z 1M9*
[6] *SCRIPT, Toronto, ON, Canada M4S 1Z9*

Correspondence should be addressed to Donna Reece, donna.reece@uhn.on.ca

Received 5 April 2012; Accepted 14 August 2012

Academic Editor: Antonio Palumbo

In Canada, lenalidomide combined with dexamethasone (Len/Dex) is approved for use in relapsed or refractory multiple myeloma (RRMM). Our expert panel sought to provide an up-to-date practical guide on the use of lenalidomide in the managing RRMM within the Canadian clinical setting, including management of common adverse events (AEs). The panel concluded that safe, effective administration of Len/Dex treatment involves the following steps: (1) lenalidomide dose adjustment based on creatinine clearance and the extent of neutropenia or thrombocytopenia, (2) dexamethasone administered at 20–40 mg/week, and (3) continuation of treatment until disease progression or until toxicity persists despite dose reduction. Based on available evidence, the following precautions should reduce the risk of common Len/Dex AEs: (1) all patients treated with Len/Dex should receive thromboprophylaxis, (2) erythropoiesis-stimulating agents (ESAs) should be used cautiously, and (3) females of child-bearing potential and males in contact with such females must use multiple contraception methods. Finally, while Len/Dex can be administered irrespective of prior therapy and in all prognostic subsets, patients with chromosomal deletion 17(p13) have less favorable outcomes with all treatments, including Len/Dex. New directions for the use of lenalidomide in RRMM are also considered.

1. Introduction

Multiple myeloma (MM), the second most common hematological malignancy in adults, is associated with various clinical manifestations including anemia, lytic bone lesions, and renal and immune impairments. According to Canadian Cancer statistics, an estimated 2300 Canadians will be diagnosed with MM and 1350 will die from this disease in 2011 [1]. While no cure for MM is available, five-year survival rates have risen substantially in Canada and elsewhere over the last decade, partly due to novel therapies such as thalidomide, bortezomib, and lenalidomide [2, 3]. Nonetheless, regardless of initial treatment, most patients will eventually relapse and require salvage therapy, often consisting of novel agents, alone or in combination.

Lenalidomide is an immunomodulatory drug with direct effects on myeloma cells as well as their microenvironment. Early clinical trials with lenalidomide as a single agent in relapsed or refractory MM (RRMM) patients demonstrated its antimyeloma activity [4]. In preclinical studies, the agent has been shown to kill myeloma cells by upregulating certain cyclin-dependent kinase inhibitors and other early response factors [5]. Lenalidomide can also induce apoptosis by the activation of the intrinsic caspase-8 pathway [6], and it is thought to be more potent than thalidomide at inhibiting MM cell line growth and inhibiting TNF-α secretion from peripheral blood cells following LPS stimulation [7, 8].

Lenalidomide also has antiangiogenic properties, manifested *in vitro* by its ability to inhibit endothelial cell migration [9]. In addition, lenalidomide has properties not shared by thalidomide, such as inhibition of T regulatory cells and enhancement of tumor immunity [10, 11].

As reported in two landmark phase III trials that are the basis of Canadian approval of lenalidomide in RRMM, the efficacy of this agent is greatest when used in combination with dexamethasone [12, 13]. This combination is supported by data showing that lenalidomide can activate caspases 3, 8, and 9 with variable efficiency in different MM cell lines and that the addition of dexamethasone is synergistic and leads to a greater induction of apoptosis [5].

Additional studies, subgroup analyses of available phase III trials, and Canadian postmarketing experiences have all informed current practice regarding the use of lenalidomide in the RRMM patient population. In this paper we aim to provide an up-to-date practical guide on the use of this novel agent in the setting of RRMM, as well as a guide to managing commonly seen adverse events. To the best of our knowledge, the current report provides the first Canadian guidance for using lenalidomide in RRMM.

2. Methods

The expert panel convened in Paris, France, on May 2, 2011, in conjunction with the 13th International Myeloma Workshop. The group met to discuss the use of lenalidomide in the management of RRMM in the Canadian environment. The Chair (DR) invited panelists to research and write individual sections of the paper.

The various sections were collected, compiled, and distributed to the group, which discussed the paper via web conference. Panelists subsequently generated a revised draft in which all sections included specific clinical guidance (i.e., practice considerations). The revised paper was discussed at a final web conference, where all practice recommendations were considered, revised as appropriate, and ultimately adopted by the full panel; any areas of disagreement are noted.

Celgene Canada provided the impetus for the panel to pursue this project freely and independently. Celgene Canada supported the process throughout, including support for the participation of a medical writer (JA) in preparing this paper. The opinions represented here are solely those of the physician-panelists.

3. Indication, Timing, Dose, and Treatment Duration

In October 2008, the combination of lenalidomide and dexamethasone (Len/Dex) was approved in Canada for the treatment of RRMM in patients who had received at least one prior therapy. This approval was based on evidence from two phase III trials, namely, MM009 [12] and MM010 [13], which showed significant benefits in response rate (RR), time to progression (TTP), and overall survival (OS) following Len/Dex therapy, compared with dexamethasone monotherapy. The benefits of Len/Dex over dexamethasone alone were seen in all age groups and were independent of previous therapy type. Based on the currently approved indication for this agent in Canada and the results of available studies, the initiation of lenalidomide therapy is not limited by the number or type of previous lines of therapy, although OS and progression-free survival are greater among patients with only one prior therapy versus those with two or more prior therapies [35].

In the MM009 and MM010 trials, lenalidomide was given at a dose of 25 mg per day on days 1 to 21 of a 28-day cycle, along with 40 mg of dexamethasone per day on days 1–4, 9–12, and 17–20. After the fourth cycle, 40 mg of dexamethasone was given daily on days 1–4 of every cycle. Cycles were continued until disease progression or until toxicity persisted, despite dose reduction. Noting the lack of prospective randomized trials specifically addressing different approaches to drug administration in the relapsed/refractory setting, the panel agreed that the recommended dose and schedule of Len/Dex therapy need not directly follow those outlined in published clinical trials.

We agreed that the starting dose of lenalidomide should remain at the current standard (25 mg daily on days 1–21) in the absence of baseline renal insufficiency and/or significant cytopenias. Specifically, the dose of lenalidomide must be adjusted based on the creatinine clearance, using standard dose adjustments (Table 1). Either the Cockcroft-Gault or the MDRD (modification of diet in renal disease) formula may be used to calculate creatinine clearance. Caution is urged in calculating the renal function based solely on serum creatinine level in older patients with MM [36].

Lenalidomide treatment should be used with caution in patients with thrombocytopenia (i.e., platelet counts $<50 \times 10^9$/L or $<30 \times 10^9$/L in those with heavy marrow infiltration with myeloma) and absolute neutrophil counts $<1.0 \times 10^9$/L; if lenalidomide is used in this setting, measures for aggressive growth factor supplementation and/or platelet transfusion support must be in place.

Although the pivotal phase III trials in RRMM used the standard high-dose (HD) pulsed dexamethasone (12 doses of 40 mg per month, on days 1–4, 9–12, and 17–20 of a 28-day schedule), it has become common practice in Canada to administer dexamethasone on a weekly schedule (four doses of 20–40 mg per month, on days 1, 8, 15, and 22 of a 28-day schedule). Although dexamethasone dose should be selected on the basis of individual clinical circumstances, the panel notes that such low-dose (LD) dexamethasone administration is particularly suitable for elderly patients, as well as those with uncontrolled diabetes, unmanageable glucocorticoid side effects, or relatively indolent relapses.

Weekly LD dexamethasone now represents the standard of care in newly diagnosed individuals. The panel's preference for LD dexamethasone administration is based in part on the results of a trial on Len/Dex in initial therapy for MM (see New Directions, below) [30]. Here, despite a somewhat lower RR compared to the HD dexamethasone group, patients receiving LD dexamethasone plus lenalidomide experienced improved OS and fewer grade ≥ 3 toxicities. A second line of evidence supporting the use of dexamethasone

Table 1: Dose adjustments at the start of therapy according to renal function [14].

Renal function	Dose*
Mild renal impairment (60 ≤ CrCl < 90 mL/min)	25 mg (normal dose) every 24 hours
Moderate renal impairment (30 ≤ CrCl < 60 mL/min)	10 mg† every 24 hours
Severe renal impairment (CrCl < 30 mL/min, not requiring dialysis)	15 mg every 48 hours
End-stage renal disease (CrCl < 30 mL/min, requiring dialysis)	5 mg once daily. On dialysis days, the dose should be administered following dialysis

* While maintaining a treatment cycle of 21 out of 28 days.
† Dose may be escalated to 15 mg once daily after two cycles if patient does not respond to and is tolerating treatment.
CrCl: creatinine clearance.

Table 2: Lenalidomide dose reduction levels with adequate renal function.

Dose level	Lenalidomide dose (mg)
Initial dose	25
First reduction level	15
Second reduction level	10
Third reduction level	5
Fourth reduction level	Discontinuation

at doses lower than employed in MM009 and MM010 comes from a post hoc analysis of stepwise dose reduction in these trials [37]. In this analysis, which did not directly test a weekly dexamethasone regimen, patients who reduced the dose of dexamethasone experienced significantly better RR, TTP, and OS. A final reason for the use of weekly LD dexamethasone is more hypothetical: one purported important mechanism of action of lenalidomide is via its immunomodulatory properties, and laboratory and clinical studies have demonstrated that dexamethasone can antagonize the potentially beneficial immunostimulatory effects of this drug [5, 38]. Therefore, LD dexamethasone may allow better immunomodulatory effects, while preserving the ability of corticosteroids to enhance the antiproliferative activities of lenalidomide.

Finally, with regard to treatment duration, lenalidomide therapy should be maintained continuously in most patients. This is in contrast to regimens in which combination therapy is given to maximal response and then discontinued to allow a treatment hiatus. One study has reported that the duration of lenalidomide therapy is directly related to a longer survival [39]. However, the optimal dose of lenalidomide when administered on a long-term basis is less certain. For example, another post hoc analysis of the MM009/MM010 study examined patients who were still on therapy 12 months after entering the trial and found that those who had dose reductions after 12 months had a significantly longer PFS than those who had reductions less than 12 months earlier, or no dose reduction [40].

The panel makes no specific recommendation on routine dose reduction after a specific period of time. However, we recommend that doses of lenalidomide and/or dexamethasone should be reduced to allow treatment to continue until disease progression occurs. Dose interruptions should occur only in situations of significant toxicities, with a plan to reinstitute therapy as soon as toxicity decreases with appropriate dose modifications. If required, dose reduction to ameliorate toxicity should follow the recommendations outlined in Tables 1, 2, 3, and 4.

Practice considerations are as follows.

(i) Len/Dex is approved for the treatment of RRMM in patients who have received ≥1 prior therapy and is appropriate irrespective of the number or type of therapies previously given.

(ii) Lenalidomide dose must be adjusted based on creatinine clearance.

(iii) Dosing should take into account pre-existing and developing cytopenias.

(iv) Dexamethasone is usually administered at doses of 20–40 mg once per week. However, this LD regimen has not been formally studied in the setting of relapsed myeloma, and the results may not be the same as those reported in the pivotal MM009/MM010 trials.

(v) Len/Dex treatment should be maintained as in the pivotal trials, that is, continued until disease progression or until significant toxicity persists despite dose reduction.

4. Treatment of Special Populations

4.1. High-Risk Multiple Myeloma. The definition of high-risk myeloma has evolved considerably over the past decade from one that predominantly relied on clinical and biochemical parameters (Durie-Salmon and ISS (International Staging System) stage, serum LDH (lactate dehydrogenase), CRP (C-reactive protein), proliferating index, etc.) to one that accounts for disease-specific cytogenetic and genomic factors. Several recurrent chromosomal aberrations—including chromosomal deletions (del(13q14), del(17p13)), translocations (t(4; 14), t(14; 16), t(14; 20)), and amplifications (1q21), as well as numerical chromosomal abnormalities (hypodiploid versus hyperdiploid karyotype)—correlate with poor disease outcomes. Similarly, genomewide gene expression profiling (GEP) studies have identified myeloma molecular subgroups with unique gene signatures that correlate with disease outcomes. In particular, a 70-gene signature was validated as a predictor of response to therapy

Table 3: Lenalidomide dose adjustment for neutropenia.

Neutrophil count	Recommendations
$<1 \times 10^9$/L on day 1 of a cycle	Delay start of the cycle for a week, until neutrophil count $\geq 1 \times 10^9$/L
$<1 \times 10^9$/L during a cycle	Interruption of lenalidomide until next cycle (dexamethasone should be continued)
Returning to $\geq 1 \times 10^9$/L on next cycle	Continue lenalidomide at same dose ± addition of G-CSF, if no other significant toxicities needing dose reduction Reduce lenalidomide to the first reduction level if other significant toxicities observed
For each subsequent drop $<1 \times 10^9$/L	Interrupt lenalidomide treatment
Returning to $\geq 1 \times 10^9$/L on next cycle	Resume lenalidomide at next dose reduction level

G-CSF: granulocyte-colony stimulating factor.

Table 4: Lenalidomide dose adjustment for thrombocytopenia.

Platelet count	Recommendations
$<30 \times 10^9$/L on day 1 of a cycle	Delay start of the cycle for a week, until platelet count $\geq 30 \times 10^9$/L
$<30 \times 10^9$/L during a cycle	Interruption of lenalidomide until next cycle (dexamethasone should be continued)
Returning to $\geq 30 \times 10^9$/L on next cycle	Reduce lenalidomide to the first reduction level
For each subsequent drop $<30 \times 10^9$/L	Interrupt lenalidomide treatment
Returning to $\geq 30 \times 10^9$/L on next cycle	Resume lenalidomide at next dose reduction level

and disease survival independently of clinical parameters and structural or numerical chromosomal abnormalities. Although most Canadian centers perform FISH cytogenetics for detection of del(13q14), del(17p13), and t(4; 14), genomic analyses are not routinely obtained.

To date, four retrospective studies have assessed the impact of cytogenetic abnormalities on outcomes of Len/Dex treatment among RRMM patients [15–18], as summarized in Table 5. The most consistent finding among these studies is that patients with del(17p13) experience less favorable outcomes when treated with Len/Dex [15, 16] or Len/Dex with bortezomib than those individuals lacking this adverse prognostic factor [18]. However, the presence of del(17p13) has repeatedly been shown to predict a shorter progression-free survival (PFS) and OS among RRMM patients, regardless of therapy [15, 16, 18]. Although patients with del(17p13) derive less benefit, the panel agreed that they may be treated with Len/Dex but should preferentially be considered for clinical trials designed for high-risk patients, if such an option is available. Innovative strategies, not yet defined, are needed for patients with a 17p13 deletion.

Although the trials of Reece et al. [15] and Klein et al. [16] suggest that Len/Dex treatment can overcome the poorer prognosis ordinarily associated with del(13q14) and t(4; 14), these conclusions are in contrast to those of Avet-Loiseau et al. [17]. In this last study, del(13q14) and t(4; 14) were associated with significantly lower RR, PFS, and OS in univariate analysis. In particular, patients with t(4; 14), compared to patients without t(4; 14), experienced significantly lower response and survival rates. However, multivariate regression analysis identified a prior history of progression while on thalidomide as the main adverse prognostic factor, and t(4; 14) per se was not retained in the model. Moreover, the patients in the Avet-Loiseau trial were more heavily pretreated. Evidence to date is also equivocal regarding the impact on Len/Dex treatment efficacy of chromosome 1q21 amplifications [16, 18].

With regard to high-risk myeloma, as defined by the 70-gene GEP signature, there are currently no studies assessing the impact of lenalidomide-based therapy on the survival of these patients when used in the relapsed setting. However, results of studies incorporating lenalidomide in the frontline treatment regimen (e.g., Total Therapy 3, incorporating multidrug induction therapy, tandem autologous stem cell transplantation, and maintenance with the combination of lenalidomide, bortezomib, and dexamethasone) suggest that the 70-gene GEP signature remains a predictor of poor survival outcomes [41].

Currently, it remains difficult to provide definite recommendations for the use of lenalidomide in relapsed patients with high-risk cytogenetics. Prospective studies in this area are clearly warranted.

Practice considerations are as follows.

(i) Based on the results of a Canadian analysis of the Expanded Access Program of Len/Dex in relapsed/refractory myeloma patients, Len/Dex may be effective in patients with t(4; 14) or del(13q14) identified by FISH (fluorescence in situ hybridization) cytogenetics.

(ii) Patients with del17(p13) have poorer outcomes with all treatments, including Len/Dex treatment, and are high-priority candidates for innovative regimens directed to high-risk patients. However, Len/Dex may be used in the absence of such alternatives.

4.2. Previous Thalidomide Treatment. Although lenalidomide has been shown to be more potent than thalidomide in preclinical studies, the two agents are structurally similar

Table 5: Adverse prognostic factors identified by multivariate analysis in patients with relapsed/refractory myeloma treated with lenalidomide and dexamethasone.

Reference	Study population	PFS/TTP	Overall survival
Reece et al., 2009 [15]	130 RRMM patients treated with Len/Dex	Del(17p13) Elevated creatinine Prior bortezomib Prior thalidomide	Del(17p13) Elevated creatinine Prior bortezomib Prior thalidomide Age >65 yrs
Klein et al., 2011 [16]	92 RRMM patients treated with Len/Dex	Del(13q) if associated with other abnormalities	Del(17p13) Amp(1q21)
Avet-Loiseau et al., 2010 [17]	207 "heavily pretreated" RRMM patients treated with Len/Dex	Progression during thalidomide Hemoglobin <100 Del 13q	Progression during thalidomide
Dimopoulos et al., 2010 [18]	99 RRMM patients treated with Len/Dex ($n = 50$) or Len/Dex + bortezomib	t(4;14) Del(17p13) Thalidomide resistance Elevated LDH Extramedullary disease	Del(13q) Amp(1q21) Del(17p13) Thalidomide resistance ISS Bortezomib resistance Elevated LDH Extramedullary disease

PFS: progression-free survival; TTP: time to progression; RRMM: relapsed or refractory multiple myeloma; Len/Dex: lenalidomide combined with dexamethasone; LDH: lactate dehydrogenase; ISS: international staging system.

Table 6: The effect of Len/Dex treatment according to prior response to thalidomide. Adapted from Wang et al. [19].

	Thalidomide sensitive[1]			Thalidomide relapsed[2]			Thalidomide resistant[3]		
	Len/Dex	Placebo/Dex	P	Len/Dex	Placebo/Dex	P	Len/Dex	Placebo/Dex	P
Overall response rates (PR or better) %	64.8	17.1	<0.001	41.9	5.9	<0.01	50	20.8	0.042
Response duration, mo (95% CI)	13.4 (7.0 to NE)	3.2 (2.3 to NE)	0.009	8.8 (5.3 to NE)	NE (8.6 to NE)	0.77	NE (6.0 to NE)	NE (6.0 to NE)	0.22
Median PFS, mo (95% CI)	9.3 (5.6 to 18.0)	4.6 (3.9 to 4.7)	<0.001	7.8 (5.2 to 11.1)	3.7 (2.8 to 6.5)	0.002	7.0 (4.9 to 16.9)	3.7 (2.1 to 8.4)	0.013

[1] Sensitive: patients with stable disease or better who did not progress while on thalidomide.
[2] Relapsed: patients with stable disease or better who progressed while on thalidomide.
[3] Resistant: patients who progressed on thalidomide but never responded to thalidomide.
Len/Dex: lenalidomide combined with dexamethasone; PR: partial response; PFS: progression-free survival; NE: not estimable.

and likely exert their antimyeloma effects through similar mechanisms. Retrospective investigations suggest that prior thalidomide exposure [15], progression during thalidomide [17], and thalidomide resistance [18] independently predict reduced PFS and OS.

The MM-009 and MM-010 phase III studies included 154 (44%) and 120 (34%) patients, respectively, who had been previously exposed to thalidomide [12, 13]. A post hoc analysis of these two studies demonstrated that, while the overall RR of lenalidomide treatment was lower in patients previously treated with thalidomide (65% versus 54%), the response duration was not statistically different [19]. Further subgroup analyses of patients with prior thalidomide exposure revealed that those who had responded to thalidomide and did not progress while on therapy had the best overall RR, median duration of response, and PFS when subsequently treated with lenalidomide (Table 6). RR and PFS among patients who failed to respond to thalidomide were better with Len/Dex than with dexamethasone alone, although duration of response to the assigned agent did not differ. Finally, PFS was superior with Len/Dex over dexamethasone monotherapy, regardless of prior thalidomide response. Another nonrandomized, prospective study of 106 previously thalidomide-treated patients suggested that the overall RR, PFS, and OS were not significantly different between patients who were thalidomide-sensitive versus thalidomide-resistant (56%, 10 months, 17 months, resp.) [42]. A third study, retrospective in nature, looked at retreatment with immunomodulatory agents in patients given this class of drugs as initial therapy for myeloma. For the subset of patients who received Len/Dex after initial thalidomide, the overall RR was 48% and the median TTP was 9 months [43].

Practice considerations are as follows.

(i) Although treatment efficacy may be somewhat reduced, Len/Dex is an appropriate treatment choice among patients previously treated with thalidomide, irrespective of their earlier response.

4.3. Elderly Patients. Up to 37% of newly diagnosed MM patients are older than 75 years [44]. Elderly patients are more likely to have significant comorbidities, tend to be frail, and have lower performance status and poorer tolerance to medications. Nevertheless, elderly patients have often been included in clinical studies of novel agents, and available evidence suggests that, with appropriate management, they can also benefit from these agents. However, a 40 mg dose of dexamethasone can be challenging to deliver to some elderly patients, and this agent may be given at a lower weekly dose of 20 mg.

Practice considerations are as follows.

(i) Among elderly patients, dexamethasone should be started at a dose of 40 mg per week, unless there are significant and/or severe comorbidities.

(ii) Dexamethasone should be started at a dose of 20 mg per week in less fit patients; an initial dose of 16 mg may be considered for very frail patients, as guided by clinical judgment. As noted above, these doses are lower than those used in the MM009/MM010 studies and the results may not be the same as when 4-day pulses are administered.

5. Toxicities and Management of Adverse Events

The safety and toxicity of lenalidomide have been evaluated in published clinical trials [12, 13], as well as in an expanded-access program for Canadian and international patients [45]. Although lenalidomide is well tolerated by most patients, some adverse effects are common during treatment. However, some of the more significant side effects associated with thalidomide are not seen with lenalidomide. Indeed, in the MM-009 and MM-010 studies, the incidences of grade 3-4 constipation, somnolence, and peripheral neuropathies were similar for the Len/Dex-treated group compared to the dexamethasone monotherapy group [12, 13]. Importantly, side effects associated with Len/Dex are not affected by the number of prior therapies [35].

5.1. Hematologic Toxicities. The most common grade 3-4 adverse events in the two phase III pivotal trials of lenalidomide were hematologic, including neutropenia; thrombocytopenia; to a lesser extent, anemia. The risk of grade 3 or 4 febrile neutropenia was slightly increased with the addition of lenalidomide (3.4% in the Len/Dex group versus 0% in the dexamethasone group). Dose reductions typically occur most frequently during the initial cycles. It is not clear whether the risks of neutropenia and thrombocytopenia per se decrease with time or whether the pattern observed is secondary to dose modifications [46, 47]. At any rate, clinicians should be particularly vigilant during the first few months after initiation of lenalidomide. Given that a standard lenalidomide dose of 25 mg among patients with renal failure is associated with more cytopenias, especially neutropenia and thrombocytopenia [48], reducing the initial dose may ameliorate these risks. Specific recommendations for laboratory monitoring are summarized below.

5.1.1. Neutropenia and Thrombocytopenia. Myelosuppression associated with lenalidomide is dose-dependent and is usually predictable and manageable [47]. To decrease risks of infection and bleeding, lenalidomide should not be started in patients with an absolute neutrophil count (ANC) below 1.0×10^9/L or a platelet count below 50×10^9/L except in exceptional circumstances and with supportive measures in place, as discussed above. Lenalidomide administration should be interrupted whenever neutrophil and platelet counts reach these cutoffs. At the next cycle, if neutropenia is the only dose-limiting toxicity, treatment may resume at the same dose, with the addition of growth factor support such as filgrastim 300 or 480 mcg administered subcutaneously once or twice weekly, in patients with ANC $>1.0 \times 10^9$/L. In the presence of other dose-limiting toxicities, dose reduction is recommended (Table 2). Treatment may also be reintroduced, albeit at a reduced level, when platelet count is over 30×10^9/L. For each subsequent grade 3-4 neutropenia and platelet count less than 30×10^9/L, lenalidomide administration should be withheld and restarted at a lower dose at the next cycle. Dose adjustments for neutropenia and thrombocytopenia associated with lenalidomide are presented in Tables 3 and 4. In some circumstances, especially during the first few cycles, significant neutropenia or thrombocytopenia can result from heavy myeloma bone marrow infiltration rather than pure myelosuppression. In these cases, lenalidomide should probably be continued with the addition of G-CSF (granulocyte-colony stimulating factor) in case of neutropenia and platelet transfusions given to manage thrombocytopenia.

5.1.2. Anemia. Anemia is rarely a significant problem in patients undergoing Len/Dex combination therapy. Thus, clinicians should follow the standard practice established by their institution for transfusions. Some concerns have been raised regarding the potential risk of venous thromboembolic events associated with concomitant use of erythropoietin. Although the MM-010 study [13] suggested that these events are unrelated, the MM-009 [12] study identified a trend toward more venous thromboembolic events with erythropoietin. Accordingly, we recommend that erythropoiesis-stimulating agents (ESAs) be used with caution in patients receiving lenalidomide; if an ESA is given, the hemoglobin level should be maintained at <120 g/L as per the Health Canada label.

5.1.3. Others. Recently, lenalidomide exposure has been associated with failure to mobilize a sufficient number of

stem cells using growth factors alone [49–51]. This negative effect on stem cell mobilization can be overcome with the addition of cyclophosphamide [52] or plerixafor [53]. Since use of lenalidomide most commonly follows relapse after autologous stem cell transplant (ASCT) and successful stem cell mobilization in eligible patients, this consideration is rarely problematic in Canada.

Practice considerations are as follows.

(i) MM patients experiencing neutropenia or thrombocytopenia should interrupt lenalidomide treatment until their ANC reaches 1.0×10^9/L and/or their platelet count reaches 30×10^9/L. Lenalidomide may then be restarted at a lower dose, as indicated in Table 2.

(ii) The timing of interrupting and restarting lenalidomide in response to neutropenia and thrombocytopenia should follow the guidance in Tables 3 and 4, respectively.

(iii) To avoid a potential increase in the risk of venous thromboembolism, ESAs should be utilized cautiously with Len/Dex, and the hemoglobin level should be maintained at <120 g/L.

5.2. Nonhematological Toxicities. Many nonhematological adverse effects reported with the combination of Len/Dex are associated with dexamethasone alone, including insomnia, peripheral edema, tremor, muscle weakness, blurred vision, dyspepsia, psychological changes, and hyperglycemia. These adverse events should be managed in the usual manner; if significant and persistent, they may necessitate dexamethasone dose reduction. Additionally, lenalidomide is potentially associated with gastrointestinal symptoms such as diarrhea, constipation, and nausea, as well as with muscle cramps, fatigue, and muscle weakness. As a general rule, for grade 3-4 nonhematological treatment-related toxicities, lenalidomide treatment should be withheld and restarted at the next lower dose level when toxicity has resolved to grade 2 or lower.

5.2.1. Infections. Despite the immunomodulatory effect of lenalidomide, the infection rate was increased with the addition of lenalidomide in both the MM-009 and MM-010 trials [12, 13]. Most infections were low-grade, with grade 3-4 infections seen in 10–20% of patients. Per study protocol, no antibiotic prophylaxis was provided in either of the two phase III trials. Due to this risk of infection, antibiotic prophylaxis may be considered for patients treated with Len/Dex, especially if HD dexamethasone is used. Unfortunately, there currently exists no recommendation for a single antibiotic class for this purpose, but our own preference is levofloxacin. Given that use of LD dexamethasone is associated with less frequent infections in newly diagnosed patients [30], it is not clear whether routine antibiotic prophylaxis is necessary.

In the MM-009 and MM-010 studies, reports of grade 3-4 viral or fungal infections were rare [54].

Practice Considerations are as follows.

(i) LD dexamethasone is associated with a lower risk of infection than HD dexamethasone among new MM patients.

(ii) Given the modest elevation in the risk of infection with Len/Dex treatment, antibiotic prophylaxis may be considered. Acceptable agents include trimethoprim/sulfamethoxazole or levaquin.

5.2.2. Thromboembolic Events. Although the risk of thromboembolic events is low when lenalidomide is administered as a single agent [4], this risk increases when it is used in combination with dexamethasone. The incidences of thromboembolic events in the MM-009 and MM-010 studies were 8.8–14.7% with Len/Dex versus 3.4–4.7% with dexamethasone alone. However, thromboprophylaxis was not required in either of these studies.

The risk of venous thromboembolism is higher within the first few months after initiation of therapy with Len/Dex, decreasing dramatically thereafter [46]. This observation might be explained in part by the administration of higher doses of dexamethasone during the first 4 cycles of therapy, followed by a significant decrease. Indeed, an Eastern Cooperative Oncology Group (ECOG) trial has shown that the incidence of thromboembolism is directly related to dexamethasone dose [30]. The risk of venous thromboembolism among MM patients treated with Len/Dex is comparable to that of other high-risk populations for whom thromboprophylaxis is commonly recommended. A number of prophylactic approaches have been suggested when immunomodulatory agents are administered, including those based on the number of potential risk factors for venous thromboembolism [55, 56]. However, a recently published phase III trial reported similar rates of thrombosis when either enoxaparin or ASA was used as thromboprophylaxis in transplant-eligible patients with newly diagnosed MM treated with lenalidomide-based regimens [57].

In the absence of randomized phase III trials comparing the thromboprophylaxis agents with a control/placebo group in an RRMM setting, it is difficult to draw conclusions concerning the real efficacy of these regimens. Nevertheless, the panel endorsed an approach in which daily ASA was suggested as thromboprophylaxis in patients not known to be at heightened risk of thrombotic events or to be allergic or intolerant to ASA. For those in whom ASA is contraindicated, prophylactic low molecular-weight heparin (LMWH)—such as enoxaparin 40 mg per day—should be used. For patients with a recent history of a thromboembolic event, full anticoagulation with LMWH is recommended, although warfarin could eventually be considered in patients with robust and stable platelet counts while on lenalidomide. Due to the low risk of venous thromboembolism associated with lenalidomide monotherapy (see New Directions, to be mentioned later), thromboprophylaxis in this scenario is not indicated.

Practice considerations are as follows.

(i) For lenalidomide monotherapy, the decision for thromboprophylaxis should be based on medical considerations. Some panel members felt strongly that thromboprophylaxis should be employed routinely in this setting.

(ii) In the absence of contraindications, all patients on Len/Dex therapy should receive thromboprophylaxis. For patients without a history of thromboembolism or other known thrombotic conditions, ASA 81 or 325 mg per day is recommended. Prophylactic doses of LMWH (e.g., enoxaparin 40 mg sc daily) represent an alternative for such low-risk patients.

(iii) Therapeutic anticoagulation with LMWH is recommended as thromboprophylaxis in patients with a recent history of thromboembolism or other known thrombotic disorder. Warfarin may be considered in patients with stable and reliable platelet counts over 100×10^9/L.

(iv) Thromboprophylaxis should be held if the platelet count drops below 50×10^9/L and restarted when patients recover over that threshold.

5.2.3. Rashes. Rashes occur in up to 29% of patients on the Len/Dex regimen [58]. These rashes occur most frequently during the first few weeks of treatment, are usually self-limited, and are severe in only a minority of patients. Nevertheless, Stevens-Johnson syndrome and toxic epidermal necrolysis have been reported and can be fatal. For localized rashes, antihistamines and topical steroids are usually sufficient. For mild but more extensive rashes, short-duration systemic low-dose steroids are usually needed. When rashes are more severe, dose interruption, reduction, or permanent discontinuation may be required, depending on clinical judgment. Importantly, patients with a past history of a severe rash associated with thalidomide should not receive lenalidomide. Of interest, one case of skin hypersensitivity reaction to lenalidomide with successful desensitization has been reported [59]. A similar case has been described for thalidomide [60], further supporting this intervention for those experiencing type I hypersensitivity to lenalidomide. Recommended management of rashes is summarized in Table 7.

Practice considerations are as follows.

(i) If a rash becomes severe, lenalidomide dose may be reduced, interrupted, or discontinued; otherwise, antihistamines and steroids are usually sufficient.

5.2.4. Teratogenicity. Since lenalidomide could potentially be teratogenic in humans, precautions in females with child-bearing potential and males are important to avoid birth defects. In order to reduce these risks, the RevAid program provides a safe access to lenalidomide by stipulating a number of conditions for potential patients. For females of child-bearing potential, birth control using complete abstinence or two contraception methods is mandatory, beginning four weeks before initiation of lenalidomide and up to four weeks after. For males, complete abstinence or use of latex condoms during sexual contact with females of child-bearing potential is mandatory. While it is unknown whether lenalidomide is excreted in breast milk, breastfeeding is generally not recommended.

Practice considerations are as follows.

(i) Females of child-bearing potential and males in sexual contact with such females who are on Len/Dex treatment must use multiple contraception methods.

5.2.5. Other. General symptoms such as fatigue and asthenia are reported at a similar frequency with Len/Dex as with dexamethasone monotherapy. However, these symptoms can become a reason for lenalidomide dose modification or discontinuation, especially in the elderly. Diarrhea and constipation have both been described, each occurring in approximately 20% of patients [45]. Although these symptoms can be routinely managed, our experience indicates that diarrhea may be particularly problematic in certain patients and that ongoing treatment with loperamide or similar agents may allow continuation of full doses of lenalidomide. The fact that lenalidomide capsules contain lactose might contribute to the gastrointestinal side effects noted in some patients.

For unexplained reasons, Len/Dex combination has been associated with a higher incidence of grade 3-4 atrial fibrillation compared to dexamethasone alone (4% versus 1.1%, resp.) [14]. Other side effects, such as loss of appetite and muscle cramps, may be bothersome to patients receiving treatment on a long-term basis. We have found that the use of quinine sulphate 200–300 mg per day is often effective in reducing the incidence and frequency of muscle cramps in significantly affected patients [61]. Anecdotally, patients with severe muscle cramps not completely controlled with quinine have derived relief from daily low doses of benzodiazepines such as clonazepam.

Tumor lysis syndrome has been described with lenalidomide, but it is more often a concern in chronic lymphocytic leukemia patients treated with this agent. Its occurrence in MM has not been well evaluated but appears to be uncommon. Nevertheless, tumor lysis syndrome can occur in any patient with a hematologic malignancy and a high tumor burden or with renal impairment. Thus, proper hydration and monitoring of electrolytes, creatinine, and uric acid is advisable in patients with a high tumor load and/or rapidly proliferating disease.

In contrast to that of thalidomide, the incidence of peripheral neuropathy with lenalidomide is very low [14]. Some cases of neurologic deterioration have been described with lenalidomide, but they might be due to the evolution of prior neuropathy. A recent observational study on the clinical course of peripheral neuropathy during lenalidomide treatment concluded that this therapy does not worsen peripheral neuropathy [62].

Table 7: Management of rashes due to lenalidomide.

Signs/symptoms	Treatment
Localized maculopapular rash	Topical steroids; antihistamines
Widespread maculopapular rash	Hold lenalidomide; topical or oral steroids depending on severity; antihistamines; after resolution, restart lenalidomide at lower dose
Generalize erythroderma or desquamation	Hold lenalidomide; oral steroids; Dermatology consultation; do not restart lenalidomide
Urticaria	Hold lenalidomide; symptomatic management with antihistamines ± oral steroids; after resolution, may attempt desensitization if reinitiation of lenalidomide is planned

Practice considerations are as follows.

(i) Patients with significant diarrhea may require agents such as loperamide on a regular basis.

(ii) Quinine sulphate 200–300 mg per day can reduce muscle cramps in affected patients.

(iii) Although tumor lysis syndrome is considerably more common in patients treated with lenalidomide for chronic lymphocytic leukemia, myeloma patients with a high tumor load and/or rapidly proliferating disease may be at risk for this complication, especially if renal insufficiency is present. Proper hydration and laboratory monitoring is advisable in such patients when lenalidomide is initiated [63, 64].

5.3. Second Primary Malignancies. Emerging data from maintenance therapy studies using lenalidomide (IFM 05-02, CALGB 100104, and MM-015) suggest that long-term use of this agent might be associated with the development of second primary malignancies (SPM). However, in RRMM, after a median followup of 48 months for surviving patients, MM-009 and MM-010 have shown a low incidence of SPM. Furthermore, SPM rates were similar for patients on Len/Dex versus those on dexamethasone alone [40].

After an exhaustive review of clinical trials and post-marketing data, the European Medicines Agency issued a statement on September 23, 2011 to the effect that "the benefit-risk balance for lenalidomide remains positive within its approved patient population but advises doctors of the risk of new cancers as a result of treatment with the medicine." This analysis found that there were 3.98 cases of new cancer for every 100 patient-years in patients receiving lenalidomide compared with 1.38 cases in those not receiving lenalidomide in the approved population (Press Release 23 Sept 2011, European Medicines Agency, http://www.ema.europa.eu/). These included skin cancers as well as hematologic malignancies and some invasive solid tumors. Health Canada issued a similar statement in May 2012, recommending careful evaluation of patients "before and during treatment in order to screen for the occurrence of new malignancies" (http://hc-sc.gc.ca/dhp-mps/medeff/advisories-avis/prof/_2012/revlimid_hpc-cps-eng.php, accessed July 2012).

The panel therefore reiterates that patients on lenalidomide should be watched for signs or symptoms of a new cancer. Routine cancer screening should be followed, per Canadian or local guidelines [65, 66].

Practice consideration are as follows.

(i) The efficacy of lenalidomide in RRMM outweighs the small risk of developing a secondary malignancy.

(ii) Physicians and patients should be aware of this small risk; routine Canadian cancer screening measures should be performed, and any signs or symptoms of a possible second cancer should be evaluated and reported, if appropriate, to the RevAid program.

5.4. Monitoring of Adverse Events. Proper monitoring is required to note emerging side effects and to prevent potential treatment complications. A complete blood count with differential should be obtained every two weeks during the first 3 cycles and subsequently every month before a new cycle. Serum creatinine should be obtained before each cycle in order to adjust the lenalidomide dose according to impaired renal function. Because of possible liver toxicity [67] or thyroid dysfunction [68] associated with lenalidomide therapy, liver function tests including aspartate aminotransferase (AST), alanine aminotransferase (ALT), and bilirubin, as well as thyroid function tests should be done periodically throughout the treatment. Because atrial fibrillation remains a relatively rare event, serial electrocardiograms (ECGs) are not routinely required. For females of child-bearing potential, two pregnancy tests must be negative before starting lenalidomide: at 7–14 days and at 24 hours before administration of the drug. During the treatment, pregnancy tests should be conducted weekly for the first four weeks, then monthly (or every two weeks if menstrual cycles are irregular) until four weeks after treatment cessation.

5.5. New Directions. Given the established efficacy and favorable toxicity profile of lenalidomide in RRMM, this agent has now been evaluated at different time points in the disease course, as well as in combination with drugs other than dexamethasone alone (combination therapy) [20–29, 31–34, 68–75]. Combination of lenalidomide with alkylators, anthracyclines, and/or bortezomib yields very high remission rates (Table 8). So far, no randomized trials have established the superiority of a 3- or 4-drug combination over Len/Dex in terms of PFS or OS. Results of

Table 8: Summary of emerging lenalidomide combination therapies in the first- and second-line treatment of multiple myeloma.

Combination	First line		≥Second line	
	Efficacy	Major toxicities	Efficacy	Major toxicities
MPR Palumbo et al., 2007 [20]	81% ≥ PR	Hematological toxicity		
MPR-R Palumbo et al., 2010 [21]			75% ≥ PR	Hematological toxicity, infections
RMPT Palumbo et al., 2010 [22]				
RVD Richardson et al., 2009, 2010 [23, 24]	100% ≥ PR	Hematological toxicity, sensory neuropathy	61% ≥ MR	Hematological toxicity
CPR Reece et al., 2010 [25]			94% ≥ MR	Hematological toxicity
CRD Schey et al., 2010 [26]			81% ≥ PR	Hematological toxicity
RVDC Kumar et al., 2010 [27]	96% ≥ PR	Hematological toxicity, sensory neuropathy		
CRd Kumar et al., 2011 [28]	85% ≥ PR	Hematological toxicity		
RVDD Jakubowiak et al., 2011 [29]	95% ≥ PR	Fatigue, constipation, sensory neuropathy, infection		

MPR: melphalan, prednisone, lenalidomide; PR: partial response; MPR-R: MPR + lenalidomide maintenance until progression; RMPT: lenalidomide, melphalan, prednisone, thalidomide; RVD: lenalidomide, bortezomib, dexamethasone; MR: minimal response; CPR: cyclophosphamide, prednisone, lenalidomide; CRD: cyclophosphamide, lenalidomide, dexamethasone; RVDC: lenalidomide, bortezomib, dexamethasone, cyclophosphamide; CRd: cyclophosphamide, lenalidomide, dexamethasone; RVDD: lenalidomide, bortezomib, pegylated liposomal doxorubicin, dexamethasone.

an induction trial, comparing Len/dex with MPT (MM020), are anticipated later this year.

In addition, even though lenalidomide—given with dexamethasone—is currently approved only for use after one prior therapy, there is considerable interest in employing this drug as part of initial therapy in newly diagnosed patients. Options in this setting include its administration in induction regimens in patients both eligible and ineligible for ASCT, in addition to its use as maintenance therapy after ASCT.

Phase III trials have now been initiated in these settings, and the available results are summarized in Table 9. Two recent randomized trials indicate that posttransplant maintenance therapy with single agent lenalidomide started 60–100 days after ASCT significantly improves PFS, and one of these trials has noted a survival advantage in the lenalidomide arm [27] . Adoption of lenalidomide maintenance as a standard of care will depend on the identification of the subgroups most likely to benefit, the risk of late complications such as SPM, and the cost implications of such a strategy. On balance, it is likely that the results of recent/ongoing randomized studies will lead to expanded applications of lenalidomide in the treatment of patients with MM.

6. Conclusions

Based on available evidence, Len/Dex appears to be an effective and safe treatment strategy for RRMM patients, regardless of the type and number of prior therapies. In order to ensure optimal balance between efficacy and tolerability, lenalidomide dose and schedule should be adjusted based on creatinine clearance and presence of neutropenia and thrombocytopenia; dexamethasone should typically be administered at weekly doses of 20–40 mg, and treatment should be continued until disease progression or toxicity, even in patients requiring dose reduction.

Although certain adverse events can occur with Len/Dex, the following precautions can significantly reduce their impact: (1) Lenalidomide interruption and dose modification should follow established guidelines, with judicious use of G-CSF and transfusions if needed to avoid potential hematological toxicities; (2) all patients should receive thromboprophylaxis unless contraindicated. In most patients without a history of thrombosis, 81 mg of ASA is sufficient; alternatively, prophylactic doses of LMWH may be administered. Patients with a recent history of thromboembolism or known thrombotic disorder require full anticoagulation while on Len/Dex, usually consisting of LMWH; patients with stable platelet counts over 100×109/L can be considered for coumadin; (3) ESAs should be used cautiously, and if this treatment is used, the hemoglobin target should be <120 g/L; (4) females of child-bearing potential and males in sexual contact with such females must use multiple contraception methods.

Future studies are needed to elucidate the role of lenalidomide as part of initial MM therapy, as well as maintenance therapy after ASCT. Also, while various three- and four-drug combinations including lenalidomide as the

Table 9: Summary of phase III trials evaluating new indications for lenalidomide in the treatment of multiple myeloma.

New indications	Trials	Regimens	Response rate	PFS	OS
Induction therapy	ECOG E4A03 Rajkumar et al., 2010 [30]	Len + HD dex	79%	19.1 mos	75% (2-yr)
		Len + LD dex	68%	25.3 mos	87% (2-yr)
	MM-015 Palumbo et al. 2012 [31] $N = 348$ (Age 65–75)	MP	47%	12 mos	65% (3 yrs)
		MPR		15 mos	~70% (3 yrs)
		MPR-R	79%	31 mos	73% (3 yrs)
	MM-020	MPT Len + LD dex until progression Len + LD dex for 18 mos	In progress	In progress	In progress
	Palumbo et al., 2011 [32] $N = 402$	Len + LD dex × 4 cycles → MPR	20%	54% (2 yrs)	87% (2 yrs)
		Len + LD dex × 4 cycles → ASCT × 2	25%	73% (2 yrs)	90% (2 yrs)
Maintenance therapy after ASCT	IFM2005-02 Attal et al. 2010 [33] $N = 614$	Len	—	42 mos	81% (3 yrs)
		Placebo	—	24 mos	81% (3 yrs)
	CALGB 100104 McCarthy et al. 2010 [34] $N = 568$	Len	—	43.6 mos	~80% (3 yrs)
		Placebo	—	21.5 mos	~80% (3 yrs)
Induction and maintenance ± ASCT in newly diagnosed patients	IFM/Dana Farber trial	VRD × 8 → Len maintenance × 1 yr (ASCT at progression) VRD × 3 → ASCT → Len maintenance × 1 yr	In progress	In progress	In progress

CR: compete response; PFS: progression-free survival; OS: overall survival; Len: lenalidomide; HD dex: high-dose dexamethasone; LD dex: low-dose dexamethasone; MP: melphalan, prednisone; MPR: melphalan, prednisone, lenalidomide; MPR-R: MPR + lenalidomide maintenance until progression; MPT: melphalan, prednisone, thalidomide; ASCT: autologous stem cell transplantation; VRD: bortezomib, lenalidomide, dexamethasone.

backbone appear promising, not enough information is available to recommend combination treatment outside of a clinical trial.

Disclosure

D. Reece research funding from Celgene, Janssen, Millennium Pharmaceuticals, Novartis, Merck, Bristol-Meyers-Squibb and honoraria from Celgene, Janssen, Novartis and Amgen. C. Tom Kouroukis received honoraria and had advisory boards from Celgene and Novartis and Honoraria from Janssen, Amgen. R. LeBlanc received research funding from Janssen, Celgene, The Binding Site and Myeloma Canada and honoraria and advisory board from Celgene, Janssen and Novartis. M. Sebag received research funding from Janssen and honoraria and advisory boards from Celgene, Janssen and Novartis. K. Song received research support from Celgene and honoraria and advisory boards from Celgene, Janssen and Novartis. The participation of J. Ashkenas was supported by Myeloma Canada. J. Ashkenas declares no other areas of possible conflicts, financial or otherwise.

Following submission of this manuscript, Chen et al., sponsored by Cancer Care Ontario. Published a guideline on Lenalidomide in Multiple Myeloma [76].

Acknowledgments

The authors thank Myeloma Canada and Celgene Canada for their support of this project through an unrestricted educational grant. They also thank Peter Janiszewski, Ph.D. SCRIPT, Toronto, Ontario, Canada, for assistance in preparing this paper.

References

[1] Canadian Cancer Society's Steering Committee on Cancer Statistics, *Canadian Cancer Statistics*, Canadian Cancer Society, Toronto, ON, 2011.

[2] S. K. Kumar, S. V. Rajkumar, A. Dispenzieri et al., "Improved survival in multiple myeloma and the impact of novel therapies," *Blood*, vol. 111, no. 5, pp. 2516–2520, 2008.

[3] C. P. Venner, J. M. Connors, H. J. Sutherland et al., "Novel agents improve survival of transplant patients with multiple myeloma including those with high-risk disease defined by

early relapse (<12 months)," *Leukemia and Lymphoma*, vol. 52, no. 1, pp. 34–41, 2011.

[4] P. Richardson, S. Jagannath, M. Hussein et al., "Safety and efficacy of single-agent lenalidomide in patients with relapsed and refractory multiple myeloma," *Blood*, vol. 114, no. 4, pp. 772–778, 2009.

[5] A. K. Gandhi, J. Kang, L. Capone et al., "Dexamethasone synergizes with lenalidomide to inhibit multiple myeloma tumor growth, but reduces lenalidomide-induced immunomodulation of T and NK cell function," *Current Cancer Drug Targets*, vol. 10, no. 2, pp. 155–167, 2010.

[6] N. Mitsiades, C. S. Mitsiades, V. Poulaki et al., "Apoptotic signaling induced by immunomodulatory thalidomide analogs in human multiple myeloma cells: therapeutic implications," *Blood*, vol. 99, no. 12, pp. 4525–4530, 2002.

[7] T. Hideshima, D. Chauhan, Y. Shima et al., "Thalidomide and its analogs overcome drug resistance of human multiple myeloma cells to conventional therapy," *Blood*, vol. 96, no. 9, pp. 2943–2950, 2000.

[8] D. Verhelle, L. G. Corral, K. Wong et al., "Lenalidomide and CC-4047 inhibit the proliferation of malignant B cells while expanding normal $CD34^+$ progenitor cells," *Cancer Research*, vol. 67, no. 2, pp. 746–755, 2007.

[9] A. De Luisi, A. Ferrucci, A. M. L. Coluccia et al., "Lenalidomide restrains motility and overangiogenic potential of bone marrow endothelial cells in patients with active multiple myeloma," *Clinical Cancer Research*, vol. 17, no. 7, pp. 1935–1946, 2011.

[10] V. Kotla, S. Goel, S. Nischal et al., "Mechanism of action of lenalidomide in hematological malignancies," *Journal of Hematology and Oncology*, vol. 2, p. 36, 2009.

[11] F. Davies and R. Baz, "Lenalidomide mode of action: Linking bench and clinical findings," *Blood Reviews*, vol. 24, supplement 1, pp. S13–S19, 2010.

[12] D. M. Weber, C. Chen, R. Niesvizky et al., "Lenalidomide plus dexamethasone for relapsed multiple myeloma in North America," *The New England Journal of Medicine*, vol. 357, no. 21, pp. 2133–2142, 2007.

[13] M. Dimopoulos, A. Spencer, M. Attal et al., "Lenalidomide plus dexamethasone for relapsed or refractory multiple myeloma," *The New England Journal of Medicine*, vol. 357, no. 21, pp. 2123–2132, 2007.

[14] Revlimid Product Monograph. Celgene Inc. July 30, 2011.

[15] D. Reece, K. W. Song, T. Fu et al., "Influence of cytogenetics in patients with relapsed or refractory multiple myeloma treated with lenalidomide plus dexamethasone: adverse effect of deletion 17p13," *Blood*, vol. 114, no. 3, pp. 522–525, 2009.

[16] U. Klein, A. Jauch, T. Hielscher et al., "Chromosomal aberrations $^+$1q21 and del(17p13) predict survival in patients with recurrent multiple myeloma treated with lenalidomide and dexamethasone," *Cancer*, vol. 117, no. 10, pp. 2136–2144, 2011.

[17] H. Avet-Loiseau, J. Soulier, J. P. Fermand et al., "Impact of high-risk cytogenetics and prior therapy on outcomes in patients with advanced relapsed or refractory multiple myeloma treated with lenalidomide plus dexaméthasone," *Leukemia*, vol. 24, no. 3, pp. 623–628, 2010.

[18] M. A. Dimopoulos, E. Kastritis, D. Christoulas et al., "Treatment of patients with relapsed/refractory multiple myeloma with lenalidomide and dexamethasone with or without bortezomib: prospective evaluation of the impact of cytogenetic abnormalities and of previous therapies," *Leukemia*, vol. 24, no. 10, pp. 1769–1778, 2010.

[19] M. Wang, M. A. Dimopoulos, C. Chen et al., "Lenalidomide plus dexamethasone is more effective than dexamethasone alone in patients with relapsed or refractory multiple myeloma regardless of prior thalidomide exposure," *Blood*, vol. 112, no. 12, pp. 4445–4451, 2008.

[20] A. Palumbo, P. Falco, P. Corradini et al., "Melphalan, prednisone, and lenalidomide treatment for newly diagnosed myeloma: a report from the GIMEMA—Italian Multiple Myeloma Network," *Journal of Clinical Oncology*, vol. 25, no. 28, pp. 4459–4465, 2007.

[21] A. Palumbo, M. Delforge, J. Catalano et al., "A phase 3 study evaluating the efficacy and safety of lenalidomide combined with melphalan and prednisone in patients >= 65 years with newly diagnosed multiple myeloma (ndmm): continuous use of lenalidomide vs fixed-duration regimens," *Blood*, vol. 116, abstract no. 622, 2010.

[22] A. Palumbo, A. Larocca, P. Falco et al., "Lenalidomide, melphalan, prednisone and thalidomide (RMPT) for relapsed/refractory multiple myeloma," *Leukemia*, vol. 24, no. 5, pp. 1037–1042, 2010.

[23] P. G. Richardson, E. Weller, S. Jagannath et al., "Multicenter, phase I, dose-escalation trial of lenalidomide plus bortezomib for relapsed and relapsed/refractory multiple myeloma," *Journal of Clinical Oncology*, vol. 27, no. 34, pp. 5713–5719, 2009.

[24] P. G. Richardson, E. Weller, S. Lonial et al., "Lenalidomide, bortezomib, and dexamethasone combination therapy in patients with newly diagnosed multiple myeloma," *Blood*, vol. 116, no. 5, pp. 679–686, 2010.

[25] D. E. Reece, E. Masih-Khan, A. Khan et al., "Phase I-II trial of oral cyclophosphamide, prednisone and lenalidomide (Revlimid) for the treatment of patients with relapsed and refractory multiple myeloma," *Blood*, vol. 116, abstract no. 3055, 2010.

[26] S. A. Schey, G. J. Morgan, K. Ramasamy et al., "The addition of cyclophosphamide to lenalidomide and dexamethasone in multiply relapsed/refractory myeloma patients; a phase I/II study," *British Journal of Haematology*, vol. 150, no. 3, pp. 326–333, 2010.

[27] S. K. Kumar, I. Flinn, S. J. Noga et al., "Bortezomib, dexamethasone, cyclophosphamide and lenalidomide combination for newly diagnosed multiple myeloma: phase 1 results from the multicenter EVOLUTION study," *Leukemia*, vol. 24, no. 7, pp. 1350–1356, 2010.

[28] S. K. Kumar, M. Q. Lacy, S. R. Hayman et al., "Lenalidomide, cyclophosphamide and dexamethasone (CRd) for newly diagnosed multiple myeloma: results from a phase 2 trial," *American Journal of Hematology*, vol. 86, no. 8, pp. 640–645, 2011.

[29] A. J. Jakubowiak, K. A. Griffith, D. E. Reece et al., "Lenalidomide, bortezomib, pegylated liposomal doxorubicin, and dexamethasone in newly diagnosed multiple myeloma: a phase 1/2 multiple myeloma research consortium trial," *Blood*, vol. 118, no. 3, pp. 535–543, 2011.

[30] S. V. Rajkumar, S. Jacobus, N. S. Callander et al., "Lenalidomide plus high-dose dexamethasone versus lenalidomide plus low-dose dexamethasone as initial therapy for newly diagnosed multiple myeloma: an open-label randomised controlled trial," *The Lancet Oncology*, vol. 11, no. 1, pp. 29–37, 2010.

[31] A. Palumbo, R. Hajek, M. Delforge et al., "Continuous lenalidomide treatment for newly diagnosed multiple myeloma," *Blood*, vol. 366, pp. 1759–1769, 2012.

[32] A. Palumbo, F. Cavallo, i. Hardan et al., "Melphalan/ Prednisone/Lenalidomide (MPR) Versus High-Dose Melphalan

and Autologous Transplantation (MEL200) in Newly Diagnosed Multiple Myeloma (MM) Patients <65 Years: results of a randomized phase III study," *Blood*, vol. 118, abstract no. 3069, 2011.

[33] M. Attal, V. Cances-Lauwers, G. Marit et al., "Lenalidomide maintenance treatment after stem-cell transplantation for multiple myeloma," *The New England Journal of Medicine*, vol. 366, pp. 1782–1791, 2010.

[34] P. McCarthy, K. Owzar, and K. Anderson, "Phase III Intergroup study of lenalidomide versus placebo maintenance therapy following single autologous hematopoietic stem cell transplantation (AHSC T) for multiple myeloma: CALGB, 100104," *Blood*, vol. 116, abstract no. 37, 2010.

[35] E. A. Stadtmauer, D. M. Weber, R. Niesvizky et al., "Lenalidomide in combination with dexamethasone at first relapse in comparison with its use as later salvage therapy in relapsed or refractory multiple myeloma," *European Journal of Haematology*, vol. 82, no. 6, pp. 426–432, 2009.

[36] M. Kleber, G. Ihorst, B. Deschler et al., "Detection of renal impairment as one specific comorbidity factor in multiple myeloma: multicenter study in 198 consecutive patients," *European Journal of Haematology*, vol. 83, no. 6, pp. 519–527, 2009.

[37] J. F. San Miguel, M. Dimopoulos, D. Weber et al., "Dexamethasone dose adjustments seem to result in better efficacy and improved tolerability in patients with relapsed/refractory multiple myeloma who are treated with lenalidomide/dexamethasone (MM-009/010 sub-analysis)," *Blood*, vol. 110, abstract no. 2712, 2007.

[38] A. K. Hsu, H. Quach, T. Tai et al., "The immunostimulatory effect of lenalidomide on NK-cell function is profoundly inhibited by concurrent dexamethasone therapy," *Blood*, vol. 117, no. 5, pp. 1605–1613, 2011.

[39] J. F. San-Miguel, M. A. Dimopoulos, E. A. Stadtmauer et al., "Effects of lenalidomide and dexamethasone treatment duration on survival in patients with relapsed or refractory multiple myeloma treated with lenalidomide and dexamethasone," *Clinical Lymphoma, Myeloma and Leukemia*, vol. 11, no. 1, pp. 38–43, 2011.

[40] M. Dimopoulos and N. R. Orlowski, "Lenalidomide and dexamethasone (LEN plus DEX) treatment in relapsed/refractory multiple myeloma (RRMM) patients (pts) and risk of second primary malignancies (SPM): analysis of MM-009/010.," *Journal of Clinical Oncology*, vol. 29, abstract no. 8009, 2011.

[41] B. Nair, F. Van Rhee, J. D. Shaughnessy et al., "Superior results of total therapy 3 (2003-33) in gene expression profiling-defined low-risk multiple myeloma confirmed in subsequent trial 2006-66 with VRD maintenance," *Blood*, vol. 115, no. 21, pp. 4168–4173, 2010.

[42] T. Guglielmelli, S. Bringhen, S. Rrodhe et al., "Previous thalidomide therapy may not affect lenalidomide response and outcome in relapse or refractory multiple myeloma patients," *European Journal of Cancer*, vol. 47, no. 6, pp. 814–818, 2011.

[43] S. Madan, M. Q. Lacy, A. Dispenzieri et al., "Efficacy of retreatment with immunomodulatory drugs (IMiDs) in patients receiving IMiDs for initial therapy of newly diagnosed multiple myeloma," *Blood*, vol. 118, pp. 1763–1765, 2011.

[44] K. C. Altekruse SF, M. Krapcho, N. Neyman et al., Eds., *SEER Cancer Statistics Review, 1975–2007*, National Cancer Institute, Bethesda, MD, USA, 2010.

[45] C. Chen, D. E. Reece, D. Siegel et al., "Expanded safety experience with lenalidomide plus dexamethasone in relapsed or refractory multiple myeloma," *British Journal of Haematology*, vol. 146, no. 2, pp. 164–170, 2009.

[46] J. D. Ishak, M. A. Weber, D. Knight et al., "Declining rates of adverse events and dose modifications with lenalidomide in combination with dexamethasone," *Blood*, vol. 112, abstract no. 3708, 2008.

[47] S. B. Lonial, R. Swern, A. S. . Weber et al., "Neutropenia is a predictable and early event in affected patients with relapsed/refractory multiple myeloma treated with lenalidomide in combination with dexamethasone," *Blood*, vol. 114, abstract no. 2879, 2009.

[48] R. Niesvizky, T. Naib, P. J. Christos et al., "Lenalidomide-induced myelosuppression is associated with renal dysfunction: adverse events evaluation of treatment-naïve patients undergoing front-line lenalidomide and dexamethasone therapy," *British Journal of Haematology*, vol. 138, no. 5, pp. 640–643, 2007.

[49] S. Kumar, A. Dispenzieri, M. Q. Lacy et al., "Impact of lenalidomide therapy on stem cell mobilization and engraftment post-peripheral blood stem cell transplantation in patients with newly diagnosed myeloma," *Leukemia*, vol. 21, no. 9, pp. 2035–2042, 2007.

[50] U. Popat, R. Saliba, R. Thandi et al., "Impairment of filgrastim-induced stem cell mobilization after prior lenalidomide in patients with multiple myeloma," *Biology of Blood and Marrow Transplantation*, vol. 15, no. 6, pp. 718–723, 2009.

[51] A. Nazha, R. Cook, D. T. Vogl et al., "Stem cell collection in patients with multiple myeloma: impact of induction therapy and mobilization regimen," *Bone Marrow Transplantation*, vol. 46, no. 1, pp. 59–63, 2011.

[52] T. Mark, J. Stern, J. R. Furst et al., "Stem cell mobilization with cyclophosphamide overcomes the suppressive effect of lenalidomide therapy on stem cell collection in multiple myeloma," *Biology of Blood and Marrow Transplantation*, vol. 14, no. 7, pp. 795–798, 2008.

[53] I. N. M. Micallef, A. D. Ho, L. M. Klein, S. Marulkar, P. J. Gandhi, and P. A. McSweeney, "Plerixafor (Mozobil) for stem cell mobilization in patients with multiple myeloma previously treated with lenalidomide," *Bone Marrow Transplantation*, vol. 46, no. 3, pp. 350–355, 2011.

[54] R. L. Baz, S. Hussein, M. Swern et al., "Lenalidomide (LEN) therapy in combination with dexamethasone (DEX) is associated with a low incidence of viral infections," *Blood*, vol. 116, abstract no. 1950, 2010.

[55] M. Carrier, G. Le Gal, J. Tay, C. Wu, and A. Y. Lee, "Rates of venous thromboembolism in multiple myeloma patients undergoing immunomodulatory therapy with thalidomide or lenalidomide: a systematic review and meta-analysis," *Journal of Thrombosis and Haemostasis*, vol. 9, no. 4, pp. 653–663, 2011.

[56] A. Palumbo, M. Cavo, S. Bringhen et al., "Aspirin, warfarin, or enoxaparin thromboprophylaxis in patients with multiple myeloma treated with thalidomide: a phase III, open-label, randomized trial," *Journal of Clinical Oncology*, vol. 29, no. 8, pp. 986–993, 2011.

[57] A. Larocca, F. Cavallo, S. Bringhen et al., "Aspirin or enoxaparin thromboprophylaxis for newly-diagnosed multiple 11 myeloma patients treated with lenalidomide," *Blood*, vol. 119, no. 4, pp. 933–939, 2012.

[58] H. P. Sviggum, M. D. P. Davis, S. V. Rajkumar, and A. Dispenzieri, "Dermatologic adverse effects of lenalidomide therapy for amyloidosis and multiple myeloma," *Archives of Dermatology*, vol. 142, no. 10, pp. 1298–1302, 2006.

[59] J. Phillips, J. Kujawa, M. Davis-Lorton, and A. Hindenburg, "Successful desensitization in a patient with lenalidomide

hypersensitivity," *American Journal of Hematology*, vol. 82, no. 11, p. 1030, 2007.

[60] E. Nucera, D. Schiavino, S. Hohaus et al., "Desensitization to thalidomide in a patient with multiple myeloma," *Clinical Lymphoma and Myeloma*, vol. 8, no. 3, pp. 176–178, 2008.

[61] S. El-Tawil, T. Al Musa, H. Valli, M. P. Lunn, T. El-Tawil, and M. Weber, "Quinine for muscle cramps," *Cochrane database of systematic reviews*, vol. 12, Article ID CD005044, 2010.

[62] R. Zambello, T. Berno, L. Candiotto et al., "Peripherial neuropathy clinical course during lenalidomide therapy for relapsed/refractory multiple myeloma: a single-centre prospective non interventional study," *Haematologica*, vol. 96, abstract no. P-399, 2011.

[63] C.-M. Wendtner, P. Hillmen, D. Mahadevan et al., "Final results of a multicenter phase 1 study of lenalidomide in patients with relapsed or refractory chronic lymphocytic leukemia," *Leukemia and Lymphoma*, vol. 53, no. 3, pp. 417–423, 2012.

[64] C. S. Chim, "Rapid complete remission in multiple myeloma with bortezomib/thalidomide/ dexamethasone combination therapy following development of tumor lysis syndrome," *Cancer Chemotherapy and Pharmacology*, vol. 62, no. 1, pp. 181–182, 2008.

[65] D. J. Leddin, R. Enns, R. Hilsden et al., "Canadian Association of Gastroenterology position statement on screening individuals at average risk for developing colorectal cancer: 2010," *Canadian Journal of Gastroenterology*, vol. 24, no. 12, pp. 705–714, 2010.

[66] J. Izawa, K. L. D. Siemens et al., "Prostate cancer screening: Canadian guidelines 2011," *Canadian Urological Association Journal*, vol. 5, pp. 235–240, 2010.

[67] S. Hussain, R. Browne, J. Chen, and S. Parekh, "Lenalidomide-induced severe hepatotoxicity," *Blood*, vol. 110, no. 10, p. 3814, 2007.

[68] M. K. Figaro, W. Clayton, C. Usoh et al., "Thyroid abnormalities in patients treated with lenalidomide for hematological malignancies: results of a retrospective case review," *American Journal of Hematology*, vol. 86, no. 6, pp. 467–470, 2011.

[69] F. Gay, S. S. Vincent Rajkumar, P. Falco et al., "Lenalidomide plus dexamethasone vs. lenalidomide plus melphalan and prednisone: a retrospective study in newly diagnosed elderly myeloma," *European Journal of Haematology*, vol. 85, no. 3, pp. 200–208, 2010.

[70] A. J. Jakubowiak, D. Dytfeld, S. Jagannath et al., "Carfilzomib, lenalidomide, and dexamethasone in newly diagnosed multiple myeloma: initial results of phase I/II MMRC trial," *Blood*, vol. 116, abstract no. 862, 2010.

[71] S. Lentzsch, A. O. 'Sullivan, R. Kennedy et al., "Combination of bendamustine, lenalidomide, and dexamethasone in patients with refractory or relapsed multiple myeloma is safe and highly effective: results of a phase i clinical trial," *Blood*, vol. 116, abstract no. 989, 2010.

[72] S. Lonial, R. Vij, J. L. Harousseau et al., "Elotuzumab in combination with lenalidomide and low-dose dexamethasone in patients with relapsed/refractory multiple myeloma: interim results of a phase 1 study," *Blood*, vol. 116, abstract no. 1936, 2010.

[73] A. Palumbo, P. Falco, A. Falcone et al., "Melphalan, prednisone, and lenalidomide for newly diagnosed myeloma: kinetics of neutropenia and thrombocytopenia and time-to-event results," *Clinical Lymphoma and Myeloma*, vol. 9, no. 2, pp. 145–150, 2009.

[74] D. J. White, N. J. Bahlis, D. C. Marcellus et al., "Phase II testing of lenalidomide plus melphalan for previously untreated older patients with multiple myeloma: the NCIC CTG MY. 11 trial," *Blood*, vol. 112, abstract no. 2767, 2008.

[75] W. Bensinger, M. Wang, R. Z. Orlowski et al., "Dose-escalation study of carfilzomib (CFZ) plus lenalidomide (LEN) plus low-dose dexamethasone (Dex) (CRd) in relapsed/refractory multiple myeloma (R/R MM)," *Journal of Clinical Oncology*, vol. 28, abstract no. 8029, 2010.

[76] C. Chen, F. Baldassarre, S. Kanjeekal et al., *Lenalidomide in Multiple Myeloma*, Program in Evidence-Based Care Evidence-Based Series no. 6-5, Cancer Care Ontario, Toronto, Canada, 2012.

Hindawi Publishing Corporation
Advances in Hematology
Volume 2012, Article ID 842945, 11 pages
doi:10.1155/2012/842945

Review Article

Biological Activity of Lenalidomide and Its Underlying Therapeutic Effects in Multiple Myeloma

Roberta Martiniani, Valentina Di Loreto, Chiara Di Sano, Alessandra Lombardo, and Anna Marina Liberati

Department of Oncohematology, University of Perugia, Santa Maria Hospital, 05100 Terni, Italy

Correspondence should be addressed to Anna Marina Liberati, marinal@unipg.it

Received 16 February 2012; Revised 1 May 2012; Accepted 2 May 2012

Academic Editor: Agostino Cortelezzi

Lenalidomide is a synthetic compound derived by modifying the chemical structure of thalidomide. It belongs to the second generation of immunomodulatory drugs (IMiDs) and possesses pleiotropic properties. Even if lenalidomide has been shown to be active in the treatment of several hematologic malignancies, this review article is mostly focalized on its mode of action in multiple myeloma. The present paper is about the direct and indirect antitumor effects of lenalidomide on malignant plasmacells, bone marrow microenvironment, bone resorption and host's immune response. The molecular mechanisms and targets of lenalidomide remain largely unknown, but recent evidence shows cereblon (CRBN) as a possible mediator of its therapeutical effects.

1. Introduction

Lenalidomide and pomalidomide are synthetic compounds derived by modifying the chemical structure of thalidomide [1]. In particular, as shown in Figure 1, lenalidomide has been synthesized from the structural bone of thalidomide molecule. Lenalidomide has been developed by adding an amino group (NH2–) at 4th position of phthaloyl ring and by removing the carbonyl group (C=O) of the 4-amino-substituted phthaloyl ring. This drug is the result of the pressing need to develop molecules with enhanced immunomodulatory and antitumor activity in comparison to thalidomide. Lenalidomide, which possesses pleiotropic properties, belongs to the second generation of immunomodulatory drugs (IMiDs).

Lenalidomide and its parental molecule thalidomide have shown therapeutical activity in various malignancies [2–21].

The US Food and Drug Administration (FDA) first approved lenalidomide for the treatment of patients suffering from 5q-myelodysplastic syndrome [22]. However, because of the proven activity of thalidomide in multiple myeloma (MM), the clinical activity of lenalidomide has been evaluated more extensively in this neoplasia [7–12], in respect to other B-cell neoplasia. The favourable toxic profile of lenalidomide and its antitumor activity emerged from phase I and phase II studies in relapsed or refractory MM patients [23–25]. These encouraging results led to the design of two large, phase III, multinational, randomized, double-blind, placebo-controlled, registration trials (MM-009 in US and Canada and MM-010 in Europe, Australia, and Israel) in this setting of patients. In both studies, patients were randomly assigned to receive 25 mg of lenalidomide or placebo on days 1 to 21 of 28-day cycles plus dexamethasone (40 mg on days 1 to 4, 9 to 12 and 17 to 20 for the first four cycles, then only on days 1 to 4). The results of these trials have shown the superiority of lenalidomide-dexamethasone combination compared to placebo-dexamethasone in terms of time to progression (11,1–11,3 months versus 4,7 months in the lenalidomide and in the placebo group, resp., $P < 0,001$), overall survival (in MM-009: 29,6 months versus 20,2 months in the lenalidomide and in the placebo group, resp., $P < 0,001$, in MM-010: hazard ratio for death 0,66, $P = 0,03$) and overall response rate (60,2–61% versus 19,9–24% in the lenalidomide and in the placebo group, resp., $P < 0,001$). At a median followup of 48 months for surviving patients, a pooled update analysis of these studies has shown

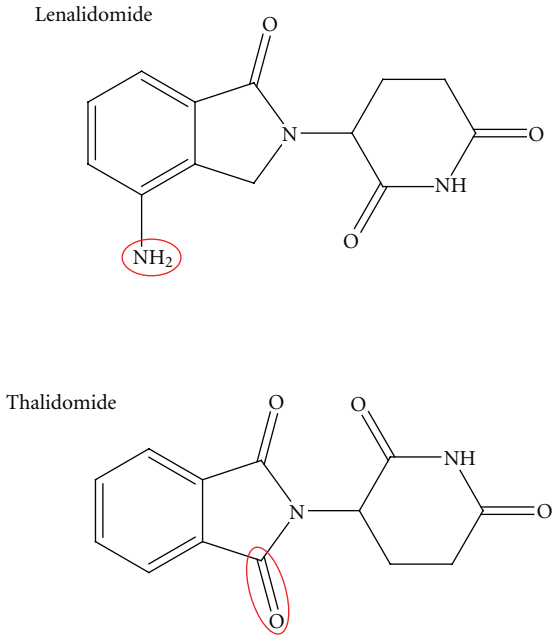


Figure 1: Lenalidomide and thalidomide structure.

a significant benefit in overall survival (38 versus 31,6 months, $P < 0,045$) for those patients initially randomized to be treated with lenalidomide-dexamethasone combination [8, 26]. It should be emphasized that the improved survival associated to lenalidomide-dexamethasone treatment was retained despite 47,6% of patients, who were initially randomized to placebo dexamethasone, received lenalidomide-based therapies after disease progression or study unblinding [27]. More recently, several studies have compared the activity of lenalidomide combined with high or reduced dose of dexamethasone in newly diagnosed MM patients [28, 29]. The results of these experiences are in favour of low dose of dexamethasone. Furthermore, clinical experience with lenalidomide indicates that early use in MM therapy is associated with a higher response rate and, possibly, prolonged survival [30]. To further improve the outcome of lenalidomide, combination regimens (BiRd, VRD, RAD, VDCR, and VRDD) [31–35] have been evaluated or are under investigation in both old and young MM patients, in transplant and non transplant settings.

MM has been chosen for this article with the purpose of showing our current knowledge on the mechanisms of antitumor activity of lenalidomide. Some of these actions are operative in other diseases too.

2. Biological Features of Multiple Myeloma

To understand the therapeutic activity of lenalidomide in MM, the knowledge of the pathophysiology of this disease and the complex crosstalk between malignant plasma cells (PCs) and their microenvironment in tumor growth and progression is relevant. In addition, the notion that survival of neoplastic cells is dependent on the escape from the host's antitumor immune response can help to explain the therapeutic role of lenalidomide.

Two major pathways are involved in the early pathogenesis of MM [36, 37]. Nearly half of these tumors are nonhyperdiploid and mostly are characterized by immunoglobulin H (IgH) translocations that involve five recurrent chromosomal loci, including 11p13, 6p21, 4p15, 16p23, and 20p11, which result in the dysregulated expression of an oncogene [36, 38]. These genetic lesions are responsible, at least in part, for anen hanced proliferative capacity of malignant PCs. In fact, the translocations lead directly (11q13 cyclin D1 and 6p21 cyclin D3) or indirectly (4p16, 16p23, 20p11 cyclin D2) to cyclin D dysregulation. In hyperdiploid tumors, cyclin D1 or less often cyclin D2 is usually dysregulated too [38]. Cyclin D, together with CDK4 and CDK6, regulates G1-S cell cycle progression by phosphorylating and inactivating retinoblastoma protein (RB). This reaction is inhibited by the CDK inhibitors p161INK4a and p181INK4c. These molecules can undergo mutations in MM and, in addition to cyclin D [39–41] dysregulation, can further facilitate the proliferation of the neoplastic clone.

Malignant PCs reside in the BM microenvironment which comprises physical and soluble factors. Physical elements of BM include extracellular matrix (ECM), glycoproteins, hemopoietic stem, progenitor, and precursor cells, as well as B, T, and NK lymphocytes, bone marrow endothelial cells, osteoclasts, and osteoblasts and bone marrow stromal cells (BMSCs). Tumor cells adhere to ECM proteins and BMSCs. These interactions are responsible for tumor cell localization in the BM milieu and moreover for multiple biologically relevant sequelae [37, 42]. Adhesion molecules, including CD44, very late antigen 4 (VLA-4), very late antigen 5 (VLA-5), leukocyte function-associated antigen-1 (LFA-1, CD11a), neural cell adhesion molecule (NCAM, CD56), intercellular adhesion molecule-1 (ICAM-1, CD54), syndecan (CD138), and monocyte chemoattractant protein-1 (MPC-1), mediate adhesion of malignant PCs to either ECM proteins or BMSCs [37, 42] Table 1.

The initial homing of neoplastic cells to the BM milieu is mediated by the binding of the stromal-derived growth factor (SDF-1α), present in the BM, to its receptor CXCR4, expressed by malignant PCs. High serum concentrations of SDF-1α correlate with a more aggressive disease. This event is the consequence of the effect of this chemokine on IL-6 and VEGF production by BMSCs. These cytokines promote PC growth and survival [43]. Furthermore, SDF-1α modulates the expression of adhesion molecules on PCs (VLA4 and LFA-1) and BMSCs (VCAM-1 and ICAM-1) and favours the adherence between these cells. Syndecan and VLA-4, expressed on malignant PCs, mediate their adhesion to collagen and fibronectin, respectively [44, 45]. Finally, adhesion of malignant PCs via syndecan to collagen induces matrix metalloproteinase-1, thereby promoting bone resorption and tumor invasion, while binding via VLA-4 to fibronectin is responsible for cell adhesion-mediated drug resistance (CAM-DR) [44, 45] (Figure 2).

Moreover, adhesion of PCs to BMSCs triggers, in these latter cells, the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) which results in

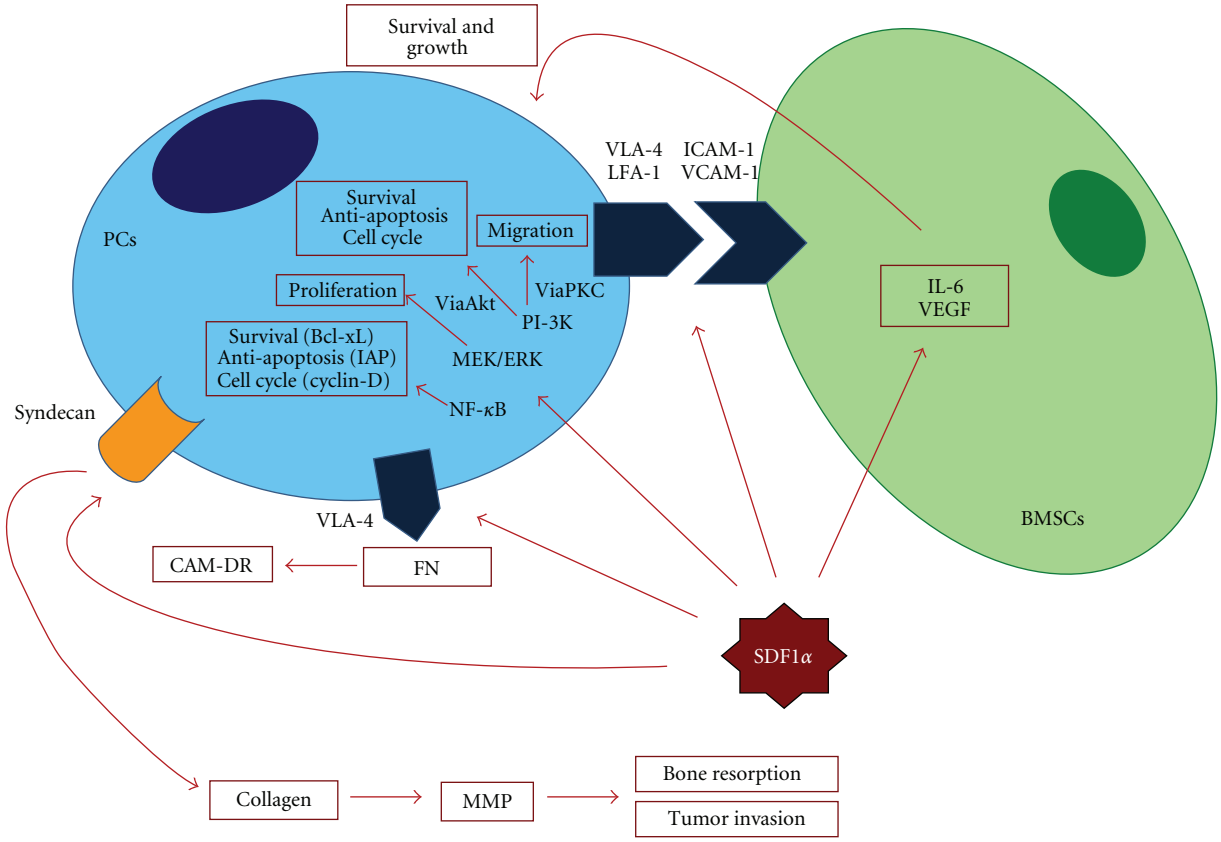


Figure 2: SDF-1α actions and its functional sequelae.

Table 1: "Crosstalk" between PC and BMSC.

Adhesion molecules
Very late activation antigens-4 (VLA-4)
Lymphocyte function-associated antigen-1 (LFA-1)
Vascular cell adhesion molecule-1 (VCAM-1)
Intercellular adhesion molocule-1 (ICAM-1)
Syndecan-1
Cytokines
Tumor necrosis factor-α (TNF-α)
Transforming growth factor-β (TGF-β)
Vascular endothelial growth factor (VEGF)
Fibroblast growth factor-2 (FGF-2)
Hepatocyte growth factor (HGF)
Angiopoietin-1 (Ang-1)
Interleukin-6 (IL-6)
Insulin-like growth factor (IGF-1)
Proteasi
Matrix metalloproteinases -2 and -9 (MMP-2 e MMP-9)
Chemokine
Macrophage inflammatory protein-1 (MIP-1)
Stromal derived factor-1 (SDF-1)

both further upregulation of adhesion molecules, transcription and secretion of interleukine-6 (IL-6) [46] and other cytokines (vascular endothelial growth factor (VEGF), basic fibroblast growth factor (b-FGF), tumor necrosis factor-α [TNFα] and insulin-like growth factor-1 [IGF-1]) within the BM milieu [47, 48]. (Figure 3).

In detail, IL-6 is a critical growth factor for normal B-cell and PC development. IL-6 is primarily produced by BMSCs and by only a few malignant PCs [49]. TNFα is secreted by both malignant PCs and BMSCs. It does not induce growth and survival of the neoplastic clone directly, but it binds to a TNFα response element of the IL-6 promoter in BMSCs inducing paracrine production of IL-6 [49, 50]. Furthermore, TNFα secreted by malignant PCs activates NF-kB pathway, which results in additional upregulation of adhesion molecules (CD49d, an integrin alpha subunit and ICAM-1) on both tumor PCs and BMSCs [50]. The final effects of this loop consist in additional paracrine secretion of IL-6, as well as that of IGF-1 and VEFG by BMSCs and in induction of CAM-DR [44, 46] (Figure 4).

Cytokine secretion in BMSCs is also upregulated by PC-derived transforming growth factor β (TGF-β) and VEGF [48]. This event, in turn, induces BMSCs to produce further TNFα, VEGF and b-FGF. Overall, these events lead to the generation of a vicious circuit responsible for continuously increased cytokine production and malignant PC clone expansion.

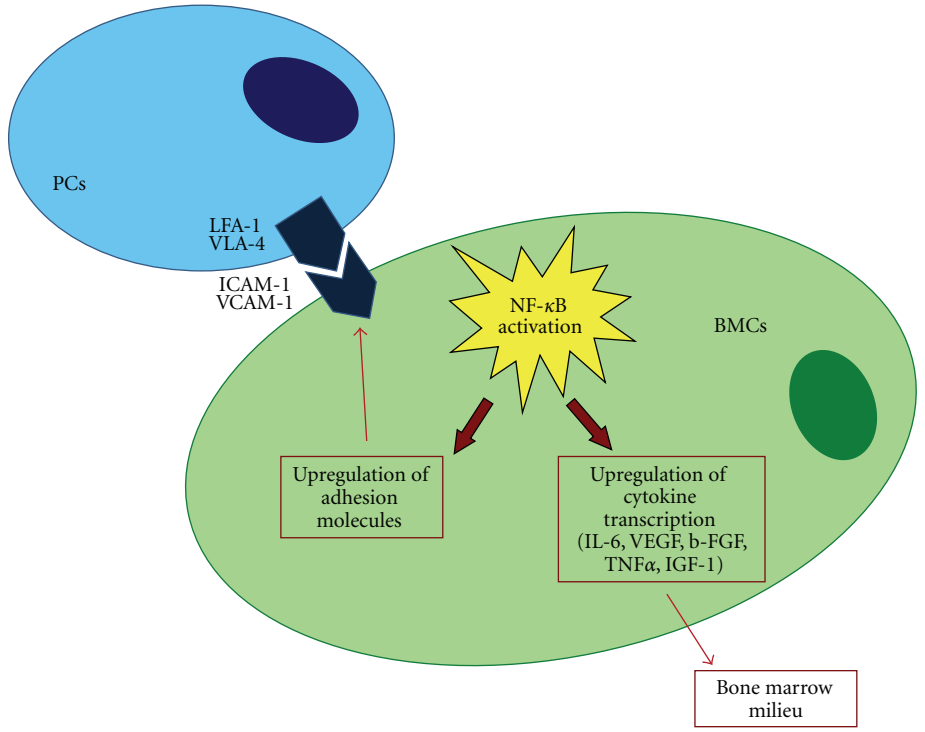


Figure 3: NF-kB activation and its functional biological sequelae.

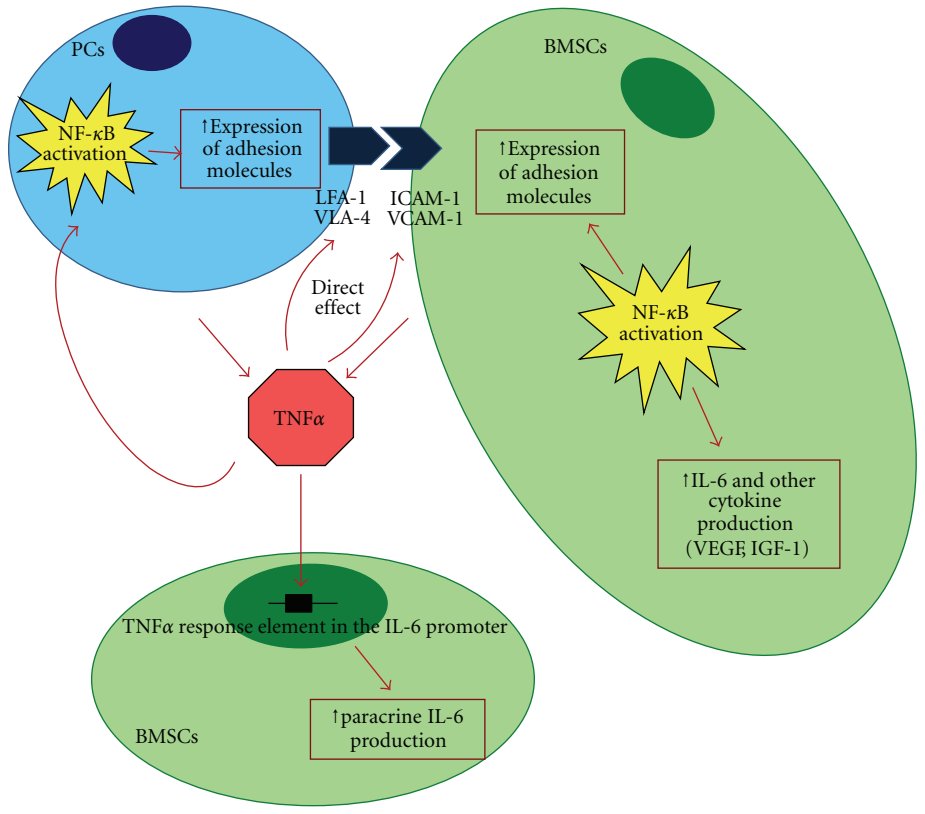


Figure 4: Induction of IL-6 secretion by TNFα and NF-kB activation.

Binding of the cytokines to their receptors, expressed on malignant PCs, leads to activation of mitogenic/antiapoptotic pathways (mitogen actived protein kinase [MAPK], janus kinase/signal transducer and activator of transcription [JAK/STAT], phosphatidylinositol 3-kinases/protein kinase B [PI-3K/Akt] and inhibitor of nuclear factor kappa-B kinase [IKK/NF-kB]) [37], which promote cell proliferation, survival, cycle progression and migration. Survival is also mediated by increased transcription of antiapoptotic molecules (B cell lymphoma gene-2 [Bcl-2] family members such as B-cell lymphoma-extra large [Bcl-xL], myeloid cell factor-1 [Mcl-1] and caspase inhibitor such as Fas-Associated protein with Death Domain-like [FADD-like] IL-1β-converting enzyme (FLICE) inhibitor protein (FLIP) and cellular inhibitor of apoptosis protein 2 (cIAP-2)) in malignant PCs which act along with disregulated cyclins whose expression is further upregulated by NF-kB activation [51–54]. Overall, cytokines present in the BM milieu, reflecting the PC-BMSC bidirectional interactions, mediate growth (IL-6, IGF-1, VEGF), survival (IL-6, IGF-1), drug resistance (IL-6, IGF-1, VEGF), and migration (IGF-1, VEGF, SDF1α) of neoplastic cells as well as angiogenesis (VEGF, b-FGF) (Figure 5).

Because of its pleiotropic properties, lenalidomide interferes with several pathogenetic relevant moments associated to different clinical phases of MM.

First of all, lenalidomide upregulates the cyclin dependent kinase inhibitor 1 (p21/waf1), a key cell cycle regulator that modulates the activity of cyclin dependent kinase (CDKs) [55]. Recently it has been demonstrated that lenalidomide mediates the increased expression of p21 by an epigenetic mechanism [56]. Lenalidomide reduces histone methylation and increases histone acetylation of the p21 promoter, thus enhancing transcription factor access to the DNA. In addition to upregulation of p21, lenalidomide-mediated growth inhibition has been demonstrated to be associated with the induction of CDK inhibitors p15, p16 and p27 and the early response transcription factors Egr1, Egr2 and Egr3 [57]. In MM derived cell lines, U266 and LP-1, reduction in CDK2 activity has been demonstrated after exposure to lenalidomide [55].

Lenalidomide inhibits the production of proinflammatory cytokines TNF-α, IL-1, IL-6, and IL-12 and increases the secretion of anti-inflammatory cytokine IL-10. IMiDs have an opposite effect on IL-12 production, depending on the different type of stimulation on peripheral blood mononuclear cells [58].

Lenalidomide also downregulates adhesion molecules. This effect is mediated by the inhibition of TNFα production [50]. Thus, lenalidomide ultimately suppresses a positive feedback loop which upregulates the expression of cell surface adhesion molecules on both BMSCs and malignant PCs. Moreover the downregulation of PC adherence to BMSCs reduces the production of cytokines by these cells (IL-6, VEGF, IGF-1) which, as previously indicated, are responsible for thegrowth and survival of neoplastic clone. Lenalidomide also reduces the production of IL-6 by a direct action [59].

Increased micro-vascular density has been reported to correlate with MM-progression [60]. VEGF is produced by malignant PCs and BMSCs and accounts, at least in part, for increased angiogenesis in the BM of MM patients [60]. All IMiDs, including lenalidomide, possess antiangiogenic activity. This effect appears to occur via the modulation of TNFα, VEGF and b-FGF, which regulate endothelial cell migration, rather than cell proliferation. Antiangiogenesis by lenalidomide correlates with reduced Akt phosphorylation in response to both VEGF and bFGF [61]. Beyond the anti-angiogenesis, the lenalidomide induced-inhibition of VEFG and bFGF production determines other biological effects. In fact, these growth factors upregulate the production by BMSCs of pro-inflammatory cytokines including IL-6.

Apoptosis is triggered by the activation of both extrinsic and intrinsic pathways. Besides, the success of this process is also related to the down-regulation of inhibitor of apoptosis protein (IAP) activity. In malignant PCs, caspase 8 is activated in response to extracellular apoptosis-inducing ligand (i.e., FADD) [62]. Lenalidomide is able to induce caspase 8 activity which in turn results in increased malignant PC apoptosis [62]. Bcl-2 homology domains (BH3) interacting domain death agonist (Bid) can mediate a cross-talk of apoptotic signaling from caspase 8 to caspase 9 [63]. On the other hand,dexamethasone induced apoptosis in MM cells is associated with caspase 9 activation and release of second mitochondrial-derived activator of caspases (Smac) [64]. Moreover, long term treatment of malignant PCs with lenalidomide determines a downregulation of NF-kB activity, which results in a reduction of antiapoptotic proteins including cIAP2 [65] and FLIP [66]. Thus, lenalidomide induced apoptosis is the result of multiple effects consisting in the direct upregulation of caspase 8 activity, indirect upregulation of caspase 9 and the downregulation of NF-kB activity which, in turn, determines the inhibition of FLIP and cIAP2 and antagonizes prosurvival effects mediated by several cytokines (IL-6 and IGF-1). NF-kB is activated by IL-6 and determines the production of antiapoptotic proteins. Consequently, lenalidomide, by inactivating NF-kB, inihibits the antiapoptotic activity induced by IL-6.

Defective host immune surveillance has a central role in the survival of malignant PCs [67]. The mechanism responsible for myeloma cell tolerance includes the immunosuppressive activity of cytokines such as TGF-β derived by malignant PCs [68], reduced numbers of CD4+ T-cells [69], impaired cytotoxic CD8+ T-cell responses [70], defective antigen presentation, disfunction of human natural killer-T (NK-T) and natural killer (NK) [71, 72] cells as well as resistance to NK cell lysis [73].

Lenalidomide acts at different levels in the immune system by modifying cytokine production, improving T-cell activity, regulating T-cell co-stimulation and augmenting the NK-T- and NK-cell cytotoxicity. Lenalidomide enhances the cytolytic activity of antigen-specific CD8+ T-cells. This effect has been demonstrated in a dendritic cell/CD8+ T-cell in vitro co-culture system. It appears to be mediated by IL-2 induced expansion of antigen-specific memory effector CD8+ T-cells [74].

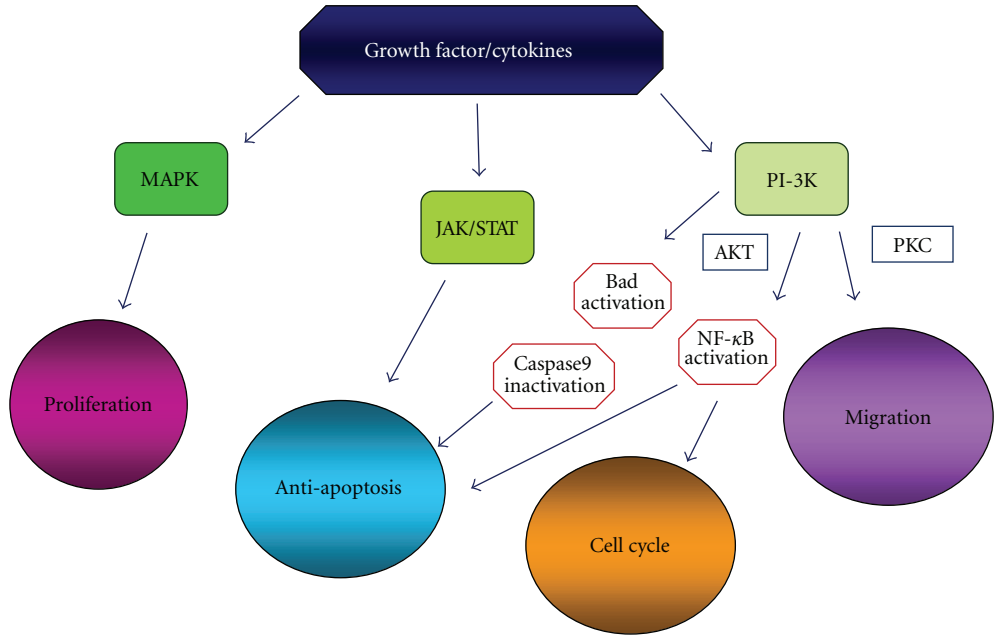


Figure 5: Signaling pathways activated by BM cytokines.

T-cell activation requires the presentation of the peptide fragments by antigen presenting cell (APC) to the T-cell receptor (TCR). Moreover to generate an effective response against the antigen, a secondary interaction is required [75]. This is mediated by the B7 family molecules on APC and CD28 molecule on the T-cell surface and provide the costimulatory signal that augments and potentiates T-cell proliferation, differentiation and survival followed by IL-2 and IFNγ production. In MM patients the number of dendritic cells (DCs) is normal, but CD80 (B7-1) expression may fail to be upregulated in the presence of trimeric human CD40-ligand (HU-CD40LT) because of the negative effect of tumor-derived TGF-β or IL-10 [76]. Impairment of T all activation by DCs is also mediated by IL-6 [77] and VEGF [78] of PC or BMSC origin. IMiDs including lenalidomide are only able to stimulate T-cells that have been partially activated by either anti-CD3 or DCs [75]. Lenalidomide induces the proliferation of partially activated CD3+ T cells obtained from human PBMC. T-cell proliferation is associated with increased IL-2 and INFγ production. The mechanism of T-cell co-stimulation by lenalidomide involves increased transcriptional activity of activated protein-1 (AP-1), a driver of IL-2 production [79]. In addition, this drug abrogates the requirement of a secondary co-stimulation signal from APCs to allow T-cell activation. In fact, it acts on T-cells via the B7-CD28 costimulatory pathway directly inducing tyrosine phosphorylation of CD28 on T-cells leading to the activation of downstream targets such as PI3K-signaling pathway and the nuclear translocation of the nuclear factor of activated T cells-2 (NFAT-2) [75, 80].

NK-T- and NK-cells belong to distinct lymphocyte lineages. However, these cells share striking similarities such as the expression of the same set of receptors (NKR-P1 and Ly49) and the capacity to rapidly release, without prior sensitization, INFγ and IL-4 (NK-T) or INFγ alone (NK) [81, 82]. IL-12 can modulate both NK-T-cells [83, 84] and NK-cells [85] to release INFγ and exert natural cytotoxicity. NK-T-cells are distinct lymphocytes, which often use a restricted T cell receptor (Vα24-Vβ11) that recognizes glicolipid ligands in the context of the major histocompatibility class 1-like CD1d molecule. The anti-tumor properties of these cells include a direct cytotoxic effect of neoplastic cells, INFγ production and interaction with DC expressing glicolipid ligands. Lenalidomide increases the NK-T-cell expansion mediated by DCs loaded with α GalCer and INFγ production from NK-T-cells [86]. Because of the cross-talk between NK-T- and NK-cells, NK-T-cells transact with NK-cells. This network of activation later involves B and T cells indicating the sequential recruitment of distinct and adaptive effector lymphocytes [87]. Lenalidomide might potentiate the function of these other immune cells, using the transactivation mediated by NK-T-cells.

Lenalidomide not only increases NK-cell proliferation, but also potentiates natural and antibody dependent cellular cytotoxicity (ADCC) of NK-cells [88]. These effects are mediated by lenalidomide-induced IL-2 production by T cells. More in detail, lenalidomide triggers PI3K activation of AP-1 and related increased IL-2 secretion by T cells [88]. IL-2 in turn activates NK-cells.

Bone remodelling is a tightly regulated process. The binding of receptor activator of NF-kB ligand (RANKL), on BMSCs and OBLs, to its receptor RANK, on mature OCLs and their precursors, stimulates OCL late differentiation and activity. Osteoprotegerin (OPG), a decoy receptor for RANKL, is produced by OBLs. OPG inhibits RANK-RANKL interaction, thus suppressing osteoclastogenesis [89]. Several cytokines and chemokines [IL-6, IL-1α, IL-1β, IL-11, macrophage-colony stimulating factor (M-CSF), TNF-α,

TNF-β, macrophage inflammatory proteins-1α and -β (MIP-1α, -β) and VEGF], which possess pro-osteoclastogenic activity, as previously mentioned, are present in the BM milieu. Other molecules, as SDF-1α, IL-3 and hepatocyte growth factor (HGF), secreted by both malignant PCs and BMSCs, stimulate the expression of RANKL by BMSCs and thus enhance osteoclastogenesis. In MM, OPG production is downregulated. In addition, malignant PCs internalize and degradate OPG. This vicious cycle determines an increased RANK-RANL binding, augments OCL differentiation and proliferation and favours bone resorption [90]. Moreover, OBL activity is impaired in MM. In fact, malignant PCs suppress OBL differentiation and induce mature OBL apoptosis through the production of dickkopf-1 (DKK-1) and soluble frizzle-related protein-2 (sFRP-2). These molecules inhibit the Wingless-type (Wnt) signaling pathway, which promotes OBL differentiation. Other molecules, such as IL-7, IL-3 and TGF-β, overexpressed in MM BM milieu, also downregulate the OBL maturation [91].

Lenalidomide has been reported to reduce osteoclastogenesis in MM [91]. This effect is achieved in a dose-dependent manner through the inhibition of the transcription factor PU.1 and extracellular signal-regulated kinase (ERK). The first one is an early activator of osteoclastogenesis; the second one plays a key role in OCL survival and differentiation. In MM patients, after treatment with lenalidomide, OPG levels were significantly higher than baseline ($P < 0,05$), whereas RANKL production was inhibited, so lenalidomide has been confirmed to reduce the serum markers of bone lytic disease.

Although all the above mentioned mechanisms explain the direct and indirect anti-myeloma effect of lenalidomide, the precise molecular mechanisms and targets through which this molecule exerts its effects remain not completely understood.

A seminal paper has recently identified cereblon (CRBN) as a primary target of thalidomide teratogenecity [92] and moreover an essential element for response to lenalidomide [93]. Human CRBN is a 51 kDa protein that is localized in cytoplasm, nucleus and peripheral membrane of cells in testis, spleen, prostate, liver, pancreas, placenta, kidney, lung, skeletal muscle, ovary, small intestine, peripheral blood leukocytes, colon, brain and retina [94]. CRBN links to DNA damage-binding protein 1 (DDB1) [92]. DDB1 is a nucleotide excision repair protein which binds to DDB2 leading to set up a heterodimer. It is part of the cullin-4 (Cul4)-based E3 ubiquitin protein ligase complex. This complex is formed by DDB1, Cul4 (Cul4A and Cul4B), regulator of cullins-1 (Roc1) and a substrate receptor. Cul4-based E3 ubiquitin protein ligase complex plays a relevant role in cell cycle regulation, carcinogenesis and embryogenesis [95, 96]. CRBN is a part of the Cul4-based complex and it competes with DDB2 in binding to DDB1. CRBN-complex has auto-ubiquitination properties, which are inhibited by thalidomide, as shown in in vitro-studies.

Several in vitro studies have shown that CRBN is also the target molecule of lenalidomide activity.

Zhu et al. have clearly demonstrated in human MM cell lines (HMMCLs) the central role of CRBN in sensitivity and resistance to lenalidomide and have identified interferon regulatory factor-4 (IRF-4) as one of the downstream targets of CRBN. IRF-4 has previously reported to also be a target of and downregulated by lenalidomide [93].

Lopez-Girona et al. have demonstrated that lenalidomide binds to CRBN-DDB1 complex in a dose-dependent manner and with a ten-fold higher affinity than thalidomide. Moreover, after reducing CRBN expression by short interfering RNAs (siRNAs) in activated human T cells, lenalidomide has increased IL-2 and TNF-α production by these cells, thus suggesting that some immunomodulatory effects of lenalidomide are mediated by CRBN complex. This study has also shown that induction of p21/waf1 cyclin-dependent kinase inhibitor protein is prevented in absence of CRBN expression, indicating a role of CRBN in mediating antiproliferative effects of lenalidomide [97].

Heintel et al. have found a significant relationship between CRBN expression and response to lenalidomide in 44 MM patients. In fact, CRBN expression resulted three times higher in responding patients compared to non-responders. Moreover, this study has shown a clear correlation between CRBN levels and quality of response. CRBN expression was lower in patients with stable or progressive disease and higher in patients with complete remission or partial responses [98].

The data emerging from in vitro studies as well as the in vivo findings about the role of CRBN in lenalidomide action wait to be confirmed.

3. Conclusions

IMiDs including lenalidomide have proven therapeutically effective molecules in several malignant diseases characterized by different hystogenetic origin of neoplastic cells, as well as by distinct phatogenetic pathways. Notwithstanding these differences IMiDs activity in the diverse neoplasia can be traced back to the pleiotropic mechanism of these molecules.

Lenalidomide exerts a direct antitumor effect, interferes with the tumor microenvironment and enhances the host's antitumor immune responses. In MM, because of the complex bidirectional cross-talk between malignant PCs and the BM milieu including the BMSCs, the ECM proteins and the multitude of cytokines secreted in the BM milieu, the final effects of lenalidomide are the results of additional or synergic actions on different relevant pathogenetic events operating in this disease.

In addition, lenalidomide activates caspase 8 and downregulates NF-kB activity induced by cytokines secreted in the BM milieu. This in turn determines reduced expression of antiapoptotic proteins. Thus, relevant in the lenalidomide apoptosis is also the modulation induced by this drug on adhesion molecules on PCs and BMSCs as well as on cytokines production. The anti-angiogenetic well known properties of IMiDs, including lenalidomide, might be relevant in MM as increased microvascular density has been reported to be associated with disease progression. Furthermore, lenalidomide acts on different host's effector immune cells. However the immune-mediated antitumor activity well

defined in vitro are not completely correlated with the clinical outcome because of the complex immunosuppressive activity of underlying disease as well as of conventional antitumor drugs.

Finally, lenalidomide downregulates bone resorption.

The molecular mechanisms and targets of lenalidomide remain largely unknown. However, CRBN has recently been identified as the possible central mediator of lenalidomide activity and IRF-4 as a downstream molecule of CRBN action. Lenalidomide resistance in MM cells which, despite CRBN depletion, are able to restore their IRF-4 levels, suggest the existence of alternative pathways.

References

[1] L. G. Corral and G. Kaplan, "Immunomodulation by thalidomide and thalidomide analogues," *Annals of the Rheumatic Diseases*, vol. 58, supplement 1, pp. I107–I113, 1999.

[2] A. List, S. Kurtin, D. J. Roe et al., "Efficacy of lenalidomide in myelodysplastic syndromes," *The New England Journal of Medicine*, vol. 352, no. 6, pp. 549–557, 2005.

[3] A. F. List, "Lenalidomide: from bench to bedside (part 1)," *Cancer Control*, vol. 13, supplement 2-3, 2006.

[4] A. F. List, A. F. Baker, S. Green, and W. Bellamy, "Lenalidomide: targeted anemia therapy for myelodysplastic syndromes," *Cancer Control*, vol. 13, supplement 4–11, 2006.

[5] A. Raza, J. A. Reeves, E. J. Feldman et al., "Phase 2 study of lenalidomide in transfusion-dependent, low-risk, and intermediate-1-risk myelodysplastic syndromes with karyotypes other than deletion 5q," *Blood*, vol. 111, no. 1, pp. 86–93, 2008.

[6] A. List, G. Dewald, J. Bennett et al., "Lenalidomide in the myelodysplastic syndrome with chromosome 5q deletion," *The New England Journal of Medicine*, vol. 355, no. 14, pp. 1456–1465, 2006.

[7] J. D. Tariman, "Lenalidomide: a new agent for patients with relapsed or refractory multiple myeloma," *Clinical Journal of Oncology Nursing*, vol. 11, no. 4, pp. 569–574, 2007.

[8] D. M. Weber, C. Chen, R. Niesvizky et al., "Lenalidomide plus dexamethasone for relapsed multiple myeloma in North America," *The New England Journal of Medicine*, vol. 357, no. 21, pp. 2133–2142, 2007.

[9] R. Baz, E. Walker, M. A. Karam et al., "Lenalidomide and pegylated liposomal doxorubicin-based chemotherapy for relapsed or refractory multiple myeloma: safety and efficacy," *Annals of Oncology*, vol. 17, no. 12, pp. 1766–1771, 2006.

[10] R. Niesvizky, D. S. Jayabalan, P. J. Christos et al., "BiRD (Biaxin [clarithromycin]/revlimid [lenalidomide]/dexamethasone) combination therapy results in high complete- and overall-response rates in treatment-naive symptomatic multiple myeloma," *Blood*, vol. 111, no. 3, pp. 1101–1109, 2008.

[11] S. V. Rajkumar, S. Hayman, G. S. Nowakowski et al., "Combination therapy with thalidomide and dexamethasone in patients with newly diagnosed multiple myeloma not undergoing upfront autologous stem cell transplantation: a phase II trial," *Haematologica*, vol. 90, no. 12, pp. 1650–1654, 2005.

[12] A. Palumbo, P. Falco, P. Corradini et al., "Melphalan, prednisone, and lenalidomide treatment for newly diagnosed myeloma: a report from the GIMEMA—Italian Multiple Myeloma Network," *Journal of Clinical Oncology*, vol. 25, no. 28, pp. 4459–4465, 2007.

[13] A. Chanan-Khan, K. C. Miller, L. Musial et al., "Clinical efficacy of lenalidomide in patients with relapsed or refractory chronic lymphocytic leukemia: results of a phase II study," *Journal of Clinical Oncology*, vol. 24, no. 34, pp. 5343–5349, 2006.

[14] A. Chanan-Khan and C. W. Porter, "Immunomodulating drugs for chronic lymphocytic leukaemia," *Lancet Oncology*, vol. 7, no. 6, pp. 480–488, 2006.

[15] A. G. Ramsay, A. J. Johnson, A. M. Lee et al., "Chronic lymphocytic leukemia T cells show impaired immunological synapse formation that can be reversed with an immunomodulating drug," *The Journal of Clinical Investigation*, vol. 118, no. 7, pp. 2427–2437, 2008.

[16] A. Ferrajoli, B. N. Lee, E. J. Schlette et al., "Lenalidomide induces complete and partial remissions in patients with relapsed and refractory chronic lymphocytic leukemia," *Blood*, vol. 111, no. 11, pp. 5291–5297, 2008.

[17] A. Dispenzieri, M. Q. Lacy, S. R. Zeldenrust et al., "The activity of lenalidomide with or without dexamethasone in patients with primary systemic amyloidosis," *Blood*, vol. 109, no. 2, pp. 465–470, 2007.

[18] M. A. Gertz, R. Comenzo, R. H. Falk et al., "Definition of organ involvement and treatment response in immunoglobulin light chain amyloidosis (AL): a consensus opinion from the 10th International Symposium on Amyloid and Amyloidosis," *American Journal of Hematology*, vol. 79, no. 4, pp. 319–328, 2005.

[19] P. H. Wiernik, I. S. Lossos, J. M. Tuscano et al., "Lenalidomide monotherapy in relapsed or refractory aggressive non-Hodgkin's lymphoma," *Journal of Clinical Oncology*, vol. 26, no. 30, pp. 4952–4957, 2008.

[20] A. Tefferi, J. Cortes, S. Verstovsek et al., "Lenalidomide therapy in myelofibrosis with myeloid metaplasia," *Blood*, vol. 108, no. 4, pp. 1158–1164, 2006.

[21] S. P. Treon, C. J. Patterson, Z. R. Hunter, and A. R. Branagan, "Phase II study of CC-5013 (revlimid) and rituximab in Waldenstrom's macroglobulinemia: preliminary safety and efficacy results," *ASH Annual Meeting Abstracts*, vol. 106, no. 11, abstract 2443, 2005.

[22] A. A. Chanan-Khan and B. D. Cheson, "Lenalidomide for the treatment of B-cell malignancies," *Journal of Clinical Oncology*, vol. 26, no. 9, pp. 1544–1552, 2008.

[23] M. T. G. Zangari, J. Zeldis, P. Eddlemon, F. Saghafifar, and B. Barlogie, "Results of phase I study of CC-5013 for the treatment of multiple myeloma (MM) patients who relapse after high dose chemotherapy (HDCT)," *Blood*, vol. 98, abstract 775a, 2001.

[24] P. G. Richardson, R. L. Schlossman, E. Weller et al., "Immunomodulatory drug CC-5013 overcomes drug resistance and is well tolerated in patients with relapsed multiple myeloma," *Blood*, vol. 100, no. 9, pp. 3063–3067, 2002.

[25] P. G. Richardson, E. Blood, C. S. Mitsiades et al., "A randomized phase 2 study of lenalidomide therapy for patients with relapsed or relapsed and refractory multiple myeloma," *Blood*, vol. 108, no. 10, pp. 3458–3464, 2006.

[26] M. Dimopoulos, A. Spencer, M. Attal et al., "Lenalidomide plus dexamethasone for relapsed or refractory multiple myeloma," *The New England Journal of Medicine*, vol. 357, no. 21, pp. 2123–2132, 2007.

[27] M. A. Dimopoulos, C. Chen, A. Spencer et al., "Long-term follow-up on overall survival from the MM-009 and MM-010 phase III trials of lenalidomide plus dexamethasone in patients with relapsed or refractory multiple myeloma," *Leukemia*, vol. 23, no. 11, pp. 2147–2152, 2009.

[28] S. V. Rajkumar, S. Jacobus, N. S. Callander et al., "Lenalidomide plus high-dose dexamethasone versus lenalidomide plus low-dose dexamethasone as initial therapy for newly diagnosed multiple myeloma: an open-label randomised controlled trial," *The Lancet Oncology*, vol. 11, no. 1, pp. 29–37, 2010.

[29] A. Palumbo, F. Cavallo, I. Hardan et al., "A phase III study to compare melphalan, prednisone, lenalidomide (MPR) versus melphalan 200 mg/m2 and autologous transplantation (MEL200) in newly diagnosed multiple myeloma patients," *Blood*, vol. 116, abstract 3573, 2010.

[30] J. A. Zonder, J. Crowley, M. A. Hussein et al., "Lenalidomide and high-dose dexamethasone compared with dexamethasone as initial therapy for multiple myeloma: a randomized Southwest Oncology Group trial (S0232)," *Blood*, vol. 116, no. 26, pp. 5838–5841, 2010.

[31] F. Gay, S. V. Rajkumar, M. Coleman et al., "Clarithromycin (Biaxin)-lenalidomide-low-dose dexamethasone (BiRd) versus lenalidomide-low-dose dexamethasone (Rd) for newly diagnosed myeloma," *American Journal of Hematology*, vol. 85, no. 9, pp. 664–669, 2010.

[32] P. G. Richardson, E. Weller, S. Lonial et al., "Lenalidomide, bortezomib, and dexamethasone combination therapy in patients with newly diagnosed multiple myeloma," *Blood*, vol. 116, no. 5, pp. 679–686, 2010.

[33] S. Knop, C. Langer, M. Engelhardt et al., "The efficacy and safety of RAD (lenalidomide, adriamycin and dexamethasone) in newly diagnosed multiple myeloma—first results of a phase II trial by the German DSMM Group," *Blood*, vol. 116, abstract 1945, 2010.

[34] S. K. Kumar, I. Flinn, S. J. Noga et al., "Novel three-and four drug combination regimens of bortezomib, dexamethasone, cyclophosphamide, and lenalidomide, for previously untreated multiple myeloma: results from the multicenter, randomized, phase 2 EVOLUTION Study," *Blood*, vol. 116, abstract 621, 2010.

[35] A. J. Jakubowiak, D. E. Reece, C. C. Hofmeister et al., "Lenalidomide, bortezomib, pegylated liposomal doxorubicin, and dexamethasone in newly diagnosed multiple myeloma: updated results of phase I/II MMRC trial," *Blood*, vol. 114, abstract 132, 2009.

[36] W. M. Kuehl and P. L. Bergsagel, "Multiple myeloma: evolving genetic events and host interactions," *Nature Reviews Cancer*, vol. 2, no. 3, pp. 175–187, 2002.

[37] T. Hideshima, P. L. I. Bergsagel, W. M. Kuehl, and K. C. Anderson, "Advances in biology of multiple myeloma: clinical applications," *Blood*, vol. 104, no. 3, pp. 607–618, 2004.

[38] P. L. Bergsagel and W. M. Kuehl, "Critical roles for immunoglobulin translocations and cyclin D dysregulation in multiple myeloma," *Immunological Reviews*, vol. 194, pp. 96–104, 2003.

[39] M. Urashima, G. Teoh, A. Ogata et al., "Characterization of p16(INK4A) expression in multiple myeloma and plasma cell leukemia," *Clinical Cancer Research*, vol. 3, no. 11, pp. 2173–2179, 1997.

[40] G. Guillerm, E. Gyan, D. Wolowiec et al., "p16INK4a and p15INK4b gene methylations in plasma cells from monoclonal gammopathy of undetermined significance," *Blood*, vol. 98, no. 1, pp. 244–246, 2001.

[41] M. S. Kulkarni, J. L. Daggett, T. P. Bender, W. M. Kuehl, P. L. Bergsagel, and M. E. Williams, "Frequent inactivation of the cyclin-dependent kinase inhibitor p18 by homozygous deletion in multiple myeloma cell lines: ectopic p18 expression inhibits growth and induces apoptosis," *Leukemia*, vol. 16, no. 1, pp. 127–134, 2002.

[42] G. Teoh and K. C. Anderson, "Interaction of tumor and host cells with adhesion and extracellular matrix molecules in the development of multiple myeloma," *Hematology/Oncology Clinics of North America*, vol. 11, no. 1, pp. 27–42, 1997.

[43] T. Hideshima, D. Chauhan, T. Hayashi et al., "The biological sequelae of stromal cell-derived factor-1alpha in multiple myeloma," *Molecular Cancer Therapeutics*, vol. 1, no. 7, pp. 539–544, 2002.

[44] J. S. Damiano, A. E. Cress, L. A. Hazlehurst, A. A. Shtil, and W. S. Dalton, "Cell adhesion mediated drug resistance (CAM-DR): role of integrins and resistance to apoptosis in human myeloma cell lines," *Blood*, vol. 93, no. 5, pp. 1658–1667, 1999.

[45] L. A. Hazlehurst, J. S. Damiano, I. Buyuksal, W. J. Pledger, and W. S. Dalton, "Adhesion to fibronectin via β1 integrins regulates p27(kip1) levels and contributes to cell adhesion mediated drug resistance (CAM-DR)," *Oncogene*, vol. 19, no. 38, pp. 4319–4327, 2000.

[46] D. Chauhan, H. Uchiyama, Y. Akbarali et al., "Multiple myeloma cell adhesion-induced interleukin-6 expression in bone marrow stromal cells involves activation of NF-κB," *Blood*, vol. 87, no. 3, pp. 1104–1112, 1996.

[47] B. Dankbar, T. Padró, R. Leo et al., "Vascular endothelial growth factor and interleukin-6 in paracrine tumor- stromal cell interactions in multiple myeloma," *Blood*, vol. 95, no. 8, pp. 2630–2636, 2000.

[48] D. Gupta, S. P. Treon, Y. Shima et al., "Adherence of multiple myeloma cells to bone marrow stromal cells upregulates vascular endothelial growth factor secretion: therapeutic applications," *Leukemia*, vol. 15, no. 12, pp. 1950–1961, 2001.

[49] B. Klein, X. G. Zhang, M. Jourdan et al., "Paracrine rather than autocrine regulation of myeloma-cell growth and differentiation by interleukin-6," *Blood*, vol. 73, no. 2, pp. 517–526, 1989.

[50] T. Hideshima, D. Chauhan, R. Schlossman, P. Richardson, and K. C. Anderson, "The role of tumor necrosis factor α in the pathophysiology of human multiple myeloma: therapeutic applications," *Oncogene*, vol. 20, no. 33, pp. 4519–4527, 2001.

[51] R. Catlett-Falcone, T. H. Landowski, M. M. Oshiro et al., "Constitutive activation of Stat3 signaling confers resistance to apoptosis in human U266 myeloma cells," *Immunity*, vol. 10, no. 1, pp. 105–115, 1999.

[52] D. Puthier, R. Bataille, and M. Amiot, "IL-6 up-regulates mcl-1 in human myeloma cells through JAK / STAT rather than ras / MAP kinase pathway," *European Journal of Immunology*, vol. 29, no. 12, pp. 3945–3950, 1999.

[53] M. Jourdan, J. L. Veyrune, J. De Vos, N. Redal, G. Couderc, and B. Klein, "A major role for Mcl-1 antiapoptotic protein in the IL-6-induced survival of human myeloma cells," *Oncogene*, vol. 22, no. 19, pp. 2950–2959, 2003.

[54] B. Zhang, I. Gojo, and R. G. Fenton, "Myeloid cell factor-1 is a critical survival factor for multiple myeloma," *Blood*, vol. 99, no. 6, pp. 1885–1893, 2002.

[55] D. Verhelle, L. G. Corral, K. Wong et al., "Lenalidomide and CC-4047 inhibit the proliferation of malignant B cells while expanding normal CD34+ progenitor cells," *Cancer Research*, vol. 67, no. 2, pp. 746–755, 2007.

[56] L. Escoubet-Lozach, I. L. Lin, K. Jensen-Pergakes et al., "Pomalidomide and lenalidomide induce p21WAF-1 expression in both lymphoma and multiple myeloma through a LSD1-mediated epigenetic mechanism," *Cancer Research*, vol. 69, no. 18, pp. 7347–7356, 2009.

[57] A. K. Gandhi, J. Kang, L. Capone et al., "Dexamethasone synergizes with lenalidomide to inhibit multiple myeloma tumor

growth, but reduces lenalidomide-induced immunomodulation of T and NK cell function," *Current Cancer Drug Targets*, vol. 10, no. 2, pp. 155–167, 2010.

[58] L. G. Corral, P. A. Haslett, G. W. Muller et al., "Differential cytokine modulation and T cell activation by two distinct classes of thalidomide analogues that are potent inhibitors of TNF-α," *Journal of Immunology*, vol. 163, no. 1, pp. 380–386, 1999.

[59] V. Kotla, S. Goel, S. Nischal et al., "Mechanism of action of lenalidomide in hematological malignancies," *Journal of Hematology & Oncology*, vol. 2, article 36, 2009.

[60] J. L. Xu, R. Lai, T. Kinoshita, N. Nakashima, and T. Nagasaka, "Proliferation, apoptosis, and intratumoral vascularity in multiple myeloma: correlation with the clinical stage and cytological grade," *Journal of Clinical Pathology*, vol. 55, no. 7, pp. 530–534, 2002.

[61] K. Dredge, R. Horsfall, S. P. Robinson et al., "Orally administered lenalidomide (CC-5013) is anti-angiogenic in vivo and inhibits endothelial cell migration and Akt phosphorylation in vitro," *Microvascular Research*, vol. 69, no. 1-2, pp. 56–63, 2005.

[62] D. Chauhan, T. Hideshima, and K. C. Anderson, "Apoptotic signaling in multiple myeloma: therapeutic implications," *International Journal of Hematology*, vol. 78, no. 2, pp. 114–120, 2003.

[63] Y. Dai, P. Dent, and S. Grant, "Tumor necrosis factorrelated apoptosis-inducing ligand (TRAIL) promotes mitochondrial dysfunction and apoptosis induced by 7-hydroxystaurosporine and mitogenactivated protein kinase kinase inhibitors in human leukemia cells that ectopically express Bcl-2 and Bcl-xL," *Molecular Pharmacology*, vol. 64, no. 6, pp. 1402–1409, 2003.

[64] D. Chauhan, T. Hideshima, S. Rosen, J. C. Reed, S. Kharbanda, and K. C. Anderson, "Apaf-1/cytochrome c independent and Smac dependent induction of apoptosis in multiple myeloma cells," *The Journal of Biological Chemistry*, vol. 276, no. 27, pp. 24453–24456, 2001.

[65] Z. L. Chu, T. A. McKinsey, L. Liu, J. J. Gentry, M. H. Malim, and D. W. Ballard, "Suppression of tumor necrosis factor-induced cell death by inhibitor of apoptosis c-IAP2 is under NF-κB control," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 94, no. 19, pp. 10057–10062, 1997.

[66] S. Kreuz, D. Siegmund, P. Scheurich, and H. Wajant, "NF-κB inducers upregulate cFLIP, a cycloheximide-sensitive inhibitor of death receptor signaling," *Molecular and Cellular Biology*, vol. 21, no. 12, pp. 3964–3973, 2001.

[67] W. Zou, "Immunosuppressive networks in the tumour environment and their therapeutic relevance," *Nature Reviews Cancer*, vol. 5, no. 4, pp. 263–274, 2005.

[68] M. Urashima, A. Ogata, D. Chauhan et al., "Transforming growth factor-β1: differential effects on multiple myeloma versus normal B cells," *Blood*, vol. 87, no. 5, pp. 1928–1938, 1996.

[69] H. Ogawara, H. Handa, T. Yamazaki et al., "High Th1/Th2 ratio in patients with multiple myeloma," *Leukemia Research*, vol. 29, no. 2, pp. 135–140, 2005.

[70] B. Maecker, K. S. Anderson, M. S. von Bergwelt-Baildon et al., "Viral antigen-specific CD8+ T-cell responses are impaired in multiple myeloma," *British Journal of Haematology*, vol. 121, no. 6, pp. 842–848, 2003.

[71] M. V. Dhodapkar, M. D. Geller, D. H. Chang et al., "A reversible defect in natural killer T cell function characterizes the progression of premalignant to malignant multiple myeloma," *Journal of Experimental Medicine*, vol. 197, no. 12, pp. 1667–1676, 2003.

[72] M. J. Smyth, D. I. Godfrey, and J. A. Trapani, "A fresh look at tumor immunosurveillance and immunotherapy," *Nature Immunology*, vol. 2, no. 4, pp. 293–299, 2001.

[73] M. Jarahian, C. Watzl, Y. Issa, P. Altevogt, and F. Momburg, "Blockade of natural killer cell-mediated lysis by NCAM140 expressed on tumor cells," *International Journal of Cancer*, vol. 120, no. 12, pp. 2625–2634, 2007.

[74] P. A. Haslett, W. A. Hanekom, G. Muller, and G. Kaplan, "Thalidomide and a thalidomide analogue drug costimulate virus-specific CD8+ T cells in vitro," *Journal of Infectious Diseases*, vol. 187, no. 6, pp. 946–955, 2003.

[75] R. LeBlanc, T. Hideshima, L. P. Catley et al., "Immunomodulatory drug costimulates T cells via the B7-CD28 pathway," *Blood*, vol. 103, no. 5, pp. 1787–1790, 2004.

[76] R. D. Brown, B. Pope, A. Murray et al., "Dendritic cells from patients with myeloma are numerically normal but functionally defective as they fail to up-regulate CD80 (B7-1) expression after huCD40LT stimulation because of inhibition by transforming growth factor-β1 and interleukin-10," *Blood*, vol. 98, no. 10, pp. 2992–2998, 2001.

[77] M. Ratta, F. Fagnoni, A. Curti et al., "Dendritic cells are functionally defective in multiple myeloma: the role of interleukin-6," *Blood*, vol. 100, no. 1, pp. 230–237, 2002.

[78] D. I. Gabrilovich, H. L. Chen, K. R. Girgis et al., "Production of vascular endothelial growth factor by human tumors inhibits the functional maturation of dendritic cells," *Nature Medicine*, vol. 2, no. 10, pp. 1096–1103, 1996.

[79] P. H. Schafer, A. K. Gandhi, M. A. Loveland et al., "Enhancement of cytokine production and AP-1 transcriptional activity in T cells by thalidomide-related immunomodulatory drugs," *Journal of Pharmacology and Experimental Therapeutics*, vol. 305, no. 3, pp. 1222–1232, 2003.

[80] T. Hayashi, T. Hideshima, M. Akiyama et al., "Molecular mechanisms whereby immunomodulatory drugs activate natural killer cells: clinical application," *British Journal of Haematology*, vol. 128, no. 2, pp. 192–203, 2005.

[81] A. Bendelac, M. N. Rivera, S. H. Park, and J. H. Roark, "Mouse CD1-specific NK1 T cells: development, specificity, and function," *Annual Review of Immunology*, vol. 15, pp. 535–562, 1997.

[82] C. A. Biron, K. B. Nguyen, G. C. Pien, L. P. Cousens, and T. P. Salazar-Mather, "Natural killer cells in antiviral defense: function and regulation by innate cytokines," *Annual Review of Immunology*, vol. 17, pp. 189–220, 1999.

[83] K. Takeda, S. Seid, K. Ogasawara et al., "Liver NK1.1+ CD4+ $\alpha\beta$ T cells activated by IL-12 as a major effector in inhibition of experimental tumor metastasis," *Journal of Immunology*, vol. 156, no. 9, pp. 3366–3373, 1996.

[84] J. Cui, T. Shin, T. Kawano et al., "Requirement for V(α)14 NKT cells in IL-12-mediated rejection of tumors," *Science*, vol. 278, no. 5343, pp. 1623–1626, 1997.

[85] G. Trinchieri and P. Scott, "Interleukin-12: a proinflammatory cytokine with immunoregulatory functions," *Research in Immunology*, vol. 146, no. 7-8, pp. 423–431, 1995.

[86] S. Fujii, K. Shimizu, R. M. Steinman, and M. V. Dhodapkar, "Detection and activation of human Vα24+ natural killer T cells using α-galactosyl ceramide-pulsed dendritic cells," *Journal of Immunological Methods*, vol. 272, no. 1-2, pp. 147–159, 2003.

[87] C. Carnaud, D. Lee, O. Donnars et al., "Cutting edge: cross-talk between cells of the innate immune system: NKT cells

rapidly activate NK cells," *Journal of Immunology*, vol. 163, no. 9, pp. 4647–4650, 1999.

[88] T. Hayashi, T. Hideshima, M. Akiyama et al., "Molecular mechanisms whereby immunomodulatory drugs activate natural killer cells: clinical application," *British Journal of Haematology*, vol. 128, no. 2, pp. 192–203, 2005.

[89] N. Takahashi, K. Maeda, A. Ishihara, S. Uehara, and Y. Kobayashi, "Regulatory mechanism of osteoclastogenesis by RANKL and Wnt signals," *Frontiers in Bioscience*, vol. 16, no. 1, pp. 21–30, 2011.

[90] E. Terpos, M. A. Dimopoulos, and O. Sezer, "The effect of novel anti-myeloma agents on bone metabolism of patients with multiple myeloma," *Leukemia*, vol. 21, no. 9, pp. 1875–1884, 2007.

[91] I. Breitkreutz, M. S. Raab, S. Vallet et al., "Lenalidomide inhibits osteoclastogenesis, survival factors and bone-remodeling markers in multiple myeloma," *Leukemia*, vol. 22, no. 10, pp. 1925–1932, 2008.

[92] I. Takumi and H. Hiroshi, "Deciphering the mystery of thalidomide teratogenicity," *Congenital Anomalies*, vol. 52, no. 1, pp. 1–7, 2012.

[93] Y. X. Zhu, E. Braggio, C. X. Shi et al., "Cereblon expression is required for the antimyeloma activity of lenalidomide and pomalidomide," *Blood*, vol. 118, no. 18, pp. 4771–4779, 2011.

[94] X. B. Chang and A. K. Stewart, "What is the functional role of the thalidomide binding protein cereblon?" *International Journal of Biochemistry and Molecular Biology*, vol. 2, no. 3, pp. 287–294, 2011.

[95] Y. Cang, J. Zhang, S. A. Nicholas et al., "Deletion of DDB1 in mouse brain and lens leads to p53-dependent elimination of proliferating cells," *Cell*, vol. 127, no. 5, pp. 929–940, 2006.

[96] Y. Cang, J. Zhang, S. A. Nicholas, A. L. Kim, P. Zhou, and S. P. Goff, "DDB1 is essential for genomic stability in developing epidermis," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 8, pp. 2733–2737, 2007.

[97] A. Lopez-Girona, D. Mendy, K. Miller et al., "Direct binding with cereblon mediates the antiproliferative and immunomodulatory action of lenalidomide and pomalidomide," in *Proceedings of the ASH Annual Meeting and Exposition*, 2011.

[98] D. Heintel, A. Bolomsky, M. Schreder et al., "High expression of the thalidomide-binding protein cereblon (CRBN) is associated with improved clinical response in patients with multiple myeloma treated with lenalidomide and dexamethasone," in *Proceedings of the ASH Annual Meeting and Exposition*, 2011.

Hindawi Publishing Corporation
Advances in Hematology
Volume 2012, Article ID 513702, 9 pages
doi:10.1155/2012/513702

Review Article

Molecular Action of Lenalidomide in Lymphocytes and Hematologic Malignancies

Jessica M. McDaniel,[1,2] Javier Pinilla-Ibarz,[2,3] and P. K. Epling-Burnette[2]

[1] *Cancer Biology PhD Program, University of South Florida, Tampa, FL 33620, USA*
[2] *Department of Immunology, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL 33612, USA*
[3] *Department of Malignant Hematology, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL 33612, USA*

Correspondence should be addressed to P. K. Epling-Burnette, pearlie.burnette@moffitt.org

Received 16 November 2011; Revised 12 May 2012; Accepted 18 June 2012

Academic Editor: Anna Marina Liberati

The immunomodulatory agent, lenalidomide, is a structural analogue of thalidomide approved by the US Food and Drug Administration for the treatment of myelodysplastic syndrome (MDS) and multiple myeloma (MM). This agent is also currently under active investigation for the treatment of chronic lymphocytic leukemia (CLL) and non-Hodgkin's lymphoma (NHL), as well as in drug combinations for some solid tumors and mantle cell lymphoma (MCL). Although treatment with lenalidomide has translated into a significant extension in overall survival in MM and MDS and has superior safety and efficacy relative to thalidomide, the mechanism of action as it relates to immune modulation remains elusive. Based on preclinical models and clinical trials, lenalidomide, as well as other structural thalidomide derivatives, enhances the proliferative and functional capacity of T-lymphocytes and amplifies costimulatory signaling pathways that activate effector responses and suppress inflammation. This paper summarizes our current understanding of T- and natural killer (NK) cell pathways that are modified by lenalidomide in hematopoietic neoplasms to inform future decisions about potential combination therapies.

1. Introduction

Lenalidomide (Revlimid, CC-5013) is a second-generation synthetic derivative of glutamic acid and thalidomide analogue with antiangiogenic, antitumorigenic, and immunomodulating activity that was realized due to anecdotal immunomodulatory activity in erythema nodosum leprosum (ENL) [1, 2] and in autoimmune disorders [3–5]. Creation of synthetic modifications to the thalidomide backbone led to the discovery of lenalidomide and pomalidomide with 500-fold greater immunomodulatory potency and safer side effect profile compared to the parent drug [6, 7]. Use of lenalidomide in proliferative neoplasms has recently intensified due to the agent's success in MM and MDS where it acts to alter immune homeostasis and modulate inflammation within the bone marrow microenvironment. Studies in relapsed and refractory B-cell chronic lymphocytic leukemia (B-CLL) as well as non-Hodgkin's lymphoma (NHL) solid malignancies such as central nervous system, ovarian, and renal cell carcinoma demonstrate the potential of this drug in diverse neoplastic processes [8, 9]. While the molecular antitumor mechanism and specificity have been extensively studied in preclinical and clinical settings, the future application and design of effective therapeutic combinations with lenalidomide is dependent on understanding the immunomodulatory mechanism and anti-inflammatory properties in the context of the bone marrow milieu, the microenvironmental interactions, and bioactivity within adaptive and innate immune cells.

2. Lenalidomide Augments T-Cell Proliferation and Activation

Immunosurveillance of cancer cells is now a well-established principle thought to contribute not only to the quantity, but also to the quality, or immunogenicity, of a tumor during development [10, 11]. Mechanisms regulating innate and adaptive immune responses are carefully orchestrated to detect and remove infected, transformed, or erratically

growing cells within the body. Immune tolerance, induced by changes in the microenvironment and within the tumor cells, contributes to neoplastic expansion. Lenalidomide is able to enhance the proliferative and functional capacity of T cells, which augments immune activity through a variety of mechanisms. Thalidomide was first shown to augment T-cell proliferation and cytokine production in the absence of costimulatory molecules without direct mitogenic activity [12]. Early reports of bone marrow lymphoid aggregates in lenalidomide-responsive MDS patients implicated immune modulation in hematological responses to this agent [13]. When a T-cell encounters cognate tumor antigens presented by antigen presenting cell (APCs), there is an increase in a variety of costimulatory molecules, most importantly CD28, that enables a fully competent signal response by T cells [14]. CD28 binds to B7-1 (CD80) and -2 (CD86) molecules on APCs to generate the appropriate response to antigen stimulation. Absence of CD28-APC interaction (Signal 2) in the presence of T-cell receptor ligation (Signal 1) leads to inactivation or anergy of naïve T cells. Thalidomide, and to a greater extent lenalidomide, induces interleukin-2 (IL-2), interferon-γ (IFN-γ), and TNF-α secretion [12] in the absence of CD28 stimulation, suggesting that the drug somehow activates the costimulatory-dependent signaling cascade initiated by Signal 2 [15].

Both Signal 1 (TCR) and Signal 2 (co-stimulation) are necessary for IL-2 production leading to the hypothesis that lenalidomide and the other IMiDs function somewhere within this costimulatory pathway [16–18]. Signaling pathways associated with IL-2 transcriptional activation are shown in Figure 1 and recently reviewed by [19]. The exact differential downstream intermediates emanating from the TCR CD28 are difficult to elucidate because the pathways are integrally connected. LeBlanc et al. showed that lenalidomide acts to increase tyrosine-phosphorylation in the intracellular domain of the CD28 receptor in the absence of costimulatory molecules [20]. Although it is not known if lenalidomide acts directly to induce phosphorylation, the presence of downstream signaling events after treatment such as NF-κB p65 translocation to the nucleus, and cytokine production, suggests that this pathway may be important for lenalidomide's immunomodulatory effect [20]. Others have shown that the activation of PKC-ζ and NFAT-2 are important mediators of cytokine production after IMiD treatment [21]. However, a conflicting report showed that PKC-θ activity and AP-1 DNA binding was increased, without an increase in NF-κB, OCT-1, and NFAT transcription factor binding, which adds to the controversy about lenalidomide's T-cell-associated molecular mechanism of action [22, 23] (see Figure 1). These controversial results, however, may be attributed to the methods used for T-cell stimulation, namely, TCR stimulation versus calcium channel activation, respectively. Görgün et al. showed that lenalidomide and pomalidomide reduce Suppressor of Cytokine Signaling-1 (SOCS1) expression in T cells, which is an important negative regulator of cytokine signaling [24]. Even when treated with IFN-γ to induce SOCS1 expression, the drug was capable of blocking this inhibitory response and potentiating TCR/anti-CD28 costimulation in effector T cells [24]. Although reduction in a suppressive signal may be important, this would not be expected to generate unique responses, such as IL-2, that specifically require a costimulatory signal.

In addition to the activation of effector T cells and NK cells, there is a valid concern about the potential effect of IMiDs on regulatory T (Treg) cells that may deter antitumor immunity by suppressing immunosurveillance [11, 25]. In this regard, lenalidomide and pomalidomide were shown to inhibit the expansion and function of Tregs by downregulating the expression of forkhead box protein 3 (FOXP3) [26, 27]. The preferential augmentation of CD8+ cytotoxic T cells and inhibition of regulatory T cells makes this drug a very interesting and potentially valuable therapeutic candidate to augment immunotherapy responses in cancer patients.

In addition to the specific effects of lenalidomide on T-cell signaling, our lab and others have shown that the drug alters homeostatic regulation of T cells [28]. In MDS and MM, lenalidomide preferentially acts on specific T-cell memory subsets to reverse immune dysfunction. We found that erythroid responsive MDS patients displayed a greater increase in naïve and central memory T-cell subsets compared to nonresponders. This increase was associated with a concurrent decrease in effector memory subsets, potentially indicating that the drug restores immune homeostasis [28]. A similar increase in central memory T cells was observed by Noonan et al. [29] in MM patients that received lenalidomide in combination with the pneumococcal 7-valent conjugated vaccine (PCV) to establish the principle of vaccine combination therapy. Interestingly, the increase in PCV-specific antibody and cellular responses was specific to the vaccination schedule favoring administration of lenalidomide prior to PCV vaccine. B-CLL, like MDS, is associated with dysfunctional T-cell activity [30, 31] with defects in actin polarization at the immune synapse [32]. Treatment with lenalidomide in CLL restored IL-2 and IFN-γ secreting CD4+ and CD8+ T cells to normal levels [33] and reversed the suppressive signals blocking lytic synapse formation [32].

Antigen-specific effector T-cell activity *in vitro* and *in vivo* after lenalidomide was demonstrated after treatment for MM, supporting the idea that T-cell reconstitution may be important for antileukemia effects and eradication of myeloma cells [34]. Our studies have shown that lenalidomide is capable of increasing proliferation and cytokine secretion in anergic MDS T cells and indicate that lenalidomide not only improves healthy T-cell function, but also reverses intrinsic cancer-related immune defects associated with deregulated cancer immunosurveillance.

The evidence from *in vitro* and *in vivo* experiments to date, therefore, indicates that lenalidomide has multiple effects on T-cell signaling, but the exact molecular target and mechanism remain elusive. Interestingly, the molecular target mediating thalidomide's teratogenic effects was identified in 2010 by Ito et al. [35]. Using thalidomide-conjugated beads, an E3 ubiquitin ligase, cereblon (CRBN), was shown to directly bind to thalidomide and mediate limb malformation in a zebrafish model. Mutations of two amino acids (Y374A and W376A) in zebrafish CRBN eliminated the drug's ability to interact with the protein

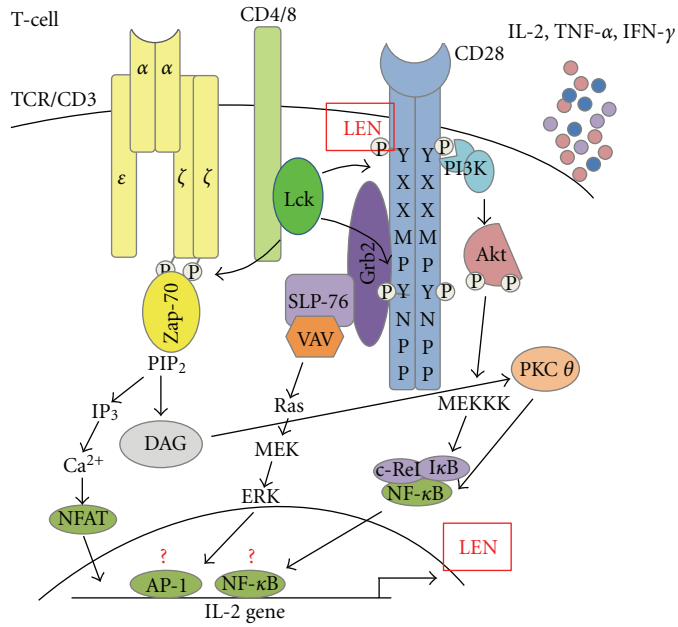


FIGURE 1: Various T-cell signaling pathways are upregulated after lenalidomide treatment. Lenalidomide is known to have no direct mitogenic activity, therefore it cannot induce proliferation directly. Upon TCR ligation, lenalidomide (LEN) increases phosphorylation of tyrosines within the intracytoplasmic tail of CD28, through an unknown mechanism, increasing downstream signaling and activation of PKC-θ, MAPK, and potentially other signaling pathways. These pathways lead to the activation of classic T-cell transcription factors like AP-1, NFAT-1, and NF-κB that induce secretion of the T helper type 1 (Th-1) cytokines interleukin-2 (IL-2), tumor necrosis factor-α (TNF-α), and interferon-γ (IFN-γ). Though it is controversial which transcription factors are ultimately increased upon lenalidomide treatment (indicated by a question mark). Upregulation of these pathways potentially reverses T-cell defects, aids in breaking tolerance, and leads to greater CD4+ T-cell help to DCs, NK cells, and CD8+ T cells, augmenting eradication of the tumor cells.

and prevented its effects on limb formation. Decreased cereblon expression in MM cells was also recently found to be associated with lenalidomide and pomalidomide resistance [36]. CRBN functions within an E3 complex containing several components including DDB1 and Cullin 4 (Cul4A or Cul4B) that polyubiquitinate (Ub) substrate proteins and mediate their degradation [35]. CRBN and other members of this E3 complex play no known role in T-cell signaling. However, increased Cul4A expression was recently linked to thalidomide response in prostate cancer [37]. Lenalidomide was shown recently by our group to stabilize mouse double minute 2 protein (MDM2) by blocking its autoubiquitination [38]. Since MDM2, like CRBN, is a RING finger E3 ubiquitin ligase, it is possible that IMiDs mediate a class-selective suppressive action against Ub-ligating enzymes, potentially mediating the increase in T-cell signaling.

3. Lenalidomide in B-CLL and MM

Lenalidomide has proven efficacy in several hematologic malignancies, including MDS, B-CLL, MM, and even some solid tumors attributed to T-cell and NK cell functional reconstitution. B-CLL is the most common leukemia in the United States, and although treatment with nucleoside analog-based chemoimmunotherapies has significantly enhanced outcomes in patients, nearly all of the patients ultimately relapse [39]. Lenalidomide combination treatments for patients with relapsed, refractory, and primary CLL, have resulted in durable hematologic improvement [40–42]. Exposure of primary CLL cells to lenalidomide *in vitro* leads to the induction of costimulatory molecules like CD80, CD86, and FASL on the tumor cells [43], restoring immunological synapse formation and improving autologous tumor cell recognition by T cells [44, 45] (Figure 2). The improved immune synapse formation between T cells and tumor cells was also evident *in vitro* when studied in NHL [46].

The ability of lenalidomide to augment IL-2, IFN-γ, and TNF-α production from T cells *in vitro* has been described extensively. Similar increases in TNF-α production in CLL have been confirmed [47]. Cytokine production and increased T-cell function in CLL is thought to contribute to the tumor flare response (TFR), which is an adverse side effect of lenalidomide treatment that is positively associated with hematologic improvement when properly managed [48]. Since TFR occurs in association with an increase in circulating CD8+ T cells and NK cells, and release of proinflammatory cytokines, it suggests that immunomodulation is important for success of the drug clinically by enhancing the reactivation of immune effector responses against the tumor [47, 48]. Continuous treatment of relapsed refractory CLL patients with lenalidomide was associated with a stable increase in T-cell number in the peripheral blood, which was indicative of a sustained immune response [42]. In B-CLL, both thalidomide and lenalidomide lead to improved tumor

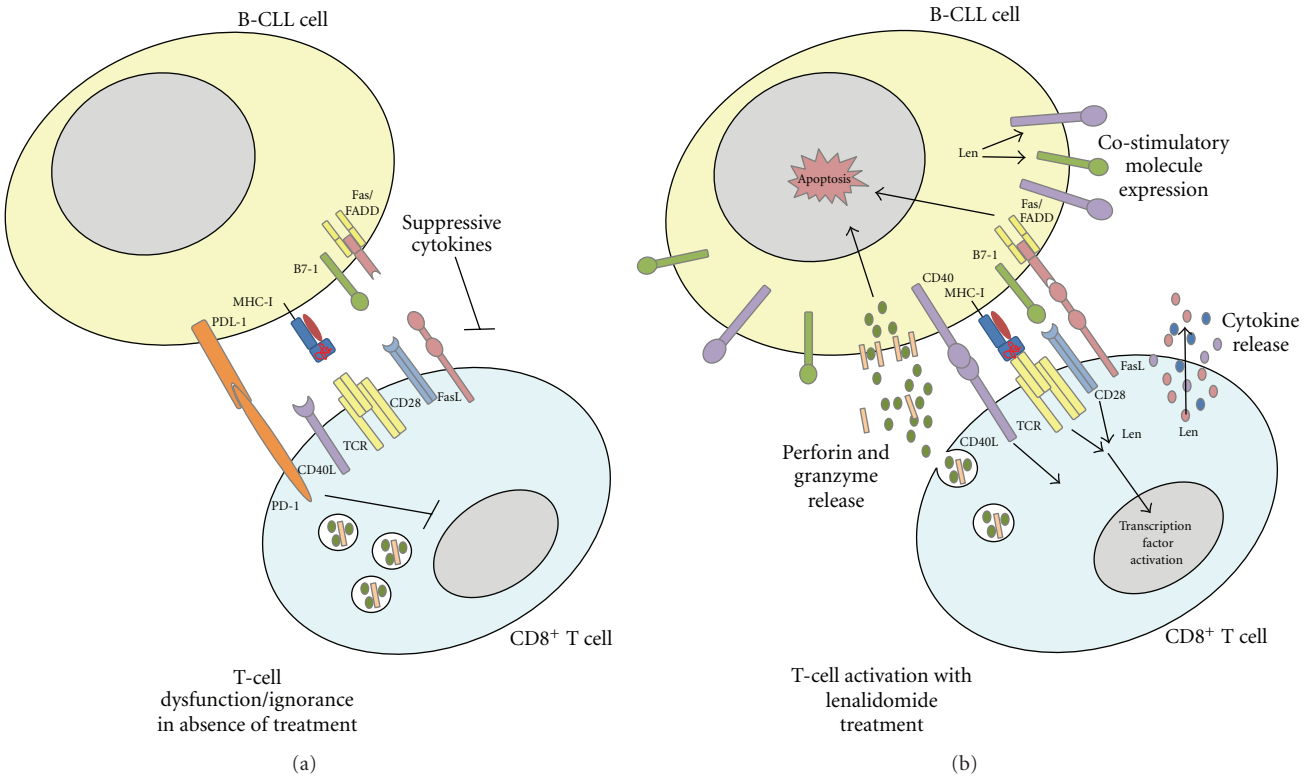


Figure 2: Lenalidomide augments direct CD8+ T-cell killing of B-CLL cells. B-CLL cells are able to evade immune detection through high levels of PDL-1, low levels of costimulatory molecules like B7-1, and a variety of immune-suppressive cytokines in the microenvironment. Lenalidomide treatment (Len) is able to overcome the immune suppression through upregulation of costimulatory molecules like CD40 and B7-1 (CD80/86) on the CLL cells, upregulation of Fas expression, as well as decreasing PDL-1. Through the alteration of surface molecule expression, as well as the increase in T-cell signaling as shown previously, lenalidomide induces better immune synapse formation allowing for increased killing by the CD8+ T cells.

recognition. The drug-induced induction of costimulatory molecules on the B-cell tumor cells in CLL resulting in enhanced immune-mediated killing, and decreased tumor burden.

Impaired differentiation and activation of T and B-cells, as well as NK and dendritic cells, is an important mediator of disease progression in MM [49, 50]. MM is, at present, an incurable B-cell malignancy with abnormal cells accumulating in bone and the bone marrow, which suppress normal hematopoiesis and disrupt the bone marrow microenvironment [51]. Lenalidomide is known in MM to disrupt cellular interactions and adherence of MM to stromal constitutions, decrease growth factors such as IL-6, and induce apoptosis of the neoplastic cells, therefore blocking disease progression [52–54]. The dysregulation of hematopoiesis and increased inflammatory cytokine milieu within the bone marrow microenvironment also contributes to impaired immune effector cell function. Lenalidomide treatment in MM, similar to B-CLL and MDS, reverses T-cell defects directly, but also reverses dendritic cell (DC) dysfunction. DCs from patients with MM have reduced expression, or even absence, of costimulatory molecules [55] and this, along with high levels of IL-6, IL-10, and TGF-β within the bone marrow microenvironment, contributes to impaired T-cell costimulation and activation [55, 56].

Although an increase in immune activation is associated with drug response and a decrease in tumor burden in CLL, efficacy of the drug has not been definitively shown to be mediated by a direct cytotoxic effect of T cells against the malignant B-cells. Christensen et al. first demonstrated such activity in MM, as lenalidomide treatment in patients *in vivo* increased the killing of *HM1.24+* myeloma cells by MART-1 specific T cells [34, 57]. Lenalidomide's action on T-cell cytokine secretion, specific tumor cell recognition, and ability to enhance costimulation derived from dendritic cells may all participate in lenalidomide's efficacy for the treatment of MM.

4. Immunomodulatory Drugs Increase Natural Killer Cell Recognition and Cytotoxicity of Leukemia Cells

In addition to the potentiating effect on T and B cells, immunomodulatory drugs have a profound effect on the innate immune response, namely, natural killer (NK) cells. NK cells are an important component of the innate immune system where they play major roles in tumor rejection, viral clearance, and DC regulation [58–60]. Thalidomide was shown to enhance the cytotoxic effects of NK cells, as

well as increase their cell numbers in MM patients [61]. This enhanced killing effect requires cytokine support from accessory lymphocytes, like T cells, as there is no measurable increase in direct killing of the K562 human leukemia cell line by purified NK cells in the presence of high doses of lenalidomide or pomalidomide [62]. PBMCs depleted of NK cells were not able to kill K562 at all, nor were PBMCs in a transwell experiment, suggesting that NK cells and their contact with the tumor cell is a necessary component of lenalidomide-mediated tumor cell apoptosis [62]. Support from T cells, in the form of IL-2 secretion, is extremely important for NK-cell-mediated cytotoxicity of MM after lenalidomide treatment [21]. Although the combination of lenalidomide with dexamethasone has been shown to have significant activity, IL-2 production was abrogated *in vivo* when MM patients received this combination simultaneously [63]. Hsu et al. demonstrated that dexamethasone treatment suppressed IL-2 production from CD4+ helper T cells, impaired NK cell-mediated cytotoxicity, and countered the immunostimulatory effects of lenalidomide in MM patients. Pharmacodynamic studies may maximize the efficacy of this combination therapy in MM.

There are multiple mechanisms postulated for increased NK cell killing in the various disease settings. Both pomalidomide and lenalidomide upregulate the expression of CD56, which normally decreases NK killing capacity, but in this setting had no detriment to NK cell killing [62]. Carbone et al. showed that the expression of natural cytotoxic receptors (NCR) and NK receptor member D of the lectin-like receptor family (NKG2D) is necessary for myeloma cell recognition [64] and NKG2D blockade abrogated the effect of lenalidomide in solid tumors [65]. It was recently shown by Benson et al. that the addition of a murine anti-inhibitory killer immunoglobulin receptor (KIR) antibody with concurrent lenalidomide therapy mediated rejection of lenalidomide-resistant tumors in a mouse model [66]. This is similar to their IPH2101 human anti-inhibitory KIR antibody that also increases *in vitro* NK cell cytotoxicity specifically against MM cell targets, but not normal cells, suggesting that clinical testing in combination with lenalidomide is warranted [66].

A schematic of the various mechanisms of NK cell-mediated killing in MM after lenalidomide treatment in combination with various monoclonal antibodies is shown in Figure 3. MM cells, like most tumor cells, express the programmed death receptor-1 ligand (PD-L1) which downregulates the immune response against malignant cells through programmed death receptor-1 interactions on T cells [67, 68]. Recently, it was shown that NK cells from MM patients express PD-1, and the PD-1/PD-L1 interaction decreased NK cell-mediated killing [69]. A novel anti-PD-1 antibody, CT-011, can increase NK cell-mediated killing of autologous MM cells from patients, without effecting normal cells [69]. This new monoclonal therapy, along with lenalidomide's action of decreasing PD-L1 on MM cells, may improve response rates to this combination therapy.

Enhanced antibody-dependent cytotoxicity (ADCC) by NK cells is also an extremely important mechanism in IMiD function in CLL, MM, and even solid tumors [21, 65, 70]. ADCC is a process where antibodies bind to their ligand antigens on target cells, which then bind to FcR-γ receptors on NK cells, and trigger cell lysis through perforin and granzyme-dependent pathways [71]. Lenalidomide- and pomalidomide-induced killing correlates with an increase in Fas ligand (FasL) and granzyme B expression in NK cells, leading to increased ADCC in multiple tumor settings [70]. Thalidomide plus rituximab (RTX), an anti-CD20 monoclonal antibody commonly used in CLL, was found to increase complete response rates in relapsed and refractory MCL patients [72]. Further study of the mechanism showed that the drug-antibody combination increased growth arrest of MCL cell lines, as well as primary cells, compared to RTX alone [73]. Mechanistically, they discovered that lenalidomide enhanced CD20-mAb-dependent apoptosis of the MCL cells by upregulating activation of caspase-3, -8, -9 and the cleavage of PARP, as well as enhanced ADCC by CD16 induction on NK cells [73]. An increase in NK-mediated ADCC is also implicated in the success of RTX and lenalidomide combination therapy in CLL and NHL, although unproven *in vivo* [74, 75]. Ofatumumab, another anti-CD20 monoclonal antibody, binds to a different epitope and induces greater complement-dependent cytotoxicity and has shown evidence of activity in fludarabine and rituximab-refractory CLL [76, 77]. Another CD20 mAb, the glycoengineered GA-101 antibody, induces greater ADCC *in vitro* than RTX and has shown promising preclinical activity in animal models of NHL and B-CLL [78–82]. Lenalidomide therapy is currently being tested with ofatumumab [83] and elotuzumab [84] in advanced, relapsed or refractory patients and has shown therapeutic potential. Therefore, concurrent lenalidomide therapy with these antibodies may prove beneficial in refractory patients to augment antitumorigenic activity through NK cell potentiating effects.

As an immunomodulatory agent in solid tumors, lenalidomide has been used to reverse tolerance to tumor antigens [85, 86]. As such, lenalidomide may prove beneficial as an adjuvant to vaccine therapies. Wu et al. demonstrated that lenalidomide enhances NK cell killing in a variety of solid tumor cell lines (breast, colorectal cancer, ovary, head and neck, lung cancer, bone sarcoma) treated with cetuximab or trastuzumab [65]. The treatment of hematologic and solid tumors with specific monoclonal antibody therapy concurrently with lenalidomide could potently increase NK cell-mediated tumor lysis and enhance response rates. Lenalidomide induces NK cells to produce granulocyte-macrophage colony-stimulating factor (GM-CSF), TNF-α, and various immune recruiting chemokines including RANTES, IL-8, MCP-1, and MIP-1α/β in response to antibody-coated tumor cell lines, which contributes to a more effective immune response [65]. The IMiDs enhance immunosurveillance in solid and liquid tumor settings through recruiting and activating T and NK cells to suppress malignant growth.

5. Summary

This paper summarizes the current information about lenalidomide in proliferative neoplasms and describes our

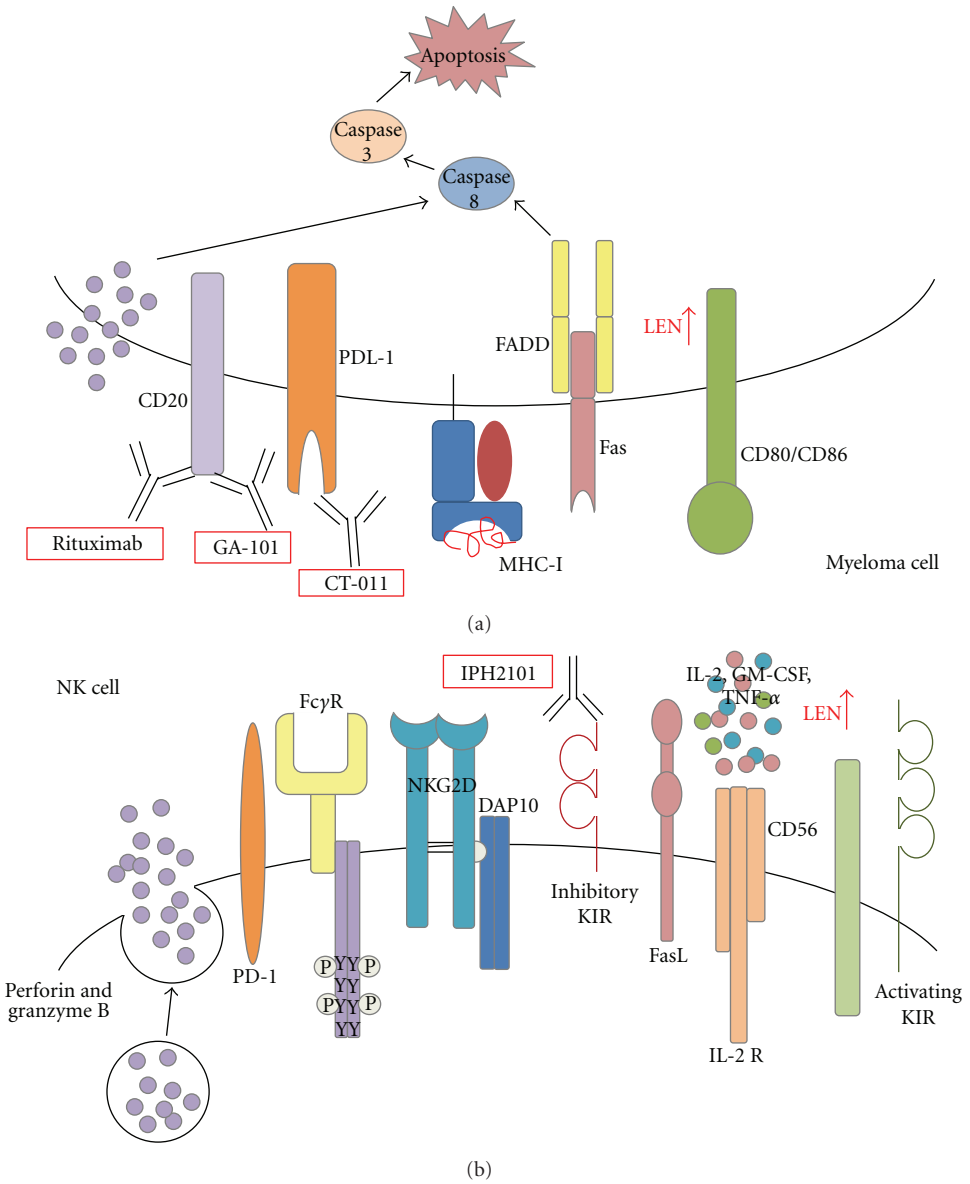


Figure 3: Lenalidomide alone, or in combination with a variety of therapeutic monoclonal antibodies, increases NK-cell-mediated killing of multiple myeloma cells. Lenalidomide (LEN) increases IL-2 secretion from by-standing T helper cells which augments NK cell activity. Lenalidomide, as described previously, upregulates Fas expression and costimulatory molecules on MM cells leading to greater Fas-mediated apoptosis. Lenalidomide has also been shown to augment the ADCC effect of various monoclonal antibodies like Rituximab (anti-CD20), GA-101 (glycoengineered anti-CD20), and CT-011 (anti-PDL-1). CT-011 blocks PD-1 ligand on the MM cells, interfering with binding to PD-1 and inhibiting NK cell activity. Binding of the anti-CD20 antibodies to their targets on MM cells increases complement-dependent cytotoxicity (CDC), as well as NK-cell recognition and killing of the MM cells. IPH2101 is an anti-inhibitory KIR that has been shown in combination with lenalidomide to increase NK-cell killing as well, as blocking the inhibitory signals allows for NK activation and detection of the tumor cells.

understanding of the molecular mechanism of action in lymphocytes. Based on the overwhelming success of lenalidomide for the treatment of several hematologic malignancies, there is potential for therapies that augment host immune responses to be extended from the relapsed and refractory setting, to primary therapy. Studies over several decades have elucidated the importance of immunosurveillance in malignancy. The seminal discoveries that lenalidomide can potently augment T-cell cytokine secretion and activation in the absence of a secondary signal and augment NK-mediated ADCC in the presence of antibody therapy have only begun to shed light on the mechanism of lenalidomide immune modulating activity. The potential in furthering lenalidomide in combination therapy with therapeutic antibodies, vaccines, and chemotherapy depends on improving our understanding of the molecular mechanism of the drug.

The mechanism of action and the important molecular and cellular determinants that mediate the immunomodulatory function are poorly understood, yet many cancer patients have benefited from this therapy. T cells and NK cells are rendered anergic or ignorant by the tumor cells through multiple mechanisms related to the lack of costimulation and immunosuppressive signals within the tumor microenvironment. Because of the importance of costimulation in determining the immune response, therapeutic manipulation with lenalidomide has generated particular interest. The mechanism of action is clearly linked to changes in the bone marrow microenvironment, cytokine secretion, regulation of angiogenesis and host antitumor immunity. Since this agent has significant activity in MM, MDS, CLL, NHL, and MCL, a better understanding of the leukemia biology and the molecular targets that mediate the immunomodulatory activity is needed to harness the full potential of this agent in combination therapies.

Acknowledgment

Investigators contributing to this work were supported by NIH R01 CA129952.

References

[1] C. G. Iyer, J. Languillon, K. Ramanujam et al., "WHO coordinated short-term double-blind trial with thalidomide in the treatment of acute lepra reactions in male lepromatous patients," *Bulletin of the World Health Organization*, vol. 45, no. 6, pp. 719–732, 1971.

[2] J. Sheskin, "The treatment of lepra reaction in lepromatous leprosy. Fifteen years' experience with thalidomide," *International Journal of Dermatology*, vol. 19, no. 6, pp. 318–322, 1980.

[3] O. Gutierrez-Rodriguez, "Thalidomide: a promising new treatment for rheumatoid arthritis," *Arthritis and Rheumatism*, vol. 27, no. 10, pp. 1118–1121, 1984.

[4] E. Atra and E. I. Sato, "Treatment of the cutaneous lesions of systemic lupus erythematosus with thalidomide," *Clinical and Experimental Rheumatology*, vol. 11, no. 5, pp. 487–493, 1993.

[5] M. H. Hamza, "Treatment of Behcet's disease with thalidomide," *Clinical Rheumatology*, vol. 5, no. 3, pp. 365–371, 1986.

[6] G. W. Muller, L. G. Corral, M. G. Shire et al., "Structural modifications of thalidomide produce analogs with enhanced tumor necrosis factor inhibitory activity," *Journal of Medicinal Chemistry*, vol. 39, no. 17, pp. 3238–3240, 1996.

[7] V. Kotla, S. Goel, S. Nischal et al., "Mechanism of action of lenalidomide in hematological malignancies," *Journal of Hematology and Oncology*, vol. 2, article 36, 2009.

[8] V. Saloura and P. D. Grivas, "Lenalidomide: a synthetic compound with an evolving role in cancer management," *Hematology*, vol. 15, no. 5, pp. 318–331, 2010.

[9] E. Carballido, M. Veliz, R. Komrokji, and J. Pinilla-Ibarz, "Immunomodulatory drugs and active immunotherapy for chronic lymphocytic leukemia," *Cancer Control*, vol. 19, no. 1, pp. 54–67, 2012.

[10] G. P. Dunn, A. T. Bruce, H. Ikeda, L. J. Old, and R. D. Schreiber, "Cancer immunoediting: from immunosurveillance to tumor escape," *Nature Immunology*, vol. 3, no. 11, pp. 991–998, 2002.

[11] V. Shankaran, H. Ikeda, A. T. Bruce et al., "IFNγ, and lymphocytes prevent primary tumour development and shape tumour immunogenicity," *Nature*, vol. 410, no. 6832, pp. 1107–1111, 2001.

[12] P. A. J. Haslett, L. G. Corral, M. Albert, and G. Kaplan, "Thalidomide costimulates primary human t lymphocytes, preferentially inducing proliferation, cytokine production, and cytotoxic responses in the $CD8^+$ subset," *Journal of Experimental Medicine*, vol. 187, no. 11, pp. 1885–1892, 1998.

[13] A. List, S. Kurtin, D. J. Roe et al., "Efficacy of lenalidomide in myelodysplastic syndromes," *The New England Journal of Medicine*, vol. 352, no. 6, pp. 549–557, 2005.

[14] P. S. Linsley, J. L. Greene, W. Brady, J. Bajorath, J. A. Ledbetter, and R. Peach, "Human B7-1 (CD80) and B7-2 (CD86) bind with similar avidities but distinct kinetics to CD28 and CTLA-4 receptors," *Immunity*, vol. 1, no. 9, pp. 793–801, 1994.

[15] P. A. Bretscher, "A two-step, two-signal model for the primary activation of precursor helper T cells," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 96, no. 1, pp. 185–190, 1999.

[16] J. A. Keene and J. Forman, "Helper activity is required for the in vivo generation of cytotoxic T lymphocytes," *Journal of Experimental Medicine*, vol. 155, no. 3, pp. 768–782, 1982.

[17] T. Mustelin and K. Taskén, "Positive and negative regulation of T-cell activation through kinases and phosphatases," *Biochemical Journal*, vol. 371, part 1, pp. 15–27, 2003.

[18] M. A. Williams, A. J. Tyznik, and M. J. Bevan, "Interleukin-2 signals during priming are required for secondary expansion of $CD8^+$ memory T cells," *Nature*, vol. 441, no. 7095, pp. 890–893, 2006.

[19] T. Nakayama and M. Yamashita, "The TCR-mediated signaling pathways that control the direction of helper T cell differentiation," *Seminars in Immunology*, vol. 22, no. 5, pp. 303–309, 2010.

[20] R. LeBlanc, T. Hideshima, L. P. Catley et al., "Immunomodulatory drug costimulates T cells via the B7-CD28 pathway," *Blood*, vol. 103, no. 5, pp. 1787–1790, 2004.

[21] T. Hayashi, T. Hideshima, M. Akiyama et al., "Molecular mechanisms whereby immunomodulatory drugs activate natural killer cells: clinical application," *British Journal of Haematology*, vol. 128, no. 2, pp. 192–203, 2005.

[22] F. Payvandi, L. Wu, S. D. Naziruddin et al., "Immunomodulatory drugs (IMiDs) increase the production of IL-2 from stimulated T cells by increasing PKC-θ activation and enhancing the DNA-binding activity of AP-1 but not NF-κB, OCT-1, or NF-AT," *Journal of Interferon and Cytokine Research*, vol. 25, no. 10, pp. 604–616, 2005.

[23] P. H. Schafer, A. K. Gandhi, M. A. Loveland et al., "Enhancement of cytokine production and AP-1 transcriptional activity in T cells by thalidomide-related immunomodulatory drugs," *Journal of Pharmacology and Experimental Therapeutics*, vol. 305, no. 3, pp. 1222–1232, 2003.

[24] G. Görgün, E. Calabrese, E. Soydan et al., "Immunomodulatory effects of lenalidomide and pomalidomide on interaction of tumor and bone marrow accessory cells in multiple myeloma," *Blood*, vol. 116, no. 17, pp. 3227–3237, 2010.

[25] M. J. Smyth, D. I. Godfrey, and J. A. Trapani, "A fresh look at tumor immunosurveillance and immunotherapy," *Nature Immunology*, vol. 2, no. 4, pp. 293–299, 2001.

[26] C. Galustian, B. Meyer, M. C. Labarthe et al., "The anticancer agents lenalidomide and pomalidomide inhibit the proliferation and function of T regulatory cells," *Cancer Immunology, Immunotherapy*, vol. 58, no. 7, pp. 1033–1045, 2009.

[27] K. Giannopoulos, M. Schmitt, P. Własiuk et al., "The high frequency of T regulatory cells in patients with B-cell chronic

lymphocytic leukemia is diminished through treatment with thalidomide," *Leukemia*, vol. 22, no. 1, pp. 222–224, 2008.

[28] J. M. McDaniel, J. X. Zou, W. Fulp, D.-T. Chen, A. F. List, and P. K. Epling-Burnette, "Reversal of T-cell tolerance in myelodysplastic syndrome through lenalidomide immune modulation," *Leukemia*, vol. 26, no. 6, pp. 1425–1429, 2012.

[29] K. Noonan, L. Rudraraju, A. Ferguson et al., "Lenalidomide-induced immunomodulation in multiple myeloma: impact on vaccines and antitumor responses," *Clinical Cancer Research*, vol. 18, no. 5, pp. 1426–1434, 2012.

[30] S. Scrivener, R. V. Goddard, E. R. Kaminski, and A. G. Prentice, "Abnormal T-cell function in B-cell chronic lymphocytic leukaemia," *Leukemia and Lymphoma*, vol. 44, no. 3, pp. 383–389, 2003.

[31] S. Kiaii, A. Choudhury, F. Mozaffari, E. Kimby, A. Österborg, and H. Mellstedt, "Signaling molecules and cytokine production in T cells of patients with B-cell chronic lymphocytic leukemia (B-CLL): comparison of indolent and progressive disease," *Medical Oncology*, vol. 22, no. 3, pp. 291–302, 2005.

[32] A. G. Ramsay, A. J. Clear, and R. Fatah, "Multiple inhibitory ligands induce impaired T cell immunological synapse function in chronic lymphocytic leukemia that can be blocked with lenalidomide," *Blood*. In press.

[33] B. N. Lee, H. Gao, E. N. Cohen et al., "Treatment with lenalidomide modulates T-cell immunophenotype and cytokine production in patients with chronic lymphocytic leukemia," *Cancer*, vol. 117, no. 17, pp. 3999–4008, 2011.

[34] B. Neuber, I. Herth, C. Tolliver et al., "Lenalidomide enhances antigen-specific activity and decreases CD45RA expression of T cells from patients with multiple myeloma," *The Journal of Immunology*, vol. 187, no. 2, pp. 1047–1056, 2011.

[35] T. Ito, H. Ando, T. Suzuki et al., "Identification of a primary target of thalidomide teratogenicity," *Science*, vol. 327, no. 5971, pp. 1345–1350, 2010.

[36] Y. X. Zhu, E. Braggio, C.-X. Shi et al., "Cereblon expression is required for the antimyeloma activity of lenalidomide and pomalidomide," *Blood*, vol. 118, no. 18, pp. 4771–4779, 2011.

[37] S. Ren, C. Xu, Z. Cui et al., "Oncogenic CUL4A determines the response to thalidomide treatment in prostate cancer," *Journal of Molecular Medicine*. In press.

[38] S. Wei, X. Chen, K. McGraw et al., "Lenalidomide promotes p53 degradation by inhibiting MDM2 auto-ubiquitination in myelodysplastic syndrome with chromosome 5q deletion," *Oncogene*. In press.

[39] F. T. Awan, A. J. Johnson, R. Lapalombella et al., "Thalidomide and lenalidomide as new therapeutics for the treatment of chronic lymphocytic leukemia," *Leukemia and Lymphoma*, vol. 51, no. 1, pp. 27–38, 2010.

[40] A. Chanan-Khan, K. C. Miller, L. Musial et al., "Clinical efficacy of lenalidomide in patients with relapsed or refractory chronic lymphocytic leukemia: results of a phase II study," *Journal of Clinical Oncology*, vol. 24, no. 34, pp. 5343–5349, 2006.

[41] C. I. Chen, P. L. Bergsagel, H. Paul et al., "Single-agent lenalidomide in the treatment of previously untreated chronic lymphocytic leukemia," *Journal of Clinical Oncology*, vol. 29, no. 9, pp. 1175–1181, 2011.

[42] A. Ferrajoli, B. N. Lee, E. J. Schlette et al., "Lenalidomide induces complete and partial remissions in patients with relapsed and refractory chronic lymphocytic leukemia," *Blood*, vol. 111, no. 11, pp. 5291–5297, 2008.

[43] A. Chanan-Khan and C. W. Porter, "Immunomodulating drugs for chronic lymphocytic leukaemia," *The Lancet Oncology*, vol. 7, no. 6, pp. 480–488, 2006.

[44] A. G. Ramsay, A. J. Johnson, A. M. Lee et al., "Chronic lymphocytic leukemia T cells show impaired immunological synapse formation that can be reversed with an immunomodulating drug," *The Journal of Clinical Investigation*, vol. 118, no. 7, pp. 2427–2437, 2008.

[45] G. Gorgun, A. G. Ramsay, T. A. W. Holderried et al., "Eμ-TCL1 mice represent a model for immunotherapeutic reversal of chronic lymphocytic leukemia-induced T-cell dysfunction," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 15, pp. 6250–6255, 2009.

[46] A. G. Ramsay, A. J. Clear, R. Fatah, and J. G. Gribben, "Lenalidomide repairs suppressed T cell immunological synapse formation in follicular lymphoma," *Blood*, vol. 112, 2008, Abstract no. 885.

[47] A. A. Chanan-Khan, K. Chitta, N. Ersing et al., "Biological effects and clinical significance of lenalidomide-induced tumour flare reaction in patients with chronic lymphocytic leukaemia: *in vivo* evidence of immune activation and antitumour response," *British Journal of Haematology*, vol. 155, no. 4, pp. 457–467, 2011.

[48] A. Chanan-Khan, K. C. Miller, D. Lawrence et al., "Tumor flare reaction associated with lenalidomide treatment in patients with chronic lymphocytic leukemia predicts clinical response," *Cancer*, vol. 117, no. 10, pp. 2127–2135, 2011.

[49] M. V. Dhodapkar, M. D. Geller, D. H. Chang et al., "A reversible defect in natural killer T cell function characterizes the progression of premalignant to malignant multiple myeloma," *Journal of Experimental Medicine*, vol. 197, no. 12, pp. 1667–1676, 2003.

[50] R. T. Perri, M. M. Oken, and N. E. Kay, "Enhanced T cell suppression is directed toward sensitive circulating B cells in multiple myeloma," *Journal of Laboratory and Clinical Medicine*, vol. 99, no. 4, pp. 512–519, 1982.

[51] R. A. Kyle and S. V. Rajkumar, "Drug therapy: multiple myeloma," *The New England Journal of Medicine*, vol. 351, no. 18, pp. 1860–1921, 2004.

[52] I. Breitkreutz, M. S. Raab, S. Vallet et al., "Lenalidomide inhibits osteoclastogenesis, survival factors and bone-remodeling markers in multiple myeloma," *Leukemia*, vol. 22, no. 10, pp. 1925–1932, 2008.

[53] H. Geitz, S. Handt, and K. Zwingenberger, "Thalidomide selectively modulates the density of cell surface molecules involved in the adhesion cascade," *Immunopharmacology*, vol. 31, no. 2-3, pp. 213–221, 1996.

[54] A. Lichtenstein, Y. Tu, C. Fady, R. Vescio, and J. Berenson, "Interleukin-6 inhibits apoptosis of malignant plasma cells," *Cellular Immunology*, vol. 162, no. 2, pp. 248–255, 1995.

[55] R. D. Brown, B. Pope, A. Murray et al., "Dendritic cells from patients with myeloma are numerically normal but functionally defective as they fail to up-regulate CD80 (B7-1) expression after huCD40LT stimulation because of inhibition by transforming growth factor-β1 and interleukin-10," *Blood*, vol. 98, no. 10, pp. 2992–2998, 2001.

[56] M. Ratta, F. Fagnoni, A. Curti et al., "Dendritic cells are functionally defective in multiple myeloma: the role of interleukin-6," *Blood*, vol. 100, no. 1, pp. 230–237, 2002.

[57] O. Christensen, A. Lupu, S. Schmidt et al., "Melan-A/MART1 analog peptide triggers anti-myeloma T-cells through crossreactivity with HM1.24," *Journal of Immunotherapy*, vol. 32, no. 6, pp. 613–621, 2009.

[58] T. Barlozzari, C. W. Reynolds, and R. B. Herberman, "In vivo role of natural killer cells: involvement of large granular

lymphocytes in the clearance of tumor cells in anti-asialo GM1-treated rats," *The Journal of Immunology*, vol. 131, no. 2, pp. 1024–1027, 1983.

[59] V. C. Huber, J. M. Lynch, D. J. Bucher, J. Le, and D. W. Metzger, "Fc receptor-mediated phagocytosis makes a significant contribution to clearance of influenza virus infections," *The Journal of Immunology*, vol. 166, no. 12, pp. 7381–7388, 2001.

[60] L. E. Wai, J. A. Garcia, O. M. Martinez, and S. M. Krams, "Distinct roles for the NK cell-activating receptors in mediating interactions with dendritic cells and tumor cells," *The Journal of Immunology*, vol. 186, no. 1, pp. 222–229, 2011.

[61] F. E. Davies, N. Raje, T. Hideshima et al., "Thalidomide and immunomodulatory derivatives augment natural killer cell cytotoxicity in multiple myeloma," *Blood*, vol. 98, no. 1, pp. 210–216, 2001.

[62] D. Zhu, L. G. Corral, Y. W. Fleming, and B. Stein, "Immunomodulatory drugs Revlimid® (lenalidomide) and CC-4047 induce apoptosis of both hematological and solid tumor cells through NK cell activation," *Cancer Immunology, Immunotherapy*, vol. 57, no. 12, pp. 1849–1859, 2008.

[63] A. K. Hsu, H. Quach, T. Tai et al., "The immunostimulatory effect of lenalidomide on NK-cell function is profoundly inhibited by concurrent dexamethasone therapy," *Blood*, vol. 117, no. 5, pp. 1605–1613, 2011.

[64] E. Carbone, P. Neri, M. Mesuraca et al., "HLA class I, NKG2D, and natural cytotoxicity receptors regulate multiple myeloma cell recognition by natural killer cells," *Blood*, vol. 105, no. 1, pp. 251–258, 2005.

[65] L. Wu, A. Parton, L. Lu, M. Adams, P. Schafer, and J. B. Bartlett, "Lenalidomide enhances antibody-dependent cellular cytotoxicity of solid tumor cells in vitro: influence of host immune and tumor markers," *Cancer Immunology, Immunotherapy*, vol. 60, no. 1, pp. 61–73, 2011.

[66] D. M. Benson Jr., C. E. Bakan, S. Zhang et al., "IPH2101, a novel anti-inhibitory KIR antibody, and lenalidomide combine to enhance the natural killer cell versus multiple myeloma effect," *Blood*, vol. 118, no. 24, pp. 6387–6391, 2011.

[67] M. J. Butte, M. E. Keir, T. B. Phamduy, A. H. Sharpe, and G. J. Freeman, "Programmed death-1 ligand 1 interacts specifically with the B7-1 costimulatory molecule to inhibit T cell responses," *Immunity*, vol. 27, no. 1, pp. 111–122, 2007.

[68] M. E. Keir, M. J. Butte, G. J. Freeman, and A. H. Sharpe, "PD-1 and its ligands in tolerance and immunity," *Annual Review of Immunology*, vol. 26, pp. 677–704, 2008.

[69] D. M. Benson, C. E. Bakan, A. Mishra et al., "The PD-1/PD-L1 axis modulates the natural killer cell versus multiple myeloma effect: a therapeutic target for CT-011, a novel monoclonal anti-PD-1 antibody," *Blood*, vol. 116, no. 13, pp. 2286–2294, 2010.

[70] L. Wu, M. Adams, T. Carter et al., "Lenalidomide enhances natural killer cell and monocyte-mediated antibody-dependent cellular cytotoxicity of rituximab-treated $CD20^+$ tumor cells," *Clinical Cancer Research*, vol. 14, no. 14, pp. 4650–4657, 2008.

[71] E. Vivier, E. Tomasello, M. Baratin, T. Walzer, and S. Ugolini, "Functions of natural killer cells," *Nature Immunology*, vol. 9, no. 5, pp. 503–510, 2008.

[72] H. Kaufmann, M. Raderer, S. Wöhrer et al., "Antitumor activity of rituximab plus thalidomide in patients with relapsed/refractory mantle cell lymphoma," *Blood*, vol. 104, no. 8, pp. 2269–2271, 2004.

[73] L. Zhang, Z. Qian, Z. Cai et al., "Synergistic antitumor effects of lenalidomide and rituximab on mantle cell lymphoma in vitro and in vivo," *American Journal of Hematology*, vol. 84, no. 9, pp. 553–559, 2009.

[74] N. H. Fowler MP, L. Kwak, F. Hagemeister et al., "Lenalidomide and rituximab for untreated indolent non-Hodgkin's lymphoma," *Journal of Clinical Oncology*, vol. 27, 15s, 2009, Abstract no. 8548.

[75] M. Veliz SR, J. E. Lancet, R. S. Komrokji et al., "Phase II study of lenalidomide in combination with rituximab for patients with $CD5^+/CD20^+$ hematologic malignancies who relapse or progress after rituximab: interim analysis," *Blood*, vol. 114, article 2376, 2009.

[76] J. Du, H. Yang, Y. Guo, and J. Ding, "Structure of the Fab fragment of therapeutic antibody Ofatumumab provides insights into the recognition mechanism with CD20," *Molecular Immunology*, vol. 46, no. 11-12, pp. 2419–2423, 2009.

[77] W. G. Wierda, S. Padmanabhan, G. W. Chan, I. V. Gupta, S. Lisby, and A. Österborg, "Ofatumumab is active in patients with fludarabine-refractory CLL irrespective of prior rituximab: results from the phase 2 international study," *Blood*, vol. 118, no. 19, pp. 5126–5129, 2011.

[78] E. Mössner, P. Brünker, S. Moser et al., "Increasing the efficacy of CD20 antibody therapy through the engineering of a new type II anti-CD20 antibody with enhanced direct and immune effector cell–mediated B-cell cytotoxicity," *Blood*, vol. 115, no. 22, pp. 4393–4402, 2010.

[79] T. Robak, "GA-101, a third-generation, humanized and glycoengineered anti-CD20 mAb for the treatment of B-cell lymphoid malignancies," *Current Opinion in Investigational Drugs*, vol. 10, no. 6, pp. 588–596, 2009.

[80] L. Bologna, E. Gotti, M. Manganini et al., "Mechanism of action of type II, glycoengineered, anti-CD20 monoclonal antibody GA101 in B-chronic lymphocytic leukemia whole blood assays in comparison with rituximab and alemtuzumab," *The Journal of Immunology*, vol. 186, no. 6, pp. 3762–3769, 2011.

[81] F. C. G. Morschhauser, T. Lamy, N. Milpied et al., "Phase I study of RO5072759 (GA101) in relapsed /refractory CLL," in *ASH Ann Meeting Abstract*, vol. 114, 2009, Abstract no. 364.

[82] L. H. Sehn AS, D. A. Steward, J. Mangel, P. Pisa, J. Kothari, and M. Crump, "A Phase I study of GA101 (RO5072759) monotherapy followed by maintenance in patients with multiple relapsed/refractory $CD20^+$ malignant disease," in *ASH Ann Meeting Abstract*, vol. 114, 2009, Abstract no. 385.

[83] X. Badoux, O. 'Brien SM, W. G. Wierda et al., "Combination of ofatumumab and lenalidomide in patients with relapsed chronic lymphocytic leukemia: initial results of a phase II trial," *Blood (ASH Annual Meeting Abstracts)*, vol. 116, 2010, Abstract no. 2464.

[84] S. Lonial, R. Vij, J. L. Harousseau et al., "Elotuzumab in combination with lenalidomide and low-dose dexamethasone in relapsed or refractory multiple myeloma," *Journal of Clinical Oncology*, vol. 30, no. 16, pp. 1953–1959, 2012.

[85] K. Staveley-O'Carroll, E. Sotomayor, J. Montgomery et al., "Induction of antigen-specific T cell anergy: an early event in the course of tumor progression," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 95, no. 3, pp. 1178–1183, 1998.

[86] P. Horna and E. M. Sotomayor, "Cellular and molecular mechanisms of tumor-induced T-cell tolerance," *Current Cancer Drug Targets*, vol. 7, no. 1, pp. 41–53, 2007.

Hindawi Publishing Corporation
Advances in Hematology
Volume 2012, Article ID 801495, 9 pages
doi:10.1155/2012/801495

Review Article

Secondary Primary Malignancies in Multiple Myeloma: An Old Nemesis Revisited

Jay Yang,[1] Howard R. Terebelo,[2] and Jeffrey A. Zonder[1]

[1] *Department of Oncology, Karmanos Cancer Institute and Wayne State University, Detroit, MI 48201, USA*
[2] *Department of Internal Medicine, Providence Hospital, Southfield, MI 48075, USA*

Correspondence should be addressed to Jay Yang, yangj@karmanos.org

Received 15 December 2011; Revised 25 May 2012; Accepted 3 June 2012

Academic Editor: Umberto Vitolo

The treatment of myeloma has undergone extraordinary improvements in the past half century. These advances have been accompanied by a concern for secondary primary malignancies (SPMs). It has been known for decades that extended therapy with alkylating chemotherapy agents, such as melphalan, carries an increased risk of therapy-related myelodysplastic syndrome and/or acute myeloid leukemia (t-MDS/AML), with a cumulative risk as high as 10–15%. High dose chemotherapy with autologous stem cell support became widely accepted for myeloma in the 1990s. Despite the use of high-doses of melphalan, the risk of t-MDS/AML with this procedure is estimated to be less than 5%, with much of this risk attributable to pretransplant therapy. Recently, lenalidomide has come under scrutiny for its possible association with SPMs. It is too soon to declare a causal relationship at this time, but there appears to be an increased number of SPMs in reports from several studies using lenalidomide maintenance. Current studies should be amended and future studies planned to better define the risk of SPMs and the risk factors and mechanisms for its development. Patients should be educated regarding this potential concern but the current use of lenalidomide should not generally be altered until further data are available.

1. Introduction

The evolution of myeloma therapy has been one of the success stories in the fight against cancer. Current treatment options for myeloma include melphalan, other cytotoxic agents, corticosteroids, high-dose therapy with autologous stem cell transplant (HDT/SCT), and more recently, novel agents such as bortezomib, thalidomide, and lenalidomide. Despite the remarkable improvement in prognosis for myeloma patients, the disease remains incurable and is characterized by multiple relapses. Therapy-related myelodysplasia and acute myeloid leukemia (t-MDS/AML) have been recognized as a consequence of treatment with alkylating agents and/or anthracyclines. Similar concerns have now been raised about the potential for an increase in secondary primary malignancies (SPMs) in myeloma patients exposed to lenalidomide, and, in particular, long-term exposures in a maintenance setting.

In this paper, we will review the developmental history of myeloma therapy, with particular emphasis on the risk of secondary cancers, and examine the available data with regard to the risk of SPMs seen with lenalidomide. We also speculate about the mechanism(s) by which lenalidomide could increase the risk of second cancers. To conclude, we make some recommendations about how our current understanding affects our treatment decisions and suggest directions for future research. As new data emerge about lenalidomide and the risk of SPMs, it is our hope that this paper will help to put that information in proper perspective.

2. Second Cancers in Multiple Myeloma

SPMs are not an uncommon occurrence among cancer patients. The NCI's SEER program analyzed its database from 1973 to 2000 and reported that the cumulative incidence of SPMs was nearly 14% at 25 years of followup for cancer patients in general [1]. Myeloma patients had a 6.1% incidence of SPMs at 20 years but the overall rate was not higher than that seen in the general population. However, increased relative risks for AML, chronic myelogenous leukemia, and Kaposi's sarcoma were noted. Leukemias,

especially AML, accounted for the largest cancer excesses and likely reflected treatment with alkylating agents.

The Finnish Leukaemia Group conducted a retrospective, long-term followup of 432 patients who were treated with conventional chemotherapy for myeloma [2]. The number and distribution of secondary solid cancers were similar to the general population but the actuarial risk of leukemia was almost 10% at 9 years. A Swedish registry database that included 8656 myeloma patients found a 5.5% risk of SPMs [3]. According to their analysis, myeloma patients had a marked increased risk of AML (standardized incidence ratio, that is, ratio of observed/expected rates = 8.19; 95% CI, 5.7–11.4), a slight increased risk of non-Hodgkin lymphoma (NHL) (SIR = 1.74; 95% CI, 1.12–2.57), and a decreased risk of solid cancers (SIR = 0.81; 95% CI, 0.73–0.90). In summary, based on older registry data, an increased risk of AML is observed in myeloma patients.

3. Chemotherapy for Multiple Myeloma

The use of chemotherapy for myeloma began in 1962 when melphalan was first reported to have activity in this disease [4]. Many patients were treated with continued courses of melphalan indefinitely until disease progression or unacceptable toxicity [5]. Combinations that added other chemotherapeutic agents such as vincristine, carmustine, doxorubicin, or nitrosureas to a melphalan/prednisone backbone showed somewhat higher response rates but no survival benefit [6–8]. Regardless of therapy given, all patients relapsed and so the concept of maintenance therapy became attractive with the goal of prolonging remissions. Trials that tested maintenance chemotherapy after responses to melphalan-based induction showed no clinical or survival benefit over observation alone [9]. Patients receiving maintenance had slightly longer remission durations which were offset by lower rates of second remissions, suggesting that maintenance therapy contributed to drug resistance at the time of relapse [10].

The practice of indefinite melphalan therapy also came under scrutiny because of a burgeoning worry about secondary malignancies. Kyle et al. were among the first to propose an association between the prolonged use of melphalan and myelodysplasia and/or acute myeloid leukemia (MDS/AML) [11]. Some questioned whether MDS/AML could be part of the natural history of myeloma, much like what is seen in other hematological conditions. Although this view was buoyed by several reported cases of untreated myeloma and concurrent AML in the literature [12, 13], the prevailing conclusion was that such cases represented a chance association. With time, MDS and AML became recognized complications after chemotherapy for other neoplasms such as Hodgkin lymphoma (HL) and ovarian cancer, and it became accepted that, regardless of the indication, chemotherapy was directly responsible for this increased risk due to mechanisms that included direct DNA damage.

Reported rates of t-MDS/AML for myeloma patients treated with melphalan ranged from anywhere from 3% at 5 years and 10% at 8 years, with estimates as high as 25% at 10 years [14]. Higher cumulative doses of melphalan increased the risk of MDS/AML [15]. Cyclophosphamide also appeared to carry a risk, but less so than melphalan [16]. The diagnosis of t-MDS/AML carried a grim prognosis with reported median survivals of less than 3 months [2, 11]. Nevertheless, the use of melphalan remained the *de facto* standard for several decades.

4. HDT/SCT for Multiple Myeloma

The use of autologous bone marrow transplantation with high-dose melphalan to treat myeloma became widely accepted as the standard of care for transplantable patients after the IFM 90 trial showed event-free and overall survivals in favor of this procedure [17]. The use of HDT/SCT for both solid tumors and hematologic malignancies, including myeloma, increased dramatically in the 1990s [18]. Cases of MDS/AML were seen after HDT and raised serious concerns about the leukemogenic risks of the procedure.

The risk of t-MDS/AML after HDT/SCT was first more clearly defined in the lymphoma patient population. Patients transplanted at the University of Minnesota for lymphoma experienced a 14.5% cumulative incidence of t-MDS/AML [19]. The risk increased with patient age and with the burden of alkylating agents prior to transplant [20]. Forrest et al. reported a 15-year cumulative incidence of 11% that indicated not only an increased risk of MDS/AML (relative risk = 47.2), but also lymphoproliferative disoders (RR = 8.1) and solid tumors (RR = 1.98) [21]. Interestingly, this analysis included 800 patients who were autotransplanted for a variety of conditions but there were no reported cases of MDS/AML in the subset of 123 myeloma patients.

Given the historical experience with melphalan-associated t-MDS/AML, concern arose over the risk in myeloma patients treated with high-dose melphalan. Govindarajan et al. reviewed 188 pts with myeloma who underwent HDT/SCT at the University of Arkansas [22]. In 117 patients who received extended courses of chemotherapy prior to tandem autotransplantation, 7 cases of MDS were seen, whereas in 71 patients who received limited chemotherapy prior to transplantation, no cases of MDS were seen. They concluded that preceding therapy was likely the cause of MDS in most cases seen after HDT/SCT, a finding that mirrored conclusions from studies in Hodgkin lymphoma (HL) [23].

The Arkansas experience was reviewed again over a decade later, this time including 3077 patients undergoing HDT/SCT for myeloma, most of whom were treated on their total therapy or total therapy-like protocols [24]. MDS-associated cytogenetic abnormalities were seen in 6%, although in roughly 2/3 of these cases, the karyotypic changes were only transient. The risk of clinically overt MDS/AML was even less, estimated at only 1% of transplanted patients. Survival after the diagnosis of t-MDS/AML in transplanted patients has been poor in most studies with a median of about 6 months [20].

In summary, contemporary studies show the rate of t-MDS/AML after HDT/SCT for myeloma to be less than 5% with higher rates in cohorts that received more previous alkyating-containing therapy. This risk is lower than in most

reported series for lymphoma, which can be explained, in part, by the earlier use of the transplant in myeloma, the emphasis on avoiding pretransplant stem cell damaging agents, and the abrogated use of total body irradiation during conditioning [25].

5. Lenalidomide Development and Mechanisms of Action

The immunomodulatory drugs (IMIDs) represent a novel class of antineoplastic agents that include thalidomide and its congeners, lenalidomide (CC-5013) and pomalidomide (CC-4043) [26]. Lenalidomide has significant activity versus myeloma and is approved as treatment in combination with dexamethasone. Lenalidomide has also been shown to have activity in a wide variety of other hematological malignancies, including MDS, non-Hodgkin lymphoma, HL, chronic lymphocytic leukemia, and myelofibrosis. It has an indication for transfusion-dependent anemia due to low or intermediate-1 risk MDS associated with a deletion 5q cytogenetic abnormality. Although approved in the United States for MDS, the European Medicines Agency (EMEA) has not yet granted approval for this indication due to initial safety concerns—namely the risk of progression to AML [27]. Recent evidence, however, including a randomized trial, has not shown an increased risk of AML in MDS patients treated with lenalidomide [28, 29].

The story of lenalidomide's development, broad antitumor activity, and drug approval is remarkable considering its unclear mechanism of action. Lenalidomide has a wide variety of effects, including antiangiogenesis and modulation of the tumor microenvironment, but it appears that its direct tumoricidal and immunomodulatory properties have the most relevance in myeloma [30, 31]. Its tumoricidal properties appear to be mediated in part by inhibiting the myeloma survival factor, IRF4, resulting in cell cycle arrest [32], as well as caspase-mediated apoptosis [33]. Its immunomodulatory properties include stimulation of immune effector cells such as NK and T cells [34]. The precise molecular target of lenalidomide in multiple myeloma has been elusive [35], but recently cereblon has been identified [36, 37]. The potential molecular targets in MDS have been reviewed elsewhere [38].

Thus, while lenalidomide itself is a simple compound, its molecular effects are pleiotropic and rather poorly understood. Furthermore, the relative contribution of these different mechanisms to its antimyeloma activity has not been well characterized [39] and may depend on multiple factors including the tumor type, dose/duration/schedule of lenalidomide, concomitant drugs given, and other preexisting patient factors.

6. Lenalidomide Therapy and Concern for SPMs

Due to disappointing results observed with maintenance chemotherapy, alternatives were eagerly sought. Interferon [40, 41] and corticosteroids [42, 43] were each tested in multiple clinical trials but were never convincingly shown to be beneficial [44]. Novel agents, with their unprecedented activity, became attractive candidates for use in the maintenance setting.

Thalidomide is the best studied of the novel agents for post-SCT maintenance, where it has prolonged progression-free survival (PFS) and/or time to progression (TTP) in multiple studies [45–49]. Despite this, only 2 studies have demonstrated a survival benefit, which may be partially explained by the shorter survivals seen after relapse in some studies [45, 49, 50]. This has again raised concerns about the selection of resistant clones at the time of relapse or progression [51]. The use of thalidomide as maintenance has never become routine by most prescribing clinicians due to its side effect profile and lack of consistent mortality benefit.

Both lenalidomide and bortezomib are being evaluated as maintenance therapy. For upfront and relapsed disease, both agents offer high levels of activity with unique but favorable side effect profiles. Lenalidomide is given orally, generally lacks the cumulative neuropathic potential of either thalidomide or bortezomib, and thus, may be the most promising drug in this setting. Combinations of novel agents are also being studied as maintenance [52, 53].

In the era of novel agents, second malignancies had been overlooked as a serious concern. Three recently published studies, however, provoked substantial interest due to the reported increased risk of SPMs (Table 1). All three were phase 3, placebo-controlled, randomized trials testing lenalidomide as maintenance therapy, either after HDT/SCT (IFM 2005-002, CALGB 100104) or after induction therapy (MM-015). These studies are of critical importance since they represent the first and only reported randomized trials to date that have prospectively and intentionally measured SPMs in multiple myeloma patients treated with maintenance lenalidomide versus placebo.

In the IFM 2005-002 study, patients under the age of 65 years with nonprogressive disease after HDT/SCT received 2 cycles of consolidation lenalidomide and then were randomized to either maintenance lenalidomide (10–15 mg daily) or placebo [54]. Lenalidomide improved median progression-free survival from 23 months to 41 months (HR = 0.5, $P < 0.001$). The 4-year overall survival was about 75% in both arms. The incidence of SPMs was 3.1 per 100 patient-years and 1.2 per 100 patient-years for patients receiving lenalidomide and placebo, respectively ($P = 0.002$). There were 13 reported hematological cancers with lenalidomide and 5 with placebo but the numbers of MDS/AML were similar (5 versus 4). Surprisingly, 7 cases of acute lymphoblastic leukemia or Hodgkin lymphoma were recorded in the lenalidomide maintenance arm and none in the placebo arm. Since all patients had received at least 2 years of lenalidomide and the optimal duration of maintenance is not known, the IFM has elected to stop the trial for safety reasons, discontinuing lenalidomide in the remaining patients still in remission.

In the CALGB 100104 study, patients under the age of 70 years with stage I-III myeloma were given induction therapy followed by HDT/SCT [55]. Those with stable disease or better were then randomized at day 100–110 posttransplant to lenalidomide (10–15 mg daily) or placebo until progression. The estimated median TTP was 46 months

for lenalidomide and 27 months for placebo, results that are similar to the IFM study. The cumulative incidence of SPMs was 8% in the lenalidomide maintenance arm versus 3% in the placebo arm, but even more striking, there were 8 cases of hematological cancers (including 6 with MDS/AML) seen with lenalidomide and only 1 with placebo. This study has now also reported a survival benefit with maintenance lenalidomide.

MM-015 randomized older patients to 1 of 3 arms: MPR-R (melphalan, prednisone, and lenalidomide induction followed by maintenance lenalidomide), MPR (melphalan, prednisone, and lenalidomide induction), or MP (melphalan and prednisone induction) [56]. After 9 cycles of induction therapy, the MPR-R arm received maintenance lenalidomide at 10 mg for 21 of every 28 days, while the other two arms received placebo maintenance. Median PFS was significantly longer with MPR-R (31 months) compared with MPR (14 months) and MP (13 months). No significant survival differences were seen. There were 12 cases of SPM in the MPR-R arm, 9 cases in the MPR arm, and 4 cases in the MP arm [57]. Ten cases of MDS/AML were seen in the lenalidomide containing arms (incidence 2.6%) and 1 case in the MP arm (0.6%). The number of solid tumors was low with no major differences seen between groups.

Several other reports have offered long-term, albeit post hoc, safety data for lenalidomide (Table 2) [58–60]. All have reported relatively low numbers of SPMs, including MDS/AML, with incidence rates ranging from 1.5 to 7.4%. The observed rates of SPMs were generally no higher than expected based on historical SEER data. An analysis of pooled data from 11 industry-sponsored trials suggested that there was no correlation between the development of SPMs and the cumulative dose or duration of lenalidomide received [58]. An analysis of the randomized MM 009/010 study noted higher rates of nonmelanoma skin cancers in patients randomized to lenalidomide compared with placebo [58]. There were no SPMs reported after the discontinuation of protocol treatment leading the authors to conclude that there are considerable obstacles in the ascertainment of second cancers during long-term followup.

7. Discussion

At the moment, there are more questions than answers, so we have designed our discussion around some of the most relevant issues.

7.1. Is There a True Risk of SPMs with Lenalidomide? When overall risks are relatively small, as they appear to be with SPMs in myeloma, it becomes more difficult to make conclusions with certainty. A number of other practical and statistical limitations exist when analyzing the data. SPMs may be underestimated if they are not specifically tracked during followup, particularly if off study. On the other hand, overreporting or overdiagnosis of SPMs may occur if they are expected on treatment arms. In retrospective or post hoc analyses, the methods of data collection may be less than desired. Finally, several reports have compared observed rates of SPMs to nonrandomized cohorts, which can sometimes be misleading. Some of these concerns are minimized when analyzing results from randomized, placebo-controlled trials with an *a priori* intention to measure SPMs. However, crossover, either on or off study, still has the potential to confound results. In the CALGB 100104 trial, the majority (67%) of the patients in the placebo arm crossed over to lenalidomide after unblinding. In the IFM 2005-002 and MM-015 trials, patients remained on their assigned treatment after unblinding, but it is likely that many of them received salvage lenalidomide at the time of progression.

Despite these limitations, there are some potentially important observations that merit attention and will need validation. The cumulative rates of invasive SPMs in the lenalidomide arms across the 3 randomized studies were quite consistent, ranging from 7 to 7.8%, thereby strengthening the observation. All 3 studies reported increased numbers of hematological malignancies in the lenalidomide arms and, in particular, 2 of the studies reported increased numbers of MDS/AML. The reported solid cancers have been heterogeneous in type. The other reviewed studies report long-term outcomes for cohorts of patients treated with lenalidomide. These studies have indicated a low risk of SPMs with rates that are no more than to be expected based on SEER data.

In aggregate, the retrospective and registry data does not support an increased risk of SPMs with chronic lenalidomide therapy. However, the recently published randomized trials demonstrate a signal suggesting otherwise. The risk of developing hematological malignancies, including MDS/AML, appears to be greater than the risk of solid cancers.

7.2. What Are the Risk Factors for the Development of SPMs? Treatment-related risk factors have received the most attention to date. There is certainly the strong possibility of an interaction between exposure to melphalan, exposure to lenalidomide, and an increased risk of MDS/AML. In the aforementioned randomized studies, patients were given melphalan either as induction therapy or as part of HDT/SCT prior to maintenance lenalidomide. There are no data at this time to suggest that an increased duration or dose of lenalidomide corresponds to an increased risk of SPMs. Whether the leukemogenicity of other chemotherapeutic agents is potentiated by lenalidomide remains to be seen. For example, results of the IFM 2005-002 trial have suggested an increased number of hematological cancers in those who received either a tandem transplant or pretransplant chemotherapy that included cyclophosphamide, etoposide, and cisplatin.

Non-treatment-related factors are less well understood but may play significant roles. Potential disease-related risk factors include baseline complex cytogenetics and the subtype of myeloma. The MM-015 study noted that 3 of the patients who ultimately developed MDS/AML in the lenalidomide arm were part of a small group of 11 patients who had complex cytogenetics at baseline [57]. IgG and IgA isotype MGUS patients have been reported to have an increased risk of MDS/AML [61]. Host factors, such as genetic polymorphisms [62], environmental factors, and behavioral factors have also been postulated as risk factors.

Table 1: Incidence of SPMs with lenalidomide (Len) maintenance versus placebo in prospective, randomized phase 3 trials.

Trial	Reference	*N*	F/u[a] (mo)	Treatment prior to randomization	Randomized arms	Invasive SPMs (no.)	Solid cancers (no.)	Heme cancers (no.)	Nonmelanoma skin cancers (no.)	Cumulative incidence of invasive cancers (%)	Incidence (per 100 patient-years)
IFM 2005-002	[54]	614	45	Induction then SCT	Len	23	10	13	5	7.5	3.1
					Placebo	9	4	5	3	2.9	1.2
CALGB 100104	[55]	460	34	Induction then SCT	Len	18	10	8	4	7.8	N/A
					Placebo	6	5	1	3	2.6	N/A
MM-015	[56, 57]	459	30	MPR or MP	MPR-R	12	5	7	1	7	1.4
					MPR	9	4	5	4	7	2.1
					MP	4	3	1	5	3	0.7

[a]Median followup from the time from randomization.

Table 2: Incidence of SPMs with lenalidomide (Len)-based therapy in selected trial cohorts based on retrospective or post hoc analyses.

Trial/Location	Reference	*N*	F/u	Patient population	Treatment	Invasive SPMs	Cumulative incidence of SPMs[a] (%)	IR[b]	SIR[c] (95% CI)	Types (number of cases)
PMH	[60]	230	N/A	RRMM[d]	Len-based	6[f]	2.6	Not reported	Not reported	MDS/AML
Cornell	[59]	68	>5 yrs	NDMM[e]	BiRD[g]	5	7.4[h]	2.85	1.36	MDS/AML (0), solid (5)
MM009-010	[58]	704	48 mo	RRMM	Len/dex	8	2.3	1.71	N/A	MDS (2), AML (0), solid (6), B-cell (0)
Pooled data	[58]	3839	N/A	RRMM	Len-based	57	1.5	2.35	0.77 (0.43–1.28)	MDS (8), AML (1), B-cell (2), solid (46)

[a]Excluding nonmelanoma skin cancers.
[b]Incidence rate per 100 person-years at risk.
[c]Standardized incidence ratio (i.e., ratio of observed/expected rates).
[d]Relapsed and/or refractory multiple myeloma.
[e]Newly diagnosed multiple myeloma.
[f]Only MDS/AML was reported.
[g]Clarithromycin, lenalidomide, and dexamethasone.

7.3. What Are the Potential Causal Mechanisms of Lenalidomide-Associated SPMs? A truly satisfactory explanation would require a better understanding of how lenalidomide works in patients with hematologic malignancies, let alone myeloma. This is an area of active research, but certainly more needs to be done. Unlike traditional cytotoxic chemotherapies, lenalidomide showed no mutagenic potential in extensive genotoxicity studies performed during its development [27]. And although carcinogenicity testing was not performed, chronic studies in rat and monkey revealed no potential for tumorigenicity. However, lenalidomide is clearly myelosuppressive and tumoricidal, and after a prolonged exposure may affect the ability to mobilize and collect stem cells. Perhaps these properties are an indication of a myelotoxicity which may predispose to MDS or AML. One could also speculate that lenalidomide's immunomodulatory properties and its effects on the tumor microenvironment may allow for the propagation of abnormal clones which can result in a malignancy. The complex mechanisms of action responsible for lenalidomide's activity, as well its unclear molecular target, make it difficult to rely on preclinical data to assess the safety and neoplastic potential of lenalidomide.

7.4. What Does the Myeloma Research Community Need to Do Now? There is a great need for additional systematic data gathering to determine whether lenalidomide is truly associated with SPMs, and if so, what types. For studies that are ongoing, amendments should be made to the protocols to include enhanced monitoring and precise measurements of second cancers. Careful monitoring for skin cancers may be important as the MM009/010 study has reported increased numbers on the lenalidomide arm. Although the large majority of skin cancers are found at an early and treatable stage, a finding of increased skin cancers would represent a proof-of-principle of the cancer promoting potential of lenalidomide.

Prospective randomized studies of lenalidomide versus placebo that include SPMs as a well-defined endpoint would be ideal. However, studies of this nature may be difficult to plan now that lenalidomide has already been established as an effective standard therapy for myeloma. However, the IMIDs, including pomalidomide, are also being evaluated in a variety of other malignancies as well as nonmalignant autoimmune mediated disorders. Careful monitoring for SPMs should be incorporated into these trials. Future protocols should include bone marrow examinations with cytogenetic analyses as part of routine monitoring.

Careful statistical analyses of the accumulating data will be critical. With the risk of SPMs being relatively low, small numbers of reported SPMs could significantly alter the results. We recommend that incidence rates of SPMs be adjusted for person-years at risk (i.e., rate per 100 person-years). This mitigates the possibility of overestimating the risk of SPMs in lenalidomide-treated patients due to longer patient survival.

Clinical and preclinical research is needed to better elucidate lenalidomide's mechanism of action and its potential role in secondary cancers. The following is a partial list of important questions to be resolved.

(i) Is there a relationship between the amount of lenalidomide exposure (dose, duration, or schedule) and the risk of SPMs?

(ii) Do baseline cytogenetic abnormalities increase the risk of developing SPMs?

(iii) Is patient age or previous history of malignancy a risk factor for the development of SPMs?

(iv) Are there other treatment (e.g., type of chemotherapy), host (e.g., SNPs), or disease (e.g., genetic aberrations) related risk factors or biomarkers for the development of SPMs?

(v) What are the characteristics, prognosis, and natural history of these SPMs? What is the time to development? For t-MDS/AML, what types of cytogenetic and molecular changes are seen and are they different than the pattern already established in cases due to cytotoxic chemotherapy or radiation?

7.5. Does This Change How We Treat Our Myeloma Patients? In our opinion, there is not enough evidence at this time to conclude that lenalidomide definitively increases the risks of second cancers, but there is a cause for concern. Patients should be informed of the potential increased risk of SPMs and an informed decision should be made keeping in mind the risks and benefits. The benefits of lenalidomide therapy for active disease in the upfront and relapsed settings are well-documented and include better and deeper responses, longer progression-free survival, and longer overall survival compared with older standard therapies [63, 64]. The information we have now should not drastically alter the decision-making process for the majority of these patients.

In the maintenance setting, the risk-benefit analysis may be more complex. The risks of extended lenalidomide therapy not only include SPMs but also well-known toxicities such as myelosuppression, fatigue, and thrombosis. Studies have consistently demonstrated that lenalidomide maintenance results in sizable improvements in disease control. The CALGB 100104 study has also recently shown a survival benefit—an exciting result that has not been confirmed yet in other studies. The risks of SPMs related to lenalidomide, if any, are currently poorly defined but may be estimated to be about 7%, for the purposes of discussion. As such, it should be kept in mind that for most myeloma patients, the competing risks of death due to disease progression exceed the risk of SPMs (Figure 1) [65]. A post hoc analysis of the CALGB 100104 trial included SPMs as primary events (along with disease progression and death) and still showed an impressive improvement in EFS (HR 0.53, 95% CI, 0.41–0.69) for patients on maintenance lenalidomide compared with placebo.

One situation which may tilt the physician/patient discussion against lenalidomide maintenance is when the patient has a known genetic predisposition to cancer, such as BRCA, or a strong personal or family history of cancer.

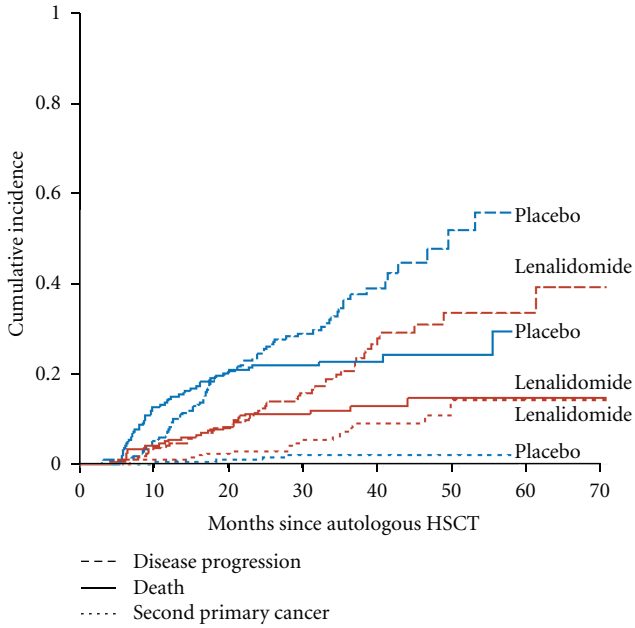


Figure 1: Competing risks in multiple myeloma patients. Cumulative incidence of second primary cancers, disease progression, and death for patients randomized to either maintenance lenalidomide or placebo post HDT/SCT in the CALGB 100104 trial. In placebo treated patients (in blue), the risk of death and disease progression far exceeds the risk of SPM. Reprinted with permission [55].

Although there is no firm data in this regard and we do not feel that this completely precludes the use of lenalidomide as maintenance, it has been our experience that such patients are reluctant to pursue this strategy and prefer to reserve the use of this drug until the time of progression.

We do recommend that all patients starting lenalidomide maintenance have a baseline bone marrow examination with cytogenetics to ensure that there is no overt evidence of dysplasia or concerning cytogenetic abnormalities. There should be a low threshold for a careful bone marrow analysis with karyotyping for patients with unexplained cytopenias that persist despite lenalidomide withdrawal. Patients should undergo age-appropriate cancer screening measures and clinicians should have a high index of suspicion when evaluating patient symptoms or findings that may represent a second malignancy.

8. Final Thoughts

Lenalidomide is an exciting drug with an impressive range and depth of activities. With the ever-expanding investigation and application of lenalidomide in myeloma and other hematological malignancies, there is a substantial need to define its possible contribution to SPMs. This concern is warranted but has come about, in large part, due to the significant improvements in survival seen in myeloma patients. The myeloma research community has much work to do to shed light on this important issue. In the meantime, our general frame-of-mind is quite similar to that of Kyle et al. who presciently opined some 35 years ago, "Late death after a long remission of myeloma is much to be preferred to early death without remission" [66].

Disclosure

H. R. Terebelo speaks on behalf of Celgene and Millennium. J. A. Zonder has received research funding and is a speaker for CME programs (no promotional speaking) for Celgene and Millennium.

Acknowledgment

The authors would like to thank Charles A. Schiffer for his review of the paper.

References

[1] R. E. Curtis, D. M. Freedman, E. Ron et al., "New malignancies among cancer survivors: SEER cancer registries, 1973–2000," NIH Publication 05-5302, National Cancer Institute, Bethesda, Md, USA, 2006.

[2] T. Olivanen, "Acute leukaemia and other secondary neoplasms in patients treated with conventional chemotherapy for multiple myeloma: a Finnish Leukaemia Group study," *European Journal of Haematology*, vol. 65, no. 2, pp. 123–127, 2000.

[3] C. Dong and K. Hemminki, "Second primary neoplasms among 53 159 haematolymphoproliferative malignancy patients in Sweden, 1958–1996: a search for common mechanisms," *British Journal of Cancer*, vol. 85, no. 7, pp. 997–1005, 2001.

[4] D. E. Bergsagel, C. C. Sprague, C. Austin, and K. M. Griffith, "Evaluation of new chemotherapeutic agents in the treatment of multiple myeloma—IV. L-Phenylalanine mustard (NSC-8806)," *Cancer Chemotherapy Reports. Part 1*, vol. 21, pp. 87–99, 1962.

[5] B. Hoogstraten, P. R. Sheehe, J. Cuttner et al., "Melphalan in multiple myeloma," *Blood*, vol. 30, no. 1, pp. 74–83, 1967.

[6] I. C. M. MacLennan and J. Cusick, "Objective evaluation of the role of vincristine in induction and maintenance therapy for myelomatosis," *British Journal of Cancer*, vol. 52, no. 2, pp. 153–158, 1985.

[7] M. M. Oken, D. P. Harrington, N. Abramson, R. A. Kyle, W. Knospe, and J. H. Glick, "Comparison of melphalan and prednisone with vincristine, carmustine, melphalan, cyclophosphamide, and prednisone in the treatment of multiple myeloma: results of Eastern Cooperative Oncology Group Study E2479," *Cancer*, vol. 79, no. 8, pp. 1561–1567, 1997.

[8] J. Blade, J. F. San Miguel, A. Alcala et al., "Alternating combination VCMP/VBAP chemotherapy versus melphalan/prednisone in the treatment of multiple myeloma: a randomized multicentric study of 487 patients," *Journal of Clinical Oncology*, vol. 11, no. 6, pp. 1165–1171, 1993.

[9] R. Alexanian, S. Balcerzak, and A. Haut, "Remission maintenance therapy for multiple myeloma," *Archives of Internal Medicine*, vol. 135, no. 1, pp. 147–152, 1975.

[10] A. Belch, W. Shelley, D. Bergsagel et al., "A randomized trial of maintenance versus no maintenance melphalan and prednisone in responding multiple myeloma patients," *British Journal of Cancer*, vol. 57, no. 1, pp. 94–99, 1988.

[11] R. A. Kyle, R. V. Pierre, and E. D. Bayrd, "Multiple myeloma and acute myelomonocytic leukemia," *New England Journal of Medicine*, vol. 283, no. 21, pp. 1121–1125, 1970.

[12] F. Rosner and H. Gruenwald, "Multiple myeloma terminating in acute leukemia. Report of 12 cases and review of the literature," *American Journal of Medicine*, vol. 57, no. 6, pp. 927–939, 1974.

[13] B. Cleary, R. A. Binder, A. N. Kales, and B. J. Veltri, "Simultaneous presentation of acute myelomonocytic leukemia and multiple myeloma," *Cancer*, vol. 41, no. 4, pp. 1381–1386, 1978.

[14] A. Dispenzieri, M. Q. Lacy, and P. R. Greipp, "Multiple myeloma," in *Wintrobe's Clinical Hematology*, J. P. Greer, J. Foerster, and G. M. Rodgers, Eds., pp. 2417–2418, Wolters Kluwer Health/Lippincott Williams & Wilkins, Philadelphia, Pa, USA, 2009.

[15] J. Cuzick, S. Erskine, D. Edelman, and D. A. G. Galton, "A comparison of the incidence of the myelodysplastic syndrome and acute myeloid leukaemia following melphalan and cyclophosphamide treatment for myelomatosis," *British Journal of Cancer*, vol. 55, no. 5, pp. 523–529, 1987.

[16] M. H. Greene, E. L. Harris, and D. M. Gershenson, "Melphalan may be a more potent leukemogen than cyclophosphamide," *Annals of Internal Medicine*, vol. 105, no. 3, pp. 360–367, 1986.

[17] M. Attal, J. L. Harousseau, A. M. Stoppa et al., "A prospective, randomized trial of autologous bone marrow transplantation and chemotherapy in multiple myeloma," *New England Journal of Medicine*, vol. 335, no. 2, pp. 91–97, 1996.

[18] M. C. Pasquini and Z. Wang, "Current use and outcome of hematopoietic stem cell transplantation: CIBMTR Summary Slides," 2011, http://www.cibmtr.org.

[19] J. S. Miller, D. C. Arthur, C. E. Litz, J. P. Neglia, W. J. Miller, and D. J. Weisdorf, "Myelodysplastic syndrome after autologous bone marrow transplantation: an additional late complication of curative cancer therapy," *Blood*, vol. 83, no. 12, pp. 3780–3786, 1994.

[20] J. Pedersen-Bjergaard, M. K. Andersen, and D. H. Christiansen, "Therapy-related acute myeloid leukemia and myelodysplasia after high-dose chemotherapy and autologous stem cell transplantation," *Blood*, vol. 95, no. 11, pp. 3273–3279, 2000.

[21] D. L. Forrest, T. J. Nevill, S. C. Naiman et al., "Second malignancy following high-dose therapy and autologous stem cell transplantation: incidence and risk factor analysis," *Bone Marrow Transplantation*, vol. 32, no. 9, pp. 915–923, 2003.

[22] R. Govindarajan, S. Jagannath, J. T. Flick et al., "Preceding standard therapy is the likely cause of MDS after autotransplants for multiple myeloma," *British Journal of Haematology*, vol. 95, no. 2, pp. 349–353, 1996.

[23] C. N. Harrison, W. Gregory, G. Vaughan Hudson et al., "High-dose BEAM chemotherapy with autologous haemopoietic stem cell transplantation for Hodgkin's disease is unlikely to be associated with a major increased risk of secondary MDS/AML," *British Journal of Cancer*, vol. 81, no. 3, pp. 476–483, 1999.

[24] B. Barlogie, G. Tricot, J. Haessler et al., "Cytogenetically defined myelodysplasia after melphalan-based autotransplantation for multiple myeloma linked to poor hematopoietic stem-cell mobilization: the Arkansas experience in more than 3000 patients treated since 1989," *Blood*, vol. 111, no. 1, pp. 94–100, 2008.

[25] P. Moreau, T. Facon, M. Attal et al., "Comparison of 200 mg/m2 melphalan and 8 Gy total body irradiation plus 140 mg/m2 melphalan as conditioning regimens for peripheral blood stem cell transplantation in patients with newly diagnosed multiple myeloma: final analysis of the Intergroupe Francophone du Myélome 9502 randomized trial," *Blood*, vol. 99, no. 3, pp. 731–735, 2002.

[26] J. B. Bartlett, K. Dredge, and A. G. Dalgleish, "The evolution of thalidomide and its IMiD derivatives as anticancer agents," *Nature Reviews Cancer*, vol. 4, no. 4, pp. 314–322, 2004.

[27] European Medicines Agency, "Withdrawal assessment report for lenalidomide Celgene Europe," 2008, http://www.ema.europa.eu/docs/en_GB/document_library/Application_withdrawal_assessment_report/2010/01/WC500065821.pdf.

[28] L. Adès, F. Le Bras, M. Sebert et al., "Treatment with lenalidomide does not appear to increase the risk of progression in lower risk myelodysplastic syndromes with 5q deletion. A comparative analysis by the Groupe Francophone des Myelodysplasies," *Haematologica*, vol. 97, no. 2, pp. 213–218, 2012.

[29] P. Fenaux, A. Giagounidis, D. Selleslag et al., "A randomized phase 3 study of lenalidomide versus placebo in RBC transfusion-dependent patients with Low-/Intermediate-1-risk myelodysplastic syndromes with del5q," *Blood*, vol. 118, no. 14, pp. 3765–3776, 2011.

[30] F. Davies and R. Baz, "Lenalidomide mode of action: linking bench and clinical findings," *Blood Reviews*, vol. 24, no. 1, pp. S13–S19, 2010.

[31] H. Quach, D. Ritchie, A. K. Stewart et al., "Mechanism of action of immunomodulatory drugs (IMiDS) in multiple myeloma," *Leukemia*, vol. 24, no. 1, pp. 22–32, 2010.

[32] A. Palumbo, J. Freeman, L. Weiss, and P. Fenaux, "The clinical safety of lenalidomide in multiple myeloma and myelodysplastic syndromes," *Expert Opinion on Drug Safety*, vol. 11, no. 1, pp. 107–120, 2012.

[33] N. Mitsiades, C. S. Mitsiades, V. Poulaki et al., "Apoptotic signaling induced by immunomodulatory thalidomide analogs in human multiple myeloma cells: therapeutic implications," *Blood*, vol. 99, no. 12, pp. 4525–4530, 2002.

[34] D. H. Chang, N. Liu, V. Klimek et al., "Enhancement of ligand-dependent activation of human natural killer T cells by lenalidomide: therapeutic implications," *Blood*, vol. 108, no. 2, pp. 618–621, 2006.

[35] V. Kotla, S. Goel, S. Nischal et al., "Mechanism of action of lenalidomide in hematological malignancies," *Journal of Hematology and Oncology*, vol. 2, article 36, 2009.

[36] T. Ito, H. Ando, T. Suzuki et al., "Identification of a primary target of thalidomide teratogenicity," *Science*, vol. 327, no. 5971, pp. 1345–1350, 2010.

[37] Y. X. Zhu, E. Braggio, C.-X. Shi et al., "Cereblon expression is required for the antimyeloma activity of lenalidomide and pomalidomide," *Blood*, vol. 118, no. 18, pp. 4771–4779, 2011.

[38] J. Boultwood, A. Pellagatti, A. N. J. McKenzie, and J. S. Wainscoat, "Advances in the 5q-syndrome," *Blood*, vol. 116, no. 26, pp. 5803–5811, 2010.

[39] C. S. Mitsiades, "How "immunomodulatory" are IMIDs?" *Blood*, vol. 117, no. 5, pp. 1440–1441, 2011.

[40] S. E. Salmon, J. J. Crowley, S. P. Balcerzak et al., "Interferon versus interferon plus prednisone remission maintenance therapy for multiple myeloma: a Southwest Oncology Group study," *Journal of Clinical Oncology*, vol. 16, no. 3, pp. 890–896, 1998.

[41] R. Alexanian, D. Weber, M. Dimopoulos, K. Delasalle, and T. L. Smith, "Randomized trial of α-interferon or dexamethasone as maintenance treatment for multiple myeloma," *American Journal of Hematology*, vol. 65, no. 3, pp. 204–209, 2000.

[42] C. Shustik, A. Belch, S. Robinson et al., "A randomised comparison of melphalan with prednisone or dexamethasone as induction therapy and dexamethasone or observation as

maintenance therapy in multiple myeloma: NCIC CTG MY.7," *British Journal of Haematology*, vol. 136, no. 2, pp. 203–211, 2007.

[43] J. R. Berenson, J. J. Crowley, T. M. Grogan et al., "Maintenance therapy with alternate-day prednisone improves survival in multiple myeloma patients," *Blood*, vol. 99, no. 9, pp. 3163–3168, 2002.

[44] A. Z. Badros, "The role of maintenance therapy in the treatment of multiple myeloma," *Journal of the National Comprehensive Cancer Network*, vol. 8, no. 1, pp. S21–S27, 2010.

[45] B. Barlogie, G. Tricot, E. Anaissie et al., "Thalidomide and hematopoietic-cell transplantation for multiple myeloma," *New England Journal of Medicine*, vol. 354, no. 10, pp. 1021–1030, 2006.

[46] M. Attal, J. L. Harousseau, S. Leyvraz et al., "Maintenance therapy with thalidomide improves survival in patients with multiple myeloma," *Blood*, vol. 108, no. 10, pp. 3289–3294, 2006.

[47] A. Abdelkefi, S. Ladeb, L. Torjman et al., "Single autologous stem-cell transplantation followed by maintenance therapy with thalidomide is superior to double autologous transplantation in multiple myeloma: results of a multicenter randomized clinical trial," *Blood*, vol. 111, no. 4, pp. 1805–1810, 2008.

[48] A. Spencer, H. M. Prince, A. W. Roberts et al., "Consolidation therapy with low-dose thalidomide and prednisolone prolongs the survival of multiple myeloma patients undergoing a single autologous stem-cell transplantation procedure," *Journal of Clinical Oncology*, vol. 27, no. 11, pp. 1788–1793, 2009.

[49] H. M. Lokhorst, B. Van Der Holt, S. Zweegman et al., "A randomized phase 3 study on the effect of thalidomide combined with adriamycin, dexamethasone, and high-dose melphalan, followed by thalidomide maintenance in patients with multiple myeloma," *Blood*, vol. 115, no. 6, pp. 1113–1120, 2010.

[50] G. J. Morgan, G. H. Jackson, and F. E. Davies, "Maintenance thalidomide may improve progression free but not overall survival; results from the myeloma IX maintenance randomisation," *ASH Annual Meeting Abstracts*, vol. 112, no. 11, 656 pages, 2008.

[51] M. Cavo, L. Pantani, P. Tacchetti et al., "Thalidomide maintenance in multiple myeloma: certainties and controversies," *Journal of Clinical Oncology*, vol. 27, no. 32, pp. e186–e187, 2009.

[52] A. Palumbo, S. Bringhen, D. Rossi et al., "Bortezomib-melphalan-prednisone-thalidomide followed by maintenance with bortezomib-thalidomide compared with bortezomib-melphalan-prednisone for initial treatment of multiple myeloma: a randomized controlled trial," *Journal of Clinical Oncology*, vol. 28, no. 34, pp. 5101–5109, 2010.

[53] M. V. Mateos, A. Oriol, J. Martínez-López et al., "Bortezomib, melphalan, and prednisone versus bortezomib, thalidomide, and prednisone as induction therapy followed by maintenance treatment with bortezomib and thalidomide versus bortezomib and prednisone in elderly patients with untreated multiple myeloma: a randomised trial," *The Lancet Oncology*, vol. 11, no. 10, pp. 934–941, 2010.

[54] M. Attal, V. Lauwers-Cances, G. Marit et al., "Lenalidomide maintenance after stem-cell transplantation for multiple myeloma," *New England Journal of Medicine*, vol. 366, no. 19, pp. 1782–1791, 2012.

[55] P. L. McCarthy, K. Owzar, C. C. Hofmeister et al., "Lenalidomide after stem-cell transplantation for multiple myeloma," *New England Journal of Medicine*, vol. 366, no. 19, pp. 1770–1781, 2012.

[56] A. Palumbo, R. Hajek, M. Delforge et al., "Continuous lenalidomide treatment for newly diagnosed multiple myeloma," *New England Journal of Medicine*, vol. 366, no. 19, pp. 1759–1769, 2012.

[57] A. P. Palumbo, M. Delforge, J. Catalano et al., "Incidence of second primary malignancy (SPM) in melphalan-prednisone-lenalidomide combination followed by lenalidomide maintenance (MPR-R) in newly diagnosed multiple myeloma patients (pts) age 65 or older," *ASCO Meeting Abstracts*, vol. 29, no. 15, supplement, 8007, 2011.

[58] M. A. Dimopoulos, P. G. Richardson, N. Brandenburg et al., "A review of second primary malignancy in patients with relapsed or refractory multiple myeloma treated with lenalidomide," *Blood*, vol. 119, no. 12, pp. 2764–2767, 2012.

[59] A. C. Rossi, T. M. Mark, D. Jayabalan et al., "Incidence of second primary malignancies (SPM) after 6-years follow-up of continuous lenalidomide in first-line treatment of multiple myeloma (MM)," *ASCO Meeting Abstracts*, vol. 29, no. 15, supplement, 8008, 2011.

[60] D. E. Reece, E. Masih-Khan, R. S. Goswami et al., "Incidence and characteristics of secondary myelodysplastic syndrome developing during lenalidomide-based regimens in relapsed and/or refractory multiple myeloma patients," *ASH Annual Meeting Abstracts*, vol. 116, no. 21, 1877, 2010.

[61] S. Mailankody, R. M. Pfeiffer, S. Y. Kristinsson et al., "Risk of acute myeloid leukemia and myelodysplastic syndromes after multiple myeloma and its precursor disease (MGUS)," *Blood*, vol. 118, no. 15, pp. 4086–4092, 2011.

[62] O. Landgren, W. Ma, R. A. Kyle, S. V. Rajkumar, N. Korde, and M. Albitar, "Polymorphism of the erythropoietin gene promotor and the development of myelodysplastic syndromes subsequent to multiple myeloma," *Leukemia*, vol. 26, no. 4, pp. 844–845, 2012.

[63] M. A. Dimopoulos, C. Chen, A. Spencer et al., "Long-term follow-up on overall survival from the MM-009 and MM-010 phase III trials of lenalidomide plus dexamethasone in patients with relapsed or refractory multiple myeloma," *Leukemia*, vol. 23, no. 11, pp. 2147–2152, 2009.

[64] J. A. Zonder, J. Crowley, M. A. Hussein et al., "Lenalidomide and high-dose dexamethasone compared with dexamethasone as initial therapy for multiple myeloma: a randomized Southwest Oncology Group trial (S0232)," *Blood*, vol. 116, no. 26, pp. 5838–5841, 2010.

[65] O. Landgren, A. Thomas, and S. Mailankody, "Myeloma and second primary cancers," *New England Journal of Medicine*, vol. 365, no. 23, pp. 2241–2242, 2011.

[66] R. A. Kyle, R. V. Pierre, and E. D. Bayrd, "Multiple myeloma and acute leukemia associated with alkylating agents," *Archives of Internal Medicine*, vol. 135, no. 1, pp. 185–192, 1975.

Hindawi Publishing Corporation
Advances in Hematology
Volume 2012, Article ID 906247, 6 pages
doi:10.1155/2012/906247

Review Article

Lenalidomide in the Treatment of Young Patients with Multiple Myeloma: From Induction to Consolidation/Maintenance Therapy

Barbara Lupo and Antonio Palumbo

Myeloma Unit, Division of Hematology, University of Torino, AOU San Giovanni Battista, 10126 Torino, Italy

Correspondence should be addressed to Antonio Palumbo, appalumbo@yahoo.com

Received 17 February 2012; Accepted 18 June 2012

Academic Editor: Anna Marina Liberati

Multiple myeloma is the second most common hematologic malignancy. It accounts for 20,580 new cancer cases in the USA in 2009, including 11,680 cases in men, 8,900 cases in women, and 10,580 deaths overall. Although the disease remains still incurable, outcomes have improved substantially over recent years thanks to the use of high-dose therapy and the availability of novel agents, such as the immunomodulatory drugs thalidomide and lenalidomide, and the proteasome inhibitor bortezomib. Various trials have shown the advantages linked to the use of novel agents in the transplant and not-transplant settings. In particular, this paper will present an overview of the results achieved with lenalidomide-containing combinations in patients eligible for high-dose therapies, namely, young patients. The advantages obtained should always be outweighed with the toxicity profile associated with the regimen used. Therefore, here, we will also provide a description of the main adverse events associated with lenalidomide and its combination.

1. Introduction

For many years, the combination vincristine-doxorubicin-dexamethasone (VAD) was the standard induction treatment for young patients with multiple myeloma (MM) eligible for autologous stem cell transplantation (ASCT). Ten years ago patients candidate for transplant used to receive VAD for 4–6 cycles before undergoing transplantation, leading to a partial response (PR) rate ranging from 52% to 63%, with 3% to 13% of complete response (CR) rate. The availability of new drugs, such as thalidomide, lenalidomide, and bortezomib, has dramatically changed the treatment paradigm of this disease and significantly increased the therapeutic options [1].

Lenalidomide is an immunomodulatory drug with higher potency than its analogue thalidomide and without sedative or neurotoxic adverse effects. Differences between lenalidomide and thalidomide activity have been shown in preclinical studies. In comparison with thalidomide, lenalidomide has more antiproliferative activity against hematopoietic tumors, including myeloma cell lines and patients' cells [2, 3], increased inhibition of tumor necrosis factor secretion from activated monocytes, and increased activation of T cells and natural killer cells [4]. In contrast, thalidomide has more antiangiogenic activity than lenalidomide in human models. Both lenalidomide and thalidomide interfere with key events in the angiogenic process, and activities of these drugs can be differentiated qualitatively depending on what component is studied [5]. Lenalidomide is administered orally, and the most common toxicities related to its therapy are neutropenia, thrombocytopenia, and thrombosis [6].

On the basis of two phase 3 clinical trials, lenalidomide has already shown additive and/or synergistic effects when used in association with dexamethasone [7–9]; therefore, this combination is at present indicated for patients with MM, who have received at least one previous therapy. [10, 11]. New and ongoing trials are assessing the benefit of lenalidomide-combination therapies in early phase of treatment. In particular, the present paper will provide an overview of the main latest combinations including

lenalidomide, used in young patients either as induction or maintenance treatment.

2. Induction Regimens Including Lenalidomide

2.1. Standard Approaches. Lenalidomide has been tested in various clinical trials as induction regimen before ASCT. In one randomized trial, lenalidomide in combination with high-dose dexamethasone (RD) showed to be superior to dexamethasone alone [12]. In that study, patients assigned to RD received lenalidomide at the dose of 25 mg/day for 28 days and dexamethasone at the dose of 40 mg/day on days 1–4, 9–12, 17–20, for three 35-day induction cycles. The same dexamethasone dose was administered in the other treatment arm. Patients assigned to treatment with RD had at least PR of 78%, with very good PR (VGPR) of 63%, while the respective figures for dexamethasone alone were 48% and 16% ($P < 0.001$). The 1-year progression-free survival (PFS) was higher with RD (78% versus 52%; $P = 0.002$), so was the 1-year overall survival (OS) (94% versus 88%, $P = 0.25$). Toxicities were higher with RD and were grade 3-4 neutropenia (21% versus 5%; $P < 0.001$), and thromboembolism despite aspirin prophylaxis (24% versus 5%; $P < 0.001$). Considering the good efficacy of the two-drug regimen, 40 eligible patients crossed over to RD arm.

The efficacy of this combination was further improved by reducing the dose of dexamethasone. A phase 3 trial also demonstrated superior survival and lower toxicity with lenalidomide plus low-dose dexamethasone (Rd) [13]. In that trial, patients who were randomly assigned to be treated with RD received lenalidomide at the dose of 25 mg on days 1–21 plus dexamethasone 40 mg on days 1–4, 9–12, and 17–20 of a 28-day cycle, while subjects in the Rd arm received the same dose of lenalidomide and dexamethasone at 40 mg on days 1, 8, 15, and 22 of a 28-day cycle. At least PR rate was 81% in the RD arm and 70% in the Rd arm ($P = 0.009$), with a CR of 5% and 4%, respectively. OS was lower with RD than Rd (2-year OS was 75% versus 87%, resp.). Toxicities were significantly higher with RD compared to Rd, in particular deep-vein thrombosis (DVT) or pulmonary embolism (26% versus 12%; $P < 0.001$), and infections (16% versus 9%; $P = 0.04$). Considering its good toxicity profile, almost all recent trials have used the lower-dose of dexamethasone (namely, 40 mg once weekly or equivalent) and high-dose dexamethasone is no longer recommended in newly diagnosed MM.

The role of Rd induction was also assessed in an Italian study [14]. Four-hundred two patients with newly diagnosed MM were included in the study. Patients received induction therapy with four 28-day cycles of Rd. Patients were subsequently randomized to receive treatment with melphalan-prednisone-lenalidomide (MPR) or high-dose melphalan (MEL200) followed by transplantation. On an intention to treat basis, best responses after Rd induction were 87% PR rate, 52% VGPR rate, and 13% CR. Induction with Rd was well tolerated: the most frequent grade 3-4 adverse events were neutropenia (8%), anemia (7%), infections (4%), and skin rash (5%). The incidence of thromboembolic events was similar in patients randomized to aspirin (2%) or low-molecular-weight heparin (1%) as thromboprophylaxis ($P = 0.45$). These results further confirm the positive role of Rd as induction regimen.

2.2. New Approaches. Based on the promising results obtained with lenalidomide in combination with dexamethasone, new combinations including lenalidomide have been tested to further improve outcomes and to achieve maximal tumour reduction.

A case-matched study compared clarithromycin-lenalidomide plus low-dose dexamethasone (BiRd) with Rd as initial therapy for newly diagnosed MM patients [15]. Seventy-two patients treated with either BiRd or Rd were included in this retrospective analysis. On intention-to-treat, CR rate was significantly higher with BiRd compared with Rd (46% versus 14%, resp.; $P < 0.001$) and so was also VGPR rate or better (74% versus 33%, resp.; $P < 0.001$). BiRd also led to longer median PFS, 48 months with BiRd compared to 28 months with Rd ($P = 0.044$). The 3-year OS was also improved in patients receiving BiRd (90% versus 73%; $P = 0.17$). Main grade 3-4 toxicities of BiRd were hematologic, in particular thrombocytopenia (24% versus 8%; $P = 0.012$). Higher rates of infections (10% versus 17%; $P = 0.218$) and dermatological toxicity (4% versus 13%; $P = 0.129$) were reported with Rd. These results demonstrate the marked benefits of adding clarithromycin to Rd. Future phase 3 trials are needed to confirm and validate these findings.

Richardson and colleagues evaluated the role of bortezomib-lenalidomide-dexamethasone (VRD) in a phase 1-2 study including 66 patients with MM [16]. Bortezomib was administered at the dose of 1.0 or 1.3 mg/m^2 on days 1, 4, 8, 11; lenalidomide at 15 to 25 mg on days 1–14; dexamethasone at 40 or 20 mg on days 1, 2, 4, 5, 8, 9, 11, 12. In the phase 2 study, bortezomib was given at 1.3 mg/m^2, lenalidomide 25 mg, and dexamethasone 20 mg. PR rate was 100% in both the phase 2 population and overall, with 74% and 67% each achieving at least VGPR. Forty-two percent of patients proceeded to transplantation. With a median followup of 21 months, estimated 18-month PFS and OS for the combination treatment with/without transplantation were 75% and 97%, respectively. Most common toxicities included sensory neuropathy (80%) and fatigue (64%), with only 27%/2% and 32%/3% grade 2/3, respectively. Moreover, 32% reported neuropathic pain (11%/3%, grade 2/3). Grade 3-4 hematologic toxicities included lymphopenia (14%), neutropenia (9%), and thrombocytopenia (6%). Thrombosis was rare (6%), and no treatment-related mortality was reported. In the light of these results, VRD proved to be effective and well tolerated in newly diagnosed MM.

The role of VRD was also confirmed in a French phase 2 trial [17]. Thirty-one patients younger than 65 years with newly diagnosed MM were enrolled and received VRD induction treatment (bortezomib 1.3 mg/m^2 (days 1, 4, 8, 11), lenalidomide 25 mg (days 1–14), and oral dexamethasone 40 mg (days 1, 8 and 14)). Patients underwent ASCT

and subsequently received VRD consolidation and lenalidomide maintenance. All patients could be evaluated for response after induction: at least PR was 97%, with a VGPR rate of 26%. Overall, the most common adverse events were sensory peripheral neuropathy (45%), only grade 1-2 events were detected, and gastrointestinal events (42%). Grade 3-4 hematologic toxicities included neutropenia (26%) and thrombocytopenia (6%). No treatment-related deaths were reported. All patients received aspirin, and no DVT nor pulmonary embolism was detected.

Lenalidomide associated with adriamycin and dexamethasone (RAD; lenalidomide 25 mg days 1–21; infusional adriamycin 9 mg/m^2 per day on days 1–4; dexamethasone 40 mg days 1–4 and 17–20) was assessed in another phase 2 study [18]. Seventy-five patients with a median age of 57 (range, 35–66) years have been enrolled. In a preliminary analysis, 17 patients were evaluated for postinduction response. Ten subjects (59%) achieved VGPR or better: 6 patients had VGPR and 2 patients each CR and stringent CR. Fifty-one patients were evaluated for toxicity during RAD induction: 31% experienced a serious adverse event, of which 68% were treatment related. Most frequent events were venous thrombosis ($n = 4$), pyrexia ($n = 3$), and syncope ($n = 2$). Neutropenia, extravasation, pleural effusion, and allergic dermatitis accounted for one serious adverse event each. These preliminary results suggest that RAD is a well-tolerated and effective novel induction upfront approach for MM. Incidence of venous thromboembolism was acceptable, while no neurotoxicity was reported.

A more intense combination including four drugs has been recently tested. Promising results were achieved in a phase 2 multicenter study comparing bortezomib-dexamethasone-cyclophosphamide plus lenalidomide (VDCR) with VDR, bortezomib-cyclophosphamide-dexamethasone (VDC), and VDC modified (VDC-mod) [19]. In all arms, the doses of bortezomib and dexamethasone were as follow: bortezomib 1.3 mg/m^2 on days 1, 4, 8, 11; dexamethasone 40 mg on days 1, 8, 15. In the VDCR, lenalidomide was given at 15 mg on days 1–14 and cyclophosphamide at 500 mg/m^2 days 1, 8; in the VDC-mod, arm cyclophosphamide was also given on day 15; in the VDR arm, lenalidomide was administered at 25 mg on days 1–14. The four induction regimens were followed by four 42-day maintenance cycles of bortezomib 1.3 mg/m^2 on days 1, 8, 15, 22. A total of 41 patients were assigned to VDCR arm, and responses were promising, with at least PR of 88% and 24% of CR, and were comparable with the responses detected in the other arms. The toxicity profile associated with VDCR was slightly higher, with serious adverse events detected in 42% of patients, resulting in treatment discontinuation in 19% of the subjects. In particular, the incidence of grade ≥ 3 peripheral neuropathy was 13%, grade ≥ 3 neutropenia was 42%, and grade ≥ 3 thrombocytopenia was seen in 10% of VDCR patients. These results show that VDCR is an effective treatment option, but it is not significantly superior to other less toxic combinations, such as VRD.

A phase 1-2 study evaluated the combination VRD plus pegylated liposomal doxorubicin (VRDD) [20]. Patients received lenalidomide at 15–25 mg on days 1–14, bortezomib 1.3 mg/m^2 on days 1, 4, 8, 11, dexamethasone 20/10 mg (cycles 1–4/5–8; days of and after bortezomib), doxorubicin 20 or 30 mg/m^2 (day 4) at 4 dose levels for up to eight 21-day cycles. Response rates in 57 patients who could be evaluated for response were as follows: 96% at least PR, 58% at least VGPR, and 30% CR/near CR. Patients treated at the maximum tolerated dose, which was determined as lenalidomide 25 mg, bortezomib 1.3 mg/m^2, dexamethasone 20 mg, and doxorubicin 30 mg/m^2, and completed at least 4 cycles, showed 100% of at least PR. Overall, toxicities were manageable, with grade 3-4 toxicities including neutropenia (18%), thrombocytopenia (7%), infections (16%), and DVT (2%). Grade 3 peripheral neuropathy was detected in 4% of patients, while no grade 4 peripheral neuropathy nor treatment-related deaths was reported. RVDD showed to be well tolerated and highly active in newly diagnosed MM.

The incidence of both venous and arterial thrombosis in newly diagnosed patients increases when lenalidomide is combined with dexamethasone or chemotherapy: thus, thromboprophylaxis is recommended for the first 6 months of therapy. For patients with standard thromboembolic risk, low-dose aspirin is indicated; for patients with high thromboembolic risk, low-molecular-weight heparin is recommended [21].

3. Impact of Lenalidomide on Stem Cell Collection

Recent reports have focused on hypothetical negative impact of new drugs on stem cell mobilization. Particularly, hematologic toxicity related to treatment with lenalidomide has raised concerns about lenalidomide use in early phase of treatment and on its possible negative effect on peripheral blood stem cell (PBSC) collection in young patients undergoing ASCT.

In fact, patients mobilized with granulocyte colony-stimulating factor (G-CSF) alone after RD induction have collected a median yield of stem cell ranging from 3.1 to 7.9×10^6 CD34$^+$/Kg [22–26].

By contrast, the use of cyclophosphamide in addition to G-CSF may overcome this problem and significantly increases the yield of stem cells collected. Different retrospective published trials on lenalidomide induction have in fact shown a median yield of PBSC ranging from 6.3 to 14.2×10^6 CD34$^+$/Kg. These data support the idea that RD is probably not a significant burden for an adequate stem cell collection, especially if the duration of induction with RD is short [22–26].

4. Consolidation and Maintenance Approaches Including Lenalidomide

Consolidation and maintenance therapies have the potential to improve the results achieved after induction treatment and transplantation [27]. Although preliminary data showed that lenalidomide as consolidation/maintenance therapy after ASCT improved responses, the role of lenalidomide

treatment as alternative to ASCT still remains uncertain, and further studies are needed.

Consolidation treatment with MPR has been tested in the Italian study, comparing MPR (six 28-day cycles of melphalan (0.18 mg/Kg days 1–4), prednisone (2 mg/Kg days 1–4), and lenalidomide (10 mg days 1–21)) versus tandem MEL200 (melphalan 200 mg/m^2) with stem cell support [14]. Response rates were similar: at least VGPR rate was 60% with MPR versus 58% with Mel200, including a CR rate of 20% versus 25%, respectively. After a median followup of 26 months, 2-year PFS was 54% in MPR and 73% in MEL200 (HR = 0.51, $P < 0.001$) and this benefit was maintained in the subgroup of patients with standard- or high-risk cytogenetic features, with a 2-year PFS of 46% in the MPR group versus 78% in the MEL200 group in patients with standard risk (HR = 0.57, $P = 0.007$), and 27% for MPR versus 71% for MEL200 in high-risk patients with $t(4;14)$ or $t(14;16)$ or del17p abnormalities (HR = 0.32, $P = 0.004$). The achievement of CR prolonged PFS, and this was more evident in the Mel200 arm. Two-year PFS in patients who achieved CR was 66% in MPR versus 87% in Mel200 group; 2-year PFS in patients who achieved PR was 56% versus 77%, respectively. PFS was significantly prolonged in patients who received a double ASCT. During consolidation therapy, the incidence of grade 3-4 neutropenia (89% versus 55%), infections (17% versus 0%) and gastrointestinal complications (21% versus 0%), was higher in MEL200 patients ($P < 0.001$). This is the first report showing a PFS advantage for ASCT in comparison with conventional therapies including novel agents; however, longer followup is needed to draw definitive conclusion. To date, more information is available on the use of lenalidomide as maintenance therapy. While maintenance including alkylating agents and interferon showed no significant impact on OS [28, 29], thalidomide maintenance seems to be an effective strategy to improve survival [30–33]. However, prolonged exposure to thalidomide may cause cumulative toxicity. Because of its better safety profile, lenalidomide could be an optimal alternative option to thalidomide as maintenance therapy.

In a phase 3 randomized double-blind, placebo-controlled study (CALGB 100104), patients with non-progressive disease after a first line ASCT were randomized to receive placebo or lenalidomide at starting dose of 10 mg daily, escalated to 15 mg daily after 3 months [34]. Final analysis showed that lenalidomide monotherapy maintenance initiated at 100–110 days after ASCT and continued until disease progression, considerably delays time to progression compared with placebo (median time to progression 42.3 months in the lenalidomide maintenance arm versus 21.8 months in the placebo arm), with a good safety profile and low discontinuation rate due to adverse events (12% in the lenalidomide group versus 2% in the placebo group). Furthermore, significant improvements in time to progression were observed in the group receiving lenalidomide maintenance regardless of $\beta 2$ microglobulin levels or choice of induction therapy (thalidomide or lenalidomide).

In the IFM2005-02 study, MM patients younger than 65 years of age, with at least standard disease after a first line ASCT, were randomly assigned to receive consolidation with lenalidomide (25 mg daily, 21 days/month, for 2 months) followed by maintenance with placebo or lenalidomide (10 to 15 mg daily) until relapse [35]. Results showed that lenalidomide maintenance significantly improved PFS, with a median PFS of 42 months from randomization in the lenalidomide group versus 24 months in the placebo group ($P < 0.01$). This benefit was observed regardless of risk factors, such as cytogenetic profile (del 13, + or −), $\beta 2$ microglobulin levels, or response after transplantation. Preliminary data published indicated that this regimen is well tolerated, with a discontinuation rate for serious adverse events similar to placebo.

Of note, both the CALGB and IFM2005-02 studies detected an improvement in PFS with lenalidomide maintenance, but only the CALGB study reported also an OS benefit with this approach [34, 35].

Recently, the higher incidence of second cancer in patients treated with lenalidomide for long time led to reconsidering the benefit and the duration of lenalidomide maintenance. In both the CALGB 100104 and IFM2005-02, the rate of second primary malignancy was 8%. An analysis of pooled data from 2459 patients enrolled in different studies including maintenance with lenalidomide was performed. Not all the studies showed an increased second cancer risk, whereas different studies confirmed that the benefits achieved with lenalidomide maintenance outweigh the risk to develop a second primary malignancy [36]. Despite the results presented above, a longer period of followup is required to draw definitive conclusion on the role of lenalidomide as consolidation/maintenance therapy, to better define the impact of lenalidomide maintenance on OS and its cumulative toxicity and to establish the optimal duration of lenalidomide maintenance therapy.

5. Conclusions

Considering its dual mechanism of action of lenalidomide, comprising tumoricidal effects which rapidly reduce the MM tumor burden, and its immunomodulatory effects which enhance the immune function and maintain disease suppression, this novel agent showed to be effective and safe in patients with newly diagnosed MM. In particular, the combination Rd proved to be a good induction treatment in this setting of patients.

Based on the promising results achieved with Rd, ongoing trials are now testing this regimen in combination with other agents upfront with the aim to increase the tumoricidal effect. Preliminary results showed the positive role of Rd in association with clarithromycin, bortezomib, adriamycin or cyclophosphamide.

In addition, studies are under way to assess the role of long-term treatment with lenalidomide as consolidation/maintenance treatment after ASCT, but longer followup is necessary to draw any definitive conclusion.

Authors' Contribution

B. Lupo and A. Palumbo wrote the paper.

Disclosure

A. Palumbo has received honoraria from Celgene, Janssen-Cilag, Merck, Amgen, and advisory committee from Celgene, Janssen-Cilag.

Acknowledgment

The authors would like to thank the Editorial Assistant Giorgio Schirripa.

References

[1] J. F. San-Miguel and M. V. Mateos, "How to treat a newly diagnosed young patient with multiple myeloma," *Hematology American Society of Hematology Education Program*, pp. 555–565, 2009.

[2] A. Gandhi, L. H. Zhang, L. Lu et al., "Effects and molecular mechanism of lenalidomide on FGFR signaling in endothelial cells and FGFR3 multiple myeloma cell lines," *Haematologica*, vol. 91, no. 274, p. 745a, 2006.

[3] T. Hideshima, D. Chauhan, Y. Shima et al., "Thalidomide and its analogs overcome drug resistance of human multiple myeloma cells to conventional therapy," *Blood*, vol. 96, no. 9, pp. 2943–2950, 2000.

[4] L. G. Corral, P. A. J. Haslett, G. W. Muller et al., "Differential cytokine modulation and T cell activation by two distinct classes of thalidomide analogues that are potent inhibitors of TNF-α," *Journal of Immunology*, vol. 163, no. 1, pp. 380–386, 1999.

[5] L. H. Zhang, L. Lu, and L. Wu, "Comparison of anti-angiogenic activities of thalidomide and lenalidomide in vitro," *Proceeding of the American Association for Cancer Research*, vol. 47, no. 1, p. 761a, 2006.

[6] S. V. Rajkumar and E. Blood, "Lenalidomide and venous thrombosis in multiple myeloma," *New England Journal of Medicine*, vol. 354, no. 19, pp. 2079–2080, 2006.

[7] M. Dimopoulos, A. Spencer, M. Attal et al., "Lenalidomide plus dexamethasone for relapsed or refractory multiple myeloma," *New England Journal of Medicine*, vol. 357, no. 21, pp. 2123–2132, 2007.

[8] D. M. Weber, C. Chen, R. Niesvizky et al., "Lenalidomide plus dexamethasone for relapsed multiple myeloma in North America," *New England Journal of Medicine*, vol. 357, no. 21, pp. 2133–2142, 2007.

[9] M. A. Dimopoulos, C. Chen, A. Spencer et al., "Long-term follow-up on overall survival from the MM-009 and MM-010 phase III trials of lenalidomide plus dexamethasone in patients with relapsed or refractory multiple myeloma," *Leukemia*, vol. 23, no. 11, pp. 2147–2152, 2009.

[10] European Medicines Agency, "Revlimid summary of product characteristics," European Public Assessment Report: Revlimid, http://www.emea.europa.eu/.

[11] Celgene Corporation, "Revlimid Package Insert," Summit, NJ, USA, http://www.revlimid.com/pdf/REVLIMID_PI.pdf.

[12] J. A. Zonder, J. Crowley, M. A. Hussein et al., "Lenalidomide and high-dose dexamethasone compared with dexamethasone as initial therapy for multiple myeloma: a randomized Southwest Oncology Group trial (S0232)," *Blood*, vol. 116, no. 26, pp. 5838–5841, 2010.

[13] S. V. Rajkumar, S. Jacobus, N. S. Callander et al., "Lenalidomide plus high-dose dexamethasone versus lenalidomide plus low-dose dexamethasone as initial therapy for newly diagnosed multiple myeloma: an open-label randomised controlled trial," *The Lancet Oncology*, vol. 11, no. 1, pp. 29–37, 2010.

[14] A. Palumbo, F. Cavallo, I. Hardan et al., "Melphalan/prednisone/lenalidomide (MPR) versus high-dose melphalan and autologous transplantation (MEL200) in newly diagnosed multiple myeloma (MM) patients < 65 years: results of a Randomized Phase III study," *Blood (ASH Annual Meeting Abstracts)*, vol. 118, abstract 3069, 2011.

[15] F. Gay, S. V. Rajkumar, M. Coleman et al., "Clarithromycin (Biaxin)-lenalidomide-low-dose dexamethasone (BiRd) versus lenalidomide-low-dose dexamethasone (Rd) for newly diagnosed myeloma," *American Journal of Hematology*, vol. 85, no. 9, pp. 664–669, 2010.

[16] P. G. Richardson, E. Weller, S. Lonial et al., "Lenalidomide, bortezomib, and dexamethasone combination therapy in patients with newly diagnosed multiple myeloma," *Blood*, vol. 116, no. 5, pp. 679–686, 2010.

[17] M. Roussel, H. Avet-Loiseau, P. Moreau et al., "Frontline therapy with bortezomib, lenalidomide, and dexamethasone (VRD) induction followed by autologous stem cell transplantation, VRD consolidation and lenalidomide maintenance in newly diagnosed multiple myeloma patients: primary results of the IFM 2008 phase II study," *Blood (ASH Annual Meeting Abstracts)*, vol. 116, abstract 624, 2010.

[18] S. Knop, C. Langer, M. Engelhardt et al., "The efficacy and safety of RAD (Lenalidomide, Adriamycin and Dexamethasone) in newly diagnosed multiple myeloma—first results of a phase II trial by the german DSMM group," *Blood (ASH Annual Meeting Abstracts)*, vol. 116, abstract 1945, 2010.

[19] S. K. Kumar, I. Flinn, S. J. Noga et al., "Novel three-and four drug combination regimens of bortezomib, dexamethasone, cyclophosphamide, and lenalidomide, for previously untreated multiple myeloma: results from the multicenter, randomized, phase 2 EVOLUTION study," *Blood (ASH Annual Meeting Abstracts)*, vol. 116, abstract 621, 2010.

[20] A. J. Jakubowiak, D. E. Reece, C. C. Hofmeister et al., "Lenalidomide, bortezomib, pegylated liposomal doxorubicin, and dexamethasone in newly diagnosed multiple myeloma: updated results of phase I/II MMRC trial," *Blood (ASH Annual Meeting Abstracts)*, vol. 114, abstract 132, 2009.

[21] A. Palumbo and K. Anderson, "Multiple myeloma," *New England Journal of Medicine*, vol. 364, no. 11, pp. 1046–1060, 2011.

[22] S. Kumar, A. Dispenzieri, M. Q. Lacy et al., "Impact of lenalidomide therapy on stem cell mobilization and engraftment post-peripheral blood stem cell transplantation in patients with newly diagnosed myeloma," *Leukemia*, vol. 21, no. 9, pp. 2035–2042, 2007.

[23] U. Popat, R. Saliba, R. Thandi et al., "Impairment of filgrastim-induced stem cell moblizaion after prior lenaliodmide in patients with multiple myeloma," *Biology of Blood and Marrow Transplantation*, vol. 15, no. 6, pp. 718–723, 2009.

[24] A. Mazumder, J. Kaufman, R. Niesvizky, S. Lonial, D. Vesole, and S. Jagannath, "Effect of lenalidomide therapy on mobilization of peripheral blood stem cells in previously untreated multiple myeloma patients," *Leukemia*, vol. 22, no. 6, pp. 1280–1281, 2008.

[25] T. Mark, J. Stern, J. R. Furst et al., "Stem cell mobilization with cyclophosphamide overcomes the suppressive effect of lenalidomide therapy on stem cell collection in multiple myeloma," *Biology of Blood and Marrow Transplantation*, vol. 14, no. 7, pp. 795–798, 2008.

[26] H. Paripati, A. K. Stewart, S. Cabou et al., "Compromised stem cell mobilization following induction therapy with lenalidomide in myeloma," *Leukemia*, vol. 22, no. 6, pp. 1282–1284, 2008.

[27] A. Palumbo, F. Gay, P. Falco et al., "Bortezomib as induction before autologous transplantation, followed by lenalidomide as consolidation-maintenance in untreated multiple myeloma patients," *Journal of Clinical Oncology*, vol. 28, no. 5, pp. 800–807, 2010.

[28] A. Belch, W. Shelley, D. Bergsagel et al., "A randomized trial of maintenance versus no maintenance melphalan and prednisone in responding multiple myeloma patients," *British Journal of Cancer*, vol. 57, no. 1, pp. 94–99, 1988.

[29] C. G. Schaar, H. C. Kluin-Nelemans, C. te Marvelde et al., "Interferon-α as maintenance therapy in patients with multiple myeloma," *Annals of Oncology*, vol. 16, no. 4, pp. 634–639, 2005.

[30] M. Attal, J. L. Harousseau, S. Leyvraz et al., "Maintenance therapy with thalidomide improves survival in patients with multiple myeloma," *Blood*, vol. 108, no. 10, pp. 3289–3294, 2006.

[31] B. Barlogie, G. Tricot, E. Anaissie et al., "Thalidomide and hematopoietic-cell transplantation for multiple myeloma," *New England Journal of Medicine*, vol. 354, no. 10, pp. 1021–1030, 2006.

[32] A. Spencer, H. M. Prince, A. W. Roberts et al., "Consolidation therapy with low-dose thalidomide and prednisolone prolongs the survival of multiple myeloma patients undergoing a single autologous stem-cell transplantation procedure," *Journal of Clinical Oncology*, vol. 27, no. 11, pp. 1788–1793, 2009.

[33] H. M. Lokhorst, B. Van Der Holt, S. Zweegman et al., "A randomized phase 3 study on the effect of thalidomide combined with adriamycin, dexamethasone, and high-dose melphalan, followed by thalidomide maintenance in patients with multiple myeloma," *Blood*, vol. 115, no. 6, pp. 1113–1120, 2010.

[34] P. L. McCarthy, K. Owzar, K.C. Anderson et al., "Phase III Intergroup Study of lenalidomide versus placebo maintenance therapy following single Autologous Hematopoetic Stem Cell Transplantation (AHSCT) for multiple myeloma: CALGB 100104," *Blood (ASH Annual Meeting Abstracts)*, vol. 116, abstract 37, 2010.

[35] M. Attal, V. Lauwers, G. Marit et al., "Maintenance treatment with lenalidomide after transplantation for myeloma: final analysis of the IFM 2005-02," *Blood (ASH Annual Meeting Abstracts)*, vol. 116, abstract 310, 2010.

[36] A. Palumbo, A. Larocca, S. Zweegman et al., "Second primary malignancies in newly diagnosed multiple myeloma patients treated with lenalidomide: analysis of pooled data in 2459 patients," *Blood (ASH Annual Meeting Abstracts)*, vol. 118, abstract 996, 2011.

Hindawi Publishing Corporation
Advances in Hematology
Volume 2012, Article ID 393864, 7 pages
doi:10.1155/2012/393864

Review Article

Lenalidomide in the Treatment of Chronic Lymphocytic Leukemia

Agostino Cortelezzi, Mariarita Sciumè, and Gianluigi Reda

Hematology and Transplantation Unit, Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico and Università degli Studi Di Milano, 20122 Milan, Italy

Correspondence should be addressed to Agostino Cortelezzi, agostino.cortelezzi@unimi.it

Received 17 February 2012; Accepted 24 April 2012

Academic Editor: Umberto Vitolo

The application of nucleoside analogue-based chemotherapy and immunotherapy with rituximab or alemtuzumab has increased both response rate and survival in patients with Chronic Lymphocytic Leukemia (CLL). However, because none of these therapies is curative, sequential therapeutic regimens are required. The majority of patients with relapsed or refractory CLL carry poor prognostic factors and show shorter overall survival and resistance to standard treatment. Numerous drugs have recently been approved for CLL therapy and many novel agents are under clinical investigation. The role of the tumor microenvironment and of immune dysfunction in CLL have allowed to enlarge the therapeutic armamentarium for CLL patients. This article will provide a comprehensive summary regarding mechanism of action, efficacy and safety of lenalidomide in CLL patients. Relevant clinical trials using lenalidomide alone or in combinations are discussed. Lenalidomide shows good activity also in relapsed/refractory or treatment-naive CLL patients. Definitive data from ongoing studies are needed to validate overall and progression-free survival. The toxicity profile might limit lenalidomide use because it can result in serious side effects, but largely controlled by gradual dose escalation. Further understanding of the exact mechanism of action in CLL will allow more efficacious use of lenalidomide alone or in combination regimens.

1. Introduction

Chronic lymphocytic leukemia (CLL) shows a remarkable heterogeneity in its clinical course, from long-term survival to fast progression and early death. Previously treated patients are known to have poor overall prognosis. In such cases, the disease almost invariably becomes resistant to various subsequent chemotherapies, leading to more toxicities and deterioration of quality of life.

Fludarabine given with cyclophosphamide and rituximab is considered the cornerstone of CLL treatment, but this effective chemoimmunotherapy cannot be given with the same success to all patients. The majority of patients with relapsed or refractory CLL carry poor prognostic features, like, deletion (17p) or TP53 mutation, which are strong predictors of shorter overall survival and resistance to first-line treatment, particularly fludarabine-based regimens [1, 2].

The management of chronic lymphocytic leukemia is currently undergoing profound changes through the introduction of new therapeutic and diagnostic tools.

A common feature of CLL patients is impairment of the immune system with hypogammaglobulinemia, defective function of B, T, NK cells and defective antigen presentation [3]. Immunomodulatory drugs, such as, lenalidomide, represent a promising approach for relapsed/refractory or treatment-naïve patients, because they show antitumor activity and promote immunostimulation.

2. Mechanisms of Action of Lenalidomide in CLL

The precise anti-CLL mechanism of action of lenalidomide is not yet completely defined. Potential mechanisms of action include antiangiogenic effect, blockade of protumor cytokines, inhibition of prosurvival interaction between bone-marrow stromal cells and CLL cells, and enhancement of T helper and cytotoxic T cells function [4].

Immunomodulatory effects of lenalidomide include a costimulatory effect on T-cell responses by increasing IL-2

and INF-gamma secretion and subsequently proliferation of IL-2-activated T cells. Effects of lenalidomide in the production of cytokines is shown by increased circulating cytokines levels, particularly, IL-6, IL-10, IL-2, and TNF-receptor-1 levels; while it is a potent inhibitor of tumor necrosis factor alpha (TNF-alfa), this inhibition may results from increased degradation of TNF-alfa mRNA [5, 6]. Data from LeBlanc suggest that lenalidomide activates T lymphocytes directly by increased phosphorylation of CD28 and increased transcriptional activity of AP-1 and indirectly by enhancing immune synapses between antigen-presenting cells and effector T cells [7–9].

Lenalidomide exposure is able to lead to immune synapses between CLL cells and T cells and promotes costimulatory activation of B cells. During treatment with lenalidomide in CLL patients an increase in circulating Ig levels has been observed. This increased production of Igs may be explained by enhanced B-cell costimulatory activity via activation of lymphocytes through phosphoinositide-3-kinase dependent upregulation of CD154 (CD40L) on CLL cells, as described by Lapalombella et al. [10].

The immune activation of CLL cells with subsequent upregulation of costimulatory molecules, such as, CD40, CD80 and CD86, might be responsible for Tumor Flare observed in CLL patients treated with lenalidomide [6]. Lenalidomide has also been shown to activate NK T cells; one of its postulated mechanisms of action is increased antibody-dependent cellular cytotoxicity (ADCC). IL-2-activated T cells are able to activate NK cells enhancing tumor cell death [5, 7, 11].

Many tumors, including CLL, are characterized by increased number of T regulatory cells and expression of CD152 (CTLA4) in T cells with a correlation with advanced disease and adverse prognostic factors. Lenalidomide reduces T regulatory cell proliferation and suppression function [12].

Lenalidomide also shows antiangiogenic properties in vitro. However, Andritsos et al. [13] reported that VEGF serum concentrations remained unchanged in patients treated with lenalidomide. Furthermore, Ferrajoli et al. [6] did not observe changes in neovascularization in the bone marrow biopsies in patients treated with lenalidomide.

3. Clinical Results Utilizing Lenalidomide in CLL

3.1. Clinical Studies with Lenalidomide as Single Agent in CLL. The second-generation immunomodulatory agent lenalidomide has shown considerable activity in CLL, either alone or in combinations and both as first-line and salvage treatment.

Activity in CLL was first demonstrated by Chanan-Khan et al. [14] in a nonrandomized phase II study that included patients with relapsed or refractory B CLL. In this trial 25 mg of lenalidomide was administered every 21 days of a 28-day cycle in 45 CLL patients. Patients were to continue treatment until disease progression, unacceptable toxicity, or complete remission. The major overall response rate (ORR) in this study was 47%, with a complete response (CR) rate of 9%. Tumor lysis syndrome (TLS) and tumor flare reaction (TFR) occurred in 5% and 58% patients, respectively. TLS is characterized by electrolyte imbalance, uremia, and renal failure, while TFR is associated with painful swelling of lymph nodes and/or splenomegaly with sometimes fever and skin rash.

Because of toxicity, including tumor lysis syndrome in two of the first 29 patients, the dosing schedule was modified and the new treatment schema stipulated a dose escalation beginning at 5 mg/day and a target dose of 25 mg/day. None of the patients (with or without the prophylaxis) required interruption, discontinuation, or dose reduction of therapy because of flare reaction.

Together with TFR, fatigue (83%) was the most common nonhematologic adverse event reported. Grades 3 or 4 neutropenia, thrombocytopenia, and anemia occurred in 70%, 45%, 18% patients during therapy, respectively. There were six episodes of febrile neutropenia. Although neutropenia was observed in a subset of patients, neither opportunistic nor bacterial infections were problematic in this heavily pretreated group of patients. Pulmonary embolism occurred in two patients.

In another clinical trial of lenalidomide in pretreated CLL patients [6], 44 patients received lenalidomide continuously at 10 mg/day with a dose escalation up to 25 mg/day. Treatment was discontinued if disease progression or excessive toxicity was observed. In this pretreated group of patients, ORR was 32%, with 7% achieving a CR. The most common toxicity was myelosuppression, and the median daily dose of lenalidomide tolerated was 10 mg. Severe neutropenia, thrombocytopenia, and anemia were reported in 41%, 15%, and 3% of patients. None of the 44 patients had grade 3 or 4 episodes of tumor lysis; however, the incidence of TFR at any grade was higher in patients with lymph nodes larger than 5 cm (53%); grade 3 tumor flare reaction was reported in 2% of the courses and grade 1 or 2 tumor flare was reported in 10% of the courses. The most common infection was pneumonia, complicating 3% of the courses. FUO was observed in 2% of the courses. The median daily dose of lenalidomide tolerated by the patients in this study was 10 mg, and only 3 patients (7%) were able to tolerate the dose of 25 mg daily for at least 1 month. Myelosuppression was the most frequent reason for treatment interruption and dose reduction. Nonhematologic toxicity consisted of grade 1 to 2 fatigue, observed in 22% of the courses and grade 3 to 4 in only 1%. Only one case of deep vein thrombosis was reported, suggesting that this complication may be more common when lenalidomide is used in association with steroids and in patients with plasma cell dyscrasias.

Treatment with lenalidomide was associated with an ORR rate of 31% in patients with 11q or 17p deletion, of 24% in patients with unmutated VH, and of 25% in patients with fludarabine-refractory disease.

Sher et al. [15] reviewed the cases of relapsed/refractory CLL patients with high-risk cytogenetics who were included in the trial of Chanan-Khan et al. [14]; clinical response was reported in 38% of these patients, with 19% CR.

The question of whether continuous exposure is superior to the 3 weeks on/1 week off schedule reported by Chanan-Khan et al. [14] will require further investigation.

While data from Ferrajoli et al. [6] and Chanan-Khan et al. [14] proposed an escalation dose up to 25 mg, a report from Andritsos et al. [13] described serious adverse events four patients with relapsed CLL treated with lenalidomide (25 mg/d for 21 days of a 28-day cycle). Tumor flare was observed in three patients and was characterized by dramatic and painful lymph node enlargement resulting in hospitalization of two patients, with one fatal outcome. Another patient developed sepsis and renal failure.

Chen et al. [16] reported results of lenalidomide therapy in 25 previously untreated patients. Lenalidomide dosing involved an escalation starting from a dose of 10 mg up to 25 mg/day. Serious toxic complications (tumor lysis and fatal sepsis) occurred in the first two patients, the protocol was modified with a 2,5 mg starting dose and an escalation up to 10 mg as target dose. Twenty-two patients were treated with this schema. The ORR was 56%, no CR. TFR was evident in 88% of patients, mostly low grade.

In a phase II study Aue et al. [17] reported a change in administration schedule of lenalidomide, which was given in pulse dosages for 3 weeks followed by 3 weeks off. This study involved high-risk pretreated patients with a poor prognosis: 52% was Rai stage III-IV, 43% had del(17p), and 64% expressed unmutated IgVH genes. Lenalidomide was administered at 10 mg daily for 21 days of a 42-day cycle, for a total of 4 cycles. Grade 3-4 neutropenia, thrombocytopenia, and anemia occurred in 56%, 30%, and 15% of cycles, respectively. No TLS cases were seen. However, the hypothesis of achieving a safer and more tolerable toxicity profile was not obtained, since TFR was observed in 78%, 48%, 38%, and 30% in 1st, 2nd, 3rd, and 4th cycle, respectively. ORR was only 16% but patients with del(17p) and bulky disease appeared to have a remarkable PR rate of 80%

Badoux et al. [18] investigated lenalidomide for elderly untreated CLL patients. Lenalidomide was administered to sixty patients 65 years of age and older at 5 mg daily with a possible dose escalation of 5 mg every 28 days up to 25 mg daily. At a median followup of 29 months, 53 patients (88%) were alive and 32 patients (53%) remained on therapy. Estimated 2-year progression-free survival was 60%. The overall response rate to lenalidomide therapy was 65%, including 10% CR, 5% CR with residual cytopenia. Neutropenia was the most common grade 3 or 4 treatment-related toxicity observed in 34% of treatment cycles. Major infections or neutropenic fever occurred in 13% of patients. There were no grade 3 or 4 episodes of tumor flare or any tumor lysis syndrome in this study. Also compared with baseline levels, the authors noted an increase in serum immunoglobulin levels across all classes.

According to these several studies, TFR is a common toxicity described with lenalidomide. Because of its high incidence, its considerable morbidity and its clinical presentation resembling disease progression, an early diagnosis, and an accurate management are critical for effective use of lenalidomide in patients with CLL.

Often TFR included a sudden onset of painful and tender enlargement of disease-involved lymph nodes, the spleen, and/or liver, which was frequently accompanied by low-grade fever, localized erythema, or generalized rash (often diffuse, erythematous, nonpruritic, and maculopapular) and occasionally associated with bone pain. Patients who develop a TFR tend to have a higher stage of disease, but mostly there is no significant difference noted with regard to the incidence of TFR among patients with bulky versus nonbulky disease.

The severity of TFR can be graded according to the National Cancer Institute Common Toxicity Criteria [i.e. grade 1, mild pain not interfering with function grade 2, moderate pain (pain or analgesics interfering with function, but not interfering with activities of daily living); grade 3, severe pain (pain or analgesics interfering with function and activities of daily living); grade 4, disabling pain].

The identification and careful characterization of a TFR are important in the treatment of CLL patients with lenalidomide to avoid unnecessary morbidity or the premature discontinuation of effective therapy. It's recommended close monitoring of patients with CLL for signs of TFR, especially during the first days of lenalidomide therapy and treatment with an NSAID,such as, ibuprofen (at a dose of 400–600 mg every 6 hours), with steroid use considered only in cases of more intense TFRs.

A slow-dose escalation strategy may reduce the intensity of TFR and should be considered, such as, reported by Chanan-Khan et al. [19] Eventually, dose adjustment of lenalidomide can be use in case of severe TFR, the dose can be increased again once the TFR subsides.

Chanan-Khan et al. [19] observed that prophylaxis with prednisone decreased the severity but not the incidence of TFR. Low-dose oral prednisone (20 mg daily for 5 days followed by 10 mg for 5 days) was used as TFR prophylaxis from treatment on days 1 to 10 of cycle 1, TFR prophylaxis was not given in subsequent cycles. Although exact clinical impact of TFR remains uncertain, the authors noted that the intensity of the TFR appeared to be correlated with a higher probability of achieving a CR; despite a higher CR rate their analysis did not demonstrate any benefit in the PFS in the TFR group. Table 1 summarizes the results from clinical trials using lenalidomide alone in CLL patients.

3.2. Lenalidomide in Combination in CLL. Lenalidomide has been shown to activate NK cells and one of its postulated mechanisms of action is increased antibody-dependent cellular cytotoxicity (ADCC). Thus, lenalidomide would seem an appealing therapeutic agent to add to rituximab treatment, which is known to induce ADCC of CD-20-expressing CLL cells [20]. However a recent laboratory study suggested a potential antagonism between these two agents if used simultaneously with CLL cells, since lenalidomide down-regulated CD20, with a reduction in NK mediated ADCC of rituximab-treated CLL cells [21].

Clinical trials report that combined treatment with lenalidomide and rituximab improves activity and decreases the toxicity of lenalidomide. Ferrajoli et al. [22] investigated the combination lenalidomide plus rituximab in 60 patients with relapsed or refractory CLL. They treated patients with rituximab weekly for 1 cycle and then once every 4 weeks during subsequent cycles. Lenalidomide was administered at dose of 10 mg daily starting on day 9 of cycle 1 and

Table 1: Selected clinical trials using lenalidomide alone for treatment of CLL. NR: not reported.

Study	Regimen	No. of patients	TLS all grades	TFR all grades	Hematologic side effects grade 3/4	OR (%)	CR (%)	OS (%)	PFS (%)
Chanan-Khanet al. [14] Phase II relapsed/refractory CLL	5 mg/d escalated to 25 mg/d	45	5%	58%	Neutropenia 70% thrombocytopenia 45% anemia 18%	47	9	NR	NR
Ferrajoli et al. [6] Phase II relapsed/refractory CLL	10 mg/d escalated to 25 mg/d	44	0	12%	Neutropenia 41% thrombocytopenia 15% anemia 3%	32	7	73 (with a median follow-up time of 14 months)	NR
Chen et al. [16] Phase II untreated CLL	2.5 mg/descalated to 10 mg/d	25	0	88%	Neutropenia 72% thrombocytopenia 28% anemia 20%	56	0	92 (estimated 2 years OS)	89 (estimated 2 years PFS)
Aue et al. [17] Phase II relapsed/refractory CLL	20 mg/d lowered to 10 mg/d	33	0	53%	Neutropenia 56% thrombocytopenia 30% anemia 15%	NR	NR	NR	NR
Badoux et al. [18] phase II (elderly) untreated CLL	5 mg/d escalated to 25 mg/d	60	0	52%	Neutropenia 34% thrombocytopenia 12% anemia <1%	65	10	88 (estimated 2 years OS)	60 (estimated 2 years PFS)

continuing daily for 12 cycles. The ORR was 64%, with 8% CR. Data suggest that this combination is superior to the single-agent lenalidomide. The most frequently observed toxicity was neutropenia, occurring in 68% of patients. Twenty-two patients experienced low-grade tumor flare.

Frontline lenalidomide plus rituximab in patients with CLL was reported by James et al. [23] in 37 patients. Lenalidomide was started at a dose of 2,5 mg daily with an escalation up to 5 mg and 10 mg on day 8, if tolerated, every 21 days of a 28-day cycle for a total of 7 cycles. Patients received lenalidomide for 21 days in 35-day cycle for the first cycle, then for 21 days in 28-day cycles for other 6 cycles. During the first cycle rituximab was given 50 mg/m^2 on day 29, and 325 mg/m^2 on day 31, 375 mg/m^{2}on day 33. During the second cycle rituximab was administered 375 mg/m^2 weekly and on day 1 during remaining cycles. Early results of the ongoing study suggest that lenalidomide-plus-rituximab immunotherapy is tolerable. The most common grade 3/4 adverse events (AE) were neutropenia (18 pts), anemia (5 pts), and thrombocytopenia (4 pts). There were no cases of neutropenic fever, sepsis, or bleeding. Nonhematologic grade 3/4 AEs included infection (3 pts), rash (2 pts), and pulmonary embolus (2 pts). The protocol was amended to include aspirin prophylaxis. Most frequent AEs (all grades) were the TFR (21 pts), fatigue (19 pts).

As a consequence of biological and clinical synergism of lenalidomide and anti-CD 20 monoclonal antibody, a combination with the humanized anti-CD20 monoclonal antibody ofatumumab was evaluated in a phase II trial [24]. Ofatumumab was given weekly, starting at 300 mg in the first week, then at 1000 mg weekly, then monthly for 6 months and lastly every 2 months up to 24 months. Lenalidomide was given at a dose of 10 mg daily continuously from day 9 of cycle 1 with treatment duration of 24 months. The ORR was 63% with 2 patients achieving a CR. Toxicity was tolerable, with 50% of patients developing grade 3 or 4 neutropenia. TFR was limited to grade 1 in 2 patients.

In the GIMEMA LLC606 phase I clinical trial patients were treated with lenalidomide, cyclophosphamide, and fludarabine [25]. The maximal tolerated dose of lenalidomide was 5 mg. The response rate observed in nine patients was 67%, with 33% CR.

Brown et al. [26] initiated a small phase I study that investigated the combination of lenalidomide with fludarabine and rituximab for untreated patients with CLL. A low dose of lenalidomide (2,5 mg) was given daily for 21 days in 28 days cycles, with fludarabine 25 mg/m^2 on days 3–5 and rituximab 375 mg/m^2 on day 1. The trial had to be closed due to significant myelotoxicity and idiosyncratic tumor flare reactions.

Another trial by Egle et al. [27] combined 6 cycles of fludarabine, lenalidomide, and rituximab. Lenalidomide was administered at a starting dose of 2,5 mg daily (days 7–21 in cycle 1) and escalated up to 25 mg/day from days 1–21 of the following cycles. After induction treatment, maintenance with lenalidomide and rituximab for 6 months was planned. Preliminary data show that all ten treated patients achieved at least a PR, except for one patient with Richter transformation; but 50% of patients received a reduced lenalidomide dose due to toxicity.

A phase I study from the German CLL study group [28] used the bendamustine-rituximab (BR) backbone and added lenalidomide to it. This might be an option for patients with relapse or even refractory CLL. Lenalidomide was given orally for 7 days followed by rituximab 375 mg/m^2 on day 1 in addition to bendamustine 90 mg/m^2 intravenously on days 1 and 2 and lenalidomide orally daily every 28 days for a total of 6 cycles. After 6 cycles of bendamustine, lenalidomide, and rituximab, lenalidomide monotherapy was administered as continued therapy for an additional 6 cycles as tolerated or until disease progression.

Blum et al. [29] conducted a phase I study in which flavopiridol was given in combination with lenalidomide in 21 patients with relapsed or refractory B-cell CLL/SLL. Flavopiridol was administered at 60 mg/m^2 on days 1,

Table 2: Selected clinical trials using lenalidomide in combination for treatment CLL. NR: not reported.

Study	Regimen	No. of patients	TLS all grades	TFR all grades	Hematologic side effects grade 3/4	OR (%)	CR (%)	OS (%)	PFS
Ferrajoli et al. [22] phase II relapsed/refractory CLL	10 mg/d Lenalidomide + rituximab weekly	59	1,7%	37%	Neutropenia 68% thrombocytopenia 22% anemia 10%	64	8	NR	NR
Badoux et al. [24] phase II relapsed/refractory CLL	10 mg/d lenalidomide + ofatumumab weekly	16	NR	13%	Neutropenia 50% anemia 13%	63	13	NR	NR
Blum et al. [29] phase I relapsed/refractory CLL	2.5 mg/d escalated to 25 mg/d lenalidomide + flavopiridol	15	14%	7%	Neutropenia 86% thrombocytopenia 38% anemia 38%	46	0	NR	NR
GIMEMA LLC 606 [25] phase I relapsed/refractory CLL	2.5 mg/d escalated to 15 mg/d lenalidomide + cyclophosphamide + fludarabine	9	0	11%	Transient grade 3-4 neutropenia in the majority of pts	67	33	NR	NR
Egle et al. [27] phase untreated CLL	2.5 mg/d escalated to 25 mg/d lenalidomide + fludarabine + rituximab	10	0	0	Neutropenia 70%	90	0	NR	NR

8, and 15 during first cycle and from second cycle at 60 mg/m^2 on days 3, 10, and 17 plus, starting at cycle 2, lenalidomide with an initial dose of 2,5 mg escalated to 25 mg for 21 days of these 28-day cycles. Thirteen patients had del(17p) and 8 patients had del (11q). Seventeen patients completed two or morecycles of therapy (median 3, range 2–8), receiving 2,5 mg (6 pts), 5,0 mg (7 pts), and 7,5 mg (4 pts) of lenalidomide. Grade 3-4 toxicitiesconsisted of neutropenia (86%), diarrhea (62%), reversible transaminitis in ≤7 days (57%), anemia (38%), thrombocytopenia(38%), hyperglycemia (38%), infection without neutropenia (24 %), febrile neutropenia (14%), TLS not requiring dialysis (14%), and fatigue (14%). Grade 1 tumor flare occurred in 1 pt and did not require additional steroids or cessation of lenalidomide. The ORR was 46% with no CRs. Partial responses were observedin 7 patients, including 4 patients with del (17p). In conclusion, combined flavopiridol and lenalidomide was well tolerated, with activity in patients with previously treated, cytogenetically high-risk CLL.

A prospective, nonrandomized, phase II study by Shanafelt et al. [30] presented data about lenalidomide consolidation following a PCR (pentostatin, cyclophosphamide, rituximab) induction. Data from this trial were compared with results of an historic trial of PCR without lenalidomide showing an improvement in quality of response and time to retreatment.

Patients with previously untreated CLL ($n = 44$) received induction therapy with the frontline regimen most commonly used at the Mayo Clinic, pentostatin 2 mg/m^2, cyclophosphamide 600 mg/m^2, and rituximab 375 mg/m^2 every 3 weeks for 6 cycles. Responding patients were eligible for consolidation therapy with lenalidomide at a starting dose of 5 mg/day with escalation to 10 mg/day as tolerated for 6 months. Thirty-four patients (77%) started lenalidomide and completed a median of 7 cycles of consolidation therapy.

Among patients who started lenalidomide consolidation following PCR, the freedom from retreatment at 12 months was 95% (95% confidence interval: 88% to 100%). The study investigators compared these findings to historic data from a trial of PCR induction therapy without lenalidomide consolidation [31]. In that study of 64 patients with CLL, the freedom from retreatment at 12 months was 86%. Cytopenias were the most frequent grade ≥3 adverse events considered possibly associated with lenalidomide consolidation: grade 3 neutropenia and thrombocytopenia occurred in 41% and 9% of patients, respectively; while grade 4 neutropenia interested 21% of patients. Therefore, the study authors concluded that lenalidomide consolidation appeared to improve the quality of response to induction therapy with PCR.

Another ongoing study which evaluates lenalidomide as maintenance therapy in CLL patients was the CONTINUUM study [32]: a phase 3, multicenter, randomized, double-blind, placebo-controlled, parallel-group study of the efficacy and safety of lenalidomide as maintenance therapy for patients with B-cell chronic lymphocytic leukemia following second-line therapy. In the experimental arm lenalidomide is given on days 1–28 of a 28 day cycle until disease progression or unacceptable toxicity. Table 2 summarizes the results from clinical trials using lenalidomide in combination regimens in CLL patients.

4. Conclusions

Lenalidomide proved to be effective in CLL as single agents or in combination with various chemo immunotherapeutic regimens. There were several concerns regarding toxicity, but modified protocols with low starting dose and gradual dose escalation suggest good tolerability. Myelosuppression was the predominant toxicity associated with lenalidomide. Tumor flare was also a problem with lenalidomide therapy, but it can be controlled by gradual dose-escalation and prophylactic corticosteroids in the patients who experienced tumor flare in previous cycles. However, further studies are needed to establish the most effective dose and schedule of this agent.

Lenalidomide might have a place in the first-line setting in older patients or as second-third line agent for patients treated with frontline chemoimmunotherapy. Currently, chemoimmunotherapy represents the standard first-line therapy for young and fit CLL patients, but patients who became refractory to fludarabine or carry deletion/mutation of TP53 and older or unfit patients could profit from alternative treatments, including, lenalidomide-based regimens. Therapeutic strategies including consolidation treatment are becoming more important in the treatment of CLL, particularly in the frontline setting. There is a greater number of complete remissions (CRs) with the new chemoimmunotherapy combinations, and therefore prolonging the duration of PFS becomes a more interesting goal. In this context, ongoing promising studies [29, 30] are evaluating the role of lenalidomide as consolidation/maintenance therapy.

References

[1] M. Hallek, K. Fischer, G. Fingerle-Rowson et al., "Addition of rituximab to fludarabine and cyclophosphamide in patients with chronic lymphocytic leukaemia: a randomised, open-label, phase 3 trial," *The Lancet*, vol. 376, no. 9747, pp. 1164–1174, 2010.

[2] T. Robak, A. Dmoszynska, P. Solal-Céligny et al., "Rituximab plus fludarabine and cyclophosphamide prolongs progression-free survival compared with fludarabine and cyclophosphamide alone in previously treated chronic lymphocytic leukemia," *Journal of Clinical Oncology*, vol. 28, no. 10, pp. 1756–1765, 2010.

[3] A. D. Hamblin and T. J. Hamblin, "The immunodeficiency of chronic lymphocytic leukaemia," *British Medical Bulletin*, vol. 87, no. 1, pp. 49–62, 2008.

[4] A. A. Chanan-Khan and B. D. Cheson, "Lenalidomide for the treatment of B-cell malignancies," *Journal of Clinical Oncology*, vol. 26, no. 9, pp. 1544–1552, 2008.

[5] L. G. Corral, P. A. J. Haslett, G. W. Muller et al., "Differential cytokine modulation and T cell activation by two distinct classes of thalidomide analogues that are potent inhibitors of TNF-α," *Journal of Immunology*, vol. 163, no. 1, pp. 380–386, 1999.

[6] A. Ferrajoli, B. N. Lee, E. J. Schlette et al., "Lenalidomide induces complete and partial remissions in patients with relapsed and refractory chronic lymphocytic leukemia," *Blood*, vol. 111, no. 11, pp. 5291–5297, 2008.

[7] R. LeBlanc, T. Hideshima, L. P. Catley et al., "Immunomodulatory drug costimulates T cells via the B7-CD28 pathway," *Blood*, vol. 103, no. 5, pp. 1787–1790, 2004.

[8] P. H. Schafer, A. K. Gandhi, M. A. Loveland et al., "Enhancement of cytokine production and AP-1 transcriptional activity in T cells by thalidomide-related immunomodulatory drugs," *Journal of Pharmacology and Experimental Therapeutics*, vol. 305, no. 3, pp. 1222–1232, 2003.

[9] A. G. Ramsay, A. J. Johnson, A. M. Lee et al., "Chronic lymphocytic leukemia T cells show impaired immunological synapse formation that can be reversed with an immunomodulating drug," *Journal of Clinical Investigation*, vol. 118, no. 7, pp. 2427–2437, 2008.

[10] R. Lapalombella, A. Gowda, T. Joshi et al., "The humanized CD40 antibody SGN-40 demonstrates pre-clinical activity that is enhanced by lenalidomide in chronic lymphocytic leukaemia," *British Journal of Haematology*, vol. 144, no. 6, pp. 848–855, 2009.

[11] F. E. Davies, N. Raje, T. Hideshima et al., "Thalidomide and immunomodulatory derivatives augment natural killer cell cytotoxicity in multiple myeloma," *Blood*, vol. 98, no. 1, pp. 210–216, 2001.

[12] M. Motta, L. Rassenti, B. J. Shelvin et al., "Increased expression of CD152 (CTLA-4) by normal T lymphocytes in untreated patients with B-cell chronic lymphocytic leukemia," *Leukemia*, vol. 19, no. 10, pp. 1788–1793, 2005.

[13] L. A. Andritsos, A. J. Johnson, G. Lozanski et al., "Higher doses of lenalidomide are associated with unacceptable toxicity including life-threatening tumor flare in patients with chronic lymphocytic leukemia," *Journal of Clinical Oncology*, vol. 26, no. 15, pp. 2519–2525, 2008.

[14] A. Chanan-Khan, K. C. Miller, L. Musial et al., "Clinical efficacy of lenalidomide in patients with relapsed or refractory chronic lymphocytic leukemia: results of a phase II study," *Journal of Clinical Oncology*, vol. 24, no. 34, pp. 5343–5349, 2006.

[15] T. Sher, K. C. Miller, D. Lawrence et al., "Efficacy of lenalidomide in patients with chronic lymphocytic leukemia with high-risk cytogenetics," *Leukemia and Lymphoma*, vol. 51, no. 1, pp. 85–88, 2010.

[16] C. I. Chen, P. L. Bergsagel, H. Paul et al., "Single-agent lenalidomide in the treatment of previously untreated chronic lymphocytic leukemia," *Journal of Clinical Oncology*, vol. 29, no. 9, pp. 1175–1181, 2011.

[17] G. Aue, S. Soto, J. Valdez et al., "Phase II trial of pulse dosed lenalidomide in previously treated chronic lymphocytic leukemia," *Blood*, vol. 116, article 1383, 2010, ASH Annual Meeting Abstracts.

[18] X. C. Badoux, M. J. Keating, S. Wen et al., "Lenalidomide as initial therapy of elderly patients with chronic lymphocytic leukemia," *Blood*, vol. 118, no. 13, pp. 3489–3498, 2011.

[19] A. Chanan-Khan, K. C. Miller, D. Lawrence et al., "Tumor flare reaction associated with lenalidomide treatment in patients with chronic lymphocytic leukemia predicts clinical response," *Cancer*, vol. 117, no. 10, pp. 2127–2135, 2011.

[20] L. Wu, M. Adams, T. Carter et al., "Lenalidomide enhances natural killer cell and monocyte-mediated antibody-dependent cellular cytotoxicity of rituximab-treated CD20+ tumor cells," *Clinical Cancer Research*, vol. 14, no. 14, pp. 4650–4657, 2008.

[21] R. Lapalombella, B. Yu, G. Triantafillou et al., "Lenalidomide down-regulates the CD-20 antigen and antagonizes direct and antibody-dependent cellular cytotoxicity of rituximab on primary chronic lymphocytic leukemia cells," *Blood*, vol. 112, pp. 5180–5189, 2008.

[22] A. Ferrajoli, X. C. Badoux, S. O'Brien et al., "Combination therapy with lenalidomide and rituximab in patients with relapsed chronic lymphocytic leukemia (CLL)," *Blood*, vol. 114, article 2376, 2009, ASH Annual Meeting Abstracts.

[23] D. F. James, J. R. Brown, L. Werner et al., "Lenalidomide and rituximab for the initial treatment of chronic lymphocytic leukemia: report of an ongoing study," *Journal of Clinical Oncology*, vol. 2010, article 6583, 2010, ASCO Annual Meeting Abstracts.

[24] X. Badoux, S. O'Brien, W. G. Wierda et al., "Combination of of atumumab and lenalidomide in patients with relapsed chronic lymphocytic leukemia: initial results of a phase II trial," *Blood*, vol. 116, article 2464, 2010, ASH Annual Meeting Abstracts.

[25] F. R. Mauro, D. Armiento, S. Orlando et al., "Fludarabine, Cyclophosphamide and Lenalidomide (FCL) for previously

treated patients with chronic lymphocytic leukemia (CLL): results of dose-finding phase of the GIMEMA LLC606 Study," *Blood*, vol. 116, no. 21, article 1377, 2010, ASH Annual Meeting Abstracts.

[26] J. R. Brown, J. Abramson, E. Hochberg et al., "A phase i study of lenalidomide in combination with fludarabine and rituximab in previously untreated CLL/SLL," *Leukemia*, vol. 24, no. 11, pp. 1972–1975, 2010.

[27] A. Egle, M. Steurer, T. Melchardt et al., "The REVLIRIT CLL5 AGMT study—a phase I/II trial combining fludarabine/rituximab with escalating doses of lenalidomide followed by rituximab/lenalidomide in untreated chronic lymphocytic leukemia (CLL): results of a planned interim analysis," *Blood*, vol. 114, article 3453, 2009, ASH Annual Meeting Abstracts.

[28] Clinicaltrials.gov, "Phase I Clinical Trial of Bendamustine, Lenalidomide and Rituximab in B-Cell Lymphoid Malignancies," http://www.clinicaltrials.gov/ct2/show/NCT00864942/.

[29] K. A. Blum, J. A. Jones, L. Andritos et al., "Phase 1 trial of flavopiridol in combination with lenalidomide in patients with relapsed or refractory B-cell CLL/SLL," *Blood*, vol. 116, no. 21, article 2472, 2010, ASH Annual Meeting Abstracts.

[30] T. Shanafelt, H. Tun, C. Hanson et al., "Lenalidomide consolidation after first-line chemoimmunotherapy for patients with previously untreated CLL," *Blood*, article 1379, 2010, ASH Annual Meeting Abstracts.

[31] N. E. Kay, S. M. Geyer, T. G. Call et al., "Combination chemoimmunotherapy with pentostatin, cyclophosphamide, and rituximab shows significant clinical activity with low accompanying toxicity in previously untreated B chronic lymphocytic leukemia," *Blood*, vol. 109, no. 2, pp. 405–411, 2007.

[32] Clinicaltrials.gov, "A study to evaluate the efficacy and safety of lenalidomide as maintenance therapy for patients with B-CLL following second line therapy (THE CONTINUUM TRIAL)," http://www.clinicaltrials.gov/ct2/show/NCT00774345/.

Hindawi Publishing Corporation
Advances in Hematology
Volume 2012, Article ID 498342, 5 pages
doi:10.1155/2012/498342

Review Article

Lenalidomide in Diffuse Large B-Cell Lymphomas

Annalisa Chiappella and Umberto Vitolo

Hematology 2, San Giovanni Battista Hospital and University, c.so Bramante 88, 10126 Torino, Italy

Correspondence should be addressed to Umberto Vitolo, uvitolo@molinette.piemonte.it

Received 17 February 2012; Revised 17 May 2012; Accepted 17 May 2012

Academic Editor: Agostino Cortelezzi

Diffuse Large B-cell Lymphomas (DLBCL) are the most frequent Non-Hodgkin Lymphomas (NHL). The addition of Rituximab to the standard chemotherapy CHOP improved the outcome in this patients, but so far 40% of patients experienced relapse or progressive disease. Lenalidomide, an immunomodulatory agent, had direct tumoricidal and antiangiogenetic actions on tumor cells and was able to modulate tumor-cell microenvironment, with the restoration of impaired T-cell activity and the formation of immuno-synapsis. Based on these actions, lenalidomide represented an active drug on aggressive relapsed NHL. In this review, the most relevant clinical trials for the use of lenalidomide in DLBCL were reported. Monotherapy with lenalidomide showed an activity in term of overall response rate, with acceptable hematological and extrahematological toxicities in relapsed/refractory aggressive NHL. The role of lenalidomide as salvage therapy in both cell of origin patterns in DLBCL (germinal center B-cell/activated B-cell) was reported in preliminary data. Preliminary data regarding the role of lenalidomide in addition to chemoimmunotherapy (R-CHOP) in first line clinical trials were discussed; data of safety, feasibility and efficacy were promising.

1. Introduction

Diffuse large B-cell lymphoma (DLBCL) represents roughly 40% of all non-Hodgkin lymphoma (NHL) (Figure 1), with a rate of incidence in continuous increase and median age at diagnosis of 55–60 years [1, 2].

The addition of monoclonal antibody anti-CD20 rituximab to standard chemotherapy CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) has improved the outcome compared to CHOP alone in untreated DLBCL elderly patients with a complete remission (CR) rate of 75% versus 63% [3]; the advantage of R-CHOP versus CHOP was maintained at a median followup of ten years; Overall Survival (OS) of 43.5% versus 27.6%; Progression Free Survival (PFS) of 36.5% versus 20.1%, respectively [4]. Also in combination with dose-dense chemotherapy CHOP14 the rituximab showed promising results in elderly untreated DLBCL [5]. In order to ameliorate prognosis, with the support of granulocyte colony-stimulating factor (G-CSF), dose-dense chemotherapy CHOP14, administered every 14 days, with or without rituximab, was tested in elderly DLBCL at diagnosis; RICOVER-60 trial showed the superiority of 6 courses of R-CHOP14 compared to CHOP14, with 3-year event-free survival 66.5% versus 47.2% and 3-year overall survival 78.1% versus 67.7%, respectively [5]. In young patients affected by poor prognosis DLBCL at diagnosis, rituximab plus dose-dense chemotherapy plus high-dose chemotherapy and autologous stem cell transplant were tested, with promising results (4-year PFS 73% and 4-year OS 80%) [6].

Despite the improvement of outcome with chemoimmunotherapy, rituximab plus dose-dense chemotherapy or high-dose chemotherapy plus autologous stem cell transplant, 30–40% of patients relapsed after first line treatment, and the rate of second CR in patients pretreated with rituximab chemotherapy is lower than 30% [7].

It will be mandatory to obtain a better CR in first line DLBCL and, in relapsed or refractory patients, to overcome chemorefractoriness; the introduction of novel drugs represents a chance to obtain these goals.

In the landscape of novel drugs, immunomodulating drugs (IMiDs) represent now a real opportunity to ameliorate prognosis in DLBCL.

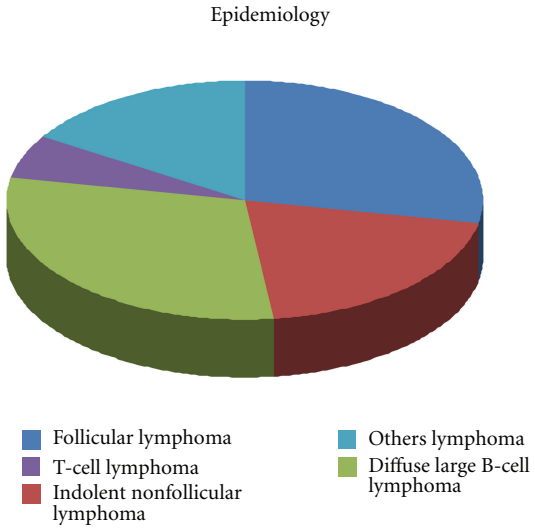


Figure 1: Incidence of Non-Hodgkin Lymphoma.

2. Lenalidomide: Mechanism of Action and Rationale

Lenalidomide, CC-5013, is an immunomodulatory agent with multiple mechanisms of action and it is an active agent on aggressive NHL, blocking tumor growth and survival with direct tumoricidal and immunomodulatory actions. This drug has both antiproliferative and antiangiogenic activities. Lenalidomide's activity is based on modulation of tumor-cell microenvironment and on stimulating the activity of effector cells, such as cytotoxic T and natural killer cells. Lenalidomide enhanced T-cell and NK-cell effector function to eliminate tumor B cells and it had a role in the restoration of impaired T-cell activity and formation of immunologic synapses [8] (see Figure 2).

Lenalidomide was initially introduced in the treatment of multiple myeloma and only in a second time was tested in lymphoma cell lines. In animal models of lymphoma, IMiDs and especially lenalidomide demonstrated a synergic action with rituximab; the addition of lenalidomide to rituximab increased median survival in mice from 45 days to 58 days compared to rituximab alone [9]. Another study demonstrated that IMiDs increased the recruitment of natural killer cells to subcutaneous lymphoma sites in mice with the stimulation of dendritic cells and modification of the cytokine microenvironment; lenalidomide, in association with rituximab, increased antibody-dependent cellular cytotoxicity [10].

3. Lenalidomide: Clinical Experience in Relapsed/Refractory DLBCL

On the basis of the *in vivo* activity of IMiDs, Wiernik et al. conducted a phase II multicenter trial to evaluate safety and efficacy of lenalidomide monotherapy in relapsed/refractory aggressive lymphomas patients. Forty-nine patients were enrolled to receive oral lenalidomide 25 mg once daily on days 1 to 21, every 28 days, for 52 weeks, until disease progression or intolerance. Median age was 65 years, 53% of patients had DLBCL, and all of them received at least four prior therapeutic regimens; 92% of patients had received prior rituximab and 29% of them had been previously transplanted. The overall response rate (ORR) was 35% for all histology and 19% for DLBCL. The estimated median duration of response was 6.2 months (range: 0 to 12.8 months) and median PFS was 4 months (range: 0 to 14.5 months). Regarding safety, the most common grade 4 adverse events were neutropenia (8.2%) and thrombocytopenia (8.2%); the most common grade 3 adverse events were neutropenia (24.5%), leukopenia (14.3%), thrombocytopenia (12.2%), and thrombocytopenia in 8.2%, resolved with dose reduction. The results showed that lenalidomide monotherapy is active in relapsed or refractory aggressive NHL, with manageable side effects [11]. The same schedule of lenalidomide was tested by Witzig in the NHL-003 international phase II trial for relapsed or refractory aggressive B-cell non-Hodgkin's lymphoma. Two hundred and seventeen patients were enrolled and 108 had DLBCL. In all histologic subgroups, ORR was 35% with CR 13%, partial remission (PR) 22%, and stable disease 21%; ORR for DLBCL was 28%. Moreover, ORR was 37% for patients who underwent prior stem cell transplantation and 33% for rituximab refractory ones. Median PFS for all 217 patients was 3.7 months; for 77 responders, the median response duration lasted 10.6 months. Despite the fact that patients were heavily pretreated, lenalidomide was well tolerated. The administered median daily dose of lenalidomide was 25 mg (range 7.1–25 mg) and 117 patients (53.9%) required at least one dose reduction or interruption due to neutropenia in 56% and thrombocytopenia in 31%. Grade 3 or 4 adverse events included neutropenia in 41%, with only 2% of febrile neutropenia, thrombocytopenia in 19%, and anemia in 9.2%. Discontinuation from study treatment occurred in 49 patients (23%). Extrahematological toxicities included tumor flares in 7 patients, 4 (1.8%) with grade 1 or 2 and 3 with grade 3, gastrointestinal events in 61.3%, rash in 18% and fatigue in 28%. Granulocyte colony stimulating factors (GCSF) were administered to 54 patients (25%) during the study [12].

In the last decades, an important role to predict the outcome in DLBCL was represented by gene expression profiling and by pattern of origin, germinal center B-cell and nongerminal, or activated B-cell-like. The outcome of the two subgroups seems to be different, with a worst outcome in activated B-cell lymphoma. In a recent study, Hernandez-Ilizaliturri retrospectively analyzed clinical outcomes of 40 patients with relapsed/refractory DLBCL, 23 germinal center, and 17 nongerminal center, treated with salvage lenalidomide as single agent. Median age was 66 years and median number of prior treatments, including rituximab plus chemotherapy, was four. Germinal center and nongerminal center B-cell subgroups were similar in terms of stage, international prognostic index score, prior number of treatments, and rituximab resistance. Results demonstrate a different antitumor responsiveness in the two biological subgroups: ORR for nongerminal (activated B cell) versus

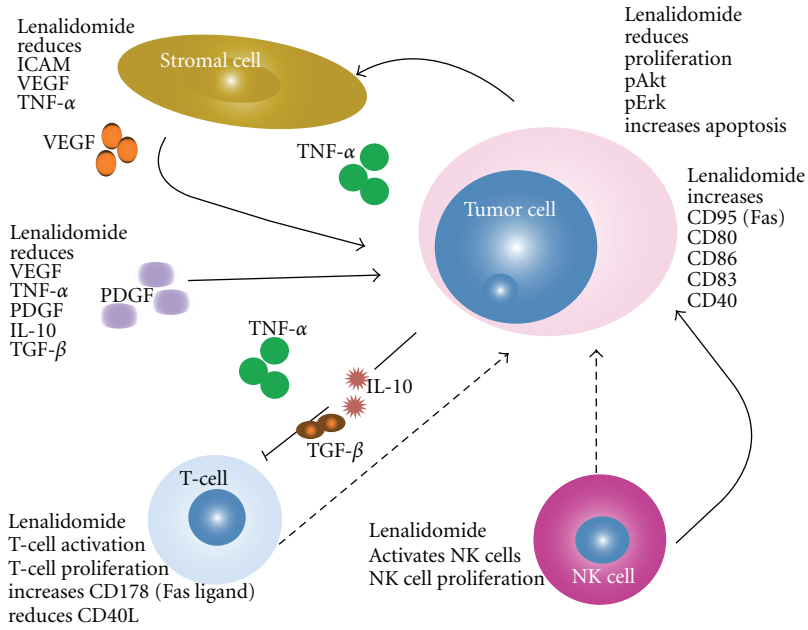


Figure 2: Action of lenalidomide.

germinal B cell was 52.9% versus 8.7% ($P = 0.006$), CR was 23.5% versus 4.3%, and median PFS was 6.2 versus 1.7 months ($P = 0.004$), respectively; no difference was observed regarding overall survival. The advantage in treatment with lenalidomide seems to be increased in nongerminal subtype compared to germinal subgroup; this hypothesis should be based on the different expression of NF-κB, targeted by IMiDs, in the two subgroups [13]. A large international trial (NCT01197560) to compare lenalidomide to investigator's choice is still ongoing; in this clinical study patients will be risk-stratified according to germinal center/nongerminal center B-cell-like in order to identify the subgroup that benefit from lenalidomide treatment.

The efficacy of lenalidomide in monotherapy and the well-tolerated profile, supported the rationale for investigating in a phase II trial the efficacy and safety of the combination of lenalidomide and rituximab in pretreated elderly patients with DLBCL. Lenalidomide was administered at the dose of 20 mg/daily from day 1 to 21 every 28 days, for four courses, in combination with 375 mg/sqm rituximab on day 1 and day 21 every 28 days for four cycles. Responsive patients (CR, PR, or stable disease) were given lenalidomide maintenance therapy at the same schedule for an additional eight months. Twenty-three elderly DLCL patients at median age 74.2 years, heavily pretreated with a median of three prior therapies (range, 2 to 8), were enrolled and treated with rituximab plus lenalidomide. After the induction phase, the ORR was 35%, with 7 patients achieving a CR, one patient a PR, 2 stable disease, and 13 progressive disease. Ten patients were eligible for lenalidomide maintenance. At the end of the entire treatment regimen, CR was 35%. At a median follow-up of 16 months, the estimated 1-year-disease-free-survival was 34.8% and the 18-month OS rate for the whole study population was 55.1%. Therapy was feasible with low rate of grade 3 or 4 toxicities [14].

Ivanov described a case report of a 65-year-old DLBCL patient relapsed after four lines of chemotherapy that included high-dose chemotherapy plus autologous stem cell transplantation. Lenalidomide was administered at the dose of 15 mg per day for 21 days every 28 days in association with 375 mg/sqm rituximab on day one and 40 mg oral dexamethasone on day, one and four. Seven courses were administered, obtaining CR; patient was in remission at 20 months after the end of treatment [15].

Lenalidomide as single agent was also tested on transformed lymphoma, such as transformed follicular lymphoma and transformed chronic lymphocytic leukemia/small lymphocytic lymphoma. Thirty-three patients were treated with 25 mg lenalidomide for 21 days every 28 days; ORR was 46%, with a median response duration of 12.8 months. Median PFS was 5.4 months. Among 23 patents with transformed follicular lymphoma, ORR was 57%, with 26% of CR; among 7 patients with transformed chronic lymphocytic leukemia/small lymphocytic lymphoma, ORR was 0 and none reached CR. Neutropenia grade 3 and 4 were observed in 33% and 15%, respectively, grades 3-4 thrombocytopenia in 5% and grade 3 pneumonia in 3% [16].

4. Lenalidomide: Clinical Experience and New Options in Untreated DLBCL

The promising results of lenalidomide in relapsed/refractory DLBCL setting, encouraged the development of trial with this drug in first line treatment.

Considering that safety and efficacy of lenalidomide in combination with standard immunochemotherapy was

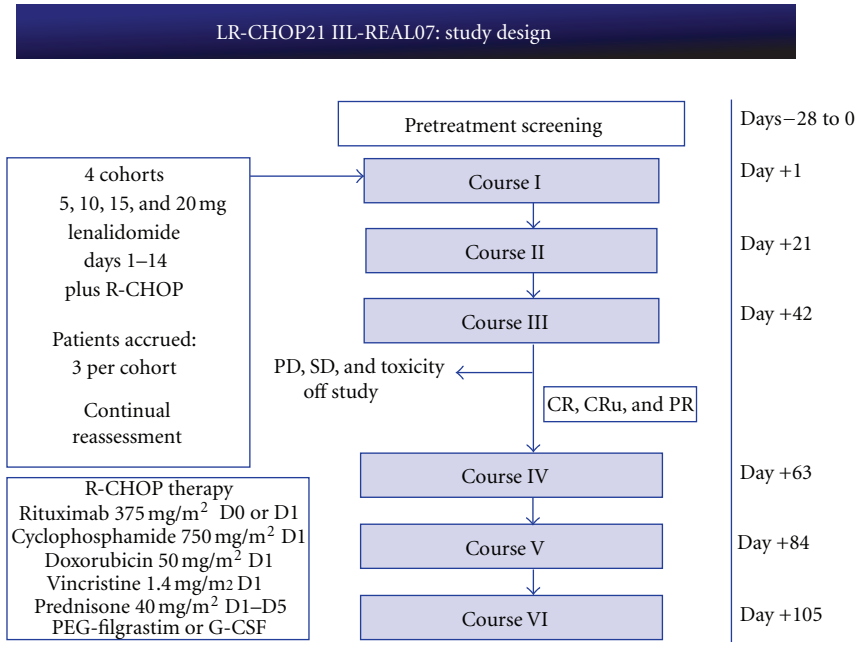


Figure 3: Phase I/II trial of Italian Lymphoma Foundation for elderly untreated DLBCL.

unknown, some phase I trials were drawn with the aim to define the Maximum Tolerated Dose (MTD) and the Dose-Limiting Toxicities (DLT) of lenalidomide in addition to standard therapy rituximab-CHOP. Nowakowski conducted a phase I/II study to define MTD and efficacy of lenalidomide administered on days 1–10 with standard R-CHOP chemotherapy (R2CHOP) in 24 newly diagnosed DLBCL and grade 3 follicular lymphomas; median age was 65 years (range 35–82) and 54% of patients were at low-intermediate IPI score. Lenalidomide dose escalation levels were 15 mg, 20 mg, and 25 mg. All patients received 6 mg pegfilgrastim on day 2 and aspirin prophylaxis. Dose-limiting toxicity (DLT) was defined as any grade 3 or higher nonhematological toxicity or a hematological toxicity resulting in a delay of the next cycle of chemotherapy. In the phase I, three patients received 15 mg, 3 patients 20 mg, and 18 patients 25 mg of lenalidomide; no DLT was found and 25 mg days 1–10 was the recommended dose for phase II. The incidence of grade 4 neutropenia or thrombocytopenia was 67% and 21%, respectively; no toxic deaths were recorded. ORR was 100% with CR in 77% of patients [17].

A similar schedule was tested by the GELA group; 27 patients affected by follicular lymphoma in 18, DLBCL in 4, mantle-cell lymphoma in 3, and indolent lymphoma in 2 were treated with oral lenalidomide on days 1–14 in association with R-CHOP given every 3 weeks for 6 cycles. Lenalidomide dose was increased from 5 mg to 25 mg (5 mg per dose level), using a 3 + 3 escalation design. Pegfilgrastim was administered on day 4 and oral aspirin prophylaxis (100 mg) was given daily during the treatment. Maximum-tolerated dose was determined by the number of DLT during the first 2 cycles. DLT was defined as grade 3 or more nonhematological toxicity, grade 3 hematological toxicity lasting more than 7 days, or grade 4 hematological toxicity lasting more than 3 days. Results showed that 25 mg was considered as the recommended dose. Most frequent adverse event was grade 3-4 neutropenia in 59%, including 7% of febrile neutropenia, and grade 3-4 thrombocytopenia in 30%. No grade 3-4 neurological toxicities were observed. One patient had pulmonary embolism of moderate severity and one patient has a deep vein thrombosis. Lenalidomide was stopped in five patients due to toxicity according to protocol defined criteria [18].

The Italian Lymphoma Foundation conducted a phase I-II trial to test the combination of lenalidomide plus R-CHOP (REAL07) in newly diagnosed DLBCL elderly patients not eligible to high-dose chemotherapy plus stem cell transplant. The treatment scheme is described in Figure 3. At the end of phase I part of the trial, MTD for lenalidomide in association with R-CHOP21 resulted as 15 mg days 1–15. The association was well tolerated, with grade 3-4 thrombocytopenia and neutropenia as expected and low rate of neurological toxicities [19].

Several studies with lenalidomide in association with standard treatment are ongoing in first line DLBCL patients. One of these, the REMARC study, is designed to demonstrate if a maintenance with lenalidomide after first line conventional chemoimmunotherapy may improve PFS compared to observation only.

5. Conclusions

The introduction of IMiDs in the treatment of DLBCL represented an improvement in the outcome of this patients. Lenalidomide represents a manageable drug, with good results in relapsed or refractory DLBCL patients heavily pretreated. The role of lenalidomide in association to standard

chemoimmunotherapy RCHOP in first line is under investigation, with promising results in term of feasibility, toxicity and with promising results in term of response. The activity of lenalidomide in histological subtypes at poor outcome, like in activated B-cell DLBCL, may be demonstrated in prospective trials.

Lenalidomide should be considered as conventional treatment in relapsed/refractory setting of patients in monotherapy or in association with rituximab and/or steroid. Ongoing trials should clarify the feasibility of lenalidomide in association with other drugs, such as with platinum containing regimens (oxaliplatinum-cytarabine or carboplatinum-idarubicine-etoposide) or with mTOR inhibitors (everolimus and temsirolimus) or with bendamustine or with monoclonal antibodies (GA-101). The role of maintenance of lenalidomide after first line chemoimmunotherapy should be established by ongoing trials.

Acknowledgment

U. Vitolo is supported by Advisory Committee of Roche Italy. Lecture-fee of Celgene and Janssen-Cilag.

References

[1] F. D. Groves, M. S. Linet, L. B. Travis, and S. S. Devesa, "Cancer surveillance series: non-Hodgkin's lymphoma incidence by histologic subtype in the United States from 1978 through 1995," *Journal of the National Cancer Institute*, vol. 92, no. 15, pp. 1240–1251, 2000.

[2] J. O. Armitage, "A clinical evaluation of the International Lymphoma Study Group classification of non-Hodgkin's lymphoma," *Blood*, vol. 89, no. 11, pp. 3909–3918, 1997.

[3] B. Coiffier, E. Lepage, J. Brière et al., "Chop chemotherapy plus rituximab compared with chop alone in elderly patients with diffuse large-B-cell lymphoma," *New England Journal of Medicine*, vol. 346, no. 4, pp. 235–242, 2002.

[4] B. Coiffier, C. Thieblemont, E. Van Den Neste et al., "Long-term outcome of patients in the LNH-98.5 trial, the first randomized study comparing rituximab-CHOP to standard CHOP chemotherapy in DLBCL patients. A study by the Groupe d'Etudes des Lymphomes de l'Adulte," *Blood*, vol. 116, no. 12, pp. 2040–2045, 2010.

[5] M. Pfreundschuh, J. Schubert, M. Ziepert et al., "Six versus eight cycles of bi-weekly CHOP-14 with or without rituximab in elderly patients with aggressive CD20+ B-cell lymphomas: a randomised controlled trial (RICOVER-60)," *The Lancet Oncology*, vol. 9, no. 2, pp. 105–116, 2008.

[6] U. Vitolo, A. Chiappella, E. Angelucci et al., "Dose-dense and high-dose chemotherapy plus rituximab with autologous stem cell transplantation for primary treatment of diffuse large B-cell lymphoma with a poor prognosis: a phase II multicenter study," *Haematologica*, vol. 94, no. 9, pp. 1250–1258, 2009.

[7] C. Gisselbrecht, B. Glass, N. Mounier et al., "Salvage regimens with autologous transplantation for relapsed large B-cell lymphoma in the rituximab era," *Journal of Clinical Oncology*, vol. 28, no. 27, pp. 4184–4190, 2010.

[8] S. Gaidarova, D. Mendy, C. Heise et al., "Lenalidomide induces capping of CD20 and cytoskeleton proteins to enhance rituximab immune recognition of malignant B-cells," *Blood*, vol. 116, abstract 2845, 2010.

[9] F. J. Hernandez-Ilizaliturri, N. Reddy, B. Holkova, E. Ottman, and M. S. Czuczman, "Immunomodulatory drug CC-5013 or CC-4047 and rituximab enhance antitumor activity in a severe combined immunodeficient mouse lymphoma model," *Clinical Cancer Research*, vol. 11, no. 16, pp. 5984–5992, 2005.

[10] N. Reddy, F. J. Hernandez-Ilizaliturri, G. Deeb et al., "Immunomodulatory drugs stimulate natural killer-cell function, alter cytokine production by dendritic cells, and inhibit angiogenesis enhancing the anti-tumour activity of rituximab in vivo," *British Journal of Haematology*, vol. 140, no. 1, pp. 36–45, 2008.

[11] P. H. Wiernik, I. S. Lossos, J. M. Tuscano et al., "Lenalidomide monotherapy in relapsed or refractory aggressive non-Hodgkin's lymphoma," *Journal of Clinical Oncology*, vol. 26, no. 30, pp. 4952–4957, 2008.

[12] T. E. Witzig, J. M. Vose, P. L. Zinzani et al., "An international phase II trial of single-agent lenalidomide for relapsed or refractory aggressive B-cell non-Hodgkin's lymphoma," *Annals of Oncology*, vol. 22, no. 7, pp. 1622–1627, 2011.

[13] F. J. Hernandez-Ilizaliturri, G. Deeb, P. L. Zinzani et al., "Higher response to lenalidomide in relapsed/refractory diffuse large b-cell lymphoma in nongerminal center b-cell-like than in germinal center b-cell-like phenotype," *Cancer*, vol. 117, no. 22, pp. 5058–5066, 2011.

[14] P. L. Zinzani, C. Pellegrini, L. Gandolfi et al., "Combination of lenalidomide and rituximab in elderly patients with relapsed or refractory diffuse large B-cell lymphoma: a phase 2 trial," *Clinical Lymphoma, Myeloma and Leukemia*, vol. 11, no. 6, pp. 462–466, 2011.

[15] V. Ivanov, E. Tabouret, G. Chuto et al., "Rituximab-lenalidomide-dexamethasone induces complete and durable remission in relapsed refractory diffuse large B-cell non-Hodgkin lymphoma," *Leukemia and Lymphoma*, vol. 51, no. 9, pp. 1758–1760, 2010.

[16] M. S. Czuczman, J. M. Vose, T. E. Witzig et al., "The differential effect of lenalidomide monotherapy in patients with relapsed or refractory transformed non-Hodgkin lymphoma of distinct histological origin," *British Journal of Haematology*, vol. 154, no. 4, pp. 477–481, 2011.

[17] G. S. Nowakowski, B. LaPlant, T. M. Habermann et al., "Lenalidomide can be safely combined with R-CHOP (R2CHOP) in the initial chemotherapy for aggressive B-cell lymphomas: phase I study," *Leukemia*, vol. 25, no. 12, pp. 1877–1881, 2011.

[18] H. Tilly, F. Morschhauser, G. A. Salles et al., "Phase I study of escalating doses of lenalidomide combined with R-CHOP (R2-CHOP) for front-line treatment of B-cell lymphomas," *Blood*, vol. 118, abstract 1632a, 2011.

[19] U. Vitolo, A. Chiappella, A. M. Carella et al., "Lenalidomide plus Rituximab-CHOP21 in elderly diffuse large B-cell lymphoma (DLBCL): results of phase I part of REAL07 trial of Italian Lymphoma Foundation (FIL)," *Annals of Oncology*, vol. 22, no. S4, abstract 331a, 2011.

Hindawi Publishing Corporation
Advances in Hematology
Volume 2012, Article ID 523842, 7 pages
doi:10.1155/2012/523842

Review Article

Therapeutic Activity of Lenalidomide in Mantle Cell Lymphoma and Indolent Non-Hodgkin's Lymphomas

Marco Gunnellini and Lorenzo Falchi

S. C. Oncohematology, Perugia University, S. Maria, 05100 Terni, Italy

Correspondence should be addressed to Lorenzo Falchi, lorenzo.falchi@gmail.com

Received 16 February 2012; Revised 29 March 2012; Accepted 16 April 2012

Academic Editor: Umberto Vitolo

Mantle cell lymphoma (MCL) comprises 3–10% of NHL, with survival times ranging from 3 and 5 years. Indolent lymphomas represent approximately 30% of all NHLs with patient survival largely dependent on validated prognostic scores. High response rates are typically achieved in these patients with current first-line chemoimmunotherapy. However, most patients will eventually relapse and become chemorefractory with poor outcome. Alternative chemoimmunotherapy regimens are often used as salvage strategy and stem cell transplant remains an option for selected patients. However, novel approaches are urgently needed for patients no longer responding to conventional chemotherapy. Lenalidomide is an immunomodulatory drug with activity in multiple myeloma, myelodisplastic syndrome and chronic lymphoproliferative disorders. In phase II studies of indolent NHL and MCL lenalidomide has shown activity with encouraging response rates, both as a single agent and in combination with other drugs. Some of these responses may be durable. Optimal dose of lenalidomide has not been defined yet. The role of lenalidomide in the therapeutic armamentarium of patients with indolent NHL or MCL will be discussed in the present paper.

1. Introduction

Non-Hodgkin's lymphomas (NHLs) are a heterogeneous group of lymphoid malignancies. The annual incidence of NHL in the United States is estimated to be 4.5% of all cancers, and they account for 3% of annual cancer-related deaths [1]. From a clinical and therapeutic standpoint, these neoplasias are subdivided into aggressive and indolent forms. Indolent lymphomas represent approximately 30% of all NHLs. Prognosis is correlated with the stage of the disease at the time of diagnosis, as well as to the international prognostic index (IPI) or other IPI-derived scores [2–5]. The current therapeutic approach for indolent NHL is based on the use of chemoimmunotherapy. Intensive treatments such as high-dose chemotherapy with autologous stem cell transplantation (ASCT) are typically reserved for relapsing patients whose disease is still chemosensitive [1].

Mantle cell lymphoma (MCL) comprises approximately 3 to 10% of NHL. It is a heterogeneous clinical entity with four recognized morphologic variants (i.e., classical, blastoid, pleomorphic and small cell, marginal zone-like). The small cell variant tends to be an indolent lymphoma, whereas both the blastoid and pleomorphic variants are associated with a clinical aggressive course.

However, the majority (80%) of MCLs show intermediate characteristics. Thus, the median survival of the majority of patients is in the range of 3 to 5 years, and very few patients are cured [2].

MCL patients typically respond well to initial treatment with an overall response rate of approximately 90%. The addition of rituximab to conventional chemotherapy has even improved both quality and durability of responses either in newly diagnosed or relapsed disease [6, 7].

However, most patients will eventually relapse, with shorter and shorter disease-free intervals, and will require multiple different therapeutic interventions during the course of their disease [8, 9]. For this reason, there is a need for new effective agents with novel mechanisms of action to be tested in these patients.

Table 1: Lenalidomide monotherapy trials.

Author	Year	Phase	Histology	*n*	Age (range)	Previous CT (range)	Rituximab refractory (%)	Schedule	ORR (%)	CR/CRu (%)	Median DR (months)
Wiernik et al. [10]	2008	II	Indolent NHL (10.2%)	49	65 (23–86)	4 (NR)	58	Lenalidomide 25 mg 21q28 days	60	20	6.2
			MCL (30.6%)						53	13.3	
Witzig et al. [11]	2009	II	Indolent NHL (100%)	43	63 (42–89)	3 (1–17)	67	Lenalidomide 25 mg 21q28 days	23	7	Not reached 16.5
Reeder et al. [12]	2009	II	MCL (100%)	54	69 (33–82)	3 (1–8)	NR	Lenalidomide 25 mg 21q28 days	43	17	NR
Habermann et al. [13]	2009	II	MCL (100%)	15	66 (45–84)	4 (2–7)	58	Lenalidomide 25 mg 21q28 days	53	20	Not reached 13.7
Witzig et al. [14]	2011	II	MCL (26.3%)	217	66 (21–87)	3 (1–13)	53.9	Lenalidomide 25 mg 21q28 days	42	21	Not reached 8.9

ORR: overall response-rate; CT: chemotherapy; CR/Cru: complete response/complete response unconfirmed; DR: duration of response; NR: not reported.

Table 2: Ongoing lenalidomide (L) monotherapy trials.

Name	Phase	Age	Histology	Drugs	Status
NCT00875667	II	>18 ys	Relapsed or refractory MCL	L	Ongoing and recruiting
NCT00737529	II	>18 ys	Relapsed or refractory MCL	L	Ongoing and recruiting
NCT00179673	II	>18 ys	Relapsed or refractory indolent NHL	L	Terminated

L: lenalidomide.

2. Rationale for and Development of Lenalidomide in Lymphoproliferative Disorders

Lenalidomide is an immunomodulatory drug (IMiD), derived from thalidomide, with increased potency and fewer side effects compared to its parent molecule. This agent has shown impressive clinical activity in patients with multiple myeloma (MM) [15] and has proven effective in chronic lymphocytic leukemia (CLL) [16] and T-cell lymphoma [17]. Preclinical models and preliminary clinical data also indicate significant antitumor activity of lenalidomide in B-cell malignancies [18, 19].

The mechanism of action of lenalidomide includes both immunomodulatory and nonimmunomodulatory effects [20–24]. It inhibits the production of proinflammatory cytokines (TNF-α, IL-1, IL-6, and IL-12) and enhances that of anti-inflammatory cytokine (IL-10) resulting in an increase of the tumor-cell apoptosis [20–22]. Lenalidomide also induces tyrosine phosphorylation of CD28, providing a costimulatory signal to T-cell activation by antigen-presenting cells via the B7 pathway [20–22]. IMiDs can decrease the expression of the angiogenic factors VEGF and IL-6 leading to a reduction of growth and survival of tumor cells [20–25]. Lenalidomide increases the number and Fc-γ receptor-mediated cytotoxicity of NK cells with an as-yet unclear mechanism of action [20]. Importantly, lenalidomide has also shown direct antiproliferative activity, in the absence of immune effectors, by decreasing erk1/2 and Akt2 and by inducing G0-G1 cell cycle arrest through inhibition of CDK2 activity [20–23]. Finally, in MM, lenalidomide has been shown *in vitro* to alter the microenvironment by downregulating cell surface adhesion molecules such as ICAM-1, VCAM-1, and E -selectin and inhibiting the adhesion of MM cell lines to the bone marrow stromal cells [20, 21].

3. Lenalidomide Monotherapy in Relapsed/Refractory Indolent and Mantle Cell Lymphoma

Oral lenalidomide monotherapy produces durable responses in patients with NHL with a manageable toxicity profile (Table 1). In a pilot study of relapsed/refractory aggressive NHL, also including 15 and 5 stage III follicular lymphoma (FL) patients, lenalidomide induced an objective response rate of 35% with 12% complete responses/unconfirmed complete responses [10]. Patients enrolled in the study had received a median of 4 prior therapies. Fifty eight percent of patients were rituximab refractory. The most frequent G3 toxicity was neutropenia. A dose reduction was necessary in 18 (37%) patients (9 patients to 20 mg, 5 patients to 15 mg, 3 patients to 10 mg, and 1 patient to a 5 mg daily dose). Eight patients (16%) discontinued treatment because of adverse events. In a second trial [11] of heavily pretreated indolent NHL patients (median number of prior lines 3 (1–17)), single-agent lenalidomide resulted in an ORR of 23% (27% in follicular and 22% in small lymphocytic lymphoma). Median duration of response was not reached with a followup of 15 to 28 months. Median PFS for the whole group was 4.4 months (95% CI, 2.5–10.4). The most frequent G3 toxicities were hematological (neutropenia, thrombocytopenia, and anemia). Seventeen patients (40%) had a dose reduction (6 patients to 20 mg, 3 patients to 15 mg, 6 patients to 10 mg, and 2 patients to a 5 mg daily dose). Eight patients (19%) discontinued treatment because of adverse events, and 1 died on treatment due to sepsis. In another study of relapsed/refractory MCL, lenalidomide showed an overall response rate (ORR) of 43%. Twenty six percent of the patients had received stem cell transplantation and 32% had been exposed to bortezomib. ORR in these two groups was 53% and 57%, respectively. The most common grade 3 or 4 adverse event was neutropenia (43%) [12]. In a smaller study including 15 patients, 58% of whom being rituximab refractory, objective responses were achieved in 53% of cases with a 20% complete remission (CR) rate [13, 26]. Eight patients (53%) had a dose reduction, but only 1 patient discontinued treatment. Finally, Witzig et al. [14] treated 217 aggressive relapsed/refractory NHLs, including 26% of patients with MCL. Median number of prior chemotherapy lines was 3 (1–13). MCL patients showed an ORR of 42% with a median progression-free survival (PFS) not reached. Fifty three (53%) of patients required dose reduction (37 patients to 20 mg, 11 patients to 15 mg, 9 patients to 10 mg, and 10 patients to a 5 mg daily dose), and 23% discontinued lenalidomide. Most G3-G4 toxicities recorded were hematological (41% neutropenia, 19% thrombocytopenia, and 9.2% anemia). Ongoing trials of single-agent lenalidomide in refractory indolent NHL and MCL are summarized in Table 2. While in untreated patients a lenalidomide daily dose of 25 mg may be appropriate (see Table 1), in patients with relapsed or refractory disease, particularly if they are elderly and/or suffering from other comorbidities, a lower dose (15 to 20 mg) appears to be a wiser choice. Of note, because of the proliferation of phase II studies with different starting doses, and the dose-escalating design of several ongoing lenalidomide trials, a dose that is considered "reasonable" may not necessarily be the optimal one.

Table 3: Lenalidomide containing regimens in relapsed/refractory indolent and mantle cell lymphoma.

Author	Year	Phase	Histology	*n*	Age (range)	Previous CT	Combination	ORR (%)	CR (%)
Wang et al. [27]	2007	I/II	MCL	15	73 (62–84)	2 (1–7)	RL	83*	17*
Dutia et al. [28]	2009	II	Indolent NHL	15	60 (50–91)	4 (1–11)	RL	83.3	41
Zaja et al. [29]	2011	II	MCL	33	68 (51–80)	3 (2–7)	LD	52	24

*: data from patients enrolled in lenalidomide 20 mg/daily arm. No response was obtained for lower dosage.
CT: chemotherapy; ORR: overall response rate; CR: complete response; RL: rituximab and lenalidomide; LD: lenalidomide and dexamethasone.

Table 4: Ongoing lenalidomide-based regime trials.

Name	Phase	Age	Histology	Drugs	Status
NCT01419795	II	>18 ys	Relapsed or refractory NHL after allo-SCT	RL	Ongoing and recruiting
NCT00238238	II	>18 ys	Relapsed follicular NHL	RL	Ongoing and recruiting
NCT00633594	I/II	>18 ys	Relapsed or refractory MCL	RVL	Ongoing and recruiting
NCT00553644 (27)	II	>18 ys	Relapsed or refractory MCL	LV	Ongoing and recruiting

RL: rituximab, lenalidomide; RVL: rituximab, bortezomib, lenalidomide; LV: lenalidomide, bortezomib.

4. Lenalidomide in Combination for Relapsed/Refractory Indolent and MCL

The Fc portion of rituximab mediates ADCC. Lenalidomide increases Fc-γ receptors on NK cell surface enhancing rituximab-mediated ADCC. Many trials have therefore evaluated the two drugs in combination (Tables 3 and 4). In a phase I/II study [27] of rituximab (375 mg/m^2 weekly for 4 doses) and escalating doses of lenalidomide (from 10 to 25 mg daily on days 1–21 of 28-day cycles for a total of 6 cycles) in relapsed or refractory MCL, no responses were observed in the 10 mg and 15 mg groups, while patients receiving 20 mg daily achieved an ORR of 83%, including 17% of complete responses. At the dose of 25 mg, a G3 hypercalcemia and a lethal neutropenic fever were observed. The recommended lenalidomide daily dose to be used in combination with rituximab in phase II trials was therefore established to be 20 mg. Dutia et al. [28] tested rituximab (375 mg/m^2 weekly for 4 doses plus 4 doses if no CR was reached) and lenalidomide (20 mg daily on days 1–21 of 28-day cycles) in heavily pretreated patients with indolent NHL. Treatment proved active and well tolerated (no dose reductions nor discontinuations were reported), particularly in patients with rituximab-refractory FL (response rate of 55%). In a recent phase II study that enrolled patients with MCL and either relapsed/refractory disease or ineligibility to intensive treatment, lenalidomide 25 mg daily for days 1–21 plus dexamethasone (40 mg on days 1, 8, 15, and 22) were given as postinduction consolidation therapy for 3 (patients in CR) or up to 12 (patients in partial remission/stable disease—PR/SD) cycles. Treatment was discontinued at CR or unacceptable toxicity. The study enrolled 33 patients. Median number of prior treatments was 3 (2–7). After a median followup of 16 months, median PFS and OS were 12 and 20 months, respectively, with median response duration of 18 months. Treatment was well tolerated, the most frequent toxicity being neutropenia (grade 3 in 25%, grade 4 in 28% of patients), leading to treatment interruption in two patients. Overall 9 serious adverse events were recorded, including one therapy-related fatal acute respiratory insufficiency [29]. Phase II trials are underway to test different combinations, notably including bortezomib plus lenalidomide [30].

5. Lenalidomide in Combination for Untreated Indolent and Mantle Cell Lymphoma

The combination of rituximab and lenalidomide has also been tested in previously untreated patients with indolent NHL and MCL (Tables 5 and 6). In an ongoing study of 30 patients with advanced-stage indolent NHL and indication for treatment, [31] rituximab (375 mg/m^2 on day 1 of each 28-day cycle) and lenalidomide (20 mg/day on days 1–21) for 6 cycles produced an ORR of 86% and an overall response rate (CRR) of 79%. Only 2 patients required treatment discontinuation due to toxicities leading the investigators to expand the originally planned accrual and include a total of 110 patients. In a study of 75 patients with indolent NHL [32], the same combination induced responses in 90% of patients with a 66% CRR. Only 5 patients discontinued treatment within the first two cycles due to toxicity. In a recent phase I trial [33], escalating doses of lenalidomide (from 5 to 25 mg once daily on days 1–14) were associated to R-CHOP21 (rituximab 375 mg/m^2, cyclophosphamide 750 mg/m^2, doxorubicin 50 mg/m^2, vincristine 1.4 mg/m^2 on day 1, and prednisone 100 mg/m^2 days 1–5 every 21 days) for 6 cycles in untreated B-cell lymphomas. Lenalidomide 25 mg was established as the recommended dose. The most frequent toxicity was hematological (grade 3-4 neutropenia in 59% of patients), 6 patients experienced cycle delay, and 5 discontinued treatment, but no toxic death occurred.

Strategies to build on the use of lenalidomide as a single agent appear the avenue to pursue. The role of dexamethasone is marginal, if any. On the contrary, the association of lenalidomide and rituximab appears to be feasible and shows encouraging activity in untreated and previously treated patients with indolent and MCL. The combination of lenalidomide with chemoimmunotherapy regimens such as R-CHOP is attracting, but both its feasibility and efficacy need to be tested in further prospective trials. Finally,

Table 5: Lenalidomide containing regimens in untreated indolent and mantle cell lymphoma.

Author	Year	Phase	Histology	*n*	Age (range)	Combination	ORR (%)	CR (%)
Fowler et al. [31]	2010	II	Indolent NHL	30	56 (36–77)	RL	86	79
Samaniego et al. [32]	2011	II	Indolent NHL	75	57 (35–84)	RL	90	66
Tilly et al. [33]	2011	I	NHL	27	NR	RL-CHOP	96	74

ORR: overall response rate; CR: complete response; NR: not reported; RL: rituximab and lenalidomide; RL-CHOP: rituximab, lenalidomide, cyclophosphamide, doxorubicin, vincristine, prednisone.

Table 6: Ongoing lenalidomide-based regime trials.

Name	Phase	Age	Histology	Drugs	Status
NCT01415752	II	>60 ys	Untreated MCL	RBV + RL	Ongoing and recruiting
NCT01316523	II	>18 ys	Untreated indolent NHL	RL	Ongoing and recruiting
NCT00695786	II	>18 ys	Untreated indolent NHL	RL	Ongoing and recruiting
FIL R2-B	II	>18 ys	Untreated indolent NHL	RBL	Ongoing and recruiting

RBV: rituximab, bendamustine, bortezomib; RL: rituximab, lenalidomide; RBL: rituximab, bendamustine, lenalidomide.

Table 7: Ongoing lenalidomide-maintenance trials.

Name	Phase	Age	Histology	Drugs	Status
NCT01035463	I/II	>19 ys	Relapsed or refractory NHL	R-BEAM + ASCT + mL	Ongoing and recruiting
NCT01035463	I/II	>18 ys	Relapsed or refractory NHL	R-BEAM + ASCT + mL	Ongoing and recruiting
NCT01254578	I	>18 ys	High-risk hematologic cancers after Allo-SCT	mL	Ongoing and recruiting
NCT01045928	I/II	>18 ys	NHL	R + mL	Ongoing not recruiting
NCT01021423	III	>18 ys	Untreated MCL	FCR or R-CHOP + mL	Ongoing not recruiting
IIL MCL0208	III	18–60 ys	Untreated MCL	R-BEAM + ASCT + mL	Ongoing and recruiting

R-BEAM: rituximab, BCNU, etoposide, ara-C, melphalan; ASCT: autologous stem cell transplantation; mL: lenalidomide maintenance; R: rituximab; FCR: fludarabine, cyclophosphamide; rituximab; R-CHOP: rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone.

the exploration of lenalidomide in other chemotherapy-free combination regimens is particularly fascinating and eagerly awaited.

6. Lenalidomide as Maintenance Therapy for MCL

With the aim of increasing disease control and survival, some authors have proposed a postinduction maintenance strategy for patients with MCL. One agent that proved successful in this context is rituximab [34]. Twenty-two untreated MCLs not candidate for autologous stem cell transplantation were treated with a maximum of 6 cycles repeated every 28 days of modified R-hyper-CVAD (rituximab, hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone) followed by rituximab maintenance (weekly doses every 6 months for a total of 4 courses). ORR and CRR were impressive (77% and 64%, resp.). In a recently presented multicenter phase III trial [35], 560 untreated elderly (>60 ys) patients not eligible for high-dose therapy were randomized to receive R-CHOP or rituximab, fludarabine, and cyclophosphamide followed by a maintenance phase with either rituximab or interferon-alfa. Rituximab maintenance doubled the remission duration compared to IFN (57% versus 26% at 4 years, resp., $P = 0.0109$). Not surprisingly, hematologic grade 3-4 toxicity was higher in the IFN arm. Overall survival did not differ between both maintenance arms ($P = 0.17$). Another randomized phase III trial evaluated the efficacy of lenalidomide versus placebo as maintenance therapy after first-line induction in patients with MCL not candidates for intensive treatment. Lenalidomide was given orally at the dose of 15 mg daily on days 1–21 every 28 days for 2 years, up to either disease progression or unacceptable toxicity, whichever occurred first. Only 9 patients (4 in CR and 5 in PR) were randomized (4 in the lenalidomide maintenance arm and 5 in the placebo). Two patients discontinued treatment due to toxicity and disease progression in the treatment and placebo arm, respectively. The study was prematurely terminated, and most analyses were not performed. A phase I/II Scandinavian trial [36] is ongoing in which lenalidomide is combined with rituximab (375 mg/m^2 on day 1) and bendamustine (90 mg/m^2 on days 1-2) as induction in untreated elderly (>65 years) MCL patients. Six 28-day induction cycles are followed by seven 28-day cycles of maintenance lenalidomide (25 mg daily on days 1–21). Recently, Ahmadi et al. [37] investigated the safety and efficacy of lenalidomide and rituximab in relapsed/refractory indolent or mantle cell lymphoma. Forty five sequential patients received two 28-day treatment cycles of lenalidomide 10 mg every day and four weekly doses of rituximab 375 mg/m2 in cycle 3 with (cohort 1) or without

(cohort 2) weekly dexamethasone. In stable and responding patients, lenalidomide and dexamethasone (cohort 1) or lenalidomide alone (cohort 2) was continued until disease progression or unacceptable toxicity (median number of prior therapies was 3 (1–7)). Thirty five patients were evaluable for response. At a median followup of 11.8 months, PFS was 73% (95% CI: 53–86%), and ORR was 60% (12 CR; 9 PR). ORR did not differ between cohort 1 and cohort 2, $P = 0.5$. Half of the patients temporarily suspended treatment, while 2 discontinued therapy. Several other trials of lenalidomide maintenance in patients with untreated or relapsed/refractory NHL are underway (Table 7).

7. Conclusions

Lenalidomide is an immunomodulatory agent with remarkable activity in a variety of lymphoproliferative disorders. Its value is well established in multiple myeloma, and increasing evidence supports its role in the management of CLL patients. Phase II data are also delineating a role for lenalidomide in NHL, including MCL and indolent NHLs. Response rates are encouraging with response lasting 6.2 to 16.5 months in relapsed NHL when used alone. The drug is generally tolerated with hematological adverse events being the most common toxicity, and no unexpected toxicities in numerically limited trials the optimal dose of lenalidomide in maintenance or in combination with other agents remains to be defined. Single-agent lenalidomide 25 mg may be appropriate in young untreated patients, while a lower dose (15 to 20 mg) should be considered in relapsed/refractory elderly patients. The route is traced out, but informative, randomized phase III trials with careful study design and adequate patient numbers will be necessary to define the role of lenalidomide in the therapeutic armamentarium of patients with NHL.

References

[1] K. Kaunshansky, M. Lichtman, E. Beutler, T. Kipps, J. Prchal, and U. Seligsohn, *Williams Hematology*, McGrow-Hill, 8th edition, 2011.

[2] S. H. Swerdlow, E. Campo, N. L. Harris et al., *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*, WHO Press, 4th edition, 2008.

[3] S. A. van de Schans, E. W. Steyerberg, M. R. Nijziel, G. J. Creemers, M. L. Janssen-Heijnen, and D. J. van Spronsen, "Validation, revision and extension of the follicular lymphoma international prognostic index (FLIPI) in a population-based setting," *Annals of Oncology*, vol. 20, no. 10, pp. 1697–1702, 2009.

[4] C. H. Geisler, A. Kolstad, A. Laurell et al., "The mantle cell lymphoma international prognostic index (MIPI) is superior to the international prognostic index (IPI) in predicting survival following intensive first-line immunochemotherapy and autologous stem cell transplantation (ASCT)," *Blood*, vol. 115, no. 8, pp. 1530–1533, 2010.

[5] L. H. Sehn, B. Berry, M. Chhanabhai et al., "The revised international prognostic index (R-IPI) is a better predictor of outcome than the standard IPI for patients with diffuse large B-cell lymphoma treated with R-CHOP," *Blood*, vol. 109, no. 5, pp. 1857–1861, 2007.

[6] H. Schulz, J. E. Bohlius, S. Trelle et al., "Immunochemotherapy with rituximab and overall survival in patients with indolent or mantle cell lymphoma: a systematic review and meta-analysis," *Journal of the National Cancer Institute*, vol. 99, no. 9, pp. 706–714, 2007.

[7] S. Sachanas, G. A. Pangalis, T. P. Vassilakopoulos et al., "Combination of rituximab with chlorambucil as first line treatment in patients with mantle cell lymphoma: a highly effective regimen," *Leukemia & Lymphoma*, vol. 52, no. 3, pp. 387–393, 2011.

[8] M. J. Kersten, "Radioimmunotherapy in follicular lymphoma: some like it hot...," *Transfusion and Apheresis Science*, vol. 44, no. 2, pp. 173–178, 2011.

[9] A. K. Gopal, J. G. Rajendran, T. A. Gooley et al., "High-dose [^{131}I]tositumomab (anti-CD20) radioimmunotherapy and autologous hematopoietic stem-cell transplantation for adults ≥ 60 years old with relapsed or refractory B-cell lymphoma," *Journal of Clinical Oncology*, vol. 25, no. 11, pp. 1396–1402, 2007.

[10] P. H. Wiernik, I. S. Lossos, J. M. Tuscano et al., "Lenalidomide monotherapy in relapsed or refractory aggressive non-Hodgkin's lymphoma," *Journal of Clinical Oncology*, vol. 26, no. 30, pp. 4952–4957, 2008.

[11] T. E. Witzig, P. H. Wiernik, T. Moore et al., "Lenalidomide oral monotherapy produces durable responses in relapsed or refractory indolent non-Hodgkin's lymphoma," *Journal of Clinical Oncology*, vol. 27, no. 32, pp. 5404–5409, 2009.

[12] C. B. Reeder, T. E. Witzig, P. L. Zinzani et al., "Efficacy and safety of lenalidomide oral monotherapy in patients with relapsed or refractory mantle-cell lymphoma: results from an international study (NHL-003)," *Journal of Clinical Oncology*, vol. 27, no. 15, supplement, abstract e19504, 2009.

[13] T. M. Habermann, I. S. Lossos, G. Justice et al., "Lenalidomide oral monotherapy produces a high response rate in patients with relapsed or refractory mantle cell lymphoma," *British Journal of Haematology*, vol. 145, no. 3, pp. 344–349, 2009.

[14] T. E. Witzig, J. M. Vose, P. L. Zinzani et al., "An international phase II trial of single-agent lenalidomide for relapsed or refractory aggressive B-cell non-Hodgkin's lymphoma," *Annals of Oncology*, vol. 22, no. 7, pp. 1622–1627, 2011.

[15] M. Roussel, T. Facon, P. Moreau, J. L. Harousseau, and M. Attal, "Firstline treatment and maintenance in newly diagnosed multiple myeloma patients," *Recent Results in Cancer Research*, vol. 183, pp. 189–206, 2011.

[16] M. Gentile, A. G. Recchia, E. Vigna et al., "Lenalidomide in the treatment of chronic lymphocytic leukemia," *Expert Opinion on Investigational Drugs*, vol. 20, no. 2, pp. 273–286, 2011.

[17] G. Dueck, N. Chua, A. Prasad et al., "Interim report of a phase 2 clinical trial of lenalidomide for T-cell non-hodgkin lymphoma," *Cancer*, vol. 116, no. 19, pp. 4541–4548, 2010.

[18] Z. Qian, L. Zhang, Z. Cai et al., "Lenalidomide synergizes with dexamethasone to induce growth arrest and apoptosis of mantle cell lymphoma cells in vitro and in vivo," *Leukemia Research*, vol. 35, no. 3, pp. 380–386, 2011.

[19] A. A. Chanan-Khan and B. D. Cheson, "Lenalidomide for the treatment of B-cell malignancies," *Journal of Clinical Oncology*, vol. 26, no. 9, pp. 1544–1552, 2008.

[20] V. Kotla, S. Goel, S. Nischal et al., "Mechanism of action of lenalidomide in hematological malignancies," *Journal of Hematology and Oncology*, vol. 2, article 36, 2009.

[21] N. Reddy, F. J. Hernandez-Ilizaliturri, G. Deeb et al., "Immunomodulatory drugs stimulate natural killer-cell function, alter cytokine production by dendritic cells, and inhibit angiogenesis enhancing the anti-tumour activity of rituximab in

vivo," *British Journal of Haematology*, vol. 140, no. 1, pp. 36–45, 2008.

[22] A. A. Chanan-Khan and B. D. Cheson, "Lenalidomide for the treatment of B-cell malignancies," *Journal of Clinical Oncology*, vol. 26, no. 9, pp. 1544–1552, 2008.

[23] D. H. Chang, N. Liu, V. Klimek et al., "Enhancement of ligand-dependent activation of human natural killer T cells by lenalidomide: therapeutic implications," *Blood*, vol. 108, no. 2, pp. 618–621, 2006.

[24] L. Wu, M. Adams, T. Carter et al., "Lenalidomide enhances natural killer cell and monocyte-mediated antibody-dependent cellular cytotoxicity of rituximab-treated $CD20^+$ tumor cells," *Clinical Cancer Research*, vol. 14, no. 14, pp. 4650–4657, 2008.

[25] L. Zhang, Z. Qian, Z. Cai et al., "Synergistic antitumor effects of lenalidomide and rituximab on mantle cell lymphoma in vitro and in vivo," *American Journal of Hematology*, vol. 84, no. 9, pp. 553–559, 2009.

[26] P. L. Zinzani, T. E. Witzig, J. M. Vose et al., "Confirmation of the efficacy and safety of lenalidomide oral monotherapy in patients with relapsed or refractory mantle-cell lymphoma: results of an international study (NHL-003)," *Blood*, vol. 112, abstract 262, 2008.

[27] M. Wang, L. Fayad, F. Hagemeister et al., "A phase I/II study of lenalidomide (Len) in combination with rituximab (R) in relapsed/refractory mantle cell lymphoma (MCL) with early evidence of efficacy," *Journal of Clinical Oncology*, vol. 25, no. 18, supplement, abstract 8030, 2007.

[28] M. Dutia, I. DeRoock, K. Chee et al., "R2: preliminary results of a phase II study of lenalidomide and rituximab in relapsed/refractory indolent non-Hodgkin's lymphoma (NHL)," *Blood*, vol. 114, abstract a1679, 2009.

[29] F. Zaja, S. De Luca, U. Vitolo et al., "Salvage treatment with lenalidomide and dexamethasone in relapsed/refractory mantle cell lymphoma: clinical results and effects on microenvironment and neoangiogenic biomarkers," *Haematologica*, vol. 97, no. 3, pp. 416–422, 2012.

[30] V. A. Morrison, S. Jung, J. L. Johnson et al., "A phase II trial of bortezomib plus lenalidomide for relapsed/refractory mantle cell lymphoma (MCL) (CALGB 50501)," *Journal of Clinical Oncology*, vol. 29, abstract TPS223, 2011.

[31] N. H. Fowler, P. McLaughlin, F. B. Hagemeister et al., "Complete response rates with lenalidomide plus rituximab for untreated indolent B-cell non-Hodgkin's lymphoma," *Journal of Clinical Oncology*, vol. 28, no. 15, supplement, abstract a8036, 2010.

[32] F. Samaniego, F. Hagemeister, P. Mclaughlin et al., "High response rates with lenalidomide plus rituximab for untreated indolent B-cell non-Hodgkin lymphoma, including those meeting GELF criteria," *Journal of Clinical Oncology*, vol. 29, abstract a8030, 2011.

[33] H. Tilly, F. Morschhauser, G. A. Salles et al., "Phase I study of escalating doses of lenalidomide combined with R-CHOP (R2-CHOP) for front-line treatment of B-cell lymphomas," *Blood*, vol. 118, no. 21, abstract a1632, 2011.

[34] V. P. Kenkre, W. L. Long, J. C. Eickhoff et al., "Maintenance rituximab following induction chemo-immunotherapy for mantle cell lymphoma: long-term follow-up of a pilot study from the wisconsin oncology network," *Leukemia & Lymphoma*, vol. 52, no. 9, pp. 1675–1680, 2011.

[35] J. C. Kluin-Nelemans, E. Hoster, J. Walewski et al., "R-CHOP versus R-FC followed by maintenance with rituximab versus interferon-alfa: outcome of the first randomized trial for elderly patients with mantle cell lymphoma," *Blood*, vol. 118, abstract a439, 2011.

[36] M. Jerkeman, A. Kolstad, A. Laurell et al., "Lenalidomide, bendamustine, and rituximab as first-line therapy for patients > 65 years with mantle cell lymphoma: the nordic lymphoma group MCL4 (LENA-BERIT) trial," *Journal of Clinical Oncology*, vol. 28, no. 18, supplement, abstract a18567, 2010.

[37] T. Ahmadi, E. A. Chong, A. Gordon et al., "Phase II trial of lenalidomide—rituximab +/− dexamethasone in relapsed or refractory indolent B-cell or mantle cell lymphomas resistant to rituximab," *Blood*, vol. 118, abstract a266, 2011.

Hindawi Publishing Corporation
Advances in Hematology
Volume 2012, Article ID 712613, 8 pages
doi:10.1155/2012/712613

Review Article

Lenalidomide before and after Autologous Hematopoietic Stem Cell Transplantation in Multiple Myeloma

S. A. Tuchman,[1] N. J. Chao,[2] and C. G. Gasparetto[2]

[1] *Division of Medical Oncology and Hematological Malignancies Program, Duke University Medical Center, Durham, NC 27710, USA*
[2] *Division of Cellular Therapy and Hematological Malignancies Program, Duke University Medical Center, Durham, NC 27710, USA*

Correspondence should be addressed to C. G. Gasparetto, cristina.gasparetto@duke.edu

Received 9 February 2012; Accepted 5 April 2012

Academic Editor: Antonio Palumbo

Although multiple myeloma remains incurable outside of allogeneic hematopoietic stem cell transplantation, novel agents made available only in the last few decades have nonetheless tremendously improved the landscape of myeloma treatment. Lenalidomide, of the immunomodulatory class of drugs, is one of those novel agents. In the non-transplant and relapsed/refractory settings, lenalidomide clearly benefits patients in terms of virtually all meaningful outcomes including overall survival. Data supporting the usage of lenalidomide as part of treatment approaches incorporating high-dose chemotherapy with autologous stem cell support (ASCT) are less mature as pertains to such long-term outcomes and toxicity, and lenalidomide is not currently approved by regulatory agencies for use in the context of ASCT in either the United States or Europe. That said, relatively preliminary efficacy data describing lenalidomide as a component of ASCT-based treatment approaches to MM are indeed promising, and consequently lenalidomide's role in ASCT-based treatment strategies is growing. In this review we summarize existing data that pertains to lenalidomide in the specific context of ASCT, and we share our thoughts on how our own group applies these data to approach this complex issue clinically.

1. Introduction

Multiple myeloma (MM) is a malignancy of plasma cells that strikes roughly 20,000 and kills 10,000 US Americans yearly [1]. Outside of allogeneic stem cell transplantation, MM remains incurable, albeit increasingly treatable, thanks to the advent of novel agents including those that are currently approved by the United States Food and Drug Administration (FDA) and European Medicines Agency (EMA)—bortezomib, thalidomide, and lenalidomide.

Regarding the latter, the initial phase one study of lenalidomide (Revlimid), then known as CC-5013, first appeared in the scientific literature in 2002 and attention rapidly focused on CC-5013's activity even in multiply relapsed and refractory MM (RRMM) [2]. That study and others led to the later definitive phase three MM-009 and 010 trials, which showed overall survival benefits for RRMM patients on lenalidomide and dexamethasone in contrast to those on dexamethasone alone [3, 4]. FDA and EMA approval for lenalidomide followed, and lenalidomide and dexamethasone became established as a standard of care for RRMM. Subsequent clinical trials have further explored the role of lenalidomide as a part of treatment strategies for newly diagnosed MM (NDMM) that both include or exclude high-dose therapy with autologous hematopoietic stem cell transplantation (ASCT), although lenalidomide remains without approval for usage in the setting of ASCT by either FDA or EMA. In the current discussion, we focus on lenalidomide specifically as part of ASCT-based therapy approaches for MM.

2. Lenalidomide Induction prior to ASCT

The combination of dexamethasone and thalidomide compared favorably, in a retrospective study, with the earlier induction standard of vincristine, doxorubicin (Adriamycin), and dexamethasone (VAD), and thalidomide plus dexamethasone became an attractive therapeutic option for NDMM. However, significant nonhematological toxicity

Table 1: Select lenalidomide-based, pre-ASCT induction regimens for NDMM.

RD	Lenalidomide 25 mg orally days 1–21 and dexamethasone 40 mg orally days 1–4, 9–12, 17–20. 28 day cycles [9]
Rd	Lenalidomide 25 mg orally days 1–21 and dexamethasone 40 mg orally weekly. 28 day cycles [9]
BiRD	Clarithromycin 500 mg orally twice daily continuously, starting on day 2 of cycle 1; lenalidomide 25 mg orally days 3–21 of cycle 1, then days 1–21 of later cycles; dexamethasone 40 mg orally days 1, 2, 3, 8, 15, and 22 of cycle 1, then days 1, 8, 15, and 22 of later cycles. 28 day cycles [10]
RVD	Lenalidomide 25 mg orally days 1–14; bortezomib 1.3 mg/m^2 IV days 1, 4, 8, 11; dexamethasone 20 mg orally days 1, 2, 4, 5, 8, 9, 11, 12. 21 day cycles [11]
CRD	Cyclophosphamide 300 mg (fixed dose) orally days 1, 8, and 15; lenalidomide 25 mg orally days 1–21; dexamethasone 40 mg orally days 1, 8, 15, 22. 28 day cycles [12]
RVCD	Lenalidomide 25 mg orally days 1–14; bortezomib 1.3 mg/m^2 IV days 1, 4, 8, 11; cyclophosphamide 500 mg/m^2 orally days 1 and 8; dexamethasone 40 mg orally days 1, 8, 15. 21 day cycles [13]
RVDDoxil	Lenalidomide 25 mg orally days 1–14; bortezomib 1.3 mg/m^2 IV days 1, 4, 8, and 11; dexamethasone 20 mg orally days 1, 2, 4, 8, 11, and 12 for cycles 1–4 and 10 mg on the same schedule for later cycles; liposomal doxorubicin 30 mg/m^2 IV on day 4. 21 day cycles [14]
CarRD	Carfilzomib 20–36 mg/m^2 days 1, 2, 8, 9, 15, 16; lenalidomide 25 mg orally days 1–21; dexamethasone 20–40 mg days 1, 8, 15, and 22. 28 day cycles (dose of carfilzomib and dexamethasone was variable in this phase 1/2 study) [15]

affected a large proportion of patients [5]. Thalidomide's efficacy and toxicity both engendered interest in lenalidomide as a possibly more potent and less toxic analog of thalidomide that could replace thalidomide in both the ASCT and non-ASCT settings.

Lenalidomide's activity in NDMM pre-ASCT was unmistakable from the outset. In its first major, phase 2 study, namely, that of the RD regimen (lenalidomide and high-dose dexamethasone; see Table 1 for details regarding regimens), 91% of patients responded and 44% proceeded to ASCT after four cycles. Toxicity was excessive, and the toxicity profile in general resembled that seen in prior trials of high-dose dexamethasone alone. The follow-up randomized trial by the Southwest Oncology Group (SWOG) of three cycles of dexamethasone alone versus RD induction, followed in each arm by the same drugs given at lower doses as maintenance, confirmed lenalidomide's activity in NDMM, manifesting as significantly increased response rates. However, that trial also provided clear evidence of lenalidomide's toxicity when used with high-dose dexamethasone, for example, a 23.5% rate of venous thromboembolic events for RD despite aspirin prophylaxis versus 5% for dexamethasone alone ($P < 0.001$) [6]. These observations of high activity and toxicity early on in the SWOG study gave rise to subsequent study of lenalidomide with lower dose, that is, weekly, dexamethasone, largely as a result of requests by patient advocacy groups [7, 8].

The ECOG's E4A03 study ($n = 445$) included both ASCT candidates and noncandidates and was designed with the primary endpoint of testing noninferiority of lenalidomide given with low-dose (weekly) dexamethasone (i.e., the Rd regimen; Table 1) to RD. Patients on RD demonstrated more objective responses than patients taking Rd (overall response rate ORR 81% versus 70%, $P = 0.008$; Figure 1), but at the price of inferior one-year overall survival (87% one-year overall survival OS for RD versus 96% for Rd, $P = 0.0002$). Closer inspection reveals that the increased mortality with RD was likely associated with the higher rate of grade 3 or greater venous thromboembolic events, infection, or cardiac complications than Rd and that toxicity occurred primarily in the first four months of therapy. In terms of ASCT, 39.5% of patients in this study attempted ASCT after four cycles of induction, 98% of whom did so successfully. Among ASCT patients, median three-year OS was 92% and similar between the RD and Rd groups. RD and Rd both emerged as clearly effective regimens for pre-ASCT induction. Although the OS one-year benefit to Rd has resulted in the more widespread usage of low-dose dexamethasone than high-dose, for patients going to ASCT, one should recall that the survival benefit with Rd was specifically in patients not going for ASCT [9].

Since initial reports on E4A03, investigators have sought to build on the lenalidomide/dexamethasone backbone to create even more efficacious pre-ASCT regimens. Several have been described, and the result comprises a significant contribution to the increasingly complex combinations that constitute modern oncology; BiRD, RVD, CRD, RVCD, and RVDDoxil are perhaps the most robustly described examples. An overview discussion of each of these regimens follows. The reader will note the paucity of head-to-head studies of most of these regimens, and this discussion hence largely limits itself to comparisons of single-arm trials. The important caveats of cross-trial comparisons therefore apply: selection bias (i.e., differences in patient selection both for trial participation and for later ASCT), variable durations of planned duration of protocol therapy and followup, and reporting of different, often surrogate endpoints, among other limitations. We offer Figure 1 partially to visually summarize available data, but also to underscore the difficulty, if not impossibility, of selecting the "correct" induction regimen based on what we know about these combinations.

Starting with BiRD, Niesvizky et al. sought to improve upon their earlier experience with the combination of thalidomide, dexamethasone, and the macrolide antibiotic clarithromycin (Biaxin), the latter of which had preclinical data supporting both independent cytotoxicity and potentiation of dexamethasone's cytotoxic effect in MM [16]. Building on Rd, this group devised BiRD—Rd plus twice daily clarithromycin (Table 1). In a single-arm trial ($n = 72$),

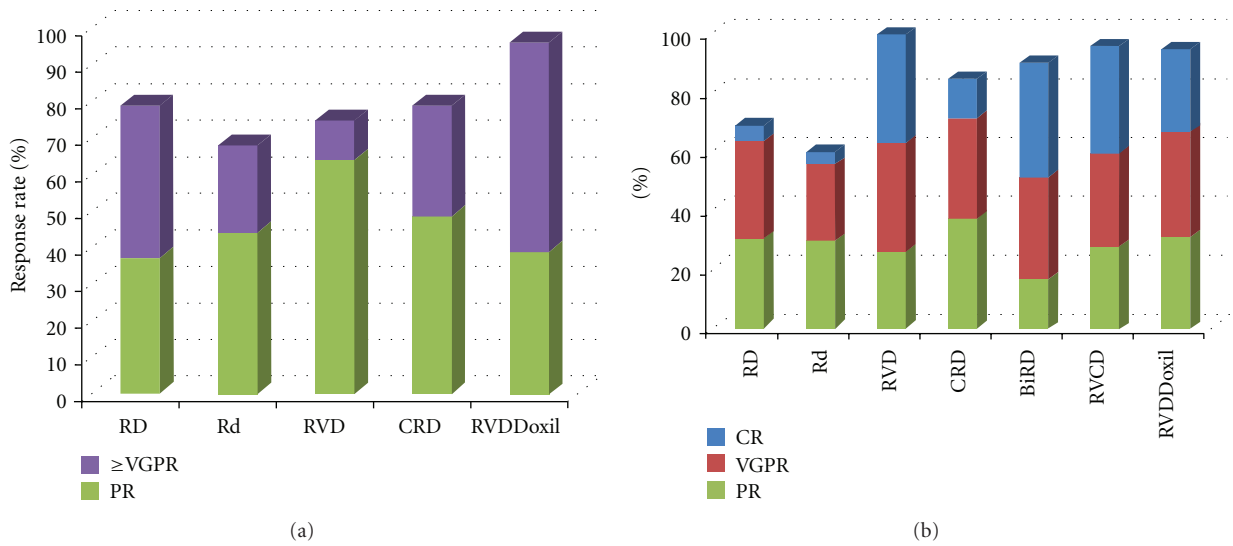


Figure 1: Reported response rates for lenalidomide-based induction regimens for MM. Rates depicted are those that could be ascertained either directly using reported data or as calculated using reported data. (a) Response rates after four cycles of therapy. Deeper response rates are not displayed due to inconsistent reporting in referenced sources. (b) Best response reached on study. Rates after four cycles could be envisioned as a measure of expected response pre-ASCT, whereas best response rate may represent a regimen's maximum potential, but only after more cycles than a patient would usually be administered as pre-ASCT induction. Data was gleaned from the following sources: RD and Rd [9]; RVD [11]; CRD [12]; BiRD [10]; and RVDDoxil [14].

90.3% of patients had an objective response with 73.6% of patients achieving a very good partial response (VGPR) or better (Figure 1). 25% of patients underwent ASCT after four or more cycles with a 5.5% (one patient) mortality rate. Two-year event-free survival for the ASCT group was 85.2% [10]. A separate case-control study comparing this cohort to matched patients who received Rd showed that BiRD was associated with notably deeper responses with induction (e.g., 73.6% versus 33.3% VGPR or better, $P < 0.001$) and a statistically insignificant trend toward improved OS. Grade 3 or greater toxicity was also increased with BiRD and was largely hematological. Important differences in these trials are worth noting, including that the median duration of therapy for BiRD was longer than Rd (11.8 versus 6 months) and BiRD patients undergoing ASCT did so much later (13.5 versus 5.9 months). The authors state that it was unclear to them why BiRD patients remained on therapy so much longer than Rd patients, but it stands to reason that the longer duration of therapy augmented both toxicity and ORR [17].

RVD (lenalidomide, bortezomib, and dexamethasone; Table 1)—a logical extrapolation of Rd to incorporate the proteasome inhibitor bortezomib—has perhaps gained the widest implementation by community oncologists after the initial phase 1/2 study demonstrated a 100% ORR and 74% VGPR or better rate in 35 phase 2 patients receiving a median of 10 cycles at the maximum tolerated dose (MTD) established in the earlier, phase 1 component of the study (Figure 1). 28 (42%) of all patients on protocol underwent ASCT at some point after cycle four with no significant difficulties reported. Among ASCT patients, the authors observed a 100% ORR with 57% of patients attaining VGPR or better. The median PFS for all patients at 18 months was 75%, and median OS had not been reached at the time of publication [11].

With CRD (cyclophosphamide, lenalidomide, dexamethasone; Table 1), investigators again sought to improve lenalidomide/dexamethasone, this time by adding weekly oral cyclophosphamide in three weeks of a four week cycle. In this single arm trial ($n = 53$), the ORR was 85% with 47% of patients achieving VGPR or better (Figure 1). 58% of patients at some point went on to attempt ASCT, but, in 25% of cases, hematopoietic stem cell mobilization with G-CSF was unsuccessful. The majority of those patients could be salvaged using either cyclophosphamide or plerixafor. In those patients that actually underwent ASCT, no unexpected complications were noted, and, in all patients, the median duration of response was 30.9 months. In general, the regimen was well tolerated, with the main toxicity being neutropenia; almost 60% of patients experienced grade 3 or 4 neutropenia [12].

RVCD has also been tested, with the idea of combining lenalidomide, bortezomib, cyclophosphamide, and dexamethasone into a single pre-ASCT induction regimen. In the initial phase 1 EVOLUTION study—the single randomized study available for all the induction regimens under discussion—patients received RVD ($n = 42$), RVCD ($n = 41$), or VCD (bortezomib, cyclophosphamide, and dexamethasone; $n = 32$) (Table 1). An update for the subsequent phase 2 component of this trial was presented at the American Society of Hematology meeting in 2010, in which additional 17 patients were given modified VCD (mVCD), in which the week three treatment break for cyclophosphamide was eliminated. Ultimately all regimens proved to have significant activity as measured by ORR, ranging from 78% (VCD) to 100% (mVCD) and ≥VGPR rates ranging from

41% (VCD) to 90% (RVCD and mVCD; Figure 1). Toxicity was similar and manageable across all groups. At the time of the first paper's publication, 13 of the original 25 patients had undergone ASCT with only one patient requiring a second attempt at stem cell collection. Specific details regarding mobilization are not yet reported, nor is longer-term followup available for this trial [13, 18].

Lastly, RVDDoxil—that is, RVD with liposomal doxorubicin (Doxil; Table 1)—has also been examined in the context of pre-ASCT induction for NDMM. In the published phase 1/2 study ($n = 72$ evaluable patients), 39 patients were treated at what was found to be the MTD. 58 patients (81%) underwent stem cell collection after a median of 3 to 8 cycles, 40 of whom (69%) received cyclophosphamide, plerixafor, or both in addition to standard G-CSF for stem cell mobilization. 49 patients (68%) proceeded to ASCT after four to eight cycles of RVDDoxil. ORR in all patients (ASCT and non-ASCT) receiving the MTD was 95% with 64% achieving VGPR or better at any point (Figure 1). ASCT proceeded without unexpected complications in all patients. Long-term followup is unavailable, but 18-month PFS for all patients was 81.6%; 93.5% for patients who underwent ASCT and 64.3% for patients who did not. Similar to the other studies discussed, hematological toxicity, neuropathy, fatigue were the primary manifestations of toxicity, although they were generally manageable with appropriate dose-reductions [14].

With the exception of perhaps EVOLUTION, these clinical trials will likely not greatly aid clinicians in sorting out the obvious question of which induction regimen is best for the patient moving toward ASCT. Future comparative studies with long-term followup of meaningful endpoints are critical, particularly as the picture becomes even more complex with upcoming trials looking at combinations of the latest generation of novel agents, such as carfilzomib and pomalidomide. Only the earliest data exist as of yet for those agents in the pre-ASCT setting, but those data suggest that these agents too can induce very deep responses pre-ASCT. Jakubowiak et al., for example, reported their pilot study in an oral abstract detailing carfilzomib, lenalidomide, and dexamethasone (CarRD—our abbreviation; Table 1) as induction therapy for NDMM. CarRD preliminarily appears to be at least as potent as the established regimens with published data, with 65% of patients reaching VGPR or better [15].

2.1. Stem Cell Mobilization and Collection after Lenalidomide-Based Induction. Stem cell mobilization into the peripheral blood and subsequent stem cell collection is the critical prelude to ASCT, with the usual aim of collecting enough cells to perform two ASCTs. Given that one of lenalidomide's most common toxicities is myelosuppression, from early on investigators have considered whether lenalidomide could damage hematopoietic stem cells and hinder G-CSF-induced mobilization. Further studies have examined whether cyclophosphamide or plerixafor could be used to overcome difficulties in mobilization that may be linked to lenalidomide-based induction.

Kumar et al. retrospectively reviewed 376 eligible patients who had undergone stem cell collection within 12 months of starting MMf therapy. 12.8% of patients had received lenalidomide and dexamethasone-based induction, whereas the others received VAD, thalidomide + dexamethasone, or dexamethasone alone. For mobilization, 64.3% of all patients received G-CSF alone and 33.6% received G-CSF with cyclophosphamide. The decision to employ the latter was made based on whether patients appeared to have "active disease," defined as the presence of circulating plasma cells at the time of mobilization. Of patients receiving G-CSF alone, three completely failed to mobilize—all had received lenalidomide for greater than six months. Furthermore, day one collection yield and total daily collection of stem cells correlated inversely with duration of lenalidomide therapy. Among patients who received cyclophosphamide-based mobilization, only five previously took lenalidomide as part of their induction. Despite impaired mobilization, however, no difference in engraftment kinetics was evident (denoting length of time until peripheral blood cell count recovery after reinfusion of stem cells) [19]. Other retrospective studies have since confirmed the link between lenalidomide and impaired mobilization. That said, the duration dependency has not been evident in all studies, meaning that a longer duration of lenalidomide therapy in some studies has not predicted greater difficulty with mobilization [20, 21]. Given the episodic difficulty of G-CSF mobilization after lenalidomide induction, subsequent studies have looked at cyclophosphamide and plerixafor as potential tools for overcoming difficulties with mobilization.

Cavallo et al. prospectively studied 346 patients who had received four cycles of Rd followed by G-CSF and cyclophosphamide for mobilization. In 21% of patients, adequate stem cells for two ASCTs could not be collected on the first attempt; they therefore went on to a second cyclophosphamide- and G-CSF-based mobilization. 8% of patients still had inadequate cells for even one ASCT after the second attempt and hence could not undergo ASCT. An additional 9% had enough cells for only one transplant, that is, 17% of patients had what would be considered a suboptimal collection using the gold standard mentioned. Engraftment kinetics were unimpaired. With 91% of patients achieving a successful mobilization at least for one ASCT, however, four cycles of Rd followed by mobilization with G-CSF and cyclophosphamide were felt by the authors to be a reasonable strategy for patients going for ASCT.

The C-X-C chemokine receptor type 4 (CXCR4) antagonist plerixafor may also mitigate lenalidomide-related impairment of stem cell mobilization. In one study, plerixafor was given with G-CSF as an initial attempt at mobilization ($n = 20$) or for remobilization in the case of an initial failed stem cell mobilization ($n = 40$) and results were retrospectively studied. Patients in both groups had received a median of roughly four cycles of lenalidomide-containing induction (range 1–20). 5% of patients receiving front-line plerixafor versus 52.5% of patients receiving it as a remobilization strategy failed to achieve the goal of collection for two ASCTs, although for most patients collection was adequate for at least a single ASCT. It appeared that patients

undergoing remobilization who had received >3 cycles of lenalidomide induction had a greater incidence of mobilization failure despite plerixafor, although small sample sizes precluded drawing definitive conclusions. Engraftment kinetics were again acceptable. In summary, it appears that plerixafor can to some degree overcome lenalidomide-related impairment of stem cell mobilization, but not entirely [22].

2.2. Lenalidomide in the Pre-ASCT Setting: Our Approach. Our approach to lenalidomide in the induction setting for ASCT patients is as follows. Existing data support, albeit not definitively, the concept that deep remissions going into ASCT are a desirable aim—in many studies, they correlate with long-term survival. Clearly, deep remissions in an individual patient could reflect either disease biology OR therapy selection, and so a causal link between induction therapy selection and OS is currently lacking. We would refer the reader to astute discussions on this controversial topic that have been published already [23–25]. Caveats notwithstanding, we believe that the extremely high response rates seen with short-course, initial induction regimens such as those discussed above, taken in combination with early hints at unprecedented post-ASCT PFS durations and manageable toxicity, will eventually translate into improvements in OS as well. Furthermore, limiting the duration of therapy limits toxicity in general, including perhaps lenalidomide-mediated impairment of stem cell collection. For that reason, we believe in "hard and fast" induction, in which we most commonly offer triple-drug regimens to fit patients prior to ASCT—either RVD as noted above, or cyclophosphamide, bortezomib, and dexamethasone, depending on clinical circumstances. We usually do not utilize four-drug regimens, such as RVCD or RVDDoxil, because response rates do not seem to be markedly improved as compared to three-drug regimens (Figure 1), yet the potential for toxicity generally rises.

Other groups have reported on other pre-ASCT triplet regimens such as bortezomib, thalidomide, and dexamethasone [26]; bortezomib, doxorubicin, and dexamethasone [27]. Those are also valid and well-tested options, but a comprehensive discussion of all described pre-ASCT induction regimens goes beyond the scope of this lenalidomide-focused paper. Truly, with the plethora of currently available data including unfortunately very few randomized trials, many of these induction regimens could be argued for. Consequently selection of a regimen presently depends heavily on provider preference and patient comorbidities. Randomized clinical trials are clearly needed to sort through the existing equipoise, so the field can move beyond personal and institutional preferences into an era of evidence-based selection of induction regimens.

Whatever the specific regimen, we optimally limit duration of therapy to four but no more than six cycles of any lenalidomide-containing induction prior to stem cell collection. For patients who do receive lenalidomide with their induction, we generally mobilize stem cells with G-CSF and cyclophosphamide 4 g/m^2, and we add plerixafor in cases of poor mobilization with the first two agents.

2.3. Lenalidomide Consolidation and Maintenance after ASCT. Early studies investigating the long-term usage of agents such as thalidomide and interferon-alpha were challenging, in the sense that interferon was overly toxic with minimal benefit [28] and thalidomide, although perhaps more efficacious, was also toxic and most patients could not tolerate it on the long term [29–31]. With the idea that lenalidomide may offer a more potent, less toxic maintenance therapy, several studies have examined the role of lenalidomide after ASCT. Followup of the two major trials driving the current discussion remains of relatively short duration, and the most recent data are only available in abstract form at the time at which we write this paper.

The CALGB 100104 trial has generated considerable excitement for lenalidomide maintenance. 568 patients who had received a variety of induction regimens usually including at least one novel agent and who had stable disease or better after single ASCT were randomized to either lenalidomide or placebo maintenance given at 5–15 mg daily, based on tolerance. An initial benefit of almost doubling of time to progression led to unblinding and cross-over to lenalidomide for 87% of placebo patients [32]. Data presented early in 2011 in an oral abstract supported an OS benefit based on an intention-to-treat analysis despite the crossover; 9% of lenalidomide patients died versus 16.1% of placebo patients with a median followup of 17.6 months ($P < 0.019$). Exploratory analyses suggest that the benefit of lenalidomide was present regardless of beta-2-microglobulin level but statistical interactions between the effect of lenalidomide and other risk factors, such as cytogenetic or fluorescent in situ hybridization (FISH) abnormalities, have not yet been reported [33].

Conversely, the Intergroupe Francophone du Myelome (IFM) 2005-02 lenalidomide maintenance trial has not confirmed the prolongation of OS despite longer median followup of 34 months. In this study, 614 patients who received either VAD +/− DCEP (dexamethasone, cyclophosphamide, etoposide, and cisplatin), or bortezomib and dexamethasone, as induction prior to ASCT were administered two cycles of lenalidomide consolidation, at 25 mg daily for three of four weeks. Subsequently patients were randomized to placebo or continuous lenalidomide 10–15 mg daily until relapse. The trial completed in mid-2010 with 34 months of median followup after randomization. Although lenalidomide almost doubled PFS (42 versus 24 months, hazard ratio HR 0.46, $P < 10^{-8}$), definitive evidence for an OS benefit has not yet been reported. To our knowledge, at the time of writing this paper, subgroup analyses (i.e., examination of outcomes in patients with high-versus standard-risk MM) are not yet available [34].

Further data will be forthcoming from ongoing trials, such the Blood and Marrow Clinical Trials Network (CTN) 0702 protocol studying patients who have completed induction and who are then randomized to single ASCT, tandem ASCT, or single ASCT followed by four cycles of RVD consolidation. Additionally, a cooperative study between the Dana-Farber Cancer Institute and IFM is investigating shorter-course RVD pre- and post-ASCT versus longer RVD induction without ASCT as part of the initial treatment

strategy. In all arms for both studies, patients will receive lenalidomide maintenance. These trials and others will help to clarify the role of lenalidomide for patients undergoing ASCT.

2.4. Lenalidomide Maintenance and Secondary Malignancies. Although maintenance lenalidomide is tolerable for patients, generally causes few symptoms, and carries likely clinical benefits as pertains to long-term outcomes, it may come at the price of secondary malignancies (SMs).

The above CALGB trial demonstrated more SMs in patients on lenalidomide maintenance: 7.7% of 231 patients on lenalidomide versus 1.7% of patients on placebo [33]. The IFM trial similarly showed SMs in 23 of 306 patients (7.5%) on lenalidomide versus 6 of 302 patients (2%) on placebo maintenance [34]. In both trials, SMs constituted a diverse collection of hematological and solid tumors. Further data from the IFM trial preliminarily hint at two key factors: (1) the increased incidence of SMs emerged most prominently 24 months after randomization; (2) in multivariate analysis, predictors of SMs included not only lenalidomide maintenance, but also advanced age, high ISS stage, male gender, and DCEP induction therapy. Cytogenetics did not predict SMs [34]. Current analyses are interrogating to what extent the inclusion of the more leukemogenic DCEP regimen on the IFM trial could explain at least part of these differences, but the continued controversy on this subject is highlighted by the fact that that placebo patients on the CALGB trial were offered cross-over into lenalidomide maintenance, whereas the IFM has stopped lenalidomide in that study, notably after patients had received 24 months of lenalidomide maintenance already. Further clues regarding the development of SMs may come from three other large trials of prolonged lenalidomide in non-ASCT candidates with MM, in which a very low incidence of SMs has been observed [35, 36]. As an example, Palumbo et al. reported their non-ASCT trial in which patients were randomized to melphalan and prednisone (MP); melphalan, prednisone and lenalidomide induction without maintenance (MPR); or MPR induction followed by lenalidomide maintenance (MPR-R). SMs occurred in 2 of 153, 6 of 152, and 4 of 150 patients on MP, MPR, and MPR-R, respectively. These rates were statistically equivalent [36]. Given these data showing virtually no increase in SMs in non-ASCT patients on lenalidomide long term, it has been hypothesized that the high-dose alkylator (i.e., melphalan) may play a key role in the development of post-ASCT SMs when lenalidomide maintenance is employed.

2.5. Lenalidomide Post-ASCT: Our Approach. Our group favors maintenance therapy after ASCT. The doubling of PFS in most trials with lenalidomide and the OS benefit in the CALGB trial weigh heavily in favor of that agent despite the small but real risk of SMs after ASCT. It is germane to the discussion of our practice to also mention that bortezomib too has growing evidence favoring its use in maintenance, especially in high-risk patients. When given at some point during ASCT-based therapy (in some trials only during induction, in others as maintenance), bortezomib mitigates, but does not eliminate entirely, the poor-prognosis implications of genetic markers such as the t(4; 14) chromosomal translocation [37], and, more recently, deletion of 17p in a trial by the HOVON cooperative group [38]. As a result of these emerging data, our general practice is to employ lenalidomide in the majority of MM patients after ASCT who have standard-risk cytogenetics and FISH, regardless of depth of response, and bortezomib in patients with high-risk markers such as 17p deletion. We do not prespecify a particular duration of maintenance with either agent, although data from ongoing maintenance trials may show in the future that limiting the time length of maintenance therapy may be beneficial.

3. Conclusions

This is an exciting time to care for MM patients. Novel agents such as lenalidomide and bortezomib have markedly lengthened survival for patients with MM and for the first time, we can begin to imagine turning MM into a chronic disease-like hypertension, diabetes, or chronic myelogenous leukemia. ASCT candidates especially enjoy a list of treatment options that continues to expand. Lenalidomide specifically is growing in importance in all stages of therapy for the ASCT patient, and rightfully so, given its capacity to induce deep remissions and extend both disease-free and overall survival without excess toxicity in most cases. How exactly to optimally incorporate lenalidomide into MM therapy is becoming clearer with time, but existing data can be difficult to interpret given the panoply of single-arm trials with relatively short followup and incomplete reporting of long-term, meaningful outcomes. Attention to long-term followup from large, randomized trials currently in progress will presumably enable us to employ this highly effective agent in a manner that achieves maximum benefit.

Acknowledgments

S. A. Tuchman is supported by National Institutes of Health KL2 RR024127. S. A. Tuchman receives research support from Novartis. C. Gasparetto has received compensation for participation in advisory boards and speakers' bureau for Celgene and speakers' bureau for Millennium. N. J. Chao has no relevant disclosures.

References

[1] Surveillance, Epidemiology, and End Results (SEER) Program and the National Center for Health Statistics, 2010, http://www.cancer.gov/aboutnci/servingpeople/snapshots/myeloma.pdf.

[2] P. G. Richardson, R. L. Schlossman, E. Weller et al., "Immunomodulatory drug CC-5013 overcomes drug resistance and is well tolerated in patients with relapsed multiple myeloma," *Blood*, vol. 100, no. 9, pp. 3063–3067, 2002.

[3] M. Dimopoulos, A. Spencer, M. Attal et al., "Lenalidomide plus dexamethasone for relapsed or refractory multiple

myeloma," *The New England Journal of Medicine*, vol. 357, no. 21, pp. 2123–2132, 2007.

[4] D. M. Weber, C. Chen, R. Niesvizky et al., "Lenalidomide plus dexamethasone for relapsed multiple myeloma in North America," *The New England Journal of Medicine*, vol. 357, no. 21, pp. 2133–2142, 2007.

[5] M. Cavo, E. Zamagni, P. Tosi et al., "Superiority of thalidomide and dexamethasone over vincristine-doxorubicin- dexamethasone (VAD) as primary therapy in preparation for autologous transplantation for multiple myeloma," *Blood*, vol. 106, no. 1, pp. 35–39, 2005.

[6] J. A. Zonder, J. Crowley, M. A. Hussein et al., "Lenalidomide and high-dose dexamethasone compared with dexamethasone as initial therapy for multiple myeloma: a randomized Southwest Oncology Group trial (S0232)," *Blood*, vol. 116, no. 26, pp. 5838–5841, 2010.

[7] S. V. Rajkumar, S. R. Hayman, M. Q. Lacy et al., "Combination therapy with lenalidomide plus dexamethasone (Rev/Dex) for newly diagnosed myeloma," *Blood*, vol. 106, no. 13, pp. 4050–4053, 2005.

[8] S. V. Rajkumar, L. Rosiñol, M. Hussein et al., "Multicenter, randomized, double-blind, placebo-controlled study of thalidomide plus dexamethasone compared with dexamethasone as initial therapy for newly diagnosed multiple myeloma," *Journal of Clinical Oncology*, vol. 26, no. 13, pp. 2171–2177, 2008.

[9] S. V. Rajkumar, S. Jacobus, N. S. Callander et al., "Lenalidomide plus high-dose dexamethasone versus lenalidomide plus low-dose dexamethasone as initial therapy for newly diagnosed multiple myeloma: an open-label randomised controlled trial," *The Lancet Oncology*, vol. 11, no. 1, pp. 29–37, 2010.

[10] R. Niesvizky, D. S. Jayabalan, P. J. Christos et al., "BiRD (Biaxin [clarithromycin]/Revlimid [lenalidomide]/dexamethasone) combination therapy results in high complete- and overall-response rates in treatment-naive symptomatic multiple myeloma," *Blood*, vol. 111, no. 3, pp. 1101–1109, 2008.

[11] P. G. Richardson, E. Weller, S. Lonial et al., "Lenalidomide, bortezomib, and dexamethasone combination therapy in patients with newly diagnosed multiple myeloma," *Blood*, vol. 116, no. 5, pp. 679–686, 2010.

[12] S. K. Kumar, M. Q. Lacy, S. R. Hayman et al., "Lenalidomide, cyclophosphamide and dexamethasone (CRd) for newly diagnosed multiple myeloma: results from a phase 2 trial," *American Journal of Hematology*, vol. 86, no. 8, pp. 640–645, 2011.

[13] S. K. Kumar, I. Flinn, S. J. Noga et al., "Bortezomib, dexamethasone, cyclophosphamide and lenalidomide combination for newly diagnosed multiple myeloma: phase 1 results from the multicenter EVOLUTION study," *Leukemia*, vol. 24, no. 7, pp. 1350–1356, 2010.

[14] A. J. Jakubowiak, K. A. Griffith, D. E. Reece et al., "Lenalidomide, bortezomib, pegylated liposomal doxorubicin, and dexamethasone in newly diagnosed multiple myeloma: a phase 1/2 multiple myeloma research consortium trial," *Blood*, vol. 118, no. 3, pp. 535–543, 2011.

[15] A. J. Jakubowiak, D. Dytfeld, S. Jagannath et al., "Final results of a frontline phase 1/2 study of carfilzomib, lenalidomide, and low-dose dexamethasone (CRd) in multiple myeloma (MM)," in *Proceedings of the ASH Annual Meeting Abstracts*, vol. 118, p. 631, December 2011.

[16] M. Coleman, J. Leonard, L. Lyons et al., "BLT-D (clarithromycin [Biaxin], low-dose thalidomide, and dexamethasone) for the treatment of myeloma and Waldenström's macroglobulinemia," *Leukemia and Lymphoma*, vol. 43, no. 9, pp. 1777–1782, 2002.

[17] F. Gay, S. V. Rajkumar, M. Coleman et al., "Clarithromycin (Biaxin)-lenalidomide-low-dose dexamethasone (BiRd) versus lenalidomide-low-dose dexamethasone (Rd) for newly diagnosed myeloma," *American Journal of Hematology*, vol. 85, no. 9, pp. 664–669, 2010.

[18] S. Kumar, I. W. Flinn, P. G. Richardson et al., "Novel three- and four-drug combination regimens of bortezomib, dexamethasone, cyclophosphamide, and lenalidomide, for previously untreated multiple myeloma: results from the multi-center, randomized, phase 2 EVOLUTION study," in *Proceedings of the ASH Annual Meeting Abstracts*, vol. 116, p. 621, December 2010.

[19] S. Kumar, A. Dispenzieri, M. Q. Lacy et al., "Impact of lenalidomide therapy on stem cell mobilization and engraftment post-peripheral blood stem cell transplantation in patients with newly diagnosed myeloma," *Leukemia*, vol. 21, no. 9, pp. 2035–2042, 2007.

[20] T. Mark, J. Stern, J. R. Furst et al., "Stem cell mobilization with cyclophosphamide overcomes the suppressive effect of lenalidomide therapy on stem cell collection in multiple myeloma," *Biology of Blood and Marrow Transplantation*, vol. 14, no. 7, pp. 795–798, 2008.

[21] A. Mazumder, J. Kaufman, R. Niesvizky, S. Lonial, D. Vesole, and S. Jagannath, "Effect of lenalidomide therapy on mobilization of peripheral blood stem cells in previously untreated multiple myeloma patients," *Leukemia*, vol. 22, no. 6, pp. 1280–1281, 2008.

[22] I. N. M. Micallef, A. D. Ho, L. M. Klein, S. Marulkar, P. J. Gandhi, and P. A. McSweeney, "Plerixafor (Mozobil) for stem cell mobilization in patients with multiple myeloma previously treated with lenalidomide," *Bone Marrow Transplantation*, vol. 46, no. 3, pp. 350–355, 2011.

[23] S. V. Rajkumar, G. Gahrton, and P. L. Bergsagel, "Approach to the treatment of multiple myeloma: a clash of philosophies," *Blood*, vol. 118, pp. 3205–3211, 2011.

[24] J. L. Harousseau, M. Attal, and H. Avet-Loiseau, "The role of complete response in multiple myeloma," *Blood*, vol. 114, no. 15, pp. 3139–3146, 2009.

[25] A. Nooka, J. Kaufman, and S. Lonial, "The importance of complete response in outcomes in myeloma," *Cancer Journal*, vol. 15, no. 6, pp. 465–472, 2009.

[26] M. Cavo, P. Tacchetti, F. Patriarca et al., "Bortezomib with thalidomide plus dexamethasone compared with thalidomide plus dexamethasone as induction therapy before, and consolidation therapy after, double autologous stem-cell transplantation in newly diagnosed multiple myeloma: a randomised phase 3 study," *The Lancet*, vol. 376, no. 9758, pp. 2075–2085, 2010.

[27] Palumbo, "Bortezomib as induction before autologous transplantation, followed by lenalidomide as consolidation-maintenance in untreated multiple myeloma patients," *Journal of Clinical Oncology*, vol. 28, no. 5, pp. 800–807, 2010.

[28] K. Wheatley, "Interferon as therapy for multiple myeloma: an individual patient data overview of 24 randomized trials and 4012 patients," *British Journal of Haematology*, vol. 113, no. 4, pp. 1020–1034, 2001.

[29] M. Attal, J. L. Harousseau, S. Leyvraz et al., "Maintenance therapy with thalidomide improves survival in patients with multiple myeloma," *Blood*, vol. 108, no. 10, pp. 3289–3294, 2006.

[30] H. M. Lokhorst, B. Van Der Holt, S. Zweegman et al., "A randomized phase 3 study on the effect of thalidomide

combined with adriamycin, dexamethasone, and high-dose melphalan, followed by thalidomide maintenance in patients with multiple myeloma," *Blood*, vol. 115, no. 6, pp. 1113–1120, 2010.

[31] A. Spencer, H. M. Prince, A. W. Roberts et al., "Consolidation therapy with low-dose thalidomide and prednisolone prolongs the survival of multiple myeloma patients undergoing a single autologous stem-cell transplantation procedure," *Journal of Clinical Oncology*, vol. 27, no. 11, pp. 1788–1793, 2009.

[32] P. L. McCarthy, K. Owzar, K. C. Anderson et al., "Phase III intergroup study of lenalidomide versus placebo maintenance therapy following single autologous hematopoietic stem cell transplantation (AHSCT) for multiple myeloma: CALGB 100104," in *Proceedings of the ASH Annual Meeting Abstracts*, vol. 116, p. 37, December 2010.

[33] P. L. McCarthy, K. Owzar, K. C. Anderson et al., "Phase III Intergroup study of lenalidomide versus placebo maintenance therapy following single autologous stem cell transplant (ASCT) for multiple myeloma (MM): CALGB ECOG BMT-CTN 100104," *Haematologica*, vol. 96, p. S23, 2011.

[34] M. Attal, P. Olivier, C. V. Lauers et al., "Maintenance treatment with lenalidomide after transplantation for myeloma: analysis of secondary malignancies within the IFM 2005-02 trial," *Haematologica*, vol. 96, p. S23, 2011.

[35] M. A. Dimopoulos, R. Z. Orlowski, R. Niesvizky et al., "Lenalidomide and dexamethasone (LEN plus DEX) treatment in relapsed/refractory multiple myeloma (RRMM) patients (pts) and risk of second primary malignancies (SPM): analysis of MM-009/010," in *Proceedings of the ASCO Meeting Abstracts*, vol. 29, p. 8009, Chicago, Ill, USA, June 2011.

[36] A. P. Palumbo, M. Delforge, J. Catalano et al., "Incidence of second primary malignancy (SPM) in melphalan-prednisone-lenalidomide combination followed by lenalidomide maintenance (MPR-R) in newly diagnosed multiple myeloma patients (pts) age 65 or older," in *Proceedings of the ASCO Meeting Abstracts*, vol. 29, p. 8007, Chicago, Ill, USA, June 2011.

[37] H. Avet-Loiseau, X. Leleu, M. Roussel et al., "Bortezomib plus dexamethasone induction improves outcome of patients with t(4;14) myeloma but not outcome of patients with del(17p)," *Journal of Clinical Oncology*, vol. 28, no. 30, pp. 4630–4634, 2010.

[38] K. Neben, H. M. Lokhorst, A. Jauch et al., "Administration of bortezomib before and after autologous stem cell transplantation improves outcome in multiple myeloma patients with deletion 17p," *Blood*, vol. 119, no. 4, pp. 940–948, 2012.