



Protocol Page

A Phase II Clinical Trial of Sapacitabine, Cyclophosphamide, and Rituximab (SCR) for Relapsed Patients with Chronic Lymphocytic Leukemia / Small Lymphocytic Lymphoma (CLL/SLL) and Deletion 11q22-23 by FISH
2010-0516

Core Protocol Information

Short Title	Phase II Sapacitabine, Cyclophosphamide, Rituximab for Rel CLL/SLL with del(11q22-23)
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Full Title:	A Phase II Clinical Trial of Sapacitabine, Cyclophosphamide, and Rituximab (SCR) for Relapsed Patients with Chronic Lymphocytic Leukemia / Small Lymphocytic Lymphoma (CLL/SLL) and Deletion 11q22-23 by FISH
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Which Committee will review this protocol?

☒ The Clinical Research Committee - (CRC)

Protocol Body



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**A Phase II Clinical Trial of Sapacitabine,
Cyclophosphamide, and Rituximab (SCR) for
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and Deletion 11q22-23 by FISH**

Version 7

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Short Title: Phase II SCR for Relapsed CLL with deletion 11q22-23

1.0 OBJECTIVES

Primary objective:

- Evaluate efficacy (2008 International Workshop on Chronic Lymphocytic Leukemia [IWCLL] overall response rate) of the SCR regimen in previously treated patients with CLL who have deletion 11q22-23 by FISH.

Secondary objectives:

- Evaluate the association between IWCLL response (CR, PR, and OR) to SCR and ATM function in previously treated patients with CLL who have deletion 11q22-23 by FISH.
- Evaluate toxicities, safety and tolerability of the SCR regimen in previously treated patients with CLL who have deletion 11q22-23 by FISH.
- Evaluate the number of courses of SCR to best response and overall number of courses SCR administered to each patient.
- Evaluate time-to-treatment failure (TTF), time-to-progression (TTP) for responders, and overall survival for previously treated patients with CLL who have deletion 11q22-23 by FISH treated with SCR. Also, evaluate the association between these time-to-event endpoints and ATM function.
- Correlate pretreatment prognostic factors, including ZAP-70 expression, CD38 expression, β -2 microglobulin, *IGHV* mutation status, response and time-to-event outcomes.
- Evaluate pharmacodynamic endpoints including 1) quantitate the proportion of proliferating cells (proliferating fraction) in blood and/or bone marrow by flow cytometry before and during treatment; and 2) quantitate DNA double-strand breaks in blood and bone marrow lymphocytes by staining for Rad51 foci and evaluating cells by confocal microscopy, and 3) measure changes in apoptosis during therapy.

2.0 BACKGROUND

2.1 CLL is the most common leukemia in Western societies (reviewed in ¹). Nearly two-thirds of patients with CLL are over 65 years of age, and there is a steady increase in the prevalence of this disease in the population over 50 years of age. Interest and development of new therapeutic agents was lagging until recently, owing to the usual advanced age of patients and often indolent course of disease. The natural history of the disease is diverse; patients with only lymphocytosis have a median survival in excess of 10 years, while those with evidence of marrow failure manifested by anemia or thrombocytopenia have a median survival of only 2 years. Intermediate survival is expected for patients with lymphadenopathy or organomegaly. The National Cancer Institute Working Group on CLL described clinical features of “active disease” which are helpful in the decision to treat^{2,3}. These indications for treatment were revised and re-reported by the International Working Group for CLL (IWCLL)⁴ and include: 1) unintentional weight loss of more than 10% body weight over the past 6 months; 2) fever or night sweats in the absence of infection; 3) extreme fatigue; 4) worsening anemia or thrombocytopenia; 5) massive (>6 cm below the left costal margin) or progressive

splenomegaly; 6) massive (>10 cm in the longest diameter) or progressive lymphadenopathy; 7) progressive lymphocytosis with rapid lymphocyte doubling time; 8) marked hypogammaglobulinemia or paraproteinemia.

- 2.2 Historically, the mainstay of therapy was systemic chemotherapy consisting of an alkylating agent and corticosteroid. Chlorambucil plus prednisone was the standard initial therapy with response rates from 40-77%. Generally, responses were not complete⁵⁻⁹. Therapy for patients refractory to alkylating agents was unsatisfactory. The response rates were substantially lower (approximately 30%) with rare complete responses (CR)⁸⁻¹².
- 2.3 Fludarabine has marked activity in several indolent lymphoproliferative disorders including CLL, low-grade lymphoma, Waldenstroms macroglobulinemia, and prolymphocytic leukemia¹³⁻¹⁷.

Treatment of previously treated patients with CLL with fludarabine resulted in 13% CR and 44% partial remission (PR)¹³. Fludarabine was also given to patients with previously untreated CLL, resulting in even higher response rates with 33% confirmed CR, 39% unconfirmed CR and 6% PR¹⁶.

Despite achieving clinical CR, most patients experience recurrence at a median of approximately 2 years after frontline fludarabine treatment¹⁸. This is likely related to the fact that even patients in CR have residual disease. Residual disease can now be assessed using highly sensitive flow cytometry or molecular methods that were previously not available. In previously untreated patients, 55% of CR patients have residual nodules on bone marrow biopsy (now referred to as nodular partial remission [nPR])¹⁶. At 2 years 87% of CR patients were progression-free, versus 55% of nPR patients¹⁸.

- 2.4 Cyclophosphamide was combined with vincristine (VCR) and prednisone (CVP) to treat lymphoproliferative disorders. Liepman and Votaw first reported on this combination to treat CLL in 1978. Thirty-six patients received this regimen, 23 were previously untreated. The response rate was 72%; 18 of 23 (78%) previously untreated patients responded; 8 of 13 (62%) patients previously receiving chlorambucil responded to CVP¹⁹. Oken and Kaplan treated 18 patients with CLL with CVP (cyclophosphamide 800 mg/m² I.V. on day 1 or 400 mg/m² orally for 5 days, VCR 2 mg I.V. on day 1 and prednisone 60-100 mg/m² on days 1-5). All patients were previously treated and 17 of 18 were refractory to chlorambucil. The response rate was 44%²⁰.
- 2.5 *In vitro* studies demonstrated that fludarabine inhibited repair of cyclophosphamide-induced DNA inter-strand cross-links in CLL B cells²¹. This was the basis for a regimen of fludarabine, 25-30 mg/m², combined with cyclophosphamide, 250-500 mg/m²; both drugs were given daily for three days in 4-week courses. Frewin et al²² first reported their experience giving fludarabine 25 mg/m² and cyclophosphamide 250 mg/m² daily for 3 days in a limited trial of 7 patients with CLL; 2 patients achieved complete remission and the overall response rate was 71%. Toxicities included nausea and vomiting, myelosuppression and infections. O'Brien et al²³ gave this treatment to 94 patients who were previously treated with either an alkylating agent, fludarabine, or both. The CR rate was 11% and an overall response rate (ORR) of 69% was reported. Patients previously

treated with an alkylating agent and were resistant to fludarabine had a markedly lower, but noteworthy response rate with 3% complete remissions and 39% ORR.

2.6 Rituximab and Chemoimmunotherapy in CLL

The Food and Drug Administration (FDA) approved Rituximab in 1997 for treatment of relapsed or refractory low-grade non-Hodgkin's lymphoma. In the pivotal trial, 33 of 166 patients had International Working Formulation (IWF) A disease or small lymphocytic lymphoma (SLL). Rituximab was given at a dose of 375 mg/m² weekly for 4 weeks, and produced an ORR of 48%. For the patients with IWF A disease, the ORR was low, 12% compared to 58% for those with IWF B, C, and D (combined) disease²⁴. There are several potential explanations for the lower activity of rituximab in patients with SLL. Leukemia/lymphoma cells from patients with CLL/SLL express lower levels of CD20 than follicular lymphoma cells. In addition, pharmacokinetic analyses conducted during the pivotal trial showed lower plasma levels of rituximab in non-responders and, effectively, in most patients with SLL. Finally, circulating CD20 has been demonstrated in the plasma of patients with CLL/SLL²⁵. Soluble CD20 may act as a sink for the therapeutic antibody, resulting in more rapid clearance and reducing delivery of the antibody to leukemia cells. Therefore, higher plasma concentrations of rituximab could potentially improve response rates in CLL.

Rituximab monotherapy has been evaluated as treatment for CLL. The standard dose of 375 mg/m² weekly for 4 weeks was given to 28 previously patients with CLL²⁶. There were no CRs; 25% of patients achieved PR, 43% had stable disease, and 32% had progressive disease. Smaller studies evaluating rituximab 375 mg/m² weekly for 4 weeks in previously treated patients with CLL report low ORR with no CRs²⁶⁻²⁸. Two studies of dose-escalated rituximab in CLL yielded encouraging results. O'Brien et al.²⁹ conducted a study of 4 weekly doses of rituximab at 500, 650, 825, 1000, 1500, and 2250 mg/m². All patients with CLL had been previously treated with chemotherapy. The ORR among the 39 evaluable patients was 36% and all remissions were PRs. Responses were seen in 5 of 24 (21%) patients who received 500-825 mg/m², 3 of 7 (43%) patients who received 1000-1500 mg/m², and 6 of 8 (75%) patients who received 2250 mg/m² (P=0.03). Byrd et al.³⁰ conducted a dose-intense study with 375 mg/m² administered thrice weekly for 4 weeks. Twenty-seven previously treated patients were evaluable for response, 10 (37%) achieved remission. There appeared to be increased activity for rituximab in CLL with dose intensified schedules; the optimal dose and schedule for rituximab monotherapy for CLL is yet to be determined.

The increased activity of fludarabine combined with cyclophosphamide (FC) and the potential chemo-sensitization between purine analogue, alkylating agent, and monoclonal antibody was the rationale for combining rituximab with FC. The efficacy, toxicity, and tolerability of chemoimmunotherapy with the combination of fludarabine, cyclophosphamide, and rituximab (FCR) were evaluated in previously treated patients with CLL³¹. The purpose of this study was to improve the CR rate for previously treated patients and evaluate the quality of bone marrow response. One hundred seventy-seven previously treated patients with CLL were evaluated. Treatment consisted of rituximab 375 mg/m² day 1 of course 1 and 500 mg/m² day 1 of courses 2 to 6; fludarabine 25 mg/m²/d days 2 to 4 of course 1 and days 1 to 3 of courses 2 to 6; and cyclophosphamide 250 mg/m²/d days 2 to 4 of course 1 and days 1 to 3 of courses 2 to 6. Courses were repeated every 4 weeks. CR was achieved in 25% of 177 patients, and nPR and partial remission (PR) were achieved in 16% and 32% of patients, respectively;

the overall response rate was 73%. Twelve (32%) of 37 complete responders tested achieved molecular remission in bone marrow. The FCR regimen was an active and well-tolerated treatment for previously treated patients with CLL. Myelosuppression was the most common toxicity. FCR induced the highest CR rate reported in a clinical trial of previously treated patients with CLL. Furthermore, molecular remissions were achieved in a third of patients achieving CR. Despite the succession of incremental improvements in the duration of response to such chemoimmunotherapy during the last decade, the relapse rate appears to be uninterrupted, and the long-term prognosis for survival remains poor³².

The FCR regimen was evaluated in two large phase III randomized trials, one in previously untreated patients (CLL8 trial³³) conducted by the German CLL Study Group (GCLLSG) and one in previously treated patients (REACH trial³⁴) conducted as an international trial sponsored by F. Hoffmann La-Roche. Both trials demonstrated superior efficacy for the FCR combination over FC in terms of longer progression-free survival (PFS) associated with higher CR and ORR. A more recent updated analysis of survival in the CLL8 trial demonstrated superior overall survival for patients treated with FCR versus FC, making this the standard frontline treatment for fit individuals with CLL³⁵. The REACH trial was conducted mainly in Europe and randomized previously treated patients with CLL to either FC or FCR. Patients could have only had one prior treatment that could not have included FC or rituximab. A total of 552 patients were randomized, 276 each received FC or FCR; patients were equally distributed between treatment arms in terms of pre-treatment characteristics. The doses of FC were the same for both treatment arms, fludarabine 25 mg/m² and cyclophosphamide 250 mg/m², both daily for 3 days of each course. For FCR, rituximab was given at 375 mg/m² x1 for course 1 and 500 mg/m² x1 for courses 2-6. The CR and ORR were 24.3 and 69.9% respectively for patients treated with FCR and 13 and 58% respectively for patients treated with FC. The median PFS for patients treated with FCR was 30.6 versus 20.6 mos for patients treated with FC. Treatment was well tolerated by both arms and there was no significant difference in the rates of grade 3 and 4 neutropenia, anemia, thrombocytopenia, infectious, or other adverse events. These phase III data clearly demonstrated superiority for the FCR regimen over FC in previously treated patients with CLL and led to FDA approval of rituximab in combination with chemotherapy for frontline and salvage treatment for CLL.

2.7 Deletion 11q22-23 and ATM in CLL

The majority of leukemia cells in blood and bone marrow are not proliferating, therefore, it is challenging to generate leukemia metaphase karyotypes for patients with CLL. Fluorescent *in situ* hybridization (FISH) enables chromosome analyses in interphase cells as well as cells in metaphase and substantially increases the sensitivity for identifying specific genomic aberrations in CLL. Indeed, FISH analysis of 325 CLL cases showed chromosome abnormalities in over 80% of CLL cases, including 13q deletion, the most common abnormality, occurring in 55% of cases³⁶. Other abnormalities, in order of frequency, were deletion 11q22-23 (18%), trisomy 12 (16%), deletion 17p13 (7%), and deletion 6q (6%). This study also reported the prognostic significance of these abnormalities. Deletion 13q14, as a single aberration, was associated with the longest median survival (133 months) while deletion 11q22-23 (79 months) and deletion 17p13 (32 months) were associated with poor prognosis, high-risk disease. Trisomy 12 and diploid karyotype were intermediate prognosis. Other studies have reported the poor clinical outcomes associated with deletion 11q22-23, particularly

in patients younger than 55 years old³⁶⁻³⁸. Characteristically, patients 11q22-23 deletion present with advanced disease and bulky lymphadenopathy³⁷.

Deletion at chromosome 11q22.3 is associated with deletion of the Ataxia Telangiectasia Mutated (*ATM*) gene. *ATM* protein plays an important role in cellular responses to double strand DNA breaks in a process involving a number of factors, including p53^{39,40}. In addition to deletions, mutations of *ATM* are not uncommon in CLL^{41,42}. One study showed that some patients with CLL have germline *ATM* mutations, suggesting that a "carrier state" may predispose to the development of CLL⁴². Mutation of *ATM* in the residual allele for patients with deletion 11q22-23 was demonstrated in 36% (19/52) of untreated patients, and additional mutations in *ATM* arising subsequent to treatment (2/10, 20%)⁴³.

We noted that 41% (50/121) of patients who relapsed after first remission from FCR had deletion 11q22-23, whereas this was present in only 10% of our frontline population⁴⁴. Although these results suggest that the region containing *ATM* is important for response to chemioimmunotherapy, our recent findings suggest an alternative treatment is needed that is effective in cells that lack *ATM* function.

2.8 Sapacitabine Preclinical Studies

Sapacitabine/
 CNDAC

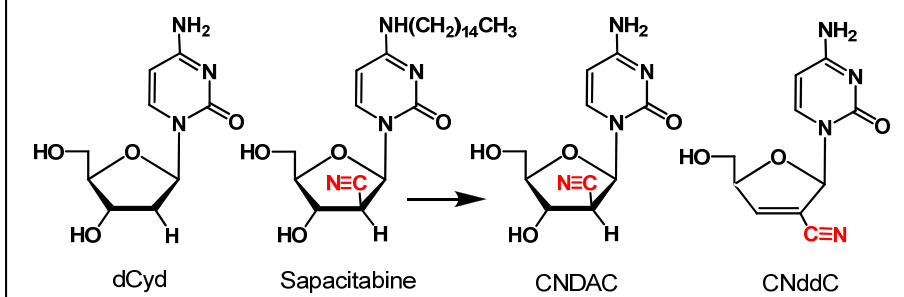
causes DNA
 double-stand
 breaks.

CNDAC (2'-
 C-cyano-2'-
 deoxy-1-beta-
 D-arabino-
 pentofuranos-
 yl-cytosine)

(Fig.1) is a

deoxycytidine analog that was conceptualized as a DNA strand breaking nucleoside⁴⁵. We recently reported that the orally administered prodrug formulation, Sapacitabine, has activity in treating relapsed acute leukemia⁴⁶. Investigations of the metabolism and mechanism of action of CNDAC demonstrated that the nucleoside is transported into the cell and phosphorylated to the tri-phosphate, which is a substrate for both DNA replication and repair⁴⁷. Upon incorporation into DNA, it becomes chemically unstable because of the strong electron-withdrawing properties of the cyano group, and undergoes β -elimination to generate a nick in one strand of DNA that is terminated on the 3'-strand by the dideoxy analog, CNddC⁴⁷. This is not a substrate for ligation, although the lesion is repaired to some extent by the transcription-coupled nucleotide excision pathway⁴⁸. We've measured as many as 7×10^4 such nicks in DNA of single cells following incubation with clinically relevant CNDAC concentrations⁴⁹.

Fig. 1. Structures of dCyd, Sapacitabine, CNDAC and CNddC.



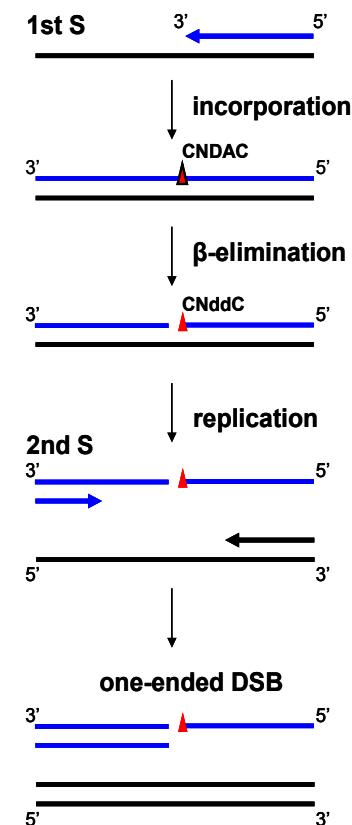
These nicks are not inhibitory to DNA synthesis; cells pass through mitosis and progress to a subsequent S phase. However, upon doing so, as the replication fork encounters a nick in the DNA, a one-ended double strand break occurs at the replication fork (Fig. 2)⁴⁹. This is evidenced by a significant increase in number of chromosomal breaks after cells pass into the second mitosis. Likely, this damage is principally repaired by the homologous recombination repair (HRR) pathway, as the clonogenicity of cells lacking genes that function in this pathway such as ATM, XRCC3 and BRCA2, is sensitized as much as 100-fold. In contrast, cells deficient in non-homologous end-joining proteins (DNA-PKcs, Ku80), an alternative mechanism of double-strand break repair, are not affected. This is likely because the non-homologous end-joining mechanism joins two double-stranded ends, whereas HRR uses the homologous sequence to initiate repair and extension of a one-ended double-strand break^{50,51}. The presence of sister chromatid exchanges, which arise more significantly after cells pass into the second S phase after exposure to CNDAC, is diagnostic of homologous recombination activity⁴⁹. Taken together, these results demonstrate that CNDAC kills cells by a unique mechanism of action, that cells are able to repair the damage to DNA to a limited extent, and that homologous recombination is likely a major factor in cell survival.

The results described above demonstrate that cells that lack homologous recombination repair function are specifically sensitized to the actions of CNDAC, the active form of the clinical prodrug, Sapacitabine. We hypothesize that patients with CLL who lack ATM function, and therefore are not able to conduct homologous recombination repair, will be specifically sensitized to treatment containing Sapacitabine.

2.9 Sapacitabine Clinical Studies

The palmitoyl side chain on CNDAC allows for improved oral absorption of Sapacitabine and protects the N4 amino group from deamination, which is a major route of inactivation of the molecule. Following oral administration, Sapacitabine is converted to 1-(2-C-cyano-2-deoxy- β (-D-arabino-pentafuranosyl) cytosine (CNDAC) by amidases and esterases in the gut, plasma, and liver. CNDAC is further converted to CNDAC-mono phosphate by deoxycytidine kinase (dCK) and this is thought to be the rate-limiting step in the formation of CNDAC-triphosphate (CNDAC-TP), the most active metabolite in terms of cytotoxicity. CNDAC-phosphates are degraded by cytidine deaminase (CDA) and 5'nucleotidase. Both Sapacitabine and CNDAC are active against a wide range of human cancer cell lines *in vitro* and animal models *in vivo*. To date, 3 phase I trials with Sapacitabine have been reported, 2 in advanced solid tumors and 1 in patients with AML/MDS. In the first phase I trial in solid tumors, increasing doses of oral Sapacitabine

Fig. 2. Generation of single- and double-strand breaks after incorporation of CNDAC into DNA.



were administered to patients once daily 3 times a week (Mon, Wed, Fri), for consecutive 4 weeks, followed by a 2-week rest period⁵². The following dose levels were evaluated: 1.5, 12, 20, 25, 30, 50, 67, 90, 120, 160, and 220 mg/m²/day. Forty patients were enrolled with a variety of solid tumors, including colorectal (most common), prostate, breast, and lung cancer. Severe hematologic toxicities (G3-4) were not uncommon, 10 patients experienced G3-4 neutropenia, 2 experienced G4 thrombocytopenia, and 2 experienced G3 anemia. Dose-limiting neutropenia was observed at 220mg/m²/day; the maximum tolerated dose (MTD) was 160mg/m²/day for the schedule in this trial. Non-hematologic toxicities (G3) related to treatment included nausea, vomiting, anorexia, asthenia, and dehydration but were not dose-limiting. No tumor responses were noted in this trial. Peak plasma concentrations of the active form CNDAC were achieved 2.2±0.9 hr after drug administration and the terminal elimination half-life was 1.7±1.5 hr. CNDAC was metabolized by cytidine deaminase to the inactive product CNDAU.

In the second phase I trial for advanced solid tumors, oral Sapacitabine was administered 5 days per week for 4 weeks, followed by 2 weeks off treatment observation (6 weeks treatment course)⁵³. The following dose levels were evaluated: 1, 2.5, 5, 7.5, 10, 13, 17.5, 23.5, 30, 50, and 67mg/m²/day. The MTD was 40mg/m²/day, 5 days each week for 4 weeks followed by 2 weeks of treatment. The most common dose-limiting toxicity (DLT) was grade 4 neutropenia, which occurred at doses of 40mg/m²/day and higher. Other G3-4 toxicities in order of frequency included asthenia and diarrhea. No tumor responses were noted in this trial.

A phase I trial was conducted in patients with acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS). In this study, oral Sapacitabine was given twice daily for 7 days every 3-4 weeks or twice daily on days 1-3 and 8-10 every 3-4 weeks. Sapacitabine was escalated from 75-375 mg twice daily for 7 days and from 375-475 mg twice daily on days 1-3 and 8-10 every 3-4 weeks. The DLT for both schedules was gastrointestinal (diarrhea, colitis, small-bowel obstruction). The MTDs were 375mg twice daily for 7 days and 425 mg twice daily on days 1-3 and 8-10 every 3-4 weeks. The recommended phase II single-agent dose for these hematologic diseases was 325 mg twice daily for 7 days and 425 mg twice daily for 3 days on days 1-3 and 8-10 of each 3-4 week course. Responses were observed in 13 patients (28%); 4 were complete remissions, and 9 were marrow complete responses.

The recommended Phase II dose for monotherapy has been established for 4 dosing schedules using the Cyclacel BL formulation: a) 50 mg *b.i.d.* x 14 days every 3 weeks (solid tumors); b) 75 mg *b.i.d.* x 7 days every 3 weeks (solid tumors); c) 325 mg *b.i.d.* x 7 days every 3 weeks (leukemias/MDS); and d) 425 mg *b.i.d.* x 3 days/week x 2 weeks every 3 weeks (leukemias/MDS). The predominant dose-limiting toxicity (DLT) was myelosuppression in solid tumor patients which consisted of neutropenia, febrile neutropenia, neutropenic sepsis, and thrombocytopenia. Myelosuppression was generally reversible after interruption of drug dosing. To date, the predominant DLTs reported in patients with advanced leukemias or MDS were gastrointestinal toxicities which included abdominal pain/small bowel obstruction, diarrhea and neutropenic enteritis. Common non-hematological toxicities were fatigue, nausea, vomiting, diarrhea, constipation, anorexia, abdominal pain, fever, pneumonia, cough, dyspnea,

dizziness, epistaxis, peripheral edema, alopecia and hypokalemia which were generally mild to moderate.

Given the clinical activity of Sapacitabine in advanced leukemia⁴⁶, we are encouraged to extend additional clinical investigations to a patient population having a deletion in 11q22-23, those defined by the loss of ATM function. The goal of this effort is to create a tumor-specific therapy that will benefit a specific patient population.

2.11 Rationale for the SCR Combination in CLL with Deletion 11q22-23

Although current chemoimmunotherapy for untreated CLL is highly effective at inducing CR, there is a steady relapse rate. Subsequent salvage treatment for these patients is associated with lower response rate, and there is now clear evidence that with repeated treatment, many patients develop disease resistant to therapy and that many of these patients succumb to their CLL. Several biological characteristics at diagnosis or initiation of treatment, including *IGHV* mutation status, serum markers, inappropriate protein expression and cytogenetic changes predict for outcome. Of these, so far cytogenetic changes provide a clue to disease etiology, or pathophysiology, as molecular studies have indicated several of the underlying factors that are affected. This trial focuses on 1 of these changes, expression of the DNA damage response gene, ATM, which is deleted in the common abnormality at chromosome 11q22-23. Remarkably, more than 40% of patients whose disease relapses after treatment with FCR, our most effective therapy, exhibit this abnormality, whereas only 15-20% of the treatment-naïve CLL population seen at M. D. Anderson exhibit this deletion prior to frontline treatment. Studies suggest as many as half of patients with deletion 11q22-23 have lost ATM function in the gene at the residual allele due to gene mutations. The investigational agent, Sapacitabine, causes DNA damage by a novel mechanism that requires ATM function for repair. Therefore, there is strong rationale to select patients with 11q22-23 deletion for Sapacitabine therapy, since their disease may be selectively sensitized to this agent. This trial will test this hypothesis.

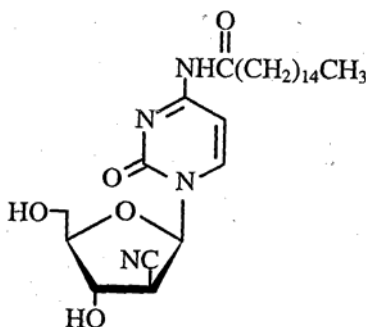
This is a phase II trial of Sapacitabine combined with cyclophosphamide and rituximab (SCR) for previously treated patients with CLL and 11q22-23 deletion by FISH. The primary objective will be to evaluate the overall response rate of the regimen; secondary endpoints will be to evaluate tolerability and toxicities, determine association between response and ATM function, determine time-to-treatment failure, progression-free survival, and overall survival. Sapacitabine will be provided by Cyclacel Pharmaceuticals. The IND under which this clinical trial will be conducted will be held by MD Anderson Cancer Center with cross-filing on the Cyclacel Pharmaceuticals master file.

3.0 BACKGROUND DRUG INFORMATION

3.1 Sapacitabine

3.1.1 Chemical Name: 1-(2-C-cyano-2-deoxy- β -D-arabino-pentafuranosyl)-N4-palmitoylcytosine

3.1.2 Chemical structure



- 3.1.3 Formulation:** The Cyclacel BL formulation comprises liquid-filled capsules of a suspension of the crystalline Form B of the active pharmaceutical ingredient in miglyol 812N and is supplied as 25 mg, 50 mg and 75 mg strength capsules. For this clinical trial, 50 mg capsules will be used. Capsules are packaged in high-density polyethylene bottles, with low-density polyethylene snap-on tamper resistant closures. The capsules should be stored at room temperature (15-25°C) in a closed container, protected from light in a secure, limited-access storage area. The 25, 50 and 75 mg capsules currently have a 60 -month retest date.

Unit Formula

Ingredient 50 mg

Sapacitabine B Form 50 mg

Miglyol 812N Ph.Eur/GRAS 200 mg

Gelatin Capsule and gelatin banding USP/Ph.Eur. Size 2

Ph.Eur = European Pharmacopoeia; GRAS: Generally regarded as safe; USP = United States Pharmacopoeia

- 3.1.4 Route of administration:** Oral

- 3.1.5 Source of Drug:** Sapacitabine is an investigational drug supplied by Cyclacel under Investigational New Drug #53748.

- 3.1.6 Drug Procurement**

FDA regulations require investigators to establish a record of the receipt, use, and disposition of all investigational agents. Investigators may delegate responsibility for drug ordering, storage, accountability, and preparation to their designees. Cyclacel's requirements for procurement, accountability, and disposition of study drug are provided below.

Drug Ordering: Sapacitabine should be requested by the Principal Investigator (or his/her authorized designees) at each participating institution. Sapacitabine may not be used outside the scope of this protocol, nor can it be transferred or licensed to any party not participating in the clinical study. Cyclacel policy requires that Sapacitabine be shipped directly to the institution where the patient is to be treated. Cyclacel does not permit the transfer of Sapacitabine between institutions.

3.1.5 **Drug Accountability**

A capsule count of the drug will be maintained on the Drug Accountability Record (DAR). All drug received, dispensed, and returned by the patient must be recorded on the DAR. Patients will be instructed to return the dispensed capsule bottle on Day 1 of each cycle. Capsule count will occur at each visit to assess patient compliance with study drug.

3.1.6 **Disposition of Unused Drug**

All unused drug, including drug returned by patients, must be retained by study site staff until verified by MDCC Investigational Pharmacy. After the drug accountability has been verified, unused drug will be destroyed according to Institutional guidelines and applicable laws and regulations.

The clinical supplies of cyclophosphamide and rituximab will be commercial drug supply.

3.2 Cyclophosphamide (Cytosan)

Cyclophosphamide is commercially available and has activity in treating a variety of malignancies. Cyclophosphamide is an alkylating agent that prevents cell division by cross-linking DNA strands and decreasing DNA synthesis. It is a cell cycle phase non-specific agent. Cyclophosphamide is a prodrug that must be metabolized to active metabolites in the liver.

Expected toxicities include myelosuppression. Cystitis can be caused by cyclophosphamide and can be dose limiting.

3.2.1 How Supplied: Supplied in quantities of 100 mg, 200 mg, 500 mg, 1 gm, and 2 gm for intravenous use. Maximum concentration is 20 mg/ml.

3.2.2 Solution Preparation: This is per standard pharmacy practice.

3.2.3 Stability: Room temperature stability is 48 hrs. Refrigeration stability is 28 days.

3.2.4 Route of Administration: Intravenous

3.2.5 Toxicities:

- **COMMON**: The most common effects observed include alopecia (40% to 60%), which usually starts 3 to 6 weeks after therapy, effects on fertility (ie, irreversible sterility, amenorrhea), gastrointestinal effects (ie, nausea, vomiting, diarrhea, anorexia, mucositis, stomatitis), a potentially fatal acute hemorrhagic cystitis (in up to 40% of patients), and thrombocytopenia, anemia, and leukopenia, which generally starts 7 days after exposure with a nadir at 10 to 14 days and recovery at 3 weeks. Less common effects (1% to 10%) in patients include facial flushing, headache, skin rashes, SIADH, renal tubular necrosis, and after rapid intravenous infusion, nasal congestion, runny eyes, rhinorrhea, sinus congestion, and sneezing.
- **RARE**: Rare but life-threatening side effects include cardiac effects (ie, congestive heart failure, cardiac necrosis, and hemorrhagic myocarditis), pulmonary effects (ie, interstitial pneumonitis and pulmonary fibrosis),

anaphylactic reactions, hepatotoxicity, electrolyte imbalances, renal injury, secondary malignancy, and toxic epidermal necrolysis. Cyclophosphamide is teratogenic (FDA pregnancy category D) and long-term use is associated with an increased risk of a variety of malignancies.

3.3 Rituximab:

3.3.1 Drug Nomenclature

- IDEC Pharmaceuticals code designation Rituxan®
- Generic Name: rituximab

3.3.2 Clinical Formulation:

Clinical supplies for this study will be manufactured by Genentech Incorporated in South San Francisco, CA.

Rituximab will be provided to the clinical sites packaged in single use 10 mL (100mg) and 50 mL (500mg) Type I glass vials at a concentration of 10 mg of protein per mL. The product is formulated in 7.35 mg/mL sodium citrate buffer, containing 7 mg/mL polysorbate 80, 9.0 mg/mL sodium chloride and Sterile Water for Injection. The pH is adjusted to 6.5.

Rituximab may be produced by the mammalian (Chinese Hamster Ovary) cell suspension culture in a nutrient medium containing 100 mg/mL of the antibiotic gentamicin. The antibiotic is not detectable in the final product.

3.3.3 Storage

Rituximab for clinical use should be stored in a secure refrigerator at 2-8°C.

3.3.4 Reconstitution and Dilution of Rituximab

Using a sterile syringe and a 21 gauge or larger needle, transfer the necessary amount of rituximab from the vial into a partially filled IV pack containing sterile, pyrogen-free 0.9% Sodium Chloride, USP (saline solution). The final concentration of rituximab should be 1 mg/mL. Mix by inverting the bag gently.

Caution should be taken during the preparation of the drug. Parenteral drug products should be inspected visually for particulate matter prior to administration. Preparations of rituximab containing visible particles should not be used. As with all parenteral drug products, aseptic procedures should be used during the preparation and administration of rituximab.

NOTE: DO NOT USE A VACUUM APPARATUS to transfer rituximab from the syringe to the infusion pack. DO NOT USE evacuated glass containers, which require vented administration sets, because this causes foaming when air bubbles pass through the solution.

3.3.5 Rituximab Side Effects: Common

- Cardiovascular: hypertension (all grades, 6%; grades 3-4, 1%); hypotension (all grades, 10%; grades 3 and 4, 1%)
- Dermatologic: pruritis

- Gastrointestinal: nausea, vomiting
- Neurologic: asthenia (non-Hodgkin's lymphoma, all grades, 26%; grades 3 and 4, 1%; rheumatoid arthritis, 2%), dizziness (all grades, 10%; grades 3 and 4, 1%), headache (all grades, 19%, grades 3 and 4, 1%), sensory neuropathy (30%)
- Other: fever (all grades, 53%; grades 3 and 4, 1%), shivering (all grades, 33%; grades 3 and 4, 3%)

Serious

- Cardiovascular: cardiac dysrhythmia, cardiogenic shock, heart failure, myocardial infarction, supraventricular arrhythmia, supraventricular tachycardia
- Dermatologic: drug-induced pemphigus, Lichenoid dermatitis, Stevens-Johnson syndrome, toxic epidermal necrolysis
- Gastrointestinal: bowel obstruction, gastrointestinal perforation
- Hematologic: anemia (all grades, 8%; grades 3 and 4, 3%), aplastic anemia, transient cytopenia, grades 3 and 4 (48%), hemolytic anemia, leukopenia (all grades 14%; grade 3 and 4, 4%), lymphocytopenia (grades 3 and 4, 40%) neutropenia (all grades, 14%; grade 3 and 4, 6%), thrombocytopenia (all grades, 12%; grade 3 and 4, 2%)
- Hepatic: relapsing type B viral hepatitis
- Immunologic: complication of infusion (first infusion, 77%); subsequent infusions, (14% to 30%), immune hypersensitivity reaction
- Neurologic: progressive multifocal leukoencephalopathy (rheumatoid arthritis, rare)
- Renal: nephrotoxicity
- Respiratory: obliterative bronchiolitis, pneumonitis, pulmonary fibrosis
- Other: infectious disease (all grades, 31%, grades 3 and 4, 4%), tumor lysis syndrome
- Hepatitis B virus (HBV) reactivation with fulminant hepatitis, hepatic failure, and death has been reported in some patients with hematologic malignancies treated with rituximab. The majority of patients received rituximab in combination with chemotherapy. The median time to the diagnosis of hepatitis was approximately 4 months after the initiation of rituximab and approximately one month after the last dose.

4.0 PATIENT ELIGIBILITY

4.1 Inclusion criteria:

1. Patients must have a diagnosis of CLL/SLL and be previously treated
2. Patients must have had FISH evaluation of leukemia cells within 3 months without intervening treatment demonstrating deletion 11q22-23
3. Patients must have an indication for treatment by 2008 IWCLL Criteria
4. Age \geq 18 years
5. ECOG/Zubrod performance status \leq 2
6. Adequate renal and hepatic function as indicated by all the following:
 - a. serum creatinine \leq 2 mg/dL AND;
 - b. alanine aminotransferase (ALT) \leq 2.5 times upper limit of normal AND;
 - c. total bilirubin \leq 2.5 times upper limit of normal

7. Patients must have an ANC $\geq 500/\mu\text{L}$, HGB ≥ 8 gm/dL, PLT count $\geq 20\text{K}/\mu\text{L}$, unless attributed to marrow infiltration with CLL
8. Patients must give written informed consent
9. Patients of childbearing potential (females who have not been postmenopausal for at least 12 consecutive months or who have not undergone previous surgical sterilization or males who have not been surgically sterilized) must be willing to practice birth control during the study

4.2 Exclusion Criteria:

1. Pregnant or breast-feeding females
2. Significant co-morbidity indicated by major organ system dysfunction
3. Active infection, uncontrolled with intravenous antibiotics
4. Uncontrolled autoimmune hemolytic anemia (AIHA) or immune thrombocytopenia purpura (ITP)
5. Treatment including chemotherapy, chemoimmunotherapy, monoclonal antibody therapy, radiotherapy, high-dose corticosteroid therapy (prednisone ≥ 60 mg daily, or equivalent), or immunotherapy within 3 weeks prior to enrollment or concurrent with this trial

5.0 TREATMENT PLAN

After patients provide informed consent, complete screening, complete all pretreatment evaluations, and eligibility is confirmed, they may begin treatment. Treatment will consist of combined Sapacitabine, cyclophosphamide, and rituximab.

Sapacitabine will be given at 350 mg oral flat dose, preferably 1 hour prior or 2 hours after a meal on Days 1,2, and 3 of each course. Cyclophosphamide 250 mg/m² will be administered IV 2 hrs following the dose of Sapacitabine on Days 1,2, and 3 of each course. Rituximab 375 mg/m² IV will be administered on Day 3 of Course 1, after cyclophosphamide, then at 500 mg/m² on Day 1, after cyclophosphamide for subsequent courses. Courses will be every 4 weeks as permitted by recovery of blood counts. The total number of courses given will be 2 courses beyond best response. Patients will be monitored closely for myelosuppression. The next course of treatment may begin 4 weeks after start of last course and when absolute neutrophil count (ANC) and platelet (PLT) count have recovered to within 20% of pretreatment levels or ANC $\geq 1,000/\mu\text{L}$ and PLT $\geq 75,000/\mu\text{L}$.

Patients who experience delayed recovery of neutrophils or platelets will have dose reduction. Delayed recovery will be defined as failure to recover counts to within 20% of pretreatment level by Day 42 or later for patients who begin treatment with baseline cytopenias and for patients who begin treatment with normal neutrophil and platelet counts, they must have < grade 2 cytopenia (IWCLL Criteria). Dose reduction may occur at the treating physicians' discretion for treatment-related, non-hematologic toxicity \geq grade 2 that occurs at any time during a treatment course. Dose reduction will be according to Table 1.

Table 1. Dose Reduction Schema for Course ≥ 2

Dose Level	Sapacitabine (PO dose, D=days)	Cyclophosphamide (IV dose, D=days)	Rituximab (IV dose, D=day, course > 1)
0	350 mg, D1-3	250 mg/m ² , D1-3	500 mg/m ² , D1
-1	300 mg, D1-3	200 mg/m ² , D1-3	500 mg/m ² , D1
-2	250 mg, D1-3	175 mg/m ² , D1-3	500 mg/m ² , D1
-3	200 mg, D1-3	150 mg/m ² , D1-3	500 mg/m ² , D1

Patients will be evaluated for response by 2008 IWCLL/NCI criteria before course 4, then after every 2 courses, and at end of treatment (2 months after last course) according to the schedule of events. Response assessment will include CT scan and bone marrow evaluations for patients considered in clinical CR. Non-hematologic toxicity will be evaluated using the National Cancer Institute Version 4.0 criteria. Patients will continue study treatment until 2 courses beyond best response; they will stop treatment early for disease progression, unacceptable toxicity, patient choice, or death.

- 5.1 Suggested premedications: Antiemetic (ondansetron 8 mg IV or equivalent) premedication will be given 30 min prior to cyclophosphamide. Premedication for rituximab will consist of 325-650 mg acetaminophen orally and 25-50 mg diphenhydramine hydrochloride oral or intravenous. Steroids may also be used at the discretion of the treating physician. Other premedications or modifications of the above may be appropriate based on the physician or patient experience.
- 5.2 Suggested supportive medications: Allopurinol is recommended for at least the first 14 days of course 1 for tumor lysis prophylaxis. Valacyclovir (or equivalent) is recommended for herpes virus prophylaxis and Bactrim DS (or equivalent) for PCP prophylaxis throughout treatment and for at least 3 months following completion of treatment. For patients who were previously exposed to hepatitis B and are seropositive, consideration should be given for lamivudine prophylaxis.

Neutrophil growth factor and erythrocyte growth factor may be used at the discretion of the treatment physician and according to appropriate standard of care guidelines.
- 5.3 Administration of rituximab: See institutional standard of care and package insert guidelines.
- 5.4 Patients achieving a stable disease, partial response or demonstrating continued response after 3 courses will continue on treatment. Responses will be evaluated every 2 courses beyond course 3 and patients will receive 2 additional courses beyond best response to complete treatment.
- 5.5 Patients demonstrating progressive disease after receiving 3 courses of treatment will come off study.
- 5.6 Dose adjustment to the next lower level may be made for pneumonia, sepsis, or other life-threatening infection or any grade ≥ 3 non-hematologic toxicity.
- 5.7 Carriers of hepatitis B should be closely monitored for clinical and laboratory signs of active HBV infection and for signs of hepatitis throughout their study participation.

6.0 PRETREATMENT EVALUATION

Patients will undergo screening to evaluate and confirm eligibility. Upon confirming eligibility, patients will proceed with treatment. Screening evaluation, including laboratory tests will be done within 3 weeks of starting treatment.

- 6.1 Screening will consist of medical history and physical examination and pertinent laboratories, including SMA12 (sodium, potassium, chloride, CO₂, BUN, creatinine, glucose, albumin, alkaline phosphatase, ALT, total protein, calcium, phosphorus, uric acid, total bilirubin, lactate dehydrogenase) and CBC with differential, as well as a pregnancy test (blood) for females of childbearing potential. This will confirm eligibility and provide baseline measurements of lymph node, spleen, and liver size and blood counts that will be used for response assessment.
- 6.2 Patients will have bone marrow aspirate and biopsy with samples sent for differential and morphology within 3 months of screening if no intervening treatment. Patients will be screened for hepatitis exposure and infection by serum HBcAb, HBsAb and AHBSAG. Patients will have their prognostic factors characterized on pretreatment blood or bone marrow including presence of cytogenetic abnormalities (FISH for 13q⁻, +12, 11q⁻, and 17p⁻), expression of ZAP70 and CD38, and serum β -2 microglobulin. If already known, the following prognostic factors do not need to be reevaluated since they are not expected to change: leukemia cell *IGHV* gene mutational status and ZAP-70 expression.
- 6.3 Any appropriate radiological and radioisotopic examinations should be performed as clinically indicated.
- 6.4 Optional blood (20 mL purple-top tube) and bone marrow (5 mL purple-top tube) will be taken to isolate and store pretreatment mononuclear cells, DNA, RNA, and plasma. For patients with WBC $\geq 10,000/\mu\text{L}$, 10 mL will be taken for pharmacodynamic studies as specified in Section 11.0. Not all samples will be collected on all patients at all time points.

7.0 EVALUATION DURING STUDY (TABLE 3)

Table 3. Schedule of Events

Tests and Evaluations	Screening Visit Day ≤ -21	C1 D1	C1 D2	C1 D3	C1 D4	C1* D8	C1* D15	C1* D22	C2-Σ* D1	C2-Σ D2	C2-Σ D3	C2-Σ* D15	Prior to C4	End of Tx
Informed consent	X													
Medical history	X													
Interval history									X*					
PE including VS	X								X*					X
HBcAb, HBsAb, AHBSAG	X													
Prognostic factors and sample for ATM function	X													
Pregnancy test (blood)	X													
Screening BM aspiration and biopsy	X													
Sapacitabine		X	X	X					X	X	X			
Cyclophos.		X	X	X					X	X	X			
Rituximab (mg/m ²)				375					500					
Adverse event screening	X					X	X	X	X			X		
CBC with diff, PLT	X	X				X	X	X	X*			X	X	X
SMA12	X	X				X	X	X	X*				X	X
Optional blood and BM samples (including PD samples)	X	X	X	X	X				X					X
Response assessment (IWCLL criteria***)													X**	2 mos after last course, then 6, 12, 24 mo****

BM=bone marrow evaluation; C=course; D=day; PE=physical examination; VS=vital signs; CBC=complete blood count; PK=pharmacokinetic; PD=pharmacodynamic; IWCLL=International Working Group for CLL; MRD=minimal residual disease; All courses are 4 weeks, depending on recovery of ANC and PLT; Tx=treatment; *indicates ± 3 days; **indicates response assessments after each 2nd course beyond course 3 and 2 months after last course; ***IWCLL criteria requires history and physical examination, blood count with differential, and for patients in clinical complete remission, confirmatory bone marrow aspirate, biopsy, evaluation for MRD, and CT scan of chest, abdomen, and pelvis to confirm CR; ****Beyond 24 months after last course, patients will be followed annually with history, physical examination, and blood counts; bone marrow examination and CT scans will be done as clinically indicated; Σ=last course

7.1 Patients will be followed with CBC, platelet count and differential weekly (±3 days) for the first course and q2 weeks (±3 days) during therapy thereafter. An SMA12 will be done weekly (±3 days) for the first course. For course 2 and beyond, SMA12 will be

done before each course and as clinically indicated. For labs done outside of MDACC phos, uric acid and LDH may be eliminated if normal on C1D8.

- 7.2 Before course 4, after every 2nd course and 2 months after last course a full evaluation for response assessment will be performed including history, physical examination, CBC with differential and platelet count to assess for clinical remission. Patients in clinical CR will have confirmatory bone marrow aspiration and biopsy with samples sent for differential, flow cytometry (4-color flow for MRD), and morphologic analysis and restaging CT scan of chest, abdomen, and pelvis. Last course of treatment will be 2 courses beyond best response.
- 7.3 Myelosuppression and associated complications are expected events during leukemia therapy and are part of the treatment success (marrow emptying of leukemia cells). Therefore, myelosuppression and associated complications such as fever, infections, bleeding, and related hospitalizations, will not be reported as individual adverse drug reactions (ADRs), but will be summarized in the annual safety and final reports. Only prolonged Grade 3-4 myelosuppression, as defined by the 2008 IWCLL criteria specific for leukemia, i.e., marrow cellularity <5% on day 42 or later (6 weeks) from start of therapy without evidence of leukemia, will be reported as ADR and considered in defining the maximum tolerated dose (MTD) and dose-limiting toxicity (DLT) of particular agents or regimens.
- 7.4 Repeat response assessments by physical examination, blood counts, and bone marrow evaluation, including for MRD, and CT scan to confirm CR will be done 6, 12, and 24 months after last treatment course until relapse. Evaluation for MRD may be done on blood. Blood or marrow for MRD evaluation may be taken by referring physician and mailed to MDACC. Follow-up will be annually thereafter with history, physical examination, blood counts, bone marrow examinations and CT scans will be done at the discretion of the treating physician for those visits. Patients will be followed until disease progression requiring alternative treatment or death.
- 7.5 Optional blood (10 ml purple- or yellow-top tube) will be taken to determine rituximab levels prior to rituximab dose with each course. Not all samples will be collected on all patients at all time points.

Samples will be delivered to:

Attention of Ruth LaPushin
MD Anderson Cancer Center
1515 Holcombe Blvd
T6.3849
Houston, Texas 77030
Phone: 713-792-3690

- 7.6 Optional blood (20 mL purple- or yellow-top tube) will be taken at response assessment (end of treatment) and follow-up visits after completion of treatment to monitor for immune reconstitution. Immune reconstitution samples will be evaluated for T cell and normal B cell populations, T cell receptor repertoire, T cell functional subsets defined by multi-color flow cytometry. Not all samples will be collected on all patients at all time points.

7.7

Information regarding concomitant medication will not be collected separately for patients treated on this study. Concomitant medication information is routinely collected and stored in Clinic Station (MDACC electronic medical record) and is routinely updated each patient visit to the MDACC out-patient clinic and during in-patient stays. Therefore, there is no need to collect this information separately.

8.0 RESPONSE CRITERIA, TOXICITY EVALUATION, AND EVENT REPORTING

Responses will be evaluated by the updated 2008 IWCLL Response Criteria (Table 5), including staging with CT scan of chest abdomen and pelvis, and bone marrow evaluation for MRD for patients in CR⁴. Bone marrow will be evaluated for MRD by 4-color flow cytometry. Lymph nodes 1.5 cm in diameter or smaller on CT scan will be considered normal and consistent with CR.

Table 5. 2008 IWCLL Response Criteria Summary

SITE	CR	PR
Nodes	None	≥ 50% decrease
Liver/Spleen	Not palpable	≥ 50% decrease
Symptoms	None	N/A
PMN	>1,500/μl	> 1,500/μl or >50% improvement from baseline
Platelets	>100,000/μl	>100,000/μl or 50% improvement from baseline
Hemoglobin (non-transfused)	>11.0 gm/dl	>11.0 g/dl or >50% improvement from baseline
Lymphocytes	<4,000/μl	>50% decrease
Bone Marrow aspirate	<30% lymphocytes	N/A for PR
Bone Marrow biopsy	No lymphocyte infiltrate	< 30% lymphocytes with residual disease on biopsy for nodular PR
Bone Marrow aspirate flow	Research	N/A
CT scan of chest, abdomen, pelvis	Lymph nodes <1.5 cm	Lymph nodes ≥ 50% reduced (sum product of lymph nodes

Non-hematologic toxicity will be described and graded by the Common Terminology Criteria for Adverse Events (CTCAE) Version 4. Hematologic toxicity will be graded according to the 2008 IWCLL criteria for grading (Table 4)⁴. Adverse events will be documented in the medical record and entered into the case report form according to the Leukemia-Specific Adverse Event Recording and Reporting Guidelines (Appendix D). PDMS/CORE will be used as the electronic case report form for this protocol. The Investigator or physician designee is responsible for verifying and providing source documentation for all adverse events and assigning the attribution for each event for all subjects enrolled on the trial. The investigator will sign and date the PDMS CRF toxicity pages for each patient at the completion of each course. Following signature, the CRF will be used as source documentation for the adverse event attribution.

Table 4. – Grading of Myelosuppression

Grade	Decrease in PLT* or HGB** (nadir) from pretreatment value, %	Absolute neutrophil count (ANC)/μl*** (nadir)
0	< 10%	\geq 2000
1	11 – 24%	\geq 1500 – < 2000
2	25 – 49%	\geq 1000 – < 1500
3	50 – 74%	\geq 500 – < 1000
4	\geq 75%	< 500

Death occurring as a result of toxicity at any level of decrease from pretreatment will be recorded as grade 5.

* PLT counts must be below normal levels for grades 1-4. If, at any level of decrease, the PLT count is < 20K/ μ l, this will be considered grade 4 toxicity, unless there was severe or life-threatening low initial PLT count (< 20K/ μ l) pretreatment, in which case the patient is not evaluable for toxicity referable to PLT count.

** HGB levels must be below normal levels for grades 1-4. Baseline and subsequent HGB determinations must be performed before any given transfusions.

*** If the ANC reaches <1000/ μ l, it should be judged to be grade 3 toxicity. If the ANC was <1000/ μ l before therapy, the patient is not evaluable for toxicity referable to the ANC.

Serious Adverse Event Reporting (SAE) for M. D. Anderson-Sponsored IND Protocols

An adverse event or suspected adverse reaction is considered “serious” if, in the view of either the investigator or the sponsor, it results in any of the following outcomes:

- Death
- A life-threatening adverse drug experience – any adverse experience that places the patient, in the view of the initial reporter, at immediate risk of death from the adverse experience as it occurred. It does not include an adverse experience that, had it occurred in a more severe form, might have caused death.
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
- A congenital anomaly/birth defect.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic

bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse (21 CFR 312.32).

- Important medical events as defined above, may also be considered serious adverse events. Any important medical event can and should be reported as an SAE if deemed appropriate by the Principal Investigator or the IND Sponsor, IND Office.

- All events occurring during the conduct of a protocol and meeting the definition of a SAE must be reported to the IRB in accordance with the timeframes and procedures outlined in "The University of Texas M. D. Anderson Cancer Center Institutional Review Board Policy for Investigators on Reporting Unanticipated Adverse Events for Drugs and Devices". Unless stated otherwise in the protocol, all SAEs, expected or unexpected, must be reported to the IND Office, regardless of attribution (within 5 working days of knowledge of the event).

- **All life-threatening or fatal events**, that are unexpected, and related to the study drug, must have a written report submitted within **24 hours** (next working day) of knowledge of the event to the Safety Project Manager in the IND Office.

- Unless otherwise noted, the electronic SAE application (eSAE) will be utilized for safety reporting to the IND Office and MDACC IRB.

- Serious adverse events will be captured from the time of the first protocol-specific intervention, until 30 days after the last dose of drug, unless the participant withdraws consent. Serious adverse events must be followed until clinical recovery is complete and laboratory tests have returned to baseline, progression of the event has stabilized, or there has been acceptable resolution of the event.

Additionally, any serious adverse events that occur after the 30 day time period that are related to the study treatment must be reported to the IND Office. This may include the development of a secondary malignancy.

Reporting to FDA:

- Serious adverse events will be forwarded to FDA by the IND Sponsor (Safety Project Manager IND Office) according to 21 CFR 312.32.

It is the responsibility of the PI and the research team to ensure serious adverse events are reported according to the Code of Federal Regulations, Good Clinical Practices, the protocol guidelines, the sponsor's guidelines, and Institutional Review Board policy.

9.0 REMOVAL FROM STUDY

9.1 Progressive or Relapsed Disease

Progressive disease (PD) will be characterized by at least one of the following:

- a. $\geq 50\%$ increase in the sum of the products of at least two nodes on two consecutive examinations two weeks apart (at least one node must be ≥ 2 cm). Appearance of new palpable lymph nodes.
- b. $\geq 50\%$ increase in the size of liver and/or spleen as determined by measurement below the respective costal margin; appearance of palpable hepatomegaly or splenomegaly, which was not previously present.
- c. $\geq 50\%$ increase in absolute number of circulating lymphocytes and at least 10,000/ μ l.

9.2 Patient request.

9.3 Active HBV infection or hepatitis.

10.0 STATISTICAL CONSIDERATIONS

10.1 Primary Endpoint and sample size

The primary endpoint is overall response rate (ORR). A maximum of 40 patients with deletion 11q22-23 will be enrolled into this study. A sample size of 40 ensures that, if the trial continues to completion, a posterior 90% credible interval (CI) of response rate will be (0.49, 0.74), assuming a response rate of 0.625 (25/40 in these 40 patients).

10.2 Safety lead-in

Sapacitabine was previously studied in 2 phase I trials for patients with solid tumors and in 1 phase I trial for patients with AML/MDS; all 3 trials evaluated different schedules and doses. In the solid tumor trials, myelosuppression was the DLT and in the AML/MDS trial, GI toxicity was DLT that defined the MTD. There are no trials yet combining Sapacitabine with other agents, therefore, there will be a safety lead-in to this trial. The schedule of Sapacitabine in this trial is daily for 3 days of each 4-week course, which is significantly less frequent administration compared to all phase I trials. The chosen Sapacitabine dose in this combination is 350 mg flat dose, which less than half the MTD dose identified in the AML/MDS trial (where the drug was administered on a more frequent schedule). This trial combines Sapacitabine with cyclophosphamide and rituximab, which may contribute to toxicity or have unforeseen associated toxicity. Therefore, there will be a lead-in phase to this trial, where 3 patients will be enrolled, treated, and monitored for toxicity for at least 4 weeks during course 1. If there are no unforeseen toxicities and no "excessive myelosuppression", defined as Grade ≥ 3 myelosuppression with delayed recovery beyond day 42 of course 1, then enrollment will

proceed without further pause. If 1 of 3 patient experiences excessive myelosuppression, then an additional 3 patients will be enrolled at dose level 0 and evaluated for at least 4 weeks during course 1. If >2 of the 6 patients enrolled at dose level 0 experience excessive myelosuppression, then enrollment will be held pending review of all cases and discussion of rational strategy for dose reduction. If exactly 2 of 6 patients experience excessive myelosuppression during course 1, then the next 3 patients will be enrolled at dose level -1. If any of these 3 patients experiences excessive myelosuppression, then enrollment will be suspended pending review of all cases and discussion of rational strategy for dose reduction. Patients who experience excessive myelosuppression during the lead-in phase may proceed to their next course of treatment with -1 level dose reduction (Table 1), when their counts have recovered to acceptable levels as defined elsewhere in this protocol.

10.3 Interim analysis on efficacy

This trial will follow a Bayesian sequential monitoring design⁵⁴ with a recommendation to stop the trial if the new treatment is unfavorable in comparison with historical data. In particular, the following decision criterion will be applied. Let $P(\text{exp})$ and $P(\text{hist})$ be probability of response in the experimental regimen and historical data, respectively, stop the trial for lack of efficacy if:

$$\text{Prob}\{P(\text{exp}) > P(\text{hist}) + 0.15 \mid \text{data}\} < 0.01$$

In a historical data set, the overall response rate is 50% in 103 patients. Thus, the prior distribution is $\text{beta}(51, 52)$ based on 103 patients for the historical data, and $\text{beta}(1, 1)$ for the new experimental regimen. Following this rule, the trial will be terminated due to futility if $[\# \text{ responses}]/[\# \text{ patients evaluated}] \leq 2/10, 7/20, 12/30$. For purposes of futility, patients will be considered evaluable if they have received at least 6 courses of treatment. If they were removed from treatment prior to 6 courses for treatment failure or toxicity, they will also be evaluable for futility. The operating characteristics for the efficacy are summarized in Table 6, based on a 1000 simulations study. For example, the probability of early stopping will be 0.93 when true response rate is 0.3; this probability will be only 0.04 if the true response rate is 0.6.

Table 6. Simulation study with maximum 40 patients:

Scenario		
True overall response rate	Prob. of early stop	Total Samples (25%, 75%)
0.30	0.93	20 (10, 20)
0.40	0.63	30 (20, 40)
0.50	0.23	40 (40, 40)
0.55	0.10	40 (40, 40)
0.60	0.04	40 (40, 40)
0.65	0.01	40 (40, 40)

10.4 Interim analysis for toxicity

The probability of toxicity (non-hematologic Grade 3 or 4) will be monitored based on a beta-binomial distribution by assuming a priori probability of toxicity following $\text{beta}(1,1)$. Accrual to the trial will be suspended if $\text{Prob}(\text{toxicity} > 0.25 \mid \text{data}) > 0.8$. Following this rule, accrual to the trial will be suspended if $[\# \text{ patients with toxicity}]/[\# \text{ patients evaluated}] \geq 4/10, 5/15, 7/20, 8/25, 10/30$, or $11/35$. All cases will be reviewed and the

PI will make a determination if the trial will be terminated or may resume accrual at reduced dose of study drugs. The operating characteristics for toxicity are summarized in Table 7.

Table 7. Operating characteristics based on 1000 simulation study

true Prob(tox)	Pr(stop)	Median # Pts (25%, 75%)
0.05	0.0	40 (40, 40)
0.15	0.09	40 (40, 40)
0.25	0.43	40 (15, 40)
0.35	0.80	15 (10, 25)
0.45	0.97	10 (10, 10)

10.5 Statistical Analyses

The primary endpoint is overall response rate and the secondary endpoints include CR rate, time-to-treatment failure, time-to-progression, and overall survival. We will also compare the outcomes between two sub-groups (with or without ATM function). Demographic and baseline laboratory results will be summarized using descriptive statistics, including means with standard deviations, or medians with ranges, histograms and box-plot. Fisher's exact test and Wilcoxon rank test will be used in the data analyses of categorical and continuous variables, respectively. Survival or times-to-failure and time-to-progression functions will be estimated using the Kaplan-Meier method. The two-sided log-rank test will be used to assess the differences of time to events between groups. Toxicity will be reported by type, frequency and severity. Worst toxicity grades per patient will be tabulated for selected adverse events and laboratory measurements.

11.0 PHARMACODYNAMIC ENDPOINTS

These investigations will be performed in Dr. Plunkett's laboratory. Patients with more than 5,000 WBC/ μ l in peripheral blood who agree to participate in pharmacodynamic investigations will be evaluated. Not all samples will be collected on all patients at all time-points.

Please page Yuling Chen (713-404-2550) or Min Fu (713-606-2212) or call 713-792-3336 to inform of registration and treatment date and to coordinate blood sample pickup.

Blood samples (10 ml) will be collected at the following times if there are circulating leukemia cells ($\geq 5,000$ WBC/ μ l) for correlative studies. If there are no circulating leukemia cells, then the sample will not be taken.

- **Screening and Course 1, Day 1 before treatment begins**
- **Course 1, Day 2**
- **Course 1, Day 3**
- **Course 1, Day 4**

These samples will be processed to collect plasma and cells. Plasma will be stored. Cells will be processed for the following endpoints:

1. Pretreatment (screening) cell samples will be used to evaluate for ATM function. This will be evaluated by determining the phosphorylation response of Nbs1 and Smc1 to radiation in CLL cells isolated from these samples will be determined by comparison of the ratios of immunoblot band intensities of the phosphorylated

protein divided by band intensity of the total protein before and after irradiation. The results will be averaged to generate standard values for the phosphorylation response to irradiation in samples with two ATM alleles (normal FISH and function). These values will be used as a denominator for assessing the ATM function of patients with deletion 11q22-23 entered on the clinical trial.

2. Development of double-strand breaks after Sapacitabine and cyclophosphamide therapy requires a cycle of DNA replication. To evaluate proliferation and the actions of Sapacitabine, the proliferative compartment will be determined in circulating CLL cells by changes in the CD38⁺, CD5⁺, and low expression of CRCX4 by multicolor flow cytometry⁵⁵. Evidence for double strand break formation will be assessed by the formation of Rad51 foci, visualized by confocal microscopy⁵⁶. We expect that cytoreductive therapy will trigger a homeostatic proliferation in surviving CLL cells that will be associated with greater strand break action in CLL that lacks ATM function.
3. Changes in apoptosis will be evaluated in circulating leukemia cells before and during treatment by measuring binding of Annexin V and by mitochondrial permeability changes in freshly obtained patient samples. For comparative analyses, each patient serves as his or her own control. Correlations will be sought between these laboratory endpoints and cytoreduction and/or clinical response to therapy.

12.0 DATA CONFIDENTIALITY PLAN

All laboratory and clinical data gathered on this protocol will be stored in a password-protected database. All patient information will be handled using anonymous identifiers. Linkage to patient identity is only possible after accessing a password-protected database. Access to the database is only available to individuals directly involved in the study.

Information gathered for this study will not be reused or disclosed to any other person or entity, or for other research. Once the research has been completed, identifiers will be retained for as long as is required by law and by institutional regulations, and at that point will be destroyed.

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