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Phase I study of the anti-CD40 humanized monoclonal antibody lucatumumab (HCD122) in relapsed chronic lymphocytic leukemia

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Abstract

Lucatumumab is a fully humanized anti-CD40 antibody that blocks interaction of CD40L with CD40 and also mediates antibody-dependent cell-mediated cytotoxicity (ADCC). We evaluated lucatumumab in a phase I clinical trial in chronic lymphocytic leukemia (CLL). Twenty-six patients with relapsed CLL were enrolled on five different dose cohorts administered weekly for 4 weeks. The maximally tolerated dose (MTD) of lucatumumab was 3.0 mg/kg. Four patients at doses of 4.5 mg/kg and 6.0 mg/kg experienced grade 3 or 4 asymptomatic elevated amylase and lipase levels. Of the 26 patients enrolled, 17 patients had stable disease (mean duration of 76 days, range 29–504 days) and one patient had a nodular partial response for 230 days. Saturation of CD40 receptor on CLL cells was uniform at all doses post-treatment but also persisted at trough time points in the 3.0 mg/kg or greater cohorts. At the MTD, the median half-life of lucatumumab was 50 h following the first infusion, and 124 h following the fourth infusion. In summary, lucatumumab had acceptable tolerability, pharmacokinetics that supported chronic dosing and pharmacodynamic target antagonism at doses of 3.0 mg/kg, but demonstrated minimal single-agent activity. Future efforts with lucatumumab in CLL should focus on combination-based therapy.

Keywords

CLL; chronic lymphocytic leukemia; lucatumumab; combination therapy; efficacy

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Introduction

Chronic lymphocytic leukemia (CLL) is the most common adult leukemia in the Western world and is a disease of older adults. The median age at diagnosis is 72 years of age, and approximately 70% of patients with CLL are over the age of 65 [1]. The median survival of patients is highly variable, with some patients exhibiting indolent disease with a life span similar to an age-matched control population, whereas others exhibit aggressive disease with a survival of less than 2–3 years. Genomic features such as immunoglobulin heavy chain variable gene (IgV_H) mutational status [2,3], β_2-microglobulin (B_2M) level [4], ZAP70 expression [5], interphase cytogenetics [6] and stimulated karyotype [7] provide further differentiation of prognosis as measured by time from diagnosis to initial treatment. While treatment options for CLL have increased over the past two decades with the introduction of fludarabine [8–10], combined chemoimmunotherapy [11–14], alemtuzumab [15], bendamustine [16] and recently ofatumumab [17], none of these therapies are curative. Therefore, identifying new therapies for CLL represents a major scientific goal.

For many years the biology of CLL was thought to be simply due to progressive accumulation of morphologically mature-appearing B lymphocytes in the blood, bone marrow and lymphatic tissues. These malignant lymphocytes typically have a CD5 +, CD23 +, CD43 + / –, CD10 –, CD19 +, CD20dim, sIg dim + immunophenotype [18]. Additionally, they express receptors to a variety of cytokines or soluble ligands including interleukins (IL-4 [19], IL-8 [20], IL-6 [21], IL-10 [22] and IL-15 [23,24]) and tumor necrosis factor (TNF) family (TNF-α [25,26], BAFF [27–31] and CD40 [32,33]) members whose soluble ligand under experimental conditions can disrupt spontaneous apoptosis commonly observed with CLL. Of these soluble factors, one of the best described is CD40 ligand (CD40L), which is produced predominantly by T-helper cells, and platelets; additionally it has been reported in natural killer (NK) cells that are part of the innate immune system. The receptor for CD40L is CD40, which is expressed on both normal and malignant B-cells including CLL cells. In normal B-cells, CD40 ligation promotes activation of both the phosphoinositol (PI) 3-kinase pathway [34,35], and nuclear factor κB (NF-κB) [32,36], thereby disrupting apoptosis; CD40 ligation also promotes activation and proliferation when administered with other cytokines. Similar PI3-kinase and NF-κB activation is observed when CLL cells are treated with CD40L, with several groups demonstrating disruption of both spontaneous and drug induced apoptosis [32,36]. Disrupting the CD40L–CD40 signaling axis represents a potential therapeutic target for the treatment of CLL and other B-cell malignancies dependent upon this pathway.

Lucatumumab (HCD122; CHIR-12.12) is one such potential therapeutic to target the CD40L–CD40 pathway. Lucatumumab is a fully human, recombinant monoclonal antibody of the immunoglobulin G1 (IgG1) isotype targeting human CD40. Preclinically, lucatumumab is a potent antagonist that blocks signaling by CD40L [37]. It binds to CD40 molecules with high affinity (Kd of 0.5 nM) and has a slow off-rate. *In vitro*, lucatumumab inhibits CD40L-mediated proliferation of normal B cells, CLL cells and non-Hodgkin lymphoma (NHL) cells. In the absence of CD40L, lucatumumab does not stimulate proliferation of either normal B cells or malignant B cells from patients with CLL or NHL. Lucatumumab also has the capacity to mediate killing and clearance of tumor cells via antibody-dependent cell-mediated cytotoxicity (ADCC) and opsonization. *In vitro* studies demonstrate that lucatumumab is not internalized after binding, remaining available on the cell surface to bind effector cells and mediate cell lysis via ADCC. Additionally, data from human lymphoma and myeloma xenograft models suggest a potential role for lucatumumab in the treatment of lymphoid malignancies. Studies with primary CLL cells demonstrated that lucatumumab could inhibit CD40L-induced protection from apoptosis. Furthermore, lucatumumab is also a potent mediator of ADCC against CLL cells, and is more potent than

rituximab [37]. These preclinical data combined with the success of other therapeutic antibodies in CLL such as rituximab, alemtuzumab and ofatumumab prompted initiation of a disease-specific phase I study of this agent that is described herein.

Materials and methods

Patients

Patient enrollment occurred from April 2005 through February 2008, with all patients giving written informed consent to an institutional review board (IRB) approved study. Patients were required to have symptomatic CLL that was relapsed or refractory to at least one fludarabine-containing regimen and that met the National Cancer Institute (NCI) 1996 criteria for treatment [38]. Other eligibility included an Eastern Cooperative Oncology Group (ECOG) performance status grade of 0–2, platelet count ≥ 75×10^9/L, hemoglobin ≥ 8.0 g/dL, serum creatinine < 2.0 mg/dL, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase less than two times normal, total bilirubin < 1.5 mg/dL, hepatitis B surface antigen negative, and 30 days since last CLL treatment. Exclusion criteria included rituximab within 90 days, alemtuzumab within 6 months, significant pulmonary or cardiac disease, infection requiring antibiotics within 1 month, history of a deep venous thrombosis or pulmonary embolus, and prior allogeneic stem cell transplant.

Pretreatment and serial laboratory assessments

Baseline laboratory assessments included complete blood count (CBC) with differential, platelet count and absolute lymphocyte count; serum chemistries, including liver functions; prothrombin time, partial thrombin time, amylase, lipase and urinalysis; direct and indirect antibody tests; immunoglobulin levels; thyroid function tests; β_2-microglobulin; interphase cytogenetics; flow cytometry; and an electrocardiogram. CBC and serum chemistry, amylase, lipase and liver function measurements were done weekly during the treatment period, and then monthly during the post-treatment follow-up period up to month 12. Patients were followed even in the setting of progression until all toxicities deemed to be possibly due to lucatumumab resolved.

Treatment

Patients were assigned to one of the five dose-escalation cohorts that were opened for enrollment, and were treated at the dose level under evaluation in that cohort. Patients were treated at 0.3 mg/kg, 1.0 mg/kg, 3.0 mg/kg, 4.5 mg/kg or 6.0 mg/kg. Premedication prior to each infusion was recommended by the protocol, and was administered at the discretion of the investigator. Typical premedications included diphenhydramine, acetominophen and hydorcortisone. Lucatumumab was formulated at 1 mg/mL and administered for the first hour of therapy at 50 mL per hour. If vital signs remained stable during the first hour of infusion, the rate could be increased by 50 mL every 30 min to a maximal rate of 400 mL/h, as long as vital signs remained stable. Other supportive care was administered at the discretion of the treating physician.

Toxicity assessment and dose-limiting toxicity

A dose-limiting toxicity (DLT) was defined as suspected to be related to lucatumumab and occurring within the first 56 days of the study; see Table I for a list of the study-specific DLTs.

Criteria for dose escalations

Patients were enrolled in a staggered fashion, one per week, in order to monitor for acute toxicity for 1 week after the previous infusion before the next subject was treated. If no DLT occurred among the first three patients after each was monitored for 2 weeks following their final infusion, enrollment into the next dose level occurred. If one DLT occurred among the first three subjects after the monitoring period, three more subjects were to be enrolled at the same dose level. If no more than one DLT was observed among the six patients in this expanded dose group, enrollment was to begin at the next dose level. The maximally tolerated dose (MTD) was defined as the highest dose at which at least six patients completed the treatment course with no more than one subject experiencing a DLT. If two or more patients experienced a DLT at a given dose level, the MTD was determined to be exceeded and no additional patients were to be treated at that level.

Response assessments

Patients were assessed for response at week 8, and responses were confirmed 2 months later. The primary efficacy variable in this study was overall response rate (ORR), defined as the percentage of subjects with response classified as complete response (CR) or partial response (PR) using the NCI-Working Group (NCIWG) 1996 criteria for CLL.

Pharmacokinetics

Samples for lucatumumab levels were drawn before the first infusion; at the end of the infusion (EOI); at 2 h post-EOI; on day 2; and on day 3. Additional samples were drawn on day 5 (± 1 day). For subsequent infusions, samples were drawn on day 8 (± 1 day); day 15 (± 1 day); day 22 (prior to infusion, at EOI, 2 h post-EOI); day 23; day 29 (± 2 days); day 43 (± 3 days); day 57 (± 5 days); and day 78 (± 5 days). After week 12 (day 78), blood samples were collected every 8 ± 2 weeks until lucatumumab levels were undetectable. Total serum concentrations of lucatumumab were determined using a validated enzyme-linked immunosorbent assay (ELISA) developed by Novartis. The assay was validated according to International Conference on Harmonization guidelines.

Pharmacodynamic studies

A sandwich electrochemiluminescence assay was used to detect antibodies against lucatumumab (at time points corresponding to pharmacokinetic [PK] samples). CD40 saturation on CD5/CD19 CLL cells and lymphocyte subset measurements were obtained by flow cytometry at pretreatment, days 1, 2, 3 post-therapy; days 8, 15, 22 pre- and post-therapy; and days 29, 43, 57, 78.

Data collection and statistical methods

Case report form data were entered in duplicate into a Clintrial® database by the Department of Biostatistics and Clinical Data Management (BCDM) at Chiron Corporation. Data quality control was performed using Procedural Language/Sequential Query Language (PL/SQL) and Statistical Analysis System (SAS)® software version 8.2 or higher (SAS Institute, Cary, NC). Analysis was to be performed by Chiron Corporation, using SAS software version 8.2 or higher. Data were summarized using descriptive statistics. No formal statistical analysis was performed comparing response to pharmacokinetics or pharmacodynamic variables. Pharmacokinetic parameters, including serum lucatumumab concentrations, area under the serum concentration–time curve (AUC) (0–inf), AUC (0–168 h), drug concentration at end of infusion (C_{max}), serum half-life ($t_{1/2}$), serum clearance (CL) and volume of distribution (V_{ss}), were calculated after single and multiple dosing using non-compartmental methods, and were summarized by collection time relative to dosing. Dose proportionality, time to attain steady state and accumulation upon multiple dosing were

evaluated. Data were also visualized using per-patient plots of serum lucatumumab concentrations over time (with respect to drug infusion). The program WinNonlin 5.2 (Pharsight, Mountain View, CA) was used to estimate the pharmacokinetic parameters.

Results

Patient characteristics

Twenty-six patients gave consent and were treated at five clinical sites. The patient characteristics are summarized in Table II. The median age was 66 (range 41–83) with 14 patients being at least 65 years old. The majority of patients (69%) had stage I/II disease and had received a median of 4 (range 1–12) prior therapies. While all had received fludarabine, only six (23%) were refractory to fludarabine at the time of lucatumumab treatment. The median B_2M value was 3.2 μg/mL (range: 1.7–7.3 μg/mL); two (8%) patients had del(17p13) and three (12%) had del(11q22.3).

Toxicity assessments

Lucatumumab was well tolerated at the first two dose levels (0.3 mg/kg and 1.0 mg/kg), with no DLTs or serious adverse events reported for the seven patients treated on these two cohorts (one patient did not complete all four doses of lucatumumab and was re-placed in the 1.0 mg/kg cohort). A total of eight patients were enrolled onto the 3.0 mg/kg cohort (third treatment cohort) because two of the patients were not evaluable for MTD/DLT determination due to early study discontinuation. Of the six evaluable patients, one developed sepsis on day 12 of therapy and died the following day. The event was deemed not related to study drug. Three patients were enrolled in the 6.0 mg/kg cohort. Of these patients, two experienced the same DLT: grade 3 or 4 amylase/lipase elevation lasting longer than 7 days. Therefore, no additional patients were enrolled at this dose level. Of the six evaluable patients enrolled onto the 4.5 mg/kg dose cohort, two patients experienced grade 3 or 4 elevation of amylase and lipase for greater than 7 days. The recommended phase II dose was identified to be 3.0 mg/kg.

The most common adverse events, regardless of relationship to lucatumumab, are summarized in Table III by grade and dose group. Of those enrolled, 14 patients (53.8%) experienced grade 3 or 4 adverse events as summarized in Table III. All grade 3 or 4 adverse events occurred in patients treated at greater than or equal to 3.0 mg/kg. The most frequently reported grade 3 or 4 adverse events were increased lipase (19.2%) and neutropenia (11.5%). No patient experienced a grade 3 or 4 infusion-related adverse event. Toxicities commonly occurring with lucatumumab treatment included infusion-related events, asymptomatic elevation of amylase and/or lipase and infection.

Of the 93 infusions of lucatumumab, 18 (19%) were interrupted (in 13 patients) due to an adverse event. Four patients with interruptions during the first infusion had one or more interruptions during subsequent infusions. Infusion-related events with lucatumumab were all grade 1 or 2 and included chills (42.3%), hypotension (23.1%), nausea (23.1%), pyrexia (19.2%) or vomiting (15.4%). The symptoms generally resolved either after a reduction in the infusion rate or with a temporary interruption of the infusion.

Elevated amylase and/or lipase was reported for 17 patients. Of these patients, seven experienced grade 3 or grade 4 elevations of amylase or lipase. Grade 3 amylase elevations and grade ¾ lipase elevations were reported at doses ≥ 3.0 mg/kg, with increasing incidence at increased doses. Among the seven patients, four patients had elevations that met the DLT criteria (asymptomatic grade 3 and/or 4 amylase or lipase for greater than 7 days) (two in 6.0 mg/kg cohort, two in 4.5 mg/kg cohort). All cases of amylase/lipase elevations were asymptomatic, with no patient developing typical signs or symptoms of pancreatitis.

Infections were reported in nine patients and the incidence was similar across all dose levels. There was one death due to infection (not related to study drug). This patient had a history of cellulitis prior to enrollment and developed a recurrence of this with associated sepsis on day 12 of treatment. Despite supportive care he died 1 day later. Pneumonia was observed in three patients. There were no opportunistic infections observed during treatment with lucatumumab or during follow-up.

Response

Of the 26 patients enrolled, 24 completed four weekly doses of lucatumumab, were observed for 4 weeks after treatment, and were evaluable for response assessment. One (3.8%) patient with predominately non-bulky lymphadenopathy without lymphocytosis attained a nodular partial response by NCI 1996 criteria that lasted for 230 days. Seventeen patients (65.4%) had stable disease (SD) (mean duration of 76 days, range of 29–504 days), and five (19.2%) had progressive disease (PD). There was no significant decline in the CD5/CD19 lymphocyte count in any patient enrolled on this study from pretreatment to day 29 of treatment.

Pharmacokinetics

Pharmacokinetic parameters obtained after the initial and the last infusions are presented in Table IV. Dose-proportional increases in overall exposure and C_{max} were seen after the first and fourth infusions for dose levels starting at 3.0 mg/kg and higher, and serum concentrations were maintained between infusions. In contrast, dose-proportional increases were not observed at the two lower dose levels (0.3 and 1.0 mg/kg) and serum concentrations of lucatumumab were not maintained between infusions. For the 3.0 mg/kg dose level, the median $t_{1/2}$ was 50 h following the first infusion, and 124 h (approximately 5 days) following the fourth infusion. For the 4.5 mg/kg dose level, median $t_{1/2}$ was 86 h following the first infusion, and 158 h (approximately 7 days) following the fourth infusion, and for the 6.0 mg/kg dose level, mean $t_{1/2}$ was 89 h following the first infusion, and 165 h following the fourth infusion. Calculated median half-lives (50–165 h) for the three highest dose levels indicate that for these three doses, a serum concentration of lucatumumab was maintained between infusions.

Pharmacodynamics

An evaluation of assessments of the percent saturation of CD40 by lucatumumab on blood CD5 + CD19 + CLL cells was performed to determine the dose level at which approximately all the CD40 molecules on CLL cells were bound by lucatumumab throughout the entire course of treatment. There was essentially 100% saturation of CD40 molecules at the end of each infusion for all dose groups, but this saturation was lost prior to the beginning of the next infusion in the 0.3 mg/kg and 1.0 mg/kg dose cohorts. In the remaining three dose cohorts ($\geq$ 3.0 mg/kg), bound lucatumumab remained on the circulating CLL cells between infusions. A substantial decrease ($\geq$ 50%) in percent saturation occurred at day 78 in the 3.0 mg/kg, 4.5 mg/kg and 6.0 mg/kg groups.

Anti-lucatumumab antibodies

Immunogenicity was assessed before and after therapy, and there were no detectable antibodies to lucatumumab in the serum collected from any patient.

Discussion

Herein, we have reported the first in-man study with lucatumumab, a fully humanized anti-CD40 antagonist antibody in relapsed and refractory CLL. We identified the recommended

phase II dose of lucatumumab as 3.0 mg/kg weekly for 4 weeks. The dose-limiting toxicity of lucatumumab in CLL was asymptomatic grade 3 and 4 elevated amylase and/or lipase that persisted for greater than 7 days. These elevated amylase and lipase levels were not associated with symptoms of acute pancreatitis. The elevations were reversible and manageable without medical intervention. Imaging studies failed to reveal signs of pancreatic inflammation. Other toxicities associated with lucatumumab were mild to moderate infusion-related events. Of the 24 patients evaluable for response assessment, one patient attained a nodular partial response, whereas 17 had stable disease at completion of therapy. While lucatumumab at doses of 3.0 mg/kg maximally saturated CD40 binding sites on peripheral blood CLL cells throughout the 4 weeks of therapy, there was essentially no clearing of these cells. Pharmacokinetics of lucatumumab demonstrated features typical of other humanized antibodies in CLL, including evidence of increasing half-life from the first to the fourth dose of therapy and a half-life of approximately 7 days. Collectively, this study establishes a safe dose of lucatumumab that also provides consistent blocking of CD40 receptor on CLL cells.

Unique to this first in-man study with lucatumumab as compared to many other therapeutic antibodies was a non-hematologic dose-limiting toxicity of asymptomatic elevated amylase and lipase. In some patients at higher doses this persisted for several weeks in the absence of signs or symptoms of pancreatitis. The etiologies of these elevated amylase and lipase levels are unknown. However, pancreatic injury does not appear to underlie these enzyme elevations given their asymptomatic presentation and the associated lack of pancreatic tissue changes as assessed by computed tomography (CT) imaging. Studies by several groups have shown that both pancreatic ductal cells [39] and pancreatic beta cells [40] have some CD40 expression, at lower levels than malignant B-cells. We hypothesize that the source of asymptomatic elevated amylase and lipase following higher doses of lucatumumab represents a target-mediated effect. This target-mediated effect may be dependent on the specific properties of the therapeutic monoclonal antibody, such as affinity, on-off rates or ADCC potency, since changes in pancreatic laboratory values have not been reported for other humanized CD40 antibodies such as SGN40 [41,42]. Future clinical studies with lucatumumab in CLL should continue to include careful amylase and lipase assessments.

This study showed that lucatumumab had minimal clinical activity against CLL in the specific patient population studied and the doses and schedules employed; there was only one partial response among 24 evaluable patients with CLL. Additionally, there was minimal evidence of tumor cell clearance as measured by the day-29 post-therapy CD19/CD5 lymphocyte counts. The observation of a sustained nodular response in one patient may suggest a more critical contribution of CD40L in the microenvironment of the lymph node. Nonetheless, the correlative binding studies demonstrated that at the recommended phase II dose of lucatumumab (3.0 mg/kg) there was sustained blocking of CD40 antigen on CLL cells; this could potentially antagonize microenvironmental CD40 ligand protection from other cytotoxic therapy commonly utilized in CLL. Additionally, while innate immune function of NK cells is defective in CLL [43], we have demonstrated that the immunomodulating agent lenalidomide enhances ADCC of CD40 antibodies against autologous human CLL target cells [44]. Consideration of combining lucatumumab with another agent for therapy in CLL will likely be the most viable option for future application of this antibody in this disease.

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References

1. Ries LAG, Melbert D, Krapcho M, et al.SEER Cancer Statistics Review, 1975 - 2004. 2007National Cancer InstituteBethesda, MDAvailable from: http://seercancergov/csr/1975_2004/ Based on November 2006 SEER data submission, posted to the SEER web site
2. Hamblin TJ, Davis Z, Gardiner A, et al. Unmutated Ig V(H) genes are associated with a more aggressive form of chronic lymphocytic leukemia. Blood. 1999; 94:1848–1854. [PubMed: 10477713]
3. Damle RN, Wasil T, Fais F, et al. Ig V gene mutation status and CD38 expression as novel prognostic indicators in chronic lymphocytic leukemia. Blood. 1999; 94:1840–1847. [PubMed: 10477712]
4. Hallek M, Wanders L, Ostwald M, et al. Serum beta(2)-microglobulin and serum thymidine kinase are independent predictors of progression-free survival in chronic lymphocytic leukemia and immunocytoma. Leuk Lymphoma. 1996; 22:439–447. [PubMed: 8882957]
5. Rosenwald A, Alizadeh AA, Widhopf G, et al. Relation of gene expression phenotype to immunoglobulin mutation genotype in B cell chronic lymphocytic leukemia. J Exp Med. 2001; 194:1639–1647. [PubMed: 11733578]
6. Dohner H, Stilgenbauer S, Benner A, et al. Genomic aberrations and survival in chronic lymphocytic leukemia. N Engl J Med. 2000; 343:1910–1916. [PubMed: 11136261]
7. Mayr C, Speicher MR, Kofler DM, et al. Chromosomal translocations are associated with poor prognosis in chronic lymphocytic leukemia. Blood. 2006; 107:742–751. [PubMed: 16179374]
8. Johnson S, Smith AG, Loffler H, et al. Multicentre prospective randomised trial of fludarabine versus cyclophosphamide, doxorubicin, and prednisone (CAP) for treatment of advanced-stage chronic lymphocytic leukaemia. The French Cooperative Group on CLL. Lancet. 1996; 347:1432–1438. [PubMed: 8676625]
9. Rai KR, Peterson BL, Appelbaum FR, et al. Fludarabine compared with chlorambucil as primary therapy for chronic lymphocytic leukemia. N Engl J Med. 2000; 343:1750–1757. [PubMed: 11114313]
10. Leporrier M, Chevret S, Cazin B, et al. Randomized comparison of fludarabine, CAP, and CHOP in 938 previously untreated stage B and C chronic lymphocytic leukemia patients. Blood. 2001; 98:2319–2325. [PubMed: 11588025]
11. Keating MJ, O'Brien S, Albitar M, et al. Early results of a chemoimmunotherapy regimen of fludarabine, cyclophosphamide, and rituximab as initial therapy for chronic lymphocytic leukemia. J Clin Oncol. 2005; 23:4079–4088. [PubMed: 15767648]
12. Hallek M, Fischer K, Fingerle-Rowson G, et al. Addition of rituximab to fludarabine and cyclophosphamide in patients with chronic lymphocytic leukaemia: a randomised, open-label, phase 3 trial. Lancet. 2010; 376:1164–1174. [PubMed: 20888994]
13. Woyach JA, Ruppert AS, Heerema NA, et al. Chemoimmunotherapy with fludarabine and rituximab produces extended overall survival and progression-free survival in chronic lymphocytic leukemia: long-term follow-up of CALGB study 9712. J Clin Oncol. 2011; 29:1349–1355. [PubMed: 21321292]
14. Byrd JC, Peterson BL, Morrison VA, et al. Randomized phase 2 study of fludarabine with concurrent versus sequential treatment with rituximab in symptomatic, untreated patients with B-cell chronic lymphocytic leukemia: results from Cancer and Leukemia Group B 9712 (CALGB 9712). Blood. 2003; 101:6–14. [PubMed: 12393429]
15. Keating MJ, Flinn I, Jain V, et al. Therapeutic role of alemtuzumab (Campath-1H) in patients who have failed fludarabine: results of a large international study. Blood. 2002; 99:3554–3561. [PubMed: 11986207]
16. Knauf WU, Lissichkov T, Aldaoud A, et al. Phase III randomized study of bendamustine compared with chlorambucil in previously untreated patients with chronic lymphocytic leukemia. J Clin Oncol. 2009; 27:4378–4384. [PubMed: 19652068]
17. Coiffier B, Lepretre S, Pedersen LM, et al. Safety and efficacy of ofatumumab, a fully human monoclonal anti-CD20 antibody, in patients with relapsed or refractory B-cell chronic lymphocytic leukemia: a phase 1 - 2 study. Blood. 2008; 111:1094–1100. [PubMed: 18003886]

18. Matutes E, Owusu-Ankomah K, Morilla R, et al. The immunological profile of B-cell disorders and proposal of a scoring system for the diagnosis of CLL. Leukemia. 1994; 8:1640–1645. [PubMed: 7523797]
19. Pu QQ, Bezwoda WR. Interleukin-4 prevents spontaneous in-vitro apoptosis in chronic lymphatic leukaemia but sensitizes B-CLL cells to melphalan cytotoxicity. Br J Haematol. 1997; 98:413–417. [PubMed: 9266941]
20. Francia di Celle P, Mariani S, Riera L, et al. Interleukin-8 induces the accumulation of B-cell chronic lymphocytic leukemia cells by prolonging survival in an autocrine fashion. Blood. 1996; 87:4382–4389. [PubMed: 8639799]
21. Moreno A, Villar ML, Camara C, et al. Interleukin-6 dimers produced by endothelial cells inhibit apoptosis of B-chronic lymphocytic leukemia cells. Blood. 2001; 97:242–249. [PubMed: 11133767]
22. Jurlander J, Lai CF, Tan J, et al. Characterization of interleukin-10 receptor expression on B-cell chronic lymphocytic leukemia cells. Blood. 1997; 89:4146–4152. [PubMed: 9166857]
23. Trentin L, Cerutti A, Zambello R, et al. Interleukin-15 promotes the growth of leukemic cells of patients with B-cell chronic lymphoproliferative disorders. Blood. 1996; 87:3327–3335. [PubMed: 8605349]
24. de Totero D, Meazza R, Capaia M, et al. The opposite effects of IL-15 and IL-21 on CLL B cells correlate with differential activation of the JAK/STAT and ERK1/2 pathways. Blood. 2008; 111:517–524. [PubMed: 17938255]
25. Trentin L, Zambello R, Agostini C, et al. Expression and functional role of tumor necrosis factor receptors on leukemic cells from patients with type B chronic lymphoproliferative disorders. Blood. 1993; 81:752–758. [PubMed: 8381309]
26. Digel W, Stefanic M, Schoniger W, et al. Tumor necrosis factor induces proliferation of neoplastic B cells from chronic lymphocytic leukemia. Blood. 1989; 73:1242–1246. [PubMed: 2539214]
27. Nishio M, Endo T, Tsukada N, et al. Nurselike cells express BAFF and APRIL, which can promote survival of chronic lymphocytic leukemia cells via a paracrine pathway distinct from that of SDF-1alpha. Blood. 2005; 106:1012–1020. [PubMed: 15860672]
28. Endo T, Nishio M, Enzler T, et al. BAFF and APRIL support chronic lymphocytic leukemia B-cell survival through activation of the canonical NF-kappaB pathway. Blood. 2007; 109:703–710. [PubMed: 16973958]
29. Enzler T, Kater AP, Zhang W, et al. Chronic lymphocytic leukemia of E{micro}-TCL1 transgenic mice undergoes rapid cell-turnover that can be offset by extrinsic CD257 to accelerate disease progression. Blood. 2009; 114:4469–4476. [PubMed: 19755673]
30. Haiat S, Billard C, Quiney C, et al. Role of BAFF and APRIL in human B-cell chronic lymphocytic leukaemia. Immunology. 2006; 118:281–292. [PubMed: 16827889]
31. Kern C, Cornuel JF, Billard C, et al. Involvement of BAFF and APRIL in the resistance to apoptosis of B-CLL through an autocrine pathway. Blood. 2004; 103:679–688. [PubMed: 14504101]
32. Furman RR, Asgary Z, Mascarenhas JO, et al. Modulation of NF-kappa B activity and apoptosis in chronic lymphocytic leukemia B cells. J Immunol. 2000; 164:2200–2206. [PubMed: 10657675]
33. Kitada S, Zapata JM, Andreeff M, et al. Bryostatin and CD40-ligand enhance apoptosis resistance and induce expression of cell survival genes in B-cell chronic lymphocytic leukaemia. Br J Haematol. 1999; 106:995–1004. [PubMed: 10520003]
34. Clayton E, Bardi G, Bell SE, et al. A crucial role for the p110delta subunit of phosphatidylinositol 3-kinase in B cell development and activation. J Exp Med. 2002; 196:753–763. [PubMed: 12235209]
35. Cuni S, Perez-Aciego P, Perez-Chacon G, et al. A sustained activation of PI3K/NF-kappaB pathway is critical for the survival of chronic lymphocytic leukemia B cells. Leukemia. 2004; 18:1391–1400. [PubMed: 15175625]
36. Romano MF, Lamberti A, Tassone P, et al. Triggering of CD40 antigen inhibits fludarabine-induced apoptosis in B chronic lymphocytic leukemia cells. Blood. 1998; 92:990–995. [PubMed: 9680368]

37. Luqman M, Klabunde S, Lin K, et al. The antileukemia activity of a human anti-CD40 antagonist antibody, HCD122, on human chronic lymphocytic leukemia cells. Blood. 2008; 112:711–720. [PubMed: 18497318]

38. Cheson BD, Bennett JM, Grever M, et al. National Cancer Institute-sponsored Working Group guidelines for chronic lymphocytic leukemia: revised guidelines for diagnosis and treatment. Blood. 1996; 87:4990–4997. [PubMed: 8652811]

39. Vosters O, Beuneu C, Nagy N, et al. CD40 expression on human pancreatic duct cells: role in nuclear factor-kappa B activation and production of pro-inflammatory cytokines. Diabetologia. 2004; 47:660–668. [PubMed: 15298343]

40. Klein D, Barbe-Tuana F, Pugliese A, et al. A functional CD40 receptor is expressed in pancreatic beta cells. Diabetologia. 2005; 48:268–276. [PubMed: 15690148]

41. Advani R, Forero-Torres A, Furman RR, et al. Phase I study of the humanized anti-CD40 monoclonal antibody dacetuzumab in refractory or recurrent non-Hodgkin's lymphoma. J Clin Oncol. 2009; 27:4371–4377. [PubMed: 19636010]

42. Furman RR, Forero-Torres A, Shustov A, et al. A phase I study of dacetuzumab (SGN-40, a humanized anti-CD40 monoclonal antibody) in patients with chronic lymphocytic leukemia. Leuk Lymphoma. 2010; 51:228–235. [PubMed: 20038235]

43. Kay NE, Zarling JM. Impaired natural killer activity in patients with chronic lymphocytic leukemia is associated with a deficiency of azurophilic cytoplasmic granules in putative NK cells. Blood. 1984; 63:305–309. [PubMed: 6607080]

44. Lapalombella R, Gowda A, Joshi T, et al. The humanized CD40 antibody SGN-40 demonstrates pre-clinical activity that is enhanced by lenalidomide in chronic lymphocytic leukaemia. Br J Haematol. 2009; 144:848–855. [PubMed: 19183192]

Table I

Dose-limiting toxicity.

Toxicity	Any of the following criteria
Hematologic	Platelet count < 25 000/mm^3 for > 7 consecutive days
	Absolute neutrophil count < 500/mm^3 for > 14 consecutive days
	Febrile neutropenia (ANC ° 1.0×10^9/L, fever ≥ 38.5 °C)
Pancreatic	Asymptomatic ≥ CTCAE grade 3 serum amylase or lipase for > 7 consecutive days
	Symptomatic serum amylase or lipase, medical intervention required
Renal	≥ CTCAE grade 3 serum creatinine
Hepatic	Total bilirubin 2-3 × ULN for > 7 consecutive days
	≥ CTCAE grade 3 total bilirubin
	CTCAE grade 3 AST or ALT for > 7 consecutive days
	CTCAE grade 4 AST or ALT
Cardiac – other	≥ CTCAE grade 3
Infusional toxicity	Infusion reaction CTCAE grade 3 and occurs despite optimal premedication
	Infusion reaction CTCAE grade 4
Other adverse events	CTCAE grade 3 adverse events for > 7 consecutive days (excluding CTCAE grade 3 elevations in alkaline phosphatase)
	CTCAE grade 4 adverse events (excluding CTCAE grade 4 elevations in alkaline phosphatase)
	≥ CTCAE grade 3 vomiting or CTCAE grade 3 nausea despite the use of standard antiemetics
	≥ CTCAE grade 3 diarrhea despite the use of optimal antidiarrheal treatments

ANC, absolute neutrophil count; CTCAE, Common Terminology Criteria for Adverse Events; ULN, upper limit of normal; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

Table II

Patient demographics.

	Total (*n* = 26)
Age, median	66
n (%) ≥ 65 years	14 (53)
Female, *n* (%)	9 (35)
Weight (kg), median (range)	78.7 (46.4–115.5)
Rai stage at study entry [*n* (%)]	
I/II	18 (69)
III/IV	8 (31)
ECOG performance status [*n* (%)]	
0	10 (39)
1	15 (58)
2	1 (4)
Organomegaly	
n (%) with splenomegaly	6 (23)
n (%) with hepatomegaly	4 (15)
n (%) with lymphadenopathy	25 (96)
Hematology, median (range)	
WBC (10^9/L)	16 (2–244)
Hgb (g/dL)	117 (72–163)
Platelets (10^9/L)	135 (53–234)
β_2-Microglobulin (μg/mL), median (range)	3.2 (1.7–7.3)
Interphase cytogenetic abnormalities	
n (%) with del(13q14.3)	10 (39)
n (%) with del(11q22.3)	3 (12)
n (%) with del (17p13.1)	2 (8)
n (%) with trisomy 12	4 (15)
Treatment history	
Prior therapies, median (range)	4 (1–12)
n (%) relapsed to fludarabine	17 (65)
n (%) refractory to fludarabine	6 (23)

ECOG, Eastern Cooperative Oncology Group; WBC, white blood cells; Hgb, hemoglobin.

Table III

Most frequent events (greater than or equal to 10% of total patients [*]) by preferred term and treatment group regardless of relation to lucatumumab.

	Dose group					All patients (*n* = 26)	
	0.3 mg/kg (*n* = 3), *n* (%)	**1.0 mg/kg (*n* = 4), *n* (%)**	**3.0 mg/kg (*n* = 8), *n* (%)**	**4.5 mg/kg (*n* = 8), *n* (%)**	**6.0 mg/kg (*n* = 3), *n* (%)**	**All grades [*]**	**Grade 3/4 [†]**
Patients with AE(s)	3 (100)	4 (100)	8 (100)	8 (100)	3 (100)	26 (100)	14 (53.8)
Preferred term [†]							
Chills	1 (33.3)	4 (100)	4 (50.0)	4 (50.0)	1 (33.3)	14 (53.8)	0 (0.0)
Nausea	0 (0.0)	3 (75.0)	3 (37.5)	4 (50.0)	2 (66.7)	12 (46.2)	0 (0.0)
Hypotension	0 (0.0)	2 (50.0)	3 (37.5)	2 (25.0)	2 (66.7)	9 (34.6)	1 (3.8)
Arthralgia	1 (33.3)	0 (0.0)	3 (37.5)	1 (12.5)	2 (66.7)	7 (26.9)	0 (0.0)
Pyrexia	1 (33.3)	1 (25.0)	2 (25.0)	1 (12.5)	2 (66.7)	7 (26.9)	0 (0.0)
Diarrhea	0 (0.0)	2 (50.0)	3 (37.5)	0 (0.0)	1 (33.3)	6 (23.1)	0 (0.0)
Fatigue	1 (33.3)	1 (25.0)	2 (25.0)	1 (12.5)	1 (33.3)	6 (23.1)	0 (0.0)
Vomiting	0 (0.0)	2 (50.0)	2 (25.0)	2 (25.0)	0 (0.0)	6 (23.1)	0 (0.0)
Lipase increased	0 (0.0)	0 (0.0)	1 (12.5)	3 (37.5)	1 (33.3)	5 (19.2)	5 (19.2)
Constipation	0 (0.0)	0 (0.0)	2 (25.0)	1 (12.5)	1 (33.3)	4 (15.4)	0 (0.0)
Dizziness	1 (33.3)	0 (0.0)	3 (37.5)	0 (0.0)	0 (0.0)	4 (15.4)	0 (0.0)
Dyspnea	0 (0.0)	0 (0.0)	3 (37.5)	1 (12.5)	0 (0.0)	4 (15.4)	2 (7.7)
Edema peripheral	1 (33.3)	0 (0.0)	1 (12.5)	1 (12.5)	1 (33.3)	4 (15.4)	0 (0.0)
Weight decreased	0 (0.0)	0 (0.0)	2 (25.0)	1 (12.5)	1 (33.3)	4 (15.4)	0 (0.0)
Anemia	0 (0.0)	0 (0.0)	1 (12.5)	1 (12.5)	1 (33.3)	3 (11.5)	0 (0.0)
Blood amylase increased	0 (0.0)	0 (0.0)	1 (12.5)	0 (0.0)	2 (66.7)	3 (11.5)	2 (7.7)
Cough	0 (0.0)	0 (0.0)	1 (12.5)	1 (12.5)	1 (33.3)	3 (11.5)	0 (0.0)
Decreased appetite	0 (0.0)	0 (0.0)	2 (25.0)	1 (12.5)	0 (0.0)	3 (11.5)	0 (0.0)
Erythema	0 (0.0)	0 (0.0)	0 (0.0)	1 (12.5)	2 (66.7)	3 (11.5)	0 (0.0)
Headache	0 (0.0)	0 (0.0)	1 (12.5)	1 (12.5)	1 (33.3)	3 (11.5)	0 (0.0)
Insomnia	0 (0.0)	0 (0.0)	2 (25.0)	1 (12.5)	0 (0.0)	3 (11.5)	0 (0.0)
Neutropenia	0 (0.0)	0 (0.0)	1 (12.5)	2 (25.0)	0 (0.0)	3 (11.5)	3 (11.5)
Night sweats	0 (0.0)	0 (0.0)	1 (12.5)	2 (25.0)	0 (0.0)	3 (11.5)	0 (0.0)
Pleural effusion	0 (0.0)	0 (0.0)	1 (12.5)	1 (12.5)	1 (33.3)	3 (11.5)	0 (0.0)
Pneumonia	0 (0.0)	0 (0.0)	2 (25.0)	2 (25.0)	0 (0.0)	3 (11.5)	2 (7.7)
Tremor	0 (0.0)	0 (0.0)	1 (12.5)	2 (25.0)	0 (0.0)	3 (11.5)	0 (0.0)

[*] Arranged by frequency in all patients, all grades.

[†] Included are only Common Toxicology Criteria (CTC) grade 3/4 AEs when the overall incidence of that AE (all CTC grades) is ≥ 10%.

Table IV

Summary of pharmacokinetic parameters of HCD122 by treatment group.

	0.3 mg/kg (*n* = 3)	1.0 mg/kg (*n* = 4)	3.0 mg/kg (*n* = 8)	4.5 mg/kg (*n* = 8)	6.0 mg/kg (*n* = 3)
Half-life, $t_{1/2}$ (h)					
1st infusion, *n*	0	4	8	8	2
Mean	–	14.542	52.405	108.970	88.676
SD	–	7.719	37.948	80.565	9.726
Median	–	16.731	50.010	85.753	88.676
Minimum	–	4.018	12.986	34.672	81.798
Maximum	–	20.689	102.772	280.430	95.553
4th infusion, *n*	0	2	5	6	3
Mean	–	20.002	198.474	761.449	175.581
SD	–	16.049	228.381	1502.719	20.810
Median	–	20.002	124.386	157.651	164.531
Minimum	–	8.654	15.487	99.676	162.627
Maximum	–	31.350	575.338	3828.216	199.585
C_{max} (μg/mL)					
1st infusion, *n*	3	4	8	8	2
Mean	2.033	14.109	63.963	100.040	125.302
SD	0.874	1.381	23.768	34.388	24.501
Median	2.252	14.246	66.571	97.214	125.302
Minimum	1.070	12.411	24.166	57.540	107.977
Maximum	2.776	15.535	92.471	156.675	142.626
4th infusion, *n*	3	3	5	6	3
Mean	2.121	15.977	107.033	163.057	213.496
SD	1.388	1.653	55.277	63.476	34.050
Median	2.565	16.929	125.835	145.108	226.962
Minimum	0.566	14.069	24.744	82.726	174.772
Maximum	3.233	16.934	157.348	256.273	238.753
$AUC_{0–168\ h}$ (μg•h/mL)					
1st infusion, *n*	3	4	8	8	2
Mean	24.392	463.028	4192.166	7493.302	9687.652
SD	10.487	223.506	2749.109	3510.210	1853.683
Median	27.024	464.066	4144.004	7224.067	9687.652
Minimum	12.840	188.764	636.688	3342.899	8376.900
Maximum	33.312	735.216	7640.840	13 900.435	10 998.404
4th infusion, *n*	3	3	5	6	3
Mean	39.040	973.100	12 153.413	16 908.049	26 829.964
SD	8.385	521.575	7341.541	8136.447	8284.051
Median	38.796	1095.226	16 942.062	14 717.737	27 513.054
Minimum	30.780	401.298	1125.866	7283.433	18 225.518
Maximum	47.544	1422.776	17 573.372	30 344.744	34 751.321

SD, standard deviation; C_{max}, drug concentration at end of infusion; AUC, area under the curve.