Maintenance Therapy for B-Chronic Lymphocytic Leukemia

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Abstract: Although modern treatment options for B-chronic lymphocytic leukemia (CLL) produce high response rates, virtually all patients relapse, presumably due to the persistence of minimal residual disease (MRD). Novel approaches that maintain response and therefore delay growth of MRD may ultimately improve survival outcomes. In CLL, any type of continued therapy must be not only well tolerated but also convenient to ensure compliance. There has been some exploration of rituximab as maintenance therapy in CLL; however, given its limited clinical activity as a single agent, other options need to be studied. One such agent is the immunomodulatory drug lenalidomide, which has demonstrated clinical activity both in patients with relapsed or refractory CLL and in the frontline setting. Other attractive agents being explored in the maintenance setting include epigallocatechin gallate, curcumin, and the citrus pectin-derived galectin-3 inhibitor GCS-100. These naturally occurring compounds are well tolerated, and they inhibit survival signals in the microenvironment necessary for tumor development, making them well suited for evaluation as maintenance therapy for CLL.

Keywords
Chronic lymphocytic leukemia, maintenance therapy, immunomodulatory drugs, lenalidomide

Introduction

Chronic lymphocytic leukemia (CLL) is the most common form of leukemia in the United States, affecting over 15,000 people and causing more than 4,000 deaths each year.[1] Although purine nucleoside-based chemotherapy regimens produce high overall response rates (ORRs),[2-5] virtually all patients will eventually relapse, presumably due to the persistence of residual leukemia cells. Initial phase II studies of triple-drug regimens that combined rituximab (Rituxan, Genentech) with chemotherapy, consisting of purine analogues and alkylating agents, significantly improved ORRs and progression-free survival (PFS) relative to historical controls in both the frontline setting[6] and in patients with relapsed/refractory disease.[5,7,8] These results were recently validated with data from 2 phase III studies. The REACH (Rituximab in the Study of Relapsed Chronic Leukemia) study showed that fludarabine, cyclophosphamide, and

rituximab (FCR) significantly improved ORRs, including complete response (CR) rates, and PFS relative to chemotherapy alone (fludarabine and cyclophosphamide [FC]) in 522 relapsed patients.[9] Patients receiving FCR attained a 10-month benefit in PFS compared with patients who received FC (30.6 vs 20.6 months; $P<.01$). In the first-line setting, definitive results on the clinical activity of FCR were presented by the German CLL Study Group.[10] At a median observation time of 37.7 months, the CR rate in the FCR arm was more than double that achieved by patients treated with FC (44.1% vs 21.8%), which appeared to ultimately translate into not only a PFS benefit but also a significant improvement in overall survival (OS; 84.1% in FCR arm vs 79.0% in FC arm; $P=.01$). However, even in this relatively younger patient population (median age, 61 years), more than 76% of patients in the FCR arm developed a grade 3/4 adverse event (62.9% in the FC arm). Also noteworthy is that infection rates were comparable between treatment arms (21.5% and 25.5% in the FC and FCR arms, respectively). Given that the median age of a typical CLL patient is 71 years, these safety results suggest that not every patient with CLL may be a candidate for fludarabine-based combination therapy, and that alternate, less-intensive strategies may need to be considered for older patients who are infirm or have comorbidities.

Despite the significant clinical activity of FCR, many patients will still relapse, and it is yet to be determined whether use of such aggressive first-line therapies changes the natural disease course of these patients.[11] A 6-year follow-up of approximately 300 patients who received first-line therapy with FCR in a study at M.D. Anderson Cancer Center showed an actuarial 6-year failure-free survival rate of 51%.[6] Further analysis of 114 patients in first relapse after FCR revealed that patients with PFS of less than 36 months had significantly worse outcomes with subsequent therapies versus patients with a 36-month or longer response to first-line therapy. Many relapsed patients exhibited characteristics associated with poor prognostic risk: 16% of patients had del(17p) and 24% had del(11q) cytogenetic mutations, 65% had unmutated immunoglobulin variable region heavy chain (IgVH) status, and 67% were ZAP-70 positive.[11] It is clear that patients who relapse are likely to relapse again, and that there is a need for safe and effective strategies that prolong response durations and delay both clinical relapse and disease progression in patients with CLL.

For patients who either do not qualify for first-line treatment with FCR or who are more likely to relapse, one potential strategy to improve survival outcomes is to improve response durability. This approach has been used with some success in the treatment of other B-cell malignancies. Similarly, in CLL, maintenance therapy has the potential to control the proliferative activity of residual leukemic cells and suppress their potential to reemerge, and to prevent clinical relapse. Perhaps, more importantly, therapy with the ability to modulate the host cell microenvironment or the immune response to CLL cells has the potential to create sustained conditions that prevent or potentially suppress the expansion of the leukemic cell clone. This change in the tumor cell microenvironment may translate into a prevention of relapse and prolongation of a patient's response, irrespective of the initial level of response, and ultimately the attainment of a survival benefit.

Drug Candidates for Maintenance Therapy in CLL

Maintenance therapy should be effective, have an acceptable safety profile, and be easy to administer to minimize the burden on the patient and on healthcare resources. Although purine analogue-based combinations are currently the most active treatments available, long-term use of these agents is associated with hematologic toxicity and increased risk of infectious complications, which precludes their use in the maintenance setting. Several studies have evaluated the effects of various types of immunotherapy as maintenance therapy or as part of other postinduction treatment strategies. These included interferon-alpha[12,13] and rituximab.[14,15] Alemtuzumab (Campath, Genzyme) has been studied in the consolidation setting, where it demonstrated a significant improvement in PFS,[16,17] but also resulted in a significant incidence of infectious complications.

These relatively small studies of postinduction approaches in CLL have failed to show a consistent improvement in response duration or OS without a significant increase in toxicity. Another drawback associated with many of the available agents in the maintenance setting is that they require intravenous or subcutaneous administration, which reduces the ease of administration and, especially in the context of long-term treatment, may have a negative effect on patient adherence to treatment.

Rituximab

The monoclonal antibody rituximab is directed against the cell surface protein CD20. Addition of rituximab to chemotherapy regimens has yielded significant improvements in survival outcomes in patients with diffuse large B-cell lymphoma (DLBCL) and in patients with follicular lymphoma (FL). Rituximab maintenance has shown no benefit after rituximab-containing induction therapies for patients with DLBCL. In 2 trials in patients with low-grade lymphoma, rituximab maintenance was shown to increase both PFS and OS.[18,19] However, rituximab was

not part of the initial regimen, and the recently presented PRIMA (Primary Rituximab and Maintenance) trial addressed the value of rituximab maintenance in patients receiving rituximab plus chemotherapy. Results from this trial demonstrated that maintenance therapy has the potential to improve PFS for patients with indolent lymphoma. The 2-year PFS rate was 82% with rituximab maintenance compared to 66% with observation (P<.001).[20] With limited follow-up, no difference in OS has yet been shown.

Clinical activity in indolent non-Hodgkin lymphoma (NHL) prompted an exploration of rituximab maintenance in patients with CLL. In a study led by Hainsworth and colleagues,[14] 44 previously untreated patients received rituximab as both first-line and maintenance treatment, consisting of 4-week rituximab courses at 6-month intervals, for a total of 4 courses. The ORR was 58%, and 9% of patients achieved a CR. At a median follow-up of 20 months, median PFS was 18.6 months.[14] This median PFS was also significantly shorter than the 36–40-month PFS achieved with rituximab maintenance in FL. These data suggest that outcomes after maintenance therapy may be partially dictated by the quality of response achieved with induction therapy.

An analysis of whether maintenance therapy can improve response duration achieved with induction chemotherapy was recently reported by Del Poeta and coworkers.[15] Responders to induction therapy with fludarabine followed by 4 weekly doses of rituximab (375 mg/m^2) were included (N=54). Those who remained minimal residual disease (MRD)-positive (n=38) were consolidated with 4 monthly cycles of rituximab followed by a maintenance regimen of 12 monthly doses of rituximab administered at a lower dose (150 mg/m^2). The group that had received postinduction therapy with rituximab achieved a significantly longer response duration compared to those who received no postinduction treatment (n=16; 75% vs 9% at 4 years; P<.00001). OS was also significantly shorter among patients who were MRD-positive but received no additional therapy after induction (0% vs 79% at 15 years; P=.0007). Although these data suggest a benefit to maintaining a good response through postinduction therapeutic approaches, such as consolidation and maintenance, it is important to note that the 2 treatment arms analyzed in this study were not balanced in terms of patient numbers or patient characteristics, and the study was not randomized. Therefore, it is difficult to draw a definitive conclusion regarding the benefit of rituximab maintenance based on the data reported.

Thalidomide

Thalidomide (Thalomid, Celgene) and the structurally related compound lenalidomide (Revlimid, Celgene) belong to the immunomodulatory class of agents; they are immunomodulatory drugs that are active in CLL, and have the added advantage of oral administration. As reviewed in Chanan-Khan and Cheson,[21] thalidomide and lenalidomide each have unique effects on the host microenvironment and immune response that may sustain a clinical response in patients with CLL. However, clinical trials evaluating thalidomide in patients with CLL have produced mixed results.[22-25] The combination of thalidomide and fludarabine yielded an ORR of 100% and a CR rate of 55% in a phase I dose-escalating study involving 13 previously untreated patients.[22] The most common adverse events were fatigue, constipation, peripheral neuropathy, and tumor flare reaction (TFR). Another early report indicated that thalidomide was active in patients with relapsed/refractory disease when given alone or in combination with fludarabine.[23] However, in a subsequent report, 4 of 5 heavily pretreated patients receiving the combination of thalidomide, fludarabine, and cyclophosphamide discontinued treatment due to disease progression; the fifth patient stopped due to neuropathy.[24] The largest trial reported to date to evaluate thalidomide in patients with CLL was coordinated by the North Central Cancer Treatment Group.[25] This study, which evaluated thalidomide monotherapy in patients with relapsed/refractory disease, was closed early due to slow accrual. Among the 28 evaluable patients, the ORR was 11%. Of note is that 22 of 28 patients (79%) experienced a significant drop in their absolute lymphocyte counts (ALC), suggesting some level of clinical activity. The most common adverse events were fatigue, myelosuppression, and TFRs. Given the relatively modest activity of thalidomide in CLL and its toxicity profile, particularly with regard to cumulative peripheral neuropathy, thalidomide is not an ideal candidate for evaluation as maintenance therapy.

Lenalidomide

Lenalidomide is an immunomodulatory agent that was primarily selected based on its more potent immunomodulatory activity relative to its parent compound thalidomide. Recent laboratory studies demonstrate that the mechanism of action of lenalidomide in CLL involves the enhancement of immune-cell function and proliferation, including that of T cells and natural killer (NK) cells,[21,26-30] and enhancement of antibody-dependent cell-mediated cytotoxicity.[31] Lenalidomide-mediated restoration of immune function is further supported by upregulation in the expression of costimulatory molecules, such as CD80 and CD86, which are involved in improving immune recognition of CLL cells and enhancing immune synapse formation between CLL B cells and T cells or natural killer NK cells.[29,30]

The activity of lenalidomide in CLL was initially observed in 2 phase II studies conducted in patients with relapsed/refractory CLL. In the first study, 45 patients received lenalidomide administered at 25 mg/day on days 1–21 of a 28-day cycle, a dose and schedule picked based on its approved indication in relapsed multiple myeloma (MM). The initial ORR was 47%, and 9% of patients achieved a CR.[32] With further follow-up, the ORR increased to 53% and the CR rate increased to 18%, suggesting that continued therapy may be required to attain maximum benefit and that optimum responses may take longer to achieve with lenalidomide than with traditional chemotherapy.[33,34] With the 25 mg/day dose, 2 patients developed tumor lysis syndrome (TLS), requiring hospitalization; grade 3/4 neutropenia and thrombocytopenia were seen in 76% and 51% of patients, respectively, and severe fatigue and TFR occurred in 10% and 8% of patients, respectively. In an effort to improve tolerability, the second study evaluated lenalidomide at a lower dose (10 mg/day), administered in a continuous schedule to 44 heavily pretreated patients.[35] The ORR was 32%, including a 7% CR rate. With the 10 mg dose, no patient developed TLS; adverse events remained primarily myelosuppression, fatigue, and TFRs.

Several studies have explored lenalidomide in the frontline setting. In the first of these, lenalidomide was initiated at a dose of 10 mg daily on days 1–21 of a 28-day cycle, with the aim of escalating the dose in 5-mg increments weekly to the target dose of 25 mg daily.[36] When 2 initial patients developed TLS, the protocol was amended to reduce both the starting and target doses to 2.5 mg and 10 mg, respectively. With the amended protocol, no further cases of TLS were observed. For 25 patients accrued to the amended study, the ORR was 56%, and at a median follow-up of 13.7 months, the median response duration was 13 months.[36] Interestingly, rebound lymphocytosis was observed during cycle days 22–28 (off therapy), which suggested that continuous dosing may be required to maintain consistent therapeutic pressure, particularly if lenalidomide is administered at low doses.

Another phase II study explored the clinical utility of lenalidomide in 60 patients older than 65 years of age with previously untreated CLL.[37] Lenalidomide was initiated at 5 mg daily for the first 56 days, after which the dose could be increased in 5-mg increments every 28 days up to a maximum of 25 mg daily. For 54 patients who completed 15 cycles of therapy, the ORR was 57%, which included a 6% CR rate, a 7% nodular partial response (nPR) rate, and a 44% partial response (PR) rate. Responses to therapy were slower than with traditional chemotherapy; the majority of CRs and nPRs were achieved between treatment cycles 9 and 15. At a median follow-up of 19 months, the median time to treatment failure had not been reached.[37] Correlative analyses were conducted to monitor changes in the lymphocyte population. Lenalidomide increased the percentage of CD4+ and CD8+ lymphocytes as well as the number of T lymphocytes in the bone marrow. Although there was a reduction in ALC, the population of normal CD19+ B cells did not change significantly, suggesting that the lymphocyte decline mostly occurred in the leukemic CD19+/CD5+ cell population. Additionally, after 15 cycles of therapy, a significant increase (from 724 mg/dL at baseline to 941 mg/dL; $P<.001$) in median serum immunoglobulin (Ig) levels was observed.[37] Eight of 16 patients with hypogammaglobulinemia at baseline had normalized their IgG levels after 15 cycles of lenalidomide.[37]

Clinical data are not yet available on the use of lenalidomide in the maintenance setting for patients with CLL. However, experience with lenalidomide in other hematologic malignancies suggests that lenalidomide may be particularly well suited for long-term use. In patients with relapsed/refractory MM treated with lenalidomide-based therapy, prolonged treatment has been associated with improved treatment outcomes. Based on an analysis of pooled data from 2 large phase III trials, prolonged treatment with lenalidomide plus dexamethasone (>10 months) after achievement of best response was associated with significantly longer median time to progression and OS.[38] Furthermore, survival outcomes were inferior in patients who achieved a clinical response but subsequently discontinued treatment due to adverse events. In a study by Dimopoulos and associates,[39] a lower maintenance dose of lenalidomide after 12 months of full-dose therapy for relapsed/refractory patients improved long-term patient tolerability and extended the treatment duration, thereby improving long-term outcomes.

The known effects of lenalidomide on the host microenvironment and immune effector cells in conjunction with its demonstrated clinical activity in patients with CLL suggest that this agent may be a good candidate for maintenance therapy. Clinical studies evaluating lenalidomide in this setting are currently under way. In an actively accruing, multinational, randomized, placebo-controlled study known as the CONTINUUM (A Study to Evaluate the Efficacy and Safety of Lenalidomide as Maintenance Therapy for Patients With B-Cell CLL Following Second Line Therapy; CLL-002; NCT00774345) trial, patients with CLL who achieved PR or better to second-line therapy are randomized to receive either placebo or maintenance with lenalidomide initiated at a dose of 2.5 mg/day on days 1–28 of a 28-day cycle, escalated to 5 mg/day on days 1–28 of the second cycle if the 2.5 mg/day dose level is well tolerated (Figure 1). The primary endpoints are OS and PFS.

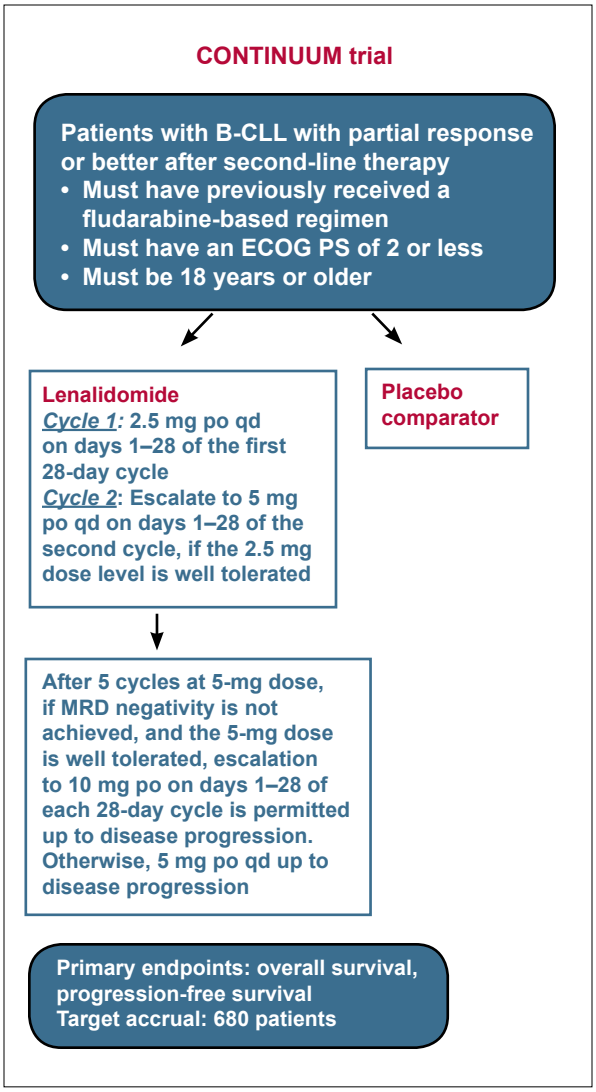

Figure 1. Design of the CONTINUUM trial (NCT00774345).

B-CLL=B-cell chronic lymphocytic leukemia; ECOG PS=Eastern Cooperative Oncology Group performance status; MRD=minimal residual disease; po=orally; qd=daily.

Table 1. Pharmacologic Targets of Naturally Occurring Compounds

Natural Compound	Mechanism of Action	Food Source Example
Epigallocatechin-3-gallate	Tumor evasion Tumor metastasis Angiogenesis Growth factor signalling	Green tea
Curcumin, resveratrol	Inflammatory enzymes (eg, cyclooxygenase-2) Transcription factor activity (eg, nuclear factor-κB, activator protein-1)	Turmeric, grapes
GCS-100	Tumor proliferation Tumor metastasis Angiogenesis	Citrus fruits
Delphinidin, ellagic acid	Growth factor receptor-mediated signal transduction	Pigmented fruits and vegetables
Diallyl disulfide	Multidrug resistance	Garlic
Genistein	Estrogenic actions Angiogenesis Tumor proliferation	Soy
Indole-3-carbinol	Metabolic activation of carcinogens via phase I enzymes	Cabbage
Phenethyl isothiocyanate	Tumor-cell apoptosis	Cabbage, watercress
Lentinan	Immune-system function	Shiitake mushrooms
Sulforaphane	Detoxification via phase II enzymes	Broccoli

Adapted from Béliveau and Gingras. *Can Fam Physician*. 2007;53:1905-1911.

Naturally Occurring Compounds as Candidates for Maintenance Therapy

Other novel compounds may be particularly well suited for evaluation as long-term maintenance therapy in patients with CLL based on their excellent tolerability profiles and promise of clinical activity in CLL. Like lenalidomide, these novel compounds appear to have pleiotropic and biologic effects on CLL cells and their microenvironment. By virtue of being naturally occurring, these compounds may have diminished toxicity profiles relative to currently established therapies, which make them good candidates for further study in the long-term maintenance setting. Although they are still in early clinical development, a number of naturally occurring compounds have shown promising results as therapeutic agents (Table 1).[40]

Delphinidin is an anthocyanin that has been recently investigated in colon,[41] breast,[42] and hepatocellular cancer models.[43] Ellagic acid is the main polyphenol found in pomegranates and it inhibits prostate cancer cell proliferation.[44] As reviewed by Powolny and colleagues, diallyl disulfide is derived from allium vegetables and exerts anti-

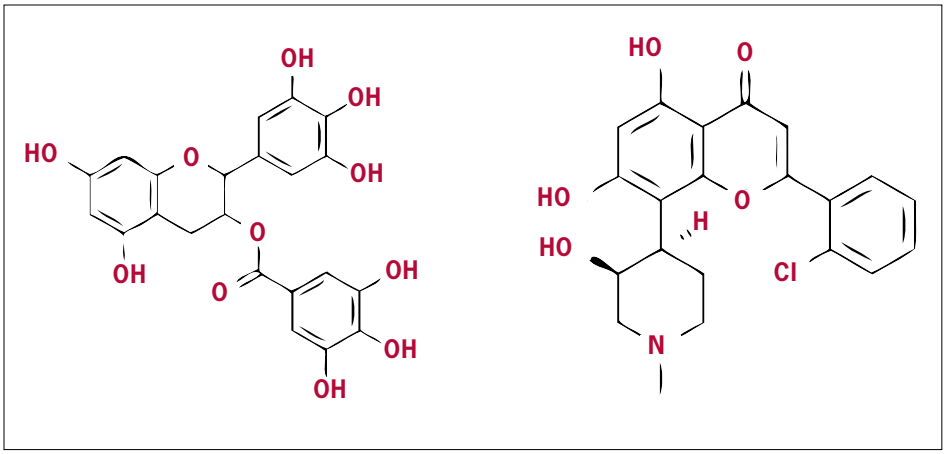

Figure 2. The chemical structures of epigallocatechin-3-gallate (EGCG) and flavopiridol.

malignant effects through various mechanisms.[45] Indole-3-carbinol appears to exert chemosensitization effects on a number of different human cancers.[46] Sulforaphane exhibits antiapoptotic properties in cervical, colon, and breast cancer cells.[47-49] The resveratrol monograph describes resveratrol as a polyphenol molecule derived from grapes, which has been investigated in various cancer models.[50] In an in vitro model of CLL, resveratrol showed antiproliferative and apoptotic effects on leukemic B cells.[51] Finally, lentinan is a beta-glucan polysaccharide that exerts immune-stimulating and anticarcinogenic effects[52]; specifically in CLL patients, lentinan caused a dose-dependent increase in NK and in antibody-dependent cellular cytotoxicity activity.[53]

The natural compounds that have been investigated for the treatment of CLL are discussed in detail below. These include the green tea component epigallocatechin-3-gallate (EGCG); the natural compound curcumin, which is found in the spice turmeric; phenethyl isothiocyanate (PEITC), which is found in cruciferous vegetables (eg, cabbage and watercress); and the galectin-3 inhibitor GCS-100.

EGCG

EGCG is a polyphenolic compound found in green tea. Its structure is similar to flavopiridol (Figure 2), a cyclin-dependent kinase inhibitor, which has both proapoptotic and antiangiogenic effects, and is under clinical development for the treatment of CLL.[54,55] In an in vitro study by Higashi and coauthors,[56] EGCG was shown to suppress activation of a member of the Rho family of small G proteins (ie, Rho in the human hepatic stellate cell line TWNT-4). Given that Rho has been shown to promote cell survival by regulating the Akt pathway,[57] and that EGCG has been reported to inhibit Akt phosphorylation in platelet-derived growth factor–treated hepatic stellate cells,[58] the naturally occurring compound EGCG is a promising potential therapeutic agent for maintenance therapy. Additionally, this compound has been reported to inhibit Hep G2 cell proliferation and induce apoptosis through p53-dependent and Fas-mediated pathways.[59] These data suggest that one mechanism of action of EGCG is the induction of apoptosis via p53-dependent and Fas-mediated pathways, or through inhibition of the Akt pathways.

In B-CLL cells, EGCG also induces apoptosis in vitro by partially inhibiting vascular endothelial cell growth factor (VEGF) receptor phosphorylation, thereby suppressing VEGF-dependent angiogenesis.[60,61] Additionally, in an in vitro study, EGCG seemed to decrease viability of CLL cells when combined with alkylating agents, purine nucleoside analogues, or the combination of both.[62] Primarily based on these laboratory observations and, to a lesser extent, anecdotal evidence that over-the-counter green tea extracts can yield responses in patients with CLL,[63] a phase I/II study of EGCG (also designated Polyphenon E) was conducted in 33 patients with asymptomatic early-stage CLL.[64] Eight dose levels were evaluated, ranging from 400–2,000 mg twice daily. Most adverse events were grade 1 and 2 in severity, and included nausea, abdominal pain, dyspepsia, and transaminitis. Grade 3 abdominal pain and diarrhea occurred in only 2 patients. Clinical activity was observed, particularly at the higher dose levels studied (1,200–2,000 mg twice daily). Although only 1 patient responded according to the National Cancer Institute Working Group criteria, signs of clinical activity were evident in 11 patients (33%), with a sustained 20% or greater reduction in ALC, and in 11 of 12 patients (92%), showing 50% or greater reduction in palpable nodes.[64] Based on data from pill diaries, it is important to note that median overall compliance was 99%. In the recently completed phase II portion of this study, early-stage CLL patients (n=42) were treated with 2,000 mg of EGCG twice daily for 6 months to

further evaluate response rate and response duration.[65] These patients received a median of 6 cycles (range, 1–6 cycles). Thirty-one patients have completed the study, and 11 patients are still on treatment. Grade 1 and 2 adverse events included transaminitis (43%), abdominal pain (28%), and nausea (57%). Only 2 of 48 (4.8%) patients experienced grade 3 toxicities, which included transaminitis (2%), abdominal pain (2%), and fatigue (2%). A sustained 20% or higher reduction in ALC was observed in 31% of patients, and 66% of patients with palpable adenopathy experienced a 50% or greater reduction in the sum of the products of all nodal areas during treatment. In total, 67% of patients attained either a sustained 20% or greater decline in ALC and/or a 50% or greater reduction in the sum of the products of all nodal areas at some point during the 6 months of active treatment, suggesting that green tea extracts may have potential as disease-stabilizing agents in patients with early-stage CLL.[65]

Curcumin

Curcumin is a naturally occurring compound that is one of the active ingredients in turmeric, which is a member of the ginger family. It has been used for centuries as a remedy for several disorders, including aches, pains, wounds, sprains, and liver disorders, and has more recently been studied extensively for its antitumor, anti-inflammatory, and antioxidative properties.[66] In primary CLL cells, curcumin has been shown to induce apoptosis through inhibition of prosurvival molecules, including signal transducer STAT3, Akt, and nuclear factor-nB (NF-nB), as well as the antiapoptotic protein myeloid cell leukemia-1 (Mcl-1).[60,66-68] Curcumin also induces caspase-mediated apoptosis, as evidenced by poly (ADP-ribose) polymerase (PARP) cleavage in primary B-CLL cells.[60] Notably, when given sequentially with EGCG, curcumin appears to overcome stromal cell-mediated drug resistance in CLL cells.[60] Thus, antagonism towards cell growth was observed when samples from patients with CLL were treated first with EGCG and then with curcumin. Sequential use of these agents led to a substantial increase in CLL B-cell death, and was shown to overcome stromal protection.[67] Clinical trials with curcumin and EGCG as individual agents, or in combination with standard chemotherapy, are already under way in a number of therapeutic areas. For example, single-agent curcumin is being investigated in advanced pancreatic cancer (NCT00094445), and the combination of curcumin and chemotherapy is currently under investigation in colorectal cancer (NCT00973869). Single-agent EGCG is under investigation in both breast cancer (NCT00917735) and prostate cancer (CT00459407).

PEITC

PEITC is an isothiocyanate found in cabbage, watercress, and other common cruciferous vegetables. This compound has been shown to inhibit carcinogenesis and tumorigenesis, and as such may be useful as a chemopreventive agent. PEITC was shown to effectively disable the glutathione antioxidant system and preferentially kill ovarian cancer cells with increased reactive oxygen species (ROS) generation.[69]

Compared to normal B cells, B-CLL cells display a substantial increase in ROS, which is associated with oxidative DNA damage and mitochondrial DNA mutations.[70-73]

A recent study by Trachootham and colleagues[74] investigated whether the biologic difference in ROS levels between normal B cells and fludarabine-refractory B-CLL cells could be exploited using the redox-modulating agent PEITC as a therapeutic agent. This study showed that PEITC treatment induced severe glutathione depletion, ROS accumulation, and oxidation of mitochondrial cardiolipin, leading to massive cell death in fludarabine-refractory B-CLL cells in vitro. Additionally, increased ROS accumulation resulted in the rapid degradation of antiapoptotic proteins due to oxidative stress. These results warrant further clinical evaluation of the therapeutic potential of PEITC in refractory B-CLL.

Genistein

Genistein is a known isoflavone that is found in a number of plants, such as fava beans, kudzu, and soy. It functions as an antioxidant and has been shown to interact with animal and human estrogen receptors. Additional mechanisms of action of genistein and other isoflavones may include antiangiogenic effects, antiproliferative effects (ie, blocking of growth factors), and direct proapoptotic effects. However, genistein primarily functions as a tyrosine kinase inhibitor, which is implicated in many cell growth and proliferation cascades.

In a study by Uckun and associates,[75] administration of genistein as an immunoconjugate with anti-CD19 was well tolerated and even showed some efficacy in patients with therapy-refractory B-lineage acute lymphoblastic leukemia. More recently, the sensitivity of ex vivo B-CLL cells to fludarabine in the presence of genistein was assessed.[76] This study showed a significant increase in B-CLL apoptosis ex vivo with the combination of genistein and fludarabine relative to fludarabine alone. Moreover, a positive correlation was found between apoptosis and the signal transduction molecule ZAP-70, which is expressed in a subset of CLL patients. In this respect, genestein was shown to inhibit ZAP-70 tyrosine kinase activity, resulting in enhanced cell death.[76]

GCS-100

GCS-100 is a modified citrus pectin (MCP) that binds to and inhibits galectin-3, whose presence is associated with poor prognosis and evasion of apoptosis in MM cells.[77]

Exposure to MCPs has been shown to inhibit cancer cell growth, angiogenesis, and metastasis in vivo, presumably via its effects on galectin-3 function.[78] GCS-100 has been shown to induce apoptosis in myeloma cells.[77] In patients with solid tumors, treatment with GCS-100 at a dose of 30–200 mg/m^2 was extremely well tolerated: treatment-related adverse events included rash, nausea, and fatigue.[79] Similar results were seen in a phase II trial of 24 elderly patients (median age, 67 years) with relapsed CLL who received GCS-100 at a dose of 160 mg/m^2 intravenously for 5 days of a 21-day cycle.[80] In a preliminary efficacy assessment, 6 patients (25%) achieved a PR and 12 (50%) had stable disease; 3 patients had more than 50% shrinkage of lymph node lesions. Nine patients discontinued therapy, 6 due to progressive disease. Although GCS-100 appears to have a good tolerability profile, the parenteral administration may not be as convenient over protracted courses of therapy.

Summary

Maintenance therapy has the potential to improve treatment outcomes in CLL through multiple mechanisms; a particularly attractive one is sustaining a microenvironment that discourages tumor regrowth or expansion in patients who respond to initial therapy. Standard chemotherapy agents like fludarabine and chlorambucil, and now more recently approved agents, such as bendamustine (Treanda, Cephalon), are highly active in CLL, but their toxicity profiles preclude long-term use as maintenance therapy. Rituximab and other monoclonal antibodies may be effective in delaying disease progression, but these agents require frequent parenteral administration, which will hinder long-term compliance. The orally administered immunomodulatory drug lenalidomide has demonstrated activity in patients with CLL and has an acceptable toxicity profile that does not appear to worsen as the number of treatment cycles increases. Lenalidomide has multiple effects on the tumor microenvironment and immune response, which may help sustain a growth-inhibiting environment. In total, these observations suggest that lenalidomide is well-suited for evaluation as maintenance therapy in CLL. Data from ongoing phase II studies and the phase III CONTINUUM trial will provide further insight into the potential role of lenalidomide as maintenance therapy in CLL. Lastly, naturally occurring compounds currently under investigation for CLL have clinical activity and good tolerability profiles. Natural agents such as EGCG, curcumin, and GCS-100 have also demonstrated promising preclinical activity in CLL. In conclusion, these naturally occurring compounds and immunomodulatory drugs such as lenalidomide are a novel and promising approach to maintain a clinical response by delaying relapse via modulation of the tumor microenvironment. In combination with their favorable safety profile, these agents may be well suited as maintenance therapy for patients with CLL.

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