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Targeting the microenvironment: a new treatment paradigm for chronic lymphocytic leukaemia

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Chronic lymphocytic leukaemia (CLL) is one of the most common leukaemias to affect adults, comprising about 30% of all leukaemia cases and 20% of leukaemia-related deaths each year.¹ The incidence is 3/100,000 per year in the western hemisphere,² and, in the USA alone, an estimated 10,000 new cases are expected in 2006 and a further 4,660 men and women are expected to die from the disease. Despite treatment advances over the last few years, CLL remains incurable, and individuals with relapsed or refractory disease have limited therapeutic options. Although patients with early or low-risk disease do not require treatment and can expect a normal quality of life for many years, all patients with intermediate- or high-risk disease eventually progress. For patients diagnosed with advanced-stage disease Binet C or Rai III–IV, the median survival is less than 2 years (Table 1) and thus these patients require therapeutic intervention.²

CLL is a clonal proliferation of lymphocytes that appear morphologically mature, but are functionally inert. More than 95% of cases are of B-cell origin (B-CLL). Survival of B-CLL cells is significantly prolonged compared with normal B-cells, which results in a low proliferative index and probably explains the reduced sensitivity of these cells to conventional cytotoxic drugs. In contrast with most other haematological malignancies, inability to undergo apoptosis is a major feature of CLL pathogenesis.³ Increasingly, evidence suggests that the prolonged survival associated with B-CLL cells relates to their ability to manipulate the tumour microenvironment, thereby resisting apoptosis and promoting growth and accumulation.^{3–6} Thus, while the primary pathogenic event responsible for transformation of normal B-cells remains undetermined, an increased

understanding of the biology of CLL, and particularly the contributing role of the tumour microenvironment, has unmasked a potential therapeutic role for immunomodulatory drugs.⁷

Current treatment options for CLL

The standard of care in patients with early disease continues to be a watch-and-wait strategy; chemotherapy is considered when the disease begins to progress rapidly or becomes symptomatic.² Treatment options are limited, particularly for patients with relapsed or refractory CLL. Current chemotherapeutic regimens include purine nucleoside analogues, such as fludarabine, and the alkylating drugs cyclophosphamide and chlorambucil, the latter being typically reserved for older patients due to its better tolerability profile in this population.^{2,8} The incorporation of fludarabine into combination regimens has resulted in improved response rates to treatment. However, overall response and clinical outcome with fludarabine-based and other chemotherapeutic regimens are heavily dependent on previous treatment, clinical stage, cytogenetic abnormalities, and immunoglobulin heavy-chain variable-region mutations.^{7,9}

In recent years, immunotherapy has had an increasingly important role in CLL by specifically targeting tumour cells. The anti-CD52 monoclonal antibody alemtuzumab has been shown to achieve high overall response rates in patients refractory to chemotherapy, including those patients previously treated with fludarabine.¹⁰ Investigational treatments in CLL include rituximab, lumiliximab, flavopiridol, anti-Bcl-2 antisense therapy, either alone or in combination with traditional chemotherapy, and myeloablative or non-myeloablative allogeneic stem cell transplantation.^{2,7,11,12} Although ritux-

Table 1. Prognosis of chronic lymphocytic leukaemia by stage. (Adapted from Hallek M, *et al.*² with permission of Oxford University Press.)

	Frequency, %	Median survival, years
Binet stage		
A	63	>10
B	30	5
C	7	1.53
Rai stage		
O (Low risk)	30	>10
I, II (Intermediate risk)	60	7
III, IV (High risk)	10	1.5

imab, an anti-CD20 monoclonal antibody, has limited efficacy as a single agent against CLL, it is associated with greater tolerability and ease of use than alemtuzumab, resulting in a wider use, especially in combination with chemotherapy.^{13,14} Aggressive treatment approaches that have combined alemtuzumab or rituximab with fludarabine-based chemotherapy have yielded further improvements in response rates, but without extending patient survival.^{15,16} Given that the clinical course of CLL is marked by frequent relapse, and that approved therapeutic options in patients with relapsed or refractory disease are limited, there is a need for additional effective, well-tolerated treatments.

Role of CLL microenvironment

The treatment paradigm that has emerged in CLL is one of specifically targeting the malignant clone only. In this regard, purine analogues, alkylating drugs, and monoclonal antibodies share a common therapeutic approach. The use of certain proprietary compounds, called IMiDs[®] compounds, offers a promising new treatment strategy by targeting the cytokine and cellular microenvironment upon which the malignant B-CLL clone is dependent for its survival (Figure 1).

As for normal B-cells, a network of cytokines and growth factors directly regulates the growth, proliferation, differentiation, and survival of B-CLL cells.⁷ However, in CLL there is evidence that the cytokine networks are aberrant, enabling malignant B-cells to resist apoptosis by offering a microenvironment that continues to foster growth and survival.^{4,5,17} Cytokines that appear to be particularly important in the survival of malignant B-CLL clones include interleukins, such as IL-4, IL-6, and IL-10, and tumour necrosis factor alpha (TNF- α), which are differentially produced by T-cells of patients with CLL compared with control T-cells.¹⁷ In addition, malignant B-CLL

clones produce their own cytokines and growth factors, such as vascular endothelial growth factor (VEGF), which are regulated in an autocrine fashion to further promote B-cell growth and resistance to apoptosis, which ultimately leads to a progressive accumulation of the tumour in the bone marrow and lymphoid tissue.⁶

Given the ability of B-CLL cells to evade immune effector cells through dysregulation of the tumour microenvironment, there is a strong rationale for a therapeutic approach that is able to counter or modulate the aberrant cytokine network, thereby removing the pro-survival signals delivered to the B-CLL clone. The encouraging anti-tumour effects shown by thalidomide in various malignant disorders have sparked an interest in developing analogues with greater potency and improved tolerability for use as particular IMiDs[®] compounds. Lenalidomide is one such drug, demonstrating potent *in vitro* activity, and proven efficacy in treating patients with multiple myeloma and myelodysplastic syndromes.^{18,19} Similar to thalidomide, lenalidomide is able to downregulate cytokines associated with B-CLL survival, such as VEGF, IL-6, and TNF- α , while promoting the expression of cytokines associated with the activation of natural killer (NK) cells and cytotoxic T-cell responses, such as IL-2 and interferon- γ .²⁰ Thus, the importance of the tumour microenvironment in the pathogenesis of CLL, and the ability of thalidomide and lenalidomide to modify this microenvironment in favour of a cytokine network that augments the immune effector cell response, provides the basis for further investigation of these drugs in the treatment of CLL.

Clinical trials of thalidomide in CLL

Several clinical trials have assessed the therapeutic potential of thalidomide in patients with CLL, including previously untreated patients and those with relapsed or refractory disease. In a phase I, non-randomized clinical trial of 13 previously untreated patients with CLL, treatment with thalidomide 100-300 mg/day in combination with fludarabine 25 mg/m² for 5 days every 4 weeks for 4-6 cycles produced a major response in 9 of 9 evaluable patients, including 5 (55%) patients with complete remission.²¹ There were no dose-limiting toxicities, and a reduction in absolute lymphocyte count (ALC) was noted on day 7 of treatment, prior to the first cycle of fludarabine treatment. A phase II continuation of this study is currently recruiting patients and is intended to establish the role

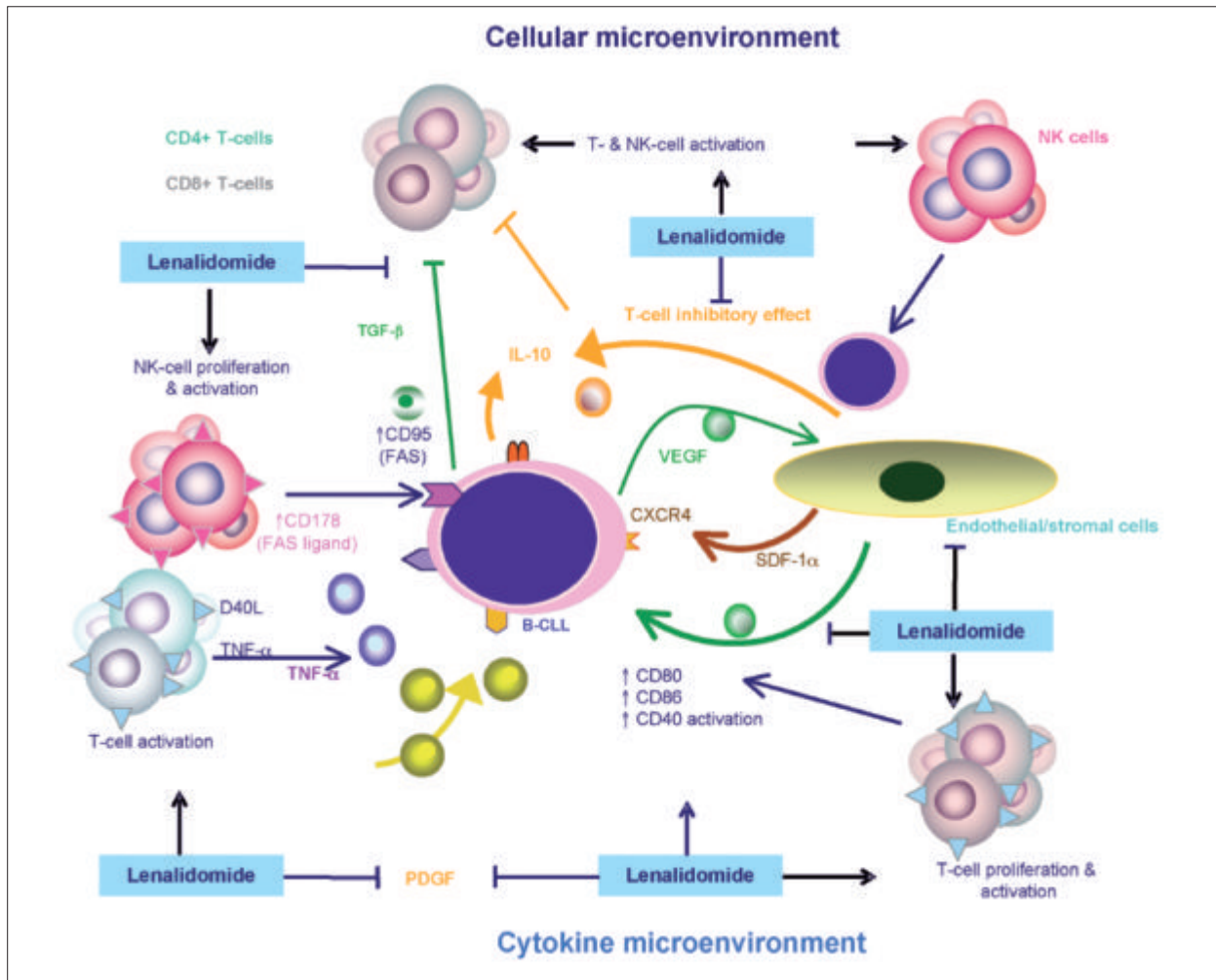


Figure 1. The immunomodulatory drug lenalidomide downregulates cytokines, and other pro-survival factors produced by malignant B-CLL clones and supporting stromal cells within the tumour microenvironment, while increasing the expression of co-stimulatory and soluble factors required for T-cell and NK-cell activation, with consequent reduction of tumour cell activity. (Reproduced from Chanan-Khan A and Porter CW⁷ with permission from Elsevier.)

Abbreviations: CLL, chronic lymphocytic leukaemia; CXCR4, chemokine receptor type 4; IL, interleukin; NK, natural killer; PDGF, platelet-derived growth factor; SDF-1 α , stromal-cell-derived factor-1 alpha; TGF- β , transforming growth factor beta; TNF- α , tumour necrosis factor alpha; VEGF, vascular endothelial growth factor.

of thalidomide in combination with fludarabine as initial treatment of CLL.

In patients with relapsed or refractory disease, thalidomide has been evaluated alone and in combination with fludarabine in phase II trials.^{22,23} In 28 patients with mostly refractory CLL, including 63% with Rai stage III or IV disease, and a median of 2 prior treatment regimens and 4 prior treatment cycles, thalidomide titrated up to a maximum of 1,000 mg/day from a starting dose of 200 mg/day produced a small anti-leukaemic effect observed as a decrease in ALC in 21 of 25 patients.²² In addition, 20 patients had stable disease after completing the first treatment cycle, and a single patient had a partial remission. However, median time to disease progres-

sion was just 8.5 months, and the study was stopped early due to slow accrual, which was potentially related to a high incidence of tumour flare. Tumour flare reaction was characterized as an increase in lymph node and spleen volume, increased ALC, and decreased haemoglobin and platelet counts. Despite the lack of response in patients with refractory disease, the finding that thalidomide maintained a stable disease state and decreased ALC in most patients suggested a potential role for this drug in combination with standard chemotherapy.

Thalidomide as a combination therapy was recently investigated in a phase II trial of 16 CLL patients previously treated with fludarabine, including 6 patients with fludarabine-refractory

Table 2. Clinical response to lenalidomide by category.

Response category	n (%)
Intent-to-treat population (n=41)*	
Overall response	17 (41)
Complete response	3 (7)
Partial response	14 (34)
Stable disease	9 (22)
Progressive disease	3 (7)
Evaluable population (n=26)†	
Overall response	17 (65)
Complete response	3 (12)
Partial response	14 (54)

*Intent-to-treat population includes all 41 patients who were enrolled in the study and received at least 1 dose of the study drug. †Evaluable population includes 26 patients who completed at least 2 cycles of treatment in order to be assessed for a response; 11 patients were not able to complete at least 2 cycles of treatment due to pulmonary embolism, thrombocytopenia, or neutropenia and were not considered evaluable; a further 4 patients are awaiting assessment of response. All responses were maintained for at least 2 months.

disease.²³ Patients were randomized to either thalidomide alone at a starting dose of 200 mg/day with titration as tolerated, or thalidomide in combination with fludarabine 25 mg/m² for 5 days every 4 weeks for a total of 6 cycles. Of 10 evaluable patients, a single patient in the combination arm achieved complete remission, and a total of 4 patients achieved partial remission, including 3 patients assigned to the combination therapy. A single patient in each arm achieved haematological improvement and a minor response, while 1 patient in the thalidomide arm progressed. Five patients treated with thalidomide only developed tumour flare reactions that were characterized by fever, lymphadenopathy, increased ALC, and decreased haemoglobin and platelet counts. Symptoms resolved spontaneously in 3 patients, and by temporarily discontinuing thalidomide in 2 patients. The investigators concluded that thalidomide, alone and in combination with fludarabine, is an active treatment for CLL. The combination offered superior efficacy compared with thalidomide alone, and also had an influence on the development of tumour flare reactions.

Clinical trials of lenalidomide in CLL

The encouraging results reported with thalidomide in CLL led to the investigation of the anti-tumour effect of lenalidomide, which offers greater potency than thalidomide, as well as a better tolerability profile. A phase II trial was conducted to investigate the role of lenalidomide in patients with relapsed or refractory CLL.²⁴ The primary objective of this study was to evaluate the

overall response rate of single-drug lenalidomide in this patient group, while a secondary objective was to evaluate safety.

To date, 41 patients with relapsed or refractory CLL, with a median age of 64 years (range 47-75 years) and a median of 4 prior therapies (range 1-10) have been recruited for this ongoing open-label study. The majority of patients recruited have been males (80%), with Rai stage III or IV disease (66%). Prior therapies included monotherapy with alkylating drugs (38%), monotherapy with fludarabine (38%), and fludarabine combination regimens (48%), such as FCR (fludarabine-cyclophosphamide-rituximab), FT (fludarabine-thalidomide), and FR (fludarabine-rituximab). Of 33 patients with an elevated ALC at baseline of at least 5,000 cells/L, 24 had a white blood cell count of at least 20,000 cells/L. Cytogenetic assessment of 32 patients with available data indicated a wide range of cytogenetic abnormalities, including 13q deletions (n=14), trisomy 12 (n=6), 17p deletions (n=3), ATM deletions (n=4), p53 deletions (n=1), normal cytogenetics (n=1), and multiple abnormalities (n=3).

Oral lenalidomide 25 mg was administered once daily for 21 days followed by 7 days rest on a 28-day cycle, with the dosing schedule based on phase II and III studies of lenalidomide in patients with myelodysplastic syndromes and multiple myeloma.^{18,19} Patients with stable disease or better were continued on treatment for a maximum of 12 months, while those with progressive disease received rituximab 375 mg/m² on days 1, 15, and 21 in addition to lenalidomide. Patients were considered evaluable for response if they completed at least 2 months of treatment.

Within the first 7 days of lenalidomide treatment, ALC significantly decreased from baseline, with the decline preceding a decrease in tumour bulk in the peripheral blood. This drop in ALC was maintained over the duration of the study. Of 26 evaluable patients, 17 (65%) achieved a major response, including 3 (12%) patients with a complete response for an overall response rate in the intent-to-treat population of 41% (Table 2). To date, just 3 patients with progressive disease have been administered rituximab in addition to lenalidomide, resulting in partial remission in all 3. Another 9 patients have stable disease and are continuing on lenalidomide therapy together with 14 patients who achieved partial remission.

Three patients had a molecular complete remission, defined as the absence of minimal residual disease using polymerase-chain-reaction-based detection of the variable heavy gene.²⁵ The benefit of ongoing lenalidomide treatment is

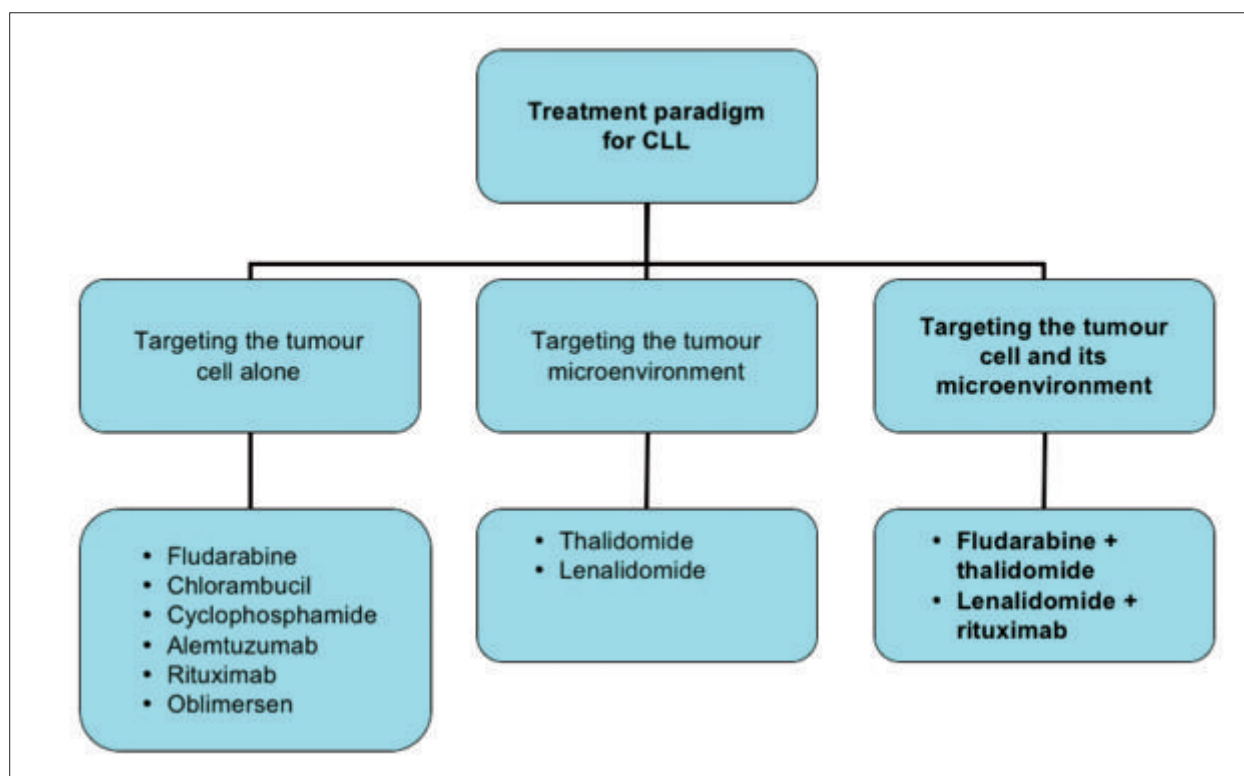


Figure 2. Targeting the tumour cell and its microenvironment: a new treatment paradigm for chronic lymphocytic leukaemia (CLL).

being investigated in the long-term follow-up of these patients. The median follow-up is currently 6 months, and the longest follow-up is >20 months. Haematological toxicities were a common adverse event, with grade 3-4 neutropenia occurring in about 70% of patients, and grade 3-4 thrombocytopenia occurring in about 50% of patients. Grade 3-4 toxicities also included anaemia (20%) and fatigue (10%), and 3 patients each experienced a grade 3-4 rash and pulmonary embolism. No thromboembolic prophylaxis was administered. Tumour flare reaction, similar to that reported with thalidomide, was another important adverse event reported. Grade 1-2 tumour flare was reported in 50% of patients, and a total of 3 patients experienced grade 3-4 tumour flare. None of the patients required dose reduction or cessation of therapy for tumour flare reaction.

Tumour flare has been further investigated in patients enrolled in this phase II study.²⁶ In an initial cohort of 29 patients, no prophylaxis was given for tumour flare, but symptoms were treated with ibuprofen and oxycodone (600 mg and 5-10 mg orally every 6 hours, respectively). In the subsequent patient cohort (n=12), the prophylactic use of oral prednisone 20 mg once daily starting 1 day prior to lenalidomide therapy and

continuing for 5 days, followed by oral prednisone 10 mg once daily for 5 days, was investigated. In the initial patient cohort, 23 of 29 (79%) patients experienced tumour flare, including 14 patients with a grade 1 flare reaction, 7 patients with a grade 2 reaction, and 2 patients with a grade 3 reaction. There were no grade 4 flare reactions, and patients were adequately managed, with symptoms resolving following treatment with ibuprofen and oxycodone. Recurrence of flare was observed in a single patient during the second cycle of lenalidomide treatment, and increased ALC was observed in 2 patients. In the patient cohort administered prednisone prophylaxis, 2 patients have so far experienced grade 1 flare that resolved within 10 days. Thus, prophylactic use of prednisone resulted in a decreased incidence and severity without impacting the clinical response to lenalidomide. It should be noted that elevation of ALC can be part of the flare reaction. Therefore, patient responses to treatment should be interpreted cautiously for disease progression.

Cytokine analysis of serum samples from 5 patients taken at baseline, and again after 7 days of lenalidomide treatment, showed significant increases in TNF- α , IL-10, and monokine induced by interferon- γ .²⁶ These preliminary

findings, together with previously recorded decreases in pro-survival cytokines,²⁷ suggest that lenalidomide induces early changes in the tumour microenvironment that are consistent with a rapid decline in ALC. Furthermore, investigation of changes in immune effector cells showed that 6 of 10 patients treated with lenalidomide experienced an increase in the proportion of NK cells, ranging from 20% to almost 200%.²⁷

Conclusions

Current approaches in the treatment of CLL are based on directly targeting the tumour cell using chemotherapies and/or antibodies. The use of certain IMiDs[®] compounds, such as lenalidomide, represents a novel treatment strategy by targeting the cytokine and cellular tumour microenvironment upon which the tumour cells are dependent for their survival. Lenalidomide is active in patients with relapsed or refractory CLL, producing high overall response rates, including complete remission in 3 of 41 patients to date. In addition, these responses have been maintained, in some cases for over a year, indicating that a durable response may be achievable with lenalidomide. Despite being adequately managed, the haematological toxicities and tumour flare reactions associated with lenalidomide remain a concern, and will be further investigated in terms of dose optimisation. However, there is some evidence that prednisone prophylaxis administered during the first 10 days of lenalidomide treatment can reduce the incidence and severity of the tumour flare reaction. Studies are planned to further investigate the efficacy and safety of lenalidomide in CLL. The ability of lenalidomide to produce durable clinical responses by targeting the tumour microenvironment provides the basis for a new treatment paradigm in CLL (Figure 2).

By altering the cytokine and cellular microenvironment from one that supports the survival of B-CLL cells to one that promotes the cytotoxic activities of immune effector cells, certain IMiDs[®] compounds such as lenalidomide may provide an effective and enduring anti-tumour effect. Moreover, the combination of lenalidomide and chemotherapy and/or antibodies that directly target the malignant B-CLL clones may offer a dual approach that could provide additional clinical benefits beyond what could be achieved with either approach alone.

References

- Jemal A, Siegel R, Ward E, Murray T, Xu J, Smigal C, et al. Cancer statistics, 2006. *CA Cancer J Clin.* 2006;56:106-30.
- Hallek M, Stahel RA, Greil R. ESMO Minimum Clinical Recommendations for diagnosis, treatment and follow-up of chronic lymphocytic leukemia. *Ann Oncol* 2005;16 Suppl 1:i50-1.
- Meinhardt G, Wendtner CM, Hallek M. Molecular pathogenesis of chronic lymphocytic leukemia: factors and signaling pathways regulating cell growth and survival. *J Mol Med* 1999;77:282-93.
- Burger JA, Burger M, Kipps TJ. Chronic lymphocytic leukemia B cells express functional CXCR4 chemokine receptors that mediate spontaneous migration beneath bone marrow stromal cells. *Blood* 1999;94:3658-67.
- Orsini E, Foa R. Cytokines and soluble molecules in CLL. In: Faugt G, editor. *Chronic lymphocytic leukemia*. New Jersey: Humana Press 2005. p 123-42.
- Kay NE, Bone ND, Tschumper RC, Howell KH, Geyer SM, Dewald GW, et al. B-CLL cells are capable of synthesis and secretion of both pro- and anti-angiogenic molecules. *Leukemia* 2002;16:911-9.
- Chanan-Khan A, Porter CW. Immunomodulating drugs for chronic lymphocytic leukaemia. *Lancet Oncol* 2006;7:480-8.
- Abbott BL. Chronic lymphocytic leukemia: recent advances in diagnosis and treatment. *Oncologist.* 2006;11:21-30.
- Byrd JC, Gribben JG, Peterson BL, Grever MR, Lozanski G, Lucas DM, et al. Select high-risk genetic features predict earlier progression following chemoimmunotherapy with fludarabine and rituximab in chronic lymphocytic leukemia: justification for risk-adapted therapy. *J Clin Oncol* 2006;24:437-43.
- Moreton P, Kennedy B, Lucas G, Leach M, Rassam SM, Haynes A, et al. Eradication of minimal residual disease in B-cell chronic lymphocytic leukemia after alemtuzumab therapy is associated with prolonged survival. *J Clin Oncol* 2005;23:2971-9.
- Cheson BD. Monoclonal antibody therapy of chronic lymphocytic leukemia. *Cancer Immunol Immunother* 2006; 55:188-96.
- Byrd JC, Peterson BL, Gabrilove J, Odenike OM, Grever MR, Rai K, et al. Treatment of relapsed chronic lymphocytic leukemia by 72-hour continuous infusion or 1-hour bolus infusion of flavopiridol: results from Cancer and Leukemia Group B Study 19805. *Clin Cancer Res* 2005;11:4176-81.
- Keating M, Coutre S, Rai K, Osterborg A, Faderl S, Kennedy B, et al. Management guidelines for use of alemtuzumab in B-cell chronic lymphocytic leukemia. *Clin Lymphoma* 2004;4:220-7.
- Lin TS, Lucas MS, Byrd JC. Rituximab in B-cell chronic lymphocytic leukemia. *Semin Oncol* 2003;30:483-92.
- Byrd JC, Peterson BL, Morrison VA, Park K, Jacobson R, Hoke E, et al. Randomized phase 2 study of fludarabine with concurrent versus sequential treatment with rituximab in symptomatic, untreated patients with B-cell chronic lymphocytic leukaemia: results from Cancer and Leukemia Group B 9712 (CALGB 9712). *Blood* 2003;101:6-14.
- Keating MJ, O'Brien S, Albitar M, Lerner S, Plunkett W, Giles F, et al. Early results of a chemoimmunotherapy regimen of fludarabine, cyclophosphamide, and rituximab as initial therapy for chronic lymphocytic leukemia. *J Clin Oncol* 2005;23:4079-88.
- Mainou-Fowler T, Miller S, Proctor SJ, Dickinson AM. The levels of TNF alpha, IL4 and IL10 production by T-cells in B-cell chronic lymphocytic leukaemia (B-CLL). *Leuk Res* 2001;25:157-63.
- List A, Kurtin S, Roe DJ, Buresh A, Mahadevan D, Fuchs D, et al. Efficacy of lenalidomide in myelodysplastic syndromes. *N Engl J Med* 2005;352:549-57.
- Richardson PG, Schlossman RL, Weller E, Hideshima T, Mitsiades C, Davies F, et al. Immunomodulatory drug CC-5013 overcomes drug resistance and is well tolerated in patients with relapsed multiple myeloma. *Blood* 2002;100:3063-7.
- Corral LG, Haslett PA, Muller GW, Chen R, Wong LM, Ocampo CJ, et al. Differential cytokine modulation and T

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- cell activation by two distinct classes of thalidomide analogues that are potent inhibitors of TNF-alpha. *J Immunol* 1999;163:380-6.
21. Chanan-Khan A, Miller KC, Takeshita K, Koryzna A, Donohue K, Bernstein ZP, et al. Results of a phase I clinical trial of thalidomide in combination with fludarabine as initial therapy for patients with treatment-requiring chronic lymphocytic leukemia (CLL). *Blood* 2005;106:3348-52.
 22. Kay N, Geyer S, Yaqoob I, Phyliky R, Kutteh L, Li C-Y. Thalidomide (Td) treatment in chronic lymphocytic leukemia (CLL): a North Central Cancer Treatment Group (NCCTG) Study [abstract 5162]. *Blood* 2003;102.
 23. Furman RR, Leonard JP, Allen SL, Coleman M, Rosenthal T, Gabrilove JL. Thalidomide alone or in combination with fludarabine are effective treatments for patients with fludarabine-relapsed and refractory CLL [abstract 6640]. *J Clin Oncol* 2005;23 Suppl:16S.
 24. Chanan-Khan A, Miller K, Musial S, Padmanabhan S, Lawrence D, Bernstein Z, et al. Antileukemic activity of lenalidomide (Revlimid) in patients with relapsed or refractory chronic lymphocytic leukaemia [abstract 0277]. *Haematologica* 2006;91 Suppl 1:103.
 25. Trainor KJ, Brisco MJ, Wan JH, Neoh S, Grist S, Morley AA. Gene rearrangement in B- and T-lymphoproliferative disease detected by the polymerase chain reaction. *Blood* 1991;78:192-6.
 26. Musial L, Miller K, Depaolo D, Swinnich D, Chanan-Khan AA. Prednisone (P) prophylaxis decreases incidence and severity of lenalidomide (L) induced flare reaction (flr) in patients with relapse (rel) or refractory (ref) chronic lymphocytic leukaemia [abstract 18512]. *J Clin Oncol* 2006;24 Suppl:18S.
 27. Chanan-Khan AA, Padmanabhan S, Miller KC, Pera P, DiMicoli L, Ersing N, et al. *In vivo* evaluation of immunomodulating effects of lenalidomide (L) on tumor cell microenvironment as a possible underlying mechanism of the antitumor effects observed in patients (pts) with chronic lymphocytic leukemia (CLL) [abstract 2975]. *Blood* 2005;106:834a.