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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.

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 take 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
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 (10²–10³ 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/p21WAF1/Cip1 dependent pathway [31]. p21WAF1/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 (Aioli) (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 p21WAF1–1 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/p21WAF1/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 TP53 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
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 (Tregs), 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 proportions 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 1. Studies with lenalidomide as therapy in patients with R/R CLL.

<table>
<thead>
<tr>
<th>Author</th>
<th>N</th>
<th>Phase</th>
<th>Treatment</th>
<th>Median age (years)</th>
<th>ORR (CR) (%)</th>
<th>OS/PFS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chanan-Khan et al. [58]</td>
<td>45</td>
<td>II</td>
<td>L* – 5–25 mg/day (D1–21/28)</td>
<td>64 (42–75)</td>
<td>47 (9)</td>
<td>1-year estimated PFS – 81 months</td>
</tr>
<tr>
<td>Ferrajoli et al. [59]</td>
<td>44</td>
<td>II</td>
<td>L* – 10–25 mg/day continuous</td>
<td>64 (49–86)</td>
<td>32 (7)</td>
<td>OS – 73 months at a median follow up of 14 months</td>
</tr>
<tr>
<td>Wendtner et al. [60]</td>
<td>34</td>
<td>II</td>
<td>L* – 5–25 mg/day (D1–21/28)</td>
<td>65.5 (32–81)</td>
<td>44 (14.7)</td>
<td>Median PFS – 96.3 weeks</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>II</td>
<td>L* – 10–25 mg/day (D1–21/28)</td>
<td>63 (39–78)</td>
<td>37 (5.7)</td>
<td>Median PFS – 117.6 weeks</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>II</td>
<td>L* – 15–25 mg/day (D1–21/28)</td>
<td>65 (48–78)</td>
<td>40 (2.9)</td>
<td>Median PFS – 89.3 weeks</td>
</tr>
<tr>
<td>Badoux et al. [61]</td>
<td>59</td>
<td>II</td>
<td>R – 375 mg/m² (weekly × 4; monthly × 12)</td>
<td>62 (42–82)</td>
<td>66 (12)</td>
<td>At 36 months, OS – 71</td>
</tr>
<tr>
<td>Maddocks et al. [62]</td>
<td>39</td>
<td>I</td>
<td>Flavo. – 60 mg/m² C1:D1, 80 mg/m² C1:DB, 15; dose escalation C2–D3,10,17;</td>
<td>62 (26–74)</td>
<td>51 (0)</td>
<td>Median PFS – 7.7 months</td>
</tr>
<tr>
<td>Costa et al. [63]</td>
<td>21</td>
<td>II</td>
<td>Ofa – 2 g C1–6; D1, L 5 mg/day continuous, dose escalation C2–8</td>
<td>63 (48–80)</td>
<td>48 (0)</td>
<td>Median OS – 21.5 months</td>
</tr>
<tr>
<td>Vitale et al. [64]</td>
<td>34</td>
<td>II</td>
<td>Ofa – 300 mg C1D1, 1000 mg C1 D8,15,22; C2–6D1, L 10 mg D9 onwards continuous</td>
<td>64 (34–82)</td>
<td>71 (18)</td>
<td>5 year OS – 53 months</td>
</tr>
</tbody>
</table>

L: lenalidomide; R: rituximab; F: fludarabine; Flavo: flavopiridol; Ofa: ofatumumab; L*: lenalidomide dose escalation; R/R: relapsed/refractory; PFS: progression-free survival; OS: overall survival; CR: complete response.
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 nonhematological 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 \( \geq 5 \) 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

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

<table>
<thead>
<tr>
<th>Author</th>
<th>( N )</th>
<th>Phase</th>
<th>Treatment</th>
<th>Median age (years) (range)</th>
<th>ORR (CR %)</th>
<th>OS/PFS (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chen et al. [65]</td>
<td>25</td>
<td>II</td>
<td>L – 2.5–25 mg (D1–21/28)</td>
<td>60 (33–78)</td>
<td>56 (0)</td>
<td>2 year OS – 92%</td>
</tr>
<tr>
<td>Badoux et al. [23]</td>
<td>60</td>
<td>II</td>
<td>L – 5–25 mg (D1–28)</td>
<td>71 (66–85)</td>
<td>65 (10)</td>
<td>2 year PFS – 89%; 2 year estimated OS – 88; PFS – 60</td>
</tr>
<tr>
<td>Brown et al. [66]</td>
<td>9</td>
<td>I</td>
<td>F – 25 mg/m(^2) (D3–5) R – 375 mg/m(^2) (D1) L* – 2.5–25 mg (D1–21/28)</td>
<td>59 (37–66)</td>
<td>56 (11)</td>
<td>NA</td>
</tr>
<tr>
<td>Flinn et al. [67]</td>
<td>51</td>
<td>I/II</td>
<td>F – 25 mg/m(^2) (D3–5) R – 375 mg/m(^2) (C1,01 + 2) 500 mg/m(^2) (C2–6/D1) L* – 2.5–5 mg (D8–28) (C1–6) F – 40 mg/m(^2) (D1–3)</td>
<td>62 (44–82)</td>
<td>63 (11)</td>
<td>OS/PFS – 88/71 at 24 months</td>
</tr>
<tr>
<td>Egle et al. [68]</td>
<td>45</td>
<td>I/II</td>
<td>F – 25 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 to a maximum of 2 months till 24 months</td>
<td>67 (43–79)</td>
<td>96 (67)</td>
<td>PFS – 89% at a median follow up of 35 months</td>
</tr>
<tr>
<td>James et al. [69]</td>
<td>40</td>
<td>II</td>
<td>R – 375 mg/m(^2) (weekly, D1) L* – 2.5–10 mg (D1–21/28)</td>
<td>56 (45–64)</td>
<td>95 (20)</td>
<td>PFS – 18 months</td>
</tr>
<tr>
<td>Chen et al. [70]</td>
<td>29</td>
<td>II</td>
<td>L* – 5–25 mg/day continuously Dex 12 mg (D1–7,14, and 21)</td>
<td>70 (65–80)</td>
<td>79 (7)</td>
<td>PFS – 20 months</td>
</tr>
<tr>
<td>Thompson et al. [71]</td>
<td>58</td>
<td>II</td>
<td>R – 375 mg/m(^2) (weekly × 4; monthly 3–12) L – 10 mg/day from D9 – till 24 months</td>
<td>66 (42–79)</td>
<td>83 (15)</td>
<td>Median OS and TTF not reached</td>
</tr>
</tbody>
</table>

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].
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 ≤ 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 (131.2 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 B-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/m² on day 29, 325 mg/m² on day 31, and 375 mg/m² 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 OS 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/m²) was given for 3–5 days and rituximab (375 mg/m²) 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/m²) given orally d1–3 q28d) and rituximab (375 mg/m² 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/m², day 1), cyclophosphamide (C) (600 mg/m², day 1) and rituximab (R) (cycle 1:100 mg, day 1; 375 mg/m², day 2, cycle 2–6: 375 mg/m² 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 correla-
tive 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 lenala-
domide 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 lenali-
domide 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 lenali-
domide 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 indefi-
nitely in patients experiencing a sustained PR or CR, but patients were discontinued from the study if there was pro-
gression 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 Fliei
drome 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 encoun-
tered was neutropenia (82%). Grade 3–4 neutropenia, throb-
ocytopenia 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 neu-
tropenia 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 ele-
vated 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 inflamma-
tory markers, such as TNF-α, soluble vascular endothelial adhe-
sion 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 individu-
ally 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 fail-
ure [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), hyper-
uricemia, hyperphosphatemia and high lactate dehydrogenase levels, suggestive of severe TLS and died due to hypoxemic respiratory failure. Another patient developed severe neutro-
penic 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/m² 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 hepatotoxicity 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 II/III 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, 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 immunorespose, 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 perspective 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 electrolyte 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.


- A large study showing efficacy of lenalidomide in elderly untreated CLL.


- This study describes lenalidomide-induced reversal of immunosynapse dysfunction in CLL.

- This study describes correlation between lenalidomide action and cereblon pathway.

- This study describes correlation between lenalidomide-induced immune activation and its unique toxicities.

- First study describing efficacy of lenalidomide in patients with R/R CLL.


This study summarizes the incidence of TFR/TLS.