Mayo Clinic Cancer Center

A Phase I/II Clinical Trial of Lenalidomide in Combination with AT-101 for the Treatment of Relapsed B-cell Chronic Lymphocytic Leukemia (B-CLL)

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Drug Company Supplied: *AT-101 (IND #113614)*
Commericial Agent: *Lenalidomide*

√Study contributor(s) not responsible for patient care.

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MCCC Addendum 1	February 5, 2015

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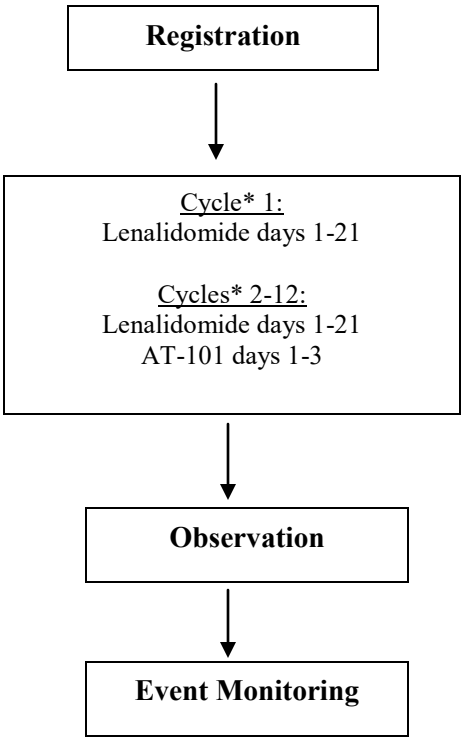
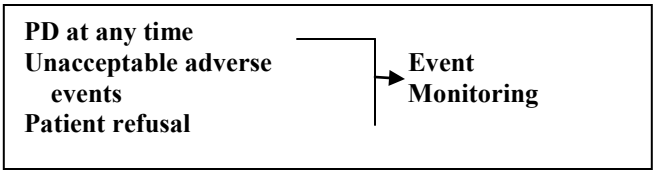
Schema

Phase I only: Prior to discussing protocol entry with the patient, call the MCCC Registration Office ([redacted]) for dose level and to insure that a place on the protocol is open to the patient.





*Cycle length = 28 days Cycles 1-11; Cycle length = 49-56 days Cycle 12 (or last cycle of treatment if patient discontinues treatment before Cycle 12)

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1.0 Background

Overview

CLL is a lymphoproliferative malignant disorder that remains incurable in majority of the patients using standard therapeutic approaches. It is manifested by progressive accumulation of functionally incompetent, mature looking lymphocytes in the blood, bone marrow and lymphoid tissues. It is a heterogeneous disease with a variable clinical spectrum ranging from an indolent variety (Rai Stage 0) where the patients enjoy a long-term survival of over 12 years vs. advance stage disease (Stage III/IV) where the median survival is about 2.5 years [1]. Treatment options have included alkylating agents (Chlorambucil, Cyclophosphamide) or purine analogues (fludarabine, Pentostatin) and/or monoclonal antibodies (rituximab, alemtuzumab, ofatumumab). These agents are often used in combination regimens with variable responses. Treatment of this disease remain palliative as the clinical course is punctuated with frequent relapses and eventual death of the patient from progressive disease. New agents are thus needed for the treatment of relapsed and refractory CLL with curative aim [2]. In reviewing the natural history of this disease it is clear that this aim is unlikely until complete remission can be achieved in higher proportions of this patient population [3].

Treatment

Due to the incurable nature of the disease and limited therapeutic options, treatment of CLL is often only instituted when patients develop symptomatic or persistently progressive disease. Criterion to consider treatment intervention is outlined in the IWCLL/Hallek, December 2008 formulation (see Appendix IV).

Chemotherapy in CLL

Initial treatment for CLL patients that require therapy[4] include either (a) single agent alkylating agent (chlorambucil) or a (b) purine analogue (fludarabine or pentostatin). Compared to chlorambucil, higher overall response rate (ORR) and complete responses are observed with fludarabine though no superiority in overall or progression free survival was noted in randomized studies.[5,6] Thus, up until very recently, the standard of care for this disease remained the use of single agent oral Chlorambucil or Fludarabine with complete remission (CR) rate of 4% and 20% respectively. Kanti Rai reported on the CALGB randomized Chlorambucil vs. Fludarabine vs. the combination of Fludarabine and Chlorambucil [6]. Among the 170 patients treated with fludarabine 20% achieved a CR while 43% with a partial remission (PR). The median duration of remission and the median progression free survival in this group was 25 and 20 months respectively. Despite improvement in the ORR no survival benefit was reported.

Combination of these agents with steroids, and or other chemotherapy agents (such as vincristine or an anthracycline) also did not result in improved survival. [7-9] Importantly, even early disease stage patients treated with these agents did not alter the overall clinical outcome. Despite evaluation of various combination regimens purine analogues are the most active agents in patients with CLL, yielding higher overall and complete response rates. [6,10,11] Eventually all patients with CLL will relapse. Although retreatment with fludarabine can result in disease, especially in those with an initial clinical response lasting for more than 1 year, continued treatment with fludarabine is difficult due to cumulative marrow toxicity resulting in prolong cytopenia and inability to further treat the patient. Patients who do not achieve durable remission after first fludarabine therapy have only a modest response with a second round of fludarabine [12]. For these patients combination with alkylating agents (cyclophosphamide) has been used with improved outcome [10,13,14]. Various investigators reported combination of fludarabine with cyclophosphamide as salvage regimen in patients with CLL.[14,15].

Monoclonal antibodies and chemoimmunotherapy

Rituximab is a chimeric humanized monoclonal antibody that showed an ORR of approximately 50-60% in patients with relapsed and refractory low-grade non-Hodgkin's lymphoma [16-18]. Since CLL is a B-cell lymphoproliferative disease with expression of CD20, rituximab was investigated for the treatment of CLL. Interestingly, despite expression of CD-20 on the CLL cells, single agent rituximab (on standard dosing schedule) failed to demonstrate any meaningful clinical response. Though more frequent dosing i.e., thrice weekly for 4 weeks (at 375mg/m^2 per dose) showed a better response rate 45% (3% CR, 42% PR) with a median duration of response of 10 months [19]. Higher doses may have more anti-tumor efficacy but are cost prohibitive, and the true long-term benefit remains undetermined. [20]

While results of single agent rituximab in CLL were disappointing, combination with fludarabine (chemoimmunotherapy) were impressive.[21,22] Clinical experience of combination of rituximab with fludarabine, or fludarabine and cyclophosphamide demonstrated higher rates of complete remission [14,19,20,23-27]. Overall response rates of fludarabine + rituximab combination are in the range of 90% with 47% CR [24,28]. This strategy of chemoimmunotherapy though yield higher response rates, is often associated with significant toxicity and morbidity to the patients. Despite the higher response rates and increase rates of reported CR, all patients eventually relapse and die of progressive disease or its complications. Nevertheless, the chemoimmunotherapy approach is now the most commonly used front-line treatment approach in CLL. Despite these high overall and complete response rates all patients eventually relapse and develop resistance to therapy. For these patients the only approved therapy is alemtuzumab.

Alemtuzumab

It is a humanized monoclonal antibody that targets CD52 and demonstrated clinical response in over 30% of CLL patients with relapsed or refractory disease. Again, the CR rate remains low (<5%). Several clinical trials are investigating combination of alemtuzumab with chemotherapy to improve upon the number of patients achieving a CR. An important and limiting side of alemtuzumab has been severe immunodeficiency resulting from depletion of both T and NK cells along with B cells. This often results in infectious complications and has limited its use in the community.

Immunomodulating agents in CLL

Cytokines which have been shown in vitro to function as survival factors for CLL lymphocytes include: interleukin-2 (IL-2), IL-4, IL-6, IL-8, IL-13, basic fibroblast growth factor (bFGF), tumor necrosis factor-alpha (TNF-α), interferon-alpha (IFN-α), and IFN-α [29,30]. Increased intracellular and plasma levels of bFGF have been demonstrated in patients with CLL and have been correlated with stage of disease. [31,32] CLL lymphocytes with high levels of bFGF are more resistant to fludarabine, and exogenously added bFGF impairs fludarabine induced apoptosis, possible through the induction of the anti-apoptotic protein bcl-2. [30,33]

Immunomodulating agents (thalidomide and lenalidomide) are a new class of anti-cancer agents with antitumor activity in multiple myeloma and myelodysplastic syndrome. The exact mechanism of action of thalidomide remains unclear though investigators have reported its effects on down regulation of IL-6, IFN-α, VEGF and bFGF. It has been reported to alter the bone marrow microenvironment of patients with multiple myeloma by changing the cytokine milieu thus rendering the environment unfavorable for tumor growth and progression.

We investigated thalidomide in combination with fludarabine for treatment of patients with newly diagnosed CLL. The phase I part of this study did not demonstrate and dose limiting toxicity of thalidomide (up to 300mg daily dose) when combined with standard dose fludarabine. The Phase II part of the study to date had 30 patients enrolled. Preliminary results of this study were reported and demonstrate overall response rate of 100% (vs. 43% with fludarabine alone – historical control). The complete response rate is over 70% (vs. 23% with fludarabine alone – historical control). An interesting observation was a sudden decrease in absolute lymphocyte counts in peripheral blood with single agent thalidomide prior to instituting fludarabine. These findings encouraged us to investigate lenalidomide, a more potent derivative of thalidomide. Like thalidomide, lenalidomide too is reported to stimulate T-cells proliferation and activation. It is noted to be 50-2000 times more potent than thalidomide in stimulating T-cell proliferation and about 50-100 times more effective in increasing the production of IL-2 and INF-γ. It is also noted to decrease the production of IL-6, TNF-α, VEGF, bFGF and IL-1β. Recently, lenalidomide was approved for the treatment of patients with multiple myeloma (another B-cell malignancy) as well as myelodysplastic syndrome with deletion 5q.

Lenalidomide in CLL

We hypothesized that lenalidomide with its ability to modulate the cytokine milieu and its immune stimulatory properties will be able to modulate the CLL cell microenvironment and thus compromise CLL survival. Preclinically our group had also observed that lenalidomide augments anti-lymphoma activity of rituximab in mouse xenograft model. Thus we initiated a phase II study investigating the antitumor activity of lenalidomide alone in patients with relapsed or refractory B-CLL. Those patients who would develop disease progression on lenalidomide alone will then have rituximab added to lenalidomide for additional 6 cycles of treatment.

In the first report of lenalidomide single agent in CLL we observed that over 47% of the patients who receive single agent lenalidomide demonstrated a clinical response and among these 7% achieved a complete remission as per the 1996 NCI-WG Cheson criteria (median follow-up of 1 year). Additionally, 4 of the 5 patients who achieved a complete response actually were noted to have a molecular response in the peripheral blood and bone marrow.

A unique side effect of lenalidomide that was also observed with thalidomide was the tumor flare reaction. Clinically this presented with sudden onset tender enlargement of the disease involved lymph nodes, spleen or liver. This was often associated with low-grade fever, rash and in some cases actually an increase in the absolute lymphocyte counts. Some people also complained of pain in bones. Typically this was a first cycle phenomenon with a median duration of 14 days (range 1-21 days) and was suggestive of an immune activation syndrome. Interestingly, severity of the tumor flare tends to correlate with the quality of clinical responses observed.

Correlative studies conducted revealed several interestingly findings: **First** we observed that *in vitro* lenalidomide have no direct proapoptotic activity on B-CLL cells, **secondly** we observed that patients who had normal or high baseline levels of NK-cells tend to have a better quality clinical responses and that this correlated with the severity of tumor flare reaction, **thirdly** we observed upregulation of costimulatory molecules (CD80, CD86 and CD40) on B-CLL cells upon treatment with lenalidomide *in vitro* or *in vivo* (samples obtained from patients before and after treatment with lenalidomide) and **lastly** we observed that lenalidomide down regulated p-Akt and p-Erk1/2 but not Bcl-2 or Bcl-xl in B-CLL cells *in vitro* or *in vivo* (samples obtained from patients before and after treatment with lenalidomide).

While the clinical responses observed with single agent lenalidomide are encouraging in the relapsed and refractory population treated, most patients remained in partial remission and only a limited number of patients were able to achieve complete remission. Thus lenalidomide based therapeutic approach needs to be further optimized possibly using combination with other therapeutic agents. To this effect our correlative studies are instrumental in defining rationale combination for further investigations.

Bcl-2 is an important therapeutic target in B-CLL

The antiapoptotic protein Bcl-2 is over-expressed in all cases of CLL and enhances the leukemia-cell survival and resistance to cell-death caused by anti-cancer drugs or immune therapy. This makes Bcl-2 is an attractive therapeutic target in CLL. CLL has been classically understood as a disease more of impaired apoptosis than excessive proliferation. B-CLL cells have numerous defects in the normal apoptotic pathways. Prominent among these are abnormalities involving members of the Bcl-2 family. This family includes proteins, such as Bcl-2, Bcl-xL, Bcl-w, Mcl-1, and A1, which inhibit apoptosis; and other proteins, such as Bax, Bak, and others. Inhibition of apoptosis by Bcl-2 appears principally due to its ability to bind the shared BH3 domain of proapoptotic members, thereby inactivating the latter. Disruption of this binding, in turn, increases a cell's susceptibility to undergo apoptosis.[34] Overexpression of the anti-apoptotic protein Bcl-2 has been observed in CLL B cells,[35-37] as has an increased ratio of Bcl-2 to the related proapoptotic protein, Bax. Increased Bcl-2 expression and an increased Bcl-2/Bax ratio have further been reported to be associated with resistance of CLL B cells to chemotherapeutic agents *in vitro* and with poor clinical prognosis.[38-41] Also, apoptosis of CLL-B cells has been reported to be associated with conformational changes of Bax and Bak.[42,43] Presence of a common polymorphism in the promoter region of the *bax* gene has been reported to be associated with lower Bax protein levels, higher Bcl-2/Bax and Mcl-1/Bax ratios, and shortened patient survival in CLL.[44] In a recently reported prospective clinical trial of standard first-line therapy of patients with CLL, two factors—elevated expression of Bcl-2 protein or presence of a p53 abnormality (either as a result of p53 mutation or del 17p)—were associated with a reduced likelihood of complete response to treatment[45]. The negative association between these two factors and treatment response was stronger than for other important prognostic factors in CLL, including the expression of another antiapoptotic member of the Bcl-2 family, Mcl-1. A small molecule BH3 mimetic, designed to inhibit the binding of Bcl-2 to proapoptotic family members, increased the susceptibility of CLL B cells to apoptosis,[45] as did treatment with an antisense oligonucleotide against Bcl-2,[46] while an antisense oligonucleotide against Bax reduced the susceptibility of CLL B cells to apoptosis.[47] Adding a Bcl-2 antisense oligonucleotide to standard therapy of patients with CLL increased their likelihood of achieving a complete response or nodular partial response in a

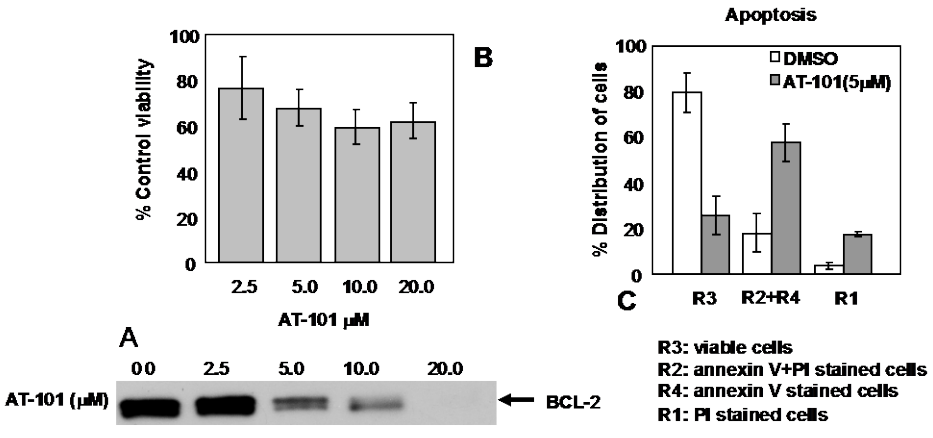


Figure 1: Effect of AT-101 on Bcl-2 expression an B-CLL cell survival: (A) AT-101 treatment decrease Bcl-2 expression in CLL cells in a dose dependent manner. (B) AT-101 treatment decrease the viability of CLL cells in a dose dependent manner and this is associated with induction of apoptosis (C).

prospective, randomized trial, although the trial design did not permit an assessment of the effects of treatment on Bcl-2 function.[48] Mcl-1 expression has also been associated with reduced response of CLL to therapy.[49-51]

Combining lenalidomide with Bcl-2 targeting drug

Since our correlative studies fail to demonstrate any effect of lenalidomide on Bcl-2 or Bcl-xl, an inhibitor of Bcl-2 with an acceptable safety and dosing profile could have a potentially beneficial effect when combined with lenalidomide for the treatment of B-CLL patients. To investigate this we conducted preclinical studies summarized that are summarized below:

Preclinical evaluation of lenalidomide in combination with AT-101

AT-101, a pan-Bcl-2 inhibitor, induces apoptosis in CLL: AT-101 is a novel, orally active BH3-mimetic that inhibits the activity of Bcl-2-family-member proteins including Bcl-2, Bcl-xL, and Mcl-1 inducing death in CLL cells. We evaluated the effect of AT-101 on Bcl-2 expression and cell survival in our CLL cell line (MO1043) and primary CLL cells obtained from patients and noted a dose dependent decrease in Bcl-2 levels **(Fig 1)**. AT-101 decreased Bcl-2 expression and cell viability of the MO1043 cell line in a dose dependent manner and this effect was validated in primary CLL cells from patients.

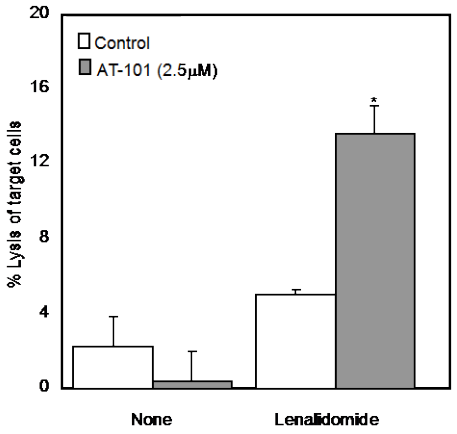


Figure 2. AT-101 primes the CLL cells for increased antibody dependent cell mediated cytotoxicity (ADCC) by lenalidomide activated effector cells: PBMCs (containing effector cells) from healthy donors were treated with 1μM lenalidomide for 48 hours and mixed with MO1043 CLL cells that were pre-treated with 2.5μM AT-101 in presence of 10mg/ml of rituximab for 4 hours. Significant increase in lysis was seen when target cells were pre-treated with AT-101. (* p<0.01)

Bcl-2 downregulation by AT-101 enhance immune killing mediated by lenalidomide: We then investigated if Bcl-2 downregulation with AT-101 can help enhance immune cell directed killing. We treated normal human PBMC (containing immune effector cells) with lenalidomide (1 μM for 48 h) and used them as effector cells to kill the MO1043 target cells that were pretreated with AT-101 (2.5 μM AT-101 for 4 h) in ADCC assay with 50:1 ratio for 4 h. We noted a significantly increased lysis of target leukemic cells when the effector cells were pretreated with lenalidomide (**Fig 2**). This suggests that downregulation of Bcl-2 with AT-101 can enhance susceptibility of leukemic cells to immune mediated killing and affirms the rationale to combine lenalidomide (an immune stimulatory agent) with AT-101 a Bcl-2 targeting agent for the treatment of CLL.

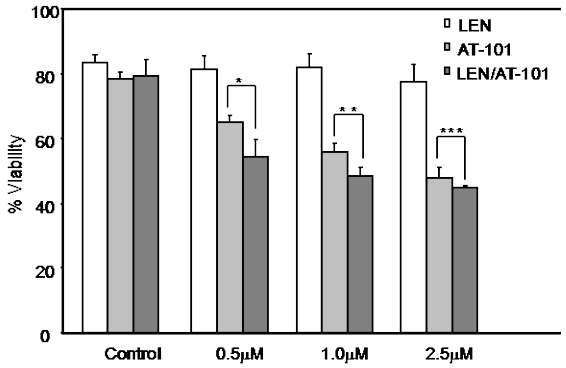


Figure 3. Impact of AT-101 on antiproliferative effect of lenalidomide in MO1043 CLL cells: MO1043 cells were treated with increasing concentrations of AT-101, lenalidomide (LEN) or both and cell viability was determined at 48h. In combination treatments, cells were treated with 1mM Lenalidomide for 48h before treating with increasing concentration of AT-101. Lenalidomide alone had minimal effect on cell proliferation while a significant decrease in leukemia cell viability was noted with the combination of lenalidomide + AT-101. *p=0.02, **p=0.05; ***p=0.2.

Lenalidomide can also enhance anti-CLL effects of AT-101: lenalidomide is not directly cytotoxic to CLL cells, however it can modulate the phenotype of the leukemic blasts. It is thus possible that lenalidomide may engage other mechanism(s) (independent of immune effector cells) that can cooperate with AT-101 and enhance CLL cell killing. To evaluate if lenalidomide treatment sensitizes CLL cells to AT-101

induced death in an immune independent manner, MO1043 CLL cell line was treated with increasing concentration of AT-101, lenalidomide and combination of both. As anticipated, in the absence of immune cells, lenalidomide did not have any significant effect on the viability of leukemic cells (determined by the trypan blue assay) while AT-101 demonstrated a dose dependant decrease in cell viability. Interestingly, pretreatment of leukemic cells with lenalidomide significantly enhanced the anti-CLL effects of AT-101 (**Fig 3**). This may suggest lenalidomide's effect on other prosurvival pathway(s) such as the Akt, Erk1/2, which independently may not be sufficient to kill CLL cells but in conjunction with downregulation of another survival pathways (Bcl-2) may compromise survival.

Summary of preliminary data and rationale for clinical trial proposal: We have found that lenalidomide is clinically active in patients with CLL. The anti-leukemic effect of lenalidomide is mediated through activation of a robust immune response that is dependent upon NK and T cells and clinically manifests as a flare reaction. The intensity of this immune reaction correlates with pretreatment levels of immune effector cells and with the depth of response. The maximal clinical benefit that can be harnessed with lenalidomide is truncated by the compromised immunity (dysfunctional T and NK cells), known to exist in CLL patients. An important component in undermining the efficacy of the anti-leukemic immune response is the inherent molecular makeup of the CLL cell and the presence of the antiapoptotic Bcl-2 protein. Since lenalidomide does not have any effect in decreasing Bcl-2 levels in CLL cells, we hypothesized that therapeutic downregulation of the antiapoptotic protein Bcl-2, may help maximize the killing potential of immune effector cells that are activated by lenalidomide. AT-101, an orally available BH-3 mimetic, was investigated and demonstrated effective downregulation of Bcl-2. The anti-leukemic effect of AT-101 in primary CLL cells revealed that AT-101 was particularly more effective in cell that had higher basal Bcl-2 protein levels. Furthermore, downregulation of Bcl-2 in CLL cells *in vitro* enhanced the killing potential of lenalidomide activated immune cells. Conversely, pretreatment of CLL cells with lenalidomide enhanced the cytotoxicity of AT101 in an immune cell independent manner. Thus our preliminary findings support the role of a Bcl-2 targeting agent(s) in augmenting clinical efficacy of immune directed therapies such as lenalidomide. The proposed clinical trial will therefore investigate the feasibility of a novel combination of an orally available Bcl-2 (AT-101) in combination with oral lenalidomide as a potential path for strategic development of AT-101.

Racemic Gossypol

Racemic gossypol has been consumed as a constituent of the human diet (primarily in cottonseed meal and oil) or as a component of folk medicines (e.g. for the treatment of chronic bronchitis and cough) for many years. After studies demonstrated that gossypol purified from cotton seeds had antispermatogenic effects in animals, racemic gossypol was evaluated in clinical trials as a potential male anti-fertility agent.[52] Studies in over 9,000 men, conducted primarily in China, demonstrated that oral racemic gossypol (7.5 to 20 mg/day) was an effective male contraceptive and was generally well tolerated. Adverse effects included fatigue, gastrointestinal symptoms, mild hypokalemia and an irreversible contraceptive effect in some patients. Hypokalemia was rare in studies conducted outside China.[53] Ovarian suppression and endometrial atrophy by racemic gossypol has also been described; racemic gossypol is approved by the Chinese State Food and Drug Administration as a treatment for endometriosis, dysfunctional bleeding of the uterus, and uterine myoma.

In the 1980's and 1990's, nonclinical studies determined that racemic gossypol had cytotoxic effects in human breast, prostate, colon, melanoma, and adrenal carcinoma cell lines.

Consequently, the safety and antitumor activity of single-agent, oral racemic gossypol acetic acid were evaluated in over 100 patients with advanced cancers (metastatic adrenal cancer, recurrent adult malignant glioma, metastatic breast cancer, advanced solid tumors) in four clinical trials conducted in the U.S. and U.K. Patients received oral doses of racemic gossypol acetic acid ranging from 30 to 180 mg weekly or 20 to 70 mg daily (primarily in divided doses), until disease progression or toxicity. [54-57]

Tumor responses and stable disease were also reported in these early-stage, uncontrolled studies in a variety of cancers. Partial responses were observed in 2/15 patients with relapsed/refractory glioblastoma multiforme, and 3/21 patients with metastatic, previously treated adrenal carcinoma. These responses were associated with improvement in clinical symptoms and, in two cases (1 glioblastoma, 1 adrenal) were reported to have a duration of a year or more with continued therapy.

Reported side effects of repeat treatment were mild or moderate and included gastrointestinal adverse events (nausea, vomiting, diarrhea, transient ileus, anorexia), transaminitis, fatigue, peripheral edema, and rash. Hypokalemia and hematologic toxicity were rare. Most patients discontinued gossypol treatment due to disease progression and not due to toxicity. The MTD for racemic gossypol was estimated in three of the studies, and ranged from 30 mg/day to 50 or 60 mg/day for daily treatment, and 120 mg/week for weekly treatment. The best estimate of the MTD for daily administration of racemic gossypol was 40 mg/day. [56]

Non-clinical *in vitro* and *in vivo* studies have demonstrated that the antitumor activity of racemic gossypol appears to reside principally in the R-(-)-enantiomer, with reduced activity observed for the S-(+)-enantiomer. Consequently, Ascenta Therapeutics, Inc. is evaluating R-(-)-gossypol acetic acid (AT-101) as a treatment for advanced cancer.

AT-101

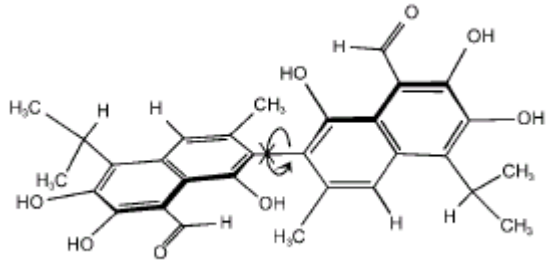
AT-101 is levorotatory enantiomer of gossypol acetic acid, R-(-)-gossypol acetic acid; the chemical name is R-(-)-1,1', 6,6',7,7'-hexahydroxy-3,3'-dimethyl-5,5'-bis (1-methylethyl) [2,2'-Binapthalene]-8, 8'-dicarboxaldehyde acetic acid (figure 1). AT-101, administered orally is a BH3-mimetic inhibitor of Bcl-2 family-member-proteins (Bcl-2, Bcl-xl, Mcl-1, Bcl-w) that induces apoptosis in CLL cells. The oral formulation makes this compound more attractive to combine with other oral compounds such as oral lenalidomide. Recently Castro et al reported the preliminary results of an ongoing phase II clinical trial of oral AT-101 in combination with rituximab among patients with relapsed or refractory B-CLL. In the preliminary report presented at the 2006 annual meeting of the American society of hematology (ASH), 12 patients have received AT-101, at 30mg/d, for 21 or 28 days during each of three 28-day cycles. Rituximab was administered at 375 mg/m2 for 12 doses (total dose = 4,500 mg/m2) on days 1, 3, 5, 8, 15, 22, 29, 31, 33, 57 59, 61. The patients' median age was 62 years (range 43-82). Six patients were high risk and five were intermediate risk based on the modified Rai classification. The median number of previous treatments was 2.5 (range 1-11). Eight patients interrupted treatment due to adverse events, most of which were transient and without residual complications. Gastrointestinal and constitutional effects were the most common adverse events. Two patients had Grade II ileus (small bowel obstruction). Six patients experienced treatment-associated fatigue (grade I-II in five patients and grade III in one patient). The only grade III/IV event believed related to AT-101 was fatigue. One patient died while undergoing treatment from community-acquired bacterial pneumonia; the patient developed grade IV neutropenia, septic shock, and renal failure during the course of the infection. These events were considered unrelated to AT-101. The combination of

AT-101 and rituximab appears active in relapsed/refractory CLL. Correlative studies presented demonstrated down regulation of Bcl-2 and Mcl-1 in tumor cells obtained from patients.

In a second report presented at the 2007 annual meeting of ASH, Castro et al presented initial results from a second cohort (n=6) treated with intermittent, pulse AT-101, 80 mg/d on days 1-3 and 15-17 of each 28-day cycle, in combination with weekly rituximab, 375 mg/m2/week. Six (6) patients have received pulse AT-101. Patient demographic characteristics and risk prognostic status (ZAP70, IgVH mutational status, and cytogenetics / FISH) are comparable between the two dose cohorts. Gastrointestinal (GI) toxicity, the most notable adverse effect of AT-101 with daily administration, appears reduced with intermittent AT-101; 2/6 patients have had NCI-CTCAE Grade 1-2 GI toxicity, and 0/6 have had Grade 3-4 ileus, compared with 11/12 and 2/12 patients, respectively, in the daily dose cohort. Apoptosis of CLL cells evaluated by Annexin V FACS at the time of maximum AT-101 concentration, was present in 18-45% of cells in 4 of the 6 patients after a single 80 mg dose of AT-101. By comparison, apoptosis after a 30 mg AT-101 dose appeared lower and was detected in approximately 1-15% of cells. After 80 mg of AT-101, plasma concentrations of up to 6.6 M have been observed compared with concentrations of approximately 0.8-1.8 M after a 30 mg dose in the daily dose cohort. In the pulse AT-101 cohort we have observed partial responses (PR) in 3 patients while the other 3 are still receiving treatment. Five (5) out of 12 patients had a PR in the previously reported AT-101 continuous administration group.

Intermittent administration of AT-101 with a pulse dose regimen appears associated with an increased pro-apoptotic effect in vivo and higher plasma concentrations, as well as reduced toxicity, when compared with daily dosing.

Chemical Structure of R-(-)-Gossypol



As summarized below racemic gossypol and R-(-)-gossypol (AT-101) bind to and inactivate anti-apoptotic members of the Bcl-2 gene family that are elevated in a variety of cancers.

Non-Clinical Pharmacology

The Bcl-2 family of proteins plays an essential role in regulating apoptosis, or programmed cell death. Over-expression of two anti-apoptotic members of this family, Bcl-X_L and/or Bcl-2, is frequently found in a wide variety of human cancers and may contribute to chemotherapy resistance. The experimental structures of Bcl-X_L and Bcl-2 showed that BH1 (Bcl-2 homology domain 1), BH2, and BH3 domains form a hydrophobic BH3 binding pocket into which the endogenous inhibitors of Bcl-2 and Bcl-X_L, bind. Through structure-based computational screening, Ascenta discovered that gossypol is a potent, small-molecule inhibitor targeting the BH3 domain of Bcl-2/Bcl-X_L proteins.

Studies by Ascenta and its collaborators have demonstrated that:

- Both gossypol enantiomers bind to the anti-apoptotic proteins Bcl-X_L and Bcl-2 *in vitro* with similar affinity and block the binding of pro-apoptotic Bcl inhibitor proteins such as Bid and Bak. Nuclear magnetic resonance (NMR) three-dimensional solution structure data confirm that both gossypol enantiomers bind to the BH3-binding pocket of Bcl-X_L where the BH3 domain of pro-apoptotic proteins bind.
- R-(-)-gossypol is more potent than S-(+)-gossypol or racemic gossypol at inhibiting proliferation and inducing apoptosis in cancer cell lines *in vitro*. For example, in the National Cancer Institute (NCI) *in vitro* screen of 60 cell lines from multiple human tumors the average 50% growth inhibition (GI_{50}) value for R-(-)-gossypol was 0.57 μM, whereas the GI_{50} value was 2.1 μM for racemic gossypol and 14.5 μM for S-(+)-gossypol. R-(-)-Gossypol induced apoptosis in a dose dependent manner in cancer cells that over-express Bcl-2 or Bcl-X_L, but has little effect on normal cells with low Bcl-X_L and Bcl-2 expression. The ability of R-(-)-gossypol to induce apoptosis in cancer cells is correlated with the level of Bcl-X_L; apoptosis is induced via caspase activation. R-(-)-gossypol's anti-proliferative activity is correlated with target cell Bcl-X_L/Bcl-X_S expression ratios.
- Gossypol inhibits mitochondrial functions regulated by Bcl-2/Bcl-X_L.
- R-(-)-gossypol acts synergistically with standard chemotherapeutic agents and radiation in inhibiting cancer cell proliferation.
- R (-) gossypol has *in vivo* tumor growth inhibition activity as a single agent in a variety of xenograft models in mice. The combination of gossypol with docetaxel, cisplatin, CHOP (cyclophosphamide, doxorubicin, Oncovin, and prednisone) or radiation achieved significantly greater tumor growth inhibition than either treatment alone in xenograft models.
- R (-) gossypol is a potent inducer of the pro-apoptotic proteins Noxa and Puma in numerous cancer cell lines, providing an additional mechanism by which AT-101 promotes apoptosis
- Stromal cells protected CLL B-cells from spontaneous and fludarabine-induced apoptosis by increasing the Mcl-1 protein levels. However, AT-101 induced similar extent of down-regulation of Mcl-1 and apoptosis in CLL lymphocytes cultured in suspension or on stroma. Stromal cells expressed undetectable levels of anti-apoptotic but high levels of activated ERK and AKT proteins and had low or no apoptosis with AT-101. Collectively, these data demonstrate that AT-101 induces apoptosis in CLL B-cells and overcomes microenvironment-mediated resistance while sparing normal stromal cells.

Clinical safety data with AT-101

As of November 2008 approximately 620 patients have been enrolled to 14 Phase 1-2a clinical trials of AT-101 as a single agent or in combination with standard agents for the treatment of patients with advanced solid tumors, lymphoma, or chronic lymphocytic leukemia. Patients have received AT-101 once or twice daily, every day for 21 out of every 28 days or using 'pulse' schedules administering AT-101 once weekly or b.i.d. for 3 days every 2 or 3 weeks in these trials. The phase 2 recommended doses are 20 mg daily for 21 of 28 days or 40 mg b.i.d. x 3 days every 21 days.

Clinical trials with AT-101 are ongoing; however, preliminary safety data show that the most common AEs, when AT-101 is administered on a once daily schedule, have been gastrointestinal (GI) and Grade 1/ 2 fatigue. The GI AEs include nausea, vomiting, abdominal pain, abdominal discomfort, diarrhea, constipation, and non-mechanical small bowel obstruction. The most common SAEs reported include nausea, vomiting, and ileus, after several months of continuous dosing. Reversible elevation of ALT and AST were dose-limiting at doses greater than 40mg/day,

when AT-101 was administered daily for 21/28 days, in the initial Phase 1 trial. Non-mechanical small bowel obstruction was the dose limiting toxicity at doses ≥ 120mg/day administered once weekly or at doses ≥ 40mg BID administered on days 1-3, every other week. Following periodic safety reviews, Ascenta has modified the dosing schedule of single-agent AT-101 to include a drug 'holiday' and has reduced the dose of single-agent AT-101, when administered daily, to 20mg/day for 21/28 days. Additionally, Ascenta is also investigating shorter periods of exposure (pulse dosing) of AT-101 as a single agent and in combination with standard agents. A preliminary comparison of SAEs and AEs among patients treated on Phase 2 trials with AT-101 at 30mg/day and 20mg/day for 21 of 28 days has demonstrated a reduction in the rate of GI SAEs and AEs. In addition, as of July 2008, approximately 160 patients have been treated with AT-101, in combination with docetaxel or docetaxel/prednisone, given b.i.d. for 3 days every 3 weeks. A preliminary review of SAEs and AEs among these patients suggests that AT-101 does not augment the cytopenias, GI toxicity, or neuropathy associated with docetaxel administration. As of July 2008, only one case of ileus has been reported among approximately 160 patients treated with AT-101 40 mg b.i.d. x 3 days every 3 weeks. As this patient was treated on a blinded trial, exposure to AT-101 is not certain.

As of June 2007, there have been 102 SAEs reported after treatment with oral AT-101. Twenty-nine patients have had events that represent, or may represent, non-mechanical small bowel obstruction. Several of these events were considered by the investigator to be unrelated to AT-101, but nonetheless prompted brief hospitalization and interruption of AT-101 dosing, with resolution of symptoms 2-10 days later in most patients. Two patients were successfully re-challenged at a lower dose. These events tended to occur at higher doses and typically had an onset later than 4 weeks after the first dose of AT-101. The dosing regimens associated with these events involved continuous daily dosing, dosing for 21 out of 28 days, and pulse schedules. Recent modifications in the dose and schedule of AT-101 have suggested a reduction in the incidence of severe GI toxicities. For example, as of November 1, 2008, among approximately 200 patients treated on 3 trials using the combination of AT-101 40 mg b.i.d. x 3 days on days 1-3 with docetaxel 75 mg/m2 on day 1, repeated every 21 day, there is only one (1) patient who developed non-mechanical small bowel obstruction. Further investigations into other doses and schedules of AT-101 are underway.

Phase I studies on the combination of AT-101 with radiotherapy and temozolomide and radiotherapy and docetaxel and 5-FU are ongoing in patients with glioblasoma multiforme and esophageal cancers, respectively. The phase 2 dose of AT-101 recommended in combination with temozolomide and radiotherapy for GBM is 20 mg daily, Monday through Friday, throughout RT administration. A phase I/II study on the combination of AT-101 with topotecan is ongoing in patients with relapsed and refractory small cell lung cancer and has enrolled 30 patients.

We also conducted *in vitro* studies of AT-101 and observed its ability to induce apoptosis in B-CLL cells. Thus, AT-101 is a novel, oral agent with ability to target Bcl-2 family of anti-apoptotic protein that has demonstrated *in vitro* and in patient anti-CLL activity. Collectively the pre-clinical and clinical data available and presented here and the data available on Lenalidomide allow us to put forward the following hypothesis:

Hypothesis: Concurrent targeting of B-CLL cell microenvironment by lenalidomide and the tumor cell itself by AT-101 will result in an enhanced antileukemic effect **(Fig 4)**. Therefore we propose to conduct a phase I/II clinical trial to first determine the maximum tolerated dose of fixed dose of AT-101 in combination with escalating doses of Lenalidomide and subsequently investigate the clinical efficacy of this combination.

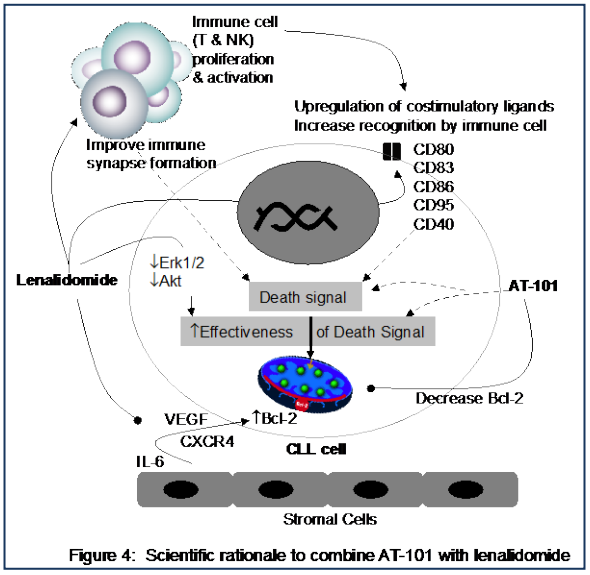


Figure 4: Scientific rationale to combine AT-101 with lenalidomide

Rationale for the combination and dosing: Developing a novel regimen for relapsed /refractory B-CLL patients is an unmet need. AT-101 and lenalidomide have been tested in phase II studies independently and have demonstrated anti-leukemic potential. Despite encouraging results most patients only achieved partial remission with single agent use of these agents. There are several important reasons that rationalize to develop the combination of lenalidomide with AT-101 these are; (1) both have clinically active in patients with B-CLL (2) They have non overlapping toxicities (major toxicity for lenalidomide is hematologic while that for AT-101 is gastrointestinal) (3) both an be delivered orally and are patient convenient (4) Both have mutually exclusive targets (lenalidomide targets the microenvironment and results in immune activation while AT-101 down regulates the prosurvival signals of Bcl-2 family of proteins i.e., Bcl-2, Mcl-1 resulting in enhanced susceptibility to undergo apoptosis).

We will initiate lenalidomide at 5mg per day, while using the sample 21 out of 28 day cycle schedule as published previously. This is the minimum dose at we were able to demonstrate antileukemic effects of lenalidomide. The dose of lenalidomide will be escalated over 4 cohorts as outlined later in the study protocol. Although the administered dose in our previous study was 25mg / day the most tolerable dose (in terms of hematologic) was 20mg / day. Therefore in this study we propose that the maximum dose to be investigated in combination with AT-101 be 20mg/day.

The maximum administered daily dose of AT-101 in a phase II study (in combination with rituximab) is 30mg/day for 21 out of 28-day schedule. Although clinical activity was noted at 10 mg / day dose as well which was considered be also the dose that had the least toxicity. Using a pulse administration schedule, 80 mg/d for 3 days or 40 mg b.i.d. for 3 days every cycle have demonstrated augmented apoptosis in CLL and other diseases. Since the premise of this study is to constantly render microenvironment unfavorable while at the same time make tumor cell vulnerable to death through interruption of the prosurvival signals mediated through Bcl-2 antiapoptotic protein, a maximal pulse dose of AT-101 that can be intermittently delivered is thus favored. In this study therefore, the dose of the AT-101 will remain stable throughout the various cohorts.

2.0 Goals

2.1 Primary

2.11 Phase I: Determine the maximum tolerated dose (MTD) of lenalidomide in combination with AT-101.

2.12 Phase II: To assess the overall response rate of lenalidomide in combination with AT-101.

2.2 Secondary

2.21 To assess the overall response rates of lenalidomide in combination with AT-101 at 6 months and 12 months.

2.22 To evaluate time to progression (TTP) for the combination of lenalidomide + AT-101.

2.23 To evaluate the safety of this combination in patients with relapsed B-CLL.

2.3 Correlative Research

2.31 To conduct correlative studies for further understanding of the mechanism of antitumor activity of lenalidomide and lenalidomide + AT-101.

3.0 Patient Eligibility

Phase I only: Prior to discussing protocol entry with the patient, call the MCCC Registration Office ([redacted]) for dose level and to insure that a place on the protocol is open to the patient.

3.1 Inclusion Criteria

3.11 Understand and voluntarily sign an informed consent form.

3.12 Age ≥ 18 years.

3.13 Able to adhere to the study visit schedule and other protocol requirements.

3.14 Diagnosis of B-CLL, confirmed by flow cytometric analysis and as per the criteria outlined by the IWCLL/Hallek December 2008. (Refer to Appendix III).

3.15 Any prior therapy for B-CLL must have been discontinued ≥ 28 days prior to registration.

3.16 Patients must have an absolute lymphocyte count (ALC) of more than 5,000 cell/mm^3.

3.17 During Phase I: All patients with relapsed disease will be eligible if they have received at least 1 prior standard CLL therapy* and no more than 4 prior therapies (one of which must be a purine analog and/or an alkylating agent).

During Phase II: All patients with relapsed disease will be eligible if they have received a minimum of 1 prior standard therapy* and a maximum of 2 prior treatments (one of which must be a purine analog and/or an alkylating agent) for B-CLL and have developed relapse disease.

Note: Patients who have refractory disease (defined as – progressive disease on last treatment, or less than 6 months of clinical response to the last treatment) will not be eligible.

* *Standard Therapies are defined as those listed in the NCCN guidelines for treatment of CLL.*

3.18 ECOG performance status of 0, 1 or 2 at registration (see Appendix I).

3.19a The following laboratory values obtained ≤ 14 days prior to registration:

- Absolute neutrophil count ≥ 1500/mm^3
- Platelet count ≥ 30,000/mm^3
- Serum creatinine ≤ 1.5 x ULN.
- Total bilirubin ≤ 1.5 mg/dL or direct bilirubin ≤ 1.0 mg/dL for patients with Gilbert's syndrome
- SGOT (AST) and SGPT (ALT) ≤ 2 x ULN or ≤ 5 x ULN if hepatic disease is present.

3.19b Females of childbearing potential (FCBP) † must have a negative serum or urine pregnancy test with a sensitivity of at least 50 mIU/mL 10 – 14 days prior to and again within 24 hours before starting lenalidomide and must either commit to continued abstinence from heterosexual intercourse or begin TWO acceptable methods of birth control, one highly effective method and one additional effective method AT THE SAME TIME, at least 28 days before she starts taking lenalidomide. FCBP must also agree to ongoing pregnancy testing. Men must agree to use a latex condom during sexual contact with a FCBP even if they have had a successful vasectomy. All patients must be counseled at a minimum of every 28 days about pregnancy precautions and risks of fetal exposure. See Appendix VI: Risks of Fetal Exposure, Pregnancy Testing Guidelines and Acceptable Birth Control Methods, AND also Appendix VII: Education and Counseling Guidance Document and Appendix VIII: lenalidomide information.

† A female of childbearing potential is a sexually mature woman who: 1) has not undergone a hysterectomy or bilateral oophorectomy; or 2) has not been naturally postmenopausal for at least 24 consecutive months (i.e., has had menses at any time in the preceding 24 consecutive months).

3.19c All study participants must be registered into the mandatory RevAssist ® program, and be willing and able to comply with the requirements of RevAssist®

3.19d Patients must require treatment for symptomatic B-Cell CLL as defined by the IWCLL/Hallek, December 2008 criterion (see Appendix IV) or as determined clinically necessary by the treating physician.

3.19e Willing to provide blood and baseline bone marrow aspirate samples for correlative research purposes (see Sections 6.31 and Section 14.0).

3.2 Exclusion Criteria

3.21 Any serious medical condition, laboratory abnormality, or psychiatric illness that would prevent the subject from signing the informed consent form.

3.22 Pregnant or lactating females.

3.23 Any condition, including the presence of laboratory abnormalities, which places the subject at unacceptable risk if he/she were to participate in the study or confounds the ability to interpret data from the study.

3.24 Use of any other experimental drug or therapy ≤28 days prior to registration.

3.25 Known hypersensitivity to thalidomide or lenalidomide.

3.26 The development of erythema nodosum if characterized by a desquamating rash while taking thalidomide or similar drugs.

3.27 Patients with history of any other cancer (except non-melanoma skin cancer or carcinoma in-situ of the cervix, unless in complete remission and off therapy for that disease for >3 years).

3.28 Patient with history of cardiac arrest within the past 6 months.

3.29a Patients with history of prior bowel resection, malabsorption syndrome, inflammatory bowel disease, prior bowel obstruction (partial or complete), Crohn's disease, or any other disease significantly effecting the gastrointestinal tract.

3.29b Prior use of gossypol or AT-101.

4.0 Test Schedule

	Active Monitoring Phase					
Tests & Procedures	Days Prior to Registration		Cycle 1	Prior to subsequent Cycles 2-12	Study Drug Discontinuation	Observation
	≤ 28 days	≤ 14 days			3-4 weeks from the last dose	Every 3 months x 2 years
Complete medical history	X					
Adverse event assessment		X		X	X	X
Physical exam, including weight and vital signs, and PS[1]		X	X	X	X	X
Height		X				
Tumor measurement by physical exam[2, 3]		X		X	X	X
CT scans (Chest, Abdomen, Pelvis)[4]	X				X	
EKG/ECHO[5]	X					
CBC with differential[6]		X	X	X	X	X
Chemistry group (SGOT [AST], SGPT [ALT], serum creatinine, total bilirubin[18], LDH, uric acid, phosphorus, alk phos)[6]		X	X	X	X	X
Quantitative Immunoglobulins	X			X	X	X
TSH[7]	X					
Coagulation (PT/PTT)[8]	X			X		
Flow Cytometry- CLL Panel[9]	X			X	X	X
Bone marrow biopsy and aspirate[10]	X			X	X	X
B-cell gene rearrangement – peripheral[11]	X					
CLL FISH Panel[16],	X					
Zap 70, CD38, IgHV[17]	X					
Urinalysis	X					
Pregnancy test[12]		X	X	X	X	
Beta2 microglobulin	X					
Research blood sample collection[13, R]		X	X	X		
Research bone marrow aspirate collection[14, R]	X		X	X	X	
Research lymph node aspirate or biopsy collection[15, R]		X	X	X		
T&B cell quantitation[13, R]		X	X	X		

1. During the first 2 cycles of treatment, patients will have a focused clinical examination weekly for monitoring tumor lysis syndrome (TLS) and tumor flare reaction (TFR). This can be done in collaboration with local MD/referring physician.
2. Physical exam should measure the spleen and liver noting the maximal distance below the respective costal margins and should record the bidimensional diameter of the largest palpable node in each area of involvement including the following sites: left neck (sub-mandibular, cervical, supra-clavicular), right neck (sub-mandibular, cervical, supra-clavicular), left axillary, right axillary, left groin (inguinal, femoral) and right groin (inguinal, femoral).
3. Response assessment: will be done as per IWCLL criteria starting with evaluation at the end of Cycle 2 and every 2 cycles thereafter during treatment. The response assessment will be done every cycle during observation.
4. CT scans should be done as clinically indicated for the management of the patient and in accordance with the IWCLL guidelines. CT scans may be done with patient's primary physician (if outside Mayo Clinic), results of these will be entered in the source document.
5. EKG testing must be done as clinically indicated ; ECHO if clinically indicated.
6. Hematology (CBC) / Serum Chemistry: During cycle 1 and 2 (day 8 and 15) will be done at the treating physician's' discretion based on patient's clinical needs and/or can be done based on risk for TLS/TFR or for conduct of correlative studies. In subsequent cycles CBC/chemistry will be done prior to beginning of each cycle as standard of care and additional testing will only be done as clinically indicated to be determined by treating physician. Laboratory workup may be done with patient's primary physician (if outside Mayo Clinic), results of these will be entered in the source document.
7. TSH level will be done at baseline and subsequent testing will be done as clinically indicated and at the discretion of the treating physician.
8. Coagulation testing: Risk assessment for thrombosis may be done at the treating physician's discretion. Patients should receive some form of thromboprophylaxis as recommended in section 9.8 and monitoring will be done as per standard clinical practice.
9. Flow Cytometry-CLL Panel: The objective of this is to confirm diagnosis as per standard of care (pretreatment) and during/post treatment to assess minimal residual disease. It may be done every 2 cycles starting with Cycle 3 for patients whose ALC is $\leq$ 5,000 and is not necessary for patients who have persistently elevated ALC (>5,000).
10. Bone marrow biopsy / aspirate: at base line will be done to confirm diagnosis, extent of marrow involvement and subsequently will be done only to document complete response as per standard guidelines (see Section 11.0).
11. B cell gene rearrangement-Peripheral Blood: to assess B-cell clone (at baseline) as per standard of care and then to assess minimal residual disease (MRD) or recurrence of clone at the discretion of the treating physician.
12. Pregnancy test – standard recommendation for patients on lenalidomide therapy. Must be done within 10-14 days of initiation of therapy, 24 hours before first dose, weekly for the first 28 days and then every 28 days (or 14 days if menstrual cycles are irregular), and 28 days (or 14 and 28 days if menstrual cycles are irregular) after the last dose of lenalidomide. These are recommended for female patients with child bearing potential. Additional testing is to be done as clinically indicated and at the discretion of the treating physician.
13. Blood collected for correlative studies (see Section 14.0) should be collected at baseline; 8 hours, 12 hours and 16 hours post treatment on Cycle 1 Day 1; Cycle 1 Days 8 and 15; Cycle 2 Days 1, 8 and 15; and Cycle 3 Day 1. Samples for the T&B cell quantitation will not be collected after Cycle 2 Day 1.
14. Mandatory bone marrow aspirate for correlative studies (see Section 14.0) should be collected at baseline, and optionally at Cycle 2 Day 1, Cycle 6 Day 21, and Cycle 12 Day 21.

15. Optional lymph node aspirate or biopsy for correlative studies (see Section 17.0) should be collected as baseline, within 24 hours of tumor flare, 48-72 hours after start of tumor flare, 7-10 days after the start of tumor flare, and at resolution of tumor flare.
16. May be performed anytime within the last year.
17. Performed at anytime in the past.
18. A direct bilirubin should be performed at baseline if the total bilirubin is > 1.5 mg/dL.

R Research funded.

5.0 Grouping Factor:

5.1 Phase: I vs. II

6.0 Registration/Randomization Procedures

6.1 Phase I

Prior to discussing protocol entry with the patient, call the MCCC Registration Office [redacted] for dose level and to insure that a place on the protocol is open to the patient.

6.11 Registration Procedures

6.111 To register a patient, fax ([redacted]) a completed eligibility checklist to the Mayo Clinic Cancer Center (MCCC) Registration Office between 8 a.m. and 4:30 p.m. central time Monday through Friday.

6.2 Phase II

6.21 Registration Procedures

6.211 To register a patient, access the Mayo Clinic Cancer Center (MCCC) web page and enter the registration/randomization application. The registration/randomization application is available 24 hours a day, 7 days a week. Back up and/or system support contact information is available on the Web site. If unable to access the Web site, call the MCCC Registration Office at [redacted] between the hours of 8 a.m. and 5:00 p.m. Central Time (Monday through Friday).

The instructions for the registration/randomization application are available on the MCCC web page [redacted] and detail the process for completing and confirming patient registration. Prior to initiation of protocol treatment, this process must be completed in its entirety and a MCCC subject ID number must be available as noted in the instructions. It is the responsibility of the individual and institution registering the patient to confirm the process has been successfully completed prior to release of the study agent. Patient registration via the registration/randomization application can be confirmed in any of the following ways:

- Contact the MCCC Registration Office [redacted]. If the patient was fully registered, the MCCC Registration Office staff can access the information from the centralized database and confirm the registration.
- Refer to “Instructions for Remote Registration” in section “Finding/Displaying Information about A Registered Subject.”

6.3 Phase I and II

6.31 Correlative Research

Mandatory:
A mandatory correlative research component is part of this study, the patient will be automatically registered onto this component (see Sections 3.19e and 14.0).

Optional:
An optional correlative research component is part of this study, there will be an option to select if the patient is to be registered onto this component (see Sections 14.0 and 17.0).

- Patient has/has not given permission to give his/her bone marrow aspirate sample for research testing.
- Patient has/has not given permission to give his/her lymph node aspirate or biopsy sample for research testing.

6.32 Prior to accepting the registration, registration/randomization application will verify the following:
- IRB approval at the registering institution
- Patient eligibility
- Existence of a signed consent form
- Existence of a signed authorization for use and disclosure of protected health information

6.33 Documentation of IRB approval must be on file in the Registration Office before an investigator may register any patients.

In addition to submitting initial IRB approval documents, ongoing IRB approval documentation must be on file (no less than annually) at the Registration Office (fax: [redacted]). If the necessary documentation is not submitted in advance of attempting patient registration, the registration will not be accepted and the patient may not be enrolled in the protocol until the situation is resolved.

When the study has been permanently closed to patient enrollment, submission of annual IRB approvals to the Registration Office is no longer necessary.

6.34 At the time of registration, the following will be recorded:
- Patient has/has not given permission to store and use his/her sample(s) for future research of CLL at Mayo.
- Patient has/has not given permission to store and use his/her sample(s) for future research to learn, prevent, or treat other health problems.
- Patient has/has not given permission for MCCC to give his/her sample(s) to researchers at other institutions.

6.35 Treatment on this protocol must commence at Mayo Clinic in Florida under the supervision of a medical oncologist or hematologist.

6.36 Treatment cannot begin prior to registration and must begin ≤ 7 days after registration.

6.37 Pretreatment tests/procedures (see Section 4.0) must be completed within the guidelines specified on the test schedule.

6.38 All required baseline symptoms (see Section 10.6) must be documented and graded.

6.39 Study drug is available on site.

7.0 Protocol Treatment

7.1 Treatment Schedule – Cycle length = 28 days Cycles 1-11; Cycle length = 49-56 days Cycle 12 (or last cycle of treatment if patient discontinues treatment before Cycle 12)

7.11 Phase I (Dose Escalation)

Cycle 1

Agent	Dose	Route	Day
Lenalidomide	As assigned by Registration Office	Oral	1-21

Cycles 2-12*

Agent	Dose	Route	Day
Lenalidomide	Assigned at time of registration	Oral	1-21
AT-101	Assigned at time of registration	Oral	1-3

*Growth factors cannot be used during Cycle 2 due to DLT monitoring.

7.12 Phase II

Cycle 1

Agent	Dose	Route	Day
Lenalidomide	MTD per Phase I findings	Oral	1-21

Cycles 2-12

Agent	Dose	Route	Day
Lenalidomide	MTD per Phase I findings	Oral	1-21
AT-101	MTD per Phase I findings	Oral	1-3

7.2 Phase I – determination of Maximum Tolerated Dose (MTD)

7.21 Dose Escalation

Dose level	Lenalidomide	AT-101
-2	5 mg	20 mg b.i.d.
-1	5 mg	30 mg b.i.d.
***1**	**5 mg**	**40 mg b.i.d.**
2	10 mg	40 mg b.i.d.
3	15 mg	40 mg b.i.d.
4	20 mg	40 mg b.i.d.

***starting dose level**

7.211 Treatment by a local medical doctor is not allowed.

7.212 Three patients will be treated at each dose level and observed during the first cycle of the combination therapy (i.e., cycle 2: lenalidomide and AT-101, up to day 28 of starting the combination) to assess toxicities, before new patients are treated. Doses will not be escalated in any individual patient.

7.213 Investigators are to contact the Study Chair as soon as any dose-limiting toxicity (DLT) occurs.

7.22 Definitions of DLT

7.221 For this protocol, dose-limiting toxicity (DLT) will be defined as an adverse event at least possibly related to treatment (definitely, probably, or possibly related) that occurs in cycle 2 of the study treatment and meets the following criteria:

Toxicity	**DLT Definition**
Hematologic	ANC $\leq 0.5 \times 10^9$/L for more than 14 days
	Febrile neutropenia of any duration (ANC $<1.0 \times 10^9$/L, fever $\geq 38.5°C$)
	Grade 4 thrombocytopenia or Grade 3 thrombocytopenia with bleeding or any requirement for platelet transfusion
	Grade 4 anemia, unexplained by underlying disease
Non-hematologic	Any grade 3 or 4 (except tumor lysis syndrome, tumor flare reaction, fatigue, nausea, hyperglycemia in diabetic patients)

8.0 Dosage Modification Based on Adverse Events

8.1 Dose Levels (Based on Adverse Events in Tables 8.*2* and 8.3)

Dose Level	Lenalidomide	AT-101
-2	5 mg	20 mg b.i.d.
-1	5 mg	30 mg b.i.d.
1*	**5 mg**	**40 mg b.i.d.**
2	10 mg	40 mg b.i.d.
3	15 mg	40 mg b.i.d.
4	20 mg	40 mg b.i.d.

* Dose level 1 refers to the starting dose.

Omit = The current dose(s) for the specified drug(s) during a cycle is skipped. The patient does not make up the omitted dose(s) at a later time

Hold/Delay = The current dose(s) of all drugs during a cycle is delayed. The patient does make up the delayed dose(s) when the patient meets the protocol criteria to restart drugs.

Discontinue = The specified drug(s) are totally stopped.

8.2 Lenalidomide

A new course of treatment may begin on the scheduled Day 1 of a new cycle if:

- The ANC is ≥ 1,000/μL;
- The platelet count is ≥ 25,000/μL;
- Any lenalidomide-related allergic reaction/hypersensitivity or sinus bradycardia/ other cardiac arrhythmia adverse event that may have occurred has resolved to ≤ grade 1 severity;
- Any other lenalidomide-related adverse event that may have occurred has resolved to ≤ grade 2 severity.

If these conditions are not met on Day 1 of a new cycle, the subject will be evaluated weekly and a new cycle of lenalidomide will not be initiated until the toxicity has resolved as described above. If lenalidomide dosing was omitted during the previous cycle and was restarted with a one-level dose reduction without requiring an interruption for the remainder of the cycle, then that reduced dose level will be initiated on Day 1 of the new cycle. **If lenalidomide dosing was omitted for the remainder of the previous cycle or if the new cycle is delayed due to toxicity newly encountered on the scheduled Day 1**, then the new cycle will be started with a one-level dose reduction.

If either drug is discontinued, patient will come off treatment and go to event monitoring.

→ → *Use the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 unless otherwise specified* ← ←			
CTCAE System/Organ/Class (SOC)	ADVERSE EVENT	AGENT	ACTION
BASED ON INTERVAL ADVERSE EVENTS (Days 2-28)			
Investigations	Platelet count decreased*: (Platelets < 10,000/mm^3)	Lenalidomide	• Omit dose. • Follow CBC weekly. • If thrombocytopenia resolves to ≤ grade 3 restart lenalidomide at the same dose level. • If thrombocytopenia < 10,000/mm^3 recurs, reduce dose by one dose level (5 mg) and continue therapy when platelet count ≥ 25,000/mm^3.
	Grade 4 Neutrophil count decreased*		• Omit dose. • Follow CBC weekly. • If neutropenia has resolved to ≤ grade 2, resume dose at same level with GCSF support.
Blood and lymphatic system disorders	≥ Grade 3 Febrile neutropenia		• Omit dose. • Follow CBC weekly. • If neutropenia has resolved to ≤ grade 2, resume dose at same level with GCSF support.
Cardiac Disorders	Sinus bradycardia/ other cardiac Arrhythmia Grade 2		• Omit dose. Follow at least weekly. • If the toxicity resolves to ≤ grade 1, reduce dose by one dose level (5mg) and continue therapy.
	≥ Grade 3		• Discontinue study treatment and go to event monitoring.
Vascular disorders	thromboembolic event ≥ Grade 3		• Omit dose and start anticoagulation; restart at investigator's discretion (maintain dose level).

→ → *Use the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 unless otherwise specified* ← ←			
CTCAE System/Organ/Class (SOC)	ADVERSE EVENT	AGENT	ACTION
BASED ON INTERVAL ADVERSE EVENTS (Days 2-28)			
Immune system disorders	Allergic reaction Grade 2-3	Lenalidomide	• Omit dose. Follow at least weekly. • If the toxicity resolves to ≤ grade 1, reduce dose by 5 mg and continue therapy.
	Anaphylaxis Grade 4		• Discontinue study treatment and go to event monitoring.
Skin and subcutaneous tissue disorders	Erythema multiforme ≥ Grade 3		• Discontinue study treatment and go to event monitoring.
	Skin ulceration ≥ Grade 2		• Discontinue study treatment and go to event monitoring.
	Other: Non-blistering rash Grade 3		• If Grade 3 omit dose. Follow weekly. • If the toxicity resolves to ≤ grade 2 continue therapy.
	Other: Non-blistering rash Grade 4		• Discontinue study treatment and go to event monitoring.
Other	Non-hematologic toxicity assessed as lenalidomide related ≥ Grade 3		• Omit dose. Follow at least weekly. • If the toxicity resolves to ≤ grade 2, implement one dose reduction step and continue therapy.

* use IWCLL/Hallek, December 2008 criteria (see Appendix V)

NOTE: Subjects who cannot tolerate the minimum dose 5 mg daily on days 1 – 21 of a 28 day cycle, are to be discontinued from the treatment phase of the study and go to event monitoring.

Note: Dose modification schedule as outlined in the table above will be applicable to all patients enrolled on the study. This schedule will not be applicable to patients during the two cycles of the combination therapy of the patients enrolled on the phase I portion of the study to clearly define MTD. Once patients on the phase I portion of the study have completed cycle #2 (1st combination cycle) they may be managed based on the dose modification criteria outlined in the table above. Dose modification due to toxicity will be based on dose de-escalation in 5 mg increments from the maximum dose obtained for the individual patient. The optimum dose in any individual patient may be determined by the investigator based on managing toxicity and maximizing response. The maximum dose of lenalidomide permitted will not exceed the MTD determined in the phase I portion of the study and is not to exceed 20 mg daily on days 1 – 21 of a 28 day cycle and the minimum dose permitted is 5 mg daily on days 1 – 21 of a 28 day cycle.

Dose re-escalation: For patients who are dose reduced either for disease or drug related toxicities, lenalidomide dose re-escalation can be considered provided all toxicity has resolved to ≤ Grade 1. Patients can be started at minimum of 5mg PO daily on days 1 – 21 of a 28 day cycle and the dose titrated up at 5mg increments weekly to the target dose not to exceed the MTD determined in the phase I portion of the study. The rate of escalation is at the PI discretion based on patient tolerability and concern for tumor lysis syndrome.

8.3 AT-101

During the study period it is the goal to prescribe the fully intended dose of AT-101. It is expected that toxicity of AT-101 can be solved with appropriate measures. However, in case of severe toxicity (grade ≥ 3) attributed (possibly, probably or definitively) to AT-101, treatment will be held for a maximum of 8 weeks. If toxicities resolve to ≤ grade 1, patients may resume treatment. Patients who are resumed on treatment will be started at a lower dose of AT-101 (25% dose reduction or 30mg BID); if the toxicity recurs (grade ≥ 3) then one more dose reduction (50% dose reduction or 20mg BID) will be allowed (upon resolution of toxicity ≤ grade 2). If toxicity recurs ≥ grade 3, patients will be removed from the study treatment and go to event monitoring.

Note: No dose reduction is allowed in cycle #2 as this is the MTD defining cycle.

→ → *Use the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 unless otherwise specified* ← ←			
CTCAE System/Organ/Class (SOC)	ADVERSE EVENT	AGENT	ACTION
BASED ON INTERVAL ADVERSE EVENTS (Days 2-3)			
Gastrointestinal disorders	≥ Grade 2 Nausea ≥ Grade 2 Vomiting Any Grade Abdominal pain	AT-101	For any of these events that last longer than 48 hours, AT-101 should be omitted and the following actions are recommended: • Physical examination, including assessment of vital signs • Screening abdominal radiography to include an abdominal series to exclude ileus, small bowel obstruction (SBO), or pneumatosis intestinalis. • Consideration for obtaining a CT scan with contrast of the abdomen should be made based on clinical judgment • If ileus, non-mechanical SBO, or pneumatosis intestinalis are excluded, AT-101 dosing may be reinstated. If a patient is determined to have ileus, SBO, or pneumatosis intestinalis, AT-101 dosing should be permanently discontinued and expectant management with supportive care is recommended, unless clinical signs or symptoms are present that suggest septicemia or abdominal catastrophe that warrants surgical management.

9.0 Ancillary Treatment/Supportive Care

9.1 Antiemetics may be used at the discretion of the attending physician.

9.2 Blood products and growth factors should be utilized as clinically warranted and following institutional policies and recommendations. Growth factors cannot be used during Cycle 2 of the Phase I portion due to DLT monitoring. The use of growth factors should follow published guidelines of the American Society of Clinical Oncology (42) Update of Recommendations for the Use of Hematopoietic Colony-Stimulating Factors: Evidence-Based, Clinical Practice Guidelines. J Clin Oncol 18(20): 3558-3585, 2000.

9.3 Patients should receive full supportive care, including transfusions of blood and blood products, antibiotics, and antiemetics when appropriate. **Any blood transfusions administered must be irradiated blood products to reduce risk of transfusion mediated graft versus host disease in CLL patients receiving T-cell suppressive therapy. Leukocyte reduction of all blood products for patients on protocol is also required.** All blood products and concomitant medications such as antidiarrheals, analgesics, and/or antiemetics received from the first day of study treatment administration until 30 days after the final dose will be recorded in the medical records.

9.4 Diarrhea: This could be managed conservatively with loperamide. The recommended dose of loperamide is 4 mg at first onset, followed by 2 mg every 2-4 hours until diarrhea free (maximum 16 mg/day).

In the event of grade 3 or 4 diarrhea, the following supportive measures are allowed: hydration, octreotide, and antidiarrheals.

If diarrhea is severe (requiring intravenous rehydration) and/or associated with fever or severe neutropenia (grade 3 or 4), broad-spectrum antibiotics must be prescribed. Patients with severe diarrhea or any diarrhea associated with severe nausea or vomiting **should be hospitalized** for intravenous hydration and correction of electrolyte imbalances.

9.5 **Tumor Lysis Syndrome (TLS) Prophylaxis and Treatment**
Subjects **must receive** Allopurinol 300 mg/day (or an equivalent standard agent for prevention of hyperuricemia) orally for the first two weeks of the first cycle of protocol therapy. Allopurinol may be continued after the first two weeks at the investigator's discretion and as clinically indicated. Subjects should be observed closely for signs and symptoms of TLS during the initial cycle of therapy. Subjects should be encouraged to drink an abundant amount of fluid prior to treatment. Subjects should maintain adequate hydration and urine output.

9.6 **Tumor Flare Reaction (TFR)**
Patients may not receive any prophylaxis for TFR. We do not anticipate severe (grade 4) TFR. It is to be noted that in our previous clinical trial (using 25mg PO QD) no grade 4 TFR was observed. Also, as occurrence of the TFR can positively impact response and progression free survival (PFS) (Chanan-Khan et al *Cancer 117(10) 2127-35*), steroid prophylaxis for TFR will not be used. We recommend the following management plan for TFR:

Grade 1-2 TFR: Grade 1 defined as mild pain not interfering with function; grade 2 defined as moderate pain; pain or analgesics interfering with function, but not interfering with activities of daily living (ADL). Patients can be treated with (a) non-steroidal anti-inflammatory agents (preferred agent - ibuprofen 400-600mg PO Q8 hours) to decrease inflammatory response and / or (b) morphine sulphate (or equivalent analgesic) to control flare associated pain.

Grade 3 TFR: Defined as severe pain; pain or analgesics interfering with function and interfering with ADL. Patients will be treated with (a) non-steroidal anti-inflammatory agents (preferred agent - ibuprofen 400-600mg PO Q8 hours) to decrease inflammatory response and (b) morphine sulphate (or equivalent analgesic) to control flare associated pain. Also, (c) low-dose steroid treatment (preferred agent prednisone 20mg PO QD x 7 days and then 10mg PO QD x 7 days) can be added to this regimen at treating physician's discretion and as clinically indicated.

Grade 4 TFR: Defined as disabling. (a) treatment will be stopped until TFR grade is ≤ grade 1. (b) steroid treatment will be initiated (preferred agent prednisone 20mg PO QD x 7 days and then 10mg PO QD x 7 days) can be added to this regimen at treating physician's discretion and as clinically indicated. High dose steroid (Prednisone 60-100mg PO QD x 5 days and then tapering doses, alternatively medrol pack can be used) maybe initiated as clinically indicated and at treating physician's discretion. (c) non-steroidal anti-inflammatory agents (preferred agent - ibuprofen 400-600mg PO Q8 hours) to decrease inflammatory response and morphine sulphate (or equivalent analgesic) to control flare associated pain may be added at treating physician's discretion.

9.7 **Lymphadenitis (cytokine release syndrome)**

Some subjects with CLL have experienced lymphadenitis characterized by swelling of the lymph nodes, fever, and pain. This syndrome is likely a result of cytokine release. If these symptoms occur, subjects should be treated with methylprednisolone 50 mg IV or dexamethasone 10 mg IV (or equivalent dose of corticosteroid therapy, either IV or PO) at least once daily until symptoms resolve.

9.8 **Anticoagulation prophylaxis:** Due to high-risk of thromboembolism with lenalidomide all subjects will receive thromboprophylaxis either with:

(a) warfarin 1 mg (for <70 kg body weight) and 2 mg (≥70 kg body weight) orally QD while on lenalidomide therapy either alone or in combination phase. Full anti-coagulation may be done if in investigators' assessment there is a higher risk of developing a venous thromboembolic event. Warfarin should be stopped if platelet count is ≤ 50,000 and should be resumed once platelet counts increase and are above ≥50,000. Patients who are on full therapeutic dose of warfarin or low-molecular heparin for prior episode of DVT or other indications can be enrolled and treated on this protocol but will not require low-dose warfarin prophylaxis. Patients developing a DVT/PE while on this study should have their treatment held and receive full therapeutic anticoagulation either with warfarin (target INR 2-3) or low-molecular weight heparin as per standard clinical practice. Once therapeutic level of anticoagulation is achieved, these patients can resume therapy on the study. Anticoagulation in these patients should be continued throughout the duration of therapy and can be stopped 1 week after the last dose of lenalidomide.

(b) Low-dose aspirin (81 mg PO QD) can be used as an alternative strategy.

Selection of either (a) vs. (b) will be at the treating physician's discretion.

9.9a Contraindicated medications: Patient should not be allowed to take concurrent oral iron supplements (including nutritional supplements containing iron) while taking AT-101.

9.9b Pneumocystis jiroveci prophylaxis: Co-trimoxazole (Bactrim®) SS, 1 tablet by mouth daily beginning Day 1 and continued for the duration of treatment until one month following completion of treatment. Co-trimoxazole intolerant patients will receive dapsone 100 mg per day by mouth or atovaquone 1,500 mg per day by mouth for the same period.

9.9c Herpes Simplex and Herpes Zoster prophylaxis: Acyclovir 400 mg by mouth twice a day OR valacyclovir 500 mg by mouth daily beginning Day 1 and continued for the duration of treatment until one month following completion of treatment. Substitution with comparable doses of famciclovir is allowed for the same period.

10.0 Adverse Event (AE) Reporting and Monitoring

10.1 Adverse Event Characteristics

CTCAE term (AE description) and grade: The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web: (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm)

10.11 Adverse event monitoring and reporting is a routine part of every clinical trial. First, identify and grade the severity of the event using the CTCAE version 4.0. Next, determine whether the event is expected or unexpected (see Section 10.2) and if the adverse event is related to the medical treatment or procedure (see

Section 10.5). With this information, determine whether the event must be reported as an expedited report (see Section 10.). Expedited reports are to be completed within the timeframes and via the mechanisms specified in Sections 10.4. All AEs reported via expedited mechanisms must also be reported via the routine data reporting mechanisms defined by the protocol (see Sections 10.6 and 18.0).

10.12 Each CTCAE term in the current version is a unique representation of a specific event used for medical documentation and scientific analysis and is a single MedDRA Lowest Level Term (LLT). Grade is an essential element of the Guidelines and, in general, relates to **severity** for the purposes of regulatory reporting to NCI.

NOTE: A severe AE, as defined by the above grading scale, is **NOT** the same as serious AE which is defined in the table in Section 10.4.

10.2 **Expected vs. Unexpected Events**

- The determination of whether an AE is expected is based on agent-specific information provided in Section 15.0 of the protocol and the study specific consent form.

- Unexpected AEs are those not listed in the agent-specific information provided in Section 15.0 of the protocol and the study specific consent form.

NOTE: "Unexpected adverse experiences" means any adverse experience that is neither identified in nature, severity, or frequency of risk in the information provided for IRB review nor mentioned in the consent form.

10.3 **Assessment of Attribution**

When assessing whether an adverse event is related to a medical treatment or procedure, the following attribution categories are utilized:

Definite - The adverse event *is clearly related* to the agent(s).
Probable - The adverse event *is likely related* to the agent(s).
Possible - The adverse event *may be related* to the agent(s).
Unlikely - The adverse event *is doubtfully related* to the agent(s).
Unrelated - The adverse event *is clearly NOT related* to the agent(s).

Events determined to be possibly, probably or definitely attributed to a medical treatment suggest there is evidence to indicate a causal relationship between the drug and the adverse event.

10.4 Expedited Reporting Requirements for IND/IDE Agents

Phase 1 and Early Phase 2 Studies: Expedited Reporting Requirements for Adverse Events that Occur on Studies under an IND/IDE within 30 Days of the Last Administration of the Investigational Agent/Intervention [1, 2]

FDA REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS (21 CFR Part 312)
NOTE: Investigators **MUST** immediately report to the sponsor **ANY** Serious Adverse Events, whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64)
An adverse event is considered serious if it results in **ANY** of the following outcomes:

1) Death
2) A life-threatening adverse event
3) An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization for ≥ 24 hours
4) A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5) A congenital anomaly/birth defect.
6) Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon 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. (FDA, 21 CFR 312.32; ICH E2A and ICH E6).

ALL SERIOUS adverse events that meet the above criteria MUST be immediately reported to the sponsor within the timeframes detailed in the table below.

Hospitalization	Grade 1 and Grade 2 Timeframes	Grade 3-5 Timeframes
Resulting in Hospitalization ≥ 24 hrs	7 Calendar Days	24-Hour 3 Calendar Days
Not resulting in Hospitalization ≥ 24 hrs	Not required	

NOTE: Protocol specific exceptions to expedited reporting of serious adverse events are found in Section 10.41 of the protocol.
Expedited AE reporting timelines are defined as:

- "24-Hour; 3 Calendar Days" - The AE must initially be reported within 24 hours of learning of the AE, followed by a complete expedited report within 3 calendar days of the initial 24-hour report.
- "7 Calendar Days" - A complete expedited report on the AE must be submitted within 7 calendar days of learning of the AE.

[1] Serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of possible, probable, or definite require reporting as follows:
Expedited 24-hour notification followed by complete report within 3 calendar days for:

- All Grade 3, 4, and Grade 5 AEs

Expedited 7 calendar day reports for:

- Grade 2 AEs resulting in hospitalization or prolongation of hospitalization

[2] For studies using PET or SPECT IND agents, the AE reporting period is limited to 10 radioactive half-lives, rounded UP to the nearest whole day, after the agent/intervention was last administered. Footnote "1" above applies after this reporting period.

Effective Date: May 5, 2011

Additional instructions:

1. Contact Ascenta and Celgene (see contact information below).
2. Use paper *Adverse Event Expedited Report – Single Agent* or *Multiple Agents* report available in forms packet. Submit to:

Ascenta: Senior Manager
Safety & Pharmacovigilence
Ascenta Therapeutics Inc.
101 Lindenwood Dr, Suite 405
Malvern PA 19355
[redacted]

Celgene: Celgene Corporation
Global Drug Safety and Risk Management
Connell Corporate Park
300 Connell Dr. Suite 6000
Berkeley Heights, N.J. 07922
[redacted]

Mayo Clinic Cancer Center (MCCC) Institutions: Provide copies, along with the UPIRTSO cover sheet, by fax ([redacted] to the MCCC Regulatory Affairs Unit (RAU) Risk Information Specialist who will determine and complete IRB reporting. The RAU will submit to the MCCC SAE Coordinator and the MCCC IND Coordinator to determine if FDA submission is needed.

EVENT TYPE	REPORTING PROCEDURE
Other Grade 4 or 5 Events and/or Any Hospitalizations During Treatment Not Otherwise Warranting an Expedited Report	Complete a Notification Form: Grade 4 or 5 Non-AER Reportable Events/Hospitalization Form electronically via the MCCC Remote Data Entry System or paper form within 5 working days of the date the clinical research associate (CRA) is aware of the event(s) necessitating the form. If an expedited written report has been submitted, this form does not need to be submitted.

10.41 Special Situations for Expedited Reporting and Submission of Notification Forms

Exceptions to Expedited Reporting and Submission of Notifcation Forms: EXPECTED Serious Adverse Events

An expedited report or notification form may not be required for specific Grade 1, 2 and 3 Serious Adverse Events where the AE is **EXPECTED**. Any protocol specific reporting procedures MUST BE SPECIFIED BELOW and will supercede the standard Expedited Adverse Event Reporting and Notification Form Requirements (Note: These adverse

events must still be reported through the routine reporting mechanisms [i.e. Nadir/adverse events form]; see footnote 1):

System Organ Class (SOC)	Adverse event/ Symptoms	CTCAE Grade at which the event will not be expeditedly reported [1].
Blood and lymphatic system disorders	Anemia	≤4
Investigations	White blood cell decreased	≤4
	Lymphocyte count decreased	≤4
	Neutrophil count decreased	≤4
	Platelet count decreased	≤4

[1] These exceptions only apply if the adverse event does not result in hospitalization. If the adverse event results in hospitalization, then the standard expedited adverse events reporting requirments must be followed.

Specific protocol exceptions to expedited reporting should be reported expeditiously by investigators **ONLY** if they exceed the expected grade of the event.

10.5 Other Required Reporting

10.51 Persistent or Significant Disabilities/Incapacities

Any AE that results in persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions (formerly referred to as disabilities), congenital abnormities or birth defects, must be reported immediately if they occur at any time following treatment with an agent under an IND/IDE since they are considered to be a serious AE and must be reported to the sponsor as specified in 21 CFR 312.64(b).

10.52 Death

Any death occurring within 30 days of the last dose, regardless of attribution to an agent/intervention under an IND/IDE requires expedited reporting within 24-hours.

Any death occurring greater than 30 days with an attribution of possible, probable, or definite to an agent/intervention under an IND/IDE requires expedited reporting within 24-hours.

Reportable categories of Death

- Death attributable to a CTCAE term.
- Death Neonatal: A disorder characterized by cessation of life during the first 28 days of life.
- Death NOS: A cessation of life that cannot be attributed to a CTCAE term associated with Grade 5.

- Sudden death NOS: A sudden (defined as instant or within one hour of the onset of symptoms) or an unobserved cessation of life that cannot be attributed to a CTCAE term associated with Grade 5.

- Death due to progressive disease should be reported as **Grade 5 "Neoplasms benign, malignant and unspecified (incl cysts and polyps) – Other (Progressive Disease)"** under the system organ class (SOC) of the same name. Evidence that the death was a manifestation of underlying disease (e.g., radiological changes suggesting tumor growth or progression: clinical deterioration associated with a disease process) should be submitted.

10.53 Secondary Malignancy

- A ***secondary malignancy*** is a cancer caused by treatment for a previous malignancy (e.g., treatment with investigational agent/intervention, radiation or chemotherapy). A secondary malignancy is not considered a metastasis of the initial neoplasm.

- All secondary malignancies that occur following treatment with an agent under an IND/IDE be reported. Three options are available to describe the event:

  - Leukemia secondary to oncology chemotherapy (e.g., Acute Myeloctyic Leukemia [AML])

  - Myelodysplastic syndrome (MDS)

  - Treatment-related secondary malignancy

- Any malignancy possibly related to cancer treatment (including AML/MDS) should also be reported via the routine reporting mechanisms outlined in each protocol.

10.54 Second Malignancy

- A second malignancy is one unrelated to the treatment of a prior malignancy (and is NOT a metastasis from the initial malignancy). Second malignancies require ONLY routine reporting.

10.55 Pregnancies

Pregnancies occurring while the subject is on study drug or within 4 weeks after the subject's last dose of study drug are considered immediately reportable events. If the subject is on study drug the study drug is to be discontinued immediately and the subject is to be instructed to return any unused portion of the study drug to the Investigator. The pregnancy must be reported to the Safety Monitor within 24 hours of the Investigator's knowledge of the pregnancy by phone and facsimile using the SAE Form.

The Investigator will follow the subject until completion of the pregnancy, and must notify the Safety Monitor of the outcome within 5 days or as specified below. The Investigator will provide this information as a follow-up to the initial SAE.

If the outcome of the pregnancy meets the criteria for immediate classification as a SAE (i.e., spontaneous abortion [any congenital anomaly detected in an aborted fetus is to be documented], stillbirth, neonatal death, or congenital anomaly), the Investigator should follow the procedures for reporting SAEs (i.e., report the event to the Safety Monitor by telephone and facsimile within 24 hours of the Investigator's knowledge of the event).

All neonatal deaths that occur within 30 days of birth should be reported, without regard to causality, as SAEs. In addition, any infant death after 30 days that the Investigator suspects is related to the *in utero* exposure to the study drug should also be reported.

In the case of a live "normal" birth, the Safety Monitor should be advised as soon as the information is available.

10.6 **Required Routine Reporting**

Adverse events to be graded at each evaluation and pretreatment symptoms/conditions to be evaluated at baseline per the CTCAE v4.0 grading unless otherwise stated in the table below:

System Organ Class (SOC)	Adverse event/Symptoms	Baseline	Each evaluation
Investigations	Platelet count decreased*	X	X
Blood and lymphatic system disorders	Anemia*	X	X
Gastrointestinal disorders	Constipation	X	X
	Ileus		X

*** Grading will be performed by the study statisticians at the time of analysis based on the CLL toxicity grading scale for blood counts in Appendix V. Anemia grade per CTCAE will also be recorded for reporting purposes.**

10.61 Submit via appropriate MCCC Case Report Forms (i.e., paper or electronic, as applicable) the following AEs experienced by a patient and not specified in Section 10.6:

10.611 Grade 2 AEs deemed *possibly, probably, or definitely* related to the study treatment or procedure.

10.612 Grade 3 and 4 AEs regardless of attribution to the study treatment or procedure.

10.613 Grade 5 AEs (Deaths)

10.6131 Any death within 30 days of the patient's last study treatment or procedure regardless of attribution to the study treatment or procedure.

10.6132 Any death more than 30 days after the patient's last study treatment or procedure that is felt to be at least possibly treatment related must also be submitted as a Grade 5 AE, with a CTCAE type and attribution assigned.

10.62 Refer to the instructions in the Forms Packet (or electronic data entry screens, as applicable) regarding the submission of late occurring AEs following completion of the Active Monitoring Phase (i.e., compliance with Test Schedule in Section 4.0).

11.0 Treatment Evaluation

Note: Formal response evaluation should occur every 2 cycles during treatment, with the first response evaluation occurring at the end of cycle 2. Formal response evaluation should occur every cycle during observation. Objective status should be classified as PD vs. Not PD on cycles when a formal response evaluation does not occur.

11.1 Schedule of evaluations: For the purposes of this study, patients should be reevaluated for progression every 28 days during therapy with lenalidomide and AT-101. Formal response evaluation will occur every 2 cycles, with the first formal response evaluation occurring at the end of cycle 2. However, patients will be evaluated prior to each cycle of therapy to identify individuals who have experienced disease progression. Prior to the first formal response evaluation, baseline on study measurements will be used to determine disease progression (e.g. **not** cycle by cycle comparisons). Once patients undergo formal response evaluation, the nadir value at either baseline or time of response evaluation will be used for evaluating future disease progression. In addition to a baseline scan, confirmatory scans should also be obtained as needed to document objective response at sites of non-palpable lymphadenopathy or organomegaly as indicated in Section 4 or as needed clinically.

NOTE: Information from CT scans is not considered in the standard classification of response.

11.2 Definitions

The NCI Working Group criteria[59] will be used to assess response to therapy.

11.21 COMPLETE RESPONSE (CR) requires all of the following for a period of at least 2 months. The first formal response evaluation for CR should occur no sooner than the end of cycle 2.

11.211 Absence of lymphadenopathy (e.g. lymph nodes >1.5 cm) by physical examination.

11.212 No hepatomegaly or splenomegaly by physical examination.

11.213 Absence of constitutional symptoms.

11.214 CBC demonstrating:

- Neutrophils >1500/ul.
- Platelets >100,000/ul (untransfused).
- Hemoglobin >11.0 gm/dl (untransfused).
- Peripheral blood lymphocytes <4000/uL.

Note: Patients who fulfill all criteria for a CR but who have a persistent anemia, thrombocytopenia, or neutropenia related to drug toxicity rather than residual CLL will be classified as **CR with incomplete marrow recovery (CRi)** according to the international criteria[59].

11.215 Bone marrow aspirate and biopsy should be performed **within two month** after documentation of clinical and laboratory evidence of a complete response to document that a **complete response (CR)** has been achieved. The marrow sample should ideally be at least normocellular with <30% of nucleated cells being lymphocytes. Samples are to be analyzed by a pathologist and the presence or absence of nodules noted. Repeat bone marrow aspirate and biopsy are not necessary to document sustained CR.

In a subset of patients who are otherwise in a complete response, bone marrow nodules can be identified histologically. In such cases, special stains will be performed to determine whether such nodules represent "regenerative nodules" or residual "clonal nodules". The presence of regenerative nodules is consistent with CR while the presence of residual clonal nodules will be classified as an **nPR (nodular PR)** which is a sub-classification of PR.

Note: In a patient who achieves clinical and laboratory evidence of a complete response, the objective status should be recorded as a CCR on the cycle where the formal response evaluation occurred until a bone marrow biopsy and aspirate have been performed. At that time, the objective status should be amended to classify the patient as a CR, CRi, or nPR **on the cycle where the formal response evaluation occurred**.

11.216 Patients who have clinical and laboratory evidence of CR but who have not yet had a bone marrow biopsy to distinguish between CR and nPR will be classified as having a **Complete Clinical Response (CCR)** until the marrow biopsy is obtained.

11.217 In some settings MRD assays may be considered as a surrogate of response as discussed in section 11.3. No other laboratory assays (e.g., quantitative immunoglobulins) will be used currently as an index for response but will be recorded for clinical correlations.

11.218 For patients whose only measurable disease at the time of enrollment is on CT scan (i.e. SLL with no palpable nodes), a CT scan is required before classifying patients a CR.

11.22 PARTIAL RESPONSE (PR) requires the patient exhibits at least two of the features in Sections 11.221, 11.222, and 11.223 below (if abnormal prior to therapy) as well as one or more of the remaining features (Sections 11.224, 11.225, 11.226) for at least 2 months. In addition to the parameters listed below, the presence or absence of constitutional symptoms will be recorded. The first formal response evaluation for PR should occur no sooner than the end of cycle 2.

11.221 ≥ 50% decrease in peripheral blood lymphocyte count from the pretreatment baseline value.

11.222 ≥ 50% reduction in the sum of the products of the maximal perpendicular diameters of the largest measured node or nodal masses in the right and left cervical, axillary, and inguinal lymph node regions on physical examination.

11.223 ≥ 50% reduction in size of liver and/or spleen as measured by physical exam noting the maximal distance below the respective costal margins of palpable hepatosplenomegaly during rest.

11.224 Neutrophils >1500/ul or 50% improvement over baseline.

11.225 Platelets >100,000/ul or 50% increase over baseline.

11.226 Hemoglobin >11.0 gm/dl or 50% increase over baseline without transfusions.

11.227 For patients whose only measurable disease at the time of enrollment is on CT scan (i.e. SLL with no palpable nodes), a CT scan demonstrating > 50% reduction of target nodes enlarged at baseline is required before classifying patients a PR.

11.228 For patients whose only measurable disease at the time of enrollment is on CT scan (i.e. SLL with no palpable nodes), a CT scan is required before classifying patients PR.

11.23 PROGRESSION (PD): Patients will receive protocol therapy unless they have evidence of disease progression according to the NCI criteria[3] as evidenced by:

11.231 ≥ 50% increase in the sum of the products of at least 2 lymph nodes on 2 consecutive determinations 2 weeks apart (at least one node must be ≥2 cm) or the appearance of new palpable lymph nodes >1.5 cm not due to a tumor flare. Enlargements or the appearance of new nodes due to a tumor flare do NOT qualify as progression.

11.232 ≥ 50% increase in the size of the liver and/or spleen as determined by measurement below the respective costal margin on 2 consecutive determinations 2 weeks apart and with a minimum of a ≥ 2 cm increase in size from baseline; or appearance of hepatomegaly or splenomegaly which was not previously present at baseline and not due to a tumor flare.

11.233 Transformation to a more aggressive histology (e.g. Richter's transformation).

11.234 ≥ 50% increase in the absolute number of circulating lymphocytes NOT due to infection or tumor flare (taking as reference for progressive disease the smallest absolute lymphocyte count recorded since the treatment started). The absolute lymphocyte count must be at least $5000/mm^3$ to qualify as disease progression.

11.235 In the absence of progression as defined by 1, 2, 3, or 4 above, the presence of a ≥ 2 g/dl decrease in HGB, or ≥ 50% decrease in platelet count, or absolute neutrophil count will NOT exclude a patient from continuing the study. Work-up of such decreases to exclude auto-immune hemolytic anemia, pure red cell aplasia, or idiopathic thrombocytopenic purpura (ITP) should be considered.

11.236 For patients who achieve a CR or nodular PR, progression will be defined as recurrence of circulating leukemia cell clone and an ALC >5000 or recurrence of adenopathy >1.5 cm not due to a tumor flare.

11.24 STABLE DISEASE (SD): Patients who do not meet criteria for CR, CRi, nPR, CCR, PR, or PD will be classified as having "stable disease". The first formal response evaluation should occur no sooner than the end of cycle 2.

11.25 Not PD: The patient was evaluated for progression only this cycle and a formal response evaluation did not occur. The patient did not meet the criteria for progression per section 11.23.

Note: Formal response evaluation should occur every 2 cycles during treatment, with the first response evaluation occurring at the end of cycle 2. Formal response evaluation should occur every cycle during observation. Objective status should be classified as PD vs. Not PD on cycles when a formal response evaluation does not occur.

11.3 EVALUATION OF MINIMAL RESIDUAL DISEASE (MRD): MRD will be assessed by flow cytometry and/or PCR for patients who achieve a CR/nPR/CRi.

11.4 Summary Definition of objective response for patients with B–CLL

	CCR[1]	CR[2]	CRi[3]	nPR[4]	PR[5]	PD[6]
PHYSICAL EXAM						
Nodes[7]	None	None	None	None	≥50% ↓	≥50% ↑, new nodes
Liver/spleen[8]	Not palpable	Not palpable	Not palpable	Not palpable	≥50% ↓	≥50% ↑, newly palpable
Symptoms	None	None	None	None	N/A	N/A
PERIPHERAL BLOOD						
ANC	>1500/µL	>1500/µL	See footnote 3	>1500/µL	>1500/µL or >50% improvement from baseline	See footnote 6
Platelets	>100,000/ µL	>100,000/µL	See footnote 3	>100,000/µL	>100,00/µL or >50% improvement from baseline	See footnote 6
Hemoglobin	>11.0 g/dL without transfusion	>11.0 g/dL without transfusion	See footnote 3	>11.0 g/dL without transfusion	>11.0 g/dL or >50% improvement from baseline without transfusion	See footnote 6
Lymphocytes	<4000/µL	<4000/µL	<4000/µL	<4000/µL	≥50% ↓	≥50% ↑ to at least 5,000/µL
BONE MARROW	N/A	<30% lymphocytes; no nodules	<30% lymphocytes; no nodules	bone marrow nodules[4]	N/A	N/A

1. Clinical complete response (CCR) requires fulfillment of all physical exam and peripheral blood criteria as noted in the table above. No bone marrow biopsy is required to call a patient a CCR; however, patients who achieve a CR/nPR/CRi should have a bone marrow within one month of the formal response evaluation where clinical and laboratory evidence of complete response was first seen as instructed in the test schedule to confirm CR.
2. Complete response (CR) requires fulfillment of all physical exam and peripheral blood criteria for a duration of ≥2 months. A bone marrow aspirate and biopsy are required to document the response as a complete within one month of the formal response evaluation where clinical and laboratory evidence of complete response was first seen (see Section 11.21).
3. Patients who fulfill all criteria for a CR but who have a persistent anemia, thrombocytopenia, or neutropenia related to drug toxicity rather than residual CLL will be classified as CR with incomplete marrow recovery (CRi).
4. Nodular partial response (nPR) is essentially a patient in who it appeared that CR had been obtained but nodules are present in the bone marrow. It requires fulfillment of all physical exam and peripheral blood criteria for CR; however, when the bone marrow is done to confirm CR, nodules of malignant lymphocytes are found. See Section 11.215 regarding the distinction between clonal and regenerative nodules.
5. Partial response (PR) requires fulfillment of at least two of the above-noted decrease in circulating lymphocytes, regression in adenopathy and regression in hepatosplenomegaly, and at least one other parameter listed above for a duration of ≥ 2 months. See Section 11 22.

Footnotes continued on next page.

6. Progression: Fulfilling the criteria as noted in section 11.23. Prior to the formal response evaluation, baseline on study measurements will be used to determine disease progression (e.g. not cycle by cycle comparisons). Once patients undergo formal response evaluation, the nadir value at either baseline or time of response will be used for evaluating future disease progression. In the absence of other indices of clinical progression, the presence of a ≥2 g/dL decrease in hemoglobin or a ≥50% decrease in platelet count and/or absolute neutrophil count will not exclude a patient from continuing on the study. Although not mandatory, bone marrow aspirate and biopsy are strongly encouraged to better define the cause of the suppressed counts (i.e. treatment- versus disease-related).
7. Measurement of lymphadenopathy will be determined on physical exam by adding the sum of the products of the maximal perpendicular diameters of measured lesion(s). No simultaneous increase in the size of any lesions or the appearance of any new lesions may occur for more than 2 consecutive cycles. Minor fluctuations are acceptable as long as they don't exceed 50% of previous measurement. However, if they do exceed 50% of the previous measurement it should be held for 2 consecutive cycles to rule out the possibility of nodes that wax and wane. For purposes of determining CCR and nPR, all nodes on physical exam need to be ≤ 1.5 cm in maximal dimension or documented to be free of CLL by biopsy. NOTE: Information from CT scans regarding lymphadenopathy is not considered in the standard classification of response with the exception of the patients fitting criteria of section 11.218 and 11.228.
8. Measurement of hepatosplenomegaly will be determined by noting the maximal distance below the respective costal margins of palpable hepatosplenomegaly during rest (e.g., not during deep inspiration). NOTE: Information from CT scans regarding hepatosplenomegaly is not considered in the standard classification of response with the exception of the patients fitting criteria of section 11.218 and 11.228.

12.0 Descriptive Factors

12.1 Rai Stage: 0 vs. 1 vs. 2 vs. 3 vs. 4. (Appendix II)

12.2 $CD38^+$ expression: Positive (≥30%) vs. negative (<30%).

12.3 Chromosomal anomalies as detected by FISH: 13q- vs. 12+ vs. 11q- vs. 17p- vs. other abnormality vs. normal karyotype.

12.4 IgV_H mutation status: Mutated (≥2%) vs. unmutated (<2%) vs. indeterminate.

12.5 ZAP-70 expression: Positive (≥20%) vs. negative (<20%).

12.6 Dose level *(to be assigned by the Registration Office)*: -2 vs. -1 vs. 1 vs. 2 vs. 3 .vs. 4.

13.0 Treatment/Follow–up Decision at Evaluation of Patient

13.1 If the patient develops PD at any time during active treatment, unacceptable adverse events, or patient refuses all further study participation, study treatment will be discontinued and the patient will go directly to event monitoring. Patients will then be followed in event monitoring per Section 18.0.

13.2 Patients not progressing on active treatment will continue treatment per protocol up to a maximum of 12 cycles. After the completion of 12 cycles, the patient will go to observation (maximum duration of observation 2 years).

13.3 Phase I Only: If a patient fails to complete the first two cycles of treatment for reasons other than toxicity, the patient will be regarded as inevaluable and will be replaced.

13.4 A patient is deemed ineligible if after registration, it is determined that at the time of registration, the patient did not satisfy each and every eligibility criteria for study entry. The patient may continue treatment off-protocol at the discretion of the physician as long as there are no safety concerns, and the patient was properly registered. The patient will go directly to the event-monitoring phase of the study (or off study, if applicable).

- If the patient received treatment, all data up until the point of confirmation of ineligibility must be submitted. Event monitoring will be required per Section 18.0 of the protocol.
- If the patient never received treatment, on-study material and the End of Active Treatment/Cancel Notification Form must be submitted. No further data submission is necessary.

13.5 A patient is deemed a *major violation*, if protocol requirements regarding treatment in cycle 1 of the initial therapy are severely violated that evaluability for primary end point is questionable. All data up until the point of confirmation of a major violation must be submitted. The patient will go directly to the event-monitoring phase of the study. The patient may continue treatment off-protocol at the discretion of the physician as long as there are no safety concerns, and the patient was properly registered. Event monitoring will be required per Section 18.0 of the protocol.

13.6 A patient is deemed a *cancel* if he/she is removed from the study for any reason before any study treatment is given. On-study material and the End of Active Treatment/Cancel Notification Form must be submitted. No further data submission is necessary.

14.0 Body Fluid Biospecimens

14.1 Summary Table of Research Blood and Body Fluid Specimens to be Collected for this Protocol

14.11 Peripheral Blood

Correlative Study (Section for more information)	Mandatory or Optional	Blood or Body Fluid being Collected	Type of Collection Tube (color of tube top)	Volume to collect per tube (# of tubes to be collected)	Baseline	Cycle 1 Day 1: 8 hours, 12 hours, & 16 hours post treatment	Cycle 1 Days 8 & 15	Cycle 2 Days 1, 8, & 15	Cycle 3 Day 1
Immune cellular microenvironment[1]	Mandatory	Peripheral Blood	Green top	5 mL (1-2)	X	X	X	X (Day 1 only)	
Effect of lenalidomide & AT-101 on molecular targets	Mandatory	Peripheral Blood	Green top	5 mL (1-2)	X	X (prior to treatment only)	X	X	X

1. Samples for T and NK cells will be sent to routine clinical lab for testing and charged to the study.

14.12 Bone Marrow Aspirate

Correlative Study (Section for more information)	Mandatory or Optional	Blood or Body Fluid being Collected	Type of Collection Tube (color of tube top)	Volume to collect per tube (# of tubes to be collected)	Visit 1 (Baseline)	Visit 2 (Cycle 2 Day 1)	Visit 3 (Cycle 7 Day 1)	Visit 4 (At end of treatment)
Immune cellular microenvironment and Effect of lenalidomide and AT-101 on molecular targets	Mandatory at Baseline and all other timepoints are optional	Bone marrow aspirate	Green top	3 mL (1)	X	X	X	X

14.2 **Collection and Processing**

14.21 **All samples will be collected and stored at Dr. Chanan Khan's laboratory** [redacted]

14.4 Background and Methodology

14.41 **Immune cellular microenvironment:** (a) To determine the effect of lenalidomide on the immune effector arm (T and NK cells) in the peripheral blood (1-2 green top tubes-5ml) we will obtain peripheral blood samples at days 0 (baseline); Cycle 1 days 1, 8 and 15; and day 1 of cycle 2. Note: during day 1 three samples will be collected (at 8, 12 and 16 hours post therapy) (b) To evaluate the changes in the immune cellular microenvironment in the bone marrow we will obtain bone marrow aspirate (3ml-green top) samples at baseline and then at day 1 of cycle 2 (participation in bone marrow studies will be optional). These samples will be retained in Dr Chanan Khan's laboratory at Mayo Clinic in Florida.

14.42 **Effect of lenalidomide and AT-101 on molecular targets:** tumor cells from peripheral blood will be obtained at baseline, day 1, 8, 15 (cycle 1) and then at day 1, 8, 15, (cycle 2) and day 1 of cycle 3. Effect of specific molecular targets including (MCl-1, Akt, Erk1/2, Bcl-2, Bcl-xl, Puma, Noxa and XIP will be determined by western blot analysis). These studies will be conducted in Dr. Chanan-Khan laboratory [redacted] Furthermore, peripheral blood samples (same time points) will be collected for gene expression profiling using microarray gene chip technology. These samples will be stored in Dr. Chanan-Khan's Laboratory and will be analyzed at Mayo Clinic in Florida's genomic core facility. Leukemia cells (CD19+) will be sorted from the aspirate using the magnetic beads, and the analysis performed on these cells.

15.0 Drug Information

15.1 Lenalidomide for Oral Administration (Revlimid®)

15.11 **Background**: Lenalidomide has antineoplastic, immunomodulatory and antiangiogenic characteristics via multiple mechanisms. Lenalidomide selectively inhibits secretion of proinflammatory cytokines (potent inhibitor of tumor necrosis factor-alpha secretion); enhances cell-mediated immunity by stimulating proliferation of anti-CD3 stimulated T cells (resulting in increased IL-2 and interferon gamma secretion); inhibits trophic signals to angiogenic factors in cells. Lenalidomide inhibits the growth of myeloma cells by inducing cell cycle arrest and cell death.

15.12 **Formulation and Dispensing**: Commercially available for oral administration as: Capsules: 5 mg, 10 mg, 15 mg and 25 mg

Lenalidomide is approved for marketing only under a FDA approved, restricted distribution program called RevAssist. Physicians, pharmacies, and patients must

be registered; a maximum 28-day supply may be dispensed; a new prescription is required each time it is filled; pregnancy testing is required for females of childbearing potential.

15.13 **Preparation, storage, and stability**: Store oral capsules at controlled room temperature between 15ºC and 30ºC (59 ºF and 86 ºF). Refer to labeling on the bottle for expiration date of the commercial tablets.

15.14 **Administration:** Refer to the treatment section for specific administration instructions. Administer with water. Swallow capsule whole; do not break, open, or chew.

15.15 **Pharmacokinetic information**:
Absorption: Rapid
Metabolism: Approximately two-thirds of Lenalidomide is eliminated unchanged through urinary excretion.
Protein binding: ~30%
Time to peak, plasma: Healthy volunteers: 0.6-1.5 hours; Myeloma patients: 0.5-4 hours
Half-life elimination: ~3 hours
Excretion: Urine (~67% as unchanged drug)

15.16 **Potential Drug Interactions**:
Increased Effect/Toxicity: Abatacept and Anakinra may increase the risk of serious infection when used in combination with Lenalidomide. Lenalidomide may increase the risk of infections associated with vaccines (live organism).
Decreased Effect: Lenalidomide may decrease the effect of vaccines (dead organisms).
Herb/Nutraceutical Interactions: Avoid echinacea (has immunostimulant properties; consider therapy modifications).

15.17 **Known potential adverse events:** Consult the package insert for the most current and complete information.

Boxed Warnings:
1. Potential for human birth defects
2. Hematologic toxicity (neutropenia and thrombocytopenia)
3. Deep Venous Thrombosis and Pulmonary Embolism

Common known potential toxicities, > 10%:
Cardiovascular: Peripheral edema
Central nervous system: Fatigue, pyrexia, dizziness, headache
Dermatologic: Pruritus, rash, dry skin
Endocrine & metabolic: Hyperglycemia, hypokalemia
Gastrointestinal: Diarrhea, constipation, nausea, weight loss, dyspepsia, anorexia, taste perversion, abdominal pain
Genitourinary: Urinary tract infection

Hematologic: Thrombocytopenia, neutropenia, anemia, myelosuppression is dose-dependent and reversible with treatment interruption and/or dose reduction
Neuromuscular & skeletal: Muscle cramp, arthralgia, back pain, tremor, weakness, paresthesia, limb pain
Ocular: Blurred vision
Respiratory: Nasopharyngitis, cough, dyspnea, pharyngitis, epistaxis, upper respiratory infection, pneumonia

Less common known potential toxicities, 1% - 10%:
Cardiovascular: Edema, deep vein thrombosis, hypertension, chest pain, palpitation, atrial fibrillation, syncope
Central nervous system: Insomnia, hypoesthesia, pain, depression
Dermatologic: Brusing, cellulitis, erythema
Endocrine & metabolic: Hypothyroidism, hypomagnesemia, hypocalcemia
Gastrointestinal: Vomiting, xerostomia, loose stools
Genitourinary: Dysuria
Hematologic: Leukopenia, febrile neutropenia, Lymphopenia
Hepatic: ALT increased
Neuromuscular & skeletal: Myalgia, rigors, neuropathy
Respiratory: Sinusitis, rhinitis, bronchitis, pulmonary embolism
Miscellaneous: Night sweats, diaphoresis

Rare known potential toxicities, <1% (Limited to important or life-threatening):
Angioedema, Stevens-Johnson Syndrome and Toxic Epidermal Necrolysis
Tumor Lysis Syndrome

15.18 **Drug procurement:** As a requirement of the REMS program, access to Lenalidomide is restriced. Lenalidomide is approved for marketing only under a FDA approved, restricted distribution program called REVLIMID REMS (www.REVLIMIDREMS.com) formerly known as the RevAssist program. Physicians, pharmacies, and patients must be registered; a maximum 28-day supply may be dispensed; a new prescription is required each time it is filled; pregnancy testing is required for females of childbearing potential.

15.19 **Nursing Guidelines:**

15.191 Myelosuppression is dose-dependent and reversible with treatment interruption and/or dose reduction. Monitor CBC w/diff regularly. Instruct patient to report any unusual bruising or bleeding (thrombocytopenia); signs and symptoms of infection (neutropenia); and energy conserving lifestyle (anemia).

15.192 Lenalidomide can have thrombotic adverse events (i.e DVT and PE). Instruct patient to report any limb swelling or pain, and to seek medical attention for shortness of breath or chest pain.

15.193 Because of the potential for birth defects patients should be instructed in effective methods of birth control. Female patients should use 2 forms of birth control during treatment and for 4 weeks after discontinuing

therapy. Males must be instructed to use a latex condom during any sexual contact with a woman of child bearing potential (even if they have had a vasectomy), because it is unknown if lenalidomide is present in semen.

15.194 Patents may experience pruritus, rash and dry skin. Because of the rare risk of Steven's Johnson Syndrome, patients should immediately report any rash to their provider.

15.195 Drug may cause hyperglycemia. Patients with diabetes or impaired fasting glucose may need to have their glucose levels monitored more closely.

15.196 Gastrointestinal side effects (diarrhea, constipation, nausea, dyspepsia, anorexia, etc) are commonly seen. Manage patient symptomatically and monitor for effectiveness.

15.197 Patients may experience myalgias, arthralgias, and other generalized pain. Administer analgesics as ordered and monitor for their effectiveness.

15.198 Upper respiratory symptoms (nasopharyngitis, cough, epistaxis, etc.) can be seen. Manage symptomatically and monitor for effectiveness.

15.2 **AT-101 (10 mg R-(-)-gossypol acetic acid)**

15.21 **Background**: Ascenta Therapeutics, Inc. is developing AT-101 [R-(-)-gossypol acetic acid] for the treatment of patients with advanced cancer. AT-101 is the levorotatory enantiomer of racemic gossypol acetic acid.

AT-101 is a small molecule inhibitor of the anti-apoptotic proteins Bcl-2, Bcl-XL, Bcl-w and Mcl-1 and a potent inducer of the proapoptotic proteins Noxa and Puma. In vitro data have demonstrated that AT-101 binds to Bcl-2, Bcl-XL, Bcl-w and Mcl-1 in the BH3 binding pocket of these proteins, thereby inhibiting their ability to interact with related, pro-apoptotic proteins, and thus promoting apoptosis. Transcriptional up-regulation of pro-apoptotic proteins Noxa and Puma contributes to the antitumor activity of AT-101. In addition, AT-101 is antiangiogenic and promotes the regression of tumor vasculature. AT-101 has been confirmed to induce apoptosis and related cellular events such as caspase activation and inhibition of mitochondrial function. AT-101 exhibits broad activity in tumor cell lines and is more potent than either racemic gossypol or S-(+)-gossypol in anti-proliferation assays.

15.22 **Formulation**: Each AT-101 tablet contains 10 mg R-(-)-gossypol acetic acid and the following excipients: silicified microcrystalline cellulose, sodium starch glycolate, and stearic acid.

15.23 **Preparation and storage**: AT-101 tablets should be stored under refrigeration, between 2 to 8ºC and protected from direct heat. To assure maximal stability, AT-101 should not be handled at ambient temperatures for more than 24 hours (i.e. for the purpose of delivery or dispensing).

15.24 **Administration:** It is recommended that AT-101 be taken at least an hour (or more) before or after food.

15.25 **Pharmacokinetic information**:

a) Absorption – Following administration of AT-101 in both the fed and fasted state, Cmax and AUC(0-8h) were approximately the same while AUC(0-tlast) was higher in the fed state. Based on the finding that food may increase AT-101 exposure, it is recommended that AT-101 be taken at least an hour (or more) before or after food. The Cmax increased with increasing dose. While the data was quite variable, the geometric mean values of the dose corrected Cmax were generally similar across dose groups while there was an apparent trend of lower values at the higher doses. This indicates there may be some dose limiting absorption of AT-101 at higher doses. Tmax was variable with a range of 0.5 to 8 hours post dose with an overall median value of approximately 3 hours.

b) Distribution - In all species tested, gossypol is widely distributed following oral or parenteral administration. Various studies with gossypol show that it has extensive protein binding potential, perhaps on the order of 90% or greater, based on a study in swine; there is also some evidence for a difference in the protein binding potential between the individual enantiomers of gossypol. The definitive assessment of *in vitro* protein binding potential for gossypol has been problematic due to logistical issues with standard assays. Plasma pharmacokinetic profiles for gossypol in various animal species are consistent with redistribution of gossypol from blood into tissues, with the distribution into these tissues likely to be a consequence of the variable degree of protein binding for gossypol in each tissue.

c) Metabolism – The potential for AT-101 to inhibit or induce major cytochrome P450 isoenzymes was assessed in in vitro studies using human liver microsomes. These studies showed that AT-101 did not induce or inhibit the CYP450 enzymes at clinically relevant concentrations. Bioavailability, metabolism and mass balance studies have not been conducted in humans. In vitro metabolism studies in human liver microsomes did not indicate that AT-101 is metabolized by cytochrome P450 isoenzymes.

d) Excretion – Excretion is thought to be primarily via feces, based on animal radioactivity studies. Urinary excretion was less than 2-3% in rodents.

15.26 **Potential Drug Interactions**: The results of studies to date show no inhibition or induction of activities for any CYP isoform tested. In induction studies, slight decreases in activities of CYP2B6, 3A4, CYP2C9 and UDPGT were actually observed, which were again considered to be a non-specific effect on these enzymes. Overall, data suggest negligible to minimal effects of AT-101 on CYP450 enzyme activities at concentrations likely to be achieved in humans.

15.27 **Known potential toxicities**: The most frequent adverse events associated with AT-101 dosing have been gastrointestinal findings such as nausea and vomiting; anorexia, dehydration, hypokalemia and fatigue have also been frequent. Most adverse events have been of low grade (Grade 1-2 on the NCI Common Toxicity Criteria scale), but a clinical picture of ileus/nonmechanical small bowel

obstruction has also been observed in approximately 10-15% of patients receiving 30 mg of AT-101 daily or daily for 21 of 28 days. Following these initial observations the dosing schedule of single-agent AT-101 was modified and the dose reduced for continuous dosing schedules over multiple cycles (from 30 mg/day to 20 mg/day for 21/28 days). The incidence of ileus/non-mechanical small bowel obstruction was reduced to approximately 5%. Please review the Investigator's Brochure for more detailed information regarding ileus/non mechanical small bowel obstruction.

15.28 **Drug procurement:** AT-101 will be provided free of charge to patients by Ascenta Therapeutics.

15.29 **Nursing Guidelines:**

15.291 AT-101 should be taken on an empty stomach (at least one hour before or after meals).

15.292 GI side effects were the most common. Nausea, vomiting, anorexia were the most common. Treat symptomatically and monitor for effectiveness.

15.293 Monitor electrolyte levels, hypokalemia is common.

15.294 Fatigue has been seen. Instruct patient on energy conserving lifestyle and monitor for effectiveness.

15.295 Monitor bowel function. Patients getting higher and prolonged doses of the agent have presented with higher (10-15% vs. 5%) rates of ileus/or mechanical small bowel obstruction.

16.0 Statistical Considerations and Methodology

16.1 Overview: This is a phase I/II study of lenalidomide in combination with AT-101 in patients with relapsed CLL. The phase I portion of this study is designed to determine the maximally tolerated dose (MTD) and toxicity profile of this combination. Following the MTD determination, the phase 2 portion of the study will assess the efficacy of this combination in patients with relapsed CLL using a one-stage design with an interim analysis.

16.11 Primary Endpoint: The primary endpoint of the phase I portion of this trial is to determine the maximum tolerated dose of this combination. For the phase II portion of this trial, the primary endpoint is the overall response rate. Throughout Section 16.0, response will be considered synonymous with "success" for the phase II portion of this study, unless specified otherwise.

16.12 The phase I portion of this study is expected to require a minimum of 9 and a maximum of 24 evaluable patients. The 6 patients treated at the MTD in the phase I portion will also be included in the phase II portion. A minimum of 8 and a maximum of 31 additional evaluable patients will be accrued at the MTD dose level for a minimum of 14 and a maximum of 37 evaluable patients in the phase II portion of this study. We anticipate accruing up to 5 additional patients (2 phase I, 3 phase II) to account for ineligibility, cancellation, major treatment violation, or other reasons. Therefore, this study is expected to accrue a maximum of 26 patients in the phase I portion and 34 patients in the phase II portion for an overall maximum sample size of 60 patients.

16.13 The anticipated accrual rate is 10-15 evaluable patients per year. At this rate, it will likely take about 6 months to enroll, treat, and evaluate each phase I cohort. The phase I portion is expected to take between 18 and 30 months (but could be as long as 48 months). The phase II portion of this study will accrue in the subsequent 3 years. The total study duration is expected to be approximately 5.5 years, or until the last patient accrued has been observed for at least 6 months.

16.2 **Phase 1:** This portion of the study is designed to determine the MTD and toxicity profile of lenalidomide in combination with AT-101.

16.21 MTD Definition: MTD is defined as the dose level below the lowest dose that induces dose-limiting toxicity (DLT) in at least one-third of patients (at least 2 of a maximum of 6 new patients). A total of 6 patients treated at the MTD will be sufficient to identify common toxicities at the MTD. For instance, those toxicities with an incidence of at least 25% will be observed with a probability of at least 82% $(1-(1-0.25)^6)$.

Refer to Section 7.24 for definition of dose-limiting toxicity (DLT).

16.22 MTD Determination:

Dose Escalation: The phase I portion of this study will utilize a standard cohort of three design. The dose levels to which patients will be assigned in sequential cohorts are described in Section 7.1. The first cohort of three patients will be

treated at dose level 1. Decisions on when and how to dose escalate are described below.

16.221 Three patients will be treated at a given dose level combination and observed for at least 2 cycles from start of treatment to assess toxicity.

16.222 If DLT is not seen in any of the 3 patients, 3 new patients will be accrued and treated at the next higher dose level. If DLT is seen in 2 or 3 of 3 patients treated at a given dose level, then the next 3 patients will be treated at the next lower dose level, if only 3 patients were enrolled and treated at this lower dose level.

16.223 If DLT is seen in 1 of 3 patients treated at a given dose level, up to 3 additional patients will be enrolled and treated at the same dose level. If DLT is seen in at least one of these additional three patients (≥2 of 6), the MTD will have been exceeded and further accrual will cease to this cohort. If dose-limiting toxicity (DLT) is not seen in any of the three additional patients, 3 new patients will be accrued and treated at the next higher dose level.

16.224 After enrolling 6 patients on a specific dose level, if DLT is observed in at least 2 of 6 patients, then the MTD will have been exceeded and defined as the previous dose unless only 3 patients were treated at the lower dose level. In that case, 3 additional patients will be treated at this lower dose level such that a total of 6 patients are treated at the MTD to more fully assess the toxicities associated with the MTD.

16.225 Dose de-escalation: If dose-limiting toxicity meets the stopping boundaries set by the above dose escalation algorithm at dose level 1 (for example, more than 1 out of 3 patients or more than 1 out of 6 patients), the next cohort of three patients will be entered at a dose level of -1. If dose level -1 meets the stopping boundaries, the next cohort of three patients will be entered at dose level -2. Further dose re-escalation will depend on the toxicity profile observed at these dose levels, and re-evaluation of the regimen by the study team may be done.

16.226 If a patient fails to complete the first 2 cycles of therapy (one cycle of lenalidomide and one cycle of lenalidomide in combination with AT-101) for reasons other than dose-limiting toxicity defined adverse events, the patient will be regarded as uninformative in regard to the primary study goal and an additional patient will be treated at the current dose level; however, all toxicity information will be utilized in the analysis.

16.227 Operating Characteristics for standard cohort of 3 design: The following table gives the probability of dose escalation at a single dose level as a function of the true probability of DLT at that level using the cohorts of 3 design described above.

True Rate of DLT (%)	Probability of Dose Escalation
10	0.91
20	0.71
30	0.49
40	0.31
50	0.17

16.23 Analysis Plans: All the relevant results pertaining to toxicity, MTD, response, timed endpoints and laboratory correlates will be examined in an exploratory and hypothesis-generating fashion. The small sample size and the heterogeneous patient population associated with phase I studies restricts the generalizability of the results. Any notable statistical result should only be viewed as preliminary evidence for further study in Phase II trials rather than a definitive finding in and of itself.

16.231 Adverse Events Profile

Platelets and hemoglobin will be graded according to the Grading Scale for Hematologic Adverse Events in CLL Studies in Appendix V. The number and severity of all adverse events (overall and by dose-level) will be tabulated and summarized in this patient population. The Grade 3+ adverse events will also be described and summarized in a similar fashion. This will provide an indication of the level of tolerance for this treatment combination in this patient group.

16.232 Toxicity Profile

The term toxicity is defined as adverse events that are classified as either possibly, probably, or definitely related to study treatment. Platelets and hemoglobin will be graded according to the Grading Scale for Hematologic Adverse Events in CLL Studies in Appendix V. All other toxicities will be evaluated via the ordinal CTC standard toxicity grading.

Overall toxicity incidence as well as toxicity profiles by dose level, patient and tumor site will be explored and summarized. Frequency distributions, graphical techniques and other descriptive measures will form the basis of these analyses.

16.233 Response Profile

A response is defined to be a CR, CRi, CCR, nPR, or PR noted as the objective status. Response will be evaluated using all cycles of treatment. All patients meeting the eligibility criteria who have signed a consent form and have begun treatment will be evaluable for response.

Responses will be summarized by simple descriptive summary statistics delineating complete and partial responses as well as stable and progressive disease in this patient population. The number of responses may indicate further evaluation for specific disease types in a Phase II setting.

16.3 **Phase 2 Statistical Design**: This is a Phase II study designed to assess the overall response rate as well as the toxicity associated with lenalidomide in combination with AT-101 in patients with relapsed CLL.

16.31 Decision Rule:

In a study of 44 patients, single agent Lenalidomide was given at a starting dose of 10 mg daily and could be escalated or de-escalated based on tolerability and response (Ferrajoli et al, 2008).[60] The overall response rate for all patients was 32%, where the median dose was 10 mg.

Lenalidomide dose	Response rate
5 mg	3/11 (27%)
10 mg	4/16 (25%)
15 mg	4/12 (33%)
20 mg	1/2 (50%)
25 mg	2/3 (67%)

With the addition of AT-101, an overall response rate of 50% would be of interest.

The largest success proportion where the proposed treatment regimen would be considered ineffective in this population is 30%, and the smallest success proportion that would warrant subsequent studies with the proposed regimen in this patient population is 50%. The following one stage-design with an interim analysis is based on a two-stage Simon[61] optimum design and uses 37 evaluable patients to test the null hypothesis that the true success proportion in a given patient population is at most 30%.

16.311 Interim Analysis: Enter 14 patients into the study. If 4 or fewer successes are observed in the first 14 evaluable patients, we will consider this regimen ineffective in this patient population and terminate this study. If at least 5 successes are observed, we will continue accrual.

16.312 Final Decision Rule: Enter an additional 23 patients into the study. If 14 or fewer successes are observed in the first 37 evaluable patients, we will consider this regimen ineffective in this patient population. If 15 or more successes are observed in the first 37 evaluable patients, we may recommend further testing of this regimen in subsequent studies in this population.

16.313 Over Accrual: If more than the target number of patients are accrued, the additional patients will not be used to evaluate the stopping rule or used in any decision making process. Analyses involving over accrued patients are discussed in Section 16.413.

16.32 Power and Significance Level: Assuming that the number of successes is binomially distributed, the significance level is .10, i.e. there is a 10% chance of finding the drug to be effective when it truly is not. The probability of declaring that this regimen warrants further study (i.e. statistical power) and the probability of stopping after the interim analysis under various success proportions can be tabulated as a function of the true success proportion as shown in the following table.

If the true success proportion is...	0.30	0.35	0.40	0.45	0.50
Then the probability of declaring that the regimen warrants further study is…	0.10	0.25	0.47	0.69	0.85
And the probability of stopping after the interim analysis is …	0.58	0.42	0.28	0.17	0.09

16.33 Other considerations: Adverse events, quality/duration of response, and patterns of treatment failure observed in this study, as well as scientific discoveries or changes in standard care will be taken into account in any decision to terminate the study

16.4 **Phase 2 Analysis Plan**: The analysis for this trial will commence at planned time points (see Section 16.3) and at the time the patients have become evaluable for the primary endpoint. Such a decision will be made by the Statistician and Study Chair, in accord with CCS Standard Operating Procedures, availability of data for secondary endpoints, and the level of data maturity. It is anticipated that the earliest date in which the results will be made available via manuscript, abstract, or presentation format is when the last patient registered has been followed for at least 6 months.

16.41 Primary Endpoint:

16.411 Definition: For the phase II portion of this trial, the primary endpoint is the overall response rate. A response is defined to be a CR, CRi, CCR, nPR, or PR noted as the objective status. The analysis will include all patients meeting eligibility criteria who signed a consent form and have begun treatment.

16.412 Estimation: The proportion of successes will be estimated by the number of successes divided by the total number of evaluable patients. Confidence intervals for the true success proportion will be calculated according to the approach of Duffy and Santner.[62]

16.413 Over Accrual: If more than the target number of patients are accrued, the additional patients will not be used to evaluate the stopping rule or used in any decision making processes; however, they will be included in final point estimates and confidence intervals

16.42 Definitions and Analyses of Secondary Endpoint:

16.421 Overall response rate at 6 and 12 months: The proportion of responses by 6 months will be estimated by the number of patients who achieve a response by 6 months divided by the total number of evaluable patients. The proportion of responses by 12 months will be estimated in a similar manner. Exact binomial 95% confidence intervals for the true success proportions will be calculated.

16.422 Time to Progression: Time to disease progression is defined as the time from registration to the earliest date of documentation of disease progression. If a patient dies without a documentation of disease progression the patient will be considered to have had tumor progression at the time of their death unless there is sufficient documented evidence to conclude no progression occurred prior to death. In the case of a patient starting treatment and then never returning for any evaluations, the patient will be censored for progression on day 1 post-registration. Patients who receive subsequent treatment for CLL before disease progression will be censored on the date of their last disease assessment prior to initiation of the subsequent treatment. The distribution of time to progression will be estimated using the method of Kaplan-Meier (Kaplan-Meier, 1958).[63]

16.423 Adverse Events: Platelets and hemoglobin will be graded according to the Grading Scale for Hematologic Adverse Events in CLL Studies in Appendix V. The maximum grade for each type of adverse event, regardless of causality, will be recorded and reported for each patient, and frequency tables will be reviewed to determine adverse event patterns. Adverse events will continue to be recorded and reported up to 30 days after the last day of study drug treatment.

16.43 Correlative Analyses: Due to the small overall sample size, the results of the following analyses will be considered exploratory and hypothesis-generating in nature.

16.431 Immune cellular microenvironment: To determine the effect of lenalidomide on the immune effector arm, immune function will be evaluated before and during treatment by assessing T and NK cell quantification in both peripheral blood and bone marrow. Changes in these values over time will be both graphically and quantitatively summarized and explored. In addition, these values will be explored in

relation to response (responder vs non-responder as well as by quality of response, i.e. CR vs PR).

16.432 Effect of lenalidomide and AT-101 on molecular targets: Specific molecular targets including MCl-1, Akt, Erk1/2, Bcl-2, Bcl-xl, Puma, Noxa and XIP will be evaluated on peripheral blood by western blot analysis before and during treatment. Changes in these values over time will be both graphically and quantitatively summarized and explored. In addition, peripheral blood samples will be collected for gene expression profiling using microarray gene chip technology. These samples will be analyzed at Mayo Clinic in Florida's genomic core facility.

16.5 Data & Safety Monitoring

16.51 The principle investigator(s) and the study statistician will review the study at least twice a year to identify accrual, adverse event, and any endpoint problems that might be developing. The Mayo Clinic Cancer Center (MCCC) Data Safety Monitoring Board (DSMB) is responsible for reviewing accrual and safety data for this trial at least twice a year, based on reports provided by the MCCC Statistical Office.

16.52 Adverse Event Stopping Rule for phase II patients: The stopping rule specified below is based on the knowledge available at study development. We note that the rule may be adjusted in the event of either (1) the study re-opening to accrual or (2) at any time during the conduct of the trial and in consideration of newly acquired information regarding the adverse event profile of the treatments under investigation. The study team may choose to suspend accrual because of unexpected adverse event profiles that have not crossed the specified rule below.

Accrual will be temporarily suspended to this study if at any time we observe events considered at least possibly related to study treatment (i.e. an adverse event with attribute specified as "possible," "probable," or "definite") that satisfy either of the following:

- if 5 or more patients in the first 14 treated patients experience a grade 4 or higher non-hematologic adverse event at least possibly related to treatment.

- if after the first 14 patients have been treated, 30% of all patients experience a grade 4 or higher non-hematologic adverse event at least possibly related to treatment.

We note that we will review grade 4 and 5 adverse events deemed "unrelated" or "unlikely to be related", to verify their attribution and to monitor the emergence of a previously unrecognized treatment-related adverse events

16.6 Inclusion of Women and Minorities

16.61 This study will be available to all eligible patients, regardless of race, gender, or ethnic origin.

16.62 There is no information currently available regarding differential effects of this regimen in subsets defined by race, gender, or ethnicity, and there is no reason to expect such differences to exist. Therefore, although the planned analysis will, as always, look for differences in treatment effect based on racial and gender groupings, the sample size is not increased in order to provide additional power for subset analyses.

16.63 The geographical region served by MCCC has a population which includes approximately 3% minorities. Based on prior MCCC studies involving similar disease sites, we expect about 3-5% of patients will be classified as minorities by race and about 40% of patients will be women. Expected sizes of racial by gender subsets are shown in the following table:

Accrual Targets			
Ethnic Category	**Sex/Gender**		
	Females	**Males**	**Total**
Hispanic or Latino	0	1	1
Not Hispanic or Latino	24	35	59
Ethnic Category: Total of all subjects	24	36	60
Racial Category			
American Indian or Alaskan Native	0	0	0
Asian	0	0	0
Black or African American	1	1	2
Native Hawaiian or other Pacific Islander	0	0	0
White	23	35	58
Racial Category: Total of all subjects	24	36	60

Ethnic Categories:	**Hispanic or Latino –** a person of Cuban, Mexican, Puerto Rican, South or Central American, or other Spanish culture or origin, regardless of race. The term "Spanish origin" can also be used in addition to "Hispanic or Latino." **Not Hispanic or Latino**
Racial Categories:	**American Indian or Alaskan Native –** a person having origins in any of the original peoples of North, Central, or South America, and who maintains tribal affiliations or community attachment. **Asian –** a person having origins in any of the original peoples of the Far East, Southeast Asia, or the Indian subcontinent including, for example, Cambodia, China, India, Japan, Korea, Malaysia, Pakistan, the Philippine Islands, Thailand, and Vietnam. (Note: Individuals from the Philippine Islands have been recorded as Pacific Islanders in previous data collection strategies.) **Black or African American –** a person having origins in any of the black racial groups of Africa. Terms such as "Haitian" or "Negro" can be used in addition to "Black or African American." **Native Hawaiian or other Pacific Islander –** a person having origins in any of the original peoples of Hawaii, Guam, Samoa, or other Pacific Islands. **White –** a person having origins in any of the original peoples of Europe, the Middle East, or North Africa.

17.0 Pathology Considerations/Tissue Biospecimens

17.1 Summary Table of Research Tissue Specimens to be Collected for this Protocol

Correlative Study (Section for more information)	**Mandatory or Optional**	**Type of Tissue to Collect**	**Block, Slides, Core, etc. (# of each to submit)**	**Visit 1:Baseline Visit 2: Within 24 hours of tumor flare Visit 3: 48-72 hours after start of tumor flare Visit 4: 7-10 days after start of tumor flare Visit 5: At resolution of tumor flare**	**Process at site? (Yes or No)**	**Temperature Conditions for Storage /Shipping**
Tumor Flare Reaction	Optional	Lymph node aspirate or biopsy	2-3 cores	X	Yes	Tissue to be transferred to pathology and parafin embeded block will be prepared

17.2 **Collection and Processing**

17.21 **A**ll samples will be collected and stored at Dr. Chanan Khan's laboratory [redacted]

17.3 Background and Methodology

17.31 **Lymph Node Aspirate/biopsy:** Lymph node biopsies (on accessible lymph nodes) will be done on all consenting participants at baseline. If patients experience tumor flare reaction, during any cycle, then lymph node biopsy/aspirates (2-3 cores) will be performed during and then after resolution of the TFR. Since the timing of the TFR varies, the treating physician in consultation with Dr. Chanan-Khan will determine the appropriate time for performing the biopsy/aspirate on thelymph node. The site of lymph node biopsy will be determined by the treating physician and /or the study chair. **NOTE:** Patients with no accessible lymph nodes may not participate in this correlative research, but can still participate in all other correlative research associated with this study.

18.0 Records and Data Collection Procedures

18.1 Submission Timetable

Initial Material(s)

CRF	**Active-Monitoring Phase** (Compliance with Test Schedule Section 4.0)
On-Study Form	≤2 weeks after registration
Baseline Adverse Event Form	
Pretreatment Measurement Form	
Baseline Research Blood Submission Form (see Section 14.0)	
Baseline Research Lymph Node Aspirate/Biopsy Submission Form (see Section 17.0)	
Baseline Research Bone Marrow Aspirate Submission Form	
Bone Marrow Biopsy Form	
FISH Results Form – Baseline	
Quantitative Flow Cytometry Form	
Immunophenotyping Reports including CD38, and ZAP-70[1, 2]	
IgVH Mutation Analysis Report[1]	
Other Lab Results Form	
CLL FISH Report[1]	
Bone Marrow Biopsy Report[1]	
CT Scan Report[1]	
B-cell Gene Rearrangement Report[1]	
End of Active Treatment/Cancel Notification Form	Submit ≤2 weeks after registration if withdrawal/refusal occurs prior to beginning protocol therapy

1. Submit copy of the report, Attention: QAS for MC128A, Fax [redacted].
2. For patients who previously had full flow immunophenotyping performed and at pre-study workup had limited repeat flow immunophenotyping, submit a copy of both reports.

Test Schedule Material(s)

CRF	**Active-Monitoring Phase** (Compliance with Test Schedule Section 4.0)		
	At each evaluation during treatment	At end of treatment	Observation
Evaluation/Treatment Form	X[2]	X	
Evaluation/Observation Form			X[1]
Nadir/Adverse Event Form	X	X	X
Measurement Form	X	X	X
Quantitative Flow Cytometry Form	X[4]		
Research Blood Submission Form	X[4] (see Section 14.0)		
Research Lymph Node Aspirate/Biopsy Submission Form *(for patients with tumor flare only)*	X[4] (see Section 17.0)		
Research Bone Marrow Aspirate Submission Form	X[4] (see Section 14.0)	X	
Bone Marrow Biopsy Form	X[4]	X[4]	X[4]
Other Lab Results Form	X	X	X
Immunophenotyping Report[3]	X[4]	X[4]	
B-cell Gene Rearrangement Report[3]	X[4]	X[4]	
Bone Marrow Biopsy Report[3]	X[4]	X[4]	
CT Scan Report[3]	X[4]	X[4]	
End of Active Treatment/Cancel Notification Form		X	
Notification Form – Grade 4 or 5 Non-AER Reportable Events/Hospitalization Form	At each occurrence (see Section 10.0)		
ADR/AER	At each occurrence (see Section 10.0)		

1. Complete at each evaluation during Observation (see Section 4.0).
2. Complete at each evaluation during Active Treatment (see Section 4.0).
3. Submit copy of the report, Attention: QAS for MC128A, Fax [redacted].
4. Complete when required per the test schedule.

Follow-up Material(s)

CRF	Event Monitoring Phase[1]				
	q. 3 months until PD or subsequent treatment for CLL[2]	At PD or subsequent treatment for CLL[2]	q. 6 mos. after PD or subsequent treatment for CLL	Death	New Primary
Event Monitoring Form	X	X	X	X	At each occurrence

1. If a patient is still alive 3 years after registration, no further follow-up is required.
2. Submit copy of documentation of response or progression to the Operations Office, Attention: QAS for MC128A, Fax [redacted].

19.0 Budget

19.1 Costs charged to patient: routine clinical care and lenalidomide.

19.2 Tests to be research funded: Correlative studies described in Section 14.0 and 17.0. The study drug (AT-101) will be provided free of charge to the patient.

20.0 References

1. Aksentijevich I, Flinn IW: Chronic lymphocytic leukemia: advances in biology and therapeutics. Curr Opin Oncol 15:16-22, 2003
2. Dighiero G, Binet JL: When and how to treat chronic lymphocytic leukemia. N Engl J Med 343:1799-801, 2000
3. Keating M: Improving the complete remission rate in chronic lymphocytic leukemia. Am J Hematol:262-9, 1999
4. Cheson B, Bennett J, Grever M, et al: National Cancer Institute-sponsored Working Group guidelines for chronic lymphocytic leukemia: revised guidelines for diagnosis and treatment. Blood 87:4990-4997, 1996
5. Keating MJ: Chronic lymphocytic leukemia. Semin Oncol 26:107-14, 1999
6. Rai KR, Peterson BL, Appelbaum FR, et al: Fludarabine compared with chlorambucil as primary therapy for chronic lymphocytic leukemia. N Engl J Med 343:1750-7, 2000
7. Chemotherapeutic options in chronic lymphocytic leukemia: a meta-analysis of the randomized trials. CLL Trialists' Collaborative Group. J Natl Cancer Institute 91:861-8, 1999
8. Schmitt B, Wendtner CM, Bergmann M, et al: Fludarabine combination therapy for the treatment of chronic lymphocytic leukemia. Clin Lymphoma 3:26-35, 2002
9. 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 98:2319-25, 2001
10. Plunkett W, Gandhi V, Huang P, et al: Fludarabine: pharmacokinetics, mechanisms of action, and rationales for combination therapies. Semin Oncol 20:2-12, 1993

11. Weiss MA, Maslak PG, Jurcic JG, et al: Pentostatin and cyclophosphamide: an effective new regimen in previously treated patients with chronic lymphocytic leukemia. J Clin Oncol 21:1278-84, 2003
12. Keating MJ, O'Brien S, Lerner S, et al: Long-term follow-up of patients with chronic lymphocytic leukemia (CLL) receiving fludarabine regimens as initial therapy. Blood 92:1165-71, 1998
13. Koehl U LL, Nowak B, et al.: Fludarabine and cyclophosphamide: synergistic cytoxicity associated with inhibition of interstrand cross-link removal. Proc Am Assn Cancer Res 38:2, 1997
14. O'Brien SM, Kantarjian HM, Cortes J, et al: Results of the fludarabine and cyclophosphamide combination regimen in chronic lymphocytic leukemia. J Clin Oncol 19:1414-20, 2001
15. Marotta G, Bigazzi C, Lenoci M, et al: Low-dose fludarabine and cyclophosphamide in elderly patients with B-cell chronic lymphocytic leukemia refractory to conventional therapy. Haematologica 85:1268-70, 2000
16. McLaughlin P, Grillo-Lopez AJ, Link BK, et al: Rituximab chimeric anti-CD20 monoclonal antibody therapy for relapsed indolent lymphoma: half of patients respond to a four-dose treatment program. J Clin Oncol 16:2825-33., 1998
17. Piro LD, White CA, Grillo-Lopez AJ, et al: Extended Rituximab (anti-CD20 monoclonal antibody) therapy for relapsed or refractory low-grade or follicular non-Hodgkin's lymphoma. Ann Oncol 10:655-61., 1999
18. Czuczman MS, Fallon A, Mohr A, et al: Rituximab in combination with CHOP or fludarabine in low-grade lymphoma. Semin Oncol 29:36-40. hDBfor=art&artType=abs&id=asonc02901c0036&target=, 2002
19. Byrd JC, Murphy T, Howard RS, et al: Rituximab Using A Thrice Weekly Dosing Schedule in B-Cell Chronic Lymphocytic Leukemia and Small Lymphocytic Lymphoma Demonstrates Clinical Activity and Acceptable Toxicity. J Clin Oncol 19:2153-2164, 2001
20. O'Brien SM, Kantarjian H, Thomas DA, et al: Rituximab Dose-Escalation Trial in Chronic Lymphocytic Leukemia. J Clin Oncol 19:2165-2170, 2001
21. Bellosillo B, Villamor N, Lopez-Guillermo A, et al: Complement-mediated cell death induced by rituximab in B-cell lymphoproliferative disorders is mediated in vitro by a caspase-independent mechanism involving the generation of reactive oxygen species. Blood 98:2771-7, 2001
22. Golay J, Lazzari M, Facchinetti V, et al: CD20 levels determine the in vitro susceptibility to rituximab and complement of B-cell chronic lymphocytic leukemia: further regulation by CD55 and CD59. Blood 98:3383-9, 2001
23. 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 101:6-14, 2003
24. Byrd JC PB, Park K: Rituximab added to fludarabine improves response in previously untreated chronic lymphocytic leukemia: Preliminary reesult from CALGB 9712. Proc Am Soc Clin Oncol 20:280a (Abstract 1116), 2001
25. Hainsworth JD, Litchy S, Barton JH, et al: Single-agent rituximab as first-line and maintenance treatment for patients with chronic lymphocytic leukemia or small

lymphocytic lymphoma: a phase II trial of the Minnie Pearl Cancer Research Network. J Clin Oncol 21:1746-51, 2003
26. Huhn D, von Schilling C, Wilhelm M, et al: Rituximab therapy of patients with B-cell chronic lymphocytic leukemia. Blood 98:1326-31, 2001
27. Marotta G, Lenoci M, Fabbri A, et al: Efficacy of rituximab in the treatment of refractory chronic lymphocytic leukemia patient with bone marrow aplasia. Hematol J 3:299-301, 2002
28. Garcia-Manero G, S. OB, Cortes J: Combination fludarabine, cyclophosphamide and rituximab for previously treated patients with chronic lymphocytic leukemia. Blood 96:757a, 2000
29. Castejon R VJ, Romero Y, Briz M, Munoz RM, Durantez A.: Modulation of apoptosis by cytokines in B-cell chronic lymphocytic leukemia. Cytometry 38:224-30., 1999
30. Mainou-Fowler T PA: Modulation of apoptosis with cytokines in B-cell chronic lymphocytic leukaemia. Leuk Lymphoma. 21:369-77, 1996
31. Duensing S AJ: Increased intracellular and plasma levels of basic fibroblast growth factor in B-cell chronic lymphocytic leukemia. Blood 85:1978-80, 1995
32. Menzel T RZ, Calleja E, et al.: Elevated intracellular level of basic fibroblast growth factor correlates with stage of chronic lymphocytic leukemia and is associated with resistance to fludarabine. Blood 87:1056-63, 1996
33. Konig A MT, Lynen S, et al.: Basic fibroblast growth factor (bFGF) upregulates the expression of bcl- 2 in B cell chronic lymphocytic leukemia cell lines resulting in delaying apoptosis. Leukemia 11:258-65, 1997
34. Letai A: BH3 domains as BCL-2 inhibitors: prototype cancer therapeutics. Expert Opin Biol Ther 3:293-304, 2003
35. Hanada M, Delia D, Aiello A, et al: bcl-2 gene hypomethylation and high-level expression in B-cell chronic lymphocytic leukemia. Blood 82:1820-8, 1993
36. Aviram A, Rabizadeh E, Zimra Y, et al: Expression of bcl-2 and bax in cells isolated from B-chronic lymphocytic leukemia patients at different stages of the disease. Eur J Haematol 64:80-4, 2000
37. Consoli U, Santonocito A, Stagno F, et al: Multidrug resistance mechanisms in chronic lymphocytic leukaemia. Br J Haematol 116:774-80, 2002
38. Robertson LE, Plunkett W, McConnell K, et al: Bcl-2 expression in chronic lymphocytic leukemia and its correlation with the induction of apoptosis and clinical outcome. Leukemia 10:456-9, 1996
39. Pepper C, Bentley P, Hoy T: Regulation of clinical chemoresistance by bcl-2 and bax oncoproteins in B-cell chronic lymphocytic leukaemia. Br J Haematol 95:513-7, 1996
40. Pepper C, Hoy T, Bentley DP: Bcl-2/Bax ratios in chronic lymphocytic leukaemia and their correlation with in vitro apoptosis and clinical resistance. Br J Cancer 76:935-8, 1997
41. Thomas A, Pepper C, Hoy T, et al: Bcl-2 and bax expression and chlorambucil-induced apoptosis in the T-cells and leukaemic B-cells of untreated B-cell chronic lymphocytic leukaemia patients. Leuk Res 24:813-21, 2000
42. Bellosillo B, Villamor N, Lopez-Guillermo A, et al: Spontaneous and drug-induced apoptosis is mediated by conformational changes of Bax and Bak in B-cell chronic lymphocytic leukemia. Blood 100:1810-6, 2002

43. Dewson G, Snowden RT, Almond JB, et al: Conformational change and mitochondrial translocation of Bax accompany proteasome inhibitor-induced apoptosis of chronic lymphocytic leukemic cells. Oncogene 22:2643-54, 2003
44. Starczynski J, Pepper C, Pratt G, et al: Common polymorphism G(-248)A in the promoter region of the bax gene results in significantly shorter survival in patients with chronic lymphocytic Leukemia once treatment is initiated. J Clin Oncol 23:1514-21, 2005
45. Kitada S, Monosov E, Chandler S, et al: Pro-apoptotic effects on CLL (chronic lymphocytic leukemia) or ABT-737, a novel fully synthetic Bcl-2/Bcl-XL antagonist. Blood, 2004
46. Pepper C, Hooper K, Thomas A, et al: Bcl-2 antisense oligonucleotides enhance the cytotoxicity of chlorambucil in B-cell chronic lymphocytic leukaemia cells. Leuk Lymphoma 42:491-8, 2001
47. Pepper C, Thomas A, Hoy T, et al: Antisense oligonucleotides complementary to Bax transcripts reduce the susceptibility of B-cell chronic lymphocytic leukaemia cells to apoptosis in a bcl-2 independent manner. Leuk Lymphoma 43:2003-9, 2002
48. Rai K, Moore J, Boyd T, et al: Phase 3 randomized trial of fludarabine/cyclosphosphamide chemotheray with or without oblimersen sodium (Bcl-2 antisense; Genasense; G3139) for patients with relapsed or refractory chronic lymphocytic leukemia (CLL). Blood, 2004
49. Kitada S, Andersen J, Akar S, et al: Expression of apoptosis-regulating proteins in chronic lymphocytic leukemia: correlations with In vitro and In vivo chemoresponses. Blood 91:3379-89, 1998
50. Bannerji R, Kitada S, Flinn IW, et al: Apoptotic-regulatory and complement-protecting protein expression in chronic lymphocytic leukemia: relationship to in vivo rituximab resistance. J Clin Oncol 21:1466-71, 2003
51. Saxena A, Viswanathan S, Moshynska O, et al: Mcl-1 and Bcl-2/Bax ratio are associated with treatment response but not with Rai stage in B-cell chronic lymphocytic leukemia. Am J Hematol 75:22-33, 2004
52. Qian S, Hu J, Ho J, et al: The first clinical trial of gossypol on male antifertility., Clin Pharmacol Ther, 1980, pp 489-492
53. Liu Z, Ziu G, Hei L, et al: Clinical trial of gollyppol as a male antifertility agent. Recent advances in fertility regulation. Atar:160-163, 1981
54. Stein RC, Joseph AE, Matlin SA, et al: A preliminary clinical study of gossypol in advanced human cancer. Cancer Chemother Pharmacol 30:480-2, 1992
55. Flack MR, Pyle RG, Mullen NM, et al: Oral gossypol in the treatment of metastatic adrenal cancer. J Clin Endocrinol Metab 76:1019-24, 1993
56. Van Poznak C, Seidman AD, Reidenberg MM, et al: Oral gossypol in the treatment of patients with refractory metastatic breast cancer: a phase I/II clinical trial. Breast Cancer Res Treat 66:239-48, 2001
57. Bushunow P, Reidenberg MM, Wasenko J, et al: Gossypol treatment of recurrent adult malignant gliomas. J Neurooncol 43:79-86, 1999
58. Chronic lymphocytic leukemia: recommendations for diagnosis, staging, and response criteria. International Workshop on Chronic Lymphocytic Leukemia. Ann Intern Med 110:236-8, 1989

59. Hallek M, Cheson BD, Catovsky D, et al. Guidelines for the diagnosis and treatment of chronic lymphocytic leukemia: a report from the International Workshop on Chronic Lymphocytic Leukemia updating the National Cancer Institute-Working Group 1996 guidelines. Blood. 2008;111:5446-5456.
60. Ferrajoli A, Lee BN, Schlette EJ, O'Brien SM, Gao H, Wen S, Wierda WG, Estrov Z, Faderl S, Cohen EN, Li C, Reuben JM, Keating MJ. Lenalidomide induces complete and partial remissions in patients with relapsed and refractory chronic lymphocytic leukemia. Blood. 2008 Jun 1;111(11):5291-7. Epub 2008 Mar 11. PubMed PMID: 18334676.
61. Simon R: Optimal two-stage designs for phase II clinical trials. Controlled Clinical Trials 10:1-10, 1989.
62. Duffy D, Santner T: Confidence intervals for a binomial parameter based on multistage tests. Biometrics 43:81- 93, 1987.
63. Kaplan E, Meier P: Nonparametric estimation for incomplete observations. J Am Stat Assoc 53:457-481, 1958.

Appendix I

ECOG Performance Status Scale	
Grade	***Descriptions***
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.

Appendix II

Rai Staging System for CLL

Modified 3-stage System	Rai Stage	Clinical Features
Low Risk	0	Lymphocytes (L) in blood > 5000/mm^3 and marrow > 30% only
Intermediate Risk	I II	L+ enlarged lymph nodes (LN) L + spleen and/or liver (LN + or -)
High Risk	III IV	L + anemia (Hbg < 11 gm/dl) L + Thrombocytopenia < 100,000/µl.

Subjects in the intermediate risk group must have evidence of active disease as demonstrated by at least one of the following criteria:

Massive or progressive splenomegaly and/or lymphadenopathy; (Massive splenomegaly is here defined by spleen tip > 6 cm below costal margin.)

Presence of weight loss > 10% over the preceding 6 month period;

Grade 2 or 3 fatigue;

Fevers > 100.5°F or night sweats for greater than 2 weeks without evidence of infection;

Progressive lymphocytosis with an increase of > 50% over a 2 month period or an anticipated doubling time of less than 6 months;

Worsening anemia or thrombocytopenia.

Appendix III
Criteria for Diagnosis of Disease

Specific diagnosis of B-Cell CLL meeting the following criteria at any time during the course of disease (i.e., at initial diagnosis, at relapse, etc.):

- An absolute lymphocytosis of > 5,000/mm3.
- Morphologically, the lymphocytes must appear mature with < 55% prolymphocytes by manual differential.
- The aspirate smear must show > 30% of nucleated cells to be lymphoid or the bone marrow core biopsy must show lymphoid infiltrates compatible with marrow involvement by CLL. The overall cellularity must be normocellular or hypercellular.
- Local institution lymphocyte immunophenotype must reveal a predominant B-cell monoclonal population sharing a B-cell marker (CD19, CD20, CD23, CD24) with the CD5 antigen, in the absence of other pan-T-cell markers. While the absence of CD23 expression will not exclude subjects, it should prompt close re-examination of the lymphocyte morphology to exclude the diagnosis of mantle cell lymphoma in the leukemic phase.

Appendix IV

Definition of Symptomatic B-Cell

Active disease should be clearly documented for protocol therapy. At least one of the following criteria should be met:

1. Evidence of progressive marrow failure as manifested by the development of, or worsening of, anemia (Hgb < 11.0 g/dL) and/or thrombocytopenia (Platelets < 100 x 10^9)
2. Massive (ie, at least 6 cm below the left costal margin) or progressive or symptomatic splenomegaly
3. Massive nodes (ie, at least 10 cm in longest diameter) or progressive or symptomatic lymphadenopathy
4. Progressive lymphocytosis with an increase of more than 50% over a 2-month period or lymphocyte doubling time (LDT) of less than 6 months. LDT can be obtained by linear regression extrapolation of absolute lymphocyte counts obtained at intervals of 2 weeks over an observation period of 2 to 3 months. In patients with initial blood lymphocyte counts of less than 30 x 10^9/L (30 000/µL), LDT should not be used as a single parameter to define a treatment indication. In addition, factors contributing to lymphocytosis or lymphadenopathy other than CLL (eg, infections) should be excluded.
5. Autoimmune anemia and/or thrombocytopenia that is poorly responsive to corticosteroids or other standard therapy.
6. Constitutional symptoms, defined as any one or more of the following disease-related symptoms or signs:
    a. Unintentional weight loss of 10% or more within the previous 6 months;
    b. significant fatigue (ie, inability to work or perform usual activities);
    c. fevers higher than 100.5°F or 38.0°C for 2 or more weeks without other evidence of infection; or
    d. night sweats for more than 1 month without evidence of infection.

Appendix V
Grading Scale for Hematologic Toxicity in CLL Studies

Non Hematologic Toxicity will be scored using NCI CTCAE (version 4.0) for toxicity and adverse event reporting.

Hematologic toxicity will be assessed using IWCLL/Hallek December 2008

Grading scale for hematologic toxicity in CLL studies

Grade*	Decrease in platelets† or Hb‡ (nadir) from pretreatment value, %	Absolute neutrophil count/μL§ (nadir)
0	No change to 10%	≥ 2000
1	11%-24%	≥ 1500 and < 2000
2	25%-49%	≥ 1000 and < 1500
3	50%-74%	≥ 500 and < 1000
4	≥ 75%	< 500

* Grades: 1, mild; 2, moderate; 3, severe; 4, life-threatening; 5, fatal. Death occurring as a result of toxicity at any level of decrease from pretreatment will be recorded as grade 5.

†Platelet counts must be below normal levels for grades 1 to 4. If, at any level of decrease, the platelet count is < 20 x 10^9/L (20 000/μL), this will be considered grade 4 toxicity, unless a severe or life-threatening decrease in the initial platelet count (eg, 20 x 10^9/L [20 000/μL]) was present pretreatment, in which case the patient is not evaluable for toxicity referable to platelet counts.

‡Hb levels must be below normal levels for grades 1 to 4. Baseline and subsequent Hb determinations must be performed before any given transfusions. The use of erythropoietin is irrelevant for the grading of toxicity but should be documented.

§If the absolute neutrophil count (ANC) reaches < 1 x 10^9/L (1000/μL), it should be judged to be grade 3 toxicity. Other decreases in the white blood cell count, or in circulating neutrophils, are not to be considered because a decrease in the white blood cell count is a desired therapeutic endpoint. A gradual decrease in granulocytes is not a reliable index in CLL for stepwise grading of toxicity. If the ANC was < 1 x 10^9/L (1000/μL) before therapy, the patient is not evaluable for toxicity referable to the ANC. The use of growth factors such as G-CSF is not relevant to the grading of toxicity, but should be documented.

APPENDIX VI

Risks of Fetal Exposure, Pregnancy Testing Guidelines and Acceptable Birth Control Methods

Risks Associated with Pregnancy

The use of lenalidomide in pregnant females and nursing mothers has not been studied nor has the effect of the lenalidomide on human eggs and sperm. The risks to a fetus are not known. However, because lenalidomide is related to thalidomide, and thalidomide is known to cause severe birth defects, the following requirements must be observed.

Females of childbearing potential (FCBP)[†] must agree to use two reliable forms of contraception simultaneously or to practice complete abstinence from heterosexual intercourse during the following time periods related to this study: 1) for at least 28 days before starting study drug; 2) while participating in the study; and 3) for at least 28 days after discontinuation from the study. The two methods of reliable contraception must include one highly effective method (i.e. intrauterine device (IUD), hormonal [birth control pills, injections, or implants], tubal ligation, partner's vasectomy) and one additional effective (barrier) method (i.e. latex condom, diaphragm, cervical cap). FCBP must be referred to a qualified provider of contraceptive methods if needed.

Before starting study drug:

Female Subjects:

- FCBP must have two negative pregnancy tests (sensitivity of at least 50 mIU/mL) prior to starting study drug. The first pregnancy test must be performed within 10-14 days prior to the start of study drug and the second pregnancy test must be performed within 24 hours prior to the start of study drug. The subject may not receive study drug until the Investigator has verified that the results of these pregnancy tests are negative.
- Will be warned that sharing study drug is prohibited and will be counseled about pregnancy precautions and potential risks of fetal exposure, and the Appendix: Education and Counseling Checklist must be completed by a certified RevAssist Counselor.
- Must agree to abstain from donating blood during study participation and for at least 28 days after discontinuation from the study.

Male Subjects:

- Must agree to use a latex condom during sexual contact with females of childbearing potential while participating in the study and for at least 28 days following discontinuation from the study even if he has undergone a successful vasectomy.
- Will be warned that sharing study drug is prohibited and will be counseled about pregnancy precautions and potential risks of fetal exposure, and the Appendix: Education and Counseling Checklist must be completed by a certified RevAssist Counselor.
- Must agree to abstain from donating blood, semen, or sperm during study participation and for at least 28 days after discontinuation from the study.

During study participation and for 28 days following discontinuation from the study:

All Subjects:

- Only enough lenalidomide for one cycle of therapy may be dispensed with each cycle of therapy.
- If pregnancy or a positive pregnancy test does occur in a study subject or the partner of a male study subject during study participation, lenalidomide must be immediately discontinued.

Female Subjects:

- FCBP with regular or no menstrual cycles must agree to have pregnancy tests weekly for the first 28 days of study participation and then every 28 days while on study, at study discontinuation, and at day 28 following discontinuation from the study. If menstrual cycles are irregular, the

[†] A female of childbearing potential is a sexually mature woman who: 1) has not undergone a hysterectomy or bilateral oophorectomy; or 2) has not been naturally postmenopausal for at least 24 consecutive months (i.e., has had menses at any time in the preceding 24 consecutive months).

pregnancy testing must occur weekly for the first 28 days and then every 14 days while on study, at study discontinuation, and at days 14 and 28 following discontinuation from the study.

- In addition to the required pregnancy testing, the Investigator must confirm with FCBP that she is continuing to use two reliable methods of birth control at each visit.
- Counseling about pregnancy precautions and the potential risks of fetal exposure must be conducted at a minimum of every 28 days. During counseling, subjects must be reminded to not share study drug and to not donate blood, and the Appendix: Education and Counseling Checklist must be completed by a certified RevAssist Counselor.
- Pregnancy testing and counseling must be performed if a subject misses her period or if her pregnancy test or her menstrual bleeding is abnormal. Study drug treatment must be discontinued during this evaluation.
- Females must agree to abstain from breastfeeding during study participation and for at least 28 days after discontinuation from the study.

Male Subjects:

- Counseling about the requirement for latex condom use during sexual contact with females of childbearing potential and the potential risks of fetal exposure must be conducted at a minimum of every 28 days. During counseling, subjects must be reminded to not share study drug and to not donate blood, sperm, or semen, and the Appendix: Education and Counseling Checklist must be completed by a certified RevAssist Counselor.

Appendix VII
Education and Counseling Checklist

Do Not Dispense lenalidomide (Revlimid®) if:

- **The patient is pregnant.**
- **A female of childbearing potential states pregnancy tests were not conducted.**
- **The patient states they did not use recommended effective birth control (unless practicing continuous abstinence.**

Checklist for females of childbearing potential

I verified that pregnancy tests were performed and were negative

I counseled adults and children on:

- Potential fetal harm
- Using 2 forms of effective birth control at the same time or abstaining from heterosexual intercourse
- Continuation of 2 forms of birth control if therapy is interrupted and for 4 weeks after therapy is discontinued
- FCBP with regular or no menstrual cycles must have pregnancy tests weekly for the first 28 days of study participation and then every 28 days while on study, at study discontinuation, and at day 28 following discontinuation from the study. If menstrual cycles are irregular, the pregnancy testing must occur weekly for the first 28 days and then every 14 days while on study, at study discontinuation, and at days 14 and 28 following discontinuation from the study.
- The need to stop taking lenalidomide right away in the event of becoming pregnant and to call their healthcare provider immediately. Female partners of males taking lenalidomide must call their healthcare provider right away if they get pregnant
- Possible side effects due to neutropenia, thrombocytopenia, deep vein thrombosis, and pulmonary embolism
- Not sharing medication
- Not donating blood while taking lenalidomide and for 4 weeks after stopping lenalidomide
- Not to break, chew, or open lenalidomide capsules
- Provided Medication Information Sheet for Subjects Enrolled in Clinical Research Studies using Investigational Drug Supply

Checklist for females NOT of childbearing potential (natural menopause for at least 24 consecutive months, a hysterectomy, or bilateral oophorectomy)

I counseled adults and children on:

- Possible side effects due to neutropenia, thrombocytopenia, deep vein thrombosis, and pulmonary embolism
- Not sharing medication
- Not donating blood while taking lenalidomide and for 4 weeks after stopping lenalidomide
- Not to break, chew, or open lenalidomide capsules
- Provided Medication Information Sheet for Subjects Enrolled in Clinical Research Studies using Investigational Drug Supply

Checklist for males

I counseled adults and children on:

- Potential fetal harm
- Need for contraception wearing a latex condom when engaging in sexual intercourse with a female of childbearing potential
- Possible side effects due to neutropenia, thrombocytopenia, deep vein thrombosis, and pulmonary embolism
- Not sharing medication
- Not donating blood while taking lenalidomide and for 4 weeks after stopping lenalidomide
- Not to break, chew, or open lenalidomide capsules
- Provided Medication Information Sheet for Subjects Enrolled in Clinical Research Studies using Investigational Drug Supply

Counselor Signature:_____Date:_____

**Keep a copy of this checklist with the prescribing/dispensing records. **

Appendix VIII
Lenalidomide Information Sheet

FOR PATIENTS ENROLLED IN CLINICAL RESEARCH STUDIES

Please read this Lenalidomide Information Sheet before you start taking lenalidomide and each time you get a new supply, since there may be new information. This Lenalidomide Information Sheet does not take the place of an informed consent to participate in clinical research or talking to your study doctor or healthcare provider about your medical condition or your treatment.

What is the most important information I should know about lenalidomide?

1. **Lenalidomide may cause birth defects (deformed babies) or death of an unborn baby.** Lenalidomide is similar to the medicine thalidomide. It is known thalidomide causes life-threatening birth defects. Lenalidomide has not been tested in pregnant women but may also cause birth defects.

If you are a female who is able to become pregnant:

- **Do not take lenalidomide if you are pregnant or plan to become pregnant**
  - for 28 days before starting lenalidomide
  - while taking lenalidomide
  - during dose interruptions of lenalidomide
  - for 28 days after stopping lenalidomide
- **Stop taking lenalidomide if you become pregnant during lenalidomide treatment**
- **Do not breastfeed while taking lenalidomide**
- **You must have pregnancy testing done at the following times:**
  - within 10 – 14 days and again 24 hours prior to the first dose of lenalidomide
  - weekly for the first 28 days
  - every 28 days after the first month or every 14 days if you have irregular menstrual periods
  - if you miss your period or have unusual menstrual bleeding
  - 28 days after the last dose of lenalidomide (14 and 28 days after the last dose if menstrual periods are irregular)
- **You must practice complete abstinence or use two reliable, separate forms of effective birth control at the same time:**
  - for 28 days before starting lenalidomide
  - while taking lenalidomide
  - during dose interruptions of lenalidomide
  - and for 28 days after stopping lenalidomide
- Female partners of males taking lenalidomide should be advised to call their own physician right away if they get pregnant.

- Study doctors, healthcare providers and patients should report all cases of pregnancy to Celgene Corporation at [redacted].

If you are a male:

It is not known if lenalidomide passes into semen.

- Male patients, including those who have had a vasectomy, must use a latex condom during sexual intercourse with a pregnant female or a female that can become pregnant:
  - While you are taking lenalidomide

- for 28 days after you stop taking lenalidomide
- **Male patients should not donate sperm or semen** while taking lenalidomide and for 28 days after stopping lenalidomide.

2. **Lenalidomide may cause a reduction in the number of white blood cells and platelets.** This can lead to increased risk of infection and bleeding. You may need a blood transfusion or certain medicines if your blood counts drop too low. You will have blood tests done as part of the clinical research trial in which you are participating. This is discussed in the informed consent document.
3. **Lenalidomide may cause an increased chance for blood clots in the veins and in the lungs.** Call your study doctor or get emergency medical care right away if you get the following signs or symptoms:
   - shortness of breath
   - chest pain
   - arm or leg swelling

4. **Lenalidomide restrictions in sharing lenalidomide and donating blood:**

   - **Do not share lenalidomide with other people**
   - **Do not give blood** while you take lenalidomide and for 28 days after stopping lenalidomide
   - You will get no more than a 28-day supply of lenalidomide at one time

   Additional information is provided in the informed consent form and you can ask your study doctor for more information.

Appendix IX

MEDICATION DIARY

Name _____ Study No. _____
Mayo Clinic No. _____

Cycle 1

Please complete this diary on a daily basis. Write in the amount of the dose of Lenalidomide that you took in the appropriate "Day" box. Lenalidomide capsules should be swallowed whole, and should not be broken, chewed or opened. If a dose is missed, it should be taken as soon as possible on the same day. If it is missed for the entire day, it should not be made up. If you take more than the prescribed dose of lenalidomide, seek emergency medical care if needed and contact study staff immediately.

If the capsules are thrown up, this should be noted on your diary but you should not take another capsule until your next scheduled dose.

On the days that you do not take any study drug, please write in "0". If you forget to take your daily dose, please write in "0", but remember to take your prescribed dose at the next regularly scheduled time.

Week of: _____

Study Drug	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Lenalidomide							

Week of: _____

Study Drug	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
Lenalidomide							

Week of: _____

Study Drug	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 21
Lenalidomide							

My next scheduled visit is: _____
If you have any questions, please call: _____

_____ _____
(Date) (Participant's Signature)

Area Below Only To Be Completed only by Coordinator

Number of pills returned_____ Study Coordinator Initials_____

Date_____ Discrepancy Yes____ No_____

MEDICATION DIARY

Name _____ Study No. _____
Mayo Clinic No. _____

Cycles 2 and beyond

Please complete this diary on a daily basis. Write in the amount of the dose of Lenalidomide and AT-101 that you took in the appropriate "Day" box. AT-101 will be taken twice a day (once in the morning and once in the evening) and you should record the time you take each dose. Lenalidomide capsules should be swallowed whole, and should not be broken, chewed or opened. If a dose is missed, it should be taken as soon as possible on the same day. If it is missed for the entire day, it should not be made up. If you take more than the prescribed dose of lenalidomide, seek emergency medical care if needed and contact study staff immediately.

If the capsules are thrown up, this should be noted on your diary but you should not take another capsule until your next scheduled dose. You should not take oral iron supplements (including nutritional supplements containing iron) while taking AT-101.

On the days that you do not take any study drug, please write in "0". If you forget to take your daily dose, please write in "0", but remember to take your prescribed dose at the next regularly scheduled time.

Week of: _____

Study Drug	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Lenalidomide							
AT-101	AM	AM	AM				
	PM	PM	PM				

Week of: _____

Study Drug	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
Lenalidomide							

Week of: _____

Study Drug	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 21
Lenalidomide							

My next scheduled visit is: _____
If you have any questions, please call: _____

_____ _____
(Date) (Participant's Signature)

Area Below Only To Be Completed only by Coordinator

Number of pills returned_____ Study Coordinator Initials_____

Date_____ Discrepancy Yes____ No_____