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Thalidomide and lenalidomide as new therapeutics for the treatment of chronic lymphocytic leukemia

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Abstract

The use of nucleoside analog-based chemoimmunotherapeutic regimens over the last decade has significantly improved outcomes in patients with chronic lymphocytic leukemia (CLL). Nonetheless, virtually all patients with CLL relapse from chemoimmmunotherapy and current available therapies are not curative. Identifying therapies that effectively eliminate CLL cells and lack immunesuppression represent an exciting new therapeutic approach. IMiDs are a class of immunomodulating drugs that increase T-cell and NK-cell directed killing of tumor cells. The first generation molecule is thalidomide followed by a second generation molecule lenalidomide that lacks neurotoxicity and is being explored more extensively in clinical trials. Lenalidomide has been shown to benefit patients with multiple myeloma, myelodysplastic syndromes, and lymphoma. Initial reports in patients with relapsed and refractory CLL have shown promising responses. In a subset of patients with CLL complete responses have been noted. Subsequent studies, however, have suggested that this class of drug can also have serious and potentially life-threatening side effects including myelosuppression, tumor flare reaction and in a small subset of patients tumor lysis syndrome. Tumor flare with both thalidomide and lenalidomide appear to be disease specific to CLL and may reflect clinical manifestation of CLL tumor cell activation. As a consequence of these disease specific effects, the optimal safe dose of lenalidomide in CLL remains to be determined but appears to be lower than that tolerated in other B-cell malignancies. To date, biomarkers for response remain poorly defined and the relationship of clinical benefit to tumor flare is uncertain. This review examines the existing literature on the use of IMiDs in patients with CLL and provides suggestions for future research in this area.

Keywords

Chronic lymphocytic leukemia; thalidomide; lenalidomide; tumor flare reaction; tumor lysis syndrome

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Introduction

The advent of nucleoside analog-based regimens over the last two decades have resulted in significant improvements in the outcomes of patients with chronic lymphocytic leukemia (CLL) which remains the most common leukemia in the western hemisphere. Advances in combination chemotherapy with fludarabine/cyclophosphamide [1–3] and the addition of rituximab to such treatments [4,5] has further improved outcome for patients with CLL. Indeed recent data from a large randomized clinical trial reported by the German CLL study group [6] have shown a benefit of the addition of antibody therapy with rituximab in the prolongation of progression-free survival (PFS) in patients with untreated CLL. This encouraging progress in therapy and our understanding of the disease has resulted in significantly improved response rates and progression-free survival, however, significant improvements in overall survival (OS) and ultimately cure, remains an elusive goal. Most patients eventually relapse with their disease and frequently with refractory disease.

Patients who relapse after combined chemoimmunotherapy have a poor outcome with subsequent therapies. Although options for these patients are present including alemtuzumab [7], bendamustine [8], high dose corticosteroids [9], flavopiridol [10], ofatumumab [11], and combination-based approaches [12], none of these therapies produce durable remissions that exceed that observed with first line chemoimmunotherapy. Several of these therapies including alemtuzumab, [13,14] and high dose steroids [9,15–17] are also associated with significant toxicity and sustained immunosuppression. These toxicities are compounded by pre-existing disease related immune dysregulation commonly seen in patients with advanced CLL and frequently result in serious and life-threatening infections. Therefore, therapies for CLL that result in minimal impairment of the immune system or alternatively recruit and enhance the immune system to effectively clear CLL cells are highly desired. The benefit and promise of immune-based therapy is best substantiated by the prolonged sustained remissions observed with allogeneic stem cell transplantation [18,19]. Other attempts to capitalize on reversing the immune defect in CLL as a therapy for CLL include the recent use of CD154 gene therapy [20] and CpG oligodeoxy-nucleotides [21] that are currently being optimized in clinical trials of CLL. Other therapies such as the anti-sense molecule Genasense (G3139) may have immunologic properties of a CpG oligonucleotide [22]. If such therapies could be sufficiently developed, they would also potentially have promise for treatment earlier in the course of disease thereby avoiding immune suppression commonly observed with fludarabine-based therapies.

The IMiDs are a class of drugs developed by Celgene Corporation (Summit, NJ) that exert their action primarily through the modulation of the immune system and their use has been shown to both reverse immune defects and also promote promising responses in patients with CLL. The IMiDs under clinical development include thalidomide and its more potent analogs lenalidomide and pomalidomide. Lenalidomide is the most widely studied agent in this class for the treatment of CLL and is approved for marketing by the FDA in patients with multiple myeloma and myelodysplastic syndromes. Clinical trials with pomalidomide in cancer have recently begun and to date there are no reports of pre-clinical or clinical application of this agent in CLL. In this article, we aim to recent clinical data on the use of thalidomide and lenalidomide for the management of CLL, both in the untreated and relapsed, refractory setting. We will then provide some background on specific relevant mechanisms of action of lenalidomide in CLL with particular attention to features unique to CLL including the mechanism of tumor flare.

Clinical activity of thalidomide

Early reports of utilizing thalidomide in patients with heavily pre-treated CLL were not very encouraging with a substantial number of patients experiencing tumor flare, neurologic toxicities, and fatigue [23–25]. This resulted in poor accrual and premature closure of the North Central Cancer Treatment Group (NCCTG) trial [23]. Tumor flare had previously been described by the National Cancer Institute in the Common Terminology Criteria for Adverse Events v3.0 as a 'constellation of signs and symptoms in direct relation to initiation of therapy'. These symptoms or signs may include tumor pain, inflammation of visible tumor, hypercalcemia, diffuse bone pain, and other electrolyte disturbances, with grade 3 and 4 tumor flares causing severe or disabling pain, respectively, enough to adversely impact the activities of daily living [26]. In the instance of CLL, the flare was evident by progressive painful lymphadenopathy, rash, low-grade fever, flu-like symptoms, and in some cases, elevation of lymphocyte count within the initial few days of starting therapy. In the eventual cohort of these relatively high-risk patients in the NCCTG study, there were only infrequent responses with an overall response (OR) rate of 11% with only one patient achieving a complete response (CR). Thalidomide did however demonstrate a promising ability of sustaining its therapeutic effects for a significant duration as evidenced by disease stabilization in 50% of the patients for a median of 8 months [23,25] (Table I).

Multiple subsequent small phase II studies also showed similar limited benefits of thalidomide with a high incidence of tumor flare that was seen mostly during the early part of therapy [24,28,29]. Interestingly, Furman *et al.* reported in their randomized phase 2 trial [32,27], comparing the combination of fludarabine and thalidomide with thalidomide alone, an absence of the tumor flare response in patients treated with fludarabine compared to a 62% incidence in patients randomized to the thalidomide only arm. It is unclear whether these early studies were confounded by the inadvertent misclassification of patients experiencing flares into the group of patients experiencing progressive disease and the resulting underestimation of the therapeutic benefits of thalidomide (Table I).

Thalidomide combination trials have also been pursued in symptomatic patients with untreated CLL. Two small studies by Chanan-Khan *et al.* [29] and Giannopoulos *et al.* [30] both utilizing similar combination regimen of fludarabine and thalidomide demonstrated 100% OR rates with CR rates of 55% and 22%, respectively. The combination was generally very well tolerated with low incidences of cytopenias and thromboemboli. Tumor flare reactions (TFR) were reported in both studies but were generally of lower grades and responded well to supportive treatments including the use of non-steroidal anti-inflammatory drugs (NSAID). The further use of thalidomide however was limited by the development of more potent IMiD analogs like lenalidomide, which have been used more extensively in the management of patients with CLL (Table I).

Clinical activity of lenalidomide in chronic lymphocytic leukemia

On the basis of its activity in multiple myeloma and myelodysplastic syndromes and preliminary evidence of efficacy with the use of thalidomide, lenalidomide was utilized in patients with CLL both in the relapsed/refractory and untreated settings. In the original report noting activity of lenalidomide, Chanan-Khan *et al.* from the Roswell Park Cancer Institute (RPCI) reported exciting and promising results with the use of lenalidomide in patients with relapsed/refractory CLL in their phase 2 study [33]. In their study that utilized a 25 mg daily dose of lenalidomide every 21 of 28 days, 45 patients were enrolled. The patient demographics included 64% with Rai stage 3–4 disease, 36% having del(11q22.3) or del(17p13.1), a median of three prior therapies with 51% being fludarabine refractory. After the first 29 patients were enrolled on this study the dosing schedule was modified due to two

patients having tumor lysis syndrome. A dose escalation beginning at 5 mg/day was the starting dose followed by escalating this dose weekly by 5 mg to a maximal dose of 25 mg. Using an intent to treat analysis, 47% of patients had an objective partial (38%) or complete (9%) response. The responses were observed in patients with poor risk cytogenetic abnormalities including del(11q22.3) and del(17p13), although the number of patients with high risk genomic features was low. The median time to best response was 5.9 months with a median progression-free survival of 19.4 months [33] (Table II).

Lenalidomide in this study [33] was associated with significant grade 3 and 4 cytopenias including 70% of patients developing grade 3 or 4 neutropenia and 45% by thrombocytopenia. Eight (20%) of patients developed grade 3 or 4 infections or febrile neutropenia were during the treatment with lenalidomide. Fatigue was noted in the majority (83%) of patients enrolled but was grade 3 and 4 in only 10% of patients. Similarly, TFR characterized by painfully enlarging lymph nodes and bone pain occurring early in treatment was noted in 58% of patients but was only grade 3 or 4 in 8% of individuals. This was often treated with corticosteroids which decreased the severity of tumor flare. No correlation between clinical response and tumor flare was noted in this trial [33]. Grade 1–2 rash was noted in 40% and diarrhea in 33% of patients. Unusual grade 3 or 4 toxicities included pulmonary embolism and tumor lysis syndrome in two patients. The cases of tumor lysis had very modest electrolyte abnormalities and predominately renal insufficiency and elevated uric acid levels. Neither of these cases required dialysis. As a result of occurrence of tumor lysis syndrome in two of the first 29 patients, the treatment protocol was revised to allow slow dose escalation in subsequent patients enrolled. Lenalidomide was started at 5 mg and escalated by 5 mg every 1 to 2 weeks (maximum of 25 mg).

A subsequent study performed at the MD Anderson Cancer Center (MDACC) utilized a lower dose of lenalidomide 10 mg daily, with a 5 mg dose escalation every 28 days upto a maximum dose of 25 mg daily in a continuous manner.[34] The MDACC enrolled 44 patients with relapsed CLL with 45% being Rai stage 3–4 disease, 56% having del(17p13.1) or del(11q22.3), and having received a median of 5 prior therapies with 27% being fludarabine refractory. This second study using a continuous schedule did not note the same drug tolerability as observed in the former trial and despite the planned dose escalation built in the protocol most of the patients were unable to escalate to 25 mg/day. A dose of 10 mg was the median delivered dose in this trial. Similar to the initial study, clinical benefit was observed with an overall response with 32% with 7% attaining a complete remission. Notably, 13% of patients with del (17p13.1) and 39% of patients with del(11q22.3) responded to therapy. Response appeared to be sustained for a median of greater than 12 months.

Similar to the Roswell Park experience [33], lenalidomide was associated with significant grade 3 and 4 hematopoetic toxicity including 41% of courses complicated by neutropenia and 15% by thrombocytopenia. Twenty grade 3 or 4 infections were noted during the treatment with lenalidomide with a small proportion of opportunistic infections. Of note, no decrease in the T-cell counts was noted in this study. Some degree of tumor flare was noted in 30% of patients with a significantly higher frequency (9 of 17, 53%) in patients with lymph nodes larger than 5 cm when compared with the remaining patients (4 of 27, 15%) with nodes less than this. No difference in response (38% versus 34%) among patients with and without tumor flare. Patients with elevated serum IL-6 and VEGF during therapy had a diminished clinical response. This was often treated with a short course of corticosteroids. Outside of tumor flare, diarrhea and rash were also commonly noted but in most cases was grade 1 or 2. One patient had a deep venous thrombosis and no patient had tumor lysis syndrome.

Based upon the promising data generated to date with lenalidomide in relapsed CLL, our group initiated a phase I/II study that incorporated a variety of biologic studies to try to understand the mechanism of action of this drug [38]. The eligibility criteria of this trial were similar to the initial trial performed by Chanan-Khan *et al.* [33] and applied the 25 mg day dose scheduling with 21 days on therapy followed by a 7-day rest period. Three patients in the first cohort had early or late dose limiting toxicity due to tumor flare ($n = 2$) or life threatening infection ($n = 1$). One patient required resection of his tonsils because of impending airway compromise. The excised tonsils were examined, and showed CLL/SLL involvement without evidence of transformation. In this trial, we were able to demonstrate that *ex vivo* activation of CLL cells measured by increased expression of CD40 correlated with development of tumor flare [38]. On the other hand, a large multi-institutional study was initiated to replicate the safety data with lenalidomide using the RPCI and MDACC mentioned schedules of lenalidomide [39]. This phase II trial randomized between 25 mg/day or continuous dosing at 10 mg/day of lenalidomide multiple US and European sites. This trial enrolled 18 patients with early suspension of accrual due to unexpected deaths due to rapid disease progression, tumor flare and atypical tumor lysis syndrome [39]. These unfortunate events in the validation study along with toxicity observed by our group [38] suggest that the 25 mg/day schedule cannot be safely administered to patients with CLL with active disease and that lower doses should be pursued. A follow-up study of this trial that ultimately was designed to a dose escalation phase I study demonstrated that initiating lenalidomide at 2.5 mg/day using continuous dosing and slowly dose-escalating as tolerated was a safe way to administer this drug [35]. Efficacy from this schedule in relapsed CLL has not been reported.

Moving forward from these studies with lenalidomide in patients with previously treated CLL, several groups have also begun exploring lenalidomide as initial therapy for CLL. The MDACC group (Table III) has reported the use of lenalidomide in elderly patients (>65 years of age) with symptomatic, previously untreated CLL [41]. Dosing began at 5 mg and was increased as tolerated to a maximum of 25 mg. The median dose administered in this trial was 10 mg. Forty-three patients were reported with a median age of 72, 42% being high risk (Rai stage III/IV disease), 30% having high risk cytogenetics (del(11q22.3 or del(17p13.1)), and 44% having un-mutated IgV_H disease. Nineteen patients achieved a partial response according to the 1996 NCIWG criteria for an overall response rate of 54%. Toxicity included grade 3 and 4 myelosuppression (26%) and infections (6%). Grade 1 or 2 TFR were observed in 17 patients (44%) and manageable with therapy. Only two patients had gone off therapy at the time of this report.

The Canadian CLL study group (Table III) reported a phase I study in previously untreated patients where a starting dose of 10 mg po daily with weekly 5 mg dose escalations to the target dose of 25 mg daily every 21 of 28 days [40]. Toxic events in the first two patients (tumor lysis requiring dialysis; neutropenic sepsis leading to death) promoted modification to include starting at a low doses (2.5 mg and 10 mg, respectively, days 1–21), slow the dose escalation rate (2.5 mg cycle 1) and escalating course 2 (5 mg) and course 3 (10 mg) with close monitoring for tumor lysis. Deep-venous thrombosis (DVT) prophylaxis with low dose acetylsalicylic acid (ASA) was mandated. Twenty-five patients with a median age of 60 and 40% being advanced Rai disease were enrolled with 32% having high risk genomic features. At the time of this report [40], 17 patients were evaluable for response with 11 (65%) attaining a partial response and no complete responses being noted. The median tolerated dose was 10 mg although 26% required dose reduction below this because of neutropenia or other toxicity. Toxicity included 10 patients (43%) with grade 3 or 4 neutropenia whereas three patients (13%) had 3–4 thrombocytopenia. Grade 1 or 2 fatigue (74%), tumor flare (78%), non-desquamating rash (48%), and grade infections (43%) were common. Only four

(16%) of patients developed infections requiring hospitalization. The relationship of tumor flare to response in this study was not reported.

Toxicity issues of lenalidomide specific to chronic lymphocytic leukemia

Across all diseases where lenalidomide has been effectively utilized, select toxicity including myelosuppression, fatigue, and skin rash have been observed. The observation of TFR has been unique to CLL that occurs commonly in patients receiving this drug. Similarly, an atypical tumor lysis syndrome that often is difficult to differentiate from and may even occur with tumor flare has also been observed in CLL. As this toxicity has delayed transition of lenalidomide into phase III studies for CLL and also represent the biggest challenge to physicians using this agent, they are reviewed below.

TFR is a unique toxicity associated with CLL and not generally observed in other B-cell malignancies, multiple myeloma, or Hodgkin disease. The exact mechanism of action of tumor flare reaction is not certain but our preliminary study [38] suggested patients having demonstrable *ex vivo* activation with lenalidomide may be at higher risk for tumor flare. A more comprehensive study that was preliminarily reported by the NHLBI [42] group appear to support this finding. This group demonstrated that the ability to *ex vivo* activation of CLL cells with lenalidomide predicted cytokine release (a manifestation of tumor flare) *in vivo* following lenalidomide treatment. In contrast, activation of T-cells *ex vivo* by lenalidomide was not predictive of cytokine release.

Clinical features and management strategies from the more mature studies with lenalidomide have also been reported. A total of 58% of the patients in the RPCI study [33] and 29% of the patients in MDACC study [34] developed tumor flare. In both instances, the TFRs were managed by supportive care, pain medications, and a short course of steroids for 5–7 days, which resulted in complete resolution of symptoms. In the RPCI study [33], the tumor flares occurred as early as the initial 24-h of starting therapy with a median time to onset of 6 days and median duration of 14 days. This resulted in amendment of the study protocol to start therapy at a lower dose of 10 mg daily with gradual escalation to 25 mg daily with steroid prophylaxis [33]. The comparison of the data between the two groups of patients treated with or without prophylactic steroids revealed a decrease in the incidence of grade 2–3 TFR from 47% to 9% and onset of TFR from 4 to 9 days [33]. Further analysis reported in the MDACC study identified lymph node size >5 cm as a risk factor for the development of TFR [34]. In both the studies however, there was no correlation of TFR with response, OR rate or PFS, possibly limiting the utility of the assessment of TFR as a surrogate marker for prediction of response. In managing this toxicity, our group has still observed this when administering lenalidomide to relapsed and refractory patients with CLL even at the dose of 2.5 mg/day. Similar to therapeutic monoclonal antibodies, it is essential to provide education to patients regarding this expected toxicity, observe patients closely during the first 1–2 weeks of therapy and either prophylactically administer a short course of corticosteroids during this time or alternatively add corticosteroids when tumor flare begins to manifest. Our practice as well is to hold lenalidomide if symptoms are present or bulky nodes are present. In most cases lenalidomide can be re-initiated within 1–2 days of starting steroids.

Tumor lysis syndrome (TLS) is another serious side effect that has been reported with the use of lenalidomide. Two patients in the RPCI study developed TLS[33] but none in the MDACC study [43]. Multiple other studies have required amendments to their dosing schedules as result of the TLS observed with the use of lenalidomide. In the study by Chen *et al.*, TLS and neutropenic sepsis leading to death in the first two patients resulted in dose modification of the study to utilize a lower starting dose with more aggressive supportive care and slow escalation [40]. In another study by Wendtner *et al.*, TLS in five out of the

first 18 patients enrolled resulted in two fatalities in the planned phase 3 trial. The study was subsequently amended to a phase I/II trial to determine a safe dose and schedule of lenalidomide and started at a dose of 2.5 mg daily, followed by slow intra-patient dose escalation of 5 mg every 28 days until maximally tolerated or a maximum dose of 25 mg daily [35]. The revised dosing parameters and supportive care strategies have reportedly eliminated the incidence of TLS at lower doses.

Mechanism of action of lenalidomide

As a second generation IMiD, lenalidomide was created using thalidomide as a template by adding an amino group to the 4th carbon of the phthaloyl ring and removal of a carbonyl group. Lenalidomide was selected for further clinical development after it was determined to be over 50 000 fold more potent inhibiting TNF-α *in vitro*, and more stable, when compared to its parent compound thalidomide [44–46]. Lenalidomide possesses multiple potential anti-tumor mechanisms of action, although it is currently unclear which mechanism(s) are responsible for clinical activity in patients responding to therapy. The mechanisms may also differ depending on the type of tumor being treated. These mechanisms include cytokine modulation, immune (T and NK) cell modulation, inhibition of angiogenesis, and direct effects on tumor cells [47].

The immunologic mechanisms of action of lenalidomide have been best described. Lenalidomide increases the proliferation of human T cells activated by CD3-crosslinking (mimicking T cell receptor engagement) [44]. In addition, lenalidomide significantly amplified T cell IL-2 and IFN-γ secretion at 0.1 μM concentrations by CD3-crosslinked human T cells *in vitro* – an effect 100–1000 times more potent than thalidomide. This result may be partially mediated by increased CD40L expression on lenalidomide treated T cells. In subsequent studies, the ability of lenalidomide to provide a co-stimulatory signal to CD3-crosslinked CD4+ and CD8+ T cells was confirmed [48]. These results were extended by using a more physiologic model whereby lenalidomide augmented dendritic cell-mediated activation of T cell proliferation and cytokine production [49]. Two potential intracellular mechanisms that could be responsible for the observed T cell co-stimulatory property of lenalidomide have been identified: increased phosphorylation of CD28 and increased transcriptional activity of AP-1 [49,50]. In patients with CLL [51] and NHL [52], lenalidomide has been demonstrated to reverse the T-cell defect observed in these disease and to promote immune synapse formation when these cells are placed in proximity of autologous tumor cells.

Davies *et al*. demonstrated that the addition of lenalidomide to IL-2 stimulated human PBMC *in vitro* increased their ability to kill the multiple myeloma (MM) cell line HSS, as well as traditional NK cell targets such as K562 [53]. This effect was abrogated when CD56+ cells were depleted from the cultures, suggesting that NK cells were mediating the observed tumor cell killing. In addition, lenalidomide increased killing of autologous MM cells *in vitro* when PBMC from MM were treated *in vitro* with IL-2 followed by lenalidomide. In a subsequent study, Hayashi *et al*. confirmed that lenalidomide augmented NK cell killing *in vitro*, in this situation via indirect T cell production of IL-2 [54]. List and coworkers reported an NK cell defect in patients with MDS consisting of defective NK cell activating receptor expression [55]. *In vitro* treatment with lenalidomide reversed the activating receptor defects and allowed NK cell killing in a re-directed killing assay, suggesting that modulation of NK receptors is a potential mechanism used by lenalidomide in patients with MDS. *In vivo* NK cell modulation has not been reported with lenalidomide therapy.

Outside of immune modulation, it is likely that lenalidomide has several alternative mechanisms of action. In myelodysplastic syndrome an erythroid signature has been identified that predicts response to lenalidomide [56]. Additionally, the induction of the tumor suppressor gene SPARC has been suggested as a mechanism of action in this disease [57]. In multiple myeloma, lenalidomide interaction with stromal cells has been observed where IL-6 production is diminished, thereby enhancing sensitivity to alternative therapies [54]. A preliminary finding in CLL also suggested that lenalidomide might be effective against antagonizing the stromal influence in protecting from apoptosis [58]. In B-cell lymphoma, the mechanism of action of lenalidomide has been studied less. Hernandez-Ilizaliturri *et al*. demonstrated in several lymphoma cell lines (Raji, DHL4, DHL10) that lenalidomide mediates growth arrest and modest apoptosis as compared to media control. No modulation of CD20 antigen was noted with lenalidomide treatment [59]. Using a Raji disseminated lymphoma xenograft model, lenalidomide had no clinical activity but modestly enhanced the efficacy of rituximab [59]. A follow up study by Reddy *et al*. from this same group using a Raji subcutaneous model demonstrated that NK cells and their interaction with dendritic cells was necessary for in vivo activity of lenalidomide [60] supporting a combined contribution of direct drug effect on cell lines with immunologic modulation as well. A recent report by Zhang *et al*. [61,62] demonstrated in mantle cell lymphoma (MCL), diffuse large-B-cell lymphoma, and follicular lymphoma (FL) cell lines that growth inhibition was observed, greatest in mantle cell lymphoma. A decrease in vascular endothelial growth factor (VEGF) with lenalidomide was noted at much lower concentrations than required for anti-proliferative effects, particularly in MCL and FL cell lines. Lenalidomide caused a decrease in VEGF and increase in the tumor suppressor genes $p21^{cip1}$ and SPARC. The elevation of SPARC mRNA significantly correlated with both the anti-proliferative and the VEGF-suppressive effects of lenalidomide on MCL cells ($p < 0.05$). The transfection of tumor cells with SPARC siRNA led to significant resistance to lenalidomide suggesting that this effect is mediated at least in part through the up-regulation of SPARC [61]. Finally, in multiple myeloma lenalidomide has also been shown to have direct anti-tumor cell apoptotic effects. Hideshima *et al*. showed that micromolar concentrations of lenalidomide inhibited DNA synthesis by multiple myeloma cell lines *in vitro* in a dose dependent fashion [54]. Mitsiades *et al*. showed that lenalidomide induces apoptosis in multiple myeloma cell lines via caspase-8, and enhanced cell death combination with TNF-related apoptosis-inducing ligand (TRAIL) ligation or bortezomib treatment [63]. Thus, direct, as well as immunologic mechanisms may be most relevant to the mechanism of action of lenalidomide *in vivo*.

Is the mechanism of action of lenalidomide different in chronic lymphocytic leukemia?

Given these clinical observations in CLL, we have sought to better understand the mechanisms underlying the disease activity of lenalidomide in CLL and to determine why tumor flares are seen so frequently [64]. We hypothesized that the tumor flare toxicity might be related to inadvertent activation of CLL cells as the clinical features of this syndrome were quite similar to what has been observed with CpG oligonucleotide therapy such as G3139 (Genasense) [65]. In our published report describing our initial experiments we demonstrated that lenalidomide does not exert any cytotoxic effect toward CLL cells but does promote up-regulation of B-cell activation markers including CD40, CD80, CD86, HLA-DR, CD95 [38]. Here, we observed both an increase in expression and/or up-regulation of antigen expression on all patients tested [38]. In this small study, activation of CLL cells *ex vivo* correlated with development of tumor flare. Consistent with B-cell activation by lenalidomide, we also observed internalization of CD20 antigen on CLL cells accompanied by diminished ADCC and direct apoptosis by the anti-CD20 antibody

rituximab [65]. In contrast, up-regulation of CD40 was demonstrated to enhance the efficacy of the anti-CD40 antibody SGN-40 [66]. Further studies by our group are ongoing to better define the exact mechanism of lenalidomide mediated activation in CLL cells. Additionally, we are currently initiating pilot studies with the TCL1 transgenic mouse model of CLL that re-capitulates many of the features associated with CLL including immune response to lenalidomide and importance of specific effector cells to the mechanism of action of this agent.

A second group at the NHLBI has preliminarily reported the observation of activation of CLL cells *ex vivo* as measured by enhanced expression of CD80 on CLL cells pre-treatment and correlation with tumor flare, cytokine release syndrome, and decrease in circulating blood lymphocyte counts in patients receiving lenalidomide [42]. Serial lymph node biopsies pre-treatment and post-treatment on lenalidomide treated patients with CLL in this study did not demonstrate evidence of nodal T-cell infiltration but the continued presence of CLL cells suggesting this tumor enlargement is not representative of T-cell infiltration. Collectively, the studies of mechanism of action of lenalidomide are important to understand if tumor flare that occurs early in the treatment of patients with CLL is related at all to treatment responses that are often delayed with this agent.

Conclusion and recommendations

Given the exciting *in vitro* data and the efficacy of lenalidomide observed in clinical trials even in patients with high risk and relapsed/refractory disease, it remains an exciting agent. However, the toxicity of lenalidomide is not trivial and has resulted in catastrophic outcomes in some unfortunate cases. Our own group is approaching the clinical investigation of lenalidomide in patients who have received alternative cytoreductive therapy and have only minimal residual disease and also in less heavily treated patients where disease and treatment related bone marrow reserve is often better. Additionally, we are in the way to understand the mechanism of action of lenalidomide so that we can develop more effective combination strategies for this agent. On the basis of these existing data, we present our recommendations for the use of lenalidomide in patients with CLL.

Lenalidomide potentially works through a variety of mechanisms in CLL making continued laboratory study of this agent essential to best apply combination strategies for this disease.

Lenalidomide should only be used in the context of a well-designed clinical trial.

Clinical trials with lenalidomide should employ a lower starting dose with gradual dose escalation.

Combination trials with lenalidomide that incorporate therapeutic antibodies should consider the influence of CLL cell activation with respect to sequence of administration of agents within such trials.

Aggressive prophylactic measures should be employed to combat tumor flare and tumor lysis. These include but should not be limited to

- appropriate patient selection – patients with high counts and bulky disease may benefit from aggressive hydration and cytoreductive therapy before initiation of lenalidomide.
- extensive education of patients and family prior to receiving lenalidomide so tumor flare does not inappropriately surprise patients leading to its early discontinuation.

- close monitoring for evidence of tumor flare reaction with prophylactic or early administration of corticosteroids for at least the first week of at least the first cycle.

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Table I

Summary of clinical trials utilizing thalidomide.

Reference	Study phase	No. pts	Median age (yrs)	Prior Rx/ median	Regimen utilized	CR (%)	PR (%)	OR (%)	Hematologic toxicity Grade 3–4 (%)	Tumor flare (%)	Other toxicities Grade 3–4 (%)
Kay *et al.* [23,25] (N9986)	2	28*	66	>1–3/2	T-200mg qHS escalated by 100–200 mg q2 wks to a maximum of 1000 mg/d q28d for upto 12 cycles (Median dose = 400 mg/ d, range 100–1000)	4	7	11†	Neutropenia (32) Thrombocytopenia (25) Anemia (11)	53‡ (18% had Grade 3–4)	Fatigue (25)
Grinblatt *et al.* [24] (CALGB 50002)	2	25§	–	≤3/−	T-200 mg/d, escalated by 100 mg/d q1–2 wks to a max of 800 mg/d (Median dose = 400 mg/d, range 50–800)	14	0¶	14	Neutropenia (17) Anemia (13)	NA	Neurologic toxicities (29) Dyspnea (13) Fatigue (8)
Furman *et al.* [27]	2-R	16-8(T) 8(FT)	60	>1/−	T-200 mg qHS and adjusted as tolerated. Or 200 mg qHS with F-25 mg/m^2 qd x 5d for six cycles (FT) (Median dose = 200 mg qHS range 50–450)	0 (T) 12 (FT)	12 (T) 37 (FT)	12 (T) 50 (FT)	Neutropenia (12)	62 (all T)‡	Infection (37) Neuropathy (25)(T)
Laurenti *et al.* [28]	2	5	67	>1/4	T-100 mg d1–180, oral F-30 mg/m^2 and oral C-250 mg/m^2 d1–3 q28d for six cycles, starting from day (10 of T**	0	0	0	–	0	–
Chanan-Khan *et al.* [29]††	1	13	65	Untreated	T-100–300 mg d0–180 + F-25 mg/m^2 for 5d q28d starting on d7, for 4–6 cycles (MTD = not reached)	55	45	100	Neutropenia (8)	46‡‡	Fatigue (31) Pulmonary VTE (15)
Giannopoulos *et al.* [30,31]	2	20	62.5	Untreated	T-100 mg d0–180 + F-25 mg/	25	55	80	Neutropenia (22) Anemia (10)	35‡	Infection (10) AIHA (10)

Reference	Study phase	No. pts	Median age (yrs)	Prior Rx/ median	Regimen utilized	CR (%)	PR (%)	OR (%)	Hematologic toxicity Grade 3–4 (%)	Tumor flare (%)	Other toxicities Grade 3–4 (%)
		20	61	≤2/−	m^2 for 5d q28d starting on d7, for upto six cycles (Median cycles = 3, Range 1–5)	0	25	25	Thrombocytopenia (10)	15[‡]	

CR, complete response; PR, partial response; OR, overall response; NA, not available; VTE, venous thromboembolism; AIHA, auto-immune hemolytic anemia; T, thalidomide; F, fludarabine; C, cyclophosphamide; R, randomized; MTD, maximally tolerated dose.

[*] 61% were Rai stage 3 and 4, 15% had 17p- and/or 11q-, 53% had unmutated disease.

[†] 71% had disease stabilization for atleast one cycle of therapy. Median time to progression was 7.3 months. Study was stopped early because of poor accrual possibly due to the high incidence of tumor flare reactions.

[‡] All grades.

[§] Eighteen patients had follicular lymphoma (FL).

[¶] 72% with FL had PR.

[**] Oral acyclovir 800 mg tid and trimethoprim-sulfamethoxazole 960 mg bid for two consecutive days a week were used for prophylaxis. None of the patients were able to complete therapy due to progressive disease.

[††] 54% were Rai stage 3 and 4, 15% had p53 deletion.

[‡‡] Grades 1–2.

Table II

Summary of clinical trials utilizing lenalidomide.

Reference	Study phase	No. pts	Median age (yrs)	Prior Rx/median	Regimen utilized	CR (%)	PR (%)	OR (%)	Hematologic toxicity Grade 3–4 (%)	Tumor flare (%)	Other toxicities Grade 3–4 (%)
Chanan-Khan *et al.* [33]	2	45*	64	>1/3	L-5–25 mg/d until molecular CR or unacceptable toxicity. Rituximab 375 mg/m^2/wk ×4 in cycle 1 and on d1 and 15 of cycles 2–6 in pts with PD or SD for 2 months (Median dose =25 mg/d)	9	38	47	Neutropenia (70) Thrombocytopenia (45) Anemia (18)	58†	Fatigue (10) Pulmonary VTE (5) TLS (5)
Ferrajoli *et al.* [34]	2	44‡	64	>1/5	L-10 mg/d for 28d continuously, based on response and toxicity, and increased by 5 mg q28d to a maximum of 25 mg/d (Median dose =10 mg/d, 7% able to tolerate 25 mg/d for at least 1 month)	7	25	32	Neutropenia (41) Thrombocytopenia (15) Anemia (3)§	29¶	Pneumonia (3)§
Wendtner *et al.* [35] (CLL-001)	1/2	17**	66	>1/4	L-2.5 mg/d escalated to 5 mg after 28d and subsequently 5 mg q28d as tolerated until MTD or 25 mg/d (MTD =not reached)	–	–	–	Neutropenia (≤66)†† Thrombocytopenia (11)	≤23.5‡‡	
Witzig *et al.* [36,37]	2	18	65	>1/3	L-25 mg d1–21 q28d for 52 wks as tolerated or until disease progression	5	16	22	Neutropenia (60) Thrombocytopenia (28)	14 (Gr 1–2)	NA

CR, complete response; PR, partial response; OR, overall response; VTE, venous thromboembolism; MTD, maximally tolerated dose; CLL, chronic lymphocytic leukemia; L, lenalidomide; PD, progressive disease; SD, stable disease; TLS, tumor lysis syndrome.

[*] 51% were fludarabine refractory, 64% had Rai stage 3–4, 6 had p53 deletion.

[†] All Grades. 50% with grade 1–2 and 8% with grade 3–4. Lenalidomide treatment was not stopped or dose reduced for flare reaction and ibuprofen 400 mg q6h was used for symptom control. No flare reaction prophylaxis was used in the first 29 patients. Subsequent 16 patients received prophylaxis with prednisone (20 mg orally for 7 days followed by 10 mg for 7 days).

[‡] 27% were Fludarabine refractory, 45% had Rai stage 3–4, 18% had 17p and 41% had 11q deletion (13% ORR in 17p and 39% in 11q deleted).

[§] All averaged over a total of 333 courses administered.

[¶] All grades. 12% grade 1–2 and 2% with grade 3–4 of 333 courses administered were complicated by tumor flare. Tumor flare was managed with a short 6-day course of oral methylprednisolone.

[**] 56.3% were fludarabine refractory.

[††] 23.5% at 2.5 mg; 42.9% at 5 mg; 66.7% at 10 mg dose, respectively.

[‡‡] All grades. 23.5% at 2.5 mg; 14.3% at 5 mg dose had grade 3 tumor flare reaction.

Table III

Trials of lenalidomide in previously untreated patients.

Reference	Study phase	No. pts	Median age (yrs)	Regimen utilized	CR (%)	PR (%)	OR (%)	Hematologic toxicity Grade 3–4 (%)	Tumor flare (%)	Other toxicities Grade 3–4 (%)
Chen *et al.* [40]	2	25*	60	L-2.5 mg d1–21 of a 28d cycle to a target dose of 10 mg (2.5 mg cycle 1, 5 mg cycle 2, 10 mg cycle 3 and thereon) +low dose ASA prophylaxis (Median dose = 10 mg/d, 26% required dose reductions to 5 mg/d)	0	65	65	Neutropenia (43) Thrombocytopenia (13)	78†	–‡
Ferrajoli *et al.* [41]	2	43§	72	L-5 mg/d for the first 56d and increased by 5 mg q28d to a maximum dose of 25 mg/d (Median dose =10 mg/d)	0	54	54	Neutropenia &/or Thrombocytopenia (26)	44†	Infections (7)

CR, complete response; PR, partial response; OR, overall response; ASA, aspirin.

*40% had Rai stage 3–4, 32% had 17p or 11q deletion.

†all grade 1–2.

‡all grade 1–2, fatigue (74%), rash (48%).

§42% had Rai stage 3–4 disease. 26% had 17p or 11q deletion.