Immunomodulatory drugs alone or with immunotherapy represent new treatment options for patients with chronic lymphocytic leukemia.

Immunomodulatory Drugs and Active Immunotherapy for Chronic Lymphocytic Leukemia

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Background: The last decade witnessed the emergence of several therapeutic options for patients with chronic lymphocytic leukemia (CLL) for first-line and relapsed settings. The vast majority of patients with relapsed or refractory CLL carry poor prognostic features, which are strong predictors of shorter overall survival and resistance to first-line treatment, particularly fludarabine-based regimens.

Methods: This article highlights the current role of immunomodulatory drugs (IMiDs) and active immunotherapy as treatment options for this select group. The rationale of using IMiDs is discussed from the perspective of lenalidomide as a novel active agent. Relevant clinical trials using IMiDs alone or in combinations are discussed. New immunotherapeutic experimental approaches are also described.

Results: As a single agent, lenalidomide offers an overall response rate of 32% to 47% in patients with relapsed/refractory disease. Recent studies have shown promising activity as a single agent in treatment-naive patients. The combination of lenalidomide with immunotherapy (rituximab and ofatumumab) has also shown clinical responses. Encouraging preclinical and early clinical data have been observed with different immunotherapeutic approaches.

Conclusions: The use of IMiDs alone or in combination with immunotherapy represents a treatment option for relapsed/refractory or treatment-naive patients. Mature data and further studies are needed to validate overall and progression-free survival. The toxicity profile of lenalidomide might limit its use and delay further studies. Immunotherapy offers another potential alternative, but further understanding of the immunogenicity of CLL cells and the mechanisms of tumor flare reaction is needed to improve the outcomes in this field.

Introduction

Prognostic factors, discussed in a separate article in this issue (Sagatys EM, Zhang L; pp 18-25), are strong predictors of progression-free survival (PFS) and overall survival (OS) in patients with newly diagnosed chronic lymphocytic leukemia (CLL). Patients with poor prognostic features are more likely to be refractory to first-line treatment, or they relapse early, requiring salvage therapies. Although several alternative therapies are available, none offers a durable response. Even with current standard therapies, there are still clearly unmet needs. Therefore, the treatment of relapsed or refractory CLL has become a challenge. For these reasons, considerable effort is aimed
toward the development and use of different therapeutic agents for this population.

Immunotherapy is an appealing alternative and appears to offer a logical approach if we consider the biology of CLL. The tumor microenvironment and different cytokines play an important role in the evolution of this disease. Immunomodulatory drugs (IMiDs) offer different biologic effects on cytokine and cell-mediated responses. This article reviews the use of this new class of drugs for the treatment of relapsed and refractory CLL and also discusses new immunotherapeutic approaches.

**Immunomodulatory Drugs**

**Thalidomide and Lenalidomide: Chemistry and Metabolism**

Thalidomide \([\alpha-(N\text{-}phthalimido)\text{glutarimide}]\) is a synthetic glutamic acid derivative, with an empirical formula \((3\,\text{H}10\,\text{N}2\,\text{O}4)\). It is formulated as a racemic mixture of two active enantiomers, \(S(-)\) and \(R(+)\). Both isoforms \(S(-)\) and \(R(+)\) are considered to have teratogenic properties.\(^1\) Thalidomide is an oral agent since it is not soluble in water. The mean plasma protein binding is 55% to 66%, and it is metabolized by nonenzymatic hydrolysis to different metabolites, which are then eliminated in the urine. The specific pharmacokinetic characteristics of this drug in the setting of renal or hepatic dysfunction are unknown. Thalidomide has a wide dosing range, with daily doses of 50 mg to 800 mg, depending on each particular disease. In searching for a less toxic and better tolerated drug, lenalidomide was created using thalidomide as a backbone. Lenalidomide is a 4-amino-gultaramide derivative of thalidomide in which an amino group was added to the fourth carbon of the phthaloyl ring of the parent compound.\(^2\) This modification led to an enhanced immunomodulatory potency drug with less neurologic toxicity. As thalidomide, it also exists as a racemic mixture of the active \(S(-)\) and \(R(+)\) forms. Like thalidomide, lenalidomide is available in oral formulation and is given every 21 to 28 days of monthly cycles. Since renal elimination predominates, adjusted doses are recommended with impaired creatinine clearance. In contrast to thalidomide, lenalidomide lacks significant neurosedative toxicity; however, they share the risk of venous thromboembolism.

**Rationale**

An understanding of CLL pathogenesis is necessary to comprehend the logistics behind the use of IMiDs. A key feature of CLL is the inability to undergo programmed cell death and subsequent increased survival of mature B cells, mostly due to the aberrations in the apoptosis pathway. The proliferation, differentiation, and apoptosis of healthy B lymphocytes are regulated directly by several cytokines and growth factors. Data suggest that malignant B cells can resist apoptosis through their ability to manipulate the microenvironment and through the production and secretion of prosurvival cytokines. One such cytokine is vascular endothelial growth factor (VEGF), which has autocrine and paracrine properties that directly affect the B-cell CLL (B-CLL) cell growth, apoptosis, and resistance to treatment. Tumor microenvironment is responsible for the accumulation of clonal malignant B cells, particularly by promoting and housing increased survival while avoiding apoptosis. The survival of the tumor cells is also promoted by the ineffective immune response of the host in the milieu of tumor antigens. These evasive strategies can be overcome by introducing IMiDs that might help defeat tumor resistance. Taking this into consideration, targeting the microenvironment as part of the treatment for CLL seems to be an attractive proposal. For these reasons, further understanding of the microenvironment and cytokines role is an important part in guiding the selection and further discoveries of new therapeutic agents.

**Mechanisms of Action**

Thalidomide, initially developed in the 1950s as an anticonvulsant medication, offered insufficient efficacy. Since sedation was a common effect of thalidomide, it was used as a sleeping and sedative drug. Thalidomide was also used as antiemetic treatment in pregnancy, but due to its detrimental teratogenic effects, the drug was withdrawn by the US Food and Drug Administration in 1961. By serendipity, thalidomide was found to be an effective treatment against erythema nodosum leprosum because it helped reduce fevers and improve skin lesions in patients with dermatologic and rheumatologic conditions.\(^3\) The effect of thalidomide on tumor necrosis factor alpha (TNF-\(\alpha\)), a cytokine that regulates the inflammatory cascade (higher levels reported on CLL patients),\(^4,5\) is responsible for this benefit. Despite the setbacks since its initial development, the beneficial effects of thalidomide on the cytokines and its enhancement influence on the immune system have prompted interest in the drug as an option in treating hematologic malignancies.\(^6,7\) Thalidomide is now considered the first immunomodulating agent. In an effort to improve efficacy and reduce toxicity, thalidomide analogs were developed using the backbone of the thalidomide structure. Lenalidomide (CC-5013) and pomalidomide (CC-4047) are two of the analogs that were later developed.

A few characteristics of thalidomide identified it as an attractive anticancer drug. Various potential antitumor mechanisms have been attributed to the IMiDs, starting with the discovery of its potent anti-inflammatory activities through the inhibition of the synthesis of TNF-\(\alpha\) by activated monocytes.\(^7\) TNF-\(\alpha\) is mainly produced by monocytes and macrophages, but lymphocytes, under stimuli, also produce it.\(^8\) This property allows the use of thalidomide for several diseases associated with increased TNF-\(\alpha\), such as autoimmune deficiency disease (AIDS), Kaposi sarcoma, and rheumatologic conditions.
Thalidomide and its analogs modulate cytokine production, with inhibitory effects in inflammation and stimulatory immune-mediated effects. This dual property is through the suppression of TNF-α production (by enhancing the degradation of TNF-α mRNA)\(^9\) from endotoxin-stimulated monocytes and macrophages\(^10\) and also through T-cell costimulation that induces increased cytokine production to enhance the immune response.\(^2\) Both lenalidomide and CC-4047 (pomalidomide) are 4-amino-glutaramide derivatives of thalidomide that have been shown to be more potent TNF-α antagonists in endotoxin models.\(^11,12\) Lenalidomide is 50,000 times more potent in inhibiting TNF-α in vitro than is its parent drug, thalidomide.\(^11\) Effects of lenalidomide in the production of cytokines have been reported that support its role as an immune-modulating agent in this disease.\(^13,14\) Lenalidomide has been shown to increase circulating cytokines, particularly interleukin 6 (IL-6), IL-10, IL-2, and TNF receptor-1 levels.\(^14\)

IMiDs have a costimulatory effect on T-cell responses that include increased production of IL-2 and IFN-α by increasing the proliferation of CD3- or IL-2-activated T cells, which activate natural killer (NK) cells enhancing tumor cell death.\(^2,15\) This costimulatory activity provides an immunologic adjuvant to promote an otherwise ineffective immune response associated with malignancies.\(^2\) In most tumors, including CLL, an increased number of T regulatory cells are present. The expression of CD152 (cytotoxic T-lymphocyte-associated antigen 4 [CTLA4]) in T cells of patients with CLL is particularly increased and can correlate with advanced disease and prognostic factors.\(^18\) Lenalidomide and pomalidomide strongly inhibit T regulatory cell proliferation and suppressor function.\(^19\) Data from LeBlanc et al\(^17\) demonstrated that lenalidomide activates CD28 and overcomes the CTLA4 immunoglobulin blockade, thereby confirming that drug-induced costimulation is mediated via the B7-CD28 pathway.

The effect of IMiDs in natural killer (NK) T cells was described by Davies et al\(^10\) when they reported that in the in vivo setting, there is an increase in the number of NK cells in patients with multiple myeloma (MM) cells who responded to lenalidomide, which was accompanied by an increase in IL-2 and IFN-α secretion. Hayashi et al\(^20\) demonstrated how the IMiDs augment NK-cell cytotoxicity in myeloma cells, triggering NK-cell-mediated tumor cell lysis. These effects were produced via the induction of IL-2 transcription and secretion in T cells. In vitro data from NK cell modulation have not yet been demonstrated for in vivo models; however, this suggested the effect of NK cells in anti-MM immune responses. Lenalidomide antiangiogenesis properties were discovered and explored for cancer treatment, mostly while the role of new blood vessel formation was defined as a crucial component for tumor growth and metastasis. Preclinical data showed that IMiDs have a potent antiangiogenic activity in vitro, and this is likely to contribute to their antitumor effects in vivo.\(^2,21\) IMiDs might help to minimize metastasis by reducing the expression of proangiogenic cytokines such as VEGF by decreasing blood-vessel density, and by affecting cell-adhesion molecules.\(^2\) However, Andritsos et al\(^22\) reported that VEGF serum concentrations remained unchanged, regardless of response, in patients treated with lenalidomide.

Hideshima et al\(^23\) have suggested that lenalidomide has proapoptotic effects that inhibit the proliferation of B-CLL, according to in vitro tumor models. Lenalidomide effects on the bone marrow microenvironment modulate the adhesive interactions and also alter tumor cell growth, survival, and drug resistance.\(^25\) In discussing the effects of lenalidomide on tumor cell microenvironment, Chanan-Khan et al\(^24\) suggested that its antileukemic effect is most likely from in vivo modulation of the tumor microenvironment as is demonstrated from changes in the cytokine milieu and the cellular immune response. In in vitro models, lenalidomide inhibits the cell proliferation of B-malignant cell lines by arresting cells in the G\(_1\) phase.\(^25\) As discussed by Chanan-Khan et al,\(^24\) the downregulation of prosurvival pathways such as the phosphatidylinositol pathway provides lenalidomide with its modulating antileukemic effects. Lapalombella et al\(^26,27\) addressed this hypothesis in several reports. Their data support that lenalidomide induces the downregulation of CD20 surface antigen expression via the enhanced internalization and the upregulation of CD40 expression on primary B-CLL cells, enhancing the efficacy of the anti-CD40 antibody SGN-40. Lenalidomide also promotes the upregulation of functional CD154 on CLL cells, which may reverse the humoral immune defect characteristic in the immunopathology of CLL. Given the modulatory effects of lenalidomide in the immune responses, as well as its antitumor effects, the novel use of IMiDs is becoming more popular in various tumor types such as multiple myeloma, myelodysplastic syndrome, renal cell carcinoma,\(^28\) and prostate cancer.\(^29,30\)

**Clinical Trials**

Several clinical trials have been conducted to assess the use of lenalidomide, either alone or in combinations, in patients with CLL. Different doses, regimens, and effects have been investigated (Table).

Chanan-Khan et al\(^35\) investigated high doses of lenalidomide in a nonrandomized phase II study that included patients with relapsed or refractory B-CLL. Among 64 patients assessed, 64% with advanced Rai stage III or IV and 51% who were refractory to fludarabine received 25 mg once daily for 21 days on a 28-day schedule. Patients were able to receive rituximab in the evidence of progressive or stable disease for 2 consecutive months. The major overall response (OR) in this study was 47%, with a complete response (CR) rate of 9%. A partial response (PR) was achieved in 38%. These data dem-
onstrated that the antitumor activity of lenalidomide was evident as early as day 8 of treatment, with 24 of 34 patients (70.5%) demonstrating a decrease in their peripheral-blood absolute lymphocyte count. Tumor lysis syndrome (TLS) and tumor flare reaction (TFR) were among the toxicities, accounting for 5% and 58%, respectively. TLS, which is characterized by electrolyte imbalance, uremia, and renal failure, caused 1 fatality. TFR, which is associated with painful swelling of the lymph nodes and/or splenomegaly with or without fever and rash, predominated as one of the most common nonhematologic toxicities, in addition to fatigue (86%).

A study by the Ferrajoli et al reported the clinical activity of lenalidomide (10 mg to 25 mg) in patients with

Table. — Selected Clinical Trials Using Lenalidomide Therapy Alone or in Combination With Rituximab and Ofatumumab for Treatment of Chronic Lymphocytic Leukemia

<table>
<thead>
<tr>
<th>Study</th>
<th>Initial Dose/Regimen</th>
<th>No. of Patients</th>
<th>TLS All Grades</th>
<th>TFR All Grades</th>
<th>Hematologic Side Effects</th>
<th>Grade 3/4</th>
<th>Overall Response (%)</th>
<th>Partial Response (%)</th>
<th>Complete Response (%)</th>
<th>Overall Survival (%)</th>
<th>Progression-Free Survival</th>
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<tbody>
<tr>
<td>Badoux et al&lt;sup&gt;31&lt;/sup&gt; Phase II (elderly)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5 mg/d L escalated to 25 mg/d</td>
<td>60</td>
<td>0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>52%</td>
<td>Neutropenia 34%</td>
<td>Thrombocytopenia 12%</td>
<td>Anemia &lt; 1%</td>
<td>65</td>
<td>43</td>
<td>10</td>
<td>88</td>
</tr>
<tr>
<td>Chen et al&lt;sup&gt;32&lt;/sup&gt; Phase II&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.5 mg/d escalated to 10 mg/d L&lt;sup&gt;1&lt;/sup&gt;</td>
<td>25</td>
<td>0</td>
<td>88%</td>
<td>Neutropenia 72%</td>
<td>Thrombocytopenia 28%</td>
<td>Anemia 20%</td>
<td>56</td>
<td>56</td>
<td>0</td>
<td>92</td>
</tr>
<tr>
<td>Maddocks et al&lt;sup&gt;33&lt;/sup&gt; Phase I&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2.5 mg/d escalated to 15 mg/d L&lt;sup&gt;1&lt;/sup&gt;</td>
<td>14</td>
<td>NR</td>
<td>14%&lt;sup&gt;f&lt;/sup&gt;</td>
<td>Neutropenia 35.7%</td>
<td>Thrombocytopenia 7%</td>
<td>Anemia 14%</td>
<td>NR</td>
<td>10&lt;sup&gt;g&lt;/sup&gt;</td>
<td>0</td>
<td>NR</td>
</tr>
<tr>
<td>Wendtner et al&lt;sup&gt;34&lt;/sup&gt; Phase I&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2.5 mg/d escalated to 5 mg/d L until MTD of 20 mg/d</td>
<td>52</td>
<td>3.8%</td>
<td>44%</td>
<td>Neutropenia 65%</td>
<td>Thrombocytopenia 33%</td>
<td>Anemia 9.6%</td>
<td>NR</td>
<td>11.5&lt;sup&gt;h&lt;/sup&gt;</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Aue et al&lt;sup&gt;31&lt;/sup&gt; Phase II</td>
<td>20 mg/d L lowered to 10 mg/d</td>
<td>33</td>
<td>0</td>
<td>53%</td>
<td>Neutropenia 56%</td>
<td>Thrombocytopenia 30%</td>
<td>Anemia 15%</td>
<td>NR</td>
<td>16&lt;sup&gt;i&lt;/sup&gt;</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Chanan-Khan et al&lt;sup&gt;35&lt;/sup&gt; Phase II</td>
<td>5 mg/d escalated to 25 mg/d L&lt;sup&gt;1&lt;/sup&gt; + R</td>
<td>45</td>
<td>5%</td>
<td>58%</td>
<td>Neutropenia 70%</td>
<td>Thrombocytopenia 45%</td>
<td>Anemia 18%</td>
<td>47</td>
<td>38</td>
<td>9</td>
<td>NR</td>
</tr>
<tr>
<td>Ferrajoli et al&lt;sup&gt;36&lt;/sup&gt; Phase II</td>
<td>10 mg/d escalated to 25 mg/d L&lt;sup&gt;1&lt;/sup&gt;</td>
<td>44</td>
<td>0</td>
<td>12%&lt;sup&gt;i&lt;/sup&gt;</td>
<td>Neutropenia 41%</td>
<td>Thrombocytopenia 15%</td>
<td>Anemia 3%</td>
<td>32</td>
<td>25</td>
<td>7</td>
<td>73&lt;sup&gt;j&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ferrajoli et al&lt;sup&gt;38&lt;/sup&gt; Phase II&lt;sup&gt;l&lt;/sup&gt;</td>
<td>10 mg/d L + R wkly</td>
<td>59</td>
<td>1.7%</td>
<td>37%</td>
<td>Neutropenia 68%</td>
<td>Thrombocytopenia 22%</td>
<td>Anemia 10%</td>
<td>64</td>
<td>39&lt;sup&gt;k&lt;/sup&gt;</td>
<td>8</td>
<td>90</td>
</tr>
<tr>
<td>Veliz et al&lt;sup&gt;37&lt;/sup&gt; Phase II</td>
<td>2.5 mg/d to 20 mg/d L + R</td>
<td>17</td>
<td>4.5%</td>
<td>27.2%</td>
<td>Neutropenia 36.3%</td>
<td>Thrombocytopenia 4.5%</td>
<td>67</td>
<td>NR</td>
<td>0</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Badoux et al&lt;sup&gt;38&lt;/sup&gt; Phase II</td>
<td>10 mg/d L + D</td>
<td>16&lt;sup&gt;p&lt;/sup&gt;</td>
<td>NR</td>
<td>13%</td>
<td>Neutropenia 50%</td>
<td>Anemia 13%</td>
<td>63</td>
<td>50</td>
<td>13</td>
<td>NR</td>
<td>NR</td>
</tr>
</tbody>
</table>

<sup>L</sup> = lenalidomide, <sup>R</sup> = rituximab, <sup>O</sup> = ofatumumab, TLS = tumor lysis syndrome, TFR = tumor flare reaction, NR = not reported, ITT= intention-to-treat population.

* estimated 2 years PFS
* Treatment-naïve, 7 fatalities reported.
<sup>a</sup> No grade 3/4 TLS, grade 1/2 not reported.
<sup>b</sup> One fatality.
<sup>c</sup> Protocol was amended; initial dose of lenalidomide 10 mg per day with 5 mg dose escalations to a target of 25 mg caused severe toxicities (TLS, fatal sepsis) in the first 2 patients enrolled.
<sup>d</sup> Protocol was amended; initial dose of lenalidomide 25 mg caused TLS in 3 patients, 1 death and 1 grade 3 neutropenia with sepsis.
<sup>e</sup> Not including 3 patients who developed TLS with initial dose of 25 mg per day.
<sup>f</sup> Ten patients evaluated for response.
<sup>g</sup> Three fatalities.
<sup>h</sup> 58% had stable disease.
<sup>i</sup> A total of 80% had del(17p).
<sup>j</sup> Protocol was amended; initial dose was 25 mg per day that caused TLS in 2 patients.
<sup>k</sup> Averaged per cycle of treatment; 53% incidence of any grade.
<sup>l</sup> Overall survival rate 73% are alive with a median follow-up time of 14 months.
<sup>m</sup> Two fatalities during treatment and 6 deaths occurred after progression of disease during subsequent therapies.
<sup>n</sup> Plus 12% nodular PR.
<sup>o</sup> Twenty-six patients accrued, data of 16 were reported.

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relapsed and refractory CLL. Of the 44 patients included in this trial, 45% had advanced Rai stage (III and IV), 59% had unfavorable cytogenetics, and 27% were refractory to prior fludarabine treatment. In this well-structured study, plasma levels of angiogenic factors, inflammatory cytokines, and cytokines receptors at baseline on day 7 and day 28 were measured. In addition of showing an OR rate of 32%, with a CR rate of 7% (3 patients) CR and a 25% rate of PR (10 patients), the authors also demonstrated an association between lenalidomide and immune activation, exemplified by changes in the levels of TNF-α and its soluble receptor TNF-R1, and increased levels of IL-6, IL-10, and IL-2. None of the 44 patients in this group had TLS; however, the incidence of TFR at any grade was higher in patients with lymph nodes larger than 5 cm (53%) and had no correlation with the OR rate. When comparing results of Chanan-Khan et al35 with those of Ferrajoli et al,14 the incidence of grade 3/4 neutropenia was 70% vs 41%, respectively, as well as 11% for grade 1/2 in the Ferrajoli study. The Chanan-Khan study used a higher dose of lenalidomide of 25 mg on a schedule of 3 weeks on and 1 week off, and the Ferrajoli study used 10 mg daily for 28 days as a starting dose. At this time, it is still not clear whether continuous vs intermittent exposure offers a better control of the disease. Further studies with different schedules — 3 weeks on and 1 week off vs 28 consecutive days using different doses — are needed to establish a better tolerated toxicity profile.

Sher et al39 reviewed the cases of relapsed/refractory CLL patients with high-risk cytogenetics who were included in the phase II clinical trial of Chanan-Khan et al.35 Patients with del(11q)(q22.3) or del(17p)(p13.1) received single-agent lenalidomide until disease progression, when rituximab was added. Durable responses with a median PFS of 12.1 months were reported, with a clinical response reported in 6 of 16 patients (38%; 95% confidence interval [CI], 15%–65%). Three patients achieved a CR (19%) and 3 achieved a PR (19%). This subgroup analysis confirmed the data presented by Ferrajoli et al14 showing lenalidomide to have durable response in patients with high-risk cytogenetics, with an OR rate of 52%.

Early-phase trials provoked significant changes in the dosage of lenalidomide. A phase I study in relapsed or refractory CLL34 and a phase II study in treatment-naive patients had protocol amendments secondary to fatalities, particularly TLS and neutropenic sepsis.32 Significant side effects were reported with the initial doses (10 mg vs 25 mg), when 5 out of 18 patients developed TLS resulting in 2 fatalities, thereby causing amendments in the protocol.34 The redesigned phase I study34 included 52 patients, of whom 69% had bulky disease and 48% had high-risk disease [del(17p) and/or del(11q)]. A dose of 2.5 mg was then used, allowing dose escalation with a maximum of 25 mg daily after 28 days. Hematologic grade 3/4 neutropenia, thrombocytopenia, and anemia were seen in 65%, 33%, and 9.6% of the patients, respectively. TLS occurred in only 2 patients receiving the lowest dose of lenalidomide. However, 23 patients developed grade 1/2 TFR, and 5 patients developed grade 3 TFR. The best response obtained was PR and stable disease. Six of the 52 patients had a PR, with a median time to response of 18.7 weeks. Stable disease was seen in 58% of the patients, regardless of lenalidomide dose (eg, 7 of 30 patients with SD received the lowest dose, 2.5 mg). These outcomes should be evaluated in the context of a heavily pretreated population with high-risk features, where stable disease or PR might be significant in the absence of alternative agents.

Similarly, after adjusting for dose and allowing for slow dose escalation, Chen et al35 accrued 25 treatment-naive patients. Of these, 32% had 17p or 11q deletion, 60% had ZAP70+, and 75% had unmutated IgVH. Mature data from this treatment-naive group of CLL patients were recently published. The hematologic toxicities included grade 3/4 neutropenia in 72%, with 5 cases of febrile neutropenia, and 28% developed grade 3/4 thrombocytopenia. A high rate of skin reactions (64%) was reported, described as maculopapular rash, nodular and urticarial. Fatigue was also a predominant side effect (72%). TFR was evident in 88% of patients, and they were treated with corticosteroids; however, no TLS was reported. A PR was reported in 56%, and 40% had stable disease. One patient progressed during treatment and died of Richter’s transformation. The estimated 2-year OS rate was 92% (95% CI, 81%–100%), and the PFS rate was 89% (95% CI, 74%–100%).

While phase II data from Chanan-Khan et al40 and Ferrajoli et al14 proposed that doses up to 25 mg offer clinical activity, a recent report documented serious outcomes with higher doses of lenalidomide.22 Four patients with relapsed/refractory CLL were treated with lenalidomide at 25 mg daily for 21 days in a 28-day cycle. Unacceptable toxicity was reported in 3 of the 4 patients, with 1 death and serious TFR that required acute hospitalization in 2 patients. The fourth patient developed sepsis and pulmonary and renal complications. These small series contrast with data from earlier phase II trials14,40 discussed above. In vitro data also showed that lenalidomide-induced B-cell activation corresponds to the degree of tumor flare, showing a relationship between B-cell activation and lenalidomide toxicity.

Considering the toxicities associated with higher doses of lenalidomide, Maddocks et al39 presented at the 2009 meeting of the American Society of Hematology the preliminary data from a small phase I dose escalation study of lenalidomide in patients with relapsed/refractory CLL. In this trial, 14 patients received lenalidomide 2.5 mg per day escalated to 15 mg per day or to a maximal tolerated dose of 25 mg (after an amendment was done to the initial starting dose of 25 mg due to significant toxicity associated with this dose). Dose-limiting tox-
The results of 34 patients who completed 15 cycles of lenalidomide, which was given in pulse dosages for 3 weeks followed by 3 weeks off. In this phase II trial, high-risk patients with a poor prognosis were represented: 52% were Rai stage III-IV, 43% had del(17p), 15% had del(11q), 70% had bulky disease, 56% were ZAP70+, and 64% expressed unmutated IgVH genes. Initial dose was reduced from 20 mg to 10 mg due to the toxicities observed in other lenalidomide trials. Grade 3/4 neutropenia, thrombocytopenia, and anemia occurred in 56%, 30%, and 15% of cycles, respectively. While TLS was not seen, an increase in grade 3 deep venous thrombosis was reported in 5 patients. Infection complications included grade 3 cytomegalovirus colitis, *Pneumocystis carinii* pneumonia, and candididemia. One patient died of streptococcal sepsis. Conversely, the hypothesis of achieving a safer and more tolerable toxicity profile was not obtained with this alternative regimen since TFR was observed in 78%, 48%, 38%, and 30% in cycles 1, 2, 3, and 4, respectively. Among the patients evaluated for response, 16% achieved a PR, 58% showed stable disease, and 26% had progressive disease. Conversely, patients with del(17p) and bulky disease appeared to have a remarkable PR rate of 80%; however, 27% of patients could not complete beyond 4 cycles.

In 2010, Badoux et al.13 designed a phase II trial with lenalidomide for elderly treatment-naïve patients. Since patients older than 65 years of age typically present with significant comorbidities or borderline performance status, front-line chemotherapy or myelosuppressive therapy can be challenging. Taking these factors into consideration, lenalidomide was administered daily at 5 mg and could be titrated up by 5 mg every 28 days to 20 mg daily. Eighteen of the 60 patients had Rai stage III and IV disease, 33% had unfavorable cytogenetics (17p deletion or 11q deletion), and 55% had unmutated IgVH. Grade 3/4 hematologic toxicities included neutropenia and thrombocytopenia in 34% and 12% of the cycles, respectively, and anemia was seen in < 1%. Only grade 1/2 TFR was reported in 52% of patients. The presence of 17p deletion was associated with shorter PFS (statistically significant) compared with other cytogenetic abnormalities (median PFS 6 months vs not reached). There was a high tendency to achieve CR in patients with unmutated IgVH; however, this was not statistically significant (P = .07). Seven fatalities occurred: 1 patient developed Richter's transformation and 2 died of unrelated malignancies. This study showed that lenalidomide offered an OR rate of 65%, including a CR rate of 10% and a PR rate of 43%. The results of 34 patients who completed 15 cycles of lenalidomide were studied to analyze the lymphocyte populations in the bone marrow and peripheral blood. Further analysis of these data suggests that lenalidomide induces a functional reconstitution of the lymphocytes in the peripheral blood and bone marrow, which might be the key phenomenon necessary for the antileukemic effect of lenalidomide.41

A review of these trials highlights the two particular nonhematologic toxicities of lenalidomide, which are also seen with the parent drug thalidomide. TLS and TFR occurred regardless of the dose, schedule, or Rai stage of CLL patients. Both were closely monitored in all the clinical trials presented. Using higher doses, TLS occurred in 2 of 45 patients, causing 1 fatality, and TFR occurred in 58% of patients.40 Most of the clinical data showed that lenalidomide was associated with a similar toxicity profile regardless of the dose used. It is important to emphasize the presentation of TFR since it can be mistaken for progression of disease. Fever, enlarged or worsening lymphadenopathy, and rash are part of the constellation of symptom associated with TFR. In order to improve pain control in TFR, the use of prophylactic corticosteroids, NSAIDs, or allopurinol was administered to patients enrolled in the trials with good response.13,31-34 Among the hematologic toxicities, myelosuppression seems to be the most common side effect associated with the use of this immunomodulatory agent.

**Lenalidomide Plus Rituximab**

Since lenalidomide offers an OR rate of 32% to 47%,14,35 two trials were conducted to evaluate its combination with rituximab in patients with relapsed or refractory CLL. Ferrajoli et al.36 treated patients with rituximab weekly for 1 cycle and then once every 4 weeks during cycles 3 to 12 combined with lenalidomide at the dose of 10 mg per day starting on day 9 of cycle 1 and continuing daily for 12 cycles. Data suggest that this combination offers a superior treatment when compared to single-agent lenalidomide, with PR and OR rates of 39% and 64%, respectively. Complete response was seen in 8% of the subjects. The toxicity profile was similar to single-agent lenalidomide, with hematologic complications and fatigue as common side effects. Grade 3 and 4 neutropenia and thrombocytopenia were reported in 68% and 22% of patients, respectively. Only 1 patient had grade 3 TLS but 22 patients (37%) had grade 1/2 TFR.

Our group recently presented preliminary data of a phase II clinical trial with the same combination.37 This study, which included patients with relapsed or refractory mantle cell lymphoma and CLL, allowed dose escalation of the lenalidomide in 28-day cycle and weekly rituximab at 375 mg/m² for 4 weeks starting on day 15 of cycle 1. Interim analysis showed that 42% of the patients with CLL had a PR and 50% had stable disease, with a median duration of response of 18 and 12 months, respectively. Unpublished data from this small trial...
showed the same hematologic toxicities, predominantly a 36.3% rate of grade 2/3 neutropenia. TFR and TLS were reported at 27% and 4.5%, respectively.

Based on these two small studies, the combination of lenalidomide and rituximab appears to offer a promising synergistic effect. Mature data and further studies are needed to confirm and validate OS and PFS.

**Lenalidomide Plus Ofatumumab**

The initial results of a phase II study evaluating the efficacy and tolerability of the combination of lenalidomide and ofatumumab in patients with relapsed CLL was presented at the ASH 2010 meeting. Treatment consisted of ofatumumab administered intravenously on a weekly basis for 4 weeks (300 mg in week 1, 1,000 mg in week 2 and all subsequent doses), then monthly for months 2 through 6 and once every 2 months for months 7 through 24. Lenalidomide was given at a dose of 10 mg daily starting on day 9 and continuing daily with a treatment duration of 24 months. Results for the first 16 out of 40 planned patients who were on study for at least 3 months have been reported. Four patients (25%) were refractory to fludarabine and all were previously treated with rituximab. Ten of 16 evaluable patients achieved a response: 2 CRs (13%) and 8 PRs (50%) for an OR rate of 63%. Four patients with stable disease were continuing on treatment. The most common grade 3/4 treatment-related adverse events were neutropenia (8 patients, 50%) and anemia (2 patients, 13%). TFR was limited to grade 1 in 2 patients (13%). Data from this study suggested that the combination of lenalidomide and ofatumumab was well tolerated and is a therapeutically active combination for patients with relapsed CLL.

**Lenalidomide Plus Chemotherapy**

In an effort to improve the efficacy of IMiDs and obtain better outcomes with their use, Brown et al initiated a small phase I trial that combined lenalidomide with fludarabine and rituximab for untreated patients with CLL. Low doses of lenalidomide (2.5 mg) were administered daily for 21 days in 28-day cycles, with fludarabine 25 mg/m² on days 3 through 5 and rituximab 375 mg/m² on day 1. Due to significant cytopenias and myalgias associated with elevated creatine phosphokinase (CPK), a side effect evident early in the trial, the lenalidomide administration was reduced to every other day. Regardless of the dose adjustment, persistent toxicities of myelosuppression and TFR led to an early closure of the trial. A 56% response rate was evaluated in 5 of the 9 patients who had objective responses by intention to treat basis. Nevertheless, the results from this negative trial showed the first attempt to combine lenalidomide with chemo-immunotherapy.

All these provocative trials have emerged in an effort to improve outcomes, develop non-chemotherapy treatment options, and discover better alternatives for patients with this incurable leukemia. Lenalidomide has been shown to be an active IMiD that offers a significant clinical response in patients with CLL who are heavily pretreated and carry unfavorable features. While lenalidomide has been shown to be an attractive alternative in CLL due to its particular effect in the tumor microenvironment, larger studies are being conducted to improve our understanding of its mechanism of action. These promising data will also help evaluate the most effective dose and schedule of this agent. In addition to its use in treating CLL, lenalidomide is being explored as a novel agent for other hematologic and solid tumor malignancies.

**Active Immunotherapy**

CLL is characterized by immune deficiency with both humoral and T cell functional defects, thus representing a potential model in which to study immunotherapeutic approaches. Its slow growth allows time to generate an immune response against the tumor cells, and tumor cells are easy to obtain in large numbers from the peripheral blood. Furthermore, B-CLL cells express major histocompatibility complex (MHC) class I and II in addition to the idiotype (Id), a tumor-specific epitope of the immunoglobulin B-cell receptor. Moreover, the chromosomal abnormalities commonly present in CLL may encode altered self-proteins that could serve as target antigens for immune recognition. Other observations that support the use of immunotherapy in CLL include the physical association of CLL and T cells in secondary lymphoid organs, which assures interactions between tumor-reactive T cells and tumor cells, spontaneous remissions associated with heightened immune activity following viral infections, clinical responses following treatment with immunomodulatory cytokines, and long-term disease-free survival after allogeneic bone marrow transplantation, possibly from a T-cell-mediated graft-vs-leukemia effect. Immune control of tumors, including CLL, is believed to be mediated mainly by CTLs that recognize tumor antigens. Cytotoxic CD8+ T cells are mainly responsible for the destruction of epithelial tumors, but both CD4+ and CD8+ CTLs may kill B-cell tumors. The purpose of cancer vaccines is to increase the number of these tumor-reactive CTLs and maintain their activity long enough to clear tumor cells. In theory, cancer vaccines should both promote tumor clearance and prevent relapse by providing long-term antitumor immunity.

A successful cancer vaccine requires tumor-associated antigens (TAAs) in the cancer cell that can be presented with appropriate costimulatory signals to T cells able to respond to these antigens. Activation of T cells requires two signals delivered by antigen-presenting cells (APCs). The first signal is mediated by the antigen MHC interacting with the T-cell receptor; the second signal is provided by costimulatory molecules expressed by the APCs, such as IL-2, CD80, or CD86, that bind to CD28 on the T cell. Dendritic cells (DCs) and macrophages
are potent professional APCs in that they are capable of providing costimulatory signals. A major goal in tumor immunotherapy is to mount a systemic CTL response of the tumor-bearing host against TAAs. A number of TAAs have been identified and overexpressed in CLL, and many functional studies have been performed to verify the existence of naturally occurring reactive T cells. These TAAs include fibromodulin, the receptor for hyaluronic acid-mediated motility (RHAMM/CD168), murine double-minute 2 oncoprotein (MDM2), telomerase reverse transcriptase (hTERT), the oncofetal antigen-immature laminin receptor protein (OFAiLRP), adipophilin, survivin, KW1 to KW14, and the tumor-derived IgVH-CDR3 region, which is specifically expressed by the tumor cells as surface membrane immunoglobulins sharing idiotypic determinants. Tumor-associated CLL antigens are likely encoded by genes that are mutated or overexpressed during oncogenesis and may be unique to each patient. Despite intense research efforts, active immunotherapy has achieved limited success, in part because of the uniqueness of CLL antigens that are patient-specific, thus requiring an individually prepared vaccine for each patient. Several potential reasons may explain why patients fail to mount an effective T cell–mediated immune response against their disease. Despite having a normal expression of MHC class I and class II molecules on the cell surface, B-CLL cells are poor APCs as they lack costimulatory and cell-adhesion molecules such as CD80, CD86, and CD54, which are essential in producing an effective T-cell response. Additionally, upregulation of MHC class I expression by B-CLL cells in response to interferon gamma (IFN-γ) was reduced. This relative MHC class I expression defect of B-CLL cells may reduce their susceptibility to CTL lysis in response to immunotherapeutic approaches. In addition, CLL cells were resistant to FAS (CD95) ligand-mediated apoptosis in vitro. Functional T-cell abnormalities have also been described, including inversion of the CD4/CD8 ratio with an increase in absolute numbers of activated CD4 and CD8 cells. Additionally, there is an altered production of cytokines including IL-4, IFN-γ, as well as downmodulation of CD154 (CD40 ligand), the zeta chain of the T-cell receptor, and the costimulatory molecule CD28. Furthermore, CLL cells are known to secrete transforming growth factor beta (TGF-β), a factor known to have potent immunosuppressive functions. Gene expression profiles of peripheral blood T cells from previously untreated patients with B-CLL revealed differentially expressed genes, mainly involved in cell differentiation in CD4 cells and defects in cytoskeleton formation, vesicle trafficking, and cytotoxicity in the CD8 cells of these patients. Suppressed T-cell function has been described as a major hurdle for the development of clinically efficient cancer immunotherapy. Inhibition of antitumor immune responses has been mainly linked to inhibitory factors present in cancer patients. More recently, increased frequencies of CD4+/CD25hi regulatory T cells (Treg cells) have been described as an additional mechanism that reduces immunity. A study of 73 patients with B-cell CLL demonstrated significantly increased frequencies of CTLA4+, Forkhead box P3 (FOXP3+), glucocorticoid-induced TNF receptor-related protein (GITR+), CD62L+, TGF-β1+, and IL-10+ Treg cells in patients with CLL. The inhibitory function of Treg cells was decreased or even abrogated in the majority of patients treated with regimens containing fludarabine or cyclophosphamide, suggesting that the use of these agents to reduce immunosuppression prior to cancer immunotherapy may be a promising strategy.

**Vaccination Approaches**

Multiple vaccination approaches as active immunotherapy for CLL have been investigated, including gene therapy, DC-based vaccines, whole modified tumor cell vaccines (eg, trioma cell vaccines), tumor-specific Id vaccines, and TAA-derived peptide vaccines.

**Gene Therapy**

Since TAAs have not been molecularly identified in most cases, vaccination protocols have been developed using whole autologous tumor cells genetically modified to express cytokines or costimulatory surface molecules. The transfer of an immunostimulatory gene into malignant tumor cells and the use of these autologous cells as vaccine have been extensively investigated in B-cell malignancies. The immunostimulatory genes studied include IL-2, IL-12, TNF-α, and granulocyte-monocyte colony-stimulating factor (GM-CSF), as well as gene-encoding immune accessory surface molecules such as CD80 and CD40-ligand (CD154).

The malignant B-CLL cells express a range of tumor-associated and tumor-specific antigens, as well as high levels of MHC class I and II molecules; however, they lack costimulatory molecules and are ineffective APCs. Their immunogenicity can be increased by manipulation of the CD40/CD40 ligand (CD40L) pathway, in which CD40L interacts with CD40 on B-CLL target cells to increase their antigen-presenting capacity through upregulation of the costimulatory molecules CD80 and CD86 as well as adhesion molecules such as CD54. One such approach involves the introduction of CD40L into CLL cells. Several strategies can be utilized to accomplish this: the use of adenovirus, recombinant adeno-associated virus, or herpes simplex virus vectors, as well as the molecular transfer from fibroblasts that overexpress the ligand, a nonviral electroporation-based gene delivery system, and a standard plasmid-carrying CD40L cDNA. CD40L also induces DC maturation in vivo, increasing their ability to take up and process antigens. A phase I clinical trial was performed to evaluate the response to intravenous administration of autologous CLL B cells transduced with a gene encoding murine CD154 us-
ing a replication-defective adenovirus vector.\textsuperscript{76} Eleven patients with progressive intermediate or high-risk CLL by the modified Rai criteria were enrolled in the study. Four of the patients were previously treated with chemotherapy. After a one-time bolus infusion of autologous Ad-CD154-transduced leukemia cells, there was increased or de novo expression of immune-accessory molecules on bystander, noninfected CLL cells in vivo. Patients also developed high plasma levels of IL-12 and IFN-γ, as well as increased numbers of leukemia-specific T cells as demonstrated by an autologous enzyme-linked immunosorbent spot (ELISPOT) assay and mixed lymphocyte reactions. These biological effects were associated with reductions in lymphocyte count, spleen size, and lymph node size. The infusion was well tolerated; however, patients developed not only antileukemic immune responses, but also antimurine CD154 antibodies. To avoid this, a phase I study was conducted in which patients were infused with autologous CLL cells transduced ex vivo to express ISF35, a humanized, membrane-stable CD154.\textsuperscript{83} Infusions were well tolerated and consistently followed by reductions in blood lymphocyte counts and lymphadenopathy. After infusion, circulating CLL cells had enhanced or de novo expression of CD95, DR5, p73, and Bid, which enhanced their susceptibility to death-receptor–mediated or drug-induced apoptosis, including CLL cells with del(17p). Two patients who had CLL with del(17p) had subsequent chemoinmunotherapy and responded well to treatment, suggesting that the vaccine might enhance the susceptibility of CLL cells with del(17p) to chemoinmunotherapy.

To examine the p53 dependency of the acquired latent sensitivity to Fas-mediated apoptosis following activation by CD154, investigators examined the in vitro responses to CD154 of CLL cells that did or did not have functional p53.\textsuperscript{84} They found that CD154 induces CLL cells to express p53 and the p53-target genes CD95, DR5, p21, and Bid. Also, CLL cells still lacking functional p53 were induced to express Bid and to acquire sensitivity to CD95-mediated apoptosis following co-culture with CD154-bearing cells. Such treatment also induced p73, a p53-related transcription factor regulated by c-Abl kinase, and enhanced the sensitivity of CLL cells lacking functional p53 to fludarabine. The transduction of CLL cells with an adenovirus encoding p73 also induced Bid and CD95 and enhanced the sensitivity of p53-deficient CLL cells to fludarabine. However, inhibition of c-Abl with imatinib suppressed CD154-induced expression of p73, p73-induced expression of Bid, and CD95 and also blocked the sensitization of p53-deficient CLL cells to CD95-mediated or fludarabine-induced apoptosis. Conversely, CLL cells transduced with an imatinib-resistant c-Abl mutant could be induced by CD154 to express p73 and Bid even when treated with imatinib. These results indicate that CD154 can sensitize leukemia cells to apoptosis via the c-Abl–dependent activation of p73 and can mitigate the resistance of p53-deficient CLL cells to anticancer drug therapy.

Human interleukin 2 (IL-2) has been shown to further potentiate the immunogenicity of human CD40 ligand (hCD40L) in preclinical murine models.\textsuperscript{77} An early-phase vaccination study of autologous B-CLL cells that expressed both hCD40L and hIL-2 was conducted in 9 patients who received 3 to 8 subcutaneous vaccinations.\textsuperscript{85} The vaccine was administered without evidence of significant local or systemic toxicity. A B-cell CLL-specific T-cell response was detected in 7 patients. Three patients produced leukemia-specific immunoglobulins. Three patients had greater than a 50% reduction in the size of affected lymph nodes, although these responses were transient. High levels of circulating CD4+/CD25+/LAG-3+/FoxP-3+ immunoregulatory T cells (Tregs) were present before, during, and after treatment. The authors speculated that the increase in Tregs might have limited the magnitude and duration of the antileukemic immune response since in vitro removal of these cells increased the antileukemic T-cell reactivity.

Due to the complexities and expense of manufacturing viral vectors, as well as their lingering safety concerns, further studies have investigated the transduction of B-CLL cells using nonviral gene delivery methods such as electroporation, a physical means of transferring CD40L and IL-2 plasmids to produce vaccines with similar biological properties in vitro and in vivo.\textsuperscript{81} A vaccine clinical trial using this strategy was conducted in which 7 patients received a total of 6 subcutaneous injections of autologous transduced cells using electroporation in the presence of DNA plasmids encoding hCD40L or hIL-2.\textsuperscript{86} Following vaccination, all patients had stable leukemia counts and 1 patient had a transient decrease of more than 50% in local adenopathy. However, using the National Cancer Institute response criteria, no CRs or PRs were observed. Compared with adenoviral vaccines, electroporation generally provides a simpler and more rapid means of preparing IL-2/CD40L-expressing B-CLL vaccines, but the cells express higher levels of IL-2 and lower levels of secondary costimulator molecules.\textsuperscript{87} Additionally, the administration of the transduced cells via subcutaneous injection limited the number of CLL cells that could be exposed to the transfected CLL cells, thereby minimizing the bystander effect seen with intravenous infusion of autologous adenovirus-CD40L–transduced CLL cells.\textsuperscript{74} A second clinical trial is currently evaluating a prolonged vaccination using 18 deltoid injections of this vaccine strategy over 52 weeks (NCT00458679).\textsuperscript{88} IL-12 is a potent cytokine that stimulates T cells and natural killer cells. Animal studies of vaccination using a murine B lymphoma cell line transfected with a replication-defective retrovirus encoding IL-12 induced T-cell–mediated antitumor immunity in mice.\textsuperscript{89} TNF-α is a cytokine that can directly induce apoptosis of some tumors and can stimulate DC maturation and function.

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Given the systemic toxicity of TNF-α, gene therapy strategies that allow local expression of TNF-α in the tumor microenvironment are being developed. The costimulatory molecules CD80 and CD86 bind to CD28 on T cells and provide a second positive signal to T cells for activation and proliferation. Animal studies with transduced cell lines have confirmed in vivo activity. GM-CSF is a hematologic growth factor that also induces DC maturation and activation. In vitro studies of murine T-cell leukemia transduced with retrovirus-encoding GM-CSF induced tumor-specific immunity. However, none of these vaccine approaches have been investigated in clinical trials of CLL patients.

An alternative vaccine strategy, called TRICOM, was created to enhance the immunogenicity of CLL cells via infection with vectors encoding for three essential costimulatory molecules: CD80, lymphocyte function-associated antigen 3 (LFA-3), and intercellular adhesion molecule 1 (ICAM-1). A recombinant-modified vaccinia virus strain Ankara (MVA), which is a highly attenuated, replication-impaired virus variant, was successful in infecting and delivering the simultaneous expression of the three human costimulatory molecules in TRICOM on the surface of CLL cells. Cytotoxic T lymphocytes, generated in vitro by stimulation of autologous T cells with MVA-TRICOM–infected CLL cells, showed cytotoxicity against unmodified, uninfected CLL cells. These findings suggest that the use of CLL cells infected ex vivo with MVA-TRICOM or via direct injection of MVA-TRICOM in patients with CLL had potential for the immunotherapy of CLL. Furthermore, following incubation with irradiated MVA-TRICOM–modified CLL cells, allogeneic and autologous CD4+ and CD8+ T cells expressed significantly higher levels of CD80, ICAM-1, and LFA-3. This increase was shown to be the result of physical acquisition from the APCs, and purified T cells that acquired costimulatory molecules from MVA-TRICOM–modified CLL cells were able to stimulate the proliferation of untreated T cells. These results demonstrated for the first time that T cells from CLL patients can acquire multiple costimulatory molecules from autologous CLL cells and can then act as APCs themselves. Given the immunodeficiencies characteristic of CLL, enhancing the antigen-presenting function of CLL cells and T cells simultaneously could be a distinct advantage in the effort to elicit antitumor immune responses. Clinical trials evaluating this vaccine approach for patients with CLL have not yet been conducted.

**DC-Based Vaccines**

As previously discussed, CLL cells are poor APCs. One way of improving immune response to antigens is to use more powerful APCs such as DCs. This strategy involves loading DCs with peptides derived from TAA, tumor lysates, RNA, or DNA from tumors or fusing DCs with tumor cells. The use of DCs transfected with in vitro amplified B-CLL mRNA elicited both HLA class I and II CLL-specific CD4- and CD8-mediated T-cell responses, suggesting that this approach might be a potent new strategy in the treatment of CLL. Based on a study showing several different clones of leukemia-reactive CD4 and CD8 cells present in CLL patients, it has been speculated that a vaccination approach using whole tumor cells was preferable to a single defined antigen. Additionally, this approach minimizes the potential selection of tumor antigen escape variants. Compared to using DCs alone, the use of DCs electrofused with CLL B cells (fusion hybrids) produced higher levels of specific cytotoxic T-cell responses to tumor cells. Additionally, the cytokine response induced by Apo-DCs (DCs that had endocytosed CLL apoptotic bodies) was significantly higher than that induced by DCs fused with tumor cells. This study showed that endocytosed apoptotic tumor cells induced a significantly stronger T-cell response than DC hybrids induced, making this strategy a better candidate for vaccine production. A clinical study evaluated the potential of allogeneic monocyte-derive DCs obtained from normal donors pulsed ex vivo with tumor cell lysates or apoptotic bodies to stimulate antitumor immunity in patients with B-CLL in early stages. Nine patients with Rai stage 0 and 1 CLL were vaccinated 5 times with a mean number of 32 × 10^6 stimulated DCs administered intradermally once every 2 to 3 weeks. No signs of autoimmunity were detected, and only mild local skin reactions were noted. A decrease in peripheral blood leukocytes and CD19+/CD5+ leukemic cells was observed during the treatment period. A significant increase of specific cytotoxic T lymphocytes against RHAMM/CD168 was detected in 1 patient after DC vaccination. A second cellular vaccination study was conducted by the same group using autologous DCs pulsed ex vivo with tumor cell lysates. Twelve patients with early-stage CLL received up to 8 intradermal vaccinations. Five patients showed a decrease in peripheral blood leukocytes and CD19+/CD5+ leukemic cells, 3 showed a stable disease, and 4 progressed despite DCs vaccination. A significant increase in specific cytotoxic CD8+ T lymphocytes against the leukemia-associated antigens RHAMM or fibromodulin was detected in 4 patients following DC vaccination. In patients with a clinical response, an increase of IL-12 serum levels and a decrease of the frequency of CD4+/CD25+/FOXP3+ T regulatory cells were observed. These results justify further investigation of this immunotherapeutic approach. A phase I/II clinical trial using Apo-DC vaccination for the treatment of previously untreated CLL patients is ongoing.

**Whole Modified Tumor Cell-Based Vaccines**

The difficulty in finding appropriate tumor antigens has led to innovative vaccination approaches in CLL. The use of polyvalent cellular vaccines using whole modified tumor cells would allow multiple antigens to be ingested by APCs. The most potent strategy is the trioma approach,
which is based on immunization with lymphoma cells modified to express an antibody against an internalizing and activating surface molecule (Fcc receptor) on APCs. This approach is based on redirection of the tumor-specific immunoglobulin Id toward professional APCs, thereby overcoming the inefficient presentation on the parental transformed B cell. In this method, malignant B cells are fused to a xenogeneic hybridoma cell line that secretes an antibody against a surface receptor of APCs, resulting in trioma cells. These cells express tumor-derived antigens and have anti-APC specificity. The trioma cell binds to the Fc receptor of an APC, resulting in uptake, processing, and presentation of the Id. In a mouse model, vaccination with trioma cells conferred long-lasting, T-cell–dependent tumor immunity and was able to eradicate established lymphomas. In a preclinical study, malignant cells from 11 patients with B-CLL were fused to an anti-Fc receptor hybridoma. In 7 cases, trioma cells could successfully be generated from B-CLL cells. Stimulation of autologous lymphocytes with trioma cells induced a leukemia-specific T-cell response in vitro. Furthermore, DCs pulsed with trioma cells effectively activated T lymphocytes against CLL in vitro. In this study, activation of T cells was more pronounced after stimulation with trioma-pulsed DCs compared with stimulation with trioma cells in the presence of APCs, and overexpressed antigens associated with malignant transformation, such as BCL-2, MDM2, and ETV5, serve as targets for those T cells. Immune escape by antigen loss or mutation is less likely to occur if immunity is directed against altered self-proteins that are involved in malignant transformation. Therefore, vaccines based on modified tumor cells such as triomas show promise for immunotherapy of CLL and other malignancies. Polyvalent vaccines originally designed as individualized therapeutics may be more broadly applicable, at least in patients showing similar antigen patterns. Clinical studies will determine the effectiveness and safety of trioma-induced immunity.

A different approach that used altered tumor cells as an antigen source was investigated by subjecting blood from 25 patients with CLL to a combination of oxidative physicochemical stressors in a blood treatment unit. It was hypothesized that this treatment would release antigen-binding heat shock proteins and free radicals that would activate APCs and increase the immunogenicity of the CLL cells in vivo. The treated blood sample was intramuscularly reinjected into the patients twice weekly for 6 weeks. After vaccination, an increase in CLL-reactive T-cell levels was seen in patients with preexisting CLL-reactive T cells, and there was an inverse correlation between disease stage and anti-CLL T-cell reactivity. A subsequent phase I/II clinical trial was conducted to evaluate the feasibility, safety, and efficacy of autologous vaccines made from oxidized tumor cells in 18 patients with earlier-stage CLL. Partial clinical responses associated with enhanced antitumor T-cell activity in vitro were observed in 5 of the 18 patients. Stable disease was observed in 6 patients, and disease progression appeared to be unaffected in the remaining patients. Toxicity was minimal. This vaccination method appears worthy of further investigation and may be a potential alternative to a “watch and wait” strategy for selected CLL patients.

In further attempts to increase immunogenicity of CLL cells, a phase I clinical study of intradermal vaccination with irradiated autologous CLL cells with Bacillus Calmette-Guérin (BCG) as an adjuvant was conducted in 17 patients with previously untreated early-stage CLL. The investigators hypothesized that inducing apoptosis in irradiated leukemic cells would increase the antigenicity of malignant cells by enhanced presentation of tumor antigens, without requiring the precise identification of antigenic targets. Proliferation studies did not show any significant activation of specific T cells. However, hematologic improvement (> 25% reduction in leukocyte count) was observed in 5 of the 17 patients, stabilization of disease in 5 patients, and no response to immunotherapy in 7 patients. Additionally, a significant increase of the lymphocyte doubling time was noted in 7 of 9 patients, suggesting that cellular immunotherapy might prolong disease progression and the need for chemotherapy. Further investigation using this vaccine method is warranted.

**Id Vaccines**

Normal B cells express an immunoglobulin with unique variable region sequence in the heavy and light chains that together form the antigen-binding site. During malignant transformation, this Id is maintained by the malignant clone and therefore can be regarded as a TAA. Animal studies have shown an effective humoral and cellular mechanism against Ids at inducing tumor regression. A potential limitation of this approach is the fact that Id vaccines must be custom-made for each patient. Furthermore, one study showed that CTL responses generated against naive immunoglobulin-derived peptides were weak. These limitations may explain why no clinical trials have been reported in patients with CLL using this approach.

**TAA-Derived Peptide Vaccines**

Generating a T-cell–mediated response targeted at the TAA represents a novel therapeutic approach for patients with CLL. Vaccination with TAA-derived peptides might allow exact monitoring of T-cell responses to these particular antigens by different methods including ELISPOT assays, flow cytometry analysis of intracellular IFN-γ, or tetramer staining. As previously noted, several TAA have been identified and are overexpressed in CLL. Encouraging results in vitro, as well as the safety, feasibility, and economic advantage of peptide vaccination in patients with other hematologic malignancies, provided
Conclusions

Clinical trials using lenalidomide as a single agent or in combination with immunotherapy in patients with relapsed and refractory CLL have shown promising response rates. More recently, compelling data from phase II trials have demonstrated significant activity in chemotherapy-naive patients. In this setting, lenalidomide appears to offer a survival benefit and improve PFS.

Even after reviewing and analyzing these provocative results, the toxicity profile associated with the use of lenalidomide makes its use challenging. Because of its potential treatment complications, particularly tumor flare reaction and tumor lysis syndrome, this drug should be used only in a clinical trial setting. Other immunotherapeutic approaches are also showing some benefit in patients with high-risk CLL for whom chemotherapy regimens are no longer an option.

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