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DRUG EVALUATION

Lenalidomide in chronic lymphocytic leukemia

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ABSTRACT

Introduction: Chronic lymphocytic leukemia (CLL) is a complex disease. Insights into its pathogenesis and the interplay between B cell receptor (BCR) signaling and tumor microenvironment (TME) have provided newer therapeutic targets for the treatment of CLL. Lenalidomide is a 2nd generation, immunomodulatory analogue with demonstrated clinical activity in CLL, both as an initial therapy and in patients with progressive disease.

Areas covered: Lenalidomide pharmacological properties, mechanisms of action, clinical activity, adverse effect profile and its potential for further use are discussed here. This review offers an insight into the unique mechanisms of action of lenalidomide and provides an overview of the clinical experience with lenalidomide in patients with CLL.

Expert opinion: Lenalidomide demonstrates efficacy among patients with CLL, both as monotherapy and as combination regimens. Its activity has been explored in patients with relapsed and previously untreated disease. Lenalidomide is best tolerated when given daily at a lower starting dose with gradual, dose escalation. Patients with CLL undergoing treatment with lenalidomide should be monitored for tumor lysis syndrome and myelosuppression. Tumor flare reactions can be observed in patients with CLL during the initial phases of treatment. Newer ongoing trials are studying combination of lenalidomide with newer monoclonal antibodies and kinase inhibitors.

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CLL; lenalidomide; mechanism of action; safety

1. Introduction

Chronic lymphocytic leukemia (CLL) is a clonal lymphoproliferative disorder characterized by a progressive accumulation of mature, neoplastic B-lymphocytes in the hematopoietic tissues [1]. Diagnosis of CLL is based on the presence of ≥5000 clonal B cells/μL in the peripheral blood (PB), and a characteristic immunophenotype on flow cytometry showing a co-expression of CD5, CD19, and CD23, expression of CD20 (dim), CD79b, surface immunoglobulin's (sIg) and kappa or lambda Ig light chain restriction [2]. CLL is considered a disease of the elderly, with a median age of 72 years at the initial diagnosis [3]. Despite the high median age at presentation, it is not rare for young adults to be diagnosed with CLL [4]. With a high prevailing incidence of 1.1% among all the newly diagnosed cancers, (approximately 18,960 estimated new cases in 2016) CLL has an age-adjusted incidence of 4.6 per 100,000 men and women per year and an age-adjusted death rate of 1.3 per 100,000 men and women per year [5]. It is a remarkably heterogeneous disease, with a diverse clinical course. Clinical staging systems introduced by Rai and Binet takes into account the progressive accumulation of neoplastic lymphocytes in hematopoietic tissues, guiding appropriate treatment decisions in affected patients [6,7]. Under current guidelines, asymptomatic patients with early stage disease do not require any treatment [2,8]. Additional prognostic tools incorporate genomic and molecular marker analysis, including the mutational status of immunoglobulin heavy chain variable regions (*IGHV*), CD38 status, zeta chain associated protein kinase 70 (ZAP-70), 17p deletion, and *TP53* mutation. These markers further stratify patients in terms of time to first treatment, duration of response and overall survival [9–11]. The pathogenesis of CLL is multifactorial in origin and involves an interplay between the B-cell receptor (BCR) signaling and the tumor microenvironment (TME) [9–11]. Research has provided therapeutic targets for treatment: Surface antigens are primarily targeted by monoclonal antibodies, BCR pathway enzymes by the kinase group of inhibitors, and the TME by immunomodulatory compounds (IMIDs) (thalidomide and lenalidomide) [12].

Lenalidomide is a second generation, thalidomide analog with significant clinical activity in lymphoid malignancies [13–17]. It is utilized in the treatment of myelodysplasia (MDS) (deletion $5q^-$), multiple myeloma (MM), and relapsed/refractory (R/R) mantle cell lymphoma [18,19]. In combination with rituximab (R), lenalidomide has shown significant efficacy in patients with R/R diffuse large B-cell lymphoma and follicular lymphoma, and as a frontline regimen in follicular, marginal zone, and small lymphocytic lymphomas [18,19]. In combination with rituximab (R), lenalidomide has shown significant efficacy in patients with relapsed/refractory (R/R) diffuse large B-cell lymphoma and follicular lymphoma, and as a frontline regimen in follicular, marginal zone, and small lymphocytic lymphomas [17,20]. In CLL, lenalidomide has shown clinical activity both as an initial therapy and in patients with R/R disease [12,13,17,21–31]. In this review, we aim to provide

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Box 1. Drug summary

Drug name (generic)	Lenalidomide
Phase (for indication under discussion)	Not applicable
Indication (specific to discussion)	Chronic lymphocytic leukemia: orphan drug designation
Pharmacology description	Thalidomide derivative
Mechanism of action	Immunomodulation
Route of administration	Oral
Chemical structure	Synthetically prepared by addition of an amino group at the fourth position and a single oxo group in phthaloyl ring of thalidomide
Pivotal trial(s)	Phase III trials have been conducted, but not published yet

an overview of the unique mechanisms of action of lenalidomide in CLL, summarize the larger clinical trials conducted in CLL, and describe ongoing research initiatives.

2. Chemical structure and pharmacological properties

Lenalidomide (L) is a thalidomide (T) analog, synthetically prepared by addition of an amino group at the fourth position and a single oxo group in phthaloyl ring (Box 1) [32–34]. Its pharmacokinetic properties allow rapid oral absorption, with peak plasma concentrations occurring between 0.77 and 1.0 h after dosing and a terminal half-life of 3 h [33,35]. It has a linear pharmacokinetic profile and is predominantly excreted unchanged through renal elimination (90% of the radioactive dose), with smaller amounts being excreted through feces (4%) and semen (0.0062%) [35,36].

3. Mechanism of action

Lenalidomide has cytotoxic, anti-angiogenic, and immunomodulatory properties [30]. It has stronger tumoricidal and immunomodulatory effects than its parent compound, thalidomide, including a stronger stimulation of T-cell proliferation, and higher levels of IL-2 and IFN-γ secretion (10^2–10^3 times) [30,34,37]. In CLL, lenalidomide is believed to act mainly through its immunomodulatory properties, involving B cells, T cells, and their interactions, as well as NK cells and dendritic cells [31,38–42]. Its known mechanisms of action are outlined in the following subsections.

3.1. Activation of Cu14A–DDB1E3 ubiquitin ligase complex and induction of p21 expression

Lenalidomide activates the Cu14A–DDB1E3 ubiquitin ligase complex through cereblon (CRBN), a direct molecular target of lenalidomide and other thalidomide analogs [31]. Treatment of CLL cells with lenalidomide results in activation of the TP53 independent, cereblon/$p21^{WAF1/Cip1}$ dependent pathway [31]. $p21^{WAF1/Cip1}$ is a negative cell cycle regulatory protein, actively induced *in vivo* in CLL cells treated with IMID (R), and cereblon is a part of the Cu14A–DDB1E3 ubiquitin ligase complex together with cullin4A and a damaged DNA binding protein 1 [43]. Once cereblon recognizes and binds the target protein, the Cu14A–DDB1E3 ubiquitin ligase complex effectively ubiquitinates it, causing proteosomic degradation of the ubiquitinated target [21,31,43,44]. Lenalidomide can also induce p21 transcription through the rapid degradation of complexes formed by essential hematopoietic transcription factors including IKZF1 (Ikaros) and IKZF3 (Aiolos) (substrates of CRBN) [45–47]. Substrate degradation of these transcription proteins also stops repression of interleukin 2 (IL-2) production from T cells, resulting in higher levels of IL-2 production [29,45–47]. Induction of $p21^{WAF-1}$ expression through lenalidomide also leads to inhibition of the cyclin-dependent kinases CDK2, CDK4, and CDK6, pRb hypophosphorylation, and cell cycle arrest at G1/S phase [48]. The combined cereblon/$p21^{WAF1/Cip1}$-dependent pathway induced by lenalidomide and its independence from the TP53 pathway could explain the clinical responses observed in chemorefractory patients with deletion 17p and *TP*53 mutated CLL [31,49,50].

3.2. Upregulation of immune co-stimulatory molecules

Lenalidomide induces dynamic *in vitro* interaction between the neoplastic B cells and T cells through upregulation of immune co-stimulatory molecules including CD80, CD86, HLA-DR, CD95, and CD40 on neoplastic B cells [42,51,52]. This upregulation has been shown to promote Ig production by non-neoplastic B cells [29]. Lenalidomide upregulates CD80 and CD95 expression solely on neoplastic B cells, and upregulates CD86 more strongly on neoplastic B cells [51]. Upregulation of CD80 has been correlated to increased T-cell activation [51]. Lenalidomide exposure also produces a time and dosage dependent upregulation of CD154 (CD40L) on CLL cells [52]. This increase in CD154 has been shown to increase IgM production by non-neoplastic B cells as well as IgG production, indicating that CD154 mediated class switching may be occurring [52].

T-cell activation by an antigen presenting cell is dependent on conjugation of T cells with antigen presenting cells, F-actin polymerization, and recruitment of a variety of molecules to the synapse [42]. This process in impaired in CLL patients through a downregulation of Rho-GTPases (RhoA and Rac1) and Cdc42 [42,53]. Lenalidomide has been shown to increase activation of the Rho-GTPases (RhoA and Rac1) and Ras-family small GTPase (Rap1) and increase both the conjugation of T cells and the F-actin polymerization at the immune synapse in CLL patients [42,53]. This upregulation of co-stimulatory molecules has also been shown to promote repair of T-cell synapses [54]. In addition, increases in these GTPases have, in turn, been shown to stimulate actin polymerization and to, therefore, induce cytoskeleton reorganization, increasing phagocytosis by Nurse-like cells [54]. This lenalidomide-induced reorganization has been speculated to change both, the adhesive and migratory properties of CLL cells [54]. The changes in Rho GTPase signaling and Rap1 trafficking improve T-cell motility and function in CLL patients [54]. Lenalidomide was

Table 1. Studies with lenalidomide as therapy in patients with R/R CLL.

Author	*N*	Phase	Treatment	Median age (years) (range)	ORR (CR) (%)	OS/PFS (%)
Lenalidomide as monotherapy						
Chanan-Khan et al. [58]	45	II	L* – 5–25 mg/day (D1–21/28)	64 (42–75)	47 (9)	1-year estimated PFS – 81 months
Ferrajoli et al. [59]	44	II	L* – 10–25 mg/day continuous	64 (49–86)	32 (7)	OS – 73 months at a median follow up of 14 months
Wendtner et al. [60]	34	II	L* – 5–25 mg/day (D1–21/28)	65.5 (32–81)	44 (14.7)	Median PFS – 96.3 weeks
	35	II	L* – 10–25 mg/day (D1–21/28)	63 (39–78)	37 (5.7)	Median PFS – 117.6 weeks
	35	II	L* – 15–25 mg/day (D1–21/28)	65 (48–78)	40 (2.9)	Median PFS – 89.3 weeks
Lenalidomide as combination therapy						
Badoux et al. [61]	59	II	R – 375 mg/m^2 (weekly × 4; monthly × 12) L – 10 mg/day from C1D9 then continuous	62 (42–82)	66 (12)	At 36 months, OS –71
Maddocks et al. [62]	39	I	Flavo. – 60 mg/m^2 C1:D1, 80 mg/m^2 C1:D8, 15; dose escalation C2–8;D3,10,17; L – 2.5 mg qd in C2; (D1–21;C2–8) (dose escalation C2–8)	62 (26–74)	51 (0)	Median PFS – 7.7 months
Costa et al. [63]	21	II	Ofa – 2 g C1–6; D1, L- 5 mg/day continuous, dose escalation from C2–6	63 (48–80)	48 (0)	Median OS – 21.5 months
Vitale et al. [64]	34	II	Ofa – 300 mg C1D1, 1000 mg C1 D8,15,22; C2–6;D1 L – 10 mg D9 onwards continuous	64 (34–82)	71 (18)	5 year OS – 53 months Median PFS-16 months

L: lenalidomide; R: rituximab; F: fludarabine; Flavo: flavoparidol; Ofa: ofatumumab; L*: lenalidomide dose escalation; R/R: relapsed/refractory; PFS: progression-free survival; OS: overall survival; CR: complete response.

also shown to increase expression of CORO1B, and to down regulate RhoH in CLL cells, inhibiting their migration [54].

3.3. Regulation of T cells

Lenalidomide activates CD8$^+$ T cells, decreases CD4$^+$ T-cell-derived cytokines, augments Th1 T-cell differentiation, and leads to a higher production of interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), IL-6, CCL-2, CCL3, and CCL4 [55,56]. It thus upregulates T-cell proliferation, reverses its functional defects, and enhances IFN-γ production [31,38–42]. Lenalidomide also promotes dendritic cell (DC) priming, mediates repair of the NK-cell lytic synapse formation, downregulates PD-L1 axis, inhibits T regulatory cells (T_R), and inhibits the crosstalk between CLL and the TME through inhibition of CXC chemokine receptor (CXCR12) migratory axis [22,53–55,57].

Lenalidomide induces an increase in the relative proportion of both CD4$^+$ and CD8$^+$ T cells, with CD4$^+$ cells increasing more significantly [22]. This results in an increase proportion of CD8$^+$ cells producing IL-2, IFN-γ, and TNF-α [56]. CD8$^+$ cells were shown to have increased cytotoxicity after treatment with lenalidomide, including through improved lytic synapse formation and granzyme B trafficking [42,53]. There is also a resultant increase in secretion of IL-2 by CD4$^+$ T cells, likely leading to an increase in NK cells in CLL patients and an increase in NK-like cells [22]. In addition, lenalidomide increases the cytotoxic activity of both NK cells and NK-like cells against CLL cells, perhaps due to the increased expression of NKP30 on NK cells [22].

4. Clinical studies with lenalidomide

Prior to being evaluated in patients with CLL, lenalidomide showed clinical activity in patients with MDS and MM. Thus, initial experience on its efficacy and toxicity in CLL has been acquired in the context of phase II trials. Relevant studies of lenalidomide as monotherapy and as part of combination trials are shown in Tables 1 and 2.

4.1. Lenalidomide as monotherapy

Chanan-Khan et al. reported the first phase II trial using lenalidomide as monotherapy in 45 patients with R/R CLL [58]. These patients had advanced disease and a median number of three prior therapies. Lenalidomide was initially given at 25 mg/day for 21 days (4-week cycle), and treatment was continued until unacceptable toxicity or disease progression. Rituximab was added at disease progression. Two of the first 29 enrolled patients developed severe tumor lysis syndrome (TLS), following which, in the subsequent 16 patients, the starting dose of lenalidomide was reduced to 5 mg/day followed by a gradual increase by 5 mg every 1–2 weeks up to 25 mg/day. Tumor flare reaction (TFR) prophylaxis with prednisolone was administered in the later 16 patients, considering the high incidence of TFR with higher doses of lenalidomide in the first 29 patients. The overall response rate (ORR) was 47% (complete response [CR] = 9%) and the estimated probability of progression-free survival (PFS) at 1 year was 81%. Grade 3–4 neutropenia and thrombocytopenia was seen in 70% and 45% of the patients, respectively. Most common non-hematologic adverse effect (AE) was fatigue (83%). A unique phenomenon called tumor flare reaction (TFR), characterized by development of swollen, painful lymphadenopathy, occasionally associated with fever and rash, was seen in 58% of patients (grade 3–4 in 8%). Other notable complication in this study was pulmonary embolism in two patients (5%) [58]. In an updated report, Chanan-Khan et al. studied the correlation between lenalidomide response and

Table 2. Studies of lenalidomide as initial therapy in patients with CLL.

Author	*N*	Phase	Treatment	Median age (years) (range)	ORR (CR) (%)	OS/PFS (%)
Lenalidomide as monotherapy						
Chen et al.[a] [65]	25	II	L – 2.5–25 mg (D1–21/28)	60 (33–78)	56 (0)	2 year OS – 92% 2 year PFS – 89%
Badoux et al.[b] [23]	60	II	L – 5–25 mg (D1–28)	71 (66–85)	65 (10)	2 year estimated OS – 88; PFS –60
Lenalidomide as combination therapy						
Brown et al. [66]	9	I	F – 25 mg/m^2 (D3–5) R – 375 mg/m^2 (D1) L*- 2.5–25 mg (D1–21/28)	59 (37–66)	56 (11)	NA
Flinn et al. [67]	51	I/II	F – 25 mg/m^2 (D3–5) R – 375 mg/m^2 (C1;D1 + 2) 500 mg/m^2 (C2–6;D1) L* – 2.5–5 mg (D8–28) (C1–6)	62 (44–82)	63 (11)	OS/PFS – 88/71 at 24 months
Egle et al. [68]	45	I/II	F – 40 mg/m^2(D1–3) L – 2.5 mg/day (C1; D7–21) 5–25 mg/day (C2-6;D1-21) R – 375 mg/m^2 (C1; D1 + 2) → L (10–25 mg/day) daily + R – 375 mg/m^2 maintenance once in 2 months	67 (43–79)	96 (67)	PFS – 89% at a median follow up of 35 months
James et al. [69]	40	II	R – 375 mg/m^2 (weekly,D1) L* – 2.5–10 mg (D1–21/28)	56 (45–64)	95 (20)	PFS –18 months
	29			70 (65–80)	79 (7)	PFS – 20 months
Chen et al. [70]	31	II	L* – 5–25 mg/day continuously Dex 12 mg (D1–7,14, and 21)	59 (40–84)	74 (6)	Median PFS – 27.8 months
Thompson et al. [71]	58	II	R – 375 mg/m^2 (weekly × 4; monthly 3–12) L – 10 mg/day from D9 – till 24 months	66 (42–79)	83 (15)	Median OS and TTF not reached

L: lenalidomide; R: rituximab; F: fludarabine; Dex: dexamethasone; L*: lenalidomide dose escalation; R/R: relapsed/refractory; NA: not applicable.
[a]Extension at a median follow up of 53.2 months from the prior 23 months, showed a 3 year OS – 85% and PFS – 65% [72].
[b]Extension showed the long-term responders having a 2-year OS of 82% at 4 years and median OS not being reached in the short-term responders [73].

TFR in 45 patients of R/R CLL [74]. The study showed a trend toward a higher CR in patients developing a TFR versus patients without a TFR, but the difference in the median PFS was not significant (19.9 vs. 19.4 months, respectively, $p = 0.92$) [74]. The intensity of TFR did correlate to the presence of adequate amount of NK cells in the peripheral blood circulation, and it was also higher in patients who initiated therapy with lenalidomide at a higher dose and without prophylaxis [74].

Ferrajoli et al. reported the activity of lenalidomide in 44 patients with R/R CLL [59]. Lenalidomide was started at 10 mg/day and given continuously, followed by a 5 mg increase every 28 days, to a maximum of 25 mg daily. ORR in the whole population was 32% (CR 3%). ORR in patients with unmutated *IgHV* was 24%, with 11q or 17p deletion was 31% and with fludarabine-refractory (Flref) disease was 25%, respectively. Grade 3–4 neutropenia, thrombocytopenia, and anemia were seen in 41%, 16%, and 3%, respectively. Most common non-hematological side effects were rash (grade 1–2 in 13%). None of the patients developed TLS, and TFR was seen in 12% (grade 3–4 = 2%, grade 1–2 = 10%). TFR was more common in patients with bulky lymph nodes >5 cm (53% vs. 15%), and its presence did not correlate with a better ORR (38% vs. 34%). The median tolerated dose of lenalidomide was 10 mg [59].

Chen et al. studied the outcome of therapy with single agent lenalidomide in 25 patients with TN CLL using an escalated dosing schedule starting from 10 to 25 mg/day. However, the first two patients incurred severe TLS and fatal sepsis. The protocol was later amended to begin with a lower starting dose of lenalidomide at 2.5 mg/day, followed by monthly dose escalation to a maximum of 10 mg/day. The ORR was 72% (CR 20%). Three year PFS and OS was 64.6% and 85.3%, respectively. Six of the eight patients with high-risk cytogenetics (deletions 17p/11q) responded (2 CR). The median response duration of all the eight patients was 35.9 months (range, 34.2–64.6 months). Myelosuppression occurred in the first year of treatment (with grade 3–4 neutropenia and thrombocytopenia in 76% and 28% of the patients, respectively) [72]. TFR was seen in 88%, and was mild (all grade 1–2). Due to the intermittent dosing schedule, recurrence of TFR was seen in 15% of the cycles, occurring as late as cycle 28.) [72]. Wendtner et al. reported on a phase II/III study evaluating the role of lenalidomide in 52 patients with R/R CLL, of which 69% had bulky disease and 48% had HR genetic abnormalities [(deletion (17p) and (11q)] [75]. Due to TLS-related complications, the protocol was subsequently amended to be a phase I study to identify the maximum tolerated dose-escalation level (MTDEL). Lenalidomide was started at a dose of 2.5 mg/day daily and was safely increased to 20 mg/day, with MTDEL not being reached. The best response seen was a partial response (PR) in six (11.5%) patients. The median PFS (intent-to-treat population) was 24.1 weeks, but the median PFS in the responders was higher at 42.1 weeks. The most common grade 3–4 AEs were neutropenia (65.4%) and thrombocytopenia (32.7%). TLS and TFR were seen in 44% and 9.6% cases, respectively [75]. Another study by Wendtner et al. evaluated the safety and outcome of different starting doses of lenalidomide in

patients with R/R CLL [60]. Patients were randomized to receive lenalidomide at initial doses of 5, 10, or 15 mg/day (n = 103), with subsequent dose escalations up to 5 mg every 28 days in each arm. ORR was 44.1%, 37.1%, and 40.0% in the 5, 10, and 15 mg/day subgroups. Patients who received/escalated to at least 15 mg/day (n = 70) of lenalidomide showed a superior median PFS (85.1 vs. 11.1 weeks) and OS (163.9 vs. 64.9 weeks, $p \leq 0.001$) than those who did not (n = 34). Patients who received/escalated to at least 20 mg/day (n = 36) of lenalidomide also showed a superior median PFS (115.0 vs. 20.6 weeks) and OS (169 vs. 81.7 weeks) than those who did not (n = 68). Patients who received/escalated to at least 25 mg/day (n = 25) of lenalidomide also showed a superior PFS (123.1 vs. 22.7 weeks) than those who did not (n = 79). Most common grade ≥3 AEs were neutropenia (74%, 88%, and 69% in lenalidomide 5, 10, and 15 mg/day groups, respectively) and thrombocytopenia (32%, 56%, and 54% respectively). Grade ≥3 TLS was seen in three patients, with one receiving lenalidomide in 5 mg/day and two receiving 15 mg/d. Grade ≥3 TFR was seen in 15 patients [60]. Sher et al. also reported the results of a phase II trial of lenalidomide monotherapy in patients with poor cytogenetic markers [deletion (11q) or (17p)], and demonstrated an ORR of 38% (CR – 19%) and a median PFS of 12.1 months [49].

Strati et al. summarized the long-term outcomes of 60 elderly patients (66–85 years) with CLL treated in a phase II study of single agent lenalidomide. Patients were treated with a lenalidomide dose of 5 mg daily for a total of 4 weeks, followed by a 5 mg per cycle up to 25 mg daily [73]. Patients remained on treatment with lenalidomide, until disease progression. With a median follow up of 4 years, time to treatment was not reached and the OS was 82%. Thirty-five patients (58%) were identified as being long-term responders (LTRs), with a response lasting >36 months (CR – 71%; PR – 29%). Twenty five patients had no response or a response <36 months [short-term responders (STRs)]. LTRs had lower plasma baseline levels of ß-2-microglobulins, were more likely to have trisomy12 and less likely to have deletion 17p. After the initial toxicity (grade 3–4 neutropenia – 34% in 1st year), all grade 3–4 neutropenia resolved with subsequent dose reduction. In addition, in these patients there was an improvement of > 50% from the baseline in the IgA, IgG and the IgM levels and normalization in the percentage of $CD4^+$ and $CD8^+$ cells and T-cell numbers [73].

4.2. Lenalidomide in combination therapy

Combination trials of lenalidomide with chemoimmunotherapy were designed with the goal to improve both efficacy and tolerability of this agent. Lenalidomide is known to improve B-cell synapse formation and upregulates B-cell co-stimulatory molecules, augmenting rituximab-induced antibody dependent NK- and T-cell cytotoxicity [52,61]. Badoux et al. reported the outcomes of a combination of lenalidomide with rituximab in 59 patients with R/R CLL [61]. Rituximab was given weekly for 1 cycle, followed by once every 4 weeks during the subsequent 12 cycles. Lenalidomide was started at a dose of 10 mg daily, continuously from day 9 of cycle 1, with each cycle of 28 days. The ORR was 66% (CR – 12%; PRn – 12%) The estimated survival at 36 months is 71%. Grade 3 TLS was seen in one patient, and grade 3–4 neutropenia in 73% [61].

James et al. reported the combination of lenalidomide and rituximab in the 69 TN patients with CLL [69]. Lenalidomide was started at the dose of 2.5 mg/day and increased to 5 mg/day in the first two cycles, for 21 days of a 28-day cycle. The dose was escalated up to 10 mg/day in the subsequent five cycles, if tolerated. Rituximab was started at 50 mg/m^2 on day 29, 325 mg/m^2 on day 31, and 375 mg/m^2 on day 33 of cycle 1. Subsequently, it was given weekly throughout cycle 2, and then on day 1 from cycle 3 to 7. Patients were assigned to the two separate treatment arms based on being younger (arm A) or older (arm B) than 65 years at time of start of therapy: 40 patients were enrolled in arm A (median age 56 years) and 29 in arm B (median age 70 years). The combined ORR was 88% (CR – 15%; PRn – 12%). At a median follow up of >20 months, the median PFS in the arm A was 19 months in comparison to 20 months in arm B. The median PFS in patients achieving a CR was significantly longer than patients achieving a PR or PRn (21 vs. 9 months, p = 0.008). The median OS was not reached in either arm. TFR was only mild to moderate and was mainly seen during cycle 1, with only one patient developing grade 3–4 TFR. Most common grade 3–4 toxicity was neutropenia, with a similar incidence in both the arms (53% vs. 66%, respectively). Anemia and thrombocytopenia were predominantly grade 1–2 in both arms [69].

Brown et al. reported a phase I study of lenalidomide in combination with fludarabine and rituximab in nine patients with TN CLL [66]. A total of six cycles of combination therapy, followed by two cycles of consolidation with lenalidomide were planned. Lenalidomide was initiated at 2.5 mg/day on days 1–21, fludarabine (25 mg/m^2) was given for 3–5 days and rituximab (375 mg/m^2) on day 1 of a 4-week cycle. The initial schedule at the dose level 1 included 2.5 mg/day of lenalidomide and 3 days of fludarabine. The response rate was 56%. At the first dose level, two patients suffered dose-limiting toxicity. One of them developed tumor flare with prolonged neutropenia lasting for 50 days. Another patient developed a complex syndrome of rash, fever, rhabdomyolysis (increased creatinine kinase), and myalgias. He initially tested positive for influenza, but his symptoms recurred on day 1 of cycle 2. He later had to be removed from the study. At all dose levels, grade 3–4 neutropenia and thrombocytopenia were seen in 67% and 22%, respectively. Grade 3–4 TFR was seen in 22% [66]. This combination of chemoimmunotherapy was poorly tolerated and the study was closed due to excessive myelotoxicity and idiosyncratic TFRs.

Egle et al. studied a combination of fludarabine, lenalidomide and rituximab in a phase I/II trial in 45 TN patients with CLL [76]. Lenalidomide was initiated at a dose of 2.5 mg/day (days 7–21 in cycle 1) and then escalated up to 25 mg/day over day 1–21 over cycles 2–6, fludarabine (40 mg/m^2 given orally d1–3 q28d) and rituximab (375 mg/m^2 d4 cycle 1). Rituximab (2 monthly doses) and lenalidomide at the highest tolerated dose were continued for a total of 6 months, as maintenance. ORR was 96% (CR – 67%), with a PFS of 89% at a median follow up of 35 months. Twenty-five percent of patients showed an improvement of their response from a

post-induction PR to a CR at the end of maintenance. Grade 3–4 neutropenia was seen in 72% [76].

Shanafelt et al. reported a phase II trial in 38 patients with TN CLL treated with six cycles of intravenous pentostatin (P) (2 mg/m^2, day 1), cyclophosphamide (C) (600 mg/m^2, day 1) and rituximab (R) (cycle 1:100 mg, day 1; 375 mg/m^2, day 2, cycle 2–6: 375 mg/m^2 day 1), every 3 weeks. This was followed by 6 months of lenalidomide consolidation at a dose of 5 mg/day initially, and increased gradually to 10 mg/day, as tolerated [77]. The data from this trial was compared with the historical experience with PCR, without lenalidomide consolidation. The initial ORR after CIT was 93% (CR – 36.8%, PR – 63.1%). Lenalidomide consolidation was given to 34 patients, of which 18 patients (52.9%) achieved CR (11 MRD$^-$ and 7 MRD$^+$). The grade 3–4 toxicities observed during lenalidomide treatment included neutropenia (58.8%), rash (6%), fatigue (3%), myalgia (3%) and autoimmune syndromes (3%). None of the patients experienced TFR or TLS. Longitudinal correlative studies showed an improvement in antitumor T-cell immune synapse activity after CIT, which was further enhanced with lenalidomide consolidation [77].

The second-generation fully human, CD20 mAb ofatumumab has also been combined with lenalidomide in patients with TN and R/R CLL. Ofatumumab triggers enhanced ADCC and complement dependent cytotoxicity (CDC). Costa et al. reported the outcomes of the combination of lenalidomide and ofatumumab in 21 R/R patients with CLL treated in a phase II study [63]. Ofatumumab was administered as 2000 mg (300 mg in cycle 1) intravenously on day 1 (cycle 1–6) and lenalidomide on days 8–28 at 5 mg/day during cycle 1 (28 days). Lenalidomide dose was increased to 10 mg/day on cycle 2 or beyond, as tolerated. The ORR was 47.6% (all PR) and the median OS was 21.5 months. Grade 3 neutropenia and thrombocytopenia was seen 47% and 19%, respectively. TFR was seen in 47%, with grade 3 in 14% [63].

Vitale et al. reported the outcome of 34R/R patients with CLL in a phase II study evaluating the combination of lenalidomide and ofatumumab [64]. Ofatumumab was administered at a dose of 300 mg on day 1, 1000 mg on days 8, 15, and 22 in cycle 1, 1000 mg on the day 1 of cycle 2–6 and then once every other cycle during cycles 7–28 (28-day cycle). Oral lenalidomide was started at 10 mg daily on day 9 and continued until clinical benefit was seen. A total of 24 courses were planned. Lenalidomide monotherapy was continued indefinitely in patients experiencing a sustained PR or CR, but patients were discontinued from the study if there was progression of disease or any form of unacceptable toxicity at any time. The ORR was 71% (CR/CRi – 24%, MRD$^-$ 9%). The median PFS was 16 months. Patients with del17p had a significantly shorter PFS than patients with other cytogenetic abnormalities (5 vs. 27 months, $p < 0.0001$). Similarly, patients with Flref disease also had a shorter median PFS duration (5 vs. 28 months, $p < 0.0001$). Patients who had not responded to their previous line of therapy before entering the study also showed a shorter response (5 vs. 22 months, $p < 0.0001$). The estimated 5-year survival was 53% for all patients, and 62% for the responding patients. The most common toxicity encountered was neutropenia (82%). Grade 3–4 neutropenia, thrombocytopenia and anemia were seen in 82%, 18%, and 6%, respectively. There were no grades 3–4 TFR or TLS seen. Pneumonia was the most common grade 3 non-hematologic toxicity, and was seen in 24% of the patients. T cell and NK cell subset distribution was analyzed at different time points in order to assess the changes and possible correlations with response to therapy. The NK cell effector function before treatment was significantly higher in patients that achieved a CR in comparison to patients with a PR or a no response (NR). NK cell function did not show improvement over time. T-cell function showed a significant improvement over time in the patients who achieved a CR. The baseline interleukin-2 and TNF-α production before treatment was also significantly higher in patients that achieved a CR when compared to patients that achieved a PR or an NR [64].

5. Safety and tolerability

CLL patients treated with lenalidomide experience several toxicities. The most commonly seen toxicity among TN as well as R/R patients with CLL is myelosuppression, with neutropenia occurring more often than thrombocytopenia and anemia [13,29,78]. Grade 3–4 neutropenia occurs in 40–80% of patients and often leads to dose reductions and delays [13,29,78]. The most common non-hematological toxicities of lenalidomide include fatigue, diarrhea, skin rash, infections, particularly pneumonias, venous thromboembolism and elevated transaminases [13,29,78]. Venous thromboembolism (VTE) has been reported in up to 5% of patients [13,58,59] and has been mechanistically linked to lenalidomide-induced endothelial damage secondary to upregulation of inflammatory markers, such as TNF-α, soluble vascular endothelial adhesion molecule 1 (sVCAM-1) and tissue factor (TF) [79]. Treating physicians should consider the concomitant administration of aspirin or low-molecular weight heparin, based on the individual risk for VTE.

TFR and TLS can occur in patients with CLL, treated with lenalidomide. TFR is characterized by rapidly enlarging, tender lymphadenopathy (± splenomegaly); sometimes associated with an erythematous rash, low-grade fever and peripheral blood lymphocytosis [74,80,81]. TLS presents with deranged metabolic parameters, such as hypocalcemia, hyperkalemia, hyperuricemia, uricosuria, hyperphosphatemia, and renal failure [13,29,78,81]. Andritsos et al. reported their single center experience with lenalidomide at a dosage of 25 mg/day for 21 days every cycle in four patients with R/R CLL [80]. Three patients suffered severe TFRs. The first patient also developed marked peripheral blood lymphocytosis (372,000/mL), hyperuricemia, hyperphosphatemia and high lactate dehydrogenase levels, suggestive of severe TLS and died due to hypoxemic respiratory failure. Another patient developed severe neutropenic sepsis due to *Pseudomonas aeruginosa* infection [80]. An additional finding in these patients was a rise in CD40/CD86 co-stimulatory molecules, which correlated with the severity of TFR. The use of prophylactic dexamethasone (12 mg/day for the first 7 days, followed by 4 mg for the next 7 days) mitigated this rise of lenalidomide-induced upregulation of CD40/CD86 among the ex vivo CLL cells [80]. Thus, serious toxicities were seen at a higher starting dose in patients with a large tumor burden, and could be related to lenalidomide-induced

B-cell activation [80]. Moutouh-de Parseval et al. summarized the total incidence of TLS/TFR in 260 patients with CLL treated with lenalidomide [81]. Seven cases of TLS were observed, two of which also had concomitant TFR. Two patients died due to accompanying unresolved, metabolic complications and renal dysfunction [81]. TFR may resolve spontaneously or require NSAIDs, such as ibuprofen with or without oral steroids for control of the symptoms, and does not warrant termination of lenalidomide [13,29,78]. Several clinical studies suggest that starting lenalidomide at a lower dose, followed by a gradual dose escalation, adequate hydration and prophylaxis with allopurinol, can help reduce the incidence of these unique toxicities [59,82].

6. Dose and schedule of lenalidomide

The initial dosing schedule of lenalidomide at 25 mg/day (21/28 days) in CLL was modeled from its use in patients with MM. Use of such dose in CLL leads to a high incidence of severe toxicities, such as myelosuppression, TFR, and TLS [58,80]. Subsequent studies used a lower starting dose (2.5–5 mg/day), a gradual dose escalation up to 10–25 mg/day and a median tolerated dose of 5–10 mg/day, resulting in improved clinical tolerability in patients with CLL [13,78].

A continuous schedule of administration of lenalidomide is preferable in patients with CLL. An intermittent schedule (3 weeks on; 1 week off) was associated with rebound peripheral lymphocytosis in the week off therapy as noted by Chen et al. [65]. The same schedule (3 weeks on; 1 weeks off) in a cohort of heavily pretreated HR patients was associated with a low ORR [83].

7. Newer combination studies and ongoing trials

BCR activates multiple integrin signaling pathways, such as the phosphoinositide 3-kinase (PI3K), nuclear factor (NF)-κB, Brutons tyrosine kinase (BTK), nuclear factor of activated T cells (NF-AT), mitogen-activated protein (MAP) kinase, and RAS, which are essential for the survival and proliferation of antigen-specific B cells. Newer small molecule inhibitors that target BCR signaling, such as ibrutinib (BTK inhibitor), idelalisib (PI3 K inhibitor), and fostamatinib (SYK inhibitor) have shown profound efficacy in various type of B-cell malignancies, including CLL [84–87]. Several combination trials of lenalidomide with these newer inhibitors are ongoing. Pollyea et al. reported the initial results of a dose escalation phase I study by combining ibrutinib with lenalidomide in a group of nine patients with R/R CLL, with a median age of 65 years (range, 49–81) [88]. Oral ibrutinib was administered as a daily dose of 420 mg for a month (cycle 0). During cycle 1, ibrutinib was given along with lenalidomide (four dose escalation cohorts of 2.5, 5, 7.5, and 10 mg) and intra-patient dose escalation was allowed. Lenalidomide was discontinued after 12 cycles, ibrutinib was continued till unacceptable toxicity or disease progression. The ORR was 100%, with all responses being PRs. Grade 3–4 neutropenia was seen in 55.5%. Grade 3–4 anemia and thrombocytopenia were seen in 11.1% each. Grade 4 neutropenia occurred at a dosage of 7.5 mg of lenalidomide and was the predefined dose limiting toxicity [88]. Cheah et al. explored a triple drug combination of lenalidomide (5 mg/day from days 8 to 21 cycle 1, days 1–21 thereafter), rituximab (375 mg/m^2 day 1), and idelalisib [150 mg twice daily from day 1] in a cohort of seven patients with indolent non-Hodgkin lymphoma (NHL) [89]. A high incidence of hepatotoxicity (elevated transaminases and bilirubin) was reported, resulting in two deaths and subsequent termination of the study [13,89]. Idelalisib is known to induce reversible hepatotoxicity in patients receiving idelalisib monotherapy or in combination with rituximab, however the mechanisms responsible for the hepotoxicitiy observed with lenalidomide and idelalisib are unknown [90–92].

A phase I trial conducted by National Cancer Institute with the combination of lenalidomide and ibrutinib in 50 patients with R/R CLL or SLL, begun in April 2013. Another phase I trial to assess safety and efficacy of combining lenalidomide and rituximab with differing doses of ibrutinib in 28 patients with R/R CLL/SLL, has begun in April 2014 (NCT02200848) is ongoing. Besides combinations with BCR targeting agents, on-going programs are evaluating lenalidomide in combination with monoclonal antibodies. A phase II trial conducted by Ohio State University Comprehensive Cancer Center combining anti-CD19 mAb MOR00208 with lenalidomide in 40 patients with R/R CLL has been initiated in December 2013 (NCT02005289). At MD Anderson Cancer Center, a clinical trial combining the activity of lenalidomide and obinutuzumab (GA101) in patients with recurrent CLL/SLL (NCT02225275) has been initiated, and will enroll 60 patients. A similar phase I/II clinical trial combining lenalidomide and obinutuzumab for the treatment of 25 TN patients with CLL has been initiated by the University of California, San Diego (NCT02371590).

8. Conclusion

CLL outcomes have improved significantly in the last decade. Among its therapeutic options, lenalidomide has a special role due to its unique action on immunosynapse restoration and TME. Clinical trials using lenalidomide have shown sustained clinical responses in patients with R/R CLL (including the HR subgroup of patients with deletion 11q, 17p ± TP53 mutations, unmutated CLL and Flref CLL), and as initial therapy. However, it is important to note that lenalidomide is not recommended for the treatment of patients with CLL outside of controlled clinical trials.

Lenalidomide-associated toxicities can be significant, particularly TFR, TLS, and myelosuppression, and it is important for the treating physicians to be familiar with these manifestations and manage patients appropriately. Responses with lenalidomide-based therapy can be slow to occur, and often best responses are seen after 9–12 months of therapy. An important aspect in developing lenalidomide-based strategies is how to recognize the group of patients most likely to benefit from its use. Both our group and others, observed a higher pretreatment numbers of NK cells and CD8$^+$ T cells in patients obtaining a response to lenalidomide. In patients treated with lenalidomide and ofatumumab, a higher pretreatment absolute number of different T and NK cell subpopulation was found in subjects who obtained a CR. This difference reached statistical significance for CD4$^+$ T cells and CD57$^+$ CD56$^+$ NK cells. In addition, functional studies

performed on T cells and NK cells also showed a better preserved function at baseline in responding patients [56,64,74]. Correlative studies have also pointed toward a relationship between higher likelihood of response or a longer response duration, and circulating levels of various cytokines and chemokines involved in immunoresponse, cellular adhesion and neoangiogenesis, such as IFN-γ, sVEGFR2, VEGF, and bFGF [13,23,56]. Plasma levels of microRNA-155 are expressed at high levels in various types of B-cell malignancies and associated with poor prognosis in patients with CLL, also correlate with response to lenalidomide therapy [93]. CRBN expression serves as an important crosslink to lenalidomide activity, although most of the research has been focused on the relationship between CRBN and CRBN-binding proteins in patients with MM [43]. Jamroziak et al. reported that higher levels of CRBN expression correlated with responses in patients with CLL and CRBN levels could be used to predict response in both patients with CLL and MM [43]. Novel and more specific IMIDs have been identified and recently entered clinical trials with the prospective of providing better therapeutic effects and additional insights in the pathophysiology of CLL.

9. Expert opinion

Many lessons have been learned during the evaluation of lenalidomide in patients with CLL. Very early in its development, we learnt that the dosages and schedule of administration that were safe and effective in patients with MM and myelodysplastic syndromes could not be tolerated by patients with CLL. Patients with CLL require lower starting doses, careful dose escalation, and close monitoring for myelosuppression. The unexpected phenomenon of TFR was also noticed in patients with CLL. This manifestation should not be confused with evidence of early progression and managed symptomatically. Since TFR has shown to be driven by an immunological response, the use of systemic corticosteroids should be avoided if possible in order not to hamper T-cell responses. Preventive measures toward TLS are also important in the initial weeks of therapy with lenalidomide and all patients should receive prophylactic allopurinol, adequate hydration, and undergo careful monitoring of their renal function and electrolytes levels. We find that the most interesting aspect of lenalidomide is the unique ability of this agent to induce changes in the microenvironment and promote immunomodulatory effects in patients with CLL. The relevance of T cells in the response to treatment with lenalidomide provides rationale for introducing an IMID (either lenalidomide or a newer generation compound) in combination with checkpoint inhibitors and to explore the integration of IMIDs within T-cell-directed therapies.

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References

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Rozman C, Montserrat E. Chronic lymphocytic leukemia. N Engl J Med. 1995;333(16):1052–1057.
2. Hallek M, Cheson BD, Catovsky D, et al. Guidelines for the diagnosis and treatment of chronic lymphocytic leukemia: a report from the International Workshop on Chronic Lymphocytic Leukemia updating the National Cancer Institute-Working Group 1996 guidelines. Blood. 2008;111(12):5446–5456.
3. Morton LM, Wang S, Devesa SS, et al. Lymphoma incidence patterns by WHO subtype in the united states, 1992-2001. Blood. 2006;107(1):265–276.
4. Mauro FR, Foa R, Giannarelli D, et al. Clinical characteristics and outcome of young chronic lymphocytic leukemia patients: a single institution study of 204 cases. Blood. 1999;94(2):448–454.
5. Howlader N, Noone AM, Krapcho M, et al. SEER cancer statistics review, 1975-2013. Bethesda (MD): National Cancer Institute. [cited 2016 Apr]. November 2015 SEER data submission, posted to the SEER web site. Available from: http://seer.cancer.gov/csr/1975_2013/.
6. Rai KR, Sawitsky A, Cronkite EP, et al. Clinical staging of chronic lymphocytic leukemia. Blood. 1975;46(2):219–234.
7. Binet JL, Auquier A, Dighiero G, et al. A new prognostic classification of chronic lymphocytic leukemia derived from a multivariate survival analysis. Cancer. 1981;48(1):198–206.
8. Zelenetz AD, Gordon LI, Wierda WG, et al. Chronic lymphocytic leukemia/small lymphocytic lymphoma, version 1.2015. JNCCN. 2015;13(3):326–362.
9. Stilgenbauer S. Prognostic markers and standard management of chronic lymphocytic leukemia. Hematology Am Soc Hematol Educ Program. 2015;2015(1):368–377.
10. Mertens D, Stilgenbauer S. Prognostic and predictive factors in patients with chronic lymphocytic leukemia: relevant in the era of novel treatment approaches? J Clin Oncol. 2014;32(9):869–872.
11. Nabhan C, Raca G, Wang YL. Predicting prognosis in chronic lymphocytic leukemia in the contemporary era. JAMA Oncol. 2015;1(7):965–974.
12. Giannopoulos K, Mertens D, Stilgenbauer S. Treating chronic lymphocytic leukemia with thalidomide and lenalidomide. Expert Opin Pharmacother. 2011;12(18):2857–2864.
13. Maffei R, Colaci E, Fiorcari S, et al. Lenalidomide in chronic lymphocytic leukemia: the present and future in the era of tyrosine kinase inhibitors. Crit Rev Oncol Hematol. 2016;97:291–302.
14. Desai M, Newberry K, Ou Z, et al. Lenalidomide in relapsed or refractory mantle cell lymphoma: overview and perspective. Ther Adv Hematol. 2014;5(3):91–101.
15. Fowler NH, Davis RE, Rawal S, et al. Safety and activity of lenalidomide and rituximab in untreated indolent lymphoma: an open-label, phase 2 trial. Lancet Oncol. 2014;15(12):1311–1318.
16. Ruan J, Martin P, Shah B, et al. Lenalidomide plus rituximab as initial treatment for mantle-cell lymphoma. N Engl J Med. 2015;373(19):1835–1844.
17. Witzig TE, Nowakowski GS, Habermann TM, et al. A comprehensive review of lenalidomide therapy for B-cell non-Hodgkin lymphoma. Ann Oncol. 2015;26(8):1667–1677.
18. FDA approval for lenalidomide. National Cancer Institute. [cited 2013 Jun 5]. Available from: http://www.cancer.gov/about-cancer/treatment/drug/fda-lenalidomide.
19. Zeldis JB, Knight R, Hussein M, et al. A review of the history, properties, and use of the immunomodulatory compound lenalidomide. Ann N Y Acad Sci. 2011;1222:76–82.
20. Galanina N, Petrich A, Nabhan C. The evolving role of lenalidomide in non-Hodgkin lymphoma. Leuk Lymphoma. 2016;57(7):1507–1516.

21. Seiffert M. Lenalidomide, an antiproliferative CLL drug. Blood. 2014;124(10):1545–1546.
22. Acebes-Huerta A, Huergo-Zapico L, Gonzalez-Rodriguez AP, et al. Lenalidomide induces immunomodulation in chronic lymphocytic leukemia and enhances antitumor immune responses mediated by NK and CD4 T cells. Biomed Res Int. 2014;2014:265840.
• **A large study showing efficacy of lenalidomide in elderly untreated CLL.**
23. Badoux XC, Keating MJ, Wen S, et al. Lenalidomide as initial therapy of elderly patients with chronic lymphocytic leukemia. Blood. 2011;118(13):3489–3498.
24. Brown JR. Immunomodulators in chronic lymphocytic leukemia: where does lenalidomide belong? Leuk Lymphoma. 2010;51(8):1382–1385.
25. Carballido E, Veliz M, Komrokji R, et al. Immunomodulatory drugs and active immunotherapy for chronic lymphocytic leukemia. Cancer Control. 2012;19(1):54–67.
26. Cortelezzi A, Sciumè M, Reda G. Lenalidomide in the treatment of chronic lymphocytic leukemia. Adv Hematol. 2012;2012:1–7.
27. Gentile M, Recchia AG, Vigna E, et al. Lenalidomide in the treatment of chronic lymphocytic leukemia. Expert Opin Investig Drugs. 2011;20(2):273–286.
28. Hallek M. Chronic lymphocytic leukemia: 2015 update on diagnosis, risk stratification, and treatment. Am J Hematol. 2015;90(5):446–460.
29. Kater AP, Tonino SH, Egle A, et al. How does lenalidomide target the chronic lymphocytic leukemia microenvironment? Blood. 2014;124(14):2184–2189.
30. Davies F, Baz R. Lenalidomide mode of action: linking bench and clinical findings. Blood Rev. 2010;24(Suppl 1):S13–S19.
31. Fecteau J-F, Corral LG, Ghia EM, et al. Lenalidomide inhibits the proliferation of CLL cells via a cereblon/p21(WAF1/Cip1)-dependent mechanism independent of functional p53. Blood. 2014;124(10):1637–1644.
32. Fink EC, Ebert BL. The novel mechanism of lenalidomide activity. Blood. 2015;126(21):2366–2369.
33. Anonymous. National center for biotechnology information [Internet]. PubChem Compoound Database; CID=216326. [cited 2016 Apr 15]. Available from: https://pubchem.ncbi.ncbi.nih.gov/compound/216326.
34. Kotla V, Goel S, Nischal S, et al. Mechanism of action of lenalidomide in hematological malignancies. J Hematol Oncol. 2009;2:36.
35. Chen N, Wen L, Lau H, et al. Pharmacokinetics, metabolism and excretion of [(14)C]-lenalidomide following oral administration in healthy male subjects. Cancer Chemother Pharmacol. 2012;69(3):789–797.
36. Kumar G, Lau H, Laskin O. Lenalidomide: in vitro evaluation of the metabolism and assessment of cytochrome P450 inhibition and induction. Cancer Chemother Pharmacol. 2009;63(6):1171–1175.
37. Hideshima T, Chauhan D, Shima Y, et al. Thalidomide and its analogs overcome drug resistance of human multiple myeloma cells to conventional therapy. Blood. 2000;96(9):2943–2950.
38. Corral LG, Haslett PA, Muller GW, et al. Differential cytokine modulation and T cell activation by two distinct classes of thalidomide analogues that are potent inhibitors of TNF-alpha. J Immunol. 1999;163(1):380–386.
39. Marriott JB, Clarke IA, Dredge K, et al. Thalidomide and its analogues have distinct and opposing effects on TNF-alpha and TNFR2 during co-stimulation of both CD4(+) and CD8(+) T cells. Clin Exp Immunol. 2002;130(1):75–84.
40. LeBlanc R, Hideshima T, Catley LP, et al. Immunomodulatory drug costimulates T cells via the B7-CD28 pathway. Blood. 2004;103(5):1787–1790.
41. Görgün G, Holderried TAW, Zahrieh D, et al. Chronic lymphocytic leukemia cells induce changes in gene expression of CD4 and CD8 T cells. J Clin Invest. 2005;115(7):1797–1805.
•• **This study describes lenalidomide-induced reversal of immunosynapse dysfunction in CLL.**
42. Ramsay AG, Johnson AJ, Lee AM, et al. Chronic lymphocytic leukemia T cells show impaired immunological synapse formation that can be reversed with an immunomodulating drug. J Clin Invest. 2008;118(7):2427–2437.
43. Jamroziak K, Szemraj J, Robak T, et al. Cereblon expression predicts clinical response in chronic lymphocytic leukemia treated with a thalidomide/fludarabine regimen. Leuk Lymphoma. 2015;56(3):808–810.
44. Lopez-Girona A, Mendy D, Ito T, et al. Cereblon is a direct protein target for immunomodulatory and antiproliferative activities of lenalidomide and pomalidomide. Leukemia. 2012;26(11):2326–2335.
45. Gandhi AK, Kang J, Havens CG, et al. Immunomodulatory agents lenalidomide and pomalidomide co-stimulate T cells by inducing degradation of T cell repressors Ikaros and Aiolos via modulation of the E3 ubiquitin ligase complex CRL4(CRBN.). Br J Haematol. 2014;164(6):811–821.
•• **This study describes correlation between lenalidomide action and cereblon pathway.**
46. Lu G, Middleton RE, Sun H, et al. The myeloma drug lenalidomide promotes the cereblon-dependent destruction of Ikaros proteins. Science. 2014;343(6168):305–309.
47. Krönke J, Udeshi ND, Narla A, et al. Lenalidomide causes selective degradation of IKZF1 and IKZF3 in multiple myeloma cells. Science. 2014;343(6168):301–305.
48. Verhelle D, Corral LG, Wong K, et al. Lenalidomide and CC-4047 inhibit the proliferation of malignant B cells while expanding normal CD34+ progenitor cells. Cancer Res. 2007;67(2):746–755.
49. Sher T, Miller KC, Lawrence D, et al. Efficacy of lenalidomide in patients with chronic lymphocytic leukemia with high-risk cytogenetics. Leuk Lymphoma. 2010;51(1):85–88.
50. Arumainathan A, Kalakonda N, Pettitt AR. Lenalidomide can be highly effective in chronic lymphocytic leukaemia despite T-cell depletion and deletion of chromosome 17p. Eur J Haematol. 2011;87(4):372–375.
• **This study describes correlation between lenalidomide-induced immune activation and its unique toxicities.**
51. Aue G, Njuguna N, Tian X, et al. Lenalidomide-induced upregulation of CD80 on tumor cells correlates with T-cell activation, the rapid onset of a cytokine release syndrome and leukemic cell clearance in chronic lymphocytic leukemia. Haematologica. 2009;94(9):1266–1273.
52. Lapalombella R, Andritsos L, Liu Q, et al. Lenalidomide treatment promotes CD154 expression on CLL cells and enhances production of antibodies by normal B cells through a PI3-kinase-dependent pathway. Blood. 2010;115(13):2619–2629.
53. Ramsay AG, Clear AJ, Fatah R, et al. Multiple inhibitory ligands induce impaired T-cell immunologic synapse function in chronic lymphocytic leukemia that can be blocked with lenalidomide: establishing a reversible immune evasion mechanism in human cancer. Blood. 2012;120(7):1412–1421.
54. Fiorcari S, Martinelli S, Bulgarelli J, et al. Lenalidomide interferes with tumor-promoting properties of nurse-like cells in chronic lymphocytic leukemia. Haematologica. 2015;100(2):253–262.
55. Jones JA, Byrd J. How will B-cell-receptor-targeted therapies change future CLL therapy? Blood. 2014;123(10):1455–1460.
56. Lee BN, Gao H, Cohen EN, et al. Treatment with lenalidomide modulates T-cell immunophenotype and cytokine production in patients with chronic lymphocytic leukemia. Cancer. 2011;117(17):3999–4008.
57. Henry JY, Labarthe MC, Meyer B, et al. Enhanced cross-priming of naive CD8+ T cells by dendritic cells treated by the IMiDs(R) immunomodulatory compounds lenalidomide and pomalidomide. Immunology. 2013;139(3):377–385.
•• **First study describing efficacy of lenalidomide in patients with R/R CLL.**
58. Chanan-Khan A, Miller KC, Musial L, et al. Clinical efficacy of lenalidomide in patients with relapsed or refractory chronic lymphocytic leukemia: results of a phase II study. J Clin Oncol. 2006;24(34):5343–5349.

59. Ferrajoli A, Lee B-N, Schlette EJ, et al. Lenalidomide induces complete and partial remissions in patients with relapsed and refractory chronic lymphocytic leukemia. Blood. 2008;111 (11):5291–5297.
60. Wendtner CM, Hallek M, Fraser GA, et al. Safety and efficacy of different lenalidomide starting doses in patients with relapsed or refractory chronic lymphocytic leukemia: results of an international multicenter double-blinded randomized phase II trial. Leuk Lymphoma. 2016;57(6):1–9.
61. Badoux XC, Keating MJ, Wen S, et al. Phase II study of lenalidomide and rituximab as salvage therapy for patients with relapsed or refractory chronic lymphocytic leukemia. J Clin Oncol. 2013;31 (5):584–591.
62. Maddocks K, Wei L, Rozewski D, et al. Reduced occurrence of tumor flare with flavopiridol followed by combined flavopiridol and lenalidomide in patients with relapsed chronic lymphocytic leukemia (CLL). Am J Hematol. 2015;90(4):327–333.
63. Costa LJ, Fanning SR, Stephenson J Jr., et al. Sequential ofatumumab and lenalidomide for the treatment of relapsed and refractory chronic lymphocytic leukemia and small lymphocytic lymphoma. Leuk Lymphoma. 2015;56(3):645–649.
64. Vitale C, Falchi L, Ten Hacken E, et al. Ofatumumab and lenalidomide for patients with relapsed or refractory chronic lymphocytic leukemia: correlation between responses and immune characteristics. Clin Cancer Res. 2016;22(10):2359–2367.
65. Chen CI, Bergsagel PL, Paul H, et al. Single-agent lenalidomide in the treatment of previously untreated chronic lymphocytic leukemia. J Clin Oncol. 2011;29(9):1175–1181.
66. Brown JR, Abramson J, Hochberg E, et al. A phase I study of lenalidomide in combination with fludarabine and rituximab in previously untreated CLL/SLL. Leukemia. 2010;24(11):1972–1975.
67. Flinn IW, Cooper RS, Thompson DS, et al. Fludarabine, rituximab, and lenalidomide in previously untreated patients with chronic lymphocytic leukemia (CLL): a phase I/II trial of the Sarah Cannon Research Institute. Blood. 2012;120(21):715.
68. Egle A, Steurer M, Gassner FJ, et al. Lenalidomide/rituximab maintenance after induction with fludarabine/rituximab in combination with escalating doses of lenalidomide in previously untreated chronic lymphocytic leukemia (CLL): the revlirit CLL5 AGMT phase I/II study, final results. Blood. 2013;122(21):4164.
69. James DF, Werner L, Brown JR, et al. Lenalidomide and rituximab for the initial treatment of patients with chronic lymphocytic leukemia: a multicenter clinical-translational study from the chronic lymphocytic leukemia research consortium. J Clin Oncol. 2014;32 (19):2067–2073.
70. Chen CI, Paul H, Snitzler S, et al. A phase 2 study of lenalidomide in combination with oral dexamethasone in previously untreated, symptomatic patients with chronic lymphocytic leukemia (CLL). Blood. 2015;126(23):4163.
71. Thompson PA, Keating MJ, Hinojosa C, et al. Lenalidomide and rituximab in combination as initial treatment of chronic lymphocytic leukemia: initial results of a phase II study. Blood. 2014;124 (21):1988.
72. Chen CI, Paul H, Wang T, et al. Long-term follow-up of a phase 2 trial of single agent lenalidomide in previously untreated patients with chronic lymphocytic leukaemia. Br J Haematol. 2014;165 (5):731–733.
73. Strati P, Keating MJ, Wierda WG, et al. Lenalidomide induces long-lasting responses in elderly patients with chronic lymphocytic leukemia. Blood. 2013;122(5):734–737.
74. Chanan-Khan A, Miller KC, Lawrence D, et al. Tumor flare reaction associated with lenalidomide treatment in patients with chronic lymphocytic leukemia predicts clinical response. Cancer. 2011;117 (10):2127–2135.
75. Wendtner CM, Hillmen P, Mahadevan D, et al. Final results of a multicenter phase 1 study of lenalidomide in patients with relapsed or refractory chronic lymphocytic leukemia. Leuk Lymphoma. 2012;53(3):417–423.
76. Egle A, Gassner F, Geisberger R, et al. A combination of fludarabine/rituximab with escalating doses of lenalidomide in untreated chronic lymphocytic leukemia: the REVLIRIT CLL5 AGMT phase 1/2 study—results from clinical and exploratory analyses of induction results. Blood. 2011;118(21):292.
77. Shanafelt TD, Ramsay AG, Zent CS, et al. Long-term repair of T-cell synapse activity in a phase II trial of chemoimmunotherapy followed by lenalidomide consolidation in previously untreated chronic lymphocytic leukemia (CLL). Blood. 2013;121(20):4137–4141.
78. Gonzalez-Rodriguez AP, Payer AR, Acebes-Huerta A, et al. Lenalidomide and chronic lymphocytic leukemia. Biomed Res Int. 2013;2013:932010.
79. Aue G, Nelson Lozier J, Tian X, et al. Inflammation, TNFalpha and endothelial dysfunction link lenalidomide to venous thrombosis in chronic lymphocytic leukemia. Am J Hematol. 2011;86(10):835–840.
80. Andritsos LA, Johnson AJ, Lozanski G, et al. Higher doses of lenalidomide are associated with unacceptable toxicity including life-threatening tumor flare in patients with chronic lymphocytic leukemia. J Clin Oncol. 2008;26(15):2519–2525.
•• **This study summarizes the incidence of TFR/TLS.**
81. Moutouh-de Parseval LA, Weiss L, DeLap RJ, et al. Tumor lysis syndrome/tumor flare reaction in lenalidomide-treated chronic lymphocytic leukemia. J Clin Oncol. 2007;25(31):5047.
82. Maddocks K, Ruppert AS, Browning R, et al. A dose escalation feasibility study of lenalidomide for treatment of symptomatic, relapsed chronic lymphocytic leukemia. Leuk Res. 2014;38 (9):1025–1029.
83. Georg Aue SS, Janet V, Diane C. Arthur, Xin Tian5* and Adrian Wiestner. Phase II trial of pulse dosed lenalidomide in previously treated chronic lymphocytic leukemia. Blood. 2010;116(21):1383–83.
84. Lamanna N. New oral small molecules in the treatment of chronic lymphocytic leukemia. Cancer. 2015;121(12):1917–1926.
85. Byrd JC, Jones JJ, Woyach JA, et al. Entering the era of targeted therapy for chronic lymphocytic leukemia: impact on the practicing clinician. J Clin Oncol. 2014;32(27):3039–3047.
86. Wiestner A. Emerging role of kinase-targeted strategies in chronic lymphocytic leukemia. Hematology Am Soc Hematol Educ Program. 2012;2012:88–96.
87. Young RM, Staudt LM. Targeting pathological B cell receptor signalling in lymphoid malignancies. Nat Rev Drug Discov. 2013;12 (3):229–243.
88. Pollyea DA, Coutre S, Gore L, et al. A dose escalation study of ibrutinib with lenalidomide for relapsed and refractory chronic lymphocytic leukemia/small lymphocytic lymphoma. Blood. 2014;124(21):1987.
89. Cheah CY, Nastoupil LJ, Neelapu SS, et al. Lenalidomide, idelalisib, and rituximab are unacceptably toxic in patients with relapsed/refractory indolent lymphoma. Blood. 2015;125(21):3357–3359.
90. Gopal AK, Kahl BS, De Vos S, et al. PI3Kdelta inhibition by idelalisib in patients with relapsed indolent lymphoma. N Engl J Med. 2014;370(11):1008–1018.
91. Furman RR, Sharman JP, Coutre SE, et al. Idelalisib and rituximab in relapsed chronic lymphocytic leukemia. N Engl J Med. 2014;370 (11):997–1007.
92. Keating GM. Idelalisib: a review of its use in chronic lymphocytic leukaemia and indolent non-Hodgkin's lymphoma. Target Oncol. 2015;10(1):141–151.
93. Ferrajoli A, Shanafelt TD, Ivan C, et al. Prognostic value of miR-155 in individuals with monoclonal B-cell lymphocytosis and patients with B chronic lymphocytic leukemia. Blood. 2013;122(11):1891–1899.